Characteristics of Photosynthetic Pigments during the Outbreak of Harmful Algal Bloom at the South Coastal Area in the Korean Sea Waters

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Abstract − *Cochlodinium polykrikoides* has occurs regularly during the summer in the South Sea of Korea. To investigate photosynthetic pigments concerned with phytoplankton community structure as bloom of *Cochlodinium polykrikoides*, the experiment was sampled at 20 stations three times between July and September 2002 in the Southern Sea of Korea. The distribution of peridinin, the biomarker of dinoflagellate, was higher at the blooming time than it was before and it disappeared after bloom. The correlative coefficients between chl. *a* and peridinin at the blooming time and out of bloom were 0.9253 and 0.1613, respectively. This result indicated that the bloom was caused by dinoflagellate. The correlative coefficients between chl. *a* and fucoxanthin were 0.3282 and 0.9759, respectively, and the correlative coefficients showed the succession from dinoflagellate to diatom. This result means that the bloom of *Cochlodinium polykrikoides* can be detected by chl. *a* information from satellite remote sensing. Therefore, if the algorithm to detect peridinin in addition to chl. *a* were to be developed, dinoflagellate red tide could be monitored more effectively.

Key words : photosynthetic pigments, harmful algal bloom, *Cochlodinium polykrikoides*, peridinin, dinoflagellate, biomarker

INTRODUCTION

Photosynthetic pigments have been use as biomarkers to trace the chemical classification (Foss *et al*. 1984; Guillard *et al*. 1986a, b; Wright and Jeffery 1987; Bjornland *et al.* 1988; Gieskes *et al.* 1988; Hooks *et al.* 1988), and to know the degradation processing and abundance of plankton (Jeffery 1974; Reptea and Gagosian 1982; Bidigare *et al.* 1986; Roy 1988; Hendry *et al.* 1987; Nelson 1989).

The HPLC analysis of photosynthetic pigments implements a useful technique to quickly analyze and provide a quantifiable estimate of algal pigment (Mantoura and Llewellyn 1983; Bidigare *et al.* 1985; Wright *et al.* 1991). In special algae, HPLC gives more information than the UV spectrophotometer and fluoro-spectrophotometer for measuring carotinoid and chlorophyll and their production of degradation as chlorophyllide, pheophytin and pheophorbide (Holm-Hansen *et al.* 1965; Lorenzen 1967; APHA 1989).

In addition, HPLC techniques have been successfully applied to estimate a fixed quantity of a pigment which occurs in sulfur bacteria in lakes (Kartchals and Steenbergen 1985. Hurley and Watras 1991). Spectro-photomatography creates a spectrum overlap problem in analyzing bacteriochlorophyll *d* and chl. *a*. We solved this problem with HPLC (Hurley and Watras 1991).

We have known since 1980 that the process of degradation and derivatives comes from photosynthetic pigments in living and dead phytoplankton through analysis of photosynthetic pigments by HPLC. Conversely, we understand the characteristics of water columns through the distribution of pigments (Gieskes and Karry 1983; Montoura and Llewellyn 1983; Wright and Sherearer 1984; Roy 1987; Zapata *et al.* 1987; Gieskes *et al.* 1988).

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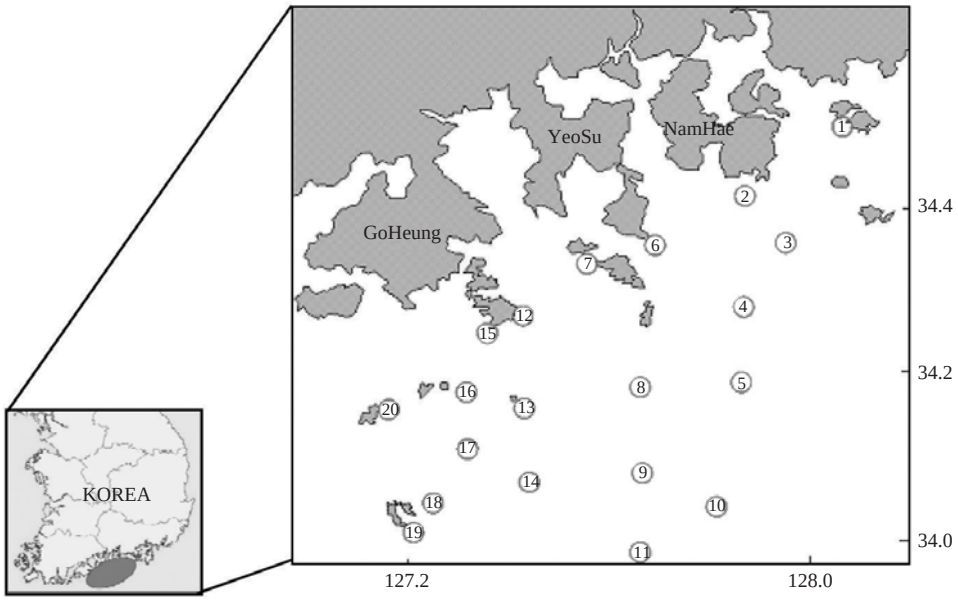

Fig. 1. Map showing the study area and sampling stations.

Therefore, these pigments can be used to understand the origin and fate of particulates which include the biogeochemical cycle of the ocean (Yacobi 1991). Namely, these study processes apply to sea areas and lakes. We'll get a lot of information from degradation processing and quantitative fluctuation of chlorophyll and multiplication, physi-ecology, degradation processing of phytoplankton.

Chl. *a* is widely used to measure phytoplankton biomass in water columns because it is the most abundant element of most phytoplankton (Sun *et al.* 1998). But we have a little information about chl. *a*, degradation production and other extra pigments during the red tide bloom in Korea. This study is focused on the distribution character of photosynthesis pigments before, during and after red tide bloom caused by *cochlodinium polykrikoides*, which has occurred annually in Korea.

MATERIALS AND METHODS

1. Study area and sampling stations

The study area is the central part of the south coast of Korea where harmful red tide continues to be spotted. We took samples at two different depths (surface and bottom) of 20 stations from July 20 ~ 22, August 14 ~ 16 and September 25 ~ 27 in 2002. The sea water for analyzing photosynthetic pigments and environments was collected at 0.5 m depth sea water.

2. Pigments analysis

The pigment extraction was performed with 100% acetone as stated in UNESCO's rule. We used four types of solvent A: Methanol (80%), Ammonium acetate (20%), 0.01% BHT, B: Acetonitrile (87.5%), H_2O (12.5%), 0.01% BHT, C: Ethyl acetate (100%), D: Methanol (100%). We injected 100 μL of an extract into HPLC, and after isolated pigments, the identification and quantification was performed by the Dual Detector (made of Waters) and the standard used Carbon14 (made of Centralen (DHI)). In addition, we used the optical microscope for the identification and quantification analysis of phytoplankton in the collected 1 L sample of sea water which was fixed with Lugol solution.

3. Identification of pigments

The photosynthesis pigments were compared and identified with a well-known pigment, which was made from Retention time and Spectrum. The standard Carbon14 (made of Centralen, DHI) was used. These photosynthesis pigments contain kinds of chlorophyll such as chl. *a*, chl. *b*, chl. *c*, chl. c_2, pheophorbide *a*, pheophytin *a* and a kind of Carotinoids such as fucoxanthin, antheraxanthin, violaxanthin, alloxanthin, 19′-butanoloxyfucoxanthin, 19′-hexanoloxyfucoxanthin, prasinoxanthin, peridinin, echinenone, diadinox-

Table 1. Identification of peaks from HPLC Chromatogram

No.	Pigments identification	Rt.
1	Chlorophyll c_3	5.7
2	Chlorophyll *c*	6.3
3	Peridinin	9.1
4	19′-butanoloxy	10.1
5	Fucoxanthin	10.9
6	19′-hexanoloxy fuco.	12.7
7	Violaxanthin	13.7
8	Diadinoxanthin	14.9
9	Alloxanthin	18.8
10	Diatoxanthin	21.1
11	Lutein	22.3
12	Zeaxanthin	23.3
13	Chlorophyll *b*	32.4
14	Chlorophyll *a*	33.3
15	Echinenone	33.6
16	Phaeophytin *a*	34.9
17	β-carotene	36.1

anthin, diatoxanthin, β-carotene at study areas (Table 1), The spectrums of each extracted standard pigment can be seen below (Fig. 2).

The selection of solvents depends on S.W. Wright's (1991) Method and the calculation for concentration of standard pigments uses the well-known Reference (Jeffery *et al*. 1997) Method. The correction of results was performed with an extinction coefficient. The equation of calculation for concentration of standard pigments is as below (Park *et al*. 1997).

$$C\,(\mu g/L)=\frac{\text{Absorbance}}{(E:\ L\ g^{-1}\ cm^{-1}\,(cm)}\times\frac{10^6\,\mu g}{g}$$

C: Concentration of pigment (Area × RF)
E: Extinction coefficient
RF (Standard response factor): Peak area/Concentration.

RESULTS

1. Environmental conditions

Sea water temperature ranged 19.24～23.25°C (mean 21.24°C) at the surface and 13.41～20.52°C (mean 15.94°C) at the bottom from July 20～22. The stratification showed strong performance between surface and bottom in July. From August 14～16, the sea water temperature showed a range of 21.42 to 23.61°C (mean 22.75°C) at the surface and 15.60～21.59°C (mean 18.44°C) at the bottom. The variations of water temperature on from September 25～27 were in the range of 22.66～23.23°C (mean 22.94°C) at the surface and 15.31～23.03°C (mean 20.80°C) at the bottom. The stratification became weaker in September than July and August.

Table 2. Solvent of HPLC

Column	C18 (Waters)
Detector	Absorbance (Waters 2487) at 436 nm Fluorescence (Waters 474) at λ_{em}=650 nm, λ_{ex}=432 nm
Flow rate	0.5～1 mL min^{-1}
Solvent	A: MeOH 80%, Ammonium Acetate (0.5 M) 20%, BHT 0.01% B: Acetonitrile 87.5%, H_2O 12.5%, BHT 0.01% C: Ethyl Acetate 100%

Table 3. Solvent gradient system

Time	A (%)	B (%)	C (%)	Flow (mL min^{-1})
0	90	10	0	1
1	0	100	0	1
11	0	90	10	1
15	0	82.5	17.5	0.5
19	0	75	25	1
27	0	60	40	1
29	0	30	70	1
30	0	30	70	1
36	0	10	90	1
37	90	10	0	1
40	90	10	0	1

The monthly variation of salinity was 31.87～33.14 (mean 32.53) at the surface and 33.36～34.99 (mean 33.84) at the bottom from July 20～22, However, from distribution character August 14～16, the salinity was 24.80～31.91 (mean 30.60) at the surface and 31.36～34.48 (mean 33.06) at the bottom and the variation of salinity from September 25～27 was 29.26-31.53 (mean 30.41) at the surface but 29.72～34.41 (mean 31.75) at the bottom. The salinity variations were mostly 32.1～34.9. Which was a smaller difference between sampling stations and study times, except the samples of coast sites because of effected by local heavy rain in August. Their salinity was 24.8～29.2 lower than other areas. (Table 4)

The diatom for good growth need for ideal ratio of Si/N/P were 16 : 16 : 1 in marine. However, the results of this study showed the ratio were 3 : 10 : 1 when before red tide. It was 11 : 27 : 1 at during red tide and 2 : 26 : 1 at the finish of red tide, so that means it was different from an ideal ratio. In addition to the Relative Enrichment factors to ideal ratio during each survey were 187 : 601 : 100, 68 : 170 : 100, 12 : 118 : 100. These means a little available used Si during the red tide than before and after of red tide. So, the concentration of silicate SiO_2-Si showed a low level compared to ideal

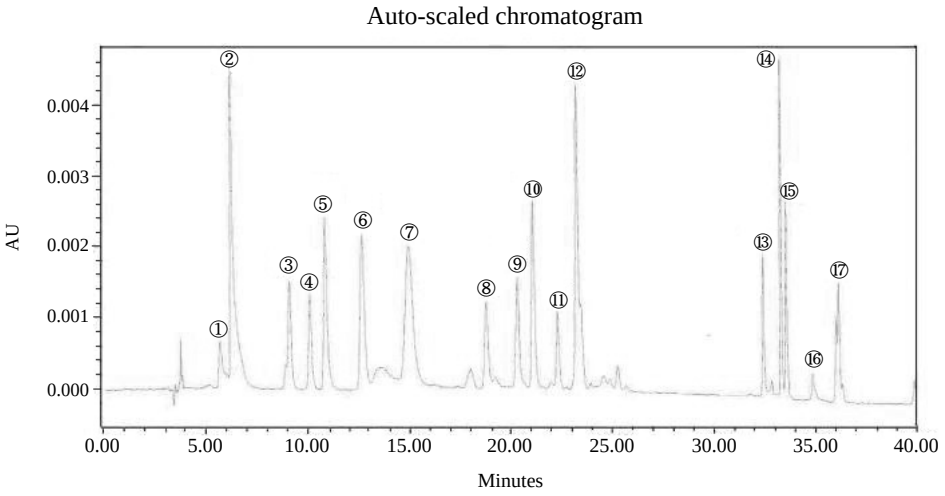

Fig. 2. Chromatogram of standard pigment.

Table 4. Temporal variations of surface water temperature, Salinity and nutrients during the study periods

Date	Layer	Temp. (°C)	Salinity	DIN (μM L^{-1})	DIP (μM L^{-1})	SiO_2-Si
20～22 July	Surface	19.24～23.25 (21.24±1.34)	31.87～33.14 32.53 (±0.33)	1.22～6.02 2.39 (±1.14)	0.12～0.6 0.30 (±0.12)	0.15～1.6 0.95(±0.63)
	Bottom	13.41～20.52 (15.94±2.41)	32.86～33.99 33.04 (±0.43)	3.87～25.17 15.29 (±6.04)	0.46～1.27 0.76 (±0.21)	
14～16 Aug.	Surface	21.42～23.61 22.75 (±0.46)	24.82～31.91 30.60 (±1.69)	0.93～62.64 8.21 (±14.15)	0.09～1.13 0.25 (±0.23)	1.23～5.44 2.86 (±1.55)
	Bottom	15.60～21.59 18.44 (±2.32)	24.82～31.91 30.60 (±1.69)	3.44～27.19 15.25 (±7.13)	0.23～0.85 0.57 (±0.21)	
25～27 Sep.	Surface	22.66～23.23 22.94 (±0.16)	29.26～31.53 30.41 (±0.57)	1.38～8.78 3.51 (±1.84)	0.05～0.39 0.17 (±0.08)	0.06～0.46 0.30 (±0.17)
	Bottom	15.31～23.03 20.80 (±3.01)	29.72～32.91 31.75 (±1.64)	1.78～31.86 10.66 (±10.45)	0.10～0.82 0.31 (±0.23)	

level during the red tide than before and after of red tide. It has been speculated that the silicate SiO_2-Si was used for the breeding and growth of diatom. It was assumed that the exceed DIN was absorbed as a constant ratio of DIN and DIP by the primary productor. If Exceed DIN has (−) of the value, the N can act as a limiting factor; conversely, it was (+) of the value means that P is the limiting factor (Wong *et al.* 1998). The results of Exceed DIN during the study as a Fig. 3. The just before red tide, the results showed (−) value of N in July which means N was a limiting factor, reversely, during the red tide, the value of P showed most positive (+) which means P was a limiting factor.

In September 27, the east side area (station 1～15) showed P acts as a limiting factor, when the red tide, *Cochlodinium plolykrikoides* still remained in this time. But, west side (station 16～20) showed N acts as the limiting factor when the red tide disappeared in this time.

2. The composition of photosynthesis pigments

The marine phytoplankton has a lot of pigments and was largely divided between the chlorophylls and carotinoids. All phytoplankton have a carotinoids as representative pigments of photosynthesis. This carotinoids consist of chlorophyll *a*, chlorophyll *b*, chlorophyll *c* and the derivatives of chlorophyll, such as chlorophyllide, pheophytin, and pheophorbide. The carotinoids contain fucoxanthin, peridinin, diadinoxanthin, diatoxanthin, lutein, zeaxanthin, alloxanthin, violaxanthin, prasinixanthin, neoxanthin, 19′-hexanoloxyfucoxanthin, 19′-butanoloxyfucoxanthin, α-carotene and β-

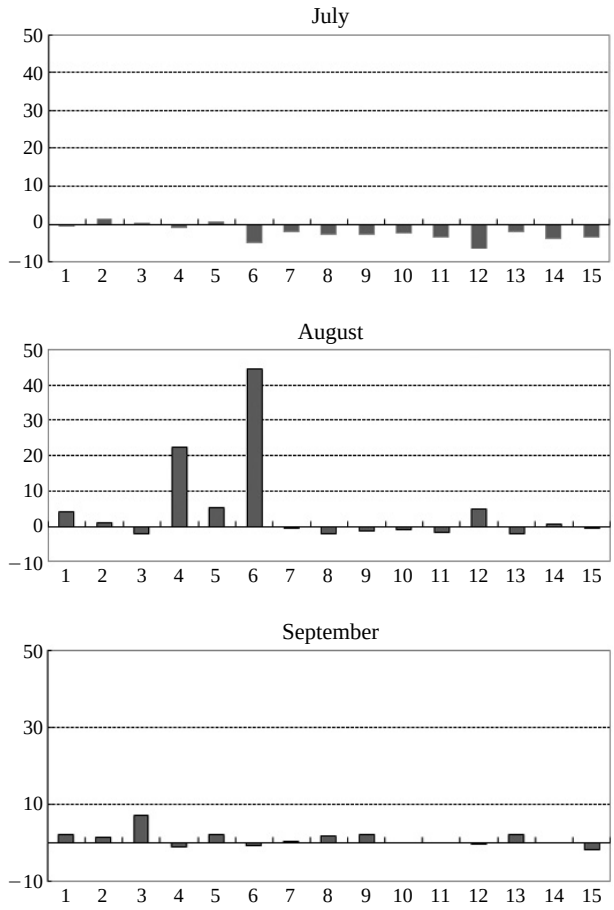

Fig. 3. Monthly variations of Excess DIN in study area.

carotene. These pigments have a unique composition in every phytoplankton as in Table 5.

These pigments are used as bio-markers without using a microscope, so we can estimate the composition of phytoplankton through pigment analysis with HPLC.

The photosynthesis pigments consist of three pigments of chlorophyll, 9 pigments of Carotenoid and the derivatives of chlorophyll asuch as pheophorbide *a* and pheophytin *a* (Table 1). Chlorophylls contains chlorophyll *a*, chlorophyll *c*, chlorophyll c_2 and chlorophyll c_3, and the Carotenoids contain Fucoxanthin, Peridinin, Diadinoxanthin, Diatoxanthin, 19-butanoloxyfucoxanthin, 19-hexanoloxyfucoxanthin, Violaxanthin, Prasinoxanthin, Alloxanthin, Zeaxanthin, Lutein and β-carotene.

The composition of assistance pigments was mostly detected with fucoxanthin as a marker of diatom besides chlorophyll *a* and the peridinin was a marker pigment of dinoflagellates, violaxanthin of chlorophytes, alloxanthin of cryptophytes, prasinoxanthin of prasinophytes, 19-hexanoloxyfucoxanthin of prymnesiophytes and β-carotene, diadinoxanthin, diatoxanthin, 19-butanoloxyfucoxanthin as detected from several algal group.

3. Distribution of photosynthesis pigment

The distribution of photosynthesis pigment was analyzed

Table 5. Summary of photosynthetic pigment distributions among the marine phytoplankton (Anderson *et al.* 1999)

Algal group	Major Accessory Pigment
Chlorophytes	Monovinyl chlorophyll *a*, chlorophyll *b*, lutein, neoxanthin, zeaxanthin, β-carotene
Chrysophytes	Monovinyl chlorophyll *a*, chlorophyll c_1 and c_2, violaxanthin, β-carotene
Diatom	Fucoxanthin, monovinyl chlorophyll *a*, diadinoxanthin, diatoxanthin, β-carotene
Dinoflagellates	Peridinin, monovinyl chlorophyll *a*, chlorophyll c_2, dinoxanthin, diadinoxanthin, diatoxanthin, β-carotene
Cryptomonads	Chlorophyll c_2, phycobilins
Cyanophytes	Phycocyanin, phycoerythrin, chlorophyll c_2, crocoxanthin, α-carotene

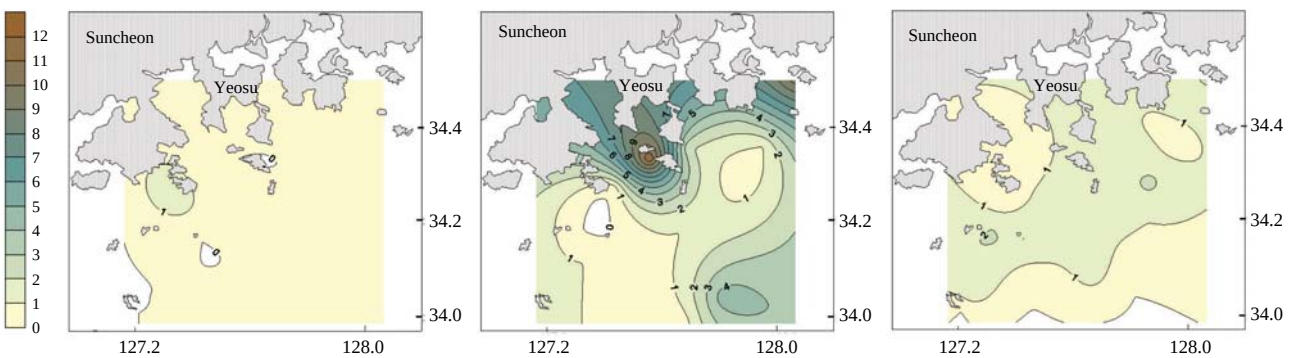

Fig. 4. Horizontal distributions of chlorophyll *a* during the study periods.

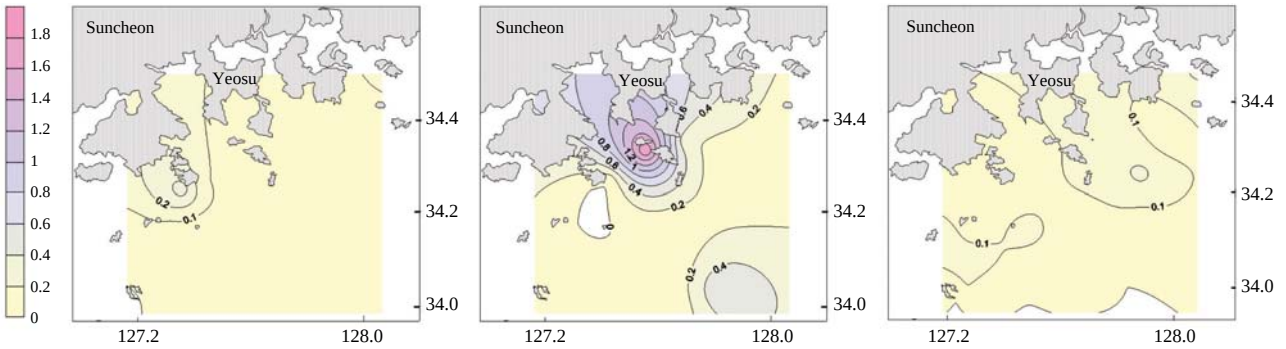

Fig. 5. Horizontal distributions of chlorophyll *c* during the study periods.

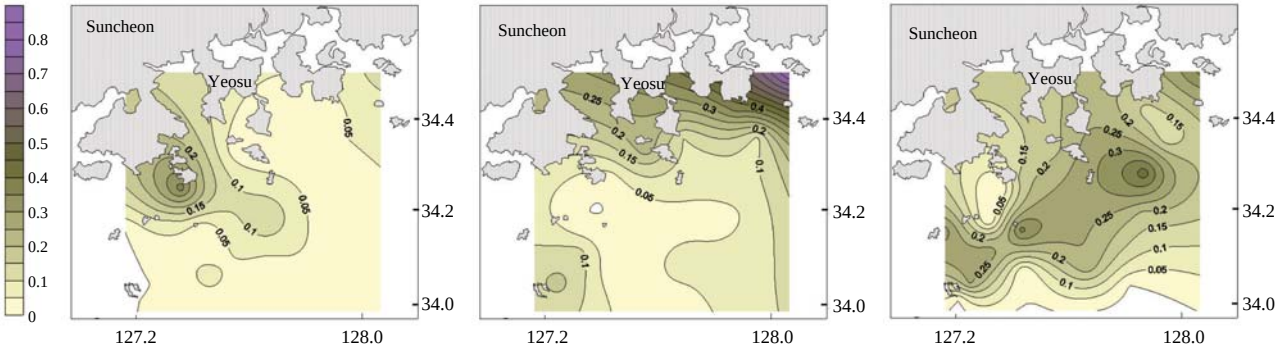

Fig. 6. Horizontal distributions of fucoxanthin during the study periods.

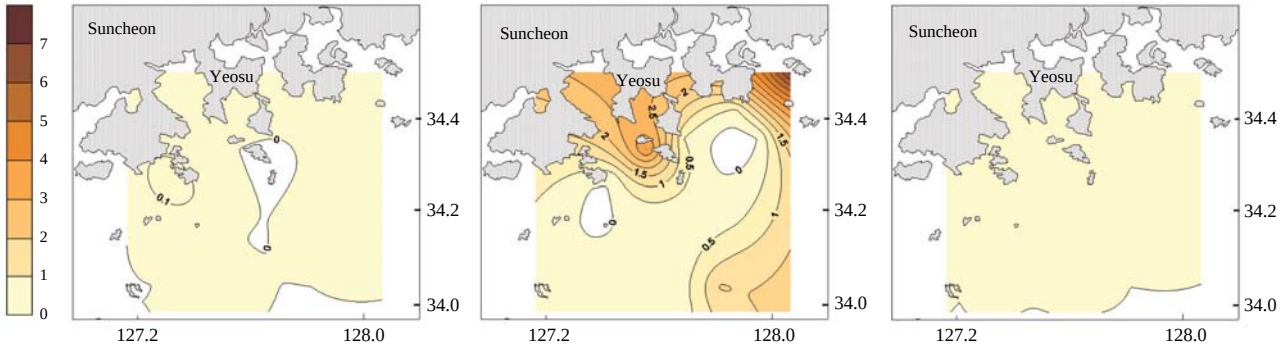

Fig. 7. Horizontal distributions of peridinin during the study periods.

a total of three times at 20 stations as in Fig. 1. before the outbreak of red tide (July 16～20), during the red tide season (August 16～20) and after extinction of red tide (September 24～28). According to the results, the chl. *a* on surface as in Fig. 4 showed 0.05～1.638 μg L^{-1} (mean 0.168 μg L^{-1}) before the outbreak of red tide, it was 0.05～11.908 μg L^{-1} (mean 2.226 μg L^{-1}) during the red tide season and it was 0.05～2.245 μg L^{-1} (mean 1.447 μg L^{-1}) at after extinction of red tide but the values of chl. *c* as in Fig. 5 showed nd～0.353 μg L^{-1} (mean 0.049 μg L^{-1}) before, it was nd～1.808 μg L^{-1} (mean 0.197 μg L^{-1}) at during and it was nd～0.228 μg L^{-1} (mean 0.08 μg L^{-1}) after extinction of red tide. On the other hand, the fucoxanthin as in Fig. 6 showed nd～0.376 μg L^{-1} (mean 0.064 μg L^{-1}) before the red tide, it was 0.004～0.803 μg L^{-1} (mean 0.122 μg L^{-1}) during red tide and its of nd～0.431 μg L^{-1} (mean 0.198 μg L^{-1}) after red ride and the peridinin as in Fig. 7 showed nd～0.173 μg L^{-1} (mean 0.0024 μg L^{-1}) before, it was 0.004～6.626 μg L^{-1}

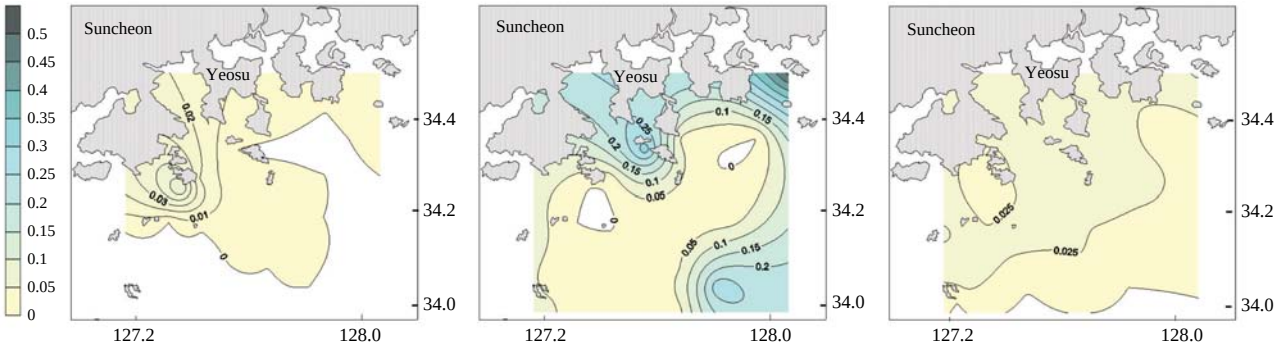

Fig. 8. Horizontal distributions of Diadinoxanthin during the study periods.

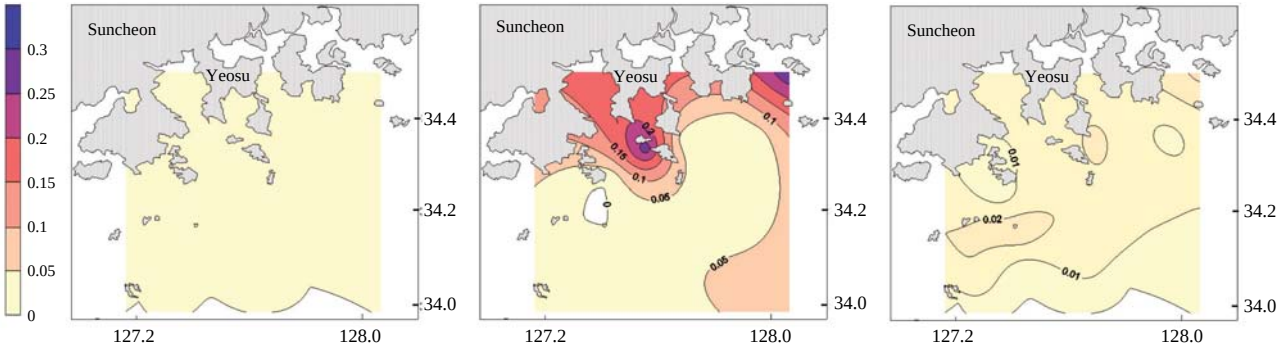

Fig. 9. Horizontal distributions of β-Carotene during the study periods.

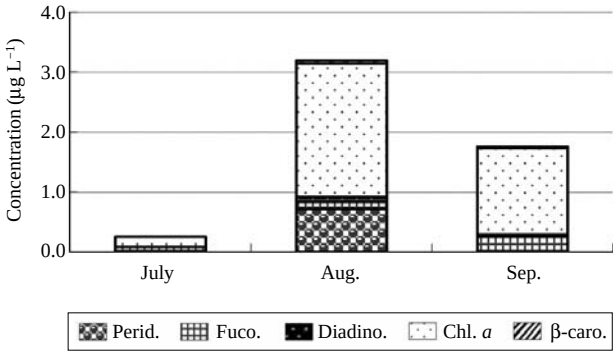

Fig. 10. Monthly variations of dominant pigments.

(mean 0.716 μg L^{-1}) during and it was nd ~ 0.041 μg L^{-1} (mean 0.020 μg L^{-1}) after the red tide and the diadinoxanthin as in Fig. 8 showed nd ~ 0.062 μg L^{-1} (mean 0.005 μg L^{-1}) before, its of nd ~ 0.516 μg L^{-1} (mean 0.079 μg L^{-1}) during, its of 0.004 ~ 0.054 μg L^{-1} (mean 0.033 μg L^{-1}) after the red tide, the β-carotene as in Fig. 9 indicated nd, before, its of 0.001 ~ 0.296 μg L^{-1} (mean 0.048 μg L^{-1}) during and its of 0.006 ~ 0.033 μg L^{-1} (mean 0.014 μg L^{-1}) after the red tide, the 19′-hexanoloxyfucoxanthin and prasinoxanthin mostly appeared during the red tide and the 19′-butanoloxyfucoxanthin mostly distributed in the middle in July, before the outbreak of red tide.

The abundance of detected main pigment showed 0.02 ~ 3.27 (mean 0.43 μg L^{-1}) on July 22 before the outbreak of red tide, it was a maximum value of 0.06 ~ 22.06 (mean 3.82 μg L^{-1}) at during and its of 0.15 ~ 3.21 (mean 1.51 μg L^{-1}) at after extinction of red tide.

The abundance of pigments indicated higher at the st. 1, Yeosu and st. 7, Tongyoung where there were more frequent outbreaks of red tide than at other stations (Figs. 10, 11).

DISCUSSION

The value of detected pigments analyzed to use the CHEMTAX (Mackey *et al.* 1996) program by taxon under MATLAB, which program analysis with taxon of phytoplankton as a degree of each of the marker pigments contribute to chl. *a*. The taxon of phytoplankton classified as diatom, dianoflagellates, prasinophytes, cryptophytes, chlorophytes,

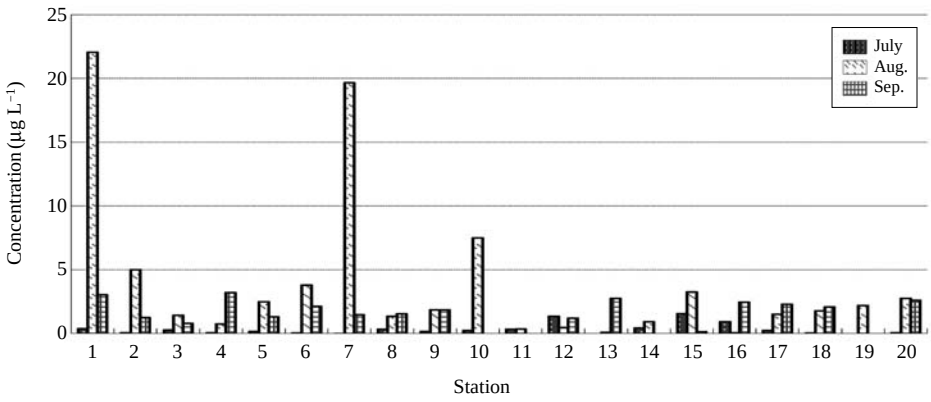

Fig. 11. Spatial and temporal variations of photosynthetic pigment abundance.

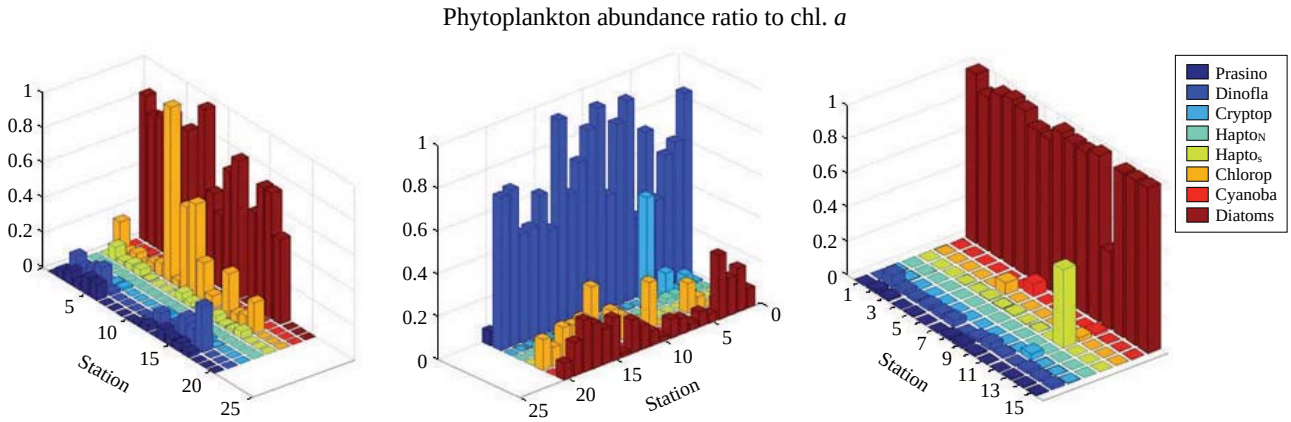

Fig. 12. Seasonal Variations of phytoplankton taxa by CHAMTEX program at Southern Coast, 2002 (L: July, M: Augst, R: September).

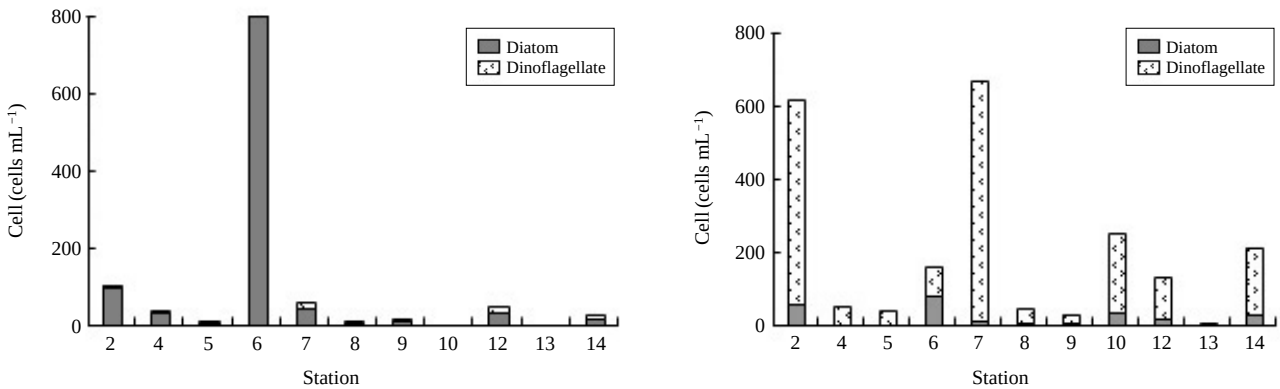

Fig. 13. Variations of dominant phytoplankton taxa by optical microscope (L: July, R: August).

Synechococcus (cyanobacteria), Haptophytes_N and Haptophytes_S.

As the results of the same as in Fig. 12-left, the diatom indicated dominant species on July 22 before the outbreak of red tide, the dianoflagellates showed dominant species during red tide with *Chochlodinium polykrikoides* as in Fig. 12-Middle and the diatom showed succession again after extinction of red tide as in Fig. 12-Right.

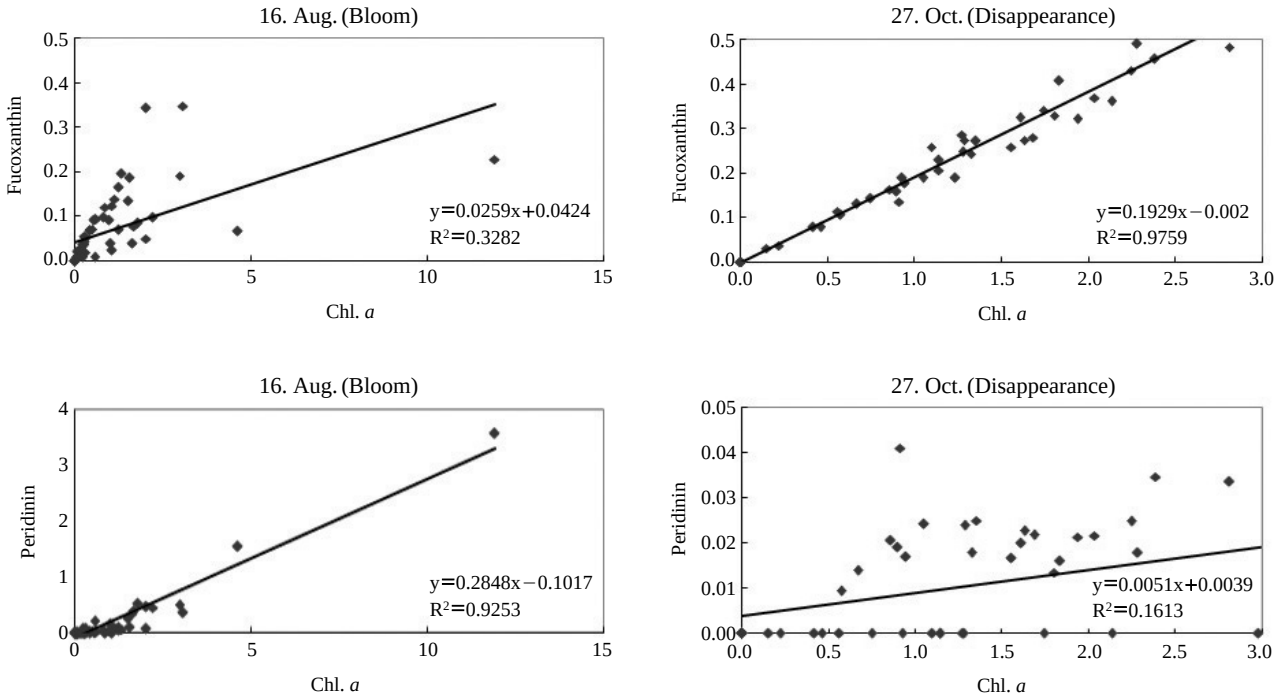

Fig. 14. Correlations between each pigment at red tide bloom and disappearance.

As the results of the identification by microscope (Fig. 13), the dominant species showed a diatom on July 22, before the outbreak of red tide and dinoflagellates during the red tide. These results accord very well with results of pigment analysis.

We investigated the relationship of dominant pigments with its of during the red tide and at the end of red tide. The peridinin showed more dominant during the red tide, *Cochlodinium polykriloides* than it's of before and after the red tide. The fucoxanthin which a marker of pigment of diatom indicated dominant pigment after extinction of red tide in the August. Using detected data of pigments, the relationship between chl. *a* and fucoxanthin was analyzed by regression analysis. The correlation coefficient (r^2) was shown to be 0.3282 during the red tide in August, Fig. 14, and 0.9759 after extinction of red tide. The diatom appeared as a dominant species, as in Fig. 14. Also, the correlation (r^2) with chl. *a* and peridinin, which was a marker of dinoflagellate, was 0.9253 during the red tide and 0.1613 after red tide. It was clearly shown to be specific of the red tide of dinoflagellate.

We performed a one-way ANOVA test using SPSS (ver. 10). The peridinin (a marker of dinoflagellate's pigment) was significantly higher ($p=0.032$) during the red tide. The result of the Pearson Correlation indicated that the relationship between the concentration of peridinin and chlorophyll *a* appeared as a positive correlation during the red tide season.

On the other hand, the fucoxanthin was significantly higher ($p=0.008$) after extinction of red tide. The concentration of chlorophyll *a* was significantly high ($p=0.001$) during the red tide. As shown by the results of the study about the relationship between pigments and investigated items of environment, the fucoxanthin showed a positive correlation ($r^2=0.404$, $p=0.009$) with water temperature and a negative correlation ($r^2=0.435$, $p=0.004$) with salinity but the chlorophyll *a* showed a negative correlation ($r^2=0.419=0.006$) with salinity.

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