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Original Paper

CARBON CONTENT OF PHYTOPLANKTON AND BACTERIA IN AN ESTUARINE SYSTEM

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ABSTRACT

Carbon content of diatom, dinoflagellates and bacteria were studied in order to estimate the living particulate organic carbon (LPOC) in an estuarine system. In the water column, diatoms were most abundant in spring than the other seasons. Dinoflagellates, cyanobacteria, and microzooplankton were present in all seasons, even low in cell numbers. The highest abundance of bacteria was at inner region of estuary and lowest at the mouth of Bekanbeusi River. The percentage of benthic diatom to total diatoms assemblages on sediment surface was higher than that in the water column. The POC in water column was composed of 13 – 24 % for diatoms and 0.6 – 1.6 % for dinoflagelate in carbon base throughout a year. The contribution of diatom carbon to total POC showed highest percentage in June (24 %) in the Akkeshi-ko estuary. In general, bacterial carbon was lower than that of diatoms carbon. On average, the contribution of bacterial carbon was 5-8 % to the total POC throughout a year.

Key word : Diatom ; dynoflagellate; bacteria ; estuary; carbon content

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INTRODUCTION

In estuaries, nutrient inputs from rivers, wastewater and other sources were able to increase the production of particulate organic matter (POM) in both water column and sediment (Galois, *et al.,* 2000; Minor, 2001; Volkman and Tanoue, 2002). Most estuaries contain high concentration of POM derived both from rivers and open marine waters (Tappin, 2002). Increasing loads of dissolved inorganic carbon will lead to an increase of diatom and bacterial biomass (Middelburg and Nieuwenhuize, 2000; Sakami, *et al.,* 2003), until diatom biomass is limited due to self-shading at high levels (Goericke and Montoya, 1998). Generally, the diatom and bacterial biomass are markedly higher in an estuarine ecosystem with some spatial differences along the estuary which is controlled by tidal flushing. Thus, the upper estuary usually has a high production than the lower one. (Middelburg and Nieuwenhuize, 2000; Revilla, *et al.,* 2000). Bacterial density is less affected than diatom biomass by river discharge to the estuary, however, the increase in POM from river runoff served as the source of carbon for bacterial growth (Revilla, *et al.,* 2000). Estimates of bacterial biomass and growth rate show that bacterial biomass turns over rapidly in high growth rates (Lovejoy, *et al.,* 2000). Diatom and bacteria are major microbial components in most of coastal ecosystem (Pinckney and Zingmacrk, 1993; Revilla *et al.,* 2000; Sakami, *et al.,* 2003). Carbon biomass of bacteria and diatom has been calculated using an estimation by converting cell volume to cellular carbon content and to determine the quantitative role of diatom and bacteria in microbial carbon cycle (Montagnes and Berges, 1994; Ribes *et al.,* 1999a; Menden-Deuer and Lessard 2000; Menden-Deuer *et al.,* 2001; Davidson *et al.,* 2002).

This research was aimd to examine carbon content of diatom, dinoflagellates and bacteria in order to estimate the living particulate organic carbon (LPOC) in an estuarine system.

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MATERIALS AND METHODS

Study sites and field methods

The field study was conducted at the Akkeshi-ko estuary, eastern Hokkaido (43^o 00’ N 144^o 51’ E), northern Japan which is almost enclosed but connected to Akkeshi Bay with a narrow channel (**Fig. 1**). Most of the estuary is relatively shallow (4 m in depth) (Kasim and Mukai, 2006, Kasim, 2006). Monthly samplings from the water column and sediment surface (less than 1 cm from the surface) were carried out at 20 stations in the Akkeshi-ko estuary for 10 months (late March – early December). Positions of all stations were determined using shipboard Global Positioning System. During January and February, almost all surface of the estuary was covered with ice, thus sampling was impossible. Additional water samples were collected in April, May, July, and October for bacterial analysis. The samplings were grouped to represent spring (March, April and May) summer (June, July, August, and September) and autumn (October, November and early December) seasons.

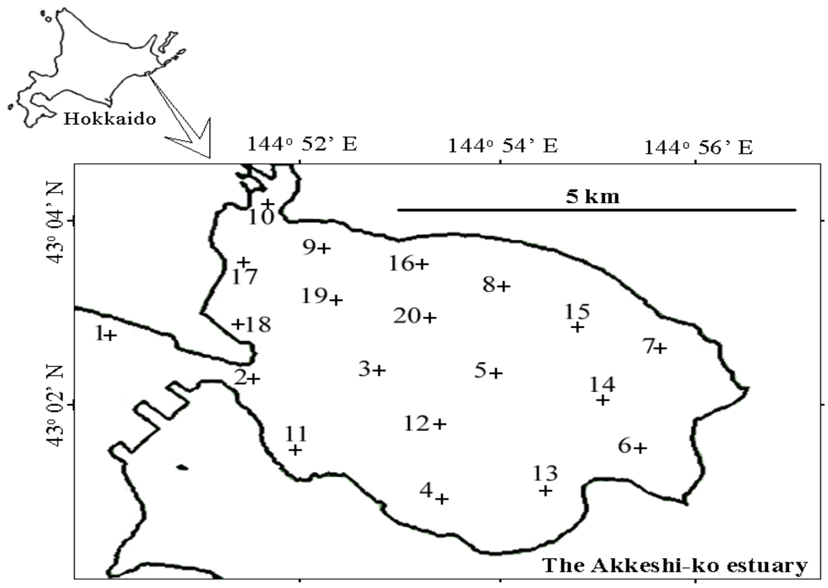

Fig. 1. Sampling stations (Sts. 1 – 20) in the Akkeshi-ko estuary, Hokkaido, Japan.

For ‘phytoplankton’ (diatom, dinoflagellates, cyanobacteria) and microzooplankton sample in water column, about 1000 mL water samples (10 cm from the water surface) were collected by bottles monthly at each station. Sediment samples were also collected by an Ekman–Birdge grab from the sediment surface. Diatoms and cyanobacteria were subsampled from the sediment surface samples by a mini core (diameter: 3 cm). For diatom studies, about 1 cm surface layer of sediment was picked up by cellophane plastic. It was restricted to 1 cm of the surface sediments because vertical change of diatom assemblages has been noted (Rathburn, *et al.*, 2001).

Bacteria enumeration

Directly after sampling, 10 ml water sample was fixed with 5% aqueous glutaraldehyde. 2-3 ml of the fixed water samples was stained with DAPI at a concentration of 1µg/mL for 15 minutes and then filtered through a black stained polycarbonate filter of 0.2 µm pore size. The filtered specimens were observed using an epifluorescence microscope.

POC analysis

For estimating the particulate organic matter of the water sample, 100 mL water samples each from 20 stations were filtered onto Whatman GF/C filter and dried at 60^{o}C. The dried filters were packed air-tight in aluminum foil and supplied to the measurement for carbon content in a C-N Analyzer (EA Flash 1112 series).

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Diatom and bacteria carbon conversion

The diatoms abundance were converted into cell carbon using the live cell volume conversion according to conversion of diatoms cell carbon (pg) = 0.288[live cell volume (µm3)]$^{0.811}$ and dinoflagellates cell carbon (pg) = 0.760[live cell volume (µm^3)]$^{0.819}$ (Montagnes *et al.,* 1994, Menden-deuer 2001, Davidson *et al.,* 2002). Diatoms cell number was estimated to biovolume (reviewed by Hillebrand *et al.,* 1999) using the average of diatoms biovolume in each shape.

The bacterial cell carbon was calculated indirectly using the conversion factor of bacterial cell to biomass (carbon base), 20 fg carbon /cell (Kirchmann, 2000).

RESULTS AND DISCUSSION

RESULTS

Plankton community

Plankton community (except bacteria) in the Akkeshi-ko estuary consisted mainly the diatoms, dinoflagellates, cyanobacteria and microzooplankton.

Each of these groups comprised, on average in water column, 89 %, 4 %, 2 % and 5 %, respectively. On sediment surface, it was dominanted by diatoms and cyanobacteria comprising 88 % and 12 %, respectively. Thus, diatoms were very abundant in both water column and sediment.

Diatom composed of benthic species (benthic diatom) and pelagic species (pelagic diatom) (Kasim and Mukai, 2009 for more detail of this category). Benthic diatoms increased in April as spring bloom and in autumn again slightly in the water column, but were most abundant in autumn season without spring bloom on the sediment surface. Dinoflagellates, cyanobacteria and microzooplankton were present in all seasons, but low in cell numbers. Cyanobacteria were in sporadically high concentration in St. 10 and 18 during spring and summer season and in St. 12 in autumn season. Dinoflagellates and microzooplankton occurred in almost all seasons although sometimes rare during these seasons (**Fig. 2**).

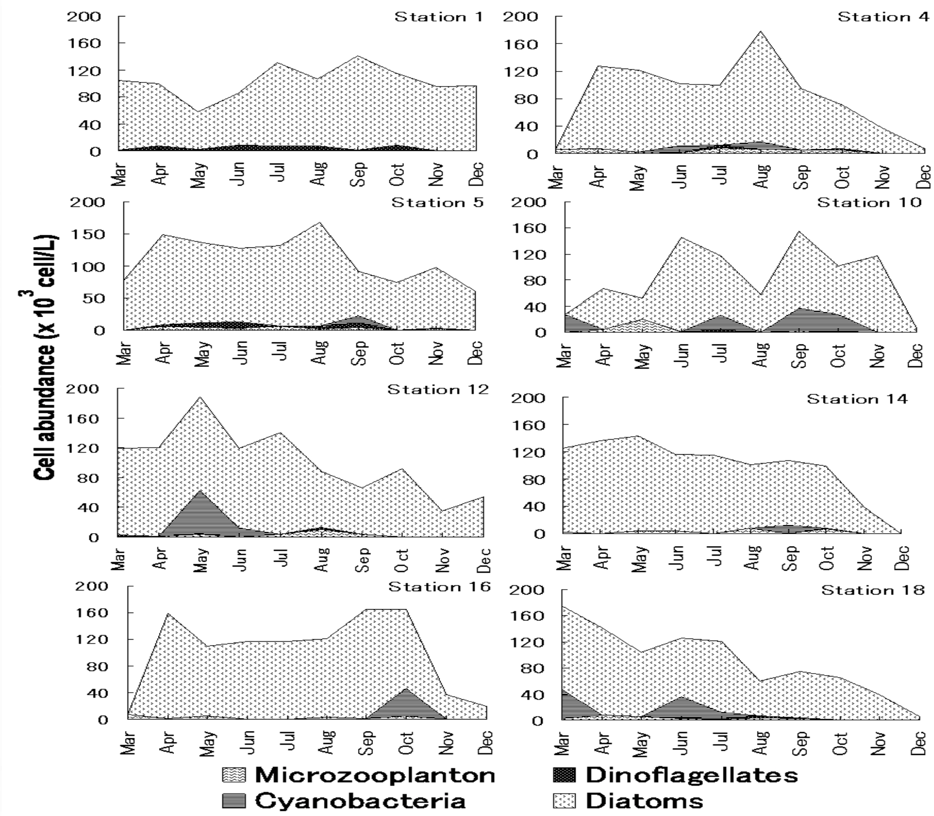

Fig. 2. The composition of diatoms, cyanobacteria, dinoflagellates and microzooplankton in the water column at selected stations in the Akkeshi-ko estuary.

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Bacteria in the Akkeshi-ko estuary

The horizontal distribution of bacteria density in the Akkeshi-ko estuary water column is illustrated in **Fig. 3**. The highest abundance of bacteria was at St. 13 (western part of estuary): 3.7±0.1 x 10^8 cell/L and lowest at St. 10 (in the mouth of Bekanbeusi River): 2.2±0.85 x 10^8 cell/L during spring season. At St. 1 which was in Akkeshi Bay and highly affected by the ocean, the bacterial abundance was 2.3±0.36 x 10^8 cell/L, lower than the average value of most stations inside the Akkeshi-ko estuary.

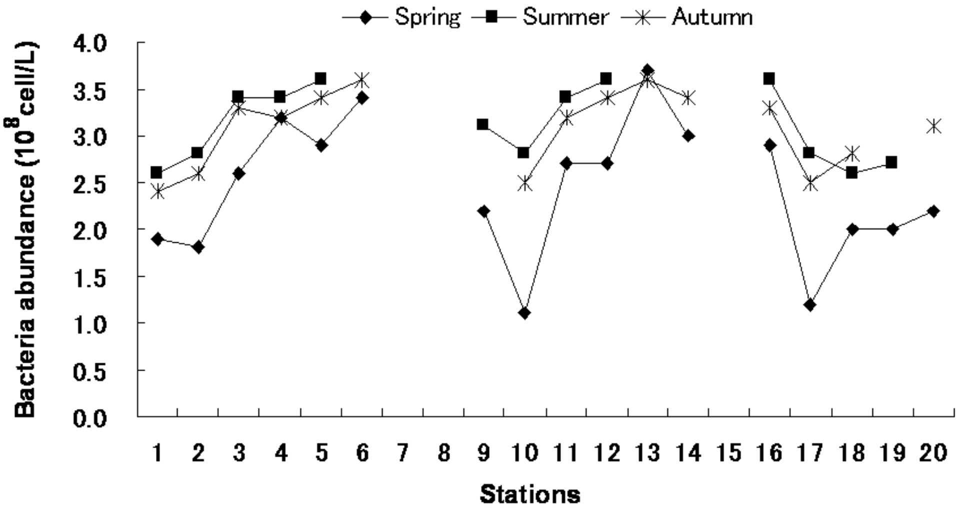

Fig. 3. Distribution of bacterial abundance in the water column during spring, summer and autumn in the Akkeshi-ko estuary.

The highest abundance was observed in summer in general. However bacteria in St. 6, 14, 18 and 20 were most abundant in autumn. In St. 13, the maximum abundance was observed in spring (**Fig. 4**).

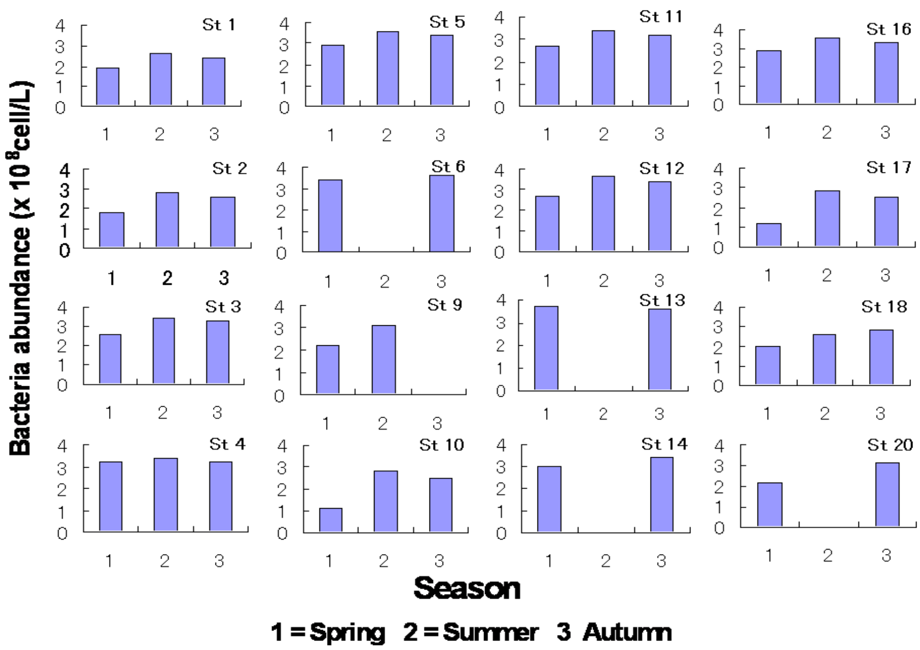

Fig. 4. Bacteria abundance and its seasonal fluctuation in the water column at selected sampling stations in the Akkeshi-ko estuary.

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Dynamics of diatom assemblages

The percentage of benthic diatom to total diatoms assemblages on sediment surface (100%) was higher than that in the water column ones (70%) in all seasons and at all sites in the Akkeshi-ko estuary (**Fig. 5**).

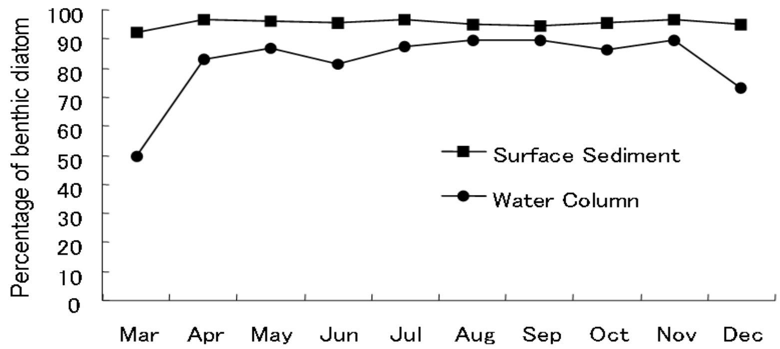

Fig.5. The percentage of benthic diatom to total diatom assembles in water column and on the sediment surface in the Akkeshi-ko estuary.

Density of diatoms (benthic and pelagic diatom) varied seasonally and to be high in spring and summer at almost all stations, except at St. 10 (near the river mouth), 16 and 20, in which there were high benthic diatoms in autumn and early winter (**Fig. 6 and 7**).

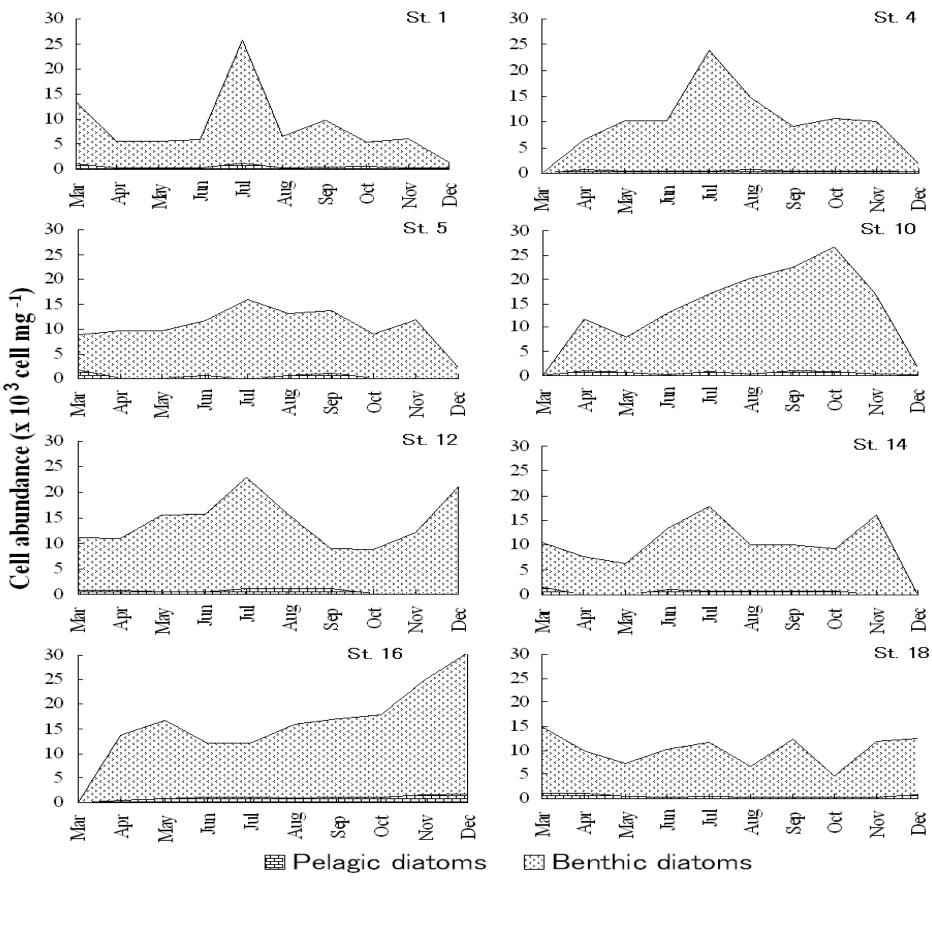

Fig. 6. The abundance of benthic and pelagic diatoms on the sediment surface at selected sampling stations in the Akkeshi-ko estuary during 2003.

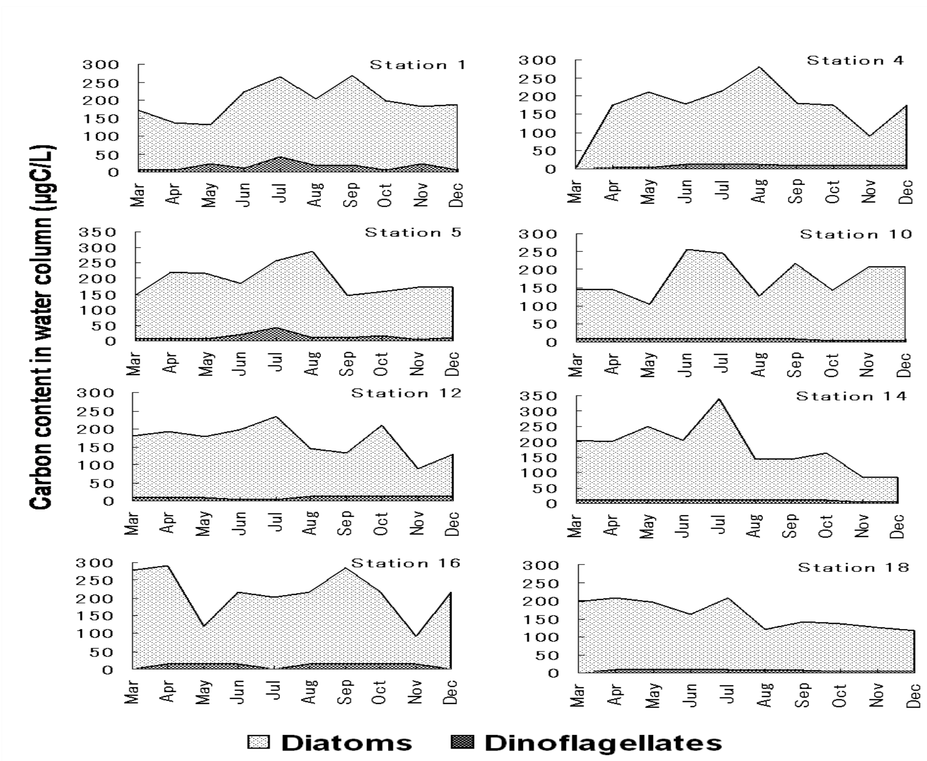

Fig. 7. The abundance of benthic and pelagic diatoms in water column at selected sampling stations in the Akkeshi-ko estuary.

Contribution of diatoms and dynoflagellates carbon

During spring and summer season, concentrations of all diatom carbon (benthic and pelagic diatom) were highest (up to 300 µgC/L) in almost all stations. Concentrations of dinoflagellate carbon were low in all seasons, except in St. 2 and 5, which had high concentration during the summer season (**Fig. 8**).

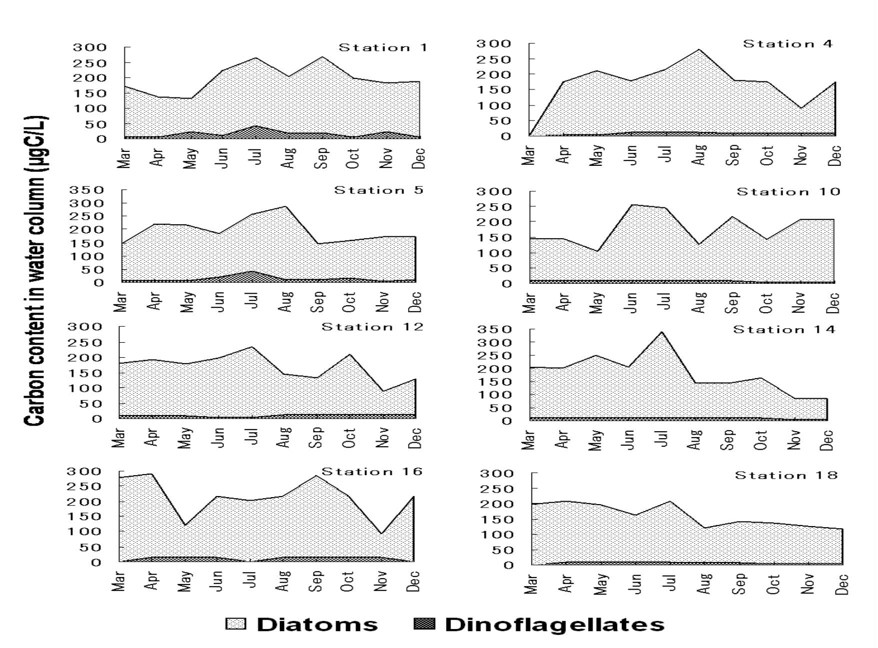

Fig. 8. The carbon content of diatoms (including benthic and pelagic diatom) and dinoflagellates in water column in the Akkeshi-ko estuary.

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The POC in water column was composed of 13 – 24 % for diatoms, 0.6 – 1.6 % for dinoflagelate and 0.03 – 0.1 % for cyanobateria in carbon base throughout a year in the Akkeshi-ko estuary. The contribution of diatom carbon to total POC showed highest percentage in June 24 % . The percentage of diatom carbon was not always similar to that of the diatom cell number, due to the different carbon value of each cell based on size and shape. In July, while the average of diatom carbon was high (219 µgC/L), the percentage to the total POC appeared to decrease (16 %). The contribution of diatom carbon showed decrease in autumn season with reduction of the total cell number of diatoms.

Contribution of bacterial carbon

In spring, summer and autumn, the averages of bacterial carbon were 48 ± 0.73 (SE) µgC/L, 62 ± 0.62 (SE) µgC/L, and 62 ± 0.46 (SE) µgC/L, and representing the contribution of bacterial carbon as 7 ±3.7 %, 5 ±1.5 % and 8 ±2.8 % to the total POC, respectively. The minimum and maximum concentration of bacterial carbon shown in spring season were 74 µgC/L and 22 µgC/L at Sts. 13 and 10, respectively. In spring, the spatial variation in concentration of bacterial carbon was high, but not in summer and autumn (**Fig. 9**). St. 10 which showed lowest bacterial contribution, was located at near river-mouth.

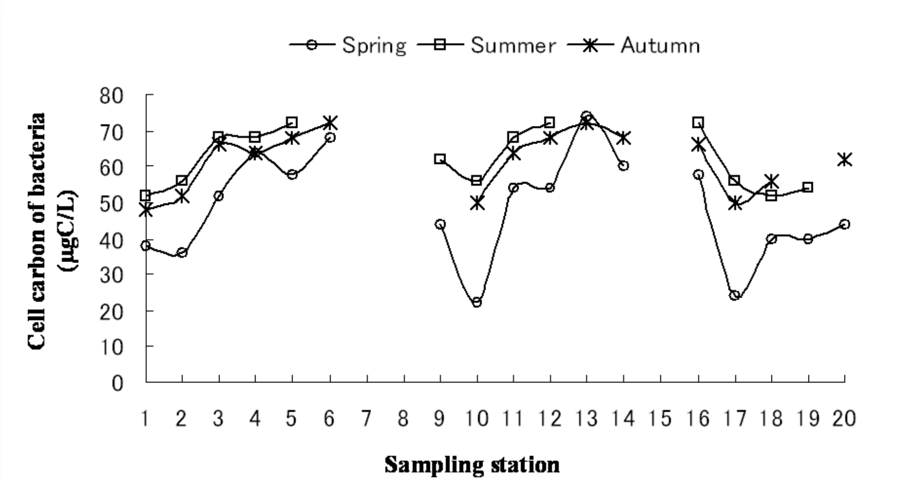

Fig. 9. The concentration of carbon cell of bacteria during spring, summer and autumn seasons in the Akkeshi-ko estuary.

DISCUSSION

POC in the Akkeshi-ko estuary were varied seasonally with a high concentration in summer season. While the biomass of epiphytic diatoms increased in mostly inner estuary (Kasim, 2006, Kasim and Mukai, 2006), the concentration of POC was high particularly in spring and summer.However, POC also related to the input of terrestrial organic matter from Bekanbeusi River during autumn. The POC concentration in estuarine systems is strongly related to the phytoplankton concentration or macrophyte abundance (Takamura, *et al.,* 2003) and also to the input of nutrient from river during spring and summer season (Hedges, *et al.,* 1997; Moon and Dustan, 1990). In San Francisco Bay, the high concentration of phytoplankton was associated with the increasing of POC concentration near the surface water during summer season and it's a lower concentration occurred during autumn season when the phytoplankton biomass was low (Wienke and Cloern, 1987).

Diatoms were the most dominant 'phytoplankton' group and their abundances varied seasonally in the Akkeshi-ko estuary. During spring and summer, the concentration of diatoms was highest in inner (upper) Akkeshi-ko estuary. The highest concentration of diatoms occurred in spring and summer season in many estuary systems, including Adriatic Sea (Blackford, 2002) and Changjiang estuary (Gao and Song, 2005). In Akkeshi-ko estuary, concentration of diatoms decreased during autumn at all stations except at St. 10 which was located at the mouth of the Bekanbeusi Rivers.

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Dominant species of diatom assemblages in the Akkeshi-ko estuary occurred in adjacent to the seagrass beds, at St 8, 9, 13, and 16 and several species of diatom community are always associated with the dense seagrass beds. Epiphytic diatoms in water column is about 20% in winter and increased to 24% and 26% respectively in May and August which are correlated to with increasing biomass of seagrasses 54 ± 14 gDW/m^2 and 168 ± 60 gDW/m^2, respectively (Kasim, 2006).

Contributions of diatoms carbon were 22 ± 0.25 %, 26 ± 0.51 % and 18 ± 0.1% to the total POC during spring, summer and autumn season, respectively in the Akkeshi-ko. There are relatively similar contributions of diatoms and bacteria to the POC occurred in the other estuary areas. For examples, in Urdaibai estuary, Northern Spain, the contribution of the phytoplankton carbon biomass was only 23 % (on average) to the total net production (bacteria plus phytoplankton), which was 17 % to the net production of organic carbon in water column.

One of the interesting aspects in the Akkeshi-ko estuary that benthic diatoms dominated the total diatoms assemblages in both water column and sediment surface representing 100 % and 90 % respectively to the total of diatom assemblages. Why did these concentrations of benthic species occur?. The fact is that diatom assemblages on surface sediments including pelagic species can be easily understood. Because, in such shallow estuaries, sinking of diatom cells usually occurs, then many living diatom cells can be found on surface sediments anytime. However, the opposite case would occur by disturbance due to tidal flows and/or - wind (Kasim and Mukai, 2006). The higher chlorophyll *a* concentration in water column is strongly addressed to the high concentration of benthic diatom in water column. Perrisinotto, *et al.*, (2002) Observed the spatial – temporal dynamics of pelagic and benthic diatoms and chlorophyll *a* concentration in Mpenjati estuary, South Africa, were higher in open estuary and directly correlated with the strong mixing as a result of strong tidal and riverine flows, causing the suspension of benthic diatoms. Benthic and epiphytic diatoms were able to suspend into water column from their substrate by wind and/or tidal currents. Magni *et al.*, (2002) described diurnal fluctuation of nutrients and suspended particulate matter including diatom assemblages in shallow estuary area in the Seto Inland Sea of Japan. They thought the fluctuation was generated by tidal flows.

In the Akkeshi-ko estuary, diatom assemblages on the surface sediment and water column were generally predominated by benthic diatoms in all seasons, as well as generally reported in several shallow estuarine water systems (Blackford, 2002. Welker, *et al.*, 2002). Benthic diatoms played a major role throughout the sampling period. On the intertidal flat in the Seto Inland Sea of Japan, the biomass of benthic diatoms increased during spring and summer (Montani, *et al.*, 2003).

The bacterial carbon concentration seemed to be high during spring and summer reflecting to high concentration of POC in the Akkeshi-ko estuary water column. In almost all stations in the estuary, the concentration of bacteria carbon was relatively high (up to 60 µgC/L), whereas the minimum concentration was found at St. 10. Although the bacteria made only a minor contribution to the total POC in the Akkeshi-ko estuary, cell numbers and POC were interraced, even though there was correlation between cell number of bacteria and POC in all sampling stations. In general, bacterial carbon was lower than that of diatoms carbon.On average, the contribution of bacterial carbon was 5-8 (5-8 are range not average) % to the total POC throughout a year. Contribution diatom carbon was 18 – 26 % to the total POC.

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Figure List

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Figure 9. The concentration of carbon cell of bacteria during spring, summer and autumn seasons in the Akkeshi-ko estuary.

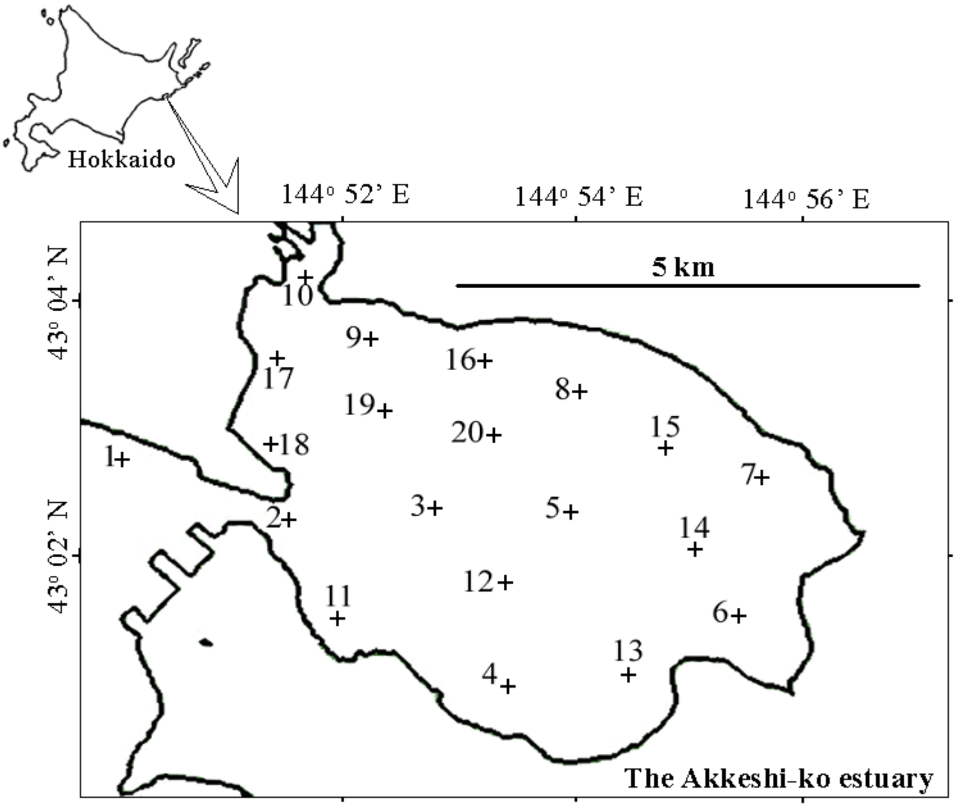

Fig 1.

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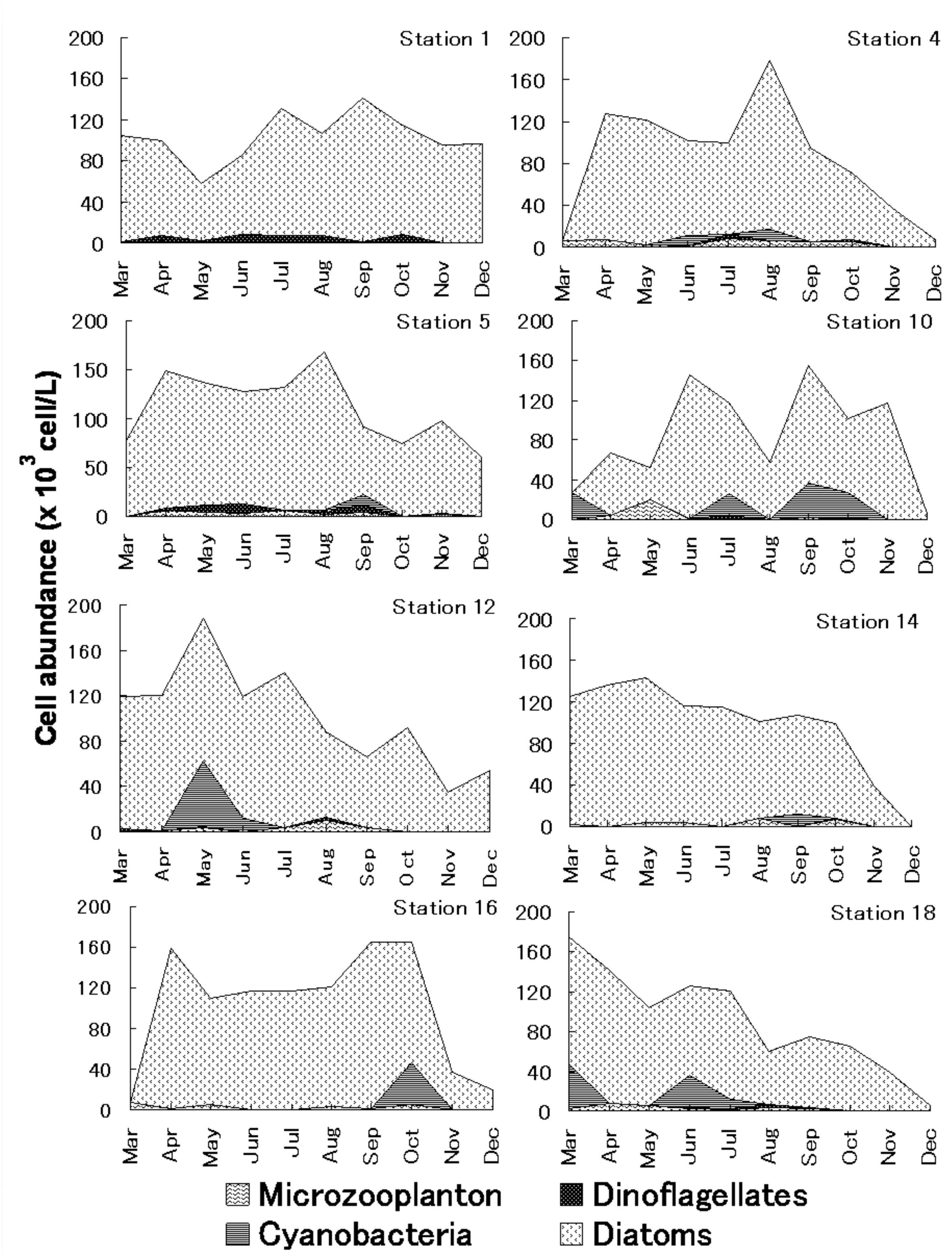

Fig 2.

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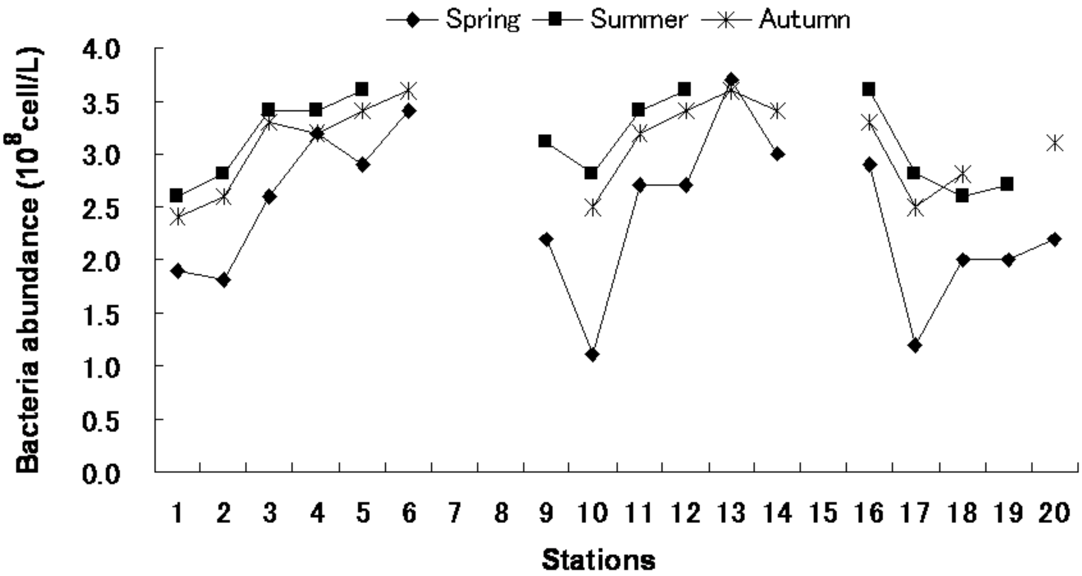

Fig 3.

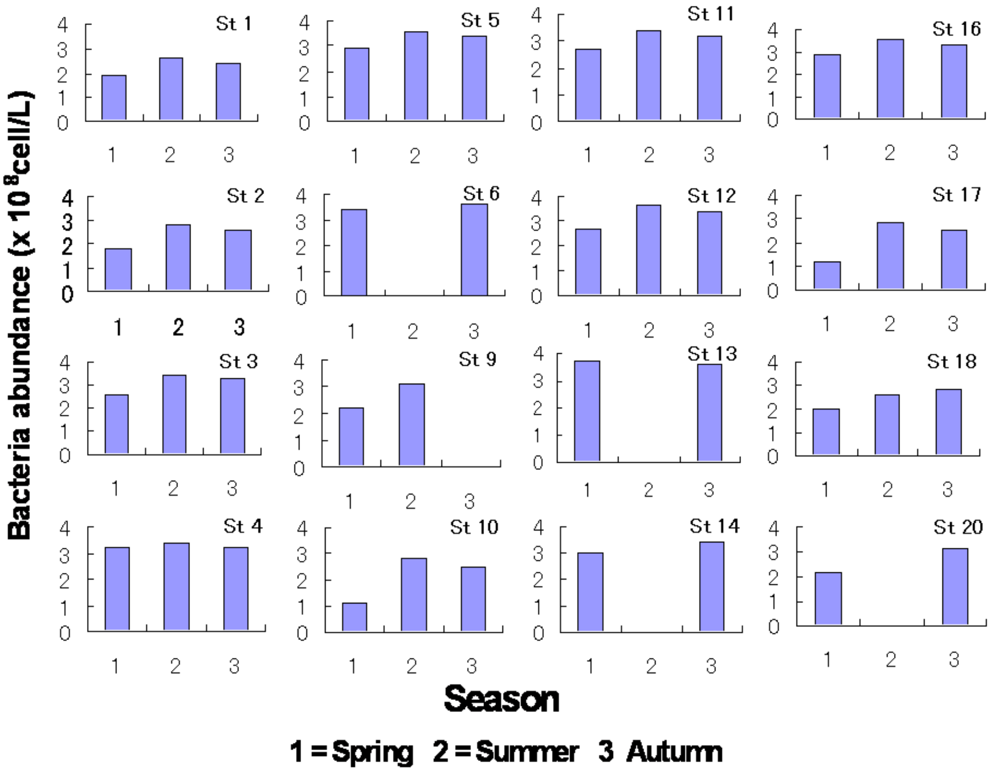

Fig 4.

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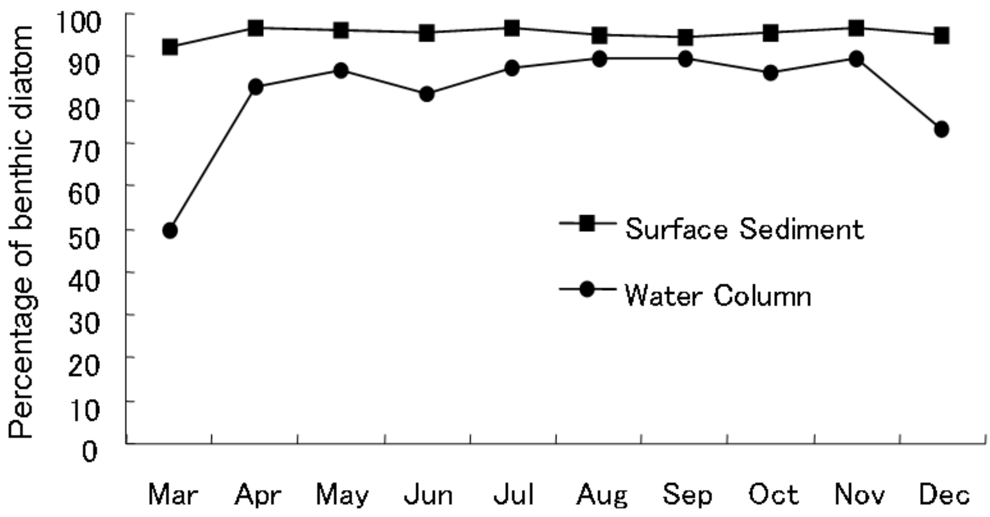

Fig 5.

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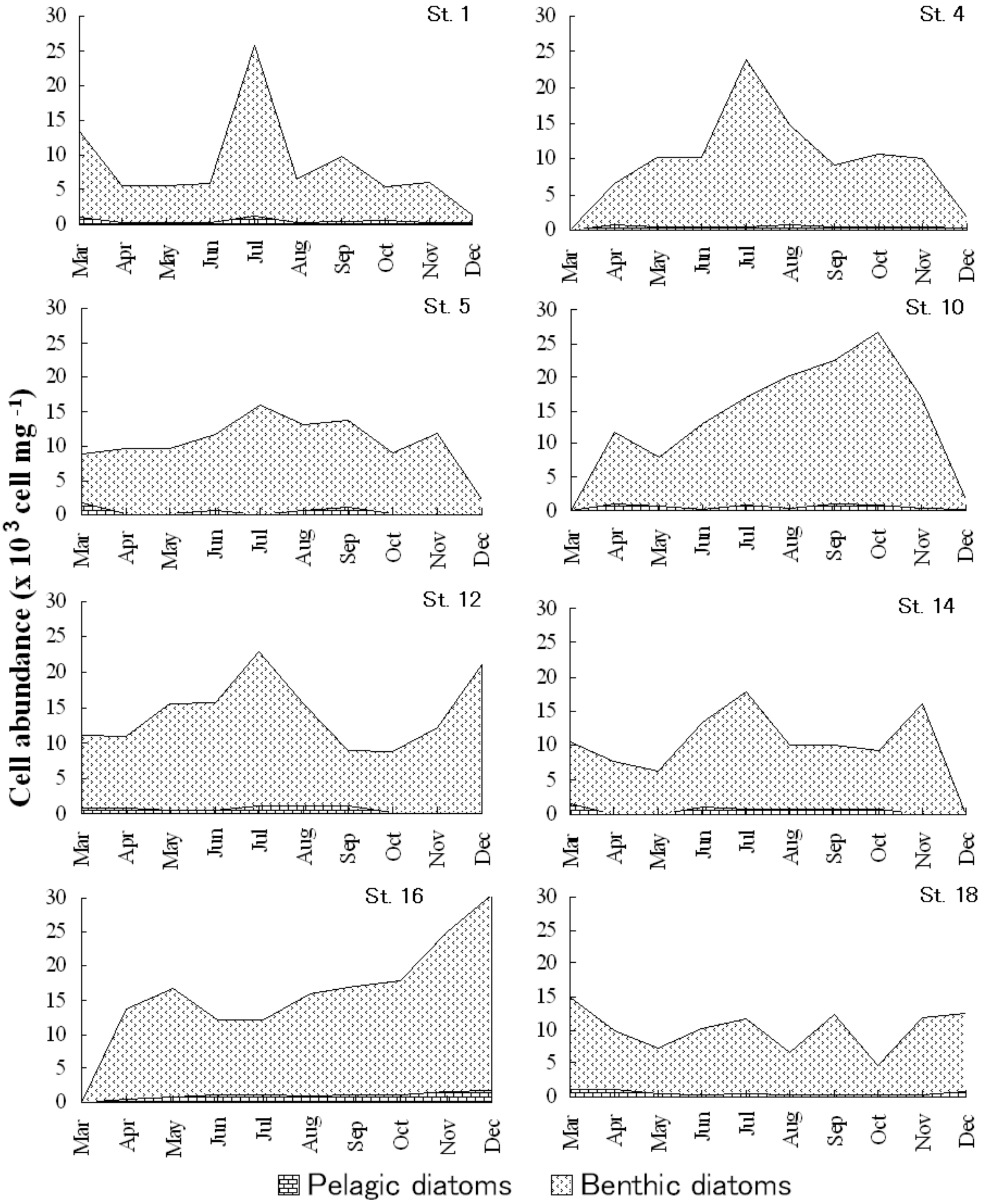

Fig 6.

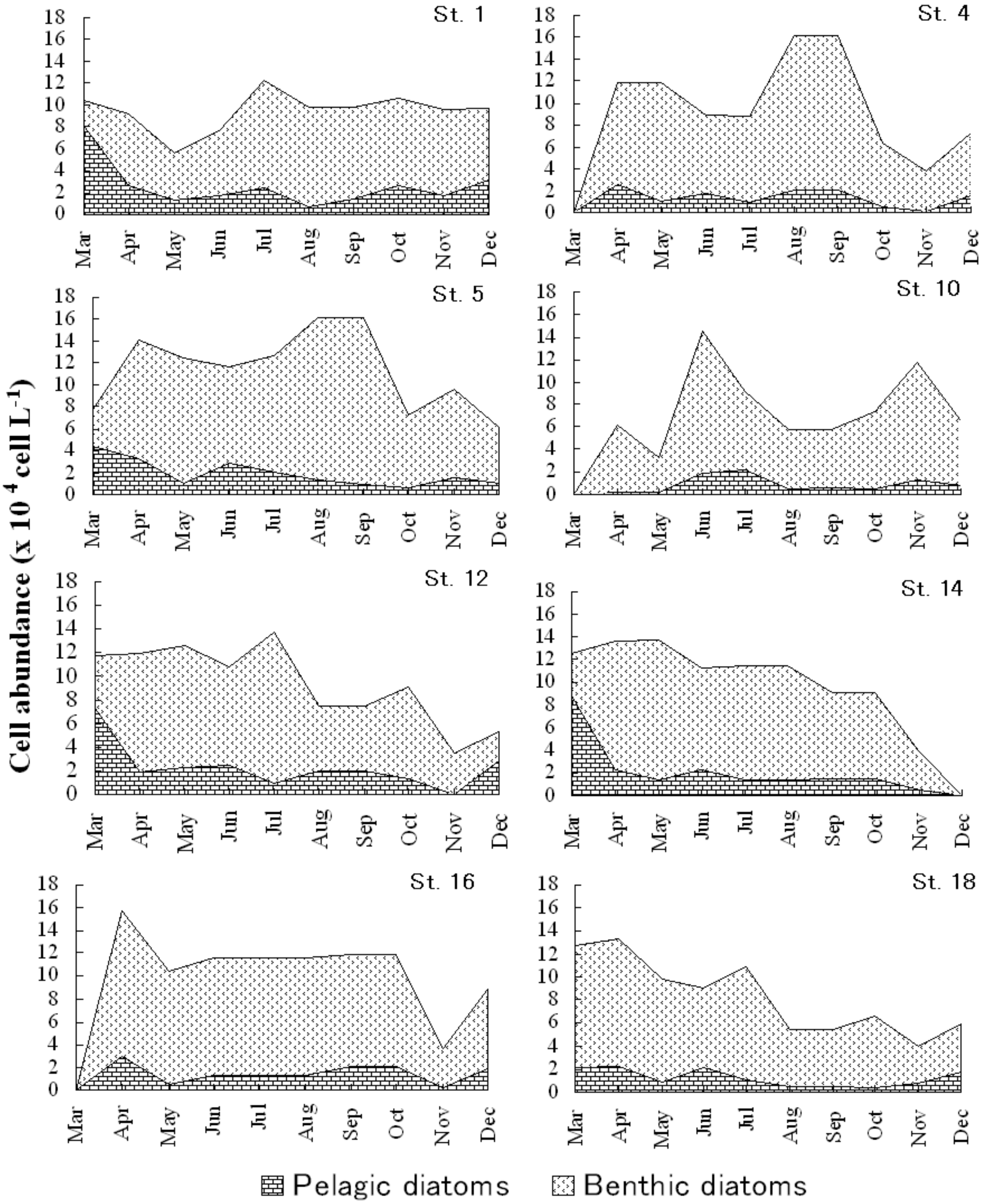
St. 1
St. 4
St. 5
St. 10
St. 12
St. 14
St. 16
St. 18
Cell abundance (x 10 ^{4} cell L^{-1})
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Pelagic diatoms
Benthic diatoms

Fig 7.

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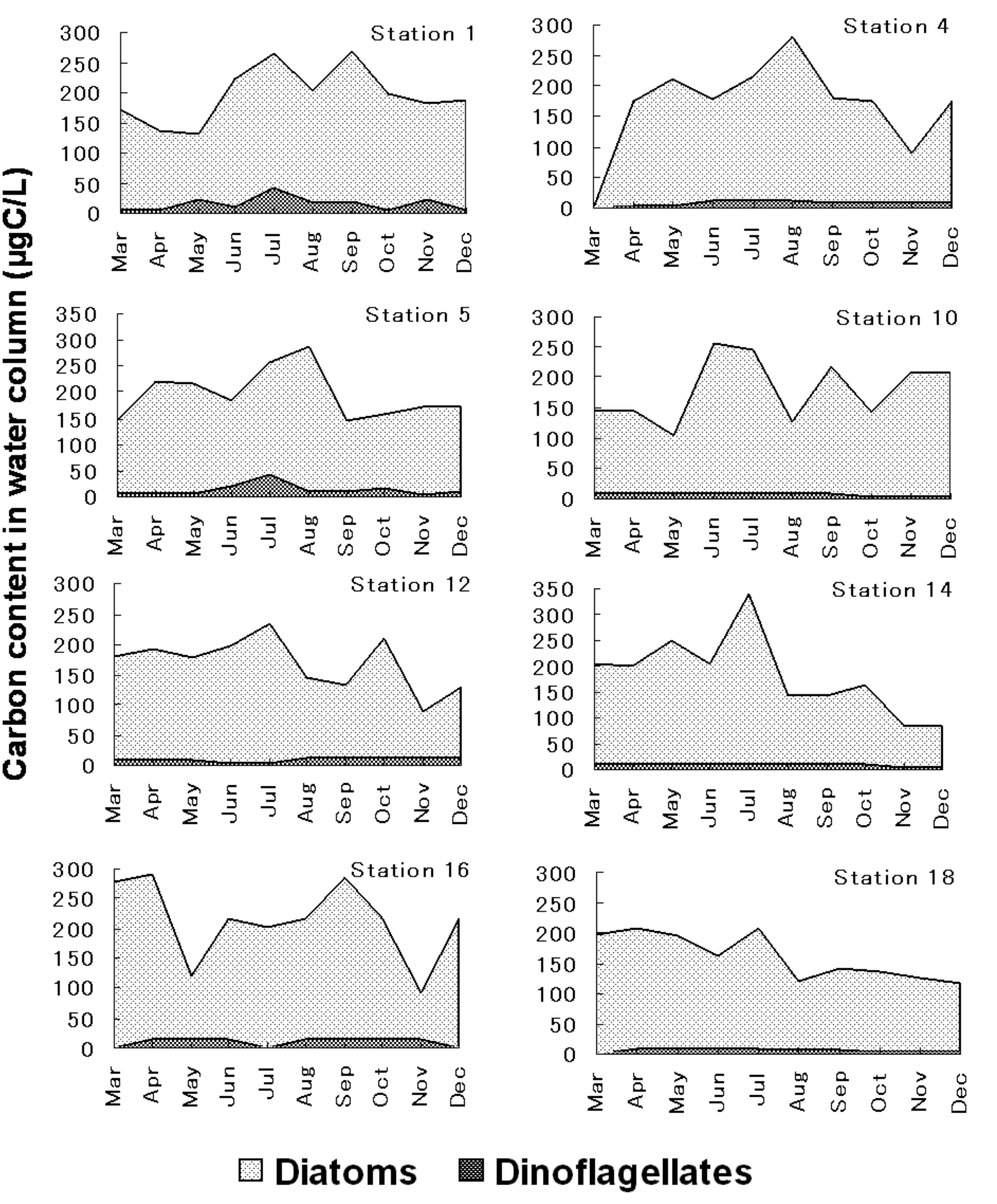

Fig 8.

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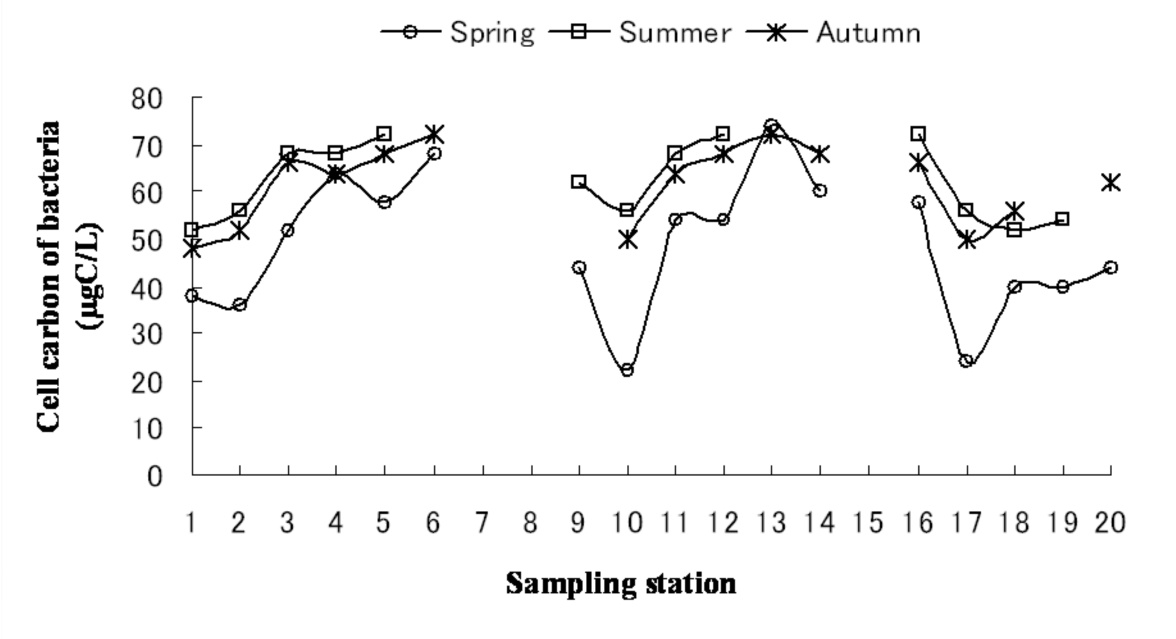

Fig 9.