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Role of Silica in Phytoplankton Succession: An Enclosure Experiment in the Downstream Nakdong River (Mulgum)

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ABSTRACT: To understand the mechanism of phytoplankton succession in the Nakdong River, the resource availability (silica) and grazing effect on the phytoplankton community were investigated in an enclosure experiment at Mulgum in March 1995. In all enclosures, *Stephanodiscus hantzchii* was dominant during the first week. Two weeks later, the diatom community in the A (river water only) and B (filtered river water) enclosures was shifted to colonial green algae (*Actinastrum* sp., *Pediastrum* spp. and *Scenedesmus* spp.) and nanoplankton (2~3 μm of diameter) due to the silica depletion. In the C (silica addition in river water, 3 mg l^{-1} $week^{-1}$) and D (silica addition in filtered water) enclosures, *Fragilaria crotonensis* and *Synedra acus* increased as the silica addition was continued. The percentage of small phytoplankton (size, 10~13 μm) in the filtered enclosures (B and D) was much higher than that of A and C enclosures. A laboratory bottle experiment conducted in the fall of 1994 also showed similar results. Therefore, it is concluded that silica and zooplankton are important regulators in phytoplankton succession during the diatom blooming season in the Nakdong River.

Key Words: Enclosure experiment, Nakdong River, Phytoplankton succession, Silica, Zooplankton

INTRODUCTION

Silica concentration and its biogenic cycling in lentic ecosystems are major factors in diatom-community regulation (Tilman 1977, Sell *et al.* 1984). During the last ten years, factors controlling the shift of dominant species within the diatom community along with the change of nutrient regimes have been a subject of the phytoplankton succession study (Moed *et al.* 1976, Donk and Kilham 1990). Kilham *et al.* (1986) proposed Si:P gradient hypothesis to explain competitive advantage of certain diatom species. Zooplankton grazing also imposes a rhythmic pattern on both loss of algae and regeneration of nutrients (Wetzel 1983, Sommer 1985, Lampert *et al.* 1986). Especially, the spring and fall reduction of silica concentration and the corresponding phytoplankton community shift from diatoms to green algae were reported in the various types of lentic environment.

In contrast to the lentic environment, mean dissolved silica concentration in the river system appears to be remarkably constant, compared with other major dissolved constituents. Silica contents of river water have been reported to be remarkably uniform and respond little to changes in discharge rates in natural river systems (Edwards and Liss 1973). In most large rivers with temperate hydrological regimes, a bloom dominated by diatoms usually occurs after the decrease of discharge in spring, whereas a mixed population of green algae and diatoms composes the early summer phytoplankton community. However, experimental approaches to study this mechanism of phytoplankton succession in large rivers are still poorly understood (Descy *et al.* 1987, Garnier *et al.* 1995).

The lower Nakdong River has been characterized by a strong seasonality of silica (SiO_2) concentration, probably due to the regulation of discharge by locks and dams (Joo 1995). In the early spring and late fall, when diatom actively reproduces, silica (SiO_2) concentration was often at its limit of detection (Ha *et al.* 1998). The previous monitoring studies in this river showed a clear seasonal change of diatom community caused by zooplankton grazing and silica dynamics in spring and fall (Joo *et al.* 1997, Ha *et al.* 1999). Thus, this study was carried to investigate the phytoplankton community shift by silica (SiO_2) availability and grazing effect through enclosure experiments in the field and

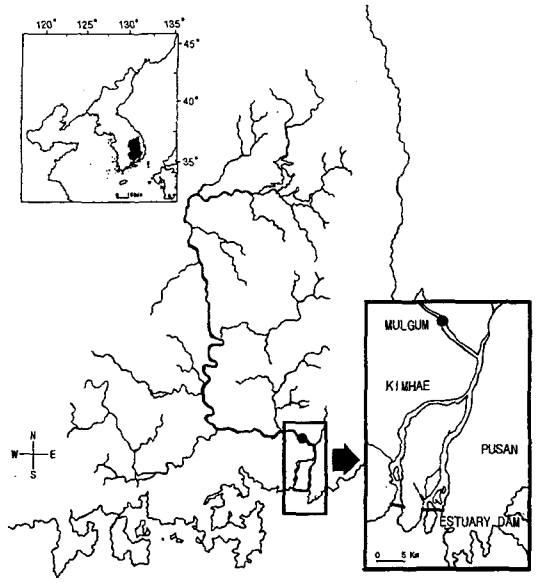

Fig. 1. Map showing the basin of the Nakdong River, major tributaries, and the study site. ●: Mulgum

laboratory.

DESCRIPTION OF THE STUDY SITE

Main channel of the Nakdong River is approximately 528 km long. The drainage area (23,817 km^2) is situated in the range of 35~37° N and 127~129° E. The study site is located 27 km upward from the estuary dam at the mouth of the Nakdong River (maximum depth of ca. 10 m; mean depth 3~4 m; river width of 250~300 m) (Fig. 1). Water intake and purification facilities are concentrated in this area. Because of high water demand in the region, modification of the river has been accelerated during the last three decades. These physical alterations have accelerated eutrophication in the lower part of the river (river kilometer (RK) 0 from the estuary dam to RK 40 upward from the estuary dam) (Song 1992). Trophic status (chl. *a*) is hypertrophic condition (58±148 μg/l, 1993~1998, n=236) according to OECD (1982) criteria (Kim *et al.* 1998).

MATERIALS AND METHODS

Twelve plastic enclosures (20 l, 40 cm × 30 cm × 20 cm) of four series (A, river water only; B, river water filtered with a 67 μm mesh size screen (Wild Co.); C, silica addition in the unfiltered water; D, silica addition in filtered river water; n=3) were set up at Mulgum in March 1995 when phytoplankton biomass and silica concentration of the river water were low (chl. *a*, 25 μg/l; silica concentration, 0.8 mg/l; water temperature, 11.3℃). Rotifer, *Brachionus angularis*, *B. calyciflorus* and *B. rubens*, were found in this season, as they raised to dominate zooplankton community. In the C and D enclosures, 3 mg/l of silica ($Na_2SiO_3 \cdot 5H_2O$, M.W.=216.16) was added once every week for four weeks. Phytoplankton species, biomass (chl. *a*), and nutrient (SiO_2, DIN, NO_3-N, NH_4-N, TP and SRP) concentrations were examined weekly for the experimental period. During the course of this experiment, all physico-chemical parameters were determined and compared with those of field situation.

A laboratory experiment was conducted in November, 1994. The river water (chl. *a*, 28 μg/l; silica concentration, 0.5 mg/l; dominant species, *Cyclotella stelligera*) was brought to the laboratory (air temperature, 17°C) and twelve plastic bottles (2 l) were set up (A, river water only; B, river water filtered with a 67 μm mesh size screen (Wild Co.); C, silica addition in the unfiltered water; D, silica addition in filtered river water; n=3). This bottle experiment was conducted after one time input of 12 mg/l of silica at silica-added enclosures (C and D enclosures). The composition of phytoplankton species was analyzed on the 3rd, 7th, 13th and 22nd day.

Nutrient (DIN, NO_3-N, NH_4-N, TP, SRP and SiO_2) concentrations were determined by filtering water samples and freezing the filtrates, which were analyzed later by QuickChem Automated Ion Analyzer® (NO_3-N, No. 10-107-04-1-O; NH_4-N, No. 10-107-06-1-B; TP, SRP, No. 10-115-01-1-B; and SiO_2, No. 10-114-27-1-A). Algal biomass (chl. *a*) was determined using a monochrometric method (Wetzel and Likens 1991, American Public Health Association 1995). Water samples for phytoplankton analyses were collected by the same method as the physico-chemical parameters. The phytoplankton samples were immediately preserved after collection with Lugol's solution. Identification of phytoplankton species was conducted under the light microscope (× 1,000) and based on the following keys: Cassie (1989), Foged (1978) and Round *et al.* (1990). Phytoplankton was enumerated using an Inverted Microscope (Zeiss) by the Utermöhl's sedimentation method (1958). Biovolumes were calculated for each species using the geometric shapes of most closely matching the cell shape.

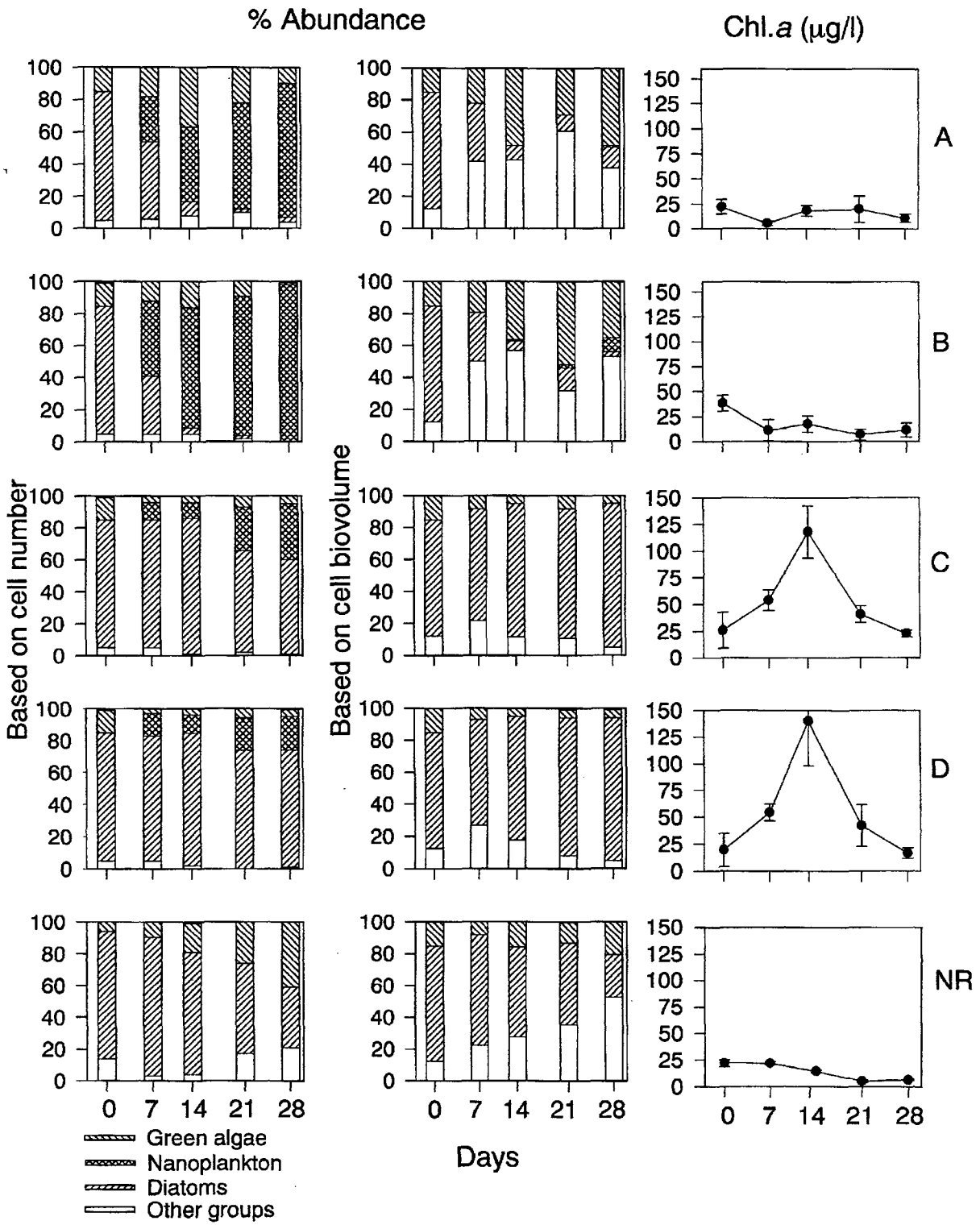

Fig. 2. Relative abundance of phytoplankton (based on cell density, cell biovolume; average of triplicate) and change of chl. *a* (mean±SD, n=3) in field enclosure experiment (day 0: March 24, 1995) (A, untreated river water; B, river water filtered with a 67 μm mesh size screen; C, silica addition in the unfiltered river water; D, silica addition in filtered river water; NR, Nakdong River water).

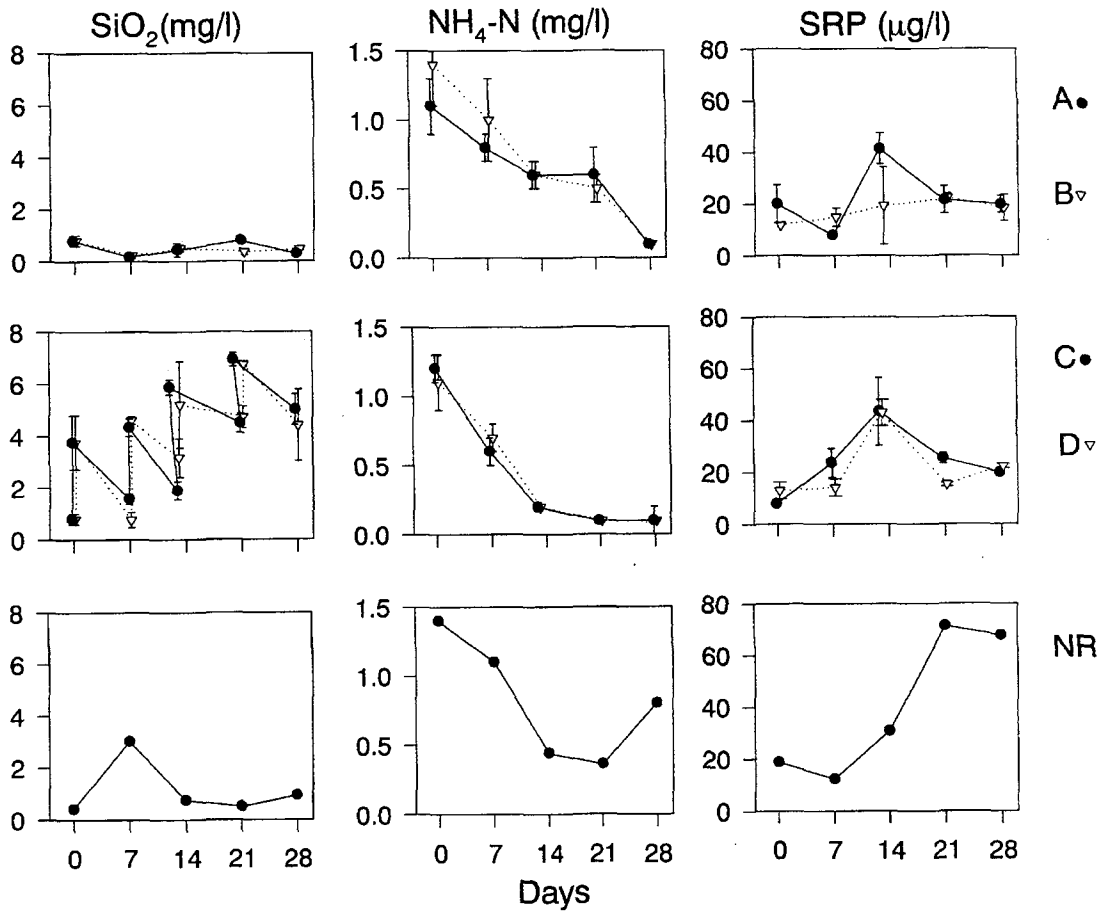

Fig. 3. Changes of nutrient (SiO_2, NH_4-N and SRP; mean±SD, n=3) concentrations for the entire experimental study period (day 0: March 24, 1995). A, B, C, D, NR: see text for further explanation.

RESULTS AND DISCUSSION

Changes in phytoplankton community

Cell density and biovolume of phytoplankton in the silica-added enclosures were quite different from those of non-added enclosures. In the C and D enclosures, diatoms continued to grow for the first two weeks, while in the A and B enclosures decline of diatom and green algae was observed (Fig. 2). The cell density and biovolume in the silica-added enclosures were several times higher than those in non-added enclosures. Changes of cell density and biovolume in the filtrate treatments (B and D) were less drastic than the effects of silica manipulation. Relatively low algal biomass (chl. *a*, 20 μg/l) was maintained in the A and B enclosures during the experiment. Chl. *a* of the C and D enclosures increased sharply in the first two weeks and declined afterwards. The peak biomass of the silica-added enclosures was 4~5 times higher than the initial ones.

In the A and B enclosures, diatoms were replaced with nanoplankton (average cell diameter, 2~3 μm) and colonial green algae. After four weeks, over 90% of the total cell was consisted of nanoplankton. Blue-green and green algae were dominant groups based on cell biovolume in A enclosures, while green algae and cryptomonads were common in the B enclosures. In the silica-added enclosures (C and D) diatom community dominated both in cell density and biovolume for the entire study period. In the second week, nanoplankton also started to appear.

Nutrient concentrations

With the addition of silica, minor changes were observed in nutrient (e.g., DIN, NO_3-N, TP and SRP) concentrations, except for ammonia (NH_4-N). Ammonia concentration in silica-added enclosures (C and D enclosures) decreased faster than the untreated enclosures (A and B

enclosures) (Fig. 3). DIN and NO_3-N were maintained at 4 and 3 mg/l, respectively, throughout the experiment. Ammonia concentration in all four series were sharply decreased in the first two weeks. Lampert *et al.* (1986) showed that the peak of ammonia coincided with the maximum density of zooplankton. However, the experiments showed that the concentration of ammonia in the C and D series decreased. It was assumed that the great amounts of diatoms consumed ammonia.

After one week, silica concentration was considerably lowered to 0.2 mg/l in the A and B enclosures. In spite of weekly addition of silica in the C and D enclosures, the concentration was relatively low (1~1.5 mg/l) for the first two weeks, but silica began to accumulate (4.5~5.5 mg/l) after three weeks. SRP concentration peaked at day 14 and then decreased gradually as the result of uptake by phytoplankton. Si:P ratio has been known to be an important factor that determines regulation of diatom species composition (Petersen 1975, Smith and Kalff 1983, Sommer 1985, Kilham and Hecky 1988). At the beginning of this experiment, the Si:P ratio of all enclosures was about 30:1. After four weeks, the ratio decreased to 10:1 in A and B and increased to 230:1 in the C and D enclosures.

Dominant species

Dominant phytoplankton species were remarkably different between the silica-added and non-added enclosures (Fig. 4). The density change of *Stephanodiscus hantzschii, Fragilaria crotonensis, Synedra acus, Scenedesmus* spp., *Actinastrum hantzschii, Pediastrum* spp. and nanoplankton were conspicuous in all four series. The effect of silica manipulation on the composition of phytoplankton species was stronger than that of zooplankton manipulation. In the A and B enclosures (silica concentration, 0.2~0.8 mg/l), only *Stephanodiscus hantzschii* increased rapidly during the first week ($1.4\times10^3 \rightarrow 1.4\times10^4$ cells/ml). After two weeks, colonial green algae *Actinastrum hantzschii, Pediastrum* spp. and *Scenedesmus* spp. became the dominant species. After four weeks, nanoplankton with circle and eyebrow forms (2~3 μm) increased to 3.2×10^5 cells/ml in the B enclosures. In silica-added enclosures (C and D), *Stephanodiscus hantzschii* was dominant (80% of total cell number). Initial density was 1.4×10^3 cells/ml and the density increased to $8.2 \sim 9.0\times10^4$ cells/ml after 2 weeks. However, the cell density of *Fragilaria crotonensis* and *Synedra acus* increased (> 85%) at the end of experiment. The shift of diatom structure may have occurred due to the interspecific competition for silica within diatom community. When the silica concentration was relatively low (0.5~1.5 mg/l), small *Stephanodiscus hantzschii* rapidly reproduced. *Fragilaria crotonensis* and *Synedra acus*, which are large and reproduce slowly, might have a competitive ability as silica concentration was gradually elevated.

Si:P ratio was used to explain the competitive ability of the diatom species (Kilham and Kilham 1990). *Synedra* spp. were at the high end with the highest Si requirements and the lowest P requirements (high Si:P), *Nitzschia* spp. were intermediate and the *Stephanodiscus* spp. were at the low end with the lowest Si requirements and highest P requirements (low Si:P) by culture experiment. Tilman *et al.* (1982) established a rank of competitive superiority for limiting P among diatoms as follows: *Synedra filiformis* > *Asterionella formosa* > *Fragilaria crotonensis* > *Diatoma elongatum* > *Stephanodiscus minuta* > *Cyclotella meneghiniana.* Particularly, large diatom species tended to choose the relatively high Si:P ratio. Sommer (1985) showed *Synedra acus* was a stable species from the Si:P=40:1 to 200:1 and *Fragilaria crotonensis* from the Si:P ratio of 40:1 to 140:1. Without the silica limitation, *Synedra acus* was known to be the most successful competitor. Our results of enclosure experiment showed similar trends. *Stephanodiscus hantzschii* was dominant in this study when Si:P ratio was much lower (10:1~30:1). Whereas, large diatom species (>150 μm) appeared when Si:P ratio was relatively high (200:1~250:1). After four weeks, 230:1 of Si:P ratio and large diatoms, such as *Fragilaria crotonensis* and *Synedra acus*, were observed in C and D enclosures.

When the zooplankton density in the A enclosures was high, density of small algae (diameter, 10~13 μm: *Cyclotella stelligera, C. meneghiniana* and nanoplankton) was reduced and the growth of large green algae (*Pediastrum* sp., *Golenkinia* sp. and *Micratinium* sp.) was stimulated. It was assumed that zooplankton in the A enclosures preferentially grazed small edible algae (nanoplankton and *Cyclotella*), whereas they could not easily feed large gelatinous colonial green algae. Selective grazing of zooplankton was also observed in the silica added C enclosures. The species composition of diatom community in the C and D enlosures was relatively similar. The proportion of large colonial green algae was larger in the zooplankton present in C enclosures. Similar study demonstrated that zooplankton had an effect on the composition of the phytoplankton species thr-

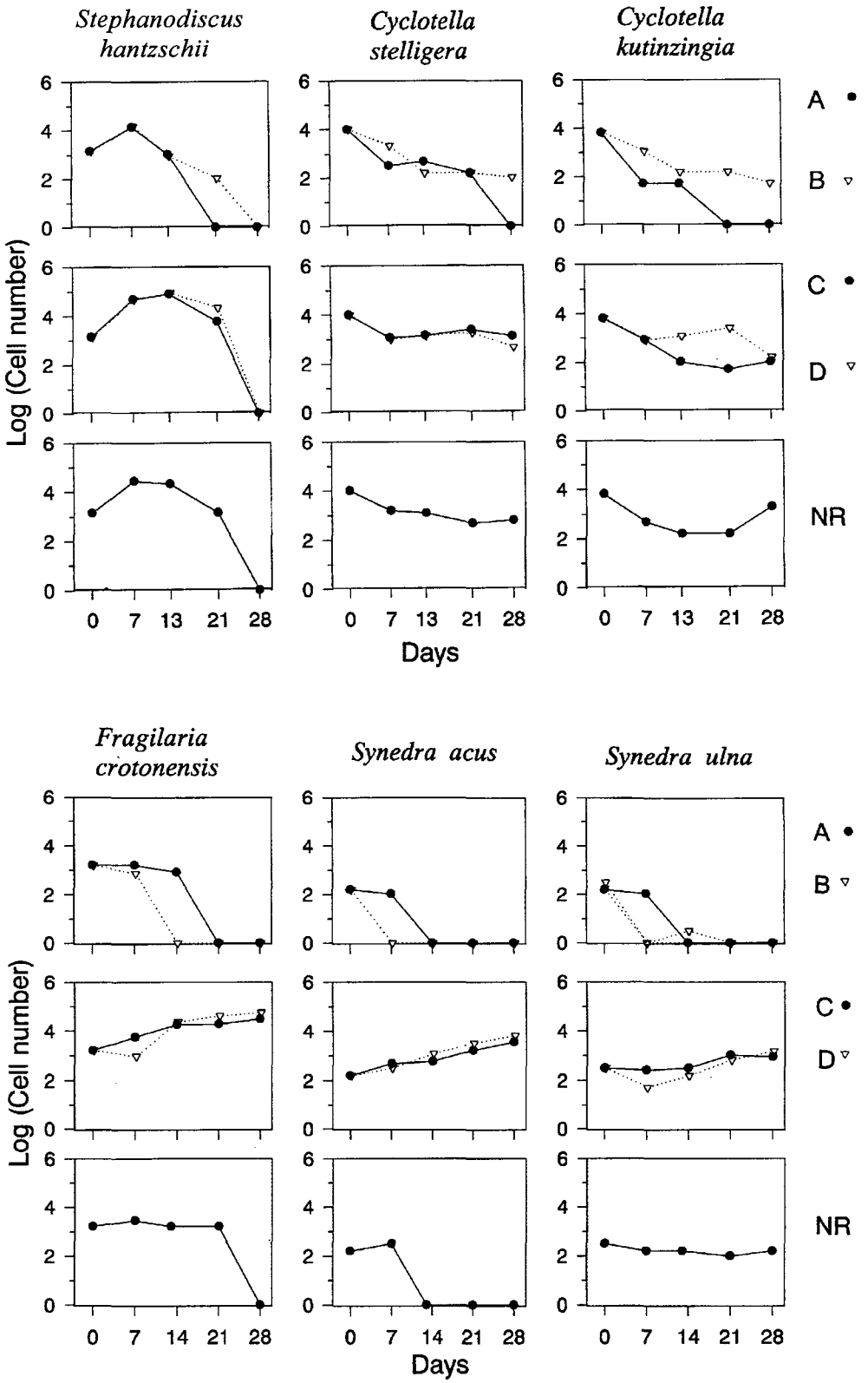

Fig. 4. Changes of dominant species in the field enclosure experiment (day 0: March 24, 1995), expressed as log cell numbers. A, B, C, D, NR: see text for further explanation.

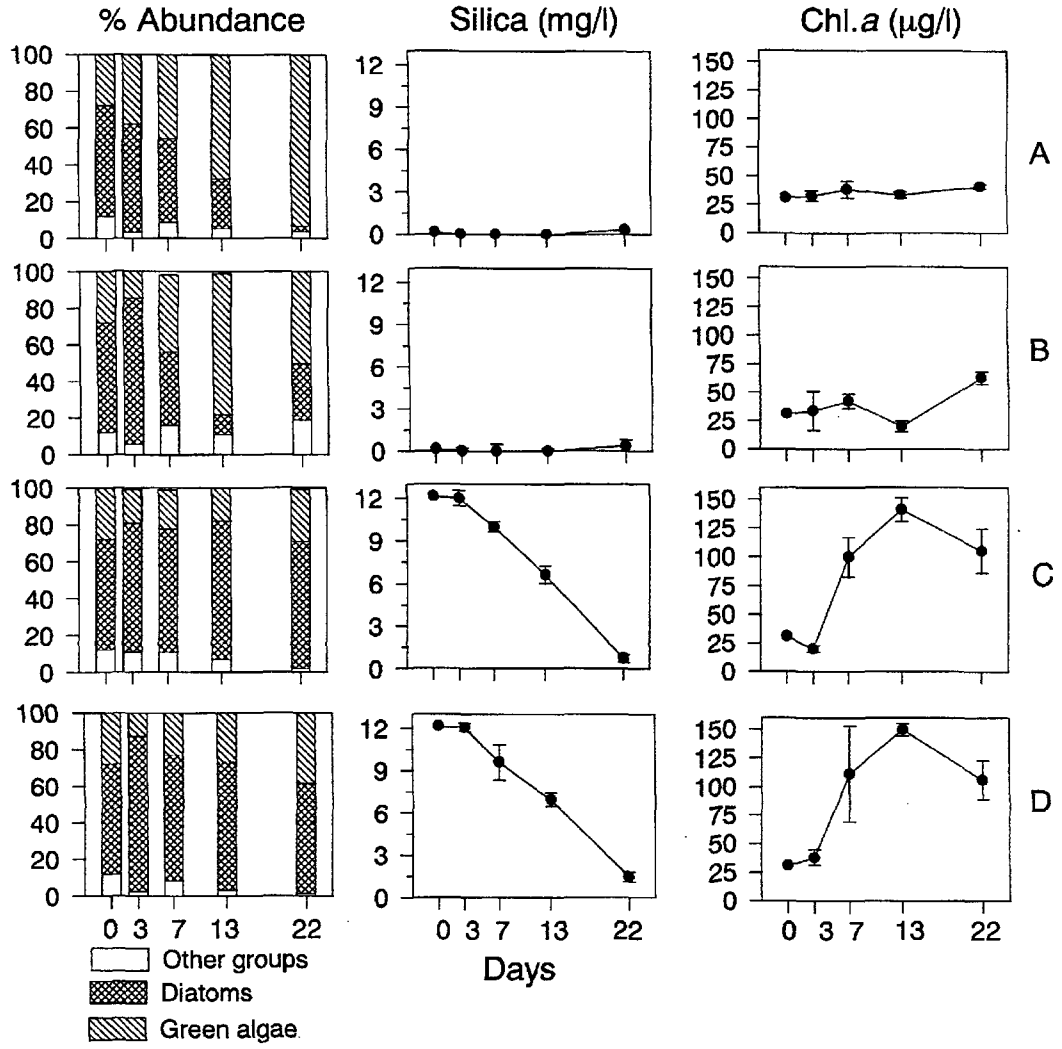

Fig. 5. Percent abundance of phytoplankton community (based on cell number) and changes of silica concentration (mean±SD, n=3) and chl. *a* (mean±SD, n=3) in laboratory bottle experiment (day 0, November 15, 1994). (A, untreated river water; B, river water filtered with a 67 μm mesh size screen; C, silica addition in the unfiltered water; D, silica addition in filtered water).

ough selective grazing and increase of nutrient by regeneration (Vyhnalek 1983, Lehman and Sandgren 1985). However, their results showed that zooplankton did not seem to strongly affect nutrients concentration, but did affect the composition of phytoplankton species.

Laboratory enclosure experiment

A laboratory experiment was conducted after one time input of 12 mg/l of silica at silica-added enclosures (C and D enclosures) in the fall of 1994 (Fig. 5). The change of the phytoplankon community in the laboratory experiment was similar to enclosure experiment performed in 1995. In the silica-added enclosures (C and D), silica concentration and species composition were not changed until day 3. After one week later, silica concentration dropped to 3 mg/l per week.

On the day 22, green algae density increased to 30~40% of the total cells. At the beginning of experiments, *Cyclotella* spp. (<12~15 μm of diameter) was dominant. After the day 13, large *Synedra acus* (150~200 μm of length) and *Cyclotella* sp. (ca. 35 μm of diameter) became dominant species (> 85%, based on cell number). In non-added enclosures (A and B), silica was almost depleted (undetectable) on the day

3. Diatom dominance (mainly, *Cyclotella* spp.) was maintained for the first week. After the day 13, diatom community shifted to green algae (*Scenedesmus* spp., *Coelastrum* sp. and *Golenkinia* sp.). From this laboratory experiment along with *in situ* study, it is concluded that silica plays an important role in regulating shift of diatom species during the diatom blooming seasons (spring and fall).

Seasonal species shift in the field situation

Seasonal changes of silica concentration in the Nakdong River were remarkable (from undetectable to over 10 mg/l) compared to the relatively conservative nature of silica concentration from other river systems (Edwards and Liss 1973, Obeng 1981, Decamps *et al.* 1984). The precipitation event and hydrologic regime would be important factors in determining silica concentration. Dominant species of diatom community (especially, *Stephanodiscus hantzschii, Synedra acus* and *Fragilaria crotonensis)* seemed to be strongly regulated by silica dynamics in the Nakdong River. When the silica level was less than 1.5 mg/l or at the detection limit (from March to April in 1994, 1995; from December of 1994 to January of 1995), *Stephanodiscus* increased. It was shown that this species had a competitive ability at a relatively low silica level (Descy *et al.* 1987)

When the 'enclosure experiment' was begun (March 24, 1995), diatom community was dominant and silica concentration was relatively low (0.8 mg/l). Increase of *Stephanodiscus hantzschii* ($1.4\times10^3 \rightarrow 2.5\times10^4$ cells/ml) and reduction of *Cyclotella* spp. ($3.9\times10^4 \rightarrow 3.2\times10^2$ cells/ml) was observed on March 24 and on April 6. Density increase of diatom would be triggered by the decrease of silica concentration. In mid April, algal biomass dropped to 7~10 μg/l due to its frequent precipitation. The shift from diatom dominance to the mixture of diatoms, green algae and cryptomonads (*Cryptomonas erosa* and *Micratinium pusillum*) was observed during this time. Water temperature, light intensity and zooplankton grazing would play an important role in phytoplankton community shift.

Peak by large diatoms occurred with a low water temperature (about 10℃) and elevated silica concentration. When silica concentration was high (over 5 mg/l) in the river, *Aulacoseira* spp., *Synedra acus* and *Fragilaria crotonensis* tended to occur. However, because of short duration of high silica concentration in the river, these species would hardly become dominant. Sharp reduction in algal biomass after the diatom bloom would be possibly related to silica depletion or zooplankton grazing. Actually, short-period (one or two weeks) of low chl. *a* and high transparency (> 100 cm of Secchi disc transparency) was repeatedly observed in mid-spring (May) in the lower Nakdong River. Grazing by different zooplankton in the river system would also be an important factor in determining the response of algal assemblage. Although the experiments were not designed to simulate river conditions, it is worthy of understanding of succession pattern under *in situ* condition. More detailed experimental approach on grazing on the changes in hydrology and trophic gradients in regulated rivers would be required to clarify.

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