Diatom-based ecoregions and diagnostic indicator species for Korean coastal waters in winter

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Abstract

Diatoms are a globally successful and eukaryotic photosynthetic group with an ornamented silica external wall. The relationship between their thecal diversity and habitat means that diatoms can be used as bioindicators to characterize the aquatic environment. To estimate the distribution and diversity of diatom communities during winter in South Korean coastal waters, we collected phytoplankton samples from 114 coastal sites. We applied the unweighted pair-group technique using the arithmetic averages clustering method to cluster the sampling sites—apart from those where the biota consisted of other groups, such as dinoflagellates—into four ecoregions: Yellow Sea (YS), Southern Sea (SS), Southern East Sea (SES), and Northern East Sea (NES). Indicator species analysis in each ecoregion led to the selection of tychoplanktonic, chain-forming, stalk-forming, and psychrophilic indicator diatoms, each of which represented a planktonic lifestyle associated with one of the four ecoregions. This study shows the diatom community to serve as bioindicators of Korean coastal water ecoregions, and the subsequent seasonal survey will provide a starting point for the improved understanding of Korean diatom-based ecoregions, in both time and space.

Introduction

Diatoms are a successful and ubiquitous phytoplankton group with over 200,000 species. They range in size from μms to mms and exist either as single cells or as chains of connected cells (Kooistra, et al. 2007). These silica-depositing microalgae have been abundant for more than 180 million years (Falkowski, et al. 2004); they contribute 40% of the total oceanic primary production and ˜20% of global primary production (Field, et al. 1998), and annual diatom photosynthesis reportedly generates approximately as much organic carbon as all rainforests combined (Field, Behrenfeld, Randerson and Falkowski 1998, Nelson, et al. 1995). Diatoms in coastal waters support the most productive fisheries, and in the open ocean, they sink rapidly from the surface to become food for deep-water organisms (Sarthou, et al. 2005). Thus, diatoms are crucial contributors to the global carbon cycle and have even been considered as a potential solution for reducing atmospheric CO_2 levels (Denman 2008). Given their ubiquitous nature, diatoms are logical subjects for researchers interested in global ecological trends such as climate change, as foreseen in the concept that "everything is everywhere, but the environment selects" ((Finlay 2002). Changes in diatom community diversity can be considered to be indicative of adaptation occurring in response to selective pressures.

The Korean Peninsula is surrounded by the Yellow Sea (YS), the Southern Sea (SS; also known as Korean coastal waters connected to the East China Sea), and the East Sea (ES, or the Sea of Japan), each with its distinguishing characteristics. The YS, located between mainland China and the Korean Peninsula, is a shallow (generally < 100 m), post-glacially submerged, epicontinental sea with one of the largest continental

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shelves in the world (Wang, et al. 2003). The SS is subjected to Kuroshio currents and receives outflows from the Changjiang River, which is among the world's largest rivers (Ichikawa and Beardsley 2002). Also bordering Japan and Russia, the ES is a typical, mid-latitude, marginal sea, approximately the size of the western Mediterranean and is somewhat unusual in exhibiting characteristics typical of both closed basins and oceans. Similarities to the former are due to its sill depths of < 150 m which limits seawater exchange with the North Pacific Ocean. ES also contains oceanic deep basins (> 3000 m), large (> 15) seasonal variations in sea surface temperatures, and highly active biological processes and exhibits a sub-arctic polar front between its sub-polar and sub-tropical seas (Kim, et al. 2001).

Diatom communities tend to dominate in well-mixed coastal and upwelling regions, as well as along sea-ice edges where sufficient light, inorganic nitrogen, phosphorus, silicon, and trace elements are available to sustain their growth (Morel and Price 2003). Therefore, the purpose of the present study is to investigate the diversity and distribution of diatom communities in the coastal areas of South Korea through a winter survey of 114 sampling sites and to clarify links to sea characteristics using diatom community cluster analysis.

Materials and Methods

Sample preparation

Diatom sampling was performed at 114 sites along the South Korean coast (Fig. 1 and Table S1) during January and February 2010. We chose sites accessible from the coast for phytoplankton netting, and that were < 40 km apart in order to achieve full coverage of Korean waters. One liter water samples were collected in clean polyethylene bottles for quantitative analysis, and phytoplankton samples were taken using a 20 μm mesh net for qualitative analysis. Collected samples were immediately fixed with 5% Lugol's solution (Sigma, St. Louis, MO, USA) and transported to the laboratory. Environmental data, including temperature, salinity, and pH were measured *in-situ*, using a YSI-6600 portable meter (YSI; Yellow Springs, OH, USA).

Diatom assemblage analysis

The fixed water samples were allowed to settle for 1 d, and then the supernatant was removed to concentrate the phytoplankton. Total diatom abundance in each 1 L water sample was determined (the minimum found was 600 cells per sample) using a Sedgwick–Rafter counting chamber under a light microscope (LM, Axioskop 40; Zeiss, Germany), and diatom diversity and sample composition were determined.

To identify the diatom species positively, cellular organic material was removed using equal amounts of $KMnO_4$ and HCl in a 70 water bath until the sample became clear, and then the acid was removed using five rinses. Selected cleaned samples were mounted in a Pleurax (cat. no.139-06682, Wako, Japan) and observed under the LM equipped with a CCD camera (AxioCamMRc5; Zeiss, German). For examination using a scanning electron microscope (SEM, JSM7600F, Jeol, Tokyo, Japan), the rest of the cleaned samples were filtered onto a polycarbonate membrane (3.0-μm pore size; TSTP02500, Millipore, Bedford, MA, USA), which was then dried in air. The filtrated membranes were attached to an aluminium stub using carbon tape and then sputter-coated with gold. The SEM was operated at accelerating voltages of 5 kV using a 10 mm working distance.

Statistical analysis

Species that contributed [?] 1% of the total diatomic assemblage in at least one sample were selected for numerical analysis resulting in 156 diatom taxa being used. Diatom assemblage diversity was calculated using the Shannon–Wiener diversity index (Shannon and Weaver 1949). The absolute abundance of each

species was transformed by its fourth root into normalizing skewed composition, data and pairwise distances between sampling sites were calculated using the Bray–Curtis similarity algorithm.

To identify spatial similarity between sampling sites, we performed eight hierarchical clustering methods based on the pairwise distance matrix. These were as follows: the single linkage method, the complete linkage method, the unweighted pair-group method using arithmetic averages (UPGMA), the weighted pair-group method using arithmetic averages (WPGMA), the unweighted pair-group method using centroids (UPGMC), the weighted pair-group method using centroids (WPGMC), and two variants of ward's minimum variance method (ward.D and ward.D2). The degrees of data distortion from the eight methods were then assessed based on cophenetic correlation coefficients (Sokal and Rohlf 1962). Pairwise distances between sampling sites were calculated in the "vegan" package ((Oksanen, et al. 2013), and clustering was visualized using the "factoextra" package (Kassambara and Mundt 2017), both in R (the R Project for Statistical Computing, supported by the R Foundation for Statistical Computing).

The indicator value method (IndVal) was applied to identify indicator species among the groups of sites using the "indicspecies" method in R (De Cáceres 2013). The IndVal values ranged from zero for "not an indicator species" to one for "maximum indicator ability."

Results

Species composition, variable characteristics, and diatom-based ecoregions

Based on the cophenetic correlation coefficient values ($r = 0.73$), the UPGMA clustering algorithm provided the best dissimilarity approximation compared to the other seven methods, and so we adopted the groupings achieved by this method to establish diatom-based regions. The UPGMA clustering, based on the Bray-Curtis similarity matrices, clustered the data into four major groups and three additional unique groups (Fig. 2). Group 1 consisted of 21 sites, mostly in the YS ecoregion but including one in the SS (site 64) and two in the ES (sites 75 and 86). Group 2 ecoregion included 51 sites located in the SS with four sites from the YS (3, 10, 12, and 13) also included in this ecoregion. Group 3 ecoregion included 25 sites in the SES, while Group 4 ecoregion had 11 sites from the NES, as well as solitary site (82) from the SES region. Three unique groups with one to two sites were separated from these four major groups: Unique Group 1 and Unique Group 2 included two sites each: 47 and 48, and 102 and 104, respectively, while Unique Group 3 included just site 105.

Abiotic variability for water temperature, salinity, and pH, was relatively low in the YS, high in the SS, and moderate in the ES (Table 1, Fig. 1 (a)–(c)). Shannon diversity indices were variable (0.25–2.82) site-by-site with low values in the western SS (Fig. 1 (e)), dominated by *Thalassiosira nordenskioeldii* (0.42–0.79 in sites 25–30), and *Eucampia zodiacus* (0.42 at site 31). Diatom richness was 7–36 with low values in the SES (7–9 at sites 89–91, 93, 100, and 101) and reduced levels of abundance (12,105–34,792 cells·l^{-1}) (Fig. 1 (f)).

Indicator species

The indicator species for the four major ecoregions were identified using calculated indicator values ($p < 0.05$), and 7, 17, 6, and 7 indicator species were found for the four ecoregions, respectively (Table 2, Fig. 3). Among the 37 indicator species, *Actinoptychus senarius* (Fig. 4 i), *Asteroplanus kariana, Cyclotella littoralis* (Fig. 4 ii), *Melosira nummuloides* (Fig. 4 iii), *Paralia sulcata* (Fig. 4 iv), *Thalassiosira eccentrica* (Fig. 4 v) and *Pleurosigma angulatum* (Fig. 4 vi) were identified as being significant for YS, while *Asterionellopsis glacialis* (Fig. 4 vii–viii), *Chaetoceros affinis, C. brevis, C. constrictus* (Fig. 4 xii), *C. contortus* (Fig. 4 ix), *C. curvisetus* (Fig. 4 x), *C. debilis* (Fig. 4 xi), *C. laciniosus, Chaetoceros* spp., *Detonula pumila* (Fig. 4 xiii), *Skeletonema dohrnii-marinoii complex* (Fig. 4 xiv), *Eucampia zodiacus* (Fig. 4 xvii), *Pseudo-nitzschia pungens* (Fig. 4 xviii), *Thalassionema nitzschioides, Thalassiosira curviseriata* (Fig. 4 xv), and *T. nordenskioeldii* (Fig. 4 xvi) were identified as being significant for SS. *Achnanthes* spp. (Fig. 4 xix–xx), *Entomoneis*

paludosa (Fig. 4 xxiv)*, Licmophora grandis* (Fig. 4 xxi)*, L. paradoxa* (Fig. 4 xxii)*, Navicula*spp. and *Odontella aurita* (Fig. 4 xxiii) were identified as being significant for SES, with *Chaetoceros radicans* (Fig. 4 xxv)*, Corethron pennatum* (Fig. 4 xxvi)*, Coscinodiscus centralis* (Fig. 4 xxvii)*, Licmophora ehrenbergii* (Fig. 4 xxx)*, Porosira glacialis* (Fig. 4 xxiv)*, Rhabdonema*spp. and *Thalassiosira pacifica* (Fig. 4 xxviii) identified as being significant for NES.

The three unique groups also had indicator species: *Amphora* spp.,*Bacillaria paxillifera* , *Grammatophora marina* ,*Lauderia annulata* , *Licmophora flabellata* , *Navicula elegans* , and *Tabularia fasciculata* species, were identified for Unique Group 1and *Licmophora debilis* and *Licmophora* spp., representing Unique Group 2. An indicator value was not calculated for Unique Group 3 as only one sample (105 site) was included, but although an indicator was not calculated here, we noted that the nearby site 105 was strongly dominated by *Chaetoceros curviseriata* .

Discussion

Abiotic variables and diatom abundance in Korean coastal waters

In the sea, abiotic environmental factors such as salinity, water temperature, light, nutrients, and tidal currents—together with the physical properties of substrates—have been reported as being the main influencers of diatom distribution in coastal waters (Desianti, et al. 2019, Trobajo and Sullivan 2010). We sampled during winter, and the water temperature did not exceed the 13 seasonal average for Korean waters. Although the water temperatures in YS were low, the diatom species found were not determined by water temperature, but rather by the relatively low pH (< 8.1), which was related to the high conductivity exhibited by the well-developed mudflat. Rather than water temperature, pH and salinity were the main influencers of diatom species occurrence in YS, as here, the dominant species such as*Actinoptychus senarius* , *Paralia sulcata* ,*Cyclotella littoralis* frequently occur year-round.

Although we did not analyze inorganic nutrients in the present study, previous research reported that rivers supply inorganic nutrients to both coastal upwelling and tidal fronts in YS and SS, and the nutrient concentrations are associated with increased diatom community abundance (Jung, et al. 2012, Yeo and Kang 1998). Warm currents from Tsushima (a branch of the Kuroshio warm currents) continuously flow into SS which also experiences coastal upwelling and front structures. Fronts in both of these seas occur at the boundary between turbid coastal waters and stratified offshore waters leading to high levels of phytoplankton abundance and primary production (Choi 1991, Seung, et al. 1990). Jung, et al. (2013) reported that in YS and SS, inorganic nutrient concentrations (dissolved nitrogen, dissolved phosphorus, and dissolved silica) were three times higher than those measured for ES, and such differences probably played a major role in increasing diatom abundance.

Overall, the data indicated that differences in the geographic, physical, and environmental factors affected diatom abundances in each coastal area. In particular, the low diatom abundance for ES, which has the characteristics of the open sea, may be related to a low inorganic nutrient flux caused by the area's deep water and lack of river mixing inputs (Kang, et al. 2004, Kim, Kim, Min, Volkov, Yoon and Takematsu 2001, Yun, et al. 2004).

Indicator species in each diatom-based ecoregion

We performed diatom indicator species analyses to identify the meaningful species from four diatom-based ecoregions in the South Korean coastal waters. This technique was used to identify diatom species that reflected the geographic and seasonal characteristics of each ecoregion.

In YS, the water column in winter is well-mixed vertically by tidal currents and winds; the NW monsoon cools the surface water and the water temperature vertical gradient becomes more uniform (Gebuehr, et

al. 2009, Hobson and McQuoid 1997, Roelofs 1984). Vertical mixing in the water column provides nutrient enrichment from the sediment to the surface and gives benthic and tychoplanktonic diatoms a chance to access both nutrients and light. The Han and Keum rivers (two of the five largest rivers in South Korea) drain into the YS region providing additional nutrient enrichment (Koh and Khim 2014, Wang, Wang and Zhan 2003).

In the YS ecoregion, the indicator species included tychoplanktonic diatoms known to be typically benthic and brackish water species (Table 2). *Paralia sulcata, Actinoptychus senarius, Pleurosigma angulatum* , *Cyclotella littoralis,* and *Asteroplanus karianus* were selected as indicator species here. *Paralia sulcata* is an environmental indicator for vertically well-mixed water due to its tychoplanktonic nature ((McQuoid and Nordberg 2003); this species is typically found in regions with frequent upwelling, and its abundance is correlated with high nutrient concentrations (Abrantes 1988). *Pleurosigma angulatum* is a typical epipelic intertidal diatom which overcomes its severe environment by undergoing periodic vertical migrations coincident with the tides and light (Happey-Wood and Jones 1988). *Cyclotella littoralis* is a euryhaline diatom frequently found in YS estuaries (Park, et al. 2013), while *Asteroplanus karianus* can rapidly uptake nutrients and form very large blooms (Yamaguchi, et al. 2014), and so nutrient inputs from sediments and rivers draining into YS provide a suitable habitat for this species. In summary, the characteristics of the YS indicator species have straightforward explanations including a liking for areas exhibiting well-established vertical mixing of the water column, freshwater inputs, and nutrient enrichment.

In the SS ecoregion, the indicator species were colony and chain-forming diatoms, such as *Asterionellopsis glacialis* ,*Chaetoceros* spp., and *Eucampia zodiacus,* and Thalassiosirales such as *Detonula pumila* ,*Skeletonema dohrnii* -*marinoii* complex,*Thalassiosira curviseriata,* and *T. nordenskioeldii* . The SS coastline of Korea is geologically a ria with many bays and islands, and is physically influenced by SW, wind-driven currents, tidal currents, and the Tsushima Warm Current. These physical factors encourage SS sediment re-suspension and the geologically complex coastline emphasizes tidal effects in bays (Bae and Kim 2012). Several theories have been advanced to explain the success of chain-forming diatoms with the advantages of chain formation being reported as including beneficial responses to physical, chemical, and biological constraints (Bjaerke, et al. 2015, Musielak, et al. 2009, Peters, et al. 2006). Recently, turbulence shear has been reported as enhancing nutrient uptake in chain-forming diatoms (Bergkvist, et al. 2018), and although the type of turbulence in SS was not studied, its physical and geological characteristics cause continuous turbulence, which may well be increasing the nutrient availability for chain-forming diatoms.

The ES has a coastal terrace with a simple, linear coastline, where diatom distribution is mainly affected by two major currents—the S-trending Liman Current, and the N-trending Tsushima Current. These currents meet and form the subpolar fronts and mesoscale eddies that influence phytoplankton community structure and distribution in this area (Choi, et al. 2016). In our study, the ES diatom-based ecoregions were divided into distinct southern and northern groups. In the SES, the indicator species were estuarine, stalk-forming diatoms, such as*Licmophora grandis* , *L. paradoxa* , *Achnanthes*spp., and *Odontella aurita* . *Licmophora* is a stalk-forming diatom that is usually found submerged in rock pools throughout the littoral zone (Honeywill 1998). Its species are known to survive on various substrates, including sediments, rocks, microalgae, vertebrates, and ice. The stalk attachment has to be strong to survive intertidal forces and wave action, as well as being pounded against rocks and macroalgae, although in sub-optimal conditions cells can easily become dislodged (Honeywill 1998). In a recent study,*Licmophora* grew well and successfully formed colonies under experimental high light intensities and low turbulence, and their growth rate showed no relationship to nutrients (Ravizza and Hallegraeff 2015). Currently, no clear explanation exists for the significant presence of attached diatoms such as *Licmophora* species, including *L. grandis* and *L. paradoxa* , in the water column. The occurrence of*Licmophora* species and *Odontella aurita* as indicator species in SES might be related to the extensive presence of the massive macroalgae habitats which are preferred by stalk-forming diatoms (Jeong, et al. 2014). It is also likely that the continuous effect of the Tsushima current may cause diatoms to become detached from substrates and suspended in the water column (Kooistra, et al. 2009).

The selected indicator species in the NES ecoregion included psychrophilic diatoms such as *Corethron pen-*

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natum ,*Coscinodiscus centralis, Porosira glacialis* , and*Thalassiosira pacifica* . *Coscinodiscuscentralis* is a large, centric diatom known to be cosmopolitan (Hasle and Syvertsen 1996) even occurring in the Arctic region (Duerksen, et al. 2014, Lovejoy, et al. 2002). *Porosiraglacialis* is a typical polar diatom (Villareal and Fryxell 1983), while *Thalassiosira pacifica* has been reported from cold to tropical regions (Park, et al. 2016), although most frequently occurring in the Arctic (Joo, et al. 2012). In the NES ecoregion, the Liman Cold Current has a more undiluted impact, while cold water and warm water probably differ in their effects. In winter, the Liman Cold Current flows strongly southward, and the indicator species are those related to the temperature transitions associated with the Liman Cold Current (Yun, Magaard, Kim, Shin, Kim and Byun 2004). Specifically, our work indicated that the NES ecoregion was probably characterized by the action of the Liman Cold Current weakening the Tsushima Warm Current (Kim and Min 2008) suggesting that this ecoregion's diatom community assemblage may be most influenced by current, rather than by geography.

Conclusion

We have investigated diatom distribution in South Korean coastal waters in winter finding four major ecoregions (YS, SS, SES, and NES). In the YS ecoregion, the selected indicator species were characterized as tychoplanktonic and euryhaline diatoms indicating that the region had uniform water column mixing with freshwater inputs. In the SS ecoregion, the dominance of chain-forming diatoms was considered to be a response to the turbulence caused by an uneven coastline and strong tidal flows and by the Tsushima Warm Current. In the SES ecoregion, having stalk-forming diatoms such as *Licmophora* and *Achnanthes* as indicator species was thought to be associated with a simpler coastline with the strong Tsushima Warm Current limiting the distribution of planktonic species in this region. In the NES ecoregion, the Liman Cold Current was considered to be the main factor influencing diatom distribution as our analysis showed that cold-water species such as*Corethron criphilum* and *Porosira glacialis*were its indicator species.

Although the influence of physical, chemical, and biological factors on diatom distribution was not investigated in this study, the diatom-based biogeographical groupings and their indicators species have reflected some factors that can influence the spatial distribution of diatoms in South Korean coastal waters, in winter.

Data Accessibility

R scripts and diatom data set for analyzing the clustering and indicator species, are able to use from the supplementary information.

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Figure legends

Fig. 1. Abiotic variables: (a) pH, (b) water temperature, and (c) salinity, and biotic variables: (d) abundance, (e) Shannon's diversity index, and (f) species richness.

Fig. 2. Hierarchical cluster analysis (UPGMA) of diatom communities based on (a) Bray–Curtis similarity, and (b) biogeographic zonation in the Yellow Sea (YS), South Sea (SS), and East Sea (ES) of Korea during winter.

Fig. 3. Heatmap graph on fourth root transformed abundance data for indicator species in the Yellow Sea (YS), South Sea (SS), Southern East Sea (SES), and Northern East Sea (NES) ecoregions. Colors for species denote the selected indicator species for each ecoregion: YS (yellow), SS (green), SES (orange), and NES (blue).

Fig. 4. Micrographs of representative indicator species for the four ecoregions identified in this study: (i) *Actinoptychus senarius* , (ii) *Cyclotella littoralis* , (iii) *Melosira nummuloides* , (iv) *Paralia sulcata* , (v) *Thalassiosira eccentrica* , (vi) *Pleurosigma angulatum* , (vii) *Asterionellopsis glacialis* , (viii–ix), *Chaetoceros contortus* , (x) *C. curvisetus* , (xi) *C. debilis* , (xii) *C. constrictus* , (xiii) *Detonula pumila* , (xiv) *Skeletonema dohrnii-marinoii* complex, (xv) *Thalassiosira curviseriata* , (xvi) *T. nordenskioeldii* , (xvii) *Eucampia zodiacus* , (xviii) *Pseudo-nitzschia pungens* , (xix–xx) *Achnanthes* spp., (xxi) *Licmophora grandis* , (xxii) *L. paradoxa* , (xxiii) *Odontella aurita* , (xxiv) *Entomoneis alata* , (xxv) *Chaetoceros radicans* , (xxvi) *Corethron pennatum* , (xxvii) *Coscinodiscus centralis* , (xxviii) *Thalassiosira pacifica* , (xxix) *Porosira glacialis* , (xxx) *Licmophora ehrenbergii* .

Table 1. Means and ranges of abiotic variables including pH, water temperature, salinity and biotic variables including number of diatoms species, abundance, Shannon's diversity index (H), evenness (E), and species richness (R).

Groups	Ecoregions	Number of sites	pH	T ()	Salinity (psu)	Number of species	Abundance ($\times 10^5$ cells·l^{-1})	H	E
Group 1	YS[1]	22	8.0 (7.7–8.3)	4.8 (0.3–12.5)	32.3 (28.6–34.1)	98	2.31±1.41 (0.07–4.81)	0.73±0.15 (0.27–0.92)	0.69±0.15 (0.28–0.91)
Group 2	SS[2]	51	8.4 (7.6–8.7)	6.4 (0.2–11.4)	33.7 (30.4–34.7)	111	5.18±3.06 (0.77–12.80)	0.68±0.21 (0.15–0.92)	0.62±0.21 (0.15–0.90)
Group 3	SES[3]	25	8.2 (8.1–8.4)	9.4 (6.0–12.2)	33.0 (26.1–33.8)	77	0.24±0.10 (0.10–0.44)	0.75±0.14 (0.45–0.93)	0.76±0.14 (0.45–0.91)
Group 4	NES[4]	11	8.1 (8.0–8.4)	5.4 (3.0–9.4)	33.2 (31.6–33.6)	90	0.23±0.12 (0.06–0.46)	0.76±0.14 (0.50–0.92)	0.69±0.15 (0.43–0.90)
Unique 1	SS	2	8.5	8.2 (8.0–8.3)	34.4 (34.2–34.5)	25	5.40±0.29 (5.10–5.69)	0.75±0.11 (0.65–0.86)	0.71±0.10 (0.61–0.82)
Unique 2	NES	2	8.2	7.5 (7.5–7.6)	33.1 (32.6–33.5)	21	0.24±0.10 (0.13–0.34)	0.03±0.08 (0.22–0.38)	0.30±0.07 (0.23–0.37)
Unique 3	NES	1	8.0	7.4	33.2	18	0.16	0.08	0.09

[1]Yellow Sea, [2]Southen Sea,[3]Southern East Sea, [4]Northern East Sea

Table 2. Indicator values of diatom species in each group.

Groups (Ecoregion)	Class	Order	Species	Stat	p-value
Group 1 (Yellow Sea)	Coscinodiscophyceae	Coscinodiscales	*Actinoptychus senarius*	0.434	0.0243
		Melosirales	*Melosira nummuloides*	0.516	0.003*
		Paraliales	*Paralia sulcata*	0.842	0.0001
	Mediophyceae	Thalassiosirales	*Cyclotella striata*	0.603	0.0005
			Thalassiosira eccentrica	0.481	0.0081
	Bacillariophyceae	Fragilariales	*Asteroplanus kariana*	0.614	0.0017
		Naviculales	*Pleurosigma angulatum*	0.462	0.0111
Group 2 (Southern Sea)	Mediophyceae	Chaetocerotales	*Chaetoceros affinis*	0.380	0.0482
			Chaetoceros brevis	0.413	0.0233
			Chaetoceros constrictus	0.613	0.0001
			Chaetoceros contortus	0.550	0.0003
			Chaetoceros curvisetus	0.490	0.0027
			Chaetoceros debilis	0.563	0.0009
			Chaetoceros laciniosus	0.538	0.0001
			Chaetoceros socialis	0.429	0.0377
			Chaetoceros spp.	0.479	0.0094
		Hemiaulales	*Eucampia zodiacus*	0.596	0.0001
		Thalassiosirales	*Detonula pumila*	0.520	0.0011
			Skeletonema dohrnii-marinoii	0.707	0.0001
			Thalassiosira curviseriata	0.657	0.0001
			Thalassiosira nordenskioeldii	0.745	0.0001

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Groups (Ecoregion)	Class	Order	Species	Stat	p-valu
	Bacillariophyceae	Bacillariales	*Pseudo-nitzschia pungens*	0.450	0.008:
		Fragilariales	*Asterionellopsis glacialis*	0.660	0.0001
		Thalassionematales	*Thalassionema nitzschioides*	0.582	0.0011
Group 3	Mediophyceae	Eupodiscales	*Odontella aurita*	0.463	0.0157
(Southern East Sea)	Bacillariophyceae	Licmophorales	*Licmophora grandis*	0.732	0.0001
			Licmophora paradoxa	0.462	0.0072
		Mastogloiales	*Achnanthes* spp.	0.400	0.0402
		Naviculales	*Navicula* spp.	0.605	0.0006
		Surirellales	*Entomoneis paludosa*	0.460	0.013:
Group 4 (Northern East Sea)	Coscinodiscophyceae	Corethrales	*Corethron pennatum*	0.521	0.0117
		Coscinodiscales	*Coscinodiscus centralis*	0.707	0.0001
	Mediophyceae	Chaetocerotales	*Chaetoceros radicans*	0.522	0.0207
		Thalassiosirales	*Porosira glacialis*	0.674	0.0009
			Thalassiosira pacifica	0.507	0.0458
	Bacillariophyceae	Licmophorales	*Licmophora ehrenbergii*	0.522	0.0171
		Rhabdonematales	*Rhabdonema* spp.	0.522	0.026:

*** = > 0.001; ** = > 0.01; * > 0.1

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