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Influence of Dissolved Inorganic Nitrogen and Phosphorus Concentrations on the Horizontal and Temporal Changes of *Microcystis* Population in Lake Kitaura.

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ABSTRACT

Influences of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentration on the horizontal distribution and temporal variation of *Microcystis* abundances were confirmed by the results of Lake Kitaura investigation. The samples were collected from the surface water at 5 stations (St. A-E) set up along a north-south transect between 2 week intervals from July to September in 2005. From the results of a present investigation, DIN concentration decreased gradually between St. A and St. E and remained constant seasonally in each station. DIP concentration increased from early July to early August, especially in St. B-D. DIN: DIP ratios were more than the Redfield ratio (=7, by weight) in St. A, and varied below 7 in St. C-E during the investigation period. These results suggested that the growth limited factor of the phytoplankton community was shifted from phosphorus to nitrogen between St. A and St. C in Lake Kitaura. *Microcystis* blooms were developed remarkably at 29 July in St. C-D. It showed that high DIP concentration and the nitrogen limited condition were associated with *Microcystis* dominance. *Microcystis* morphospecies were observed including 4 morphospecies such as *M. aeruginosa*, *M. viridis*, *M. wesenbergii* and *M. ichthyoblabe* in all samples. The previously studies reported that *M. aeruginosa* and *M. viridis* are known strains that produce toxic cyclic heptapeptide called microcystin. The relative abundance of *M. aeruginosa* and *M. viridis* decreased between St. A and St. E during the exponential growth phase. The result suggested that at high DIN concentrations, toxic morphospecies resulted in higher relative abundance than non-toxic morphospecies. We concluded that the horizontal and temporal changes of *Microcystis* blooms were affected by the supply ratio of DIN and DIP, and that the toxic morphospecies of *Microcystis* needed high DIN concentration for their growth more than non-toxic morphospecies in Lake Kitaura.

Keyword: Microcystis bloom, *Microcystis* morphospecies, macro-nutrient concentration, DIN: DIP ratio and microcystin.

INTRODUCTION

Cyanobacterial (blue-green algal) bloom is a serious environmental problem reported from eutrophic lakes in the world. *Microcystis* is a cosmopolitan genus of occurring cyanobacterial bloom. A large number of *Microcystis* blooms containing toxic cyclic heptapeptide named microcystin are reported from various countries (Kardinaal and Visser, 2005). Microcystin have caused the death of many aquatic organisms, wild animals, livestock and also implicated in human illness (Sivonen and Jones, 1999). Microcystin production of *Microcystis* is affected by a wide variety of physico-chemical factors, including temperature, light, nutrients concentration and others (Sivonen and Jones, 1999). Especially, nitrogen and phosphorus are the important factors which control both the abundance of *Microcystis* and microcystins production (Sivonen and Jones, 1999). Several field studies have been initiated for the investigation of relationship between temporal variation of nutrients and *Microcystis* abundance (Kardinaal and Visser, 2005). The most of these studies monitored nutrients and *Microcystis* bloom at one station in each study area. Therefore, the influences of horizontal variation of nutrients on *Microcystis* abundance were not clarified enough by previous studies. The present study investigated the temporal and horizontal changes of *Microcystis* bloom and nutrients conditions in eutrophic lake, Kitaura. We described that influences of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentration on the horizontal distribution and temporal variation of *Microcystis* abundances in Lake Kitaura.

METHODS

Study area, Sampling design and limnological analysis

Lake Kitaura, shallow eutrophic lake (water level area: 34.4km^2, mean depth: 4.5m) located 60km north of

Tokyo, was investigated for the occurrence of *Microcystis* bloom and environmental factors from July to September in 2005. Investigations occurred approximately at 2 week intervals during this study period. The samples were collected from the surface water at 5 stations (St. A-E) set up along a north-south transect (Fig.1) using a 6L Van-Dorn bottle (B type, Miyamoto Riken, Japan). But, sample was not collected in St. E at 16 July in 2005. Lake water samples collected for DIN and DIP analysis were filtered through a glass-fiber filter (GF/C, Whatman, UK).

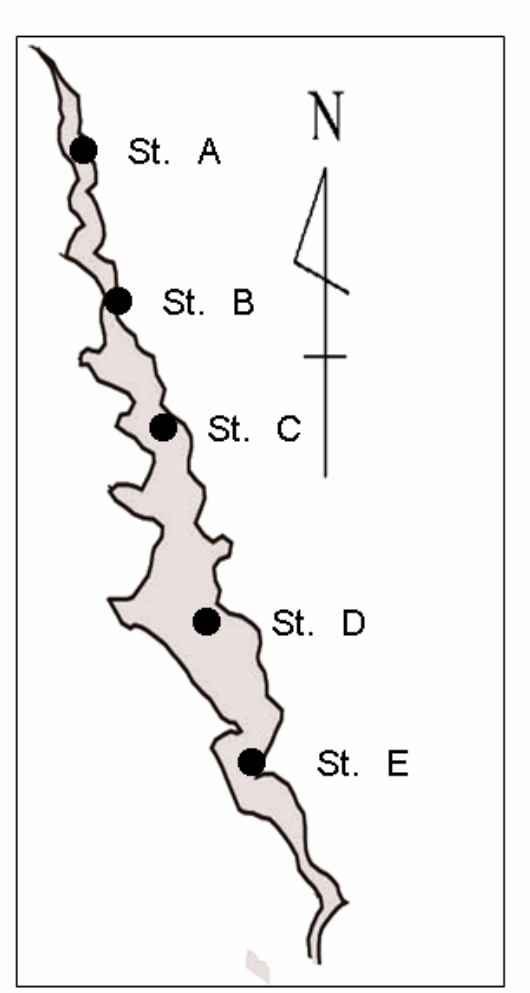

Figure 1. The map of Lake Kitaura showing sampling stations.

Filtrated lake water samples were measured for DIN (nitrate, nitrite and ammonia) and DIP (mainly, phosphate) by the autoanalyzer (AACS-□, BRAN+LUEBBE, German). Phytoplankton abundance was estimated as chlorophyll, which was determined with a water sample filtered through a glass-fiber filter (GF/C, Whatman, UK). The filter was frozen and extracted in ethanol overnight in a dark freezer. After centrifugation of the extract, the concentration of chlorophyll a was determined spectrophotometrically. *Microcystis* cells counting samples were preserved with formaldehyde solution to a final concentration 2% and stocked in dark place. Total *Microcystis* cell concentration was counted by using a hemocytometer (Fuchs-Rosenthal, KAYAGAKI Works, Japan) under a microscope (BX51, Olympus, Japan). *Microcystis* morphospecies classification was done according to Komárek (1991). Over 100 *Microcystis* colonies were identified and counted cell number of each colonies to determine the relative abundance of *Microcystis* morphospecies.

RESULTS

Figure 2 shows the horizontal and temporal variation of DIN and DIP concentration. DIN concentration decreased gradually between St. A and St. E in each sampling date (Fig.2A). Nitrate conc were always detected to be high in St. A (738-2,520μg L^{-1}) rather than in other sampling station, which decreased gradually between St. A and St. E. Nitrate concentration in Sts. B-E constantly showed low values, not detected in samples collected from Sts. C-E at 29 July in 2005, Sts. B-E in 11 August and Sts. D and E at 27 August in 2005. Nitrite and ammonia concentrations varied from >10 to 257μg L^{-1} and from 13 to 158μg L^{-1}. The variation of nitrite and ammonia concentrations was less than nitrate. DIP concentration at St. A was lower than other sampling stations except for result of 27 September in 2005 (Fig.2B). DIP concentration of each sampling stations was recorded minimum values at the first sampling date and maximum at 27 August in 2005 except for St. A. DIP concentration in St. A had been increased during this study period and reached 75 μg L^{-1} at 27 September in 2005.

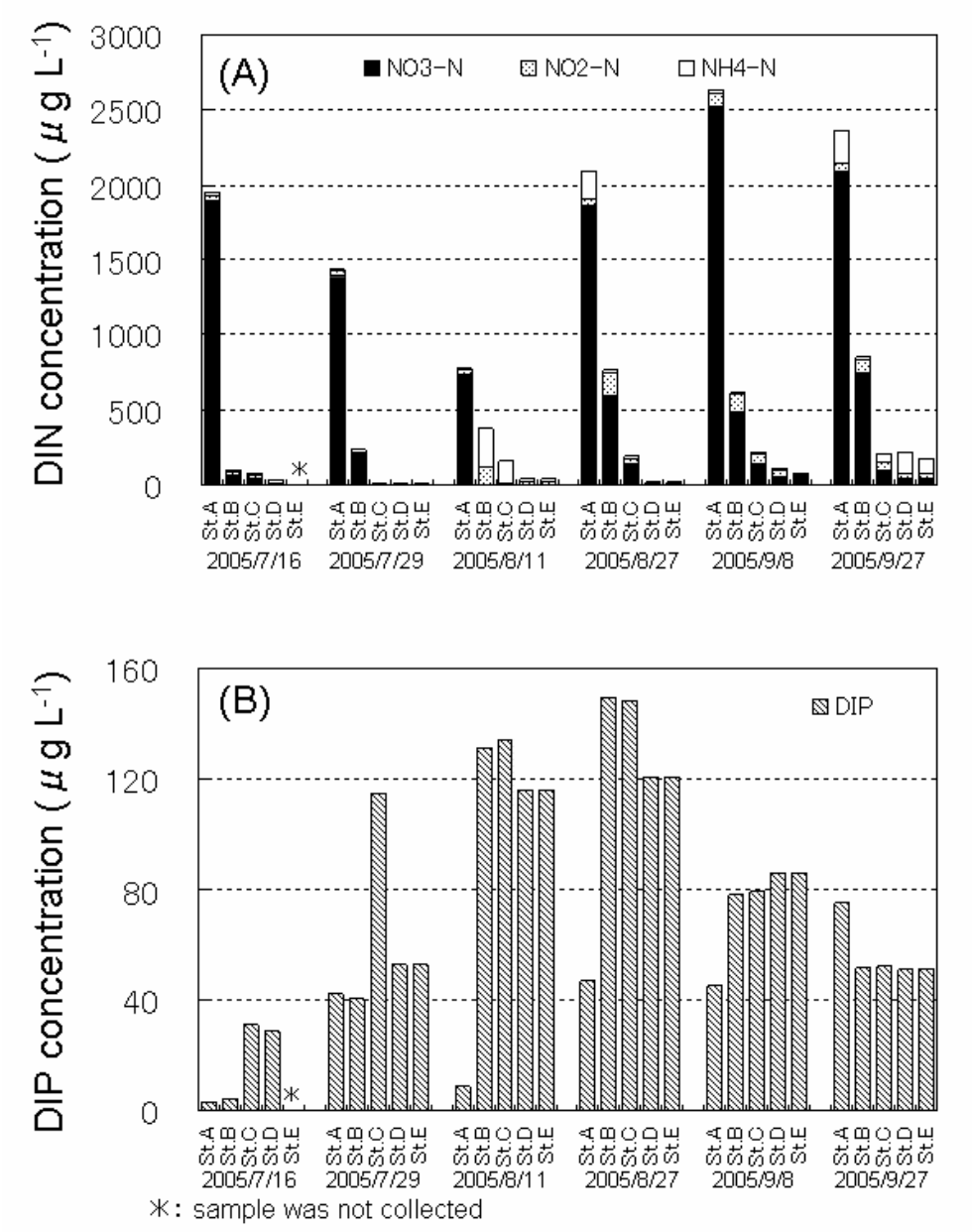

Figure 2. The horizontal and temporal variation of (A) DIN and (B) DIP concentration in lake water in Lake Kitaura. Samples were collected from July to September at 2weeks intervals in 2005.

Figure 3 shows the horizontal and temporal variation of chlorophyll a concentration, *Microcystis* cell density and morphospecies composition in Lake Kitaura. The horizontal and temporal variation of *Microcystis* cell density and morphospecies composition varied in the pattern of horizontal and temporal. The variation of chlorophyll a concentration was correlated with *Microcystis* cell density except for results of investigation in 16 July and 27 September in 2005 when *Microcystis* was low cell density in samples collected from Lake Kitaura (Fig. 3A). In all sampling stations, *Microcystis* cell density increased between 16 July (the start of this study period) and 29 July in 2005 (Fig. 3A). Highest *Microcystis* cell density in each station was observed in 29 July in 2005 except for St. A. *Microcystis* cell density at St. B, C and D reached 1.52×10^5 cells mL^{-1}, 6.50×10^5 cells mL^{-1} and 4.25×10^5 cells mL^{-1} respectively. *Microcystis* abundance of Lake Kitaura decreased gradually between 29 July and 27 September (the end of this study period) in 2005.

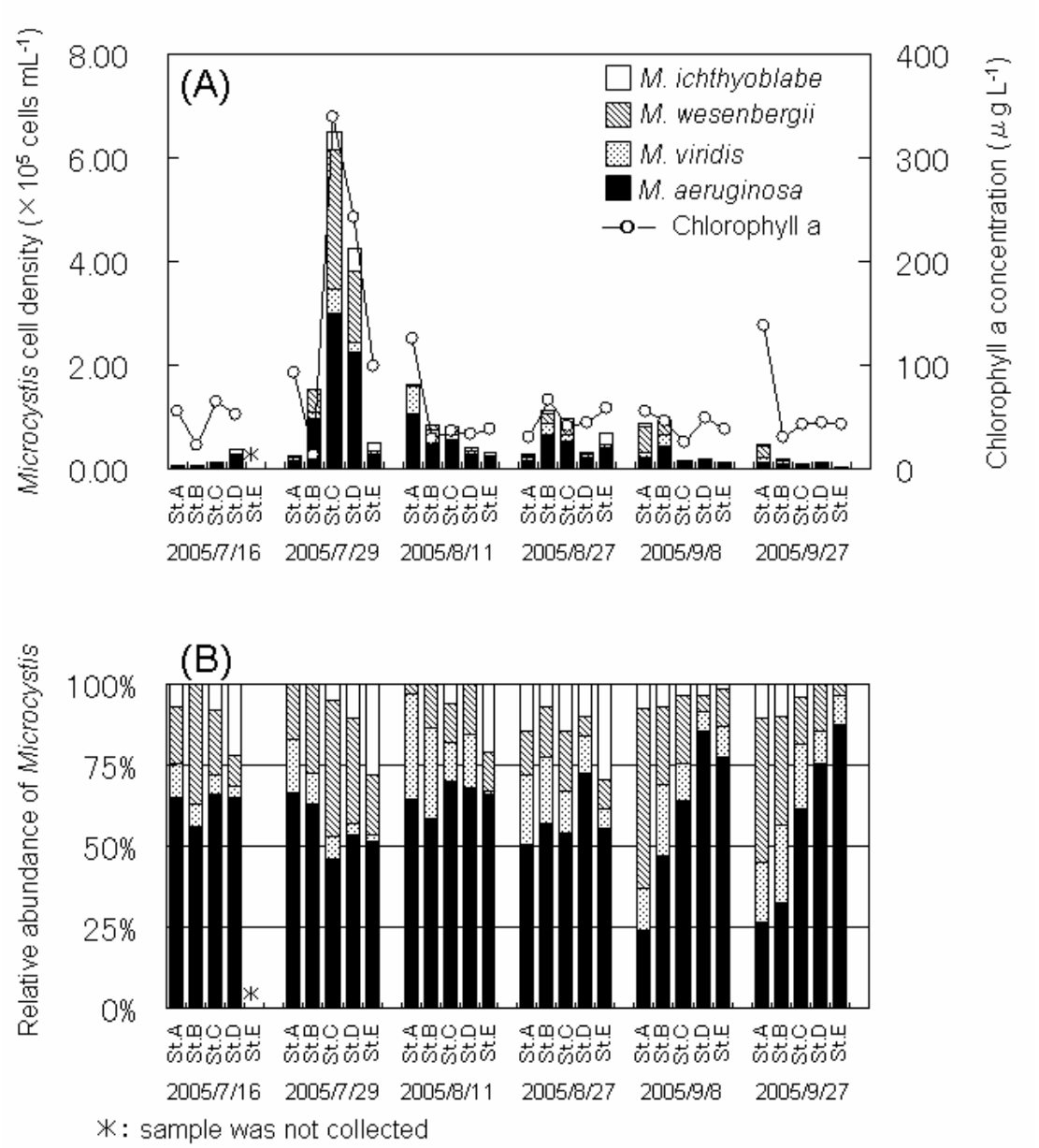

Figure 3. The horizontal and temporal variation of (A) chlorophyll a concentration, *Microcystis* cell density and (B) morphospecies composition in lake water in Lake Kitaura. Samples were collected from July to September at 2weeks intervals in 2005.

Four *Microcystis* morphospecies (*M. aeruginosa*, *M. ichthyoblabe*, *M. viridis* and *M. wesenbergii*) could be identified in all samples collected during this study period. *M. aeruginosa* dominated in the most of sampling station in Lake Kitaura during this study period (Fig. 3B). The relative abundance of *M. aeruginosa* and *M. viridis* tended to decrease gradually from St. A to St. E at July 29 in 2005, when maximum *Microcystis* density was observed.

DISCUSSION

The study on relationship between horizontal variation of nutrients and the development of *Microcystis* bloom are very few. In this study, the influences of temporal and horizontal variation of nutrients on *Microcystis* abundance were confirmed by the results of investigation of Lake Kitaura.

Focus on the result of 29 July in 2005, when maximum *Microcystis* cell density observed in each

sampling stations except for St. A. *Microcystis* cell density was higher in middle regions (St. C and D) than north regions (St. A and B) and south region (St. E). Generally, the abundance of freshwater phytoplankton is limited by nitrogen or phosphorus and both (Elser et al., 1990). The DIN:DIP ratio of lake water is less than the redfield ratio (=7, by weight), so nitrogen is limiting relative to phosphorus on the abundance of phytoplankton (Dodds, 2002). Several studies revealed that the optical DIN:DIP ratio of cyanobacteria is lower than the eukaryotic algae and that the nitrogen-limited condition may promote the dominance of cyanobacteria (Tilman et al., 1982; 1986; Smith, 1983). Jacoby et al. (2000) reported that *Microcystis* blooms are also favoured by low DIN:DIP ratio (below 10). Figure 4 shows the temporal and horizontal variation of DIN:DIP ratio in lake Kitaura. The variation of DIN:DIP ratio was always varied above 7 in St. A and below 7 in Sts. C, D and E. These results indicated that the limited factor of phytoplankton abundance shifted from phosphorus to nitrogen between St. A and St. C in Lake Kitaura. *Microcystis* cell density was higher in nitrogen-limited condition except for St. E than phosphorus limited condition (St. A) at 29 July in 2005.

Moreover, Blomqvist et al. (1994) postulated that eukaryotic algae are favoured by nitrate, non-nitrogen fixing cyanobacteria by ammonium and nitrogen fixing cyanobacteria by nitrogen deficiency. In St. E, DIN concentration was very low and nitrogen-fixing cyanobacteria (*Anabaena* and *Aphanizomenon*) were high abundance rather than other sampling stations (data not shown) during this study period. These results suggested that low *Microcystis* cell density in St. E might be caused by serious nitrogen deficiency. This study concluded that the horizontal variation of DIN:DIP ratio and DIN source composition could affect on the horizontal distribution of *Microcystis* bloom in Lake Kitaura. In most cases, *Microcystis* blooms are composed by some *Microcystis* morphospecies (Watanabe et al., 1994; Park et al., 1993 and 1998; Homma and Park, 2005). Four morphospecies were identified in *Microcystis* bloom during this study period. Several studies reported that the strains of *Microcystis* morphospecies were closely related to availability of microcystin production (Watanabe et al., 1994; Park et al., 1993; Ozawa et al., 2005). Results of these studies demonstrated that the most of strains of both ***M. aeruginosa*** and ***M. viridis*** are toxic and strains of ***M. wesenbergii*** are non-toxic.

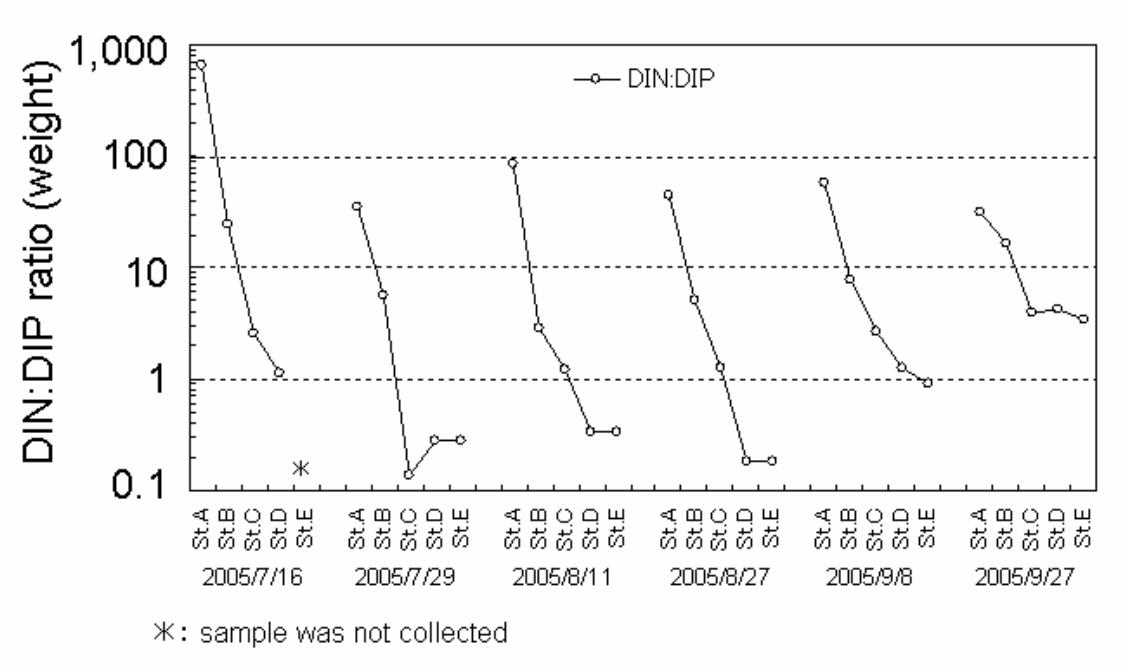

Figure 4. The temporal and horizontal variation of DIN:DIP weight ratio in lake water in Lake Kitaura.

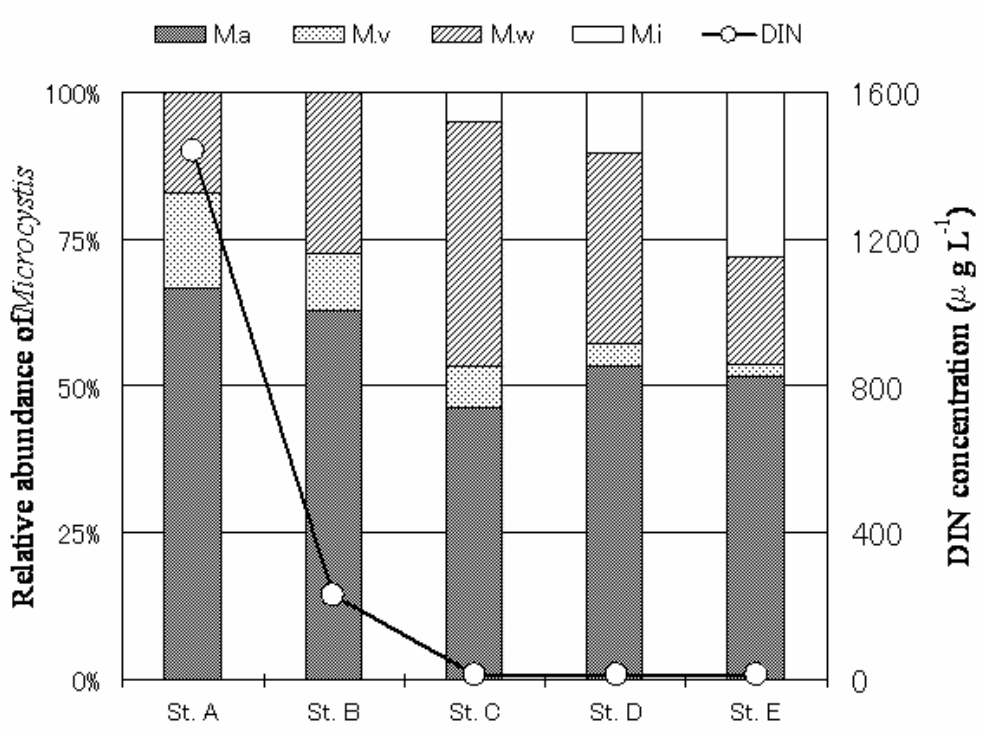

Figure 5 The relationship between the horizontal variation of the relative *Microcystis* abundance and DIN concentration in 29 July 2005.

Strains of *M. ichthyoblabe* have both toxic and non-toxic strain, it seems that the number of non-toxic strains were more than toxic strains. Several studies on the relationship between *Microcystis* morphospecies composition and microcystin concentration in lake water reported that microcystin content of *Microcystis* bloom dominated by *M. aeruginosa* or *M. viridis* was very higher than by *M. wesenbergii* or *M. ichthyoblabe* (Park et al., 1993, 1998; Oudra et al., 2002; Homma and Park, 2005). Homma and Park (2005) suggested that the major source of variation of microcystin contents of *Microcystis* bloom seems to be related to changes in dominance of *Microcystis* morphospecies. Figure 5 shows the horizontal variation of the relative abundance of *Microcystis* morphospecies composition and DIN concentration. The relative abundance of *M. aeruginosa* and *M. viridis* decreased depended on DIN concentration decreasing between St. A and St. E. In the studies by Hesse and Kohl (2001) and Vezie et al. (2002), high nutrient concentration could promote the dominance of toxic *Microcystis*. Therefore, this result suggested that at high DIN concentrations, toxic morphospecies resulted in higher relative abundance than non-toxic morphospecies. We concluded that the toxic morphospecies of *Microcystis* (*M. aeruginosa* and *M. viridis*) needed high DIN concentration for their growth more than non-toxic morphospecies (*M. wesenbergii* and *M. ichthyoblabe*) in Lake Kitaura.

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