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Evaluation of phytoplankton community composition in the eutrophic Masan Bay by HPLC pigment analysis

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Abstract

To assess the spatiotemporal changes in phytoplankton community composition in relation to the environment of Masan Bay, a semi-enclosed bay on the southern coast of Korea, photosynthetic pigments and environmental variables were analyzed in seawater, every month between March and November 2010. The level of dissolved inorganic nutrients was highest between July and September when the freshwater influx was at its peak, whereas chlorophyll *a* level was highest in April and August. Phosphate concentration was low in April (average: 0.22 ± 0.17 µM), indicating the role of phosphate as a growth-limiting factor for phytoplankton. The results of pigment analysis indicate that dinoflagellate blooms occurred under favorable conditions, where competition with diatoms occurred. Fucoxanthin- and chlorophyll *b*-containing phytoplankton dominated the surface layer of Masan Bay from July to September. The composition of phytoplankton community in Masan Bay changed dramatically each month according to variations in the amount and composition of nutrients introduced through surface runoff.

Key words

Phytoplankton, Nutrient, Masan Bay, HPLC

Introduction

Masan Bay is a semi-enclosed sea surrounded by Masan, Changwon, and Jinhae, which are large cities, populated by over 1 million inhabitants. Since 1970s, the development of large-scale free-trade zones and industrial complexes has led to a continuous increase in aquatic pollution. The continuous input of anthropogenic pollutants has led not only to the contamination of marine environment, but also to frequent biochemical changes such as red tides and hypoxic water masses (Yim *et al*., 2005).

Pollutants released into Masan Bay mainly originate from nearby streams and ocean outfalls or are released from bottom sediments into water column. Cho and Chae (1998) reported that pollution arising from the cities of Masan and Changwon accounts for 80–90% of the total marine pollution load in Jinhae and Masan Bay. The continuous discharge of pollutants into Masan Bay had led to an oxygen-deficient bottom water mass in spring and summer, with hypoxic water mass forming in mid-May that persists until October (Kim *et al*., 2012). The formation of hypoxic water masses has been reported to directly affect the respiration and physiology of necton and benthos resident in Masan Bay (Kim *et al*., 2012). Red tides in Masan Bay can be caused by single species (diatoms or dinoflagellates) or multiple species. Until 1980s, the blooms occurred mostly in summer (July and August), but recently, the blooms have been occurring every year, between March and November (Kim and Shin, 1997; Cho *et al*., 1998; Kwak *et al*., 2001). The subsequent death of plankton and the accompanying decomposition process exacerbates the formation of hypoxic water masses after red tide (NFRDI, 2009).

Most of the research on the phytoplankton species composition in Masan Bay has involved microscopic examinations of spatial and temporal distribution of major red-tide species. Choi *et al*. (2000) reported the development and distribution of *Prorocentrum* in the Masan-Jinhae Bay and Kwak *et al*. (2001) studied the frequency and magnitude of red tides. Although, several studies have been conducted regarding phytoplankton species composition and the development of red tides, studies on the annual changes of phytoplankton community

composition based on changes of marine environment are required.

Although studies on phytoplankton community composition through photosynthetic pigment analysis using high-performance liquid chromatography (HPLC) has limitations (i.e., it can only classify the community at the class level), it has been widely used as an effective tool for examining not only the spatiotemporal distribution of phytoplankton community composition, but also changes in phytoplankton community composition during dramatic changes in marine environment (Paerl *et al*., 2003; Hassen *et al*., 2008; Kim *et al*., 2010).

The aim of the present study was to elucidate spatio temporal changes in phytoplankton community composition through photosynthetic pigment analysis in relation to changes in marine environmental conditions in Masan Bay.

Materials and Methods

Field survey and sample analysis : To examine changes in phytoplankton community composition in Masan Bay attributable to environmental perturbations, 11 stations we selected within Masan Bay and field surveys were conducted between March and November 2010 (Fig. 1). During the summer months of July and August, which is the peak season for eutrophication, surveys were conducted twice a month. Water temperature, salinity, dissolved oxygen (DO), and pH values were measured on-site using a multiparameter water quality sonde (YSI 6920, YSI Inc., USA). Seawater samples were taken the surface and bottom layers of the bay to measure nutrients, chemical oxygen demand (COD), suspended particulate matter (SPM), particulate organic matter (POM), and photosynthetic pigments. Samples for nutrient analysis were filtered through pre-ashed GF/F filters at 450°C for 4 hr. COD was calculated by alkalizing the sample and adding

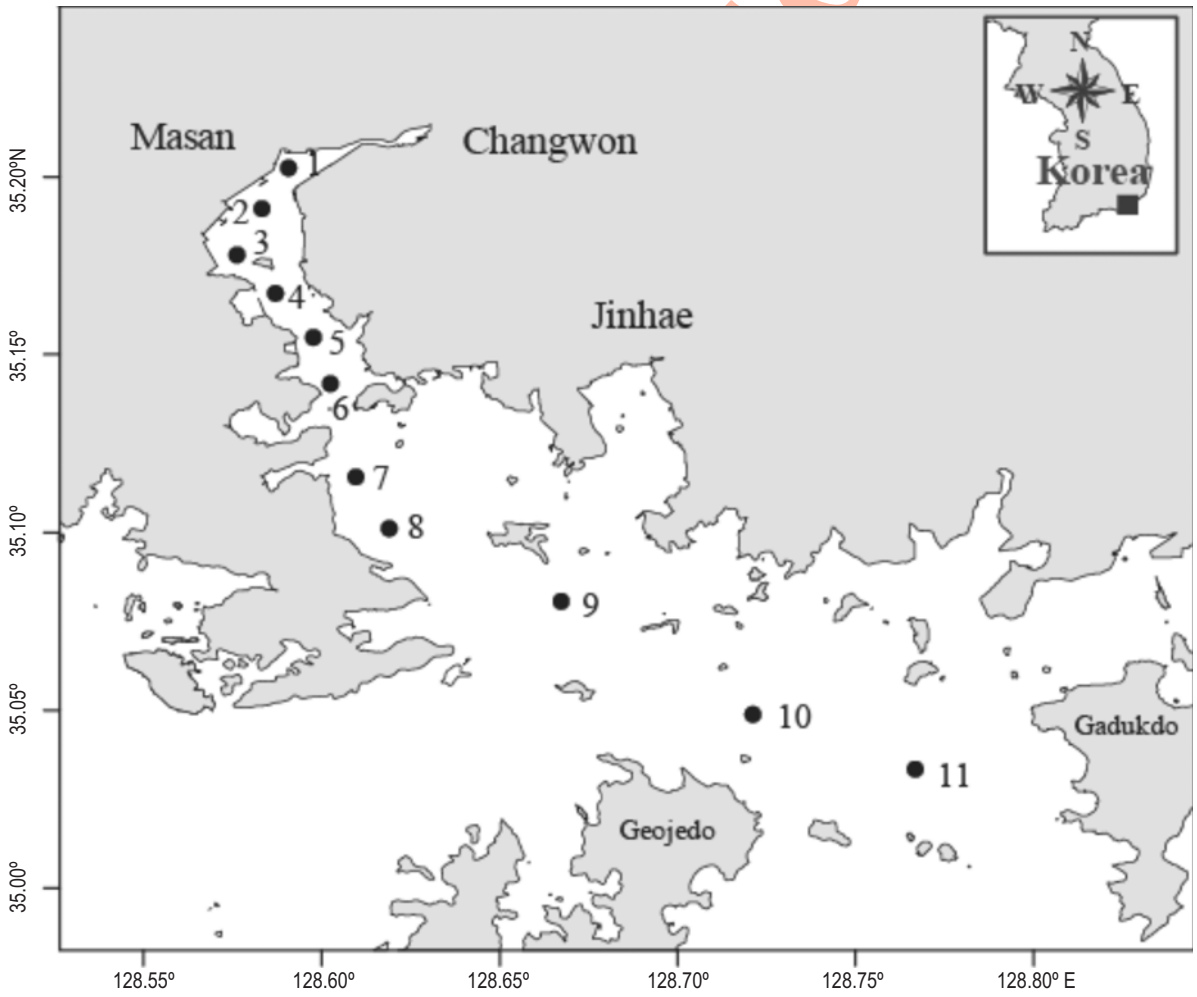

Fig. 1 : Sampling locations in Masan Bay

potassium permanganate ($KMnO_4$) to oxidize organic compounds, thereby measuring the oxygen consumed. SPM was determined by filtering 500 ml of seawater through a pre-weighed GF/F filter (47 mm diameter), followed by washing with approximately 10 ml of deionized water three times to remove salts from filter and filtering apparatus. Samples for photosynthetic pigment analysis were prepared by filtering 500 ml of seawater through GF/F filter (47 mm diameter); the filter was refrigerated until it was transported to the laboratory. The samples were then stored in a deep freezer (–80°C) for photosynthetic pigment analysis. Ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), and silicate ($Si(OH)_4$) concentrations were measured using a nutrient AutoAnalyzer (Bran+Luebbe, Quaatro, Germany). SPM was calculated by comparing the difference in weight of the filter before and after 24 hr of drying at 60°C in a dryer. POM was determined after ashing the filter and then weighed for SPM analysis at 550°C for 5 hr.

Analysis of photosynthetic pigments : A filter paper was inserted into a 10 ml culture tube, and 5 ml of 100% acetone and 50 µl of canthaxanthin (an internal standard) was added. The sample was then extracted at –20ºC for 24 hr in a cold dark room (Jeffrey, 1997). During the extraction process, the filter was manually pulverized with a spatula and then further processed with a sonicator for 5 min. The sample was then centrifuged at 2,000 rpm for 10 min. Approximately, 1 ml of the supernatant was thoroughly mixed with 300 µl of HPLC-grade water, and the sample was injected into a 100-µl HPLC loop.

The photosynthetic pigment concentration was analyzed using a HPLC system (2690, Waters, USA). The standard pigments used were chlorophyll *a*, *b*, *c*, and phaeophytin *a,* and the auxiliary pigments included nine types of carotenoids (i.e., fucoxanthin, 19'-hexanoyloxy-fucoxanthin, lutein, alloxanthin, prasinoxanthin, peridinin, 19'-butanoyloxy-fucoxanthin, zeaxanthin and violaxanthin). A solvent system for pigment analysis established by Park and Park (1997) was used.

Results and Discussion

The monthly changes in the marine environmental factors of Masan Bay in 2010 are listed in Fig. 2. The surface and bottom seawater temperature gradually increased from March (average surface: 9.7 ± 0.4ºC; average bottom: 9.6 ± 0.5ºC), and were highest during late August (average surface: 27.4 ± 0.5°C; average bottom: 22.1 ± 1.4°C). The difference between surface and bottom seawater temperature was small in March and largest in August (approximately 5°C). The horizontal distribution of water temperature during summer months (June–August) displayed a decreasing trend from inside of the bay towards the outside.

The salinity of surface seawater decreased from March (average: 32.78 ± 0.88) and was lowest in early July (average: 21.92 ± 6.53), which was attributable to the increased influx of freshwater during heavy precipitation. The difference between salinity of the surface and bottom seawater was largest in early July (>10). The horizontal distribution of salinity in the bottom seawater showed only small differences among stations, whereas the difference in the surface seawater was larger among stations. Salinity displayed an increasing trend from inside of the bay towards the outside.

The average SPM concentration at the surface and bottom seawater of the bay was 9.40 ± 3.45 (4.82–15.99) mg l^{-1} and 11.48 ± 6.30 (5.08–30.24) mg l^{-1}, and was highest during early July, when the freshwater influx was high. In March, April and September, the concentration was high between stations 3 and 7.

The average POM concentration was 4.48 ± 2.14 (1.95–7.77) mg l^{-1} at the surface seawater and 2.91 ± 1.25 (1.58–6.33) mg l^{-1} at the bottom seawater. As with the SPM concentrations, POM was highest in early July, (6.33 mg l^{-1}). The concentration was high as some stations in March and April.

The average DO concentration at the surface and bottom seawater was 8.98 ± 1.70 (5.49–11.62) mg l^{-1} and 4.83 ± 1.67 (2.78–9.69) mg l^{-1}, respectively, with DO of the bottom seawater approximately half that of the surface seawater. DO levels of the bottom seawater rapidly decreased after May (Fig. 2). Hypoxic water masses, with a DO of 2 mg l^{-1} or less, formed between stations 1 and 7 in May, and the range of hypoxic water masses varied from month to month. The hypoxic water mass of the bottom seawater formed in May persisted until early October (Kim *et al*., 2012).

The pH range of surface and bottom seawater was 8.00–8.38 (average: 8.19 ± 0.15) and 7.58–8.21 (average: 7.86 ± 0.11), respectively. Change in pH was greater at the bottom seawater as compared to the surface seawater, and the pH was relatively lower for bottom seawater in summer, when DO was lower (Fig. 2). The average COD of surface layer was 2.21 ± 0.99 (average: 0.65–5.30) mg l^{-1} and was highest in April. The average COD of the bottom seawater was relatively low, with a range of 0.55–2.29 (average: 1.02 ± 0.42) mg l^{-1}.

The monthly distribution of nutrients displayed an increasing trend after May and was highest in July, when freshwater influx was pronounced (Fig. 2). The monthly average concentration of NH_4^+ among stations during the survey period was within the range of 1.45–13.38 (average: 6.85 ± 9.89) µM for surface seawater and 2.74–19.72 (average: 8.57 ± 6.05) µM for the bottom seawater. The concentration of NO_2^- as the surface and bottom seawater was 0.80 ± 0.68 (0.22–1.62) and 0.73 ± 0.25 (0.21–1.37) µM, respectively, whereas for NO_3^-, the concentration was 6.83 ± 7.42 (0.60–29.63) µM for surface seawater and 4.99 ± 4.31 (1.56–22.69) µM for bottom seawater.

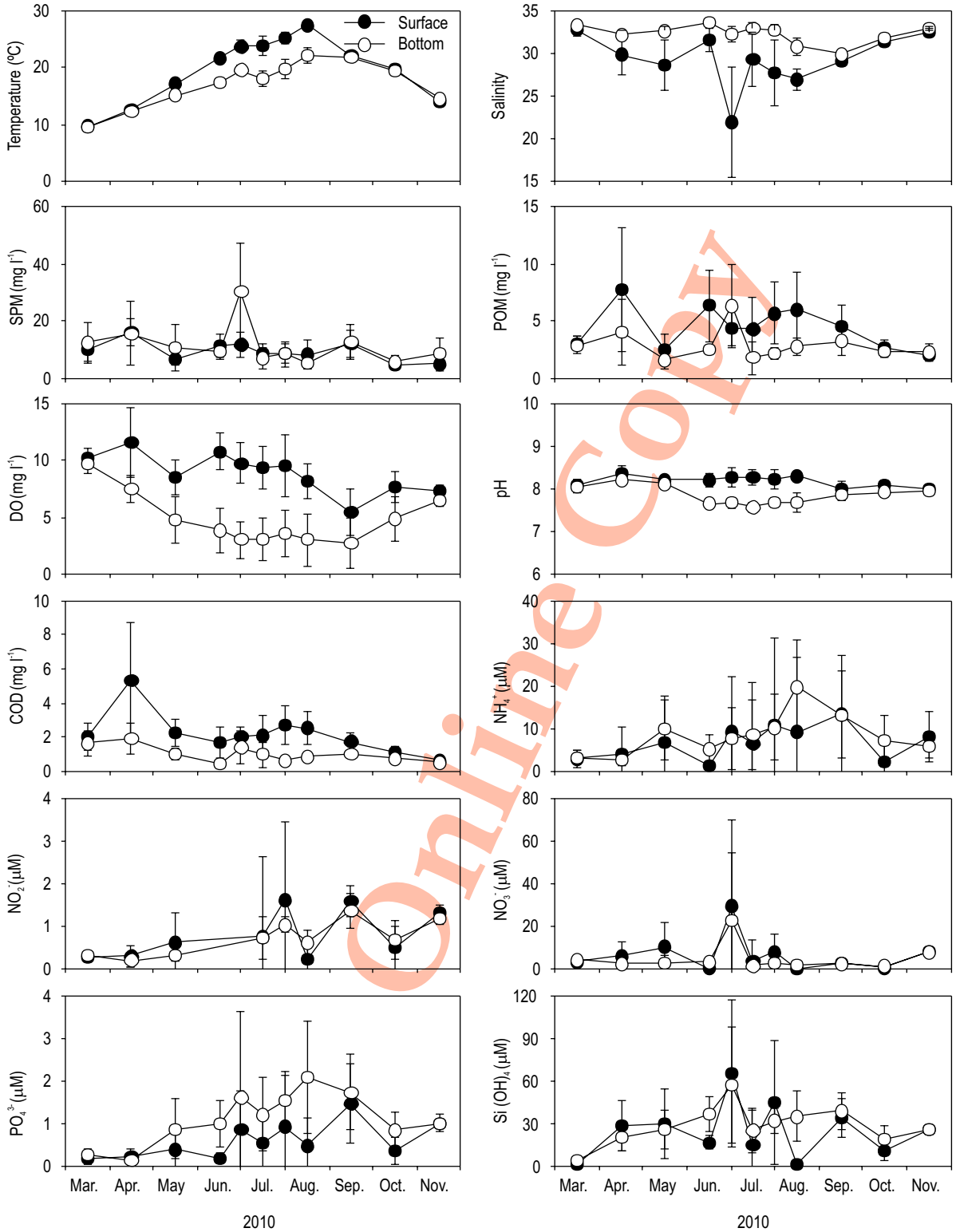

Fig. 2 : Monthly variations in temperature, salinity, SPM, POM, DO, pH, COD, and nutrients at the surface and bottom layers of Masan Bay in 2010

The values were highest in early July, when the freshwater influx was high. NH_4^+ and NO_3^- level was higher at the bottom layer as compared to the surface, whereas the concentration of NO_2^- was higher at the surface. It is believed that due to the formation of hypoxic water masses the bottom seawater of Masan Bay during summer, the reductive environment led to denitrification, resulting in a decrease in NO_2^- at the bottom of the bay (Fig. 2). The monthly average concentration of PO_4^{3-} at the surface and bottom layers were 0.60±0.53 (0.17–1.47) and 1.11±0.70 (0.14–2.08) µM, respectively. The average $Si(OH)_4$ concentration was 24.8±17.5 (0.9–64.8) µM at the surface and 29.0±13.2 (4.0–57.2) µM at the bottom layers. The concentration of dissolved inorganic nutrients was higher at the bottom as compared to the surface of the bay, and in terms of horizontal distribution, the concentration was higher within the bay and decreased in regions farther from the bay. In July, when the influx of freshwater was high, NO_3^- and $Si(OH)_4$ levels increased substantially. However, a relatively smaller change in PO_4^{3-} concentration was observed, indicating that the main source of NO_3^- and $Si(OH)_4$ in Masan Bay was freshwater influx. $Si(OH)_4$ discharged in July was observed until August, but had decreased dramatically by the end of the same month. This decreasing trend may be attributable to consumption by phytoplankton. For PO_4^{3-}, the difference between the concentration in surface and bottom layers was large. In particular, during summer when water temperature increased, the concentration at the bottom layer was twice higher than at the surface (Fig. 2). Lee *et al*. (2009) monitored the amount of PO_4^{3-} introduced into Masan Bay through submarine groundwater discharge using radium isotope tracing and reported that the amount introduced through this mechanism was approximately 3.8-fold higher in May and 2.4-fold higher in August, as compared to the amount introduced by river discharges. Therefore, PO_4^{3-} in Masan Bay is mainly supplied by diffusion from the bottom sediments or introduced through submarine groundwater discharge, rather than river discharges.

The monthly average concentration of chlorophyll *a* at the surface seawater of Masan Bay was highest in April (26.0 ± 23.6 µg l^{-1}) and lowest in November (4.29 ± 1.05 µg l^{-1}) (Fig. 3). The monthly average concentration of chlorophyll *a* at the bottom seawater of Masan Bay was highest in March (19.6 ± 7.9 µg l^{-1}) and lowest in June (0.9 ± 1.0 µg l^{-1}). The horizontal distribution of chlorophyll *a* displayed very high levels between stations 3 and 7 in March and April and SPM and POM concentration was also high during this period.

High concentration of chlorophyll *a* at the surface seawater between March and April decreased in May. This may be attributable to the consumption of DO at the bottom layers through decomposition of organic matter that had accumulated at the bottom sediments following the decline of phytoplankton blooms, resulting in the development of hypoxic water at the bottom layer in May.

Between May and early October, when hypoxic water was produced, chlorophyll *a* concentration at the surface of Masan Bay was high (>10 µg l^{-1}). However, chlorophyll *a* concentration at the bottom layer was low, which may be attributable to an increase in opacity following increase in SPM during influx of freshwater in summer and the growth of phytoplankton, thus hindering photosynthesis of phytoplankton at the bottom layer (Son *et al*., 2012).

The results from the mid-July (July 19) survey showed that the nutrient concentration of Masan Bay was high due to influx of freshwater from land, whereas chlorophyll *a* level was not high. In contrast, the results from the survey conducted in late July (July 29–30) showed that the nutrient content had dramatically decreased and the concentration of chlorophyll *a* was high. These observations may be attributable to the oversupply of nutrients from freshwater influx into Masan Bay in July, which resulted in a large increase in phytoplankton biomass.

To examine the changes in phytoplankton community composition in Masan Bay in relation to changes in the marine environment, photosynthetic pigments of phytoplankton at the surface and bottom seawater were analyzed. Analysis of photosynthetic pigments in the surface seawater revealed that the concentration of fucoxanthin, peridinin, alloxanthin and chlorophyll *b* was high, whereas the concentration of 19'-butanoyloxy-fucoxanthin, 19'-hexanoyloxy-fucoxanthin, zeaxanthin, violaxanthin and lutein was relatively low at 5 µg l^{-1} or less (Fig. 3). At the surface, the concentration of phaeophytin *a*, which is a decomposition product of chlorophyll *a* that serves as an indicator of the physiological state of phytoplankton, was highest in April and May when chlorophyll *a* concentration was highest (>2 µg l^{-1}; Fig. 3).

Although the pigment analysis cannot identify phytoplankton species in Masan Bay, it can provide information on the phytoplankton community composition at class level. During the survey period, concentration of fucoxanthin, the main pigment of diatoms, was highest, with an average monthly concentration ranging between 0.51 (April) and 5.46 µg l^{-1} (August). The concentration was highest during summer, between August and October. In contrast, the range of peridinin concentrations, the main pigment of dinoflagellates, and chlorophyll *b*, the main pigment of green algae or euglenophytes, ranged from 0.07 (March) to 2.97 µg l^{-1} (April) and 0.06 (March) to 17.4 µg l^{-1} (April), respectively; both pigments were highest during spring (April). The concentration of alloxanthin, the main pigment of cryptophyta, ranged from 0.01 (March) to 1.21 µg l^{-1} (May) and was highest in May. Based on the overall distribution pattern of photosynthetic pigments, it exhibited a decreasing trend from the inside of the bay towards the outside.

Masan Bay, which is frequently associated with red tides, is predominantly inhabited by phytoplankton and dynamic

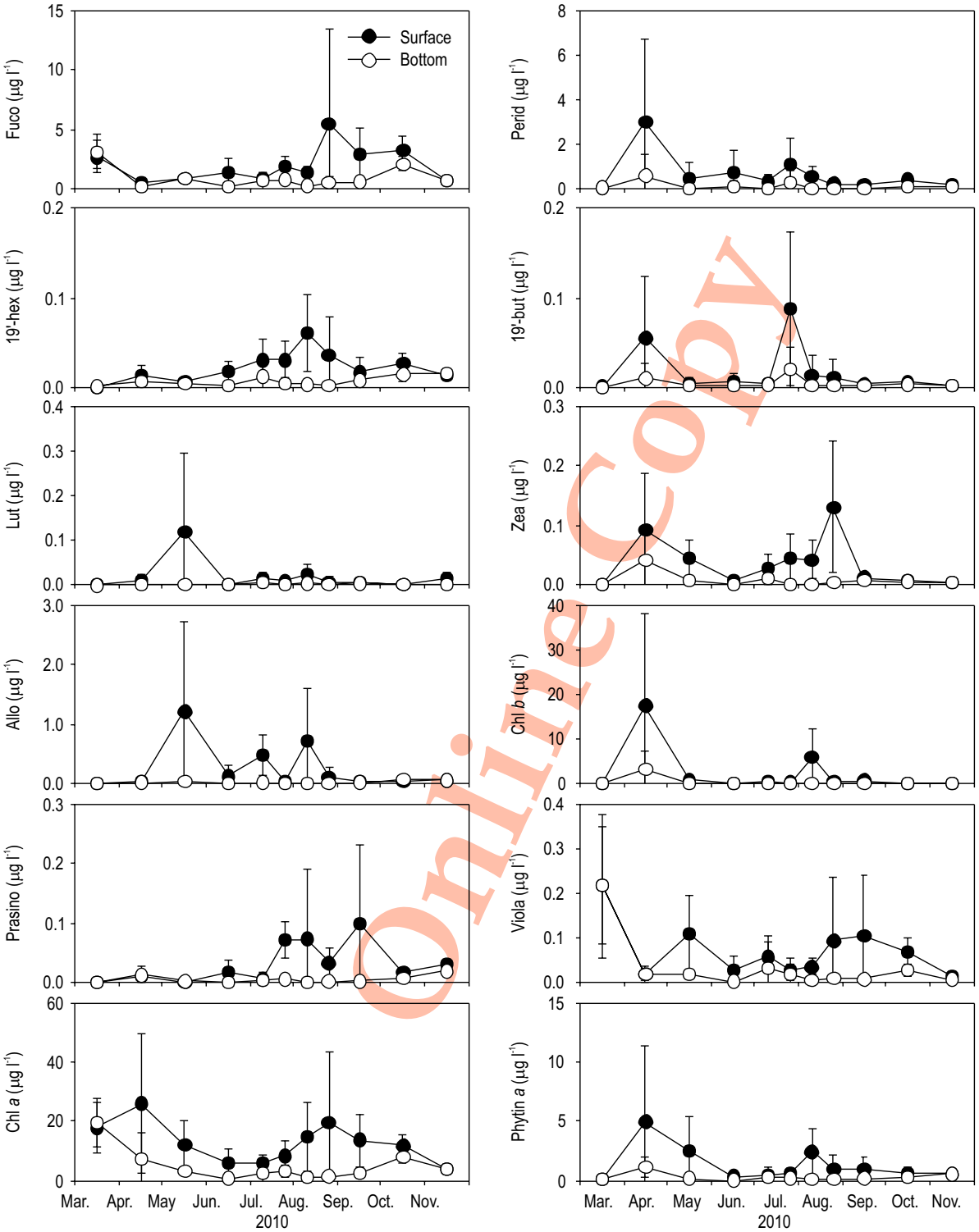

Fig. 3 : Monthly variations in each markers pigment concentrations at the surface and bottom layers of Masan Bay in 2010. Abbreviations: Fuco: fucoxantin; Perid: peridinin; 19'-hex: 19'-hexanoyloxy-fucoxanthin; 19'-but: 19'-butanoyloxy-fucoxanthin; Lut: lutein; Zea: zeaxanthin; Allo: alloxanthin; Chl *b*: chlorophyll *b*; Prasino: prasinoxanthin; Viola: violaxanthin; Chl *a*; chlorophyll *a*; Phytin *a*: phaeophytin *a*

seasonal successions have been observed. In April, when chlorophyll *a* concentration was highest, peridinin and chlorophyll *b* concentrations were also highest. High chlorophyll *a* concentration during this time may be attributable to a bloom of dinoflagellates and green algae or euglenophytes. Conversely, high chlorophyll *a* concentration of blooms occurring in July and August coincided with high fucoxanthin concentrations and was thus likely to be due to proliferation of diatoms.

In April, when the dissolved inorganic phosphorus (DIP) concentration (average: 0.22 ± 0.17 μM) dramatically decreased, the community of dinoflagellates increased. This may be attributable to limitation of growth of various phytoplankton as a response to decrease in DIP, leading to a bloom of dinoflagellates, which can grow by utilizing dissolved organic phosphorus (DOP) (Glibert *et al*., 2001; Oh *et al*., 2002; Lee *et al*., 2010).

Between May and early October, when hypoxic water was produced at stations 1 and 7, the concentration of fucoxanthin, the main pigment of diatoms, was high at the surface seawater. The concentration of fucoxanthin was highest at the bottom seawater, although the total biomass of phytoplankton was low. The growth of diatoms was significantly correlated with the distribution of dissolved inorganic silicate (DSi) (Wu and Chou, 2003). When hypoxic water was present, the DSi concentration at surface seawater was maintained at a high level after influx of freshwater in early July, indicating that diatoms flourished due to their interspecific dominance over dinoflagellates bloom in spring.

In early August, chlorophyll *a* concentration of surface seawater between stations 1 and 6 was high, with levels of more than 10 (12.7–38.1) μg l^{-1}, which coincided with high fucoxanthin and chlorophyll *b* concentrations. During this period, a mixed red tide consisting of diatoms and green algae or euglenophytes likely occurred. Jeong *et al*. (2011) reported that during a 2004 survey of Masan Bay, a mass of euglenophyte (*Eutreptiella gymnastica*) increased in early August. Therefore, high chlorophyll *b* concentration in August 2010 may be attributable to increase in *Eutreptiella gymnastica*, which contains chlorophyll *b* (Schlüter *et al*., 2000).

In late August, chlorophyll *a* concentration at surface water between stations 1 and 4 was high (14.8–81.1 μg l^{-1}), and the concentration of fucoxanthin was highest. Low concentration of DSi in seawater in late August may be attributable to the growth of diatoms during this period. The biomass and community composition of phytoplankton are affected by nutrient distribution and composition (Wu and Chou, 2003; Baek *et al*., 2008, 2009).

The results indicate that the biomass and community composition of phytoplankton are associated with the dynamics of DSi in summer and DIP in spring. Analysis of photosynthetic pigments can serve as a useful tool for predicting changes in phytoplankton community composition in coastal areas impacted by large changes in the marine environment.

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