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Diversity of Cyanobacteria *Synechococcus* spp. based on DNA Analysis and Phycoerythrin Chromophores in the East Sea, Korea

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한국 동해의 DNA 분석과 Phycoerythrin Chromophores 의 따른 Cyanobacteria *Synechococcus* spp. 의 다양성

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by

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Abstract

Distribution patterns and diversity of *Synechococcus* related to environmental factors in the East Sea were investigated using three different methods: flow cytometry, pyrosequencing for the DNA analysis, and PE chromophores excitation ratio (PUB_{EX}:PEB_{EX} ratio). *Synechococcus* distribution showed high seasonal variation with the highest value in autumn ($2.6\pm3.3 \times 10^3$ cells ml^{-1}) and the lowest in winter ($0.2\pm0.1 \times 10^3$ cells ml^{-1}). The abundance changed dynamically with season and the environmental factors influencing the distribution differently in each season. DNA analysis showed high diversity of *Synechococcus* with 22 clades identified. Distinct seasonal contribution of the clades were observed in which clades I and IV contributed higher (~ 50 %) in spring, whereas clade II contributed more in autumn. Based on PUB_{EX}:PEB_{EX} ratio, three different populations of *Synechococcus* were found: high PUB_{EX}:PEB_{EX} ratio (> 1) type that dominated the study areas and were found mostly at the deeper layers, low PUB_{EX}:PEB_{EX} ratio (< 1) type that was found

dominated in the upper layer in autumn and spring, and PUB-lacking cells which were rare. This study is one of the few studies that used DNA analysis and PUB_{EX}:PEB_{EX} ratio method to understand the diversity and ecotypes of *Synechococcus* and the relationship with the environmental factors. Moreover, it is the only study that used natural samples where most of the studies have been using culture samples. The result showed an agreement in which PE-rich type of *Synechococcus* that is represented with the high occurrence of *Synechococcus* subcluster 5.1 dominated in the East Sea. The distribution of *Synechococcus* clades showed close relationship with temperature, while the distribution of PUB_{EX}:PEB_{EX} ratio related to the water masses and light.

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Chapter 1

Introduction

1.1 . Research background

Picophytoplankton is a fraction of plankton composed by cells between 0.2-2 μm. They are among the smallest free-living cells and possess the photosynthetic apparatus that occupies about half of the cell volume (Raven 1998). Picophytoplankton was not discovered until the early 1980s and it is an important contributor to primary production (Chisholm et al. 1988). It has been in the ecosystem for a long time, however due to lack of instrument to identify this extremely small organism, it is not well known until recently. Before the development of the instruments to identify picophytoplankton were discovered, scientists always believed that mostly photosynthesis in the ocean was conducted by microscopic eukaryotic algal species such as diatoms and dinoflagellates. However with the increasing knowledge about picophytoplankton, scientists started to consider that this organism also responsible for the most of photosynthesis in the surface oceans (Partensky et al. 1999; Agawin et al. 2000).

There are probably relatively few species of picophytoplankton with very wide biogeographical ranges in marine or freshwater habitats. The small size and low biodiversity means large numbers of individuals worldwide (Raven 1998). There are three groups of autotroph picophytoplankton: *Synechococcus*, *Prochlorococcus*, picoeukaryote (Zhang et al. 2007). *Synechococcus* and *Prochlorococcus* are prokaryotes, while picoeukaryote

is a eukaryotic cell. *Synechococcus* (average size about 0.9 μm) can be found in almost everywhere in the ocean and much more abundant in nutrient-rich water, while *Prochlorococcus* (average size about 0.6 μm) is known to be abundant in the oligotrophic waters (Partensky et al. 1999).

This study focused on *Synechococcus* due to the following reasons: *Synechococcus* can be easily recognized by the orange fluorescence of their phycoerythrin and this pigment can be used to identify genetically distinct population of *Synechococcus*; using flow cytometry, it is easy to discriminate phycoerythrin-containing *Synechococcus* from other fluorescent phytoplankton species. Other than using phycoerythrin, *Synechococcus* showed high diversity based on the DNA composition. Thus, since the diversity of *Synechococcus* in the molecular level, it is very sensitive to the changing of the environmental conditions. Ecological study of *Synechococcus* will provide more knowledge about the importance of environmental factors that control the distribution of this organism in the oceans.

1.2. The role of picophytoplankton

Synechococcus, together with *Prochlorococcus* are known to be responsible for about half of the world's photosynthesis. Due to their ubiquity and abundance, they are widely recognized as a major contributor

to global photosynthetic biomass and primary production (Partensky et al. 1999; Agawin et al. 2000). Nybakken and Bertness (2005) had emphasized that picophytoplankton contributed to primary production of the ecosystem in the open ocean significantly when the bigger size organisms (> 20 μm) do not thrive. In tropical and subtropical oceanic waters, picophytoplankton account for ~80 % of total Chl *a* and phytoplankton biomass as well as ~70 % of total primary production (Li and Harrison 2001). A review study of the importance of picophytoplankton in coral reef ecosystem stated that *Synechococcus* contributed the most, up to 90 % (Charpy 2005, and the references therein).

Picophytoplankton is an important food source. It has been suggested that *Synechococcus* occupies an important trophic position in the transfer of new nitrogen into the oceanic food web as they are responsible to the elevated nitrate concentrations and transferred this new productions to grazers (Glover et al. 1988). In the coral reef ecosystems, picophytoplankton is actively grazed by sponges, ascidians, zooxanthellae soft corals and scleractinian. It helps maintain a positive carbon balance and therefore their availability and grazing rate by benthic community have been taken into account when estimating the new production of coral reef ecosystems (Charpy 2005). A study of trophic interactions between picophytoplankton and micro and nanozooplankton conducted in the

western Arabian Sea during the northeastern monsoon in 1993 found that picophytoplankton was the major food source of small heterotrophic nanoflagellates (HNF) and it was assumed that HNF could satisfy their daily carbon demand only from picophytoplankton (Reckermann and Veldhuis 1997).

1.3. *Synechococcus* distribution

1.3.1. Pacific Ocean

There have been many studies of *Synechococcus* in the Pacific Ocean (Blanchot et al. 2001; Blanchot and Rodier 1996; Odate and Fukuchi 1994; Olson et al. 1990). Blanchot et al. (2001) studied the distribution of *Synechococcus* in two distinct regions of equatorial Pacific: the western warm pool and the equatorial upwelling that was characterized by high-nutrient-low-chlorophyll (HNLC) conditions. The result showed that in the warm pool, *Synechococcus* was homogenously distributed in the 0-80 m layer, and then decreased dramatically at depth. In the HNLC waters, *Synechococcus* distribution showed a homogenous pattern in the 0-40 m layer and the population densities decreased sharply to 90-100 m. The study of *Synechococcus* (refer to as phycoerythrin-fluorescing cyanobacteria) in the western north Pacific Ocean showed large distributions in the upper water column and the most abundant

Synechococcus can be found in the subtropical surface water (~10^8 cells l^{-1}) (Odate et al. 1990). Olson et al. (1990) in their study in Pacific Ocean found higher cell concentrations near coastal areas (> 4 x 10^4 cells ml^{-1}) and lowest in the central oligotrophic ocean (< 0.5 x 10^4 cells ml^{-1}). Another study was conducted during a temporary weakening El Nino event (1991-1994) in the tropical Pacific Ocean in the absence of equatorial upwelling and in the presence of an equatorial eastward flow (Blanchot and Rodier 1996). The result showed *Synechococcus* was abundant when the nitrate was detectable and decline sharply around 1% light level. However, in other part of the study region, significant *Synechococcus* was also found in the nitrate-depleted layers, under the 1% light level (Blanchot and Rodier 1996).

1.3.2. Atlantic Ocean

The study conducted by Zubkov et al. (1998) from the British Islet to the Falkland Island in Atlantic Ocean found that *Synechococcus* was present in the photic layer at almost all stations, but the concentration varied depending on the region. In the oligotrophic waters, the concentration did not exceed 4 x 10^3 cells ml^{-1}, while higher abundance of *Synechococcus* occurred in the upwelling region, that can reached up to 200 x 10^3 cells ml^{-}

[1]. In the equatorial region, *Synechococcus* formed a deep maximum layer of up to 23 x 10^3 cells ml^{-1} at 60-70 m.

Partensky et al. (1996) compared three different sites of the tropical northeastern Atlantic Ocean on a gradient between eutrophic and oligotrophic waters at different seasons (winter, late spring and fall) and various results were observed. In the near coastal site, there was a dramatic increase of *Synechococcus* in winter when the upwelling less developed. In oligotrophic site, there was an important nutrient enrichment event that favored the blooming of diatoms and decreased population of *Synechococcus*. Another study in Sargasso Sea was conducted to observe long-term distribution of *Synechococcus* (1989-1994) showed the highest abundance was found during the spring bloom each year when the water column was deeply mixed and nutrients were detectable in surface waters (DuRand et al. 2001).

1.3.3. East Sea

Synechococcus studies in the East Sea have been conducted in the southwestern part of the sea (Kim 2002; Kang et al. 2004; Shim et al. 2008), in the south part of the sea (Choi et al. 2011), and in the near-shore areas around Japan (Shiomoto et al. 2004). Kim (2002) in his study at the coast of Gampo, southeastern coast of Korea had found low abundance of

Synechococcus in the surface and increased at depth between 10 and 20 m. *Synechococcus* abundance ranged from 0.2 to 23.2 x 10^3 cells ml^{-1} in August 2000 and lower abundance was found in July 2001 ranged from 0.027 to 3.9 x 10^3 cells ml^{-1}. On the other hand, Choi et al. (2011) found higher *Synechococcus* abundance in the south sea due to the influence of the water masses from East China Sea that can reach up to 84 x 10^3 cells ml^{-1}. Another study in near-shore areas around Japan which was also influenced by the Tsushima Warm Current found higher *Synechococcus* abundance during spring season in the range of 10^2 to 10^4 cells ml^{-1} (Shiomoto et al. 2004).

1.3.4. Other locations

There have been many studies of *Synechococcus* conducted in Mediterranean Seas (Agawin et al. 1998; Uysal 2006; Polat and Uysal 2009; Carillo et al. 2008; Uysal and Koksalan 2010). Mediterranean Sea is oligotrophic and considered to be one of the least productive seas of the world. The basin-wide cyclonic circulation of nutrient-depleted water, hot and dry climate and low land runoff contribute to the low productivity levels (Turley et al. 2000). In their study, Polat and Uysal (2009) found *Synechococcus* in high number in autumn in the shallower water (23.09 x 10^4 cells ml^{-1}) of the bay and least observed during winter (0.8 x 10^4 cells

ml^{-1}). The same result was also observed in the Ebro River estuary outflow to the Mediterranean Sea in Spain where the abundance of picophytoplanktonic cyanobacteria increases from summer to autumn, and decreases from autumn to winter in the upper fresh water and lower brackish water layers (Carillo et al. 2008).

Odate and Fukuchi (1994) sampled *Synechococcus* in the coastal waters of Southeast Asia and the eastern Indian Ocean and found higher abundance of cell concentration in the coastal waters of Southeast Asia rather than the one in the eastern Indian Ocean. Another study in a tropical coastal ecosystem in Philippines, South China Sea found a high abundance of *Synechococcus* in the coastal ecosystem ranged from 0.31 to 21 x 10^6 cells l^{-1} with higher biomass occurring near river sources rich in inorganic nutrients (Agawin et al. 2003).

Table 1. *Synechococcus* abundance in many different locations

Location	Study area	*Synechococcus* abundance (10^3 cells ml^{-1})	Reference
Pacific Ocean	Western north Pacific Ocean	110-510	Odate et al. 1990
	Western tropical Pacific Ocean	64	Blanchot and Rodier 1996
	The western warm pool (oligotrophic) The equatorial upwelling (HNLC)	1.5 ± 0.1 8.9 ± 1.5	Blanchot et al. 2001
Atlantic Ocean	Mauritanian upwelling region	200	Zubkov et al. 1998
	Sargasso Sea	33-56	DuRand et al. 2001
	Southern Atlantic Ocean	59	Doolittle et al. 2008
East Sea	Southeastern coast of Korea	0.027-23.2	Kim 2002
	Near shore areas around Japan	0.1-10	Shiomoto et al. 2004
	Sea waters around Ulleung Island	9.2-251	Shim et al. 2008
	South Sea	84	Choi et al. 2011
Other locations	Mediterranean Sea	155	Polat and Uysal 2009
	Coastal ecosystem in Philippines	0.31-21	Agawin et al. 2003

1.4. Environmental factors controlling distribution of *Synechococcus*

1.4.1. Physico-chemical factors (temperature, salinity, and nutrient level)

Temperature is one of the most important factors that influence the abundance, distribution and also growth rate of *Synechococcus* (Chang et al. 1996; Jiao et al. 2005; Pan et al. 2007; Uysal and Kosalan 2010). There is a general trend that the abundance of *Synechococcus* is decreasing as

latitude increases and temperature decreases. *Synechococcus* was absent in the very cold water of the Southern Ocean with temperature < 1 °C (Doolittle et al. 2008). In detail, Doolittle et al. (2008) in their study in the Southern Ocean during winter time observed that *Synechococcus* did not appear consistently until the water temperature exceeded 1.26° C and the abundance went up incrementally with the increasing temperature, exceeding 10^2 cells ml^{-1} at temperatures > 2 °C, 10^3 cells ml^{-1} at temperatures > 4 °C, and 10^4 cells ml^{-1} at temperatures > 9 °C.

There have been many seasonal studies of *Synechococcus* in order to understand the importance of temperature in controlling *Synechococcus* abundance (Olson et al. 1990; Partensky et al. 1996; Agawin et al. 1998). *Synechococcus* population in subtropical northeastern Atlantic showed low concentration in late springtime that was characterized by homogenously cool (17.5 °C) water and slightly increased in winter when the stratification was well marked (Partensky et al. 1996). Seasonal study in the Bay of Blanes (NW Mediterranean) found high concentration of *Synechococcus* in summer (6 x 10^4 cells ml^{-1}) with temperature > 15 °C and low concentration (5 x 10^2 cells ml^{-1}) in low temperature (< 5 °C) in winter (Agawin et al. 1998).

Cyanobacteria are known to tolerate and acclimate to high salt concentrations, in more specific, *Synechococcus* species are known to be abundant in transitional and freshwater areas (Powell et al. 2005). A study in the South Australian coastal lagoon from the brackish waters to the hypersaline waters found that salinity was identified as the main factor structuring the distribution of *Synechococcus* along the lagoon (Schapira et al. 2010). Another study in the highly eutrophic sea of Black Sea found that salinity seemed to have the greatest impact on the surface distribution of *Synechococcus* in which the concentration progressively increased towards more saline and colder offshore waters to a maximum of 1.45 x 10^5 cells ml^{-1} (Uysal 2006).

Light availability or water transparency may influence the distribution of *Synechococcus* regarding their growth rate (Pan et al. 2007). Light influence the distribution of *Synechococcus* where higher cell densities always found at depth corresponding to the 2.0 to 0.5 % incident irradiation (I_o) level (Veldhuis and Kraay, 1990). Moreover, the presence of the different types of pigment composition of *Synechococcus* might influence the adaptation of this organism to the different light condition and allowed them to grow through the whole array of light levels found in the euphotic zone. For example, with the presence of the high amount of protective pigment Zeaxanthin, allows *Synechococcus* to survive high

irradiance levels (Veldhuis et al. 2005) or in other words, *Synechococcus* cells had photosynthetic characteristics typical of high light populations (Glover et al. 1988).

The distribution and biomass of picophytoplankton is mainly related with nutrient conditions. *Synechococcus* can be found in both nutrient-depleted stratified waters (Olson et al. 1990; Blanchot and Rodier 1996; Partensky et al. 1996) and nutrient-rich mixed waters (Hall and Vincent 1990; Partensky et.al. 1999; Blanchot et al. 2001). In less productive waters, picophytoplankton fraction had higher biomass than the nano and microphytoplankton fraction, whereas in more productive waters, the larger phytoplankton had higher biomass than the picophytoplankton (Agawin et al. 2000). High abundance of *Synechococcus* was found in nutrient-depleted waters, in case of oligotrophic conditions. From many studies, it has been concluded that picophytoplankton play a greater role in oligotrophic waters (Glover et al. 1988; Campbell and Vaulot 1993; Partensky et al. 1996; Agawin et al. 2000). Agawin et al. (2000) in their comprehensive review of phytoplankton communities growing under different nutrient regimes at different geographical areas found that picophytoplankton dominate (≥ 50 %) the biomass and production in oligotrophic nutrient poor (NO_3+NO_2 < 1 μM), and warm (> 26 °C) waters,

but represent < 10 % of autotrophic biomass and production in nutrient rich and cold (< 3 °C) waters.

High abundance of *Synechococcus* in nutrient-rich water related with the upwelling events (Hall and Vincent 1990; Partensky et al. 1996). Hall and Vincent (1990) found significantly high abundance of picophytoplankton in the west coast upwelling-region off the South Island of New Zealand during winter in the range of 6.3 x 10^5 to 2.1 x 10^7 cell l^{-1}. Along the African coast of Mauritania, high concentration of *Synechococcus* was also found in the presence of permanent upwelling (Zubkov et al. 1998). However, the opposite result also occurred in the tropical northeastern Atlantic Ocean, in which a dramatic increase of the cell abundance of *Synechococcus* was observed when the upwelling was less developed (Partensky et al. 1996).

1.4.2. Biological factors (specific growth rate, grazing)

Growth rate of *Synechococcus* may be related with temperature (Moore et al. 1995), nutrient conditions (Glover et al. 1988), and also the presence of accessory pigment, i.e. phycoerythrin (Veldhuis et al. 2005). A study of cultured *Synechococcus* from Sargasso Sea found that the optimal growth temperature for *Synechococcus* was about 24 °C, and they would not grow below 15 °C (Moore et al. 1995). In addition, nutrient level also determine

the growth rate of *Synechococcus* as Glover et al. (1988) had found larger size of *Synechococcus* populations was the typical types of the oceanic and neritic nitracline with the sufficient supply of nitrogen. They observed that higher nitrate concentration cause the shifting of *Synechococcus* cell-size toward larger cells, and at the end leading to higher cellular rates of photosynthesis.

Grazing is one of the significant factors controlling the distribution and biomass of *Synechococcus*. To some extent, grazing can be responsible for a succession of different sub-population of *Synechococcus* in the same water mass (Reckermann and Veldhuis, 1997). Heterotrophic (including mixotrophic) nanoflagellates and small ciliates have been recognized as the most important grazers of picophytoplankton (Simek et al. 1996). Gonzales et al. (1998) in their study in the coral reef waters in the Tikehau lagoon (Tuamotu) found that phagotrophic nanoflagellates were the main grazer of picophytoplankton. However, another study had found that grazing by heterotrophic nanoflagellates appeared inadequate to control the abundance of *Synechococcus* (Guillou et al. 2001).

1.5. Phycoerythrin study of *Synechococcus*

Phycoerythrin is one pigment that belongs to the biliproteins. Biliproteins are light-harvesting pigments used in photosynthesis and found primarily in red-algae, cyanobacteria and cryptomonads. While chlorophylls can be found in the thylakoid membranes, biliproteins are located on the exterior of these membranes. Biliproteins absorb better blue-green light than Chl *a*. Light absorbed by biliproteins migrates from the biliproteins to the chlorophyll and then ultimately to the photosynthesis reaction centers where it is transduced to chemical energy. Other than phycoerythrin, biliproteins are also comprised of phycocyanin and allophycocyanin. Different types of organisms have different type of biliproteins pigments. Cyanobacteria and red-algae may have all of the three basic pigments, while cryptomonads do not posses allophycocyanin.

Among phycobiliproteins, phycoerythrin (PE) is the most common and abundant accessory pigment in marine environment (Olson et al. 1988; Lantoine and Neveux 1997). It is a light harvester that absorbs blue light and consists of two chromophores, which are phycourobilin (PUB) and phycoerythrobilin (PEB) (Olson et al. 1990). PUB is an orange molecule that shows an absorption maximum spectrum around 495-500 nm and it efficiently absorbs blue-green light and is not found in all marine cyanobacteria. PEB is found in all PE-containing marine cyanobacteria

which has absorption maximum spectrum around 540-570 nm (Wood et al. 1999). Both of these chromophores have a single emission maximum at lower wavelength around 563-570 nm (Lantoine and Neveux 1997).

The diversity of spectral characteristic of PE could result from the presence of different genotype *Synechococcus* populations having PEs with different PUB/PEB content, each with different responses to environmental factors such as light intensity, nutrient conditions and also temperature (Lantoine and Neveux 1997). Decreasing irradiance with the depth can increase fluorescence intensity of phycoerythrin, while high nutrient level can increase fluorescence intensity (Algarra and Vaque 1989). Water clarity also influenced the distribution of pigment where PE lacking PUB can be found in more turbid water and PE containing PUB were high in transparent waters (Olson et al. 1990; Katano et al. 2004).

Spectral characteristics of PE based on the different chromophores present (PUB_{EX}:PEB_{EX} ratio) can be used to identify different type of *Synechococcus* populations. There are some studies about the different types of phycoerythrin chromophore of *Synechococcus* in various marine environments (Olson et al. 1988; Olson et al. 1990; Campbell and Vaulot 1993; Campbell et al. 1998; Katano et al. 2004). Low PUB_{EX}:PEB_{EX} ratio had been found in the North Atlantic Ocean and Pacific Ocean (Olson et al. 1990) and also along the coast of Arabian Sea with relatively low

temperature and less saline water (Campbell et al. 1998). It is also coincided with the presence of cool, upwelled water in the upper levels of the water column (Wood et al. 1999). On the other hand, high PUB_{EX}:PEB_{EX} was associated with higher salinity and warm waters (Wood et al. 1998). PUB_{EX}:PEB_{EX} ratio increases with the depth and the distance from the coast indicating an increase in the proportion of the PUB chromophore and this was due to PUB chromophore absorbs more blue-green light (Lantoine and Neveux 1999). Study in the Northeastern Atlantic Ocean showed that high PUB-contain populations occurred in the oligotrophic areas and this was due to the maximum light transmission in these areas (490 nm), which appeared more favorable to the development of high PUB-dominant populations (Lantoine and Neveux 1997). Culture studies showed that different types of *Synechococcus* reacted differently to different environmental condition. For example, two different strains of *Synechococcus* isolated from the same water samples (California Current) were able to respond to changing light conditions differently. Strains CC9311 increased its PUB_{EX}:PEB_{EX} ratio, while strains CC9317 did not significantly change their PUB_{EX}:PEB_{EX} ratio when grown from white light to blue light (Palenik 2001).

Olson et al. (1988) had classified *Synechococcus* population into “bright” and “dim” where “bright” population represented cells with greater

phycoerythrin per cell and hence greater phycoerythin fluorescence. From the data analysis, it appeared that the bright cells had much higher relative PUB contents than the dim cells (Olson et al. 1988). Cells contain both PUB and PEB chromophores have higher fluorescence twice as those cells containing only PEB. The distribution of low PUB content or dim population was restricted to the shallower depth (Olson et al. 1990) and this means that high PUB-containing PE organisms were distributed in the open ocean (Olson et al. 1988).

There are two different instruments and methods that have been used to determine the relative content of PUB and PEB chromophores. The first one is using spectrofluorometer (Lantoine and Neveux 1997; Wood et al. 1998, 1999; Katano et al. 2007; Choi and Noh 2009) and the second one is using dual laser flow cytometry (Olson et al. 1988; Campbell and Vaulot 1993; Katano et al. 2007). Spectrofluorometer method is based on the peak of the excitation spectra of PUB and PEB in which the relative height of the peaks or shoulders can be used as an indicator of the relative concentration of the two chromophores (Wood et al. 1999). The PUB_{EX}:PEB_{EX} ratio is calculated by determining the ratio of relative fluorescence from the PUB peak (excitation around 495 nm) to relative fluorescence from the PEB peak (excitation around 545 nm) (Toledo et al. 1999). On the other hand, dual laser flow cytometry method uses two

different wavelengths (488 nm for blue light and 515 nm for green light) to excite the fluorescence intensity of the chromophores in the range of 564-586 nm. The orange fluorescence excited by 488 nm (FL2) is mainly related to the PUB contents and the orange fluorescence excited by 515 nm (FL4) is related to the PUB + PEB content. At an excitation of 514 nm, PUB and PEB absorb almost equally, while, at an excitation of 488 nm, PUB absorbs more efficiently than PEB (Olson et al. 1988). The relative content of PUB:PEB is then determined from the ratios of FL2:FL4.

The study of PUB_{EX}:PEB_{EX} ratio had been conducted either in the natural seawater samples (Lantoine and Neveux 1997; Wood et al. 1998, 1999; Katano et al. 2007) or in isolated strains (Toledo et al. 1999; Palenik 2001; Fuller et al. 2003; Choi and Noh 2009). Wood et al. (1999) had categorized three different types of PE excitation spectra in their study in the surface waters of Arabian Sea during the northeast Monsoon which are very low PUB_{EX}:PEB_{EX} ratio (< 0.6), intermediate PUB_{EX}:PEB_{EX} ratio, and very high PUB_{EX}:PEB_{EX} ratio (~ 1.8). They found the most common PUB_{EX}:PEB_{EX} ratio was the low ratio (~0.7); 20 % of the samples had ratio ~1.5; and another 20 % showed intermediate PUB_{EX}:PEB_{EX} ratio. In addition, Lantoine and Neveux (1997) found distribution patterns of the PUB_{EX}:PEB_{EX} ratio in three different study sites (eutrophic, mesotrophic, and oligotrophic) in the tropical northeastern Atlantic Ocean. At the

eutrophic sites, above 30 m, the ratio was about 0.56 and below it there was weak increase of about 0.63. Toward mesotrophic sites, the ratio increased significantly below thermocline that reaches a value of 1.33. At the oligotrophic sites, the ratio was relatively constant throughout the water column (0-75 m) with a ratio ranging from 1.8 to 2. Another study based on different water masses was also conducted in the southwestern Japan and found PUB_{EX}:PEB_{EX} ratio in the range of 0.6-1.8 (Katano et al. 2007). Higher PUB_{EX}:PEB_{EX} ratios were found in the study sites which experienced the most of the intrusion of warm surface water from the Kuroshio region (Kyucho events), whereas vertical distribution showed fairly similar ratio throughout the water column (Katano et al. 2007).

Choi and Noh (2009) found four different PE pigment types of *Synechococcus* cultures isolated from the East China Sea and the East Sea. Type 1 pigment which carried phycocyanin only from strains belonging to clade VIII; type 2 which has PEB only, but not PUB from all strains belonging to clade V; type 3 exhibited low PUB:PEB ratio ranged of 0.46-0.62 from strains in clade VI, and type 4 which has high PUB:PEB ratio ranged of 1.10-1.31 from strains belonging to clade III, WPC2 and sub-cluster 5.3. Fuller et al. (2003) in their study also found similar types of PUB_{EX}:PEB_{EX} ratio with the lowest ratio was 0.4 (strains from clade I

isolated from Mediterranean Sea) and the highest was 2.3 (strains from clade II isolated from Gulf of Aqaba).

1.6. DNA study of *Synechococcus*

Genetic studies have been used to understand more in detail about the distribution of small unicellular cyanobacteria. The molecular approaches have been developed (1) to better understand the diversity of these organisms, (2) to define the niches occupied by specific populations, and (3) to define how populations, indeed single cells, are affected specially and temporally by biological and physical factors of the ocean environment (Scanlan and West 2002).

There are several methods that can be used to study genetic diversity of picocyanobacteria with the advantages and disadvantages. Most of the studies have been using dot-blot hybridization (Fuller et al. 2003; Mella-Flores et al. 2011) and real time quantitative polymerase chain reaction (qPCR) (Tai and Palenik 2009). Dot-blot hybridization is used to quantify the abundance of certain RNA or DNA in the extracted nucleic acid, while the qPCR used the rpoC1 (a subunit of DNA-dependent RNA polymerase) phylogeny to assess the abundance of *Synechococcus* (Tai and Palenik 2009). Both of these methods could not reveal the diversity of clades without the availability of specific primer/probes (Choi et al. 2013b).

Other methods that have been used recently are the clone library sequencing and the metagenomic analysis. Clone libraries are constructed from the phylogenetic marker genes and this technique requires a selection of appropriate target genes (Leigh et al. 2010). It is a labor intensive and expensive method, but able to provide an unparalleled level of phylogenetic resolution. Metagenomic is defined as the direct genetic analysis of genome contained with an environmental sample. It provides access to the functional gene composition of the microbial communities and thus gives a much broader description than phylogenetic surveys, which are often based only on a diversity of one gene, for instance the 16S rRNA gene (Thomas et al. 2012). Pyrosequencing is another method that has been widely used to reveal the microbial diversity in marine environments. It is a method of DNA sequencing based on the sequencing-by-synthesis principle which employs a series of four enzymes to accurately detect nucleic acid sequences during the synthesis (Fakhruddin et al. 2012). It allows the analyses of many samples at a time and produces a large number of sequences for community analysis (Choi et al. 2013b).

Some studies have used cultured organisms (Robertson et al. 2001; Fuller et al. 2003; Choi and Noh 2009) while others used population from natural environments (Ferris and Palenik 1998; Mella-Flores et al. 2011; Choi et al. 2013a) to study genetic diversity of *Synechococcus*. Culture cells were

normally isolated from the natural samples and grown in certain temperature and illuminated from fluorescent lamp. However, by using natural samples, a large number of distinct clades could be found which are not represented by isolates in the culture (Ferris and Palenik 1998).

Recent studies using 16S RNA gene sequence analysis had further widen the taxonomy of *Synechococcus* at genus level and had proven that *Synechococcus* is polyphyletic-derived from more than one ancestor (Robertson et al. 2001). DNA studies of *Synechococcus* have revealed the existence of diverse types of this very small organism that can be defined as clade. Based on their general distribution patterns, *Synechococcus* can be classified into ten different types of clade (Ferris and Palenik 1998; Rocap et al. 2002; Fuller et al. 2003; Mella-Flores et al. 2011). Clades I and IV generally co-occur at latitudes above 30° N/S and seem to be restricted to near coastal waters in the lowest part of their latitudinal distribution. Clade II seems to be abundant in warm, coastal or shelf areas and is thought to be the (sub) tropical counterpart of clade I/IV, although some overlap may occur in the boundary zone (i.e. between 30° and 35°, in both the southern and northern hemisphere. Clade III distribution seems to prefer oligotrophic, offshores waters, while all other clades are generally found at lower concentrations than clade I and IV and their distribution patterns are therefore less clearly defined.

1.7. Research objectives

As small organisms that take an important role in the microbial food web and ocean primary productivity, it is very important to understand more about *Synechococcus* and its distribution all around the ocean. Little was known about the distribution of *Synechococcus* in relation to the environmental factors in the East Sea, Korea. Moreover, very few studies of the diversity of *Synechococcus* in the East Sea based on its phycoerythrin chromophores and also the DNA composition and what environmental factors which influence the diversity were published. Therefore, the purposes of this study are to describe seasonal distribution pattern of *Synechococcus*, to identify different types of *Synechococcus* based on phycoerythrin (PE) chromophores and DNA analysis, and to describe what environmental factors influence the diversity of *Synechococcus*. Three different methods were used to identify the diversity of *Synechococcus*: (1). flow cytometry was used to discriminate *Synechococcus* from the other picophytoplankton types and determine the abundance, (2). PE excitation ratio was used to identify *Synechococcus* different types based on the chromophores (PUB and PEB), (3). pyrosequencing method was used to identify genetic diversity of *Synechococcus* based on its DNA composition.

The studies of *Synechococcus* based on its light harvesting pigment and also DNA analysis have shown that *Synechococcus* is very diverse. PUB:PEB ratio exhibits strong variations among PE-containing *Synechococcus*, resulting in wide variations in the optical properties and also colour of *Synechococcus* cultures. DNA analysis shows large genetic variability of *Synechococcus* that correlate with specific physiological adaptation. The diversity of *Synechococcus* is highly adapted to the environmental factors. Since the diversity of *Synechococcus* is in the molecular level, they are very sensitive to the changing of the environmental conditions. Therefore, with the understanding of the diversity of *Synechococcus*, it is possible to obtain information about the changes of water conditions and the effect of environmental changes to the presence of *Synechococcus*.

Chapter 2
Materials and Methods

2.1. Environmental characteristics of the study area

East Sea, Korea is located at the northeast of the Asian Continent and west of the Pacific Ocean. East Sea is characterized with varying water mass properties, such as warm water in the southern part, cold water in the northern part, sub-polar front and eddies (Kim et al. 2010). It is mixing among three different water masses which are Tsushima current (sub-tropical water), Liman Current (sub-arctic water) and also water from East China Sea (Shiomoto et al. 2004). Tsushima current bring along the warm water from the subtropical Kuroshio Current, while cold water originates from the Liman Current. The meeting of these two different water masses forms the sub-polar front at 40°N (Kim et al. 2010) and it occurs particularly in the surface water layer, from 0-50 m (Toya et al. 1988).

East Sea can be categorized as a closed basin which due to the presence of the sill depth of less than 150 m limited the exchange of sea water with the North Pacific Ocean. The surface area is about 10^6 km^2 with the average of water depth about 1700 m. It has three deep basins: the Yamato Basin to the southeast, Ulleung Basin to the southwest with depths greater than 2000 m, and the Japan Basin to the north with a maximum depth of about 4000 m. East Sea shows a very complex ocean characteristic despite its small size compare to the other big oceans. The typical ocean characteristics of East Sea are the seasonal surface temperature that varied

largely in the range more than 15 degrees centigrade, active biological processes, and a sub-arctic polar front between sub-polar and sub-tropical seas (Kim et al. 2001).

The southwest part of the East Sea (Ulleung Basin) is characterized as a nutrient poor region. In this area, the nutrient level does not undergo much seasonal change due to the low nutrient level of Tsushima Current which is the branch of Kuroshio (Yoo and Kim 2004). The model study tested in the mixed layer depth of the East Sea suggested that low nitrate concentration, caused by strong stratification and intense consumption of nitrate by phytoplankton in spring, limits phytoplankton growth during summer, while grazing by zooplankton suppresses the phytoplankton biomass after the spring bloom (Liu and Chai 2009). Hence, the availability of nitrogen is generally regarded as the primary limiting factor to the primary production in the East Sea (Lee et al. 2009).

The nutrient supply into the East Sea is influenced by several factors which are the amount of volume transport through the Korea Strait, nutrient concentration from source water (East China Sea), and biological and physical processes along the pathways. One important sources of nutrient in the southern East Sea is from the water mass flowing through the Korea Strait and the characteristics of this water mass is closely related to those of the northern East China Sea (ECS). The nutrient concentration

of the ECS is influenced by the intrusion of nutrient rich Kuroshio Sub-Surface Waters and the Changjiang discharge (Zhang et al. 2007).

There are two types of phytoplankton bloom in the East Sea: (1) the spring blooms that occur earlier in the southern part of the sea associated with earlier seasonal increases in solar radiation and stratification, (2) the fall blooms that associated with the weakening of the seasonal thermal stratification (Yamada et al. 2004). This is the general patterns observed in temperate waters where light and nutrients become the limiting factor for phytoplankton growth in winter and summer, respectively. The spring bloom usually occurs in April or May, and fall bloom some time from October to December (Kim et al. 2000).

2.2. Sampling sites

This study was conducted in the southwestern part of East Sea, Korea. There were 4 sampling lines (A-D) and each line consists of 3 to 4 stations spaced about 20-40 km for each station in the same transect (Figure 1). Water samples were taken in every season from 2011 to 2012 which are summer (25-26 July 2011), autumn (26-28 September 2011), winter (30-31 January 2012), spring (30-31 May 2012) and one additional autumn season (13-15 November 2012) for DNA study.

Temperature and salinity data were obtained using CTD attached to a rosette sampler. Seawater samples were collected for Chl *a* size fraction, nutrient and picophytoplankton abundance at standard depths (0, 10, 20, 30, 50, 70 and 100 m).

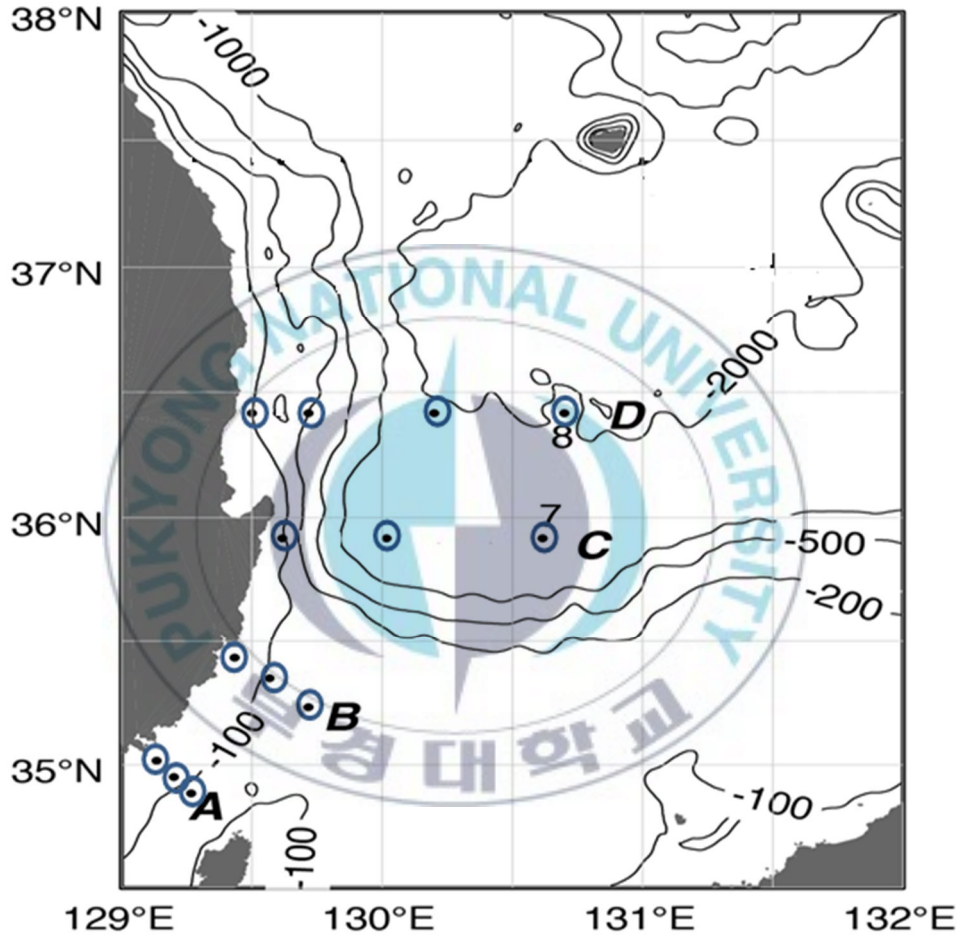

Fig. 1. Map of east coast of Korea showing sampling sites of the cruise. The circled dots showing the sampling sites of this study

2.3. Chlorophyll *a* analysis

Chl *a* size fraction was carried out using Whatman nuclepore polycarbonate membrane filter with two different membrane sizes (3 µm and 0.4 µm). Three liters of seawater was filtered on a parallel filtration where 3 µm membrane filter was placed on top of the 0.4 µm membrane filter. Micro-size fraction of Chl *a* were collected using 3 µm membrane filter, while 0.4-3.0 µm membrane size collected pico-size fraction of Chl *a*. Both filters were covered with aluminium foil and stored in the deep freeze until further analysis in the laboratory. Chl *a* size fraction data was collected only in autumn (September 2011) and spring (May 2012).

Chl *a* was extracted in 10 ml of 90 % acetone by immersing the filter fully into acetone solution and was placed in a dark refrigerator for 8-24 hours. After that period of time, the samples were centrifuged at 2000 rpm for 10 minutes and concentration values were measured using 10-AU Fluorometer Turner Design. Chl *a* concentration was calculated using the following equation (Stricland and Parsons 1972):

$$Chl\,a\left(\mu g/l\right) = Fd * \frac{T}{T-1} * \left(R_b - R_a\right) * \frac{v}{V}$$

Rb : fluorescence before acidification
Ra : fluorescence after acidification
T : Rb/Ra ratio of pure Chl *a*
Fd : appropriate calibration factor
v : volume of acetone extract
V : volume of seawater filter

2.4. Nutrient analysis

Water samples for analysis of nutrient were stored frozen in 125 ml plastic bottles after filtration through Whatman GF/F filters. Nitrate + nitrite and phosphate analysis were conducted in the Marine Biogeochemistry Laboratory, Pukyong National University.

2.5. Flow cytometry analysis of *Synechococcus*

1.8 ml of seawater was preserved by the addition of 0.2 ml of 10 % paraformaldehyde, placed in the vial and stored at -80 ºC until analyzed. The addition of preservatives i.e. paraformaldehyde has been proved to prevent minimal cell loss and suitable for quantitative studies of picophytoplanktonic population in the field (Vaulot et al. 1989). Samples were analyzed at the highest flow rate (approximately 60 μl/min) of a Coulter EPICS XL flow cytometry. Before analyzing, fixed samples were thawed and kept in the dark at room temperature for less than an hour and a known concentration of standard beads (1,09 μm) is added to each sample. The recorded data were analyzed using CXP program that is developed by Applied Cytometry System.

Synechococcus abundance was calculated using the following equation (Campbell 2003):

$$N = C / (T * R) * CF * 1000 \mu l / ml$$

N = cell abundance (cell ml^{-1})
C = number of events acquired (cells)
T = duration of analysis (min)
R = sample delivery rate (µl min^{-1})
CF = correction factor to account for sample dilution owing to preservation, bead addition or staining

2.6. PUB_{EX}:PEB_{EX} ratio analysis

Two liters of seawater was filtered onto 47 mm Whatman GF/F filters and kept frozen until analysis. The extraction method of PUB and PEB chromophores was modified from Neveux et al. (2006). The filters were extracted in 7 ml of phosphate buffer and maintained 3 hours at 4 ºC in the dark. Phosphate buffer keep the stability of the pigments (Moreth and Yentsch 1970). Filters were grinded and centrifuged for 10 minutes at 2500 rpm. The fluorescence of about 3.5 ml of the supernatant was measured using Fluoromax-4 spectrofluorometer. The excitation of Phycoerythrin (PE_{EX}) was recorded at 0.2 nm intervals between 450 and 580 nm (emission fixed at 605 nm). Slit widths were set at 5 nm and 10 nm at excitation and emission, respectively. Excitation spectra of PUB

appeared around 495-500 nm and the one of PEB appeared around 540-560 nm (Fig. 2).

PE excitation spectra were used because it is sensitive to the presence or absence of the PUB (Wood et al. 1999). In the excitation spectra, PUB and PEB appear as separate peaks and/or shoulders and it provides a basis for distinguishing among different PUB-containing PEs. The relative height of the peaks and shoulders can be used as an indicator of the relative concentration of the two chromophores (PUB_{EX}:PEB_{EX} ratio).

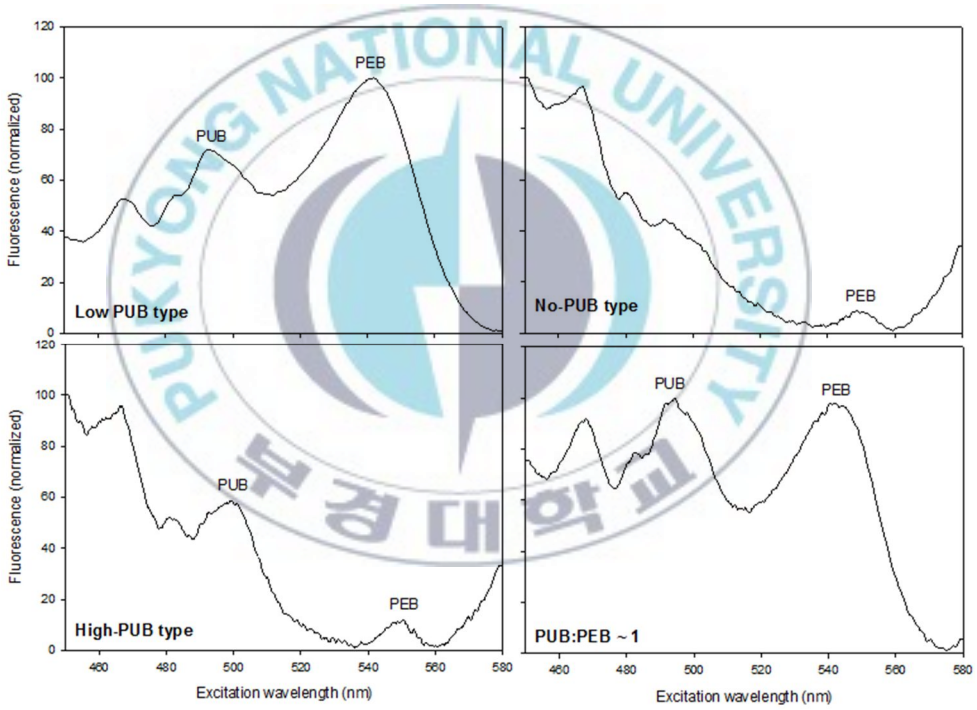

Fig. 2. Normalized fluorescence excitation spectra of phycoerythrin (PE). Excitation spectra of PUB appeared around 495-500 nm and PEB excitation spectra was around 540-560 nm. Emission was fixed at 605 nm.

2.7. Diversity of picocyanobacteria using a molecular approach

2.7.1. Extraction

One liter water samples were passed through 0.2 μm Suporfilter (Gelman Sciences, Ann Arbor, MI, USA) and the filter was placed in a vial with the addition of 1 ml STE buffer (100 mM NaCl, 10 mMTris-HCl, 1 mM EDTA, pH 8.0). STE buffer is added to maintain pH of the solution which prevents denaturation of DNA. Vials were stored at -80 °C before analyses. DNA extraction method was conducted according to Choi et al. (2013a). To extract the DNA, the filters were cut into small pieces with sterilized scissors and placed in 50 ml sterilized conical tube with the remaining lysis buffer and 1 ml of fresh buffer. The tubes were then heated up at 37 °C for 30 minutes after the addition of 80 μl Lysozyme (5 mg/ml Tris-HCl). Lysozyme is an enzyme to degrade the cell wall. The tubes were again heated up at 55 °C for 2 hours after the addition of 45 μl 10% SDS and 56 μl Proteinase K (20 mg/ml). SDS is a detergent that is able to denature proteins while proteinase K is an enzyme that will degrade most of type proteins impurities in DNA because proteins are contaminating agents in DNA.

2.7.2. Purification

One ml of the extraction liquid was placed in the 15 ml conical tube and then DNA was purified using Qiagen DNeasy Tissue Kit (Qiagne) according to the manufacturer`s instruction. The tubes were heated up at 70 ºC for 10 min after the addition of 1 ml AL buffer and placed in a vortex. One ml of 100 % ethanol was added and the liquid were placed in to spin column and centrifuged at 8000 rpm to obtain the lysate. AL buffer is added to break open the cells and ethanol is used to precipitate the DNA. 500 µl of each AW1 and AW2 buffer were added to allow all the components except DNA to pass through the filter. 100 µl of AE buffer was added to remove the DNA from the filter. 100 µl of the DNA template were collected and kept in the freezer for the PCR process.

2.7.3. PCR amplification

PCR amplifications were performed in a volume of 50 µl, containing PCR buffer (10x buffer), deoxynucleotide triphosphates (dNTP), Taq DNA polymerase, forward-primer and reverse-primer, distilled water, and DNA template (Choi et al. 2013b). The volume of each component can be changed in order to obtain maximum result of PCR. The PCR thermal cycling protocol includes an initial denaturation at 94 ºC for 5 min, followed by 35 cycles at 94 ºC for 1 min, an annealing temperature of 50

°C for 1 min, an elongation at 72 °C for 2 min, and a final 7 min extension step at 72 °C. PCR products were confirmed by electrophoresis in 2 % agarose in TAE buffer and visualized under the UV light.

DNA QuantLadders (Lonza Rockland Inc., Rockland, ME, USA) were used for quantification of each PCR product and identical quantities of the products were pooled and purified. Using 2% agarose gel, the region between 200 and 400 bp (base pair) was excised and extracted using the Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Pyrosequencing (on a 1/8 PicoTiterPlate) of PCR products was performed at Macrogen.

2.7.4. Analysis of pyrosequencing data

Pyrosequencing data were analyzed using the Mothur software (Schloss et al. 2009) and the analyzing was according to Choi et al. (2013a). Raw reads were filtered to remove reads associated with errors by allowing only those with perfect match to barcode and forward primer sequences. The allowed number of homopolymer length maximum was 6 bp. Reads with initial noisy signal (flow intensity, 0.5-0.7) before 150 were removed and flows beyond 350 were ignored and flowgram data were grouped by samples based on their barcodes. Command 'shhh.flows' which is the Mothur-based re-implementation of Pyronoise was used to de-noise the filtered reads (Quince et al. 2011; Schloss et al.2009) and the

‘chimera.perseus’ command was used to identify chimeric sequences. Needleman algorithm and the reference alignment were used to align the remaining reads. Then, reads showing similarities less than 90 % to the reference sequences were screened to remove nonspecifically amplified reads and to avoid inconsistent classification by size difference, short reads not covering the full alignment were removed. The aligned reads were clustered to remove sequences that were likely due to pyrosequencing errors using the ‘pre.cluster’ command (with the option of diffs=4) and the ‘chimera.uchime’ command was used to remove the chimeric sequences. The remaining reads were classified to each corresponding clade by means of the ‘classify.seqs’ command (k-nearest neighbor approach with an option of k=1) using the reference sequence and its taxonomy files.

Chapter 3

Results

3.1. Hydrographic conditions

3.1.1. Temperature

3.1.1.1. Surface distribution of temperature

Surface temperature showed varied values among seasons (Fig. 3). Temperature decreased to the northern region of the study area in all seasons. In summer 2011, temperature ranged from 20.1-25.1 ˚C. Warmer temperature was in the southern areas and temperature decreased to the northern part. Small eddy was observed with the core centered at station C4. Lower temperature inside the eddy core showed the characteristic of cyclonic eddy event.

In autumn 2011, temperature ranged from 21.8-25.6 ˚C. This study was conducted in the early autumn; therefore, high temperature from summer can still be observed. Temperature decreased toward northern areas. The water mass at the southern part seems to be affected by warm Kuroshio water (along transects A and B). Along transects C and D, temperature difference occurred between coastal and offshore with higher temperature was in the offshore.

In winter 2012, temperature ranged from 12.3-15.5 ˚C. Three distinct temperature conditions can be identified in this season in which warmer and homogeneous water temperature was observed between transect A and

B, rapid changing of temperature between transect B and C, and homogeneous colder water toward transect D.

In spring 2012, temperature ranged from 17.7-19.8 ˚C. During this season, temperature differences were observed from coastal to offshore for all transects. Temperature increased toward offshore areas.

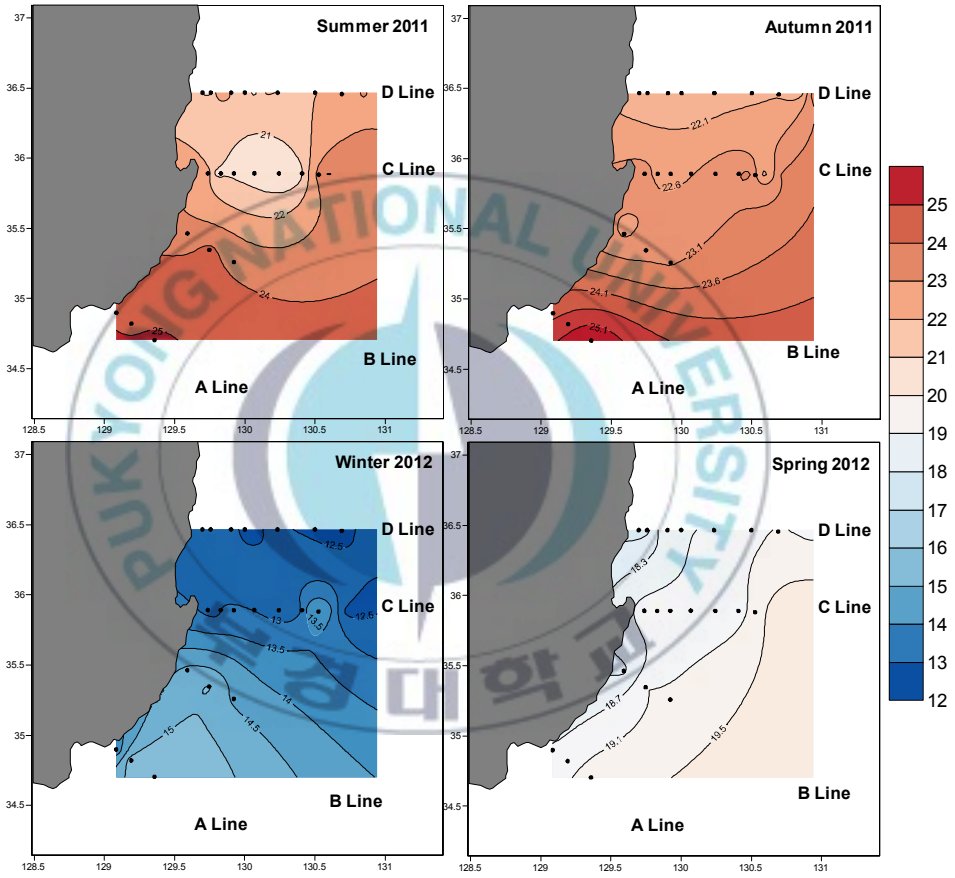

Fig. 3. Surface distribution of temperature (ºC) for all seasons

3.1.1.2. Vertical distribution of temperature

Vertical distribution of temperature for all seasons was shown in Figure 4. Wide range of temperature can be seen from surface to the bottom layers for all seasons, especially during summer and autumn. In summer 2011, temperature distribution ranged from 2.9-28.4 ˚C. Temperature decreased toward northern regions and bottom layers. Transect A showed fairly similar range of temperature in the coastal and offshore stations in the upper 50 m, and at deeper depth, lower temperature was at the coastal areas. Weak stratification layer was observed above 30 m. Rapid decreased of temperature was seen between 30 m to 50 m, and below that temperature decreased slowly to the depth. Slightly similar pattern occurred at transects B and C. However, at transect B, weak stratification was in the upper 30 m and below that thermocline started to appear. At transect C in the upper 30 m, temperature was lower in the core of eddy area (station C4) compared to coastal (station C1) and offshore (station C7). Thermocline occurred below 30 m. Transect D showed stratification from the surface layer for all stations except at the offshore station which has higher temperature in the upper 20 m.

Temperature distribution in autumn 2011 ranged from 2.6-25.7 ˚C. Thicker mixed layers started to appear at transect A that reached 50 m depth. Rapid decreased of temperature was seen between 50 m to 70 m,

and below that temperature decreased slowly to the depth. Thicker mixed layers can be seen at transect B and C only in the offshore stations that reached a depth of 50 m, while in the coastal areas mixed layer was observed only until 30 m depth. Thermocline developed below those depths. Transect D showed shallower mixed layer (30 m depth) similarly from coastal to offshore and thermocline appeared from 30 m depth.

Temperature distribution in winter 2012 ranged from 5.0-15.5 ˚C. Very deep mixed layers over 100 m appeared at transect A and B with very clear temperature difference between coastal and offshore. In contrast, at transect C, mixed layers of temperature were in the upper 70 m and below it temperature started to drop constantly. Transect D showed well mixed above 70 m at the coastal stations and deeper mixed layer at the offshore stations. Thermocline occurred below 70 m of the coastal stations.

Temperature distribution in spring 2012 ranged from 2.5-19.8 ˚C. Well mixed layers can still be observed in some of the stations. Weak stratification layer started to appear below 10 m at transect A. At transect B, coastal stations showed strong stratification from the upper layer, while at the offshore station, strong mixed layer still occurred in the upper 30 m. Transect C displayed very weak stratification layer from 10 m depth at all stations, while transect D started to show strong stratification layers at the coastal. However, warm temperature from the coastal areas flowed to the

deeper part of the offshore station created weak stratification along this area.

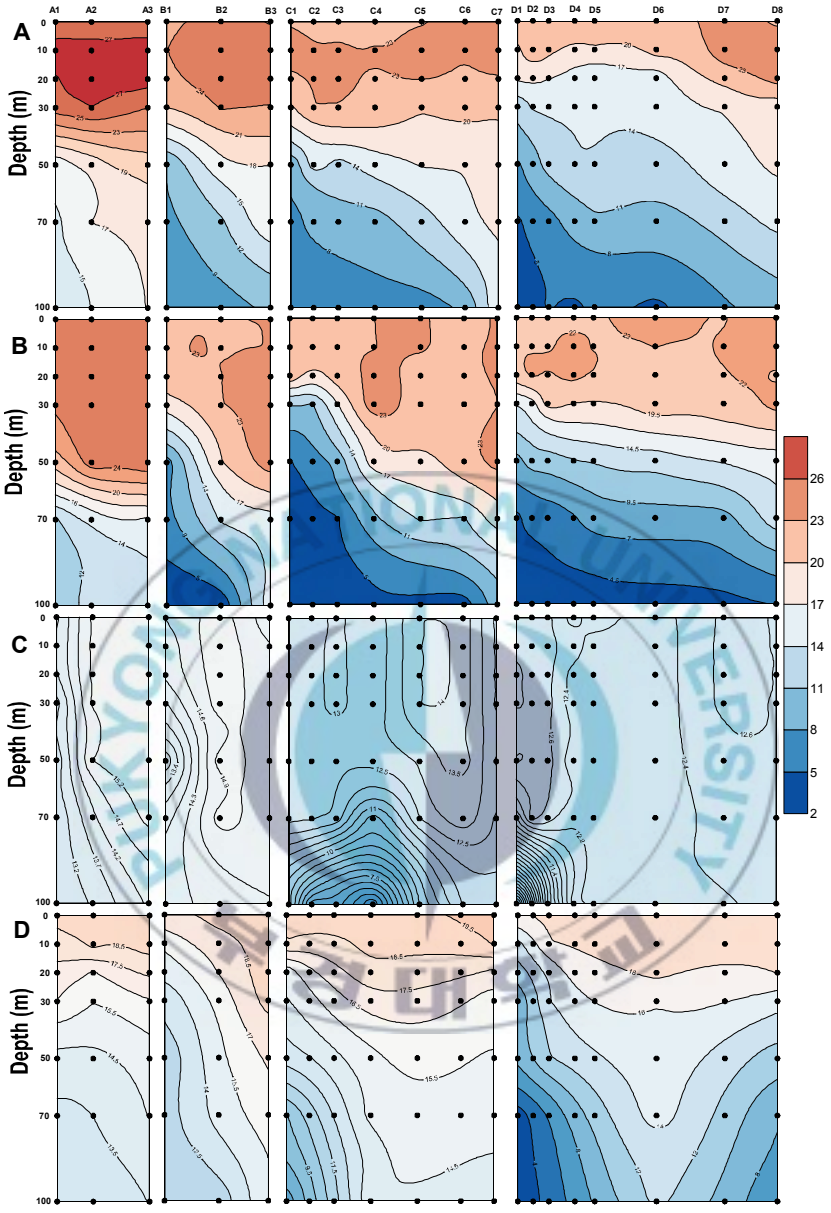

Fig. 4. Vertical distribution of temperature (°C) in summer 2011 (A), autumn 2011 (B), winter 2012 (C), and spring 2012 (D)

3.1.2. Salinity

3.1.2.1. Surface distribution of salinity

Surface salinity showed varied values among seasons (Fig. 5). In summer 2011, salinity ranged from 32.6-33.2 psu. Salinity increased to the northern region and the highest was at station D8. Lower salinity was in coastal areas of transect A and it increased toward offshore in which it started to spread homogeneously toward transect B. More saline water began to distribute from transect B to transect C and it was shown with the dense salinity gradient between those transects. Like the one in transect A, higher salinity was observed in the offshore areas of transect D.

In autumn 2011, salinity ranged from 32.6-34.0 psu. Salinity started to increase from summer and the most significant increasing value occurred at transect A above 33 psu. In contrast to summer, dense salinity gradient during autumn was found from transect A to transect B. At transects C and D, homogeneous distribution of salinity was observed in the northern region and more saline water can be seen in the offshore areas.

Salinity distribution in winter showed quite similar pattern with the one in summer, except that the concentrations were higher. Higher salinity was observed in the southern part of the study areas and salinity decreased toward upper region. Rapid increase of salinity can be seen from coastal to offshore along transect A and it moved toward transect B. Rapid increase

of salinity was observed also between transect B and C, while between transect C and D, salinity difference occurred from coastal to offshore. Less saline water was found in the offshore stations.

In spring 2012, warm saline water was in the offshore along the study areas, while in the coastal areas, less saline water with some patches were observed. Low salinity center was seen at station B2.

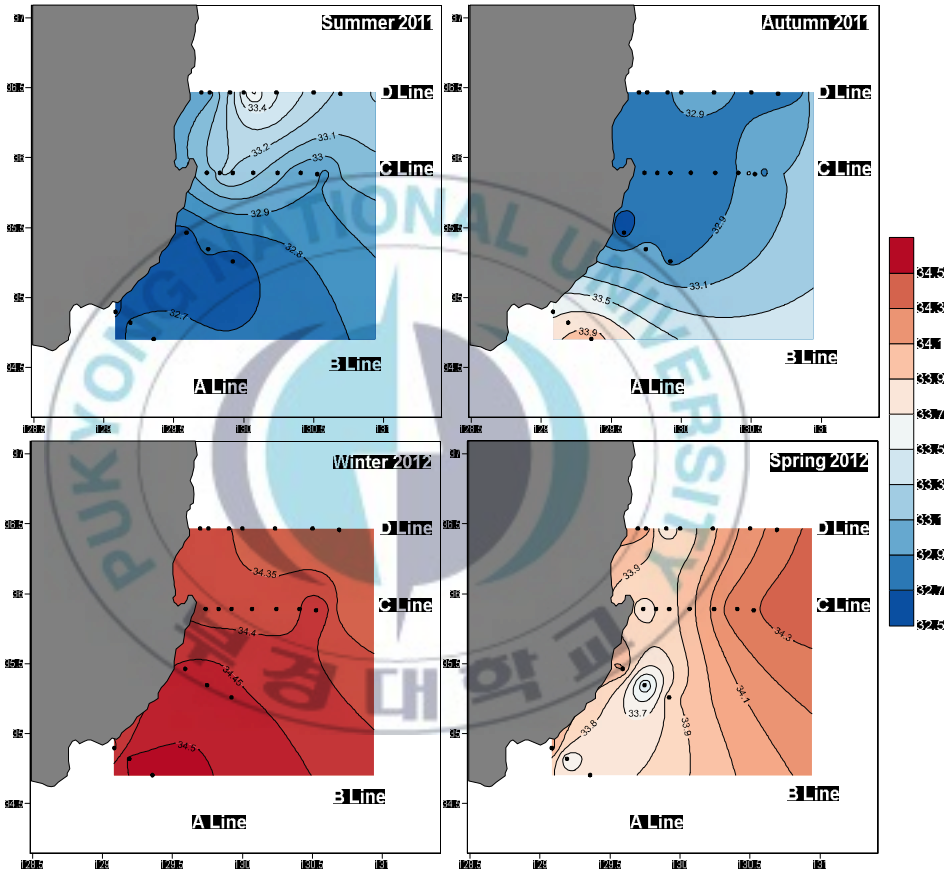

Fig. 5. Surface distribution of salinity (psu) for all seasons

3.1.2.2. Vertical distribution of salinity

Vertical distribution of salinity was shown in Figure 6. Wide range of salinity was in summer followed by autumn, spring, and winter. In summer 2011, salinity ranged from 30.3-34.5 psu. All transects except transect D showed decreased values from the surface layer to the middle layer, before it started to increase again to the deep with varied depth between stations. Similar pattern was displayed at transect A, B, and C in which strong stratification layers were in the upper 10 m. Vertically homogeneous salinity occurred between 10-30 m and salinity started to increase rapidly to 50 m depth. Below 50 m less uniform values were observed. Transect D showed homogeneous vertical distributions of salinity from station D1 to D6 and dense salinity gradient can be seen toward offshore. At station D1 to D6 rapid increase of salinity was observed in the upper 30 m and below that salinity changed in relatively low variation. On the other hand, station D8 showed less saline water with rapid decreased of salinity in the upper 10 m. Salinity started to increase after 20 m depth.

In autumn 2011, high variation pattern of salinity occurred among stations with salinity ranged from 32.6-34.5 psu. At transect A, vertically well mixed salinity were distributed homogeneously above 50 m with higher salinity was in the offshore station than in the coastal stations. Haloclines

appeared below 50 m in all stations and salinity varied in small range after 70 m to the deep. Shallower haloclines were observed at transect B. It occurred at 20 m at station B1 and at 30 m at the offshore stations. Similar pattern was observed at transect C in which shallower halocline was at the coastal station from 10 m to 30 m. At the offshore station, well-mixed salinity layer was in the upper 30 m and salinity increased slowly below it. Transect D displayed well-mixed salinity layer in the upper 20 m for all stations. Rapid stratification layer of salinity can be seen below it and after 50 m depth less uniform values of salinity were observed.

In winter 2012, the distribution of salinity was in a narrow range, ranging from 34.1-34.5 psu. Both at transects A and B, thick mixed layers were distributed vertically with more saline waters were in the offshore areas than in the coastal stations. At transect C, salinity distribution showed well mixed salinity layers in the upper 50 m and dense salinity gradient can be seen below it for all stations. Similar pattern can be seen in the coastal areas of transect D, whereas offshore station displayed very well mixed salinity layer from surface to the bottom.

In spring 2012, the distribution of salinity ranged from 33.4-34.5 psu. Well-mixed salinity layer was observed in the upper 30 m along transect A and homogeneous less saline water was in the stations toward offshore.

Dense salinity gradient started from the surface layers to 50 m depth of transect B and salinity varied in relatively narrow range below 50 m.

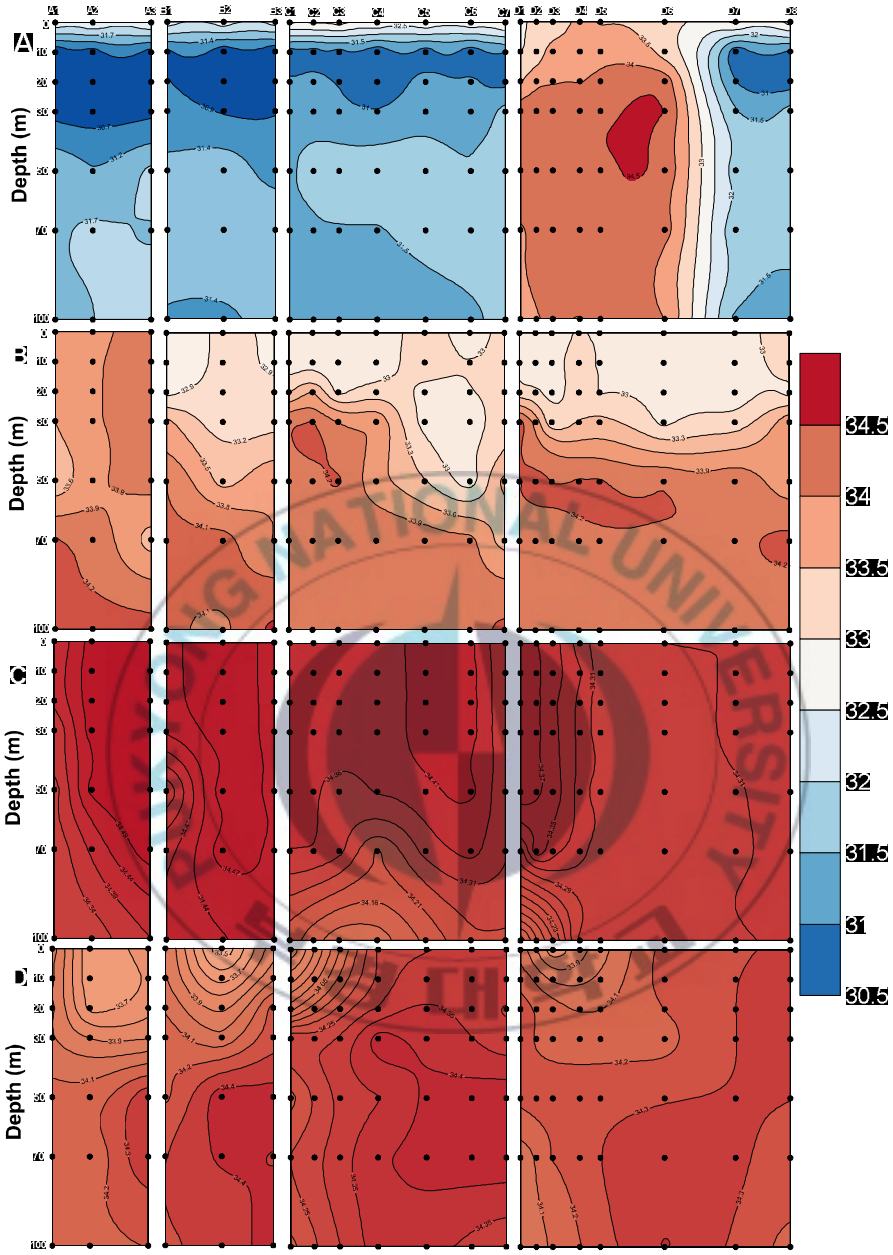

Fig. 6. Vertical distribution of salinity (psu) in summer 2011 (A), autumn 2011 (B), winter 2012 (C), and spring 2012 (D)

3.1.3. Nitrate + nitrite

3.1.3.1. Surface distribution of nitrate + nitrite

Surface distribution of nitrate + nitrite for all seasons was shown in Figure 7. The highest was in winter followed by summer, autumn and spring. Surface distribution of nitrate + nitrite during summer 2011 ranged from 1.8-5.2 µM with the highest was at station D8 and the lowest was at station B1. The distribution showed higher concentration in the offshore than in the coastal areas for all transects, especially at transect B and D in which rapid increased can be seen toward the open ocean. Transect A and C displayed similar distribution pattern with similar values of nitrate + nitrite concentration.

In autumn 2012, surface distribution of nitrate + nitrite ranged from 1.2-3.7 µM. The distribution showed closely similar distribution pattern with the one in summer. The highest concentration was at station B3 and the lowest was at station C1. In winter 2012, nitrate + nitrite concentration increased significantly in the range of 2.8-7.0 µM. Higher concentration was observed toward the open ocean with the highest was in the offshore of transect C. In spring 2012, nitrate + nitrite concentration decreased from the previous season. Higher concentration was in the coastal areas than in the offshore for all transects, except at transect C. The highest concentration was found at station A1 and the lowest was at station D6.

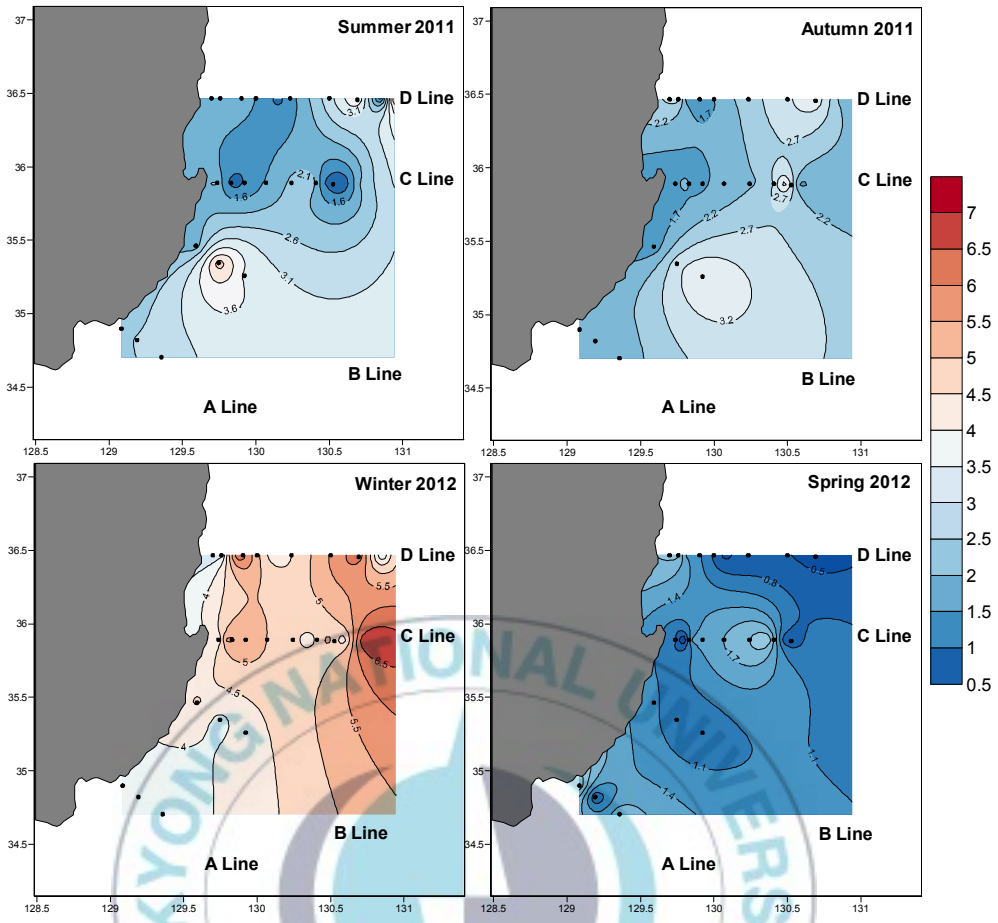

Fig. 7. Surface distribution of nitrate + nitrite (μM) for all seasons

3.1.3.2. Vertical distribution of nitrate + nitrite

Vertical distribution of nitrate + nitrite was shown in Figure 8. Unlike the order of the surface distribution, vertical distribution showed wide range of nitrate + nitrite concentration was in summer followed by autumn, winter, and spring. Nitrate + nitrite distribution in summer 2011 ranged from 0.6-21.5 μM. Shallow mixed layers of nitrate + nitrite concentration can be

seen in the upper 30 m of transect A and B, whereas at transect C, nitracline started to appear from the surface layer. On the other hand, rapid increased of nitrate + nitrite concentration occurred in the upper 50 m of the coastal areas at transect D, while in the offshore areas, vertical distribution showed less varied values.

In autumn 2011, nitrate + nitrite concentration ranged from 0.6-18.6 µM. At transect A, very well mixed layer was found in the upper 50 m and nitracline started to appear below it. The other three transects showed similar pattern in which nitracline was in the shallower depth around 20 m.

In winter 2012, nitrate + nitrite concentration ranged from 2.8-19.7 µM. Very well-mixed layers were seen at transect A, just like the one in the offshore of transect B. In the coastal areas of transect B, well-mixed layer was found in the upper 30 m and below it the concentration increased rapidly. The distributions of both transect C and D showed markedly stratification layers below 50 m.

Concentration of nitrate + nitrite in spring 2012 ranged from 0.4-18.7 µM. Mixed layer still present in the upper layer of transect A, and below 30 m, stratification started to appear. Transect B and D showed similar distribution pattern in which surface mixed layers were in the offshore stations whereas coastal areas showed stratification layers from the surface.

On the other hand, transect C showed variability of nitrate + nitrite concentration started from the upper layer to the bottom.

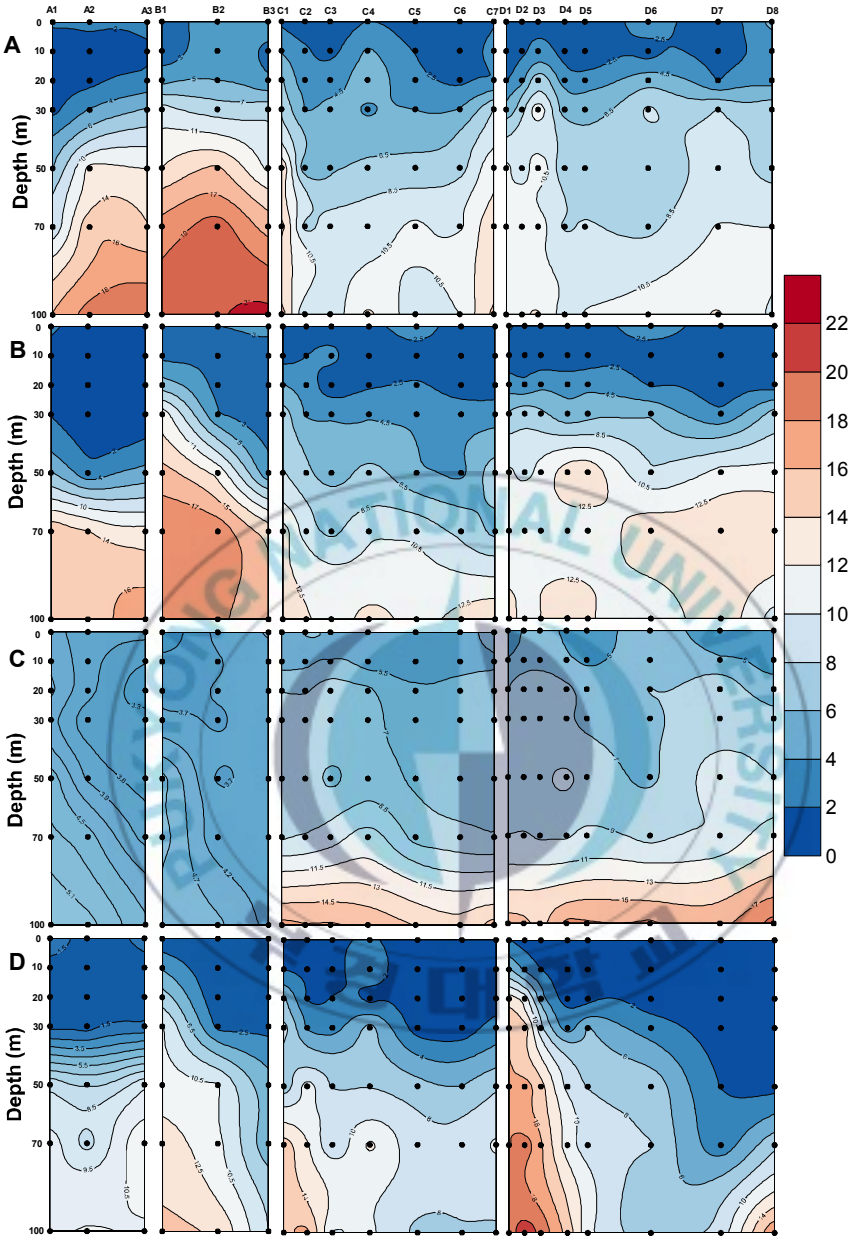

Fig. 8. Vertical distribution of nitrate + nitrite (μM) in summer 2011 (A), autumn 2011 (B), winter 2012 (C), and spring 2012 (D)

3.1.4. Phosphate

3.1.4.1. Surface distribution of phosphate

Surface distribution of phosphate for all seasons was shown in Figure 9. Unlike surface distribution of nitrate + nitrite, surface distribution of phosphate showed higher concentration occurred during summer, followed by winter, spring and autumn. In summer 2011, phosphate concentration ranged from 0.5-0.8 μM in which the highest was at station B1 and the concentration decreased toward northern region.

In autumn and winter, phosphate data only available for transect C and D. Phosphate concentration in autumn 2011 ranged from 0.3-0.5 μM, while the one in winter ranged from 0.5-0.7 μM. Both seasons showed higher concentration was in the coastal areas rather than in the offshore. In spring 2012, phosphate concentration ranged from 0.2-0.7 μM. The concentration decreased toward northern region. The highest concentration was at station B1 and the lowest was at station C1.

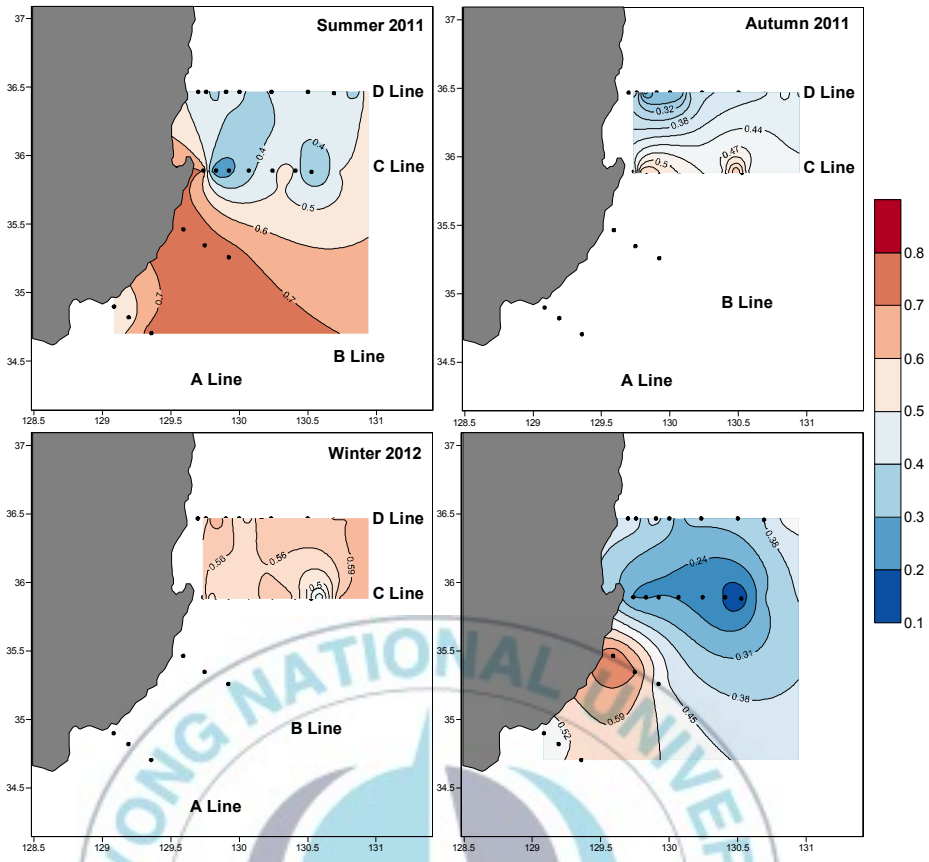

Fig. 9. Surface distribution of phosphate (μM) for all seasons

3.1.4.2. Vertical distribution of phosphate

Vertical distribution of phosphate concentration was shown in Figure 10. The complete phosphate data for all transects were only available during summer 2011 and spring 2012, whereas during autumn 2011 and winter 2012, only data for transect C and D available. In summer 2011, phosphate concentration ranged from 0.4-1.8 μM. Well mixed water layer of phosphate concentration were observed in the upper 30 m of transect A

and B, and the concentration decreased gradually below that depth. Both transects showed higher concentration in the coastal areas than in the offshore at the upper layers and at the bottom the concentration shifted to be higher in the offshore areas. Transect C and D exhibited similar distribution patterns among stations. From surface layer to 10 m depth, phosphate concentrations were distributed homogeneously and stratification started to appear below it. However, at station D6, very well mixed layer can be seen below 30 m to the depth.

In autumn 2011, phosphate concentration ranged from 0.3-1.2 μM. The concentration range decreased from the one in summer. Transect C showed clear input of phosphate from the deeper layer of offshore to the surface layer of the coastal areas. Transect D showed well-mixed layers in the upper 20 m in all stations and the concentrations increased rapidly to the bottom layers.

Phosphate concentration in winter 2012 ranged from 0.5-1.0 μM. Both transects showed deeper mixed layer. At transect C, higher phosphate concentrations in the upper region were observed in the coastal areas, while the opposite occurred at transect D in which higher concentrations were in the offshore. At the bottom region, both transects showed similar concentration between coastal and offshore areas.

In spring 2012, phosphate concentration ranged from not detected to 1.5 μM. Very low concentration of phosphate was at station C7-70 m. During this period of the time, the distribution showed stratification layers that occurred toward offshore. Except for transect A, the other three transects exhibited higher concentration of phosphate in the coastal areas compared to the one in the offshore. Deeper well mixed layer can be seen at station C4.

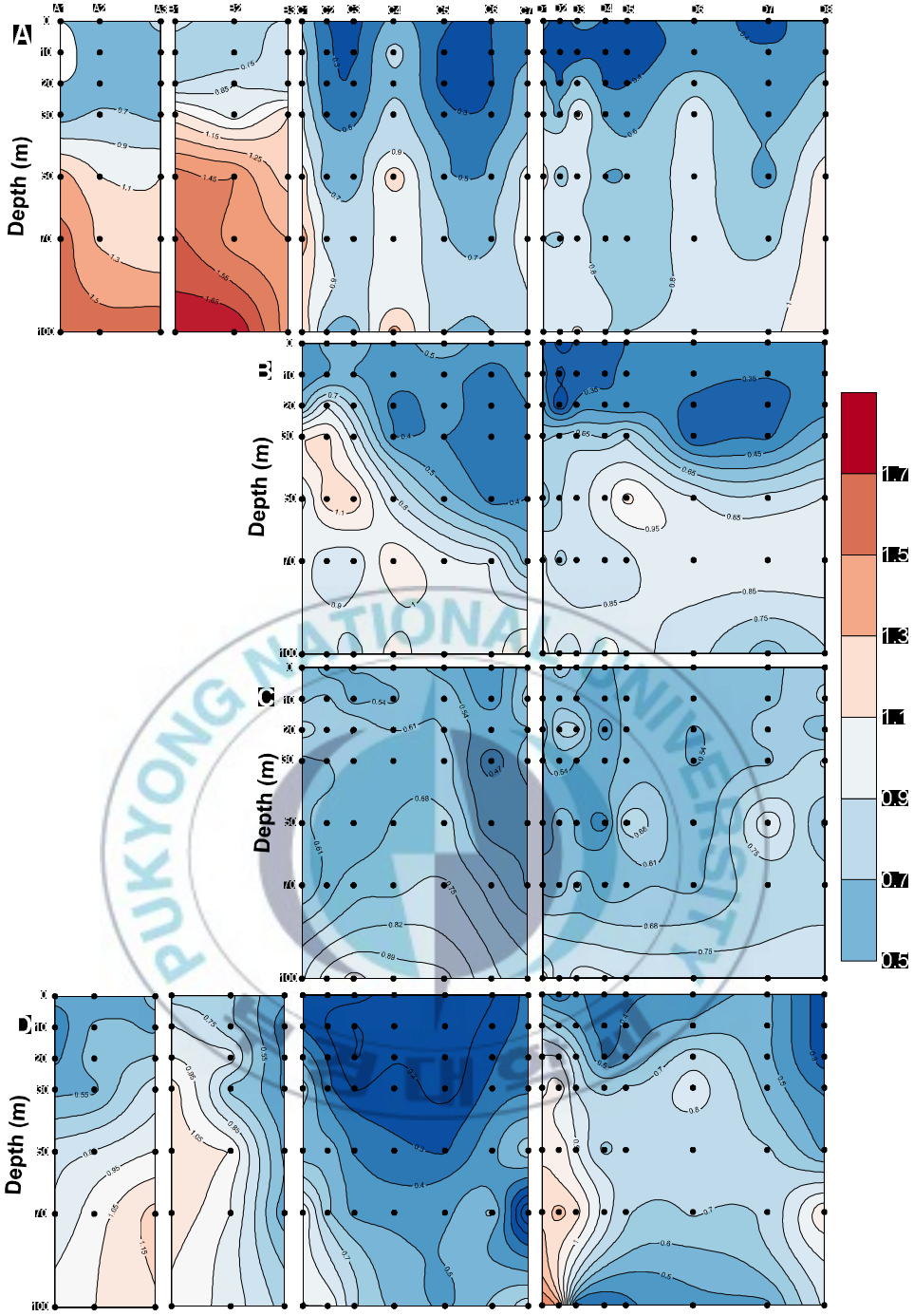

Fig. 10. Vertical distribution of phosphate (μM) in summer 2011 (A), autumn 2011 (B), winter 2012 (C), and spring 2012 (D)

3.2. Chlorophyll *a*

3.2.1. Total Chlorophyll *a*

3.2.1.1. Surface distribution of total Chlorophyll *a*

Surface distribution of total Chl *a* for autumn 2011 and spring 2012 were shown in Figure 11. Higher concentration of Chl *a* was in autumn rather than in spring. Total Chl *a* for autumn 2011 was in the ranged of 0.11-1.27 μg l^{-1}. High Chl *a* was found in coastal areas of transects B and C. Chl *a* concentrations decreased toward offshore waters of transects B and C. Transects A and D showed lower range of concentrations of Chl *a* compared to the one along transects B and C.

In spring 2012, total Chl *a* concentration ranged from 0.04-0.74 μg l^{-1}. Transect B had the highest concentration of Chl *a* among the other transects. Moreover, rapid increased of Chl *a* concentrations were observed from station B1 to B2. The concentration decreased and varied within narrow range in the offshore waters.

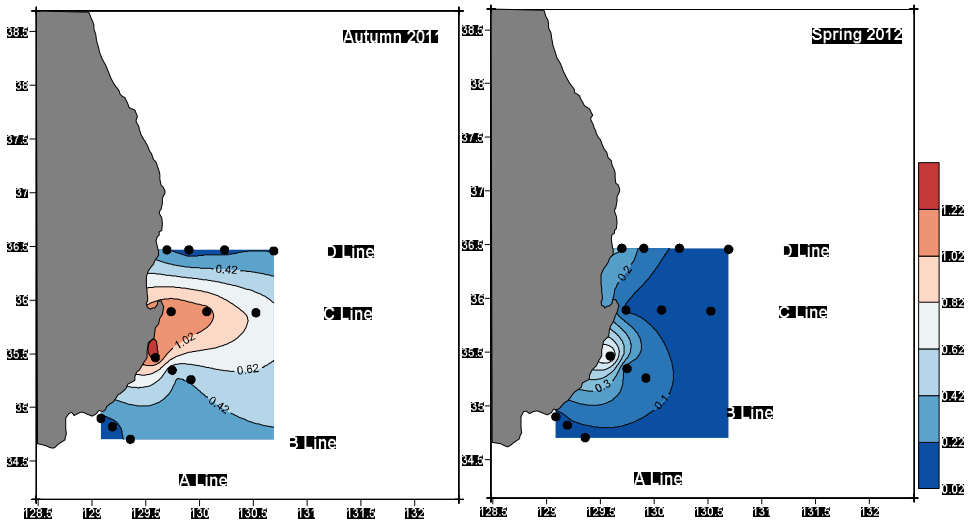

Fig 11. Surface distribution of total Chl *a* (μg l^{-1}) in autumn 2011 and spring 2012

3.2.1.2. Vertical distribution of total Chlorophyll *a*

Vertical distribution of total Chl *a* was shown in Figure 12. Between autumn and spring, higher concentration was found during autumn. Total Chl *a* concentration in autumn ranged from 0.02-1.64 μg l^{-1}, while the one in spring ranged from 0.02-1.37 μg l^{-1}. Along transect A, Chl *a* was relatively low in the surface water, increased and forming a sub-surface Chl *a* maximum layer (10-30 m) and then progressively decreased to the deeper layer. In the upper 30 m, Chl *a* showed an increase concentration toward offshore. In the upper 20 m of transect B, significantly high concentration of Chl *a* was at coastal areas with rapid decreased of concentration was displayed from station B1 to B2. Between station B2

and B3, vertically uniform concentration of Chl *a* was observed in the upper 20 m. Below 30 m concentration decreased rapidly and after 50 m the distribution was quite uniform. Transect C showed vertically uniform concentration of Chl *a* in the upper layer and rapid decreased values were observed in the middle layers. Throughout the water column, higher concentration was in the coastal than in the offshore station. Station C4 exhibited sub-surface Chl *a* maximum layer at 20 m and the concentration decreased rapidly with depth. Transect D showed two distinct high Chl *a* centers at both coastal and offshore station at around 20 m depth. From both of the centers sharp decreased of the concentration occurred horizontally and below 30 m concentration decreased vertically for all stations.

Sub-surface Chl *a* maximum layers were observed in some stations in spring. It occurred at varying depth between 10 to 50 m with the highest sub-surface Chl *a* maximum layer was at 10 m depth of station B1. Coastal area of transect A showed rapid changes of Chl *a* in the upper 50 m, while in the offshore, vertically uniform Chl *a* was shown throughout the water column. At transect B, strong stratification of Chl *a* occurred in the upper 30 m at the coastal areas. In contrast, offshore stations showed relatively weak stratification of Chl *a* in the upper 20 m and quite strong stratification can be seen between 20 to 50 m. Concentration decreased

progressively below 50 m to the depth for all stations. Transects C and D showed distinct stratification of Chl *a* from the upper layer. Sub-surface maximum layer was observed at 20 m in both coastal and offshore stations of transect C. At transect D, sub-surface Chl *a* maximum layer was seen at shallower depth (10 m) at coastal station and at the deeper depth (50 m) at station D6.

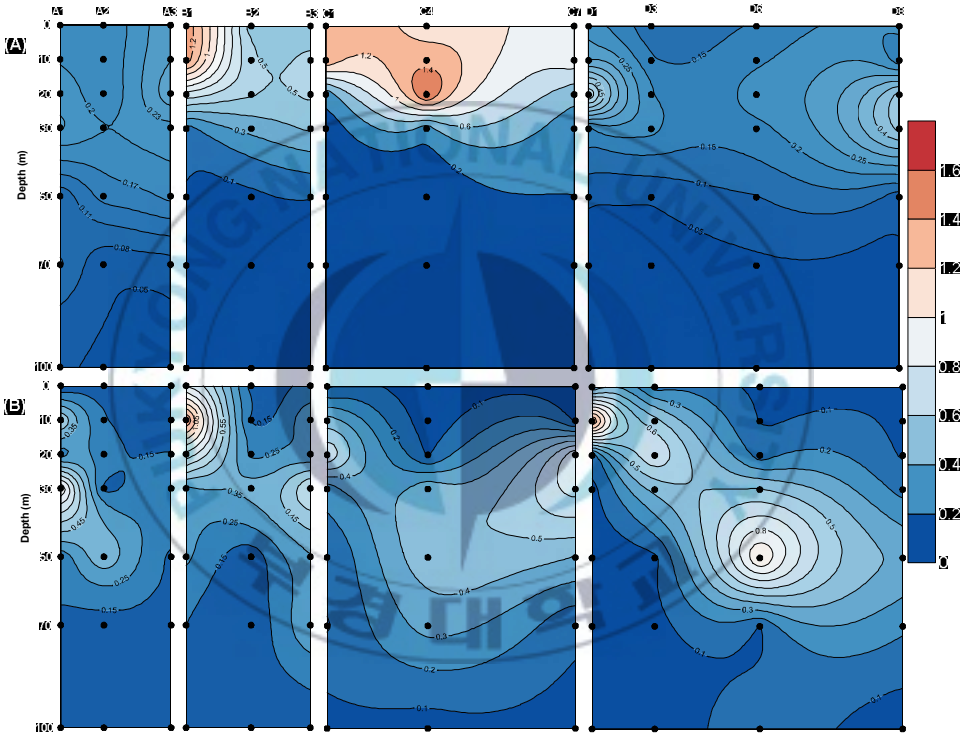

Fig. 12. Vertical distribution of total Chl *a* (µg l^{-1}) in autumn 2011 (A) and spring 2012 (B)

3.2.2. Size fraction of Chlorophyll *a*

3.2.2.1. Surface distribution of pico-size fraction of Chlorophyll *a*

Horizontal distribution of pico-size fraction of Chl *a* was shown in Figure 13. Higher concentration was found in autumn rather than in spring. Concentration of pico-size fraction of Chl *a* in autumn 2011 was in the range of 0.04-0.67 μg l^{-1}, and the one in spring 2012 was in the range of 0.02-0.24 μg l^{-1}. The distribution displayed fairly similar pattern between autumn and spring with the highest was at station B1 and concentration decreased toward offshore. Lower concentration was observed at transect A and D compared to the concentration at transect B and C for both seasons. Transect C in autumn showed Chl *a* concentration gradient from coastal to offshore station with lower concentration was in the middle station. Spring showed more homogeneous Chl *a* concentration along the offshore areas.

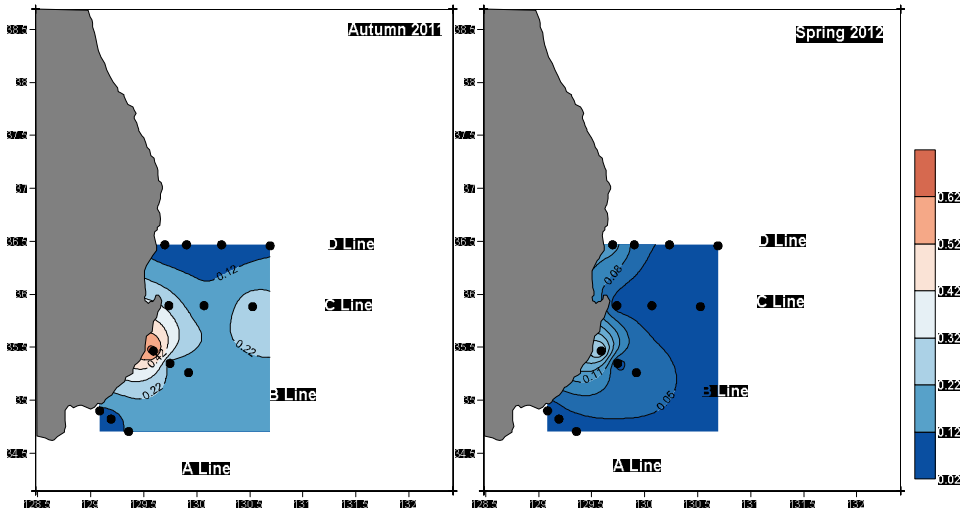

Fig 13. Surface distribution of pico-size fraction of Chl *a* (μg l^{-1}) in autumn 2011 and spring 2012

3.2.2.2. Vertical distribution of pico-size fraction of Chlorophyll *a*

Vertical distribution of pico-size fraction of Chl *a* was shown in Figure 14. Chl *a* concentration in autumn 2011 ranged from 0.002-0.66 μg l^{-1}. Higher concentration was found along transect B and the lowest was along transect A. sub-surface Chl *a* maximum layer was found at 30 m depth of station A1 and at 20 m depth of station C4. Vertically uniform Chl *a* concentration was observed in the upper 50 m of offshore areas of transect A. Transect B showed the highest concentration of Chl *a* in the surface layer of station B1 and then progressively decreased with the depth. Strong stratification layers of Chl *a* was observed at transects C and D in the upper 50 m.

Chl *a* concentration in spring 2012 ranged from 0.01-1.22 μg l^{-1}. Strong stratification layer of Chl *a* was in the upper 50 m at the coastal station of transect A. Compared to the coastal stations, offshore station showed weaker stratification layer of Chl *a* and sub-surface Chl *a* maximum layer can be seen between 20-30 m. At transect B in the coastal area Chl *a* concentration was higher in the upper layer and decreased to the depth. On the other hand, offshore stations showed relatively low concentration of Chl *a* in the surface layer, increased and forming a sub-surface Chl *a* maximum layer (20-50 m) and then decreased with the depth. Transect C showed relatively strong vertical gradient in all depths with sub-surface Chl *a* maximum layer was at 30-50 m depth. Transect D showed weak stratification layers of Chl *a* for all stations. Coastal stations showed shallower sub-surface Chl *a* maximum layer (20-30 m) while deeper sub-surface Chl *a* maximum layer (50 m) was at the offshore station.

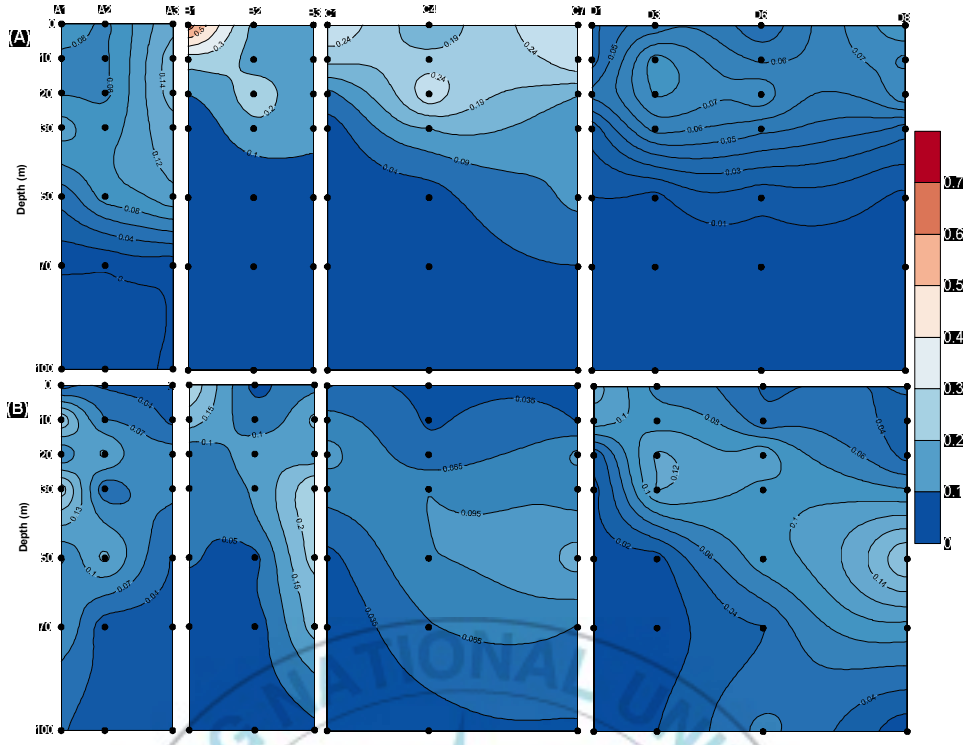

Figure 14. Vertical distribution of pico-size fraction of Chl *a* (μg l^{-1}) of each transect in autumn 2011(A) and spring 2012 (B)

3.2.3. Percentage contribution of pico-size fraction to total Chl *a*

Surface distribution of size fraction of Chl *a* and percentage contribution of pico-size to total Chl *a* was shown in Fig. 15. Chl *a* concentration for both size fraction (micro and pico-size of Chl *a*) was found higher in autumn compared to the one in spring. On the other hand, percentage contribution of pico-size organisms to total Chl *a* was higher in spring rather than in autumn. In autumn, high concentration of Chl *a* for both size fractions was in all stations of transect C and yet these stations have quite

low contribution of pico-size fraction to total Chl *a* (< 50%). In contrast, transect A showed significantly low concentration of both size fraction of Chl *a*, but pico-size fraction contributed fairly high to total Chl *a* (> 50%). High concentration of pico-size fraction of Chl *a* and followed with its high contribution was observed at station B1. Transect D was found to have quite similar pattern with transect A with low concentration of both size fraction of Chl *a*, but quite high contribution of pico-size fraction to total Chl *a*. In spring, transect A and D showed constantly high contribution of pico-size fraction to total Chl *a* regardless their low concentration of Chl *a* for both size fraction. The highest contribution of pico-size fraction to total Chl *a* was at station C4 (74%). However, this station had the lowest concentration of Chl *a* for both size fraction.

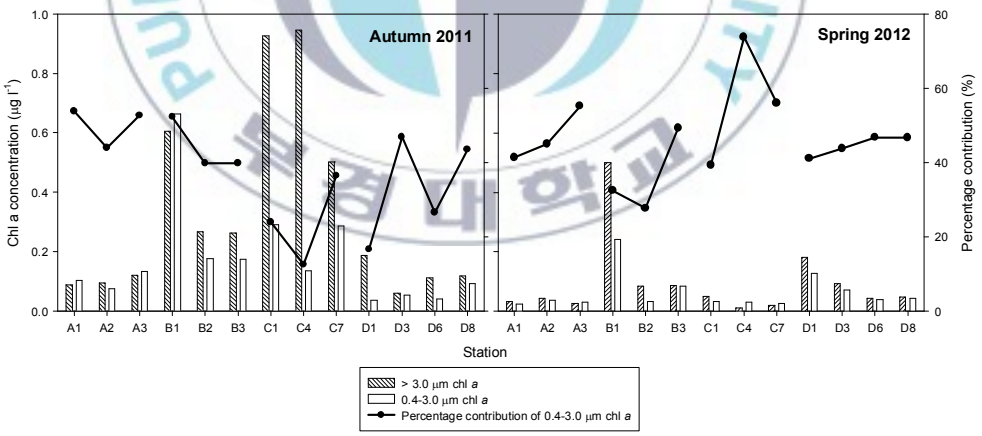

Fig 15. Surface distribution of size fraction of Chl *a* and percentage contribution of pico-size fraction to total Chl *a* in autumn 2011 and spring 2012

3.3. *Synechococcus* seasonal distribution

3.3.1. Surface distribution of *Synechococcus* cells abundance

Surface distribution of *Synechococcus* abundance showed a great variation among seasons (Fig. 16). The highest abundance of Synechococcus was in autumn for almost all stations and the lowest range of abundance was in winter.

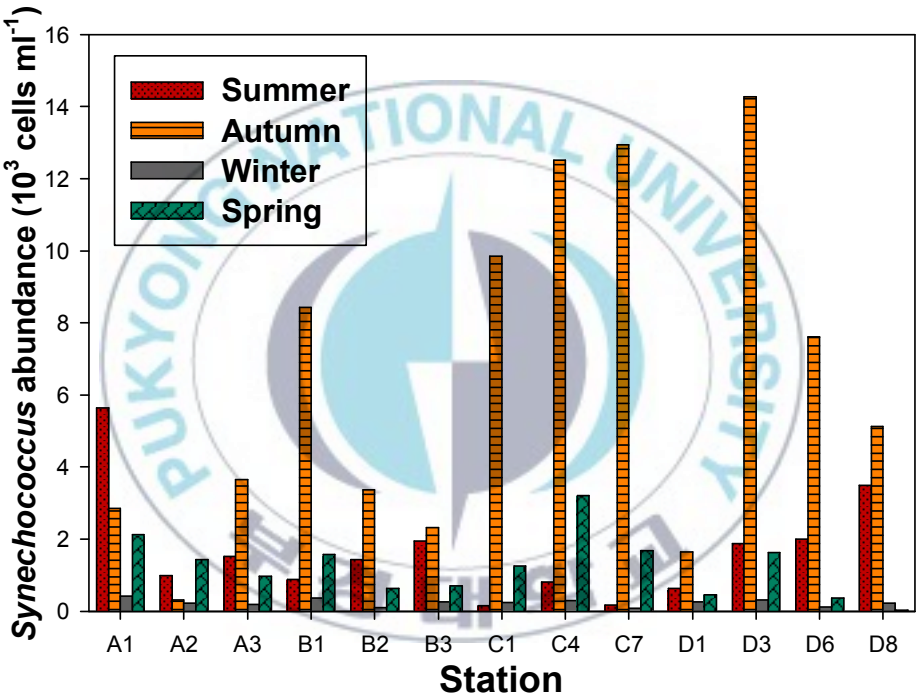

Fig. 16. Surface distribution of *Synechococcus* abundance (cells ml^{-1}) for all seasons

In summer 2011, *Synechococcus* abundance ranged from 0.15 to 5.64 x 10^3 cells ml^{-1} (Fig. 16 and 17A). Distinct distribution pattern of *Synechococcus* can be seen in this season, in which higher abundance of *Synechococcus* occurred in the most southern part and northern part of the study areas (coastal station of transect A and offshore station of transect D). The abundance decreased rapidly in the middle part of the study areas (transects B and C). The highest abundance was found at station A1 and the lowest was at station C1.

In autumn 2011, *Synechococcus* abundance ranged from 0.32 to 14.27 x 10^3 cells ml^{-1} (Fig. 17B). The abundance increased to the northern part of the study areas with the highest was at station D3 and the lowest was at station A2. Both transects A and C showed an increased abundance of *Synechococcus* toward offshore stations, while transect B and D showed higher abundance was in the coastal areas. At transect B, significantly high abundance of *Synechococcus* was observed in the coastal area and declined to the offshore stations. In winter 2012, *Synechococcus* abundance ranged from 0.08 to 0.41 x 10^3 cells ml^{-1} (Fig. 17C). The abundance decreased to the northern region. Higher abundance was observed in the coastal areas rather than in the offshore for all transects.

In spring, *Synechococcus* abundance ranged from 0.45 to 3.21 x 10^3 cells ml^{-1} (17D). The abundance was relatively high in the coastal areas of

transects A and B when the phosphate concentration was higher (Fig. 9). The highest abundance was at station C4 and the lowest was at station D8.

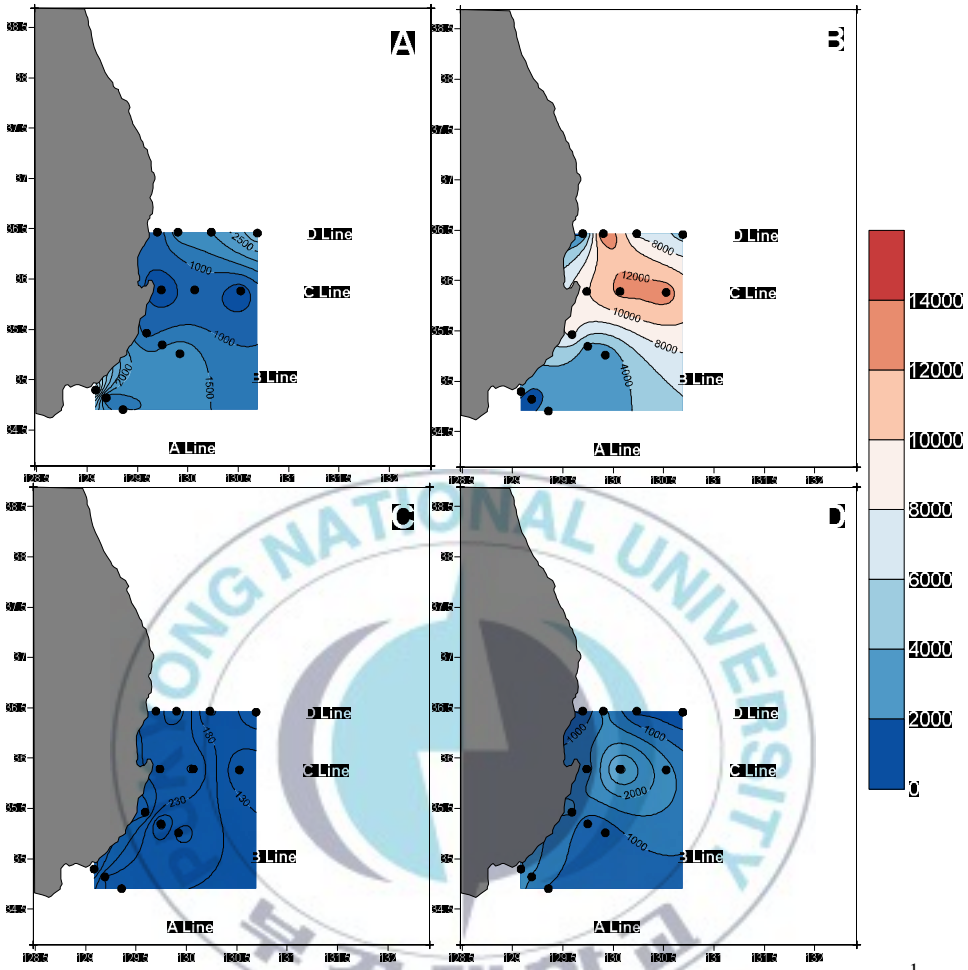

Figure 17. Surface distribution of *Synechococcus* abundance (cells ml^{-1}) in summer 2011 (A), autumn 2011 (B), winter 2012 (C), and spring 2012 (D)

3.3.2. Vertical distribution of *Synechococcus* cells abundance

Vertical distributions of *Synechococcus* for all seasons were shown in Figure 18. The highest abundance was found in autumn, followed by spring, summer and winter. *Synechococcus* abundance in summer ranged

from 0.01 to 5.64 x 10^3 cells ml^{-1}. At transect A, higher abundance was in the surface layer of station A1 and in the middle layer of station A3. Below 30 m the abundance decreased significantly in all stations. Similar ranges of *Synechococcus* abundance was in all stations of transect B which higher abundance was observed in the upper 30 m. Unlike any other stations with higher abundance in the surface layer, transect C showed higher abundance in the middle layers with the highest abundance occurred in the 50 m of C4 station.

Synechococcus abundance in autumn ranged from 0.02 to 14.27 x 10^3 cells ml^{-1}. Higher abundance was found in the upper 50 m for almost all of the stations. At transect A, stratification layers was in the upper 50 m for both station A1 and A3, while at station A2, the abundance was quite uniform from the upper part to the bottom. Sub-surface maximum layer was shown at station A1 at 30 m. At transect B, significantly high abundance of *Synechococcus* and strong stratification layers were found in the upper 20 m of station B1. Below 20 m, the abundance decreased to a value that was similar to the concentration range of the other stations. In contrast with station B1, stations B2 and B3 showed well mixed layer in the upper 20 m depth. Sub-surface maxima were found in both station B1 and B2 but in different depth (10 m at B1 and 30 m at B2). Stratification layers can be observed at transect C for all stations in the upper 50 m. In this transect,

there was no difference of the distribution of *Synechococcus* between coastal and offshore stations. The three stations showed progressively decreased of abundance below 30 m. Transect D showed significantly high *Synechococcus* abundance in the upper 50 m for all stations. Stratification appeared from coastal to offshore. At this transect, sub-surface maxima were at all stations between 10-30 m.

Compared to the other seasons, winter had the lowest range of *Synechococcus* abundance. The abundance ranged from 0.01 to 0.46 x 10^3 cells ml^{-1}. Due to the deeper well mixed layer during this season, the abundance can be found almost in equal number from surface to the bottom layer for all transects. At transect A, rapid changes of abundance can be seen from coastal to offshore stations which higher abundance was in the coastal station. Sub-surface maximum layers (about 30 m) can still be observed in this transect despite its slightly higher number from the surface layers. Higher abundance of *Synechococcus* was found at stations B1 and B3 and significantly low abundance was at B2 station. Sub-surface maximum layer can be found at station B1 and none was observed at the other two stations. B3 station showed considerably high abundance at the surface layer. Transect C showed decreasing concentration from coastal to offshore stations. Clear sub-surface maximum layers were observed in all stations at 10-30 m depth. Similar to transect C, transect D exhibited

higher *Synechococcus* abundance in the coastal areas rather than in the offshore areas. Vertical mixed layer was not as strong as the other transects. Moreover, stratification can be observed in the coastal stations (D1 and D3).

Synechococcus abundance in spring ranged from 0.01 to 6.56 x 10^3 cells ml^{-1}. Sub-surface maximum layers were observed at all stations in the depth between 10 m and 30 m. Station A2 showed two maximum layers at 10 m and 30 m depth. The abundance decreased from coastal to offshore station and rapid changing of abundance can be seen in the upper 50 m at all stations along transect A. On the other hand, transect B showed lower abundance in the coastal station compared to the one in the offshore. In the coastal station, stratification layer started at deeper depth (below 20 m) while in the offshore stations, strong stratification layer can be seen from the surface. Mixing started to happen in the middle layer of offshore stations and the highest abundances were observed in these areas at around 30 m depth for both B2 and B3 station. At transect C, rapid increase of abundance occurred from the surface layer to the middle layer and centered at station C4. Below 30 m, the abundance decreased rapidly. At transect D, a very distinct pattern can be observed. Well mixed layer occurred among three stations (D1, D3, and D6) with markedly low *Synechococcus* abundance from surface to the bottom. These stations even

had the lowest abundance among all stations during spring. However, station D8 showed different pattern in which above 20 m the abundance was also observed at low value. However, below 20 m to 70 m depth, *Synechococcus* abundance started to increase dramatically with the very strong stratification layers. The highest abundance was found at 50 m that reached a value of 6.32 x 10^3 cells ml^{-1}.

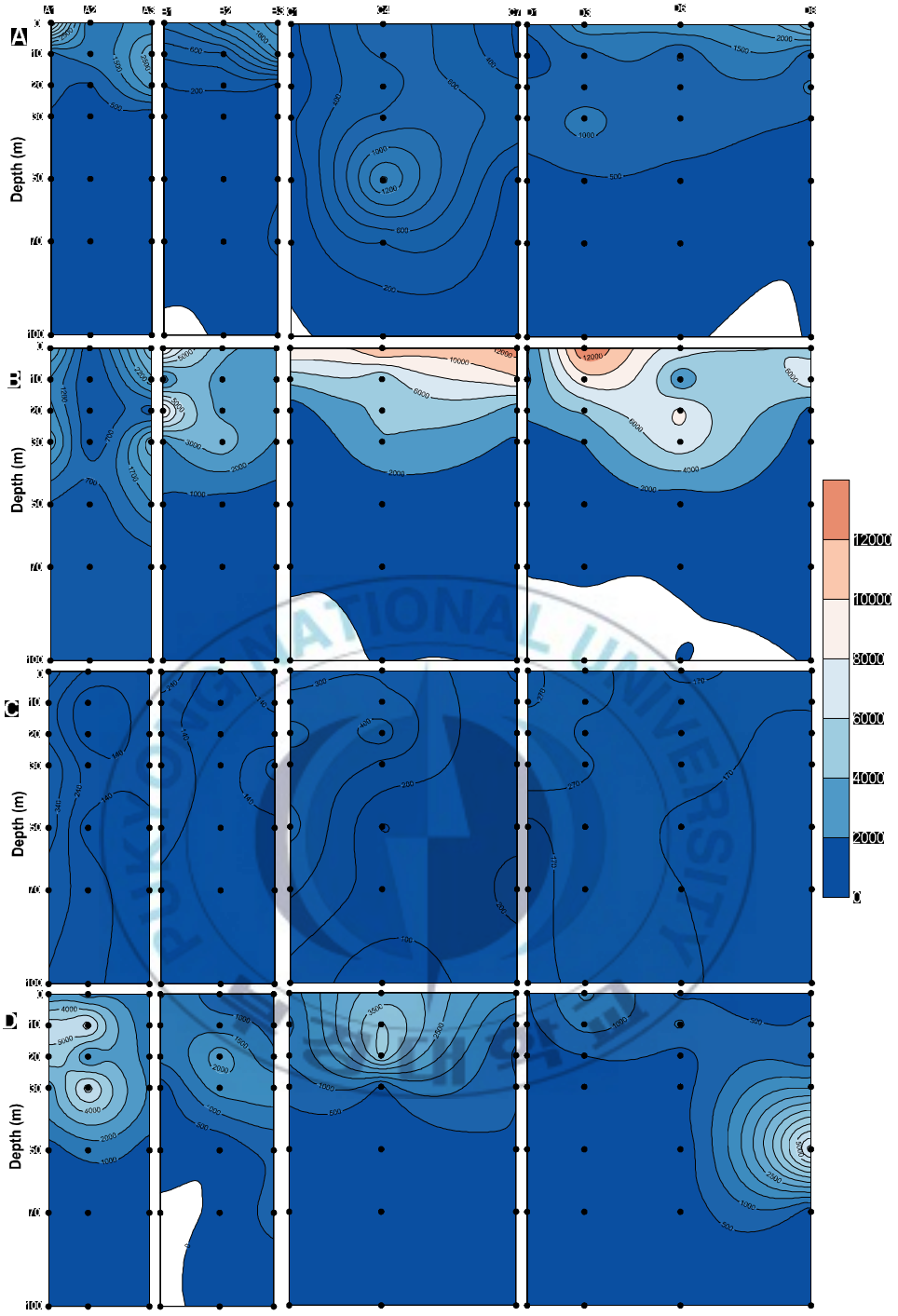

Figure 18. Vertical profiles of *Synechococcus* abundance (cells ml^{-1}) at each transect in summer 2011 (A), autumn 2011 (B), winter 2012 (C), and spring 2012 (D)

3.4. Seasonal variation of PUB_{EX}:PEB_{EX} ratios

3.4.1. Surface variation of PUB_{EX}:PEB_{EX} ratios

Surface distributions of PUB_{EX}:PEB_{EX} ratio for all seasons were shown in Figure 19. In summer 2011, the distribution of PUB_{EX}:PEB_{EX} ratio ranged of 1.05-1.34. The highest ratio was found in the coastal area of transect A.

In autumn 2011, the distribution of PUB_{EX}:PEB_{EX} ratio ranged of 0.83-1.45. Lower PUB_{EX}:PEB_{EX} ratio was found in the northern region and higher ratio was in the southern region of the study areas. Significantly high ratio was in transect A compare to the ratio of the other transects (> 1.4). PUB_{EX}:PEB_{EX} ratio less than 1 was found in all stations of transect C and in the offshore station of transect B.

In winter 2012, the distribution of PUB_{EX}:PEB_{EX} ratio ranged of 1.18-1.48. The ratio changed from coastal to offshore almost in all transects. Transects A and B showed decreasing ratio toward offshore. On the other hand, transect D showed fairly similar ratio from coastal to offshore.

In spring 2012, the distribution of PUB_{EX}:PEB_{EX} ratio ranged of 0.82-1.34. Higher ratios were in the offshore rather than in the coastal areas for all transects. PUB_{EX}:PEB_{EX} ratio less than 1 was found only at station B1 and D3 and the highest ratio was at station A3 (1.34).

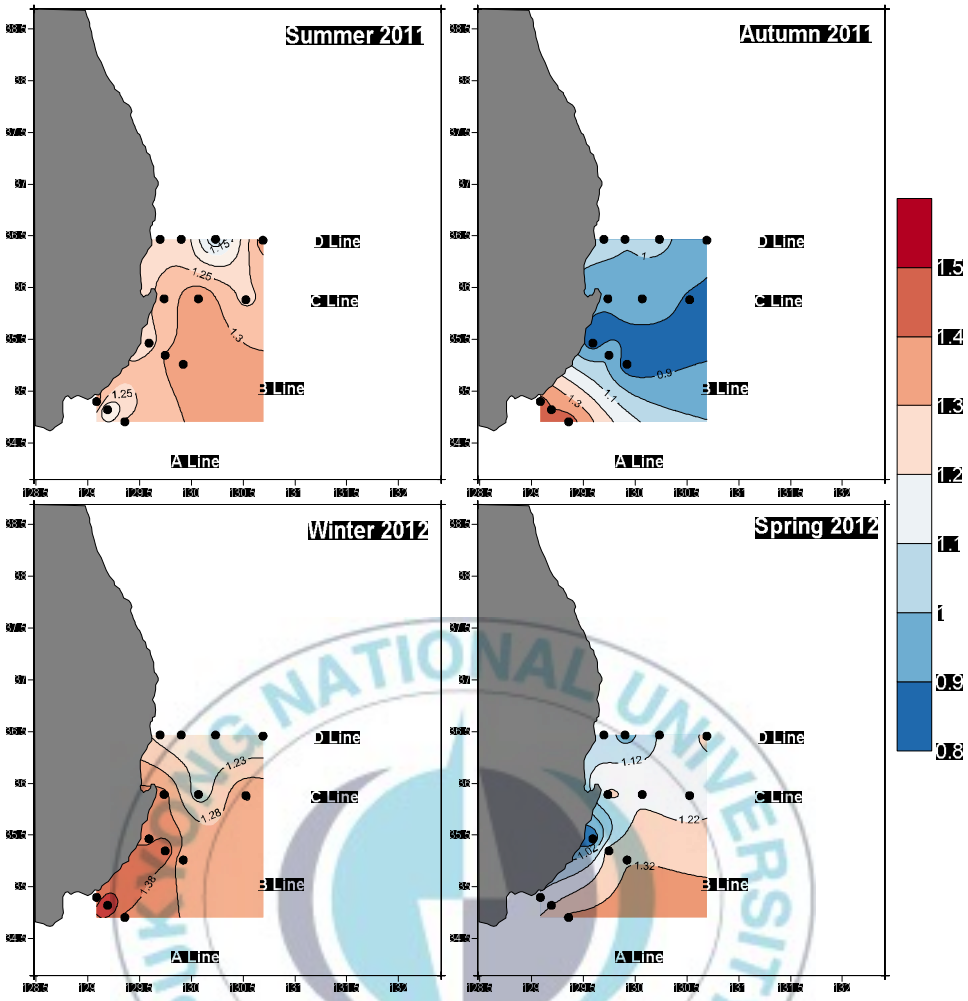

Fig. 19. Surface variation of PUB_{EX}:PEB_{EX} ratio in different seasons

3.4.2. Vertical variation of PUB_{EX}:PEB_{EX} ratios

Vertical distributions of PUB_{EX}:PEB_{EX} ratio for all seasons were shown in Figure 20. Seasonal distribution showed slightly similar range of the ratio among the seasons. Winter had the highest average of PUB_{EX}:PEB_{EX} ratio (1.30±0.08) followed by summer (1.24±0.13), autumn (1.20±0.22) and

spring (1.19±0.15). Ratio less than one were found in the upper 30 m for most of the seasons except during winter which can be found at the deeper layer of station A2 (70 m). The highest occurrence of ratio less than 1 was in autumn (17 out of 84 samples) followed by spring (12 out of 87 samples), and summer (4 out of 88 samples). This study was also observed undetected-peak of PUB chromophore. There are two possibilities that PUB excitation peak cannot be detected. Either there were not any *Synechococcus* contained the PUB chromophore or there were ones but with very low number that is difficult to distinguish its excitation peak with the instrument noise (Wood et al. 1999). Three stations with undetected PUB excitation peak were found in summer, one station during autumn, and one station in spring.

PUB_{EX}:PEB_{EX} ratio in summer 2011 ranged from 0.95-1.61. Ratio less than one was found at station A1 at 10 m depth. The ratio increased in the middle layer and homogeneous distribution can be seen between 30 m to 50 m of stations A2 and A3. Below 70 m, the ratio decreased progressively. The variability of PUB_{EX}:PEB_{EX} ratio at transect B showed lower values in the offshore stations compared to the one at the coastal stations especially in the middle layer. Ratio less than 1 was not detected in this transect and PUB-lacking peak was detected at station B3 at 70 m depth. At transect C, well stratified layer from surface to the bottom was seen at station C4. The

ratio showed decrease value from surface to 10 m depth, increased to 30 m, decreased to 50 m and increased to the deeper depth. On the other hand, coastal and offshore stations showed decrease value from surface to the middle layer (30 m) increase to the depth. Similar to the distribution pattern of transect B, ratio less than 1 was also not detected in this transect. On the other hand, at transect D, three samples were found to have ratio less than 1 (at 20 m, 10 m and 20 m for station D3, D6 and D8, respectively). Ratio distribution showed well stratified layer from the surface layer to the deeper depth. The ratio increased to the depth and coastal stations had higher values compared to the one at offshore.

PUB_{EX}:PEB_{EX} ratio in autumn 2011 ranged from 0.77-1.57. At transect A, the ratio decreased from coastal to offshore. PUB_{EX}:PEB_{EX} ratio less than 1 was not found in this transect. On the other hand, quite many PUB_{EX}:PEB_{EX} ratio less than 1 was found at transect B (5 samples). It appeared in the upper 20 m for most of the stations. Below 30 m, the ratio increased progressively to the bottom with higher ratio was observed in coastal compared to the one in offshore areas. One station was found to have undetected-peak of PUB in this transect which is station B2 at 70 m. Like transect B, PUB_{EX}:PEB_{EX} ratio less than 1 was also found in the upper layers of transect C. Along coastal station, the ratio increased significantly at shallower depth (20 m). Toward offshore stations, the ratio

started to increase at deeper depth (30-50 m). At transect D, well mixed layers of PUB_{EX}:PEB_{EX} ratio less than 1 was observed above 30 m for all stations. Among the other transects, transect D have the most number of PUB_{EX}:PEB_{EX} ratio less than 1 (about 7 samples). Below 30 m, the ratio increased to the bottom layer in the similar range between coastal and offshore stations.

PUB_{EX}:PEB_{EX} ratio in winter 2012 ranged from 0.93-1.48. The ratios decreased to the bottom at transect A. Ratio less than 1 was found at 70 m of station A2. In contrast to transect A, transects B, C and D showed an increasing trend of ratio to the deeper layer. At transect B, strong vertical stratification of the ratio occurred at the upper 30 m for all stations.

PUB_{EX}:PEB_{EX} ratio in spring 2012 ranged from 0.82-1.42. There were three different types of PUB_{EX}:PEB_{EX} ratio variability along transect A. First was the ratio higher than 1 that can be found in the surface layers of all the stations. Second was the ratio less than 1 that appeared in the middle layer (10-30 m). Third was the rapidly increased ratio below 50 m in all stations. At transect B, PUB_{EX}:PEB_{EX} ratio less than 1 was observed in the surface layer of B1 and B2 stations. At the coastal station, stratification layers of PUB_{EX}:PEB_{EX} ratio started at shallow depth (20 m) while at the offshore station, this layer started below 50 m. At transect C, stratification layer of PUB_{EX}:PEB_{EX} ratio occurred in the upper 50 m at the

coastal station. At transect D, PUB_{EX}:PEB_{EX} ratio less than 1 was observed only at station D3 at 0 m and 10 m. In addition, stations D1 at 20 m showed undetected-peak of PUB.

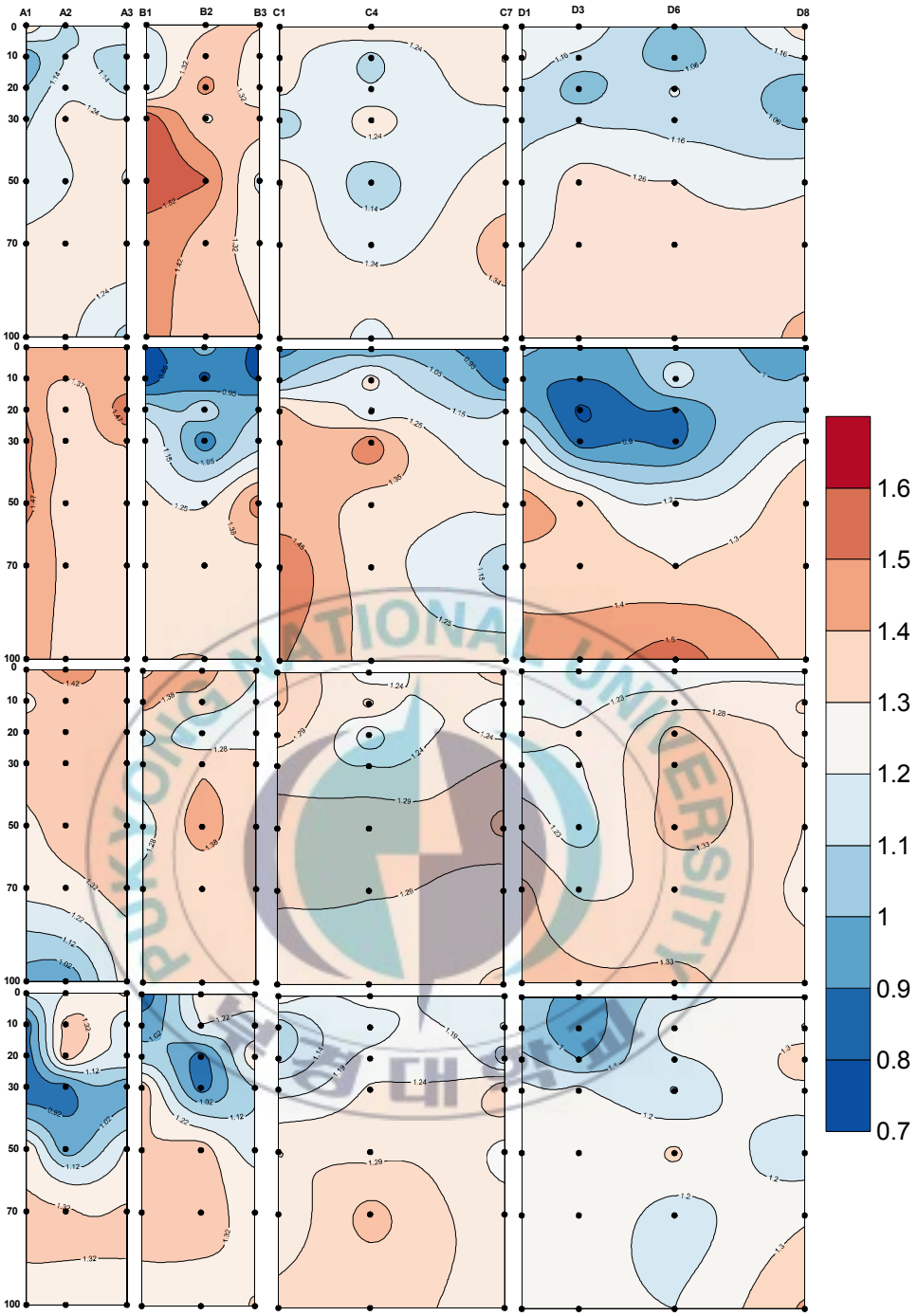

Fig. 20. Vertical variation of PUB_{EX}:PEB_{EX} ratio in summer 2011 (A), autumn 2011 (B), winter 2012 (C), and spring 2012 (D)

3.5. *Synechococcus* clade distribution

Based on the database that has been constructed by Choi et al. (2013a) using ITS sequences from cultures and clone libraries from previous studies, mothur software was able to classify the sequences in this study into corresponding clades. They were 15 clades belong to *Synechococcus* subcluster 5.1, one clades belong to *Synechococcus* subcluster 5.2, and six clades belong to *Synechococcus* subcluster 5.3. In total, 22 clades were found. The differentiation of *Synechococcus* into three different subclusters was based on the composition of the major light harvesting pigment, an ability to perform a novel swimming motility, whether there is an elevated salt requirement for growth, and also G+C content (Fuller et al. 2003).

3.5.1. Surface distribution of *Synechococcus* clade

Synechococcus diversity was found lower in spring 2012 compared to the one in autumn 2012. Surface distribution of *Synechococcus* clades in spring 2012 was shown in Table 4. In spring, four clades (contribution > 1 %) from *Synechococcus* subcluster 5.1 were found, with the highest diversity was at station C7. There were three dominant clades: Syn5.1-I, Syn5.1-II and Syn5.1-IV (Fig. 21). *Synechococcus* clade 5.1-I dominated the study areas in spring and the percentage occurrence was higher in the

coastal areas in all transects. The highest occurrence of clade Syn5.1-I was at station B1 and B2 (~ 70 %), while clade Syn5.1-IV was found markedly high at station A2 (52 %) and C1 (60%). Among the other stations, clade Syn5.1-II dominated only at station C7 with contribution about 41 %. Syn5.1-VII was observed only at station C7 with the occurrence of 1.5 %.

Horizontal distribution of *Synechococcus* clades in autumn 2012 was shown in Table 5. Due to weather conditions during sampling period, the data for autumn was only at transect A and B and one station of transect C (station C1). With lesser sampling stations, however, higher diversity of *Synechococcus* clades was in autumn rather than in spring. There were six clades found during autumn from *Synechococcus* subcluster 5.1 and subcluster 5.3. Based on Figure 22, Syn5.1-II was the dominating clade with percentage occurrence > 50 %, followed by Syn5.1-I (10-50 % contribution). The highest contribution of Syn5.1-I was at station B3 (43.9 %), while Syn5.1-II was found highest at station B1 (68.1 %). Two different clades found in slightly high values in autumn and were not dominating in spring were Syn5.1-III and Syn5.3-I/II. On the other hand, Syn5.1-IV that was dominant in spring was only found at one station (station B3) with percentage of 1.5 % in autumn.

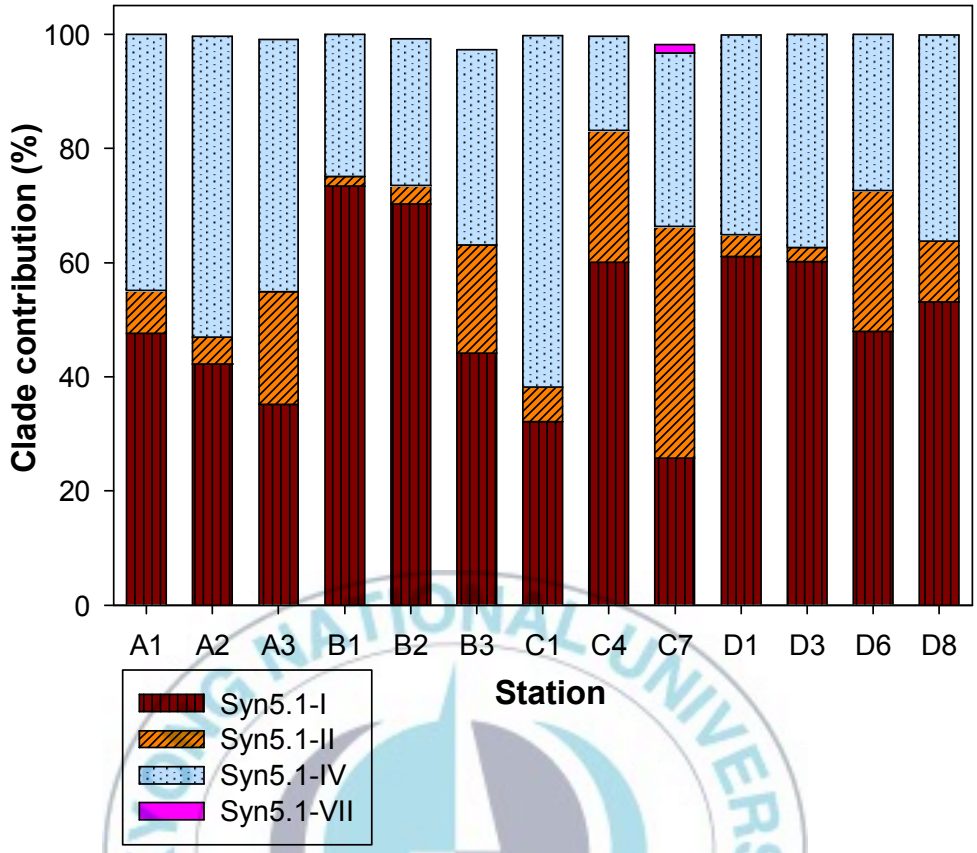

Fig. 21. Surface distribution of the percentage occurrence of *Synechococcus* clade (%) in spring 2012

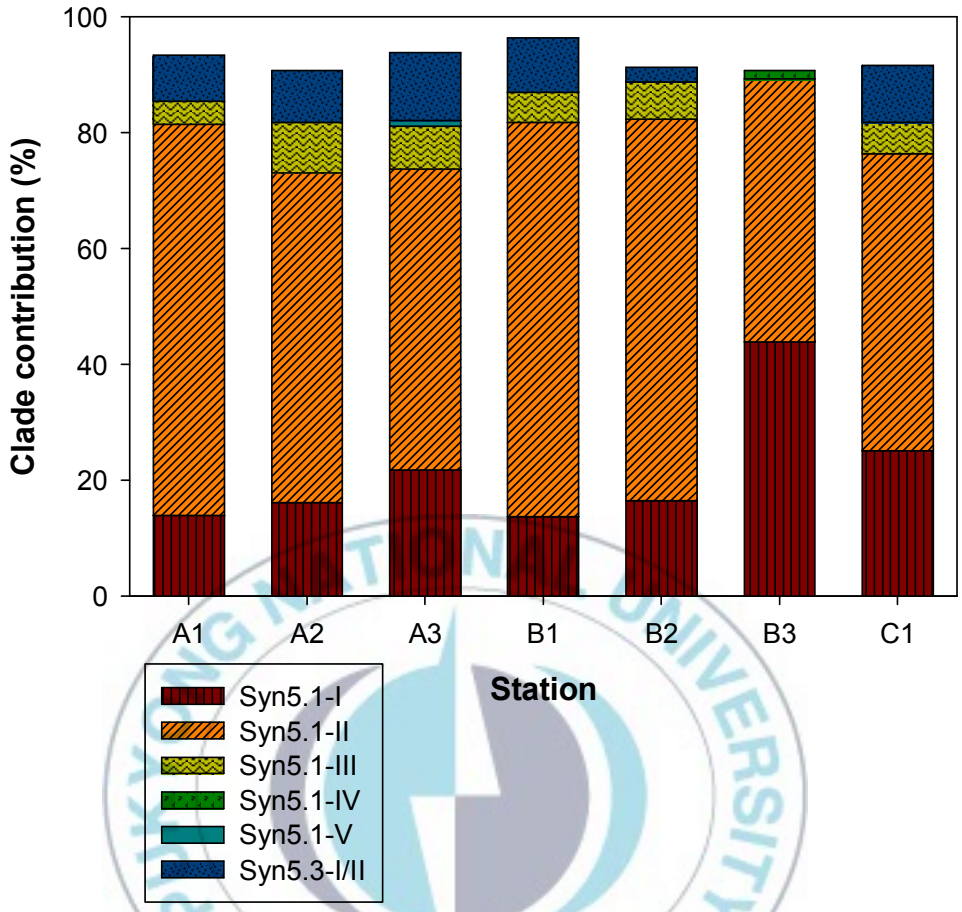

Fig. 22. Surface distribution of the percentage occurrence of *Synechococcus* clade (%) in autumn 2012

3.5.2. Vertical distribution of *Synechococcus* clade

Spring 2012

Vertical distribution of *Synechococcus* clade in spring 2012 showed higher diversity in the offshore than in the coastal areas. The most dominant clades present in almost all stations and every depth were Syn5.1-I, Syn5.1-II, and Syn5.1-IV. The other clades were present at low percentage (< 1 %). Syn5.1-I was always found as the highest contributor (the average of 61.9±13.1 %) among the other clades with the highest contribution was found at transect D compared to the other transects. It did not appear only at Station C4 at 70 m. Syn5.1-IV was found in all transects with the highest contribution was at transect A (the average of 33.2±10.9 %).The highest contribution of Syn5.1-II was found at transect C (~ 22 %) among the other transects.

Vertical distribution of *Synechococcus* clades along transect A was shown in Fig. 23. Syn5.1-I appeared in lower percentage at the upper layers and increased to the depth. The highest can be found at station C7 at 100 m with contribution up to 80 %. Unlike Syn5.1-I, Syn5.1-IV was found higher in the upper layers and decreased to the depth, while Syn5.1-II was found in the similar range between upper and deeper layers. Both Syn5.1-XVII and Syn5.1-CRD1 was found with

contribution of 3.8 % at station A3 at 70 m and Syn5.1-XVI contributed 1.9 % at 20 m depth of station A2.

Vertical distribution of *Synechococcus* clades along transect B was shown in Fig. 24. Transect B showed sub-surface maximum value (~70 %) of Syn5.1-I around 10-30 m. The lowest was found at 50 m depth of station B3 with contribution of 33.7 %. Syn5.1-IV showed an increase contribution to the depth in the coastal areas but in the offshore areas, vertical distribution varied in a small range. Syn5.1-II also showed small variation between upper and deeper layers in all stations, however, the contribution was higher in the offshore station (station B3) compared to the one in the coastal areas. Low percentage of Syn5.1-XVI (1 %) appeared in the upper layer of station B3, while Syn5.1-CRD1 contributed (1-3 %) from 30 m to the 100 m.

Vertical distribution of *Synechococcus* clades along transect C was shown in Fig. 25. Vertical distribution of Syn5.1-I showed a slight variation between coastal and offshore areas. Sub-surface maximum values can be observed at station C1 and C4 at 10-20 m, while station C7 showed low contribution of Syn5.1-I in the upper layer and increased significantly to the deeper layers. The highest contribution was found at 100 m depth of station C7 that reached a value of 86.3 %. The average contribution of Syn5.1-IV was about 25 % with the highest was up to 60 %

on the surface layer of station C1. Station C1 showed lower contribution to the deeper layers, on the contrary station C4 and C7 showed higher contribution in the upper layers. Syn5.1-II showed similar trend from surface to the deeper layer at station C1 and C4. At these stations, the contribution of Syn5.1-II was less than 20 %. However, at station C7, Syn5.1-II showed higher contribution in the upper 10 m (~ 40%). It was coincided with the warm water masses flowing along the offshore (Fig. 3) Three clades with contribution > 1 % found were Syn5.1-VII (was found in the upper layer of station C7), Syn5.1-XVI (was found in the deeper layer of station C4), and Syn5.1-CRD1 in the middle layer of station C1 and C4.

Vertical distribution of *Synechococcus* clades along transect D was shown in Fig. 26. The distribution of Syn5.1-I showed an increase contribution to the deeper layers at all stations. On the other hand, Syn5.1-IV showed an inverse pattern in which the contribution decreased to the deeper layers. *Synechococcus* subcluster 5.3 clade I/II was not found in the other transects, however, at transect D it contributed around ~ 4 % at the deeper layer.

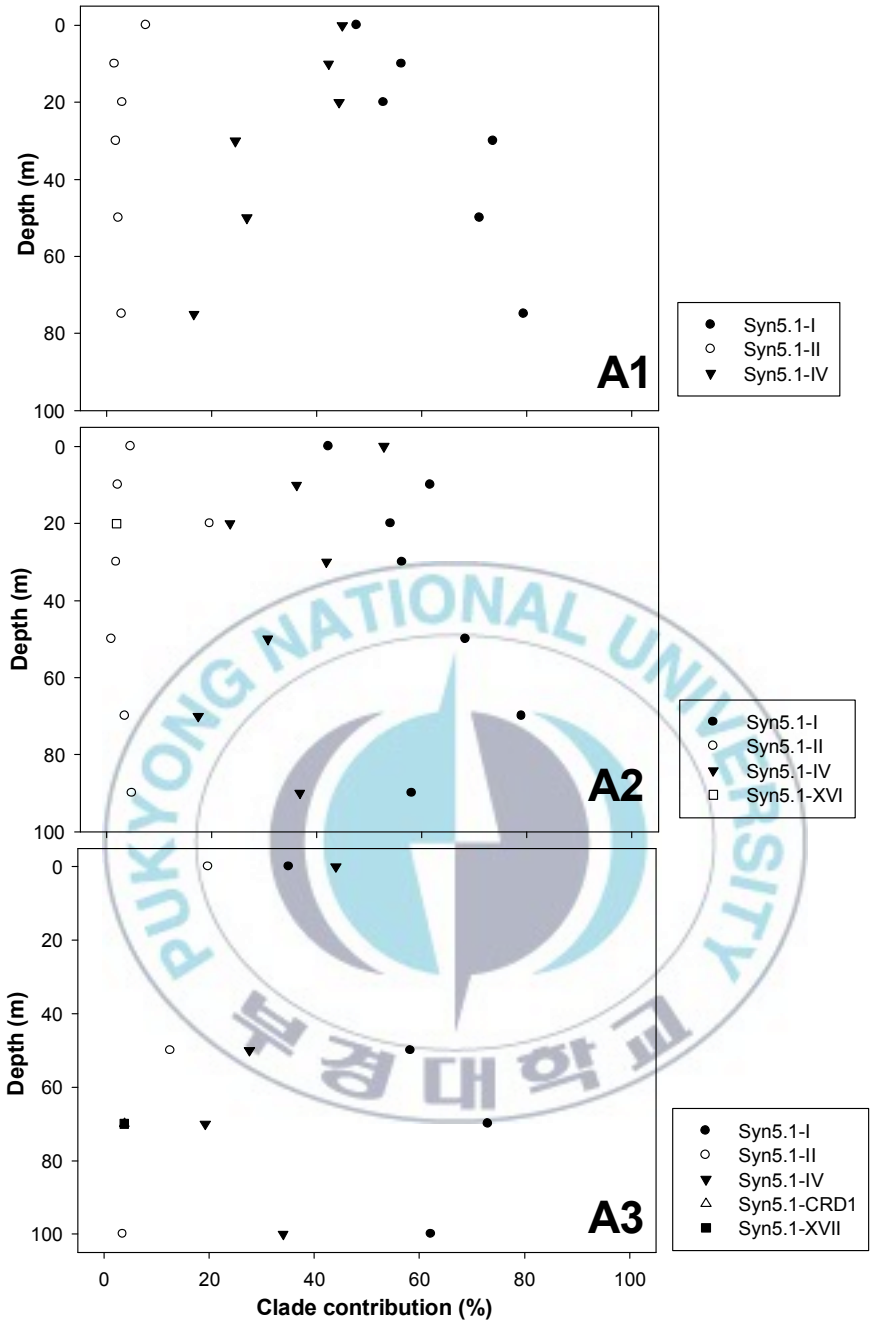

Fig. 23. Vertical distribution of percentage occurrence (%) of *Synechococcus* clades along transect A in spring 2012

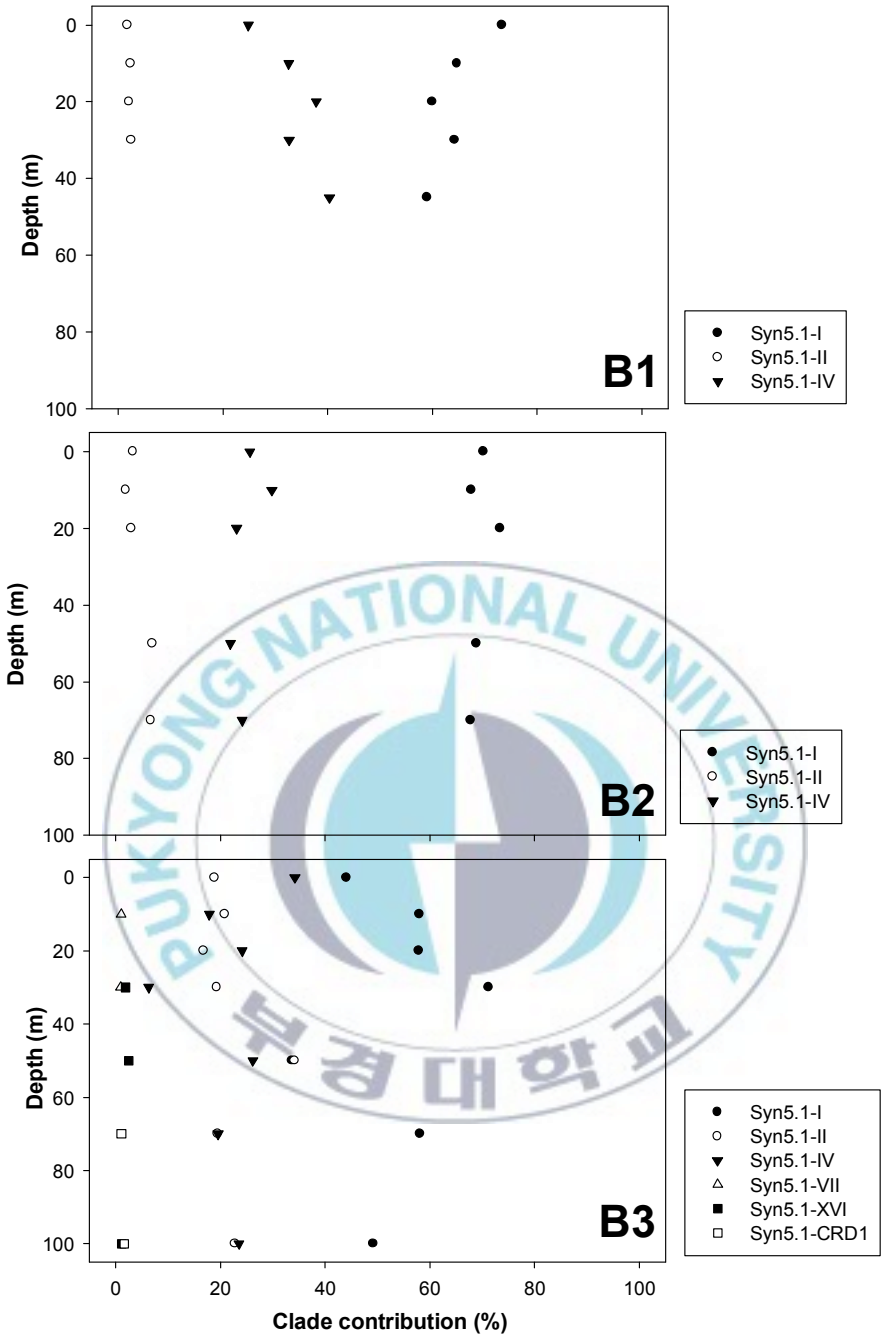

Fig. 24. Vertical distribution of percentage occurrence (%) of *Synechococcus* clades along transect B in spring 2012

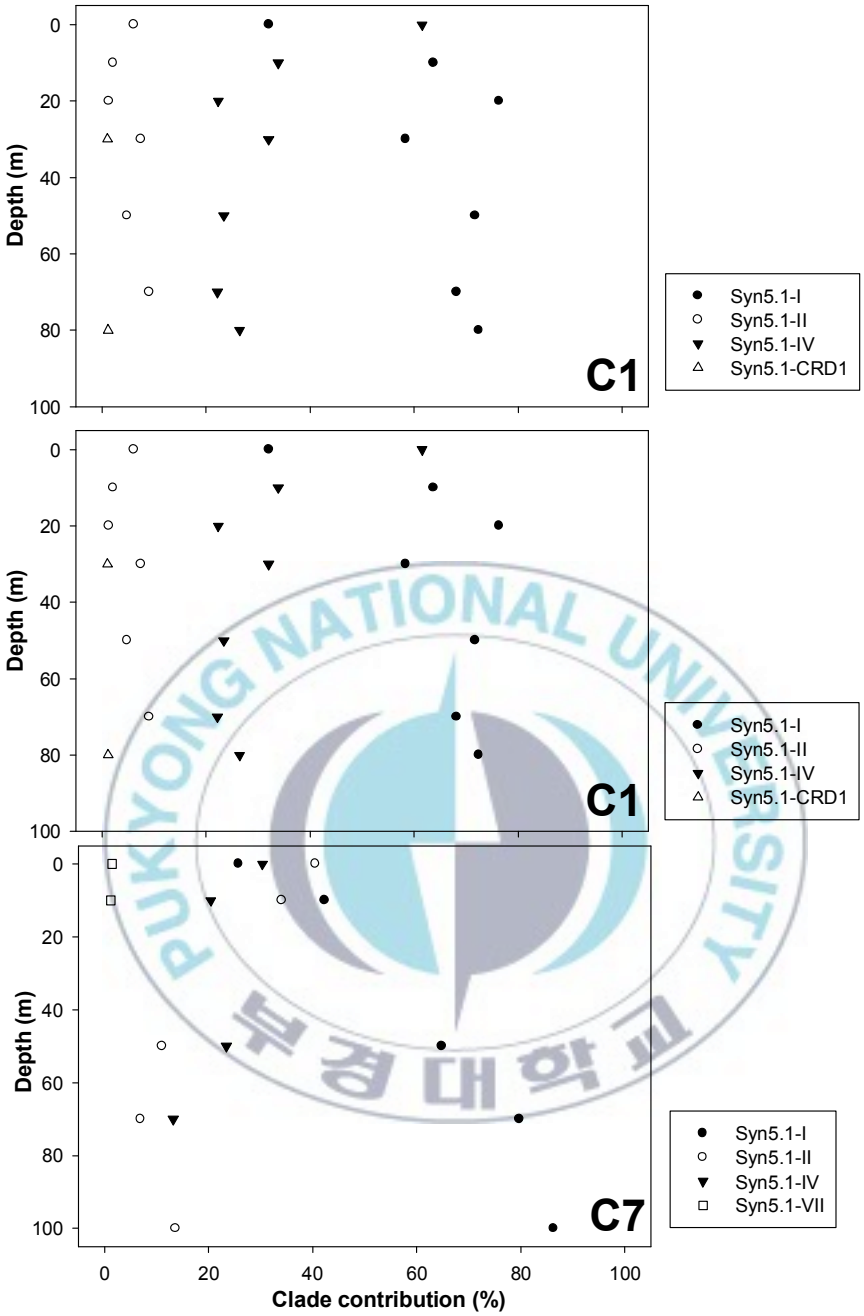

Fig. 25. Vertical distribution of percentage occurrence (%) of *Synechococcus* clades along transect C in spring 2012

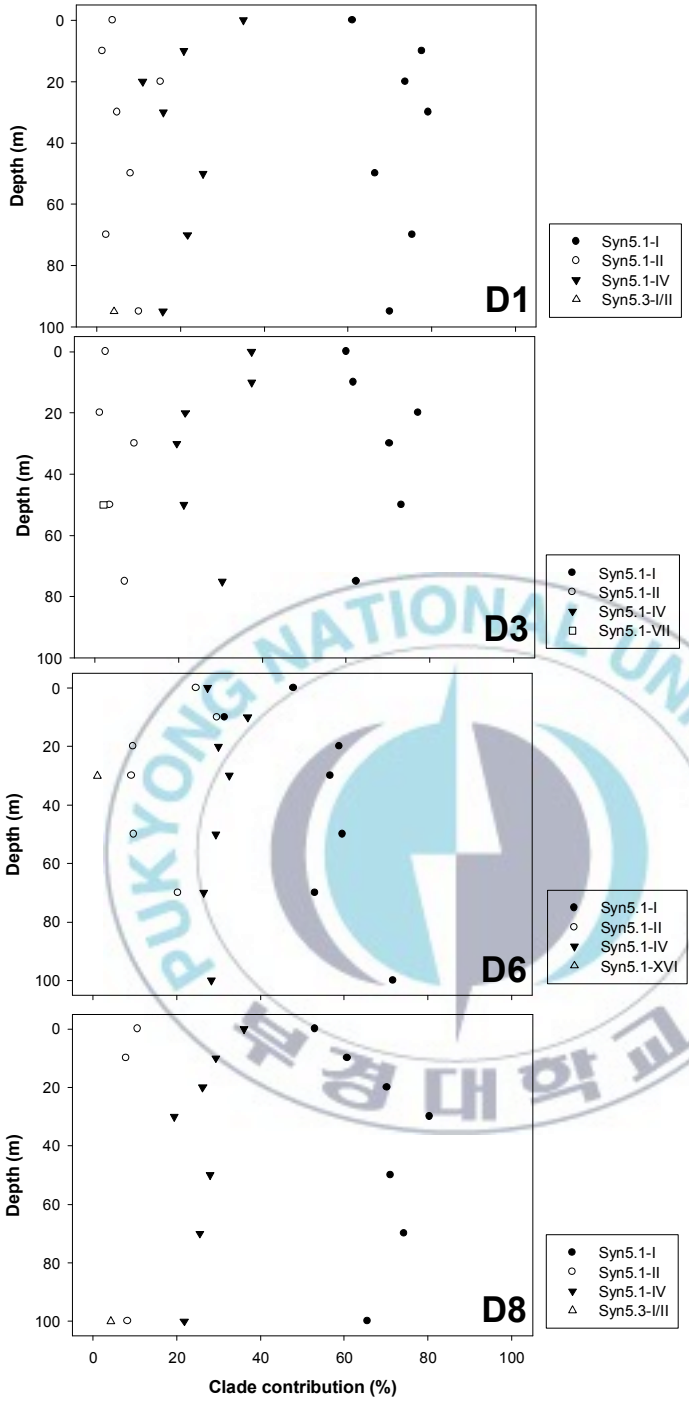

Fig. 26. Vertical distribution of percentage occurrence (%) of *Synechococcus* clades along transect D in spring 2012

Autumn 2012

Vertical distribution of *Synechococcus* clade in autumn 2012 showed higher diversity compared to the one in spring 2012. Syn5.1-II was found to be the dominating clade (~ 40 %) during this season. Vertical distribution of *Synechococcus* clades along transect A was shown in Figure 27. Syn5.1-II was found higher in the upper 30 m and the contribution decreased rapidly to the depth. In contrary of Syn5.1-II, Syn5.1-I have lower contribution in the upper layer and increased at the deeper layer. Syn5.3-I/II was found in all stations and distributed in similar range from upper to the deeper layer and also from coastal to offshore station. It was the third highest dominating clade with the average of 7.2±2.4 % after Syn5.1-II (49.4±17.8 %) and Syn5.1-I (22.6±10.7 %).The other clades found with the average contribution > 1 % were Syn5.1-III (5.2±2.1 %), Syn5.1-VI (2.8±1.2 %), Syn5.1-V (3.4±3.4 %), and Syn5.1-IV (3.0±4.4).

Vertical distribution of *Synechococcus* clades along transect B was shown in Figure 28. Syn5.1-II contributed lower in this transect compared to the contribution along transect A. However, the distribution showed similar pattern in which the contribution was higher in the upper layer, and decreased rapidly to the depth. On the other hand, Syn5.1-I showed significantly higher contribution at transect B (37.5±24.7 %) compared to

transect A and the variations were relatively high between upper and deeper layers. Noticeable high contribution of Syn5.2-CB5 was at station B1 at 50 m depth that reached a value up to 50 %. Ten clades with the average contribution of > 1 % were Syn5.1-III, Syn5.1-IV, Syn5.1-V, Syn5.1-VI, Syn5.1-VII, Syn5.1-IX, Syn5.1-XVII, Syn5.1-CRD1, Syn5.1-CRD2, and Syn5.3-I/II.

Vertical distribution of *Synechococcus* clades at station C1 was shown in Figure 29. The highest contribution of Syn5.1-II was found at this station (52.3±3.5 %). The distribution showed slightly narrow range between upper and deeper layer. Moreover, the distribution of this clade showed a mirror image with the one of Syn5.1-I. Seven clades with the average contribution of > 1 % were Syn5.1-III, Syn5.1-IV, Syn5.1-VI, Syn5.1-VII, Syn5.1-XVII, and Syn5.3-I/II.

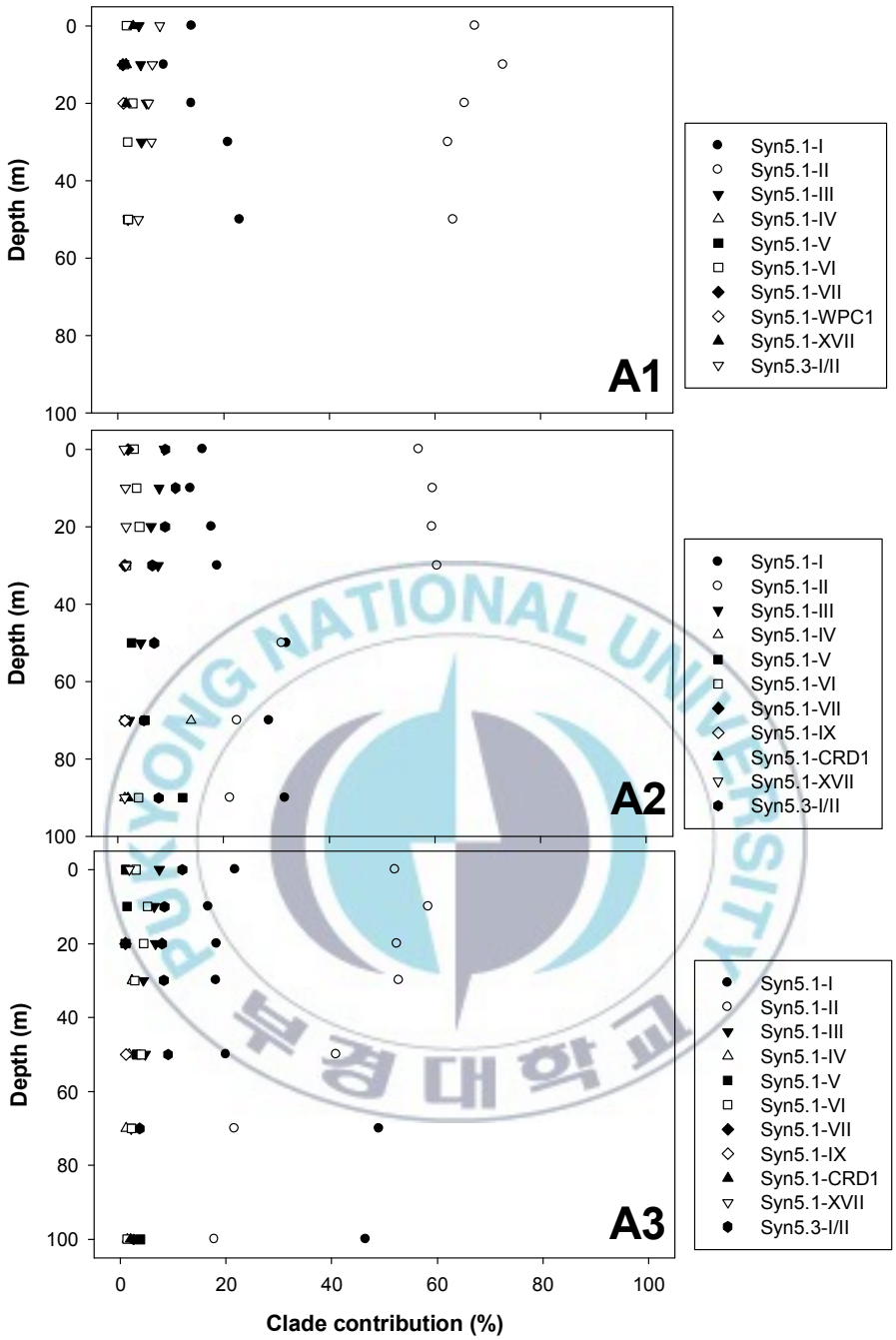

Fig. 27. Vertical distribution of percentage occurrence (%) of *Synechococcus* clades along transect A in autumn 2012

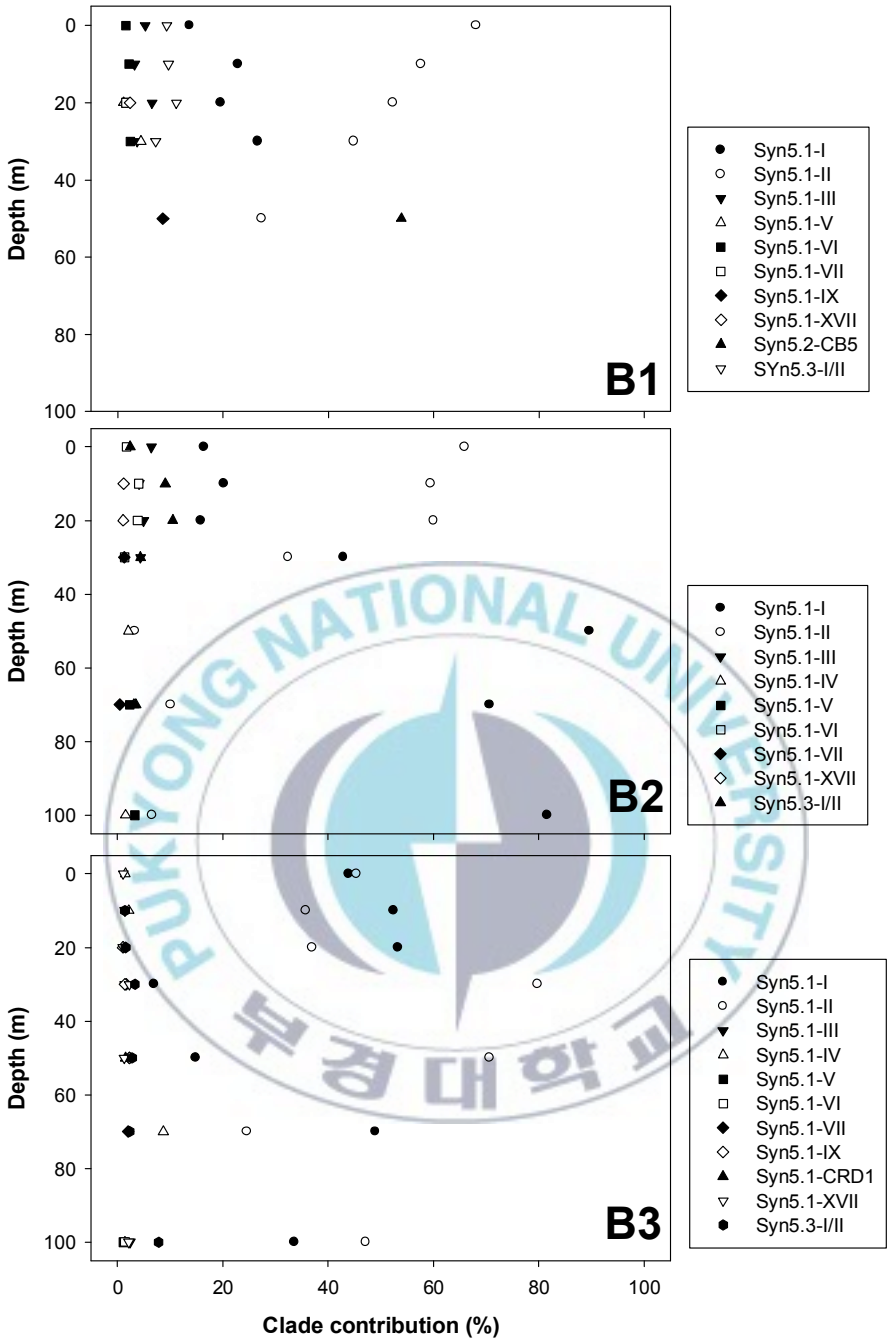

Fig. 28. Vertical distribution of percentage occurrence (%) of *Synechococcus* clades along transect B in autumn 2012

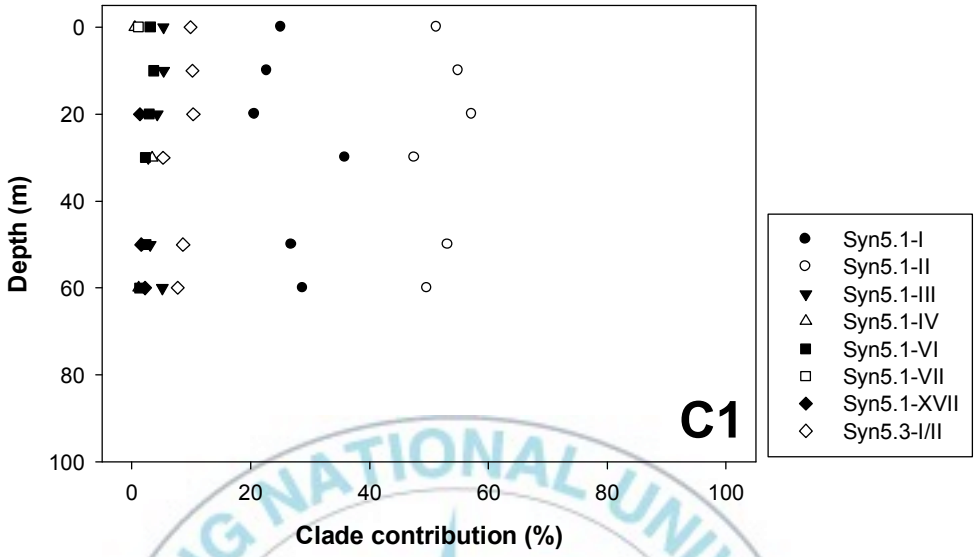

Fig. 29. Vertical distribution of percentage occurrence (%) of *Synechococcus* clades at station C1 in autumn 2012

Table 2. Mean values and standard deviation of surface distribution of *Synechococcus* abundance, PUB_{EX}:PEB_{EX} ratio and the environmental factors in the study areas. Brackets include the maximum and minimum value for each data

	Summer 2011	Autumn 2011	Winter 2012	Spring 2012
Temperature (^{0}C)	23.07±1.58 (20.05-25.12)	23.19±1.17 (21.76-25.60)	13.59±1.20 (12.30-15.50)	18.88±0.51 (17.78-19.83)
Salinity (psu)	32.90±0.23 (32.57-33.23)	33.12±0.43 (32.64-34.01)	34.40±0.08 (34.31-34.52)	33.96±0.29 (33.40-34.34)
Nitrate concentration (μM)	2.930±1.141 (1.810-5.167)	2.236±0.813 (1.217-3.694)	4.531±1.293 (2.790-6.970)	1.288±0.702 (0.391-2.580)
Phosphate concentration (μM)	0.603±0.123 (0.450-0.800)	0.391±0.066 (0.262-0.452)	0.588±0.035 (0.532-0.650)	0.451±0.149 (0.190-0.741)
0.4-3.0 μm chl *a* size fraction (μg L^{-1})	–	0.174±0.169 (0.037-0.663)	–	0.063±0.061 (0.023-0.241)
> 3.0 μm chl *a* size fraction (μg L^{-1})	–	0.330±0.315 (0.060-0.946)	–	0.094±0.130 (0.011-0.500)
Total chl *a* (μg L^{-1})	–	0.505±0.431 (0.114-1.268)	–	0.157±0.190 (0.041-0.741)
Synechococcus abundance (cells ml^{-1})	1656±1497 (154-5641)	6530±4717 (318-14270)	235±99 (82-409)	1232±851 (36-3206)
PUB_{EX}:PEB_{EX} ratio	1.25±0.09 (1.05-1.34)	1.06±0.22 (0.83-1.45)	1.31±0.1 (0.18-1.48)	1.16±0.14 (0.82-1.34)

Table 3. Mean values and standard deviation of vertical distribution of *Syn* PUB_{EX}:PEB_{EX} ratio and the environmental factors in the study areas. Brac minimum value for each data

	Summer 2011	Autumn 2011	Winter 2012
Temperature (^{0}C)	18.45±6.791 (2.88-28.43)	18.19±7.21 (2.58-25.69)	13.21±1.66 (5.03-15.50)
Salinity (psu)	31.99±1.27 (34.49-30.30	33.56±0.54 (32.64-34.48)	34.38±0.09 (5.03-15.50)
Nitrate concentration (μM)	8.037±5.617 (0.638-21.538)	6.500±5.377 (0.603-18.556)	6.328±3.522 (2.792-19.721)
Phosphate concentration (μM)	0.911±0.354 (0.350-1.750)	0.621±0.264 (0.248-1.162)	0.628±0.113 (0.472-0.945)
0.4-3.0 μm chl *a* size fraction (μg L^{-1})	–	0.088±0.101 (0.002-0.663)	–
> 3.0 μm chl *a* size fraction (μg L^{-1})	–	0.217±0.283 (0.015-1.355)	–
Total chl *a* (μg L^{-1})	–	0.305±0.356 (0.018-1.637)	–
Synechococcus abundance (cells ml^{-1})	601±859 (9-5641)	2589±3300 (18-14270)	202±108 (9-463)
PUB_{EX}:PEB_{EX} ratio	1.24±0.13 (0.95-1.61)	1.20±0.22 (0.77-1.57)	1.30±0.08 (0.93-1.48)

Table 4. Surface distribution of *Synechococcus* clade contribution in spring 2012.

Station	Spring 2012			
	Clade contribution (%)			
	Syn-5.1			
	I	II	IV	VII
A1	47.6	7.5	44.9	
A2	42.2	4.7	52.8	
A3	35.1	19.8	44.1	
B1	73.3	1.8	24.9	
B2	70.2	3.3	25.6	
B3	44.1	18.9	34.2	
C1	32.1	6.1	61.5	
C4	60.0	23.1	16.5	
C7	25.8	40.6	30.4	1.5
D1	61.1	3.8	35.0	
D3	60.1	2.5	37.4	
D6	47.9	24.7	27.4	
D8	53.1	10.7	36.1	

Tabel 5. Surface distribution of *Synechococcus* clade contribution in autumn 2012.

Station	Autumn 2012					
	Clade contribution (%)					
	Syn-5.1					Syn-5.3
	I	II	III	IV	V	I/II
A1	13.9	67.6	4.0			7.9
A2	16.1	57	8.7			9
A3	21.7	52	7.4		1.1	11.7
B1	13.7	68.1	5.2			9.3
B2	16.4	66	6.4			2.5
B3	43.9	45.4		1.5		
C1	25.1	51.3	5.3			9.9

Chapter 4

Discussion

4.1. Hydrographic conditions

The dynamic of physical oceanographic processes of East Sea is affected by the inflows and outflows through the straits, surface currents, the Sub Polar Front, and vigorous eddies (Lee et al. 2009). However, due to shallow connections with the surrounding oceans the water exchange is limited to the upper layer. The southern part of the East Sea is characterized with the warm and saline waters of Tsushima Warm Current (TWC). This study showed typical pattern of temperature and salinity in temperate waters among seasons, with wide range of variation for both horizontal and vertical distribution (Table 2 and 3).

Surface temperature and salinity showed similar distribution patterns for all seasons. Surface temperature in the East Sea are driven by the surface wind forcing and air-sea heat flux (Shimada and Kawamura 2006; Liu and Chai 2009). Based on the surface heat flux, the heating seasons in the East Sea are from March to August, and the cooling seasons are from September to February (Liu and Chai 2009). In summer, the solar heating at the surface increased as well as the sea surface temperature and resulted in the intense stratification. However, shallow mixed water column can still be seen in the upper part along transect B and C and weak stratification was in the upper 30 m of transect A and D (Fig. 4A). Deeper mixed layer was observed in winter up to > 90 m, while autumn and spring

showed a shallower mixed layer (about 30-50 m). Yoo and Park (2009) had stated that in the East Sea, the depth of the seasonal thermocline increases to 100-150 m in the winter and decreases to 10-20 m in the summer. In spring, winter mixing ended and the summer stratification started in the upper water column and can be seen very clear in the coastal areas (Fig. 4D and 6D).

The Tsushima Warm Current that flows into East Sea had a characteristic of low-nutrient water masses, with nitrate concentrations < 3 μM year-round (Yoo and Kim, 2004). In general, similar concentration was also found in this study. However, high concentration of surface nitrate + nitrite was observed in some stations especially during summer and winter (Table 2). A study in Ulleung Basin conducted by Kim et al. (2011) had found that high nitrate concentration during summer might be influenced by the intra thermocline eddy (ITE) that efficiently mixed surface and deep-ocean waters. Another possible reason for high concentration of nitrate is the stable upper layer formed by TWC that is thin enough to allow the mixing of nutrient from below (Yoo and Kim, 2004). Also, when nitracline is shallow and the thermocline is deep, there are more nutrients in the upper waters (DuRand et al. 2001).

The typical distribution of nitrate and phosphate in the East Sea is the relatively depleted concentrations in surface waters and increased

gradually with depth. Vertical distribution of nutrient showed the highest concentration was in summer and it was coincided with the warmest and least saline water compared to the other seasons (Table 3). One reason that can be used to explain higher concentration of nutrient in summer was the coastal upwelling events and it has been observed in many studies (Kim 2002; Yoo and Park 2009). Coastal upwelling occurs in the southeastern part of the Korean Peninsula from spring to autumn, as the monsoon wind changes its direction from northwesterly to southwesterly (Yoo and Park 2009). This process supplied nutrient to the upper layer during summer.

4.2. Chlorophyll *a*

4.2.1. Total Chlorophyll *a*

Yoo and Park (2009) had studied climatologically seasonal pattern of surface Chl *a* in the period 1998-2006 and found very clear seasonal changes of Chl *a* in the East Sea with a large spring bloom and a smaller autumn bloom followed by winter and the lowest occurred in summer. Following this pattern, Chl *a* in this study was only sampled during spring and autumn. However, the result showed different pattern where higher concentration of surface Chl *a* was found in autumn compared to the one in spring. Similar result was found from the study in northern South China Sea (Liu et al. 2007). They found high vertical cellular chlorophyll

fluorescence variation for *Synechococcus* occurred in late summer to early autumn and the lowest occurred in winter to spring months. Moreover, spring period conducted in this study was in May that seems to be post bloom season and resulted in low nutrient and Chl *a* concentration (Choi 2013, personal communication).

Higher surface Chl *a* was always found in the coastal areas rather than in the offshore areas. Higher concentration of surface Chl *a* in the coastal areas was likely due to the sources of active production from local origin (Yoo and Park 2009). Although turbidity was not measured in this study, nutrients runoff from the land might also be one of the reasons for higher concentration of Chl *a* in the coastal areas.

Vertical distribution of Chl *a* showed sub-surface chlorophyll maximum (SCM) and SCM is one of the characteristic of the distribution of chlorophyll in East Sea. It can be found at depth around 30-40 m. This study found SCM occurred at depth around 10-40 m. The distribution of chlorophyll with depth was determined by the combination of the vertical mixing of the water column (Nagata 1998) and nutrient availability at depth (Liu et al. 2007). Rich nutrient availability results in accelerated phytoplankton production which reflected in the high Chl *a* values (Nagata 1998).

Vertical distribution of Chl *a* at transect B and C in autumn varied in a wide range compared to the one at transect A and D. It showed that these study areas have higher productivity and transect B and C which is located in the Ulgi and Gampo have been known as the areas where cold water masses appear because of coastal upwelling (Park and Kim 2010). In spring, high concentration of vertical distribution of total Chl *a* (> 1 μg L^{-1}) was at 10 m depth of station B1 and D1 and at 50 m of station D6. These three stations showed varied mixed layer depths with high input of nutrient from deeper layers. Higher concentration of Chl *a* can also be observed at deeper layer rather than near surface when the surface waters are stratified. Similar result was found in the Sargasso Sea and was followed by the spring phytoplankton bloom (DuRand et al. 2001).

4.2.2. Size fraction of Chlorophyll *a*

Size fraction of Chl *a* in the surface layers showed higher concentration of micro-size fraction of Chl *a* occurred in almost all stations in both seasons (autumn and spring) (Fig. 15). Exception occurred at some stations, in which concentration of pico-size fraction of Chl *a* was slightly higher than concentration of the micro-size fraction of Chl *a*. High concentration of micro-size fraction of Chl *a* indicated that the study area was dominated by bigger size of phytoplankton rather than picophytoplankton.

In general, low concentration of pico-size fraction of Chl *a* was followed by high contribution of picophytoplankton to total Chl *a* in all stations. Conversely, high concentration of total Chl *a* was followed by low contribution of picophytoplankton to total Chl *a* (along transect C in autumn and at station B1 in spring). Therefore, in low phytoplankton biomass, picophytoplankton biomass becomes significant. It is proven that *Synechococcus* contributed highly in less productive water (Olson et al. 1990; Agawin et al. 2000). Moreover, lower contribution of pico-size fraction of Chl *a* in autumn rather than in spring was also related with higher concentration of nutrient found in this season. An inverse relationship between primary production rate of picophytoplankton and nutrient concentration has been stated, in which the contribution of *Synechococcus* to total phytoplankton biomass declines with increasing concentrations of nutrients (Agawin et al. 2000).

Picophytoplankton contributed higher in the offshores rather than in the coastal areas for both seasons, except for station B1 in autumn. Iriarte and Purdie (1994) have proposed the gradient of picophytoplankton contribution from the land margin to the open ocean in relation with eutrophication: > 50% offshore, ~ 20% in the coastal areas, and < 10% in estuaries. This study had found high contribution of picophytoplankton up to 73 %. High contribution of picophytoplankton up to 80 % had been

found in the oligotrophic waters (Odate and Maita 1988; Agawin et al. 2000; Park 2006). Thus, picophytoplankton shows the relatively major importance in oceanic oligothrophic waters with lower content of nutrient (Iriarte and Purdie 1994).

4.3. *Synechococcus* abundance

The result of this study has been in agreement with results from other studies in seasonal distribution of *Synechococcus* (Polat and Uysal 2009; Anfuso et al. 2013). The low values of *Synechococcus* (up to 10^3 cells ml^{-1}) as observed in this study were also observed in some studies (Kim 2002; Blanchot et al. 2001; Veldhuis and Kraay 1990). Veldhuis and Kraay (1990) found relatively low numbers of *Synechococcus* (a few hundred per ml) except in the surface water layers where up to 2500 cells ml^{-1} were found. Study in the equatorial Pacific Ocean found *Synechococcus* abundance was about 1.5 x 10^3 cells ml^{-1} in the warm pool and about 8.9 x 10^3 cells ml^{-1} in the high nutrient low chlorophyll waters (Blanchot et al. 2001). Another study in the coastal ecosystem in the tropical South China Sea (Philippines) found low abundance of *Synechococcus* ranged from 0.13 to 21 x 10^3 cells ml^{-1} (Agawin et al. 2003). They suggested that *Synechococcus* likely to be minor contributors to the tropical coastal phytoplankton community in the South Cina Sea. Kim (2002) found

Synechococcus abundance in southwestern coast of East Sea Korea in the range of 0.27 to 23.2 x 10^2 cells ml^{-1} and Shim et al. (2008) in their study in the sea water around Ulleung Island found the average abundance of Synechococcus was 9.3 x 10^3 cells ml^{-1}.

Higher concentrations of *Synechococcus* have been found near the upwelling region in the Atlantic Ocean that reached a maximum value up to 10^5 cells ml^{-1} due to the dynamic of water masses with the presence of frontal system between Brazil and Falkland Currents (Zubkov et al. 1998). High *Synechococcus* abundance was also observed in the northern South China Sea during winter to early spring period that reached a value as high as 10^5 cells ml^{-1} (Liu et al. 2007). Another high abundance of *Synechococcus* that occurred during spring bloom was reported in Sargasso Sea that reached maxima of 3.3 to 5.6 x 10^4 cells ml^{-1} (DuRand et al. 2001).

Low abundances of *Synechococcus* in the East Sea (this study; Kim 2002) were because East Sea can be considered an oligotrophic ocean. Lee et al. (2009) stated that the southern part of East Sea is a warm region contains oligotrophic, warm and saline water.

4.3.1. Surface distribution of *Synechococcus*

Surface variation showed the highest abundance of *Synechococcus* was in autumn, followed by summer, spring, and winter. The distribution patterns highly related with the distribution patterns of environmental factors (Table 2). Higher nitrate + nitrite concentration in summer, might be the reason of slightly more abundance found in this season rather than in spring. The pumping of nitrate + nitrite from deeper layer to the surface layer especially along transect D increased the productivity of the water mass. Higher phytoplankton biomass during summer has been reported due to coastal upwelling in the Ulleung region (along transect D in this study) by Yoo and Park (2009).

In summer, higher abundance of *Synechococcus* was found at the coastal station of transect A and offshore station of transect D (Fig 17A). Surface distribution of temperature showed the appearance of small eddy event with the core was at station C4 (Fig. 3) which has low abundance of *Synechococcus*. There have been some studies about the impacts of eddy event to the distribution of phytoplankton (Moran et al. 2001; Vaillancourt et al. 2003; Baltar et al. 2010). The eddy events have been known to enhance nutrient inputs to the surface ocean increasing new production (Moran et al. 2001) and Chl *a* (Tarran et al. 2001), therefore increased the productivity of phytoplankton. However, photosynthetic bacteria were

found to be numerically abundant outside the eddy as compared to inside (Vaillancourt et al. 2003). This low abundance of *Synechococcus* may be because this organism outcompeted by larger phytoplankton such as diatom in nutrient absorption in the eddy areas (Kim 2002).

Horizontal distribution of *Synechococcus* in autumn was shown to be related more to the different water masses rather than temperature and salinity independently. Different water masses have been reported to influence *Synechococcus* abundance in near-shore areas around Japan (Shiomoto et al. 2004), in the Uchiumi Bay, Japan (Katano et al. 2005) and in the East China Sea (Zhao et al. 2013). This study found two different water masses based on the distribution of temperature and salinity in autumn (Fig. 3 and 5). Colder and less saline water mass in the northern area (from transect B upward) and warm and more saline water in the southern area (along transect A). Higher distribution of *Synechococcus* (> 7.5 x 10^3 cells ml^{-1}) found in the northern area was coincided with lower temperature (~ 22 °C) and lower salinity (~ 32 psu). In addition, this area was observed to have high concentration of total Chl *a*. Therefore, high abundance of *Synechococcus* and high phytoplankton biomass based on high concentration of total Chl *a* in the northern part of this study areas may have been due to active mixing of surface water. On the other hand, lower abundance of *Synechococcus* in the surface layer in the southern

area (along transect A) especially in the coastal areas was coincided with the relatively high temperature (~ 25 °C) and more saline water (> 33 psu). In addition, both Chl *a* size fraction was also rather low at transect A but the contribution of pico-size fraction of Chl *a* was high up to 60 %. High contribution of picophytoplankton (> 50%) has been the characteristic of the oligotrophic waters (Agawin et al. 2000) and it seems related with the oligotrophic condition brought by the Kuroshio water masses (high temperature and high salinity).

Compared to autumn, cooler (< 20 °C) and more saline (> 33 psu) water masses were observed during spring. It showed the characteristic of Kuroshio water masses in the late spring and early summer as found by Shiomoto et al. (2004) with temperature exceeding 18 °C and salinity at the surface exceeding 34.0 psu. Spring experienced oligotrophic condition brought by the Kuroshio water masses and it is supported with the finding of this study, low abundance (~ 0.4 x 10^3 cells ml^{-1}) but high contribution of *Synechococcus* (> 50 %). Surface distribution of *Synechococcus* (Fig. 17D) showed similar pattern with the distribution of surface nitrate + nitrite (Fig. 7). The highest abundance was at station C4 (3.2 x 10^3 cells ml^{-1}) coincided with high concentration of nitrate + nitrite (2.27 μM).

In winter, *Synechococcus* abundance was more to temperature-dependent. Low temperature caused very low abundance of *Synechococcus*, even though nutrient levels were higher during this season (Table 2). The abundance was found higher in the southern region when the temperature was warmer (~ 15 °C) compared to the other parts of study areas. *Synechococcus* has been known to be able to tolerate a broad range of temperature. However, when temperature is low, their abundance and growth rates are decreasing (Huang et al. 2012; Mackey et al. 2013).

4.3.2. Vertical distribution of *Synechococcus*

Different patterns contributed to the vertical distribution of *Synechococcus*, but one of the most important is the depth to which photosynthetically active light penetrates with 1 % transmittance (Algarra and Vaque 1989). This depth is depend on the seasons and geographic of the study sites. Below that depth, the abundance of *Synechococcus* begins to decrease. Their high light requirement for growth restricts their high abundance to the upper well-lit layers (Blanchot et al. 2001).

Depth profiles of *Synechococcus* cell abundance in this study area can be generally summarized into four types (Fig. 30): surface maximum and gradual decreased with depth; sub-surface maximum (between 10-30 m); concentrated and homogeneously distributed in the upper layer (usually in

the upper 30 m); and the two maxima abundance. Depth profiles of abundance had also been proposed by Jiao and Yang (2002) but for *Prochlorococcus* vertical distribution.

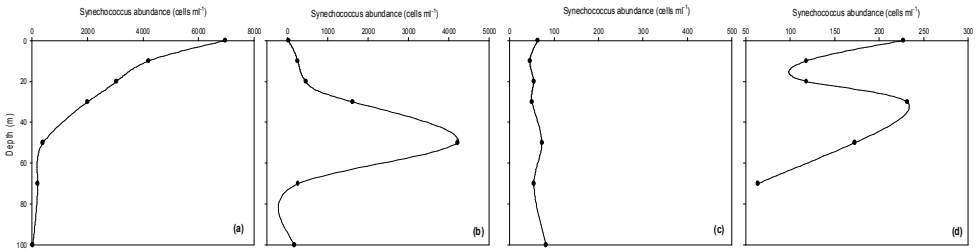

Fig. 30. Typical vertical distribution patterns of *Synechococcus* in this study areas. (a) surface maximum and gradual decrease with depth; (b) sub-surface maximum; (c) concentrated and homogeneously distributed in the upper layer; (d) two maxima abundance

Surface maximum and gradual decreased with depth of *Synechococcus* abundance distribution pattern was observed the most in autumn along transect C. Surface maximum occurred when there was a weak stratification. Similar result was also found off coast of Oman in the Arabian Sea and it was suggested that surface maximum occurred because of cold and nutrient-rich sub-surface water may have been entrained into the euphotic zone (Jochem 1995). Jiao and Yang (2002) found that surface maximum was associated with the interaction of different water masses or currents mix, as well as upwelling areas. Thus, it explained the result of

this study in which transect C that experienced different water masses on its surface layers had higher *Synechococcus* abundance at the surface layer. Overall, vertical distribution of *Synechococcus* abundance in this study was the sub-surface maximum type. This type was found frequent in spring and the least in winter. Sub-surface maxima of vertical distributions of *Synechococcus* have been described by many studies (Olson et al. 1990; Jochem 1995) and it can be found both at open-ocean and coastal areas. It was suggested that higher concentration of *Synechococcus* in the sub-surface occurred during strong water column stratification (Jochem 1995) and it supported the result of this study in which spring was experiencing slightly strong water column stratification compared to the other seasons. Jiao et al. (2005) in their study in East China Sea also found more curved and distinct peaks of *Synechococcus* abundance during summer due to stratification, whereas in the winter, the upper water columns were mixed very well and *Synechococcus* abundances were less variable (no sub-surface maximum layer). Moreover, nutrient supply or grazing pressures may have influenced on the sub-surface maxima distribution (Olson et al. 1990).

Concentrated and homogeneously distributed in the upper layer of *Synechococcus* distribution pattern type showed higher abundance in the upper 30 m and can even reach a depth of 100 m in winter. This type did

not dominate the study areas and was not even found in spring. It was associated with well-mixed water column. Similar distribution pattern was found in the warm pool and HNLC waters in the equatorial Pacific Ocean (Blanchot et al. 2001). In those study areas, *Synechococcus* abundance was homogeneously distributed in the 0-80 m in the warm pool and slightly shallower (0-40 m) in the HNLC waters. In the warm pool, nutrient depletion at the surface and light availability restricted the distribution to a deep and relatively thin layer, while in the HNLC waters, nutrient was sufficient to distribute *Synechococcus* throughout the photic zone (Blanchot et al. 2001).

The two maxima of *Synechococcus* abundance was observed more in winter with the first peak was at the surface and the second one was generally at 30 m. Similar result was also found at the shelf edge off Pakistan at 23° 30' N, 66° 30' E in the Arabian Sea (Jochem 1995). This type of distribution was the combination of weak and strong stratification that caused the abundance peak at the surface and sub-surface layer, respectively. Therefore, it was found more frequent in winter in which well-mixed water layer occurred in the upper part and stratification started to develop in the deeper water column.

4.4. Surface variation of PUB_{EX}:PEB_{EX} ratio

Seasonal distribution showed the highest average of PUB_{EX}:PEB_{EX} ratio in the surface layer was in winter (1.31±0.1) followed by summer (1.25±0.09), spring (1.16±0.14), and autumn (1.06±0.22). Both summer and winter did not display low ratio of PUB_{EX}:PEB_{EX} (ratio < 1).

During summer, only one type of pigment present in the study areas, the high ratio PUB:PEB (> 1). Looking at the distribution patterns, all of the environmental factors (temperature, salinity, nitrate + nitrite, and phosphate) might work together instead of just one factor in influencing the variability of the PUB_{EX}:PEB_{EX} ratio. Other than that, there might be another factor that influences the distribution of this ratio other than those mentioned above. High light level during summer may be a significant factor that influenced the distribution of PUB_{EX}:PEB_{EX} ratio and also the reason there was no low ratio found. Six et al. (2004) in their study on the marine *Synechococcus* sp. clone WH8102 cultured under continuous white light had found that as light increased, the relative contribution of PEB decreased tremendously. In addition, warmer temperature during summer might also the reason of only PUB_{EX}:PEB_{EX} ratio > 1 was found. The influence of warm water to the distribution of PUB_{EX}:PEB_{EX} ratio has been studied by Katano et al. (2007). Their study in southwestern Japan found high-PUB (ratio > 1.5) type of *Synechococcus* in the study areas that

experienced intrusion of warm surface water from the Kuroshio region and concluded that temperature highly affected the distribution of PE chromophores.

Similar to the variability of PUB_{EX}:PEB_{EX} ratio during summer, ratio variability during winter consists of only one type of PE pigment (ratio > 1). However, the cause of distribution might be different. Winter with its high salinity range can cause high ratio found. Salinity might be one of the important factors that influenced the ratio of PUB_{EX}:PEB_{EX} as one study conducted in the Sargasso Sea and Gulf Stream found PUB-containing cells were associated with higher salinity (Wood et al. 1998). In this study, winter experienced the lowest temperature (13.6 ℃±1.2) yet the highest salinity (34.04 psu±0.08) and nitrate + nitrite concentration (4.53 μM±1.29) among seasons. Therefore, those might be the reasons of the high variability of PUB_{EX}:PEB_{EX} ratio during winter.

Autumn and spring displayed distinct distribution patterns of PUB_{EX}:PEB_{EX} ratio. In autumn (Fig. 19), the distributions were separated into two patterns: high PUB_{EX}:PEB_{EX} ratio (> 1) in the southern part that was coincided with warmer and more saline water masses, and low PUB_{EX}:PEB_{EX} ratio (> 1) in the northern part that was coincided with colder and less saline water masses.

In spring, lower PUB_{EX}:PEB_{EX} ratio occurred in the coastal areas and the ratio increased toward offshore. The distribution was related to the water clarity. PUB-containing PE occurred almost exclusively in very transparent water with high transmissivity for blue light, while coastal areas are subject to river outflow, intense land runoff and may contain large amounts of suspended sediments that caused low transmissivity of seawater and preferable to the PEB-type of *Synechococcus* (Wood et al. 1998). Lower PUB_{EX}:PEB_{EX} ratio in the coastal areas was also coincided with lower temperature and lower salinity distribution. Thus, it is confirm the general pattern that low PUB_{EX}:PEB_{EX} ratio associated with cooler waters (Wood et al. 1999) and high PUB_{EX}:PEB_{EX} ratio associated with higher salinity and warm waters (Wood et al. 1998).

4.5. Vertical variation of PUB_{EX}:PEB_{EX} ratio

PUB_{EX}:PEB_{EX} ratio found in this study were in similar range with the other studies (Lantoine and Neveux 1997; Wood et al. 1998, 1999; Katano et al. 2007) as shown in Table 6. PUB_{EX}:PEB_{EX} ratio was found increased with depth in all stations and all seasons. Neveux et al. (2006) in their study in eastern tropical Australian waters found *Synechococcus* PE showed a homogeneous vertical distribution with a low PUB_{EX}:PEB_{EX} ratio (0.61) at the lowest station in the coastal areas. However, at the other

stations, the PUB_{EX}:PEB_{EX} ratio of *Synechococcus* generally increased with depth. The ability of *Synechococcus* to capture low light intensity in deeper layers to increase its PUB chromophore has been proved by marine *Synechococcus* strain WH8102 which increased its PUB_{EX}:PEB_{EX} ratio from 1.5 at high light to 1.95 at low light (Six et al. 2004).

Table 6. Distribution of PUB_{EX}:PEB_{EX} ratio from various studies

Study Area	Sample type	PUB_{EX}:PEB_{EX} ratio	Reference
Tropical northeastern of Atlantic Ocean	natural	0.56-2.0	Lantoine and Neveux 1997
Surface waters of Arabian Sea	natural	0.6-1.8	Wood et al. 1999
Atlantic Ocean & California Current	culture	no-PUB; 0.4-2.0	Toledo et al. 1999
Gulf of Aqaba	culture	0.4-2.3	Fuller et al. 2003
Southwestern Japan	natural	0.6-1.8	Katano et al. 2007
East China Sea and East Sea	culture	no-PUB; 0.46-1.31	Choi and Noh 2009
Southwestern areas of East Sea	natural	no-PUB; 0.77-1.61	this study 2014

Vertical distribution showed three different types of *Synechococcus* based on the PUB_{EX}:PEB_{EX} ratio, which were high PUB_{EX}:PEB_{EX} ratio (> 1); low PUB_{EX}:PEB_{EX} ratio (< 1); and PUB-lacking *Synechococcus*. *Synechococcus* with high PUB_{EX}:PEB_{EX} ratio type was dominating the study areas and found mostly at the deeper layers and also in warm surface layers. In general, high PUB_{EX}:PEB_{EX} ratio (> 1) found in the deeper layer was considered to be influenced the most by the light condition rather than the environmental factors (e.g. temperature, salinity and nutrient levels).

Although we did not measure light intensity, many studies had found the dominance of blue light in the deeper layers increased the PUB chromophore of *Synechococcus* (Olson et al. 1990; Lantoine and Neveux 1997; Wood et al. 1999; Katano et al. 2004). In addition, the increasing ratio of PUB_{EX}:PEB_{EX} to the deeper layer was also related to the decreasing concentration of small size fraction of Chl *a* (0.4-3.0 μm size fraction). It showed that in the deeper layer when the light intensity reduced, phycoerythrin takes place in capturing the light and transferred it to Chl *a* (Mizuta et al. 2002).

Synechoccus with low PUB_{EX}:PEB_{EX} ratio type was generally found in the upper layer of the study areas and was influenced by more complex interaction among the environmental factors. Low ratio of PUB_{EX}:PEB_{EX} (ratio < 1) was found when salinity was lower and temperature was higher compared to the deeper layer. In this study, it can be seen that when salinity was less than 33 psu and temperature was about 25 ^{o}C resulted in low PUB_{EX}:PEB_{EX} ratio. Low ratio of PUB_{EX}:PEB_{EX} (ratio < 1) in the upper layer could also be influenced by the light environment as one of the important factors affecting pigment-type composition in *Synechococcus* (Katano et al. 2004). The upper layer with plenty of light available favors the growth of low-PUB type *Synechococcus* (Olson et al. 1990; Katano et al. 2004). Low PUB_{EX}:PEB_{EX} ratio (< 1) was also related with the

distribution of Chl *a* of the bigger size organisms. Ratio less than 1 always coincided with higher concentration of > 3.0 μm size fraction Chl *a* and this might be related with the light level. The abundance of bigger size of phytoplankton that is shown from the higher concentration of its Chl *a* is one of the factors that influence the changing of water color and lead to the reduce of the blue light penetration which preferable for the PUB chromophores (Wood et al. 1998; Olson et al. 1990).

This study found only a few PUB-lacking *Synechococcus* compared to total samples. Among 343 samples, only 6 samples were found (3 samples in summer, 1 sample in autumn and 1 samples in spring) as no PUB-containing cells. Only few studies reported the finding of PUB-lacking *Synechococcus* (Campbell et al. 1998; Wood et al. 1999) and they were also rare. Low number of PUB-lacking cells in our study supported the fact that *Synechococcus* in the open ocean mostly are PUB-containing cells (Wood et al. 1999) with rather high PUB content and suggested that light absorption by PEB is relatively insignificant in the open ocean (Olson et al. 1988).

4.6. Distribution patterns of *Synechococcus* clades

The distribution of *Synechococcus* clades exhibited higher diversity in autumn than in spring and *Synechococcus* subcluster (SC) 5.1 was the most common ecotype of *Synechococcus* found in this study area. In spring, two subclusters were found (Syn5.1 and Syn5.3) while in autumn the three subclusters were present (Syn5.1, Syn5.2, and Syn5.3). These three subclusters have their own characteristics. Syn5.1 has been found to be ubiquitous in marine environments (Mella-Flores et al. 2011). It is a dominant group within the euphotic zone of both open-ocean and coastal waters (Olson et al. 1990; Ferris and Palenik 1998) and have an elevated salt requirement for growth with phycoerythrin as their major light-harvesting pigment (Rocap et al. 2002). Syn5.2 was dominant in the high latitude water suggesting a possible cold adaptation (Huang et al. 2011) and contains mostly halotolerant strains isolated from coastal waters and some of them are lack of phycoerythrin (Jing and Liu 2012). Syn5.3 showed niche partitioning with depth (Huang et al. 2011). However, much less is known about the biogeography of *Synechococcus* subcluster 5.2 and 5.3 compared to subcluster 5.1. In this study, Syn5.3 can be seen in remarkable contribution in the surface layers only in late autumn with lower temperature (~16 °C) which showed that this subcluster can be associated with colder water masses.

In spring, horizontal and vertical distribution showed that *Synechococcus* was dominated with Syn5.1-I and Syn5.1-IV and showed higher contribution in the coastal areas. In general, the contribution of Syn5.1-I in the coastal areas reached values over 50 %. Surface distribution in autumn showed that Syn5.1-I was still dominant but with lower contribution, while Syn5.1-IV was found in very low number with the average contribution ~ 3 %. It has been known that Syn5.1-I and Syn5.1-IV adapted to cold and coastal waters (Tai and Palenik 2009; Post et al. 2011; Choi et al. 2013a). Moreover, Choi et al. (2013a) stated that in the temperate marginal sea, Syn5.1-I and Syn5.1-IV seems to be the major *Synechococcus* lineages in winter and spring with temperature below 18 ºC and the average temperature of this study in spring was 18.9±0.5 ºC. *Synechococcus* population was dominated by Syn5.1-I and Syn5.1-IV most of the time but in general Syn5.1-I always dominated over Syn5.1-IV. Surface distribution in spring showed that Syn5.1-IV was found to dominate over Syn5.1-I along transect A and at station C1. A study in the mesotrophic waters of the Mediterranean Sea had also found higher contribution of Syn5.1-IV over Syn5.1-I (Mella-Flores et al. 2011). These two clades generally co-occur and the dominance of one clade over the other is dynamic over time and exhibits seasonal patterns (Tai and Palenik 2009). Vertical distribution showed that Syn5.1-I and Syn5.1-IV displayed a mirror image pattern.

Syn5.1-I has lower contribution in the surface layer and increased with the depth, while Syn5.1-IV showed the opposite pattern. Choi et al. (2013a) suggested that these two clades occupy distinct niches in cold waters and the availability of nitrogen may be one of the factors determining their relative abundances. Moreover, Syn5.1-I was considered to be better adapted to the variability experienced by coastal/mesotrophic environments and Syn5.1-IV contributed higher in nutrient-rich areas (Tai and Palenik 2009; Mella-Flores et al. 2011).

Syn5.1-II was found to be the dominating clade during autumn. Many studies have found that Syn5.1-II is highly competitive in warm waters such as the study in the subtropical Gulf of Aqaba in the Red Sea (Post et al. 2011), in the surface of subtropical northwestern Pacific Ocean (Choi et al. 2011), and in temperate water of East China Sea and East Sea (Choi et al. 2013a). However, this study was conducted in late autumn (November) in which temperature was slightly lower compared to spring temperature. The average temperature during this season was 16.95±1.41 ℃. Similar result was also found in the coastal Pacific site of the Southern California Bight in which Syn5.1-II appeared in the winter when the water is colder (Tai and Palenik 2009). Therefore, warmer water mass does not always become the reason of high abundant of this clade. Mella-Flores et al. (2011) found low abundance of Syn5.1-II in warm waters of Mediterranean Sea

and had suggested that some environmental factors other than temperature possibly inhibit the proliferation of this clade. Surface distribution showed a clear pattern of significantly high contribution of Syn 5.1-II in the offshore stations in spring. Vertical distribution of Syn5.1-II varied in narrow range between upper and deeper layers in both seasons; however, it showed a relatively higher abundance in the upper layers and decreased with depth especially in autumn. Many studies have found that Syn5.1-II was abundant in the offshore waters and the contribution decreased with depth (Zwirglmaier et al. 2008; Huang et al. 2011; Choi et al. 2013b). Moreover, Syn5.1-II has been known to be predominant in the oligotrophic waters with a distinct depth distribution pattern (Choi et al. 2013b).

Horizontal and vertical distribution of Syn5.1-III showed that this clade was found in all stations in autumn, when in spring it did not appear. The contribution at the surface layers ranged from 4.0 to 8.7 % with the highest was at station A2. Vertical distribution showed the contribution decreased with the depth. In the Mediterranean Sea, Syn5.1-III was most abundant in the surface layer and the contribution was much higher in the surface than mi-depth (Mella-Flores et al. 2011). Syn5.1-III appears to contribute in broader latitudinal distribution, but it seems to prefer oligotrophic, offshore waters (Mella-Flores et al. 2011). Similar distribution pattern

between Syn5.1-II and Syn5.1-III showed that these clades may have similar mechanisms that influence their dynamics as suggested by Tai and Palenik (2009).

Surface distribution of Syn5.1-VI was found only in autumn with contribution > 1 % almost in all station. Vertical distribution showed the contribution decreased with depth. Similar study in the East Sea conducted by Choi et al. (2013a) also found similar contribution range of this clade in the lower water temperature, while in the East China Sea, Syn5.1-VI contributed higher in warmer water temperature. In other studies using qPCR and dot blotting, Syn5.1-VI was always targeted together with Syn5.1-V and Syn5.1-VII (Fuller et al. 2003). The combine detection of Syn5.1-V/VI/VII in tropical and subtropical waters showed that there was no obvious latitudinal preference of these clades. However, these clades appear to be widely distributed in oceanic waters and are considered to be generalists or opportunists (Zwirglmaier et al. 2008).

Surface distribution of Syn5.1-VII showed that this clade was found only in spring and only at station C7 (1.4 %). It did not appear at all during autumn. Both horizontal and vertical appearance of Syn5.1-VII showed that this clade was found in the warmer water temperature (~ 19.5 ºC). Choi et al. (2013a) found high dominance of Syn5.1-VII (34 %) in the station that is affected by warm water from the Kuroshio Current almost

year-round. Although they found this high dominance was during winter, however, water temperature was about 21 °C. Therefore, the distribution of Syn5.1-VII was restricted to the warmer water conditions.

Syn5.3-I/II was found abundant in the surface layer in autumn with the contribution > 5 % except for stations B2 and B3. Lower temperature in autumn was the reason this clade appeared in autumn and not in spring. Choi et al. (2013a) in their study found an increasing contribution of Syn5.3-I in the colder season and concluded that this clade may be more competitive in mesotrophic and relatively low-temperature conditions. Vertical distributions showed that Syn5.3-I/II appeared only at deeper layer of transect D in spring. Vertical distribution of temperature at transect D in spring (Fig. 4D) showed that very low temperature (< 10 °C) was at the coastal and offshore areas below 70 m and the only depth Syn5.3-I/II appeared. In autumn, Syn5.3-I/II was present in almost all depth. Higher contribution of Syn5,3-I/II at transect A compared to the one at transect B was observed because of lower temperature range at this transect. Thus, the distribution of Syn5.3-I/II was highly related to low temperature conditions.

One clade from *Synechococcus* subcluster 5.2 (clade CB5) were found only at 50 m of station B1 in autumn. It contributed very high that reached a value of 50 %. *Synechococcus* subcluster 5.2 is known to have an

elevated salt requirement for growth (Chen et al. 2006). Choi et al. (2013a) found the highest contribution of Syn5.2-CB5 (10.3 %) when temperature was low in the sub-surface-chlorophyll maximum layer in the East China Sea that was seasonally affected by Changjiang River Diluted Water (CRDW) and oligotrophic Kuroshio Water.

4.7. *Synechococcus* diversity in the East Sea

This study used three different methods to understand the distribution of *Synechococcus* diversity and to determine environmental factors which influenced the diversity seasonally. Pyrosequencing method and PUB_{EX}:PEB_{EX} ratio method were used to identify the diversity of *Synechococcus* and showed an agreement result on the diversity of *Synechococcus* in the East Sea, Korea.

The result from DNA analysis showed high diversity of *Synechococcus* in the East Sea and it is comparable with the other studies (Table 7). Among 30 different *Synechococcus* clades that have been found (Rocap et al. 2002; Fuller et al. 2003; Mella-Flores et al. 2011; Choi et al. 2013a) this study found 22 clades with the dominant clades from subcluster 5.1. A study in Mediterranean Sea conducted by Mella-Flores et al. (2011) found about 9 clades from subcluster 5.1 and one clade from subcluster 5.3. However, their study only targeted specific clades designed by Fuller et al. (2003).

Another study was carried out in the East China Sea and the East Sea based on different season (Choi et al. 2013a). They found 2-19 clades which also showed large variation in diversity among samples.

Table 7. *Synechococcus* clade diversity from different studies

Study area	Clade diversity	Reference
Chesapeake Bay	9 clades from 5.1 2 clades from 5.2	Chen et al. 2006
Mediterranean Sea	9 clades from 5.1 1 clade from 5.3	Mella-flores et al. 2011
Global Oceans (North Atlantic Ocean, Pacific Ocean, South China Sea, Bering Sea and Chukchi Sea)	19 clades from 5.1 2 clades from 5.2 6 clades from 5.3	Huang et al. 2011
East China Sea and East Sea	13 clades from 5.1 1 clade from 5.2 2 clades from 5.3	Choi et al. 2013
East Sea	15 clades from 5.1 1 clade from 5.2 6 clades from 5.3	This study 2014

Based on both the phycoerythrin pigment study and DNA study, *Synechococcus* without phycoerythrin pigment was not found in the study areas. Syn5.1-VIII has been found to carry only phycocyanin and did not exhibit absorption maxima characteristics of phycoerythrin (Fuller et al. 2003; Choi and Noh 2009). In this study, with pyrosequencing study, Syn5.1-VIII was not detected at all and PUB_{EX}:PEB_{EX} ratio method showed all the data contain either phycoerythrobilin only or both

phycoerythrobilin and phycourobilin. Thus, it can be concluded that *Synechococcus* in the East Sea contains phycoerythrin.

Syn5.1-V is the *Synechococcus* clade exhibits only phycoerythrobilin (PEB) peak but not phycourobilin (PUB) (Choi and Noh 2009). This study did not find Syn5.1-V during spring but had found this clade in low contribution in autumn. PUB_{EX}:PEB_{EX} ratio also showed that PUB-lacking cells were found in very low number in the study areas. Therefore, PUB-lacking type of *Synechococcus* was a rare type in the East Sea.

Synechococcus in the East Sea was dominated with the cells that possess both PEB and PUB chromophores. Based on pyrosequencing study, it can be proven with the high occurrence of *Synechococcus* subcluster 5.1. Marine *Synechococcus* subcluster 5.1 has been known to be rich in the phycoerythrobilin and phycourobilin (Ting et al 2002). This *Synechococcus* subcluster is found to have two distinct forms of phycoerythrin antennae that enhance light absorption in the green or blue regions of the light spectrum (Six et al. 2007).

There are two types of *Synechococcus* based on the PUB_{EX}:PEB_{EX} ratio. Low PUB_{EX}:PEB_{EX} ratio (ratio < 1) was represented by Syn5.1-II, Syn5.1-VI, Syn5.1-WPC1 (Choi and Noh 2009; Rocap et al. 2002). In this study, low- PUB type of *Synechococcus* and those clades mentioned dominated during autumn. The distribution pattern of this *Synechococcus* type was

associated with salinity and also light levels. Syn5.1-II was found to contribute higher in the upper layers and Syn5.1-VI was found abundant in less saline water and both are the characteristics of low-PUB type of *Synechococcus*.

High PUB_{EX}:PEB_{EX} ratio (ratio > 1) was represented by Syn5.1-III. Syn5.1-III has been said to exhibit high PUB type of *Synechococcus* (Choi and Noh 2009; Everroad and Wood 2012). This study had found Syn5.1-III only in autumn and not in spring. Unlike with the PUB_{EX}:PEB_{EX} ratio method which found high PUB_{EX}:PEB_{EX} ratio in the deeper layers, Syn5.1-III was found to be abundant more in the upper layers. Moreover, based on phycoerythrin pigment analysis, the study areas were dominated with PUB_{EX}:PEB_{EX} ratio > 1 and this might not be represented only with Syn5.1-III. Syn5.1-I was the most dominant clade found especially in spring. Rocap et al. (2002) found that this clade is capable of chromatic adaptation which means capable of increasing their PUB/PEB chromophore ratio when growing under blue light. In addition, Everroad and Wood (2012) had also found that Syn5.1-I had a variable ratio of PUB/PEB. Therefore, high PUB_{EX}:PEB_{EX} ratio type of *Synechococcus* in the East Sea might be from the contribution of Syn5.1-I.

4.8. Future Studies

This study intended to understand the relationship between the molecular level of *Synechococcus* and its different ecotype based on phycoerythrin chromophores. However, using natural samples it is not easy to exactly relate the distribution of the *Synechococcus* clade with the presence of the PUB and PEB type of *Synechococcus*. Most of the studies have been using specific *Synechococcus* clade in culture studies to understand its pigment composition. Therefore, for future studies, it is suggested to first identify the PUB_{EX}:PEB_{EX} ratio for each clade of *Synechococcus* found in the East Sea using the cultural samples. With the specific understanding of the PUB_{EX}:PEB_{EX} ratio for each clade of *Synechococcus*, it will be easier to relate the distribution of PUB_{EX}:PEB_{EX} ratio with the diversity of *Synechococcus* clades using natural samples in the East Sea.

This study had also used filter paper that was not designed only to filter *Synechococcus*, thus, there is a possibility of the contamination of Cryptomonads that also possess phycoerythrin. Therefore, specific experiment that only targeted *Synechococcus* type of phycoerythrin is needed in the future studies.

Conclusion

The discovery of phototrophic picophytoplankton in the late 1970s provided new insight into the productivity of the world`s ocean and since then our understanding of their taxonomy, physiology and ecology has increased rapidly. There have been many studies performed to determine the abundance and distribution patterns of *Synechococcus* and the environmental factors that influence them. However, it is not easy to conclude that certain environmental factors will influence the abundance of *Synechococcus* in specific ways in the different locations because spatial and temporal factors resulted in the differentiation of *Synechococcus* distribution patterns.

This study showed a large seasonal variability of *Synechococcus* abundance. The abundance ranged from the lowest in winter (0.2±0.1 x 10^3 cells ml^{-1}) to the highest in autumn (2.6±3.3 x 10^3 cells ml^{-1}). The abundance changed dynamically with season and the environmental factors influenced the distribution differently in each season. The physical mixing of the water masses is important in determining the physicochemical properties of water that lead to the variability of the distribution of *Synechococcus*. Temperature has significant effect in controlling the abundance of *Synechococcus*, especially in winter. In other

seasons, temperature with the other factors, such as salinity, nutrient and light, influenced the abundance of *Synechococcus*. Vertical distribution showed the dominance of sub-surface maximum layer of *Synechococcus* abundance. This type of distribution pattern occurred during strong water column stratification which was found frequent in spring.

The variation in pigmentation from the phycoerythrin chromophores has been used to understand the diversity of *Synechococcus*. This study is one of the very few studies that examined PUB_{EX}:PEB_{EX} ratio in the natural samples. Based on the excitation ratio of PUB and PEB chromophores, there are three different types of *Synechococcus* population: high PUB type (ratio > 1); low PUB type (ratio < 1) and PUB-lacking type. High PUB type was the dominant type of *Synechococcus* in the East Sea. High PUB type of *Synechococcus* was associated with warm temperature and saline water. Surface distribution showed summer with its warm temperature and winter with its high salinity was observed to have only high PUB type of *Synechococcus* population. Vertical distribution showed that PUB_{EX}:PEB_{EX} ratio increased with the depth where blue light dominated. Low PUB type dominated the upper layer with the most occurrence was in autumn. Low PUB type was coincided with the colder and less saline water masses. The presence in the upper layer showed that

PEB is higher when green light is still dominating. PUB-lacking type of *Synechococcus* was found to be rare in the East Sea.

Synechococcus has been known to be genetically diverse and the diversity correlates with the environmental factors. DNA analysis in this study reported high diversity of *Synechococcus* with 22 clades were found. The most dominant clades were from *Synechococcus* subcluster 5.1. Higher diversity was in autumn compared to spring with different clade dominated different season. Clades I and IV dominated the study areas during spring, whereas clade II contributed higher in autumn. The distribution of *Synechococcus* clades showed close relationship with temperature. Clade I contributed higher in the coastal areas in spring and the contribution increased with the depth when the temperature is lower. On the other hand, clade II showed decreased contribution with the depth. Clade II has been known to be highly competitive in warm waters. Some clades were found specific in one season such as clade III and subcluster 5.3 clade I/II. The results of this study showed that *Synechococcus* diversity in the East Sea is dominated with the subcluster 5.1 that has been known to be rich in the phycoerythrobilin and phycourobilin.

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Abstract in Korean

동해의 환경 요인과 관련된 *Synechococcus* 의 다양성 및 분포특성 연구는 3 가지 방법을 사용하여 수행되었다. : flow cytometry, DNA 분석을 위한 pyrosequencing, PE chromophore 의 여기 비율. *Synechococcus* 의 분포는 높은 계절적 변동을 보였으며, 가을에 가장 높았고, 겨울에 가장 낮은 분포를 나타내었다. 그 풍부도는 계절에 따라 역동적으로 변화했다. DNA 분석은 *Synechococcus* 의 높은 다양성이 확인되었다. 클레이드는 계절적으로 다른 독특한 기여들이 관측되었는데 클레이드 I 과 IV 봄에 높게(~ 50 %) 기여하는 것으로 관측되었고, 클레이드 II 는 가을에 더 기여하는 것으로 나타났다. PUB_{EX}:PEB_{EX} 비를 바탕으로, *Synechococcus* 의 세 가지의 다른 개체군들이 발견됐다: PUB_{EX}:PEB_{EX} 비가 1 보다 높은 종류들은 대부분 심층에서 우점하는 것으로 발견되었고, PUB_{EX}:PEB_{EX} 비가 1 보다 낮은 종류들은 가을과 봄에 상층에서 우점하는 것으로 발견되었다. 그리고 PUB-lacking 세포들은 수가 적었다. 이 연구는 DNA 분석 및 PUB_{EX}:PEB_{EX} 비율 방법 Synechococcus 의 다양성 과 생태현을 이해하고 환경 요인과의 관계를 사용하는 몇 가지 연구 중 하나이었다. 또한, 이 연구는 천연 샘플을 사용하는 유일한 연구이었다. 대부분의 연구는 배양 샘플을 사용했다. DNA 분석과 PUB_{EX}:PEB_{EX} 비, 두가지 방법은 *Synechococcus* subcluster 5.1 가 높게 발생하는 것으로 대표되는 동해에서 *Synechococcus* 의 PE-rich 가 우점하는 것으로 일치하는 결과를 보였다. *Synechococcus* 풍부도와 다양한 분포사이에는 환경적인 요소들과 함께 복잡한 상호 작용이 있다. 그리고 환경적인 요소들은 각 계절마다 다른식으로 분포에 영향을 미쳤다. *Synechococcus* 클레이드의 분포는 온도와 밀접한 관계를 보였다. 그리고 PUB_{EX}:PEB_{EX} 비의 분포는 수괴와 빛과 관련이 있었다.

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