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Case Studies on the Seasonal Changes of Diatom Community in Paddy Fields

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Abstract

As a case study, the seasonal changes of diatom community in flooded water of paddy fields were discussed. The investigations were conducted on Andosols (Field 1) at Field Science Center, Tohoku University, and on Fluvisols (Field 2) at Furukawa Agricultural Experimental Station, located at Miyagi Prefecture, Japan, during the rice-growing season of 2004. The results obtained are as follows:

- 1) Diatom cell density at Field 1 ranged between 2.1×10^5 and 1.1×10^6 cells L^{-1} . There was no large difference during the experimental period in Field 1. Diatom cell density in Field 2 ranged between 4.3×10^5 and 5.3×10^6 cells L^{-1} . Diatoms in Field 2 were low in the end of May, and increased gradually thereafter.
- 2) Nineteen genera were observed in Field 1 and twenty-four genera in Field 2. In general, *Nitzschia* was predominated genus in the both fields. In Field 1, *Melosira* became predominated from June to August. In Field 2, *Navicula* was also predominated in July.

Introduction

Rice is a known silicon (Si) accumulator, and the plant benefits from Si nutrition. There is a definitive need to consider Si as an agronomically essential element for increasing and sustaining rice production (Savant et al., 1997).

Recently, Si concentration in rivers and coastal region had decreased. Kumagai et al. (1998) reported that Si in the river that irrigated to paddy fields has been decreased significantly in last 40 years. Humborg et al. (1997) investigated long-term data sets of water and nutrient discharge from the

River Danube to the Black Sea. This data revealed a reduction in the dissolved Si load of the river by about two-thirds due to dam construction in the early 1970s.

It is reasonable that diatom (*Bacillariophyceae*) is responsible for the process of reduction in the dissolved Si load. They are very important primary producer in aquatic ecosystems, and often became predominant among phytoplanktons (Iwasa, 1976). Planktonic diatoms take dissolved Si from water column to make their frustules. Much of the Si taken by diatoms would be difficult to return to the water column because it is deposited on the bottom and is not readily dissolved (Miyajima et al., 1995).

Above phenomena may also happen in the paddy fields, which use a large amount of irrigation water and act as water reservoir. This can be supposed with the investigation on the changes of Si concentration in flooded water particularly in terraced and enlarged paddy fields where rice plants were cultivated (Saigusa and Kobayashi, 2002, Saigusa et al., 2004). In the terraced paddy field conducted plot-to-plot irrigation, the Si concentration of the flooded water decreased from the upper field to the lower field (Saigusa and Kobayashi, 2002). Likewise, the enlarged paddy field, Si concentration decreased gradually with a distance from the water inlet to the water outlet (Saigusa et al., 2004). These phenomena seem to result from Si consumption by diatoms or rice plants and might be one of the reasons to decrease Si concentrations in the river and the coastal regions.

Biomass and seasonal growth pattern of paddy field diatoms provide a basis for estimating their Si consumption. Nevertheless, only few studies

have focused on the diatom community in paddy field. Thus, this study was conducted to describe the seasonal changes of diatom communities in two paddy fields.

Materials and Methods

Experimental design

The research was conducted in two paddy fields located in Miyagi prefecture, northeastern Japan.

Field 1 was located at the Field Science Center of Tohoku University (38°44.4 N, 140°45.3 E, altitude 180m) in Naruko town. It was a terraced paddy field composed of six fields. This investigation was conducted at second field from top field. The soil in the paddy field was Andosols and the area was 2,640m². Paddy field irrigation was conducted from 26 April to 30 August 2004. Water was maintained at 2–7 cm depth and irrigated from a neighbor stream through the fallow paddy field. Rice seedlings were transplanted with an average density of 24.2 seedlings m⁻² on 18 May. Coated fertilizers (N:P₂O₅:K₂O=14:20:14) were applied at the rate of 70kgN ha⁻¹ at transplanting time. Herbicides were applied on 27 May (pretilachlor) and 30 June (pyriminobac-methyl). Fungicide (probenazole) was applied on 23 July. Rice was harvested on 12 October.

Field 2 was located at Furukawa Agricultural Experiment Station (38°35.5 N, 140°54.5 E, altitude 26m) in Furukawa city. The soil in the paddy field was Fluvisols and area was 96,000m². Paddy field irrigation was applied from 20 May to 25 August 2004. Water was maintained at 3–5.5 cm depth and irrigated from agricultural water through the pond. Direct seeding culture of rice in the paddy field was conducted. Rice seeds were sowed 15 May. Coated fertilizers (N:P₂O₅:K₂O:Mg=12:16:14:3) were applied

at the rate of 50kgN ha⁻¹. Herbicide was applied on 4 June (pyriminobac-methyl) and 29 June (bentazone). Fungicide was applied on 2 July (probenazole) and 23 July (pyroquilon). Rice was harvested 4 October.

Methods of sampling and counting diatoms in flooded water

Flooded water was collected six times in the field water inlet during May–August. Samplings at Field 1 were conducted on 25 May, 27 May, 21 June, 7 July, 23 July and 8 August. Samplings at Field 2 were conducted on 26 May, 28 May, 10 June, 14 July, 28 July and 12 August. Water samples of 250 ml were taken with three replications and fixed using formalin (37%). These were concentrated 8.3–25 times. They were put into prepared slide of 100mm³ volume for counting algae cells (MATSUNAMI Co. MPC-200). Living diatom cells, as distinguished from empty frustules by the presence of chloroplasts, were counted under an inverted light microscope (LM) (Nikon Co. ECLIPSE TE300) at magnification of 400. Diatoms were identified at the genus level by using following references: Round *et al.* (1990), Round and Bukhtiyarva (1996) and Cox (1996). The concentrated samples were heated with 1 mol L⁻¹ hydrochloric acid and hydrogen peroxide to clean the diatom frustules from organic materials. Micrographs of the diatom frustules were taken with a scanning electron microscope (SEM) (HITACHI Co. S-4206).

Results

The seasonal changes of diatom cell density in flooded water of Field 1 and 2 are shown in Fig.1. Diatom cell density ranged between 2.1x10⁵ and 1.1x10⁶ cells L⁻¹ at Field 1 and between 4.3x10⁵ and 5.3x10⁶ cells L⁻¹ at Field 2. Diatom assemblage in Field 2 had a low cell density in flooded water just after rice transplanting, and increased gradually thereafter. In contrast, the diatom cell density in Field 1 did not largely fluctuate during experimental period. The diatom cell density in Field 2 was from two to five times higher than that at Field 1, except in May.

The list of diatom genera and the changes of diatom cell density in genus level are shown in Table 1. Nineteen and twenty-four diatom genera were found in Field 1 and Field 2, respectively in this study. *Stauroneis*, *Neidium*, and *Eunotia* were observed just only in Field 1, whereas *Asterionella*, *Aulacoseira*, *Gyrosigma*, *Rhoicosphenia*, and *Cymbella* were

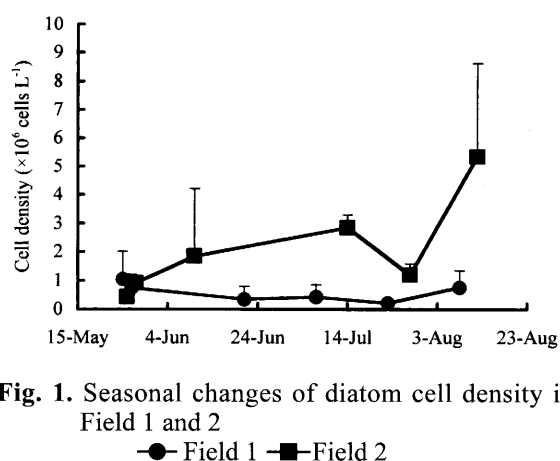


Fig. 1. Seasonal changes of diatom cell density in Field 1 and 2
 ●—Field 1 ■—Field 2

observed just only in Field 2.

In Field 1, *Nitzschia* was generally predominant; it was $7.2 \times 10^4 - 6.6 \times 10^5$ cells L^{-1} in cell density and occupied 18 – 89 % of the total diatom cells. It was the most numerous just after rice transplanting, and decreased rapidly thereafter (Table 1). From June to August, *Melosira* was also dominant with the cell densities of $6.8 \times 10^4 - 3.8 \times 10^5$ cells L^{-1} (Table 1). Eight genera were also abundant with the cell densities of more than 1.0×10^4 at least in one sampling. *Navicula*, *Pinnularia/Caloneis*, and *Surirella* were abundant throughout the sampling period. *Cyclotella* was abundant in May and August. *Rhopalodia* was abundant only in June. *Fragilaria* (sensu lato), *Synedra* and *Diploneis* were abundant only in August.

In Field 2, *Nitzschia* was also predominant with $9.4 \times 10^4 - 2.7 \times 10^6$ cells L^{-1} in cell density and occupied 7 – 61 % of the total diatom cells. Unlike in Field 1, it was not abundant in May and increased thereafter (Table 1). *Navicula* was also dominant in July and August with the cell density of $5.2 \times 10^5 - 2.6 \times 10^6$ cells L^{-1} (Table 1). Seven genera were also abundant with the cell densities of more than 1.0×10^5 at least in one sampling. *Pinnularia/Caloneis* was abundant throughout the sampling period, except May. *Asterionella* and *Alulacoseira* were abundant only in May and occupied 68-77% of the total diatom cells. *Surirella* was abundant only in June. *Fragilaria* (sensu lato), *Melosira* and *Synedra* were abundant only in August.

Figure 2 shows the SEM and LM micrographs

Table 1. List of diatom genera and the changes of diatom cell density ($\times 10^4$ cells L^{-1}) in genus level in Field 1 and Field 2.

Genera	Field 1						Field 2					
	25-May	27-May	21-Jun	7-Jul	23-Jul	8-Aug	26-May	28-May	10-Jun	14-Jul	28-Jul	12-Aug
1 <i>Nitzschia</i>	74.3	66.1	7.8	23.9	7.2	13.3	9.4	11.1	113.0	19.4	28.3	266.3
2 <i>Navicula</i>	4.9	1.2	0.5	4.4	1.7	5.8	0.2	1.2	10.7	255.2	52.3	124.1
3 <i>Fragilaria</i>	0.4	0.4		0.6	+	2.4		1.0	1.5			64.8
4 <i>Melosira</i>	0.1		20.6	6.8	8.6	37.7	1.3	3.0			0.4	13.5
5 <i>Asterionella</i>							18.5	48.3	+	0.2	0.2	0.4
6 <i>Pinnularia/Caloneis</i>	11.6	0.8	0.5	1.7	1.1	2.9	0.4	0.3	34.2	6.0	19.7	20.5
7 <i>Surirella</i>	10.6	0.5	0.5	1.1	0.5	1.0	1.3	1.5	13.4	0.4	1.0	0.7
8 <i>Aulacoseira</i>							10.7	22.5	3.0		2.6	1.7
9 <i>Synedra</i>	0.8	0.8	0.5	0.9	0.6	6.8	0.3	0.4	3.5	0.3	3.2	11.9
10 <i>Cyclotella</i>	0.3	3.7	+	+	+	1.5	0.4	0.8	1.1	0.1	5.3	1.1
11 <i>Diploneis</i>	0.3		+	0.1	0.1	1.2			0.2		0.2	5.4
12 <i>Rhopalodia</i>			2.7	0.4	0.1		+		2.0		1.8	1.8
13 <i>Encyonema</i>	+	0.1	0.2	0.5	0.2	+	0.1	1.0	0.6		1.0	8.4
14 <i>Placoneis</i>	0.2			0.2	0.1	0.7	+	0.1	+	0.2	0.1	4.5
15 <i>Gyrosigma</i>									0.2	1.3	1.1	3.7
16 <i>Amphora</i>	0.2	+	+	0.2	0.2	0.2	+	0.1	0.2		0.2	1.5
17 <i>Gomphonema</i>	0.1	0.3	0.4	0.2	0.1	0.1	0.1	+	0.1	0.1	0.5	0.3
18 <i>Tryblionella</i>	0.2		0.1		0.1	0.2					0.5	
19 <i>Planothidium</i>									0.3	0.1	0.3	0.5
20 <i>Rhoicosphenia</i>												0.5
21 <i>Cymbella</i>											0.3	
22 <i>Stauroneis</i>	0.2				+							
23 <i>Neidium</i>	0.1	+										
24 <i>Sellaphora</i>									0.1			
25 <i>Cocconeis</i>											0.1	
26 <i>Eunotia</i>	+	+			+	+						

* + This symbol means that the diatom cell density is below 1.0×10^2 cells L^{-1} .

of predominant and characterized diatom genera in Field 1 and Field 2. Fig.2-1, 2-2, and 2-3 show *Nitzschia*, it was the most predominant genus in both fields. *Nitzschia nana* (Fig.2-3) was abundant in Field 2. Fig.2-4 and 2-5 show *Navicula*. It was also the predominant genus in Field 2, and was abundant during experimental period in Field 1. *Navicula lanceolata* (Fig.2-4) is taken from Field 1, and *Navicula gregaria* (Fig.2-5) is taken from Field 2. *Melosira* (Fig.2-6) was also dominant in Field 1, *Melosira varians* was observed in both fields. *Pinnularia* (Fig.2-7), *Caloneis* (Fig.2-8) and *Surirella*

(Fig.2-9) were abundant in both fields. *Cyclotella* (Fig.2-10), *Rhopalodia* (Fig.2-11) and *Diploneis* (Fig.2-12) were abundant in Field 1. *Rhopalodia gibba* (Fig.2-11), *Stauroneis* (Fig.2-13) and *Neidium* (Fig.2-14) were observed just only in Field 1. *Asterionella formosa* (Fig.2-15) and *Aulacoseira* (Fig.2-16) were observed just only in Field 2, and were dominant in May.

Discussion

In previous papers, diatom cell density in flooded water has been reported as on the order of 1.0×10^5

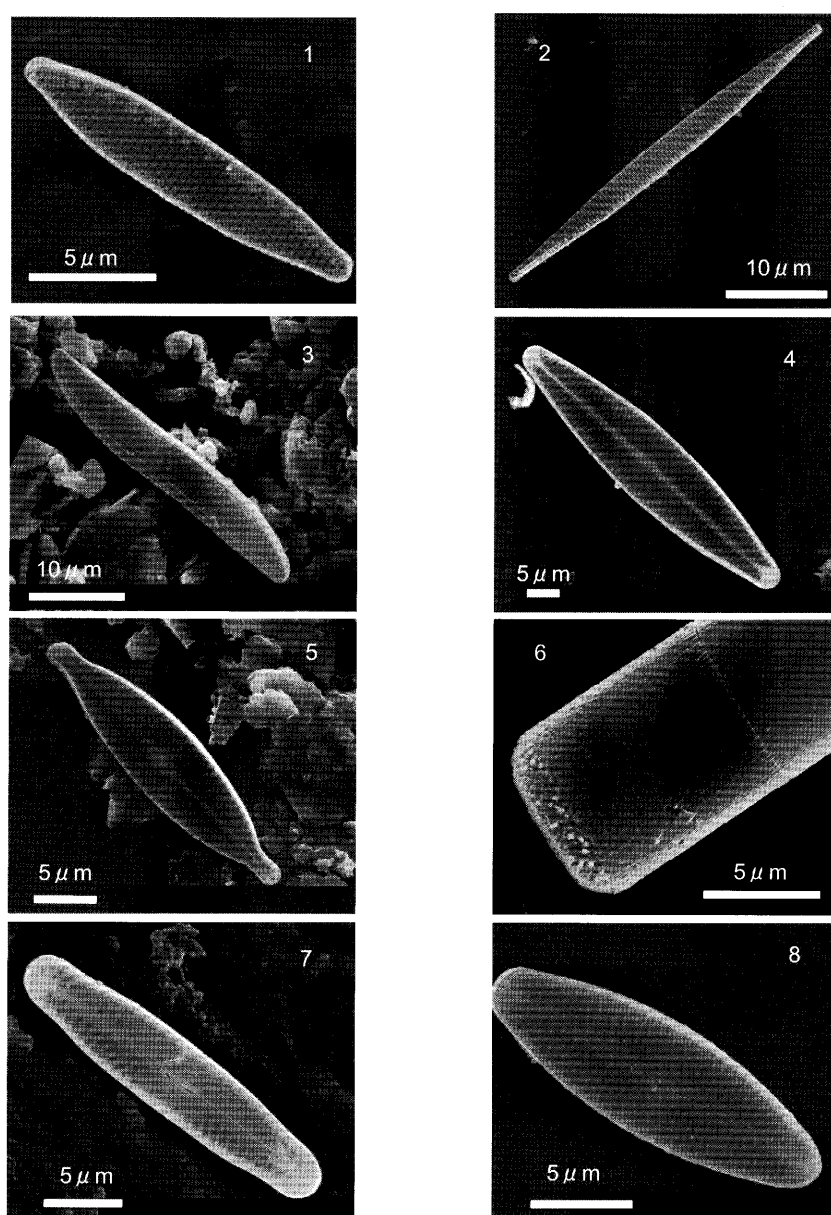


Fig. 2. The SEM and LM micrographs of predominant and characterized diatom genera in Field 1 and Field 2. 1: *Nitzschia hantzschiana* (SEM x5000). 2: *Nitzschia subacicularis* (SEM, x2000). 3: *Nitzschia nana* (SEM, x2000). 4: *Navicula lanceolata* (SEM, x1300). 5: *Navicula gregaria* (SEM, x2500). 6: *Melosira varians* (SEM, x4500). 7: *Pinnularia* sp. (SEM, x3000). 8: *Caloneis bacillum* (SEM, x4000).

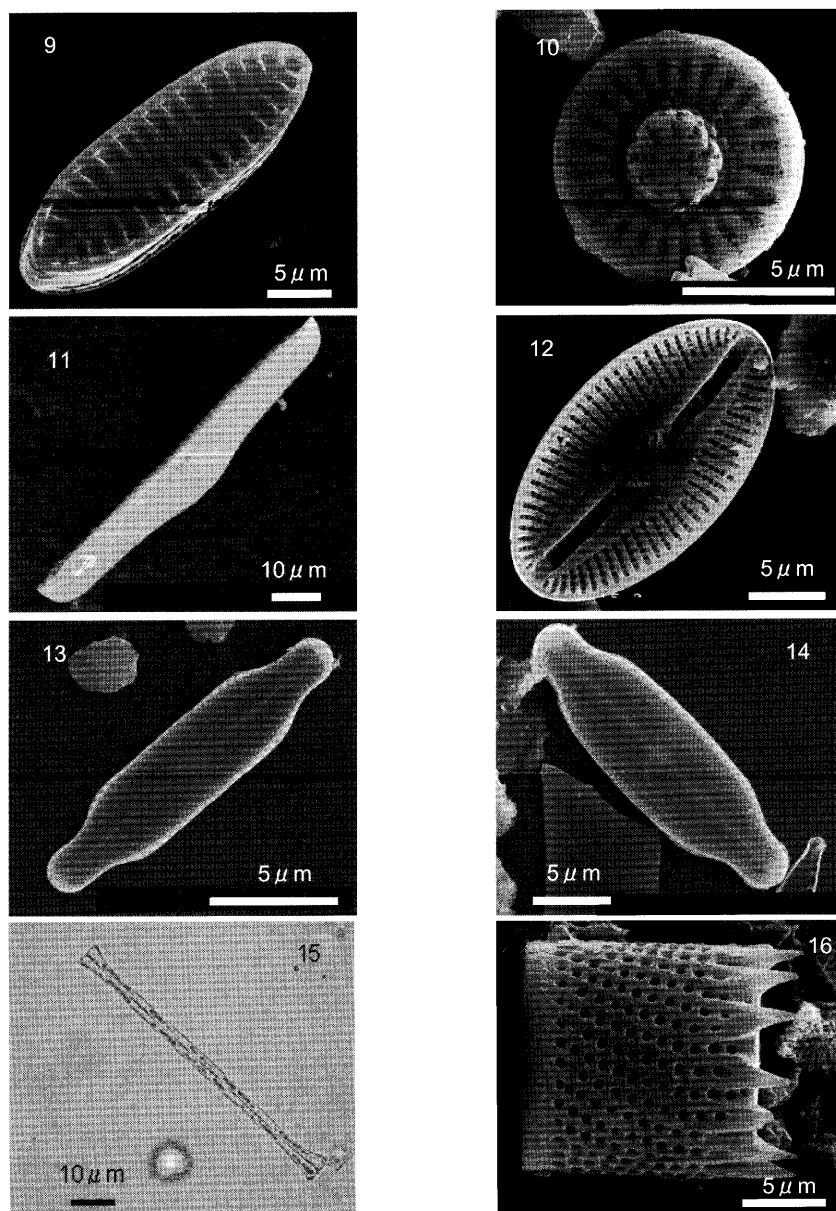


Fig. 2. continued. 9: *Surirella angusta* (SEM, x2500). 10: *Cycloella pseudostelligera* (SEM, x6000). 11: *Rhopalodia gibba* (SEM, x1000). 12: *Diploneis* sp. (SEM, x3000). 13: *Stauroneis thermicola* (SEM, x5000). 14: *Neidium longiceps* (SEM, x3000). 15: *Asterionella formosa* (LM, x800). 16: *Aulacoseira* sp. (SEM, x5000).

and 1.0×10^6 cells L^{-1} (Kurasawa, 1957, Taira et al., 1987). Results of this study showed within the range as stated above, diatom cell densities ranged from 2.1×10^5 to 1.1×10^6 cells L^{-1} in Field 1 and from 4.3×10^5 to 5.3×10^6 cells L^{-1} in Field 2. In the present study, seasonal pattern of diatom cell density in the flooded water were largely different between Field 1 and 2. In previous papers, the diatom cell density was the highest just after rice transplanting and decreased thereafter (Kurasawa, 1957, Taira et al., 1987, Fujita and Nakahara, 1999). Whereas, in Field

2, diatoms had a low cell density in flooded water just after rice transplanting, and increased gradually thereafter, which showed unusual pattern for flooded water in paddy fields. The difference of diatom cell density between Field 1 and 2 might be caused the irrigation method. Because Field 1 was conducted the plot-to-plot flow irrigation. On the other hand, Field 2 was stored the flooded water, and irrigated it when it decreased. However, we could not give sufficient interpretation for this difference of seasonal patterns with reference to the environmental factors

or agricultural managements.

Diatom genera reported from paddy fields were both planktonic and benthic, and among them, epipelagic ones were usually predominant (Kobayasi, 1950, Negoro, 1954, Kanetsuna, 1957, 1958, 1960, 1961, Mori, 1963, Negoro and Higashino, 1986, Ohtsuka and Fujita, 2001). The diatom genera identified in this study was 90% consistent with the previous studies conducted. However, *Asterionella* and *Rhoicosphenia* in Field 2 were not observed in previous studies.

Most studies reported that *Nitzschia* were predominant genus in the paddy field (Kurasawa, 1957, Taira and Hougetsu, 1987, Fujita and Nakahara, 1999, Ohtsuka and Fujita, 2001). From this study we also supported that *Nitzschia* is usually the most dominant genus in paddy fields.

The difference of genera composition between two fields was partly explained by the different origin of irrigation water. In Field 1, the water was irrigated from small stream gushed from neighbor mountains, which is thought to contain small amount of diatoms. On the other hand, Field 2 was irrigated from the large river with a dam; probably the irrigation water contains many planktonic and stream benthic diatoms. Among the genera observed just only in Field 2, *Asterionella* and *Aulacoseira* were representative planktonic genera (with some exceptional species) which are common in eutrophied lakes containing reservoirs (Krammer and Lange-Bertalot, 1991). *Rhoicosphenia abbreviata*, general species among *Rhoicosphenia*, was periphyton and abundant in the river. Therefore, the seed populations of these genera might be brought through the irrigation water.

This study has provided the basis to clarify the influence of diatoms on Si concentration of flooded water in paddy fields. To clarify the influence, however, it needs more study, especially quantitative studies on Si assimilation by diatoms and accumulation of their frustules in the paddy soil. We will elucidate them in the next studies.

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