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Monthly Variations of Microphytobenthos Pigments and Photo-Physiological Characteristics in the Middle Intertidal Zone of Geunso Bay, West Coast of Korea

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Abstract

In this study, the surveys were carried out from October 2016 to October 2017 along the tidal flat of Geunso Bay, Taean Peninsula of the western edge of Korea. The sampling trips were carried out for a total of 16 times, once or twice a month. In order to investigate the monthly variation of the microphytobenthos (MPB) biomass and community composition, microphytobenthic pigments on the surface of the sediment were analyzed by HPLC (High performance liquid chromatography). The total chlorophyll a (TChl a) concentrations have used as an indicator of MPB biomasses in the upper 1 cm sediment layer ranged from 40.4 to 218.9 mg m^{-2} throughout the sampling period. TChl a concentrations showed the maximum level on 24th of February and remained high throughout March after which it started to declined. The biomass of MPB showed high values in winter and low values in summer. The monthly variations of pheophorbide a concentrations suggested that the low grazing intensity of the predator in the winter may have partly attributed to the MPB winter blooming.

As a result of monthly variations of the MPB community composition using the major marker pigments, the concentrations of fucoxanthin, the marker pigment of benthic diatoms, were the highest throughout the year. The concentrations of most of the marker pigments except for chlorophyll b (chlorophytes) and peridinin (dinoflagellates) increased in winter. However, the concentrations of fucoxanthin increased to the highest, and therefore, the relative ratios of the major marker pigments other than fucoxanthin to the TChl a decreased during this period. The vertical distribution of chlorophyll a and oxygen concentrations in the sediments using a fluorometer and an oxygen micro-optode chlorophyll a concentrations decreased with oxygen concentrations with increasing depth of the sediment layers. Moreover, this tendency became more apparent in winter. The chlorophyll a was uniformly vertical down to 12 mm from May to July, but the oxygen concentration distribution in May decreased sharply below 1 mm. The increase in pheophorbide a concentration observed at this time is likely to be caused by increased oxygen consumption of zoobenthic grazing activities. This could be presumed that MPB cells are transported downward by bioturbation of zoobenthos.

Measuring chlorophyll fluorescence via Diving PAM (Pulse amplitude modulated fluorometer) at every hour was perform in order to investigate the vertical migration and photo-physiological characteristics of MPB. The steady-state fluorescence in ambient light (F') increased as time passes during the daytime emersion periods, which suggests that MPB moves upward to the surface of the sediments. The light utilization efficiency of PS II ($\Delta F/F_m'$) and the relative electron transport rate (rETR) was negatively and positively correlated to irradiance.

KEY WORDS: Microphytobenthos; Marker pigments; Diving PAM; Vertical distribution; Geunso Bay

한국 서해 근소만 중부조간대 내 서식하는
저서미세조류 색소와 광생리적 특성의 월별 변동

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요약

본 연구에서는 태안반도 근소만 갯벌에서 저서미세조류(MPB)의 현존량, 군집조성에 대해 알아보기 위하여 2016년 10월부터 2017년 10월까지 월 1~2회씩 총 16회에 걸쳐 갯벌 표층에 분포하는 저서미세조류 색소를 HPLC (High performance liquid chromatography)를 이용 분석하였다. 갯벌 표층 1 cm 깊이에 분포하는 저서미세조류의 광합성 색소 중 현존량의 지표로 사용되는 총 chlorophyll a (TChl a) 농도는 연중 40.4~218.9 mg m^{-2} 의 범위를 보였다. TChl a 농도는 2월 24일에 최대값이 나타났고 3월에도 높은 값을 보인 뒤 이후 감소하였다. 저서미세조류의 현존량은 동계에 높고 하계에 낮은 값을 나타냈다. Pheophorbide a 농도의 월별 변동을 통해 동계에 상위 포식자의 낮은 포식압이 저서미세조류 동계번성에 일부 기여한 것으로 사료된다.

또한 주요지시색소를 이용한 저서미세조류 군집조성의 분석한 결과 저서규조류의 지시색소인 fucoxanthin의 농도가 연중 가장 높게 나타났다. Chlorophyll b(녹조류), peridinin(와편모조류)을 제외한 대부분의 지시색소의 농도는 동계에 증가하였으나, fucoxanthin의 농도 증가율이 가장 높아 fucoxanthin을 제외한 TChl a에 대한 주요지시색소의 상대비는 동계에 감소하는 경향이 있었다. 형광광도계와 산소미세전극을 이용하여 측정한 퇴적물 내 chlorophyll a와 산소 농도의 연직분포 특성은 퇴적층 표면에서 깊이가 깊어질수록 chlorophyll a와 산소 농도가 함께 감소하는 경향을 보였고, 동계로 갈수록 이런 경향이 더욱 뚜렷하게 나타났다. 하지만 5~7월의 chlorophyll a 농도는 다른 기간에 비해 12 mm까지 연직으로 유사하게 나타났으나, 5월의 산소 농도 분포는 1mm이하에서 급격하게 감소하였다. 같은 시기에 pheophorbide a 농도가 증가하는 것으로 보아 저서동물의 포식활동에 의한 산소 소비량이 증가하였을 가능성이 있으며, 저서동물의 생물교란에 의해 저서미세조류의 세포가 아래로 옮겨진 것으로 추측된다.

Diving PAM (Pulse amplitude modulated fluorometer)으로 엽록소 형광을 시간별로 측정하여 저서미세조류의 수직 이동과 광생리적 특성에 대해 조사하였다. 낮 동안 간조시 시간이 지남에 따라 자연광 조건에서의 평형 상태 형광 발생량(F')은 증가하여, 저서미세조류가 퇴적물 표면으로 수직 상승을 하는 것으로 추정된다. 제 2 광계의 유효양자수율($\Delta F/F_m'$)과 상대 최대 전자전달율(rETR)은 일사량에 따라 각각 양의 상관관계와 음의 상관관계를 보였다.

주제어: 저서미세조류; 지시색소; Diving PAM; 수직 분포; 근소만

Chapter 1. Introduction

1.1 Avoidance strategies to reduce the light stress of MPB and necessity of research

Microphytobenthos (MPB) living in the upper layer of sediments are believed to be important primary producers, contributing up to 50% of the primary productivity of the estuaries (Underwood & Kromkamp, 1999). MPB undergoes severe environmental changes such as strong light, temperature and drying twice a day depending on the tidal cycle. Especially, MPB living in the sediment layer is exposed to more light stress than phytoplankton inhabiting the water column. Therefore, MPB has various behavioral and photo-physiological strategies to reduce such stress. The former is that MPB moves up to the surface to receive more light through vertical migration or move deeper to avoid photoinhibition. The vertical movement of MPB is active enough to be observed within 8 to 15 minutes (Perkins et al., 2002). The latter, in the form of fluorescence, extinguishes excessive light energy, changes the distribution or composition of the pigment, and regulates the density or size of the photosynthetic unit (Chan, 1978; Prézelin & Sweeney, 1978; Riper et al., 1979; Falkowski & Owens, 1980; Perry et al., 1981; SuKenik et al., 1987). Since MPB has a different avoidance strategy for each class, it influences the photosynthetic activity of MPB inhabiting the area and its productivity depending on the population composition and physiological condition of MPB (Kromkamp et al., 1998). Hence, understanding the community structure and biomass variability of MPB and the photo-physiological characteristics is crucial in order to understand the tidal flat ecosystem (Middelburg et al., 2000).

1.2 Advantages of pigments analysis using HPLC and preceding studies

Since the 1980s, biomass and community structure of phytoplankton and

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microphytobenthos have been studied in Korea. Microscopic studies on benthic diatom assemblages were conducted at the Incheon coastal tidal flats (Shim & Joe, 1984), Geum estuary intertidal zone (Kim & Cho, 1985), Songdo tidal flats (KOSEP, 1991) and Mankyeong Dongjin tidal flats (Oh, 1990). However, since the microscopic method requires a lot of time and expertise of the experimenter, research on MPB communities using HPLC has been carried out recently. Pigment analysis via HPLC allows quantitative analysis of each pigment. It is also easy to estimate the biomass through the major pigment (chlorophyll *a*) as well as the identification of the phytoplankton community composition at the class level using marker pigments (Lee et al., 2009). The chlorophyll degradation products are also useful for understanding the physiological state of the MPB community and the grazing intensity by the predators (Bidigare et al., 1986; Burkill et al., 1987). In Korea, such researches were conducted at Gomso Bay (Lee, 2001), Ganghwa island tidal flat (Yoo & Choi, 2005), Saemangeum tidal flat (Oh et al., 2004) and Gwangyang Bay tidal flat (Lee et al., 2009).

1.3 Advantages of PAM measurement

Chlorophyll fluorescence analysis using PAM (Pulse amplitude modulated fluorometer) can estimate the biomass of microalgae in a fast and non-destructive way in the field, and the effective quantum yield of the Photosystem II ($\phi_{PS\,II}$) and the electron transport rate (ETR) can be calculated (Perkins et al., 2002; Morris & Kromkamp, 2003). So far, studies have been carried out to quantify MPB biomass with fluorescence measurements obtained through PAM or to estimate photosynthetic efficiency (Serôdio et al., 1997; Consalvey et al., 2004), and primary productivity (Barranguet & Kromkamp, 2000; Serôdio, 2003). In addition, studies on the vertical movement of MPB through PAM were also conducted (Serôdio et al., 1997; Kromkamp et al., 1998; Serôdio et al., 2003; Longphuirt et al., 2006). However, most of these studies were conducted mainly in North America and Europe. In Korea, studies are limited to laboratory experiments using Diving PAM to investigate the variability of the photo-physiological characteristics of four benthic diatoms in terms of temperature (Yun et al., 2009), and the seasonal variations of phytoplankton primary

productivity in Beopsu Marsh (Kim, 2008). It is still necessary to conduct field works on the dynamics of the photo-physiological characteristics of MPB.

1.4 Aims of this study

Therefore, what we want to know in this study is as follows: The investigation of the changes of monthly biomass and community composition of MPB in the Geunso Bay; the investigation of the temporal change of photo-physiological characteristics through fluorescence measurement; the observation of the vertical distribution patterns of MPB.

Chapter 2. Materials and Methods

2.1 Sampling sites

This study was carried out 16 times from October 2016 to October 2017 in the Geunso Bay tidal flat of Taean peninsula (126.1799° E, 36.7367° N). The sampling site is shown in Figure 1. The Geunso Bay is semi-enclosure bay located just between Geun-Heung-myeon and Song-won-myeon of Taean-gun, and is controlled by tidal fluctuation. The total area is about 87 km^2, the average tidal range is 6 meters, and the mean sea level is 3.55 m. The bay is formed in the southwest-northeast direction with a total length of 10 km and a width of 2.5 to 6 km. 70~90% of the area of tidal flats are exposed to the atmosphere during the emersion period and maintain a depth of 2~4 m during immersion period Most sedimentary deposits of the bay are composed of sandy silt. Because there is no river directly flowing from the land to the mainland, a limited seawater circulation is achieved through the open sea and the bay entrance of about 2 km (Kim & Kim, 2008; Noh, 2008; Lee et al., 2010; Choi et al., 2016; An et al., 2017).
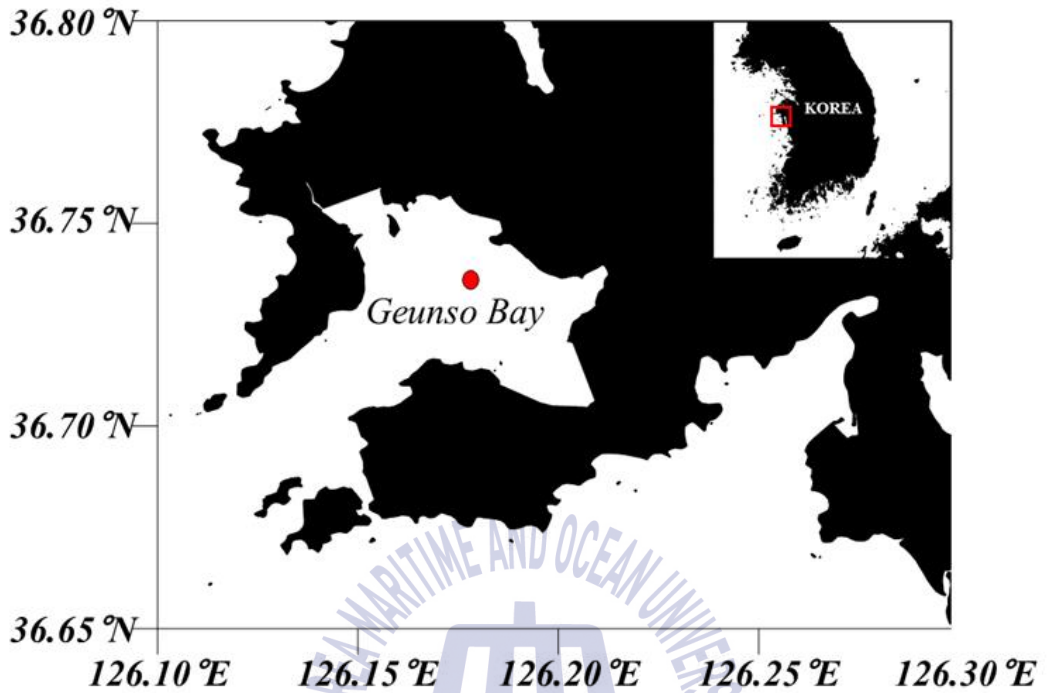
Fig. 1 Location of tidal flat at the Geunso Bay and sampling site (red point).

2.2 Photosynthetic pigments analysis

The three replicate sediment samples were collected from the research station before the end of the emersion period using an acrylic core with a diameter of 2 cm and a length of 15 cm. For the homogenization of the samples, 1 cm of the upper part of each core was cut and collected in one conical tube, followed by mixing and sub-sampling according to the purpose of the sample. All sub-samples were stored in a Deep freezer (-80℃) until HPLC analysis. For quantitative analysis of photosynthetic pigments, all samples were freeze-dried (Freeze dryer, FDZ-1200, Tokyo Rikakikai Co., Japan) at -45℃ for 8~10 hours. The water content of the sediment samples was calculated by comparing the weight before and after freeze drying. 95% acetone was used as a solvent for the MPB's photosynthetic pigments extraction. Ultrasonic sonication was performed for 5 minutes for cell disruption and stored in a dark place at -2℃ for 24 hours. The extracted samples were filtered using a syringe filter (PTFE, 0.2 μm, Hydrophobic) and used for HPLC measurement. Shimadzu LC-20A HPLC system (Shimadzu, Japan) was used for photosynthetic pigments analysis. It was operated according to the method described in Zapata et al. (2000) (Table 1). For the HPLC analysis, 1 mL of the extracted sample and 400 μL of HPLC water (J. T. Baker, USA) were mixed. To verify the analytical results, 50 μL of internal standard (canthaxanthin) was added. The injection volume was set to 200 μL. The detector was qualitatively and quantitatively analyzed using a Photodiode array detector (PAD). Identification of the peak of the chromatogram was determined by comparing the retention times with 22 standard pigments (DHI co., Denmark). (;chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll c_2 (Chl c_2), chlorophyll c_3 (Chl c_3), divinyl chlorophyll a (DV Chl a), chlorophyllide a (Childe a), pheophytin a (Phytin a), pheophorbide a (Phide a), α-carotene (α-Car), β-carotene (β-Car), 19-butanoyloxy-fucoxanthin (But-fuco), 19-hexanoyloxy-fucoxanthin (Hex-fuco), fucoxanthin (Fuco), diatoxanthin (Diato), diadinoxanthin (Diadino), peridinin (Perid), neoxanthin (Neo), prasinoxanthin (Pras), violaxanthin (Viola), alloxanthin (Allo), zeaxanthin (Zea), lutein (Lut))

Standard pigments equation was calculated according to the Woods Hole (1997). The response factor (RF) value was calculated according to the following equation 1 with

reference to the standard pigments concentration provided by DHI Company.

$$RF = \frac{(Cs)(IV)}{A}$$ Eq. 1

RF = standard response factor (ng unit-area^{-1})

Cs = pigment standard concentration (ng μL^{-1})

IV = injection volume (μL)

A = integrated peak area

Each pigment was quantified by the following equation 2 & 3.

$$C_i = A(RF)(\frac{1}{IV})(EV)(\frac{1}{SV})(D)$$ Eq. 2

C_i = individual pigment concentration (ng L^{-1})

A = integrated peak area

RF = standard response factor (ng unit-area^{-1})

IV = injection volume (mL)

EV = extraction volume (mL)

SV = sample filtration volume (L)

D = dilution factor

$$D = \frac{\dfrac{IV}{V_o}}{\dfrac{IV}{V_s}}$$

<div align="right">Eq. 3</div>

IV = injection volume of standard or sample

V_o = Total standard solution volume (μL)

V_s = Total sample solution volume (μL)

Total Chl a obtained by HPLC was calculated by the following equation 4 (NASA, 2012).

$$TChla = Chlda + ChlaAllomer + DVChla + MVChla + ChlaEpimer$$

<div align="right">Eq. 4</div>

In order to identify the monthly variation of the vertical distribution of MPB, one sediment core was collected, cut at 2 mm intervals from the top to 14 mm. Since we could not collect samples on February 1th and July 26th of 2017, we surveyed 14 times in one year. Sediment samples were freeze-dried (Freeze dryer, FDZ-1200, Tokyo Rikakikai Co., Japan) at -45℃ for 8~10 hours prior to the measurement to improve extraction efficiency. Chl a was measured using a 10-AU fluorometer (Turner designs, USA). The acid-based oxidation fluorescence method was used (EPA, 1997). The Chl a concentration is calculated using the fluorescence values before and after addition of 65% of 10% HCl (Eq. 5).

$$Chla = K(\frac{F_m}{F_m - 1}) \times (F_b - F_a) \times (\frac{v}{V})$$

<div align="right">Eq. 5</div>

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K = sensitivity coefficient, equal to 1 on 10-AU

F_m = max acid ratio F_b/F_a of pure chlorophyll *a* standard

F_b = fluorescence before acidification

F_a = fluorescence after acidification

v = extract volume (L)

V = volume filtered (L)

Table 1 HPLC column and solvent system (Zapata et al., 2000).

Column	Waters Symmetry C_8 column (150 × 4.6 mm, 3.5 μm particle size)
Solvent A	Methanol : Acetonitrile : Aqueous pyridine (50:25:25 v:v:v)
Solvent B	Methanol : Acetonitrile : Acetone (20:60:20 v:v:v)

2.3 Chlorophyll fluorescence measurement

The chlorophyll fluorescence was measured using Diving PAM (Walz, Germany). Measurements were taken at 1 cm from the surface of the sediment and at an angle of 60 degrees. The fluorescence values (F) measured by PAM are useful for estimating biomasses of MPB (Longphuirt et al., 2006). The biomass distributed on the surface of the sediment appears heterogeneous due to biofilm forming MPB. Consequently, to obtain the representative value at the study station, the fluorescence parameters during a emersion period was measured 3~4 times a day at 64 times per hour in the quadrate.

After the fluorescence measurement, 16 samples of acrylic cores were sampled at the same interval in the quadrate (Fig. 2). Sediment samples were taken to the laboratory and analyzed using an HPLC. The pigment contents of the 16 sediment samples were averaged and used as a representative value of the study station.

At the tip of the PAM probe, a red light-emitting diode (LED) is emitted (650 nm, pulse width 3 μs, Frequency 0.6 KHz). When this light is irradiated on the sediments living in the MPB, light energy is transferred to the chloroplast. Light energy is quenched by a total of four processes. The remaining light energy used in the other three processes is emitted as fluorescence, which is a form of lower energy (Maxwell & Johnson, 2000; Consalvey et al., 2004). At this time, steady-state fluorescence in ambient light (F') can be obtained under natural light conditions. The maximum fluorescence value (F_m') in the light acclimated state was measured by irradiating a saturation pulse at each measurement point. The light utilization efficiency of PS II ($\Delta F/F_m'$) and the relative electron transport rate (rETR) of the Photosystem II be calculated by Equations 6 and 7, respectively (Genty et al., 1989). $\Delta F/F_m'$ represents the ratio of the light energy absorbed by the chlorophyll located in Photosystem II to the photochemical process (Yun et al., 2009). rETR is the transport speed of the electron and it is useful for estimating the gross primary production (Davoult et al., 2009). The equations $\Delta F/F_m'$ and rETR are followed by Yun et al. (2009).

$$\Delta F/F_m{'} = \frac{(F_m{'} - F{'})}{F_m{'}}$$

<div align="right">Eq. 6</div>

$$rETR = \Phi_{PS\,\mathrm{II}} \times PAR$$

<div align="right">Eq. 7</div>

The minimum fluorescence yield (F_o) in the dark condition is highly correlated with the Chl a concentration, which can represent the phototrophic microorganism biomass (Serôdio et al., 1997; Barranguet & Kromkamp, 2000; Honeywill et al., 2002). In addition, F_o is least affected by temperature or species as compared to other fluorescence measurements (Serôdio et al., 2001). However, since it is very difficult to make repeated measurements by making dark conditions in the field, only $F{'}$ and $F_m{'}$ values are measured in this study. $F{'}$ can be used as a proxy for the biomass of MPB instead of F_o (Kromkamp et al., 1998; Longphuirt et al., 2006). Therefore, $F{'}$ has used as the proxy of MPB biomass in this research. PAR (photosynthetic active
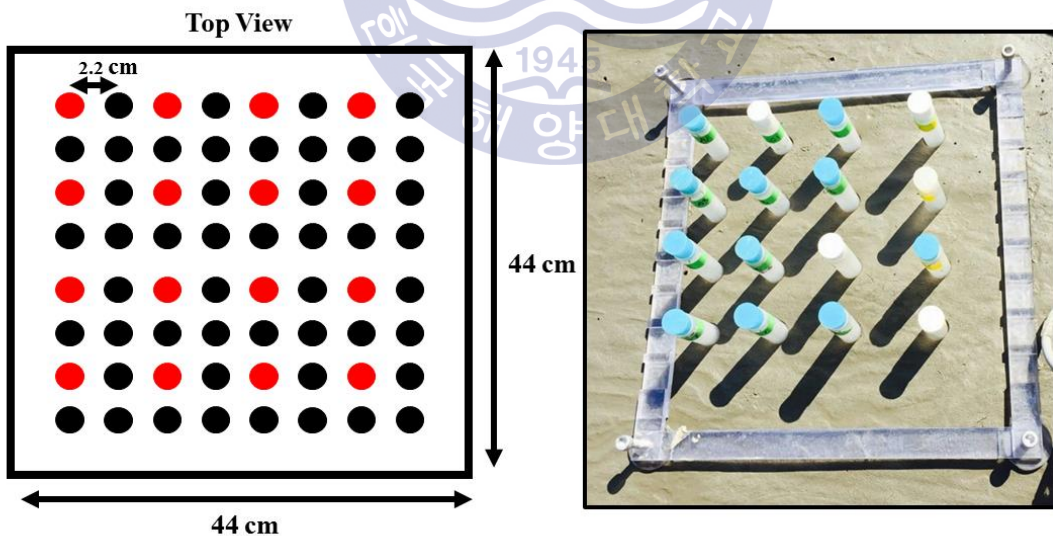
Fig. 2 The figure in the left shows the quadrate containing 64 measuring points and the right shows the 16 cores sampled at the red dot position.

radiation) was measured with a Licor 1400 data logger connected to a LI-190R Quantum sensor (LI-COR, USA).

2.4 Oxygen concentration measurement

Oxygen microoptodes has been extensively used since the 1990s because it measures oxygen concentration. This method is minimally invasive and has a high spatial resolution (Lee et al., 2010). In this study, oxygen concentrations in the sediment were measured with O_2 Microoptode (Opto-MR, Unisense, Denmark). Oxygen Microoptode sensor utilizing In Situ Stand (IS19, Unisense, Denmark) connected with Field Motor (Unisense, Denmark) was installed facing the sun so that the data is unaffected by shadows. The sensor was fixed vertically on the sediment surface. The data measured at intervals of 50~100 μm, hourly, over 3 mm depth, until there is no significant change in oxygen concentration. The data was saved in a Field Microsensor Multimeter (Unisense, Denmark).
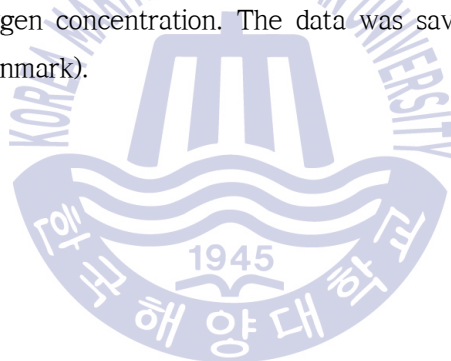
Chapter 3. Results

3.1 Temporal variation in pigment concentrations

From October 2016 to October 2017, the contents of TChl a from the surface of the sediment to 1 cm were ranged from 40.4 to 218.9 mg m^{-2}. The minimum content of TChl a have been observed in July 26th and the maximum content appeared on February 24th (Fig. 3). Phide a contents were the highest on November 10th, low from February 15th to March 15th, and increased in May.

MPB Biomasses are highly variable in a small space. Therefore, the TChl a contents obtained from the quadrate were compared with the TChl a contents of the three replicate sediment samples to identify if these values were representative of this study site (Guarini et al., 1998; Seuront and Spilmont, 2002). Looking at the monthly TChl a content on average of the 16 cores obtained in the quadrate, The TChl a contents range was 52.6~189.2 mg m^{-2}, which is lower than the TChl a content range obtained with three replicate sediment cores (Fig. 4). This is because we could not conduct experiments on February 24th using the quadrate when winter blooming occurred at the immersion period in the daytime. Likewise, TChl a values on November 10th, January 10th, June 5th, July 19th and October 16th have been missed for the same reason. The temporal variation of TChl a content of three replicate sediment core samples and the mean values of the 16 cores obtained in the quadrate were highly correlated (R^2=0.87).

In order to identify the monthly variation of the vertical distribution of MPB biomass in the sediment layer, the measured values are shown in Fig. 5. The change in Chl a content from 0 mm to 10 mm was similar to that of TChl a obtained from 3 cores (R^2=0.81). Although Chl a contents tended to decrease with increasing the sediment depths, the depth at which Chl a contents showed the maximum of content

varied temporally. On October 20th (2016) and January 3th (2017), the Chl a content was the maximum at 2~4 mm, Chl a contents peaked at 10~12 mm on May 29th (2017). In the rest of the period, Chl a content at 0~2 mm shows the maximum. The Chl a content was more concentrated at 0~2mm depth at 49% of the total concentrations on February 24th (2017) when the TChl a content was the maximum. In contrast, the Chl a content distribution has uniformly appeared in the 0~10 mm depth range on May 29th when the TChl a content was low.

The relative ratio in each accessory pigments of TChl a was obtained from three replicate sediment core samples during October of 2016~2017 (Table 2) where 20 pigments were detected. Fuco being the biggest proportion in accessory pigments, followed by Phide a which is used as a marker pigment for predation by the predator, and Diadino which are the photo-protective pigment.

Of the total 22 accessory pigments, nine were selected as the marker pigment and these monthly contents were plotted in Fig. 6. Fuco, the marker pigment of bacillariophytes, showed the highest contents of other accessory pigments during the year. The annual contents variations of Fuco, Zea and Allo were similar to those of TChl a. Zea is the marker pigment of cyanobacteria. Allo is the marker pigment of cryptophytes. The contents of But-fuco (pelagophytes) and Hex-fuco (prymnesiophytes) were the highest on February 24th. Chl b, the marker pigments of chlorophytes and euglenophytes, retained high concentration in October and November with the highest concentration of 3.305 mg m^{-2} in October 2017. Lut, the marker pigment of chlorophytes, has not shown significant changes of contents during this year other than that it has shown the maximum value of 1.446 mg m^{-2} on February 17th. Perid, the marker pigment of dinoflagellates, have shown maximum contents in May of 1.434 mg m^{-2}. Viola, the marker pigment of chrysophytes with Fuco, have shown high contents in November, January, February and showed the maximum value in March.

Fig. 7 shows the monthly variation of the contribution of each marker pigments to TChl a. The relative ratios Fuco to TChl a were the highest for one year. The relative ratios of other major marker pigments to TChl a were all lower than 0.10. The But-fuco/TChl a increased in November, January and May. The Zea/TChl a and

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Perid/TChl *a* were the highest values in May and June. The Chl *b*/TChl *a* and Lut/TChl *a* were the maximum in October of 2016, the minimum in February, and increased on summer. The Viola/TChl *a*, Hex-fuco/TChl *a* and Allo/TChl *a* were detected in the low values throughout the year.
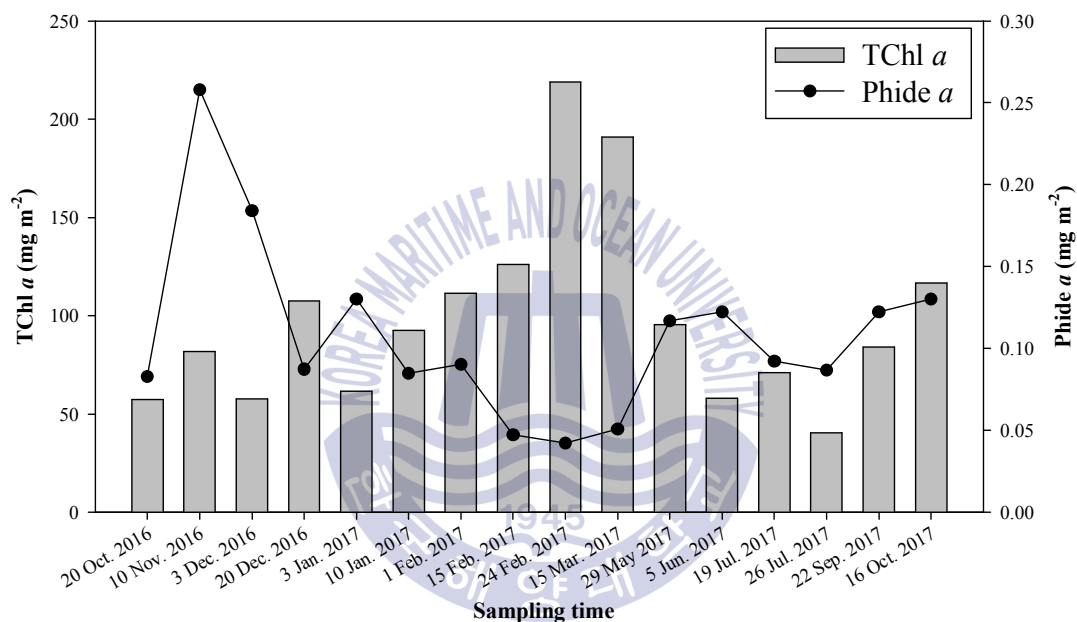
Fig. 3 Monthly variation of the total chlorophyll *a* and pheophorbide *a* contents from Oct. 2016 to Oct. 2017 in Geunso Bay tidal flat. It was the result of cutting 1 cm of the upper part of 3 cores and mixing.
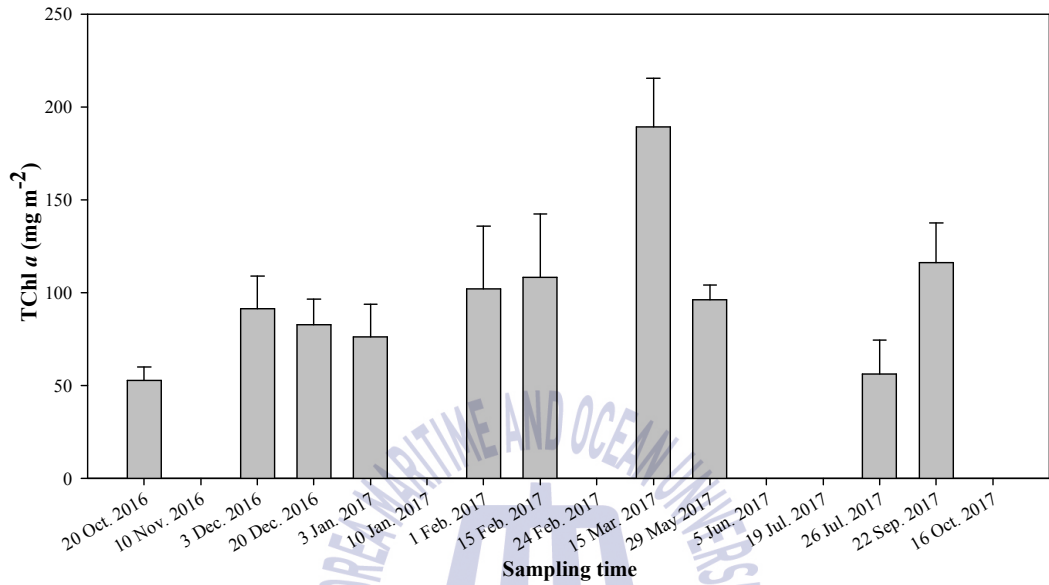
Fig. 4 Total chlorophyll *a* contents measured by using HPLC in Geunso Bay tidal flat. The results obtained by cutting 1 cm of the upper part of 16 cores in the quadrate were averaged.
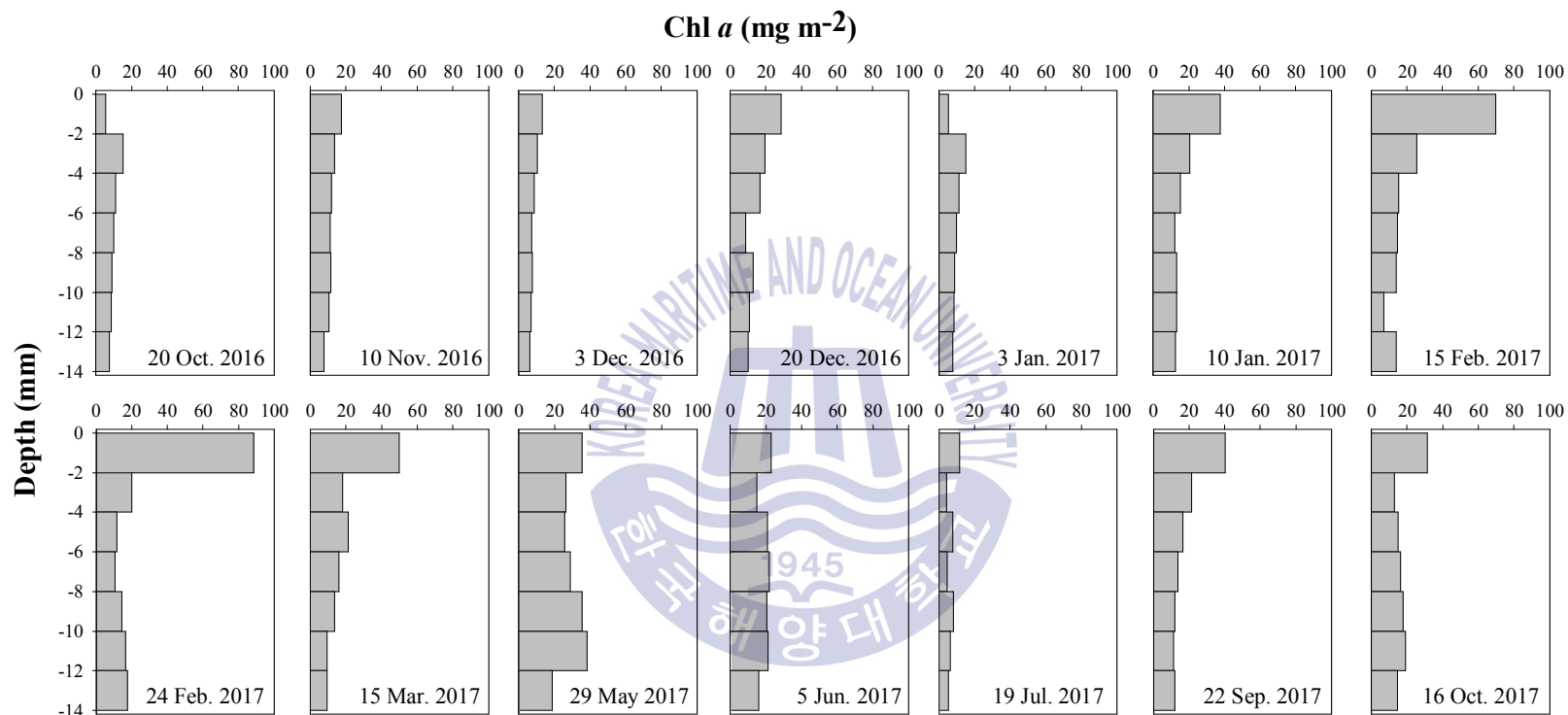
Chl *a* (mg m⁻²)

Fig. 5 Vertical distribution of monthly chlorophyll *a* (mg m⁻²). Using Turner 10-AU fluorometer. The sediment core sample has been sliced 2 mm at a time for a total of 14 mm.

Table 2 The annual average contents of photosynthetic pigments and the relative ratios of accessory pigments to total chlorophyll a in Geunso Bay tidal flat.

Pigment	Average (±S.D) [mg m^{-2}]	Relative rate to TChl a
Total chlorophyll a (TChl a)	98.22 (±48.55)	1.0000
Fucoxanthin (Fuco)	29.99 (±18.43)	0.2993
Pheophorbide a (Phide a)	7.944 (±4.729)	0.0793
Diadinoxanthin (Diadino)	7.553 (±6.949)	0.0754
β-Carotene (β-Car)	6.319 (±1.870)	0.0631
Diatoxanthin (Diato)	4.141 (±2.240)	0.0413
Zeaxanthin (Zea)	3.907 (±2.107)	0.0390
Chlorophyllide a (Chlide a)	3.383 (±1.530)	0.0338
19'-butanoyloxyfucoxanthin (But-fuco)	3.241 (±1.570)	0.0324
Pheophytin a (Phytin a)	2.976 (±0.848)	0.0297
Alloxanthin (Allo)	2.812 (±1.529)	0.0281
Chlorophyll b (Chl b)	2.120 (±0.877)	0.0212
19'-hexanoyloxyfucoxanthin (Hex-fuco)	1.061 (±0.348)	0.0106
Chlorophyll c_2 (Chl c_2)	0.969 (±0.375)	0.0097
Prasinoxanthin (Pras)	0.750 (±0.513)	0.0075
Lutein (Lut)	0.705 (±0.212)	0.0070
Peridinin (Perid)	0.577 (±0.319)	0.0058
Violaxanthin (Viola)	0.517 (±0.331)	0.0052
Neoxanthin (Neo)	0.354 (±0.109)	0.0035
α-Carotene (α-Car)	0.319 (±0.105)	0.0032

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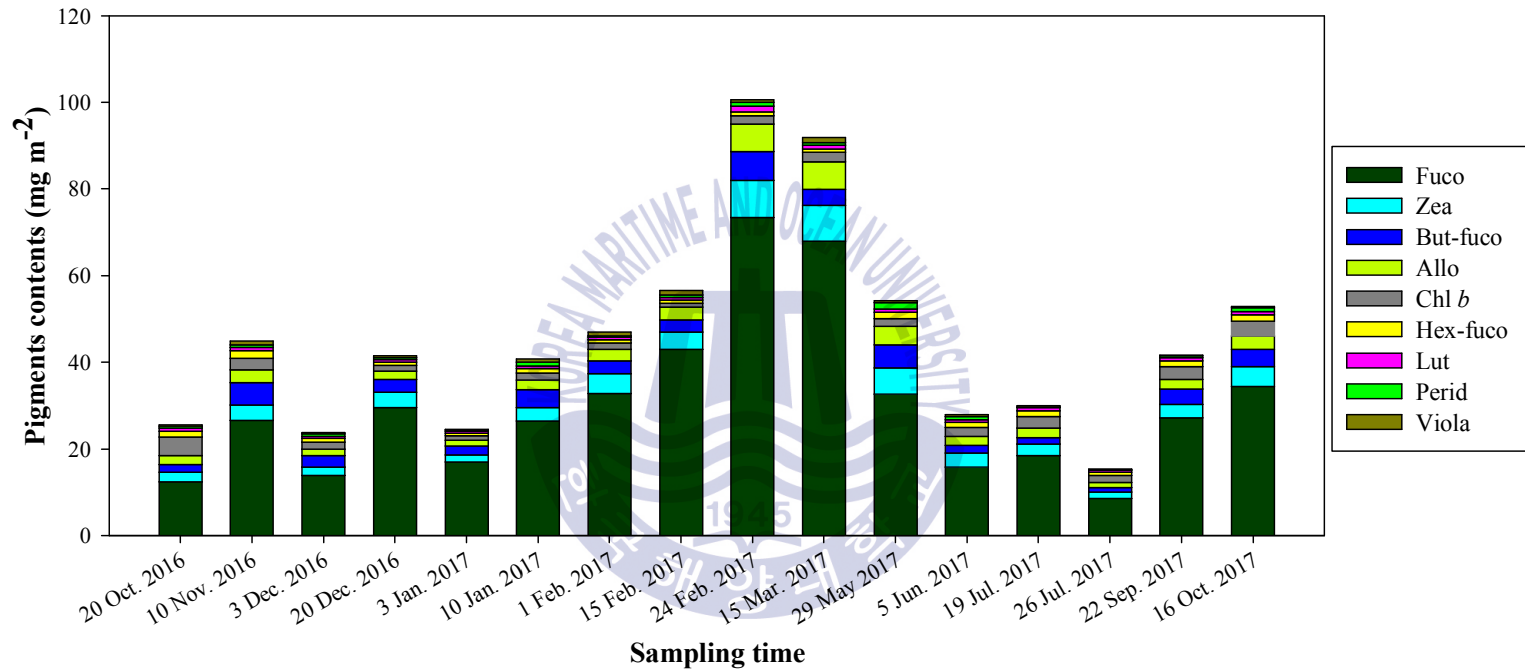
Fig. 6 Monthly variation of marker pigments contents from Oct. 2016 to Oct. 2017 in Geunso Bay tidal flat. It was the result of cutting 1 cm of the upper part of three cores and mixing.

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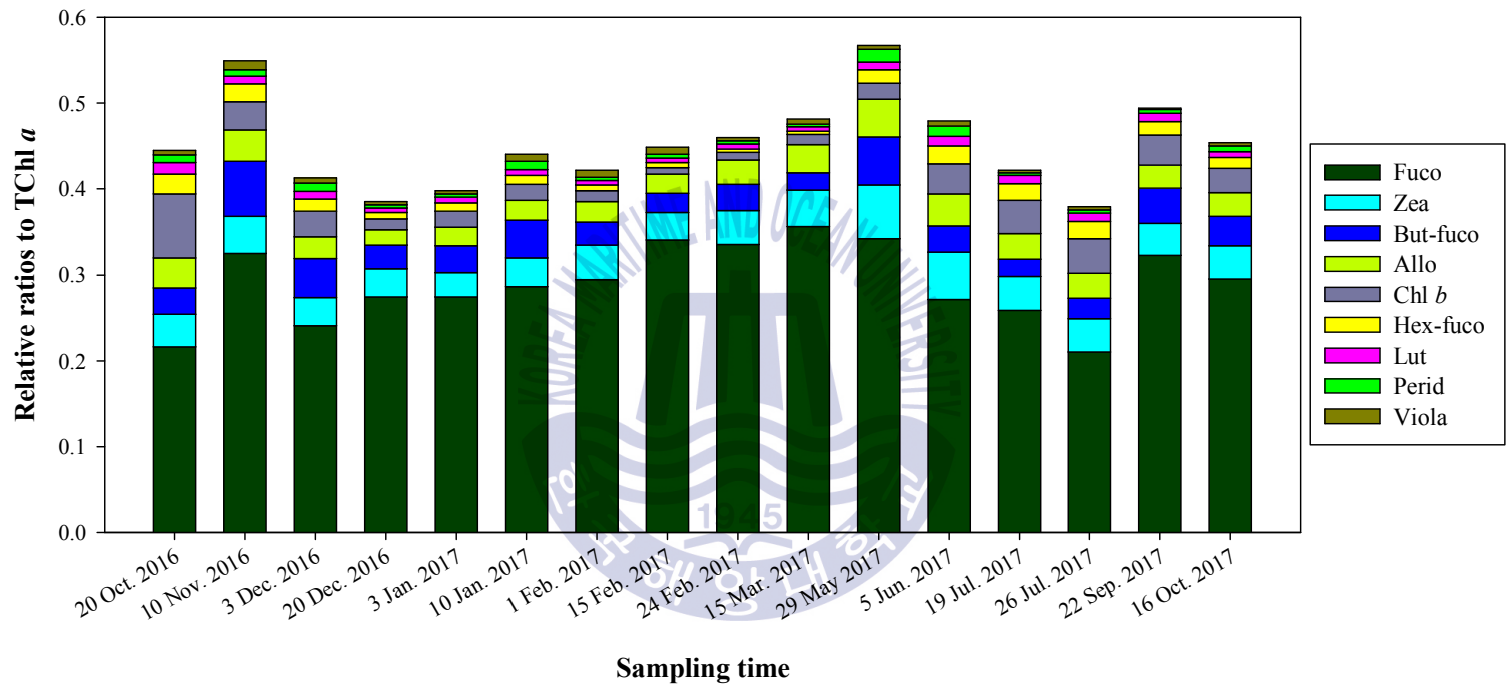
Fig. 7 The relative ratios of marker pigments to total chlorophyll *a* at sampling site in Geunso Bay. It was the result of cutting 1 cm of the upper part of three cores and mixing.

3.2 Temporal variation in fluorescence parameters

The sediment exposed to the atmosphere was measured 4~5 times hourly using Diving PAM. Increased area of patchiness proportional to the time was observed via F' value (Fig. 8). As the difference between biomass concentrated site and non-concentrated site increases with time, the area of which biomass is concentrated becomes larger; given that F' value from 15:00 in October 20th of 2016 be excluded.

Table 3 shows the PAR values (μmol m^{-2} s^{-1}) measured with LI-1400 and fluorescence parameters (F', F_m', $\Delta F/F_m'$, rETR). The F' value increased slightly over time throughout the day other than the values of October 20th at 15:00 and December 3rd at 10:40 and March 15th at 11:00. The daily variation of $\Delta F/F_m'$ and rETR was not large. However, The $\Delta F/F_m'$ value tends to be decreased and rETR value increased according to PAR (Fig. 9).
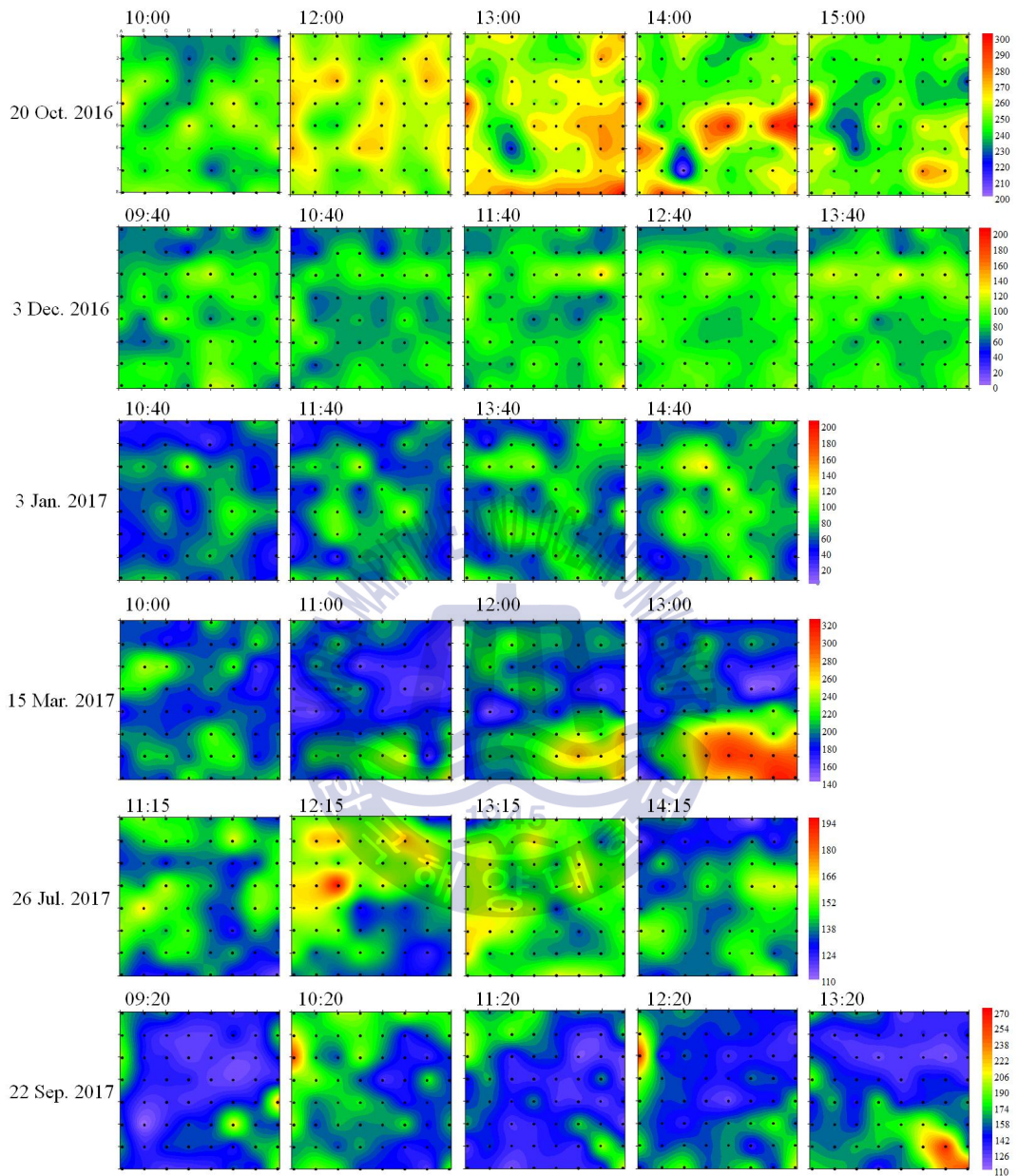
Fig. 8 Contour graphs showing the change of the steady-state fluorescence in ambient light (F') measured using Diving PAM. The dots mean the measuring point in the quadrate.

Table 3 Temporal variations of the steady-state fluorescence in ambient light (F'), the maximum fluorescence value (F_m'), the light utilization efficiency of PS II ($\Delta F/F_m'$), the relative electron transport (rETR, μmol electrons m^{-2} s^{-1}) and the photosynthetic active radiation (PAR, μmol m^{-2} s^{-1}).

Date	Time	F'	F_m'	$\Delta F/F_m'$	rETR	PAR
20 Oct. 2016	10:00	149.7	173.9	0.138	74.4	539.0
	12:00	162.8	203.7	0.199	124.7	626.0
	13:00	168.4	231.1	0.265	56.7	213.2
	14:00	163.5	200.0	0.180	38.2	215.6
	15:00	156.3	192.0	0.182	129.5	714.2
3 Dec. 2016	9:40	151.0	188.1	0.322	72.6	374.6
	10:40	146.9	177.1	0.290	100.3	683.2
	11:40	154.7	185.4	0.274	123.7	862.4
	12:40	157.5	191.3	0.284	145.0	936.5
	13:40	157.9	194.7	0.303	152.6	916.7
3 Jan. 2017	10:40	154.9	185.2	0.161	83.9	523.6
	11:40	160.2	189.2	0.152	116.9	773.3
	12:40	168.1	198.3	0.150	141.9	947.1
	13:40	170.2	204.9	0.166	159.0	957.1
15 Mar. 2017	10:00	193.4	230.3	0.157	129.6	824.6
	11:00	185.2	208.9	0.110	126.3	1144.8
	12:00	201.1	228.0	0.116	158.2	1360.1
	13:00	207.6	235.1	0.114	170.0	1493.8
26 Jul. 2017	11:15	139.5	165.8	0.155	105.5	682.3
	12:15	145.8	174.4	0.159	59.3	1008.6
	13:15	148.2	175.0	0.150	114.7	749.0
	14:15	137.5	158.6	0.131	126.9	969.3
22 Sep. 2017	9:20	145.7	201.1	0.254	126.3	497.8
	10:20	166.1	204.2	0.181	56.2	824.2
	11:20	148.5	176.7	0.153	169.6	1102.3
	12:20	156.8	193.0	0.181	255.7	1235.3
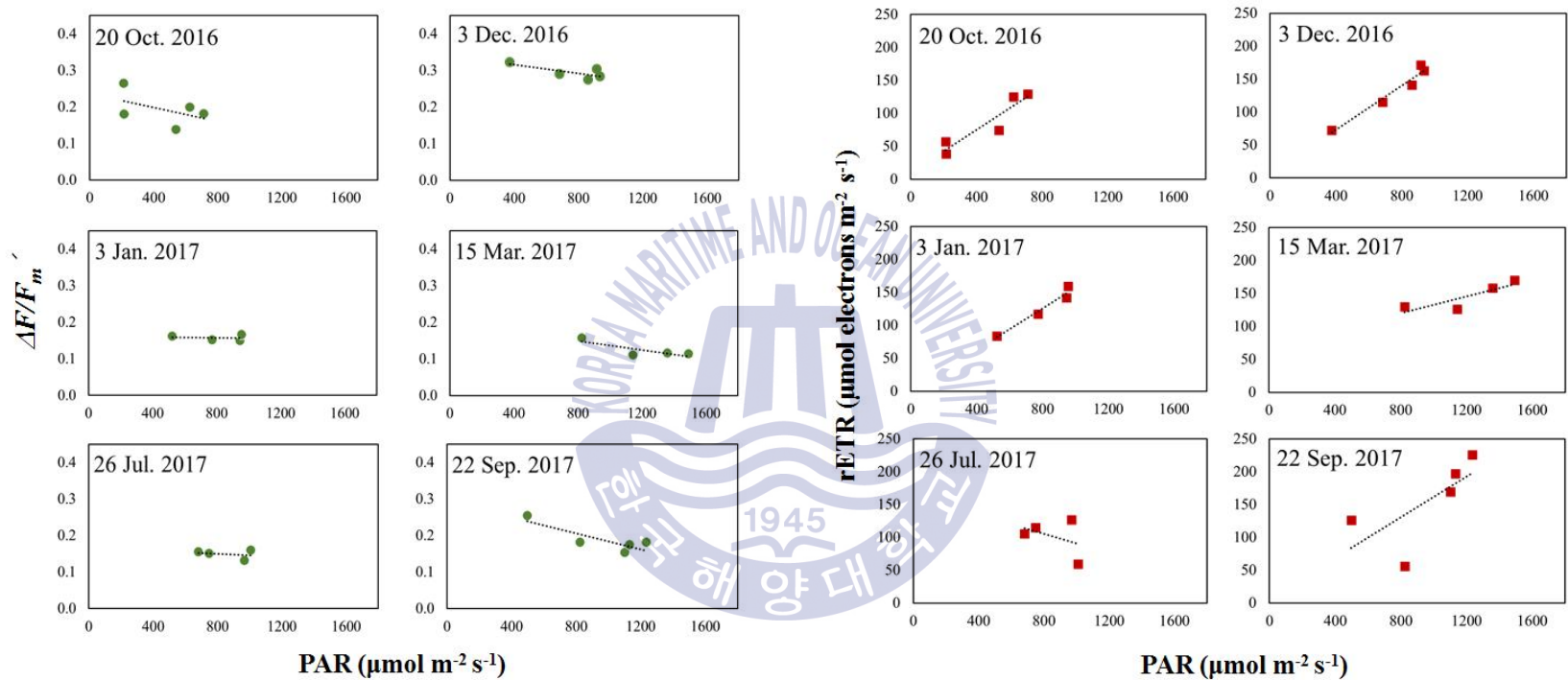
Fig. 9 Change in effective quantum efficiency ($\Delta F/F_m{}'$, closed circle) and relative photosynthetic electron transport (rETR, closed square) with photosynthetic active radiation (PAR) during emersion periods.

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3.3 Distribution of oxygen concentration in sediments

The distribution of oxygen concentration in sediments was concentrated in the range of 0~1 mm from the surface of the sediment ($71.5 \pm 12.6\%$). The oxygen permeation depth showed a maximum value of 4.5 mm in January and February of winter, and a minimum value of 1.5 mm in May. The temporal variation of oxygen concentration in the upper part of the sediment increased with time (Fig. 10).
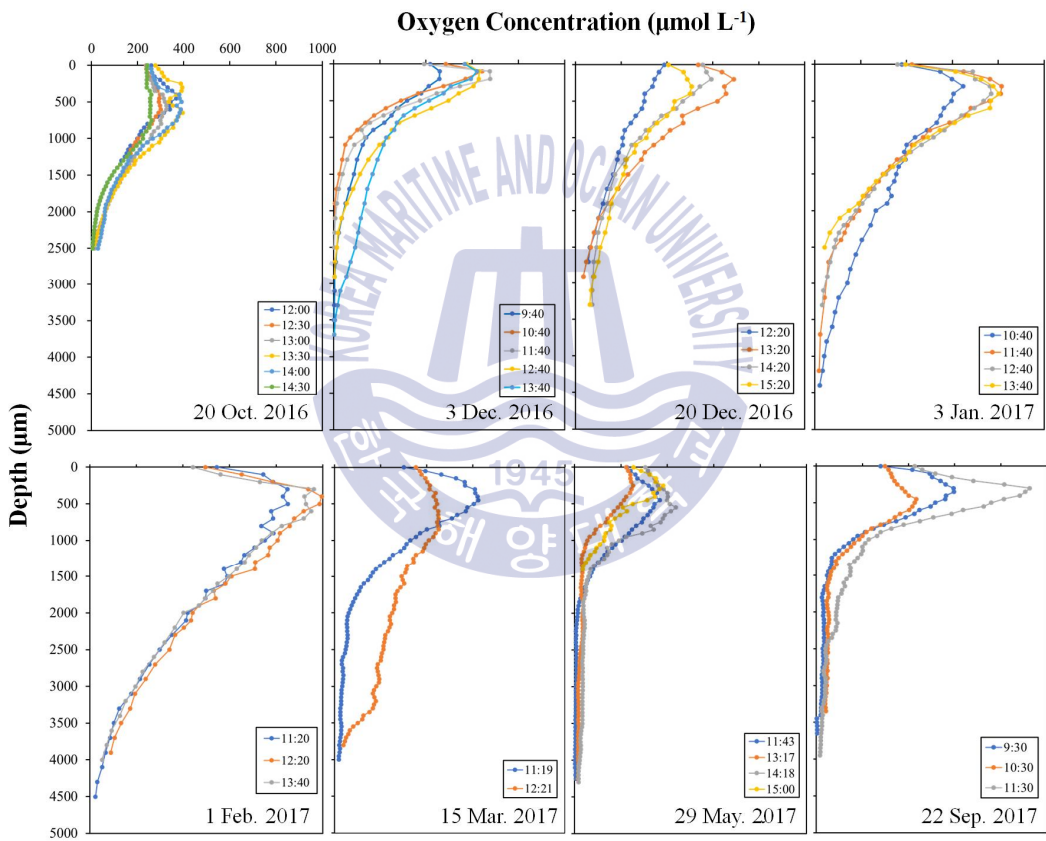
Fig. 10 Oxygen concentration profiles measured in sediment cores from Geunso Bay tidal flat.

Chapter 4. Discussion

4.1 Monthly variations of MPB biomass

Compared with the preceding research both domestically and internationally, the Chl *a* content range in this research was similar or higher than the results of other studies (Table 4). There may be differences in Chl *a* values depending on the extraction method of the pigment and the calculation method of the pigment concentration. In this study, freeze-drying was carried out before the pigments extraction to enhance the extraction efficiency and to minimize the influence of moisture content of the sediment layer. The total Chl *a* was calculated for the purpose of obtaining accurate biomass. However, the difference between the ranges of Chl *a* and TChl *a* values was not significant. Therefore, the various environmental factors affecting the MPB biomass include the exposure time of the sediment depending on the tidal cycle, the grain size of the sediment, irradiance, temperature, nutrients, and the predation pressure should be considered (Lee et al., 2009).

In this research, the content of TChl *a* has also shown highest in winter. The TChl *a* contents decrease during the spring and summer and slowly increase during the fall. In case of the temperate zone of the Northern Hemisphere, low concentration is shown in the biomass of phytoplankton living in the water column during the winter. In contrast, generally high concentration has shown in the biomass of MPB living in the sediment during the winter (Colijn & de Jonge, 1984; de Jonge & Colijn, 1994). On the other hand, Kwangyang Bay has shown low biomass during the winter and high during the summer. This is due to increased irradiance and temperature during the summer (Lee et al., 2009). Tidal flat in Ganghwa Island has shown MPB bloom during winter and low biomass during spring which concurs with this study (Yoo & Choi, 2005). According to the study by Oh et al. (2004), tidal flat in Saemangeum also shows MPB bloom during winter and smaller bloom during fall.

When Chl a is degraded by the acidic environment in the stomach of the predators, the magnesium departs from the center of the chlorophyll and becomes Phytin a, and if it falls off to a phytol chain it becomes Phide a (Bidigare et al., 1986). In previous studies, the contents of Phide a is used as an index to estimate the degree of grazing intensity by predators (Bidigare et al., 1986; Burkill et al., 1987). Phide a contents were low on February 15th, February 24th, and March 17th (Fig. 3). In general, the inhabitation density of zoobenthos that feeds on MPB in the west coastal tidal flats is known to be high in summer and low in winter due to the low temperature (Kim et al., 2005; Min et al., 2006). The similar changes were observed in the seasonal inhabitation density of meiofauna (Shin et al., 2016). Therefore, The low grazing intensity of predators seems to contribute to the winter bloom of MPB.

Table 4 Reported values of sediment chlorophyll content. Some part of data followed by MacIntyre et al. (1996). The contents in brackets mean the average values of chlorophyll *a* content.

Location	Latitude	Duration (months)	Thickness (mm)	Chlorophyll *a* content (mg m^{-2})	Source
Dutch Wadden Sea, Netherlands	53° 05 ' N	57	10	30-420 (102)[a]	Cadée and Hegeman (1974)
Netarts Bay, US	45° 25 ' N	12	10	10-315 (138)[a]	Davis and McIntire (1983)
Golfe de Fos, France	43° 23 ' N	28	10	<1-75 (53)[a]	Plante-Cuny and Bodoy (1987)
Ria de Arosa, Spain	42° 24 ' N	14	10	30-103 (14)[a]	Varela and Penas (1985)
Boston Harbor, US	42° 19 ' N	14	10	50-330 (26)[a]	Gould and Gallagher (1990)
Potter Pond Lagoon, US	41° 23 ' N	12	10	10-460 (30)	Nowicki and Nixon (1985)
Long Island Sound, US	41° 00 ' N	13	10	2-225 (30)[a]	Sun et al. (1994)
Delaware Estuary, US	38° 45 ' N	12	10	79-166 (36)	Sullivan and Daiber (1975)
Isshiki Tidal Flat, Japan	34° 46 ' N	12	10	83-149 (112.4)	Goto et al. (2000)
Peel-Harvey Estuary, Australia	32° 21 ' N	16	10	30-560 (115)[a]	Lukatelich and McComb (1986)
Ganghwa Island, Korea	37° 60 ' N	8	10	1.18-34.25 (7.60)	Yoo and Choi (2005)
Geunso Bay, Korea	**36° 44 ' N**	**16**	**10**	**40-219 (98)[b], 36-214 (96)[c]**	**Values of this research**
Mangyung Tidal Flat, Korea	35° 55 ' N	4	5	0.69-32.11 (8.27)	Oh et al. (2004)
Hampyeong Bay, Korea	35° 05 ' N	24	1	27-65 (32.2)	Lee (2013)
Gwangyang Bay, Korea	34° 55 ' N	10	5	3.44-169 (37.5)	Lee et al. (2009)

[a] Estimated from a figure [b] Total chlorophyll *a* [c] Chlorophyll *a*

4.2 The community composition of MPB

The monthly composition of photosynthetic pigments in the sediment layer of Geunso Bay was analyzed (Fig. 6). MPB community composition consisted of bacillariophytes (;benthic diatoms), chlorophytes, cryptophytes, dinoflagellates, cyanobacteria, pelagophytes, euglenophytes, chrysophytes and prymnesiophytes. Most marker pigment contents tended to increase in winter except for Chl *b* and Perid, Chl *b* contents were the lowest in winter, but the highest in fall, Perid contents were the highest on May. It was found that the relative ratios of all marker pigment except Fuco decreased in winter (Fig. 7). Therefore, although the biomass of MPB taxa other then chlorophytes and dinoflagellates increased in winter, biomass of benthic diatom had the greatest influence on winter bloom.

The contribution of benthic diatoms to MPB community composition in winter increases because benthic diatoms are strong at low temperature and can survive even in Polar Regions. Hejduková (2016) exposed polar diatoms and temperate diatoms to freezing temperatures. As a result, the difference in survival between polar and temperate strains of diatoms was not significant. All the strains survived the −4℃ treatment. Most of the strains survived −20℃ treatment. Larras et al. (2014), not only the MPB community of winter showed high biomass, Species richness, diversity, and evenness of benthic diatoms were also higher than those of summer. In order to survive at such low temperatures, benthic diatom produced high amounts of Extracellular polymeric substances (EPS). EPS is composed of polysaccharides, amino acids, and proteins. EPS plays the key role to protect diatom cells against the severe environment especially in winter conditions by increasing the volume of the inhabitable liquid phase (Welsh, 2000; Krembs et al., 2011; Hejduková, 2016).

4.3 Monthly Chl *a* vertical distribution

There are several factors that change the depth of sediment where MPB appear. The main factor is the difference in the depth of light transmission depending on the characteristics of the sediments. Other factors include temperature fluctuation

exceeding 10℃ throughout the day and tidal cycle as well as the depth of the anaerobic layer in the sediment. It is also altered by the difference in the endogenous migratory rhythm of MPB or the vertical mobility according to the MPB taxa (Serôdio et al., 1997; Underwood & Kromkamp, 1999; Perkins et al., 2002; Yun et al., 2009; Du et al., 2010).

Diatoms, euglenoids, cyanobacteria, dinoflagellates and chrysomonad have the ability of vertical migration (Round & Palmer, 1966; Paterson, 1986; 1989; Happey-Wood & Jones, 1988; Raven et al., 1992; Hay et al., 1993; Jönsson et al., 1994; as cited in Consalvey et al., 2004). The euphotic zone depth in the sediment depends on the grain size and organic content. In the case of sandy sediment, It was 2~3 mm on (Rasmussen et al., 1983). Therefore, if light penetrates deeper, photosynthesis might occur at the deeper depths, and even two layers might appear, vertically separated into two layers of diatoms and cyanobacteria. The layer of filamentous cyanobacteria appears below the diatom's layer (Wiltshire, 2000). This vertical distribution difference is due to the different physiological characteristics of the MPB taxa (Ploug et al., 1993; Kromkamp et al., 1998). However, benthic diatoms are dominant during the year in this study site. Therefore, the monthly changes in the biomass of other taxa are not large enough to have an effect (Barranguet et al., 1997). In addition, the sediment core sample of February 24th HPLC analysis was performed together. The vertical contents distribution of TChl a and Fuco showed a high correlation (R^2=0.99), and the Fuco contents was the highest among the accessory pigments at the depths of 0~14 mm (data not shown).

According to preceding study, more than half of the benthic diatoms found at the top of the sediments are the motile epipelon, and the 90~97% of benthic diatoms at 2~10 mm depth are the episammon (Barnett et al., 2015). The grain size of the sediments is seasonally changed, and the species of benthic diatom also changed together (An et al., 2018). *Nitzschia* and *Navicula* reported high relative frequency in spring and *Amphora* mostly in fall and summer (An, 2016). *Navicula* and *Nitzchia* belong to the motile epipelon and *Amphora* belongs to the motile epipsammon .

In this study, monthly Chl a vertical distribution patterns were found to be deeper

than in other periods from May to July of 2017 (Fig 5). According to the survey conducted in the Nakdong River estuary, The Chl *a* in the sedimentary layer shows a high content up to 1 cm depth from the surface; the seasonal variation of the Chl *a* content showed up to 2 cm depth. In summer and fall, the concentrations of pheopigments-*a* were higher than those of Chl *a* in some sites. The predation pressure on the upper predator was higher. Although the exact cause is unknown, the Chl *a* content was detected at 5 to 6 mm depths in April of 2007, even though it was measured during the daytime during emersion period (Du et al., 2010).

In this research, the Phide *a* contents were also relatively high at that time when the Chl *a* content appeared to a comparatively deep depth (Fig. 3). Therefore, it is possible that the predator feed on MPB and then they moved below the sediment layer and we measured the Chl *a* value, which was not decomposed (Decho, 1988). Also, the mixing effect by bioturbation can be considered. In addition, benthic diatoms are able to survive in dark sediment for months or even years utilizing low metabolic activity in a form of facultative heterotrophy and by forming resting cells (Veuger & van Oevelen, 2011).

The distribution of oxygen concentration in sediments was concentrated in the range of 0~1 mm from the surface of the sediment (Fig. 10). In other articles, the depth of maximum oxygen concentration was reported to be mostly observed at the depth of 0.1 to 0.5 mm in the surface layer, which is also similar to the results of preceding research (Nielsen et al., 1990; Kühl et al., 1996; Hwang & Cho, 2005). The oxygen concentration in the upper part of the sediments increased as time progressed from morning to afternoon, which suggest that the MPB photosynthetic rate has increased as well as vertical migration of MPB to the upper layer. Similarly, Denis et al. (2012) has observed that the pattern of increases in photosynthetic rate of the upper part of the sediments during immersion periods and the opposite pattern of rapid decreases in photosynthetic rate before the emersion periods. In this study oxygen microoptode was used to track the vertical distribution of MPB. The Chl *a* content was uniformly distributed vertically in May~July (spring~summer), and the oxygen concentration decreased sharply at less than 1 mm depths in May. Therefore, it is highly likely that the MPB cells in the sediment layer were mixed by the

bioturbation in May, and corresponding biomasses of MPB were uniformly distributed to deeper sediment layers.

4.4 Daily vertical migration of MPB

MPB's second ecological avoidance strategy to avoid photoinhibition is vertical migration. Vertical migration of MPB occurring during the tidal cycle usually move up to the surface of the sediments during emersion period in the daytime and move downward to the surface of the sediments during immersion period in the nighttime (Round & Palmer, 1966; Happey-Wood & Jones, 1988; Consalvey et al., 2004). As shown in figure 8, It implies that the MPB beneath the sedimentary layer climbed up to the surface of the sediments for photosynthesis, considering that the F' reached the maximum value after 1~2 hours in the local noon throughout the observation period.

Serôdio et al. (2006) describes the biomass variation of MPB communities using F_o as a function of the irradiance in the range 0 to 1500 μmol m^{-2} s^{-1}. In the range of 0~250 μmol m^{-2} s^{-1}, the F_o intensity was rapidly increased and there was a sharp decrease in the range of 250~500 μmol m^{-2} s^{-1} The decrease of F_o gradually occurred in 500~1500 μmol m^{-2} s^{-1}. The increase and decrease MPB biomass were made within 60 minutes according to the change of irradiance, and thereafter biomass was stable. In this study, the irradiance was 215.6 μmol m^{-2} s^{-1} at 14:00 with the sudden increase of 714.2 μmol m^{-2} s^{-1} at 15:00 in October of 2016 (Table 3). Therefore, it indicates that the F' decreases as the Irradiance increases at that time. It implies that the MPB community had a rapid vertical movement below the sediment surface. It is also assumed that the decrease in the F' values at 9:40 to 10:40 on December 3th occurred for the same reason.

4.5 Temporal variation of photo-physiological characteristics of MPB

$\Delta F/F_m'$ varies by about 30% per day depending on the day-night cycle. It shows the steepest increase when it changes from night to day and maintains a constant value during daytime (Longphuirt et al., 2006). Therefore, the daily $\Delta F/F_m'$ change

was not significant because it was always performed during the daytime. The daily change in rETR was not significant either (Table 3).

Irradiance was sufficient to show photoinhibition over 1000 μmol m^{-2} s^{-1} from January, though photoinhibition has not appeared during the observation period (Fig. 9). It turns out to be a result of vertical movement or optical physiological adaptation to avoid photoinhibition of MPB. Perkins et al. (2002) found that benthic diatoms go downward to avoid photoinhibition as PAR increases. If the MPB, which received strong light intensity, go deeper from the surface of the sediments to avoid photoinhibition, the amount of light energy actually entering the cell will be reduced. Diving PAM is capable of measuring fluorescence from a cell located at a depth of about 200 μm from the surface of the sediment. Therefore, if the PAR is increased enough to cause photoinhibition, $\Delta F/F_m'$ must also decrease, but $\Delta F/F_m'$ increased by the MPB moving downward. It is considered to be measured at rETR higher than the actual value.

The $\Delta F/F_m'$ and rETR were negatively and positively correlated to the irradiance, respectively. Therefore, as the PAR increases, the efficiency with which chlorophyll absorbs light energy decreases, while the gross primary production increases. However, It showed the different variation pattern in July. It might be the result of vertical migration of MPB, and might be interpreted as a change in photosynthetic characteristics and photosynthetic ability with changes in species composition in benthic diatoms. According to Yun et al. (2009), the photosynthetic capacities of four diatoms (*Navicula* sp., *Nitzschia* sp., *Cylindrotheca closterium, Pleurosigma elongatum*) have shown differences in temperature. For example, in *Pleurosigma elongatem* the efficiency of photosynthesis continued to increase even at 35℃. Therefore, further investigation on the temporal variations of the species composition and photosynthetic characteristics in benthic diatoms will be necessary.

Chapter 5. Conclusions

In this study, We investigated the pigment composition, vertical distribution and photosynthetic characteristics of MPB in the sedimentary layer of the middle intertidal zone of Geunso Bay. TChl a concentrations representing biomass were high throughout the year. Seasonally, MPB biomass was highest in winter and minimum in summer and increased again in fall. The benthic diatom having Fuco as a marker pigment dominated throughout the year, but the relative ratios of other marker pigments have shown low values, and these differences were more prominent in winter. The monthly vertical distribution of MPB in the sediment layer was investigated, and MPB were shown to deeper depths from May to July. It is presumed that MPB cells are transported downward by bioturbation of zoobenthos. $\Delta F/F_m{}'$ and rETR values, which reflect the photosynthetic characteristics of MPB, showed little change during the day. Each of $\Delta F/F_m{}'$ and rETR was negatively and positively correlated to irradiance. Therefore, as the PAR increases, the efficiency with which chlorophyll absorbs light energy decreases, while the gross primary production increases.

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