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Baseline

Phytoplankton assemblage responses to massive freshwater inputs and anthropogenic toxic substances contamination in the Geum River Estuary, South Korea

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ABSTRACT

This study investigated the relationships between phytoplankton assemblages and water contamination by persistent toxic substances (PTSs) and nutrients in an estuary with an artificial dam over one year. The distribution of PTSs, including 15 polycyclic aromatic hydrocarbons, 6 alkylphenols, and 8 metal(loid)s, along with nutrients, exhibited relatively high concentrations with irregular temporal fluctuations in the inner estuary. During winter and spring, phytoplankton communities showed good ecological quality, with an average of 28 species and a density of 1750 cells L^{-1}. In contrast, during summer, there was a significant increase in the density of freshwater species (max 45,000 cells L^{-1}). These assemblages were categorized into three seasonal groups, featuring dominant taxa like blue-green algae and diatoms. Temperature and nutrient levels were the principal factors influencing phytoplankton assemblages, while PTSs had a minor impact. Overall, phytoplankton assemblages displayed strong seasonal variation, mainly influenced by freshwater input and nutrient availability.

Estuaries are areas where freshwater and seawater mix, having great economic value (Costanza et al., 1997). Freshwater, which contains both nutrients and persistent toxic substances (PTSs), is discharged through estuary dams, resulting in high productivity and a complex environment in terms of physical, chemical, and biological aspects (McLuski and Elliot, 2006). This discharge, however, also results in decreased salinity and the deterioration of coastal ecosystem health (Kim et al., 2020; Shin, 2013; Yang et al., 1999). With the increase in human activities in coastal areas, eutrophication (harmful algal blooms) may occur due to the inflow of enrichment nutrients and the development of hotspots where PTSs accumulation is accelerated (Lee et al., 2022; Yoon et al., 2021). The United States and South Africa have implemented estuary management programs to control water quality and to protect coastal ecosystem health (Poole, 1996; Van Niekerk et al., 2013).

Phytoplankton serve as primary producers through photosynthesis and play an important role in carbon and nutrients [nitrogen (N) and phosphorus (P)] cycling in the coastal ecosystems. The phytoplankton community is highly sensitive to changes in water quality (Harper, 1992) and is more susceptible to disturbances compared to other organisms (Rocha et al., 2023). Thus, it serves as an excellent indicator of pollution and overall ecosystem health (Lee et al., 2023; Suthers and Rissik, 2009). Information regarding the density and composition of phytoplankton communities is valuable for assessing external influences on aquatic ecosystems (Suthers and Rissik, 2009) and monitoring coastal ecosystem health.

The Geum River Estuary, one of the four representative estuaries in South Korea, is surrounded by urban areas that contribute to significant environmental impact. Yoon et al. (2021) recently reported sediment contamination in the Geum River Estuary with PTSs, including polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and metal(loid)s, affecting the macrobenthic faunal community. This PTSs contamination may also pose potential hazards to the water column

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organisms, including phytoplankton. Previous studies of the health of the Geum River Estuary ecosystem have primarily focused on surveys of phytoplankton distribution patterns and environmental factors, such as water temperature, salinity, and dissolved oxygen (DO) (Kim and Hwang, 2018; Kim et al., 2020; Shin, 2013). South Korea experiences over 50 % of its annual precipitation during the rainy season (June–August), with freshwater inputs impacting water quality and phytoplankton communities in the estuary (Kim et al., 2000).

Most previous studies have primarily focused on assessing the effects of PTSs contamination at the individual and population levels rather than considering the response at the community level (Albarico et al., 2022; Xia et al., 2022; Zhao et al., 2015). Furthermore, these investigations have focused on the accumulation of PTSs facilitated by processes such as reduction, circulation, and bioaccumulation, wherein they serve as biological pumps. However, a gap exists in the current knowledge concerning the environmental factors that influence and alter the community, especially when considering the comprehensive analysis of PTSs and environmental parameters, including nutrient levels. While comprehending the impact of diverse environmental factors is crucial in intricate estuarine ecosystems, studies addressing this aspect are limited. To date, only one study has specifically examined the interaction between environmental estrogens and nutrient dynamics in river systems (Long et al., 2020). To gain a more comprehensive understanding of ecosystem health, it is necessary to assess the seasonal and spatial distribution of PTSs and phytoplankton communities in the estuarine area, considering changes due to freshwater discharge and the temporary influx of terrestrial substances.

In the present study, we investigated the impact of an artificial dam on the water column of the Geum River Estuary. The specific objectives were to: (1) determine the spatiotemporal distribution of PTSs and nutrients, (2) characterize phytoplankton assemblage patterns, and (3) investigate the relationships between chemical and biological variables in order to identify the key factors influencing spatiotemporal changes in the phytoplankton community.

Phytoplankton and seawater samples were collected from eight stations, including inner (stations 1–3) and outer regions (stations 4–8) of the Geum River Estuary, at two-month intervals from February to December 2015 (Fig. 1). South Korea typically experiences a predominantly dry climate, except during the rainy season (June–August). Surface seawater was collected using pre-cleaned Niskin bottles and glass bottles for the analysis of organic pollutants, metal(loid)s, phytoplankton, and nutrients. Phytoplankton samples were immediately fixed in Lugol's solution, and the water samples were stored at −20 °C until analysis. Phytoplankton was identified by microscopy after concentration by siphoning. Detailed information on the water quality parameters is presented in Yoon et al. (2021).

PAHs and APs were extracted from 1-L seawater samples using a liquid-liquid extraction with 60 mL dichloromethane (Burdick & Jackson, Muskegon, MI, USA). Five surrogate standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, perylene-d_{12}, and bisphenol A-d_{16}) were added prior to extraction for quality control. The extracts were concentrated and dehydrated with anhydrous sodium sulfate. After concentration with N_2 gas, an internal standard (2-fluorobiphenyl) was added. PAHs and APs were quantified using an Agilent 7890B gas chromatography equipped with a 5977B mass selective detector (GC-MSD, Agilent Technologies, Santa Clara, CA, USA). Detailed operating conditions are presented in Table S1 of Supplementary Materials. The method detection limits for PAHs and APs ranged from 0.28 to 0.91 ng L^{-1} and 0.10 to 0.91 ng L^{-1}, respectively. The recovery of the five surrogate standards was generally within an acceptable range (62–121 %, mean: 90 %).

For the analysis of metals (Cd, Cr, Cu, Ni, Pb, and Zn), 1 L seawater was filtered through a membrane filter and acidified with HNO_3. Then, 400 mL of seawater was adjusted to pH 4 and mixed with ammonium pyrrolidine dithiocarbamate/diethylammonium diethyldithiocarbamate. After shaking the extracts with chloroform, the samples were heated with HNO_3, and absorbed metals were eluted with 1 N HNO_3. Concentrations of six metals (Cd, Cr, Cu, Ni, Pb, and Zn) were measured using an Elan 6100 inductively coupled plasma-mass spectrometer (ICP-MS, Perkin-Elmer SCIEX, Norwalk, CT, USA). As and Hg in seawater were quantified using the PSA Millennium Merlin satellite (PS Analytical Ltd., Kent, UK). The recoveries of the seawater standard reference materials (NASS-6, National Research Council of Canada, Ottawa, ON, Canada) were generally acceptable (103–117 %; mean: 107 %; Table S2).

The water quality index (WQI) was calculated according to the method outlined by Park et al. (2019) using reference values derived from the central West Sea, which includes the Geum River Estuary. Briefly, Bottom oxygen saturation, chlorophyll-*a* (Chl-*a*) concentration, transparency (Secchi Disk), dissolved inorganic nitrogen (DIN) concentration, and dissolved inorganic phosphorus (DIP) concentration were scored and calculated by comparing them with the reference values of the Central West Sea. The detailed method and scores of each parameter for calculation was provided in a previous study (Jang and Park, 2015). In the original data matrix, species with abundances <0.1 % of the total phytoplankton were excluded, and a log (x + 1) transformation was applied. The assessment of the ecological quality of the phytoplankton assemblage involved the utilization of evenness (J′) and the integrated phytoplankton index (IPI) (Sheldon, 1969; Spatharis and Tsirtsis, 2010). Various statistical analyses were conducted, including Pearson correlation analysis using SPSS 25.0 (SPSS INC., Chicago, IL, USA), cluster analysis (CA), permutational multivariate analysis of variance (PERMANOVA), permutational analysis of multivariate

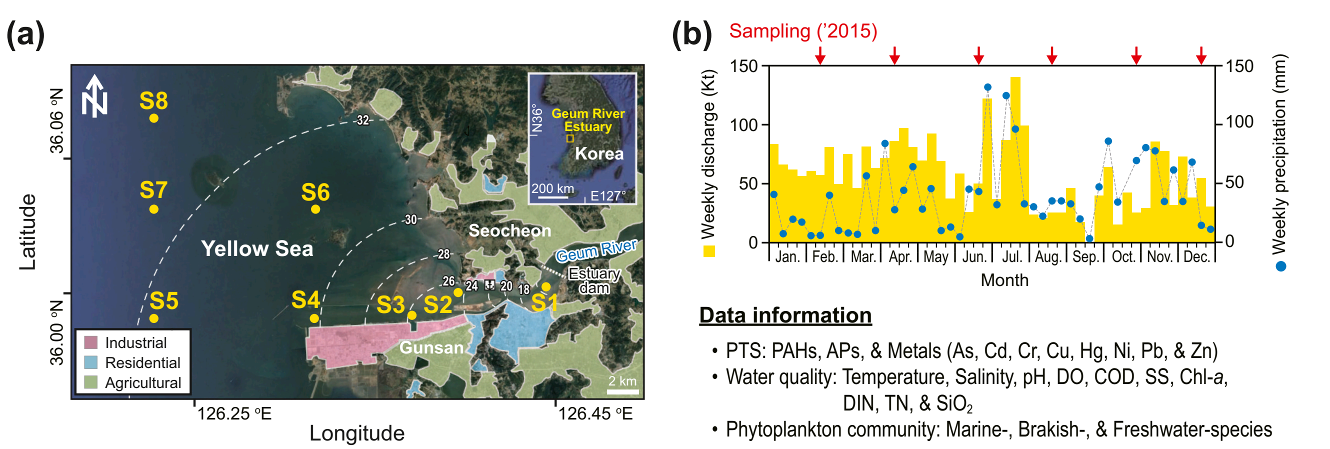

Fig. 1. (a) Map of seawater and phytoplankton sampling stations in the Geum River Estuary, Korea. The dotted line represents the annual mean salinity. (b) Weekly freshwater discharge and precipitation in the Geum River Estuary area.

dispersions (PERMDISP), distance-based linear modeling (DistLM), distance-based redundancy ordination analysis (dbRDA), canonical analysis of principal coordinates using PRIMER 6 (PRIMER-E Ltd., Plymouth, UK), and indicator value analysis with R package indicspecies using R studio version 3.6.3 (R Development Core Team). Details of the statistical analyses are presented in Yoon et al. (2021).

All PTSs were detected in all seasons and stations, except for PAHs, which had a detection frequency of 77 % (Table 1 and Fig. S1). The concentrations of PTSs in seawater exhibited variability across stations and seasons. Irregular monthly differences in concentrations were observed for most PTSs except PAHs and APs ($p < 0.05$; Table S3). Specifically, concentrations of Cu, Ni, and Cd were significantly higher during the dry season (e.g., April, February, and December), while As and Cr concentrations were significantly higher during the late rainy season (e.g., August and October). Concentrations of PAHs were higher during the rainy season, while concentrations of APs were higher during the dry season, with no significant monthly variation. The seasonal variation in PTSs was more pronounced in seawater than in sediments from the Geum River Estuary (Yoon et al., 2021), reflecting the dynamic nature of the aquatic environment. Furthermore, these findings suggest the potential influence of combined effects from discharged freshwater from an estuary dam, sources of the inner estuary, and low rainfall during the rainy season (Cheng et al., 2013; Niu et al., 2018). The spatial distribution of PTSs also differed significantly, with higher concentrations of PAHs, APs, Ni, Cu, and As in the inner estuary (stations 1–3) and higher concentrations of Cr and Cd in the outer estuary (stations 4–8). Significant differences in spatial distribution were observed primarily for PAHs, APs, As, Cu, and Ni (Table S4), indicating that harbors, industrial areas, and residential areas in the inner estuary are potential sources of PTSs (Huang et al., 2015; Park et al., 2020a, 2020b).

The composition of PAHs and APs showed variations depending on the seasons and stations (Fig. S2). In the inner estuary (stations 1–3), high-molecular-weight (HMW: 4–6 rings) PAHs were predominant, while in the outer estuary (stations 4–8), low-molecular-weight (2–3 rings) PAHs were more prevalent. These results are consistent with the previous finding that the major source of sedimentary PAHs was diesel and gasoline combustion in the study area (i.e., HMW; Yoon et al., 2021). Regarding APs, nonylphenols and nonylphenol ethoxylates dominated from February to August, while octylphenol and octylphenol ethoxylates became predominant in October and December. The prevalence of octylphenol ethoxylates in October and December, coinciding with relatively high AP concentrations, suggests a fresh source input of octylphenols during that period.

The relationships between environmental parameters and PTSs concentrations varied significantly (Table S5). PTSs were primarily associated with DO, temperature, and chemical oxygen demand (COD), with notable seasonal variations, implying that the distribution of PTSs in seawater is affected by seasonal factors (Lau et al., 2009; Niu et al., 2018). Among the parameters representing spatial variability, significant correlations were found predominantly in discharged freshwater, and only a few PTSs were correlated with low salinity and suspended solids (SS). The correlation between PTSs and precipitation was not statistically significant ($p > 0.05$), suggesting that it is rapidly diluted by inflow or influenced mainly by point sources, such as discharged freshwater (Niu et al., 2018).

The concentrations of all PTSs in the Geum River Estuary were similar to or lower than those in the surrounding waters (Cheng et al., 2013; Liu et al., 2019; Niu et al., 2018; Park et al., 2020a, 2020b). However, a few PTSs concentrations in the estuary exceeded the environmental quality guidelines of South Korea and the Canadian Council of Ministers of the Environment (CCME, 2002; MOF, 2018; Kalf et al., 1997). The concentrations of Cu exceeded the seawater quality threshold in February, April, October, and December. In addition, the concentrations of APs and Ni each exceeded the thresholds once, indicating that potential adverse effects on coastal ecosystem health due to PTSs exceeding environment quality guidelines in seawater might have adverse effects on coastal ecosystems.

Spatial and seasonal variability in water quality parameters was observed separately or simultaneously depending on the parameters (Table 1, Figs. 2, S3, and S4). Significant seasonal differences were recorded for water temperature, pH, and DO concentration, while significant spatial differences were recorded for salinity and SS, DIN, and total nitrogen (TN) concentrations ($p < 0.05$; Table S6). Significant variations were observed in both seasonal and spatial aspects for other parameters, with some parameters showing significant correlations ($p < 0.05$; Tables S6 and S7). Water temperature was relatively high (not significantly correlated with freshwater discharge), and DO concentration was low in June and August. The concentrations of DIN and TN were relatively high from February to June, with no significant seasonal variation. Meanwhile, the concentrations of SS, DIP, and total phosphorous (TP) were generally elevated in the estuary between June and October, coinciding with increasing freshwater discharge. The concentrations of Chl-*a* were relatively high in February, June, and October, and the high concentrations in only the inner estuary in June and October suggested the influence of freshwater input. These results reflect the direct impact of freshwater inputs from the estuary dam, which carries high amounts of nutrients and Chl-*a* (Fig. S5). Similar trends have been observed in other Korean estuaries, suggesting the influence of the four seasons in mid-latitude regions and the influx of freshwater from artificial dams (Park and Sin, 2022; Sin and Yu, 2018; Lee et al., 2021).

The N/P ratios exceeded the Redfield ratio of 16 from February to August but fell below 16 from October to December. These results indicated that nutrient concentrations and limited nutrients are influenced by seasonality. Previous studies suggested that the potential limitation of phosphorus in spring and winter and the potential limitation of nitrogen in fall observed from the Yeongsan River, Bach Dang, and Pearl River Estuaries (Cho, 2010; Chu et al., 2014; Tao et al., 2021; Yoon et al., 2023). The water quality parameters in the Geum River Estuary exhibit typical patterns seen in mid-latitude estuaries depending on the levels of freshwater inputs, with both DIN and DIP playing a significant role as potential nutrient-limiting factors.

The WQI for the Geum River Estuary was mainly 'moderate' in February, April, and December, 'poor' in June and October, and 'bad' in August (Fig. 2). The water quality deteriorated within the estuary in summer. The annual average of WQI of Geum River Estuary was 'moderate', corresponding to the bottom 30 % of Korean coastal waters (Park et al., 2019). These results can be primarily attributed to the lower DO concentrations in the bottom water during summer. Reductions in DO concentration are known to be influenced by several factors, including increased water temperature, freshwater input, and the decomposition of organic matter (Yin et al., 2004). Overall, the water quality in the Geum River Estuary was notably 'bad' during summer, and it is expected that significant seasonal variations could have adverse or positive effects on the coastal ecosystem.

The phytoplankton assemblages showed spatial and seasonal specificity (Table 2). Differences between sampling months were significant ($p < 0.05$), with dispersion in the taxon diversity and density. Spatially, only density differed significantly ($p < 0.05$). The interaction of the spatial and seasonal distributions significantly affected the taxon diversity and density ($p < 0.05$). These results indicate that phytoplankton assemblages in the Geum River Estuary are affected mainly by the season and that the variability of species and density is high (Wu et al., 2022).

The distributions of freshwater, brackish, and marine species in the phytoplankton assemblages varied according to the location and season (Figs. 3a and S6). The number of species and density were relatively high in the winter/spring and low in the summer/autumn, except in cases of irregular input through estuary dams (Fig. 3b). Freshwater species appeared throughout the estuary during the year, and relatively high density was found in the inner estuary. Most freshwater species belonged to the classes Chlorophyceae, Bacillariophyceae, and

Table 1
Statistics for environmental variables and phytoplankton community structure in the Geum River Estuary from Korea.

Target analytes	All (year)			February			April			June			August			October			December		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Seawater																					
As (μg L^{-1})	0.24	1.06	0.56	0.24	0.81	0.46	0.35	0.86	0.62	0.27	0.36	0.32	0.52	0.68	0.60	0.53	0.68	0.62	0.59	1.1	0.70
Cd (μg L^{-1})	0.01	0.13	0.05	0.04	0.07	0.06	0.02	0.07	0.05	0.01	0.02	0.02	0.03	0.04	0.04	0.03	0.13	0.07	0.04	0.05	0.05
Cr (μg L^{-1})	0.02	0.29	0.17	0.02	0.11	0.08	0.11	0.15	0.14	0.06	0.11	0.09	0.22	0.29	0.25	0.21	0.26	0.23	0.19	0.25	0.23
Cu (μg L^{-1})	0.17	1.71	0.79	0.63	1.5	0.96	0.91	1.4	1.1	0.17	0.32	0.25	0.40	0.82	0.58	0.59	1.3	0.77	0.73	1.7	1.1
Hg (ng L^{-1})	0.29	1.26	0.72	0.29	1.1	0.62	0.43	1.1	0.76	0.41	0.58	0.51	0.66	0.84	0.76	0.65	0.83	0.75	0.79	1.3	0.91
Ni (μg L^{-1})	0.12	1.84	0.67	0.50	1.8	0.90	0.75	1.5	1.1	0.12	0.27	0.19	0.29	0.61	0.43	0.61	1.4	0.80	0.51	0.81	0.64
Pb (μg L^{-1})	0.03	0.42	0.13	0.08	0.30	0.19	0.20	0.42	0.32	0.05	0.09	0.07	0.05	0.11	0.07	0.03	0.04	0.04	0.04	0.08	0.06
Zn (μg L^{-1})	0.11	1.58	0.70	0.11	0.63	0.44	0.59	0.91	0.80	0.20	0.43	0.33	0.74	0.83	0.80	0.63	1.3	0.79	0.82	1.6	1.0
PAHs (ng L^{-1})	N·D.	103	11.6	2.5	19.5	7.6	N.D.	6.0	4.1	0.78	42.1	9.7	N.D.	103	26.5	N.D.	2.1	1.6	N.D.	28.9	14.0
APs (ng L^{-1})	16.4	1777	143	22	1777	290	29.6	104	54.7	31.1	201	74.1	31.9	48.5	38.1	16.4	683	274	45.4	226	129
Temperature (°C)	2.7	28.2	15.0	2.7	5.5	3.6	8.4	11.9	10.1	20.9	24.1	22.1	26.6	28.2	27.1	17.0	19.4	18.4	7.3	10.4	8.7
Salinity (psu)	4.7	32.8	28.4	18.3	32.5	28.2	14.9	32.2	26.6	11.6	32.1	27.1	23.2	32.2	29.6	4.7	32.8	28.5	24.3	32.6	30.5
pH	7.7	8.7	8.1	8.0	8.3	8.2	8.2	8.5	8.4	7.8	8.3	8.0	7.7	8.2	8.0	7.8	8.7	8.0	7.9	8.1	8.1
DO (mg L^{-1})	2.9	12.9	8.5	11.0	19.2	14.1	9.1	13.0	11.3	5.4	7.9	6.7	4.1	7.4	6.0	6.7	8.7	7.6	9.1	10.5	9.7
COD (mg L^{-1})	0.8	8.4	3.8	1.2	3.2	2.0	0.8	3.2	1.9	3.6	8.4	5.9	2.9	6.0	4.3	1.8	6.0	4.1	2.9	5.9	4.6
SS (mg L^{-1})	4.8	67.2	14.5	5.0	14.8	9.5	6.0	22.8	10.9	6.4	18.4	10.2	4.8	24.0	12.7	5.0	67.2	22.4	12.8	42.8	21.5
Chl-*a* (μg L^{-1})	0.7	19.0	4.6	2.3	9.6	6.0	1.5	7.3	4.5	1.6	15.7	5.6	2.4	8.6	4.7	1.1	19.0	5.7	0.68	1.5	1.1
DIN (μg L^{-1})	14.1	1510	439	226	1510	698	14	958	277	21	1293	442	26	1073	379	115	1171	377	189	1061	461
TN (μg L^{-1})	356	1771	833	362	1771	946	355	1754	854	412	1314	751	507	1196	812	419	1283	710	510	1607	922
PO_4 (μg L^{-1})	0.2	88.5	23.4	10.8	30.6	20.6	0.21	15.6	4.2	1.7	44.7	17.4	3.7	88.5	17.8	27.2	58.0	39.7	29.3	49.6	40.5
TP (μg L^{-1})	17.7	213	56.7	34.6	54.0	40.8	22.2	75.4	37.2	17.7	90.7	43.2	28.7	118	59.7	37.9	213	94.6	53.4	87.1	64.9
SiO_2 (μg L^{-1})	12.	2078	566	275	1140	530	12.0	274	80.4	161	1347	561	171	2078	780	360	1140	661	456	1363	781
Plankton community structure																					
Number of taxa	7	44	19	21	28	25	18	44	31	11	17	14	11	23	18	9	17	12	7	15	12
Density (cells L^{-1})	16	45,148	1831	620	1752	1225	1427	3567	2274	426	45,148	6243	152	1040	580	16	3638	545	35	114	70
Dominant taxa																					
1st	Bac (76.5 %)			Bac (74.2 %)			Bac (74.2 %)			Bac (47.4 %)			Bac (91.6 %)			Bac (76.4 %)			Bac (94.9 %)		
2nd	Cry (11.2 %)			Cry (18.6 %)			Cry (18.0 %)			Cry (27.2 %)			Din (6.4 %)			Cya (20.2 %)			Cry (2.9 %)		
3rd	Cya (6.7 %)			Cya (3.1 %)			Chl (4.9 %)			Cya (15.3 %)			Cya (1.7 %)			Chl (2.4 %)			Dic (1.3 %)		
Ecological indices																					
Diversity (H′)	0.8	2.8	1.9	2.2	2.6	2.4	1.5	2.8	2.1	0.8	2.1	1.8	1.0	2.3	1.5	1.0	2.3	1.6	1.1	2.3	1.8
Evenness (J)	0.3	1.0	0.7	0.7	0.8	0.8	0.5	0.7	0.6	0.3	0.8	0.7	0.4	0.7	0.5	0.4	1.0	0.6	0.6	0.9	0.7
Richness (R)	0.9	5.7	3.0	2.8	3.8	3.4	2.2	5.5	3.9	0.9	2.4	1.9	1.8	4.0	2.8	1.0	3.6	2.6	1.4	3.5	2.6
Dominance (D)	0.3	1.0	0.6	0.6	0.9	0.7	0.4	0.9	0.6	0.1	0.6	0.5	0.6	1.5	0.9	0.1	2.8	1.4	0.8	2.2	1.5

Bac: Bacillariophyceae; Cry: Cryptophyceae; Cya: Cryptophyceae; Chl: Chlorophyceae; Din: Dinophyceae; Dic: Dictyochophyceae; N.D: Not detected.

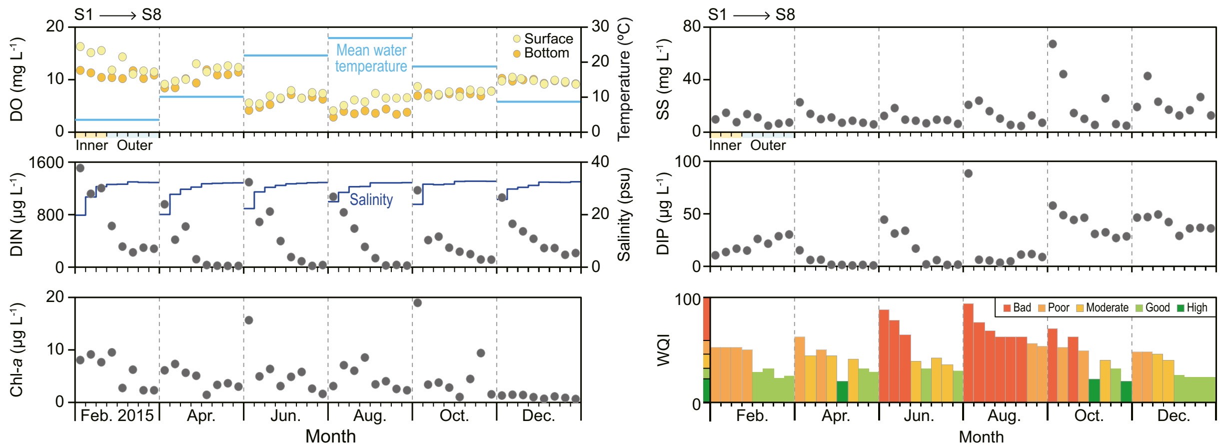

Fig. 2. Spatiotemporal distribution of water parameters and water quality index (WQI) in the Geum River Estuary area.

Table 2
Result of the PERMANOVA and PERMDISP test based on the data of taxon diversity and density of phytoplankton in the Geum River Estuary, Korea (Zo: Zone (inner/outer), Sm: Sampling month, df: degree of freedom, P-F: Pseudo-F, ECV: estimate components of variation, Sqrt: square root of ECV; bold values, $p < 0.05$).

Target	Term	PERMANOVA					PERMDISP	
		df	P-F	ECV	Sqrt	*p*	P-F	*p*
Taxon diversity	Zo	1	1.7	0.3	0.56	0.213	2.98	0.099
	Sm	5	32.3	42.3	6.50	**0.001**	2.90	**0.048**
	Zo × Sm	5	4.3	8.9	2.98	**0.005**		
	Res	36		10.1	3.18			
Density	Zo	1	4.0	161	12.7	**0.001**	0.67	0.466
	Sm	5	12.5	1868	43.2	**0.001**	3.87	**0.026**
	Zo × Sm	5	2.2	401	20.0	**0.001**		
	Res	36		1214	34.8			

Cyanophyceae. In particular, the density of Cyanophyceae accounted for 96 % of the total density in June, with a concentration of 45,000 cells mL^{-1} corresponding to a harmful algae warning (NIFS, 2023). In summer, freshwater species accounted for 93 % of the total density at station 1, suggesting the effects of high temperature and major freshwater input. Brackish species appeared in the entire estuary throughout the year and belonged only to Cryptophyceae, Bacillariophyceae, and Cyanophyceae. The density of brackish species accounted for about 20 % of the whole estuary and was almost entirely (97 %) Cryptophyceae, the density of which was high only from February to June. Marine species appeared throughout the estuary in all periods, in the order of Bacillariophyceae, Dinophyceae, and Dictyochophyceae. Marine species showed the highest composition throughout the year in the estuary except at station 1, and Bacillariophyceae accounted for 97 % of these species. These results suggest a change in the phytoplankton assemblages, with brackish and freshwater species entering or increasing in winter and summer by freshwater input, although marine species remained predominant.

The ecological indices of the phytoplankton assemblages varied by sampling month and estuary zone (Fig. 3c). The diversity (H) and richness (R) were significantly high in winter and spring (February and April), and low in summer (June and August) ($p < 0.05$). Similarly, evenness (J) was significantly high in winter (February and December) and low in August ($p < 0.05$). In contrast, the dominance (D) was significantly high in the summer and low in the winter ($p < 0.05$). In ecological indices, IPI was 'poor' at only the inner estuary in June, 'high' in December, and 'moderate' in most other times (Fig. 3c). J' was generally 'good' in February, October, and December, 'moderate' in June, and 'poor' in April and August. Although IPI appeared to have rough values compared to J', these results reflect the major input of freshwater species in summer and the low number of species and low Chl-*a* concentrations in winter. A similar trend was observed in an estuary in an area with a monsoon climate, and the community parameters were determined by the influence of the river (Bezuidenhout, 2011; Chowdhury, 2019).

The CA revealed three distinct groups in phytoplankton assemblages, dividing them into three groups: one from February to June, another in August, and a third from October to December (Fig. 4). There was a slight separation between the inner and outer estuary within the same group, indicating density-based differences as reflected in the PERMANOVA (Table 2). Throughout the study period, 9 out of the 10 most dominant species appeared mainly from February to June. Among these dominant species, the freshwater species *Oscillatoria* sp. and *Aphanizomenon* sp. were prevalent in the inner estuary in June. These species belong to the Cyanophyceae, which undergo maximum growth in premonsoon and monsoon periods, and they serve as indicators of eutrophication in upper estuaries (Bezuidenhout, 2011; Chowdhury, 2019). These results indicate that these two species at high densities were introduced together with discharged freshwater. Additionally, *Eucampia zodiacus*, *Skeletonema marinoi*, *Chaetoceros* sp., *Leptocylindrus minimus*, *Parmelia sulcata*, *Thalassiosira nordenskioeldii* (Bacillariophyceae), and *Cryptomonas* spp. (Cryptophyceae) were also dominant species from February to June. *E. zodiacus*, a large diatom species, is known to form blooms in coastal and estuarine areas during spring (Martin et al., 2007; Yoshida et al., 2011). *Cryptomonas* spp. are fast-growing in nutrient-rich environments and exhibited in winter-spring blooms (Pinckney et al., 2000). *S. marinoi* is a chain-forming species and also blooms in nutrient-rich water from estuary during winter to spring (Santos et al., 2022). The other dominant species appear in estuary areas in winter and spring with abundant DIN and DIP, with N/P ratios > 16, and their episodic peak occurrence is an indicator of eutrophication (Guinder et al., 2010; Liu et al., 2022; Morelle et al., 2018; Viličić et al., 2008). *Microcystis* spp., a freshwater species known for producing hepatotoxic microcystins, only dominated in October (Kim et al., 2021). Some dominant species were

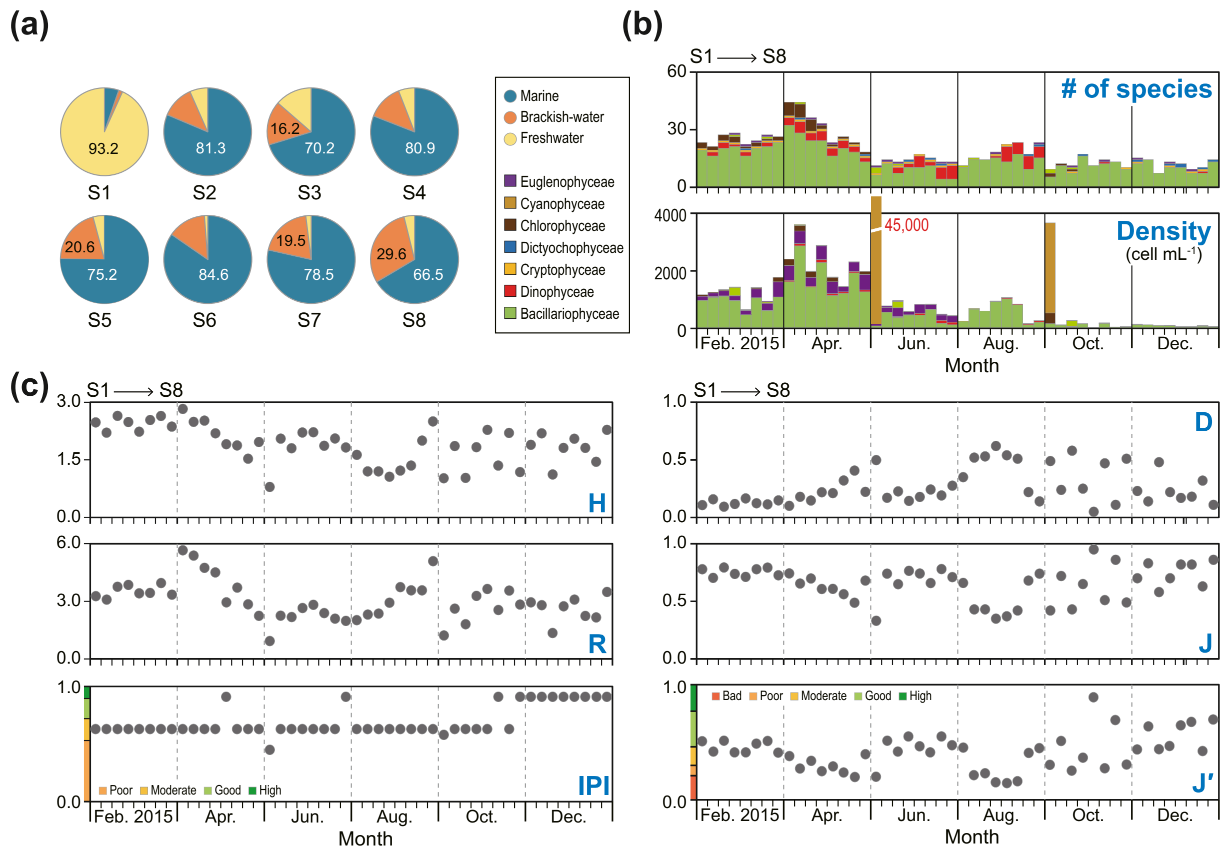

Fig. 3. (a) Spatial variation in phytoplankton composition. (b) Spatiotemporal distribution of phytoplankton by the number of species and density. (c) Spatiotemporal distribution of ecological indices [diversity (H), dominance (D), richness (R), evenness (J)] and quality [integrated phytoplankton index (IPI) and evenness (J′)].

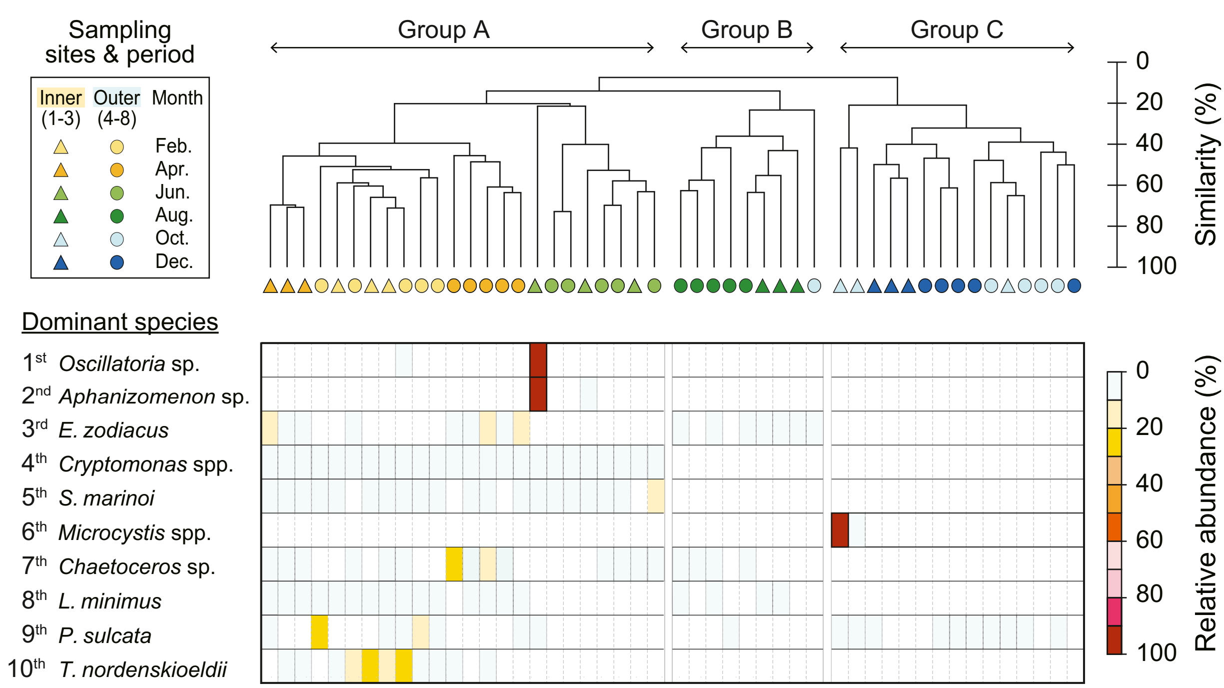

Fig. 4. Phytoplankton assemblage clusters and relative abundance of the 10 dominant species over a 1-year period.

observed in August, but their relative abundances were low. Overall, the dominant species in the Geum River Estuary consisted of both episodically flowing freshwater species and marine species responsible for winter-to-spring blooms, with their presence mainly from February to June.

The DistLM results indicated that 10 out of the 24 environmental parameters had significant impacts on the spatiotemporal distributions of phytoplankton assemblages. These influential parameters included the concentrations of Cr, Pb, Cd, and Zn, water temperature, pH, freshwater discharge, TP and silicate concentrations, and COD (Table S8). These physical-, nutrient-, and contaminant-related parameters collectively accounted for 62 % of the variability observed in the phytoplankton assemblages. Further analysis using dbRDA categorized the phytoplankton assemblages into three distinct groups based on the influence of the 10 environmental parameters (Fig. 5a). The indicator species associated with these groups were as follows: *Cryptomonas* spp. and *S. marinoi* for group A (February–June), *Pseudo-nitzschia* sp. and *Bacteriastrum* sp. for group B (August), and *Synedra* sp. for group C (October and December; Table S8). Cryptomonads and *S. marinoi* were dominant species throughout the study period and were known to thrive in nutrient-rich water (Pinckney et al., 2000; Santos et al., 2022). *Pseudo-nitzschia* sp. and *Bacteriastrum* sp. grow rapidly in response to nutrient increases in late summer (Mozetiĉ et al., 1998; Viličić et al., 2008). *Synedra* sp., on the other hand, generally appears in estuarine areas after monsoon periods and flourishes until winter (Roshith et al., 2018). These indicator species reflect the seasonal succession of species, implying that temperature was a primary determinant of phytoplankton assemblages.

The primary factors affecting the phytoplankton assemblages were temperature and freshwater discharge, followed by nutrient (silicate and TP) and metals (Pb, Cr, Zn, and Cd) concentrations. The phytoplankton assemblages were classified according to the primary factor gradients at the class level (Fig. S7). Temperature and nutrients distinctly separated the phytoplankton assemblages along the slope, with canonical correlations (δ values) of 0.94 ($p < 0.001$) and 0.88 ($p < 0.001$), respectively. The separation of phytoplankton assemblages along the PTSs gradient was ambiguous ($\delta = 0.67$, $p < 0.001$). The relative abundance of Cyanophyceae was high under high temperatures and nutrient conditions, implying that rich nutrients in summer promoted the growth of these species. The relative abundance of Dinophyceae increased with decreasing nutrient concentrations; Dinophyceae use organic nutrients such as urea and amino acids to maintain growth in the presence of limited inorganic nutrients (Davidson et al., 2012). Taxon abundance showed no clear tendency according to PTS concentrations, an expected result given that contamination with individual PTSs is seasonally specific or insufficient to affect phytoplankton assemblages (Jia et al., 2020).

The relationships between phytoplankton and individual environmental parameters varied at both class and species levels (Fig. 5b). At the class level, significant correlations were mainly found in Bacillariophyceae, Dinophyceae, and Cryptophyceae ($p < 0.05$). Most dominant species also had significant relationships with environmental parameters, but there were few significant relationships between indicator species and environmental parameters. These findings suggest that determining the responses of taxa and species that appear infrequently and only at specific times to environmental parameters can be challenging. Among all parameters, significant correlations were identified with the silicate concentration, Cr concentration, DIP concentration, freshwater discharge, pH, Pb concentration, and Chl-*a* concentration ($p < 0.05$). In general, both class- and species-level correlations with silicate and DIP concentrations were negative. Rapid increases in phytoplankton density, such as *E. zodiacus* and *Cryptomonas* spp. have been associated with negative correlations with nutrient levels (Egerton et al., 2014; Nishikawa et al., 2007). *Chaetoceros* sp., a dominant diatom found in the outer estuary where nutrient concentrations were low, also showed a negative correlation with nutrient concentrations (Ault et al., 2000). However, only *S. marinoi* and *T. nordenskioeldii* exhibited significant positive correlations, which were associated with DIN and TN concentrations. These results suggest that these two species were more prevalent in the upper reaches of the estuary compared to other species and were well adapted to nutrient-rich conditions (Tas and Yilmaz, 2015; Santos et al., 2022).

The observed positive correlation with discharge can be attributed to the increased influx of freshwater into the estuary during the winter and spring when dominant species were more prevalent. Significant relationships with pH and COD indicate the influence of seasonal changes, as they are correlated with temperature variations. Among heavy metals, phytoplankton density showed a negative correlation with Cr concentration and a positive correlation with Pb concentration. These correlations are more likely related to the seasonal distribution of phytoplankton rather than the direct effects of Cr and Pb on phytoplankton growth. In the previous study, hexavalent Cr and Pb were found to negatively affect phytoplankton growth and primary productivity by destroying cells or inhibiting protein and nucleic acid synthesis in an area about 100 times more contaminated than the present study area (Jia et al., 2020). In an open estuary without a dam, metals have been known to interact with phytoplankton, acting as biological filters during bloom events (Chaudry and Zwolsman, 2008). However, this

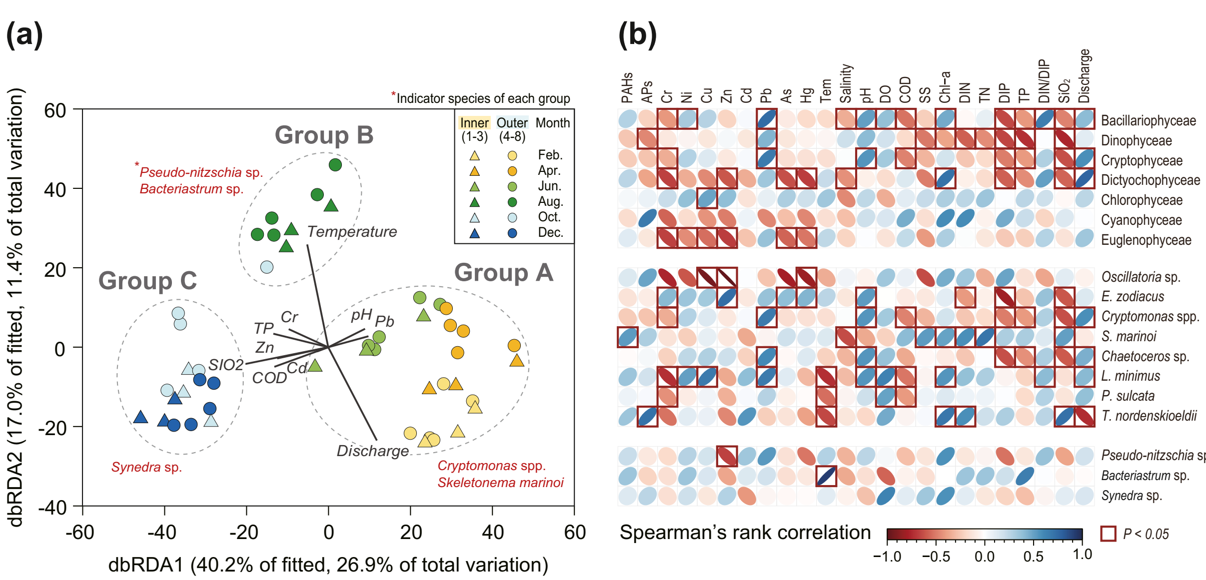

Fig. 5. (a) Results of a dbRDA based on environmental parameters and phytoplankton density. Significant environmental factors are presented as vectors. Indicator species were determined by indicator value analysis. (b) Correlations between environmental parameters and phytoplankton density.

particular trend was not observed in our study. Additionally, there were few significant correlations found for organic pollutants such as PAHs and APs, primarily due to their low contamination levels in the Geum River Estuary. The concentrations of these pollutants in our study area were significantly below the lower threshold known to affect marine microalgae, which was approximately five times higher than the maximum concentration observed in this study (Othman et al., 2023). Overall, phytoplankton assemblages in the Geum River Estuary were affected mainly by temperature and nutrients, with the impact of PTSs contamination being relatively minor.

The present study provides year-round contamination and phytoplankton response data for the Geum River Estuary. Levels of nutrients and contamination by PTSs reflect the inflow of freshwater through the estuary dam and industrial development of the inner estuary. The phytoplankton assemblages showed spatial and seasonal variation. We conducted a simultaneous analysis of estuarine contamination and various environmental parameters within the water column to identify the factors controlling the phytoplankton assemblages at taxon and species levels. These findings provide information at the genus or species levels, facilitating a better understanding of the factors governing phytoplankton assemblages in estuarine environments. Overall, our study provides valuable insights into the intricate dynamics of the estuarine water quality and ecological components, even though no significant relationship was identified between the phytoplankton assemblages and PTSs concentrations.

CRediT authorship contribution statement

Seo Joon Yoon: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Junghyun Lee:** Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft. **Hyeong-Gi Kim:** Formal analysis, Investigation. **Bong-Oh Kwon:** Investigation, Project administration. **Jaeseong Kim:** Formal analysis, Investigation, Project administration. **Seongjin Hong:** Conceptualization, Formal analysis, Project administration, Visualization, Writing – review & editing. **Jong Seong Khim:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Visualization, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.116020.

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<Supplementary Materials>

Phytoplankton assemblage responses to massive freshwater inputs and anthropogenic toxic substances contamination in the Geum River Estuary, South Korea

Seo Joon Yoon [1], Junghyun Lee [1], Hyeong-Gi Kim, Bong-Oh Kwon, Jaeseong Kim, Seongjin Hong *, Jong Seong Khim *

This file includes:

Number of pages: 17

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References

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Supplementary Tables

Table S1. Instrumental conditions of gas chromatograph equipped with a mass selective detector for analyses of persistent toxic substances.

GC/MSD system	**Agilent 7890A GC and 5975C MSD**
Column	DB-5MS UI (30 m long, 0.25 mm i.d., 0.25 μm film thickness)
Gas flow	1 mL/min He
Injection mode	Splitless
Injection volume	2 μL
Injector temperature	300 °C
Ionization	EI mode (70 eV)
MS temperature	180 °C
Detector temperature	230 °C
Oven temperature (PAHs)	60 °C hold 2 min Increase 6 °C/min to 300 °C 300 °C hold 13 min
Oven temperature (APs)	60 °C hold 5 min Increase 10 °C/min to 100 °C Increase 20 °C/min to 300 °C
Targeted PAHs (16)	Acenaphthylene (Acl), Acenaphthene (Ace), Fluorene (Flu),, Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Fl), Pyrene (Py), Benzo[*a*]anthracene (BaA), Chrysene (Chr), Benzo[*b*]fluoranthene (BbF), Benzo[*k*]fluoranthene (BkF), Benzo[*a*]pyrene (BaP), Perylene (Pery), Indeno[*1,2,3-c,d*]pyrene (IcdP), Dibenz[*a,h*]anthracene (DbahA), and Benzo[*g,h,i*]perylene (BghiP)
Targeted APs (6)	4-tert-Octylphenol (OP), 4-tert-Octylphenol monoethoxylate (OP1EO), 4-tert-Octylphenol diethoxylate (OP2EO), Nonylphenols (NPs, isomer mix), Nonylphenol monoethoxylates (NP1EOs, isomer mix), and Nonylphenol diethoxylates (NP2EOs, isomer mix)

Table S2. Certified and measured concentrations for selected metals in standard reference material (SRM; NASS-6) to check the accuracy of the method.

Metals	**Certified concentration ($\mu g\ L^{-1}$)**	**Measured concentration ($\mu g\ L^{-1}$)**	**Recovery (%)**
Cd	0.0311 ± 0.0019	0.0320 ± 0.0026	103 ± 8.4
Cu	0.248 ± 0.025	0.261 ± 0.008	105 ± 3.2
Ni	0.301 ± 0.025	0.317 ± 0.004	105 ± 1.3
Pb	0.006 ± 0.002	0.007 ± 0.002	117 ± 16.7
Zn	0.257 ± 0.020	0.267 ± 0.006	104 ± 2.3

Table S3. Statistical relationships of seasonal differences among months. The bold text highlights statistically significant relationships ($p < 0.05$).

PTSs	Kruskal-Wallis test		Month (*Post hoc* Mann-Whitney)									
	F-value	P value	2 – 6	2 – 8	2 – 10	2 – 12	4– 6	4– 8	4– 10	6– 8	6 – 10	6 – 12
As	21.4	**0.001**					**0.022**			**0.027**	**<0.001**	
Cd	31.4	**<0.001**	**<0.001**				**0.008**				**<0.001**	
Cr	39.8	**<0.001**		**<0.001**	**0.002**	**0.003**				**<0.001**	**0.002**	**0.004**
Cu	32.3	**<0.001**	**0.003**	**0.027**			**<0.001**					**<0.001**
Hg	24.0	**<0.001**				**0.008**						**<0.001**
Ni	35.4	**<0.001**	**0.001**				**<0.001**		**0.002**		**0.001**	
Pb	40.3	**<0.001**			**<0.001**	**0.002**		**0.016**	**<0.001**			
Zn	36.6	**<0.001**				**<0.001**	**0.006**			**0.013**		**<0.001**
PAHs	9.23	0.100										
APs	11.1	**0.049**										

Table S4. Mann-Whitney test for monthly inner- and outer-stations comparison. Values in bold indicate that the correlation was significant at $p < 0.05$.

	PAHs	APs	Cr	Ni	Cu[a]	Zn[a]	Cd	Pb	As	Hg
February	**0.036**[a]	0.143	0.393	0.250	**0.036**[a]	0.143	0.786	1.000	**0.036**[a]	0.143
April	0.400	0.393	**0.036**[b]	0.786	**0.036**[a]	0.786	**0.036**[b]	1.000	0.571	0.393
June	**0.071**[a]	**0.036**[a]	0.071	**0.036**[a]	**0.036**[a]	0.071	**0.036**[b]	0.393	0.393	0.571
August.	0.114	1.000	**0.036**[b]	**0.036**[a]	0.071	0.071	0.860	0.786	0.250	0.250
October	1.000	0.143	0.071	**0.036**[a]	**0.036**[a]	0.786	0.786	0.571	0.786	0.071
December	1.000	0.786	0.250	0.143	0.393	1.000	0.571	0.571	0.571	0.143

[a] Concentrations in inner stations were higher than outer stations.
[b] Concentrations in outer stations were higher than inner stations.

Table S5. Pearson correlation analysis between the persistent toxic substances and environmental parameters in seawater. Values in bold indicate that the correlation was significant at $p < 0.05$.

	PAHs	APs	Cr	Ni	Cu	Zn	Cd	Pb	As	Hg
Temperature	0.276	-0.205	**0.383****	**-0.545****	**-0.639****	-0.063	**-0.385****	**-.493****	-0.151	-0.159
Salinity	**-0.354***	-0.264	0.196	-0.278	**-0.390****	-0.119	-0.048	-0.200	-0.189	-0.037
DO	-0.238	**0.356***	**-0.418****	**0.540****	**0.601****	-0.019	**0.383****	**0.622****	0.176	0.182
pH	**-0.437****	0.012	-0.202	**0.541****	**0.414****	0.218	**0.454****	**0.620****	0.103	0.018
COD	0.210	-0.045	0.161	**-0.495****	**-0.365***	-0.029	**-0.411****	**-0.560****	-0.097	-0.006
SS	0.105	0.238	0.242	0.200	**0.305***	**0.362***	0.254	-0.250	0.276	0.245
Chlorophyll-*a*	0.185	0.169	-0.221	0.253	0.089	-0.108	0.217	0.142	-0.069	-0.212
WQI	**0.420****	0.179	0.188	-0.058	0.033	0.059	**-0.302***	-0.099	0.120	0.014
Precipitation	-0.021	0.243	0.218	-0.160	**-0.300***	-0.055	-0.060	**-0.462****	-0.090	-0.046
Discharge	**-0.300***	0.122	**-0.506****	**0.524****	0.275	-0.222	0.178	**0.454****	-0.170	-0.256

* $P < 0.05$, ** $P < 0.0$

Table S6. Results of Kruskal-Wallis and Mann-Whitney test for seasonal and spatial differences of water quality parameters, respectively. Values in bold indicate that the correlation was significant at $p < 0.05$.

	Kruskal-Wallis test		**Mann-Whitney test**						
	F-value	**P value**	**All**	**Feb.**	**Apr.**	**Jun.**	**Aug.**	**Oct.**	**Dec.**
Tem	45	**0.001**	0.992	**0.036**	**0.036**	0.071	**0.036**	0.143	**0.036**
Sal	5.5	0.355	**0.001**	**0.036**	**0.036**	**0.036**	**0.036**	**0.036**	**0.036**
pH	23.8	**0.001**	0.183	0.143	0.071	**0.036**	0.143	1.000	0.071
DO	40.7	**0.001**	0.489	**0.036**	**0.036**	0.143	0.071	0.786	0.143
COD	30.2	**0.001**	**0.046**	0.250	0.250	0.393	0.571	0.250	0.250
SS	10.4	0.066	**0.001**	0.393	0.071	**0.036**	**0.036**	0.071	0.143
Chl-a	19.2	**0.002**	**0.005**	0.250	**0.036**	0.071	0.571	0.393	**0.036**
DIN	6.3	0.276	**0.001**	**0.036**	**0.036**	**0.036**	**0.036**	**0.036**	**0.036**
TN	1.3	0.939	**0.001**	**0.036**	**0.036**	**0.036**	**0.036**	0.143	**0.036**
PO4	28	**0.001**	**0.013**	0.071	**0.036**	**0.036**	0.786	0.071	**0.036**
TP	16.4	**0.006**	**0.001**	**0.036**	**0.036**	**0.036**	**0.036**	**0.036**	**0.036**
SIO2	21.2	**0.001**	**0.001**	**0.036**	**0.036**	**0.036**	**0.036**	0.071	**0.036**
WQI	13.6	**0.018**	**0.001**	0.071	0.071	**0.036**	0.393	**0.036**	**0.036**

Table S7. Pearson correlation analysis between environmental parameters from seawater. Values in bold indicate that the correlation was significant at $p < 0.05$.

	Sal	**pH**	**DO**	**COD**	**SS**	**Chl-*a***	**DIN**	**TN**	**DIP**	**TP**	**DIN/DIP**	**SiO_2**
Tem	-0.022	**-.440****	**-.861****	**.518****	0.004	0.128	-0.150	-0.158	-0.015	0.179	-0.082	0.211
Sal		-0.187	-0.059	-0.217	**-.480****	**-.747****	**-.806****	**-.746****	-0.240	**-.339***	**-.385****	**-.448****
pH			**.558****	**-.472****	0.075	0.203	-0.133	-0.060	**-.409****	**-.348***	0.159	**-.538****
DO				**-.577****	-0.124	0.035	0.218	0.216	-0.230	**-.352***	**.336***	-0.244
COD					0.278	0.202	0.208	0.174	**.313***	**.338***	-0.173	**.441****
SS						**.321***	**.398****	**.371****	**.532****	**.706****	-0.063	**.499****
Chl-*a*							**.558****	**.435****	-0.004	0.194	**.392****	0.251
DIN								**.879****	**.404****	**.396****	**.446****	**.727****
TN									0.252	**.321***	**.526****	**.561****
DIP										**.698****	**-.484****	**.693****
TP											-0.128	**.653****
N/P												0.131

Table S8. Results of the DISTLM analysis used to explore the relationship between phytoplankton and environmental variables. *P*-values were obtained using 999 permutations of residuals under the best model (forward selection based on AIC test). Bold values indicate significant value; V: Variables; AIC: Akaike information criterion; P-F: Pseudo-F; Cum: Cumulation; B.S: Best Solution.

V	**AIC**	**P-F**	***P***	**Cum.**
Cr^{6+}	377.0	11.32	**0.001**	0.20
Pb	372.1	6.91	**0.001**	0.30
Temperature	367.3	6.73	**0.001**	0.40
Discharge	365.0	4.00	**0.001**	0.45
TP	363.31	3.39	**0.001**	0.49
COD	361.81	3.11	**0.001**	0.53
Cd	360.9	2.50	**0.003**	0.55
Zn	360.1	2.37	**0.003**	0.58
pH	359.0	2.49	**0.001**	0.60
SiO_2	358.6	1.96	**0.007**	0.62
B.S	AIC	R^2	V	
	359	0.62	10	

Table S9. IndVal analysis listing the indicator taxa in the specific environmental groups for phytoplankton. Bold values indicate significant value. The environmental groups were defined by the result of dbRDA routine (Fig. 5).

Group	Indicator Species	IndVal	*P*
1	Cryptomonads (<20mm)	0.99	**0.001**
	Skeletonema marinoi	0.96	**0.001**
2	*Pseudo-nitzschia* sp.	0.78	**0.001**
	Bacteriastrum sp.	0.75	**0.001**
3	*Synedra* sp.	0.56	**0.01**

Supplementary Figures

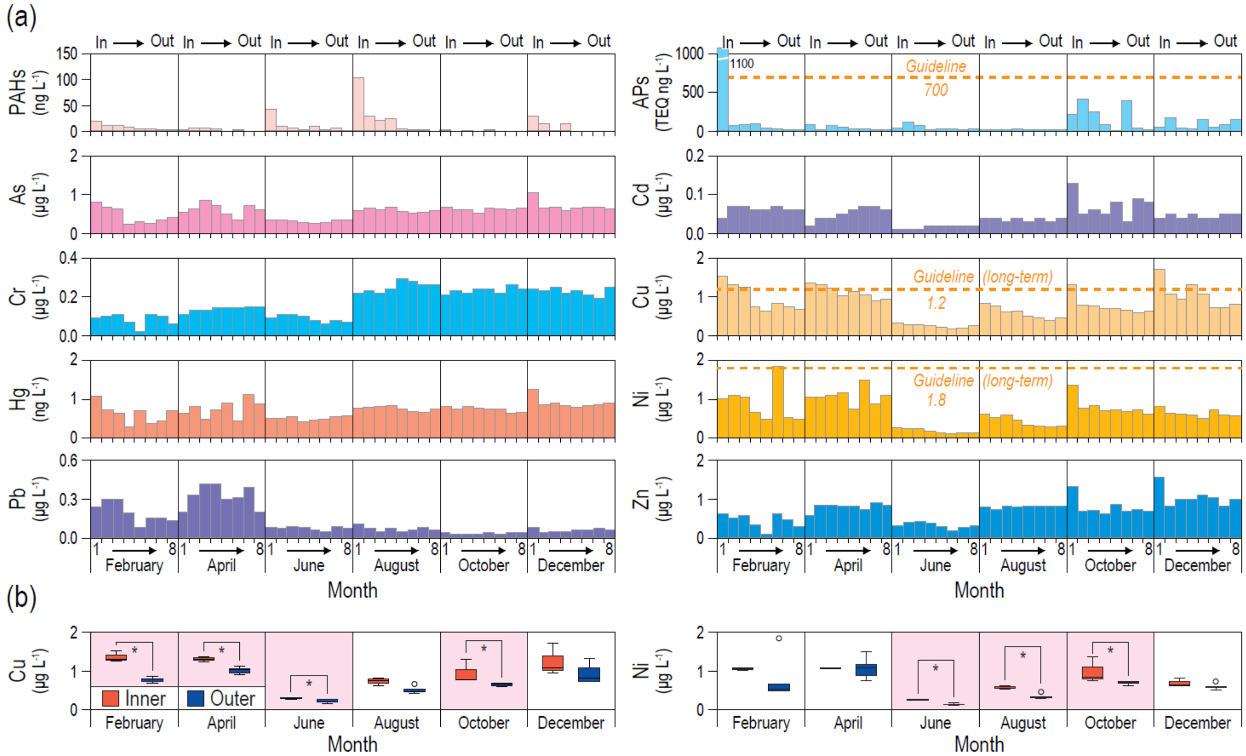

Fig. S1. Spatiotemporal distribution of PTSs in the seawater of Geum River Estuary. The long-term guideline is the standard applied as the mean value of at least four seasons. The red background on the graph indicates a case where the concentration between the inner and outer parts of the estuary was significantly different.

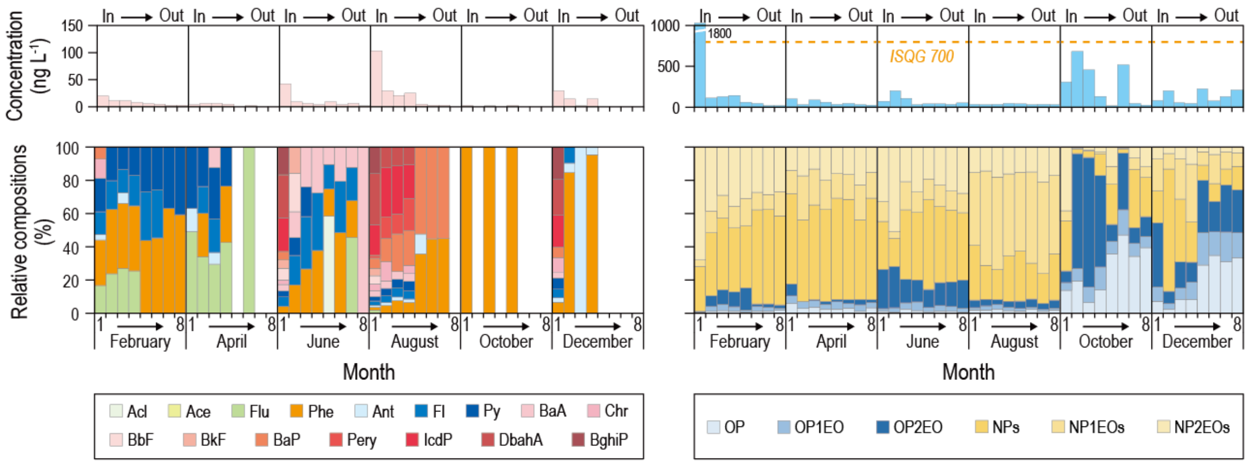

Fig. S2. Relative compositions of PAHs and APs in seawater of Geum River Estuary, Korea.

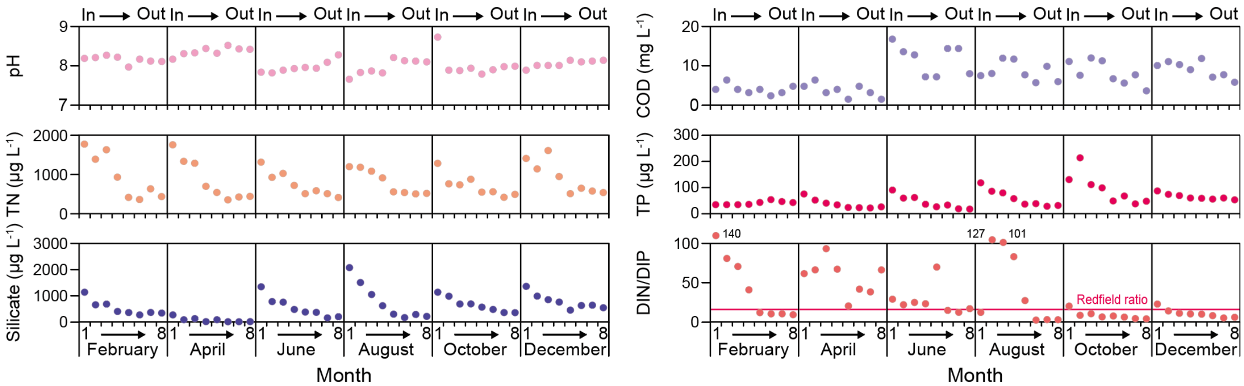

Fig. S3. Water quality parameters (pH, COD, TN, TP, silicate, and DIN/DIP) in seawater from the Geum River Estuary.

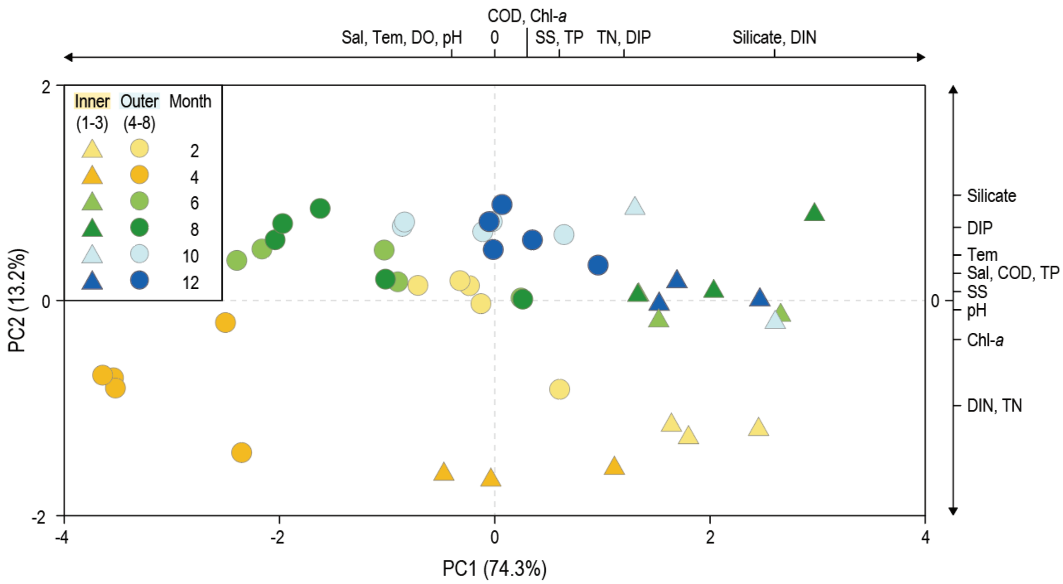

Fig. S4. Result of principal component analysis on water quality parameters of one year in seawater from the Geum River Estuary.

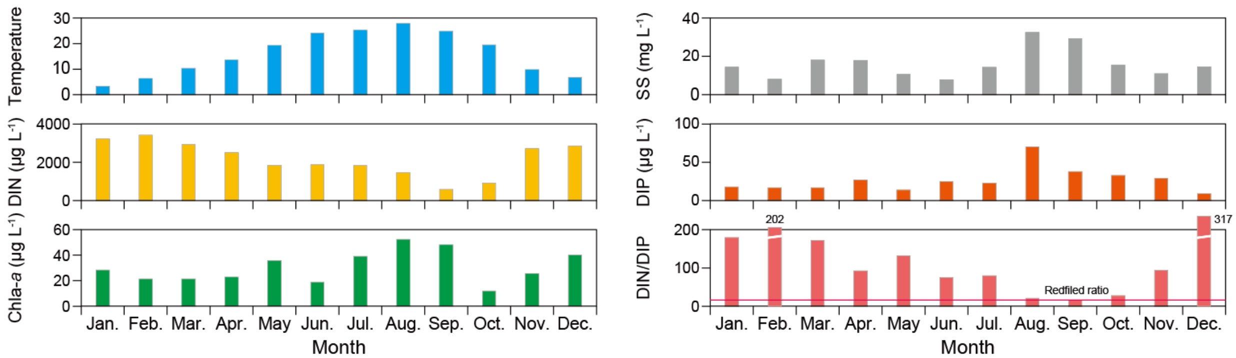

Fig. S5. Water quality parameters in freshwater from the Geum River.

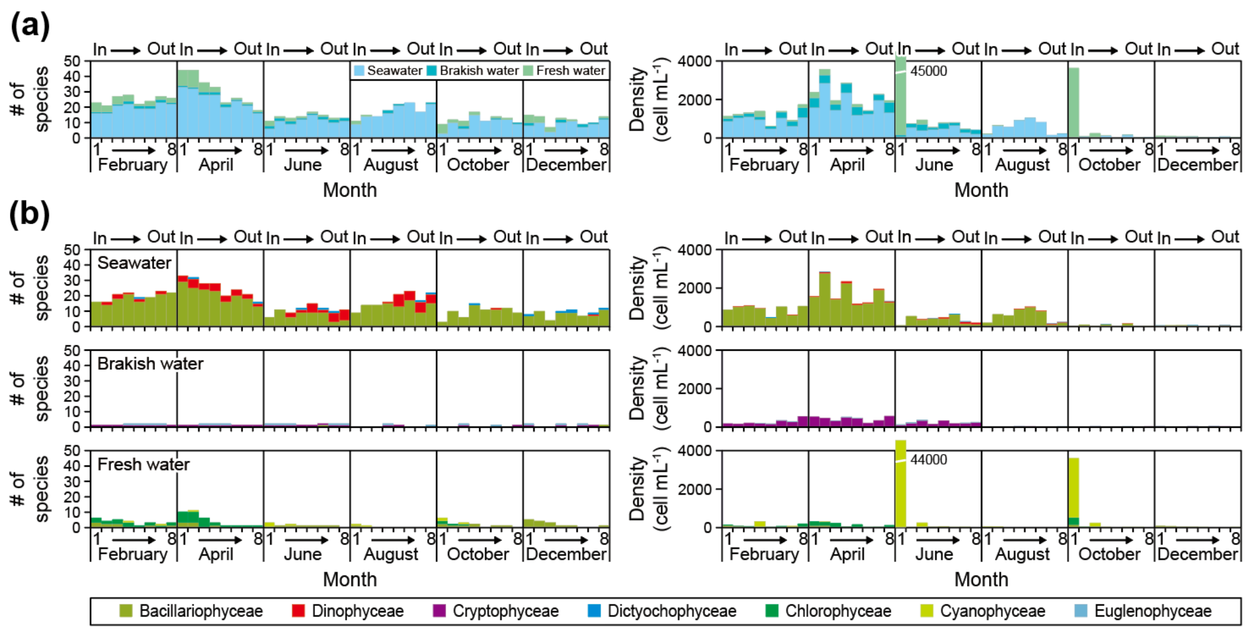

Fig. S6. The number of species and density of **(a)** total community and **(b)** species by taxa from seawater, brackish water, and freshwater.

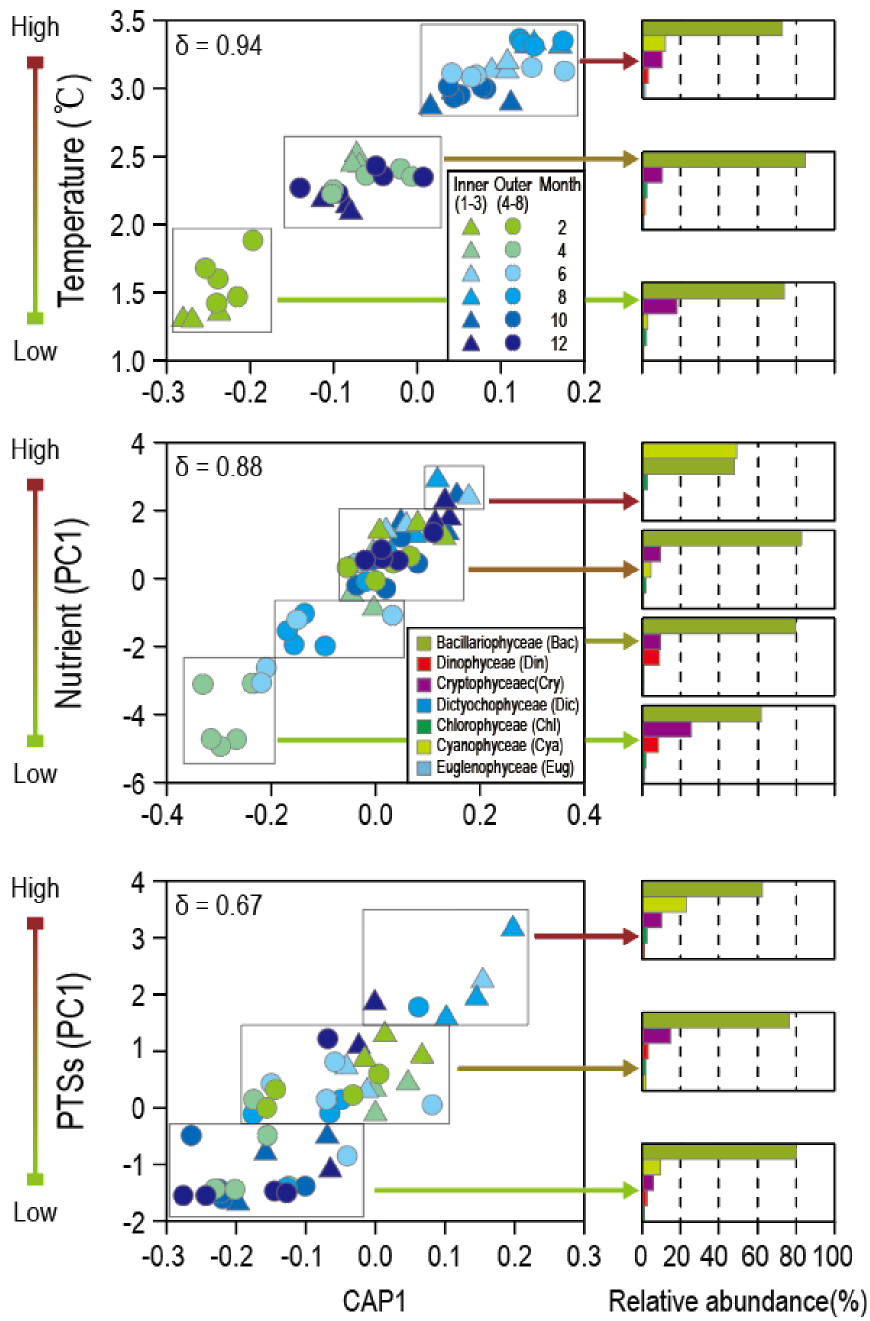

Fig. S7. Variation in plankton assemblages along the canonical gradient in relation to Temperature, nutrient, and PTSs. Bar graph on the right side indicates the proportional abundance for five taxa at the Phylum level.