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Composition and Distribution of Phytoplankton with Size Fraction Results at Southwestern East/Japan Sea

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Abstract – Abundance and distribution of phytoplankton in seawater at southwestern East/Japan Sea near Gampo were investigated by HPLC analysis of photosynthetic pigments during summer of 1999. Detected photosynthetic pigments were chlorophyll *a*, *b*, c_{1+2} (Chl *a*, Chl *b*, Chl c_{1+2}), fucoxanthin (Fuco), prasinoxanthin (Pras), zeaxanthin (Zea), 19'-butanoyloxyfucoxanthin (But-fuco) and beta-carotene (ß-Car). Major carotenoid was fucoxanthin (bacillariophyte) and minor carotenoids were Pras (prasinophyte), Zea (cyanophyte) and But-fuco (chrysophyte). Chl *a* concentrations were in the range of 0.16-8.3 μg/land subsurface chlorophyll maxima were observed at 0-10m at inshore and 30-50 m at offshore. Thermocline and nutricline tilted to the offshore direction showed a mild upwelling condition. Results from size-fraction showed that contribution from nano+picoplankton at Chl *a* maximum layer was increased from 18% at inshore to 69% at offshore on average. The maximum contribution from nano+picoplankton was found as 87% at St. E4. It was noteworthy that contribution from nano+picoplanktonic crysophytes and green algae to total biomass of phytoplankton was significant at offshore. Satellite images of sea surface temperature indicated that an extensive area of the East/Japan Sea showed lower temperature (<18 °C) but the enhanced Chl *a* patch was confined to a narrow coastal region in summer, 1999. Exceptionally high flux of low saline water from the Korea/Tsushima Strait seemed to make upwelling weak in summer of 1999 in the study area. Results of comparisons among Chl *a* from SeaWiFS, HPLC and fluorometric analysis showed that presence of Chl *b* cause underestimation of Chl *a* about 30% by fluorometric analysis but overestimation by satellite data about 30-75% compared to HPLC data.

Key words – Chl *a*, Chl *b*, But-fuco, photosynthetic pigments, upwelling, HPLC, picoplankton, size fraction, East/Japan Sea

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1. Introduction

Until recently, studies on the East/Japan Sea were rarely focused on its biological aspects (such as primary production, composition of phytoplankton community, and temporal/spatial variations in biomass of phytoplankton) partly because it was regarded as oligotrophic. Relatively few biological studies has been reported, compared to physical oceanographic investigations including international collaboration work on the East/Japan Sea. Among the biological studies, phytoplankton related studies are scarce and understanding of interaction between phytoplankton population and its physico-chemical environmental factors is poor. However, there are a few studies reported recently on phytoplankton researches in the East/Japan Sea. Shim *et al.* (1989) reported the distribution and composition of phytoplankton based on microscopic observations in southwestern East/Japan Sea. Chung and Kang (1996) isolated and identified the prasinophyte, *Pycnococcus provasoli* with HPLC pigment data from the Korea/Tsushima Strait. Park and Park (1997) and Park *et al.* (1999) also reported the results of HPLC analysis from the Korea/Tsushima Strait and high ratios of Chl *b*/Chl *a*. More recently, the flow cytometric cell countings of *Synechococcus* and *Prochlorococcus* was reported by Kang *et al.* (2004) at Ulleung Basin of the East/Japan sea.

In this paper, the well-known upwelling phenomena at the southwestern part of the East/Japan Sea during summer period are investigated from a biological aspect. The upwelling events transport nutrient-rich water from bottom

layer to euphotic layer and support phytoplankton blooms at coastal region off Gampo and near the Subpolar Front (38N). The increased flux of Tsushima Current (TC) during the summer season, in turn, transports this surface water to the north along the coast and eventually leaves at around the Subpolar Front to an offshore direction. Due to fast advection of the TC, these annual regional phytoplankton blooms do not last long. However, the dissolved organic carbon resulting from the blooms are transported by TC to the oligotrophic water in Ulleung Basin of the East/Japan Sea. Although the impact of the phytoplankton blooms in southwestern East/Japan Sea on the ecological aspects in East/Japan Sea is not known, this pulse type input of organic carbon from the annual phytoplankton bloom must act as an important source for the primary production in oligotrtophic water in East/Japan Sea. Thus, it is important to understand abundance and composition of phytoplankton community and upwelling impact on its changes in this area for extended periods.

Andersen *et al.* (1996) identified the eukaryotic cells from oligotrophic water by SEM and compared them with the results from HPLC analysis based on the classification of species by marker pigments. According to his comparison, observation by SEM and pigment composition data showed significant similarities on class level.

After HPLC analysis was proven to be useful for providing an accurate estimation of Chl *a* and qualitative information on community composition of phytoplankton, HPLC analysis of photosynthetic pigments was used widely for many oceanographic researches in the past 30 years (Jeffrey *et al.* 1997). Especially, the importance of picoplankton was studied using HPLC along with flow cytometric method, because HPLC analysis could provide additional information on contribution from class levels to total biomass and kinds of picoplankton (Goericke and Repeta 1992). HPLC analysis of photosynthetic pigments was used to measure chlorophylls, degradation products and tens of carotenoids, in order to estimate biomass, community composition and physiological state (Jeffrey *et al.* 1997; Park *et al.* 1999; Furuya *et al.* 2003).

In this paper, therefore, in order to estimate accurate Chl *a* of phytoplankton and to find the effects of upwelling on composition and distribution of phytoplankton near Gampo in the Southwestern East/Japan Sea in summer 1999, the photosynthetic pigments of phytoplankton were analyzed by HPLC and compared with fluorometric data. In addition, monthly composite of satellite image for surface seawater temperature (SST) and Chl *a* was compared with *in situ* measurement of Chl *a* by HPLC analysis. Size fractionation of phytoplankton was also carried out to find the importance of nano+picoplankton for total biomass of phytoplankton in the study area.

2. Materials and Methods

Sampling and CTD measurement

From July 19 to 21, 1999, CTD measurements and collection of water samples were performed by R/V Tamyang, for transects B, C, D, and E (Fig. 1) at Southwestern East/Japan Sea. Seawater samples were collected by Rosette sampler at 10 standard depths (0, 10, 20, 30, 50, 75, 100, 125, 150, 200 m) for nutrients and HPLC analysis. Temperature and salinity data were obtained by CTD (SBE 911) measurement at all stations. Nutrients and DO data was obtained from Kim *et al.* (2002).

HPLC analysis

1 *l* of sea water collected from Rossette sampler was filtered on GF/F (47 mm) for total biomass of phytoplankton. Nano+picoplankton fraction was collected by filtering out

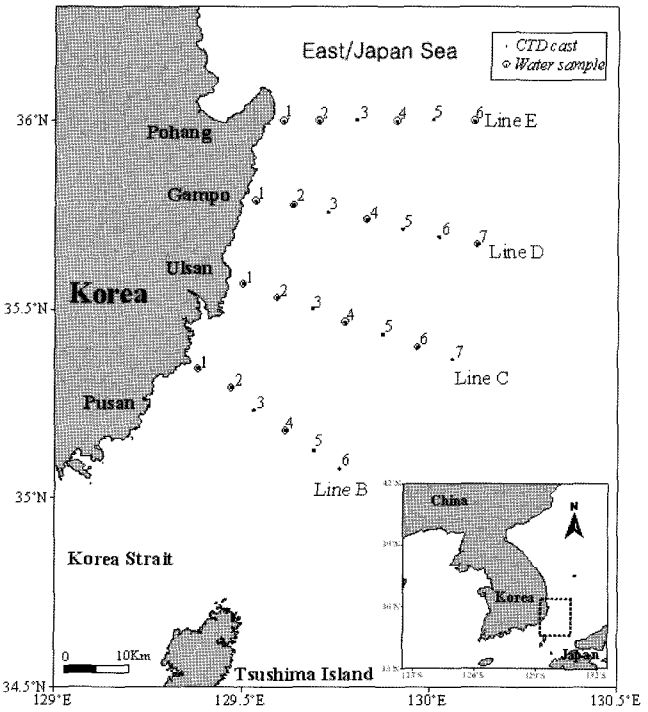

Fig. 1. Study area showing the sampling stations. (1999. 7.19 - 21).

Table 1. List of pigment standards and their abbreviations.

Standard Pigments	SCOR abbreviations	Class of phytoplankton
Chlorophyll *a*	Chl *a*	-
Chlorophyll *b*	Chl *b*	-
Chlorophyll c_1+c_2	Chl *c*1+*c*2	-
Divinyl Chlorophyll *a*	DV-Chl *a*	-
Divinyl Chlorophyll *b*	DV-Chl *b*	-
Alloxanthin	Allo	Cryptophyte
19'-Butanoyloxyfucoxanthin	But-fuco	Crysophyte
19'-Hexanoyloxyfucoxanthin	Hex-fuco	Prymnesiophyte
Fucoxanthin	Fuco	Bacillariophyte
Peridinin	Perid	Dinophyte
Prasinoxanthin	Pras	Prasinophyte
Viloaxanthin	Viola	-
Zeaxanthin	Zea	Cyanophyte

plankton larger than 20 μm and anlaysed by HPLC for size fractionation. The filters were wrapped in aluminum foil and kept in liquid nitrogen until HPLC analyses. The analysis procedures and calibration method were reported in Park and Park (1997). The name of pigment standards and related class names of phytoplankton groups are listed in Table 1. And abbreviations determined by SCOR (Scientific Committee on Oceanic research) working group 78 (Jeffrey *et al.* 1997) were used in this paper.

The concentrations of standard pigments were calibrated by spectrophotometer with the known specific extinction coefficients from Jeffrey *et al.* (1997). The absorbances were measured at both wavelengths at maximum absorbance and minimum absorbance (750 nm). The concentrations of standard pigments were calculated according to Eq. 1 (Park *et al.* 1999).

$$\text{pigment conc. } (\mu g/l) = \frac{\text{absorption}}{(E; 1\ g^{-1}cm^{-1}) \times (cm)} \times \frac{10^6 \mu g}{g}$$

(E: extinction coefficient) (1)

Standard response factors (Rf) for the pigments were obtained from the ratio between the peak area of chromatogram of HPLC (Oromtech, Korea) and the concentrations of corresponding standard pigments used in calibration. The equation for quantification of pigments in samples follows:

$$C = \text{Area} \times Rf \times (Ve / Vs)$$

C= concentration [μg/l]

Area = area of the peak [area]

Rf = standard response factor [μg l^{-1} $area^{-1}$]

$$Ve \times \frac{AIS}{(\text{peak area of IS added to sample})}$$

(volume of IS added to sample)[*l*]

AIS : peak area of the internal standard (IS) when 1 m*l* IS is mixed with 300 μ*l* of H_2O

Vs = volume of filtered water sample [*l*]

3. Results

Hydrography

Vertical profiles of salinity and temperature from CTD measurement for transects B, C, D, and E at the southwestern East/Japan Sea are shown in Fig. 2. Three different water masses were identified in the study area: Tsushima Surface Water (TSW), Tsushima Middle Water (TMW), and North Korean Cold Water (NKCW) (Table 2). Temperature and salinity of the surface water was 22.0-23.4 °C and 32.7- 33.6 psu.

The horizontal distribution of salinity showed the salinity gradients parallel to coast line and the salinity of coastal seawater showed lower salinity (< 33.1 psu) with lower SST (22.0-23 °C) compared to offshore seawater (33.1-33.6 psu and about 23.3 °C) except B transect. Boundary of these two different water masses followed the contour of the shelf break. And the regions with lower temperature and salinity near the coast tells the boundary of East Korean Warm Current (EKWC) flowing from south to northeastern direction. The vertical distribution of salinity profiles showed that TSW (<33.8 psu) is extended above depth of 20-50 m and TMW (>34.2 psu) at depth of 50-100 m.

The bottom cold water (NKCW) flowing from north to south encountered the shallow sill at transect B and approached close to the coast. As a result, the two layer of TSW and TMW overlying on the NKCW seems tilted toward an offshore direction at transect B. Typcial TMW appeared at depth 30 m at St. B1 but 75 m at St. B4. Compared to St. C1-E1 (32.66-32.86 psu and 22.6-23 °C), high saline and cold water (33.10 psu and 22 °C) at the surface of St. B1 was observed. It showed the influence of cold water (TMW and/or TMW+NKCW) from the bottom layer to the surface layer. High DO (>5.0 mg/*l*) at

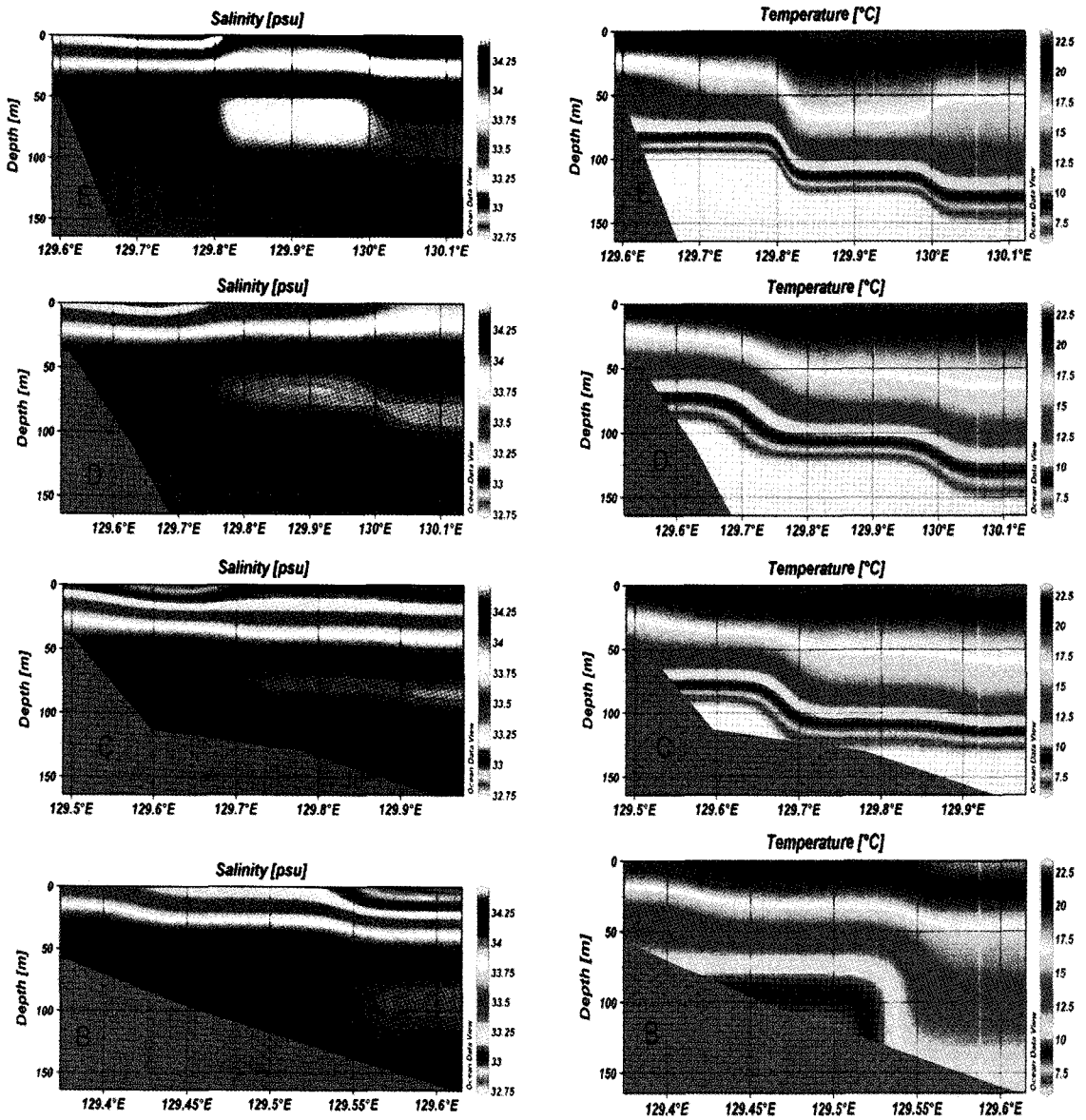

Fig. 2. CTD profiles of temperature and salinity for transects B-E.

Table 2. The characteristic temperature, salinity and DO for TSW, TMW and NKCW.

Water mass	Temperature (°C)	Salinity (psu)	DO (O_2 ml/L)	Reference
TSW (Tsushima Surface Water)	>20	<33.80	5.00~5.50	Yang *et al.*(1991)
TMW (Tsushima Middle Water)	10~17	34.20~34.43	3.66~5.00	Yang *et al.*(1991)
NKCW (North Korea Cold Water)	1~7	34.06~34.20	6.00~6.45	Yang *et. al.*(1991)

St. B1 showed enhanced primary production at this surface layer.

However, TMW appeared at 30m at both St. E1 and St. E6, which means well stratified structure. The TSW+TMW flowing from south to north were laid on top of the NKCW (120 m) and the depth of TMW decreased from 75 m to 30 m while TC passed B transect to E transect. The high nutrients from TMW pumped into well-lit surface layer (50 m) and mixed with warm TSW at an area offshore from transects D-E. The high DO (4.2-5.0 m*l*/*l*) at St. D7 and St. E6 with high concentration of Chl *a* showed the enhanced growth of phytoplankton in

this offshore subsurface region.

Annually, the observation of SST at this upwelling area showed high saline and cold water (<20 °C) at the coast off the Gampo area. But in summer of 1999, the distribution of salinity and temperature showed a different pattern compared to the upwelling condition observed in 2001 (Kim. 2002). The distribution of low saline water at the surface of coast in July 1999 seems to have originated from EKWC as branch of TC. In 1999, a huge flux of freshwater from a flood at the Changjiang River was introduced to EKWC through the Korea/Tsushima Strait (Choi *et al.* 2004). The variation in flux of TC through the Korea/Tsushima Strait was considered a cyclic phenomenon. Choi *et al.* (2004) suggested that it has an oscillation of 7 or 8 year cycle for change in volume of flux through the Korea/Tsushima Strait.

Nutrients

The vertical distribution of nutrients, especially nitrate + nitrite profiles, showed higher concentration at the surface than its concentrations below. The concentrations were 0.70-2.57 μg-at/*l* at the shallow surface layer and less than 0.7 μg-at/*l* below the surface. The vertical profile of nutrients (nitrate + nitrite) doesn't follow the typical pattern (low limit concentrations at the surface and increase slowly to depth) nor the upwelling pattern (high concentrations at the surface layer). The higher concentrations at the surface were decreased at the level beneath 10-20 m and increased again. So this inversion of nutrients distribution at vertical profile is unusual in 1999. The source of this enhanced nutrients at the surface seems to originate from the large flux of freshwater in July 1999. As Choi *et al.* (2004) reported, maximum flux through Korea Strait to East/Japan Sea was observed and this freshwater affects the salinity and nutrients, especially at the surface. So it resulted in less salinity and slightly increased nutrients at the coastal surface layer compared to the surrounding water.

Photosynthetic pigments

The measured photosynthetic pigments were Chl *a*, *b*, c_{1+2}, Fuco, Pras, But-fuco, Zea, and ß-car. The range of concentrations of Chl *a* was 0.16-8.3 μg/*l* and the maximum concentration was measured at St. D1 (10 m). The highest concentrations of Chl *a* were found along the coast. Chl *a* maximum layer was found at 10 m near the coast and got deeper to 30-50 m in the offshore direction. The concentration of Chl *a* at the surface layer sharply decreased to less than 10% below this maximum layer. The distribution pattern of Chl *a* at the surface layer was quite different from the pattern at 10 m depth (Fig. 3, 4). Although the area of high concentrations of Chl *a* was very narrow near the coast, concentrations of Chl *a* were high compared to the concentrations observed in non-upwelling summer (<2.0 μg-at/*l*) in the study area.

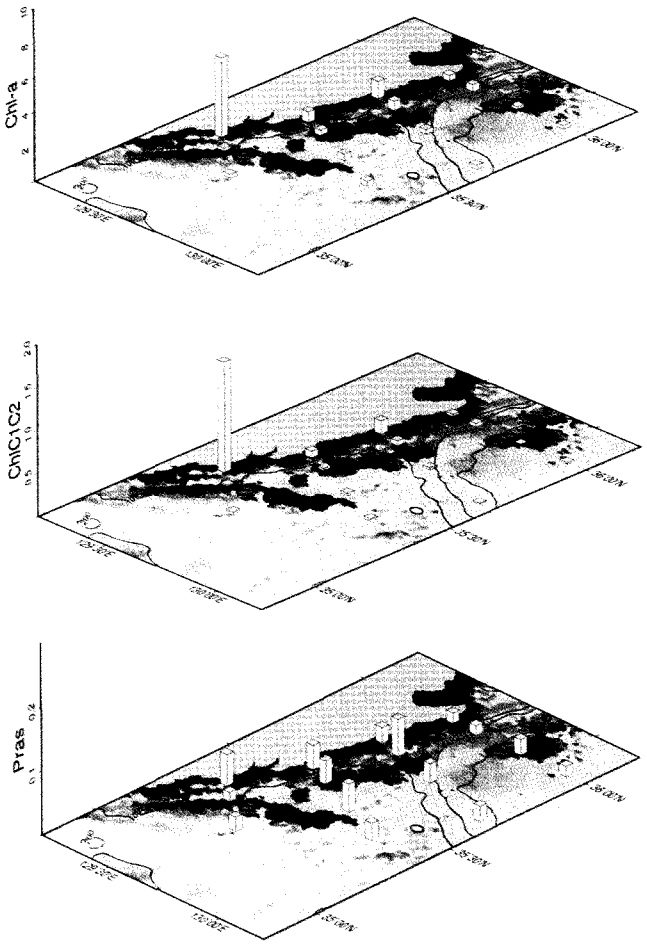

Fig. 3. Horizontal distribution of Chl *a*, Chl c_{1+2} and prasinoxanthin at the surface layer.

Fuco, Chl *b*, Chl c_{1+2}, Pras, But-fuco, Zea were detected at all stations. Fuco was in the range of nd-1.98 μg/*l* and showed the highest concentrations among the carotenoids. Fuco showed the same distribution pattern with total Chl *a* and concentrated at mixed layer depth (10-50 m). Distribution of Chl *b* was relatively homogeneous and showed maximum concentration at depth 10 m and 50 m. Chl *b* was detected at almost all depths except 200 m.

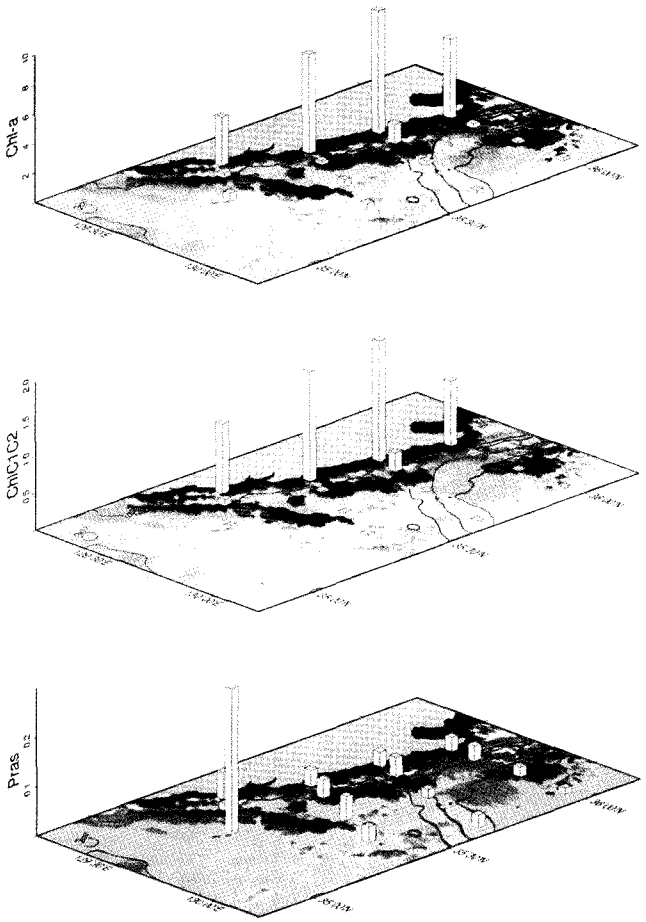

Fig. 4. Horizontal distribution of Chl *a*, Chl c_{1+2} and prasinoxanthin at 10 m depth.

The average concentration of Chl *b* was about 0.2 μg/*l* in most of the study area. However, concentrations of Chl *b* (nd-1.0 μg/*l*) were comparable or higher than Fuco at offshore stations. To the offshore direction and north, Chl *b* was increased up to two times higher than in coastal regions. At St. D4 (10 m), the highest concentration of 1.0 μg/lof Chl *b* was detected. Between the coast and offshore waters, enhanced concentrations of Chl *b* were observed in the middle of the transects. And most of Chl *b* was associated with nano+picoplankton fraction. Below 50 m depth, Chl *b* was detected, but no Pras and violaxanthin were detected. Pras was concentrated at the surface layer within depth 30 m and negligible below 75 m. Pras was detected at depth 0-20 m at coast and 0-75 m at offshore. In Fig. 6, ratio between Chl *b/a* and Pras/Fuco are plotted. In coastal regions, Chl *b/a* was low (< 0.2), but the ratios were increased up to 0.5 in offshore regions. And at few stations, quite high concentration (>0.5 μg/*l*) of Chl *b* was

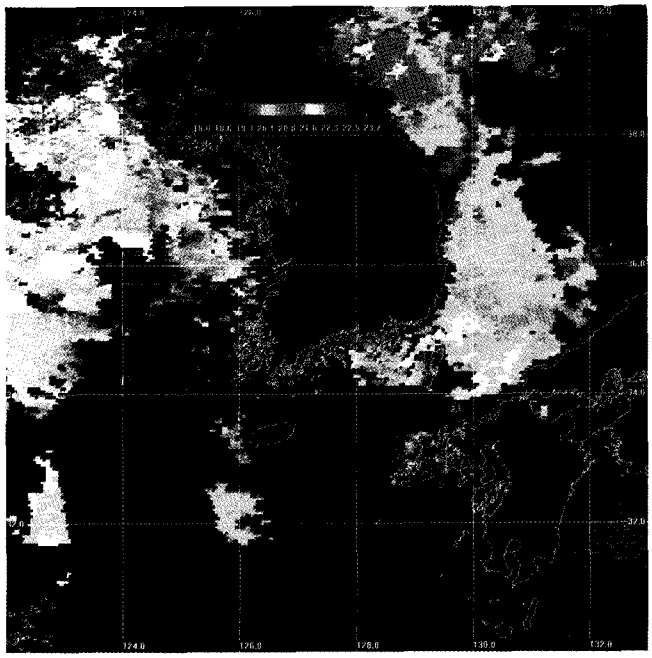

Fig. 5. Satellite image of SST from NOAA (1999.6.24-7.1).

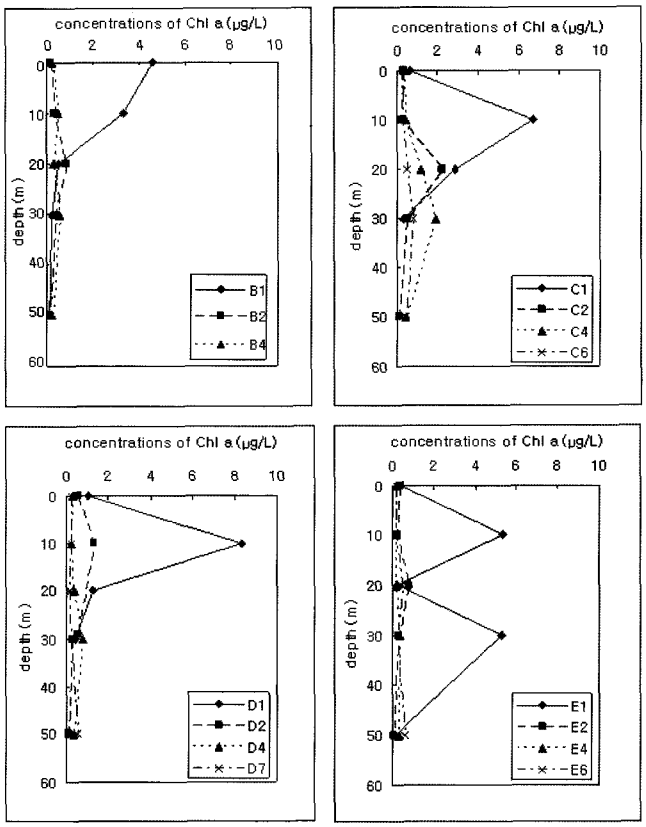

Fig. 6. Ratios of Chl *a*/ Chl *b* and Prasinoxanthin / Fucoxanthin at Chl *a* maximum layer.

measured at offshore and depth 50-125 m.

But-fuco was measured in the range of nd-0.9 μg/*l*, and

mostly about 0.02 μg/*l*. Distribution of But-fuco was relatively homogeneous and showed its maximum at 20-30 m depth. Depth for maximum concentrations of But-fuco was deep, up to 50 m offshore. Although the concentration of But-fuco decreased sharply below the subsurface Chl *a* maximum layer, it was still detected at low concentration levels up to 100 m.

Zea was concentrated at the surface layer (<30 m) and its concentration was in range of nd-0.05 μg/*l*. Like Pras, Zea was under detection limit below 50 m. From coast to offshore, concentrations of Zea increased by 2-4 times. Horizontally high concentrations were found in middle of the transects, like the distribution of Chl *b*. The composition of photosynthetic pigments in the study area was relatively simple compared to the Yellow sea, South sea or Korea Strait (Song 1999). Peridinin (Perd), 19'-hexanoyloxyfucoxanthin (Hex-fuco) and alloxanthin (Allo) were not detected. However it is noteworthy that Chl *b* and Pras were significant in composition of total Chl *a* at offshore.

Horizontal distribution of phytoplankton

The distribution pattern of dissolved oxygen (DO) and Chl *a* showed two different regions of high population of phytoplankton with high DO. The first patch was found near the coast, centered St. B1 at 0-10 m depth, with highest DO of 6.21 m*l*/*l*. This patch was spread north to St. C1-St. E1 along the coast. The second patch was observed offshore, at St. D7 with DO 5.0 m*l*/*l* and St. E6 with DO of 4.21 m*l*/*l* at 30-50 m depth. The first patch was composed mostly of diatoms and silicoflagellates (Fuco+But-fuco), but the second patch was composed of nano+picoplanktonic green algae (Chl *b* and Pras), crysophytes (But-fuco) and blue-green algae. Nutrients, N/P ratios were also very different: N/P ratio was 10 at the coastal patch and 3-4 at the offshore patch (Nitrogen deficient conditions).

Based on the concentrations of the photosynthetic pigments, bacillariophyte (diatom) was the major phytoplankton group (Chl c_{1+2} and Fuco), and minor groups were chlorophyte (Chl *b*), prasinophyte (Pras.), crysophyte (But-fuco) and cyanophyte (Zea). Chlorophyte and prasinophyte were detected in most areas and were important groups for the total biomass of phytoplankton next to bacillariophyte. Although diatoms were the most abundant in coastal regions, the populations decreased less than one tenth offshore. Chlorophyte (Chl *b*) and prasinophytes (Chl *b* and Pras) were abundant offshore (Fig. 6) compared to the coast, but prasinophytes were aberrantly high at the middle of transect (St. D4). Prasinophytes were distributed 0-20 m at coast and distributed abundantly 0-70 m offshore. Most of the cyanophyte (Zea) populated the shallow depths in the surface layer (0-20 m). The distribution pattern of cyanophyte was relatively low at the coast and high in the middle of the transects and offshore. In offshore regions with low concentration of nitrate+nitrite (< 1 μg/*l*), cyanophyte (Zea) increased its populations up to 5 times compared to the coast, at offshore stations. Meanwhile, populations of crysophytes (But-fuco) were low or not detected at the surface (0-10 m) layer, but distributed relatively evenly and concentrated at 30-50 m. By size fraction data, it was revealed that silicoflagellates were dominant but coexist with nano- and picoplanktonic cyrsophytes at the coastal region. However, most crysophytes were nano+picoplankton without silicoflagellate in the offshore region. The marine nano- and picoplanktonic crysophytes with But-fuco are Parmales and *Pelagococcus*, respectively.

Vertical distribution of phytoplankton

The vertical distribution pattern of Chl *a* at transects B-E in July 1999 is shown in Fig. 7. Only at St. B1, inverted distribution of Chl *a* was observed. Corresponding higher dissolved oxygen (5.45 mg/*l*) concentration was observed. However, the maximum concentration of Chl *a* at each transect was found at 10 m depth along the coast. At St. E1 two maximum Chl *a* were observed at 10 m and 30 m. According to pigment data with size fraction, the first maximum at 10 m was from diatoms and second maximum at 30 m was from silicoflagellate. The depth of Chl *a* maximum increased from 10 m at the coast to 30 m offshore.

The distribution patterns of Chl *a* and carotenoids at the surface and 10 m were very different (Fig. 3, 4). Except St. B1, which showed high abundance of phytoplankton at the surface, phytoplankton was most abundant at 10 m depth along the coast. The abundance of phytoplankton offshore was getting deeper to 30 m and 50 m. The abundance of phytoplankton was controlled by diatom and silicoflagellates at shallow mixed layer of coastal region, but controlled by green algae and prasinophytes below mixed layer with dim light conditions However, in offshore regions,

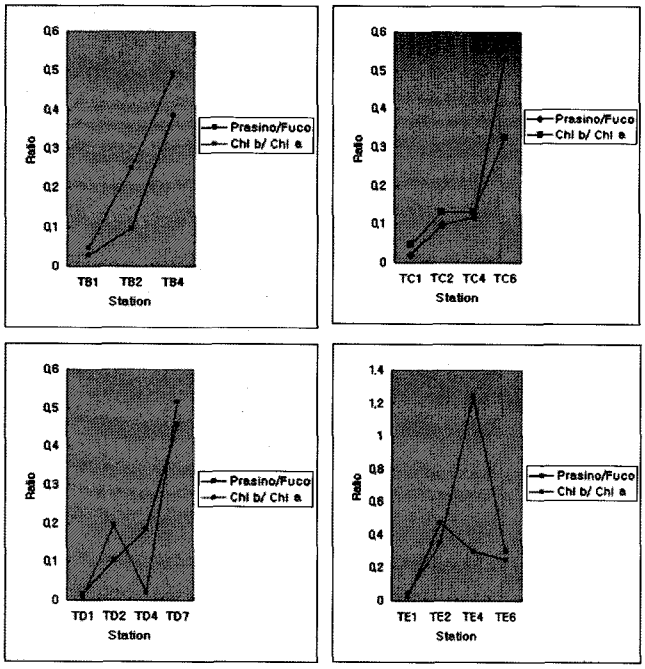

Fig. 7. Vertical distribution of Chl *a* at 4 transects (B-E line).

phytoplankton was mainly composed by nano+picoplankton. Offshore, two layered distribution was observed: top surface layer (0-30 m) was occupied by cyanophyte and lower layer (20-50 m) by prasinophytes and nano+picoplanktonic crysophyte (pelagophytes).

Chlorophytes were distributed homogeneously at all depths and stations. High concentrations of Chl *b* were observed at depths of 10 m and 50 m. The Chl *b* at 50 m originated mostly from nano+picoplankton fraction. Prasinophyte was abundant at top 30 m depth and very rare below 75 m.

Cyanophyte (Zea) was ubiquitous but concentrated at shallow depth (0-30 m). It was very rare below 30 m. Crysophytes was composed of net plankton (silicoflagellate) and nano+picoplankton (pelagophytes). Silicoflagellate was dominant at surface layer above 20 m near coast and pelagophytes was dominant at 30-50 m offshore.

At most stations, especially below 50 m depth, Chl *a* was decreased to the detection limit. The euphotic depths in this area was about 50 m or less in June 1999 (personal communication). The Chl *a* subsurface maximum was observed below the surface, from 10 m to 50 m at most. In the study area, southwesterly wind is predominant periodically and induces the upwelling annually in summer (Fig. 5). Upwelling phenomena bring deep water, either NKCW or mixed water of TMW and NKCW with high nutrients to the surface euphotic layer. This subsurface Chl *a* maximum layer was found at the top of the shallow thermocline and seems supported by the nutrients input from the bottom.

Size fractionation

The percentages of net- and nano+picoplankton for the total integrated biomass were obtained to show the size distribution pattern of phytoplankton in the study area (Fig. 8). The highest fraction of net plankton was observed at St. C2 (78%) and the highest fraction of nano+picoplankton was 87% at St. E4. And the depths for maximum Chl *a* and nano+picoplankton were identical (10 m) except St. B1,

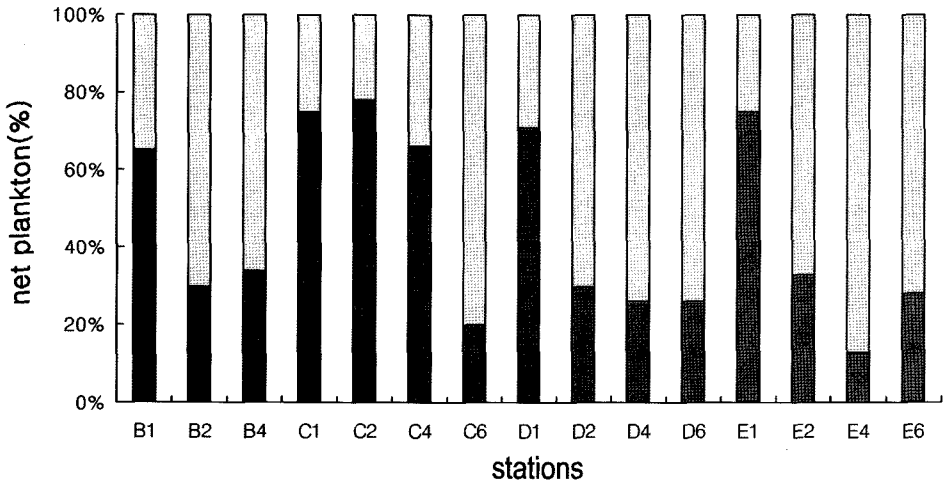

Fig. 8. The average percentage of net plankton to total integrated Chl *a* at each stations.

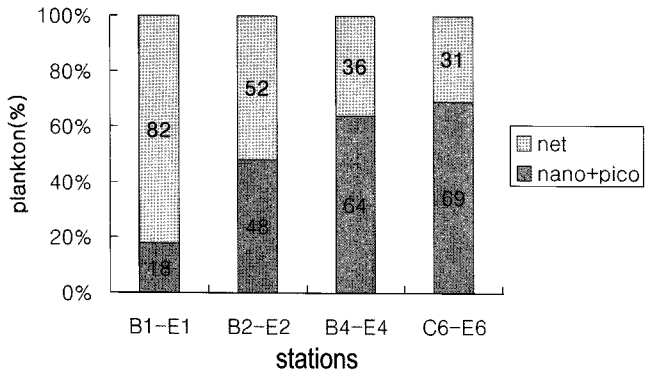

Fig. 9. Size fraction result of phytoplankton at Chl *a* maximum layer.

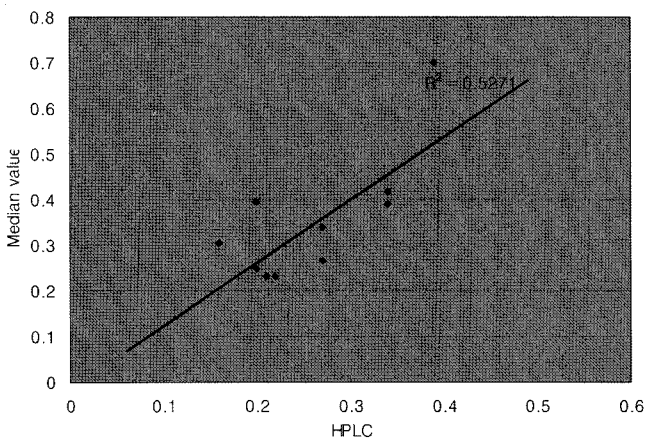

Fig. 10. Comparison between Chl *a* from SeaWiFs and HPLC analysis.

St. E1 and St. E4, where their nano+picoplankton maximum appeared at depth 10-30 m.

In Fig. 9, the percentage of net plankton at Chl *a* maximum layer was 82%, 52%, 36%, 31% in average for B-E transects from near coast to the offshore direction. The nano+picoplankton fraction was lowest at Chl *a* maximum layer. The lowest fraction by nano+picoplankton was 18% for average St. B1-E1(coast) and increased to the offshore direction up to 69% for average St. C6-E6 at the Chl *a* maximum layer. On the other hand, at the surface layer, the percentage of net plankton was 60%, 34%, 11%, 3% (Table 4). This shows net plankton is the dominant group at the coastal region and Chl *a* maximum layer, but nano+picoplankton fraction became dominant at the surface and oligotrophic water at offshore stations. Thus the contribution of nano+picoplankton was minimum (18%) at the Chl *a* maximum layer and coastal region and maximum (97%) at the surface of offshore regions.

Table 3. The percentage of net plankton for integrated Chl *a* at each station.

	1	2	4	6
B	65	30	34	
C	75	78	66	20
D	71	30	26	26
E	75	33	13	28

Table 4. Percentage of net plankton and nano+picoplankton at the Chl *a* maximum layer.

	B1-E1	B2-E2	B4-E4	C6-E6
nano + pico	18	48	64	69
net	82	52	36	31

Match up Chl *a* from HPLC wtih satellite image by Sea WiFS

Fig. 10 shows the comparison between concentrations Chl *a* from HPLC and from SeaWiFS. Clouds and fog above the sea surface made it difficult to get enough Chl *a* data for comparison. SeaWiFs data point was collected from 48 hrs composite and used median values. However, the concentration of Chl *a* from SeaWiFs was higher by about 30-75% compared to the concentrations by HPLC analysis. Absorbance of the colored dissolved organic matter in sea water seems to be the cause for discrepancy between Chl *a* concentrations from HPLC and satellite data (personal communication with G. Mitchell).

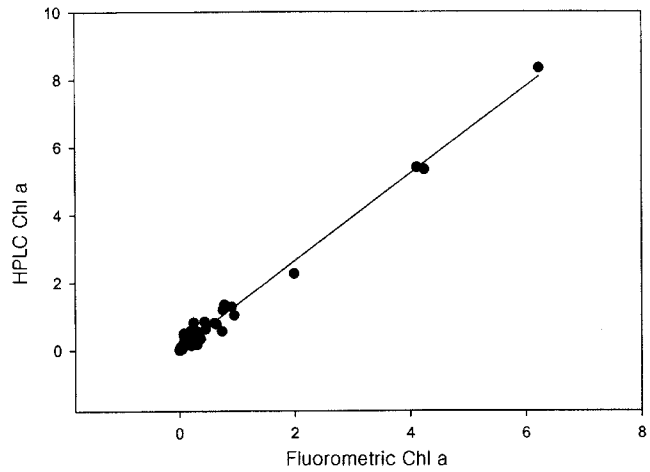

Fig. 11. Comparison between Chl *a* from fluorometric method and HPLC analysis.

Table 5. Percentage of net plankton and nano+picoplankton at the surface layer.

	B1-E1	B2-E2	B4-E4	C6-E6
nano + pico	40	66	89	97
net	60	34	11	3

Comparison of Chl *a* with fluorometric measurement with HPLC

Comparison of Chl *a* from HPLC analysis with fluorometric measurement was made (Fig. 11). The Chl *a* from HPLC analysis and fluorometric measurement showed a linear correlation with a Chl *a* (HPLC)=0.0568+1.289 Chl *a* (Fluor.) with 5 outliers. However, the Chl *a* by fluorometric measurement were about 30% less than Chl *a* from HPLC.

4. Discussion

Concentrations of Chl *a* were very high in coastal regions compared to concentrations reported at the Korea/Tsushima Strait (Park and Park 1999). Rarely, the concentrations of Chl *a* were higher than 2 μg/*l* in the study area, but maximum concentrations of Chl *a* were 4-8 μg/*l* in this study. SST in July near the coastal region was high (>22 °C) and temperature difference between inshore and offshore was very small compared in June. Near 37°N, cold surface water, which spread wide to northeastern direction in June, was observed by satellite image. Wind data measured (Lee *et al.* 2006) in June also showed the southwestern wind lasted more than 4 days and satellite image also showed enhanced Chl *a* at the coastal region. During early summer in June, 1999, it seems that an upwelling event provided rich nutrients to the surface layer and enhanced growth of phytoplankton in the study area. 15-day composite satellite images (from June to August) clearly showed the initial enhancement of growth of phytoplankton in June and the later decrease in the study area.

Two different regions of high populations of phytoplankton were found. One was located at coastal surface layer centered at St. B1. The other was located offshore (St.D7-St.E6). Two different compositions of phytoplankton community was also recognized by pigment data and size fraction. At the coastal regions, net plankton, diatoms and silicoflagellate were abundant. At this region, inverted Chl *a* or shallow Chl *a* maximum at 10 m with highest concentrations was observed and reflected the mild upwelling condition. In offshore regions, mixed composition of nano+picoplankton including cyanophyte, chlorophyte and pelagophyte was confirmed and its Chl *a* concentrations were 10% of the Chl *a* of coastal maximum layer.

Compared to the Yellow Sea (Song 1999), the composition of phytoplankton in study area in East/Japan Sea was simple. For example, dinoflagellate (Perd), prymnesiophytes (Hex-fuco) and cryptophyte (Allo) were not detected. Either Perd was too low to be detected by HPLC or the dinoflagellate was heterotrophic, without Perd in July 1999. It seems the water turbulence in this area was not favorable for growth of dinoflagellate in summer of 1999. As reported in Smayda (2000), low turbulence favors relatively dinoflagellate compared to diatoms. Very shallow mixed layer (high nutrients, low temp and salinity) and high flux of freshwater through Korea/Tsushima Strait seemed to make water column turbulent at the study area. According to Choi *et al.* (2004) the SSH (Sea Surface Hight) increases gradually from Oct. 1992 to Feb. 2002 with slope of 0.58 cm/yr and is lowest in Feb. 1996 and highest in October, 1999. According to Takikawa *et al,* (2005), a large volume transport, exceeding 4 Sv was observed in Tsushima Strait in autumn 1999. And Asian Disaster Reduction Center (ADRC) reported that precipitation over East Asia increased and river floods occurred frequently in July 1999.

The high nutrients and turbulent conditions in the coastal region favored net plankton (diatoms) such as St. B1, St. D1 and St. E1. And at all depths and stations, Chl *b* was detected. It means that green algae present as a significant group contributing to the total biomass in the study area. Although the presence of prasinophyte was reported by Chung and Kang (1996), data about the presence and significance of green algae in East/Japan Sea was scarce. Park *et al* (1999) reported an importance of Chl *b* at bottom cold water in summer (August) compared to fall (October) in the Korea/Tsushima strait. The concentrations of Chl *b* were 0.04-0.12 μg/*l* in the Korea/Tsushima strait in 1996 and nd-1.2 μg/*l* in summer 1999 in the study area. However, the high concentration above 0.5 μg/*l* were measured only at few stations. Still, concentrations of Chl *b* in summer of 1999 are about 2-3 times higher than concentrations in 1996. The increase of Chl *b* containing algae in the study area might be a sign

of an ecological change in recent warming of the East/Japan Sea. Yoon (2006) summarized the 10 yr studies about the oceanic circulation of the East/Japan Sea: that the temperature of the deep layer increased at a rate of about 0.01 °C/30 years and the oxygen minimum layer become 1000 m deeper layer 1930's.

The concentration of Chl *b* below 50 m was decreased to less than 50% of the surface, but increased again up to 1.25 μg/*l* below 75 m. Pras was not detected at these depths. This implies that Chl *b*-containing algae would be green algae, adapted to these low temperature and light conditions and smaller than nano-size prasinophyte. The fact that Pras and Viola were not detected when significant concentrations of Chl *b* were detected suggests that the Chl *b* might be from *Prochlorococcus*. since *Prochlorococcus* has neither Viola nor Pras, but DV-Chl *b* of *Prochlorococcus* which could be measured as Chl *b* in our HPLC system. Since DV-Chl *b* coelutes with Chl *b* and the same absorption coefficient with Chl *b* is used for HPLC analysis.

The green algae and cyanophyte seem to thrive in the transition region (mixed water), which is a neither eutrophic nor oligotrophic state. In future, the origin of this small Chl *b* containing microscopic algae and relationship with tropic phytoplankton reported by Furuya *et al.*(2003) at East China Sea is worth investigating with HPLC pigment analysis and flow cytometric measurement. Although this Chl *b* containing algae contribution to total integrated Chl *a* is much less than diatoms, their importance is increased up to 50% of total Chl *a* at the active mixing area. As confirmed in this study, Park *et al.* (2006) reported that the spectrofluorometric measurement with size fraction showed the Chl *b* in the transition region was higher than 50% of total Chl *a* and related with picoplankton fraction at the southwestern East/Japan sea.

Below 50 m depth, Chl *a* was decreased to the detection limit. The euphotic depths in this area was about 50 m or less in June 1999 (personal communication with Greg Mitchell). Turbulent conditions seems to limit the availability of light and steep temperature gradient made it unfavorable for growth of phytoplankton at depth below 50 m. The Chl *a* subsurface maximum was observed below the surface, from 10 m to 50 m. In the study area, southwesterly wind is predominant periodically and induces the upwelling annually in summer. Upwelling phenomena bring deep water, either NKCW or mixed water of TMW and NKCW with high nutrients to the surface euphotic layer. This subsurface Chl *a* layer was found at the top of the shallow thermocline and seems to be supported by the nutrients input from the bottom.

The average percentage of net plankton and nano+picoplankton to total integrated Chl *a* was 44% and 56%, respectively. Average 56% fraction of nano+picoplankton for the total integrated Chl *a* is comparable to 2000 (53% in June, 60% in August), when there was no upwelling event. When intensive upwelling was observed in 2001, average percentage of nano+picoplankton was less than 50% which was estimated by fluorometric measurement. The 56% fraction of nano+picoplankton in July 1999 is comparable to June (53%) and August (60%) of 2000. Thus composition of phytoplankton community in size fraction is similar to stratified conditions in 2000, not to upwelling conditions in 2001, which implies active upwelling was damped in July 1999.

The satellite image of SST in June, 1999 showed that the wide area of East/Japan Sea was covered by cold water (<18°C) at the surface near 37N and wind data showed strong southwesterlies wind in June. However the upwelling phenomena in July 1999 was not confirmed by satellite image, because clouds and fog in the study area prevented the data acquisition.

The vertical distribution of nutrients in July 1999 showed the slightly high concentrations of nitrate + nitrite (> 1 μg-at/*l*) at the surface. Horizontal distribution of temperature showed slightly low temperature near coast compared to offshore, but the difference was small (ΔT<1 °C). However, vertical temperature gradients were significant and 3-5 °C decrease for every 10 m was observed. Salinity distribution didn't support the upwelling event, because low saline water was observed near coastal regions, except St.B1. In case of upwelling event, high saline TMW and/or mixed water between NKCW and TMW rise and high saline water compared to surrounding water was observed at the surface. Although the physical parameters (temperature and salinity) or satellite image do not support the upwelling phenomena in July 1999, the concentrations of Chl *a* were very high at coastal regions up to 8.3 μg/*l* in shallow depth layer (0-10 m). The highest Chl *a* measured in 2000 was 2.96 μg/*l* (June) and 2.85 μg/*l* (August) (Kim 2002) and 6.90 μg/*l* (July) in 1999 by fluorometry method. Along the coast, high DO and Chl *a*, which was comparable to the active upwelling

period in 2001 (4.25 μg/*l*), indicated mild upwelling conditions in July 1999. In fact, 15 days composites of Chl *a* at the study area showed highly enhanced Chl a before and after the month of July. Thus it is thought that high speed and flux of low saline water from TC in summer of 1999 made the upwelling event weak at the study area.

As an evidence, the vertical CTD profile at transect B showed the tilted profiles of salinity and temperature toward offshore direction and inverted Chl *a* distribution. Sometimes there were two maximum layers above the euphotic layer, 10 m and 50 m. The subsurface Chl *a* maximum layer was above the nutricline, which was the border depth between TSW and TMW. The nutrients input was supplied both from the surface and also from the bottom.

Comparison with Chl *a* from HPLC and satellite image showed overestimation of Chl *a* by satellite data up to 30-70%. This discrepancy might have been attributed by colored dissolved organic matter (CDOM), which is known to be present in high concentration in water columns in the East/Japan Sea. Comparison with Chl *a* from HPLC and fluorometric measurement showed linear relationship between data sets. However, the concentrations from fluorometric method was much lower (30%) compared to Chl *a* obtained from HPLC analysis. It is known that Chl *a* is overestimated about 30% by fluorometric method compared to HPLC analysis when diatom is the major group, because of degradation products. It was the case in St. B1 in this study. However, in general, the concentration of Chl *a* from fluorometric analysis was 30% lower than HPLC data. In presence of Chl *b*, it is possible to underestimate Chl *a* by fluorometric method. As Jeffrey (1997) pointed out, acid-fluorometric method of Holm-Hansen underestimates Chl *a* in sea water in the presence of Chl *b* due to spectral intereference. Moreover, the Chl *a* concentrations in the East/Japan sea compared to Yellow sea is relatively low and with significant fraction of total biomass contributed from green algae with Chl *b*. It seems the discrepancy among HPLC, fluorometric method, and satellite data increases the gap due to the presence of Chl *b* and CDOM. Thus, routinely used fluorometric method for comparison with satellite image of Chl *a* should be calibrated to get an accurate assesment of phytoplankton biomass without over- and underestimation. In future, the importance and origin of Chl *b* in the study area need to be confirmed: whether it is from green algae or *Prochlorococcus*.

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