



Temporal dynamics and spatial heterogeneity of microalgal biomass in recently reclaimed intertidal flats of the Saemangeum area, Korea

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ABSTRACT

Trophodynamics of intertidal mudflats are significantly driven by microphytobenthos (MPB) production but spatial and temporal dynamics of this production source is poorly known. To understand the temporal dynamics and spatial heterogeneity of intertidal MPB, benthic chlorophyll *a*, phaeopigments, and sediment properties were determined in Gyehewa (sandy) and Gwanghwal (muddy) tidal flats of Saemangeum area over a year at 97 stations. This study set out to: (i) characterize the spatial-temporal patterns in MPB biomass on a year-round basis, (ii) identify the abiotic and biotic factors associated with MPB distributions, (iii) investigate the use of satellite-derived chlorophyll *a* data and verify with in field measurements, and (iv) determine minimum required sample size for *in situ* biomass measurement. Concentrations of benthic chlorophyll *a* and phaeopigments were greater in winter and spring with a high magnitude of variance than in summer and fall at both areas. Benthic chlorophyll *a* and phaeopigments tended to decrease approaching lower tidal zone, being associated with the corresponding decrease in shore level and/or exposure duration. Compared to available data on macrozoobenthos distribution, the spatial variation of microalgal biomass seems to be attributed to distribution of deposit-feeders. A significant positive correlation ($p < 0.001$) between *in situ* MPB biomass and satellite-derived normalized difference vegetation index (NDVI) values was observed, but was much weaker in the lower tidal zone. Mirroring algal heterogeneity, the minimum required sample size for *in situ* biomass measurement were greater in blooming season and sandy bottom, suggesting that sampling design for spatio-temporal mapping of MPB should consider the sampling season and/or abiotic and biotic features of study area. Overall, spatio-temporal dynamics of intertidal MPB seem to be influenced by a combination of abiotic and biotic factors.

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1. Introduction

Microphytobenthos (MPB) contributes significantly to water column production via resuspension, particularly in turbid shallow water systems such as intertidal flats (de Jonge, 1992; de Jong and de Jonge, 1995; de Jong and de Jonge, 1995). Previous reports indicated that resuspended MPB in tide or wind dominated regions could account for between 30 and 85% of total planktonic biomass in the water column (de

Jong and de Jonge, 1995; Koh et al., 2006). MPB is considered key food sources in the benthic-pelagic coupling system, thus the understanding of the spatio-temporal dynamics of intertidal MPB is of fundamental concern in tidal flat ecology.

In fact, many studies have endeavored to address the spatio-temporal distributions of microalgal biomass or production in tidal flats on various time scales (Brotas et al., 1995; de Jonge et al., 2012; Méléder et al., 2005; Underwood and Kromkamp, 1999). One of the key questions would be looking for the temporal changes of microalgal biomass (and production), say changes in <hour, day, month or season, and over a year (Brotas et al., 1995; Brotas and Plante-Cuny, 1998; Colijn and Dijkema, 1981; de Jonge and Colijn, 1994; Koh et al., 2007), but such temporal changes have not been well addressed in association with spatial dynamics, particularly spatial heterogeneity. While several studies

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have reported the heterogeneity characteristics of MPB distributions (Sandulli and Pinckney, 1999; Seuront and Spilmont, 2002; Weerman et al., 2012), they lacked time-series understanding of microalgal dynamics in terms of tide-associated algal zonation. While the distribution of MPB has often been studied in macroscale environments (Mélédér et al., 2005; Orvain et al., 2012), previous studies investigating spatio-temporal distribution patterns of MPB have focused on either one of the spatial or temporal aspects. Studies with strong replication or high resolution across both spatial and temporal scale are still limited.

The spatio-temporal distribution of MPB could be driven by many abiotic parameters but their association with macrofauna (Chapman et al., 2010), meiofauna (Pinckney and Sandulli, 1990), and bacteria (van Duyl and Kop, 1994) have also been recognized. Especially, some earlier studies indicated that deposit-feeders play an important role in controlling microalgal biomass (Levinton and Bianchi, 1981; MacIntyre et al., 1996). However, it is also true that such biological interactions have not received much attention compared to abiotic influences such as physical or geochemical parameters or properties of the habitat (Saburova et al., 1995). Thus, in order to understand the mechanism of spatio-temporal MPB dynamics in macroscale environments, the role of biological drivers such as grazing effects should be carefully addressed (Pratt et al., 2015).

One of the most important issues in MPB biomass and production mapping would be minimizing the spatial heterogeneity effects in the field, depending on the size of target area (Saburova et al., 1995). Accordingly, a proper random sampling approach based on the fine determination of relevant *in situ* MPB distribution would be a key step in characterizing MPB dynamics. However, there is little information available to test hypotheses concerning algal heterogeneity in time and space. More recently, a few studies have highlighted the utility of remote sensing techniques in estimating microalgal biomass and production, which might greatly advance productivity mapping of MPB in large coastal ecosystems (Brito et al., 2013; Murphy et al., 2008; van der Wal et al., 2008). Thus, the combined analysis with satellite-derived microalgal index and *in situ* measured biomass should be encouraged, which might help to reduce the heterogeneity effects (Park et al., 2014).

In the present study, we designed a year-round field sampling program and analyses in two distinctive tidal flats (i.e., sandy and soft muddy bottoms, respectively). The type of sedimentary topography common among typical tidal flats, i.e. with sandy and soft muddy bottoms, was one important issue guiding our study design regarding sediment stability and/or nutrients concentration (Underwood, 2009). To overcome the lack of MPB biomass data with high resolution and strong spatial and temporal replication, a systematic grid sampling approach was applied for measuring benthic chlorophyll *a* (Chl *a*) and phaeopigments (Phaeo). The specific purposes of the present study were to: (i) trace the spatio-temporal distribution of MPB biomass, (ii) understand the association of spatio-temporal distribution of MPB with abiotic (i.e., shore level, mud contents, and exposure duration) and biotic (i.e., relative abundance of deposit-feeders and filter-feeders) factors, (iii) compare the relationship between satellite-derived normalized difference vegetation index (NDVI) values and in-field measurements of benthic Chl *a*, and (iv) determine the sample size necessary for testing hypotheses on the MPB assemblages based on the observed heterogeneity. Altogether, we aimed to characterize the monthly and/or seasonal dynamics of intertidal microalgal biomass by addressing its association with macroscale spatial variations and algal heterogeneity.

2. Materials and methods

2.1. Study area and sampling

The Saemangeum tidal flat located on the west coast of Korea (35° 30' N, 125° 40' E) covers a total intertidal flat of 233 km², with a width of >5 km in many places, extending to a maximum of 15 km (Fig. 1a).

Of note, the entire tidal flat area disappeared in the late 2000s after the present study had been completed, as a result of the embankment constructed as part of the Saemangeum reclamation project (Koh and Khim, 2014). The third largest tidal wetland in Yellow Sea has been reclaimed due to this project (Koh and de Jonge, 2014). The sea-dike construction was ~90% complete during the study period (2003–04) and finally completed in Apr. 2006, following complete blockage of the tidal connection (Ryu et al., 2014).

We selected two typical tidal flats, respectively in the Gyehwa (Fig. 1b, A1–A64, number of stations = 64) and Gwanghwal (Fig. 1c, B1–B33, number of stations = 33) areas, representing two types of distinctive bottom topography. First, Gyehwa (~50 km²) was located in the southern part of Saemangeum and has a substrate of mixed mud and sand but is considered primarily sandy compared to the other site in the present study. The second site, Gwanghwal (~45 km²), was located in the center of Saemangeum, and has a soft muddy substrate. The bottom topography of the two areas also differs in terms of shore level and shore slope, which could be a key factor influencing variations in the composition of macrofaunal assemblages between the areas (Ryu et al., 2011a).

The sampling, samples, and associated measurement details are summarized in Tables 1 and S1 in Supplementary Materials (S). In brief, field campaigns were carried out from Dec. 2003 to Nov. 2004 on a monthly basis in Gyehwa (500 m intervals) and from Dec. 2003 to May 2004 on a monthly basis, early Sep. (summer), and Nov. (fall) 2004 on a seasonal basis in Gwanghwal (500 m intervals). Surface sediment samples were collected during the low tide using a syringe (depth = 0.5 cm, i.d. = 1.4 cm) for analyses of benthic Chl *a*, Phaeo, and other geochemical properties. Triplicates of three pooled subsamples were analyzed in order to obtain representative values at each station.

2.2. Laboratory analyses

The sediment samples were frozen in the field and brought to the laboratory and benthic chlorophylls were immediately extracted with 100% acetone (15 ml) for 24 h in the dark at 4 °C. Samples were then centrifuged at 1500 rpm for 5 min and the supernatant aliquot was measured for benthic Chl *a* and Phaeo by the Lorenzen (1967) method (Kwon et al., 2012). The basic sediment properties such as mud contents (% of particles <62.5 μm) and water contents were simultaneously determined according to the methods described in our earlier studies (Ryu et al., 2011a, 2011b). Shore level was estimated from the time of the tidal front arriving at each sampling station relative to the tide tables for Gunsan Harbor, the nearest tidal observation station (~25 km away). According to their shore level (Gyehwa, from –25 to 190 cm above approximate lowest low level; Gwanghwal, from –212 to 272 cm), the study areas were divided into three tidal zones, viz., upper (>100 cm), middle (50–100 cm), and lower tidal flat (<50 cm).

2.3. Satellite data processing

All available Normalized Difference Vegetation Index (NDVI) data from Landsat 7 with 30 m spatial resolution were obtained for the study area (Saemangeum) in 2004 (<http://landsat.usgs.gov/>). The Surface Reflectance CDR is generated using Landsat Ecosystem Disturbance Adaptive Processing System (LEDAPS) developed by NASA Making Earth System Data Records for Use in Research Environments (MEASUREs) (Masek et al., 2006). NDVI is calculated as a ratio between the reflectance values at red (Band 3) and infrared (NIR) (Band 4) wavelengths as follows (Eq. 1):

$$\text{NDVI} = (\text{Band 4} - \text{Band 3}) / (\text{Band 4} + \text{Band 3}) \quad (1)$$

Wavelengths of Band 3 and Band 4 from Landsat 7 are in 630–690 nm and 760–900 nm, respectively.

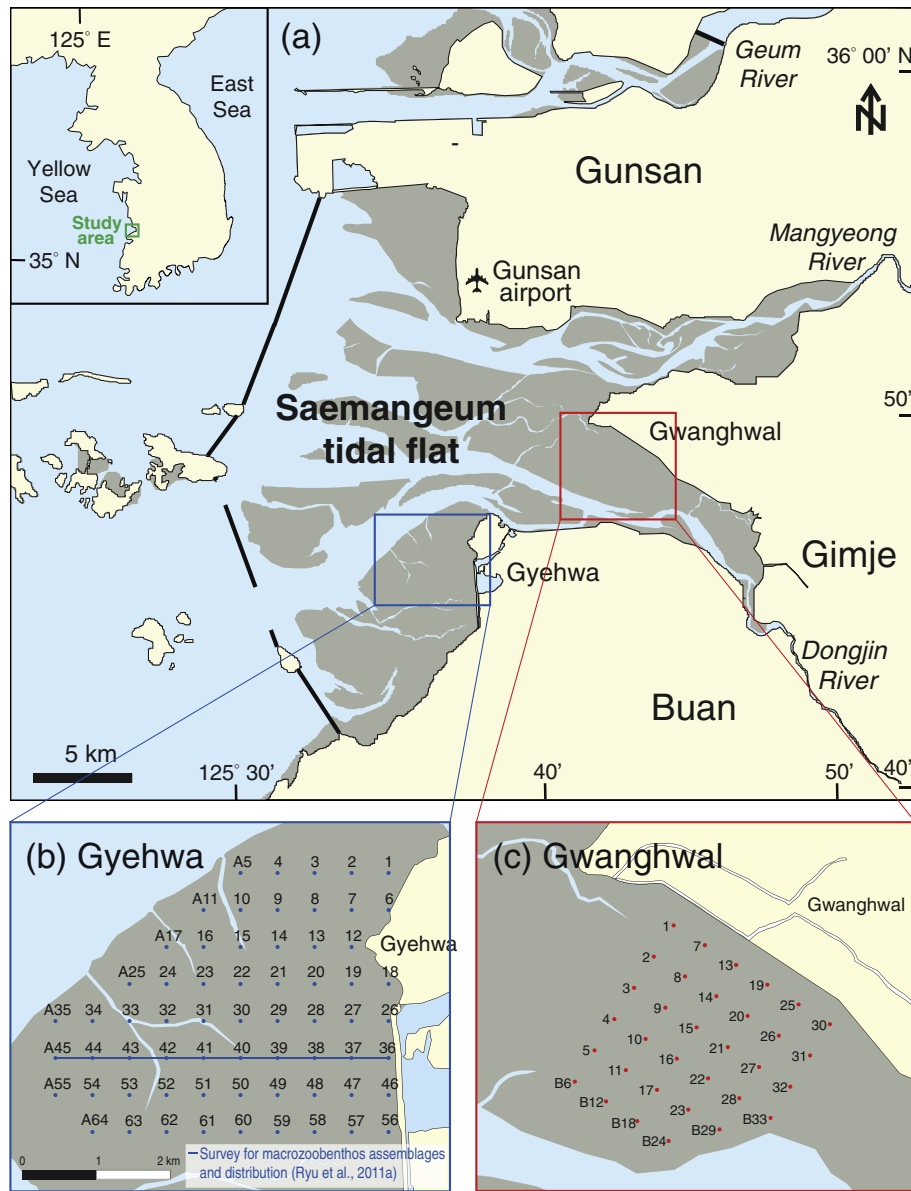


Fig. 1. (a) Map of the Saemangeum tidal flat (in 2004), (b) Gyehwa area showing the 64 sampling stations (A1–A64, 500 m intervals; transect line: data of macrozoobenthos assemblages (Ryu et al., 2011a) used for analysis of grazing activity associated with microalgal biomass distribution), and (c) Gwanghwal area showing the 33 sampling stations (B1–B33, 500 m intervals).

2.4. Data package and analyses

The data obtained from the series of monthly surveys at the two sites were categorized into five groups (Data Sets I, II, III, IV, and V), addressing specific purposes and questions (Table 1). The fundamental question of the spatio-temporal dynamics of MPB will be addressed by the first two data packages (Data Set I and II), viz., a year-round survey at the two areas. Systematic grid sampling at both sites covering the entire area of the intertidal zone enabled determination of the macroscale distribution of microalgal biomass while considering possible masking effects of spatial heterogeneity (Table S1). As part of the study, the macrozoobenthos assemblages were investigated in Gyehwa, and corresponding meta-data (Ryu, 2006; Ryu et al., 2011a) was selectively used to address the relationship between the spatial distribution of MPB and macrofaunal assemblages (Data Set III). This analysis was limited to the transect data to better describe their association with the zonal distribution of the macrozoobenthos. The dominant macrozoobenthos

in Gyehwa include *Umbonium thomasi* (deposit-feeder, 29%), *Ilyoplax pingi* (deposit-feeder, 13%), *Moerella rutila* (filter-feeder, 9%), *Sinocorophium japonicum* (deposit-feeder, 6%), and *Glycera subaenea* (carnivore, 6%) (Ryu et al., 2011a).

Further, selected benthic Chl *a* data at both areas were used to test site-specific correspondence between a satellite-derived algal index and MPB biomass measured *in situ* (Data Set IV). In order to increase the accuracy of comparison, the benthic Chl *a* data showing the greatest variations (Feb. 2004) among the satellite image data were utilized for the analysis. Finally, another meta-data set (Data Set V) obtained from randomly selected samples from Data Set II was used to determine the minimal required sample size for representative sampling of MPB biomass with statistical rigor.

The differences in MPB biomass or sediment properties among the groups were determined using ANOVA or *t*-test with SPSS 22.0 for Windows. Multiple linear regression (MLR) analysis was also used to identify important abiotic and/or biotic factors associated with MPB

Table 1
Overview of the study design for microphytobenthos dynamics in the Saemangeum tidal flat, Korea, summarized by data packages with specific progressive purposes from Data set I to V.

	Data Set I	Data Set II	Data Set III	Data Set IV	Data set V
Main subject with specific purposes	Characterization of temporal dynamics of MPB	Characterization of spatial dynamics of MPB	Macrofaunal association to microalgal distribution	Validation of satellite-derived NDVI in microalgal mapping	Sample size determination in microalgal mapping
Sampling area	Gyehwa (64 stations) ^a Gwanghwal (33 stations)	Gyehwa (64 stations) Gwanghwal (33 stations)	Gyehwa (10 stations; A36–A45)	Gyehwa (19 stations) Gwanghwal (23 stations)	Gyehwa (64 stations) Gwanghwal (33 stations)
Sampling season	Dec. 2003–Nov. 2004 (monthly data)	Winter (Feb. 2004) Spring (May 2004) Summer (Aug. or early Sep. 2004) Fall (Nov. 2004)	Sep. 2004	Feb. 2004	Winter (Feb. 2004) Spring (May 2004) Summer (Aug. or early Sep. 2004) Fall (Nov. 2004)
Key parameters	Chl <i>a</i> Phaeo Phaeo/Chl <i>a</i> ratio	Shore level Mud content Water content Chl <i>a</i> Phaeo	Chl <i>a</i> Macrofaunal distribution based on feeding type ^b	Chl <i>a</i> + Phaeo Chl <i>a</i> NDVI	Chl <i>a</i> concentration of randomly selected samples
Data presented	Tables 2, S2, and S3	Tables 2, 3, and S4 Figs. 2, S1, and S2	Tables 3 and S5 Figs. 3, S3, and S4	Fig. 4	Fig. 5

^a Details of the total number of samples examined given in Table S1.

^b Modified from Ryu, 2006 and Ryu et al., 2011a.

distributions. The shore level (substrate topography), mud contents (sediment granulometry), and exposure duration (tidal condition) were considered as abiotic variables; relative abundances of deposit-feeders and filter-feeders were considered as biotic variables. All the data were initially standardized by z-scale transformation to eliminate the effects of different units (Lee et al., 2015). The non-significant variables were removed by stepwise regression. To assess the algal heterogeneity, coefficient of variation (CV; magnitude of variance) and Moran's I (degree of spatial autocorrelation) were determined (Li and Reynolds, 1995). The Moran's I was calculated in R software with *ape* package (Paradis et al., 2004). The regression analysis and curve fitting between NDVI and benthic Chl *a* were performed using SigmaPlot software version 10.0. The determination of minimal required sample size was done using the Monte-Carlo sampling technique (Grinham et al., 2007). Twenty replicate sets of each sample size were randomly selected from all stations and the standard error was calculated for each set (20 SEs per sample size). The 95% confidence interval (CI) of Chl *a* concentrations was used in the determination of the minimal required sample size. When the Chl *a* concentrations of nineteen replicate sets of each sample size ranged within 95% CI of *in situ* values, the minimum number of sample size was considered as a "minimum required sample size" in this study.

3. Results

3.1. Temporal variations of MPB biomass

The benthic Chl *a* and Phaeo measured in Gyehwa showed significant monthly variations over a year (ANOVA, $p < 0.05$). The mean monthly concentration of Chl *a* increased from Dec. to Feb. (winter). The greatest mean was found in Mar. (27 mg m^{-2}), then tended to decrease through Apr. to Nov. while Phaeo showed the greatest values (21 mg m^{-2}) in Jul. and the smallest in Dec. (6 mg m^{-2}). The spring (Mar. to May) and summer (Jun. to Aug.) peaks were observed in concentrations of Chl *a* and phaeopigments, respectively, with clear seasonal fluctuations (Tables 2 and S2). The benthic Chl *a* and Phaeo from Gwanghwal exhibited a slightly different seasonal pattern, with strong spring (Mar. to May) peaks (Tables 2 and S3). However, the concentrations of both Chl *a* and Phaeo through the winter time (Dec. to Feb.) were comparable to the corresponding spring peaks. The ratio of Phaeo to Chl *a* (Phaeo/Chl *a*) showed a summer peak at both areas.

3.2. Spatial variations of sediment properties and MPB biomass

The significant differences were found in mud contents and Phaeo concentrations between the two areas (Student's *t*-test, $p < 0.01$). Of note, one additional distinct difference between the areas was the geographical location, which might result in different condition in shore level. However, water contents and benthic Chl *a* concentrations were not significant different between areas (Table 2).

The spatial distribution of benthic Chl *a* and Phaeo in Gyehwa significantly varied cross the sampling stations (Chl *a* ranging from 1.3 to 93 mg m^{-2} (mean = 20) whereas Phaeo ranged from 0.26 to 51 mg m^{-2} (mean = 13)). In Gwanghwal, benthic Chl *a* and Phaeo showed significant spatial variations (Chl *a* ranging from 2.4 to 56 mg m^{-2} (mean = 22) whereas Phaeo ranged from 4.4 to 57 mg m^{-2} (mean = 26)). The distinct zonal distributions were evident, with a decreasing trend toward the offshore stations (Fig. 2). The lowest concentrations of Chl *a* and Phaeo were consistently observed at the lower tidal zone where the shore level, proportion of mud in sediment, and duration of exposure were low (Table 2 and Figs. S1 and S2). Such a microalgal zonation pattern was consistently observed throughout the year and became even more apparent in the winter and spring blooming season. Relatively greater seasonal variations were found for the Phaeo/Chl *a* ratio, although spatial variations somehow weakened between the upper and lower zones (Table 2).

The CV and Moran's I values were slightly greater in the Gyehwa than in the Gwanghwal throughout four seasons (Table S4). The greatest CV values were observed in winter and summer, respectively, in sandy and muddy bottoms. On the other hand, Moran's I values were greatest in summer and fall, respectively, for the same areas.

3.3. Spatio-temporal distribution characteristics of macrozoobenthos

The biotic interactions between MPB and the macrofauna were assessed by an analysis of the compositional changes in macrofaunal abundances according to feeding type in the Gyehwa area (details in Table S5). Looking closely at the feeding type of the dominant macrozoobenthos in the study area indicated that the greater abundance (both absolute and relative) of deposit-feeders such as crabs or polychaetes were observed in summer season (Fig. S3). Further, the deposit-feeders were extremely dense in the upper tidal zone and gradually decreased to offshore. On the other hand, filter-feeders and carnivores were the major groups in the lower tidal zone (Fig. 3).

Table 2

Sediment properties and chlorophyll data in the Gyehwa and Gwanghwal areas of Saemangeum, with mean values compared cross the upper, middle, and lower tidal zones at the two areas. The detailed monthly data are provided in Tables S2 and S3.

Parameters	Gyehwa				Gwanghwal		
	Upper (N ^a = 26)	Middle (N ^a = 20)	Lower (N ^a = 18)	Total (N ^a = 64)	Upper (N ^a = 30)	Lower (N ^a = 3)	Total (N ^a = 33)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Sediment properties							
Shore level (cm)	145 ± 4.5	77.2 ± 3.2	4.39 ± 4.7	84.5 ± 7.7	248 ± 2.4	-162 ± 26	210 ± 21
Exposure duration (h day ⁻¹)	16 ± 0.2	14 ± 0.1	11 ± 0.1	14 ± 0.2	20 ± 0.05	6.2 ± 0.9	18 ± 0.7
Mud content (%)	43 ± 2.3	15 ± 1.5	12 ± 1.1	26 ± 1.4	63 ± 3.6	11 ± 1.4	58 ± 3.0
Water content (%)	28 ± 0.3	29 ± 0.3	29 ± 0.2	28 ± 0.1	28 ± 0.5	27 ± 0.8	28 ± 0.2
Sediment chlorophyll							
Chlorophyll <i>a</i> (mg m⁻²)							
Annual mean	20.9 ± 0.64	24.4 ± 0.75	13.8 ± 1.0	20.0 ± 0.48	23.0 ± 0.63	14.2 ± 2.2	22.2 ± 0.63
Seasonal mean							
Winter ^b	22.8 ± 1.3	23.2 ± 1.5	16.7 ± 3.3	21.2 ± 1.1	25.3 ± 0.89	19.2 ± 1.3	24.7 ± 0.91
Spring ^c	26.6 ± 1.4	28.1 ± 1.8	15.6 ± 2.5	24.0 ± 1.1	26.9 ± 1.0	14.1 ± 1.1	25.8 ± 1.0
Summer ^d	20.1 ± 1.1	26.4 ± 1.3	12.9 ± 1.0	20.0 ± 0.75	13.1 ± 0.77	5.64 ± 0.80	12.4 ± 0.82
Fall ^e	14.3 ± 0.92	20.0 ± 1.2	9.80 ± 0.66	14.8 ± 0.62	14.0 ± 0.68	7.72 ± 0.69	13.4 ± 0.71
Phaeopigments (mg m⁻²)							
Annual mean	17.4 ± 0.51	12.4 ± 0.56	6.45 ± 0.45	12.7 ± 0.34	27.8 ± 0.68	7.16 ± 0.77	25.9 ± 0.72
Seasonal mean							
Winter ^b	12.5 ± 0.58	6.75 ± 0.30	3.69 ± 0.32	8.24 ± 0.38	28.7 ± 1.1	7.97 ± 0.40	26.8 ± 1.2
Spring ^c	20.0 ± 1.1	13.7 ± 1.0	6.78 ± 0.87	14.3 ± 0.73	30.1 ± 1.1	6.47 ± 0.46	27.9 ± 1.2
Summer ^d	23.5 ± 0.96	20.1 ± 1.3	11.1 ± 1.2	19.0 ± 0.75	23.6 ± 1.3	6.92 ± 0.76	22.1 ± 1.5
Fall ^e	13.5 ± 0.78	8.84 ± 0.65	4.30 ± 0.54	9.46 ± 0.49	22.4 ± 1.7	7.07 ± 0.40	21.0 ± 1.8
Phaeo/Chl <i>a</i> ratio							
Annual mean	1.1 ± 0.04	0.62 ± 0.03	0.61 ± 0.03	0.80 ± 0.02	1.4 ± 0.04	0.71 ± 0.1	1.3 ± 0.04
Seasonal mean							
Winter ^b	0.66 ± 0.04	0.40 ± 0.04	0.42 ± 0.04	0.51 ± 0.02	1.2 ± 0.05	0.45 ± 0.01	1.1 ± 0.05
Spring ^c	0.94 ± 0.07	0.63 ± 0.06	0.57 ± 0.05	0.74 ± 0.04	1.3 ± 0.07	0.58 ± 0.05	1.2 ± 0.07
Summer ^d	1.5 ± 0.09	0.86 ± 0.07	0.95 ± 0.09	1.1 ± 0.05	2.0 ± 0.2	1.4 ± 0.09	1.9 ± 0.1
Fall ^e	1.3 ± 0.1	0.59 ± 0.07	0.48 ± 0.06	0.84 ± 0.06	1.6 ± 0.1	1.2 ± 0.2	1.5 ± 0.1

^a Number of stations.

^b Mean of Dec., Jan. and Feb.

^c Mean of Mar., Apr., and May.

^d Mean of Jun., Jul., and Aug. in the Gyehwa and early Sep. in the Gwanghwal.

^e Mean of Sep., Oct., and Nov. in the Gyehwa and Nov. in the Gwanghwal.

3.4. Multiple linear regression of benthic chlorophyll *a* on abiotic and biotic variables

The contributions of three abiotic (i.e., shore level, mud content, and exposure duration) and/or two biotic variables (i.e., relative abundances of deposit-feeders and filter-feeders) toward benthic Chl *a* and/or Phaeo were evaluated using multiple linear regression analysis (Table 3). Based on the grid sampling (data type A in Table 3; 97 stations; from Dec. 2003 to Nov. 2004; independent variables: three abiotic parameters), the variations in benthic Chl *a* were explained by three abiotic variables from 17 to 58% in Gyehwa and from 11 to 25% in Gwanghwal. The smallest R² values were observed in winter at both areas. On the other hand, the variations in Phaeo were explained by abiotic variables from 57 to 85% in Gyehwa and from 54 to 74% in Gwanghwal. Meanwhile, in case of transect line sampling (data type B in Table 3; 8 stations; Sep. 2004; independent variables: three abiotic and two biotic parameters), 78% of the variation in benthic Chl *a* was explained by mud content and relative abundance of deposit-feeders. Of note, both variables had negative effect on benthic Chl *a* (Table 3 and Fig. S4).

3.5. Comparison NDVI values with *in situ* measured MPB biomass

As part of this study, we analyzed satellite images to obtain NDVI values corresponding to the *in situ* measured data (Feb. of 2004 data selected) for comparison. In general, significant positive correlations ($p < 0.001$) were observed between NDVI and benthic Chl *a* and Phaeo (log transformed) at the Gyehwa and Gwanghwal areas (Fig. 4). When Phaeo were included in the correlation analysis, the regression coefficient further increased from 0.34 to 0.59.

3.6. Determination of minimum required sample size for *in situ* MPB biomass measurement

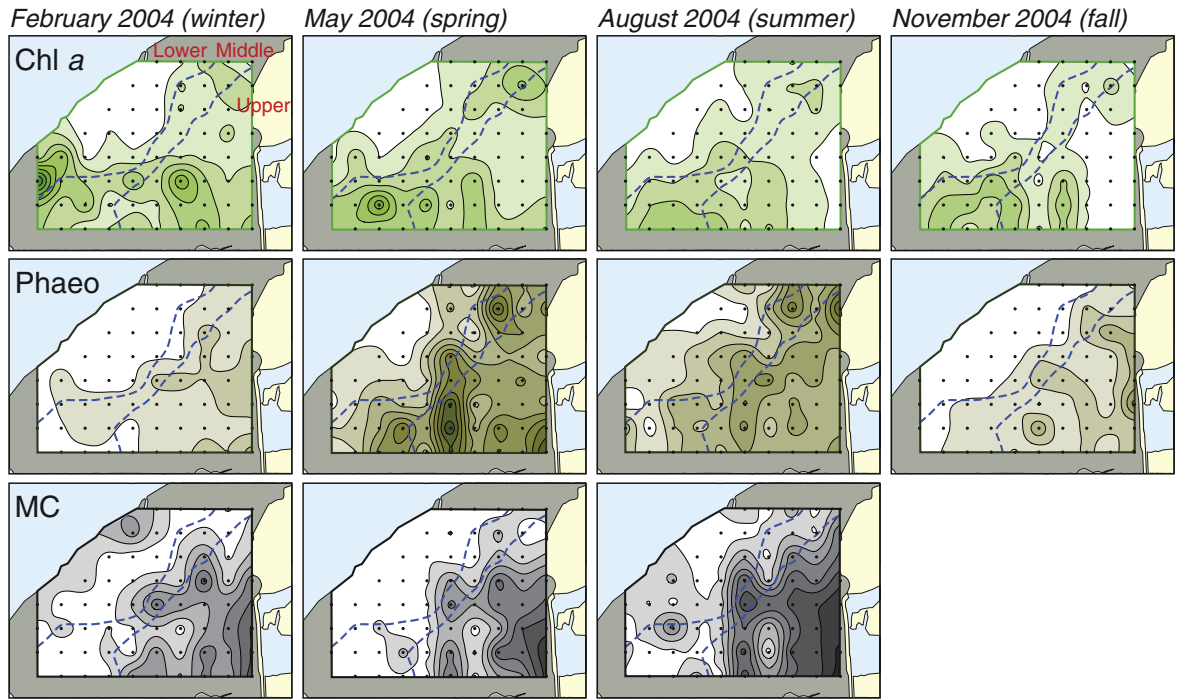
We determined the proper sample size for measurement of benthic Chl *a* that would statistically minimize the heterogeneity effects. Random combinations of sample size covering the entire area of Gyehwa and Gwanghwal generated the mean, minimum, and maximum standard errors according to the sample size across the seasons (Fig. 5). The result, as expected, indicated that standard errors decreased as the sample size increased, and minimum and maximum values approached the mean standard error at certain point. In Gyehwa (sandy bottom), minimum of 30, 25, 18, and 15 replicate samples were appropriate in winter, spring, summer, and fall, respectively. While the minimum required sample size in Gwanghwal was consistent across the seasons with 10, 13, 11, and 12, for the same seasons.

4. Discussion

4.1. Temporal trends in microalgal distribution

The temporal trend found in this study (i.e., winter to spring peaks of microalgal biomass) was consistent with the previous finding on a Japanese tidal flat (Nanaura area), where the important role of grazing activity in the benthic-pelagic coupling of resuspended MPB biomass was highlighted (Koh et al., 2007). Seasonal fluctuations of microalgal biomass and production have long been reported worldwide and accumulated data support their temporal pattern being regional and/or site-specific (de Jonge and Colijn, 1994; de Jong and de Jonge, 1995; Underwood and Kromkamp, 1999). For example, the widely distributed

(a) Gyehwa



(b) Gwanghwal

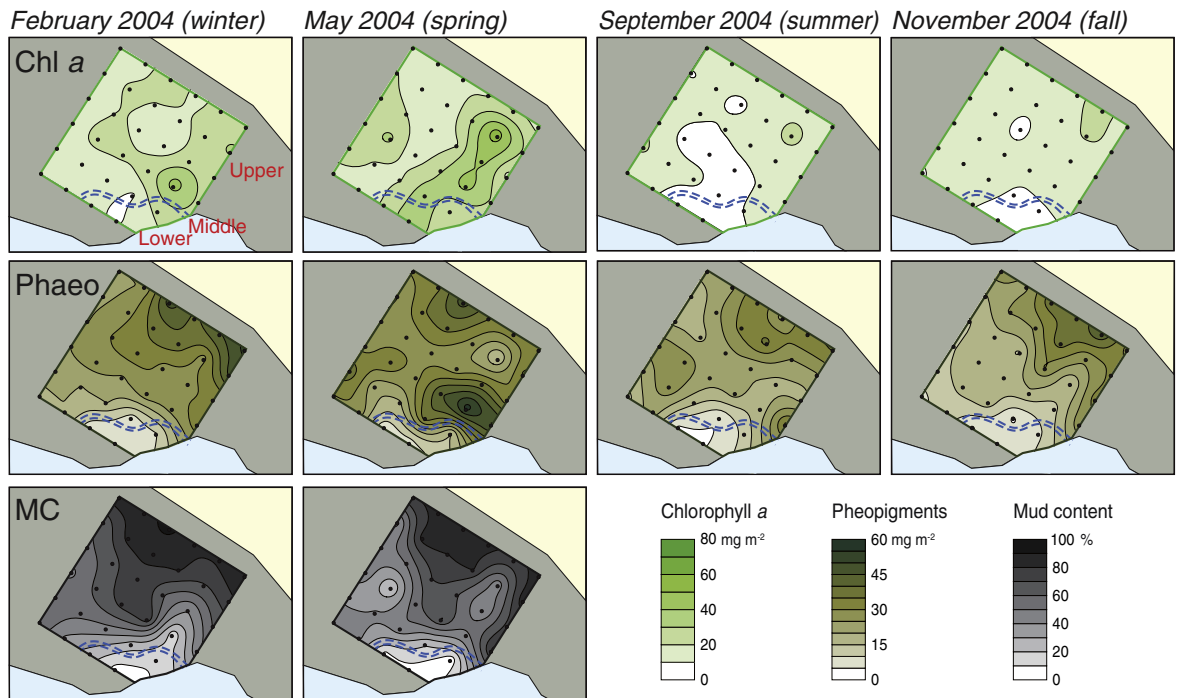


Fig. 2. Spatio-temporal distributions of benthic chlorophyll *a* (Chl *a*), phaeopigments (Phaeo), and mud content (MC) in the (a) Gyehwa and (b) Gwanghwal areas of the Saemangeum.

microalgal habitats common on the European tidal flats, primarily peak in spring (to summer) (de Jong and de Jonge, 1995; Underwood and Kromkamp, 1999). However, an irregular seasonal trend of benthic Chl *a* has been reported in the European tidal flat (de Jonge and Colijn, 1994). In other words, although the summer peak was observed in most of the stations in the Ems estuary, one station was characterized

by high MPB biomass in spring. Meanwhile, winter peaks of microalgal biomass have been reported for the Asian tidal flats, where a significant contribution of tide-induced resuspension of MPB was evident (Koh et al., 2007). Winter microalgal blooming observed in the present study and several earlier reports (Brito et al., 2013; Liu et al., 2013; Koh et al., 2007) seems to be a characteristic of temporal algal dynamics, but

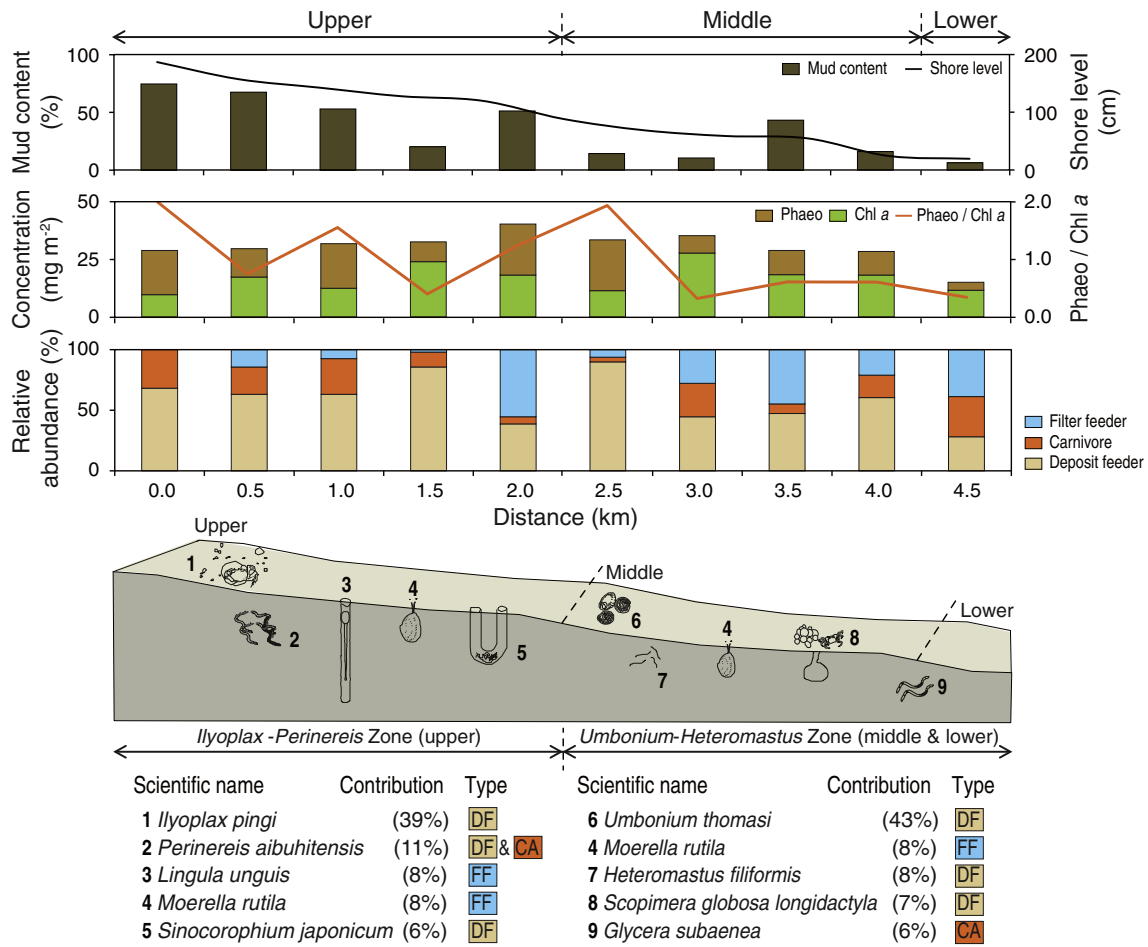


Fig. 3. The association of macrozoobenthos distribution with microalgal biomass distribution; data descriptively given for mud content (MC), shore level (SL), benthic chlorophyll *a* (Chl *a*), phaeopigments (Phaeo), Phaeo/Chl *a* ratio (Phaeo/Chl *a*), and relative abundance of macrozoobenthos by feeding type (deposit-feeder, carnivore, and filter-feeder) along the transect line (see Fig. 1b) in the Gyehwa area of the Saemangeum (Sep. 2004). Refer to Ryu et al. (2011a) for original macrozoobenthos data. Information on feeding type selection is provided in Table S4.

the general mechanism(s) responsible for this pattern remains unclear. Such winter blooming could be explained by certain changes in temperature or climate condition that are not yet fully documented in the field (Underwood and Paterson, 1993; de Jonge et al., 2012). Further, the greatest magnitude of variance (CV) for MPB biomass were recorded in blooming season (winter and spring) at both areas. It seems lead to an increase in the minimum required sample size for *in situ* MPB biomass measurement and heterogeneity during winter and/or spring.

Thermo-inhibition of photosynthetic capacity of MPB (Blanchard et al., 1997) or increased grazing pressure of consumers (Cadée and Hegeman, 1974; Cariou-Le Gall and Blanchard, 1995) during the summer have been reported previously. For example, Blanchard et al. (1997) reported that the optimum temperatures for photosynthesis of MPB do not change significantly throughout the year (~25 °C). Generally, on the Korean mudflats, the temperature of surface sediment usually exceeds 25 °C in summer (Koh and Shin, 1988). Further, exposure duration had strong negative effects on MPB biomass in Gyehwa area. This phenomenon may play a role in summer depression of MPB biomass. Meanwhile, during the summer time, relatively great macrofaunal abundance and diversity were often observed on the Korean tidal flats (Choy et al., 2008; Kang et al., 2007). The similar temporal trend also found in the Gyehwa tidal flat. Especially, the greatest abundance and relative abundance of deposit feeders were observed in summer. The Phaeo/Chl *a* ratio showed summer peak, which provide information about grazing and/or microbial activities (Cariou-Le Gall and

Blanchard, 1995). These results suggest that the greater abundances of grazer during the summer season could also partly contribute to the decreased concentration of MPB biomass.

4.2. Spatial characteristics of microalgal distribution

In general, characterizing the spatial distribution of MPB is difficult due to spatial and temporal heterogeneity, which has not been adequately addressed in the field. In the present study, although the MPB biomass did not differ significantly between both areas, the magnitude of variance (CV) and degree of spatial autocorrelation (Moran's I) for benthic Chl *a* were slightly greater in the sandy bottom than in the muddy one throughout the four seasons. These results indicate that the MPB biomass in Gyehwa area (sandy bottom) was more heterogeneous than that in Gwanghwal area (muddy bottom), based on the greater CV and Moran's I values (Li and Reynolds, 1995). Several previous studies have shown greater biomass and spatial heterogeneity of MPB in muddy bottom than in sandy one (Orvain et al., 2012; Perkins et al., 2003; Pratt et al., 2015; Ubertini et al., 2012). However, similar patterns were not observed in the present study. Considering that MPB biomass could be influenced by not only sediment grain-size but also biofilm age (Ubertini et al., 2015), other factors such as concentration and/or composition of extracellular polymeric substances could also affect the results. More complementary studies are necessary for

Table 3
Results of the multiple linear regressions of benthic chlorophyll *a* and phaeopigments using abiotic and/or biotic variables (i.e., shore level, mud content, exposure duration, and/or relative abundances of deposit-feeder and filter-feeder) in Gyehwa and Gwanghwal areas, from December 2003 to November 2004. The greatest variance inflation factors were smaller than 3.

Data type	Dependent variable	Sampling period	Gyehwa						Gwanghwal									
			Independent variables	Regression coefficient	Standard error	<i>t</i>	<i>p</i>	R ²	Independent variables	Regression coefficient	Standard error	<i>t</i>	<i>p</i>	R ²				
A ^a	Chlorophyll <i>a</i> ^c	Mean	Constant	3.92E ⁻¹⁶	0.0908				0.490	Constant	2.45E ⁻¹⁶	0.153				0.256		
			SL	0.935	0.133	7.02	0.000			ED	0.506	0.155	3.26	0.003				
			MC	-0.957	0.133	-7.19												
		Winter ^e	Constant	-4.34E ⁻¹⁷	0.116			0.172	Constant	2.18E ⁻¹⁶	0.167						0.106	
			SL	0.569	0.170	3.36	0.001		MC	0.325	0.170	1.91	0.065					
			MC	-0.551	0.170	-3.25	0.002											
		Spring ^f	Constant	-7.36E ⁻¹⁶	0.0981			0.404	Constant	1.06E ⁻¹⁶	0.153						0.250	
			SL	0.897	0.144	6.24	0.000		SL	0.500	0.156	3.22	0.003					
			MC	-0.807	0.144	-5.61	0.000											
		Summer ^g	Constant	-1.93E ⁻¹⁵	0.0831			0.579	Constant	8.54E ⁻¹⁹	0.156						0.224	
			SL	4.93	1.36	3.64	0.001		SL	0.547	0.156	2.99	0.005					
			MC	-0.826	0.137	-6.05	0.000											
	ED		-4.15	1.34	-2.97	0.004												
	Fall ^h	Constant	-5.74E ⁻¹⁶	0.0893			0.506	Constant	-7.16E ⁻¹⁶	0.154						0.245		
		SL	0.865	0.131	6.60	0.000		MC	0.495	0.156	3.17	0.003						
		MC	-1.02	0.131	-7.78	0.000												
	Phaeopigments ^c	Mean	Constant	-1.36E ⁻¹⁵	0.0593			0.786	Constant	-4.79E ⁻¹⁶	0.0885						0.749	
			SL	5.45	0.969	5.63	0.000		MC	0.866	0.0899	9.63	0.000					
			MC	0.705	0.0974	7.24	0.000											
			ED	-5.22	0.999	-5.22	0.000											
			Winter	Constant	-9.71E ⁻¹⁷	0.0485			0.854	Constant	-5.63E ⁻¹⁶	0.0907						0.737
				SL	0.471	0.0711	6.62	0.000		MC	0.859	0.0921	9.33	0.000				
				MC	0.524	0.0744	7.36	0.000										
			Spring	Constant	-1.07E ⁻¹⁵	0.0599			0.782	Constant	3.99E ⁻¹⁶	0.110						0.627
SL				6.07	0.977	6.07	0.000		MC	0.531	0.152	3.49	0.002					
MC				0.824	0.0983	8.39	0.000		ED	0.329	0.152	2.16	0.039					
Summer			Constant	-1.30E ⁻¹⁵	0.0845			0.565	Constant	8.18E ⁻¹⁷	0.120						0.542	
			SL	8.34	1.38	6.05	0.000		MC	0.736	0.121	6.06	0.000					
		MC	0.576	0.139	4.15	0.000												
		ED	-8.23	1.42	-5.79	0.000												
Fall		Constant	-2.58E ⁻¹⁶	0.0762			0.640	Constant	1.39E ⁻¹⁶	0.105						0.648		
		SL	0.367	0.112	3.28	0.002		MC	0.805	0.107	7.55	0.000						
		MC	0.493	0.112	4.41	0.000												
B ^b		Chlorophyll <i>a</i> ^d	Sep. 2004	Constant	4.462E ⁻¹⁶	0.196			0.780									
				Mud content	-0.540	0.210	-2.57	0.050										
				%DF	-0.737	0.210	-3.51	0.017										

Abbreviations: SL: shore level; MC: mud content; ED: exposure duration; %DF: relative abundance of deposit-feeder; %FF: relative abundance of filter-feeder.

^a Data from grid sampling in Gyehwa (number of stations = 64) and Gwanghwal (number of stations = 33) areas from December 2003 to November 2004.

^b Data from transect line sampling in Gyehwa area, September 2004.

^c Independent variables: SL, MC, and ED.

^d Independent variables: SL, MC, ED, %DF, and %FF. The station A39 (outlier in abundances of *Ilyoplax pingi* and *Perinereis aibuhitensis*) and A45 (lower tidal zone) were excluded from the regression analysis.

^e Mean of Dec., Jan. and Feb.

^f Mean of Mar., Apr., and May.

^g Mean of Jun., Jul., and Aug. in the Gyehwa and early Sep. in the Gwanghwal.

^h Mean of Sep., Oct., and Nov. in the Gyehwa and Nov. in the Gwanghwal.

a better understanding of the relationship between distribution of MPB biomass and various factors, including abiotic and biotic variables.

The decreasing trend in benthic Chl *a* and Phaeo, and Phaeo/Chl *a* ratio across the tidal zones consistently found at both areas could be explained by the combined changes in abiotic (i.e., shore level, mud content, and exposure duration; Underwood and Paterson, 1993) and biotic parameters (macrofaunal distribution; Anderson, 2008; Thrush et al., 2003; Pratt et al., 2015). MPB biomass is usually greater on muddy substrates (Jesus et al., 2009; MacIntyre et al., 1996; Underwood, 2009) or upper tidal flats (de Jonge, 1992; de Jonge and Colijn, 1994; van der Wal et al., 2010), which have high stability and/or low resuspension rate. In the present study, among the three abiotic factors, shore level and mud content were important variables for explaining the MPB biomass in Gyehwa area (sandy). Notably, contrary other studies (MacIntyre et al., 1996; Jesus et al., 2009), mud content had a negative effect on MPB biomass, particularly in upper shore. Oh and Koh (1995) reported that the dominant microphytobenthos species were different between Gyehwa and Gwanghwal areas. Of note, *Achnanthes hauckiana*, which was a dominant species in Gyehwa area,

showed high abundance in low mean grain size (viz. sandy bottom). These results indicate that different dominant species of microphytobenthos could influence on aforementioned trend in given area. On the other hand, although main factor was different between seasons, shore level and mud content had a positive effect on benthic Chl *a* concentration in Gwanghwal area (muddy), consistent with the previous findings (de Jonge, 1992; de Jonge and Colijn, 1994; Jesus et al., 2009; MacIntyre et al., 1996; Underwood, 2009; van der Wal et al., 2010). The three abiotic variables, which are known as the most important factor in determination of large-scale heterogeneity of MPB (Underwood, 2009), explained <50% of the variation in MPB biomass at both areas.

In fact, the differences in sediment properties such as grain size (or mud content) and organic content have long been considered as important characteristics linked to assemblage structure and dynamics of MPB (Jesus et al., 2009; MacIntyre et al., 1996) as well as of the macrozoobenthos (Anderson, 2008; Thrush et al., 2003). The benthic animals could play important roles in controlling the MPB biomass through grazing (Hillebrand, 2008) or enhancing the resuspension of

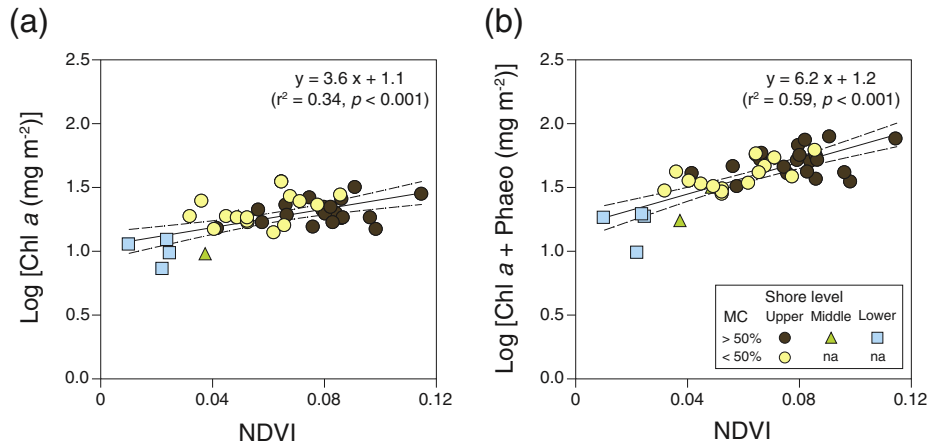


Fig. 4. Relationships between satellite-derived algal index and *in situ* measured benthic chlorophyll *a* (Chl *a*) and phaeopigments (Phaeo): (a) NDVI and log Chl *a* and (b) NDVI and log (Chl *a* + Phaeo), data presented for the Gyehwa and Gwanghwal areas of the Saemangeum, for comparison (Feb. of 2004 data), dashed line represents 95% confidence intervals (na: not available).

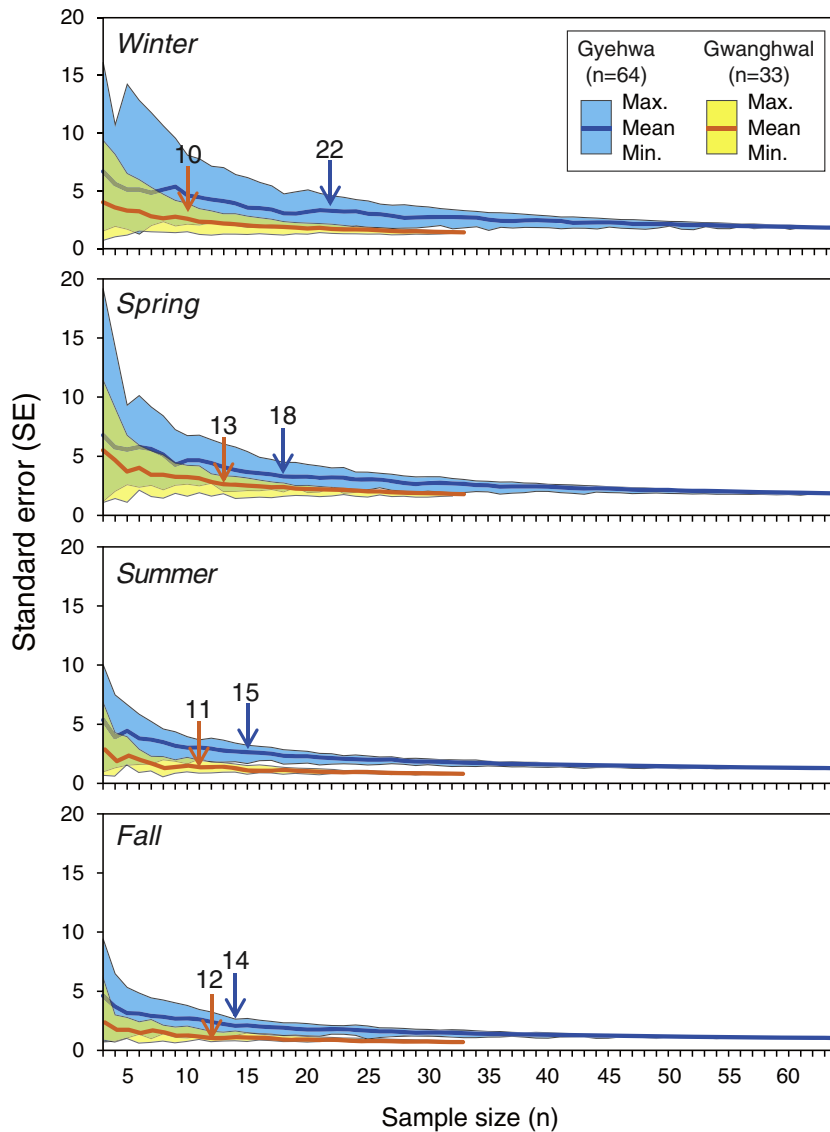


Fig. 5. Determination of proper sample size for *in situ* measurement of microalgal biomass at the Gyehwa and Gwanghwal areas of the Saemangeum. The minimum required sample size was determined through the analysis given (see numbers in the panels) for site- or season-specific comparisons.

sediment (de Jonge, 2000). Although the effects of abiotic and/or biotic components are not usually separately delineated, the analysis of macrofaunal feeding type indicated the significant role of macrozoobenthos in driving MPB dynamics in the given area. A multiple linear regression analysis, using the data from transect line sampling in Gyehwa area (Sep. 2004), showed that mud content and relative abundance of deposit-feeders significantly negative effect on benthic Chl *a*. These result indicate that, based on the transect line data, spatial distribution of MPB were influenced by combined effects of abiotic and biotic parameters. In addition, the positive relationship between mud content and relative abundance of deposit-feeders could partly explain the smaller concentration of benthic Chl *a* in high mud content stations (or areas). Meanwhile, three abiotic variables have strong influences on phaeopigments concentration and explained together 79% and 75% of the variability in Phaeo, respectively, at both areas. Particularly, the mud content had a positive effects on Phaeo. These results indicate that areas favorable to storage of fine sediments particles are also favorable for storage of dead microphytobenthos (Fagherazzi et al., 2014).

The seasonal change in the macrofaunal communities would be much more dynamic compared to those of the environmental parameter(s), thus biotic drivers could be also important when interpreting the spatial distribution characteristics of intertidal MPB. The consistent zonal distribution characteristics of microalgae over the season further questioned the relationship between algal distribution and typical zonation of the macrozoobenthos. The apparent macrofaunal zonation found in this study could be explained by changes in sediment characteristics (viz., mud content); in contrast, the grazing activity (Hillebrand, 2008; Smith and Underwood, 2000) or destability effects (de Jonge, 2000) should be associated with microalgal biomass suppression. The grazing index of the Phaeo/Chl *a* ratio had a zonal pattern similar to that of the relative abundances of deposit-feeders found in the given area, therefore supporting the importance of general biotic interaction in influencing MPB dynamics. In this respect, abiotic factors and biological drivers seemed to jointly influence the zonal distribution and heterogeneity characteristics of the MPB. The spatial heterogeneity observed in the study areas could be a possible masking effect in the determination of reliable, repeatable, and comparable biomass, and thus, accurate production estimates on a large spatial scale (Underwood and Kromkamp, 1999; Kelly et al., 2001).

4.3. Microalgal mapping and heterogeneity

Generating accurate estimates of microalgal biomass (or production) on intertidal flats has been a continuing challenge, as sediment type might be a driver of algal heterogeneity (Barillé et al., 2011; Carrère et al., 2004). Although the remote sensing technique is a powerful and cost-effective tool for estimating coastal marine production mapping on regional and/or global basis, careful application of this approach combined with *in situ* validation for the accuracy of estimates has not been well exercised. In the present study, the NDVI values and *in situ* measured MPB biomass showed significant positive correlations at both areas, thus heterogeneity effects across the sediment types seemed to be negligible. The NDVI obtained in the winter blooming season (viz., Feb.) would be less influenced by a lower masking effect of the smaller occurrence of macrophytes across the intertidal zones during this time (van der Wal et al., 2008). However, due to the limited data for satellite image analysis, the wide application of NDVI-based estimates of MPB biomass could not be extended further. For example, negative NDVI values, which were excluded from the analysis, corresponded to the turbid water and bare sediment (Benyoucef et al., 2014) that were recorded at many stations of the lower zone stations. These results were possibly due to increased resuspension of sediments and/or interference from many randomly distributed small tidal channels, which could result in technical challenges in the wide application of satellite image analysis (Brito et al., 2013). Nevertheless, among the various coastal vegetation indices, the NDVI seems to be a good proxy

for microalgal biomass (and possibly production) in the present study area (Barillé et al., 2011; Benyoucef et al., 2014; Brito et al., 2013).

Meantime, to verify these macroscale algal biomass (or production) estimates, the fundamental issue of masking effects due to heterogeneity should be resolved both in the field and laboratory. The required sufficient sample size corresponding to the minimal heterogeneity was found to be site- and/or season-specific in the study area. One interesting point is that the minimum required sample size tended to decrease from the algal blooming season (winter) to low seasons in Gyehwa. These results were consistent with changes in magnitude of variance (CV) for MPB biomass, which showed decreasing trend from winter to fall. On the other hand, the apparent lower patchiness cross the widely muddy substrate could result in relatively homogeneous distributions of MPB in Gwanghwal. In particular, the minimal required samples size in Gwanghwal was ~1 sample per km², which seems to support such homogeneous distributions of MPB in the given area. However, it should be acknowledged that single measured value in the present study was actually obtained from three pooled sub-samples of triplicate sampling (i.e., nine replicates for one value), which could initially minimize sampling bias in terms of heterogeneity (Grinham et al., 2007). Overall, site- and season-specific determination of sample size would be necessary for reliable mapping of microalgal biomass in the regional scale (say <25 km²).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.seares.2016.08.002>.

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Temporal dynamics and spatial heterogeneity of microalgal biomass in recently reclaimed intertidal flats of the Saemangeum area, Korea

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Table S1. Summary of field surveys and measurements in the Gyehwa and Gwanghwal areas, Saemangeum, from December 2003 to November 2004.

Surveys	Date		Gyehwa (number of stations = 64)						Gwanghwal (number of stations = 33)						
	Year & Month	Day	Season	MC ^a	WC ^b	Chl <i>a</i> ^c	Phaeo ^d	Macro ^e	NDVI ^g	MC	WC	Chl <i>a</i>	Phaeo	NDVI	
s-1	2003	Dec.	27-28	Winter	V	V	V	V		V	V	V	V		
s-2	2004	Jan.	27-28			V	V	V			V	V	V		
s-3		Feb.	23-24			V	V	V			V	V	V		
			20						V					V	
s-4		Mar.	22-23	Spring		V	V	V			V	V	V		
s-5		Apr.	21-23		V	V	V	V		V	V	V	V		
s-6		May	25-27			V	V	V			V	V	V		
s-7		Jun.	22-23	Summer			V	V							
s-8		Jul.	20-23				V	V							
s-9		Aug.	20-24		V		V	V							
		Sep.	03								V	V			
s-10		Sep.	20-22	Fall			V	V	V						
s-11		Oct.	19-22				V	V							
s-12		Nov.	16-19				V	V				V	V		
Total number of samples					192	320	768	768	11 ^f	19	66	165	264	264	23

^aMC: mud content; ^bWC: water content; ^cChl *a*: chlorophyll *a*; ^dPhaeo: phaeopigments; ^eMacro: macrofaunal distribution; ^fModified from Ryu et al. (2011);

^gNDVI: satellite-derived normalized difference vegetation index.

Table S2. Sediment properties, benthic chlorophyll *a* (Chl *a*), phaeopigments (Phaeo), and ratio of Phaeo/Chl *a*. Sediment samples were collected during aerial exposure in the Gyehwa area, Saemangeum, from December 2003 to November 2004 (12 surveys).

Parameters	Survey	Date			Season	Upper	Middle	Lower	Total
		Year & Month		Day		(N ^a = 26)	(N ^a = 20)	(N ^a = 18)	(N ^a = 64)
						Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Mud content (%)	s-1	2003	Dec.	28	Winter	33.8 ± 3.5	11.9 ± 2.4	11.3 ± 2.1	20.7 ± 2.2
	s-5	2004	Apr.	22-23	Spring	36.9 ± 4.5	11.6 ± 2.3	7.40 ± 1.6	20.7 ± 2.6
	s-9		Aug.	20-24	Summer	53.4 ± 4.5	18.0 ± 3.8	14.9 ± 3.3	31.5 ± 3.3
Water content (%)	s-1	2003	Dec.	28	Winter	28.8 ± 0.3	29.5 ± 0.5	28.3 ± 0.5	28.9 ± 0.3
	s-2	2004	Jan.	27-28		29.2 ± 0.4	29.2 ± 0.4	29.5 ± 0.3	29.3 ± 0.2
	s-3		Feb.	23-24		28.0 ± 0.3	28.3 ± 0.3	28.2 ± 0.3	28.2 ± 0.2
	s-4		Mar.	23	Spring	28.0 ± 0.4	28.7 ± 0.5	28.5 ± 0.3	28.3 ± 0.2
	s-5		Apr.	22-23		27.2 ± 0.3	27.4 ± 0.4	27.9 ± 0.3	27.5 ± 0.2
Chlorophyll <i>a</i> (mg m ⁻²)	s-1	2003	Dec.	28	Winter	21.0 ± 1.7	26.5 ± 2.4	14.6 ± 4.8	20.9 ± 1.8
	s-2	2004	Jan.	27-28		21.6 ± 2.7	20.7 ± 3.0	19.3 ± 6.0	20.7 ± 2.2
	s-3		Feb.	23-24		25.8 ± 2.0	22.5 ± 2.5	16.2 ± 5.3	22.1 ± 1.9
	s-4		Mar.	23	Spring	29.0 ± 2.7	29.7 ± 3.7	21.8 ± 6.3	27.2 ± 2.4
	s-5		Apr.	22-23		28.6 ± 2.2	25.8 ± 2.8	11.6 ± 2.1	22.9 ± 1.6
	s-6		May	26-27		22.2 ± 2.0	28.8 ± 2.8	13.5 ± 1.8	21.8 ± 1.5
	s-7		Jun.	22-23	Summer	19.5 ± 2.0	26.5 ± 2.5	11.6 ± 1.8	19.5 ± 1.4
	s-8		Jul.	20-23		24.1 ± 1.8	30.3 ± 2.1	15.6 ± 1.8	23.6 ± 1.3
	s-9		Aug.	20-24		16.5 ± 1.7	22.6 ± 1.6	11.5 ± 1.4	17.0 ± 1.1
	s-10		Sep.	20-22	Fall	17.8 ± 1.2	22.2 ± 2.3	10.1 ± 1.2	17.0 ± 1.1
	s-11		Oct.	19-22		11.8 ± 0.92	17.3 ± 1.4	9.60 ± 0.91	12.9 ± 0.73
	s-12		Nov.	16-18		13.4 ± 2.2	20.6 ± 2.3	9.80 ± 1.3	14.6 ± 1.3
Phaeopigments (mg m ⁻²)	s-1	2003	Dec.	28	Winter	9.20 ± 0.63	6.10 ± 0.54	2.70 ± 0.41	6.41 ± 0.47
	s-2	2004	Jan.	27-28		14.6 ± 1.0	6.90 ± 0.55	4.40 ± 0.66	9.31 ± 0.74
	s-3		Feb.	23-24		13.8 ± 1.0	7.30 ± 0.47	4.00 ± 0.49	8.99 ± 0.68
	s-4		Mar.	23	Spring	20.0 ± 1.4	11.7 ± 1.2	4.80 ± 0.58	13.1 ± 1.0
	s-5		Apr.	22-23		13.6 ± 1.6	10.4 ± 1.3	5.30 ± 1.2	10.3 ± 0.93
	s-6		May	26-27		26.4 ± 2.1	19.1 ± 2.2	10.3 ± 2.0	19.6 ± 1.5
	s-7		Jun.	22-23	Summer	25.6 ± 1.5	21.4 ± 2.1	9.20 ± 1.9	19.7 ± 1.3
	s-8		Jul.	20-23		24.6 ± 2.1	23.3 ± 2.6	13.7 ± 2.5	21.2 ± 1.5
	s-9		Aug.	20-24		20.3 ± 1.2	15.6 ± 1.6	10.3 ± 1.8	16.0 ± 1.0
	s-10		Sep.	20-22	Fall	15.6 ± 1.5	11.9 ± 1.4	6.10 ± 1.1	11.8 ± 1.0
	s-11		Oct.	19-22		14.2 ± 1.2	7.90 ± 0.72	4.20 ± 0.88	9.40 ± 0.79
	s-12		Nov.	16-18		10.8 ± 1.1	6.70 ± 0.73	2.50 ± 0.51	7.20 ± 0.68
Phaeo/Chl <i>a</i> ratio	s-1	2003	Dec.	28	Winter	0.52 ± 0.06	0.32 ± 0.06	0.31 ± 0.06	0.39 ± 0.03
	s-2	2004	Jan.	27-28		0.86 ± 0.08	0.52 ± 0.09	0.41 ± 0.08	0.62 ± 0.05
	s-3		Feb.	23-24		0.60 ± 0.08	0.43 ± 0.07	0.48 ± 0.1	0.52 ± 0.04
	s-4		Mar.	23	Spring	0.85 ± 0.05	0.55 ± 0.09	0.41 ± 0.1	0.62 ± 0.05
	s-5		Apr.	22-23		0.59 ± 0.09	0.59 ± 0.1	0.54 ± 0.1	0.58 ± 0.06
	s-6		May	26-27		1.4 ± 0.09	0.85 ± 0.2	0.76 ± 0.2	1.0 ± 0.08
	s-7		Jun.	22-23	Summer	1.6 ± 0.1	1.0 ± 0.2	0.92 ± 0.2	1.2 ± 0.09
	s-8		Jul.	20-23		1.1 ± 0.07	0.87 ± 0.1	0.85 ± 0.2	1.0 ± 0.07
	s-9		Aug.	20-24		1.6 ± 0.2	0.95 ± 0.2	1.0 ± 0.2	1.2 ± 0.1
	s-10		Sep.	20-22	Fall	1.1 ± 0.09	0.80 ± 0.2	0.69 ± 0.2	0.88 ± 0.09
	s-11		Oct.	19-22		1.4 ± 0.2	0.67 ± 0.2	0.55 ± 0.2	0.88 ± 0.1
	s-12		Nov.	16-18		1.2 ± 0.1	0.66 ± 0.2	0.42 ± 0.2	0.75 ± 0.1

^aNumber of stations.

Table S3. Sediment properties, benthic chlorophyll *a* (Chl *a*), phaeopigments (Phaeo), and ratio of Phaeo/Chl *a*. Sediment samples were collected during aerial exposure in the Gwanghwal area, Saemangeum, from December 2003 to November 2004 (8 surveys).

Parameters	Survey	Date			Season	Upper	Lower	Total
		Year & Month	Day	(N ^a = 30)		(N ^a = 3)	(N ^a = 33)	
						Mean ± SE	Mean ± SE	Mean ± SE
Mud content (%)	s-1	2003	Dec.	27	Winter	64.8 ± 3.5	15.1 ± 5.7	60.3 ± 4.1
	s-5	2004	Apr.	21	Spring	60.5 ± 3.8	7.42 ± 0.77	55.7 ± 4.4
Water content (%)	s-1	2003	Dec.	27	Winter	29.3 ± 0.6	29.1 ± 1	29.3 ± 0.5
	s-2	2004	Jan.	27		29.3 ± 0.6	28.9 ± 0.9	29.3 ± 0.5
	s-3		Feb.	23		27.8 ± 0.4	28.3 ± 1	27.9 ± 0.4
	s-4		Mar.	22	Spring	28.7 ± 0.3	28.1 ± 0.9	28.6 ± 0.3
	s-5		Apr.	21		27.0 ± 0.4	22.7 ± 5	26.6 ± 0.5
Chlorophyll <i>a</i> (mg m ⁻²)	s-1	2003	Dec.	27	Winter	28.6 ± 1.2	21.0 ± 11	28.0 ± 1.4
	s-2	2004	Jan.	27		26.6 ± 1.8	21.6 ± 8.4	26.2 ± 1.8
	s-3		Feb.	23		20.5 ± 1.2	15.1 ± 4.5	20.0 ± 1.2
	s-4		Mar.	22	Spring	24.0 ± 1.4	6.52 ± 2.3	22.4 ± 1.5
	s-5		Apr.	21		33.1 ± 1.5	9.77 ± 1.6	31.0 ± 1.8
	s-6		May	25		23.7 ± 1.9	25.9 ± 5.1	23.9 ± 1.8
	s-9		Sep.	03	Summer	13.1 ± 0.77	5.64 ± 2.5	12.4 ± 0.82
s-12		Nov.	19	Fall	14.0 ± 0.68	7.72 ± 2.2	13.4 ± 0.71	
Phaeopigments (mg m ⁻²)	s-1	2003	Dec.	27	Winter	26.4 ± 1.9	7.38 ± 2.8	24.6 ± 2.0
	s-2	2004	Jan.	27		29.9 ± 2.1	9.01 ± 2.9	28.0 ± 2.2
	s-3		Feb.	23		29.9 ± 1.7	7.52 ± 1.5	27.7 ± 1.9
	s-4		Mar.	22	Spring	33.7 ± 2.0	6.41 ± 2.4	31.2 ± 2.3
	s-5		Apr.	21		23.9 ± 1.7	3.55 ± 2.0	22.0 ± 1.9
	s-6		May	25		32.7 ± 1.6	9.45 ± 2.7	30.6 ± 1.9
	s-9		Sep.	03	Summer	23.6 ± 1.3	6.92 ± 2.4	22.1 ± 1.5
s-12		Nov.	19	Fall	22.4 ± 1.7	7.07 ± 1.3	21.0 ± 1.8	
Phaeo/Chl <i>a</i> ratio	s-1	2003	Dec.	27	Winter	0.91 ± 0.05	0.39 ± 0.06	0.87 ± 0.06
	s-2	2004	Jan.	27		1.2 ± 0.06	0.43 ± 0.06	1.1 ± 0.06
	s-3		Feb.	23		1.5 ± 0.08	0.52 ± 0.05	1.4 ± 0.09
	s-4		Mar.	22	Spring	1.5 ± 0.1	0.97 ± 0.3	1.4 ± 0.1
	s-5		Apr.	21		0.76 ± 0.07	0.35 ± 0.2	0.73 ± 0.07
	s-6		May	25		1.6 ± 0.1	0.40 ± 0.2	1.5 ± 0.1
	s-9		Sep.	03	Summer	2.0 ± 0.2	1.4 ± 0.3	1.9 ± 0.2
s-12		Nov.	19	Fall	1.6 ± 0.1	1.2 ± 0.5	1.5 ± 0.1	

^aNumber of stations.

Table S4. The coefficients of variation (CV) and spatial autocorrelation (Moran's I) for benthic chlorophyll *a* in the Gyehwa and Gwanghwal areas of Saemangeum, from December 2003 to November 2004.

Study area (bottom type)	Index	Study period															
		Month (from Dec. 2003 to Nov. 2004)													Season		
		Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	W ^a	Sp ^b	Su ^c	F ^d
Gyehwa (sandy)	CV	0.67	0.85	0.69	0.70	0.57	0.54	0.58	0.44	0.50	0.51	0.45	0.71	0.74	0.63	0.52	0.58
	Moran's I	0.05**	0.07**	0.08**	0.06**	0.09**	0.08**	0.07**	0.12**	0.12**	0.04**	0.06**	0.10**	0.08**	0.08**	0.11**	0.08**
Gwanghwal (muddy)	CV	0.28	0.39	0.35	0.39	0.33	0.43				0.38		0.30	0.37	0.40	0.38	0.30
	Moran's I	-0.01	0.01	0.05**	-0.01	0.04**	0.03*				0.05**		0.09**	0.03*	0.01	0.05**	0.08**

* $p < 0.05$; ** $p < 0.01$.

^a Winter: mean of Dec., Jan. and Feb.; ^b Spring: mean of Mar., Apr., and May; ^c Summer: mean of Jun., Jul., and Aug. in the Gyehwa and early Sep. in the Gwanghwal;

^d Fall: mean of Sep., Oct., and Nov. in the Gyehwa and Nov. in the Gwanghwal.

Table S5. Dominant species of macrozoobenthos found in the Gyehwa area, Saemangeum, given by feeding type for the analysis for its seasonal and zonal distributions association to microalgal biomass in the study area.

Species name	Feeding type ^b
Crustacea (Decapoda)	
<i>Macrophthalmus japonicus</i>	surface deposit feeder
<i>Ilyoplax pingi</i>	surface deposit feeder
<i>Scopimera globosa longidactyla</i>	surface deposit feeder
<i>Macrophthalmus dilatatus</i>	surface deposit feeder
Gastropoda	
<i>Umboonium thomasi</i>	surface deposit feeder
<i>Nassarius festivus</i> ^a	surface deposit feeder/carnivore
Crustacea (Amphipoda)	
<i>Sinocorophium japonicum</i>	subsurface deposit feeder
<i>Mandibulophoxus mai</i>	subsurface deposit feeder
<i>Monoculodes koreanus</i>	subsurface deposit feeder
Polychaeta	
<i>Periserrula leucophryna</i> ^a	surface deposit feeder/carnivore
<i>Heteromastus filiformis</i>	subsurface deposit feeder
<i>Perinereis aibuhitensis</i> ^a	subsurface deposit feeder/carnivore
<i>Lumbrineris nipponica</i>	subsurface deposit feeder
<i>Mediomastus californiensis</i>	subsurface deposit feeder
<i>Scoloplos armiger</i>	subsurface deposit feeder
<i>Glycinde</i> sp. ^a	subsurface deposit feeder/ carnivore
<i>Aricidea pacifica</i> ^a	subsurface deposit feeder/filter feeder
<i>Spio filicornis</i> ^a	subsurface deposit feeder/filter feeder
<i>Glycera subaenea</i>	carnivore
<i>Nephtys polybranchia</i>	carnivore
Nemertina (Anopla)	
<i>Lineus</i> sp.	carnivore
Brachiopoda	
<i>Lingula unguis</i>	filter feeder
Bivalvia	
<i>Moerella rutila</i>	filter feeder
<i>Meretrix petechialis</i>	filter feeder
<i>Mactra veneriformis</i>	filter feeder

^a Counted in both groups; ^b Classified based on previously published data (Choi et al., 2006; Doi et al., 2005; Fauchald and Jumars, 1979; Gaston et al., 1988; Kanaya et al., 2008a, 2008b; Koo et al., 2008; Lee and Koh, 1994; Wang et al., 2002) and personal communication with experts.

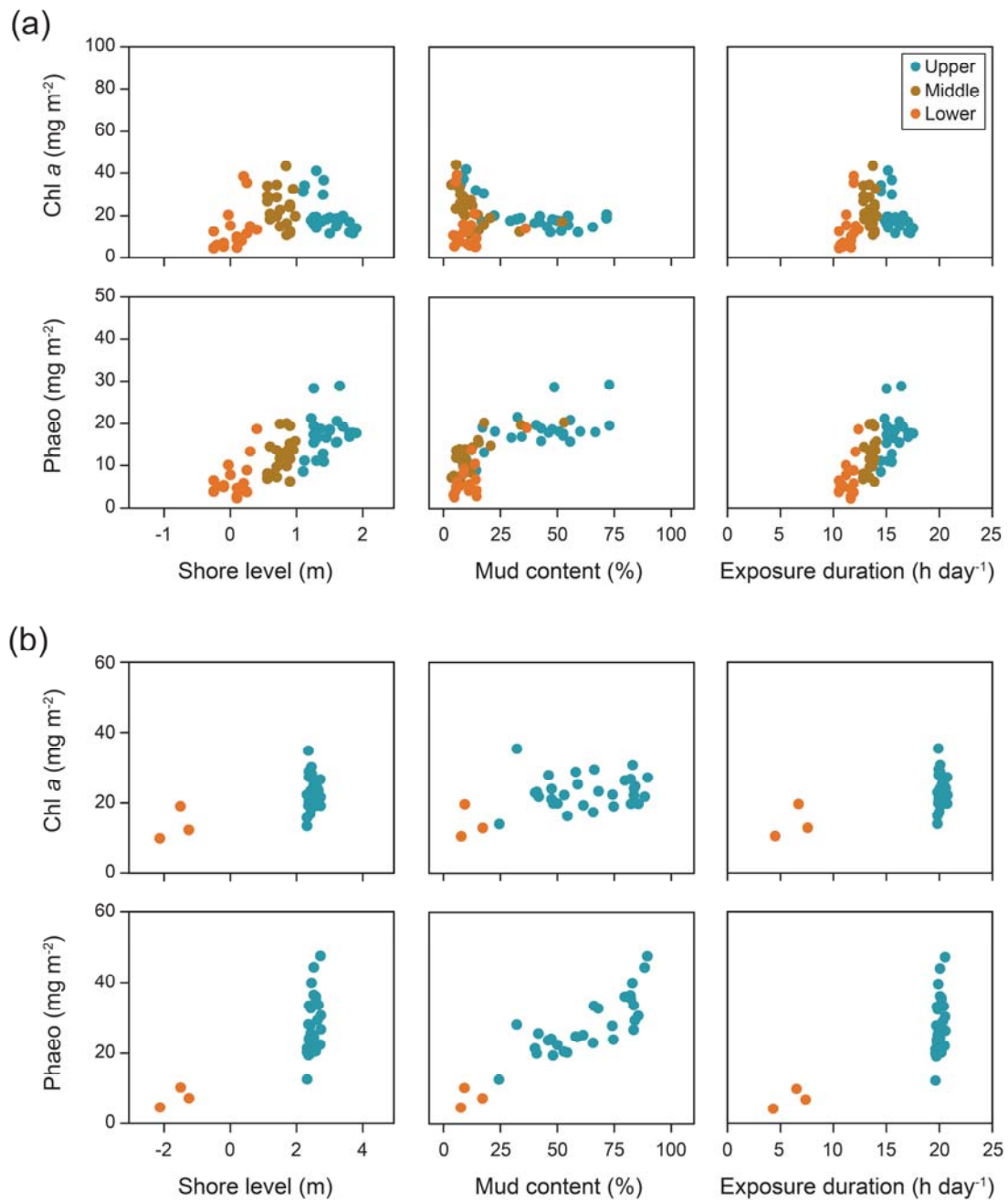


Fig. S1. Scatter plots of annual mean benthic chlorophyll *a* and phaeopigments vs. three abiotic variables (i.e., shore level, mud content, and exposure duration) in the (a) Gyehwa and (b) Gwanghwal areas, Saemangeum, from December 2003 to November 2004.

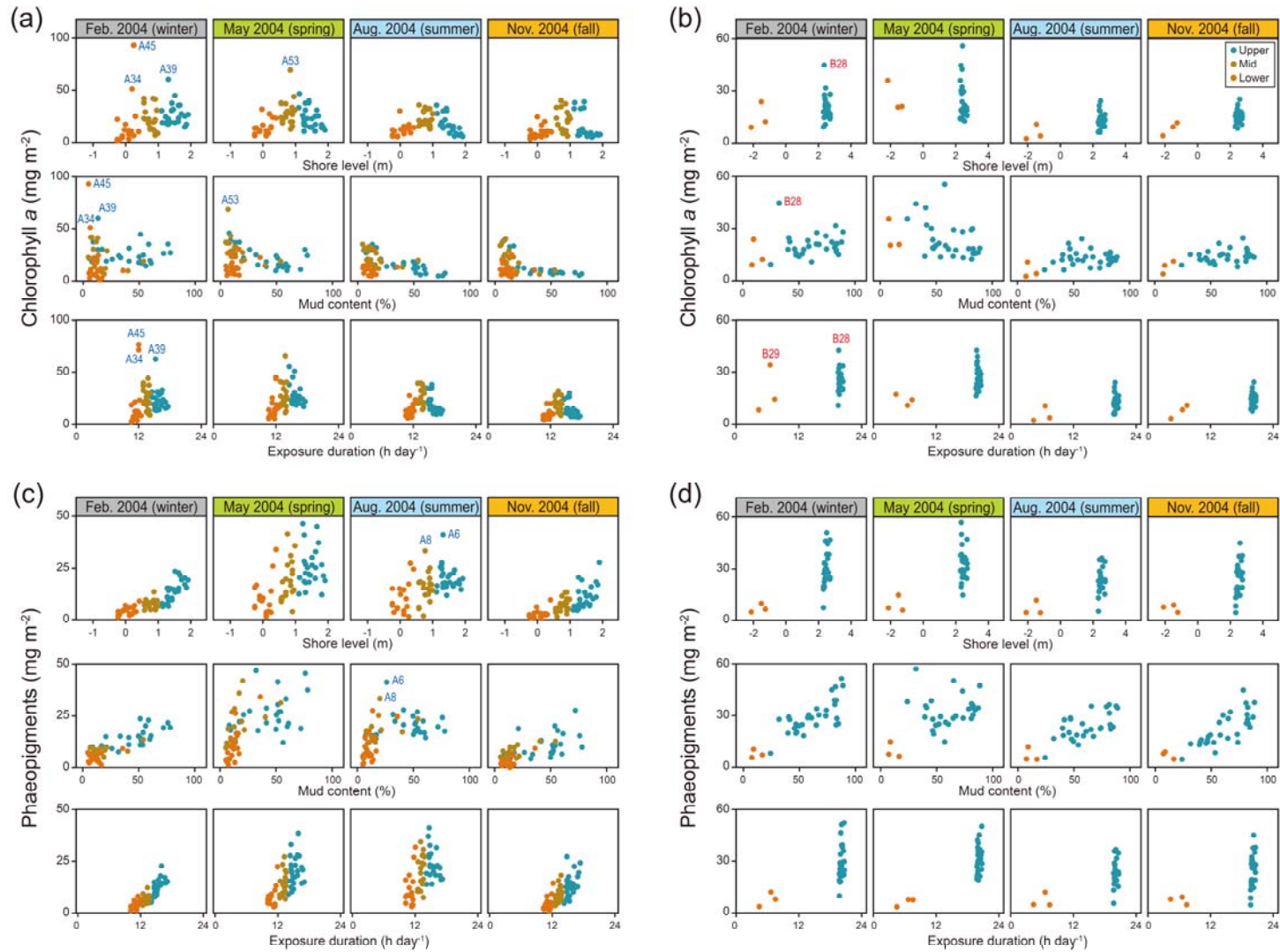


Fig. S2. Scatter plots of seasonal benthic chlorophyll *a* and phaeopigments vs. three abiotic variables (i.e., shore level, mud content, and exposure duration) in the (a, c) Gyehwa and (b, d) Gwanghwal areas, Saemangeum.

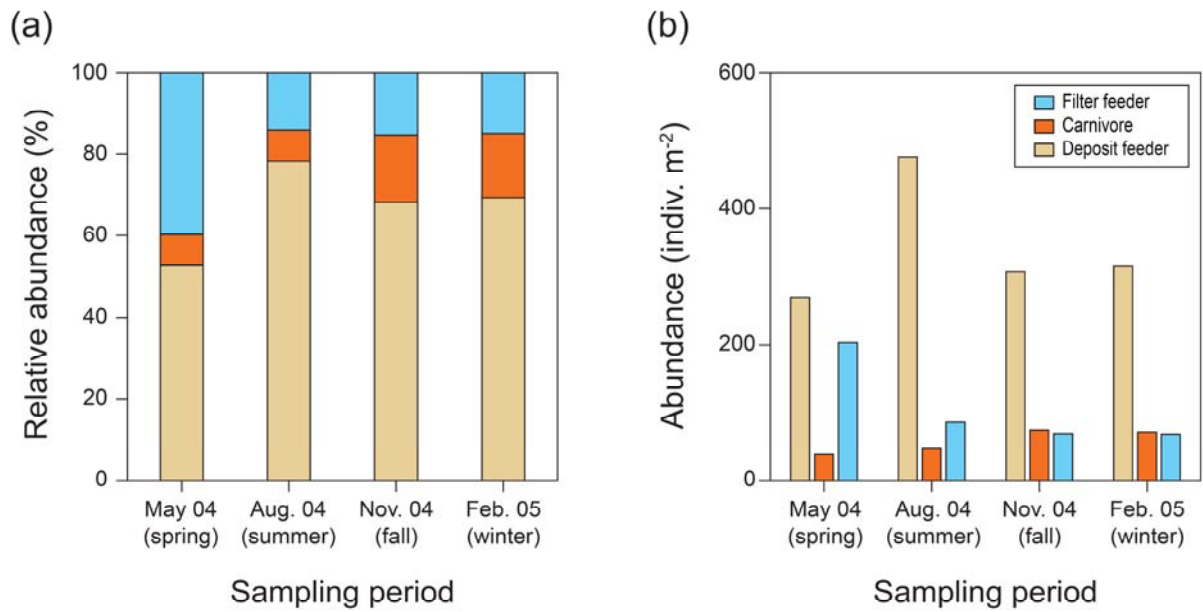


Fig. S3. Seasonal variation of macrozoobenthos in the Gyehwa tidal flat (23 stations), from May 2004 to February 2005. Refer to Ryu (2006) for original macrozoobenthos data.

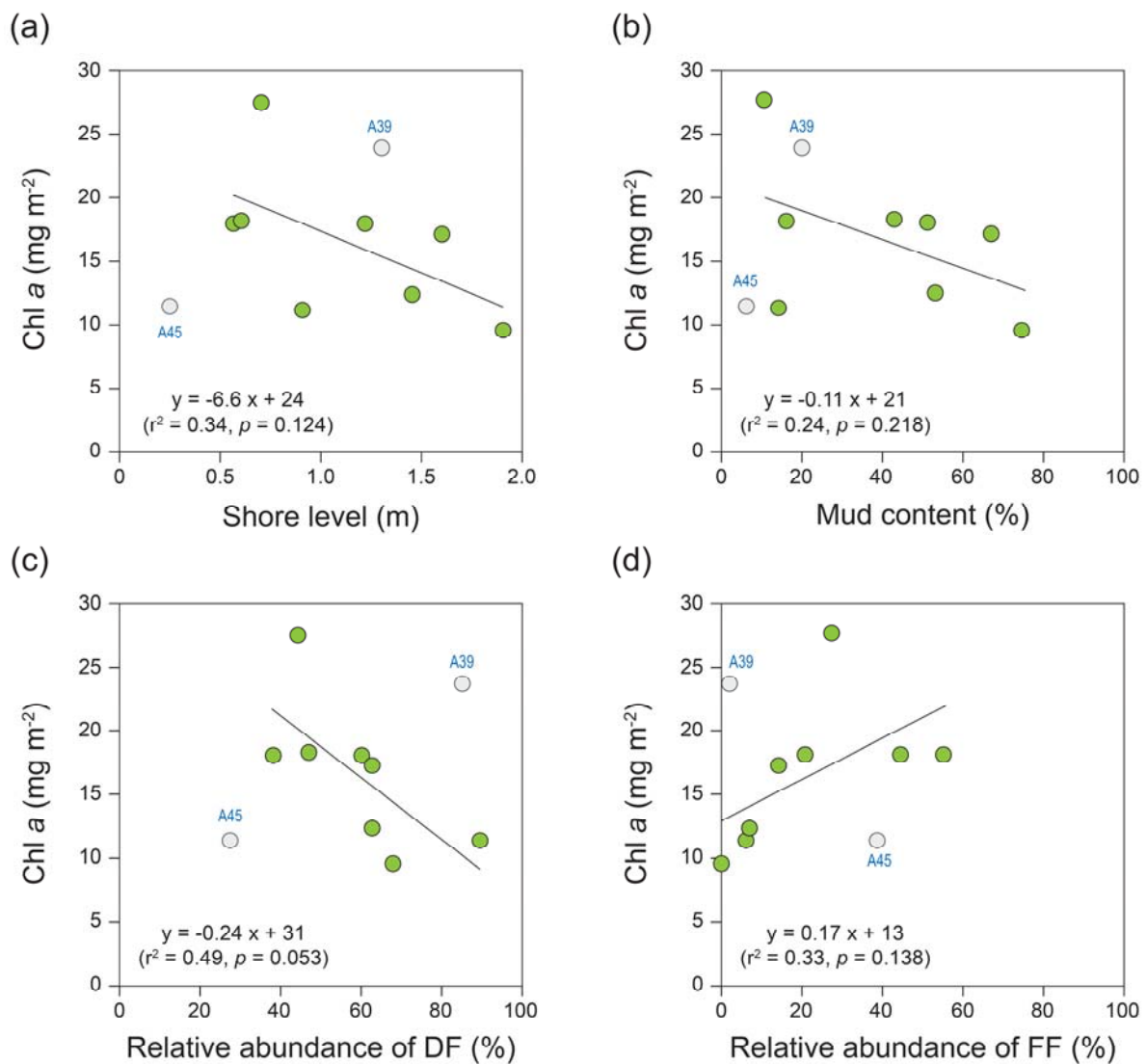


Fig. S4. Scatter plots of benthic chlorophyll *a* vs. (a) shore level, (b) mud content, (c) relative abundance of deposit-feeders, and (d) relative abundance of filter-feeders. Data from transect line sampling in Gyehwa, Saemangeum (September 2004). The station A39 (outlier in abundances of *Ilyoplax pingi* and *Perinereis aibuhitensis*) and A45 (lower tidal zone) were excluded from the regression analysis.

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