

Original Paper

## CORRELATION OF ENVIRONMENTAL FACTORS AND DIATOM ASSEMBLAGES IN AKKESHI-KO ESTUARY SYSTEM

Ma'ruf Kasim

Faculty of Fishery and Marine Science, Haluoleo University, Indonesia  
Kampus Baru Unhalu, Jln. Mokodompit, Andounhalu, Kendari, Indonesia

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### ABSTRACT

*The correlation between diatoms and environmental factor were studied in Akkeshi-ko estuary, eastern part of Hokkaido, Japan. This studied were aimed to clarify whether the dynamic and distribution pattern can be made by correlating for environmental variation and diatoms assemblages in an estuarine system. During this research, there are positive correlations between pelagic diatom abundance in water column (PDWC), depth and salinity ( $r = 0.623$  and  $r = 0.652$ ; respectively). There are positive relationship between nitrite + nitrate and the abundance of diatom on the surface sediment. Seasonal variation of the relationship between abundance of diatoms assemblages and ammonia, nitrite+nitrate and phosphate showed occurs in most stations, the high abundance of benthic and pelagic diatoms in summer followed by the increasing of ammonia, nitrite and nitrate and phosphate.*

**Keyword** : Diatoms; surface sedimen; water column; environmental factor.

**Correspondence** : Phone : +62-04013193782 ; email : [marufkasim@hotmail.com](mailto:marufkasim@hotmail.com)

### INTRODUCTION

Diatoms are unicellular microscopic algae, which play most important role in carbon cycling of estuarine food web (Pinckney and Zingmacrk, 1993). They are sensitive to wide range of environmental variables, and the species composition may quickly response to changing physical, chemical and biological conditions in the environment (Harrison and Turpin, 1982; Lapointe, 2000; Mitbavkar and Anil, 2002). The structure of benthic diatom assemblages is related to the sediment characters, light, temperature, salinity and depth (Colijn and Dijkema, 1981; Jonsson, 1987; Miller and Florin, 1989; Zalat, 1995; Nozaki *et al.*, 2002), aside the grazing pressure by benthic suspension feeder (Kasim and Mukai, 2009; Mukai, 1992; Yusoff *et al.*, 2002). The concentration of nutrient affects phytoplankton growth (Smith, 1982; Hillebrand *et al.*, 2000; Kormas *et al.*, 2001). Nutrients (ammonia, nitrate, nitrite, silicate and phosphate) are strongly

addressed to the diatom abundance and species composition (Egge, 1998; Lim *et al.*, 2001, Chiba and Saino, 2002). Nitrogen and phosphate are usually limiting factors of growth and abundance of diatoms (Hashimoto and Nakano, 2003). Availability of nutrients in estuary is strongly affected by terrestrial inputs, including human sewage and river inputs (Tappin, 2002). In the most estuaries, the fluctuation of diatom assemblages is characterized by an abundance of diatoms during spring, which corresponds with the increasing of nutrient concentration in water column and perhaps nutrient-inputs from the rivers (Andren, 1999; Ganesella *et al.*, 2000; Nozais *et al.*, 2001; Townsend and Thomas, 2002).

Biological factors such as epiphytic diatoms on seagrass leaves affect the biomass of diatoms in both surface sediment and water column surrounding the habitats. They are playing an important role and most

contributors to a primary production of seagrass ecosystems (Klumpp *et al.*, 1992) and they have a possibility of a food source of some benthic suspension feeders. In fact, the epiphytes including epiphytic diatoms have been bigger contributors for herbivores than seagrass leaves (Kitting *et al.*, 1984). Epiphytic diatoms were indirectly utilized by benthic suspension feeder (Jernakoff *et al.*, 1996). Current and/or any other physical factors are able to tear off epiphytic microalgae from seagrass leaves into water column (Sawai, 2001) and suspended epiphytes have been available for benthic suspension feeders (Cognie *et al.*, 2001).

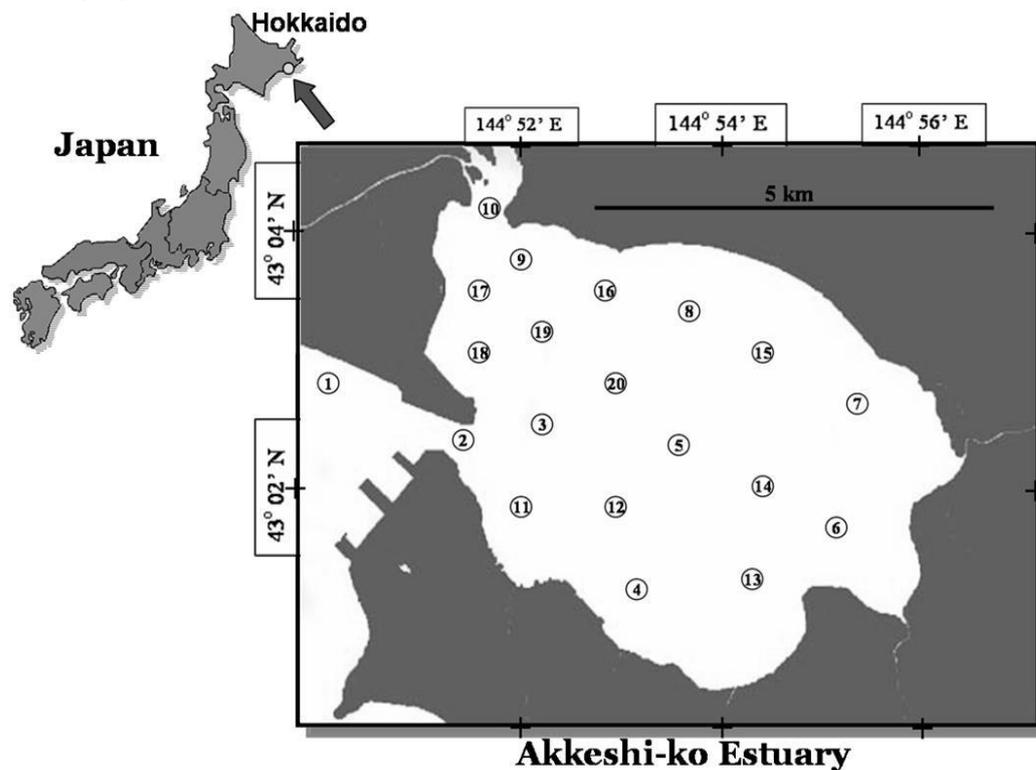
The purpose of this research is to identify and evaluate the environmental variables related to diatom assemblages' in estuary system and to examine whether the

dynamic and distribution pattern can be made by correlating for environmental variation and diatoms assemblages.

## MATERIALS AND METHODS

### Study sites

The field study was conducted at the Akkeshi-ko estuary, eastern Hokkaido ( $43^{\circ} 00' N$   $144^{\circ} 51' E$ ), 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) and is covered with extensive eelgrass beds of *Zostera marina* L. and *Z. japonica* Aschers. (Kasim and Mukai, 2006).



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

### Field methods

Diatom samples were collected from the water column in the field; 1 L water samples were carried out monthly at 20 stations for 10 month (March – December) in tidal shallow

Akkeshi-ko estuary. Three seasons were considered (spring: March – June; summer: July - September and autumn: October - December). In winter season, all surface water was covered by ice, so impossible to do water sampling.

Sediment samples were also collected by an Ekman–Birdge grab at 20 stations in the Akkeshi-ko estuary. Benthic diatoms were sub sampled from the sediment by a mini core (diameter: 3 cm). About 1 cm surface layer of sediment was picked up by cellophane plastic. I restricted 1 cm of the surface sediments for observation, because vertical change of diatom assemblages has been known (Rathburn *et al.*, 2001, Kasim, and Mukai, 2009).

### *Sample analysis*

Immediately after sampling, 100 ml water samples were preserved with formaldehyde solution of 0.2 % final concentration. Diatoms in the water samples were collected by the concentration method from 100 ml to 10 ml using hand centrifuge. The samples were pipetted 2 ml and examined under a microscope. All the living diatom samples were enumerated under light microscopic examination using phase contrast optics under 40x and 100x objective. The diatoms from water samples and sediments were identified and counted to the lowest taxonomic level as well as possible. The taxonomic identification of diatoms was carried out using the text of Yamaji (1977), Kato *et al.*, (1977), Kawamura and Hirano (1989), Mizuno and Saito (1990), Sawai and Nagumo (2003), and online publication by; <http://www.marbot.gu.se/sss/diatoms>, [http://www.bio.mtu.edu/~jkoyadom/algae\\_w ebpage/HOME.htm](http://www.bio.mtu.edu/~jkoyadom/algae_w ebpage/HOME.htm), <http://www.protist.i.hosei.ac.jp/PDB/Galleries/indexE.html>

### *Physical and chemical parameters*

Physical parameters such as water temperature, salinity, and depth were measured *in situ* by electronic apparatus ‘Chlorotec’, (Alec electronic Co), and current

was measured by a current-meter, ‘Compact EM’ (Alec electronic Co).

Ammonia ( $\text{NH}_4^+$ ), nitrite + nitrate ( $\text{NO}_2^- + \text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^-$ ) concentrations in the surface sea water were measured. Ammonia ( $\text{NH}_4^+$ ) concentration was obtained by using a phenol hypochlorite method, as soon as possible within 4 hours after collection (Strickland and Parsons 1968). Nitrite and nitrate ( $\text{NO}_2^- + \text{NO}_3^-$ ) concentrations were measured with an autoanalyzer (BRAN+LUEBE, TRAACS800, Cd-Cu methods). Phosphate was determined colorimetrically by the method of Murphy and Riley (1962).

### *Data analysis*

The MINITAB 10 software package for statistical analyses were performed in this research. Standard correlation analysis was addressed to statistically evaluate on these data. Correlation coefficients  $\geq 0.3$  or  $\leq -0.3$  are established significant.

## RESULTS AND DISCUSSION

### RESULTS

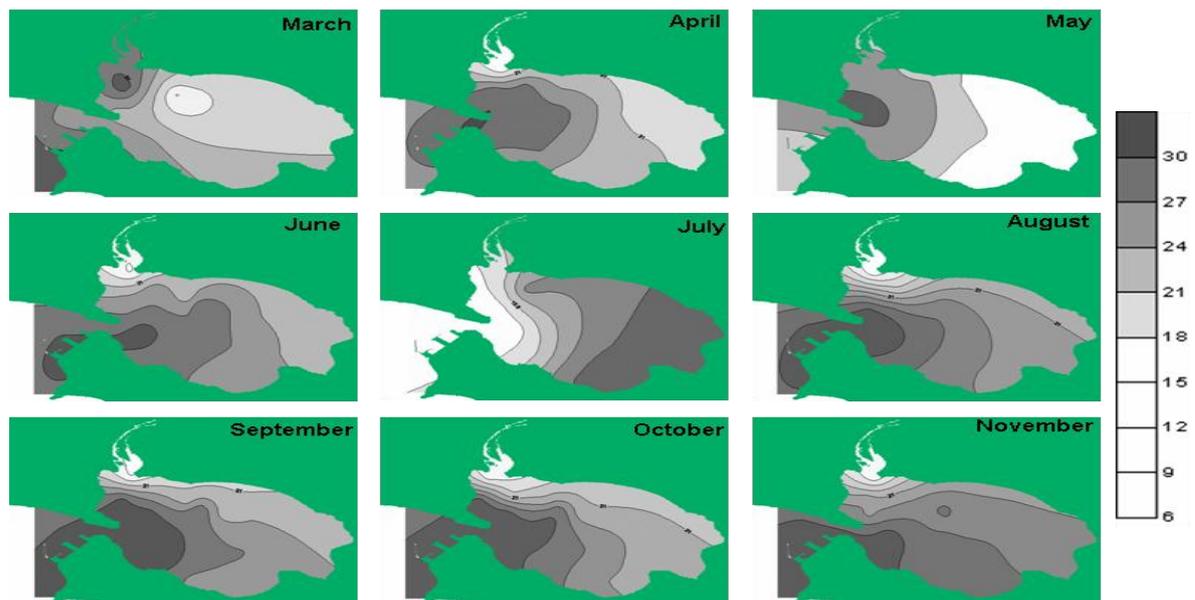
#### *Distribution of physical factors*

Yearly means of physical parameters and diatoms density in water column and sediments derived from March to December in the Akkeshi-ko estuary are presented in **Table 1**.

The depths of the estuary were generally within the range 0.3 – 3.0 m, except the Sts. 2 and 3 (4.7-5.0 m). Salinity was lowest in St. 10 (14.5 psu), which was located in the river-mouth, and a maximum in Sts. 2 and 3 (30.5 psu) which were located in or face to the narrow channel between Akkeshi Bay and the estuary (**Fig. 2**).

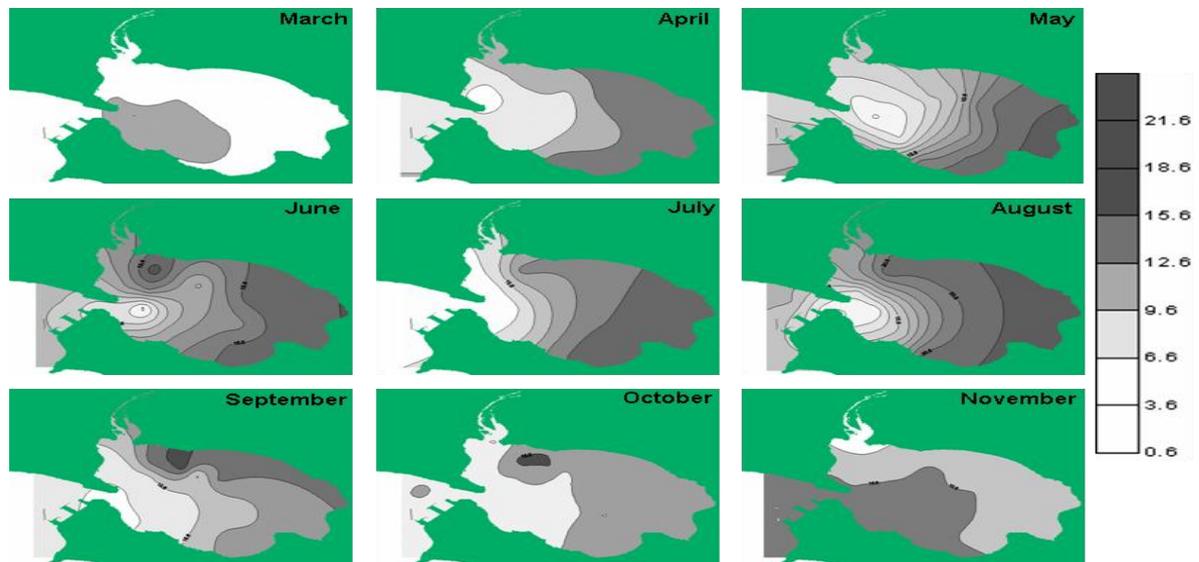
**Table 1.** Summary characteristics of the 20 station in the Akkeshi-ko estuary.

No Station	average of			Diatoms in Water column (cell.L <sup>-1</sup> )	Diatoms in Sediment (cell.mg <sup>-1</sup> )	
	depth (m)	Salinity (psu)	Temperature (°C)			Current velocity (cm.s <sup>-1</sup> )
1	1.17	29.8	9.9	17.9	94670	94250
2	5.03	30.5	10.0	46.6	80490	No data
3	4.73	30.5	9.0	32.9	83380	No data
4	0.62	26.3	13.0	16.5	91180	106250
5	3.82	27.8	10.7	14.6	114030	119850
6	0.56	24.4	10.2	24.9	55050	51050
7	1.04	23.4	9.2	14.4	50150	34450
8	1.51	24.8	9.1	10.4	57050	140200
9	1.73	24.3	11.6	10.5	104050	181000
10	0.29	14.5	11.3	12.0	70400	162100
11	2.61	29.2	10.3	17.6	89950	49750
12	3.21	28.8	10.0	24.0	93550	108000
13	0.50	24.3	13.1	11.0	90270	136250
14	0.92	25.9	11.4	21.2	75550	91700
15	0.76	24.3	11.3	18.1	46750	67200
16	0.89	25.4	9.6	15.3	62420	164050
17	0.88	27.5	10.1	21.9	64750	104550
18	0.92	28.7	9.1	21.9	83100	100750
19	0.54	28.9	10.2	14.7	84450	158850
20	0.78	28.2	7.6	11.5	70200	122950



**Fig. 2.** Distributions of salinity (psu) of surface seawater in the Akkeshi-ko estuary from March to November.

Temperature varied depending on the season. Water temperature increased during summer season. Generally, the water temperature was between 7.6 and 13.1 °C in summer and autumn season (**Fig. 3**).



**Fig. 3.** Distributions of temperature (°C) of surface seawater in the Akkeshi-ko estuary from March to November.

Correlation analysis between physical environmental factors and abundance of benthic and pelagic diatom assemblages in both, surface sediment and water column, was performed on these data. From these analyses, positive correlations between pelagic diatom abundance in water

column (PDWC) and depth and salinity ( $r = 0.623$  and  $r = 0.652$ ; respectively) were found. Correlation between benthic diatom abundance in sediment (BDSed) and in water column (BDWC) and correlation between benthic diatom abundance and depth or salinity were not significant (**Table 2**).

**Table 2.** Correlation coefficients (R) between physical environmental factors and the abundance of benthic and pelagic diatom assemblages in both, surface sediment and water column (BDWC = benthic diatoms in water column, PDWC = pelagic diatoms in water column, BDSed = benthic diatoms on the sediment, and PDSed = pelagic diatoms on the sediment).

	<i>depth (m)</i>	<i>Salinity (‰)</i>	<i>Temperature (°C)</i>	<i>Current vel. (ms<sup>-1</sup>)</i>
<i>BDWC</i>	0.244	0.179	0.431	-0.171
<i>PDWC</i>	0.623	0.652	-0.148	0.41
<i>BDSed</i>	-0.502	-0.409	0.248	-0.731
<i>PDSed</i>	-0.469	-0.195	-0.016	-0.622

### Distribution of Chemical Factors

Ammonia and Nitrate and nitrite ranged from 0.6 - 2.4  $\mu\text{M}$  and 0.3 - 5.5  $\mu\text{M}$ , in the Akkeshi-ko estuary, respectively. Ammonia concentration was highest in Sts. 10, 17, 18 and 19 (near the river mouth) than other stations in almost all seasons (Fig. 4).

Nitrate and Nitrite showed highest concentration in Sts. 9, 10 and 19 (Fig. 5). This suggests that the Sts. 9, 10, 17, 18 and 19 in the front of the river-mouth were influenced by terrestrial input of ammonia and nitrate and nitrite from the Bekanbeusi River.

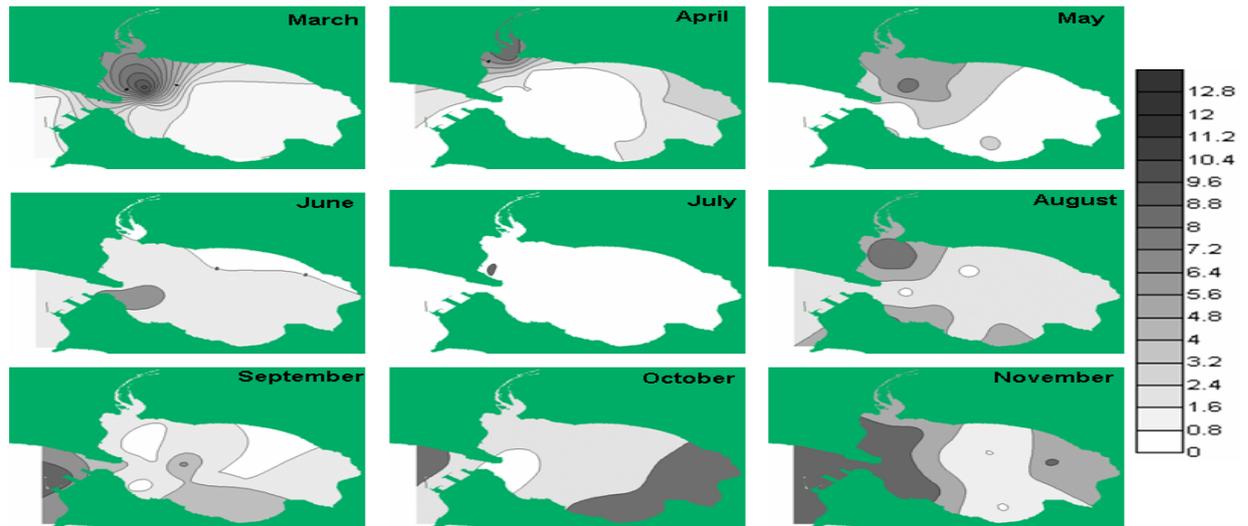


Fig. 4. Distributions of ammonia ( $\mu\text{M}$ ) of surface seawater in the Akkeshi-ko estuary from March to November.

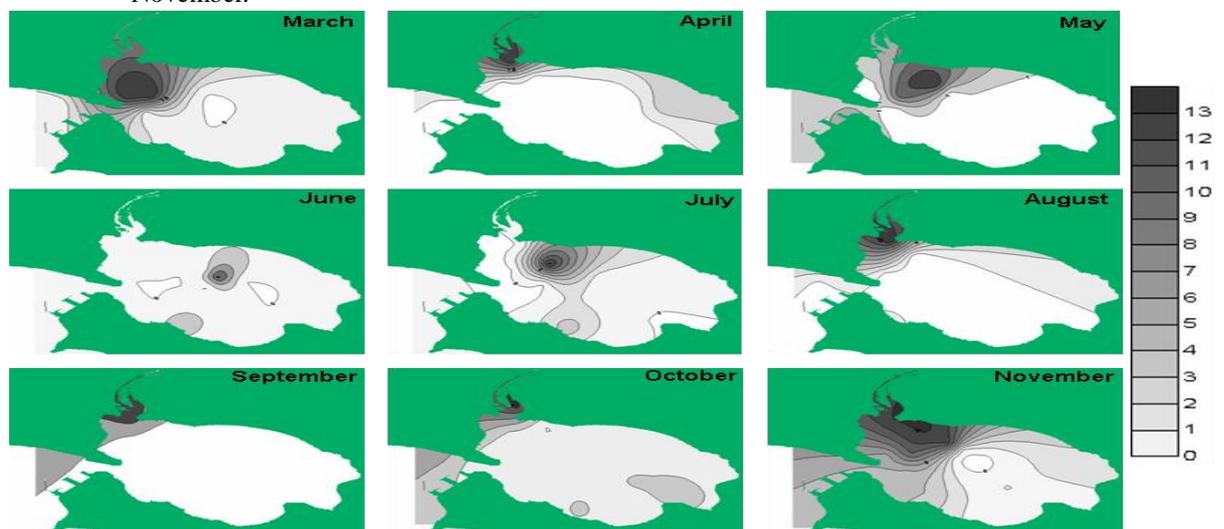


Fig. 5. Distributions of nitrite+nitrate ( $\mu\text{M}$ ) of surface seawater in the Akkeshi-ko estuary from March to November.

For chemical factors, correlation coefficients between annual mean abundance of diatom assemblages and annual mean concentration of ammonia ( $\text{NH}_4$ ), nitrite and

nitrate ( $\text{NO}_2^- + \text{NO}_3^-$ ) and phosphate ( $\text{PO}_4$ ) were calculated. Nitrite and nitrate had positive relationship with the abundance of diatom on the surface sediment (Table 3).

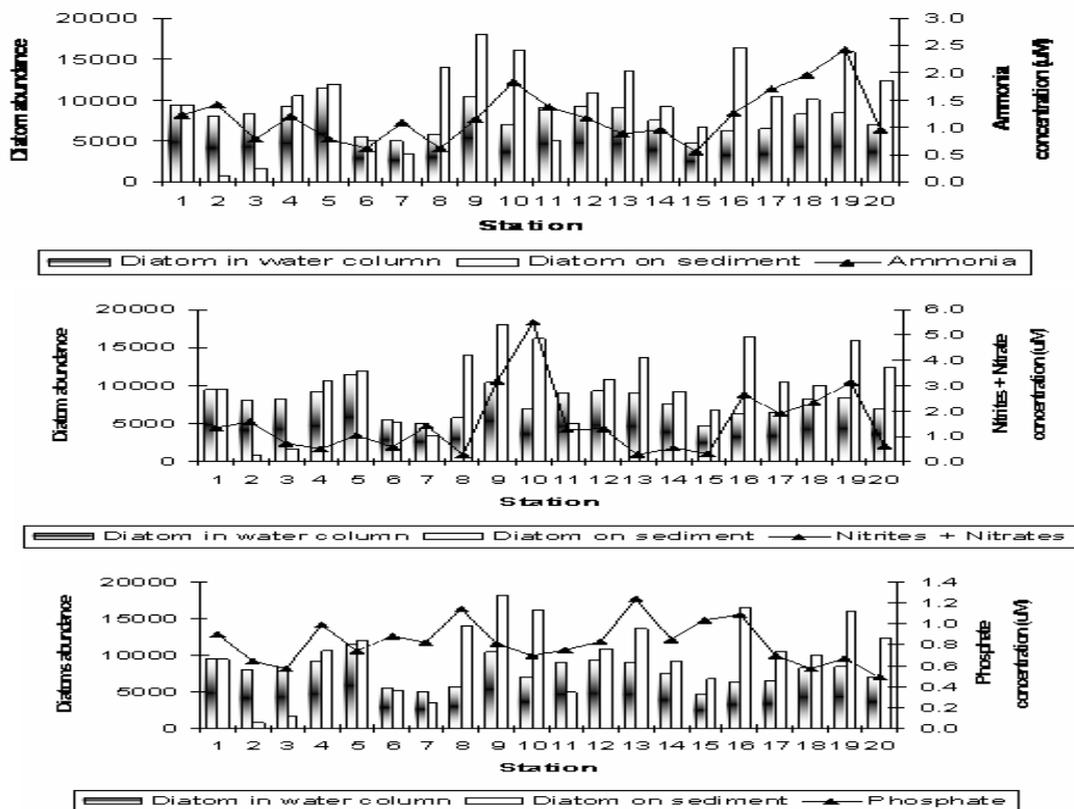
**Table 3.** Correlation coefficients (R) between chemical factors and the abundance of benthic and pelagic diatom assemblages in both, surface sediment and water column (BDWC = benthic diatoms in water column, PDWC = pelagic diatoms in water column, BDSed = benthic diatoms on the sediment, and PDSed = pelagic diatoms on the sediment).

	Ammonia ( $\mu\text{M}$ )	Nitrate + Nitrite ( $\mu\text{M}$ )	Phosphate ( $\mu\text{M}$ )
<b>BDWC</b>	0.283	0.238	-0.145
<b>BDSed</b>	0.289	0.475	0.308
<b>PDWC</b>	-0.086	-0.307	-0.264
<b>PDSed</b>	0.279	0.408	-0.032

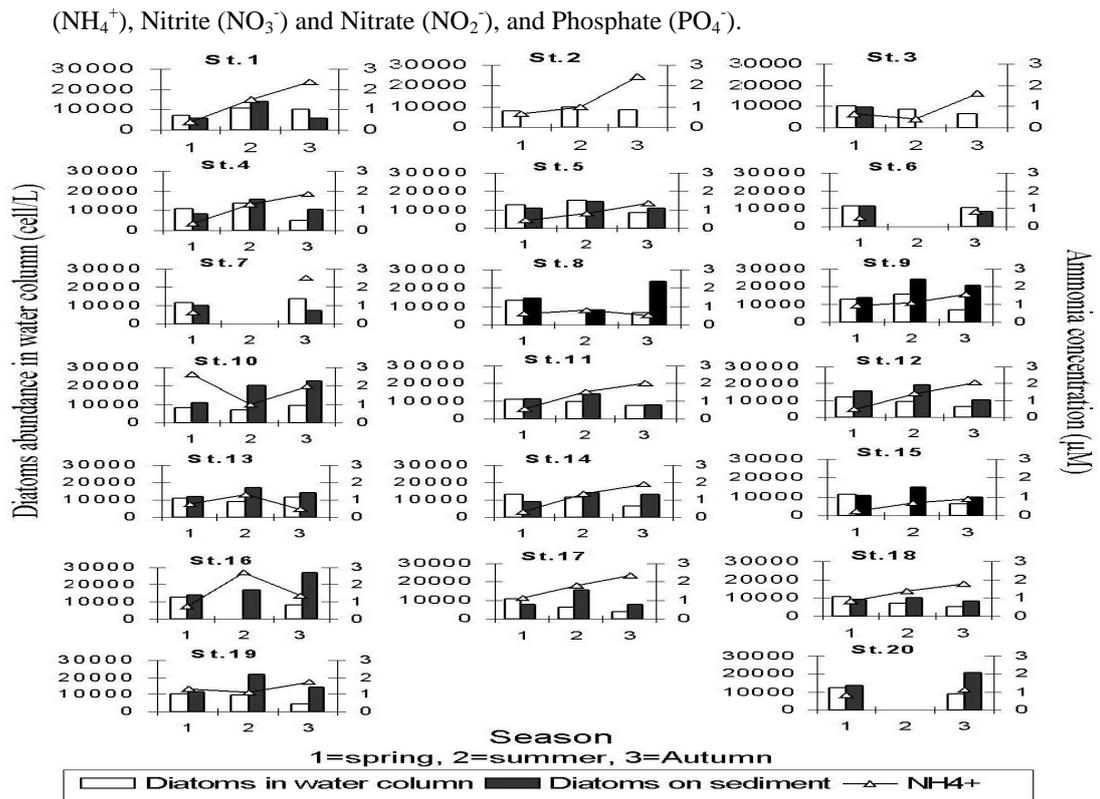
Cell number of diatoms was high in Sts 9, 10, 16 and 19 when ammonia and nitrite and nitrate had high concentration. Phosphate concentration showed opposite trends in relation to the abundance of benthic diatoms (Fig. 6).

On the other hand, seasonal variation of the relationship between abundance of

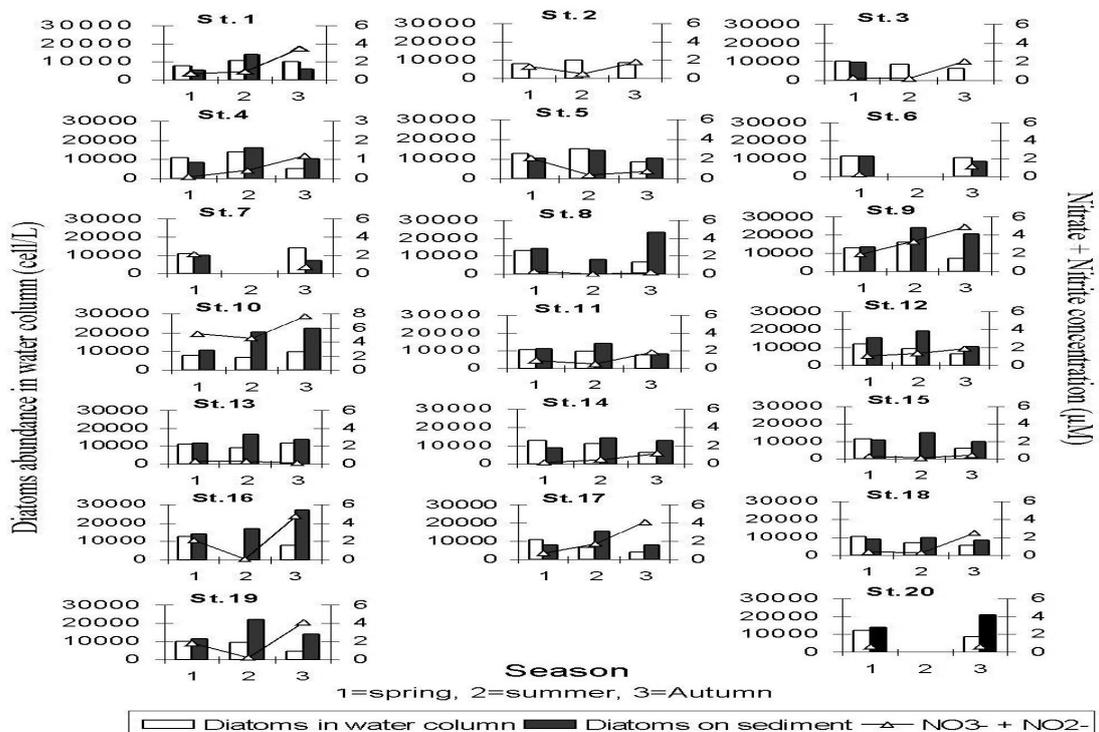
diatoms assemblages and ammonia, nitrite and nitrate and phosphate showed that generally in most stations, the high abundance of benthic and pelagic diatoms in summer followed by the increasing of ammonia, nitrite & nitrate and phosphate (Figs. 7, 8, and 9).



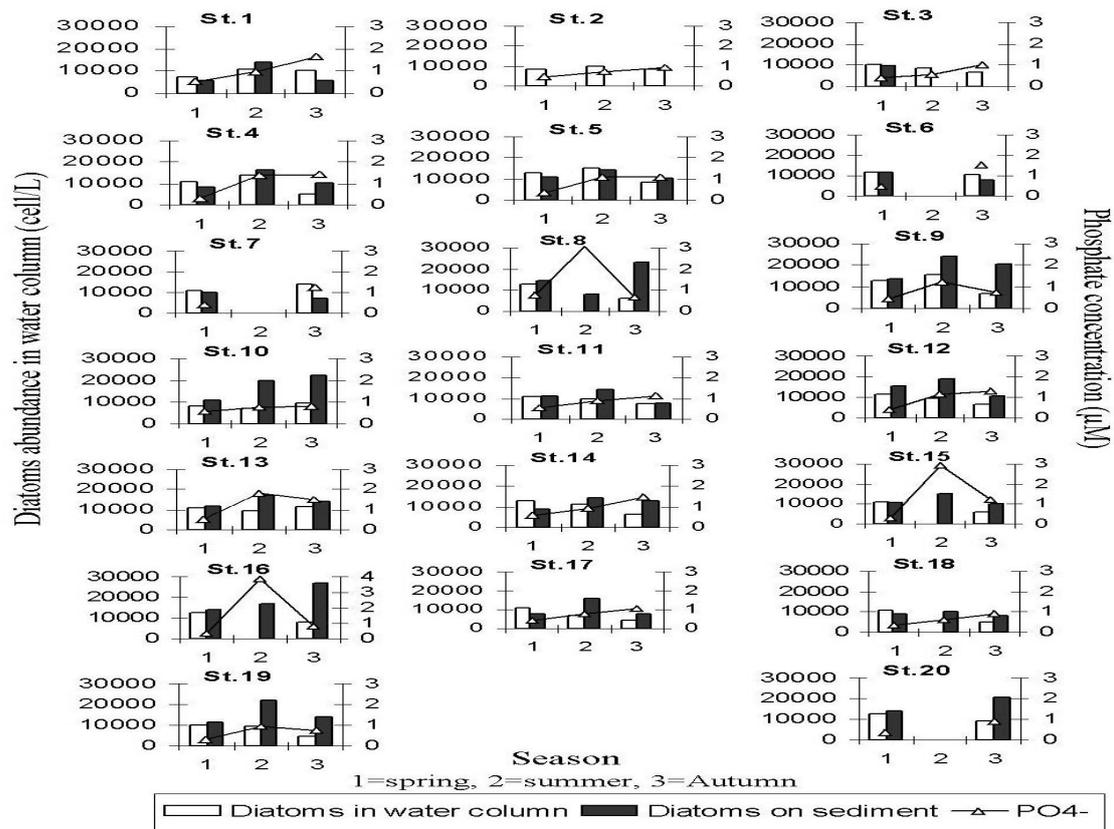
**Fig 6.** The relationship between annual mean of diatoms abundance in water column (cell/L) and surface sediment (cell/mg) and annual mean concentrations of chemical factors; ammonia



**Fig.7.** Seasonal variation of diatoms abundance and correlation with ammonia (NH<sub>4</sub><sup>+</sup>) in each stations in the Akkeshi-ko estuary



**Fig. 8.** Seasonal variation of diatoms abundance and correlation with nitrate and nitrite (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>) in each stations in the Akkeshi-ko estuary



**Fig 9.** Seasonal variation of diatoms abundance and correlation with Phosphate ( $PO_4^-$ ) in each stations in the Akkeshi-ko estuary.

## DISCUSSION

The abundance of diatoms in water column fluctuates by many abiotic and biotic factors. It is influenced by light and nutrient availability. Dissolved inorganic nitrogen, as ammonia, nitrate, nitrite, phosphate, silicate and so on, are basically important factors on diatom production (Hillebrand *et al.*, 2000; Lim *et al.*, 2001). These nutrients are supplied from rivers fundamentally. The nutrient concentration and availability for diatoms would be changed with seasons and precipitation in the Akkeshi-ko estuary (Mukai, unpublished). Dominant species of benthic diatom assemblages in the Akkeshi-ko estuary were occurred also adjacent to Bekanbeusi River mouth in Sts. 9, 10, 16 and 19. These observations suggest that dynamic of diatom assemblages was always associated with the availability of nutrient particularly from a river. It is interesting to find out that, several stations near the river, had high

diatom abundance.

The spatial and temporal fluctuation of benthic and pelagic diatoms generally addressed to light and nutrient availability (Kormas *et al.*, 2001, Welker *et al.*, 2002), tidal current (Perrisinotto *et al.*, 2002) and grazing pressure by suspension feeders (Blackford, 2002). Basically, benthic and pelagic diatoms would be controlled by nutrient concentration as bottom-up effect and or by top down effects such as feeding process by suspension feeder (Bennett *et al.*, 2000; Kasim and Mukai, 2009). In mid summer, the biomass of benthic diatoms was higher than pelagic diatoms in several stations in Adriatic Sea, with the nutrient and light availability as key factors for limiting fluctuation of diatoms abundance (Blackford, 2002). The inorganic nutrient concentration at the water column near the sediment surface was importance factor influenced the spatial fluctuation of diatoms. In the Gulf of Trieste (northern Adriatic Sea), there were

significant correlations between diatom abundance and ammonium, silicate and phosphate concentrations. And also the fluctuation of diatoms in water column near the surface bottom layer is influenced by not only the total annual regeneration of ammonium, silicate and phosphate, but also the characteristic sediment and bioturbation that may displace diatoms to water column from the surface layer caused by benthic faunal activity (Welker *et al.*, 2002).

In the Akkeshi-ko estuary, occupation ratio of diatom species in water column diatom assemblage was about 20% in winter and it increased in May to July with increasing biomass of seagrasses (Kasim and Mukai, 2006). On the other hand, some pelagic diatom species were included in the diatom assemblages on surface sediments. Furthermore, diatoms have to compete the nutrients with marine macrophytes, in particular eelgrasses, *Zostera marina* and *Z. japonica*, in Akkeshi-ko. *Z. marina* is a strong competitor for 'phytoplankton' during spring-summer, because the biomass and production rate of *Z. marina* is very high in these seasons in the Akkeshi-ko estuary (Mukai *et al.*, unpublished). Riaux-Gobin and Bourgoin (2002) reported that the increase of several benthic diatom species such as *Cocconeis* sp. and *Grammathopora* sp. during summer in Kerguelen Archipelago (Indian Ocean) were strongly related to the dense macroalgal canopy as important source of these diatoms. Facca *et al.*, (2002) found out low diatom concentration in several sites of Venice lagoon, Italy, when macroalgae disappeared in season. It suggests that epiphytic diatoms are important for sustaining 'phytoplankton' assemblages in the water column.

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Table 1. Summary characteristics of the 20 station in the Akkeshi-ko estuary.

No Station	average of				Diatoms in	Diatoms in
	depth (m)	Salinity (psu)	Temperature (°C)	Current velocity (cm.s <sup>-1</sup> )	Water column (cell.L <sup>-1</sup> )	Sediment (cell.mg <sup>-1</sup> )
1	1.17	29.8	9.9	17.9	94670	94250
2	5.03	30.5	10.0	46.6	80490	No data
3	4.73	30.5	9.0	32.9	83380	No data
4	0.62	26.3	13.0	16.5	91180	106250
5	3.82	27.8	10.7	14.6	114030	119850
6	0.56	24.4	10.2	24.9	55050	51050
7	1.04	23.4	9.2	14.4	50150	34450
8	1.51	24.8	9.1	10.4	57050	140200
9	1.73	24.3	11.6	10.5	104050	181000
10	0.29	14.5	11.3	12.0	70400	162100
11	2.61	29.2	10.3	17.6	89950	49750
12	3.21	28.8	10.0	24.0	93550	108000
13	0.50	24.3	13.1	11.0	90270	136250
14	0.92	25.9	11.4	21.2	75550	91700
15	0.76	24.3	11.3	18.1	46750	67200
16	0.89	25.4	9.6	15.3	62420	164050
17	0.88	27.5	10.1	21.9	64750	104550
18	0.92	28.7	9.1	21.9	83100	100750
19	0.54	28.9	10.2	14.7	84450	158850
20	0.78	28.2	7.6	11.5	70200	122950

Table 2. Correlation coefficients (R) between physical environmental factors and the abundance of benthic and pelagic diatom assemblages in both, surface sediment and water column (BDWC = benthic diatoms in water column,

PDWC = pelagic diatoms in water column, BDSed = benthic diatoms on the sediment, and PDSed = pelagic diatoms on the sediment).

	<i>depth (m)</i>	<i>Salinity (‰)</i>	<i>Temperature (°C)</i>	<i>Current vel. (MS-1)</i>
<b><i>BDWC</i></b>	<b>0.244</b>	<b>0.179</b>	<b>0.431</b>	<b>-0.171</b>
<b><i>PDWC</i></b>	<b>0.623</b>	<b>0.652</b>	<b>-0.148</b>	<b>0.41</b>
<b><i>BDSed</i></b>	<b>-0.502</b>	<b>-0.409</b>	<b>0.248</b>	<b>-0.731</b>
<b><i>PDSed</i></b>	<b>-0.469</b>	<b>-0.195</b>	<b>-0.016</b>	<b>-0.622</b>

Table 3. Correlation coefficients (R) between chemical factors and the abundance of benthic and pelagic diatom assemblages in both, surface sediment and water column (BDWC = benthic diatoms in water column, PDWC = pelagic diatoms in water column, BDSed = benthic diatoms on the sediment, and PDSed = pelagic diatoms on the sediment).

	<b>Ammonia (µM)</b>	<b>Nitrate + Nitrite (µM)</b>	<b>Phosphate (µM)</b>
<b>BDWC</b>	0.283	0.238	-0.145
<b>BDSed</b>	0.289	0.475	0.308

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PDWC	-0.086	-0.307	-0.264
PDSed	0.279	0.408	-0.032

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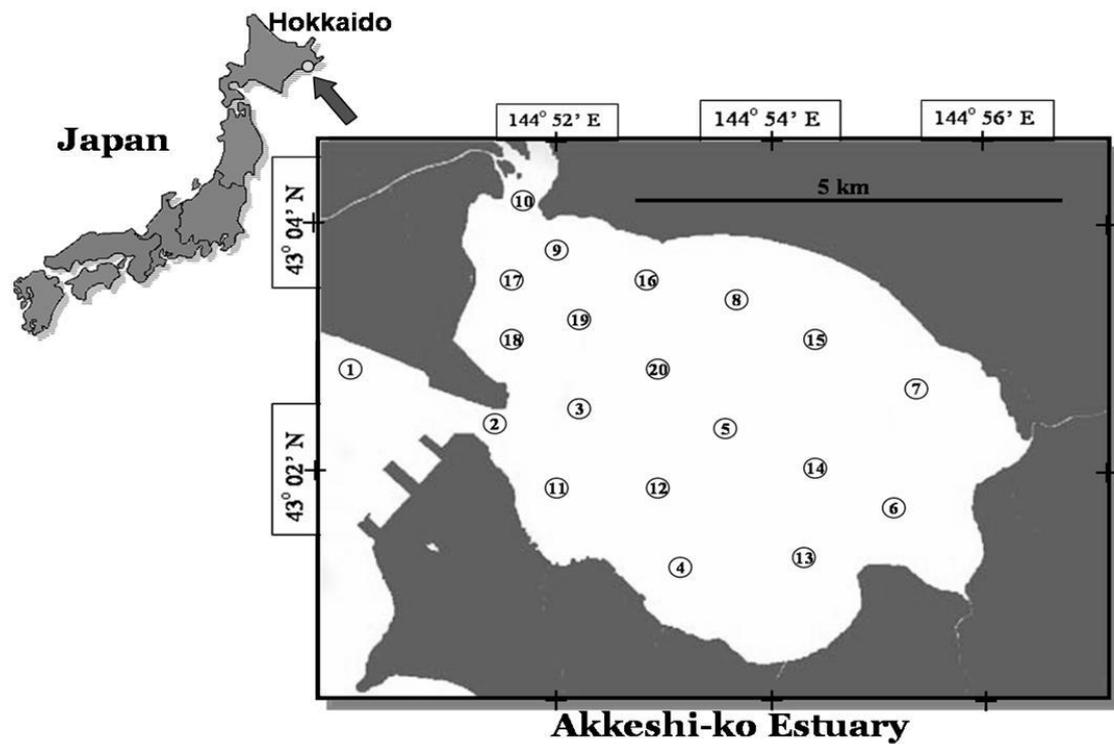


Figure 1. Sampling stations (Sts. 1 – 20) in the Akkeshi-ko estuary, Hokkaido, Japan.

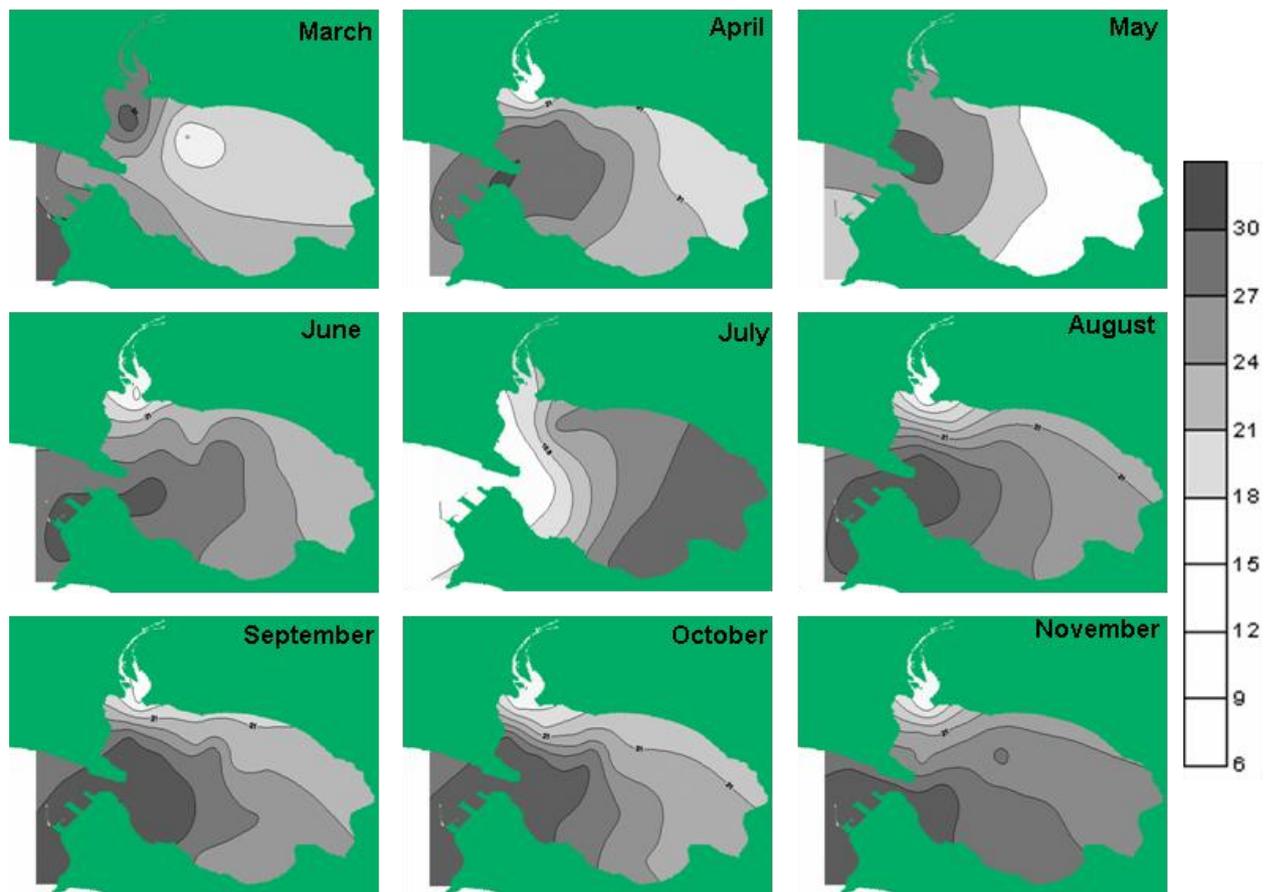


Figure 2. Distributions of salinity (psu) of surface seawater in the Akkeshi-ko estuary from March to November.

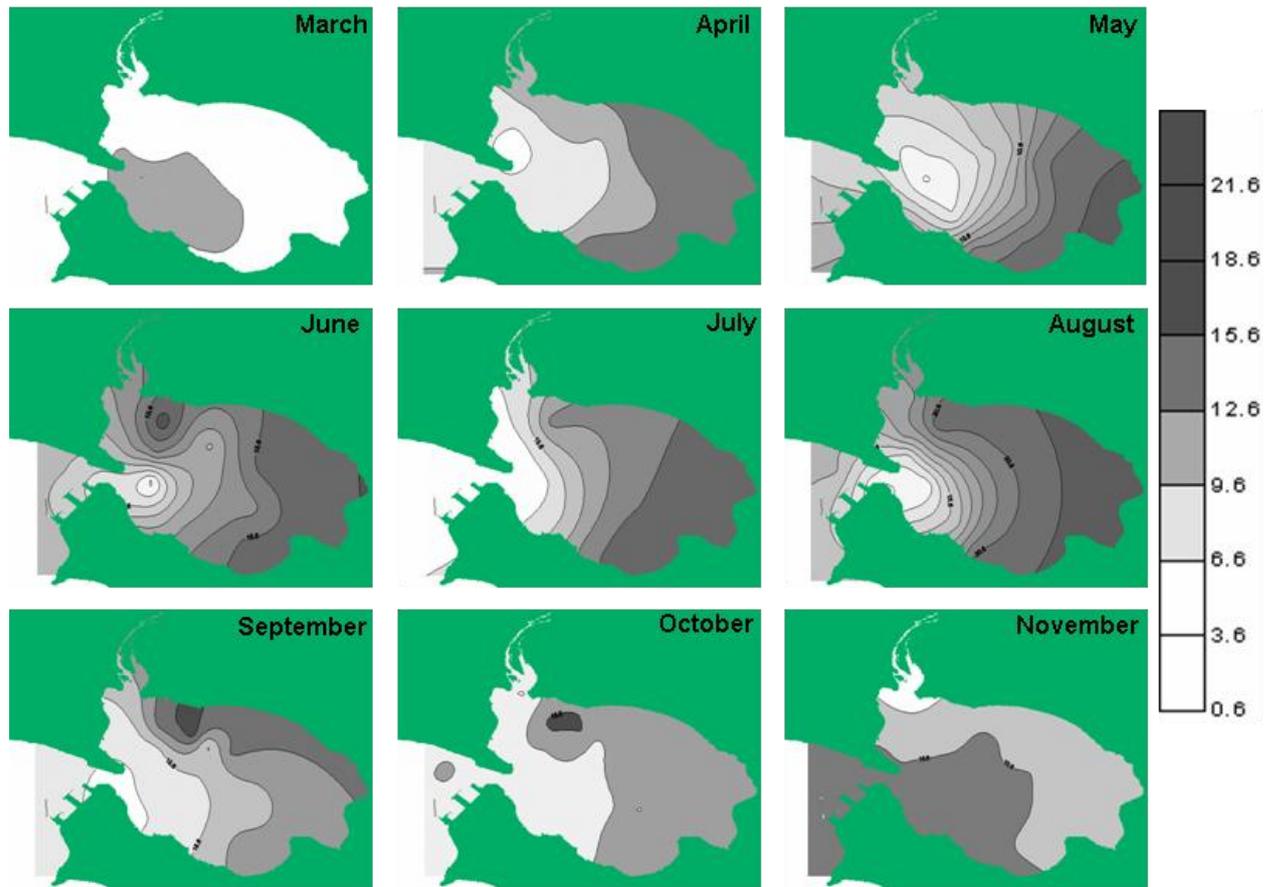


Figure 3. Distributions of temperature ( $^{\circ}\text{C}$ ) of surface seawater in the Akkeshi-ko estuary from March to November.

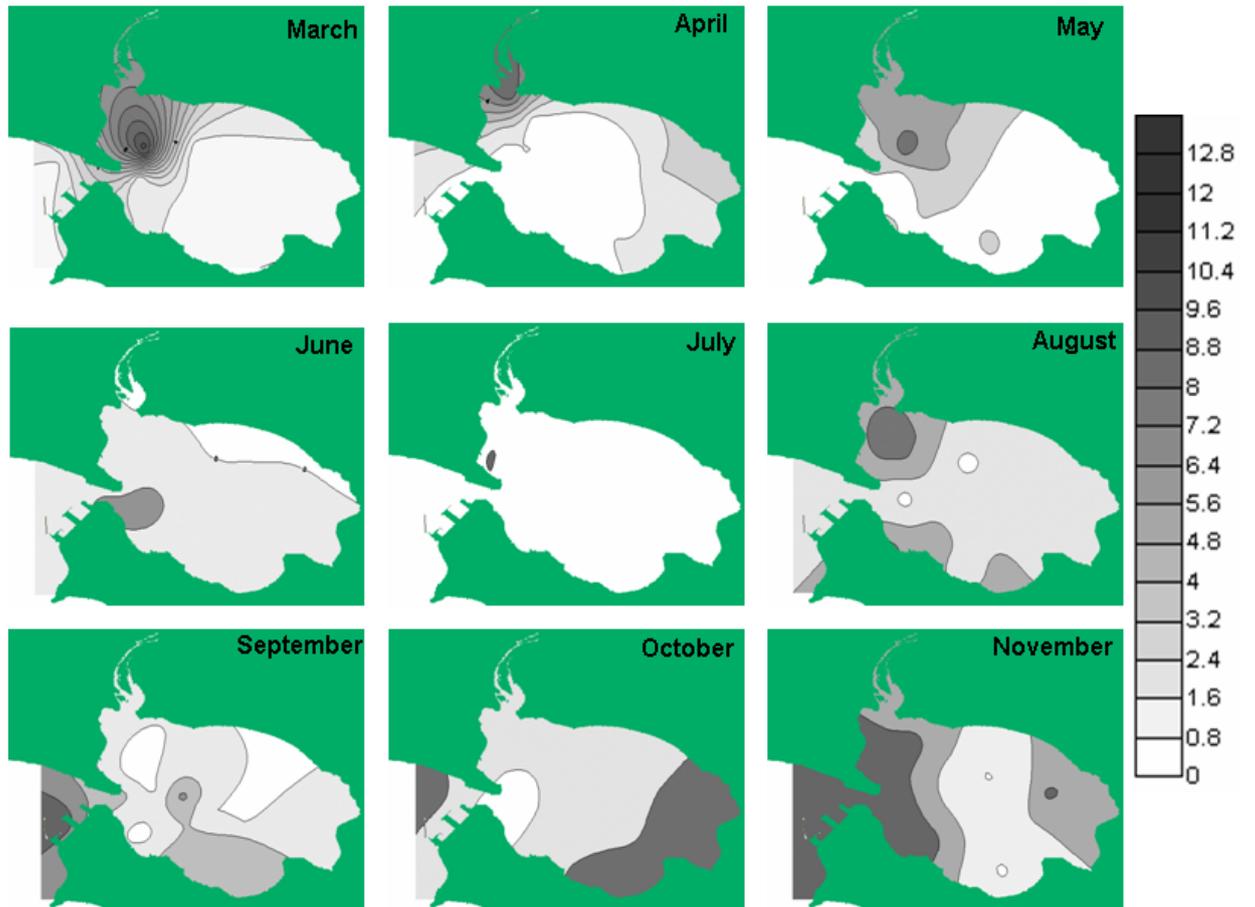


Figure 4. Distributions of ammonia ( $\mu\text{M}$ ) of surface seawater in the Akkeshi-ko estuary from March to November.

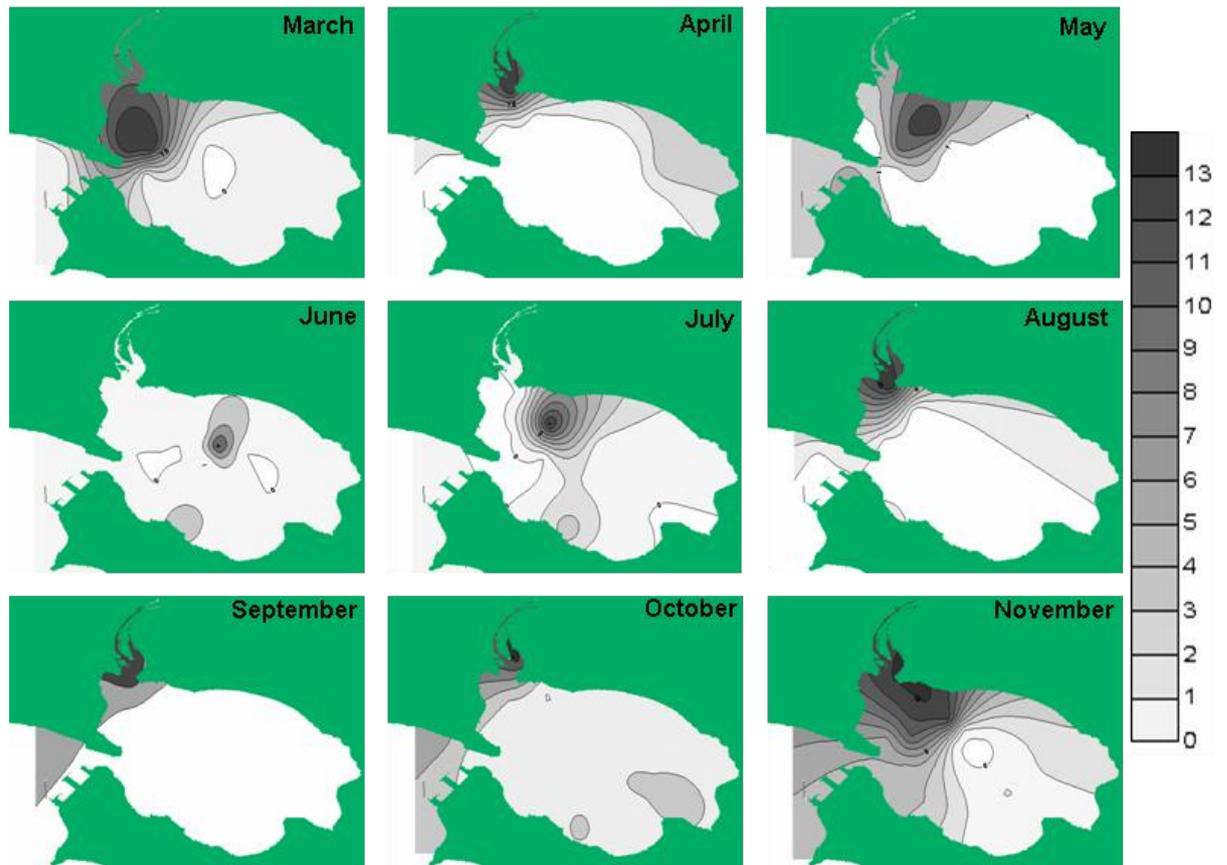


Figure 5. Distributions of nitrite+nitrate ( $\mu\text{M}$ ) of surface seawater in the Akkeshi-ko estuary from March to November.

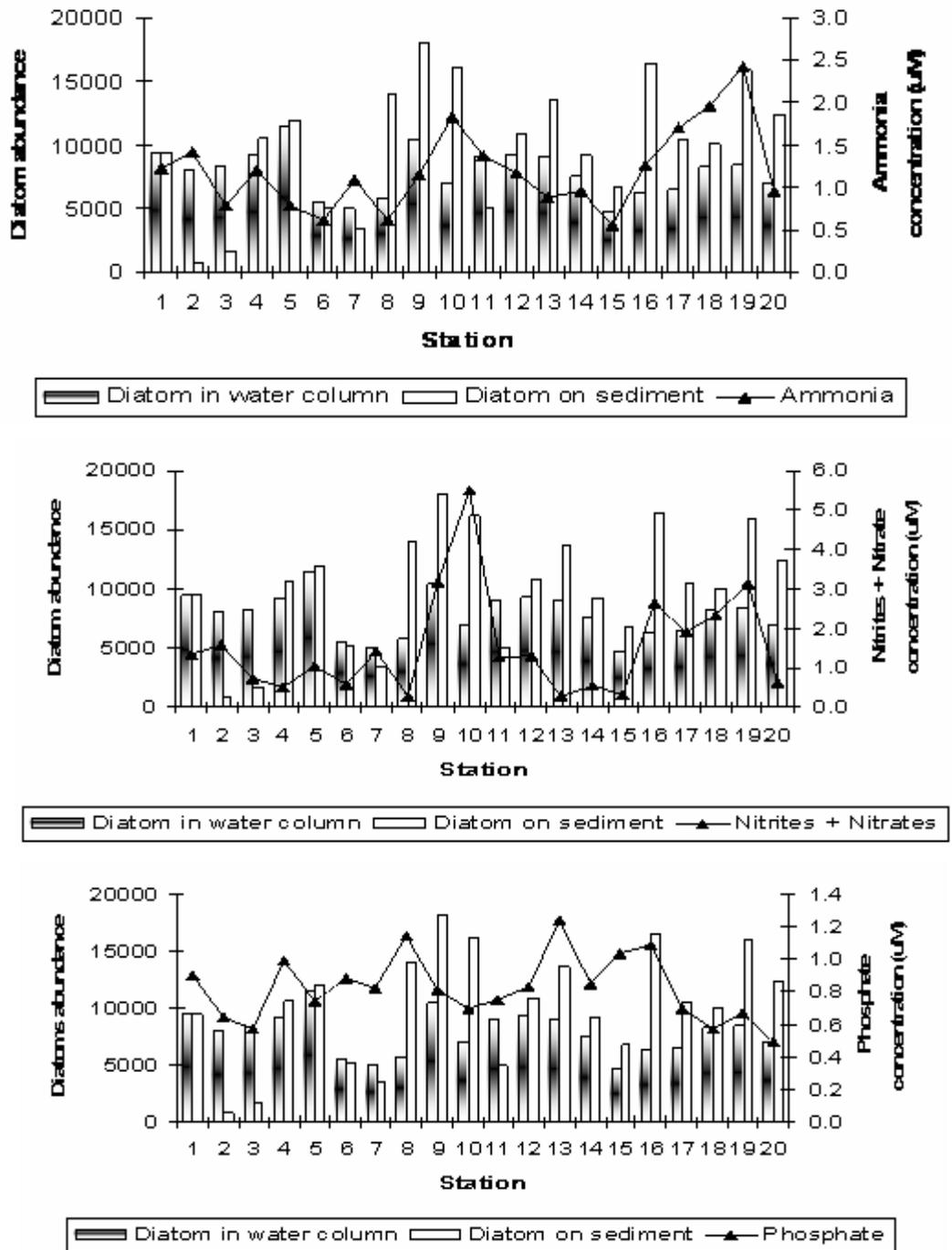


Figure 6. The relationship between annual mean of diatoms abundance in water column (cell/L) and surface sediment (cell/mg) and annual mean concentrations of chemical factors; ammonia ( $\text{NH}_4^+$ ), Nitrite ( $\text{NO}_3^-$ ) and Nitrate ( $\text{NO}_2^-$ ), and Phosphate ( $\text{PO}_4^-$ ).

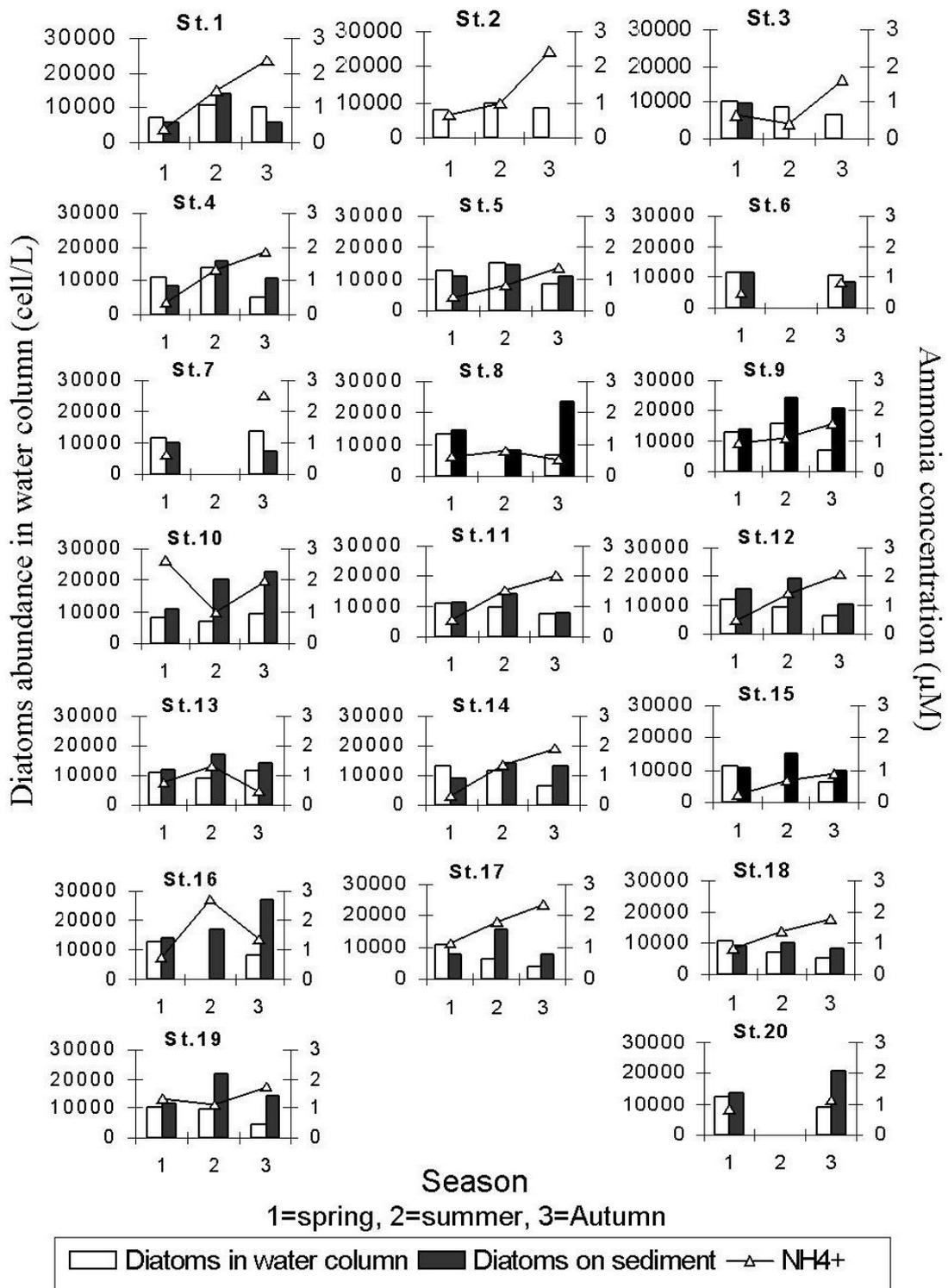


Figure 7. Seasonal variation of diatoms abundance and correlation with ammonia ( $\text{NH}_4^+$ ) in each stations in the Akkeshi-ko estuary

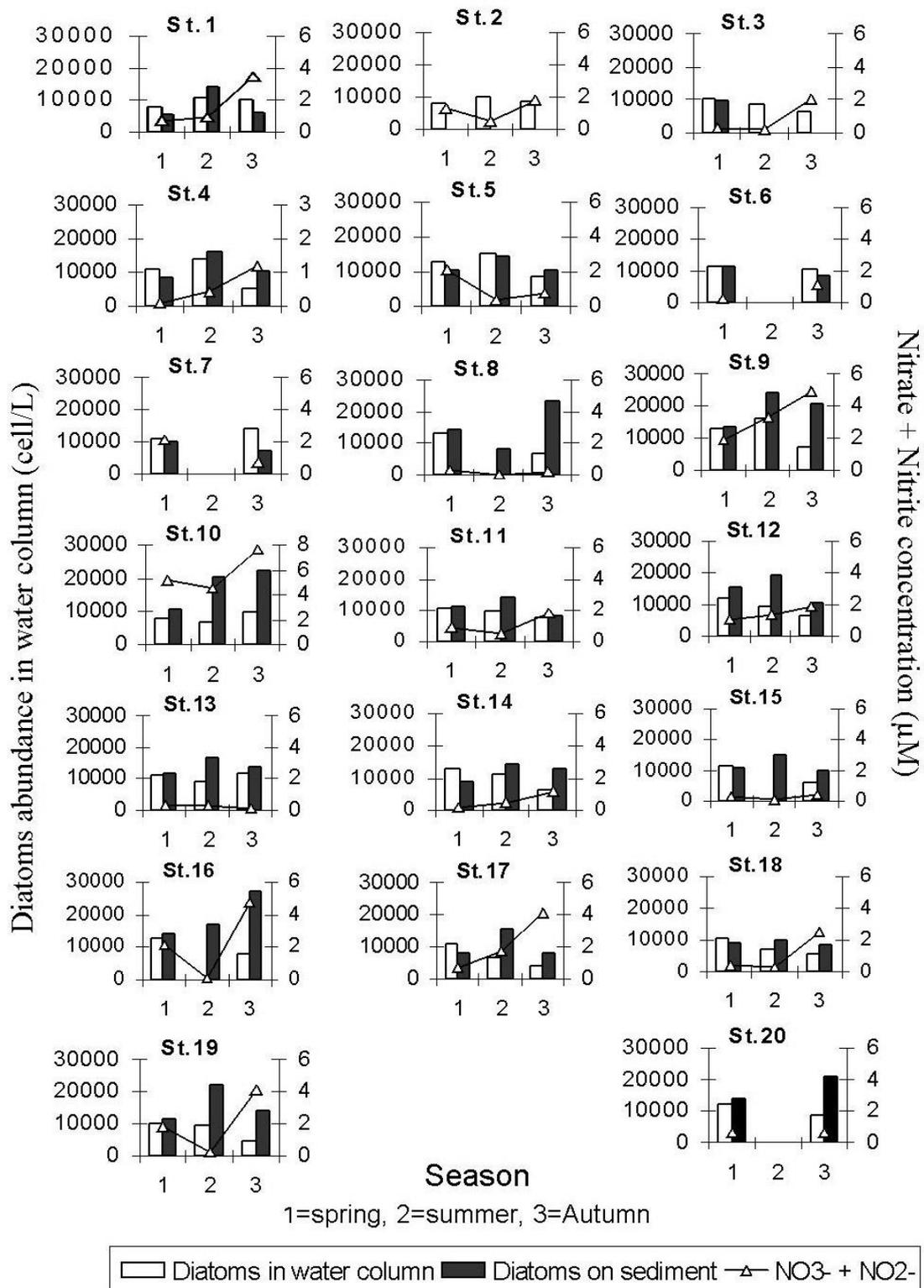


Figure 8. Seasonal variation of diatoms abundance and correlation with nitrate + nitrite (NO<sup>3</sup>- + NO<sup>2</sup>-) in each stations in the Akkeshi-ko estuary

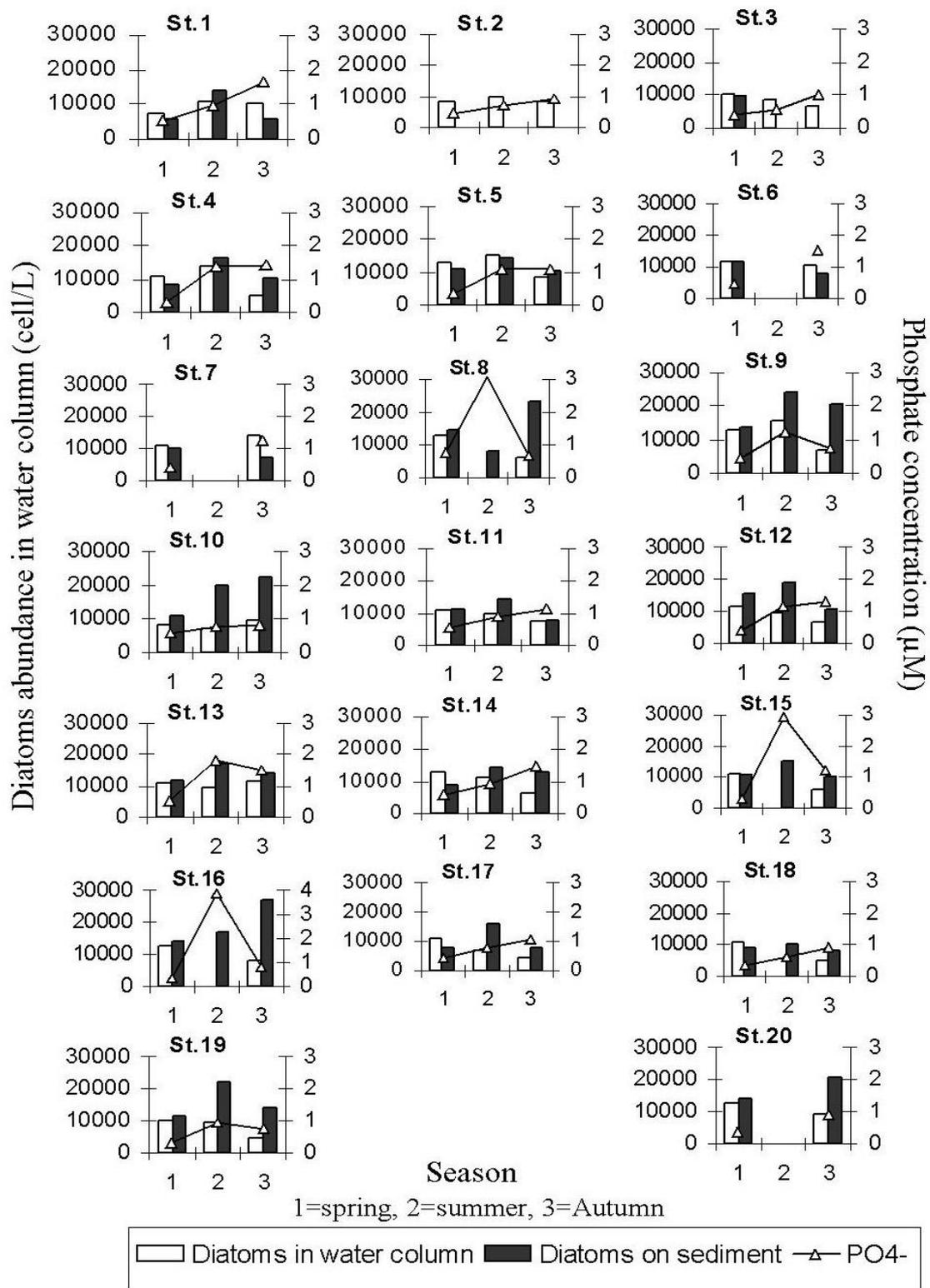


Figure 9. Seasonal variation of diatoms abundance and correlation with Phosphate (PO<sub>4</sub><sup>-</sup>) in each stations in the Akkeshi-ko estuary.

