Korean J. Limnol. 41 (2) : 137~143 (2008)

Effect of Different Substrate Characteristics on Abundance and Community Structure of Epilithic Diatoms in Two First-Order Streams

Ishida, Noriko*, **Yumi Iyoda**[1], **Osamu Mitamura**[2] **and Jun-Kil Choi**[3]

(Faculty of Literature, Nagoya Women's University, Nagoya 468-8507, Japan,
[1]Takashima Elementary School No. 3, Itabashi 175-0082, Japan,
[2]School of Environmental Science, University of Shiga Prefecture, Hikone 522-0057, Japan,
[3]Department of Biological Science, Sangji University, Wonju 220-702, Korea)

The abundance and community structure of epilithic diatoms grown on different substrata were investigated in two first-order streams located in a limestone and granite area, north of the Suzuka Mountains in Central Japan. Experiments were conducted as follows: limestone and granite without algae were submerged in their own streambed or another stream station and incubated for seven weeks, while limestone and granite with algae were transferred to another stream station and incubated at the same time. The diatom biomass was consistently high in the limestone station experiments compared to those at the granite station. In addition, there was more diatom biomass on granite substrata than on the limestone substrata at both stations. The present results suggested that the difference in water chemistry including the major nutrient concentrations was the limiting factor for algal growth in these two streams; however, when the water chemistry was the same in each stream, the difference in substratum characteristics became the important factor affecting the diatom abundance. The diatom community grown on the transferred substrata with and without algae became similar to those grown on the original substrata in each stream during the incubation period. It was suggested that the effect of the substrata characteristics on the diatom community structure was rather small.

Key words : substratum characteristics, epilithic diatom, first-order streams, geology

INTRODUCTION

The importance of geology as a determinant of algal community structure in rivers was suggested in some studies (e.g. Lay and Ward, 1987; Biggs and Gerbeaux, 1993; Leland and Porter, 2000). In the upper area of rivers, variation in water chemistry and the substratum characteristics (e.g. rock type, roughness, grain size) caused by the difference in the geological character of bedrock may affect the abundance and species composition of diatom communities. We investigated the species compositions of epilithic diatoms collected at the upper area of the rivers located in different geological regions in a small area of central Japan, and found that the diatom communities were classified as characterizing groups in relation to geological character of bedrock (Ishida and Mitamura, in preparation). Few studies have investigated the relation of the effects of water chemistry and the substratum on the abundance and species composition of diatom community in natural streams.

* Corresponding author: Tel: +81-52-801-6068, Fax: +81-52-805-3875, E-mail: ishida@nagoya-wu.ac.jp

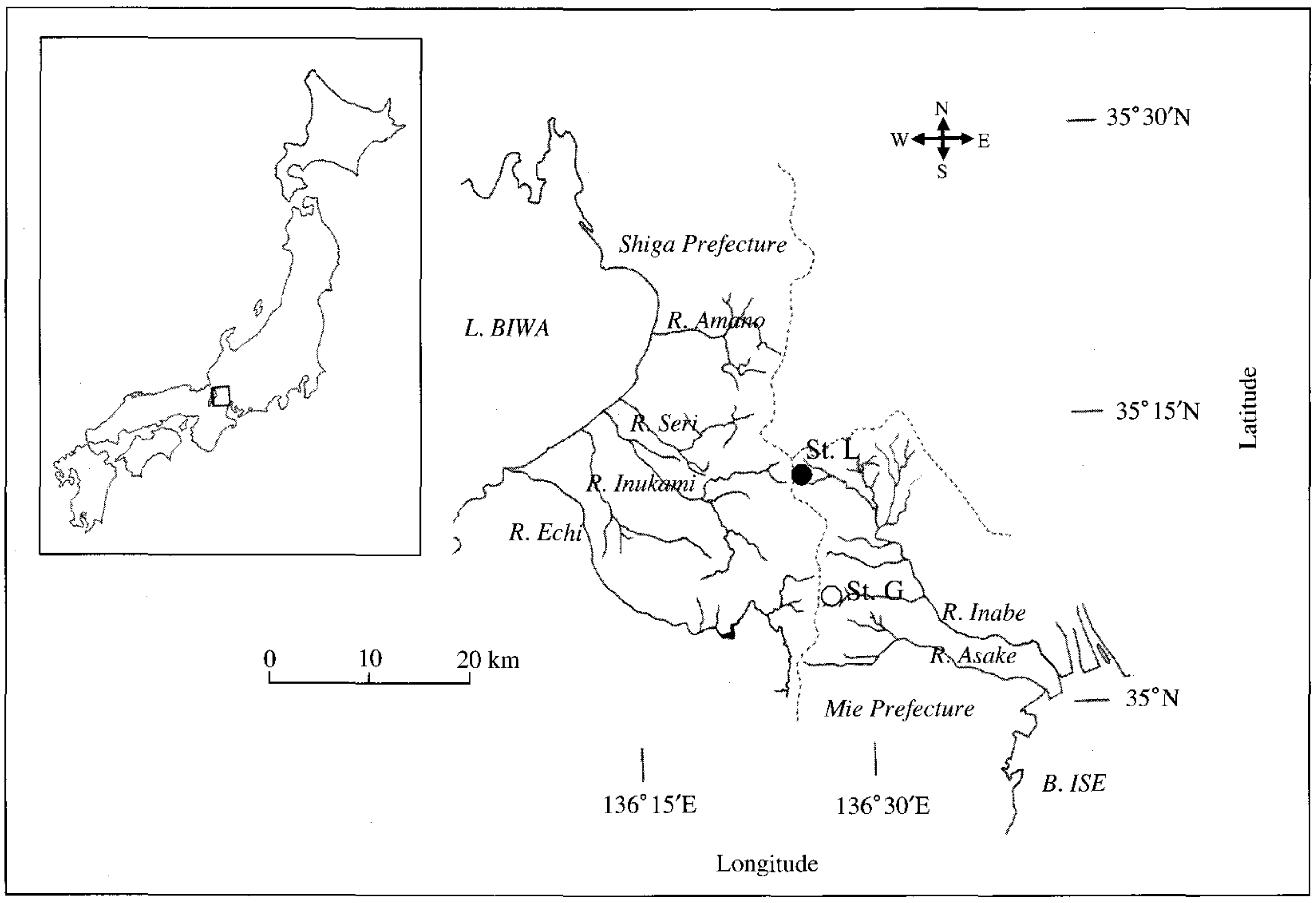

Fig. 1. A map of the investigation sites located in first-order streams in the northern part of the Suzuka Mountains, Central Japan. Sts. L and G were located on limestone and granite areas, respectively.

The aim of this study is to test the effect of the difference of mineral substratum characteristics on the diatom abundance and community structure in two first-order streams originating from a geologically different area (limestone and granite area).

MATERIALS AND METHODS

Investigations were conducted at two stations located in first-order streams from October to November 2001. St. L (35° 11′N; 136° 27′E) was located in the limestone area and St. G (35° 6′N; 136° 27′E) in the granite area (Fig. 1).

Diatom accrual experiments were carried out as follows. At St. L, about forty stones (ca. 5 ~ 10 cm in diameter) were collected from the streambed and all attached substances including algae were removed with a wire brush, and then sprayed with 70% alcohol and dried. Stones were then put into four stainless steel baskets (ca. 40 cm sides). Two baskets were submerged again in their own streambed, and two baskets were then immediately put into a container filled with river water and carried to another stream station and submerged. These baskets were set in 20 ~ 30 cm water depth under the condition without the limitation of light by terrestrial vegetation. Simultaneously, stones from St. L and G with attached algae were transferred to the other station and submerged in the same manner. Therefore, six experiments were arranged as follows: Exp. L-l: limestone submerged at St. L, Exp. L-g: granite submerged at St. L, Exp. G-g: granite submerged at St. G, Exp. G-l: limestone submerged at St. G, Exp. G → L: granite submerged at St. L, Exp. L → G: limestone submerged at St. G. In the former four experiments, substrata without algae were used, while in the latter two experiments they were with algae. In each experiment, epilithic diatoms were collected every week from the five stones with a plastic brush for seven weeks. Simultaneously, at every sampling time, the species composition of the *in situ* diatom community at each station was determined using stones randomly collected from each streambed.

Each sample was added to a glutaraldehyde solution at a final concentration of 0.2% and stored in a refrigerator until microscopic analysis.

Cell numbers of living diatom were counted using a light microscope at 200～400× magnification. Cells containing protoplasts were considered to be alive. For the classification of diatoms and determination of diatom community structure, permanent slides were prepared according to the procedure of Nagumo (1995). About 500 diatom cells were counted from permanent slides using a light microscope at 1,000× magnification, and species were identified. To determine the actual biovolume of each algal taxon, the shape of each species was measured microscopically using the graphic analysis system by Mac SCOPE ver. 2.5. The numbers of diatom cells were converted to biovolume using the average biovolume of each taxon. The relative abundances of diatom taxa were calculated by their biovolume. Identification of diatom was performed mainly according to the description by Krammer and Lange-Bertalot (1986, 1988, 1991a, b), also with reference to Patrick and Reimer (1966) and Kobayasi *et al.* (1986).

Water temperature and electric conductivity were measured using a conductivity meter (Yokogawa, Model SC-51). Values of pH were obtained by the colorimetric method. To determine the concentration of nutrients, the collected water samples were immediately filtered through Whatman GF/C glass fiber filters treated by ignition at 420°C for 2 h and stored at −20°C in a deep freezer until chemical analysis. Ammonia was determined by the method of Sagi (1966), nitrite by Bendshneider and Robinson (1952), nitrate by Mitamura (1997), and phosphate by Murphy and Riley (1962).

To estimate the substrata size of the streambed at Sts. L and G, the specimens of stone and sand were retrieved from the streambed with two 50×50 cm quadrates at each station. The x, y and z dimensions of each collected particle over 1 cm in diameter were measured, and the second highest value was used for the substratum particle size. Particles under 1 cm in diameter were filtered through two different mesh-sized sieves (0.4 mm and 0.2 mm) to divide into three sizes, and the specimen of each size was weighed separately. From the results of the determination of substratum size, coarse gravel and pebbles dominated in the streambed of St. L, while cobbles, gravel and sands were major constituents at St. G. The judgment of particulate size was based on the description by Cushing and Allan (2001).

Table 1. Physico-chemical parameters measured at each station during the investigation period from October to November 2001.

	Days	WT (°C)	pH	EC ($mS\ m^{-1}$)	CV ($cm\ s^{-1}$)	DIN (μM)	DIP (μM)
St. L	0	12.0	7.5	17.3	26.9	57.8	0.34
	7	14.3	7.7	17.1	25.2	50.0	0.36
	14	11.5	7.5	17.5	ND	51.3	0.37
	21	11.8	7.4	16.3	21.7	52.8	0.38
	28	13.5	7.7	17.3	19.6	50.1	0.32
	35	12.9	7.7	17.3	13.6	49.3	0.31
	42	14.9	7.7	16.2	15.9	50.4	0.36
	49	12.4	7.6	16.5	12.9	47.8	0.27
St. G	0	14.3	6.9	5.1	22.9	30.0	0.05
	7	13.5	6.7	4.6	21.2	31.9	0.06
	14	11.6	6.7	4.8	ND	31.8	0.04
	21	12.2	6.7	4.5	20.0	26.9	0.03
	28	13.8	6.7	4.9	19.4	30.9	0.03
	35	16.2	6.8	4.6	15.5	29.5	0.02
	42	16.0	6.6	3.7	14.4	29.2	0.08
	49	16.3	6.7	4.1	10.2	29.7	0.03

*CV: Current velocity

RESULTS

1. Physico-chemical variables

From the results of previous study in 1997～1998 (Ishida and Mitamura, in preparation), the Ca and HCO_3 concentrations were high at St. L, showing average values of 1.56 and 1.44 meq L^{-1}, respectively, whereas they were low at St. G, averaging 0.21 and 0.17 meq L^{-1}, respectively.

Mean values of water temperature, pH, electric conductivity, dissolved nitrogenous compounds and phosphate during the investigation period were 13.8°C, 7.7, 17.1 mS m^{-1}, 50 μM, and 0.36 μM at St. L, and 12.9°C, 6.7, 4.5 mS m^{-1}, 30 μM and 0.03 μM at St. G, respectively (Table 1). Those parameters except for water temperature showed obviously high values at St. L. Each parameter was generally constant throughout the investigation period.

2. Increase of diatom cells in each experiment

Numbers of diatoms at St. L (Exp. L-l, L-g) and St. G (Exp. G-g, G-l) during the incubation time were shown in Fig. 2. Increase rates of diatom cells in Exps. L-l and L-g were 4,020 and 6,740 cells cm^{-2} day^{-1}, respectively, during the first three weeks. Those values in Exps. G-g and G-l

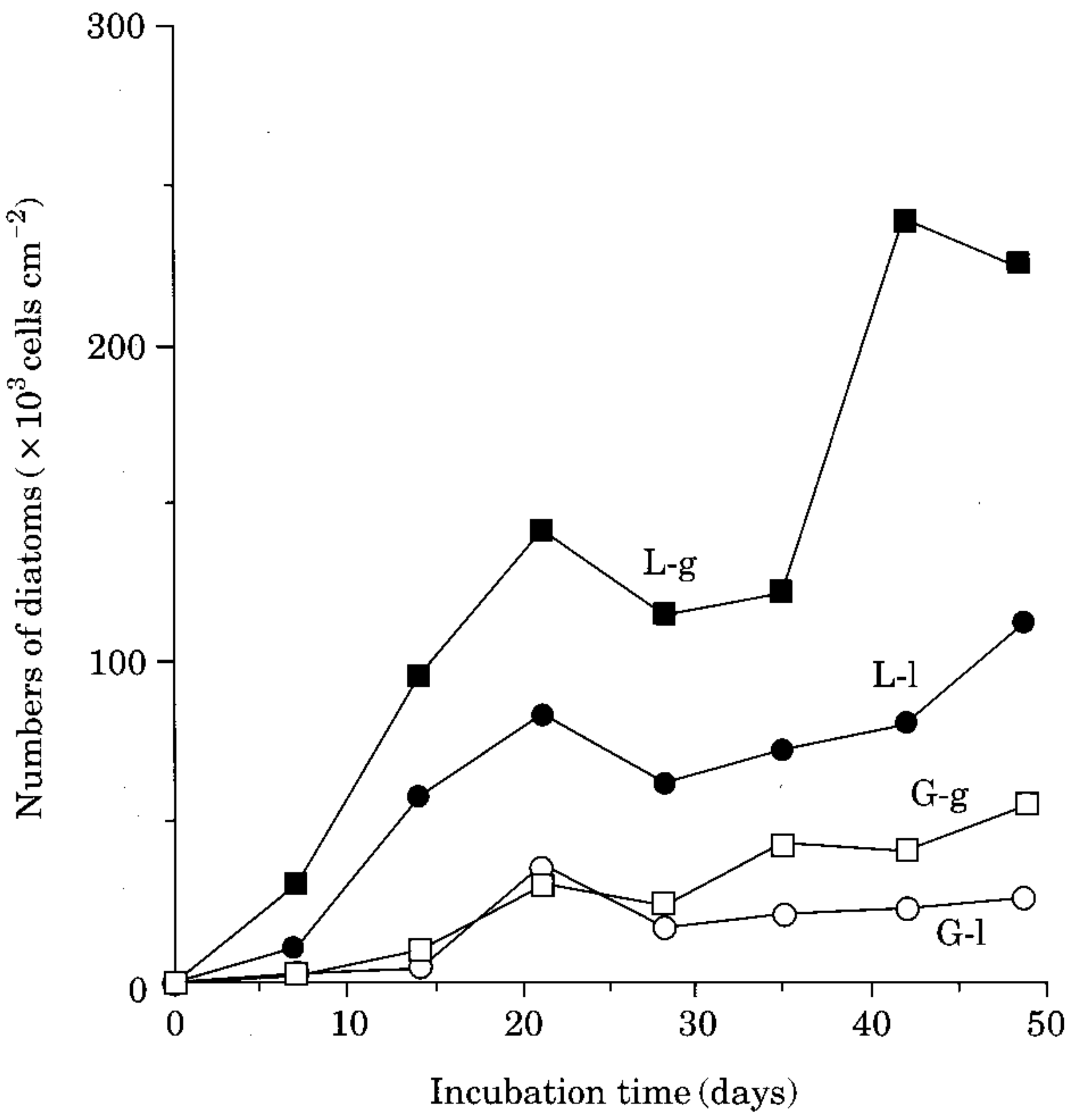

Fig. 2. Numbers of diatoms in the Exps. L-l (closed circle: limestone without algae submerged at St. L), L-g (closed square: granite without algae submerged at St. L), G-g (open square: granite without algae submerged at St. G) and G-l (open circle: limestone without algae submerged at St. G).

were low, at 1,710 and 1,450 cells cm^{-2} day^{-1}, respectively. Maximum numbers of diatom cells in Exps. L-l, L-g, G-g and G-l were 113,000, 239,000, 56,000 and 26,000 cells cm^{-2}, respectively. Increase rates of diatom cells and the maximum numbers were both high in the experiments of St. L. In addition, diatom biomass was larger on the granite substrata than on the limestone at both stations.

3. Diatom community structure in each experiment

Changes of the species composition in each experiment at St. L and St. G were shown in Fig. 3. Dominant taxa (above 5% relative abundance) were indicated. The diatom communities of Exps. L-l and L-g after three weeks were similar to each other. Dominant taxa were *Achnanthes lanceolata* (Brebisson) Grunow, *Achnanthes minutissima* Kutzing, *Cocconeis placentula* var. *euglypta* Ehrenberg and *Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck. The relative abundances of these four taxa were 21, 15, 28 and 30% in Exp. L-I, 21, 17, 26 and 25% in Exp. L-g, respectively (Fig. 3a, 3b). In the first two weeks, many taxa appeared in each community of Exps. L-l and L-g. There were no clear differences between those communities.

Table 2. Relative abundance (%; mean (SD)) of dominant diatoms found in the streambed of Sts. L and G during the investigation period from October to November 2001.

Taxa	St. L	St. G
Achnanthes japonica		15.6 (6.3)
Achnanthes convergens		16.7 (12.2)
Achnanthes minutissima	13.1 (3.9)	35.6 (19.1)
Achnanthes lanceolata	15.2 (8.2)	
Cocconeis placentula var. *euglypta*	33.4 (8.5)	
Cocconeis placentula var. *lineata*	30.1 (7.2)	7.6 (5.6)

Achnanthes japonica H. Kobayasi and *A. minutissima*, small-sized taxa, dominated in Exps. G-g and G-l. The relative abundances of these taxa were 70% in Exp. G-g, and 62% in Exp. G-l (Fig. 3d, 3e). Species composition of diatoms in Exp. G-l in the first two weeks resembled those of St. L, because of the import of cells with transferred substrates.

As for the Exp. L → G, two varieties belonging to *Cocconeis placentula*, which was the dominant taxa at St. L, dominated by five weeks after incubation. In Exp. G → L, on the other hand, the diatom community structure transferred from St. G was consistent with those of the experiments of St. L (Exps. L-l and L-g) by three weeks. Finally, the diatom communities carried from other streams became similar to those in each stream during the incubation period (Fig. 3c, 3f).

Dominant diatoms found in the streambed of Sts. L and G during the incubation period were shown in Table 2. *Achnanthes lanceolata, Achnanthes minutissima, Cocconeis placentula* var. *euglypta* and *Cocconeis placentula* var. *lineate* were dominant, with the latter two taxa accounting for more than 30%, throughout the investigation period at St. L. In the streambed of St. G, *Achnanthes japonica* and *Achnanthes minutissima* dominated, and the mean relative abundances of these two taxa were more than 50%. The diatom community structure in the latter period of the present experiment is similar to the original structure in both streambeds.

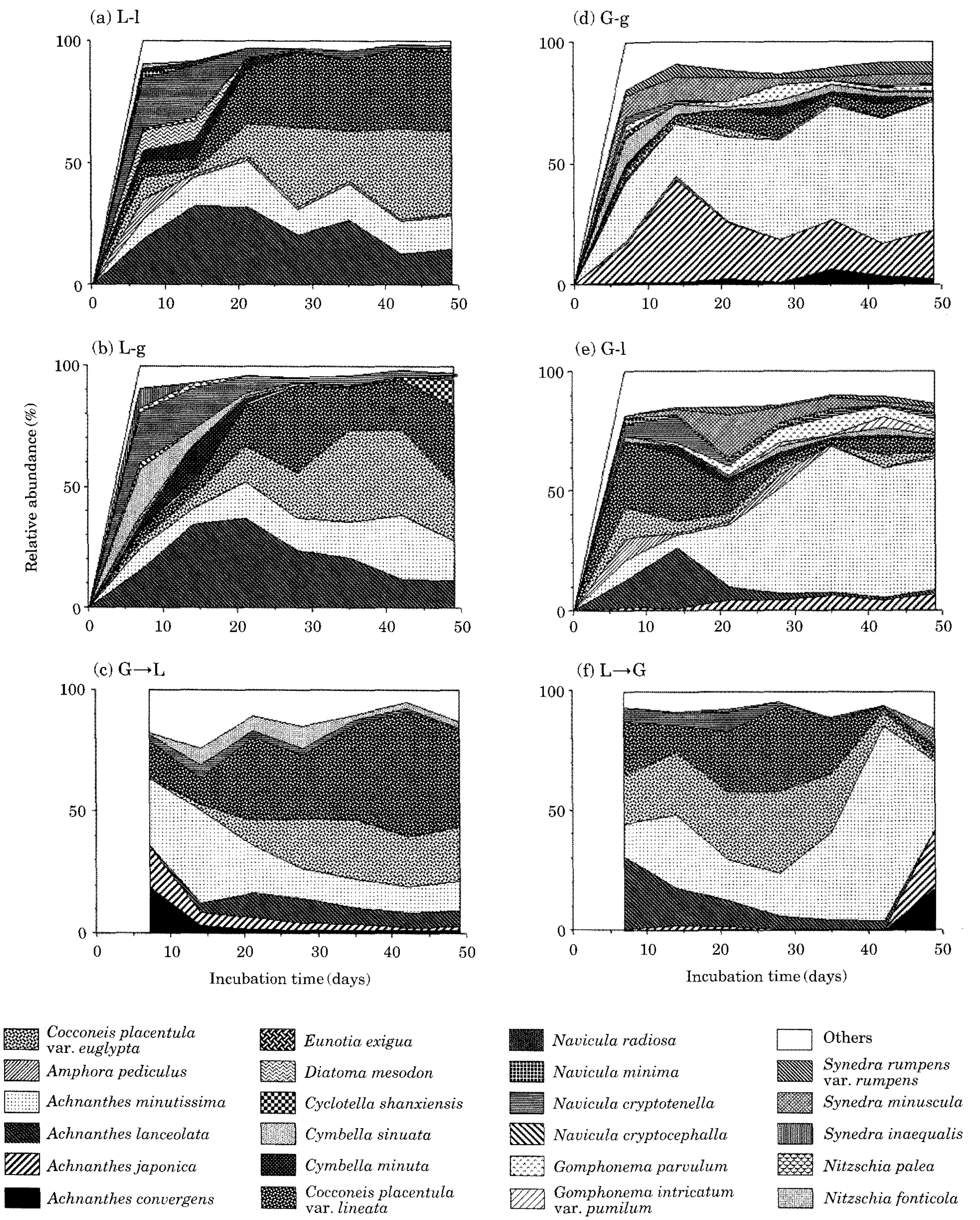

Fig. 3. Changes in the relative abundance of each dominant diatom in each experiment during the investigation period. Exps. L-l (limestone without algae submerged at St. L), L-g (granite without algae submerged at St. L), G→L (granite with algae submerged at St. L), G-g (granite without algae submerged at St. G), G-l (limestone without algae submerged at St. G) and L→G (limestone with algae submerged at St. G) were shown in a, b, c, d, e and f, respectively.

DISCUSSION

Blinn *et al.* (1980) found that the standing crop and community structure were significantly similar on all substrata (limestone, sandstone and basalt) over 5 weeks except early in the colonization period. On the other hand, some reports indicated that chlorophyll *a* levels were consistently higher in hard waters than in soft waters (Marker, 1976; Shamsudin and Sleigh, 1994). Ferris and Fyfe (1989) found that the counts of heterotrophic bacteria and cyanobacterial algae on blocks of cut limestone were greater than those on granite, and were submerged in the Speed River, Ontario, Canada. In the present study, the diatom biomass obtained from two experiments at St. G in the granite area was consistently smaller than those at St. L in the limestone area. The water originating from granite indicated the rather oligotrophic character of nutrients compared with that from limestone. From the results of this study, dissolved inorganic nitrogenous compounds (NO_3 accounted for the major parts) and phosphate concentrations showed 2 and 9 times higher at the limestone area than the granite area, respectively. The present results suggested that water chemistry, probably the major nutrient concentrations, was the limiting factor for algal growth in these two streams. Another possible important factor was the release of nutrients from the stones. Some investigators indicated that the weathering of bedrock (such as dolomite and limestone) was the dominant source of inorganic nitrogen and phosphorus in the water (Lay and Ward, 1987; Mulholland, 1992). Pringle (1990) demonstrated that the nutrients from the nutrient-diffusing artificial substrata were effective in increasing growth and maintaining the species diversity of the attached algae. The influence of nutrients supplied from the weathering of stones on the abundance and species of epilithic algae should be investigated in future. In the present study, diatom biomass was large on the granite substrata in both streams. Substrata characteristics such as the difference of roughness between limestone and granite might affect the abundance of diatom. Bergey (2005) reported that a rougher substrate such as pumice and schist had a greater total algal biomass than smoother substrata such as glass and greywacke, because of more algal biomass in crevices of the rougher substrata. Ferris and Fyfe (1989) also indicated that the abraded granite (rougher surface) was colonized more heavily than the polished granite.

Diatom community structures of all incubation experiments in each station were similar to each community of St. L and G reported in the previous study (Ishida and Mitamura, in preparation). After two or three weeks from the beginning of incubation, the species composition of diatom community grown on the transferred substrata without algae became similar to that on the original substrata at the two stream stations. Little difference was found between the species composition of diatom communities on the transferred substrata with algae and on the original substrata of each stream at the end of the investigation period. The present results thus suggested that the effect of the difference of the substratum characteristics on the diatom community structure was rather small. The establishment of an organic matrix might provide relatively similar attachment surfaces for microbial invasion (Blinn *et al.*, 1980).

The biovolumes of *Achnanthes japonica*, *Achnanthes minutissima*, *Achnanthes lanceolata*, *Cocconeis placentula* var. *euglypta* and *Cocconeis placentula* var. *lineata* averaged 44, 51, 227, 355, 1,278 μm^3, respectively. The first two taxa were dominant at St. G, and the other three were representative at St. L. At the relatively oligotrophic St. G, small size diatoms dominated, however, more larger size diatoms dominated at St. L. Physicochemical factors such as trophic level of stream water and surface characteristics of substratum may therefore relate to the appearance of different-sized diatom.

ACKNOWLEDGEMENT

We wish to thank the members of the laboratory staff of Nagoya Women's University for their valuable assistance in the field investigations.

LITERATURE CITED

Bendshneider, K. and R.J. Robinson. 1952. A new spectrophotometric method for the determination of nitrite in sea water. *J. Mar. Res.* **11**: 87-89.

Bergey, E.A. 2005. How protective are refuges? Quantifying algal protection in rock crevices. *Freshw. Biol.* **50**: 1163-1177.

Biggs, B.J.F. and P. Gerbeaux. 1993. Periphyton development in relation to macro-scale (geology) and micro-scale (velocity) limiters in two gravel-bed rivers, New Zealand. *N. Z. J. Mar. Freshw. Res.* **27**: 39-53.

Blinn, D.V., A. Fredericksen and V. Korte. 1980. Colonization rates and community structure of diatoms on three different rock substrata in a lotic system. *Br. Phycol. J.* **15**: 303-310.

Cushing, C.E. and J.D. Allan. 2001. Streams: Their ecology and life. Academic Press.

Ferris, F.G. and W.S. Fyfe. 1989. Effect of mineral substrate hardness on the population density of epilithic microorganisms in two Ontario rivers. *Can. J. Microbiol.* **35**: 744-747.

Kobayasi, H., T. Nagumo and S. Mayama. 1986. Observation on the two rheophilic species of the genus *Achnanthes* (Bacillariophyceae), *A. convergens* H. Kob. and *A. japonica* H. Kob. *Diatom* **2**: 83-93.

Krammer, K. and H. Lange-Bertalot. 1986. Bacillariophyceae 1 Naviculaceae. *In*: Susswasser flora von Mitteleuropa 2/1 (Ettl, H., J. Gerloff, H. Heynig and D. Mollenhauer eds.). Gustav Fischer Verlag, Jena.

Krammer, K. and H. Lange-Bertalot. 1988. Bacillariophyceae 2 Bacillariaceae, Epithemiaceae, Surirellaceae. *In*: Susswasser flora von Mitteleuropa 2/2 (Ettl, H., J. Gerloff, H. Heynig and D. Mollenhauer eds.). Gustav Fischer Verlag, Jena.

Krammer, K. and H. Lange-Bertalot. 1991a. Bacillariophyceae 3 Centrales, Fragilariaceae Eunotiaceae. *In*: Susswasser flora von Mitteleuropa 2/3 (Ettl, H., J. Gerloff, H. Heynig and D. Mollenhauer eds.). Gustav Fischer Verlag, Jena.

Krammer, K. and H. Lange-Bertalot. 1991b. Bacillariophyceae 4 Achnanthaceae, Kritische Erganzungen zu Navicula (Lineolatae) und Gomphonema. *In*: Susswasser flora von Mitteleuropa 2/4 (Ettl, H., G. Gärtner, J. Gerloff, H. Heynig and D. Mollenhauer eds.). Gustav Fischer Verlag, Jena.

Lay, J.A. and A.K. Ward. 1987. Algal community dynamics in two streams associated with different geological regions in the southeastern United States. *Arch. Hydrobiol.* **108**: 305-324.

Leland, H.V. and S.D. Porter. 2000. Distribution of benthic algae in the upper Illinois River Basin in relation to geology and land use. *Freshw. Biol.* **44**: 279-301.

Marker, A.F.H. 1976. The benthic algae of some streams in southern England. I. Biomass of the epilithon in some small streams. *J. Ecol.* **64**: 343-358.

Mitamura, O. 1997. An improved method for the determination of nitrate in fresh waters based on hydrazinium reduction. *Mem. Osaka Kyoiku Univ. Ser III* **45**: 297-303.

Mulholland, P.J. 1992. Regulation of nutrient concentrations in a temperate forest stream: Roles of upland, reparian, and instream processes. *Limnol. Oceanogr.* **37**: 1512-1526.

Murphy, J. and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **27**: 31-33.

Nagumo, T. 1995. Simple and safe cleaning methods for diatom samples. *Diatom* **10**: 88. (in Japanese)

Patrick, R. and C.W. Reimer. 1966. The Diatoms of the United States. 1. Academy of Natural Sciences of Philadelphia, Philadelphia.

Pringle, C.M. 1990. Nutrient spatial heterogeneity: Effects on community structure, physiognomy and diversity of stream algae. *Ecology* **71**: 905-920.

Sagi, T. 1966. Determination of ammonia in sea water by the indophenol method and its application to the coastal and off-shore waters. *Oceanogr. Mag.* **18**: 43-51.

Shamsudin, L. and M.A. Sleigh. 1994. Seasonal changes in composition and biomass of epilithic algal floras of a chalk stream and a soft water stream with estimates of production. *Hydrobiologia* **273**: 131-146.

(Manuscript received 28 January 2008,
Revision accepted 22 May 2008)