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Seasonal Variations of Epilithic Biofilm Biomass and Community Structure at Byeonsan Peninsula, Korea[1a]

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한국 변산반도 암반생물막의 생물량과 군집구조의 계절 변화[1a]

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ABSTRACT

The community structure and abundance of epilithic biofilm were bimonthly examined to know spatial and temporal patterns of biofilm biomass and taxonimical composition at the two study sites, Gosapo and Gyeokpo with different degrees of wave exposure levels from November 2010 to September 2011. Biomass was estimated by using chlorophyll a contents (Chl *a*), normalized difference vegetation index (NDVI), and vegetation index (VI). Cyanobacteria such as *Aphanotece* spp. predominated in the proportion of 57.53% at Gosapo and of 61.12% at Gyeokpo and they are abundant in mid shore and in summer at both study sites. The diatoms *Navicula* spp., *Achnanthes* spp. and *Licmophora* spp. were common species and they showed an increasing trend from high to low shore. NDVI, VI, and chl a contents were the greatest at mid shore for Gosapo (0.44, 3.05, 24.56 ㎍/㎠) and at low shore for Gyeokpo (0.41, 2.73, 17.98 ㎍/㎠). NDVI, VI, and chl *a* content were all maximal in January and minimal in March at the both sites. Average NDVI, VI, and chlorophyll a contents of biofilms were greater at Gosapo (0.43, 2.89, 22.84 ㎍/㎠) than Gyeokpo (0.38, 2.48, 15.48 ㎍/㎠).Of three shore levels(high, mid, and low) Chl *a* contents were positively correlated with NDVI and VI at the two study sites indicating that non-destructive NDVI and VI values can be used in stead of destructive Chl *a* extraction method. In conclusion, epilithic biofilm was more abundant seasonally in winter, vertically in mid and low intertidal zone, and horizontally at wave exposed shore than in summer, at high and sheltered shore in Korea.

KEY WORDS: CHLOROPHYLL, EPILITHIC BIOFILM, VEGETATION INDEX, WAVE EXPOSURE

요 약

암반생물막의 군집구조와 생물량의 시, 공간적인 변화를 확인하기 위하여, 파도에 대한 노출이 다른 고사포와 격포에

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서 11월부터 2011년 9월까지 격월로 암반조각을 채집하였다. 군집구조는 채집된 암반조각을 칫솔로 긁어 광학현미경하에서 미세조류의 분류군별 개체수를 계수하여 분석하였고, 생물량은 NDVI, VI, 엽록소 *a* 농도를 측정하여 확인하였다. 고사포와 격포의 조간대 암반생물막에서 가장 우점하는 분류군은 *Aphanotece* spp., *Lyngbya* spp.를 포함하는 남조류였으며, 환경스트레스가 적은 조간대 하부에서는 규조류의 출현율이 높게 나타났다. 암반생물막에서 우점하는 규조류는 *Navicula* spp., *Achnanthes* spp.와 *Licmophora* spp.로 확인되었다. 식생지수와 엽록소 *a* 농도는 격포에 비해 고사포 생물막에서 높게 나타났다. 식생지수인 NDVI와 VI는 고사포에서 각각 0.49-0.40(평균 0.43), 2.64-3.22(평균 2.90)였으며, 격포의 암반생물막은 NDVI와 VI가 각각 0.32-0.41(평균 0.38), 2.03-2.86(평균 2.48)으로 확인되었다. 엽록소 a의 농도는 고사포에서 12.79-32.87 ㎍/㎠(평균 22.84 ㎍/㎠)였고, 격포에서는 11.14-18.25 ㎍/㎠(평균 15.48 ㎍/㎠)로 식생지수와 마찬가지로 1월(겨울)에 최대, 3월(봄)에 최소인 계절 변화를 보였다. 엽록소 *a* 농도는 NDVI, VI와 양의 상관관계를 보여 비파괴적인 식생지수 측정방법이 파괴적인 엽록소 a 추출 방법을 대체할 수 있음을 알려준다. 결론적으로 암반생물막은 여름보다 겨울에, 조간대 상부보다 중부와 하부에서, 파도에 보호된 해안보다 노출된 해안에서 높은 값을 보였다.

주요어: 엽록소, 암반생물막,식생지수, 파도노출

INTRODUCTION

Epilithic biofilms are ecologically important components of rocky shore intertidal areas, where they exhibit high spatio-temporal variability because of tidal cycles and wave actions (Hawkins and Hartnoll, 1983; Leigh *et al*., 1987; Lamontagne *et al*., 1989; Thompson *et al*., 2004). The biofilms are taxonomically diverse and composed of bacteria, cyanobacteria, diatoms, euglenoids and macro-algal germlings (MacLulich, 1987; Murphy *et al*., 2005). They contribute important ecological roles as primary producers, food resource for herbivorous animals, and induction for larval dispersal settlement of many sessile invertebrates (Castenholz, 1961; Underwood, 1984a; Yallop *et al*., 1994; Thompson *et al*., 1998; Jackson *et al*., 2010).

It is known that epilithic biofilms show seasonal and spatial distribution, and that their abundances are closely linked to environmental conditions even in different tidal levels of the same rocky shore (MacLulich, 1987; Dye and White, 1991). The biofilms are typically more abundant in the low shore than in the high one (Castenholz, 1963; Underwood, 1984b; Thompson *et al*., 2004; Jackson *et al*., 2010). Such biofilm vertical distribution is caused by environmental gradients of desiccation, temperature and light (Underwood, 1984b; Thompson *et al*., 2004). Biofilms also show seasonal variation, with biofilm biomass being higher between late autumn- winter and generally being higher on temperate shores in comparison to tropical areas (Underwood, 1984b; MacLulich, 1987; Ruban and Horton, 1995; Jenkins and Hartnoll, 2001; Jackson *et al*., 2010). Biomass is also greater on wave-exposed shores as compared to sheltered shores (McQuaid and Branch, 1984; Thompson *et al*., 2004, 2005). Thus, the distribution and abundance of intertidal epilithic biofilms are important biological parameters that reflect specific environmental conditons (McQuaid and Branch, 1984; Thompson *et al*., 2005).

Quantification of epilithic biofilm is complex and typically is estimated directly by counting cell numbers using microscopy (Jones, 1974; Underwood, 1984b; Hill and Hawkins, 1991; Nagarkar and Williams, 1997; Norton *et al*., 1998; Chan *et al*., 2003) and indirectly by measuring the amount of chlorophyll a (Underwood, 1984b; Jackson *et al*., 2010). Chlorophyll a measurement by using spectrophotometry or High Performance Liquid Chromatography (HPLC) is difficult, expensive and time-consuming. Thus, there is a growing interest in using simpler and non-destructive techniques to estimate epilithic microalgal abundance as the ones recently used in microphytobenthos biofilm studies. These include pulse amplitude modulated (PAM) fluorometry and spectroradiometry (Kromkamp *et al*., 1998; Honeywill *et al*., 2002; Perkins *et al*., 2002; Murphy *et al*., 2005; Barillé *et al*., 2011). Murphy *et al*.(2008) reported some advantages of those remote-sensing techniques for quantifying the abundance of microalgae.

The aims of the current study were to examine the community structure and abundance of epilithic biofilms along shore gradients and to detect seasonal patterns of biofilm biomass and taxonimical composition at two study sites with different degrees of wave exposure. Epilithic biofilm biomass was examined using a combination of direct cell counts and indirect chlorophyll a extraction method. Furthermore, biofilm biomass was non-destructively quantified by spectral reflectance using the normalized difference vegetation index (NDVI) and a simple vegetation index (VI) in order to evaluate the possibility of using remote-sensing technique.

MATERIALS AND METHODS

1. Sampling

Rock chips with epilithic biofilms were collected bi-monthly from the intertidal zone of Gosapo (35°39′ N, 126°30′ E) and Gyeokpo (35°38′ N, 126°27′ E), Byeonsan, Korea, from November 2010 to September 2011. The levels of wave exposure at the two study sites were measured using a dynamometer, which was made by the following protocol of Bell and Denny(1994). Relative levels of wave exposure, from zero (no movement) to 1 (full tie length), were calculated using the moving distance of the rubber indicator connected to the practice golf ball with a nylon cable tie. The Gosapo shore was relatively more exposed than Gyeokpo shore. From each sites, rock chips with microbial biofilms were obtained by using a chisel at three intertidal shore levels (high, mid, and low) and transferred to the laboratory. The chips were sprayed with seawater occasionally and kept overnight exposed to air at room temperature (ca. 20℃). At each sampling date, a total of 66 rock chips, including 44 chips for chlorophyll a extraction and 18 chips for community structure analyses, were sampled.

2. Reflectance

Reflectance spectra were measured using a spectroradiometer (USB2000, Ocean Optics, USA). Reflectance was determined from the light spectrum reflected from the undisturbed rock chips, normalized to spectrum reflected from a reference white panel. A reflectance spectrum measured in the darkness was subtracted to the sample and to the white reference spectra in order to remove machine dark current noise. Reflectance spectra were measured on eight rock chips at each shore level. Reflectance measurements were used to estimate epilithic biofilm biomass by calculating the normalized difference vegetation index (NDVI, Rouse *et al*., 1973) and vegetation index (VI, Jordan, 1969). The NDVI and VI were calculated as follows (Jordan, 1969; Rouse *et al*., 1973):

NDVI = (Infrared - Red) / (Infrared + Red)
VI = Infrared / Red

where Infrared is the average reflectance in the range of 748-752 ㎚ and Red is the average reflectance in the range of 673-677 ㎚.

3. Chlorophyll *a* concentration

To examine temporal-spatial variation in the chlorophyll a content and the correlations between vegetation indices (NDVI or VI) and chlorophyll a, a total of 48 rock chips (eight replicates on each shore level at two study sites) were measured for reflectance followed by chlorophyll a extraction. This was a necessary calibration step to be able to use the reflectance indices to trace seasonal and vertical changes in the epilithic microalgal biomass. Chlorophyll a was extracted following the method of Thompson *et al*.(1999) using 100% methanol solvent. For efficient chlorophyll a extraction, the rock chips were immersed in seawater for 30 min prior to processing (Thompson *et al*., 1999). Each rock chip was placed into a wide mouth screw-top jar (250 ㎖) and left at room temperature (ca. 20℃) in the darkness for 12 h. Absorbance for the extracts was measured at Å665 and Å750 using a spectrophotometer (Libra S22, Biochrom, England). Rock chip surface area was measured with an Image J program. Chlorophyll a concentration was calculated as follows:

$$\text{Chlorophyll } a \text{ concentration } (\mu g/cm^2) = \frac{13.0 \times Å_{net} \times v}{d \times a}$$

where 13.0 is a constant for methanol, Ånet = Å665 - Å750, v = final volume of solvent, d = path length of spectrophotometer cell (usually 1 ㎝), a = area of rock chip that the biofilm covered.

4. Community structure

Community structure of epilithic biofilms was examined from the three rock chips, which were randomly collected at each intertidal shore level, from January to September 2011. Epilithic biofilm was removed from each rock chip using a tooth brush and placed into a petri dish (Ø 6 ㎝) containing seawater. The biofilm solution was thoroughly mixed with a plastic pipette, sub-sampled twice, identified, and counted epilithic microalgal cells under a light microscope (Olympus CX41, Philippines). A minimum of 100 cells were counted in each petri dish and the relative abundance of major taxonomic groups (cyanobacteria, diatoms and green algae) were determined. Abundant species of each taxon group were further identified at genus levels by following the classification of previous researchers (Shim, 1994; Ray, 2006; Al-Thukair *et al*., 2007).

5. Derivative analysis

Epilithic biofilms including various taxonomical microalgal groups exhibit in mixed spectral reflectance spectra with some overlapping pigment absorption features (Murphy *et al*., 2005), which are difficult to differentiate using spectral reflectance. Second derivative analysis solves some of these problematic features and allows distinguishing the different pigments present in the biofilm (Murphy *et al*., 2005; Jesus *et al*., 2008). Pigment absorption features are detected in the second derivative spectra as derivative peaks where peak centres correspond to the maximum absorption wavelengths of the pigment responsible for that particular peak. Second derivative spectra were calculated by following the method of Laba *et al*.(2005).

6. Statistical analysis

Statistical analysis was carried out using STATISTICA version 10.0 software. A one-way ANOVA (analysis of variance) was used to test the difference in biofilm biomass between the two sites over the study period. For each a site, two-way ANOVA was used to determine the difference in biofilm biomass between the season and tidal levels. The significance of the differences between mean values was evaluated with the Tukey HSD test (Sokal and Rohlf, 1981). Cochran's test was used to verify homoscedasticity, and data transformations were applied when necessary.

RESULTS

1. Biofilm Biomass

Bimonthly NDVI values varied between 0.40-0.49 at Gosapo and from 0.32 to 0.41 at Gyeokpo (Figure 1). Maximal NDVI were found in January and minimal in March in the both sites. Annual average NDVI values were greater at Gosapo (0.43±0.01, mean±SE) than Gyeokpo (0.38±0.01) and there was significant differences between the sites ($F1,10 = 6.74$, $p < 0.05$). Similarly, VI values of epilithic biofilms were higher at Gosapo ranging from 2.64 to 3.22 (2.90±0.08, mean±SE) as compared to the values of at Gyeokpo shore, which were between 2.03-2.86 (annual average, 2.48±0.12). Significant difference

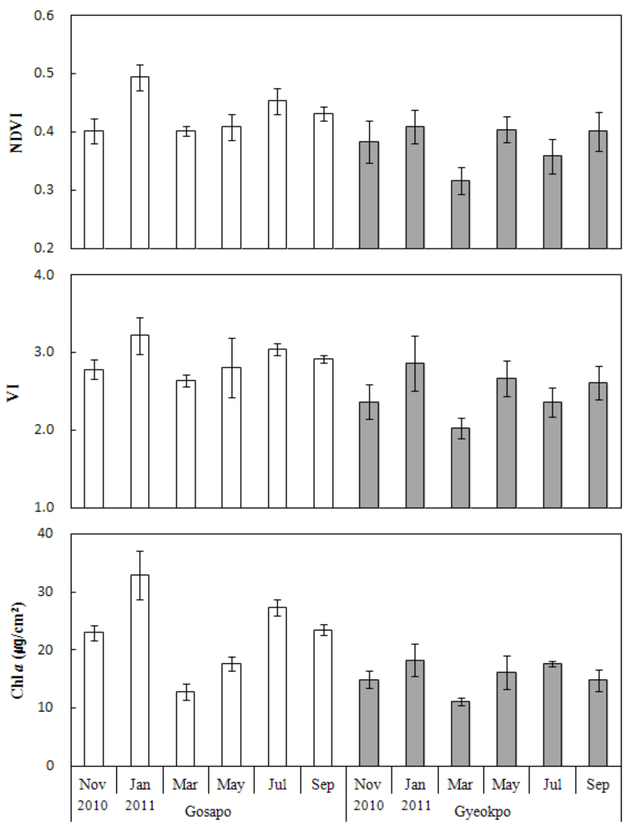

Figure 1. Biomonthly variations of average NDVI, VI, and chlorophyll a concentration (㎍/㎠) of epilithic biofilms collected at the two study sites of Byeonsan Peninsula, Korea during the study period. Vertical bars represent standard errors (n = 3 replicates)

was found in annual average VI value between the two study sites ($F_{1,10}$ = 8.31, $p < 0.05$). Chlorophyll a concentration fluctuated in the range from 12.79 to 32.87 ㎍/㎠ for Gosapo shore and between 11.14-18.25 ㎍/㎠ for Gyeokpo (Figure 1). Annual average Chl *a* contents of epilithic biofilm were 22.84 ㎍/㎠ and 15.48 ㎍/㎠ at Gosapo and Gyeokpo shore, respectively. It was significantly different between the two sites ($F_{1,10}$ = 5.36, $p < 0.05$).

At Gosapo, NDVI values of epilithic biofilm varied from 0.39 to 0.46 at high shore, from 0.40-0.53 at mid, and 0.37-0.50 at low shore (Figure 2). Average NDVI of Gosapo was greatest at mid shore (0.44±0.02, mean±SE), followed by low shore (0.43±0.02) and high shore (0.42±0.01). However, NDVI values were very variable between shore levels over the study period and no significant differences were found (Table 1). VI values of Gosapo biofilm were maximal at mid (3.05±0.16) and minimal at high shore (2.80±0.07). Seasonal patterns of VI values were very similar to NDVI patterns and the values fluctuated in the range of between 2.51-3.01 at high shore, 2.48-3.60 at mid shore, and 2.35-3.28 at low shore. Two-way ANOVA test revealed that there were no significant differences in the VI values among the three shore levels (Table 1). Chl *a* concentrations of Gosapo biofilm were 24.56 ㎍/㎠ for mid and 23.83 ㎍/㎠ for low shore and they were significantly greater than 20.13 ㎍/㎠ of high shore. Seasonally, chl a contents of biofilms varied from 10.12 to 24.76 ㎍/㎠ at high shore, from 14.48 to 38.85 ㎍/㎠ at mid, and from 13.76 to 34.98 ㎍/㎠ at low rocky shore of Gosapo. At the three shore levels, chl a contents showed a clear seasonal pattern: maximal in January and minimal in March (Figure 2). ANOVA test revealed that chl a concentration was significantly different among collection times and among shore levels but no interactions were found between season and shore level (Table 1).

In case of Gyeokpo shore, NDVI values were significantly

Table 1. Results of two-way ANOVA and Tukey HSD tests (p = 0.05) for the effects of seasons and shore heights on NDVI, VI and chl a concentration of epilithic biofilms collected at exposed Gosapo and sheltered Gyeokpo shores. Chl a concentration data of Gosapo shore and VI data of Gyeokpo shore were log(x) transformed

Source of variation	df	NDVI			VI			Chl a concentration		
		MS	F	p	MS	F	p	MS	F	p
Gosapo (Exposed)										
Season (S)	5	0.03	1.11	0.36	1.13	0.61	0.69	1198.92	24.45	<0.001
Shore height (H)	2	0.00	0.07	0.93	0.90	0.48	0.62	271.28	5.53	<0.01
S × H	10	0.01	0.27	0.99	0.65	0.35	0.97	67.01	1.37	0.20
Residuals	126	0.03			1.86			49.03		
Total	146									
Tukey HSD test (p=0.05)										
Season		Nov=Jan=Mar=May=Jul=Sep			Nov=Jan=Mar=May=Jul=Sep			Jan=Jul, Nov=Jul=Sep, Jan>Nov=Sep>May>Mar, Jul>May>Mar		
Shore height		High=Mid=Low			High=Mid=Low			High<Mid=Low		
Gyeokpo (Sheltered)										
Season (S)	5	0.03	1.33	0.25	2.01	1.57	0.17	157.08	4.20	<0.01
Shore height (H)	2	0.11	4.81	<0.01	6.63	5.18	<0.01	352.40	9.42	<0.001
S × H	10	0.00	0.11	1.00	0.24	0.19	1.00	36.46	0.98	0.47
Residuals	126	0.02			1.28			37.39		
Total	146									
Tukey HSD test (p=0.05)										
Season		Nov=Jan=Mar=May=Jul=Sep			Nov=Jan=Mar=May=Jul=Sep			Nov=Jan=Mar=May=Jul=Sep, Jan>Mar, Jul>Mar		
Shore height		High<Mid=Low			High<Mid=Low			High<Mid=Low		

higher at low shore (0.41±0.02, mean±SE) than at high shore with 0.32±0.01 (Table 1). NDVI values of epilithic biofilm ranged between 0.27-0.36 for high shore, between 0.33-0.44 for mid, and between 0.35-0.45 for low shore (Figure 2). However, there was no significant interaction between season and shore level (Table 1). VI value of epilithic biofilm was 2.73±0.15 at low and 2.05±0.07 at high shore and they were significantly different among the shore levels (Table 1). Seasonal fluctuations of VI values were found in the range of between 1.78-2.19 at high, 2.11-3.24 at mid, and 2.22-3.18 at low shore (Figure 2). However, no interactions between season and shore level were found (Table 1). Chl a contents of Gyeokpo shore fluctuated from 12.60 to 17.98 ㎍/㎠ along the shore gradients and they were significantly greater at low shore than high rocky shore (Table 1). At high shore, chl a concentration was minimal (10.24 ㎍/㎠) in March and maximal with 16.85 ㎍/㎠ in July. At mid and low shore,

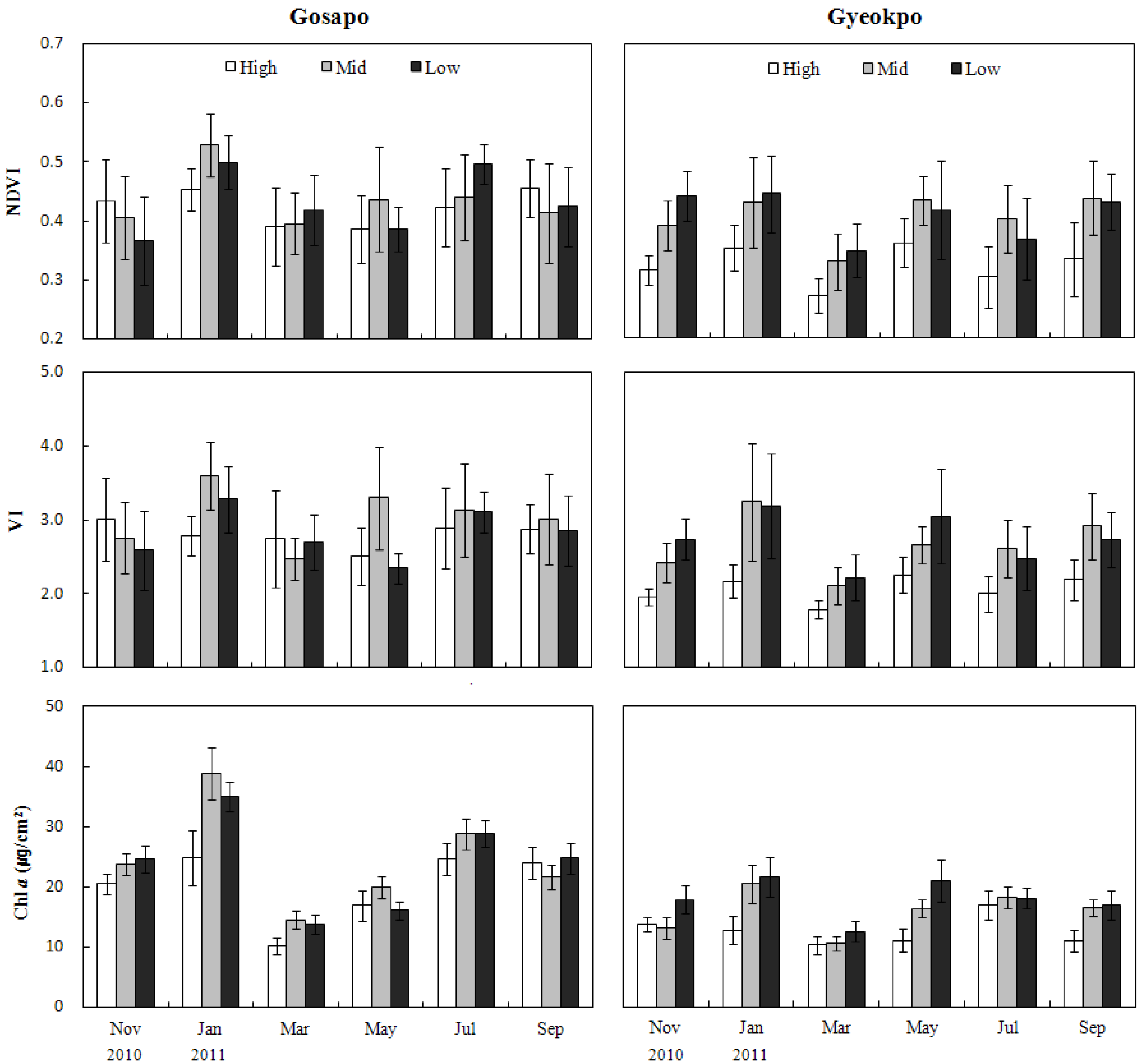

Figure 2. Bimonthly variations of NDVI, VI and chlorophyll a concentration (㎍/㎠) of epilithic biofilms collected from different shore heights of Gosapo and Gyeokpo shores, Byeonsan Peninsula, Korea over the study period. Vertical bars represent standard errors (n = 8 replicates)

chl a contents of epilithic biofilms showed similar seasonal pattern; minimal in March and maximal in January in the range from 10.62 to 20.45 ㎍/㎠ at mid and from 12.55 to 21.55 ㎍/㎠ at low shore (Figure 2).

2. Chl *a* contents vs. NDVI, or VI

Chl *a* content of epilithic biofilms was positively correlated with NDVI and VI at the two study sites. Correlations between Chl *a* concentration and NDVI were stronger at Gosapo (r^2 = 0.58, n = 18) than at Gyeokpo (r^2 = 0.52, n = 18) (Figure 3A, C). Whereas, the correlations of Chl *a* concentration and VI were higher at Gyeokpo (r^2 = 0.53, n = 18) than at Gosapo (r^2 = 0.70, n = 18) (Figure 3B, D).

3. Community structure

Average relative proportions were greater in cyanobacteria (59.33%), followed by green algae (32.40%) and diatoms (8.27%). Cyanobacteria predominated at the two study sites, over the study period. The proportion of cyanobacteria ranged from 41.53% in September to 69.38% in July (mean 57.53%) at Gosapo and from 54.73% in September to 74.48% in March (mean 61.12%) at Gyeokpo shore. Relative abundance of green algae ranged from 11.70% in July to 44.81% in May (mean 30.86%) at Gosapo and 20.56% in March to 39.61% in September (mean 33.95%) at Gyeokpo shore. On the Gosapo rocky shore, the proportions of diatoms varied from 5.79% to 20.19% (mean 11.61%) and were minimal in January and maximal in September. Diatoms were less abundant at Gyeokpo shore exhibiting a relative variation from 4.02% to 5.66 with a mean value of 4.93% and were minimal in July and showed maximal value in September. Relative abundance of diatoms was about two times greater at Gosapo than at Gyeokpo shore, but proportion of green algae was no different among the two study sites.

Along Gosapo shore gradient, the annual average propo-

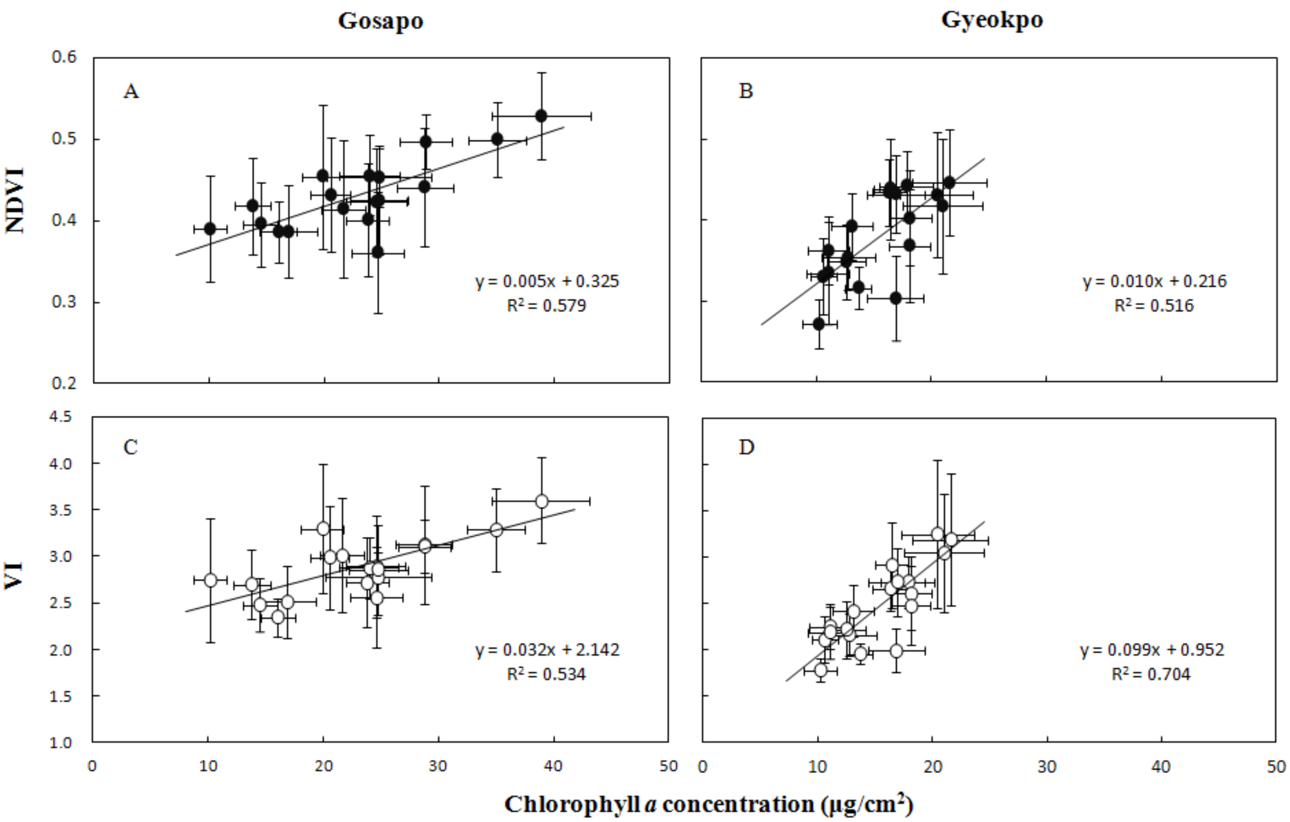

Figure 3. Correlations between chlorophyll a concent vs spectral reflectance index (NDVI and VI) of epilithic biofilms collected from Gosapo (A, C) and Gyeokpo (B, D). Data presented in average value of eight rock chips sampled at each sampling time from each shore height. Bars show standard errors

rtion of cyanobacteria was greatest (59.39%) at mid shore (from 41.42% in September to 69.40% in July), followed by 58.47% at high (from 36.76% in May to 73.48% in July), and 54.74% at low shore (from 30.79% in September to 70.52% in March). Green algae were more abundant at high shore (36.15%) followed by mid shore (29.49%) and low shore (26.93%). At high shore of Gosapo, the relative proportion of diatoms fluctuated seasonally from 1.80% in January to 12.49% in July (mean 5.38%). Green algae in the relative proportion were also changed from 4.21% (January) to 20.91% (July) with mean value of 11.12% at mid shore and from 9.69% (March) to 35.45% (September) with mean value of 18.33% at low shore. Diatoms showed an increasing trend from high to low

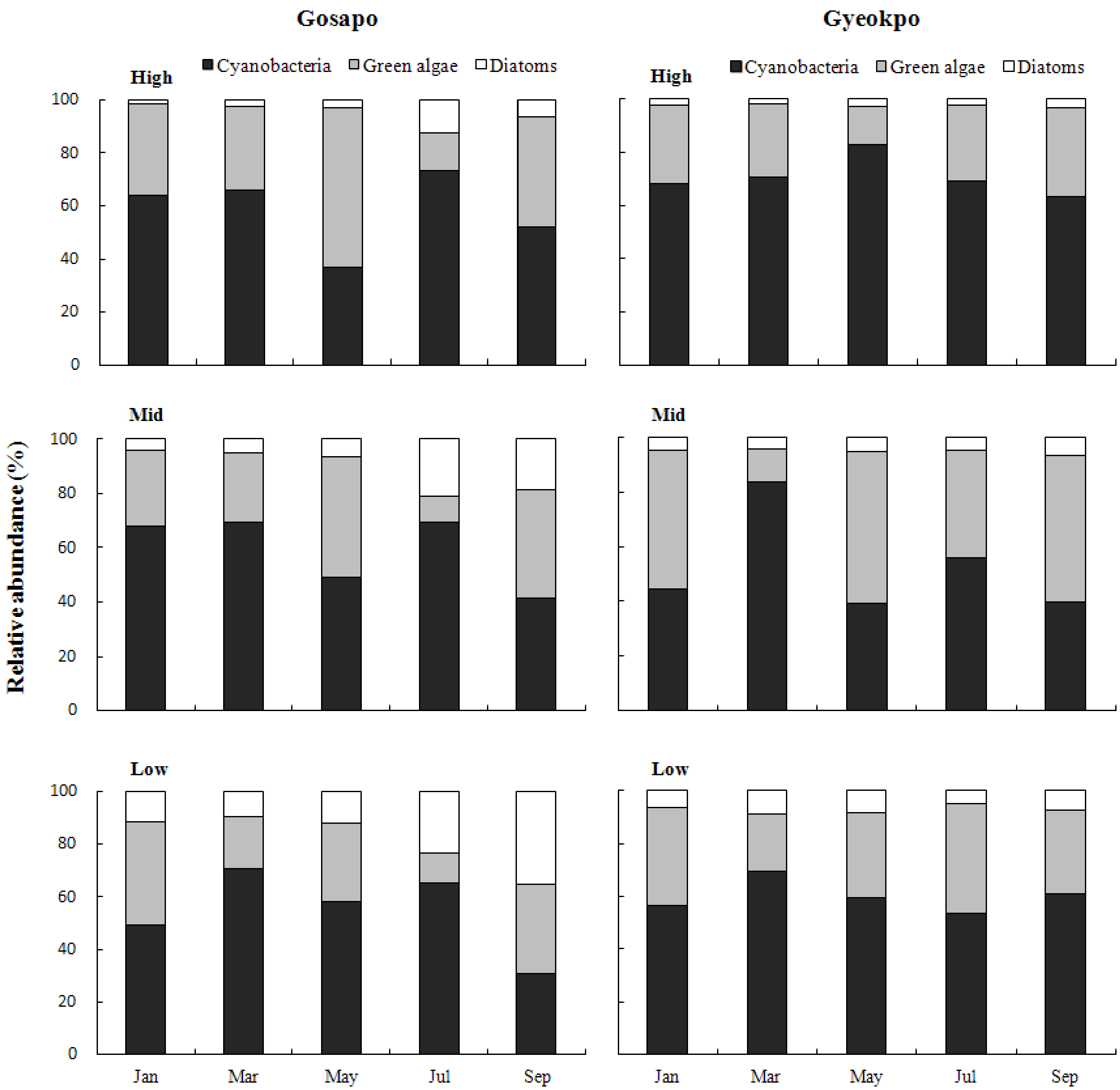

Figure 4. Seasonal variations of relative abundance of the major taxonomic groups of three intertidal shore levels at the two study sites of Byeonsan Peninsula, Korea, from January to September 2011. Data showed as mean values of three replicates

shore during the study period (Figure 4). In case of Gyeokpo shore, annual relative abundance of cyanobacteria was 70.74% at high shore (from 63.16% in September to 82.96% in May) and minimal 52.64% at mid shore (from 39.32% in May to 83.54% in March). The proportion of green algae was greater on mid shore (42.23%) than high (26.81%) and low shore (32.82%). The relative abundance of diatoms varied from 1.82% in March to 3.11% in September (mean 2.46%) on high shore, from 4.12% in March to 6.69% in September (mean 5.13%) on mid shore and from 4.74% in July to 8.96% in March (mean 7.20%) on low shore. Similar to Gosapo, the greatest proportion of diatoms was observed at low shore and decreased to high shore (Figure 4).

Cyanobacteria Aphanotece spp. was found to be the dominant genus at the two study sites, whereas *Lyngbya* spp. was the most representative genus in May and July. The diatoms, *Navicula* spp., *Achnanthes* spp. and *Licmophora* spp. were the most common species at the both sites over the study period. Several diatom planktonic species, such as *Coscinodiscus* spp. and *Paralia sulcata* were also observed.

4. Derivative analysis and pigments

The second derivative spectrum showed a double peak at between 660 and 700 ㎚ (Figure 5). The most dominant feature in the second derivative spectra is the sharp peak at about 680 ㎚ (peak 10 in Figure 5). Average height of the derivative peak at ~ 680 ㎚ was 0.022 at Gosapo and 0.015 at Gyeokpo.

At the two study sites, average second-derivative spectra were very variable with eight peaks at wavelengths of between 430 and 650 ㎚ (Figure 5). In the range of wavelength, three prominent chlorophyll (a, b, c) and chlorophyllide a absorption features were located at 432, 469, 597 and 646 ㎚ (peaks 1, 2, 6 and 8). An important feature was observed at 541 ㎚ (peak 4) indicating absorption by fucoxanthin, a pigment found primarily in diatoms. Average height of the derivative peak 4 was greater at Gosapo (0.0004) than at Gyeokpo (0.0003). Also, phycoerythrin and phycocyanin absorption features were located at 576 and 618 ㎚ (peak 5 and 7, respectively), a pigment found in cyanobacteria. Similar to peak 4, height of peak 5 was six times greater at Gosapo (0.0006) than at Gyeokpo (0.0001) and peak 7 was about two times greater at Gosapo (0.0012) than at Gyeokpo (0.0005) (Figure 5).

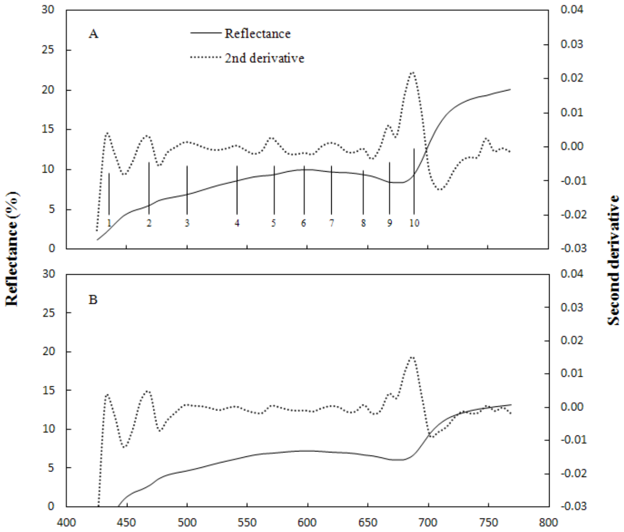

Figure 5. Average annual reflectance spectra and second derivative spectra of epilithic biofilms collected from Gosapo (A) and Gyeokpo (B) rocky shore of Byeonsan Peninsula, Korea, from November 2010 to September 2011 (n = 6 replicates). 1, 432 ㎚ (chlorophyll a); 2, 465 ㎚ (chlorophyll c); 3, 498 ㎚ (diadinoxanthin); 4, 541 ㎚ (fucoxanthin); 5, 576 ㎚ (phycoerythrin); 6, 597 ㎚ (chlorophyll c); 7, 618 ㎚ (phycocyanin); 8, 646 ㎚ (chlorophyll a, chlorophyll c, chlorophyllide a); 9, 667 ㎚ (chlorophyll a), and 10, 687 ㎚ (chlorophyllide a)

DISCUSSION

Cyanobacteria were the dominant microbial group in epilithic biofilms on a tropical shore in Hong Kong (Nagarkar and Williams, 1997, 1999) and in the southern Gulf of Mexico (Ortega-Morales *et al*., 2005). This taxonomical group was also recorded as the most abundant group on the southern temperate shore of Australia (MacLulich, 1987; Jackson *et al*., 2010). However, diatoms were the dominant taxonomical group in biofilms from temperate rocky shores around UK (Hill and Hawkins, 1991). In the present study, epilithic microalgal assemblages of Gosapo and Gyeokpo shores of Byeonsan Peninsula were mainly composed of cyanobacteria with an average of 59.33%. Epilithic biofilm composition was different even in the temperate rocky shore of between UK and Korea in northern hemisphere, which might result from different environmental condition such as seawater temperature. Also, dominant species in cyanobacteria was different; *Anacystis* sp. in Australia (MacLulich, 1987) and *Aphanotece* spp. and *Lyngbya* spp. in the present study sites. Thus, it is worth to note that cyanobacteria group is the major taxon and dominant epilithic biofilm species are *Aphanotece* spp. and *Lyngbya* spp. in the temperate Korean coasts of northern Pacific region.

The biomass of epilithic biofilm, which were estimated with chlorophyll a concentration as a proxy, fluctuated seasonally and spatially (Thompson *et al*., 2005; Jackson *et al*., 2010). In temperate rocky shore, biofilm was abundant in the winter and died back in the summer (Underwood, 1984b; Thompson *et al*., 2005; Jackson *et al*., 2010). In this study, Chl a concentration showed a clear seasonal pattern as maximal in January and minimal in March at the two study sites. Also, Chl a content was greater in the mid and low intertidal zone (20.21 ㎍/㎠ and 20.91 ㎍/㎠, respectively) than in the high shore (16.36 ㎍/㎠). Such a vertical biomass distribution of epilithic biofilms might be negatively correlated to the strength of environmental stress because less biomass was recorded on upper shore showing more severe stressful conditions (Thompson *et al*., 2005). Especially, abundance of diatoms was increased with decreasing stress such as insolation-exposure (Castenholz, 1963; Thompson *et al*., 2004). They are consistent, which is maximum relative proportion of diatoms on lower shore.

Annual average Chl *a* content was 22.84 ㎍/㎠ at Gosapo and 15.48 ㎍/㎠ at Gyeokpo shore in our result. The amount of Chl *a* content was very variability and low on intertidal rock surfaces in Portugal ranged from 1.66 to 2.80 ㎍/㎠ (Boaventura *et al*., 2002). Chlorophyll a contents were between 4-9 ㎍/㎠ on artificial panels established in St Lawrence estuary, Canada (Lamontagne *et al*., 1989). In Chile, Chl *a* concentration of epilithic microbial biofilm was slightly different; 5.36 ㎍/㎠ in Santo Domingo (contaminated site) and 4.2 ㎍/㎠ in non-contaminated site, Bandurrias (Farina *et al*., 2003). Also, Chl a content of epilithic biofilm in UK was 5.54 ㎍/㎠ and 7.32 ㎍/㎠ in sheltered and exposed shore, respectively (Thompson *et al*., 2005). Our results showed seasonal and vertical variation in chlorophyll a contents, but it was three times greater than the other results described above. Such a higher Chl *a* contents may result from the eutrophicated water supply from the two rivers, Mankyeong and Donjin that located near to our study site because the abundance of microalgae is positively correlated with nutrient concentration (Kim *et al*., 2009; Choi *et al*., 2013)

Chlorophyll a content was extensively used to estimate biofilm biomass because chl a provided a reliable index of the number of microalgal cells (Dye and White, 1991; Jenkins and Hartnoll, 2001; Boaventura *et al*., 2002). However, Chl *a* extraction methods have demerit of larbour-intensive and destructive (Murphy *et al*., 2005). Thus, remote sensing technique (spectroradiometer, vegetation index) recently used to quantify Chl *a* in epilithic and benthic biofilm (Murphy *et al*., 2005; Jesus *et al*., 2006). Many previous researches revealed correlations between different vegetation indices (NIR: red ratio, VI, NDVI and SAVI) and Chl *a* (Murphy *et al*., 2004, 2006). In the present study, NDVI was positively correlated with Chl a content in the range of 52-58% (r^2 = 0.52 for Gyeokpo and r^2 = 0.58 for Gosapo) and correlation values of VI vs Chl a content were between 53-70% (r^2 = 0.70 for Gyeokpo and r^2 = 0.53 for Gosapo). These results well explained that the proportion of Chl *a* content among various chlorophyll pigments. Also, reflectance spectra obtained from NDVI and VI data can be utilized to identify pigment composition and to measure the amount of each pigment (Louchard *et al*., 2002; Stephens *et al*., 2003; Serôdio *et al*., 2009). Many researchers identified absorption features (peaks) related to several pigments using second-derivative analysis (Murphy *et al*., 2005, 2008; Jesus *et al*., 2006; Serôdio *et al*., 2009). In this study, absorption features showed double peak at between 660 and 700 ㎚. The first peak representing at 667 ㎚ are the effects of Chl a fluorescence (Zarco-Tejada *et al*., 2003; Serôdio *et al*., 2009) and the second peak of 687 ㎚ and this feature is well-described in the literature and has been used to quantify amount of Chl *a* (Louchard *et al*., 2002; Murphy *et al*., 2005). In the second derivative analysis, Chl *a* content of Gosapo (0.022) was greater than that of Gyeokpo (0.015) and this pattern was also correspond with the results of Chl a extraction; 22.84 ㎍/㎠ at Gosapo 15.48 ㎍/㎠ at Gyeokpo shore. Cyanobacteria are distinguishable by phycobiliprotein pigments (phycocyanin, phycoerythrin and allophycocyanin) and diatoms are by fucoxanthin pigment from the other microalgal groups (Stephens *et al*., 2003; Murphy *et al*., 2005). Two second-derivative peaks at 576 ㎚ (peak 8, phycoerythrin) and 618 ㎚ (peak 10, phycocyanin) indicate the presence of cyanobacteria in the present study. Also, absorption feature at 519 and 541 ㎚ (peak 6 and 7, fucoxanthin) showed the presence of diatom. Average peak height at 576 and 618 ㎚ presenting cyanobacteria and peak of fucoxanthin showing diatoms were greater at Gosapo than at Gyeokpo biofilm, which are coincide abundance of each taxon.

In the present study, epilithic biofilm productivity was greater at wave-exposed Gosapo shore than at sheltered Gyeokpo shore based on biofilm biomass estimated NDVI, VI, and Chl a content. This result is consistent with previous findings by MacLulich(1987) and Thompson et al.(2004, 2005) that found the same type of patterns in Australia and UK, respectively. Thompson *et al*.(2005) suggested that marine epilithic biofilms are abundant in the wave exposed area as a result of increased nutrient supply caused by stronger water movements. There are also some evidence that fast water flow conditions enhance biofilm production (Lock, 1993) and epilithic microbiota is more abundant in regions of higher flow in freshwater streams and rivers (Sabater and Roca, 1990). Thus, we suggest that similar processes occur in our sites and that biofilm biomass of Korean rocky shores is influenced by the nutrient variation related to increased wave action.

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