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# Low blue light enhances growth rate, light absorption, and photosynthetic characteristics of four marine phytoplankton species

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ABSTRACT

The effects of blue light on the growth rate, biochemical properties, light absorption, and photosynthetic characteristics based on chlorophyll *a* fluorescence were studied in the marine phytoplankton prymnesiophytes *Isochrysis galbana*, chlorophytes *Dunaliella salina*, diatoms *Chaetoceros gracilis*, and dinoflagellates *Heterocapsa circularisquama* to understand the variability in ecological roles of blue light among the four species. Monocultures were acclimated to blue and white light at the same irradiance of 60 μmol photons $m^{-2}$ $s^{-1}$ for more than three dilutions in semi-continuous culture before the comparative experiment. Under our experimental conditions, growth rates under blue light condition were significantly higher (1.6–2.0 fold) than those rates under the white light condition ($p < 0.05$), although the cell volume was not influenced. In addition, cellular contents of Chl *a* per cell volume significantly increased by more than 1.2-fold under blue light, excluding *C. gracilis*. Moreover, the cellular contents of both carbon and nitrogen per cell volume significantly increased by more than 1.8-fold under blue light in only *H. circularisquama*. The significant increase in the ratio of Chl *a* to carbon was associated with an enhanced growth rate in *I. galbana* and *H. circularisquama*, whereas the growth rate of the other two species had an inverse correlation with these conditions. Chlorophyll *a*-specific absorbance for blue light (400–550 nm) for the cells that were exposed to blue light were significantly higher (1.3-fold) than those absorbance of PAR (400–700 nm) for the cells that were exposed to PAR ($p < 0.05$). The amount of light that was absorbed by chlorophyll *a* under the blue light condition was significantly higher (3-fold) than that under the white light condition ($p < 0.05$). The potential quantum efficiency ($F_v/F_m$) was not influenced by blue light in all species, whereas the dependence of $rETR_{max}$ or $\alpha$ on the Chl *a*/C ratios suggests the potential usefulness of the Chl *a*/C ratio as a photoacclimation index, even for the chlorophyll *a* fluorescence analysis. Among the species that were examined in the present study, *D. salina* was highly sensitive to the blue light and indicated a significant enhancement in the initial slope $\alpha$, the maximum *rETR*, and the $E_k$, which were estimated from the *rETR* vs. *E* curve ($p < 0.05$). The high utilization efficiency of blue light may suggest an ecological advantage for some species of phytoplankton that live in habitats where blue light prevails.



## 1. Introduction

### 1.1. Ecological significance of blue light in the sea

Solar radiation consists of a wide range of wavelengths of photons, even within the photosynthetically active radiation (PAR, 400–700 nm), which ranges from violet-blue to red. Once solar radiation penetrates into the sea, the spectral composition narrows to a certain waveband, with an optical depth that depends on the optical type of the water. Blue light becomes dominant toward the bottom layer of the euphotic zone in the open sea. However, ecologically speaking, the maximum optical depth at which phytoplankton can photosynthesize is defined conventionally as 1–0.1% of the surface light, regardless of whether the spectral composition shifts to predominantly blue light from the surface white light in the open water (Falkowski and Raven, 2007; Tilzer et al., 1994). Near the bottom layer of the euphotic zone, subsurface Chl *a* maxima have often been observed for the natural assemblage of phytoplankton (Cullen, 1984). Recently, some specific populations, such as *Prochlorococcus* spp. and *Synechococcus* spp., have been recognized as forming the subsurface maximum at deeper depths than the conventional depth of the euphotic zone, e.g., 1% (Partensky et al., 1996; Prezelin et al., 1989). Studies regarding the photo-adaptation and spectral aspects of the competition for light may, therefore, provide important next steps toward a further understanding of phytoplankton

*Abbreviations:* $a_{ph}^*$, integrated absorption of phytoplankton chlorophyll *a* from 400 to 700 nm; $a_{ph}^*{}_{(\lambda)}$, absorption of phytoplankton chlorophyll *a* at a given wavelength λ; $\alpha$, the initial slope of *ETR* vs. *E* curve; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Chl *c*, chlorophyll *c*; Chl *a*/C, ratio of chlorophyll *a* to carbon; DD, diadinoxanthin; DT, diatoxanthin; $E_a$, total amount of light that was absorbed by Chl *a*; $E_k$, adaptation index; $E_{(\lambda)}$, irradiance at a given wavelength λ; Fuco, fucoxanthin; *F*, minimum fluorescence yield in the light-adapted state; $F_0$, maximum fluorescence yield in the dark-adapted state; $F'_m$, maximum fluorescence yield in the light-adapted state; $F_v/F_m$, potential quantum efficiency; $F'_q/F'_m$, PSII operating efficiency; PAR, photosynthetically available radiation; Peri, peridinin; PFD, photon flux density; PUR, photosynthetically usable radiation; *rETR*, relative electron transport rate; $rETR_{max}$, the maximum *rETR*.

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photosynthesis (Huiman et al., 1999) and even benthic diatom competition (Mercado et al., 2004). Light at the bottom layer of the euphotic zone could be quite often less than 1% of the surface light for particular species and dominated more significantly by blue light than previously thought. The low blue light can be ecologically significant to maintain some groups of phytoplankton in the sea because the contribution of certain groups of phytoplankton has been demonstrated to be significant to daily primary production in a water column (Li, 1994; Weston et al., 2005) and these groups also play a key role in energy transfer with the microbial loop in the marine ecosystem (Scanlan, 2003).

### 1.2. Significance of blue light in photosynthesis

One of the most important light-harvesting pigments is chlorophyll *a* (Chl *a*) in microalgae. Chlorophyll *a* has two major absorption peaks at 662 nm and 430 nm (Bricaud et al., 2004), with a blue to red ratio of 1.3 (Smith and Benitez, 1955). Major photo-harvesting pigments, such as chlorophyll *b* (Chl *b*), chlorophyll *c* (Chl *c*), fucoxanthin (Fuco), $\beta$-carotene ($\beta$-caro), and peridinin (Peri), have peaks that are dominant at short wavelengths between 475 nm and 420 nm in certain groups of prymnesiophytes, chlorophytes, diatoms, and dinoflagellates. Light absorption of Chl *a* ($a_{ph}^*$) is the highest at the peak of shorter wavelengths, at approximately 443 nm (Johnsen et al., 2011). When the light absorbance at a given wavelength is calculated as the percent $a_{ph}^*(\lambda)$ at 443 nm of total $a_{ph}^*$ at 400–700 nm, the light absorbance between 400 and 550 nm is higher than those between 550 nm and 700 nm. High light absorbance at a given band of wavelength can also be expected when phytoplankton are exposed to blue light only, with the same numbers of photons between 400 nm and 700 nm (PAR). Photosynthetically usable radiation (PUR) that is absorbed by phytoplankton is highest in the blue light region even when a flat irradiance is provided (Morel and Bricaud, 1981). Therefore, the amount of light that is absorbed by phytoplankton depends on the spectral absorption characteristics of the cells and on the spectral irradiance of their ambient light field.

### 1.3. Variability in the responses to light quality

The effect of blue light on the growth rate of phytoplankton could be misleading when the comparison is based on the light-saturating irradiance for white light cultures and on the light-limiting irradiance for blue/green light cultures by using a blue filter to remove non-blue light from a white light source. To avoid a difference in energy between blue and white light, the light intensity is usually adjusted to obtain approximately the same number of quanta from each light source (e.g., Aider et al., 1994; Gostan et al., 1986).

For example, an enhancing effect for blue light at 50 μmol photons $m^{-2}$ $s^{-1}$ on growth rate has been reported for *Chaetoceros gracilis*, whereas a depressing effect has been reported for *Emiliania huxleyi* (Schofield et al., 1990). Because the methodology is not similar among studies, the comparison should be taken with caution, as suggested by Markager and Vincent (2001). Even when all methodologies are similar except for the light intensity, the choice of light intensity is critical to detect the effect of blue light, as suggested by Schofield et al. (1990). Under the saturated light condition for growth rate, the enhancement effect by blue light can be minimal, despite the fact that this effect can be detected under the light-limited condition. It is critical to use the light-limited growth condition to maximize the difference in the effect of light quality.

### 1.4. Objectives

The comparative incubation experiments were designed to study the variability in the growth rate, biochemical properties, light absorption, and chlorophyll *a* fluorescence between white light (PAR, 400–700 nm) and blue light (400–550 nm), with the same number of photons, by using four taxonomically different groups of phytoplankton with different pigment profiles. A light-limited growth condition was employed to maximize the difference in the effect of light quality. The potential quantum efficiency ($F_v/F_m$), the initial slope ($\alpha$) and the maximum *ETR* ($rETR_m$), which were estimated from the r*ETR* versus *E* curve, were examined to determine the cells which have high growth rates and light absorption efficiencies as specified by Chl *a*, and $rETR_m$, that were induced by blue light (400–550 nm) at 60 μmol photons $m^{-2}$ $s^{-1}$. However, for any given species, the composition of the light-harvesting pigments was not influenced by the blue light.

## 2. Materials and methods

### 2.1. Culture

Cultures of *Isochrysis galbana* (prymnesiophytes), *Dunaliella salina* (chlorophytes), and *C. gracilis* (diatoms) were obtained from the North East Pacific Culture Collection at the University of British Columbia, Canada, and *Heterocapsa circularisquama* (dinoflagellates) was obtained from the Culture Collection Center at the National Institute of Environmental Science, Japan. The axenic stock cultures were routinely maintained at 25 °C, with a white and blue light of 60 μmol photons $m^{-2}$ $s^{-1}$ at the center of culture vessels under a 12:12 h light and dark (L:D) cycle in a light- and temperature-controlled incubator (Eyela, FLI-301N, Japan) for more than three dilutions in a semi-continuous culture mode during the log-phase of growth in filtered, aged seawater enriched with f/2 medium (Guillard and Ryther, 1962). The culture was replaced with a fresh medium (30% in volume) at the middle of light period. The white and blue lights were provided through one layer of neutral density filter (Clark #300, Japan) with cool-white fluorescence tubes (Toshiba, FL40SW, 40W, Japan) and tubes with a single layer of blue filter (Taffcal, 4515C, Japan), respectively, to obtain the same irradiance at 60 μmol photons $m^{-2}$ $s^{-1}$. The photon flux density (PFD) in the center of the culture vessel, which contained distilled water using a 4π-scalar quantum sensor (QSL-100, Biospherical Instruments, San Diego, California, USA). The light intensity spectrum of white light, which was provided by the cool-white fluorescence tubes, showed six peaks (406, 436, 490, 545, 588, and 612 nm) from 400 nm to 700 nm, which were determined using a spectral radiometer (TrisOS Optical-Sensors, Ramses-Acc-UV, Germany) (Fig. 1). The light intensity spectrum of blue light showed four peaks (406, 436, 490, and 545 nm) from 400 nm to 550 nm (Fig. 1).

### 2.2. Incubation experiments and sampling

Triplicate of incubation bottles were prepared using 1 or 2 L polycarbonate bottles (Nalgene, USA). Experimental incubation was conducted by transferring cells during the log-phase into the incubation bottles to ensure subsequent exponential growth. Cell density was

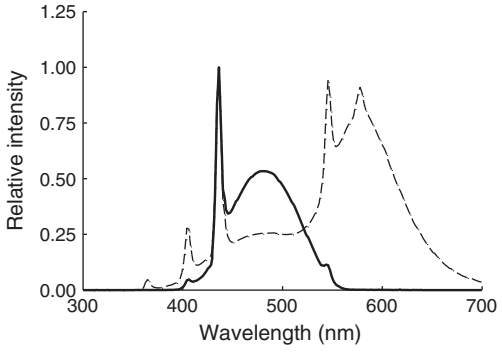


**Fig. 1.** Relative intensity of light sources with (solid line) or without a blue filter (broken line). The intensities were standardized at 435 nm.