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Summer Pattern of Phytoplankton Distribution at a Station in Jangmok Bay

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Abstract – Daily changes in phytoplankton abundance and species composition were monitored from July to September 2003 (n=47) to understand which factors control the abundance at a station in Jangmok Bay. During the study, the phytoplankton community was mainly composed of small cell diatoms and dinoflagellates, and the dominant genera were *Chaetoceros, Nitzschia, Skeletonema* and *Thalassionema*. Phytoplankton abundance varied significantly from 6.40×10^4 to 1.22×10^7 cells/*l*. The initially high level of phytoplankton abundance was dominated by diatoms, but replacement by dinoflagellates started when the N/P ratio decreased to < 5.0. On the basis of the N/P and Si/N ratios, the sampling period could be divided into two: an inorganic silicate limitation period (ISLP, 14th July-12th of August) and an inorganic nitrogen limitation period (INLP, 13th of August - the end of the study). Phosphate might not limit the growth of phytoplankton assemblages in the bay during the study period. This study suggests that phytoplankton abundance and species composition might be affected by the concentrations of inorganic nutrients (N and Si), and provides baseline information for further studies on plankton dynamics in Jangmok Bay.

Key words – inorganic nutrients, silicate limitation, nitrogen limitation, phytoplankton

1. Introduction

In diverse marine ecosystems, diatoms and dinoflagellates have been studied because of their tendency to form massive blooms, often referred to as harmful algal blooms (HABs). In the world, about 300 species can cause HABs, while about 80 species are known to have the capacity to produce potent toxins (Hallegraeff 2003). Every year, many cases of human poisoning by HABs are reported and the economic loss incurred is usually in the millions in US dollar terms (Gilbert and Pitcher 2001). In Korea, there are about 60 causative species (Kim *et al.* 1993) and HABs have frequently been reported in most parts of Korean coastal waters, especially in the southern part of Korea (*e.g.* Park and Kim 1967; Park *et al.* 1989; Kim *et al.* 1998, 2002; Lee *et al.* 2002). The economic losses have been estimated and the most severe economic loss from HABs was in 1995 (Kim *et al.* 1998). The study of HAB species has therefore become an important part of the research in marine coastal areas in Korea. In order to understand the occurrence mechanism of HABs, it is necessary to know the role of the causative species in a certain ecosystem or food web. It is therefore important to know which factors control their abundance and composition. These factors may include temperature, salinity, illuminations, pH, inorganic nutrients, and biological interactions such as species competition and predation. Previous studies (*e.g.* Hecky and Kilham 1988; Howarth *et al.* 1988; Del Amo *et al.* 1997; Diaz *et al.* 2001) have verified that inorganic nutrients may have an effect on the abundance and composition of phytoplankton and may determine the dominant species. The abundance and growth of organisms such as phytoplankton and zooplankton are viewed as being controlled by resource limitation and predation, which may act simultaneously or alternately (*e.g.* Gasol

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and Vaqué 1993).

HABs species have frequently been observed in Jangmok Bay, but there are no previous studies on the changes in abundance and composition of HABs or phytoplankton in Jangmok Bay. There was a report on the changes in mesozooplankton abundance and composition by Jang *et al.* (2002) who discussed seasonal distribution of mesozooplankton. Jang *et al.* (2004) showed that chlorophyll-a varied with seasonality, but it was not well correlated with inorganic nutrients. Kim *et al.* (2004) reported on the grazing of microzooplankton assemblage on microphytoplankton. Therefore, studies for understanding the role of HABs in the ecosystem of Jangmok Bay have been required.

The present study was conducted as a pilot study to understand the role of HABs/phytoplankton within the microbial food web of Jangmok Bay. Here, we report the estimates of the abundance of phytoplankton during the summer of 2003 at a single station in Jangmok Bay. We also measured the abundance of microzooplankton such as heterotrophic ciliates (HCI) and heterotrophic dinoflagellates (HDNF), and mesozooplankton, and measured 8 physico-chemical factors such as dissolved oxygen, salinity, temperature, inorganic nutrients (TIN, P, and Si), pH and secchi disk transparency (SDT). We used this information to understand which factors controlled phytoplankton abundance and composition in Jangmok Bay.

2. Materials and Methods

Study site and water sample collection

The present study was carried out from 14th July 2003 to 4th September 2003 at a single station in Jangmok Bay. The station was close to the tip of the pier in the South Sea Institute of the Korea Ocean Research and Development Institute (KORDI) located in the southern part of Korea (Fig. 1). The maximal tidal range in the bay is approximately 2.2 m and the mean water depth of the sampling station is about 8.5 m. There is no inflowing river into the bay, but the bay directly receives water and suspended solids from nearby mountains following rainfall. There were somewhat frequent rainfalls during the sampling period (Fig. 2), and such frequent heavy rainfall is typically experienced in the summer in Korea. Water samples (20 liters) were collected daily from surface water at high tide (day time) using a bucket.

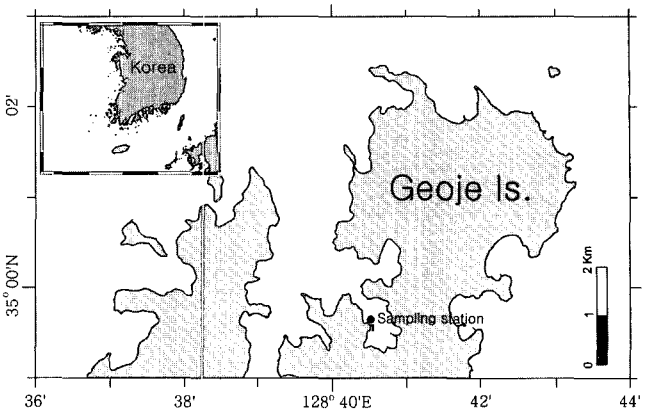

Fig. 1. Map showing the sampling station.

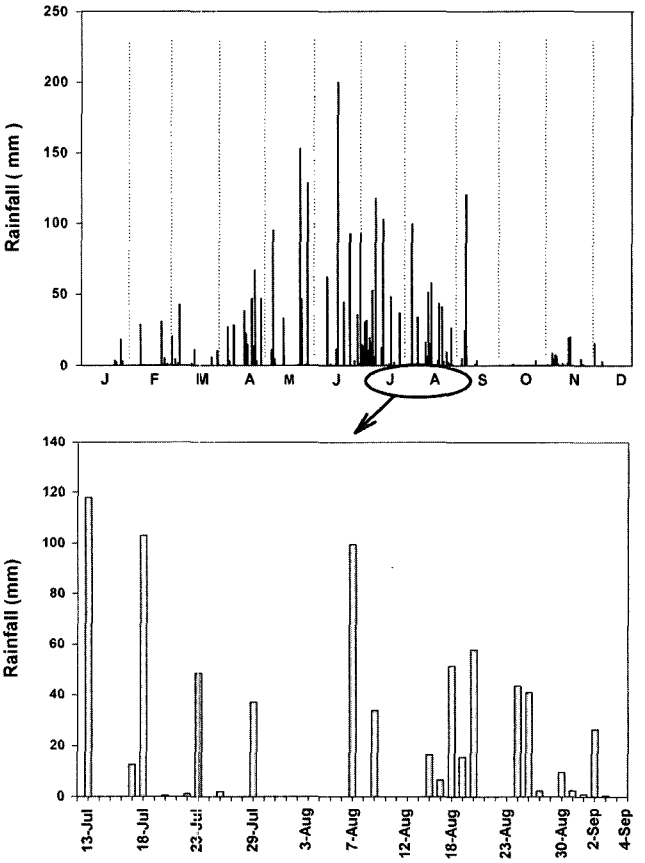

Fig. 2. Rainfall in Jangmok region during 2003.

Environmental factors

Water temperature and salinity were measured *in situ* with a Ocean Seven 319 CTD (Idronaut), and dissolved oxygen (DO) and pH were measured *in situ* with a DO meter (YSI, Model 58) and a pH meter (ISTEK, Model 720P), respectively. Samples for inorganic nutrients were collected when chlorophyll-*a* samples were filtered

through GF/F filters (retention size ca. 0.7 μm, Whatman). The samples were preserved deep frozen at -20 °C in the dark until analysis. Inorganic nutrients (total inorganic nitrogen, TIN; phosphate, P; silicate, S) were analyzed with duplicate samples using a spectrophotometer (HP 8354) and an automatic UV Recording Spectrophotometer (LACHAT, Quick Chem 8000) according to Solozano (1969) and Parsons *et al.* (1984).

Biological factors

Five hundred to 1000 ml the 20 L of water samples for analysis of chlorophyll-*a* were filtered through GF/F filters, and the filter was placed in a 50ml tube. 20 ml of 90% acetone were added to the tube and kept in a refrigerator for 24 hrs to extract pigments (Parsons *et al.* 1984). After 24 hrs, the samples were centrifuged at 1500 rpm for 5 min and the supernatant were used to measure chlorophyll-*a* with an UV spectrophotometer (HP 8354). Chl-a was fractionated through 3 μm and 20 μm in order to obtain three fractions: the smallest or picoplanktonic faction (<3 μm), the nanoplanktonic fraction (3-20 μm) and the largest or microplanktonic fraction (>20 μm).

A 500 ml portion of the 20 L water samples for phytoplankton and heterotrophic ciliates (HCI) was fixed with a Lugol acid solution at a final concentration of 1%, and allowed to settle for at least 24 hrs. Phytoplankton and HCI were enumerated with a Sedgwick-Rafter Chamber under a light microscope (Zeiss Axioplan) at a magnification of 200 x. Another 500 ml of the 20 L water samples for dinoflagellates (heterotrophic dinoflagellates: HDNF, photosynthetic dinoflagellates: PDNF) were preserved in a formaldehyde solution at a final concentration of 0.5%, stored and settled for 24hrs in a refrigerator (4°C), while the supernatants were disposed of. Each sample was transferred into a 50 ml conical tube (Greiner) and then settled again for 24hrs in the refrigerator. To distinguish HDNF from PDNF, the sample was stained with 5 μg/ml of DAPI (final conc.) in a Sedgwick-Rafter Chamber, and then HDNF and PDNF were enumerated and identified using an epifluorescence microscope (Zeiss Axioplan) with UV and G excitation filters (BP 365/12 and BP 450-490, respectively). HDNF abundance was excluded from phytoplankton abundance. A 10 L portion of the water sample for zooplankton was taken from the 20 L water samples and filtered through a 200 μm mesh to separate microzooplankton from mesozooplankton. Zooplankton was preserved in a formaldehyde solution at a final concentration of 1% and enumerated under a dissecting microscope (Zeiss Stemi SV11).

3. Results and Discussion

Environmental factors

The results of the measurements of environmental factors are shown in Fig. 3. Water temperature was in the range of 20.3-28.8°C, and salinity was in the range of 18.0-27.8 psu and relatively low in July. DO concentration varied

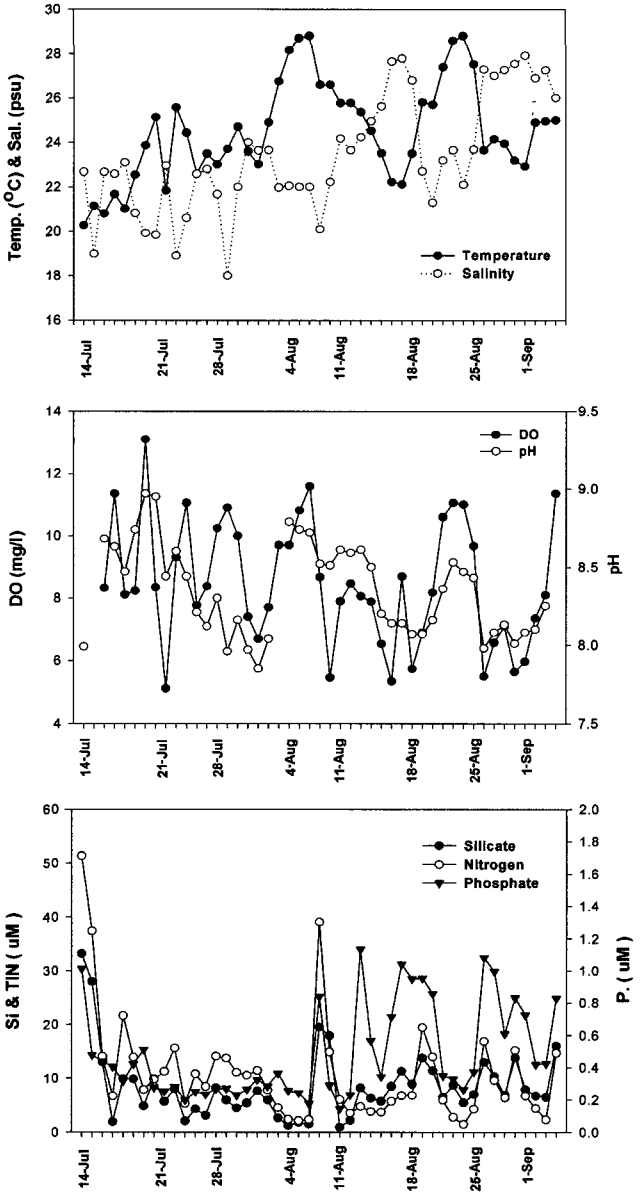

Fig. 3. Daily variations of environmental factors measured during the study.

from 5.1 to 13.1 mg/l and pH value varied from 7.85 to 8.97. Concentrations of total inorganic nitrogen (TIN), phosphate (P) and silicate (Si) were in the ranges of 1.48-51.31 μM, of 0.15-1.13 μM and of 0.96-33.16 μM, respectively. The sampling site appeared to be influenced by freshwater input after rainfall, because TIN & Si concentrations were significantly correlated with rainfall (TIN: $r = 0.744$, $P < 0.001$; Si: $r = 0.612$, $P < 0.01$) and salinity was negatively correlated with rainfall ($r = -0.350$, $P < 0.5$). Therefore, it was likely that all water samples were undergoing similar effects of freshwater input during summer, probably due to rainfall.

Total and size-fractionated chlorophyll-*a* pattern

The estimates of chlorophyll-*a* concentration are shown in Fig. 4. Chlorophyll-*a* concentration varied dramatically with a range of 1.25-23.4 (mean 7.48±5.03) μg/*l*. Contributions of nanophytoplankton (3-20 μm) and picophytoplankton (< 3 μm) to the total chlorophyll-a concentration were in the range of 3.06-70.9% (mean 40.0±20.2%) and of 2.98-74.7% (mean 21.3±17.0%), respectively. Chain-forming diatoms such as *Skeletonema costatum* and *Chaetoceros* spp. (< 20 μm) dominated throughout the sampling period, with the exception of the period of 18-28 August, when high chlorophyll-a concentrations associated with picophytoplankton were encountered and dinoflagellates dominated. The contribution of the nanophytoplankton might therefore be underestimated because the chain-formers might not have passed through the 20 μm mesh. This suggests that nanophytoplankton might be a major component of phytoplankton and that picophytoplankton was also not negligible in Jangmok Bay during the summer in 2003.

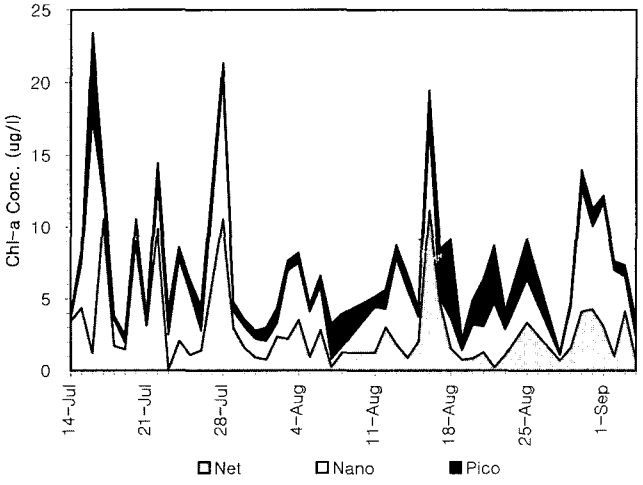

Fig. 4. Daily variations of size fractionated chlorophyll-*a* concentrations in Jangmok Bay.

Micro- and mesozooplankton

In this study, the zooplankton community was largely composed of microzooplankton and mesozooplankton, but mainly occupied by microzooplankton in abundance (Fig. 5). Total zooplankton abundance varied remarkably from 1.42×10^3 to 6.05×10^4 (mean $1.61 \pm 12.4 \times 10^3$) cells/*l*, and heterotrophic protists such as heterotrophic dinoflagellates (HDNF) and heterotrophic ciliates (HCI) accounted for over 95% of the microzooplankton and total zooplankton, while copepods and other groups of zooplankton occupied small parts of the total zooplankton abundance. This suggests that heterotrophic protists in the zooplankton community of Jangmok Bay may play an important role as consumers of small plankton such as bacteria, cyanobacteria and flagellates as previous studies indicate (*e.g.* Porter *et al.* 1985; Pierce and Turner 1992; Sherr and Sherr 1992).

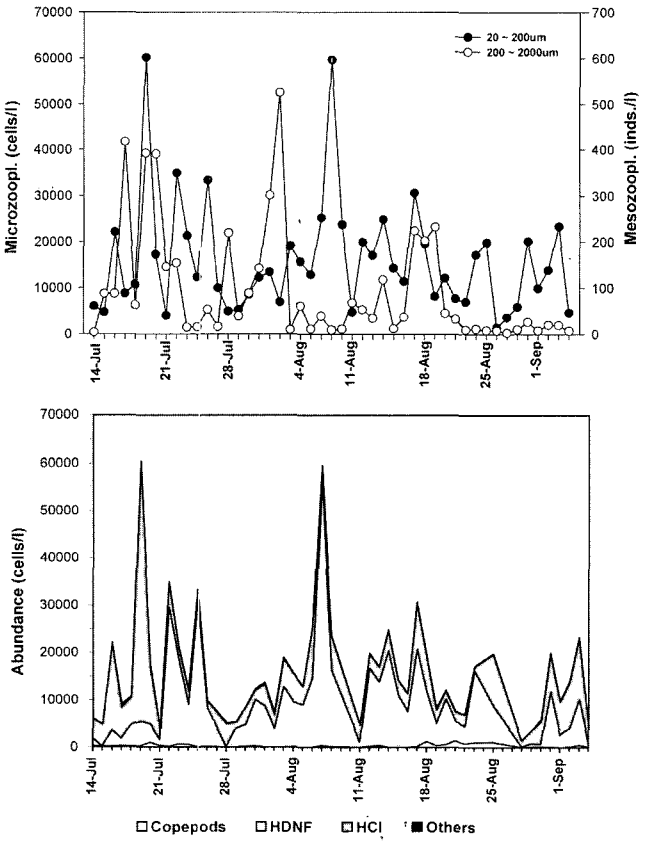

Fig. 5. Daily variation of zooplankton in Jangmok Bay.

Species composition and abundance of phytoplankton

During this study, 105 species from 45 genera, including 9 unidentified diatom species, were encountered. HDNF was excluded from the abundance and lists of species composition. Four major phytoplankton groups were represented; Bacillariophyceae, Chrysophyceae, Euglenophyceae and Dinophyceae. The most dominant phytoplankton throughout the sampling period belonged to the genera *Chaetoceros*, *Nitzshia*, *Skeletonema* and *Thalassionema*, which are chain-forming diatoms. The most dominant species causing high phytoplankton abundance were *Chaetoceros* sp2 and *Skeletonema costatum* and these are coastal species, which can grow quickly under eutrophic conditions. The number of species accounting for over 0.5% of total phytoplankton abundance was 21, and among these 21 species only 4 species contributed over 5% to the total abundance; *Chaetoceros* spl., *Cheatoceros* sp2., *Skeletonema costatum* and *Thalassionema nitzschioides* (Table 1).

Daily variation of phytoplankton in Jangmok Bay was apparent and the average abundance of phytoplankton was 2.63×10^6 cells/*l*, with a range of 6.40×10^4-1.22×10^7 cells/*l* (Fig. 6). Although the phytoplankton abundance varied significantly daily, we could not find any obvious regular patterns in daily changes. High abundances with $>3\times10^6$ cells/*l* were observed 12 times during the study (Figs. 4 and 6). These high abundances were mainly due

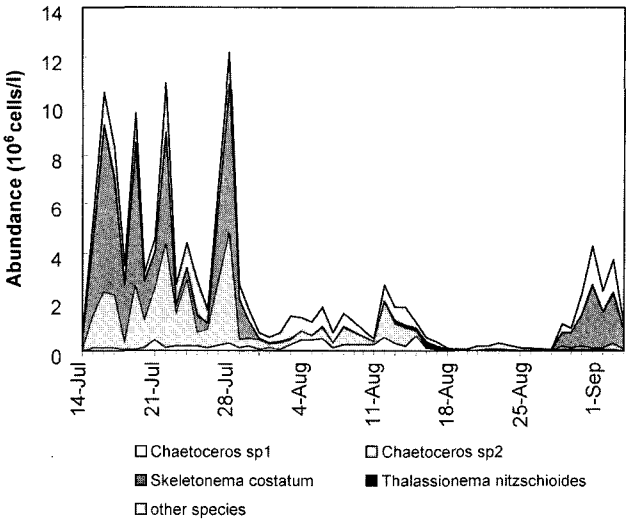

Fig. 6. Daily variation of phytoplankton in Jangmok Bay.

Table 1. 21 dominant species accounting for over 0.5 % of total abundance during the study.

Species	Average (%)	Concentration		
		<1×10^6 cells/l (%)	1-5×10^6 cells/l (%)	> 5×10^6 cells/l (%)
Ceratium fusus	1.1	2.9	0.0	0.0
Prorocentrum micans	0.9	2.3	0.1	0.0
P. minimum	1.6	2.1	1.7	0.1
P. triestinum	4.3	11.3	0.5	0.1
Cerataulina compacta	1.3	1.4	1.5	0.3
Chaetoceros affinis	1.1	0.4	1.6	0.9
Chaetoceros compressus	2.4	1.4	3.5	0.5
Chaetoceros debilis	0.8	0.6	0.5	2.7
Chaetoceros pelagicus	2.2	1.8	2.9	0.6
Chaetoceros spl.	9.1	8.6	11.4	1.4
Chaetoceros sp2.	20.2	11.8	23.9	29.0
Dactyliosolen fragillissimus	3.6	2.7	5.0	0.1
Dictyocha fibula	1.0	2.6	0.0	0.0
Navicula spl.	0.8	1.7	0.3	0.1
Nitzschia spl.	1.0	1.5	0.7	0.4
Nitzschia sp2.	4.6	5.8	4.7	0.5
Pseudonnitzschia serata	1.4	3.4	0.3	0.1
Skeletonema costatum	25.4	11.6	27.8	54.5
Thalassionema nitzschioides	5.2	11.8	1.6	0.8
Thalassiosira gravida	1.7	0.7	2.7	0.2
Thalassiosira rotula	0.9	0.7	0.4	3.7
Total	90.8	87.3	93.0	97.0

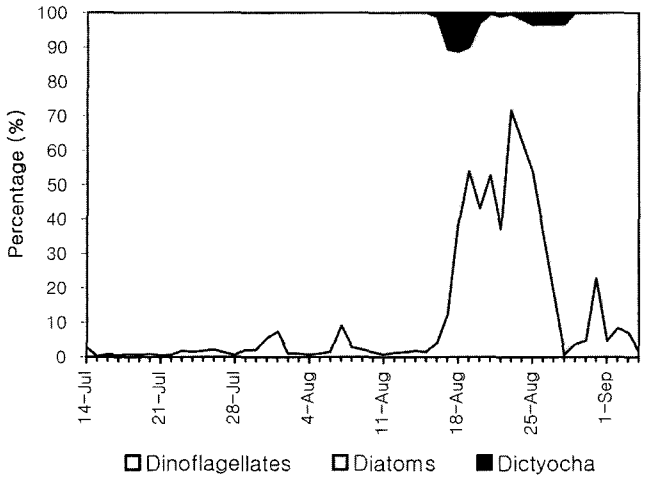

Fig. 7. Variation of phytoplankton components.

to 4 dominant species, and the average contribution of the 4 dominant species to the total phytoplankton abundance was 60%, with a range of 17-89%. The level of contribution was higher at high phytoplankton abundance than at lower abundance (Fig. 6, Table 1), indicating that high abundances were occupied by only a few dominant species. The composition of the dominant species/groups varied among sampling occasions (Figs. 6-7). At the beginning of the study (July 15), a high abundance of phytoplankton was dominated by diatoms such as *Chaetoceros* sp2 and *Skeletonema costatum*, and was maintained for two weeks with $>2\times10^6$ cells/*l*. Since then phytoplankton abundance decreased until August 16, but diatoms still occupied a high portion (>90%) of the total phytoplankton abundance (Fig. 7). After August 16, the portions occupied by dinoflagellates and *Dictyocha fibula* increased and dinoflagellates were dominant. *Ceratium fusus*, *Prorocentrum micans*, *P. minimum* and *P. triestium* were the dominant dinoflagellate species. After the dominance of dinoflagellates, diatom abundance increased again at the end of the study. This result indicates that the initial high phytoplankton abundance dominated by diatoms was replaced by dinoflagellates during this study period.

Facotrs controlling phytoplankton abundance

Correlation coefficients

Pearson's correlation coefficients among organisms and environmental factors are shown in Table 2. Phytoplankton abundance was significant and negatively correlated with temperature and salinity, suggesting that low temperature and salinity may be important factors for increasing phytoplankton abundance during the study. The phytoplankton abundance had weak negative relationships with Si and P, and a significantly positive relationship with NH_4. *Chaetoceros* sp1 had a negative relationship with salinity and inorganic nutrients, and a weak positive relationship with temperature. The abundance of *Chaetoceros* sp2 was negatively and significantly correlated with temperature, salinity, P, NO_3 and NH_4, and had a significantly positive relationship with NO_2 only. *Skeletonema costatum* had a significantly negative relationship with temperature, weak negative relationships with salinity, P and NH_4, and significantly positive relationships with NO_2 and mesozooplankton. *Thalassionema nitzschioides* had weak positive relationships

Table 2. Pearson's correlation coefficients between organisms and environmental factors. Significant correlations were defined as *P<0.05, **P<0.01, ***P<0.001.

	Temp.	Sal.	Si	P	NO_3	NO_2	NH_4	Microzoo-plankton	Mesozoo-plankton
Si (μM)	-0.382*	-0.049							
P (μM)	-0.258	0.493***	0.533***						
NO_3 (μM)	-0.452**	-0.304*	0.884***	0.313*					
NO_2 (μM)	-0.793***	-0.144	0.614***	0.214	0.695***				
NH_4 (μM)	-0.068	-0.037	0.457**	0.411**	0.550***	0.171			
Chl-a (μg/l)	-0.323*	0.218	-0.070	0.016	-0.15	0.211	-0.297*		
Microzooplankton	0.024	-0.151	0.042	0.023	0.085	-0.040	0.238		
Mesozooplankton	-0.291	-0.107	-0.115	-0.056	-0.045	0.201	-0.170	0.121	
Phytoplankton	-0.450**	-0.304*	-0.026	-0.284	0.101	0.467***	-0.207	0.228	0.327*
Chaetoceros sp1	0.274	-0.193	-0.357*	-0.408**	-0.273	-0.290	-0.289	0.054	-0.149
Chaetoceros sp2	-0.321*	-0.366*	-0.089	-0.325*	0.043	0.322*	-0.229	0.198	0.310*
Skeletonema costatum	-0.549***	-0.222	0.104	-0.155	0.191	0.584***	-0.113	0.168	0.369*
Thalassionema nitzschioides	-0.278	0.230	0.032	0.217	-0.119	0.025	-0.255	0.285	0.158

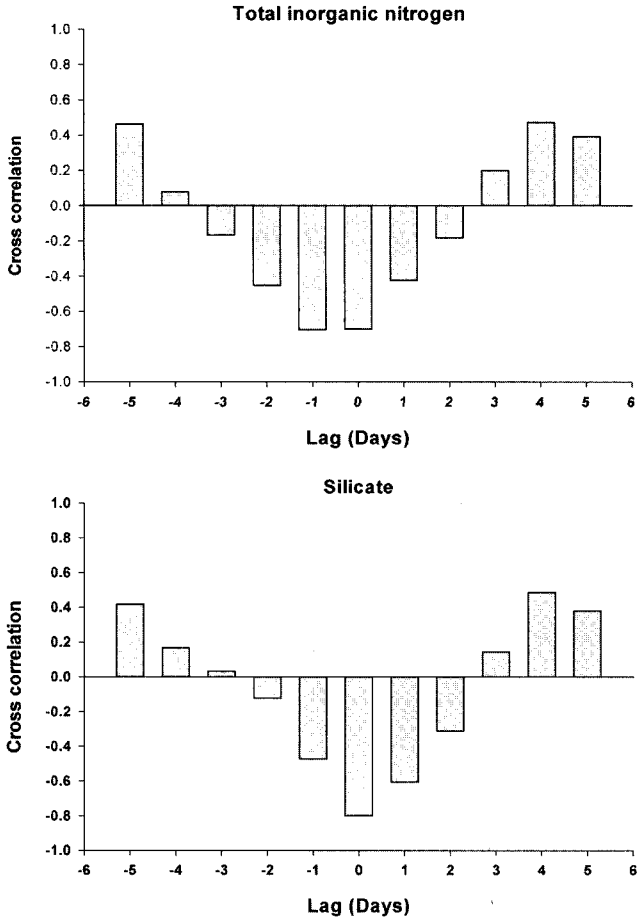

Fig. 8. Cross-correlation of the time series of the chl-a concentration and nutrients (silicate and TIN).

with Si and microzooplankton. Although no direct measurement of grazing on phytoplankton was performed in this study, there might be grazing impacts on phytoplankton by mesozooplankton because phytoplankton had a positive relationship with mesozooplankton and mesozooplankton appeared to follow a step behind phytoplankton.

In order to verify the relationship between phytoplankton and inorganic nutrients, we did a cross correlation analysis using the data from the zero rainfall period of July 29-August 6, as our understanding of the relationship can be distorted by frequent rainfall. The time series of chl-*a* concentration had a significant inverse correlation with the time series of silicate concentration and TIN concentration at a lag of 0 (day) and lags of 0 and -1 (day), respectively (Figs. 8). That is, if phytoplankton abundance starts to increase, then silicate and TIN concentrations decrease because phytoplankton uses up instantly available nutrients to achieve growth. This suggests a high possibility of silicate and TIN limitations for phytoplankton (specially diatoms) growth in coastal areas.

Molar ratios of N/P and Si/N

It is believed that diatoms require an N/P ratio of 16:1 and a Si/N ratio of 1:1 for growth (*e.g.*, Harris 1986). During the study period, the ratios of N/P and Si/N varied dramatically from 4.23 to 78.5 and from 0.16 to 3.69, respectively (Fig. 9). Generally, the N/P ratios were higher than 16:1 until August 12, and after this the ratios were lower, while the Si/N ratios were in reverse. During the entire sampling period, the frequency of the lower N/P ratio than 16:1 was 20, and among the 20, 15 cases appeared before August 13. The frequency of Si/N ratio lower than 1:1 was 29, and 25 cases appeared before August 13. On the basis of these results, the sampling period could be broadly divided into two: an inorganic silicate limitation period (ISLP, 14 July-12 August) and an inorganic nitrogen limitation period (INLP, 13 August - the end of the study), suggesting that phosphate might not limit the growth of phytoplankton assemblages in the bay during the study period (Fig. 9). Twelve high concentrations (>3×10^6 cells/*l*) and eight low concentrations (<3×10^5 cells/*l*) of the phytoplankton abundance were encountered during the ISLP and the INLP, respectively. The initially high phytoplankton abundance was dominated by diatoms and maintained for 2 weeks. During this period, diatoms might have depleted inorganic nitrogen and silicate. This might lead to nitrogen limitation and to all phytoplankton and silicate limitations due to diatoms in the surface layer. The replacement of diatoms by dinoflagellates appeared to start when N/P ratio decreased

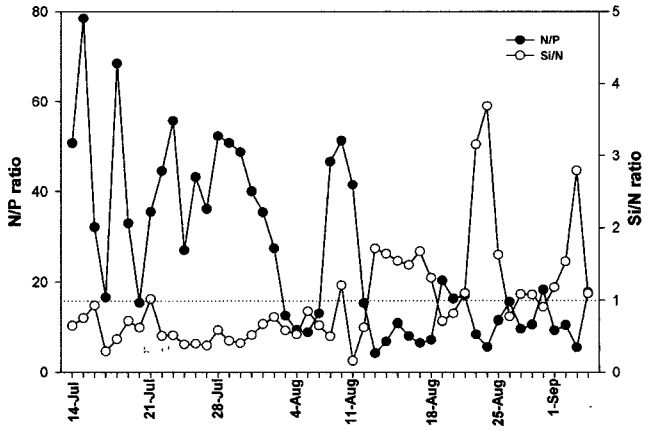

Fig. 9. Molar ratios of N/P and Si/N in Jangmok Bay.

to <5.0 and then dinoflagellates dominated during the greater part of the INLP (Figs. 2 and 7). According to previous studies (Anderson and Stolzenbach 1985; Olli 1999; Park *et al.* 2001), dinoflagellates migrate vertically and may take up inorganic nutrients in the bottom layer. Thus, dinoflagellates may dominate under an environment such as that of N-limitation. These previous studies may support our result that dinoflagellates were dominant during the INLP. During the INLP, silicate concentration was enough to sustain phytoplankton growth (high Si/N ratio). It might lead to a high abundance of phytoplankton (mainly diatoms) at the end of the study. Our results support the view that ratios and concentrations of inorganic nutrients are particularly important in controlling phytoplankton abundance and composition, and the nutrients may determine dominant species (Ryther and Dunstan 1971; Hecky and Kilham 1988; Howarth *et al.* 1988; Dederen 1992; Del Amo *et al.* 1997; Diaz *et al.* 2001).

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