Absorption and scattering properties of micro- and nano-size phytoplankton

マイクロ及びナノ植物プランクトンの吸収特性と散乱特性

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SYNOPSIS

海洋の植物プランクトン群集内の細胞の粒径分布とその植物プランクトン群集の生理特性は、海洋生態系における炭素循環を解明する要因の 1 つである。特にマイクロ、及びナノ植物プランクトンは、季節的、及び局所的に大増殖を起こし、植物プランクトン群集の 1 次生産量の変動に大きく寄与している。本研究は、光学機器を用いて、マイクロ、及びナノサイズの植物プランクトンを含む植物プランクトン群集の細胞サイズ組成の変動を検出する為に、細胞サイズが植物プランクトン群集の光学特性に与える影響を明らかにすることと、細胞サイズと光学特性の関係に影響を与える植物プランクトン群集の生理状態を、光学特性を用いて明らかにすることを目的とした。まず培養実験において、光学特性に関する研究例の少ない渦鞭毛藻を用い、マイクロ、及びナノ植物プランクトンの単一種での光学特性を調べた。マイクロ植物プランクトンである渦鞭毛藻 *Prorocentrum micans* のクロロフィル *a*（Chl *a*)濃度で標準化した吸収係数 $a^*_{ph}(676)$ と散乱係数 $\underline{b}^*_{ph}(676)$は、ナノ植物プランクトンである *P. minimum* よりも低い値を示した。これらの結果を過去の研究結果に加え、マイクロ、及びナノ植物プランクトンは、大きな細胞サイズになるほど $a^*_{ph}(676)$と $\underline{b}^*_{ph}(676)$が減少することを明らかにした。培養実験で得られた細胞サイズと $a^*_{ph}(676)$、細胞サイズと $\underline{b}^*_{ph}(676)$の関係から、それぞれ細胞サイズが $a^*_{ph}(676)$と $\underline{b}^*_{ph}(676)$に与える影響の指標である、吸収係数と散乱係数に関する重み係数を算出した。次に現場実験においてサイズ分画を行ったマイクロ、及びナノ植物プランクトン群集の光学特性を調べた。吸収係数と散乱係数に関する重み係数とそれぞれのサイズ分画した相対的な Chl *a* の比から推定した植物プランクトン群集のサイズ指数（SI_{abs}, SI_{scat}）は、それぞれ $a^*_{ph}(676)$と $\underline{b}^*_{ph}(676)$の増加に対して有意に減少した。この SI_{abs} と $a^*_{ph}(676)$、SI_{scat} と $\underline{b}^*_{ph}(676)$の関係の負の傾きは、培養実験におけるサイズと光学特性の関係の傾きとは、有意差はなかった。このことから、$a^*_{ph}(676)$と $\underline{b}^*_{ph}(676)$を用いた、植物プランクトンのサイズ組成（$SI_{abs}$, SI_{scat}）の推定が妥当であることが示された。また、植物プランクトン群集の生理状態の指標である光保護カロテノイドに対する全てのカロテノイド比(PPC:TC)は、a^*_{ph} の 488nm から 532nm の傾き(a^{*slope}_{ph})の減少とともに減少し、Chl *a* に対する粒状有機炭素の比(POC:Chl *a*)は、$\underline{b}^*_{p}(676)$の増加とともに増加した。これは植物プランクトン群集の光学特性を用いて、その細胞サイズ組成とともに、PPC:TC と POC:Chl *a* で示される生理状態を推定できることが示された。以上のことから、本研究で示した様々な生理状態での植物プランクトン群集の細胞サイズと光学特性の関係性は、人工衛星による観測等の光学観測による植物プランクトンのサイズ組成の推定に寄与できることが示唆された。

Keywords: absorption, ac-9, cell size, dinoflagellate, inherent optical properties, photophysiology, phytoplankton, ratio of carbon to chlorophyll *a*, scattering

Introduction

Size distribution of natural phytoplankton assemblages and the physiological properties are one of the fundamental features in marine carbon cycle. Particularly, large-cell plankton, such as micro- (20 – 200μm in diameter) and nano-size phytoplankton (2 – 20μm in diameter) can influence significantly on the production due to the occurrence of opportunistic and sporadic large blooms. One of the bloom-forming species is dinoflagellate. To monitor the size distribution of phytoplankton or the blooms, optical approach would be one of the most effective ways. The optical approach is based on Inherent Optical Properties (IOPs) of phytoplankton, such as absorption and scattering coefficients of phytoplankton ($a_{ph}[\lambda]$ and $b_{ph}[\lambda]$). Both $a_{ph}(\lambda)$ and $b_{ph}(\lambda)$ are influenced by cell volume which is the most critical parameter in the geometrical characteristics of natural assemblage of phytoplankton.

Cell volumes of micro- and nano-size phytoplankton indicate 10^3 fold variations. Because of the wide difference in the cell volume, the non-linear relationships between phytoplankton biomass, such as chlorophyll *a* (Chl *a*) and IOPs, such as $a_{ph}(\lambda)$ and $b_{ph}(\lambda)$ are observed. Based on the large set of *in situ* data, $a^*_{ph}(\lambda)$ can be used to distinguish cell sizes in natural phytoplankton assemblages (Ciotti et al. 2002), whereas $b^*_{ph}(\lambda)$ have not been used yet to differentiate cell sizes.

In a water column, the IOPs of phytoplankton would be influenced by the physiological properties. The $b^*_{ph}(\lambda)$ is influenced by the intracellular carbon contents, and therefore the $b^*_{ph}(\lambda)$ could be indexed on the ratio of particulate organic carbon to Chl *a* (POC:Chl *a*) as physiological properties of natural assemblages of phytoplankton. In addition, a molar ratio of photoprotective carotenoids (PPC) to total carotenoids (TC) is one of the index of the physiological properties of natural assemblages of phytoplankton. The variations in relative proportions of PPC alter the shape of a^*_{ph} spectra from 490 to 530nm normalized by 676nm (Johnsen et al. 1994). The relationships between $b^*_{ph}(\lambda)$ and POC:Chl *a*, and a^*_{ph} spectra and PPC:TC could be utilized to evaluate the physiological properties of natural assemblages of

phytoplankton.

A parameterization of IOPs for micro- and nano-size phytoplankton provides a simple tool to monitor the size distribution in the ocean and the physiological properties by using optical measurements. Establishment of relationships between phytoplankton biomass, such as Chl *a* and the IOPs by including dinoflagellate species to each size group will improve our understanding of the optical characteristics of natural assemblages of phytoplankton in relation to cell size.

The objectives in this study are to investigate the relationship between cell size and IOPs of micro- and nano-size dinoflagellates in culture in relation to published data (Study 1), and to investigate the cell size effect on IOPs in the natural assemblages of phytoplankton (Study 2). Furthermore, physiological properties of natural assemblages of phytoplankton are investigated by using IOPs of phytoplankton.

Materials and Methods

Study1. Absorption and scattering properties of dinoflagellates

Dinoflagellates *Prorocentrum micans* and *P. minimum* were incubated at 20°C, 35 PSU salinity in a modified f/2 medium without silicate. Light was provided by cool fluorescent light on on a 12 h light: 12 h dark cycle. Light intensity was 300 and 600 μmol photons m^{-2} s^{-1} which were the saturated and supra-saturated light conditions, respectively. The supra-saturated light intensity could induce the photoprotective acclimation by PPC. At the middle of exponential growth phase usually on day 2, subsamples from each experimental bottle were taken after 6 hour of the light phase. Phytoplankton pigments were determined by high performance liquid chromatography (HPLC). Cellular carbon (C) concentration was determined by elemental analyzer. The ratio of cellular carbon to cellular Chl *a* contents (C:Chl *a*) was calculated based on a weight basis.

Absorption coefficients of phytoplankton ($a_{ph}[\lambda]$) were measured by Quantitative Filter Technique (QFT) using spectrophotometer with an integration sphere. Chlorophyll *a* specific $a_{ph}(\lambda)$ ($a^*_{ph}[\lambda]$) were determined by dividing with Chl *a* concentration.

Scattering coefficients of phytoplankton ($\underline{b}_{ph}[\lambda]$, where underline indicates coefficient measured by absorption-attenuation meter [ac-9]), were calculated as the difference between the absorption and the attenuation coefficient of phytoplankton ($\underline{a}_{ph}[\lambda]$ and $\underline{c}_{ph}[\lambda]$). The $\underline{a}_{ph}(\lambda)$ and $\underline{c}_{ph}(\lambda)$ were measured at nine wavelengths; 412, 440, 488, 510, 532, 555, 650, 676, and 715nm, which were commonly employed in the current satellite. Chlorophyll *a* specific $\underline{b}_{ph}(\lambda)$ ($\underline{b}^*_{ph}[\lambda]$) were determined by dividing with Chl *a* concentration.

Study 2. Absorption and scattering properties of micro- and nano-size fractionated phytoplankton assemblages

Natural assemblages of phytoplankton were obtained at the optical depths of 0.0, 2.3, and 4.6 in open and coastal waters. The optical depths were determined from the underwater measurement of photosynthetically available radiation (PAR) by lowering the underwater irradiance meters. Open water samples were collected at 16 stations in the Indian Sector of the Southern Ocean (SO) during the cruise of the TR/V Umitaka-Maru (Tokyo University of Marine Science and Technology) in the austral summer of 2010/2011 and 2011/2012. Coastal water samples were collected aboard the research vessel "Tachibana" at station M (35º09'47"N, 139º10'33"E) off the Manazuru Peninsula in Sagami Bay (SB) every month during the period from July 2009 to December 2010. The water samples in SB were prescreened through 183 μm mesh of plankton net cloth (bulk sample). The bulk samples in SO and SB were further size-fractionated with 20 μm mesh plankton net cloth and 2 μm pore size membrane filters.

Phytoplankton pigments were determined by HPLC. The ratio of photoprotective carotenoids to total carotenoids (PPC:TC) was calculated based on a molar basis. The pigment concentration of micro-size fraction (bulk–20μm) and nano-size fraction (20–2 μm) were estimated by subtracting <20 μm from bulk fractions, and <2 μm from <20 μm fractions, respectively. The relative proportion of micro- and nano-size fractions to bulk fractions (%) were determined by relative Chl *a* concentrations to bulk Chl *a* concentrations. Bulk particulate organic carbon (POC) was determined by elemental analyzer. The POC:Chl *a* was calculated based on a weight basis.

Absorption coefficients of particle ($a_p[\lambda]$) and phytoplankton ($a_{ph}[\lambda]$) of bulk, <20 μm and <2 μm fractions were measured as in Study 1. To evaluate the spectral characteristics of a^*_{ph}, the a^*_{ph} spectra from 488 to 532nm were normalized by a^*_{ph} at 676nm which is the absorption peak by Chl *a*. Slope of a^*_{ph} spectra was calculated as follows:

$$a^*_{ph}{}^{slope} = (a^*_{ph}[488] - a^*_{ph}[532]) / (a^*_{ph}[676] \times [488 - 532]) \quad (1).$$

Scattering coefficients of particles ($\underline{b}_p[\lambda]$) were calculated as the difference between absorption and attenuation of particle ($\underline{a}_p[\lambda]$ and $\underline{c}_p[\lambda]$) at nine wavelengths. In SO, vertical profiles of $\underline{a}_p(\lambda)$ and $\underline{c}_p(\lambda)$ of bulk sample were measured by ac-9 which was set up as a profiling instrumentation. In SB, the $\underline{a}_p(\lambda)$ and $\underline{c}_p(\lambda)$ of bulk, <20 μm, and <2 μm fractions at three optical depths were measured by using ac-9 which was set up as a bench-top instrumentation in a fixed tilt position at 45º. To estimate the scattering coefficient of phytoplankton ($\underline{b}^*_{ph}[\lambda]$) from $\underline{b}_p(\lambda)$, the ratio of $a^*_{ph}(555)$ to $a^*_p(555)$ measured by QFT were used as follows:

$$\underline{b}^*_{ph}(676) = \underline{b}^*_p(676) \times a^*_{ph}(555) / a^*_p(555) \quad (2).$$

Size Indices (SI) of natural assemblages of phytoplankton were derived from relative Chl *a* proportion of micro-, nano-, and pico-size fraction to bulk fraction (%) and weighed value for absorption and scattering of three cell size class, micro-size (M_{abs} and M_{scat}), nano-size (N_{abs} and N_{scat}), and pico-size fraction (P_{abs} and P_{scat}), respectively, as follows:

$SI_{abs}=(M_{abs}\times[\text{micro-size}(\%)]+ N_{abs}\times[\text{nano-size}(\%)] + P_{abs}\times[\text{pico-size}(\%)]) /100$ (3)

$SI_{scat}=(M_{scat}\times[\text{micro-size}(\%)]+ N_{scat}\times[\text{nano-size}(\%)] + P_{scat}\times[\text{pico-size}(\%)]) /100$ (4).

The weighted values were derived from the cultural relationships between the cell size and $a^*_{ph}(\lambda)$ and/or $\underline{b}^*_{ph}(\lambda)$ when the number of cell of natural assemblage of phytoplankton was assumed as a power function of d with exponent of –4.

Differences in optical properties between micro- and nano-size fractions were tested with a non-parametric tested with Mann-Whitney Rank Sum Test. Analysis of covariance were carried out to compare the slopes and intercepts of regression lines of the cultural relationships between IOPs and cell size, and *in situ* relationships between IOPs and size index.

Results and Discussions

Study1. Absorption and scattering properties of dinoflagellates

Equivalent spherical diameters (d) of *P. micans* and *P. minimum* of both light conditions were 25.0 ± 0.22 and 12.6 ± 0.24 μm, respectively. The d of *P. micans* was about 2-fold larger than that of *P. minimum*. The a^*_{ph} (676) and $\underline{b}^*_{ph}$ (676) of *P. micans* were approximately 20% and 35% lower than that of *P. minimum*, respectively.

I enumerated the d, $a^*_{ph}(676)$, $\underline{b}^*_{ph}(676)$, and C:Chl a of phytoplankton species with various cell sizes in published data to evaluate those of *P. micans* and *P. minimum*. Both $a^*_{ph}(676)$ and $\underline{b}^*_{ph}(676)$ significantly decreased with increasing d (p<0.05, Fig. 1A and B). The decreasing $a^*_{ph}(676)$ with increasing d is due to pigments self-shading in the cell (package effect, Berner et al. 1989). The decreasing $\underline{b}^*_{ph}(676)$ with increasing d could be induced by the increasing Chl a contents per cell (Motokawa and Taguchi 2015).

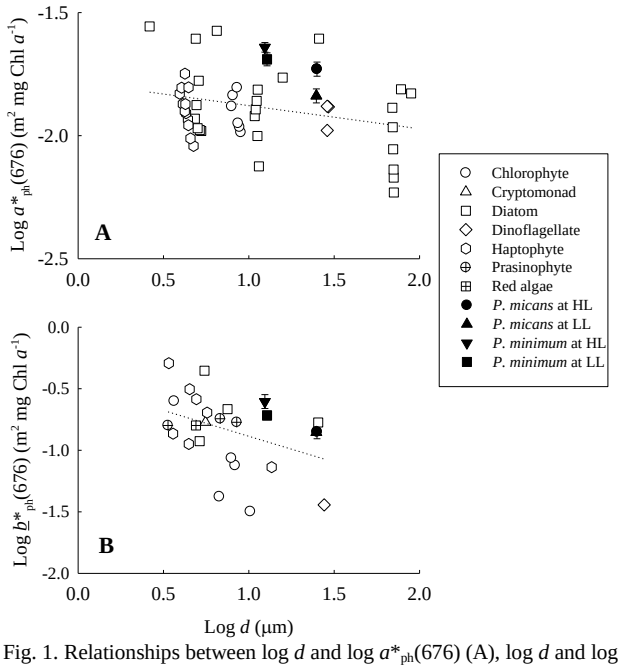

Fig. 1. Relationships between log d and log $a^*_{ph}(676)$ (A), log d and log $\underline{b}^*_{ph}(676)$ (B). Dotted lines indicate regression lines. Open and closed symbols indicate literature and this study, respectively.

The $\underline{b}^*_{ph}(676)$ of various species including *P. micans* and *P. minimum* increased significantly with the C:Chl a (p<0.001, Fig. 2). The C:Chl a and the $b^*_{ph}(676)$ of *P. micans* and *P. minimum* exhibited relatively high values. Although the intracellular C and Chl a contents are dependent on cell size under given growth conditions, the C:Chl a is independent of cell size. The significant relationship between the $b^*_{ph}(676)$ and C:Chl a suggests that the C:Chl a could play a role of the variation in the $b^*_{ph}(676)$ as a function of cell size.

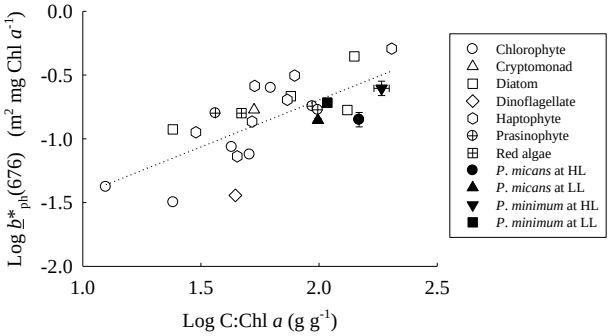

Fig. 2. Relationships between log C:Chl a and log $\underline{b}^*_{ph}(676)$. See Fig. 1 for the symbols and dotted line.

Study 2. Absorption and scattering properties of micro- and nano-size fractionated phytoplankton assemblages

Relationships between size index and $a^*_{ph}(676)$ *and/or* $\underline{b}^*_{ph}(676)$

Investigations of size distribution of phytoplankton assemblage and the optical properties were divided into four regions according to the differences in the water mass: at the North of Antarctic Convergence (AC) in SO (NAC), at the South of AC in SO (SAC), in SB during winter (from December to February, WSB), and in SB from spring to autumn (from March to November, SSB).

Bulk Chl a concentrations ranged from 0.15 to 3.8 mg m^{-3}. The highest and lowest bulk Chl a concentrations were observed in SSB and NAC, respectively. The micro- and nano-size fractionated Chl a concentrations increased with increasing bulk Chl a concentrations. The relative proportion of micro-size fractions increased with increasing bulk Chl a concentrations, whereas that of nano-size decreased.

The SI_{abs} and SI_{scat} in all regions were similar because the weighted value of each size class was similar between the absorption and scattering analyses. The average SI_{abs} and SI_{scat} in NAC, SAC, and WSB fell in the range of the nano-size phytoplankton, whereas the average SI_{abs} and SI_{scat} in SSB fell in the range of the micro-size phytoplankton.

The $a^*_{ph}(676)$ and $\underline{b}^*_{ph}(676)$ decreased significantly with increasing SI_{abs} and SI_{scat}, respectively, when the all regions were considered together. The slopes of the *in situ* relationships between $a^*_{ph}(676)$ and SI_{abs} were not significantly different from the slopes of the cultural relationships between $a^*_{ph}(676)$ and d derived from Study 1 (Table 1). The similarity suggests that the effect of cell size on $a^*_{ph}(676)$ of natural assemblage of phytoplankton with various cell sizes could be evaluated by the SI_{abs}. The higher

intercept of the *in situ* relationship compared with that of the cultural relationship suggests that the *in situ* relationship could be influenced by the physiological properties of phytoplankton which could covary with the environmental conditions.

The slopes of the *in situ* relationships between $\underline{b}^*_{ph}(676)$ and SI_{scat} were not significantly different from the slopes of the cultural relationship between $\underline{b}^*_{ph}(676)$ and *d* derived from Study 1 (Table 1). This similarity confirm that decrease in $\underline{b}^*_{ph}(676)$ could be determined by not only size distribution of cells, but also cumulative cell volume of phytoplankton assemblage. The intercept of the *in situ* relationship was significantly higher than that of the cultural relationship. The higher intercept of the *in situ* relationship could be due to the high light conditions because the high light conditions could induce the decreasing Chl *a* per cell, and the $\underline{b}^*_{ph}(676)$ increased consequently. In addition, the difference in $\underline{b}^*_{ph}(676)$ of natural assemblages of phytoplankton could be induced by the difference in the carbon contents. The higher intercept of the *in situ* relationships could be due to the carbon contents other than phytoplankton, such as detritus.

Table 1. Regression analyses between $a^*_{ph}(676)$ and *d* and/or SI_{abs}, $\underline{b}^*_{ph}(676)$ and *d* and/or SI_{scat} from cultural experiment (Study 1) and *in situ* experiment (Study 2). n, r^2, and *p* indicate the number of sample, determination coefficient, and probability, respectively. Alphabets indicate significant difference at $p<0.01$.

Population	Equation	n	Y_{int}	Slope	r^2	*p*
Culture	$\text{Log } a^*_{ph}(676)$	50	-1.78^a	-0.10^e	0.08	<0.05
	$= Y_{int} + \text{Slope} \times \text{Log } d$					
In situ	$\text{Log } a^*_{ph}(676)$	77	-1.46^b	-0.11^e	0.06	<0.05
	$= Y_{int} + \text{Slope} \times \text{Log } SI_{abs}$					
Culture	$\text{Log } \underline{b}^*_{ph}(676)$	26	-0.46^c	-0.42^f	0.16	<0.05
	$= Y_{int} + \text{Slope} \times \text{Log } d$					
In situ	$\text{Log } \underline{b}^*_{ph}(676)$	58	-0.20^d	-0.39^f	0.07	<0.05
	$= Y_{int} + \text{Slope} \times \text{Log } SI_{scat}$					

Relationships between optical properties and physiological properties

The bulk $a^*_{ph}{}^{slope}$ decreased with decreasing bulk PPC:TC (mol mol^{-1}) of phytoplankton assemblage. The PPC:TC increased with increasing optical depths, so that the PPC:TC could indicate the photoprotective response to light changes in a water column (Motokawa et al. 2014). The composition of PPC and TC in phytoplankton cell is different among phytoplankton species, however the PPC:TC as a function of light intensity was similar between micro-size and nano-size fractions. The $a^*_{ph}{}^{slope}$ and PPC:TC was similar between micro-size and nano-size fractions, and therefore the $a^*_{ph}{}^{slope}$ could be utilized to evaluate the photoprotective acclimation of phytoplankton without the size effect on the $a^*_{ph}{}^{slope}$.

The bulk $\underline{b}^*_{ph}(676)$ increased significantly with the POC:Chl *a* ($p<0.001$, Fig. 3) when all regions were considered together. As the physiological response to the environmental conditions, the C:Chl *a* of phytoplankton decrease with the decrease in light intensity or increase in temperature (Geider 1987).

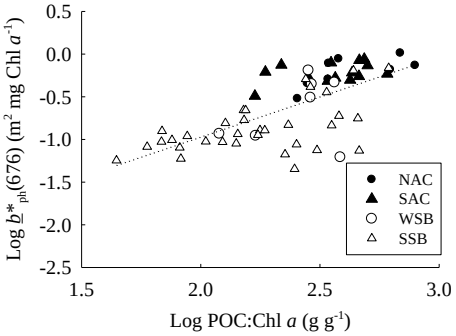

Fig. 3. Relationship between POC:Chl *a* and $\underline{b}^*_{p}(676)$. Dotted lines indicate regression lines. Open and closed symbols indicate the Southern Ocean and Sagami Bay, respectively.

Assuming that the scattering efficiency of detritus is similar to that of phytoplankton, the similar slopes of the relationships between $\underline{b}^*_{ph}(676)$ and POC:Chl *a* and/or C:Chl *a* (Table 2) suggest that the relative amount of detrital carbon increase with decrease in C:Chl *a* of phytoplankton cell.

Table 2. Regression analyses between $\underline{b}^*_{ph}(676)$, C:Chl *a*, and POC:Chl *a* in cultural experiment (Study 1) and *in situ* experiment (Study 2). See Table 1 for n, r^2, and *p*. Alphabets indicate significant difference at $p<0.01$.

Population	Equation	n	Y_{int}	Slope	r^2	*p*
Culture	$\text{Log } \underline{b}^*_{ph}(676)$	26	-2.16^a	0.74^b	0.52	<0.001
	$= Y_{int} + \text{Slope} \times \text{Log C:Chl } a$					
In situ	$\text{Log } \underline{b}^*_{ph}(676)$	62	-2.85^a	0.94^b	0.47	<0.001
	$= Y_{int} + \text{Slope} \times \text{Log POC:Chl } a$					

Conclusions

This study presented that both $a^*_{ph}(676)$ and $\underline{b}^*_{ph}(676)$ decreased with cell size, such as *d* of single species with various cell size (Study 1) and size index derived from the relative Chl *a* abundance and the weighed values (study 2). The relationships could assist the understanding for inverting remotely sensed data to the size distribution of phytoplankton assemblage. In addition, the difference in the intercepts of the relationships between the IOPs and *d* and/or size index suggests that the more accurate evaluation of the effect of cell size on the IOPs would require the knowledge of physiological properties of natural assemblage of phytoplankton. The significant relationships between $a^*_{ph}{}^{slope}$ and PPC:TC, and $b^*_{ph}(676)$ and POC:Chl *a* in the present study suggest that the $a^*_{ph}{}^{slope}$ and $b^*_{ph}(676)$ can assist to correct the physiological effect of the cell size. The estimated size distribution of natural assemblage of phytoplankton and physiological properties from remotely sensed data could contribute to the understanding marine carbon cycle.

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