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Effect of Light Wavelength on Biomass, Growth, Photosynthesis and Pigment Content of *Emiliania huxleyi* (Isochrysidales, Cocco-Lithophyceae)

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Abstract: Light wavelength is a critical abiotic factor in modulating the development and pigment accumulation of microalgae. In the present study, we investigated the influences of white, red, blue, yellow, and green light on biomass (cell density), growth (cell diameter and dry weight), net photosynthetic rate, and pigment contents (chlorophyll *a*, fucoxanthin, and lutein) of the coccolithophore *Emiliania huxleyi*. The effects of light wavelength change on its cell density and fucoxanthin content were also evaluated. The results showed that blue light significantly stimulated the cell proliferation and photosynthetic activity of *E. huxleyi*. The cell diameter, dry weight, net photosynthetic rate, and the content of fucoxanthin under red light were significantly greater than under white light. *E. huxleyi* could not effectively utilize green light and yellow light for growth, photosynthesis, and pigment synthesis. Compared with white, blue, and red light, significantly greater cell density and fucoxanthin content were found under blue light, followed by red light. These findings indicated that light wavelength could significantly affect the growth, photosynthesis, and pigments of *E. huxleyi*. The combination of blue and red light is likely to be an effective measure to enhance its biomass and fucoxanthin production.

Keywords: biomass; *Emiliania huxleyi*; fucoxanthin; growth; light wavelength

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1. Introduction

Light is one of the most significant abiotic factors that modulate the diverse physiological processes in freshwater and marine microalgae [1]. One feature of light is that the light wavelength can play a significant role in their growth and development [2,3]. For instance, blue light promoted the growth of *Tisochrysis lutea*, *Chlamydomonas reinhardtii*, and *Dunaliella* sp. by regulating their physiological metabolisms or increasing DNA or RNA synthesis for cell division [4–6]. Blue light may also generally enhance the photosynthetic activities of various microalgal species by activating their photosynthetic electron transfer chain reactions, especially the activity of ribulose—1,5—diphosphate carboxylase [7,8]. Additionally, it grew fastest for *Haematococcus lacustris* (Girod-Chantrans) Rostafinski under red light because the energy of red photons might be the most suitable to meet its photosynthesis requirements [9]. However, the amino acids of *Amphora* sp. under yellow light may be mainly used for the synthesis of pigments rather than for cell proliferation, thereby resulting in the lowest cell concentration [10]. Therefore, the selection of appropriate light wavelength is very important to optimize the biomass production of microalgae and support their high-density cultures.

Numerous studies have clarified that light wavelength can greatly impact the pigment content of microalgae. Zhao et al. [11] indicated that blue light-stimulated lutein synthesis in *Chlamydomonas* sp. by regulating its protein synthesis and related enzyme activities. For *Chlorella* sp., both red light and blue light could increase its lutein content, and maximal lutein production was found under red light [12]. The astaxanthin content of *H. lacustris*

under blue light was two to eight times higher than under red or white light [13]. Mixing red and blue light could also significantly promote the astaxanthin accumulation of *H. lacustris* [14,15]. Therefore, the manipulation of light wavelength may be an effective way to improve the content of economically valuable pigments. Similar to lutein and astaxanthin, fucoxanthin is also a commercial carotenoid that has received widespread attention. Due to its function as an antioxidant, anti-cancer, and anti-obesity, fucoxanthin has been used as a nutritional supplement in foods, nutraceuticals, and pharmaceuticals, and has been verified to be safe for human consumption [16–18]. Light conditions have also been verified to play a crucial role in improving the fucoxanthin content in microalgae. For *Odontella aurita*, *Isochrysis zhangjiangensis* and *Stephanocyclus cryptica* (Reimann, Levin & Guillard) Kulikovskiy, Genkal & Kociolek, low light intensity accelerated their fucoxanthin accumulation by inducing light-harvesting mechanisms and by modulating the diadinoxanthin cycle [19–21]. However, little information is currently available on the impact of light wavelength on fucoxanthin accumulation in microalgae and its potential application.

Emiliania huxleyi is one of the most abundant marine coccolithophores and is widely distributed from polar to tropical waters worldwide [22–24]. This species plays a significant role in the carbon cycle of seawater by fixing carbon from photosynthesis and calcification [25–27]. *E. huxleyi* is deemed to be an important raw material in biomolecules such as alkenones, neutral and polar lipids, and polyunsaturated fatty acids, which are widely applied in the food and pharmaceutical industries [28–30]. It is also an ideal candidate for fucoxanthin production because of its richness in pigments and rapid proliferation [22,31]. Given the commercial and ecological significance of this species, a great number of studies exploring the influences of various environmental factors on its physiological and biochemical characteristics have been performed [32–35]. However, the effects of light wavelength on this species, particularly its growth and pigment synthesis, have rarely been investigated.

In this study, we examined the effects of light wavelength on the biomass (cell density), growth (cell diameter and dry weight), net photosynthetic rate, and pigment contents (chlorophyll *a*, fucoxanthin, and lutein) of *E. huxleyi*. The effects of the change to light wavelength on its cell density and fucoxanthin content were also evaluated. Our findings will provide important information to improve fucoxanthin production by light wavelength regulation.

2. Materials and Methods

2.1. Microalgal Strain and Experimental Preparation

The strains of naked cells *Emiliania huxleyi* (Lohmann) W.W.Hay & H.Mohler (LAMB244) used in this study were obtained from the microalgae culture collection at the Laboratory of Applied Microalgal Biology, Ocean University of China. The stock was grown in 3000 mL Erlenmeyer flasks containing 2500 mL aseptic IMK liquid medium [36] under the conditions of 20 ± 0.5 °C, 100 µmol photons m^{-2} s^{-1} and a 12:12 h light/dark cycle. In subsequent experiments, *E. huxleyi* was maintained in its exponential growth phase by transferring it to fresh aseptic IMK mediums every week. The IMK liquid medium contained 200 mg L^{-1} $NaNO_3$, 1.4 mg L^{-1} Na_2HPO_4, 5 mg L^{-1} K_2HPO_4, 2.68 mg L^{-1} NH_4Cl, 0.52 mg L^{-1} Fe-EDTA, 0.0332 mg L^{-1} Mn-EDTA, 37.2 mg L^{-1} Na_2-EDTA, 0.023 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.014 mg L^{-1} $CoSO_4 \cdot 7H_2O$, 0.0073 mg L^{-1} $Na_2MoO_4 \cdot 4H_2O$, 0.0025 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 0.0018 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 0.2 mg L^{-1} Thiamin-HCl, 0.0015 mg L^{-1} Biotin, and 0.0015 mg L^{-1} Vitamin B12.

2.2. Single Light Wavelength Experiment

A culture experiment was carried out for 9 days under five single light wavelengths (LS-DD34, LEESA, Guangzhou, China): red light (620–630 nm), blue light (400–470 nm), green light (510–515 nm), yellow light (590–595 nm), and white light. There was a total of five experimental treatments, and each treatment was performed for nine replicates. Each of the three replicates was used for the determination of growth parameters, photosynthesis,

and pigments. This experiment used 45 Erlenmeyer flasks (500 mL), with each flask containing 450 mL of fresh aseptic IMK medium. An initial cell density of 1.5×10^4 cells mL^{-1} was set for each replicate. During the experiment, a temperature of 20 ± 0.5 °C, an irradiance of 100 µmol photons m^{-2} s^{-1}, and a 12:12 h light/dark cycle were held constant. The light intensity was determined using a Quantitherm Light Meter (QRT1, Hansatech Instruments, UK). These flasks were manually shaken several times per day.

During this experiment, the cell density of *E. huxleyi* in each replicate was determined using a phytoplankton counting chamber (XKJ-01B, Xiamen Kexi Instrument Company, China) every 3 days. To immobilize these cells, a 1 mL suspension from each replicate was transferred to centrifuge tubes (1.5 mL), to which 10 µL Lugol's iodine was added. These samples were homogenized, and a 0.1 mL sample was transferred to the phytoplankton counting chamber using a pipette. Sixty fields of view were randomly selected, and cell counting was performed under an inverted light microscope (IX73, Olympus, Japan) at ×400 magnification. The cell density (N; cell mL^{-1}) was calculated using the following Equation (1) [37]:

$$N = Cs \cdot V \cdot Pn/(Fs \cdot Fn \cdot U), \quad (1)$$

where Cs is the area of the total grid on the chamber, V is the volume of the total sample, Pn is the number of counted cells, Fs is the area of the measured grid, Fn is the number of measured grids, and U is the volume of the chamber.

After 9 days, the diameters of 120 cells from each replicate were measured under a light microscope (CX31, Olympus, Japan) at ×1000 magnification after calibration.

The algal cells were used to measure the dry weight after the culture experiment. A 200 mL suspension from each replicate was collected and centrifuged at 7000 rpm for 5 min. The deposits were adequately rinsed and centrifuged again in the same way. The pellets were then flash-frozen in liquid nitrogen and lyophilized for 24 h. Finally, dry deposits were weighed.

After 9 days, the net photosynthetic rate was determined using a Fiber-Optic Oxygen Meter (FSO2-C4, PyroScience GmbH, Germany). A 350 mL *E. huxleyi* suspension from each replicate was moved to the oxygen electrode cuvette, and the cell density was adjusted to approximately 1.0×10^5 cells mL^{-1}. The suspension was then magnetically stirred to ensure the even diffusion of oxygen. The temperature and irradiance levels were the same as for the above-mentioned experiment. The oxygen increase in the suspension was regarded as the net photosynthetic oxygen evolution. Prior to conducting measurements, these cells were allowed to acclimate to the conditions in the cuvette for 5 min. The oxygen concentration was recorded every 1 min for 15 min. The net photosynthetic rate was normalized to µmol O_2 $\times 10^5$ $cell^{-1}$ mL min^{-1}.

To obtain the Chl *a* measurement, a 5 mL suspension was collected from each replicate and centrifuged at 7000 rpm for 5 min. An amount of 3 mL precool methanol (100%) was added to the deposits, which were then resuspended for 2 h under low temperature and dark conditions. Extracts were centrifuged at 7000 rpm for 5 min. The absorbance was determined to be 665 and 750 nm using a spectrophotometer (U-2900, HITACHI, Japan). The concentration of Chl *a* (mg L^{-1}) was calculated using the following Equation (2) [38]:

$$\text{Chl } a \text{ (mg L}^{-1}) = 13.3 \times (OD_{665} - OD_{750}) \times V_m/V_t, \quad (2)$$

where 13.3 is the extinction coefficient for Chl *a* in 100% methanol, OD_{665} and OD_{750} are the absorptions of extracts at 665 nm and 750 nm, respectively, V_m is the volume of the measured suspension, and V_t is the volume of the total suspension.

The extraction and measurements of lutein and fucoxanthin were carried out according to [39]. Specifically, a 200 mL suspension from each replicate was collected and centrifuged at 7000 rpm for 5 min. The deposits were adequately rinsed and centrifuged again in the same way. The deposits were then flash-frozen in liquid nitrogen and lyophilized for 24 h. Next, 5 mL of a precooled mixture of methanol and acetone (1:1, *v*/*v*) was added to a 20 mg freeze-dried sample and mixed under a low temperature and dark condition for 2 h. After

5 min centrifugation at 3000 rpm, the supernatant was filtered using 0.22 μm polycarbonate filters for high-performance liquid chromatography (HPLC) analysis. The contents of filtered pigments were determined using an Agilent 1200 HPLC (Agilent Technologies, CA, USA) equipped with an Rx-C18 analytical column (4.6 mm × 250 mm; Agilent Technologies, USA). The standard commercial lutein and fucoxanthin used for quantification were from Sigma-Aldrich (USA). Processing of the mobile phase, which was composed of I (water/methanol/acetonitrile, 3:6:11, *v*/*v*/*v*), II (methanol/acetonitrile, 3:17, *v*/*v*), III (water/methanol/acetonitrile/ethyl acetate, 3:3:7:7, *v*/*v*/*v*/*v*), and IV (methanol/ethyl acetate, 3:7, *v*/*v*), was performed as follows: 0–15 min, linear gradient from I to II; 15–17 min, linear gradient from II to III; and 17–40 min, linear gradient from III to IV. The flow rate was 0.75 mL min^{-1}, and the column temperature was 50 °C. The pigments were detected using their absorbance at 443 nm.

2.3. Light Wavelength Change Experiment

To investigate the impact of light wavelength change on *E. huxleyi*, it was cultured under blue light for 3 days, and then changed to red light for 6 days. The cell density and fucoxanthin content of *E. huxleyi* cultured under these conditions were compared with those cultured under three single light wavelengths of white, blue, and red light for 9 days. Cell density and fucoxanthin content were determined in the same way as described above. A total of 12 Erlenmeyer flasks (500 mL) were prepared. Each flask contained 450 mL of fresh aseptic IMK medium. An initial cell density of 1.5×10^4 cells mL^{-1} was set for each replicate. During the experiment, a temperature of 20 ± 0.5 °C, an irradiance of 100 μmol photons m^{-2} s^{-1}, and a 12:12 h light/dark cycle were maintained. These flasks were manually shaken several times per day.

2.4. Statistical Analysis

The significant effects of light wavelength condition on cell density, cell diameter, dry weight, net photosynthetic rate, and pigment contents were analyzed using a one-way analysis of variance. Before analyses, the data variances were subjected to the homogeneity test. A Duncan multiple range test was applied when significant differences were found between means. Differences were significant at a probability of 5% ($p < 0.05$). Data analysis was conducted using SPSS statistical software (version 26.0).

3. Results

3.1. Single Light Wavelength Experiment

A culture experiment was performed for 9 days under five single light wavelengths. During this experiment, the cell density of *E. hurleyi* in each replicate was determined every 3 days. After the culture, the growth (cell diameter and dry weight), net photosynthetic rate, and pigment contents (chlorophyll *a*, fucoxanthin, and lutein) of *E. huxleyi* were measured.

3.1.1. Cell Density

The cell densities of *E. huxleyi* were significantly different between light wavelengths on the 3rd, 6th, and 9th day (one-way ANOVA, the 3rd day: df = 4, F = 747.3789, $p < 0.001$; the 6th day: df = 4, F = 321.786, $p < 0.001$; the 9th day: df = 4, F = 242.214, $p < 0.001$; Figure 1). On the 3rd day, the cell density under blue light was significantly greater than under the other light wavelengths. The cell density under red light was significantly lower than under white light and green light. On the 6th day, the cell density under blue light was significantly greater than under the other light wavelengths. The cell density under red light was significantly greater than under white light and green light. On the 9th day, the cell density under white light was significantly greater than under the other light wavelengths. The cell density under green light was significantly lower than under red light and blue light. On each day, the cell density under yellow light was significantly lower than under the other light wavelengths.

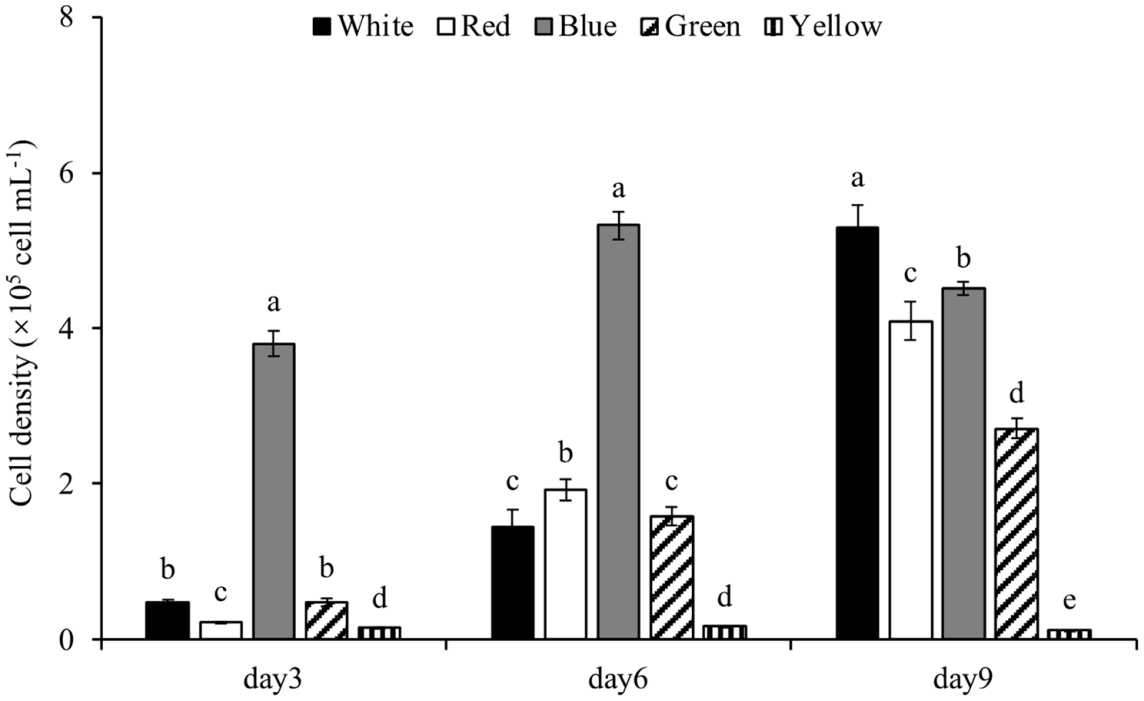

Figure 1. Cell density of *E. huxleyi* cultured under different light wavelengths (white, red (620–630 nm), blue (400–470 nm), green (510–515 nm) and yellow (590–595 nm) light) on the third, sixth, and ninth day. Data are presented as mean ± SE (n = 3). Lowercase letters indicate significant differences between light wavelengths at $p < 0.05$.

3.1.2. Growth

The cell diameters of *E. huxleyi* also differed significantly between light wavelengths (one-way ANOVA, df = 4, F = 78.288, $p < 0.001$; Figure 2). The cell diameter of *E. huxleyi* was significantly greater under red light than under the other light wavelengths. Additionally, the cell diameters under white light and green light were significantly greater than under blue light and yellow light. The cell diameter under yellow light was significantly lower than under blue light.

The dry weights of *E. huxleyi* were significantly different between light wavelengths (df = 4, F = 3172.595, $p < 0.001$; Figure 2). After 9 days, the dry weight under red light reached the maximum value of 864.20 ± 7.16 mg L^{-1}, which was significantly greater than under the other light wavelengths. The dry weights under blue, green, and yellow lights were significantly lower than under white light.

Our data showed that the cell diameter and the dry weight of *E. huxleyi* were significantly greater under red light than under the other light wavelengths, indicating that red light could significantly contribute to the cell growth of *E. huxleyi*. However, yellow light exhibited the greatest inhibition to the cell diameter and the dry weight of *E. huxleyi*, implying that this species might not effectively harvest yellow light.

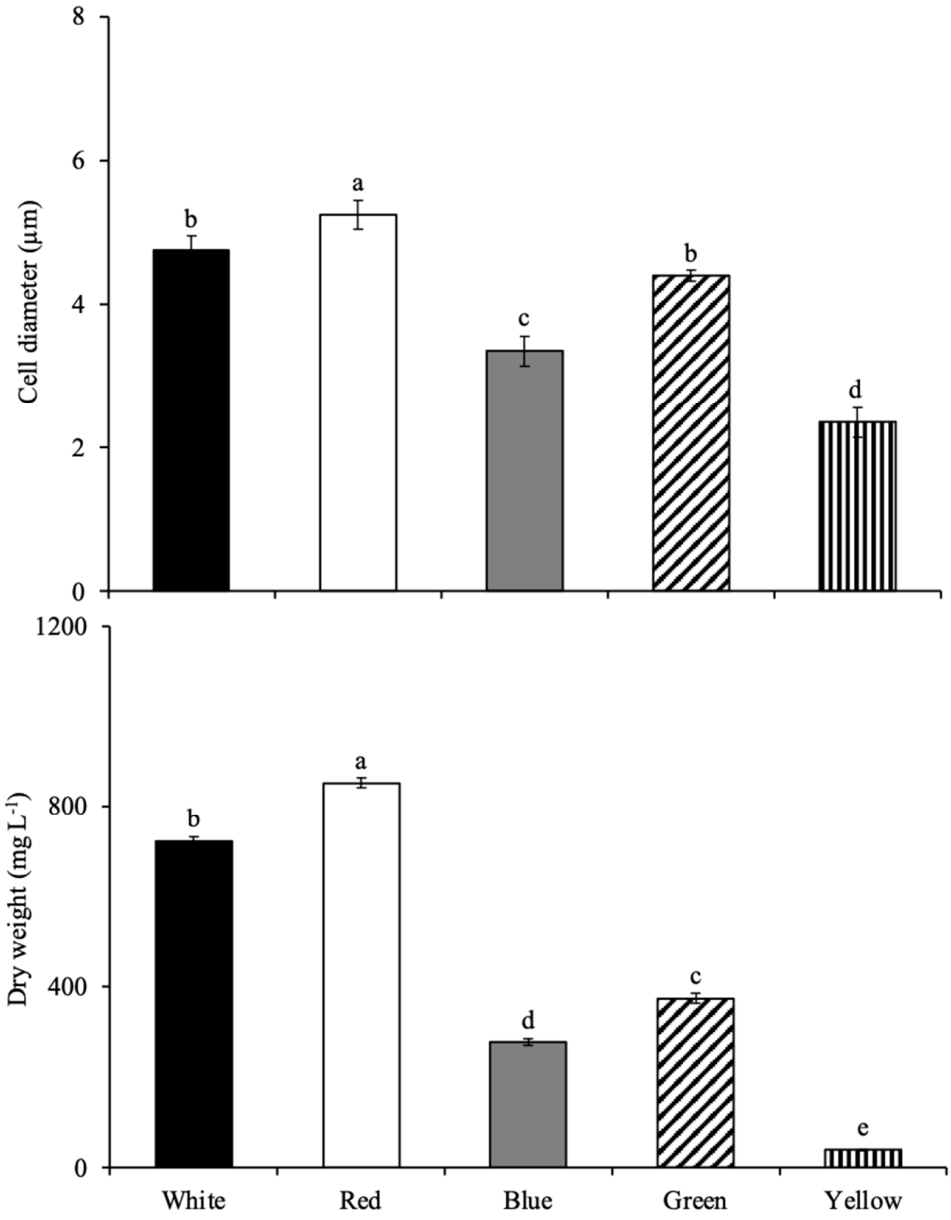

Figure 2. Cell diameter and dry weight of *E. huxleyi* cultured under different light wavelengths (white, red, blue, green, and yellow light) on the 9th day. Data are presented as mean ± SE (n = 3). Lowercase letters indicate significant differences between light wavelengths at $p < 0.05$.

3.1.3. Net Photosynthetic Rate

The net photosynthetic rate of *E. huxleyi* was significantly affected by light wavelength (df = 4, F = 87.631, $p < 0.001$; Figure 3). The net photosynthetic rate showed its maximum value under red light. The net photosynthetic rates under red light and blue light were significantly greater than under the other light wavelengths. The net photosynthetic rates under green light and yellow light were significantly lower than under white light. The net photosynthetic rate under yellow light was significantly lower than under the other light wavelengths.

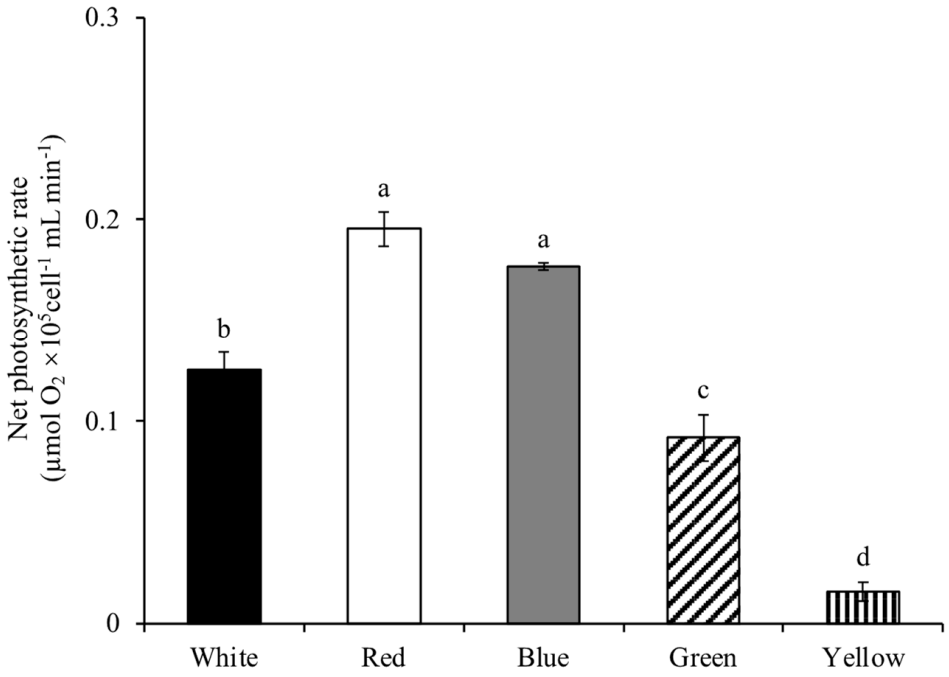

Figure 3. Net photosynthetic rate of *E. huxleyi* cultured under different light wavelengths (white, red, blue, green and yellow light) on the 9th day. Data are presented as mean ± SE (n = 3). Lowercase letters indicate significant differences between light wavelengths at $p < 0.05$.

3.1.4. Pigment Contents

The Chl *a* content was significantly affected by light wavelength (one-way ANOVA, df = 4, F = 364.175, $p < 0.001$; Figure 4). The Chl *a* content under blue light was significantly greater than under the other light wavelengths. The Chl *a* content under red, green, and yellow light was significantly lower than under white light. The Chl *a* content under yellow light was significantly lower than under the other light wavelengths.

The fucoxanthin content differed significantly between light wavelengths (df = 4, F = 679.564, $p < 0.001$; Figure 4). The fucoxanthin content under red light was significantly greater than under the other light wavelengths. The fucoxanthin content under blue, green, and yellow lights was significantly lower than under white light. The fucoxanthin content under blue light and yellow light was significantly lower than under the other light wavelengths.

There were significant differences in lutein content between light wavelengths (df = 4, F = 548.092, $p < 0.001$; Figure 4). The lutein content showed a significant continuous decrease in the following order: white, red, blue, green, and yellow light.

3.2. Light Wavelength Change Experiment

In the single light wavelength experiment, blue light could effectively enhance the cell proliferation of *E. hurleyi*, and red light had a stimulating effect on its cell growth and fucoxanthin synthesis. Given the positive role of the two light wavelengths, a light wavelength change experiment was performed. During this experiment, the cell density of *E. hurleyi* in each replicate was determined every 3 days. After the experiment, the fucoxanthin content of *E. huxleyi* was measured.

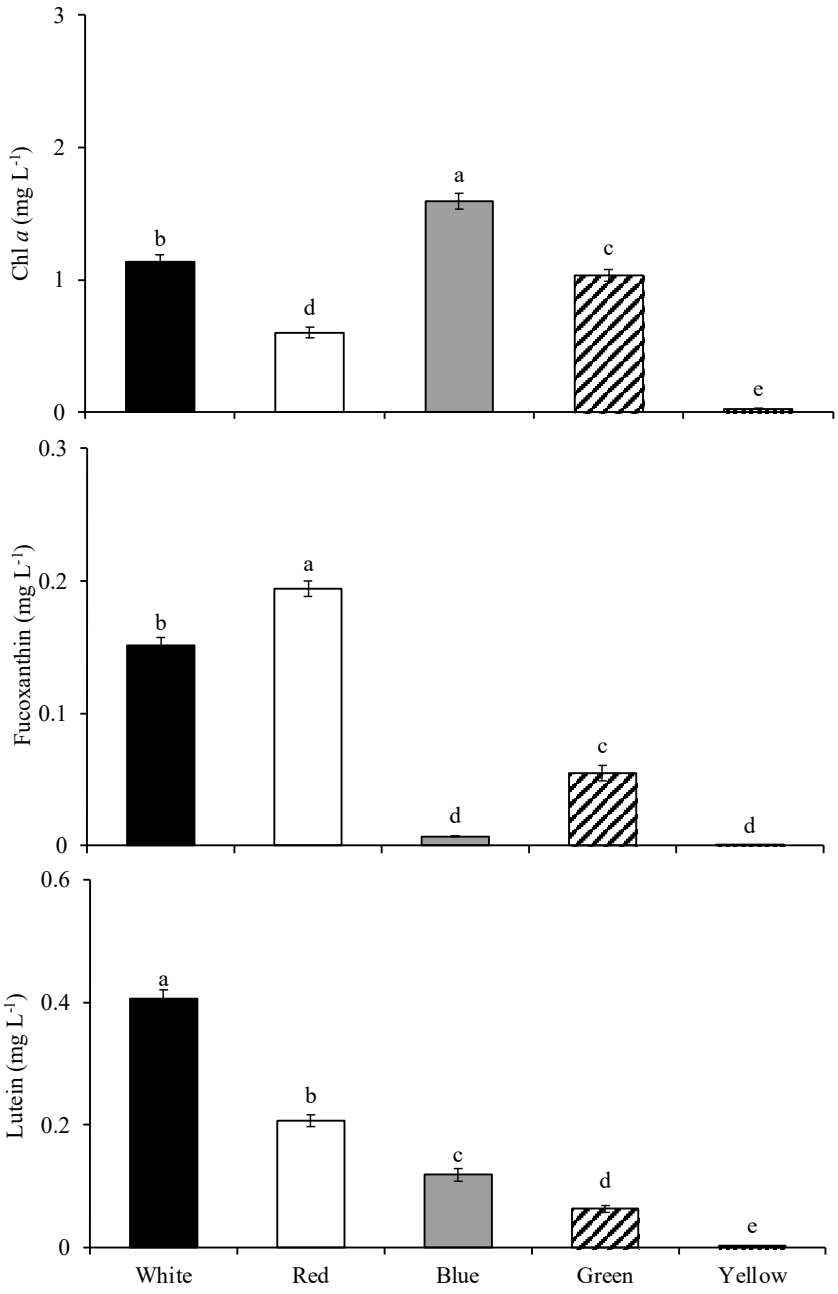

Figure 4. Content of Chl *a*, fucoxanthin and lutein of *E. huxleyi* cultured under different light wavelengths (white, red, blue, green, and yellow light) on the 9th day. Data are presented as mean ± SE (n = 3). Lowercase letters indicate significant differences between light wavelengths at $p < 0.05$.

3.2.1. Cell Density

The cell density of *E. huxleyi* was significantly different between light wavelength conditions on the 3rd, 6th, and 9th day (one-way ANOVA, the 3rd day: df = 3, F = 732.291, $p < 0.001$; the 6th day: df = 3, F = 179.763, $p < 0.001$; the 9th day: df = 3, F = 46.004, $p < 0.001$; Figure 5). On the 3rd and 6th days, the cell density under blue light and the light wavelength change were significantly greater than under white light and red light. On the 3rd day, the cell density under red light was significantly lower than under white light. On the 9th day, the cell density under light wavelength change reached the maximum value of $0.2830 \pm 0.0065 \times 10^5$ cell mL^{-1}, which was significantly greater than under the other single light wavelengths. The cell density under white light was significantly greater than under red light and blue light.

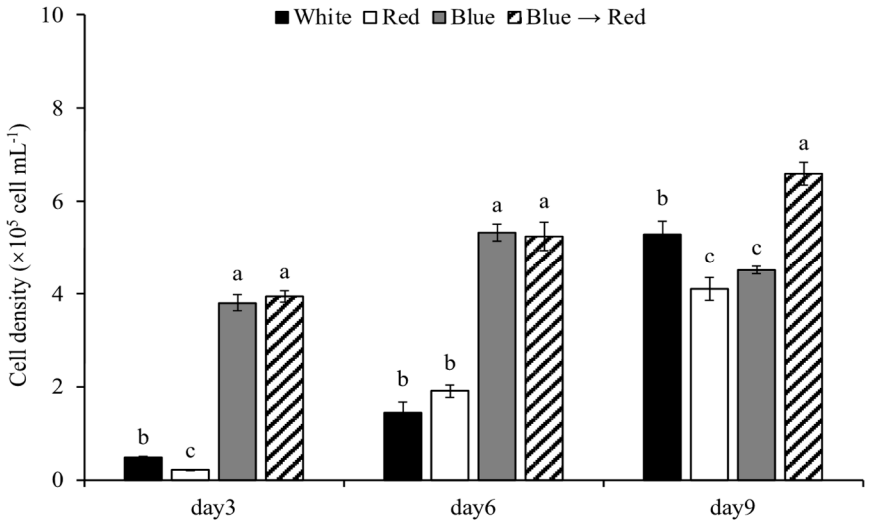

Figure 5. Cell density of *E. huxleyi* cultured under constant light wavelengths (white, red, and blue light) and light wavelength change (from blue to red light) at the third, sixth and ninth day, respectively. Data are presented as mean ± SE (n = 3). Different lowercase letters indicate significant differences between different treatments at $p < 0.05$.

3.2.2. Fucoxanthin Content

The fucoxanthin content differed significantly between light wavelength conditions (one-way ANOVA, df = 3, F = 613.777, $p < 0.001$; Figure 6). After 9 days, the fucoxanthin content under light wavelength change reached the maximum value of 0.2717 ± 0.0113 mg L^{-1}, which was significantly greater than that of the other single light wavelengths. The fucoxanthin content under red light was significantly greater than under white light and blue light. In addition, the fucoxanthin content under blue light was significantly lower than under the other light wavelength conditions.

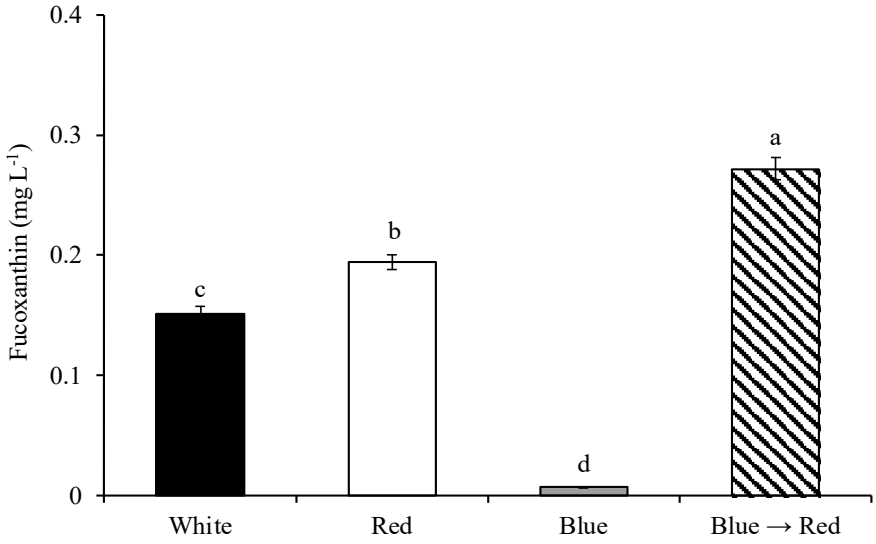

Figure 6. Fucoxanthin content of *E. huxleyi* cultured under constant light wavelengths (white, red, and blue light) and light wavelength change (from blue to red light) on the 9th day. Data are presented as mean ± SE (n = 3). Lowercase letters indicate significant differences between different treatments at $p < 0.05$.

4. Discussion

Our study showed that the cell density of *E. huxleyi* on the third and sixth day was significantly greater under blue light than under the other light wavelengths, indicating that blue light could stimulate its cell proliferation. Similar findings have been documented for *Tetraselmis* sp. and *Nannochloropsis* sp. Specifically, it was shown that the cell density of these species was the highest under blue light, probably due to the promotion of gene transcription that was related to photosynthetic enzymes and carbohydrate degrading enzymes and the regulation of activated enzymes [40]. Additionally, the maximum growth rate of *Rhodomonas* sp. was detected under blue light because its phycoerythrin and photosynthetic pigments could effectively absorb blue light to obtain energy for cell division [41]. Coincidentally, the Chl *a* content of *E. huxleyi* reached the maximum value under blue light. This is basically accordant with the results of previous studies and implies that blue light significantly enhanced the photosynthetic pigment syntheses in microalga species, including *Isochrysis galbana*, *Dunaliella salina*, *Chaetoceros gracilis*, and *Nannochloropsis* sp. [42,43]. Similar to the above species, blue light is likely to promote the energy absorption of *E. huxleyi*, which leads to its hypermetabolism and gene expression modulation, thereby stimulating its cell proliferation. Further studies are needed to identify the correlative physiological mechanisms of this finding.

As we know, carotenoids play a critical role in the photosynthetic system of microalgae, such as by protecting the photosynthetic apparatus against photooxidative damage, and are greatly affected by light wavelength [44,45]. Red light has been proven to significantly enhance the total carotenoid content of *Dunaliella* sp. and *Cyanobium* sp., probably due to the fact that red light induced their photoreceptors to accumulate carotenoids and activated correlative metabolic pathways [5,46,47]. Amaro et al. [48] demonstrated that red light could remarkably increase the β-carotene content of *Tetradesmus obliquus* (Turpin) M.J.Wynne under low cell density conditions because of its role in enhancing the function of the photosynthetic complex and, as such, avoidance of possible photodamage. Several previous studies have suggested that the change in the contents of different pigments, especially fucoxanthin and 19′-hexanoyloxyfucoxanthin, enhanced the photoacclimation capacities of microalgae including *E. huxleyi*, and that the light wavelength had a significant effect on the balance between the content of fucoxanthin and 19′-hexanoyloxyfucoxanthin [49,50]. In addition, Garrido et al. [49] have suggested that *E. huxleyi* had a higher content of fucoxanthin under red light in order to maintain its high photosynthetic performance. Similarly, the fucoxanthin content of *E. huxleyi* was significantly greater under red light than under the other light wavelengths, suggesting that red light is optimal for fucoxanthin production by this species. As it is a type of light stressor, red light could inhibit the biomass growth of *E. huxleyi*; however, it may motivate the specific photoprotective mechanisms of this species to eliminate this photodamage, thereby resulting in the enhancement of fucoxanthin accumulation. In addition, compared with the inhibition of cell proliferation by red light, our results showed that the cell diameter, dry weight, and net photosynthetic rate of *E. huxleyi* reached the maximum value under red light, which indicates that red light could significantly contribute to the cell growth of *E. huxleyi*. Similarly, the dry weight of *Chlorella vulgaris* reached the maximum value under red light because it could enhance the activity of photosystem II, thereby promoting microalgal growth [51].

The dry weight and the net photosynthesis rate of *E. huxleyi* under green light were significantly lower than under white light, which indicates that green light is not appropriate for the cultivation of *E. huxleyi*. This is coincident with the previous finding that the dry weight of microalgal species, for instance, *Microchloropsis salina* (D.J.Hibbard) M.W.Fawley, I.Jameson & K.P.Fawley and *Gloeothece membranacea*, decreased significantly under green light, which may be due to the reflection of green light by these species that led to insufficient light energy and growth inhibition [22,52]. In addition, the Chl *a* content of *E. huxleyi* under green light was significantly lower than under white light. This provides additional support for the possible interference of green light on its photosynthetic activity. Therefore, we suggest that *E. huxleyi* may not be able to effectively utilize green light through relevant

pathways to generate the photosynthetic energy that contributes to its growth. Moreover, compared with the other light wavelengths, all the measured physiological parameters of *E. huxleyi* were the lowest under yellow light, which implies that this species might not harvest yellow light. Similar to our results, yellow light exhibited the greatest inhibition of the cell density and growth rate of *Coscinodiscus granii* of all light wavelength settings [53]. However, the physiological mechanism of the negative effect of yellow light on microalgae is still unclear.

The combined application of various light wavelengths may strongly impact microalgae biomass by activating the correlative photoreceptors and promoting availability of energy [54]. For example, a ratio of blue-to-red light of 50:50 was optimal for increasing the cell density of *I. galbana* and *Phaeodactylum tricornutum*. This combination could induce and enhance the activation of photosystems, thereby producing more photosynthetic energy for cell division [55,56]. In addition, this light combination can significantly impact biomolecule synthesis. The combination of blue and red light could promote the lipid synthesis of microalgae such as C. *vulgaris*, T. *obliquus*, *Diacronema lutheri*, and *Arthrospira platensis* [57,58]. The conversion from red to blue light promoted the synthesis of lipids in *P. tricornutum*, while the conversion from blue to red light increased the content of carbohydrates [59]. In our study, on each experimental day, *E. huxleyi* had significantly greater cell density under blue light followed by red light compared with white light, while its cell density was significantly lower under blue light than under white light on the ninth day. These results suggest that the conversion from blue to red light can more effectively promote the sustainable cell proliferation of *E. huxleyi* compared with single blue light. Similarly, Granata et al. [60] demonstrated that the combination of blue and red light could remarkably increase the biomass of *E. huxleyi*, probably due to the Emerson effect for algae, which promotes the photosynthetic rate. As it is a physiological basis for the biomass enhancement of this alga, we speculate that the application of combined light with different spectra is likely to separately stimulate the two photosystems to generate sufficient energy. Furthermore, microalgal species can respond to light wavelength pressure by modulating the syntheses of relevant antioxidant pigments. Compared with single blue or red light, the mixed light composed of both induced greatly higher lutein content in *D. salina* [61]. The combination of blue and red light provided moderate external stress that stimulated lutein synthesis, thereby avoiding irreversible damage to this species [61]. Similarly, our results showed that the fucoxanthin content under blue light followed by red light was significantly higher than under the other single light wavelengths. The fucoxanthin synthesis in diatoms has been shown to be primarily supported by the photosynthetic energy from absorbed blue light and red light [62]. Hence, the change in light wavelength from blue to red light is considered to be an effective technical measure to enhance the biomass and fucoxanthin production of *E. huxleyi*.

5. Conclusions

In conclusion, we have clarified that light wavelength is a very significant variable that affects the growth, photosynthetic activity, and pigment synthesis of *E. huxleyi*. Blue light significantly stimulated the cell proliferation of *E. huxleyi*, while red light could promote its cell growth and fucoxanthin synthesis. Compared with all single light wavelength regimes, the combination of blue and red light significantly increased the cell density and fucoxanthin content of *E. huxleyi*. Our findings are likely to be highly valuable in the cultivation management and commercial exploitation of this productive species. Because of our limited data, further studies are needed to determine the correlative physiological and molecular mechanisms.

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