Korean J. Limnol. 44 (4): 337~346 (2011)

Estimation of Nutrient Loading and Trophic States in a Coastal Estuary

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We investigated nutrient loading and trophic states in a coastal estuarine system in the Asan estuary by assessing phytoplankton biomass and using the trophic index (TRIX). The monthly and yearly nutrient loading (TN, TP) from freshwater discharge from the Asan and Sapgyo reservoirs into the estuary were estimated and analyzed with related factors. Monitoring data (physio-chemical and biological variables) collected at five estuary stations were used to assess trophic states. Descriptive statistics of total phytoplankton cells, chl *a* concentrations and primary productivity were also used to assess seasonal trophic status. N loading from freshwater ranged $1.0 \sim 1.3 \times 10^4$ ton yearly. The yearly P loading ranged between 350 and 400 ton during 2004~2006, increasing to 570 ton in 2007. Regression results suggest that DIN and DSi were correlated with freshwater discharge at the upper region. Based on phytoplankton biomass and total cell abundance, the trophic state of the estuary was found to be eutrophic during spring due to phytoplankton bloom. Primary productivity level was remarkably high, especially in summer coinciding with high nutrient loading. Pheopigments increased during warm seasons, i.e. summer and fall. Trophic index results indicate that the trophic state varied between mesotrophic and eutrophic in the estuary water body, especially in the upper region. The results suggest that phytoplankton production was regulated by nutrient loading from freshwater whereas biomass was affected by other properties than nutrient loading in the Asan Estuary ecosystem.

Key words : coastal estuary, nutrient loading, trophic states, phytoplankton

INTRODUCTION

Coastal aquatic environments are disturbed by nutrient enrichment from inland waters (Bishop *et al.*, 2006; Paerl, 2006; Martins *et al.*, 2007): (1) the accumulations of biomass and oxygen depletion in bottom water, (2) the outbreak of toxic phytoplankton (dinoflagellate), (3) changes in the plankton community and size structure, (4) changes in macroalgae productivity, (5) changes in the benthic organism structure.

Total nitrogen and total phosphorus loadings are influenced by their geographic locations relevant to discharge, watershed land use and water runoff (Caccia and Boyer, 2007; Hyfield *et al.* 2008). The control of nutrient loading from point and non-point sources have been implemented in North America and Europe coasts (Whitall *et al.*, 2004; Kronvang *et al.*, 2005). A study on estuarine trophic state may be important since nutrient loading and coastal estuarine water quality change by freshwater input in Korea. Understanding the linkage between nutrient loading and coastal water quality is required to manage the water quality and ecosystem. The trophic index (TRIX)

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was introduced by Vollenweider *et al.* (1998) to evaluate the trophic condition of seawater. The TRIX index has been applied to characterize coastal waters in Europe (Giovanardi and Vollenweider, 2004; Pettine *et al.*, 2007; Lušić *et al.*, 2008). The index was used in this study since it is a composite index including four state variables (Chl-*a*, DIN, TP and oxygen) that represent stressor, biological response and environmental disturbance in water column.

The study site, Asan estuary is under influence of monsoonal force and also experiencing environmental disturbance from the construction of embankments (reservoirs) in its coastal regions. The trophic state of the estuary based on phytoplankton community and trophic index, however, has not been documented especially related to nutrient loadings. The aim of this study is to evaluate the trophic state of Asan Estuary receiving freshwater inputs from lakes by assessing phytoplankton biomass, phytoplankton cells, primary productivity and trophic index.

MATERIALS AND METHODS

1. Study site and sample collection

Sapgyo, Asan, Daeho, Seokmoon and Namyang embankments were constructed in the upper region of the Asan estuary since 1970s (Fig. 1). The large scaled national industrial complex was constructed along the coastal of the Asan estuary.

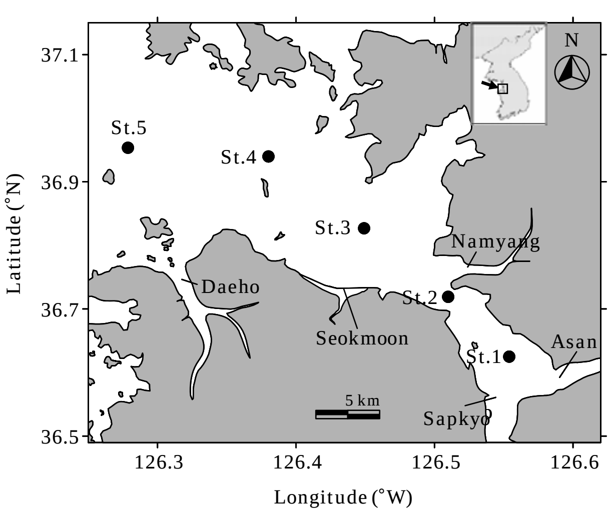

Fig. 1. Field study stations and embankments in the Asan Estuary upper region (South Korea).

The freshwater from embankments interacts with seawater when the gates of embankments are opened. Five stations were selected along the axis of the Asan Estuary from February 2004 to November 2007. Water samples were collected monthly or seasonally 0.5 m below surface by using Niskin water sampler.

2. Environmental variables

Salinity and DO were measured by YSI® Model 6600 multiparameter probe. Ambient nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, DSi) were analyzed by using Bran Luebbe autoanalyzer (Parsons *et al.*, 1984). Monthly data TN, TP concentrations from freshwater in Asan and Sapgyo lakes were obtained from the water quality monitoring program (Korea Ministry of Environment). Precipitation data were collected by Korea Meteorological Administration. Data for freshwater discharge from the embankment were collected by Korean Agricultural Administration. Nutrient loadings from freshwater were estimated by multiply of monthly nutrient concentrations at the stations near dikes of Asan and Sapgyo lakes with monthly freshwater discharge of each lake through the gates of dikes.

3. Biological variables

For determination of chl *a*, 200 mL of sampled water was filtered through Whatman® 25 mm GF/F glass microfibre filters (0.7 μm) under minimal vacuum (< 100 mm Hg). The filters were placed in dark test tubes pre-filled with 8 mL extraction solution (90% acetone and 10% distilled water). After storage for 12 h at 4°C, chl *a* was measured on a Turner Designs® 10-AU Fluorometer. The determinations of pheopigments were applied by acidification method (adding two drops of HCl (2N)). Pheopigments can be detected in egested fecal materials of grazers or during senescence as a result of poor growth environments or prolonged exposure to the dark due to converted chl *a*. The ratio of chl *a* and pheopigments (Chl : Pheo ratio) (the lower the ratio, the higher the grazing rates or senescence) is an indirect measure of gazing activity or poor condition for phytoplankton growth (Welschmeyer and Lorenzen, 1985).

Phytoplankton samples for each station were dispensed into 1 L in the bottle with 5 mL Lugols solution (I_2 final concentration: 250 mg L^{-1}). After sampling, the samples were kept in the laboratory for at least 48 h to allow phytoplankton to settle down. After the storage, 800 mL surface water of the samples were slowly decanted. The remaining 200 mL sample was mixed and 1 mL of it was examined under Sedgwick-Rafter counting chamber (50 × 20 × 1 mm). Phytoplankton cells were counted in 200 chambers using Axioskop® 2 MAT (ZEISS).

Primary productivity was conducted by Kwangju University using ^{14}C-uptake technique. Surface water samples at noontime were dispensed into 250 mL polycarbonate bottles. Samples were then inoculated with 0.5 mL ^{14}C-$NaHCO_3$ solution and incubated for 3 h. After the *in situ* incubation, unincorporated ^{14}C was filtered through GF/F filters. Each filter was fumed overnight in 0.25 mL 0.5N HCl to remove residual ^{14}C activity. ^{14}C radioactivity was determined using liquid scintillation counter after inserting 10 mL of scintillation cocktail. Primary productivity was integrated by radioactivity, total C and incubation time.

4. Trophic index and statistic analysis

The trophic status classification of coastal water in Asan Estuary was evaluated using trophic status index (TRIX) developed by Vollenweider *et al.* (1998).

$$\mathrm{TRIX} = (\mathrm{Log}_{10}[\mathrm{Chl}\text{-}a * \mathrm{a\%DO} * \mathrm{N} * \mathrm{P}] + 1.5)/1.2 \quad (1)$$

The trophic index (TRIX, Eq. 1) is a linear combination of the logarithms of four state variables (Chl *a*, N, P and the absolute percentage deviation of oxygen saturation from 100% (a%DO)). We used DIN for N and DIP for P in this study. The index is scaled from 0 to 10 for oligotrophy-hypertrophy, wide range of trophic conditions and divided into four classes: $2 < \mathrm{TRIX} < 4$ (high), $4 < \mathrm{TRIX} < 5$ (good), $5 < \mathrm{TRIX} < 6$ (moderate), $6 < \mathrm{TRIX} < 8$ (poor). Excessive 5 TRIX units of water body are considered as not good state.

Simple linear regression analysis was applied

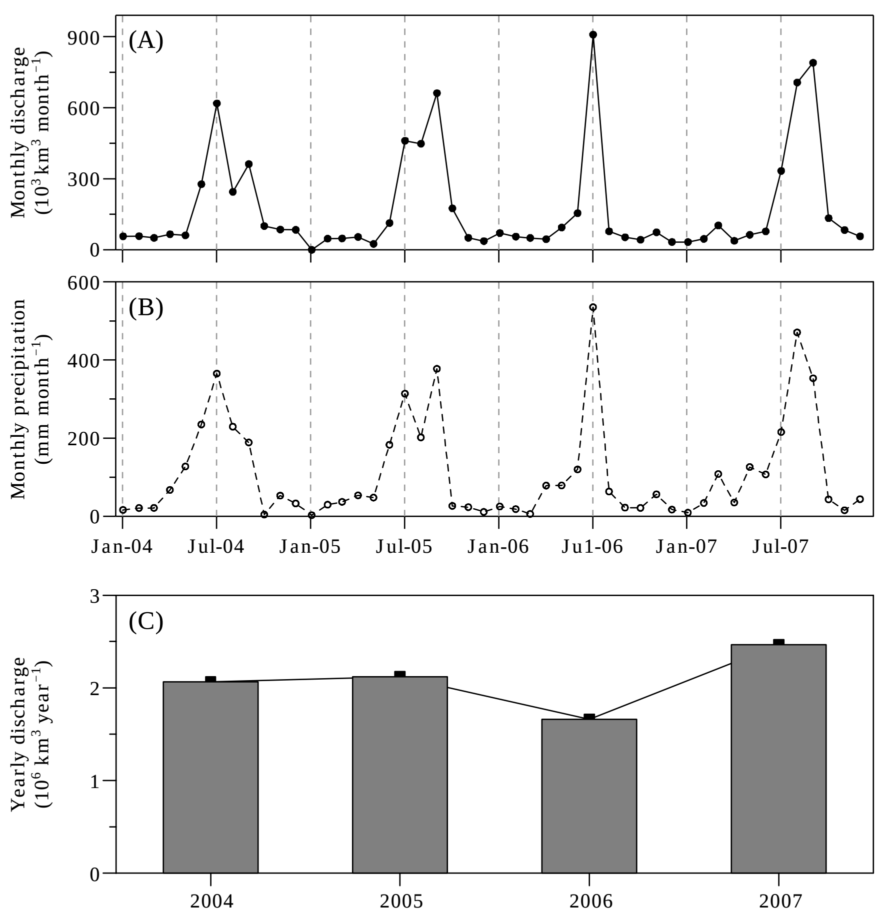

Fig. 2. Monthly (A) and yearly (C) freshwater discharge (Asan and Sapgyo dikes) into the Asan Estuary between 2004 and 2007, monthly precipitation (B) in the upper area.

to investigate correlation between salinity versus concentrations of $NO_2^-+NO_3^-$, NH_4^+, DIN, PO_4^{3-}, DSi. Descriptive statistics were applied to explore the seasonal and spatial variations of chl *a* concentrations, total phytoplankton cells, primary productivity, Chl : Pheo ratio and TRIX index using box and whisker plots. ANOVA analysis was used for statistical significance of spatial variations during each season.

RESULTS

1. Nutrient loading

Water discharge and precipitation peaked from July to September (Fig. 2A, 2B). Monthly TN, TP loadings were shown in Fig. 3A, 3B. The loadings were generally high during the wet season (July

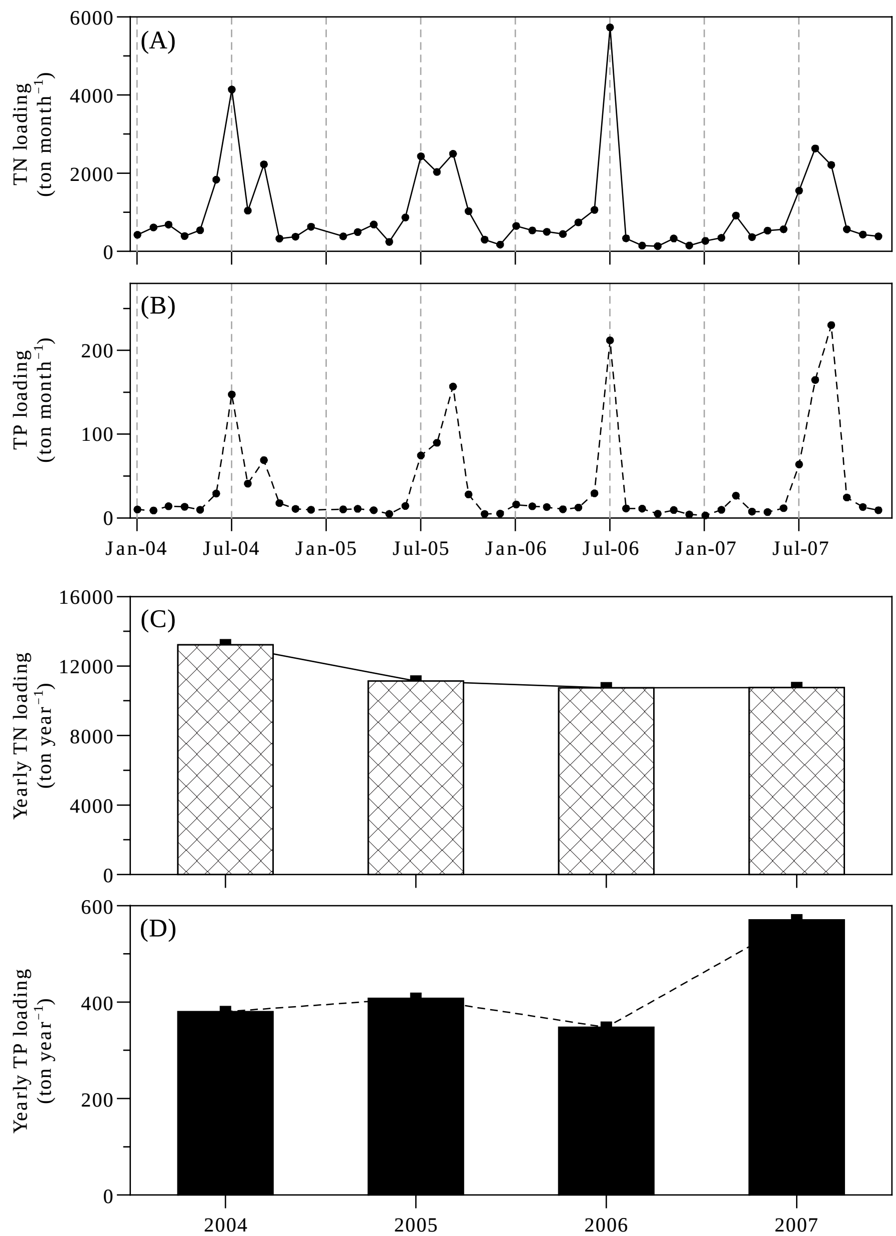

Fig. 3. Monthly and yearly TN, TP loading from freshwater discharge (Asan and Sapgyo dikes) into the Asan Estuary between 2004 and 2007.

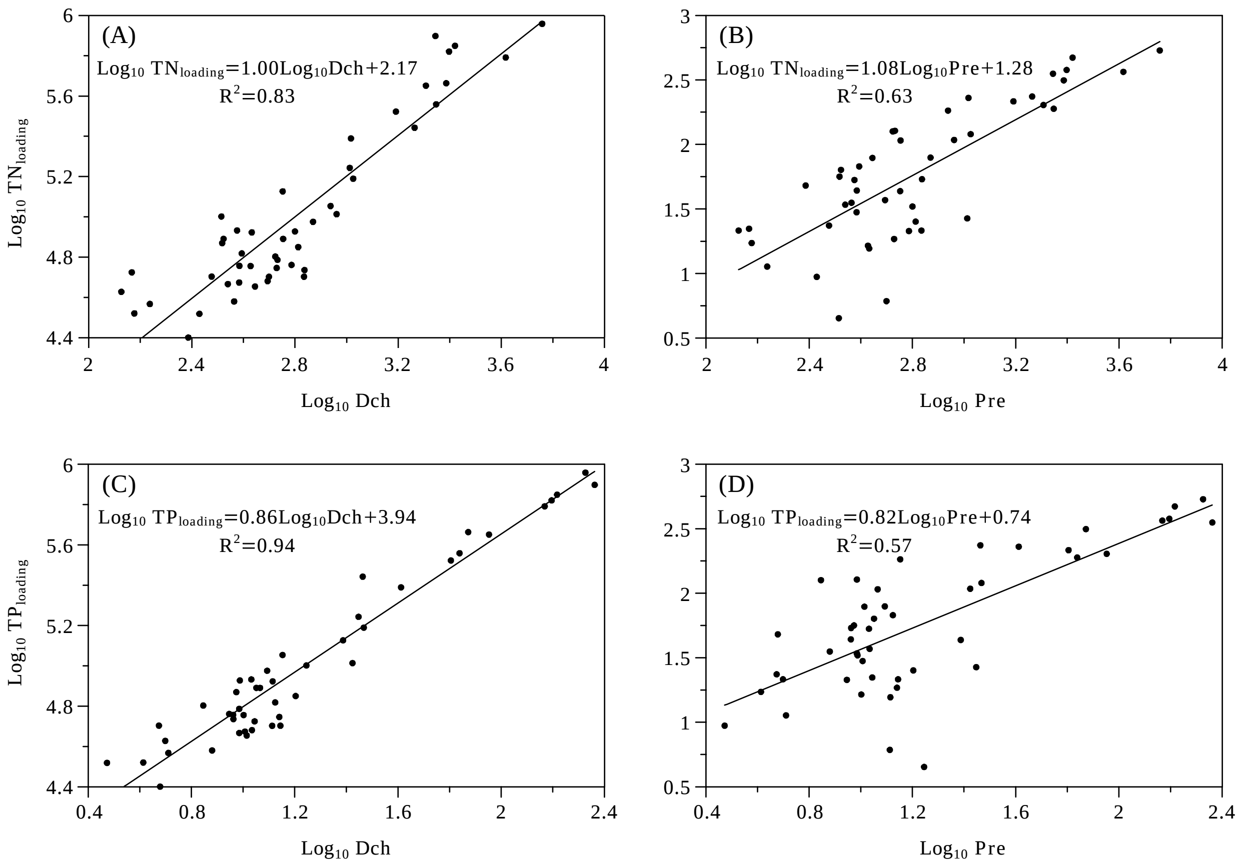

Fig. 4. Monthly relationship of log-transformed TN, TP loading (ton) versus log-transformed discharge (Dch, $\times 1{,}000\ m^3$) and precipitation (Pre, mL).

~September) coinciding with increase of discharge as well as high precipitation (Fig. 2A, 2B). Similar pattern was observed between yearly discharge and TP loading (Fig. 2C, 3D). Yearly TP loading dramatically increased to ~570 (ton year^{-1}) in 2007 from 350~400 (ton year^{-1}) in 2004~2006. However, TN loading in 2007 decreased despite that the discharge had increased (Fig. 2C, 3C). The relationships of TN, TP loading with discharge and precipitation were shown in Fig. 4. The effect of discharge on TN loading (r^2=0.83) was more significant than that of precipitation (r^2=0.63) in the watershed area (Fig. 4A, 4B). The difference between the effect of discharge and precipitation on TP loading was presented in Fig. 4C and Fig. 4D. Table 1 shows correlation between salinity (hydrodynamic variable) versus ambient nutrients. Nitrite+nitrate was negatively and significantly correlated with salinity at the upper region (r^2= 0.37~0.44, $P<0.01$). Ammonium was also correlated in this region (r^2=0.28~0.34, $P<0.01$). DIN (nitrite, nitrate and ammonium) also had a significant correlation with salinity. Orthophosphate was not correlated with salinity. However, dissolved silicate was evidently affected by dilution at upper region (r^2=0.26~0.54, $P<0.01$). DIN and DSi concentrations in Stations 1, 2, 3 were negatively correlated with salinity (Fig. 5).

2. Trophic characteristics

Phytoplankton cells were higher during spring (February~May) and decreased during summer (June~August) and fall (September~November) (Fig. 6). Based on total cells and trophic criteria for phytoplankton cells (Fig. 6), the trophic state of the Asan Estuary was mesotrophic or eutrophic. Median values reached over eutrophic/mesotrophic level (1.9×10^5 cells L^{-1}). Maxima of total phytoplankton cells at the upper region were 7.0~7.5 $\times10^5$ cells L^{-1} in spring. Chl *a* concentrations were high during spring (up to 44 μg L^{-1}) (Fig. 7).

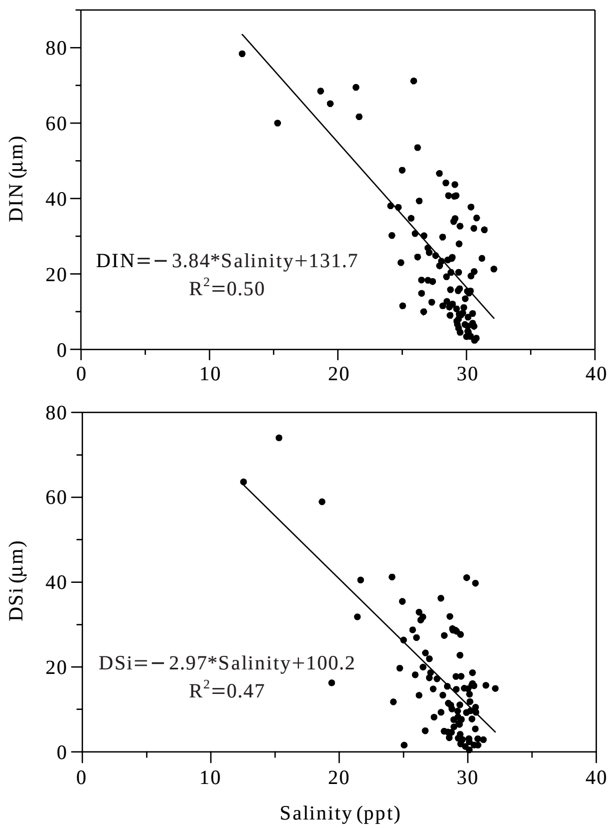

Fig. 5. Relationship between ambient DIN, DSi concentrations versus hydrodynamic variable (surface salinity) at the upper region (station 1, 2, 3), monitored between 2004 and 2007 in the Asan Estuary.

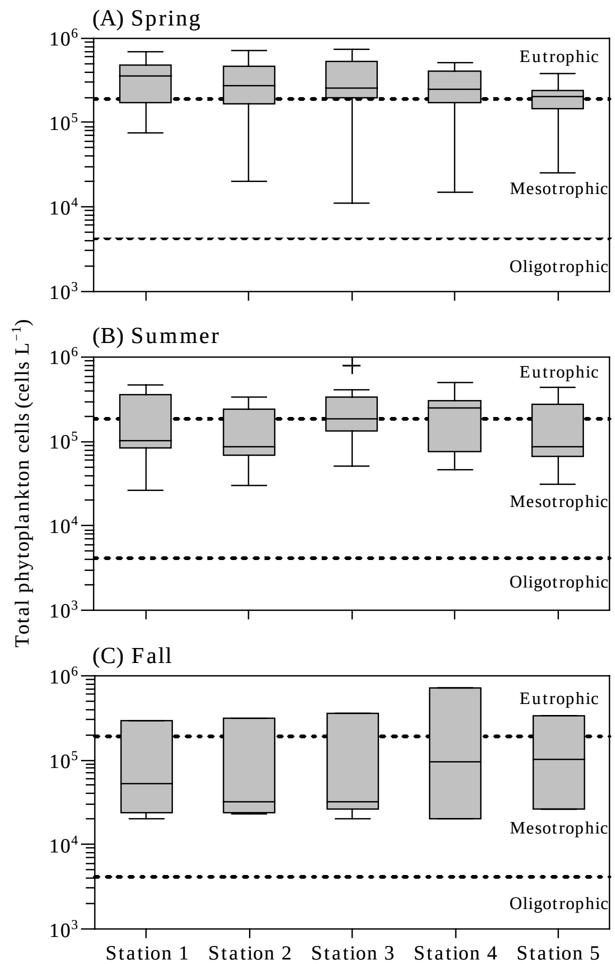

Fig. 6. Box and whisker plots of seasonal total phytoplankton cells (2004～2007) in surface water from five stations in the Asan Estuary (upper and low dashed line are phytoplankton cell numbers for Eutrophic/Mesotrophic (188,334 cells L^{-1}) and Mesotrophic/Oligotrophic (4,160 cells L^{-1}) levels in ecological indices (Kitsiou and Karydis, 2000)).

The concentrations reached to eutrophic level ($>7\ \mu g\ L^{-1}$) at the upper region. In summer, chl *a* concentrations decreased to mesotrophic level (>2 and $<7\ \mu g\ L^{-1}$). In fall, chl *a* concentrations futher decreased to oligotrophic level. Surface primary productivities were high at Station 1 during spring whereas the values increased during summer and fall, especially at the lower region (Fig. 8). Fig. 9 showed that the Chl : Pheo ratios were lower during summer and fall than spring at all stations.

The surface TRIX values in the Asan Estuary were presented in Fig. 10. The TRIX values were general higher than 6 (reaching up to 7.2 in spring) at the upper region. TRIX values were higher in spring than in summer and fall. TRIX values were low at the lower region. At station 5, TRIX values were generally within the range of 5～6 (Fig. 10).

The results from ANOVA analyses shows that chl *a* concentrations were significantly different between stations during spring and all seasons and TRIX values were significantly different during spring, summer, fall and all seasons (Table 2).

DISCUSSION

Nutrient loading from freshwater is considered as one of the main sources of eutrophication in estuary coasts (Fisher *et al.*, 2006; Clarke *et al.*, 2006). Increase in nutrient loading to coastal waters is related to rapid increase in the total and urban population (Billen *et al.*, 2007) and to

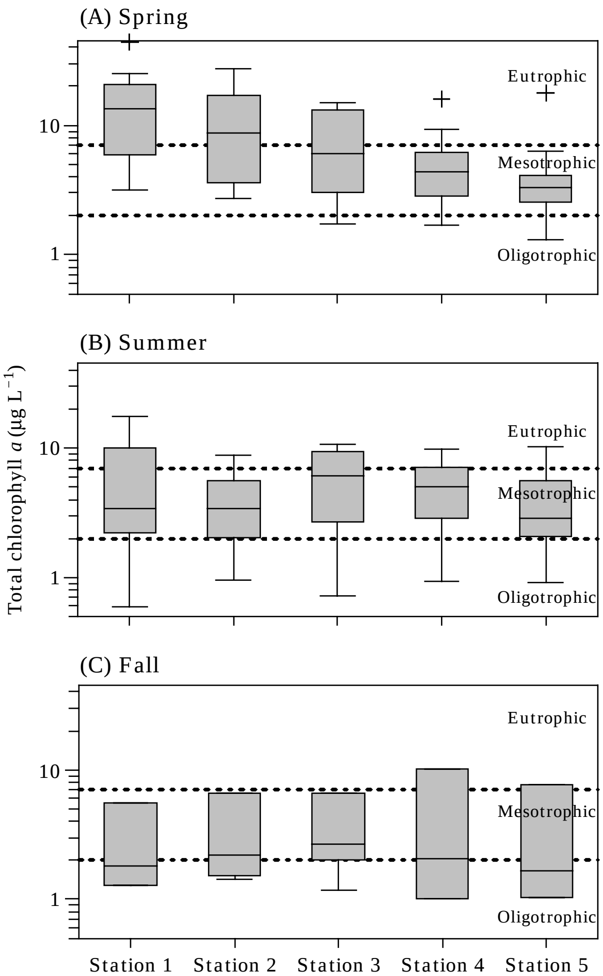

Fig. 7. Box and whisker plots of seasonal total phytoplankton biomass (chlorophyll *a*) (2004～2007) in surface water from five stations in the Asan Estuary (upper and low dashed line are chlorophyll *a* concentrations for Eutrophic/Mesotrophic (7 μg L^{-1}) and Mesotrophic/Oligotrophic (2 μg L^{-1}) levels (Molvaer *et al.*, 1997)).

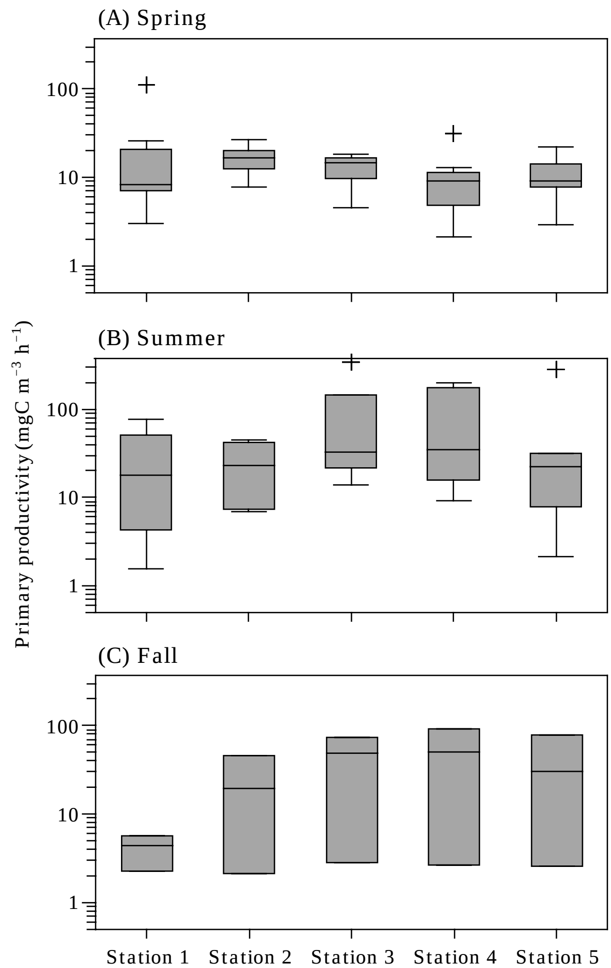

Fig. 8. Box and whisker plots of seasonal primary productivity (2004～2007 measurements) in surface water from five Asan Estuary stations.

the increase of atmospheric nitrogen deposition rate (Scudlark *et al.*, 2005), frequency and intensity of precipitation (Yin and Harrison, 2008). In this study, TN and TP loadings were more closely related to the discharge from Asan and Sapgyo lakes. The relationship between ambient nutrients and salinity in Fig. 5 and Table 1 suggests that freshwater discharge directly affected nutrient enrichments in the Asan Estuary. The discharge was related with precipitation in watershed area. Wen *et al.* (2008) also documented that nitrogen and phosphorus come mainly from urban discharge but silicate is produced from erosion of rocks in watersheds.

In the Asan Estuary, nutrient loading was high during June-September whereas total phytoplankton cells and chl *a* concentrations were high during spring from February to May. The chl *a* concentrations spatially, varied (Table 2) suggesting that upper regions have a better condition for phytoplankton growth than lower regions. No direct effect of nutrient loading on phytoplankton biomass has been detected in the Asan Estuary water. This pattern is different from other estuaries where phytoplankton responded to nitrogen and phosphorus enrichment (e.g. Smith, 2006). However, the loading may be an important mechanism to increase primary productivity. In this study, primary productivity was significantly high (up to 344 mg C m^{-3} h^{-1}) in summer (June～August),

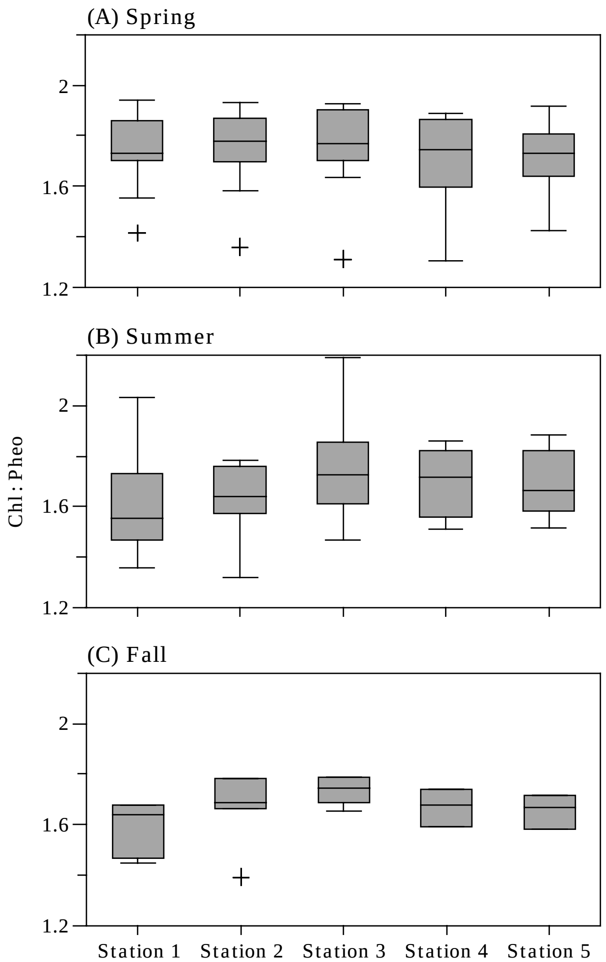

Fig. 9. Box and whisker plots of seasonal Chl *a*: Pheopigments ratios (2004～2007 measurements) in surface water from five Asan Estuary stations.

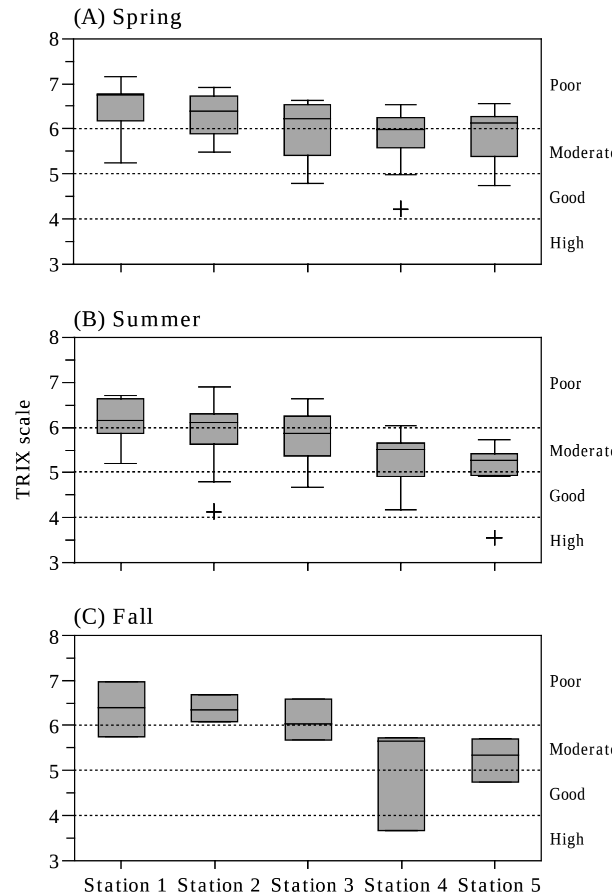

Fig. 10. Box and whisker plots of seasonal TRIX (2004～2007) in surface water from five Asan Estuary stations.

especially at the lower region. The results suggest that nutrient loading from freshwater may cause the increase of phytoplankton productivity at the lower region where turbidity and flushing rates are lower than the upper region turbidity and flushing rates are high. The low Chl : Pheo ratios during summer and fall suggest that rapid changes of water properties or enhanced grazing activities limit the increase of phytoplankton biomass during the warmer season. Especially high flushing rate may induce the decrease of phytoplankton biomass at the upper region during the wet season.

Assessment of trophic states to define eutrophication of coastal estuarine systems is important for coastal environmental management and protection. Assessment of trophic conditions of coastal waters is based on: (1) the biochemical composition of sediment organic matter, (2) chl *a* and primary productivity scales, (3) ecological indices, (4) implement of estuarine trophic status model (ASSETS), (5) log-transformation of chl *a*, oxygen

Table 1. Results (r^2) of linear regression analysis of surface salinity (ppt) versus ambient nutrients, including NH_4^+ (μM), NO_2^- + NO_3^- (μM), DIN (μM), PO_4^{3-} (μM), and dissolved Si (μM) in the Asan Estuary, monitored between 2004 and 2007 monitoring period. "−" denotes a negative relationship.

Station	NO_2^-+NO_3^-	NH_4^+	DIN	PO_4^{3-}	DSi
Station 1	−0.44[b]	−0.34[b]	−0.51[b]	−	−0.54[b]
Station 2	−0.37[b]	−0.28[b]	−0.42[b]	−	−0.49[b]
Station 3	−0.41[b]	−0.28[b]	−0.44[b]	−	−0.26[b]
Station 4	−0.18[a]	−0.21[a]	−0.21[a]	−	−
Station 5	−	−	−	0.10	−

[a]$P < 0.05$; [b]$P < 0.01$.

Table 2. Results (P values) of ANOVA analysis of data sourced from stations measured seasonally throughout 2004～2007.

Parameters	Season			
	Spring	Summer	Fall	All
Cell abundance	0.261	0.565	0.930	0.476
Chl *a*	**0.001**	0.621	0.961	**0.010**
Primary production	0.524	0.432	0.288	0.427
TRIX	**0.008**	**0.002**	**0.030**	$<$**0.001**
Chl : Pheo	0.885	0.156	0.263	0.253

saturation, nitrogen and phosphorus integration (TRIX index) (Vollenweider *et al.*, 1998; Kitsiou and Karydis, 2000; Ignatiades, 2005). In this study, we assessed trophic condition along axis of the Asan Estuary using chl *a*, total phytoplankton cell, primary productivity and TRIX index. In spring, the chl *a* level was eutrophic, especially at the upper region compared with eutrophic/mesotrophic level (7 $\mu g\ L^{-1}$) in seawater (Molvaer *et al.*, 1997). Total phytoplankton cell abundance was also high in spring. Ignatiades (2005) reported that primary productivity for eutrophic range of seawater was 3.51～5.55 mg C $m^{-3}\ h^{-1}$. In the Asan Estuary with freshwater inputs, the range of primary productivity was 2.1～344 mg C $m^{-3}\ h^{-1}$ in the surface water indicating that eutrophication was severe in the Asan Estuary. According to Vollenweider *et al.* (1998), Giovanardi and Vollenweider (2004), TRIX values range from 0 to 10 for the trophic scale and a system with TRIX value higher than 6 is highly productive. Asan Estuary is evidently productive at the upper region since TRIX values were generally higher than 6 (Fig. 10). The TRIX values probably need to be compared with TBRIX including water transparency to explore the effect of freshwater input further since turbidity is expected to increase in the estuary during the monsoon.

In summary, phytoplankton biomass and cell abundance were high and the water body was eutrophic in spring although small volumes of freshwater was introduced at the upper region. Nutrient loadings were significantly increased during the wet season by freshwater discharge from lakes suggesting that freshwater input enriched ambient nutrients in the Asan Estuary. In the lower region, primary productivity was high when nutrient inputs increased during the wet season (summer and fall) whereas chlorophyll *a* concentrations were not increased probably because the change of hydrologic conditions and grazing of consumers might cause the decrease of phytoplankton biomass in Asan Estuary.

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(Manuscript received 23 November 2011,
Revised 14 December 2011,
Revision accepted 21 December 2011)