

Relationship between watershed environments and growth of coastal diatoms

Masahiko SAIGUSA¹, Daisuke KUNII¹ and Genya SAITO²

¹Field Science Center, Tohoku University, Naruko Onsen, Osaki City, Miyagi 989-6711, Japan

²Graduate School of Agriculture Science, Tohoku University 1-1, Amamiya, Tsutsumidouri, Aoba, Sendai, Miyagi 981-8555, Japan

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Abstract

Effect of watershed environments on river water quality and the subsequent influence of water quality on the growth of diatoms in coastal seawater were studied. Land use in the upper and lower site of the Ohkawa River (O-up and O-low) and the upper site of the Nanakita River (N-up) were dominated by forestry, whereas the lower site of the Nanakita River (N-low) was characterized by urbanization. Seasonal changes in nutrients in the Ohkawa and Nanakita Rivers suggested that the concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and acid extractable-Fe were influenced by human activities, while Si concentration reflected geological conditions. The average concentrations of fulvic acid-like Fe (FA-Fe), closely associated with the growth of coastal diatoms, were 1 and 16 $\mu\text{g L}^{-1}$ at the O-up and O-low sites, respectively, while those of the Nanakita River were 5 $\mu\text{g L}^{-1}$ (N-up) and 53 $\mu\text{g L}^{-1}$ (N-low). For each river, FA-Fe concentrations of the lower sites were much higher than the upper sites. Moreover, the concentration of FA-Fe at N-low was much higher than at O-low. Therefore, it was concluded that FA-Fe originates not only from forest vegetation but also from urban activity. The growth of the *Skeletonema*, a typical diatom of coastal waters, was stimulated by the addition of O-low river water compared to addition of O-up, reflecting the FA-Fe content. Diatom growth stimulation with the addition of lower river water was much more prominent in the Nanakita River, whose watershed is characterized by runoff from Sendai city.

Introduction

Since the late 1960s, the production of the both

oyster and laver has been decreasing in Kesenuma Bay in Miyagi Prefecture. Fishermen considered that some nutrients from upstream sites, and transported to the coast by the Ohkawa River, may have been reduced by deforestation in mountainous areas. Therefore, they started the afforestation movement “Mori wa Umi no Koibito”, meaning “Forest is a sweetheart for Sea”, by transplanting broadleaf trees on the mountain areas of Mt. Murone and Mt. Yagoshi, which are the watersheds of the Ohkawa River (Hatakeyama, 2003).

Phytoplankton plays an important role in primary production in the sea and nutrients for their growth are supplied from rivers flowing into the coastal areas. Of these nutrients, recent research has shown that iron-binding organic matter played significant role in the growth of diatoms and seaweeds in coastal regions (Matsunaga et al., 1998a, 1998b). These authors have suggested that the form of iron is a fulvic acid-like iron (FA-Fe), originating from forest ecosystems. Generally, it has been considered that river water quality affects coastal ecosystems to a significant degree. Therefore, the influence of watershed environments on river water quality and how nutrients in river water affect primary production in coastal ecosystems deserves further study. However, there is little research on the relationship between watershed environments and coastal sea ecosystems.

The seasonal changes in water quality in the Ohkawa and Nanakita Rivers, which have different watershed environments, and especially the concentration of FA-Fe in river water, were studied in detail. The effect of water quality, e.g. FA-Fe, on the growth of a typical coastal diatom (*Skeletonema*) was also inves-

tigated.

Materials and Methods

Study area

Figs. 1 and 2 showed the watershed of the Ohkawa and Nanakita River, respectively. The source of the Ohkawa River lies near Mt. Toishi in the south of Iwate Prefecture and the river flows into Kesenuma Bay in the north of Miyagi Prefecture. The river is about 25 km long and the area of the watershed is about 16 700 ha. Forest vegetation occupies a considerable area of the Ohkawa River watershed and the

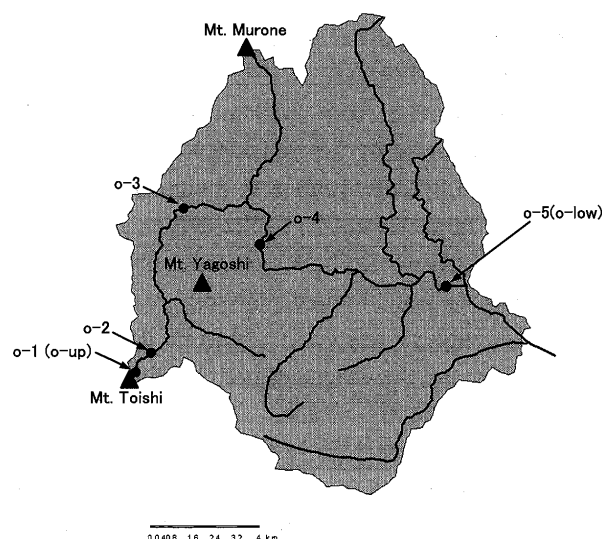


Figure 1. Watershed of the Ohkawa River

■ Watershed — River ● Sampling site

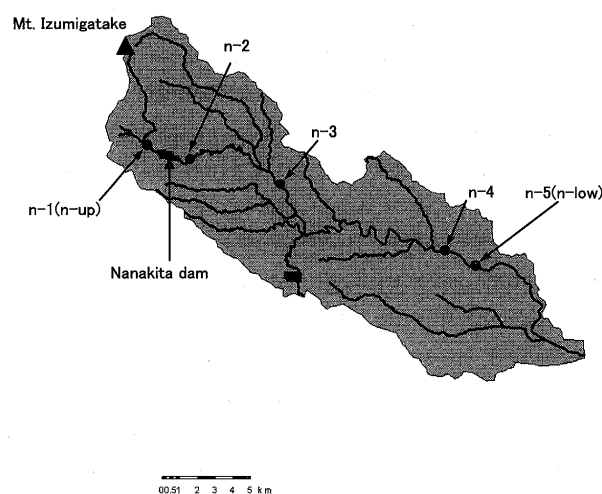


Figure 2. Watershed of the Nanakita River

■ Watershed — River ● Sampling site

well-known, reforestation strategy has been implemented at Mt. Yagoshi since 1993. The surface geology at the upper river region is mostly plutonic rock.

The source of the Nanakita River lies near Mt. Izumigatake in Miyagi Prefecture and the river flows into Sendai Bay. The river is about 40 km long and the area of the watershed is about 22 300 ha. Forest occupies the upper region but urban areas also lie along the middle and lower regions, with paddy and upland fields also developed along the river. The Nanakita dam lies on the upper river. The surface geology of the upper region is mostly volcanic rock.

Nutrient analysis of river water

River water was sampled from June 2003 to April 2004. The sampling sites on the Ohkawa and Nanakita Rivers are shown in Figs. 1 and 2, respectively, listed as o-1 to o-5 for the Ohkawa river and n-1 to n-5 for Nanakita river. The concentrations of Si, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and acid extractable-Fe (Acid-Fe), pH and EC were recorded. Water samples were collected directly in polyethylene bottles at each site. The samples, except for Acid-Fe, were filtered through 0.45- μm Millipore filters and kept in a refrigerator at 5°C until analyses. HNO_3 was added to the samples for Acid-Fe analysis at a final concentration of 10% and kept in a refrigerator at 5°C. All elements were analyzed following the established methods (The Japanese Society for Analytical Chemistry, 1994).

Iron analysis

River water samples were collected at the up- and down-stream sites in each river from June to November 2005. The concentration of acid-dissolved iron (Acid-Fe), total dissolved iron (D-Fe) and fulvic acid-like iron (FA-Fe) were analyzed by the ferrozine method (Stookey, 1970) after the following treatments. To 100-ml of river water for Acid-Fe analysis was added 10 ml of 3 M HCl and boiled; then the samples were analyzed. The river water for both D-Fe and FA-Fe analysis were filtered through 0.45 μm Millipore filters. FA-Fe is the fraction in the D-Fe adsorbed by the anion exchange resin. The method for elution of FA-Fe followed the procedure of Igarashi *et al.* (1982).

Cultivation of the diatom

Coastal water was collected at Kesenuma Bay in Miyagi Prefecture in November 2005. Salinity of the

seawater was 31‰. River water samples were collected at the upper and lower sites of the Ohkawa River (O-up and O-low) and Nanakita River (N-up and N-low) on the same day. *Skeletonema* from stock species was cultivated in media treated with different river water samples. The treatments for cultivation are shown in table 1. The cultivation media were

prepared by adding river water to the coastal seawater (1:10). Diatom was cultured at 20°C under 61 $\mu\text{mol}^{-1} \text{m}^{-2}$ fluorescent light (12-h light/dark cycle) for 5 days. The samples were stirred three times a day. After 5 days, samples were placed on a 100-mm³ glass chamber for counting diatom cells (MATSUNAMI Co. MPC-200) and counted under an inverted light

Table 1. Treatment of diatom

Treatment	media	added river water	diatom
DW	coastal water	DW	<i>Skeletonema</i>
o-up	"	collected at o-up site	"
o-low	"	collected at o-low site	"
n-up	"	collected at n-up site	"
n-low	"	collected at n-low site	"

Coastal water was sampled at the Kesennuma Bay (Salinity = 30‰). DW means deionized water. *Skeletonema* was the stocked species.

o-up: Sampling site at upper river of the Ohkawa River

o-low: Sampling site at lower river of the Ohkawa River

n-up: Sampling site at upper river of the Nanakita River

n-low: Sampling site at lower river of the Nanakita River

microscope (Nikon Co. ECLIPSE TE300).

Results and Discussion

Nutrient dynamics in river water

Figure 3 shows the nutrient dynamics for the Ohkawa and Nanakita Rivers. The abscissa shows the distance of sampling site from the source of the stream; the vertical axis shows the concentration of each nutrient. In both rivers, the variation of Si concentration was small versus distance and seasons, but the Si concentration of down-stream water tended to decrease compared to up-stream water. $\text{NH}_4\text{-N}$ concentration of the Ohkawa River was low and varied little; however, but in the Nanakita River it was also low in the upper sites but showed a high concentration and large variation in the lower sites. $\text{NO}_3\text{-N}$ concentration of the Ohkawa River showed little variation, except in June, while in the Nanakita River it was low in upper sites but showed a high concentration and large variation in the lower sites. $\text{PO}_4\text{-P}$ concentrations showed no steady patterns but large seasonal variations were recorded in both rivers. Acid-Fe concentration showed a large variation for both distance and season.

Large variations in nutrient concentration seem to

be influenced by extraneous factors, such as precipitation, drainage from agricultural fields, urbanization, river conservation works, etc. However, the amount of precipitation on the sampling days was small (0-13 ml day⁻¹) and, thus, the level of water in the river was unchanged. Therefore, the variation in $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and Acid-Fe concentrations may originate from human activities, while that of Si does not.

Relationship between watershed environment and FA-Fe concentration

The FA-Fe, D-Fe and Acid-Fe concentrations at the upper and lower sites of both rivers are shown in Fig. 4. FA-Fe concentrations at upper site of the Ohkawa River (O-up) for each month ranged 0-2 $\mu\text{g L}^{-1}$ with an average of 1 $\mu\text{g L}^{-1}$, whereas those at lower site (O-low) were 9-26 and 16 $\mu\text{g L}^{-1}$, respectively. The FA-Fe concentration at upper and lower site of the Nanakita River (N-up and N-low) were 4-7 $\mu\text{g L}^{-1}$ (average: 5 $\mu\text{g L}^{-1}$) and 43-73 $\mu\text{g L}^{-1}$ (average: 53 $\mu\text{g L}^{-1}$), respectively. Similar data were reported from the lower site in a previous paper: 7-28 $\mu\text{g L}^{-1}$ (average: 16 $\mu\text{g L}^{-1}$) (Matsunaga et al. 1998b). In both the Ohkawa and Nanakita River, the FA-Fe concentra-

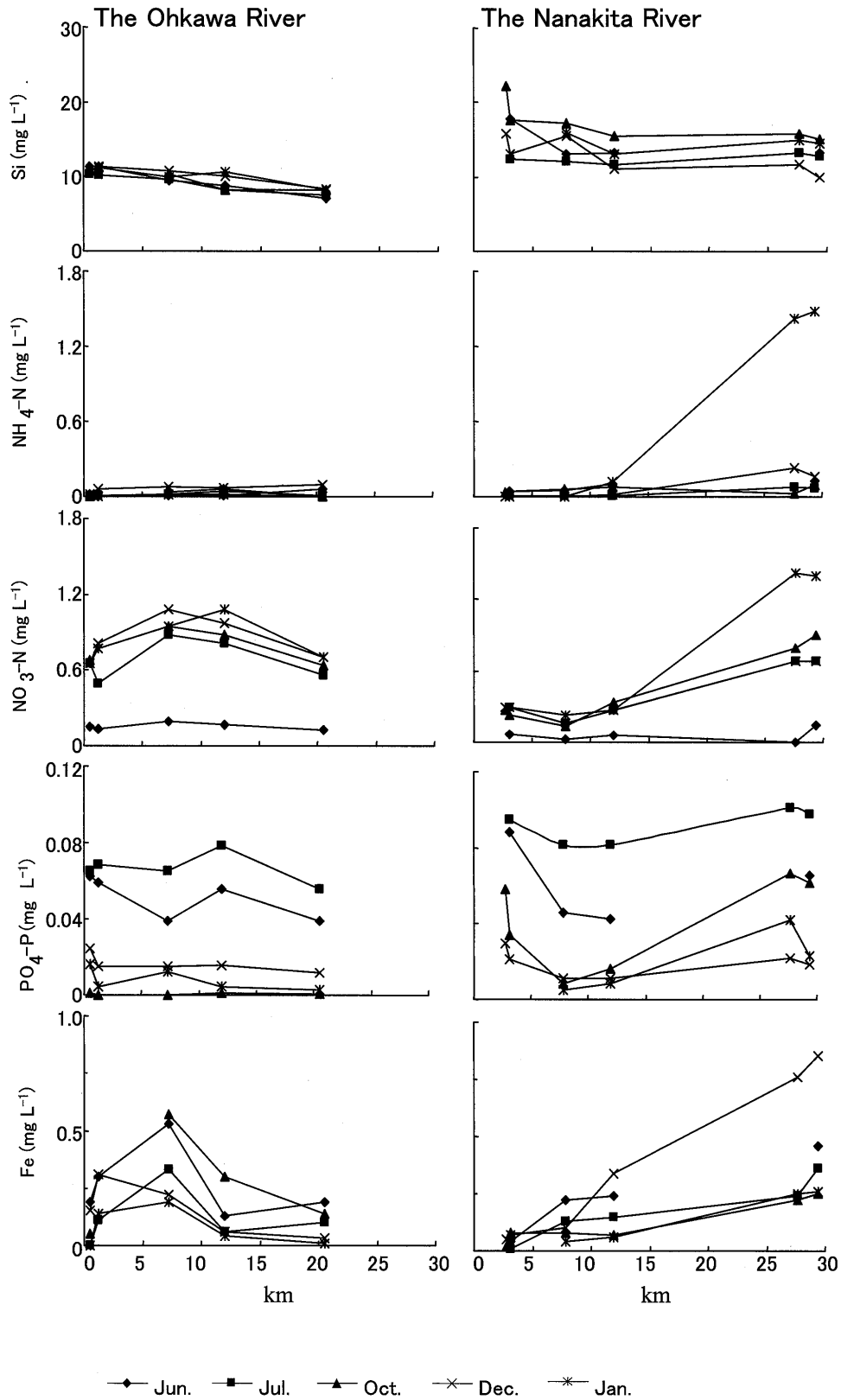


Figure 3. Nutrient dynamics in the river water

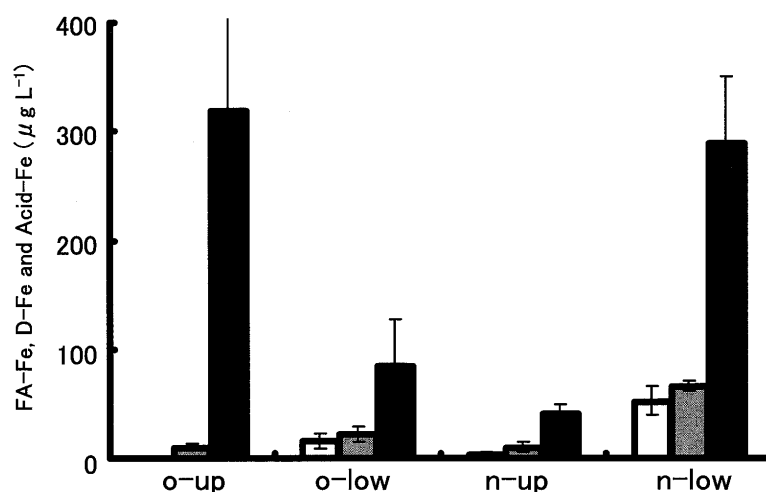


Figure 4. Concentration of FA-Fe, D-Fe and FA-Fe in the river water

FA-Fe
 D-Fe
 Acid-Fe
 * Error bars mean SD

tions at the lower site were, on average, more than 10 times higher than those of the upper stream site. Furthermore, the FA-Fe concentration at lower site of the Nanakita River was 3.3 times higher than that of the Ohkawa River. Both acid-Fe and D-Fe concentration in each site showed a similar tendency to that shown

for FA-Fe concentration.

Land use in the watersheds of each sampling site is shown in table 2. In up-stream sites of both rivers, forest vegetation was dominant. On the other hand, land use at the watershed of the O-low site was also dominated by forestry (9185 ha or 73% of water-

Table 2. Land use area of each river watersheds

the area of land use (ha)	o-up		o-low		total	
forest area	29	(100)	9185	(73)	12179	(73)
agricultural area	0	(0)	2235	(18)	2840	(17)
urban area	0	(0)	179	(1)	550	(3)
other area	0	(0)	929	(7)	1175	(7)
total	29	(100)	12529	(100)	16744	(100)

the area of land use (ha)	n-up		n-low		total	
forest area	797	(70)	7923	(47)	8090	(36)
agricultural area	117	(10)	2662	(16)	3498	(16)
urban area	10	(1)	4756	(28)	8647	(39)
other area	216	(19)	1459	(9)	2068	(9)
total	1140	(100)	16800	(100)	22303	(100)

Data was obtained from HP of Ministry of Land, Infrastructure and Transport Japan and the land use was divided into 4 groups ; forest, agricultural area, urban area and other area. The parentheses mean the rate of land use in the watershed (%)

o-up: Sampling site at upper river of the Ohkawa River

o-low: Sampling site at lower river of the Ohkawa River

n-up: Sampling site at upper river of the Nanakita River

n-low: Sampling site at lower river of the Nanakita River

shed), but that of N-low site was characterized by urban areas (forest: 7923 ha or 47% and urban area: 4756 ha or 28%). At the watershed of the O-low site, both the afforested acreage and forest proportion to total land use were higher than those of the N-low site, while the urban area acreage and utilization rate were the converse. Recently, the existence of fulvic-like organic matter, originating from human activity in river water near urban areas, has been reported (Takahashi et al., 2003). Therefore, it seems that the FA-Fe in river water originated, not only from forest vegetation in up-stream natural ecosystems, but also from human activity in urban areas.

Effect of river water quality on the growth of diatoms in coastal region

Fig. 5 showed the nutrient status of the river water added to the media used for diatom culture. N, P, Si and FA-Fe were focused on in this study as nutrients of diatoms. The concentration of all nutrients in river water, except FA-Fe at the O-up site, were higher than those of the O-low site, while those at the N-low site, except Si, were higher than those at the N-up

site. The effect of added river water on the growth of *Skeletonema*, a representative diatom in coastal seawater, is shown in Fig. 6. The initial number of diatoms was $2110 \text{ cells ml}^{-1}$. After 5 days, cell numbers for each treatment increased in following order: $9.8 \times 10^4 \text{ cells ml}^{-1}$ (N-low) > $8.0 \times 10^4 \text{ cells ml}^{-1}$ (N-up) > $6.9 \times 10^4 \text{ cells ml}^{-1}$ (O-low) > $6.0 \times 10^4 \text{ cells ml}^{-1}$ (O-up) > $5.5 \times 10^4 \text{ cells ml}^{-1}$ (DW). The effects of all added river waters on the growth of diatom (cell numbers) were greater than that of control (distilled water). Among the river water, the water collected at lower sites seemed to be more effective for diatom growth than that from upper river sites, especially in Nanakita River. Regardless of the higher nutrient concentration (N, P, Si) in the O-up water, the growth of diatoms in the O-low water treatment was significantly increased compared to O-up water treatment. This is due to the fact that FA-Fe concentration of O-low water was 10 times higher than that of O-up water, as shown in a previous report suggesting the importance of FA-Fe on the growth of diatoms (Matsunaga et al., 1998b). The numbers of diatoms in N-low water treatment was remarkably higher than that of the O-low water

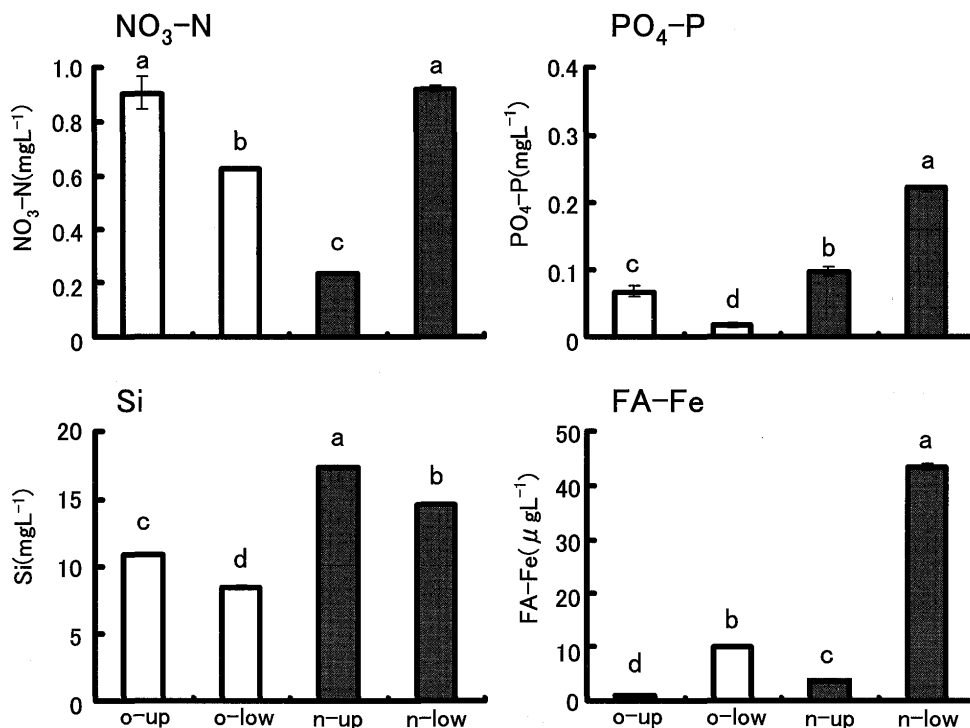


Figure 5. Nutrient status of the river water added to the media for diatom culture

* Error bars mean SD

* $\alpha=0.1$ (Tukey method)

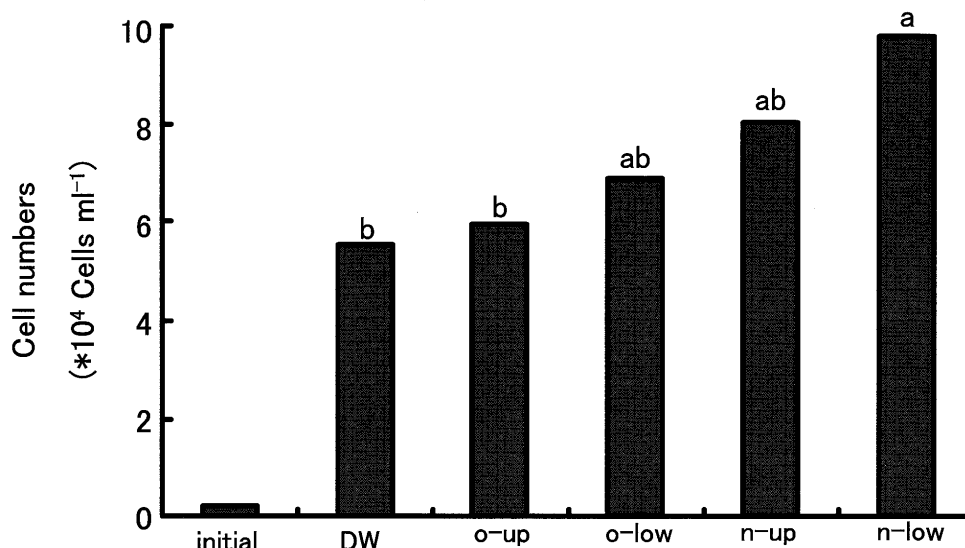


Figure 6. Influences of river water added on the growth of *Skeletonema*

■ *Skeletonema*

* $\alpha=0.1$ (Tukey method)

treatment, also suggesting the possible importance of other nutrients on the growth of coastal diatoms. In particular, Si is an important element for the growth of diatoms. The Si concentration of the Nanakita River was significantly higher than the Ohkawa River due to the surface geology of volcanic rock in upper region; in the Ohkawa River the surface geology was plutonic rock. It has also been reported that water originating from the watershed dominated by volcanic rock was much higher in Si than that dominated by plutonic rock (Kobayashi, 1961). This suggests that the quality of river water may greatly influence the growth of coastal diatoms. In particular, FA-Fe originating from urbanized drainage water may have as important a role as afforestation in terms of watershed environment. Further detailed study is required to clarify the effects of forest vegetation on coastal primary production.

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References

Hatakeyama, S. (2003) Forest is Marine's Lover, Journal of Japan Society on Water Environment, 26: 18-21.

Hokkaido branch, Japanese society of analytical chemistry (1994) Analysis of Water (4th edition). (in Japanese)

Igarashi, K., K. Matsunaga, K. Koike, K. Toya and S. Fukuse (1982) Determination of organically-bound iron in fresh and coastal sea water, Bulletin of the Faculty of Fisheries, Hokkaido University, 33: 51-55.

Kobayashi, J. (1961) Research of the average water qualities and characteristics of Japanese river (in Japanese). Agricultural Research, 48: 63-106.

Matsunaga, K., G. Nigi, Y. Suzuki, H. Yasu and G. Deein (1998a) Effect of fulvic acid-Fe derived from the forest on the growth of *Laminaria relifosa* Miyabe and *Undaria pinnatifida* Suringan, Bulletin of the Society of Sea Water Science Japan, 52: 315-318.

Matsunaga, K., J. Nishioka, K. Kuma, K. Toya and Y. Suzuki (1998b) Riverine input of bioavailable iron supporting phytoplankton growth in Kesenuma Bay (Japan), Water Research, 32: 3436-3442.

Stookey, L. L. (1970) Ferrozine-a new spectrophotometric reagent for iron, Analytical Chemistry, 42: 779-781.

Takahashi, M., N. Kaiga and R. Sudo (2003) Fluorescence excitation spectrometric analysis and evaluation of fulvic-like organic matter in river water, Journal of Japan Society on Water Environment, 26: 153-158.