Seasonal Variation of Phycoerythrin Chromophores of *Synechococcus* spp. in the East Sea/Japan Sea

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**Abstract**

The very small size of *Synechococcus* spp. as one of the important contributors to ocean primary productivity can be identified using its phycoerythrin pigment chromophores, phycourobilin (PUB) and phycoerythrobilin (PEB). Seasonal variation of the excitation (EX) ratio of PUB and PEB in the surface water of East Sea/Japan Sea was observed. This study aimed to describe the effects of environmental factors during the different season on the excitation ratio of PUB and PEB (PUB\(_{EX}\):PEB\(_{EX}\)) contained *Synechococcus* spp. Summer and winter seasons showed a slightly similar distributional pattern with PUB\(_{EX}\):PEB\(_{EX}\) ratio > 1, while PUB\(_{EX}\):PEB\(_{EX}\) ratio < 1 could be found in autumn and spring. The results of this study showed that seasonal patterns of phycoerythrin pigment from *Synechococcus* were highly related with the variability of environmental factors. High light intensity during summer and high salinity during winter were the reasons of high PUB\(_{EX}\):PEB\(_{EX}\) ratio of *Synechococcus* spp. Moreover, high PUB type of *Synechococcus* spp. was also dominated the offshore study areas as the result of higher water clarity compared to the one in the coastal areas.

**INTRODUCTION**

*Synechococcus* spp., the unicellular marine cyanobacteria, is one of the dominant contributors to primary production in the world’s oceans. It can be widely found in the ocean in both nutrient-depleted stratified [1–3] and nutrient-rich mixed waters [4–6]. Due to its tiny size, phycoerythrin (PE) pigment (as the biomarker pigment) of *Synechococcus* spp. is a useful tool to find species diversity of *Synechococcus* spp.

Phycoerythrin (PE) is one of the pigments that belong to the biliprotein. Among phycobiliprotein pigments, PE is the most common and abundant accessory of pigments in the marine environment [7,8] and it absorbs blue light. It consists of two chromophores, which are phycourobilin (PUB) and phycoerythrobilin (PEB) [2]. These chromophores absorb different wavelengths in which the absorption maximum of PUB is at 495-500 nm; while the maximum absorption of PEB is at 540-570 nm [9]. Emission maximum for both chromophores is at lower wavelength around 563-570 nm [10].

The diversity of spectral characteristics of PE comes from the different contents of the PUB and the PEB chromophores and it is related with the environmental factors such as light intensity, nutrient conditions and also temperature [8]. Decreasing irradiance with the depth can increase the fluorescence intensity of PE, while high nutrient level can increase the fluorescence intensity [11]. Water clarity also influenced the distribution of pigments; PE lacking PUB can be found in more turbid water while PE containing PUB were high in transparent waters [2,12].

There have been many studies about the different types of PE chromophore of *Synechococcus* in various marine environments [2,7,12–14] and also in the culture environments [15,16]. The study conducted in the North Atlantic Ocean and Pacific Ocean [2] and also along the coast of the Arabian Sea during the southwest monsoon had found low ratio of the excitation spectra of PUB and PEB (PUB\(_{EX}\):PEB\(_{EX}\)) [13]. Cool and upwelled water masses in the upper levels were the reason of low PUB\(_{EX}\):PEB\(_{EX}\) ratio that were found in those study areas [9]. Another study was conducted in the oligotrophic areas of the Northeastern Atlantic Ocean. It was found high PUB-containing *Synechococcus* and it was related with the maximum...
light transmission in these areas (490 nm). As a result, it was more favorable to the development of high PUB-dominant populations [8]. The PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio increases with the depth and the distance from the coast, indicating an increase in the proportion of the PUB chromophore. This result was due to the PUB chromophore that absorbs more blue-green light [10].

The study of PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio had been conducted either in the natural seawater samples [8, 9, 17, 18] or in isolated strains [15,16,19,20]. Wood et al. (1999) had categorized three different types of PE excitation spectra in their study in the surface waters of the Arabian Sea during the northeast Monsoon which had very low PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio (< 0.6), intermediate PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio, and very high PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio (~ 1.8) [9]. They found that the most common PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio on the Arabian Sea was the low ratio (~ 0.7); 20% of the samples had ratio ~ 1.5; and another 20% showed intermediate PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio. In addition, Lantoine and Neveux (1997) have found distribution patterns of the PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio in three different study sites (eutrophic, mesotrophic, and oligotrophic) in the tropical northeastern Atlantic Ocean [10]. 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 part of Japan and it was found that the PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio in the range of 0.6-1.8 [18]. Higher PUB\textsubscript{EX}:PEB\textsubscript{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 [18].

Choi and Noh (2009) have found four different PE pigments types of Synechococcus cultures isolated from the East China Sea and the East Sea [20]. Type 1 pigment which carried phycocyanin only from strains belonging to clade VIII, while 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\textsubscript{EX}:PEB\textsubscript{EX} ratio ranged of 1.10-1.31 from strains belonging to clade III, WPC2 and sub-cluster 5.3 [19]. In their study, they also found similar types of PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio with the lowest ratio was 0.4 (strains from clade I isolated from the Mediterranean Sea) and the highest was 2.3 (strains from clade II isolated from the Gulf of Aqaba).

From those studies mentioned above, it can be summarized that the PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio can be used to identify different types of Synechococcus spp. This study aims to describe the seasonal distribution pattern of PE chromophores of Synechococcus spp. which based on the excitation ratio and examine the effects of environmental factors that influence the distribution PUB\textsubscript{EX}:PEB\textsubscript{EX} ratio seasonally in the East Sea/Japan Sea.

**EXPERIMENTAL General**

Seawater samples in this study were taken using rosette sampler attached to a Conductivity, Temperature and Depth (CTD) sensors. Whatman Glass Microfiber Filters (GF/F) (Sigma Aldrich, USA) were used to filter seawater samples. The filtered papers were then extracted using phosphate buffer and the supernatants were measured using Fluoromax-4 spectrophluorometer (Horiba Scientific, Japan) at 0.2 nm intervals between 450 and 580 nm as well as 5 and 10 nm slit widths for excitation and emission.

**Sampling Sites**

This study was conducted in the southwestern part of East Sea/Japan Sea. There were four sampling transects (A-D) and each transect consists of 3 to 4 stations spaced about 20-40 km for each station in the same transect (Figure 1). Surface water samples were taken in summer and autumn 2011 and winter and spring 2012. Temperature and salinity data were obtained using CTD attached to a rosette sampler.

**PUB\textsubscript{EX}:PEB\textsubscript{EX} Ratio Analysis**

About two liters of seawater from the surface water was filtered onto 47 mm Whatman GF/F filters (Sigma-Aldrich, USA). The filter papers were then kept frozen until analysis. In the laboratory, the filter papers were extracted for the PUB and PEB chromophores analysis using the method as reported previously [21]. Phosphate buffer (7 mL, pH 6.5) was used to extract the filters and maintained for 3 hs at 4 °C in the dark. Phosphate buffer will keep the stability of the pigments [22]. Filters were ground and centrifuged for 10 min at 2,500 rpm. About 3.5 mL of the supernatant was measured using Fluoromax-4 spectrophluorometer. The excitation of phycoerythrin (PE\textsubscript{EX}) was recorded using an emission wavelength at 605 nm. Excitation spectra of PUB appeared around 495-500 nm and the one of PEB appeared around 540-570 nm (Figure 2).

**RESULTS AND DISCUSSION**

**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 [23]. However, due to shallow connections with the surrounding...
The temperature ranged from 12.3 to 21.8 °C in the southern part of the East Sea, characterized by the warm and saline waters of Tsushima Warm Current (TWC). This study showed the typical pattern of temperature and salinity in temperate waters among the seasons.

Figure 3 shows surface temperature among seasons. The temperature decreased to the northern region of the studied area in all seasons. In summer 2011, the temperature ranged from 20.1 to 25.1 °C. The warmer temperature was in the southern areas and the temperature decreased to the northern part. Small eddy was observed with the core centered at transect C. Lower temperature inside the eddy core showed the characteristic of cyclonic eddy event.

In autumn 2011, the temperature ranged from 21.8 to 25.6 °C. This study was conducted in the early autumn; therefore, high temperature from summer could still be observed. The temperature was 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, the temperature difference occurred between coastal and offshore with higher temperature was in the offshore. In winter 2012, the temperature ranged from 12.3 to 15.5 °C. Three distinct temperature conditions can be identified in this season in which warmer and homogeneous water temperature was observed between transects A and B, rapid changing of temperature between transect B and C, and homogeneous colder water toward transect D. In spring 2012, the temperature ranged from 17.7 to 19.8 °C. During this season, temperature differences were observed from coastal to offshore for all transects. The temperature increased toward offshore areas.

Similar to the temperature, the variability of surface salinity could also be observed among seasons (Figure 4). In summer 2011, salinity ranged from 32.6 to 33.2 psu. Salinity was increased to the northern region. Lower salinity was in coastal areas of transect A and it was increased toward offshore in which it was started to spread homogeneously toward transect B. More saline water was 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, the 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 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 a 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 was decreased toward the upper region. The 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 was 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 transect B.

**Distribution of PUBEX:PEBEX Ratio**

Surface distributions of PUBEX:PEBEX ratio for all seasons were shown in Figure 5. In summer 2011, the distribution of PUBEX:PEBEX ratio ranged of 1.05-1.34. The excitation intensity of PUBs was found between 9.09 x 10^4 to 1.1 x 10^5, while for PEBs were between 4.45 x 10^4 to 8.78 x 10^5. The highest ratio was found in the coastal area of transect A.

In autumn 2011, the distribution of PUBEX:PEBEX ratio ranged of 0.83 - 1.45. The ratios were calculated from the excitation intensity of PUB in the range of 8.22 x 10^4 - 2.91 x 10^5 and the excitation intensity of PEB in the range of 5.84 x 10^4 - 5.14 x 10^5. Lower PUBEX:PEBEX ratio was found in the northern region and higher ratio was in the southern region of the study areas. The significantly high ratio was in transect A.
compared 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.

Seasonal distribution of PUB_{EX}:PEB_{EX} ratio in the surface layer showed that the highest average of the ratio 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 pigments presented in the study areas, the high ratio PUB_{EX}:PEB_{EX} (> 1). High light level during summer may be a significant factor that influenced the distribution of PUB_{EX}:PEB_{EX} ratio. It is also the reason where no low ratio was found. Six et al. [24] 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, the warmer temperature during summer might also the reason of why only PUB_{EX}:PEB_{EX} ratio > 1 was found. The influence of warm water on the distribution of PUB_{EX}:PEB_{EX} ratio has been studied previously [18]. Their observation 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. Finally, they have 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 that high ratio can be 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 [17]. In this study, winter experienced the lowest temperature (13.6 ± 1.2 °C) yet the highest salinity (34.04 ± 0.08 psu) among seasons. Therefore, those might be the reasons for 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, 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 was 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* [17]. Lower PUB_{EX}:PEB_{EX} ratio in the coastal areas was also coincided with lower temperature and lower salinity distribution. Thus, it confirms the general pattern that low PUB_{EX}:PEB_{EX} ratio was associated with cooler waters [80] and high PUB_{EX}:PEB_{EX} ratio was associated with higher salinity and warm waters [17].

**CONCLUSION**

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.
ABSTRACT


Kata kunci: East Sea/Japan Sea, fikoeritrin, variasi musim, Synechococcus

References


