The Journal of the Korean Society of Oceanography
Vol. 32, No. 1, pp. 46-55, March 1997

HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton

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The quantitative determination of carotenoids, chlorophylls and their degradation products from marine phytoplankton was performed by reverse-phase high performance liquid chromatography (HPLC). Separated and quantified chlorophylls and their degraded products are chlorophyll a, b, c, chlorophyllide a, phaeophytin a, b and phaeophorbide a. Concentrations of six carotenoids including fucoxanthin, 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, prasinoxanthin, alloxanthin, zeaxanthin/lutein were also determined from natural field samples by absorbance. Results of pigment analysis of field samples collected from the southern waters of the East Sea on October 8th, 1996 were reported. Concentration of chl a ranged from 7.2 to 180.4 ng/l. Concentration of chl b and chl c ranged from 22.7 to 53.7 ng/l and from 3.3 to 58.5 ng/l, respectively. Significant concentrations of phaeophytin a, and chlorophyllide a were also detected at different depths. Fucoxanthin, 19'-hexanoyloxyfucoxanthin and zeaxanthin/lutein were the most abundant carotenoids and 19'-butanoyloxyfucoxanthin, prasinoxanthin and alloxanthin were also detected relatively at low concentrations. These results of pigment analyses suggest predominance of diatom, prymnesiophytes and cyanobacteria and presence of crysophyte, green algae (prasinophyte and prochlorophyte), and cryptophyte in the study area on October 8th, 1996. We report prymnesiophyte for the first time as an important biomass component of marine phytoplankton in the study area. Vertical profiles for the concentration changes of the major pigments were also given.

INTRODUCTION

Chlorophyll a concentrations have been routinely measured by spectrophotometric (Strickland *et al.*, 1972) or spectrofluorimetric (Holm-Hansen *et al.* 1965; Lorenzen and Jeffrey, 1980) methods, in order to estimate biomass and primary productivity of marine phytoplankton. However, as reported by Mantoura and Llewellyn (1983) and Rowan (1991), these methods are inaccurate when degradation products of chlorophyll are present (Gieskes and Kraay, 1983). These methods can under- or overestimate chlorophyll a because of the overlap of absorption and fluorescence bands of accessory pigments and degradation products of chlorophylls (Gieskes and Kraay, 1983; Trees *et al.*, 1986).

In order to solve this problem, separation of pigments by chromatographic methods (TLC: Thin-layer Chromatography and HPLC) was employed. Both methods can give accurate estimation of chlorophylls and their degradation products. However, TLC method has not been widely adopted for marine samples, because it is slow and labor-intensive. Most of all, TLC requires 0.15~1.0 μg of each pigment, which means that 10~20 l of oligotrophic seawater has to be filtered to obtain sufficient material. On the other hand, reverse-phase HPLC system offers significant advantage over TLC including speed, sensitivity, selectivity, simple procedure for sample work-up. The detection limits for carotenoid and chlorophylls are 0.5 ng and 1 ng, respectively with absorbance detection, and 0.1 ng with fluorescence detection for chlorophyll. The number of carotenoids which can be separated and quantified is more than 40.

Although various HPLC methods for determination of phytoplankton pigments have been published during the last decade, no one method is ideal for all pigments. As a result of efforts to improve HPLC techniques for separation of chlorophyll and carotenoids, which act as an indicator of microscopic plant biomass, and biological markers

본 연구는 1996년도 교육부 학술 연구조성비(해양·수산과학분야)의 지원에 의한 것임.

for algal types and recycling processes, Wright *et al.* (1991) recently reported improved HPLC method using a ternary gradient system. Although this improved method can separate and identify carotenoids including zeaxanthin and lutein, it has a very complicated solvent system and uses expensive solvent. And Mg 2,4-D (Mg 2,4-divinylphaeoporphyrin a5 monomethyl ester like) and chlorophyll c_1 and c_2 are eluted at the same time. These compounds cannot be separated and quantified when they present as a mixture of different phytoplankton groups.

In this study, we report simple reverse-phase HPLC method using binary gradient system employed to estimate the accurate concentrations of phytoplankton pigments and results of applications of the method to the marine phytoplankton assemblages in the study area of the southern waters of the East Sea. Recently, recognition of importance of picoplankton in the open ocean for primary production raised the need to investigation of composition of marine phytoplankton by chemotaxanomic marker compounds rather than by microscopic observation. Shim *et al.* (1991) and Park and Moon (1992) reported that populations of picoplankton are important for total biomass of phytoplankton community and primary productivity in coastal area of Korea. Shim *et al.* (1991) reported that picoplankton (< 3 μm) fractions contributed to total chlorophyll a concentration upto 83.2%. Despite the ecological importance of these phytoplanktons, their taxonomy is poorly understood. It is because of small size and fragility of picoplankton. The photosynthetic pigments analysis by HPLC can give useful information to distinguish various classes of phytoplankton in natural waters, even for eukarotic picoplankton which cannot differentiate by epifluorescence microscopy. There has been a report about coccoid eukaryotic marine ultraplankters which showed 4 different HPLC pigment signatures (Hooks *et al.*, 1988). Based on presence or absence of specific pigments in HPLC analysis, structure of the community of phytoplanktons was examined for two stations (A1 and A2) in the study area. Vertical profiles of various carotenoids, chlorophylls and their degradation products were obtained to investigate taxanomic diversity and physiological state of phytoplankton and grazing intensity at different depths. For low concentration of pigment analysis of field samples, effects of the extraction by 100% and 90% acetone were investigated to select efficient extraction method with well-resolved peaks.

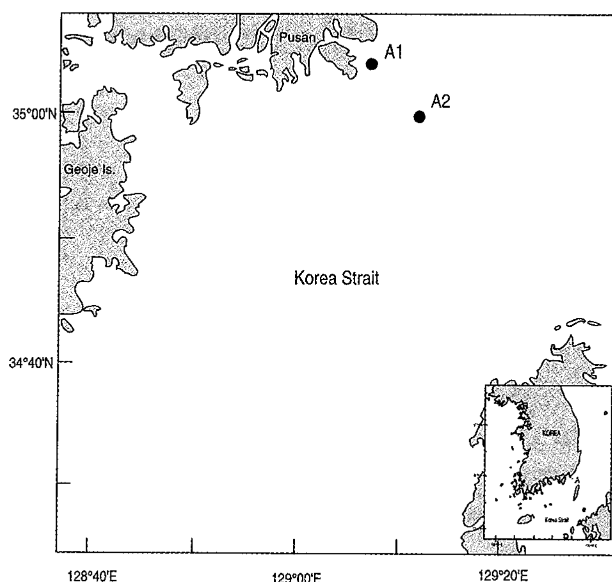

Fig. 1. Map of the study area.

MATERIALS AND METHODS

Reagents

The solvent for extraction (acetone) and chromatography (methanol, water) were HPLC-grade (J. T. Baker). The ion pairing (ip) solution consisted of 7.5 g tetrabutylammoniumacetate (Aldrich, 97%) and 38.5 g ammonium acetate (Junsei Chemical, Japan) dissolved in 500 ml distilled water.

Sampling and Extraction Procedures

Water samples (0~200 m) were collected aboard the R/V *Tamyang* with a rosette sampler on October 8th, 1996. Location of stations in the study area is shown in Fig. 1. The particulate matters from water samples (2 l) was collected by filtration through 47 mm GF/F filters. Then the filters were wrapped with aluminum foil and stored at liquid nitrogen temperature before extraction in 90% acetone (Strickland and Parsons, 1972). Details of extraction procedures are listed in Fig. 2. The filters were ground in 15 mL glass tube by Glas-col homogenizer (Terra Haute, USA) and centrifuged for 10 min at 2,000 rpm. After kept filters with the 90% acetone for 2 hours (< -20°C, in the dark), the extracted solution was filtered with 27 mm GF/F filters to remove debris and concentrated under nitrogen gas flow. Before injection, a 1 ml aliquot of samples was mixed with 100 μl of ip solution. Two hundred μl of the extract was injected.

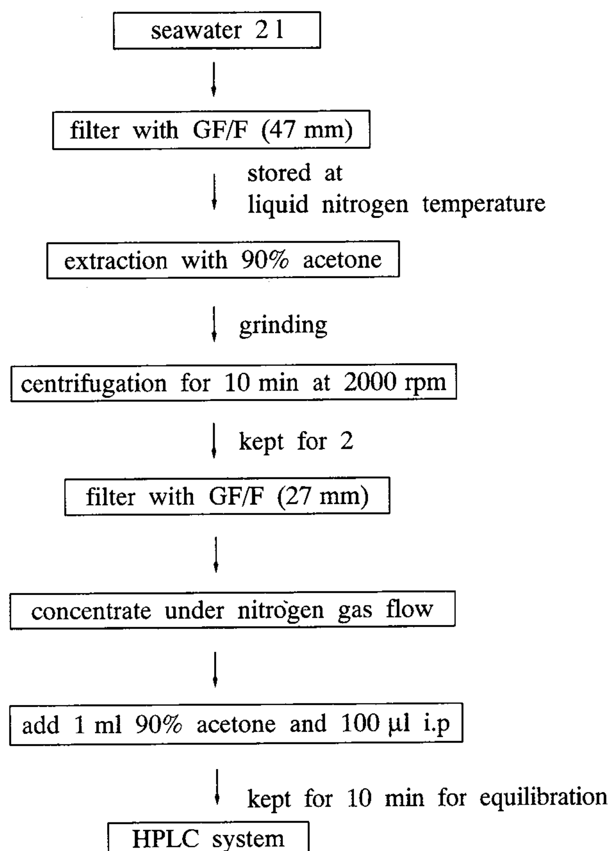

Fig. 2. Extraction procedure of pigments.

Apparatus

The pigments were separated with Orom Vintage 2000 HPLC system (Orom Tech, Korea) which composed of a programmable variable-wavelength absorbance detector (Orom UV-200). In addition, fluorescence detector (Shimadzu, RF-551) was fitted downstream from the absorbance detector. System and conditions for HPLC analysis of pigments are shown in Table 1. Pigment detection was at 432 nm for all chlorophylls and carotenoids for absorbance. For fluorescence detection, the system has been programmed to monitor at emission wavelength (650 nm) with two different excitation wavelengths (432 nm: 0~24 min, 405 nm: 24~35 min) in order to obtain enhanced peak intensity for phaeophytin a. The gradient solvent system used is shown in Table 2.

Pigment identifications

Peak identities are assigned by comparing retention times of the authentic standards (co-injection analysis) and algal extracts of known pigment composition which is available (*Phaeodactylum tricornutum*, KMCC B-14, Korea). Standard solutions of chlorophyll a, b (Sigma chemical) and carotenoids including fucoxanthin, canthaxanthin, zeaxanthin (VKI, Denmark), 19'-butanoyloxyfucoxanthin (19'-bf), 19'-hexanoyloxyfucoxanthin (19'-hf), prasinoxanthin, alloxanthin (donated by R.R. Bidigare) were prepared in 90% acetone. Diadinoxanthin was confirmed by known retention time from chromatogram of extracts of *Phaeodactylum tricornutum*, but quantitative determination was omitted. Canthaxanthin was added to the 1 ml aliquot as internal standard to compare the changes of retention time. Phaeophytin a and b were prepared from the chlorophyll a and b standards by acidification with 1 M HCl and extraction into diethyl ether, washing with water and back-extraction into acetone (Mantoura and Llewellyn, 1983). Chlorophyllide a was obtained by enzymatic de-esterification of chlorophyll a in 50% acetone suspension of the diatom *Phaeodactylum tricornutum* according to Barrett and Jeffrey (1971). Pheophorbide a prepared was converted from chlorophyllide a by the acidification-ether extraction procedure used for the phaeophytin.

Table 1. HPLC system used for analysis of pigments

System

HPLC: Orom vintage 2000 (Oromtech, Korea)
Detector: absorbance (Orom UV-200, Korea) at 432 nm
fluorescence (Shimadzu RF-551, Japan)
at λ_{ex}=432, 405 nm, λ_{em}=650 nm
Colum: Rexchrom-S5-100-ODS (octadecyl-silane bonded phase, Regis, 250×4.6 mm, 5 μm particle size USA)
Sample loop: 200 μl (Rheodyne 7125 injector, USA)

Condition

Sfolvent system
solvent A-8:1:1 (methanol: ion paring reagent*: water)
solvent B-100% methanol

*ion paring reagent: 1.5 g of tetrabutylammonium acetate and 7.7 g of ammonium acetate in 1000 ml water.

Table 2. HPLC solvent system program

Solvent program-gradient elution

Time	B(%)	Flow rate (ml/min)
0	0	1.5
10	100	1.5
18	100	2.0
19	100	3.0
20	100	4.0
21	100	5.0
35	100	5.0

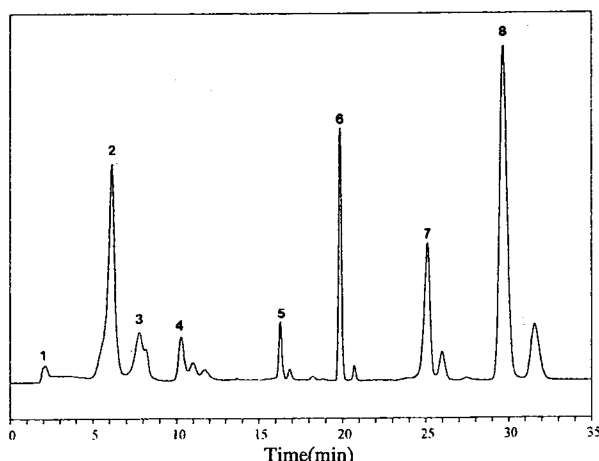

Fig. 3. HPLC chromatograms of chlorophylls and their degradation products from mixed standards (fluorescence signal, 432 nm excitation, 650 nm emission); 1: chlorophyllide a, 2: chlorophyll c_1, 3: chlorophyll c_2, 4: phaeophyorbide a, 5: chlorophyll b, 6: chlorophyll a, 7: phaeophytin b, 8: phaeophytin a.

Quantification

Standards which were commercially available and purified from algal culture were calibrated spectrophotometrically in 90% acetone (for carotenoids, in 100% ethanol) using published extinction coefficients (Jeffrey, 1972; Lorenzen and Downs, 1986; Davies, 1976; Bidigare, 1991). Chromatograms (Fig. 3) and relevant extinction coefficients for pigments are given in Table 3. Absorption was measured at the maximum absorption wavelength and 750 nm, in order to correct for light scattering. Concentrations of the standards are calculated as following (E: extinction coefficient):

$$\frac{\text{Pigment conc.}}{\text{mg/l}} = \frac{(\text{Corrected Absorption})}{(\text{E; lg}^{-1}\text{cm}^{-1}) \times (\text{cm})} \times \frac{1000\text{ mg}}{1\text{ g}}$$

After determining pigment concentrations, standards were injected into HPLC system to calculate standard response factor. The response factor was calculated as the weight of standard injected divided by the area (or height) of the pigment. Calibration curves were constructed by plotting fluorescence peak heights against standard pigment concentrations (determined spectrophotometrically) to establish the system linearity. The concentration of pigments in samples can be calculated with the sample volume filtered, the injection volume, and the extraction volume.

RESULTS AND DISCUSSION

This reverse-phase HPLC system with binary gradient system could successfully separate and quantify chlorophylls, their degradation products and carotenoids in mixture of standard solutions and extracts of marine phytoplanktons and detritus in natural sea water samples (Figs. 3 & 4). The identified chlorophylls and their degradation products which were monitored by fluorescence were chlorophyll a, b, and c and phaeophytin a and b, phaeophorbide a and chlorophyllide a. The allomer and epimer of chlorophyll a could be also separated. Detection limit for chlorophyll a concentrations was as low as 3.3 ng/l. Detection limits for the other photosynthetic pigments were in the range of 1.5~10.6 ng/l (Table 4).

Chromatograms for two different solvent extracts of *Phaeodactylum tricornutum* and for 100% ace-

Table 3. Elution order of photosynthetic pigments and their extinction coefficients

peak no.	pigments	wavelength (solvent)	$E(l\ g^{-1}\ cm^{-1})^a$
1	chlorophyllide a	664 (90% acetone)	128.0
2	chlorophyll c_1	631 (90% acetone)	44.8
3	chlorophyll c_2	631 (90% acetone)	40.4
4	19'-butanoyloxyfucoxanthin	446 (ethanol)	160.0
5	fucoxanthin	449 (ethanol)	160.0
6	19'-hexanoyloxyfucoxanthin	447 (ethanol)	160.0
7	prasinoxanthin	454 (ethanol)	160.0
8	alloxanthin	453 (ethanol)	262.0
9	zeaxanthin	450 (ethanol)	254.0
10	canthaxantin	474 (ethanol)	222.9
11	chlorophyll b	647 (90% acetone)	51.36
12	chlorophyll a allomer		
13	chlorophyll a	664 (90% acetone)	87.67
14	chlorophyll a epimer		
15	chlorophyll a	665 (90% acetone)	49.5

[a]refer to Bidigare (1991) and Latasa *et al.* (1996)

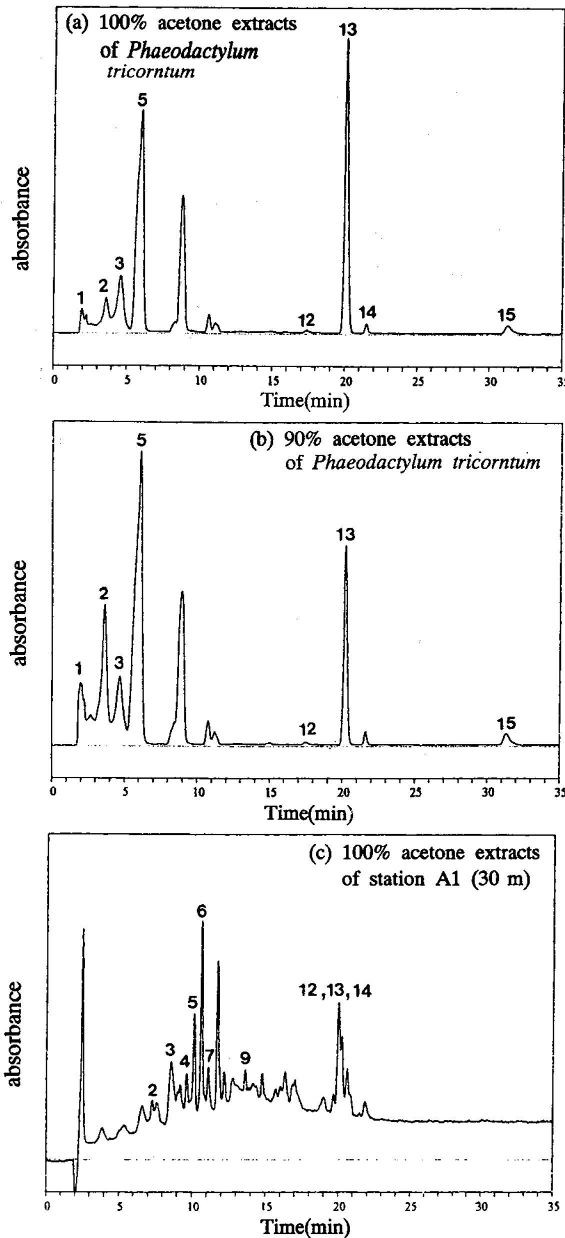

Fig. 4. Effect of different solvent extraction on chromatograms for extracts of *Phaeodactylum tricornutum* (diatoms) and seawater sample. Peak numbers refer to pigments listed in Table 3. (a) 100% acetone extracts of *Phaeodactylum tricorntum*, (b) 90% acetone extracts of *Phaeodactylum tricorntum*, (c) 100% acetone extracts of station A1 (30 m).

tone extract of a field sample are shown in Fig. 4. Before analysis of field samples, appropriate conditions for better extraction and resolution by HPLC system were tested. Since the concentrations of pigments in depth below 50 m are very low, the extract has to be concentrated by nitrogen flow. However, it takes a lot of time to evaporate due to the water content in 90% acetone. In order to reduce

Table 4. Detection limits by HPLC

pigments	detection limits[a] (ng liter^{-1})
chlorophyll a	3.3
chlorophyllide a	1.5
chlorophyll b	9.7
chlorophytin a	10.6
chlorophyll c_1+c_2	5.5
19'-bf[a]	4.1
fucoxanthin	8.3
19'-hf[b]	7.0
prasinoxanthin	7.0
alloxanthin	6.6
zeaxanthin	6.2

[a]S/N=2
[b]19'-butanoyloxyfucoxanthin
[c]19'-hexanoyloxyfucoxanthin

the time for evaporation, an effect of the extraction by 100% acetone was investigated to compare with 90% acetone extraction for this HPLC system. An apparent difference was a decreased intensity of chlorophyll c peaks compared to chlorophyll a peak when 100% acetone was used as an extraction solvent. Even though the intensity of peaks observed by absorbance and fluorescence were enhanced for 100% acetone extraction, poor resolution of chlorophyll a was shown for field samples (Fig. 4-(c), at low concentration of pigments) by 100% acetone extraction compared to 90% acetone extraction. Thus, 90% acetone is chosen for extraction solvent for all pigment analysis of the field samples.

The established HPLC system was used for pigment analysis of field samples collected from the southern waters of the East Sea on October 8th,

Table 5. Summary of pigments concentration (ng liter^{-1}) measured at station A1 (34° 00.07' N, 129° 12.20' E) and A2 (34° 57.56' N, 129° 15.29' E) in the Southern Waters of the East Sea, Korea (8 October, 1996)

pigments	Range	
	A1	A2
chlorophyll a	7.2-180.4	8.7-139.6
chlorophyllide a	10.9-39.2	14.9-34.7
chlorophyll b	25.1-53.7	22.7-35.4
phaeophytin a	9.9-28.7	11.8-58.7
chlorophyll c_1+c_2	3.3-29.2	3.8-58.5
19'-bf[a]	3.9-14.8	3.9-16.7
fucoxanthin	7.7-70.8	18.4-211.6
19-hf[b]	5.4-35.7	7.0-75.0
prasinoxanthin	5.5-29.4	8.1-19.4
alloxanthin	0-22.5	6.1-13.6
zeaxanthin	3.9-34.1	5.8-22.1
chlorophyll a related compounds	30.2-308.5	38.4-227.1

[a]19'-butanoyloxyfucoxanthin
[b]19'-hexanoyloxyfucoxanthin

1996. The pigment data by UV and fluorescence detection are summarized in Table 5. The chlorophyll a concentrations ranged between 7.2 and 180.4 ng/l. Chlorophyll b concentration was in the range of 25.1~53.7 ng/l, which is about 23% of chlorophyll a. However, relatively constant concentration of chlorophyll b was observed throughout the water column. Chlorophyll c_1+c_2 concentrations were measured between 3.3 and and 58.5 ng/l. The pattern for vertical distribution of chl c_1+c_2 was similar

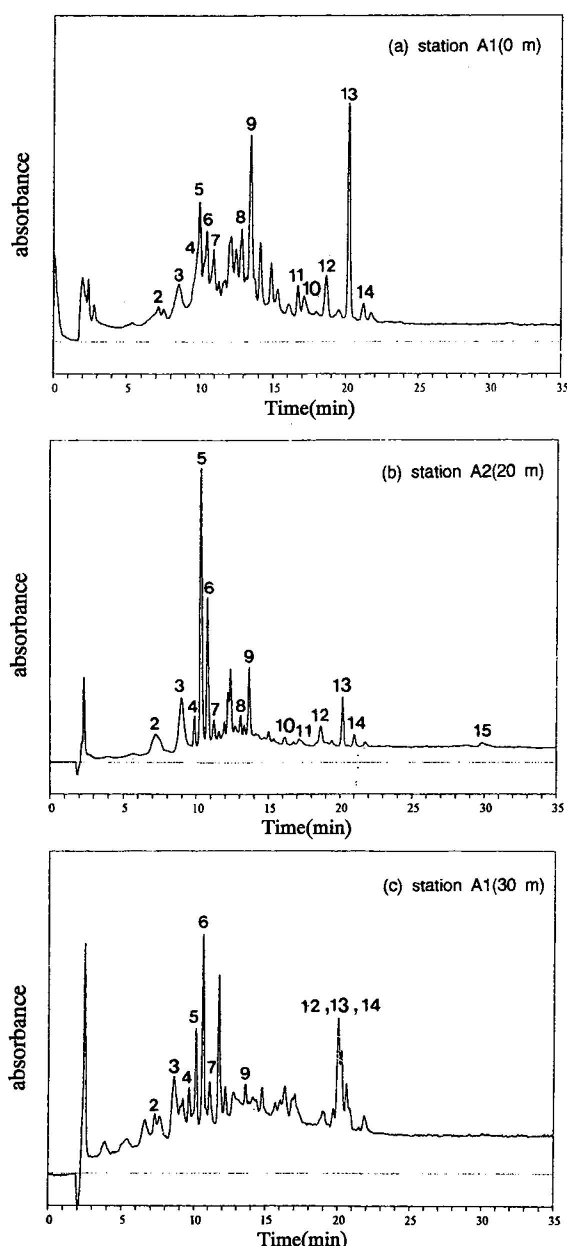

Fig. 5. HPLC chromatograms of field samples from the Southren Waters of the East Sea. Peak numbers refer to pigments listed in Table 3. (a) station A1 (0 m), (b) station A2 (20 m), (c) station A1 (30 m).

to chlorophyll a. Chlorophyllide a was detected at all depth for both Stn. A1 and A2. The highest concentration was found at the surface of both stations and at Stn. A2, subsurface peak of chlorophyllide a at 30 m depth was found.

Vertical profiles of chlorophylls and their degradation products are shown in Figs. 6 and 7. The chlorophyll a and b maxima were at depth between 20 and 30 m. This depth is about the bottom of well-mixed layer with high temperature (> 23°C) and low sigma-t (22.5). The vertical distribution of chlorophyll a showed that more than 80% of phytoplankton was present above 30 m. The subsurface maximum depth of chlorophyll a was found near the bottom of the euphotic layer. At Stn. A1, the vertical distribution of chlorophyll a is different from that observed at Stn. A2. The highest concentration of chl a was found at the surface of Stn. A1, but at Stn. A2 it was at 20 m depth. The chl c / chl a weight ratio calculated at chlorophyll a maximum depth at Stn. A1 was 0.17 and 0.42 at Stn. A2. The calculated chl c / chl a weight ratio at Stn. A2 falls within the range of values reported for diatoms and dinoflagellate cultures (Jeffrey, 1972). The calculated chl c / chl a weight ratio at Stn. A1 is relatively low. And, in fact, concentrations of chl b and prasinoxanthin were higher at Stn. A1 than Stn. A2 upto two times. This indicates high propotion of chl b containing phytoplankton in total biomass of marine phytoplankton at Stn. A1.

The concentration of chlorophyll a (b and c) at Stn. A1, was highest at the surface and sharply decreased at depth of 20 m, but at the same time phaeophytin a was slightly increased at this depth. This phenomena seemed to implicate the active grazing by zooplankton at this depth. Very high concentration of allomer of chlorophyll a was measured at 10~20 m depth of Stn. A1. Allomer of chlorophyll a was known to be made by artifact of methanol extraction or chemical treatment for better preservation. However, it is a very rare case in which higher concentration of allomer of chlorophyll a than chlorophyll a was measured as in this study. Since all the same extraction procedure with 90% acetone was used, it doesn't seem to occur because of an artifact of extraction procedure. In order to obtain total chlorophyll a, vertical changes of chlorophyll a related compounds at Stn. A1 and A2 are plotted in Fig. 6(c) and 7(c). The chlorophyll a related compounds included chlorophyll a, allomer and epimer of chlorophyll a, and chlorophyllide

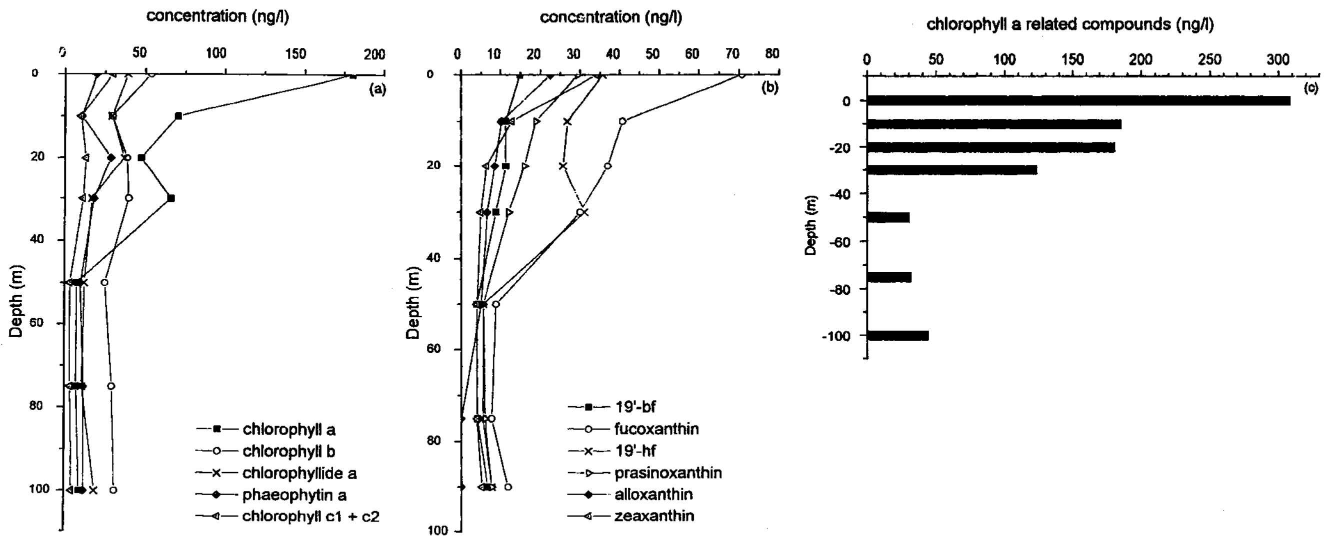

Fig. 6. Vertical profiles of pigments measured at station A1 (35° 00.07' N, 129° 12.20' E) in the Southern Waters of the East Sea, Korea (8 October, 1996); (a) depth profiles of chlorophylls and their degradation products, (b) depth profiles of carotenoids, (c) depth profiles of chlorophyll a related compounds.

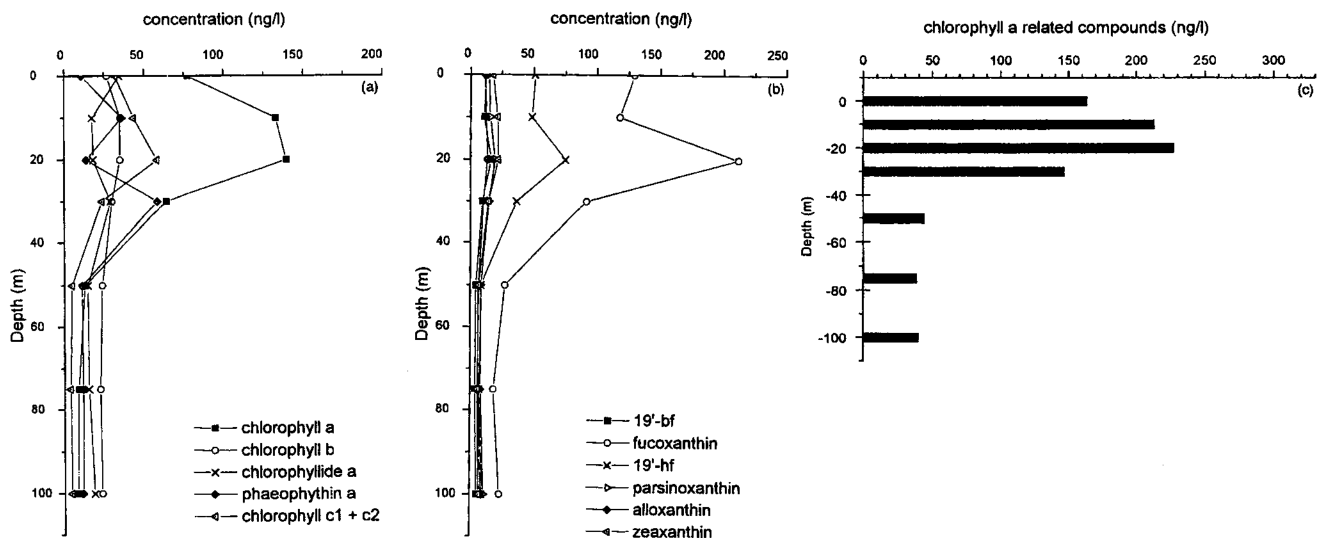

Fig. 7. Vertical profiles of pigments measured at station A2 (34° 57.56' N, 129° 15.29' E) in the Southern Waters of the East Sea, Korea (8 October, 1996); (a) depth profiles of chlorophylls and their degradation products, (b) depth profiles of carotenoids, (c) depth profiles of chlorophyll a related compounds.

a. At Stn. A1 maximum concentration of chlorophyll a related compounds was found as 308.5 ng/l at the surface and its concentration was decreased with depth. At Stn. A2, maximum chlorophyll concentration was measured at 20 m depth and its concentration was about than 230 ng/l. At both stations, concentrations of chlorophyll a related compounds decreased sharply upto 50 ng/l, below 50 m.

Concentration of chlorophyll c_1+c_2 ranged from 3.3 to 58.5 ng/l. At Stn. A1, concentration of chlorophyll c_1+c_2 was maximum at the surface and decreased sharply near detection limit (< 2 ng/l) below 50 m. Phaeophorbide was not detected at Stn. A1. The phaeophorbide a maximum was at about 50 m at Stn. A2 (not shown). Phaeophytin and phaeophorbide a are metabolic products of chlorophyll a when phytoplankton is digested by zooplankton. Maxima concentrations of phaeophytin a were found at 10~30 m and these indicate intense zooplankton grazing activity (Jeffrey, 1974) or presence of senescent phytoplankton at this depth. Chlorophyllide a was detected at all depth for Stn. A1 and A2. Concentrations of chlorophyllide a

ranged between 10.9 ng/l and 39.2 ng/l. The maximum concentration of chlorophyllide a was found at the surface, and its concentration decreased with depth. At the surface, 2~4 times more chlorophyllide a was found compared to minimum concentration at different depth.

The characteristic carotenoids identified from natural field samples by absorbance were fucoxanthin, diadinoxanthin, 19'-bf, 19'-hf, prasinoxanthin, alloxanthin, zeaxanthin/lutein. In our system, zeaxanthin and lutein cannot be separated each other. At the surface, very strong peaks of fucoxanthin, 19'-hf and zeaxanthin/lutein were observed at Stn. A1 and fucoxanthin and 19'-hf at Stn. A2. The dominant group of phytoplankton at the surface of two stations are likely different based on the detected characteristic carotenoids. Fucoxanthin was the major carotenoid at all depth of both stations which indicates that eukaryotes with chlororophyll c containing phytoplankton including diatoms and prymnesiophytes, crysophytes (Jeffrey and Vesk, 1981) can be dominant phytoplankton groups in this water column. The secondary important carotenoids were 19'-hf and zeaxanthin/lutein. At subsurface chlorophyll a maximum depth (30 m) of Stn. A1, 19'-hf was the most abundant carotenoid peak. And at Stn. A2, 19'-hf was also abundant next to fucoxanthin. 19'-hf is the major carotenoid of the coccolithophorid *Emiliania Huxleyi* (Arpin *et al.*, 1976). Abundance of 19'-hf, particularily at upper 20~30 m layer, indicates presence of coccolithophorids in the study area. Venrick (1982) has also reported that coccolithophorids (prymnesiophytes) are particularily abundant at the depth of the Pacific chlorophyll maximum.

At the surface of Stn. A1, high concentration (34.1 ng/l) of zeaxanthin/lutein was measured. Even though our HPLC sytem cannot separate peaks of Zeaxanthin and lutein, high concentration of Zeaxanthin/lutein measured at stations far from estuary tells us that contribution from lutein to Zeaxanthin/lutein peaks is negligible. Zeaxanthin was ascribed as a marker for blue-green algae (Guillard *et al.*, 1985), but is also known to be present in prasinophytes and prochlorophytes (Anderson *et al.*, 1996). The presence of prochlorophytes can be confirmed by peaks of divinylchlorophyll a and b, which are good marker compounds of prochlorophytes. However, since it is impossible to resolve chl a and divinylchlorophyll a with our HPLC system, flow cytometry was used to confirm the presence of prochlorophytes.

In order to find the source of zeaxanthin/lutein, seawater samples were collected at the same places, and 1 ml of seawater samples fixed with formaldehyde was analysed by flow cytometry. The result of the analysis by flow cytometry for water samples collected on Feb. 1997 showed the presence of synechococcus, prochlorococcus and eukaryotic picoplanktons. The high population (3,000~9,000 cell/ml) of eukaryotic picoplankton (2~5μm) and prochlorococcus(< 3 μm) was confirmed (Park, unpublished). Relatively small numbers (< 250 cell/ml) of cyanobacteria (Synechococcus) were also found. The other possibility is the increased zeaxanthin content in cyanobacteria to protect against photosensitized oxidation under high light intensity. It has been known that carotenoids protect chlorophylls from potentially harmful effects of light which occur in the presence of oxygen (Hager, 1980). Recent study reported that excessive amount of zeaxanthin is produced by phytoplankton under the strong sun light to protect from the harmful effect (Paerl *et al.*, 1983). The chromatic adaption of cyanobacteria in the upper mixed layer is well known that they contain as much as zeaxanthin relative to chl a to protect cells under high light intensities (Gieskes and Kraay, 1986). Since only at the surface, concentration of zeaxanthin was high, the light induced de-epoxidation could be a source for zeaxanthin at the surface. However, as shown from flow cytometry data, cyanobacteria, green algae including prochlorophyte and prasinophyte were found. Thus, mixed population of all three classes of phytoplankton might attribute to high concentration of zeaxanthin/lutein at the surface of Stn. A1.

Lower concentrations of 19'-bf, alloxanthin and prasinoxanthin were also measured at both stations.

Summing up, the pigment analysis by HPLC provides following informations about population of phytoplankton in the study area: The group of pigments chlorolophyll a and c, fucoxanthin, diadinoxanthin indicates the presence of diatoms and 19'-hf indicates presence of prymnesiophyte (coccolithophorids) for all depths of st A1 and A2. And group of pigments including chlorophyll b, zeaxanthin and prasinoxanthin indicates green algae (prasinophytes). In fact, Chung and Kang (1996) recently reported the isolation of the coccoid prasinophyte, *Pycnococcus provasolii* Guillard from Korea Strait. Even though it is not significant con-

centration, 19'-bf denotes the presence of crysophytes at depth between 10 and 30 m of both stations and alloxanthin denotes cryptophytes.

SUMMARY

In order to estimate the accurate concentrations of phytoplankton pigments and to know the community composition of marine phytoplankton assemblage of the southern waters of the East Sea, photosynthetic pigment analysis by reverse-phase HPLC was performed. The chlorophyll and their degradation products were well separated and determined by HPLC using binary gradient system. Chlorophyll a, b and c were detected in the range of 7.2~180.4 ng/l, 22.7~53.7 ng/l and 3.3~58.5 ng/l, respectively. Except the highest concentration of chlorophyll a at the surface of Stn. A1, chlorophyll maximum depth was found between 20~30 m, which corresponding to the bottom of euphotic zone. Chlorophyllide a concentrations were high at the surface layer and decrease rapidly. Phaeophytin a was measured high at depth below the chlorophyll maximum depth. At the depth of 20 m for A1 and 30 m for A2 with highest concentration of phaeophytin a, the grazing activity were considered to be most intensive. Among carotenoids, fucoxanthin and 19'-hexanoyloxyfucoxanthin were the dominant pigments. As minor pigments, zeaxanthin, prasinoxanthin, alloxanthin, diadinoxanthin and 19'-bf were detected. The result of pigment analysis clearly showed the dominance of diatoms and prymnesiophyte (coccolithophorids) and presence of green algae (prasinophyte and prochlorophytes), cyanobacteria, cryptophytes and crysophyte in the marine phytoplankton community in southern waters of East Sea on October 8th, 1996. Especially 19'-hf which was abundant at depth of 30 m of both stations is reported here indicating that prymnesiophyte comprised an important part of community of phytoplankton in the study area.

ACKNOWLEDGEMENTS

We thanks Dr. Huh and Dr. Hwang for providing a valuable culture (*phaeodactylum tricornutum*) and pigment standands.

For sincere cooperation by the captain J.C. Kim and his crew members og the R/V Tam Yang for the sampling, we also express our gratitude. We also express our thanks to Dr. Bidigam for their assitance to obtaim flow cytometry data. Close reading and critical comments on manuscript by two referees were also helpful.

REFERENCES

Anderson, R.A., R.R. Bidigare, M.D. Keller, and M.Latasa, 1996. A comparison of HPLC pigment signatures and electron microscopic observations for waters of the North Atlantic and Pacific Oceans. *Deep Sea Res. II*. **43**: 517-537.

Arpine, N., Svec, W. and Liaaen-Jensen, S., 1976. New fucoxanthin-related carotenoids from *Coccolithus huxlei*. *Phytochemistry*. **15**: 529-32.

Barrett, J. and S.W. Jeffrey, 1971. A note on the occurrence of chlorophyllase in marine phytoplankton. *J. Exp. Mar. Bio. Ecol.*, **7**: 255-262.

Bidigare, R.R., 1991. Analysis of algal chlorophylls and carotenoids. In *Geophysical Monograph*, **63**: 119-123.

Chung, I.K. and Y.H. Kang, 1996. A marine picoplankton from Korea: Pycnococcus Provasolii Guillard. *J. Korean. Soc. Oceano.*, **31**: 150-154.

Davies, B.H., 1976. Carotenoids, In: Chemistry and Biochemistry of Plant Pigments, edited by T.W. Goodwin Academic Press, N.Y.

Gieskes, W.W. and G.W. Kraay, 1983. Unknown chlorophyll a derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis, *Limnol. Oceanogr.*, **28**: 757-766.

Gieskes, W.W. and G.W. Kraay, 1986. Floristic and physiological differences between the shallow and the deep nanophytoplankton community in the euphotic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. *Marine Biology*, **91**: 567-576.

Guillard, R.R.L., Murphy, L.S., Foss, P. and Liaaen-Jensen, S., 1985. *Synechococcus* spp. as likely zeaxanthin-dominant ultraplankton in the North Atlantic. *Limnol. Oceanogr.*, **30**: 412-4.

Hager, A., 1980. The reversible, light-induced conversions of Xanthophylls in the chloroplast. In: Pigments in Plants, 2nd edited by Czygan, F.C. Fisher.

Holm-Hansen, O., C.J. Lorenzen, R.W. Holmes, and J.D.H. Strickland, 1965. Fluorimetric determination of chlorophyll. *J. Cons. perm. int. Explor. Mer.*, **30**: 3-15.

Hooks, C.E., R.R. Bidigare, M.D. Keller and R.R.L. Guillard, 1988. Coccoid eukaryotic ultraplankters with four different HPLC pigment signatures. *J. Phycol.* **24**: 571-580.

Jeffrey, S.W. 1972. Preparation and some properties of crystalline chlorophyll c_1 and c_2 from marine algae. *Biochim. Biophys. Acta*. **279**: 15-33.

Jeffrey, S.W. 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. *Marine. Biol.* (Berl.) **26**: 101-10.

Jeffrey, S.W. 1980. Algal pigment systems. In: Primary Productivity in the Sea. edited by Falkowski, P., Plenum Press, New York.

Jeffrey, S.W. and M. Vesk, 1981. The phytoplankton-systematics, morphology and ultrastructure. In: Marine Botany-an Australians Perspective. edited by M.N. Clayton, and R. J., King, Longman-Cheshire.

Latasa, M., R.R. Bidigare, M.E. Ondrusek and M.C. Kennicutt II, 1996. HPLC analysis of algal pigments: A comparison exercise and among laboratories and recommendations for improved analytical performance *Marine Chmistry*, **51**: 315-

324.

Lorenzen, C.J. and S.W. Jeffery, 1980. Determination of chlorophyll in seawater, *UNESCO Tech. pp. Mar. Sci.* **35**: 1-20.

Lorenzen, C.J. and J.N. Downs, 1986. The specific absorption coefficients of chlorophyllide a and phaeophorbide a in 90% acetone, and comments on the fluorometric determination of chlorophyll and phaeopigments, *Limnol. Oceanogr.*, **31**: 449-452.

Mantoura, R.F.C. and C.A. Llewellyn, 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Anal. Chim. Acta*, **151**: 297-314.

Park, M.O. and C.H. Moon, 1992. Abundance of a unicellular Chroococcoid picoplankton in the Nokdong River estuary, Korea. *J. Korean. Soc. Oceano.* **27**: 137-144.

Paerl, H.W., J. Tucker and P.T. Bland, 1983. Carotenoid enhancement and its role in maintaining blue-green algal (Microcystis aeruginosa) surface blooms. *Limnnol. Oceanogr.*, **28**: 847-857.

Rowan, K.S. 1991. Photosynthetic Pigments of Algae. Cambridge University Press, 95-110 pp.

Shim, J.H., H.G. Yeo and Y.K. Shin, 1991. Ecological Effect of Themal Effluent in the Korean Coastal waters. I Significance of Autotrophic Nano and picoplankton in the Adjacent Waters of Kori Nuclear Power Plant. *J. Korean. Soc. Oceano.* 26:77-82.

Strickland, J.D. and T. R. Parsons, 1972. A Practical Handbook of Seawater Analysis. 2nd ed., *Bull. Fish. Res. Bd. Can.* 167 p.

Trees, C.C., R.R. Bidigare and J.M., Brooks, 1986. Distributions of chlorophylls and phaeopigments in the Northwestern Atlantic Ocean. *J. Plan. Res.*, **8**: 447-458.

Venrick, E.L., 1982. Phytoplankton in an oligotrophic ocean: observations and questions. *Ecol. Monogr.* **52**: 129-154.

Wright, S.W., S.W. Jeffrey, R.F. Mantoura, C.A. Llewellyn, T. Bjornland, D. Repeta, & N. Welschmeyer, 1991. Improved HPLC methods for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.*, **77**: 183-196.