



# High-throughput sequencing analysis reveals dynamic seasonal succession of diatom assemblages in a temperate tidal flat

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## ABSTRACT

Benthic diatoms are a major phytoplankton group, and play pivotal roles in coastal biogeochemical cycles and food webs. However, their community structure and diversity remain poorly understood. We investigated the variability in their taxonomic composition and diversity over 5 years in a temperate tidal flat based on high-throughput sequencing of the *rbcl* gene. Chao1 and Shannon species richness and diversity estimates in this study ranged from 412 to 9582 and from 2.7 to 6.3, respectively, showing higher diversity than previous estimates based on traditional morphological analyses. At the genus level, *Navicula*, *Nitzschia*, and *Amphora* were dominant in all seasons. However, at the 98% identity operational taxonomic unit level, seasonal changes in community structure were remarkable. A number of taxa were found only during a specific season, and only a small portion of taxa were commonly found in all seasons. This seasonal succession was mainly influenced by sediment temperatures and sand ratios. Our research provides a solid basis for the high diversity of tidal flat diatoms.

## 1. Introduction

Diatoms are extremely diverse and play an ecologically significant role in the global ecosystem (Mann and Vanormelingen, 2013). They are distributed throughout almost all aquatic environments. In the intertidal zone in particular, benthic diatoms are known to be very abundant and play important roles in primary production and food web dynamics (Colijn and Van Buurt, 1975; Sullivan and Moncreiff, 1988). They are responsible for over 50% of primary production, and are a major food source for upper trophic level organisms in intertidal flats (Underwood and Kromkamp, 1999; Underwood and Barnett, 2006).

Most diatoms inhabiting tidal flats are pennate, and representative genera include *Navicula*, *Nitzschia*, *Amphora*, *Cocconeis*, *Fragilaria*, and *Achnanthes* (Oh and Koh, 1995). The genera *Navicula*, *Amphora*, and *Nitzschia* are known to dominate intertidal regions worldwide (Admiral, 1984; Oppenheim, 1991; Underwood, 1994; Cibic et al., 2012). However, the occurrence and distribution of each taxon depends on the survey area (Underwood and Paterson, 1993; Du et al., 2009; Sin et al., 2009). In a study by Du et al. (2009) in the Nakdong Estuary, although the distance between sampling sites was not large, the seasonal changes in the community structures of benthic diatoms differed at each site. In general, the distribution and growth of diatoms is influenced by diverse

physicochemical factors such as salinity, exposure time, sediment temperature, nutrient concentration of pore water, and sediment particle size composition, as well as biological factors such as interspecific competition and predation (Sullivan, 1975; Amspoker and McIntire, 1978; Admiral, 1984; Underwood and Paterson, 1993).

DNA metabarcoding has provided new insights into diatom diversity by revealing the distribution and biogeographic patterns of species that are difficult to discern by optical methods (Piredda et al., 2017). Therefore, morphology-based approaches are being rapidly replaced by molecular approaches in diatom diversity and taxonomic studies. Recently, next-generation sequencing (NGS) analyses of diatoms have been applied to assess water quality in rivers (Kermarrec et al., 2014; Zimmermann et al., 2015; Visco et al., 2015; Apothéoz-Perret-Gentil et al., 2017) and to reveal the distribution and diversity of toxic harmful algae such as the genus *Pseudo-nitzschia* (Ruggiero et al., 2015; Grzebyk et al., 2017).

To date, most studies of benthic diatoms have been carried out in freshwater environments, and few have been conducted on tidal flats. Diversity studies on benthic diatoms in tidal flats are limited owing to several obstacles, such as small size, simple forms, cryptic species, and low accessibility due to tides (Round et al., 1990; Ribeiro et al., 2013; Zimmermann et al., 2015; An et al., 2017).

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A previous study demonstrated that the *rbcl* gene is a good genetic marker for the identification and classification of benthic diatoms (An et al., 2017). Subsequently, an NGS technique using *rbcl* genes was applied to examine benthic diatom diversity at a fine taxonomic resolution (An et al., 2018). In this study, we applied this NGS technique to elucidate the temporal variation in benthic diatom community composition and diversity and to better understand the environmental factors regulating these changes in tidal flats.

## 2. Materials and methods

### 2.1. Study area and sample collection

Sediment samples were collected from a tidal flat in Guenso Bay (126°10'47.52" E, 36°44'12.06" N), on the west coast of Korea (Fig. 1). A total of 80 samples were collected from May 2009 to May 2015. Surface sediments with a depth >3 cm were sampled using a transparent acrylic corer (2 cm internal diameter, 15 cm in length). Because benthic diatoms form a mat on the sediment surface, five replicate samples were collected to minimize bias due to spatial heterogeneity at each sampling station. Samples were immediately stored in a cooler and transported to the laboratory. The top 1 cm of sediment samples from five replicates were pooled in a conical tube and thoroughly mixed. Subsamples of the mixed sediment sample were stored at −80 °C and used for DNA extraction, measurement of chlorophyll *a* (chl *a*) concentration, and grain size analysis.

### 2.2. DNA extraction, next-generation sequencing, and data analyses

Genomic DNA from 80 sediment samples was extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), following the manufacturer's protocol. In this study, the *rbcl* gene region was used for NGS analysis. The first PCR amplification was performed using a diatom-specific primer pair (Drbcl265\_f\_MiSeq, 5'-TAYCGYGTAGATCCAGTTCCA-3'; Drbcl695\_r\_MiSeq, 5'-GCACGRT-TRATASCTTCCAT-3') modified with adapter overhangs. These primers amplify a 431-bp fragment of the *rbcl* gene. The overall experiment and analyses for NGS were performed following the protocol described in An et al. (2018). The sequences obtained in this study were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA, <http://www.ncbi.nlm.nih.gov/Traces/sra>; accession number PRJNA421862).

### 2.3. Other analyses

Sediment temperature was measured using a thermometer with a stainless steel probe. Chl *a* and indicator pigment concentrations in

sediments were measured using a Shimadzu LC-20A HPLC system (Shimadzu, Kyoto, Japan) after extraction with 95% acetone for 24 h (Zapata et al., 2000), and converted to mass per unit area ( $\text{mg m}^{-2}$ ). Particle size analysis was conducted on 71 samples. The sediments were pretreated with 30% hydrogen peroxide and 10% hydrochloric acid, and analyzed in triplicate using a Mastersizer 3000 grain size analyzer (Malvern Instruments, Worcestershire, UK). Grain size and statistic parameters were analyzed using the GRADISTAT software package (v.8.0; Blott and Pye, 2001; <http://www.kpal.co.uk/gradistat.html>). The data on air temperature, solar irradiance, and duration of sunshine used in the study were collected by the Korea Meteorological Administration (<http://www.kma.go.kr/>).

### 2.4. Statistical analysis

One-way ANOVA and correlation analyses were undertaken using SPSS v.18 (SPSS Inc., Chicago, IL, USA). The Brown–Forsythe and Welch tests were used to examine homogeneity of variance between groups. If significant differences were found between the groups, a post-hoc analysis was performed using the Tukey test (when group variances are homogeneous) or Games–Howell test (inhomogeneous). Multivariate analyses were undertaken using PRIMER-e v7.0.13 (Clarke and Gorley, 2015). Differences in community compositions among samples were tested using permutational multivariate analysis of variance (PERMANOVA) using the add-on package PERMANOVA+ for PRIMER (Anderson et al., 2008). Canonical correspondence analysis (CCA) and variance partitioning were done using Canoco v.4.5 (ter Braak and Smilauer, 2002).

## 3. Results

### 3.1. Temperature, irradiance, and particle analysis

Fig. 2a–d shows monthly variations in environmental factors, which was significant ( $p < 0.05$ ) using ANOVA. Air and surface sediment temperatures showed the typical seasonal trends of temperate regions, with wide ranges from −8.2 °C to 28.0 °C and from −2.8 °C to 34.5 °C, respectively. Similarly, duration of sunshine and solar radiation showed the typical annual patterns of mid-latitude areas, with maximums during summer and minimums during winter.

The sediment at the study site was mostly sandy silt; gravel was not observed. However, silty sand was sometimes observed in summer. The mean grain size of the sediment samples ranged from  $2.89\phi$  to  $6.06\phi$ , corresponding to the range from medium silt to fine sand, but the monthly variation was not significant (ANOVA,  $p > 0.05$ ). The samples ranged from 12.4% to 97.4% sand and the monthly variation was also not significant (ANOVA,  $p > 0.05$ ). An unusually high sand ratio was observed in August, when artificial sand spraying was conducted.

### 3.2. Chl *a* and algal pigments

The monthly mean chl *a* concentration ranged from 44.6 to 120.4  $\text{mg m}^{-2}$ , with a maximum in early spring and the monthly variation was significant (ANOVA,  $p < 0.001$ ). Chl *a* concentrations gradually decreased with time after winter, then slightly rebounded in late summer. The concentration of fucoxanthin, an indicator pigment of diatoms, was overwhelmingly higher than that of other pigments, ranging from 6.7 to 65.0  $\text{mg m}^{-2}$  (Fig. 2e). Furthermore, the monthly variation in fucoxanthin was similar to that of chl *a*, and there was a significant positive correlation between the two concentrations ( $r = 0.641$ ,  $p < 0.001$ ). The concentration ratios of fucoxanthin to chl *a* were high (mean  $\pm$  SD  $0.34 \pm 0.12$ ) indicating that diatoms account for the majority of the algal biomass in the tidal flat.

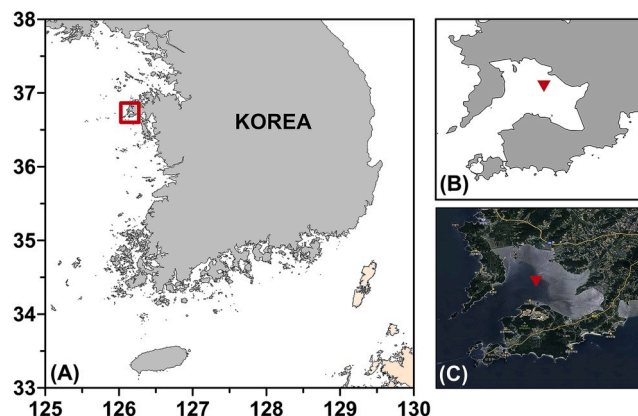
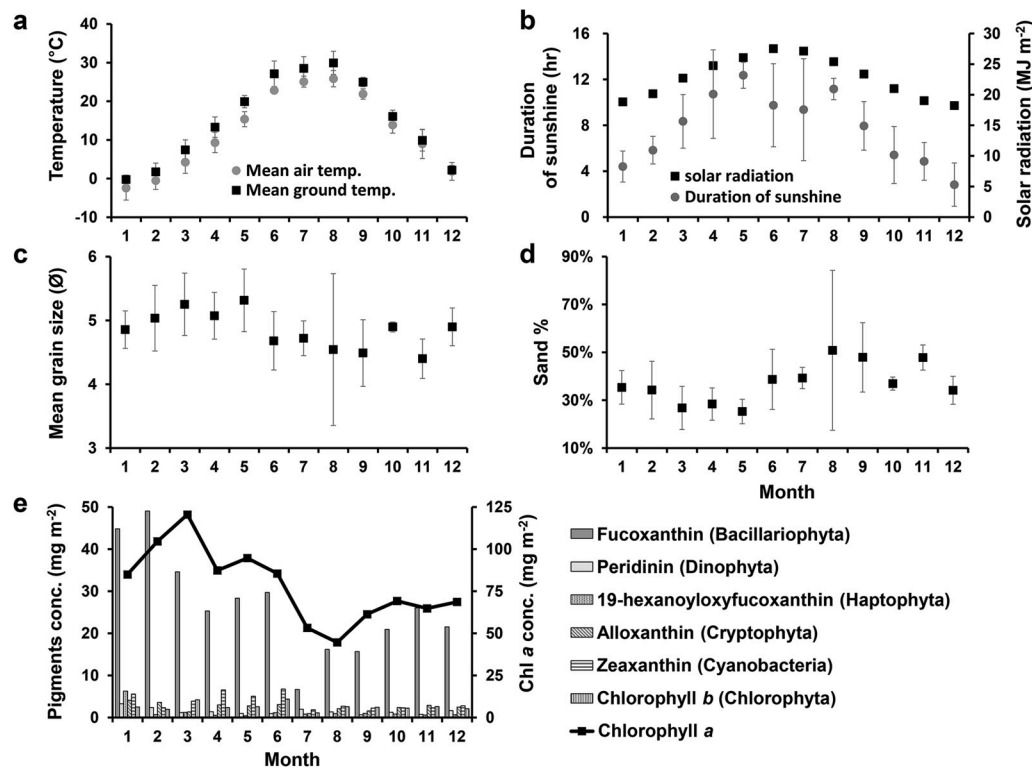


Fig. 1. Sample collection sites in Guenso Bay, on the west coast of the Republic of Korea.



**Fig. 2.** Monthly variation in environmental factors at the sampling sites. (a) Air and water temperature, (b) mean grain size, (c) percentage of sand in sediment, (d) duration of sunshine and solar radiation, and (e) major pigment concentrations in sediments at the sampling sites. The taxonomic groups represented by each indicator pigment in Fig. 2e are shown in parentheses.

### 3.3. Species richness and diversity indices

From 80 samples, we obtained 3,629,474 raw reads with an average length of 428 bp. After removing reads with low sequencing quality or internal nonsense codons, and suspected chimeras, the remaining 1,362,183 reads were used for further analyses. The number of reads obtained from each sample ranged from 4522 to 59,526. At a clustering criterion of 98% sequence identity, a total of 23,303 operational taxonomic units (OTUs) were observed. For further diversity analyses, the number of reads was randomly normalized to 4522. At the subsampling size, Good's coverage (ranging from 0.82 to 0.95) and the rarefaction curves indicated that some samples were still under-sampled (Fig. 3, Supplementary Table S1 and Supplementary Fig. S1). The Chao1 OTU richness ranged from 1148 to 4,699, showing significant monthly variation (ANOVA, Brown-Forsythe,  $p < 0.05$ ). Similarly, the Shannon diversity index showed large variation among samples, ranging between 2.47 and 6.32, and the monthly variation was significant (ANOVA,  $p < 0.001$ ), but only one subset remained significant with the Tukey *post-hoc* tests ( $\alpha = 0.05$ ). The lowest species richness and diversity indices were observed in February and March, and the highest values were observed in January.

### 3.4. Temporal changes in benthic diatom compositions

Among the 23,303 OTUs obtained in this study, most were classified into 3 classes, 28 orders, 56 families, and 138 genera. However, a small number of OTUs (1035 OTUs) either could not be classified or were *incertae sedis*. Of the total sequences, >80.5% belonged to 10 families, and the top 15 genera accounted for >64.2% of the sequences (Fig. 4). However, their relative abundances varied seasonally. In winter and spring, two families, Naviculaceae and Bacillariaceae, were the most dominant, accounting for >40% of the total sequences; however, the proportion of Bacillariaceae began to gradually decrease from June, and

then rebounded from October. Similarly, the proportion of Naviculaceae tended to decrease in the summer, but they remained dominant. By contrast, Catenulaceae, Sellaphoraceae, and Suriellaceae gradually increased from June and July. Catenulaceae surpassed Bacillariaceae and became comparable to Naviculaceae in warm seasons.

At the genus level, 52 genera were represented by  $\geq 1\%$  of sequences in at least one sample. Among them, 13 genera (*Navicula*, *Nitzschia*, *Amphora*, *Entomoneis*, *Gyrosigma*, *Halamphora*, *Parlibellus*, *Surirella*, *Haslea*, *Licmophora*, *Planothidium*, *Berkeleya*, and *Cymbella*) represented >10% of sequences in at least one season. *Navicula* was the most dominant genus throughout the year, with a relative abundance ranging from 9.5% to 30.2% (Fig. 4), although *Amphora* frequently surpassed *Navicula* in summer and autumn. *Nitzschia* was the second-most dominant genus in winter, but decreased markedly in summer, and *Amphora* appeared to be one of the dominant genera between summer and autumn in the tidal flat. *Sellaphora*, *Halamphora*, and *Planothidium* showed seasonal trends similar to that of *Amphora*, whereas *Entomoneis* and *Berkeleya* showed trends similar to those of *Navicula* and *Nitzschia*.

Among the 23,303 OTUs, 200 OTUs had a relative abundance  $\geq 1\%$  in at least one sample. The number of OTUs ranged from 131 to 1,742, depending on the sample, suggesting a great monthly variation of diatom diversity in the tidal flat (Fig. 3a). A total of 54 OTUs had relative frequencies  $\geq 1\%$  in a representative seasonal sample (February, May, August, or November). Interestingly, among the 54 OTUs, 34 (62%) appeared in only one season. However, 11 (20%) and 7 (13%) OTUs were found in two and three successive seasons, respectively. Only two OTUs (4%) were found in all four seasons (Fig. 5). Similar trends were found when all unique OTUs were considered. In summary, the benthic diatom communities in the tidal flat showed clear seasonal variation in composition. Statistical analyses of community structure (PERMANOVA) demonstrated significant monthly and seasonal variation ( $p < 0.001$ , Table 1) in diatom compositions.

Maximum likelihood (ML) phylogenetic trees of the 44 OTUs with

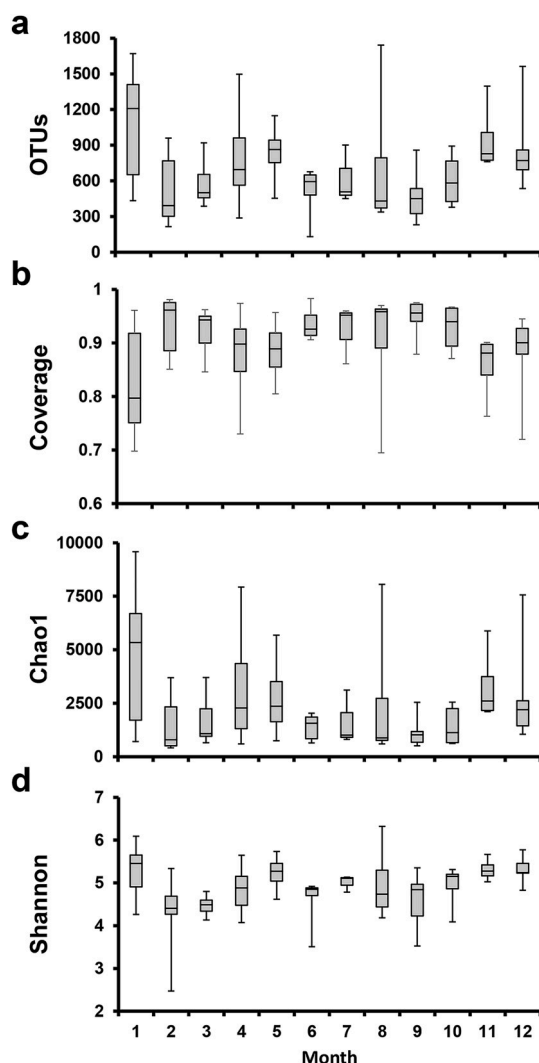


Fig. 3. Monthly variation in (a) number of operational taxonomic units (OTUs), (b) Good's Coverage, (c) Chao1 richness, and (d) Shannon diversity index.

relative frequencies  $\geq 5\%$  in at least one sample were constructed, and their relative frequencies are presented as a heatmap in [Supplementary Fig. S2](#). The major OTUs were affiliated with *Nitzschia bergii* (Otu00006), *Nitzschia aequorea* (Otu00008), *Navicula salinarum* (Otu00001), *Navicula salinicola* (Otu00014), and *Halamphora coffeiformis* (Otu00002), and exhibited maximal proportions ranging from 17.4% to 48.9%.

### 3.5. Correlation analysis between environmental factors and benthic diatom community structure

CCA analysis revealed that 78.2% of the variation in benthic diatom assemblages could be accounted for by the first two axes (54.0% for the first axis and 24.2% for the second axis), and chl *a*, sediment temperature, grain size, and sand % were statistically significant variables affecting the distribution of benthic diatoms ([Fig. 6](#)). Variance partitioning was performed on the two most important variables, sediment temperature and sand %, and suggested that the effect of sand % on benthic diatom composition (50.4%) was larger than that of sediment temperature (39.8%).

## 4. Discussion and conclusion

Dramatic changes in the benthic diatom community were elucidated through high-throughput sequencing of *rbcl* genes. At the family and

genus levels, several groups were dominant throughout the year, despite wide seasonal fluctuations. However, at the OTU level, the majority of taxa were observed in only one or two seasons, and very few taxa were found year-round, indicating a great successional change in the benthic diatom community with changing seasons.

### 4.1. Benthic diatom diversity

Benthic diatoms are known to have wide tolerance for environmental change ([Admiraal, 1984](#)). In the study area, located in a temperate region, air and sediment temperatures, as well as solar light intensity, varied greatly across the four seasons. Seasonal differences in sediment temperature were extremely great (37.4 °C). In this regard, changes in diatom diversity and community composition among seasons could be expected. However, their small and similar morphologies would prevent diatoms from being precisely classified using a primarily morphological classification approach. In previous morphological studies, diatom compositions at the genus level have been reported to be similar among seasons ([Haubois et al., 2005](#); [Du et al., 2009](#)). Furthermore, species diversity could not be precisely estimated due to the difficulty and inaccuracy of classification using morphological keys. Although a sequence identity threshold has not been firmly established for species-level classification using the *rbcl* gene, a 98% identity threshold has been suggested to be reasonable based on previous studies ([Hamsher et al., 2011](#); [Kermarrec et al., 2014](#); [An et al., 2018](#)). Using this threshold, the Chao1 richness estimate of the number of diatom species inhabiting the tidal flat at each sampling occasion (412–9582 species) was much higher than previously estimated (136–357 species; [Stief et al., 2013](#)). Similarly, the Shannon diversity values (2.7–6.3) were also higher than estimates from several previous studies (0.7–5.0; [Agatz et al., 1999](#); [Woelfel et al., 2007](#); [Du et al., 2009](#); [Park et al., 2013](#); [Ribeiro et al., 2013](#)). Benthic diatoms are known to be more numerous and diverse than planktonic diatoms ([Round et al., 1990](#)). Consistently, the diversity of benthic diatoms observed in this study was higher than observations for planktonic diatoms (Shannon diversity: 0.5–4.1; [Hobson and McQuoid, 1997](#); [Olguín et al., 2006](#)). Furthermore, the diversity also appeared to be higher than that of planktonic diatoms estimated using NGS techniques ([Endo et al., 2018](#)).

Benthic diatom biomass and species diversity are known to be negatively correlated ([Colijn and Dijkema, 1981](#); [Admiraal, 1984](#)), and the diversity of benthic microalgae is generally reduced during the colonization period ([Brown, 1973](#); [Hillebrand and Sommer, 2000](#)). In this study, the diversity of benthic diatoms was lowest in February but highest in January, even though chl *a* concentrations were very high and intense algal mats developed at that time. Although the mean Chao1 richness estimates in December and February were 2687 and 1450 OTUs, respectively, seven of the eleven surveys in January showed estimates of  $>4000$  OTUs, suggesting that this was not a temporary phenomenon. However, it is difficult to explain the reason for the high richness observed in January in this study, and further investigations will be necessary. By contrast, the species richness was lowest in the summer season, despite the average Shannon diversity, suggesting a more even distribution of diatom species in summer than in winter. [Vinson and Rushforth \(1989\)](#) reported that species diversity and richness increased in the temperature range from 14 to 25 °C, and decreased at higher temperatures. The high diversity of benthic diatoms in spring and autumn in this study is consistent with previous findings. Thus, seasonal changes in temperature affect the community structure and diversity of benthic diatoms. In addition, [Round \(1971\)](#) concluded that diatom diversity is higher in sandy sediment than in silty sediment. However, the diversity indices did not exhibit a significant correlation with grain size in this study.

### 4.2. Seasonal succession of the benthic diatom community

Tidal flats periodically experience submergence and exposure due to



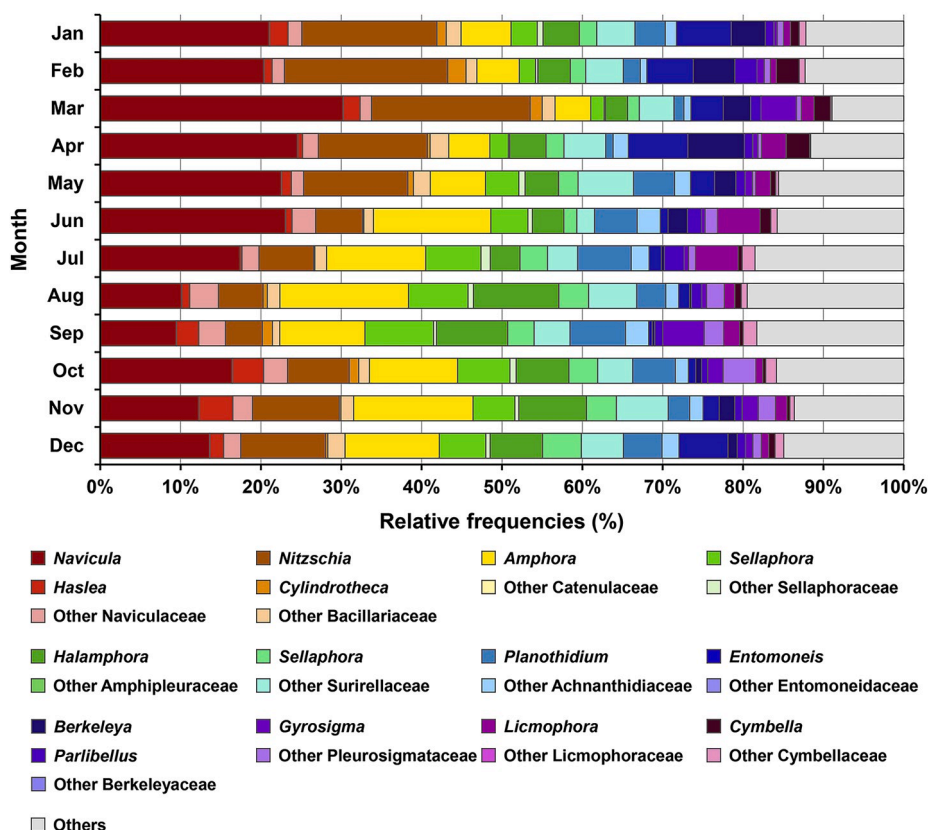


Fig. 4. Monthly variation in the relative abundance of major families and genera at sampling sites.

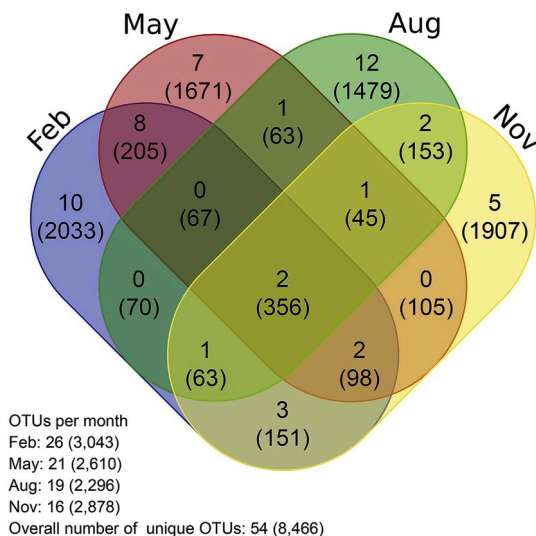


Fig. 5. The distribution of OTUs among the seasonal benthic diatom communities in tidal flat at Guenso Bay. Venn diagram showing OTU numbers with relative abundances >1% and all OTUs for four representative seasons. The values in parentheses represent the results of analysis using all OTUs.

tidal action. Despite the rapid environmental changes, benthic diatoms have been a predominant winner in tidal flats due to their high tolerance for environmental changes (Admiraal, 1984). Adaptation to rapid and large environmental changes has resulted in the outstanding resistance of benthic diatoms to such changes (Koh, 1991).

Consistently, algal pigment analyses indicated that diatoms contribute most of the algal biomass in the study area throughout the year (Fig. 2e), but what is the reason for their great success in tidal flats?

Table 1

Results of PERMANOVA analyses (based on Bray-Curtis dissimilarity matrices) of NGS data to compare the monthly and seasonally changes in the community structure of benthic diatoms. Pseudo-F: multivariate analog of Fisher's F statistic (i.e., ratio of variance), P (perm): permutational P value (i.e. proportion of permuted pseudo-F statistics  $\geq$  the original pseudo-F), Unique perms: the number of permutations used in PERMANOVA to obtain the P value, P (MC) P value of Monte Carlo randomization.

Groups	df	Pseudo-F	P(perm)	Unique perms	P (MC)
Month	11	2.1503	0.0001	9640	0.0001
Season	3	4.1777	0.0001	9811	0.0001

The broad temperature tolerance of some diatom species is one possible explanation. Souffreau et al. (2010) reported that some species of benthic diatoms are able to grow at temperatures ranging from  $-20^{\circ}\text{C}$  to  $40^{\circ}\text{C}$ . However, the high diversity of benthic diatoms and their rapid succession during environmental changes clearly demonstrated in this study are reasonable explanations for their dominance in tidal flats.

In this study, 23,303 unique OTUs were obtained, and the average species richness was estimated at 2403 (Supplementary Table S1), suggesting a high genetic diversity of benthic diatoms. However, the OTUs commonly found during all four representative seasons accounted for only 4% of the total OTUs, whereas most OTUs (64–67%) found during one month were not observed in any other months (Fig. 5). The low percentage of common OTUs among seasons might be due to the small sample number used to normalize the read numbers in this study. However, the relatively high mean value (0.9) of Good's coverage indicates that the number of reads was sufficient to identify the overall diversity. Therefore, the fact that the majority of diatom taxa were not abundant in successive seasons suggests that the diatom community is composed of genetically diverse species with very specific niches to adapt to seasonal changes in environmental factors.

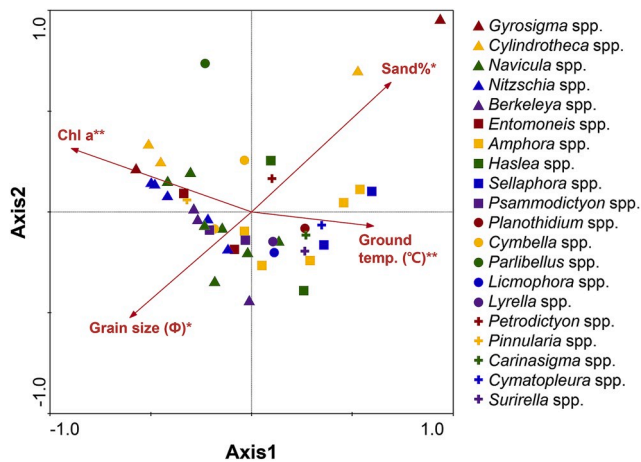


Fig. 6. Canonical Correspondence Analysis ordination diagrams of the 44 OTUs with >5% relative abundance with respect to environmental variables. Statistically significant variables revealed by permutation testing are marked with asterisks (\*\* $p < 0.01$  and \*  $p < 0.05$ ).

Only 18 of the 44 OTUs with relative frequencies  $\geq 5\%$  in at least one sample could be classified to a known species based on 98% sequence similarity of the *rbcL* gene (Fig. S2), indicating that there are still insufficient sequence data in the diatom reference database.

#### 4.3. Factors affecting diatom community structure

Benthic diatoms that inhabit tidal flats are faced with rapid changes in various environmental factors such as nutrient concentrations, temperature, salinity, and light intensity. Nonetheless, they have high biomass throughout the year and show fewer seasonal changes compared to planktonic microalgae (Colijn and Dijkema, 1981; Lee et al., 2009). The grain size of sediments is also known to significantly affect the growth rate and cell density of benthic diatoms (Watermann et al., 1999; Mitbavkar and Anil, 2002; Oh et al., 2009; De Sève et al., 2010). The relative abundances of epipelagic and epipsammic species have been closely linked with sediment grain size (De Sève et al., 2010). Furthermore, grain size can affect light intensity, porosity, water content, and dissolved nutrients in sediments, all of which are important factors affecting benthic algae (Kuhl et al., 1994; Underwood and Kromkamp, 1999; Bale and Kenny, 2005). Another major factor affecting the phytoplankton community structure is temperature (Wetzel, 1983; Sherwood et al., 2000; Muñoz et al., 2018). Temperature varies widely in surface sediments, which undergo extreme temperature changes daily due to tidal action, especially in summer and winter. In addition, sediment temperatures show great seasonal changes in temperate areas, as observed in this study (seasonal variation of 37.4 °C). Thus, temperature may be a dominant factor regulating seasonal changes in community composition. Consistently, the CCA analysis showed that sediment temperature is an important factor regulating the distribution of benthic diatom taxa, together with grain size.

In this study, *Navicula* and *Nitzschia* exhibited higher relative abundance in winter and showed similar seasonal variation patterns, consistent with previous studies (Kim and Cho, 1985; Haubois et al., 2005; Scholz and Liebezeit, 2012). This is thought to be related to the low temperature tolerance and small cell size of these taxa. They can grow rapidly in response to a slight increase in temperature at low temperature environment (Admiraal, 1976), and maintain their growth during relatively long periods at low temperatures (10–15 °C) (Watermann et al., 1999). de Jong and Admiraal (1984) also showed that *N. salinarum* dominated at low temperatures (8 °C) in a mixed culture experiment of three benthic diatoms (*N. salinarum*, *Cylindrotheca closterium*, and *Amphiprora* cf. *paludosa*). Furthermore, the small size of diatoms is beneficial for acquiring nutrients, cell division, and

colonization (Hudon and Legendre, 1987; Scholz and Liebezeit, 2012). In addition, *Navicula* and *Nitzschia* have been reported to show increased growth rate and biomass as sediment particle size decreases (Mitbavkar and Anil, 2002; Oh et al., 2009). In this study, the sedimentary facies did not show any significant seasonal changes, but the sediment grain size was small and the sand ratio was low in winter and spring. *Navicula* and *Nitzschia* also showed high relative abundances in these seasons; therefore, the sedimentary facies also seemed to have some influence on their relative abundance.

By contrast, *Amphora* exhibited high relative abundance in summer and autumn, suggesting a preference for high temperatures. Some *Amphora* spp. are considered to be thermophilic due to positive correlations with sediment temperature and resistance to high temperatures (Sheehan et al., 1998; Haubois et al., 2005; Du et al., 2009; Kwon et al., 2011; Salleh and McMinn, 2011; Csavina et al., 2011). In addition, the relatively high grain size and sand ratio in the summer season may have affected the high relative abundance of *Amphora*, given that *Amphora* has been reported to show increased growth when the sediment grain size is large or the sand fraction is high (Oh and Koh, 1995; Mitbavkar and Anil, 2002; Du et al., 2009). Meanwhile, variance partitioning analysis showed that grain size (50.4%) was more important than sediment temperature (39.8%), but both sediment temperature and grain size exert complex influences on the community structure and diversity of benthic diatoms.

In August 2011, *Amphora* had the highest relative frequency when an abnormal increase in the proportion of sand (97.4%) occurred due to artificial spraying of sand, which was conducted over a large area to increase the productivity of Manila clams (*Venerupis philippinarum*) (Yoon et al., 2014). (Supplementary Fig. S3). The Manila clam is a very important species among shellfish produced in Korean fisheries due to their high productivity, along with oysters (Han et al., 2012; Choi et al., 2014). As mentioned above, there was also a significant positive correlation ( $r = 0.511$ ,  $p < 0.001$ ) between the relative frequency of *Amphora* and the proportion of sand in sediments. This suggests that anthropogenic changes in sediment particle size may lead to changes in benthic diatom assemblages. Eventually, this may affect the structure and function of the environment and tidal ecosystem.

Although *Navicula*, *Nitzschia*, and *Amphora* dominate the tidal flat throughout the year, other taxa also showed obvious seasonal variations. *Entomoneis* and *Berkeleya* showed patterns similar to those of *Navicula* and *Nitzschia*, whereas *Sellaphora*, *Halammphora*, and *Planothidium* showed similar patterns to that of *Amphora*, suggesting dynamic seasonal variation in the benthic diatom composition in this temperate tidal flat.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### CRediT authorship contribution statement

**Sung Min An:** Investigation, Formal analysis, Visualization, Writing - original draft, Funding acquisition. **Dong Han Choi:** Methodology, Visualization, Writing - review & editing, Validation. **Jae Hoon Noh:** Supervision, Conceptualization, Funding acquisition.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2020.106686>.

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