Korean J. Limnol. 41 (1) : 26~33 (2008)

The Effect of Water Temperature on Proliferation of *Stephanodiscus* sp. *in vitro* from the Nakdong River, South Korea

Kim, Myoung-Chul, Geung-Hwan La, Hyun-Woo Kim[1], Kwang-Seuk Jeong, Dong-Kyun Kim and Gea-Jae Joo*

(Department of Biology, Pusan National University, Busan 609-735, Korea, [1]Department of Environmental Education, Sunchon National University, Suncheon 540-742, Korea)

To understand the effect of water temperature on growth pattern of *Stephanodiscus* sp., we weekly or biweekly investigated in the lower part of the Nakdong River from 1994 to 2006 and performed a laboratory experiment. *Stephanodiscus* was the most dominant species among phytoplankton in winter when low flow persisted and the high abundances of the species were maintained from December to February. Three strains of *Stephanodiscus* sp. were isolated for the *in vitro* experiment from the Nakdong River in January 2005. Over the water temperature range of 4°C to 20°C, the growth patterns of *Stephanodiscus* sp. were different in the short-term batch culture. The maximum cell density of *Stephanodiscus* sp. was observed at approximately 5°C in the river systems, but the optimum water temperature of *Stephanodiscus* sp. was 10°C for the growth in the laboratory experiment. However, the proliferation of *Stephanodiscus* sp. was related to low water temperature in the Nakdong River.

Key words : *Stephanodiscus*, diatom, water temperature, zooplankton grazing, Nakdong River

INTRODUCTION

Phytoplankton proliferation in river systems was well known as important environmental problems, reported in diverse rivers in the world (Paerl, 1987; Marker and Collett, 1997; Rose and Balbi, 1997; Mitrovic *et al.*, 1999). Eutrophication and channel modification in river systems are recognized as crucial causes for the proliferations of plankton communities (Ha, 1999; Kim, 1999). The Korean rivers are intensively utilized and modified by human beings in order to satisfy high water demand and reduce damages by summer flooding. These circumstances resulted in serious proliferations of phytoplankton in river systems (Ha *et al.*, 1998).

The centric diatom *Stephanodiscus* is a characteristic phytoplankton, considered as an important indicator species of eutrophication in large rivers during the winter (Ha *et al.*, 2002). The *Stephanodiscus* blooms are reported annually in South Korea, especially in the Paldang Reservoir and Nakdong River (Ha *et al.*, 2002; Han *et al.*, 2002; Hong *et al.*, 2002). The magnitude of *Stephanodiscus* blooms in the Korean freshwater systems is rare case compared with other freshwaters in the world (Ha *et al.*, 1998). The species

* Corresponding author: Tel: 051) 510-2258, Fax: 051) 581-2962, E-mail: gjjoo@pusan.ac.kr

proliferation causes high cost at water treatment facilities such as filter clogging and low efficiency of coagulation and sedimentation (Lim *et al.*, 2000).

From field studies, centric diatoms were believed to become dominant in water bodies by increased nutrient concentrations, low water temperature, and flow conditions in river systems (Ha *et al.*, 2002). An empirical modeling work provided simulated results for the environmental characteristics (Jeong *et al.*, 2003). Among the environmental parameters, water temperature is considered as the primary factor for the proliferations of centric diatoms, however, up to date there were rare evidences for the relationship between water temperature and proliferations of *Stephanodiscus* in river systems.

In this study, a field monitoring and laboratory experiments were simultaneously conducted to determine the influence of water temperature on the changes of *Stephanodiscus* growth. The water temperature resulting in proliferations of the species were detected from a long-term monitoring data, and it was compared with the optimal temperature from the laboratory experiments.

MATERIALS AND METHODS

1. Description of the study site

The Nakdong River, located in the southeastern region of the Korean Peninsula, is the second largest river system in South Korea (river length, 526 km; drainage area, 23,817 km^2). Its watershed lies between 35°N~37°N and 127°E~129 °E. Over 60% of the total annual precipitation (1,000~1,200 mm) occurred in summer (from June to September) due to the monsoon and several typhoons. This river has a typical characteristic of 'flow regulation', meaning hydrologically regulated by a series of multipurpose dams in the tributaries and middle part of the river, and by the estuarine barrage. The flow regulation by dams and barrage was responsible for the acceleration of eutrophication (Joo *et al.*, 1997). The study site (Mulgum) is located 27 km upstream from the estuarine barrage (mean depth, 4~5 m; river width, 250~350 m) (Kim and Joo, 2000).

2. Limnological parameters and phytoplankton collection

A weekly monitoring programme was maintained from 1994 to 2006 in order to observe the limnological dynamics at the study site. Among the data set, we utilized water temperature (measured by YSI model 58). Basic hydrological information was considered, and the local precipitation data for the study period were obtained from the Korea Meteorological Administration, and the amount of precipitation was the average value of 5 regions (i.e., Miryang, Andong, Daegu, Hapchun, Jinju). The discharge data at the Samrangjin station were obtained from the Nakdong River Flood Control Office. Phytoplankton samples at the study site were immediately preserved with Lugol's solution. Utermöhl's sedimentation method (Utermöhl, 1958) was used to identify and enumerate phytoplankton. Biovolume was estimated from mean cell dimensions and cellular shape of each species (Wetzel and Likens, 1991). Phytoplankton identification was based on morphological characteristics.

3. Organism and culture conditions

The strain of *Stephanodiscus* sp. used in this study was isolated from the lower Nakdong River in January 2005. Water samples were collected and stored in the shade at ambient temperatures until return to the laboratory. Three strains of *Stephanodiscus* sp. were isolated from water samples. We used modified macro-pipette method (Throndsen, 1969; Guillard, 1973) on an inverted microscope for isolation of *Stephanodiscus* sp. single cell. The separated single cells were cultured in well plates with diatom medium (DM). After 10 days, we selected three strains which were well grown in each plate and moved to 300 mL flasks for mass culture respectively. We designated NRS (Nakdong River *Stephanodiscus*)-1, 2, 3 of each strains as replications. Axenic cultures were conducted by the treatment of streptomycin sulfate that were gradient concentrations. *Stephanodiscus* sp. cultures were maintained by serial transfer of the above medium every 14~16 day until experiments. The stock cultures were maintained in 300 mL conical flask containing 150 mL of sterilized diatom medium at 15°C, 50 μmol photons m^{-2} s^{-1} with 12 : 12 (L : D) cycle. The physiological responses of the diatom *Stephanodiscus* sp. to temperature were determined over the temperature range of 4°C to 20°C using short-term batch culture methods. To determine the effect of acclimation on behavior, cells were cultured at different temperatures (4, 10, 15 and 20°C). We used the each stock culture in different temperatures on exponential phase. Initial inoculation cell densities were about 1,000 cells mL^{-1} in all conical flasks. The experiment was conducted for 20 days. The conical flasks were shaken manually three times a day and their locations were randomly changed in growth chamber once a day. *Stephanodiscus* sp. cells were counted in every second day using the Sedgwick-Rafter chamber (McAlice, 1971). The growth rate (μ) was calculated by Fogg and Thake (1987) from 0 to 20th day.

$$\mu=\frac{\ln N_t-\ln N_0}{dt}$$

where N_0 is initial cell number, N_t is cell number

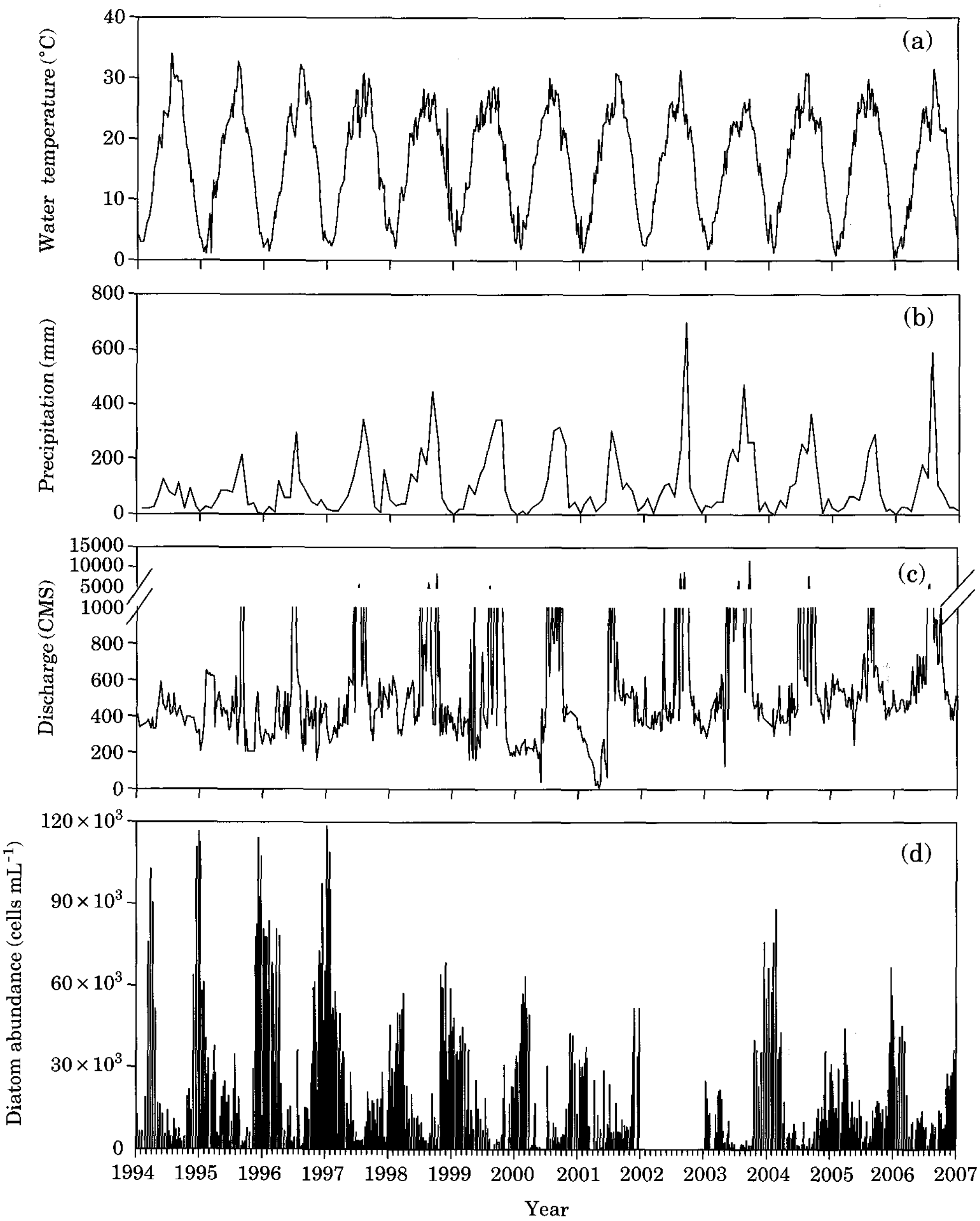

Fig. 1. Long-term changes of limnological parameters (a-water temperature, b-precipitation, c-discharge, d-diatom abundance) (no data in 2002).

at t^{th} day, t is the number of day.

RESULTS

1. Physico-chemical environments and diatom succession in the study sites

The diatom dynamics were apparently influenced by the temperature and river flow (Fig. 1). There were shown the seasonal changes of centric diatom during the study periods. The high abundances of diatom was occurred and maintained at low water temperature (4~8°C) during the winter (December~February; Fig. 1a). In winters, water flow was highly stable than that of summer, which was concentrated more than 50% of total annual precipitation (Fig. 1b, c). The diatom was repeatedly proliferated under the conditions of low water temperature and low discharge (Fig. 1d, no data in 2002). The bio-volume of *Stephanodiscus* sp. occupy ca. 42% of total diatom bio-volume, and especially over 90% in winters.

There was high probability of *Stephanodiscus* sp.'s proliferations when water temperature was below 10°C (Fig. 2). The peak cell density of *Stephanodiscus* sp. was found mostly from December to February. Water temperature average in the late autumn (November) was about 12°C and then *Stephanodiscus* abundance was drastically increased. High abundance of *Stephanodiscus* was persisted until the next March.

2. The growth curves of *Stephanodiscus* sp.

The growing pattern of *Stephanodiscus* sp. in the temperature 4°C is shown in Fig. 3a. The lag phase was observed up to the eighth days and after then the exponential phase was persisted until the twentieth days and there was no stationary phase during this experiment. In the twentieth days, the cell densities of NRS-1, 2 and 3 were 127,180 cells, 121,980 cells, 110,220 cells per milliliter respectively. The growth rates (μ) of NRS-1, 2 and 3 were 0.105, 0.104 and 0.102 per day respectively. In the temperature 10°C, lag phase was observed up to the tenth days and after then the exponential phase was persisted until the eighteenth days. In the twentieth days, the cell densities of NRS-1, 2 and 3 were 219,180 cells, 140,700 cells and 246,260 cells per milliliter. The growth rates (μ) of NRS-1, 2 and 3 were 0.117, 0.107 and 0.119 per days respectively (Fig. 3b). In the temperature 15°C, lag phase was observed up to the eighth days. In the twentieth days, the cell densities of NRS-1, 2 and 3 were 186,140 cells, 189,860 cells and 161,610 cells per milliliter. The growth rates (μ) of NRS-1, 2 and 3

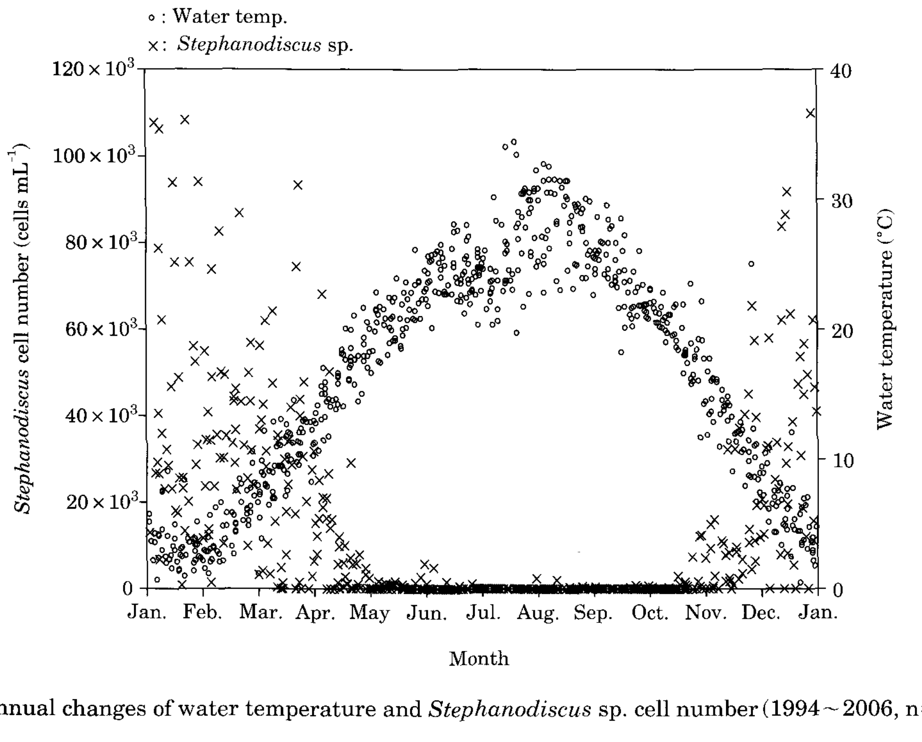

Fig. 2. Annual changes of water temperature and *Stephanodiscus* sp. cell number (1994~2006, n=680).

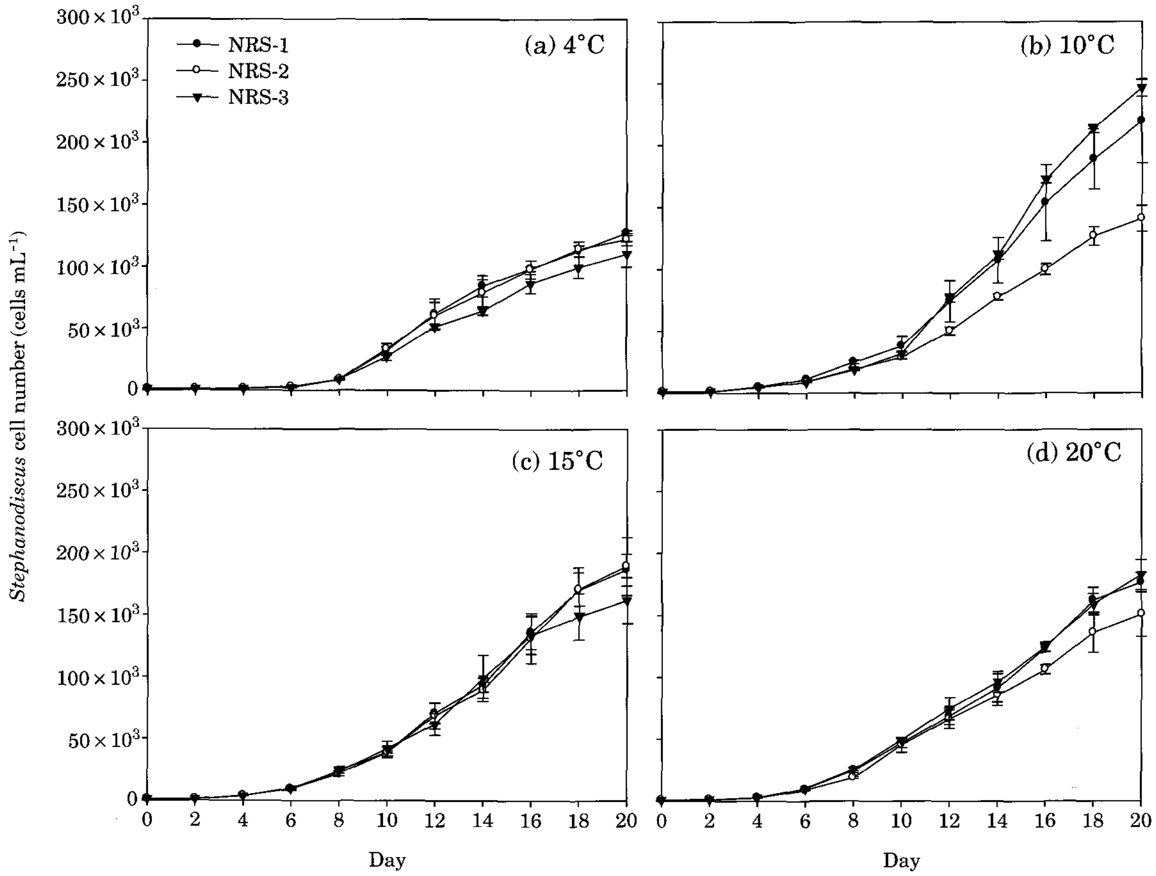

Fig. 3. Growth curves of *Stephanodiscus* sp. in different temperatures (a-4°C, b-10°C, c-15°C, d-20°C).

were 0.113, 0.113 and 0.110 per day respectively (Fig. 3c). In the temperature 20°C, lag phase was observed up to the sixth days and after then the exponential phase was persisted until the eighteenth days. The overall trends of growth curves were quite similar with the temperature 4°C. In the twentieth days, the cell densities of NRS-1, 2 and 3 were 176,200 cells, 150,660 cells and 182,320 cells per milliliter respectively. The growth rates (μ) of NRS-1, 2 and 3 were 0.112, 0.109 and 0.113 per day respectively.

The growing patterns of *Stephanodiscus* sp. in various temperature settings were distinctly differed (Fig. 3). The growth rates were different along temperatures (μ=0.104 in 4°C, 0.114 in 10 °C, 0.112 in 15°C, 0.111 in 20°C respectively) and the highest growth of *Stephanodiscus* sp. could be observed at 10°C. However, the growth patterns of each strain have been statistically different since 12th day of *in vitro* experiment at 10°C ($p > 0.05$, ANOVA).

DISCUSSION

The seasonality of diatom community (especially *Stephanodiscus*) in the lower part of the Nakdong River is influenced by complex interactions between each species and environmental factors. Among the environmental factors, hydrological and physical parameters such as precipitation, river flow, and water temperature were the most important for the growth of *Stephanodiscus* sp. Some scientific works provided similar pattern of the species in the Paltang Reservoir (Lee and Han, 2004). They concluded that low discharge or stable water column with moderate mixing could be the primary basis for the explosive growth of diatoms. The low water temperature in the winter that was favored by the species physiologically triggered the exponential growth in freshwaters. The dominance of Bacillariophyceae in the lower Nakdong River could be explained by the hypothesis (see Ha *et al.*, 2002).

These species occupied the phytoplankton community when relatively low temperature persisted (below 10°C). Jeong *et al.* (2001) suggested that the summer phytoplankton community in the large river of the region affected by monsoon climate was strongly related to the pattern of discharge. However, in summer, the blue-green algae was proliferated when the low precipitation and low discharge due to the high illumination and water temperature (Ha, 1999). While the discharge of multipurpose dam in the upper part of the Nakdong River decreased in the late autumn (October ~ November), the amount of inputting pollutants to the river were maintained with another season. Furthermore, the steady flow of the water body and high retention time affected the proliferation of winter diatom in the Nakdong River. Kim *et al.* (2007) reported that the low flow of Nakdong River during winter contributed the proliferation of *Stephanodiscus*. Kim *et al.* (2000, 2005) suggested that the edible small centric diatoms were increased from late October however; the activity and density of zooplankton in the Nakdong River were decreased due to the low temperature in winter. The proliferation of diatom in winter was not controlled by zooplankton predation activity. Algal bloom may lead not only to deterioration of water quality, but also to adverse transformation of the ecosystem and its degradation (Sorokin *et al.*, 1996). Indeed the diatom (*Stephanodiscus* sp.) in every winter degrades water quality in South Korea (Cho *et al.*, 1998; Ha *et al.*, 2002; Kang *et al.*, 2005).

Van Donk and Kilham (1990) suggested that water temperature can alter the maximum growth rate (μ_m) of algae. Ahlgren (1987) reviewed the various formulations used to describe the effects of temperature on algal growth constants and concluded that the effect of temperature on μ_m of algae was essentially linear over the range 0 ~ 40°C. Van Donk and Kilham (1990) indicated that μ_m of *S. hantzschii* (*S. minutus*; the taxonomy was in confusion) increased with the increasing temperature between 5°C to 20°C and below 20°C no real optimum was reached. Our laboratory studies of *Stephanodiscus* sp. presented the different effect with natural river system for the growth of the diatom. The cell density of *Stephanodiscus* sp. was observed maximum in approximately 5°C in the river system. However, the optimum water temperature was 10°C for the growth of *Stephanodiscus* sp. in the laboratory studies. For a long time, temperature was thought to have played a relatively minor role in seasonal succession because only maximum growth rate was considered to be set by temperature, although in nature this maximum growth probably seldom reached due to the growth limitation by effect of nutrient concentration (Eppley, 1972). The growth of unialgal clonal cultures in laboratory can be different with the species within algal community in nature ecosystem condition due to the different physico-chemical factors and biological interactions (e.g. competition for the nutrients, allelophatic activity with other species, and zooplankton grazing).

Van Donk and Kilham (1990) suggested that the *Fragilaria crotonensis* having apparently lower mortalities due to the wall growth, and *S. hantzschii* having higher mortalities at 20°C due to the selective predation of zooplankton. In our results, *Stephanodiscus* sp. was dominated during low temperature when the zooplankton community filtering rates on phytoplankton was quite low. The high cell density of *Stephanodiscus* sp. could be maintained during the winter due to the low clearances rates of zooplankton grazing. O'Brien (1974) theoretically explored the question of differential mortality on the abilities of species to compete for nutrients and found that species-specific mortality can change the outcome of nutrient dependent competition between algal species. Furthermore, *Stephanodiscus* sp. can get an advantage for uptake of nutrients because of having more tolerance to low temperature than the other algal species.

Most other rivers in South Korea are also in a highly regulated condition, and have characteristics similar to that of the Nakdong River. Therefore, the dynamics of the important diatom species *Stephanodiscus* sp. observed in the river, provide a good baseline for further studies on winter phytoplankton for efficient management during low temperature. Further experimental designs for the complex relationship among temperature, predation and resource competition are assigned to provide evidence of *Stephanodiscus*'s growth in the river system.

ACKNOWLEDGEMENT

This study was supported by the Ministry of

Environment (Long-Term Ecological Research Programme 2006), Republic of Korea. We would like to thank all the participants for the interesting discussions on the subject of this study.

LITERATURE CITED

Ahlgren, G. 1987. Temperature functions in biology and their application to algal growth constants. *Oikos* **49**: 177-190.

Cho, K.J., J.K. Shin, S.K. Kwak and O.H. Lee. 1998. Diatom genus *Stephanodiscus* as eutrophication indicator for water quality assessment. *Korean J. Environ. Biol.* **31**: 204-210.

Eppley, R.W. 1972. Temperature and phytoplankton growth in the sea. *Fish, Bull.* **70**: 1063-1085.

Fogg, G.E. and B. Thake. 1987. Algal cultures and phytoplankton ecology. The University of Wisconsin, London.

Guillard, R.R.L. 1973. Methods for microflagellates and nanoplankton. *In*: Stein, J.R. (eds.) Handbook of phycological methods, culture methods and growth measurements. Cambridge University Press, New York, p. 69-85.

Ha, K., H.W. Kim and G.J. Joo. 1998. The phytoplankton succession in the lower part of hypertrophic Nakdong River (Mulgum), South Korea. *Hydrobiologia* **369/370**: 217-227.

Ha, K. 1999. Phytoplankton community dynamics and *Microcystis* bloom development in a hypertrophic river (Nakdong River, Korea). PhD dissertation. Pusan national University, Busan.

Ha, K., M.H. Jang and G.J. Joo. 2002. Spatial and temporal dynamics of phytoplankton communities along a regulated river system, the Nakdong River, Korea. *Hydrobiologia* **470**: 235-245.

Han, M.S., H.R. Lee, S.S. Hong, Y.O. Kim, K. Lee, Y. K. Choi, S. Kim and K.I. Yoo. 2002. Ecological studies on Togyo reservoir system in Chulwon, Korea. V. Seasonal changes of size-fractionated standing crops and chlorophyll *a* of phytoplankton in Kyungan Stream of Pal'tang river-reservoir systems and Togyo Reservoir, Korea. *Korean J. Environ. Biol.* **20**: 91-99.

Hong, S.S., S.W. Bang and M.S. Han. 2002. Effects of rainfall on the hydrological conditions and phytoplankton community structure in the riverine zone of the Pal'tang Reservoir, Korea. *J. Freshwater Ecol.* **17**: 507-519.

Jeong, K.S., G.J. Joo, H.W. Kim, K. Ha and F. Recknagel. 2001. Prediction and elucidation of phytoplankton dynamics in the Nakdong River (Korea) by means of a recurrent artificial neural network. *Ecol. Model.* **146**: 115-129.

Jeong, K.S., F. Recknagel and G.J. Joo. 2003. Prediction and elucidation of population dynamics of a blue-green alga (*Microcystis aeruginosa*) and diatom (*Stephanodiscus hantzschii*) in the Nakdong River-Reservoir System (South Korea) by artificial neural networks. *In*: Ecological Informatics (Recknagel, F. eds.), Springer, Berlin, p. 195-213.

Joo, G.J., H.W. Kim, H. Kyong and J.K. Kim. 1997. Long-term trend of the eutrophication of the lower Nakdong River. *Korean J. Limnol.* **30**: 472-480.

Kang, Y.H., J.D. Kim, B.H. Kim, D.S. Kong and M.S. Han. 2005. Isolation and characterization of a bio-agent antagonistic to diatom, *Stephanodiscus hantzschii. J. Appl. Microbiol.* **98**: 1030-1038.

Kim, D.K., K.S. Jeong, P.A. Whigham and G.J. Joo. 2007. Winter diatom blooms in a regulated river in South Korea: explanations based on evolutionary computation. *Freshwater Biol.* **52**: 2021-2041.

Kim, H.W. 1999. Water quality, plankton community dynamics, and trophic regulation in the microbial food web by zooplankton in a hypertrophic river (Nakdong River, Korea). Ph. D. dissertation, Pusan National University, Busan.

Kim, H.W. and G.J. Joo. 2000. The longitudinal distribution and community dynamics of zooplankton in a regulated large river: a case study of the Nakdong River (Korea). *Hydrobiologia* **438**: 171-184.

Kim, H.W., S.J. Hwang and G.J. Joo. 2000. Zooplankton grazing on bacteria and phytoplankton in a regulated large river (Nakdong River, Korea). *J. Plankton Res.* **22**(8): 1559-1577.

Kim, H.W., K.H. Chang and G.J. Joo. 2005. Characteristics and inter-annual variability of zooplankton dynamics in the middle part of the river (Nakdong River). *Korean J. Limnol.* **38**(3): 412-419.

Lee, W.S. and M.S. Han. 2004. Community structure of plankton in eutrophic water systems with different residence time. *Korean J. Limnol.* **37**(3): 263-271.

Lim, Y.S., W.S. Song, J.S. Cho, H.J. Lee and J.S. Heo. 2000. The effect of algae on coagulation and filtration of water treatment process. *Korean J. Environ. Agric.* **19**: 13-19.

Marker, A.F.H. and G.D. Collett. 1997. Spatial and temporal characteristics of algae in the River Great Ouse. I. Phytoplankton. *Regulated Rivers: Research & Management* **13**: 219-233.

McAlice, B.J. 1971. Phytoplankton sampling with the Sedgwick-Rafter cell. *Limnol. Oceanogr.* **16**(1): 19-28.

Mitrovic, S.M., P.R. Hawkins, L.C. Bowling, R.T. Buckney and D.H.M. Cheng. 1999. Low nitrate concentrations in a tidally mixed river allow replacement of green algae by the cyanobacteria *Microcystis. Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* **27**: 924-929.

O'Brien, W.J. 1974. The dynamics of nutrient limitation of phytoplankton algae: a model reconsidered. *Ecology* **55**: 135-141.

Paerl, H.W. 1987. Dynamics of blue-green algal (*Microcystis aeruginosa*) blooms in the Lower Neuse River, North Carolina: causative factors and potential controls. Water Resources Research Institute of the University of North 14 Carolina, UNC-WRRI-87-229.

Rose, M. and D. Balbi. 1997. Rivers Nene and Great Ouse Eutrophication Studies: Final Report. Environment Agency, Peterborough, UK.

Sorokin, Y.I., P.Y. Sorokin and D. Ravagnan. 1996. On the bloom of toxic dinoflagellate *Gonyaulax tamarensis* lebour in the lagoons of the Adriatic Sea. *Zh. Obshch. Biol.* **57**(3): 381-387.

Throndsen, J. 1969. A simple micropipette for use with the Wild M40 and the Zeiss plankton microscopes. *J. Cons. CIEM.* **32**(3): 430-432.

Utermöhl, H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik. *Mitt. int. Ver. theor. angew. Limnol.* **9**: 1-38.

Van Donk, E. and S.S. Kilham. 1990. Temperature effects on cilicon- and phosphorus-limited growth and competitive interactions among three diatoms. *J. Phycol.* **26**: 40-50.

Wetzel, R.G. and G.E. Likens. 1991. Limnological analysis. New York, Springer-Verlag.

(Manuscript received 8 October 2007,
Revision accepted 22 February 2008)