

Artificial light source selection in seaweed production: growth of seaweed and biosynthesis of photosynthetic pigments and soluble protein

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Seaweed growth is often limited by light. Light limitation in coastal waters is exacerbated by coastal development and has been associated with the loss of natural seaweed and decreased seaweed aquaculture yield. There is an urgent need to innovate seaweed cultivation through artificial light supply. In this study, the effects of five artificial light sources (white, red, green and blue LEDs and fluorescent light) on a brown alga *Sargassum fusiforme* and a green alga *Ulva pertusa* were investigated. Seaweed growth, accumulation of photosynthetic pigments (chlorophyll a and carotenoid) and soluble protein were evaluated. Our results indicated that biomass accumulation of both seaweeds was favored by white-LED light. In general, compared to fluorescent light, LED light promoted the synthesis of Chlorophyll a, carotenoid and soluble protein in both species. Specifically, blue-LED light was optimal supplementary light when cultivating *U. pertusa* and *S. fusiforme*, because it promoted pigment and protein production while maintained the seaweed yield. Seaweeds accumulated more biomass under LED light as revealed by modelling approach. LEDs would be promising supplementary light sources for seaweed cultivation.

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Abstract

Seaweed growth is often limited by light. Light limitation in coastal waters is exacerbated by coastal development and has been associated with the loss of natural seaweed and decreased seaweed aquaculture yield. There is an urgent need to innovate seaweed cultivation through artificial light supply. In this study, the effects of five artificial light sources (white, red, green and blue LEDs and fluorescent light) on a brown alga *Sargassum fusiforme* and a green alga *Ulva pertusa* were investigated. Seaweed growth, accumulation of photosynthetic pigments (chlorophyll a and carotenoid) and soluble protein were evaluated. Our results indicated that biomass accumulation of both seaweeds was favored by white-LED light. In general, compared to fluorescent light, LED light promoted the synthesis of Chlorophyll a, carotenoid and soluble protein in both species. Specifically, blue-LED light was optimal supplementary light when cultivating *U. pertusa* and *S. fusiforme*, because it promoted pigment and protein production while maintained the seaweed yield. Seaweeds accumulated more biomass under LED light as revealed by modelling approach. LEDs would be promising supplementary light sources for seaweed cultivation.

Key words: light-emitting diodes(LEDs), pigment; soluble protein; seaweed growth model; light sources; macroalgae.

Introduction

Coastal ecosystem provides a variety of ecosystem goods and services which support the sustainable development for human beings (Bennett et al., 2016; Mehvar et al., 2018). Seaweeds cover a large area of coastal zone, providing high-value ecosystem services (i.e. globally significant carbon fixation, absorb contaminants and regulate coastal biodiversity and fisheries) and raw material for food, fertilizer and medicine industries (Duarte et al., 2017; Xiao et al., 2017, 2019; Pan et al., 2018). However, localized human activities and global climate change are currently putting high pressure on the near-shore ecosystems (Xiao et al., 2015; Smale et al., 2019). e. pollutants and nutrients flow into coastal waters (Zhao et al., 2017). Therefore, natural seaweeds are facing the threat of ecological degradation, and the harvest of natural seaweed cannot satisfy the daily needs of human beings. This in turn leads to increasing demand for large-scale seaweed aquaculture (Xiao et al., 2019).

Nevertheless, problems such as warming, high sediment loading, epiphyte cover, disease and fish grazing seriously affect the development of large-scale seaweed farming (Ateweberhan, Rougier & Rakotomahazo, 2015). A fundamental factor affecting seaweed growth is light limitation (Xiao et al., 2019). The intensification, high-density and high-output of modern mariculture and excessive fertilizer application retain large amounts of nutrients and contaminants in seaweed cultivation area, increasing the turbidity of the seawater (Lu, Wang & Feng, 2017). Light availability, which is limited by water transparency, directly determines the photosynthesis activity of seaweed and reduces their biosynthesis ability, causing ecological and economic loss

to seaweed farm (Orfanidis, 1992). For instance, Zhoushan Island in the East China Sea, situated at the mouth of the Yangzi River, is experiencing turbid water, and seaweeds cannot grow well in large scale in such coastal waters (Tseng, 1987). Hence, artificial lighting is needed to favor the growth of seaweed under the light-limited situations (Xiao et al., 2019), and nutrients could be removed through seaweed harvest (Xiao et al., 2017).

Light-emitting diodes (LEDs) produce monochromatic light in a highly energy-efficient way, suggesting its potential to provide supplementary light for seaweed growth (Bourget, 2008; Kim et al., 2015). By filtering fluorescent light with band-pass filters, monochromatic lights have been produced to promote seaweed growth, and its influence was tested on several seaweed species (Figueroa & Niell, 1990; Figueroa et al., 1995; Korb, Figueroa & Aguilera, 2005; Kim et al., 2015; Bonomi Barufi, Figueroa & Plastino, 2015). However, although LED light has been proposed as light source for *Gracilaria* cultivation (Kim et al., 2015; Bonomi Barufi, Figueroa & Plastino, 2015), its influence on a broader variety of seaweed species and on the seaweed biosynthesis remains poorly understood.

In this study, the use of white, blue, green and red LED light and fluorescent light were tested as artificial light sources to support the cultivation of two common and economically important seaweeds - *Ulva pertusa* and *Sargassum fusiforme*. We assessed effects of light sources from perspectives of seaweed growth, photosynthesis pigments and soluble protein accumulation. In addition, we calculated the growth rate of seaweeds cultivated under LED and fluorescent lights and constructed a seaweed growth model for comparison. Our results will facilitate the selection of artificial light sources for seaweed cultivation under light-limited conditions.

Materials & Methods

Seaweed species and cultivation

The juveniles of two seaweed species, *Sargassum fusiforme* and *Ulva pertusa*, were collected from Dongtou County, Wenzhou City, Zhejiang Province, China (27°51'42"N, 121°11'06"E). All the collected seaweeds were initially maintained in glass jars containing filtered, sterile natural seawater (33‰) where the temperature (16 °C) was controlled by a chiller (LS16-600, JLLN, Shenzhen, China). Illumination was provided by fluorescent lamps (120 μmol photons m⁻² s⁻¹, 12 dark: 12 light) for acclimation. After 3 days of acclimation, equally sized and healthy individuals were selected and used in the experiments.

Light sources

Fluorescent light and four LEDs emitting white, red, green and blue light were used as light sources for seaweed cultivation, providing 120 μmol photons m⁻² s⁻¹ on the surface of seaweed thalli. The lighting system was assembled in PVC tubes (height: 25 cm, diameter: 20 cm), with flexible rope LED lights (3 m length for each tube) affixed to the inner walls. For all the LED lighting, diodes (Oppl Co. Ltd., Shanghai, China) were driven by a 220V power supply. Light was supplied from 6:00 to 18:00 every day (12-h per day). The spectral wavelengths of lights were measured with optical spectrum analyzer (CMS-2S, Inventfine Co. Ltd., Hangzhou, China).

Emission spectral distribution of light sources

The peak wavelengths of the red, green and blue LEDs were 632 nm, 517 nm and 462 nm, respectively, and all peaks had a narrow emission spectrum (80 - 100 nm) (Fig. 2). White LED had a continuous spectrum and emitted two peaks, one in the blue light region (left) which was narrower than the other in the green light region (right). The fluorescent lamp showed a continuous emission spectrum but with many narrow peaks (10 - 15 nm).

Light incubation experiments

The cultivation lasted for 18 days. Five individuals of seaweed (approx. 5 g fresh weight) were initially placed into one cylindrical plastic bottle (1000 ml, diameter: 100 mm). Juvenile seaweeds were used in the experiments since they are more susceptible and sensitive to the changes in cultivation environment. Three replicate bottles were settled inside PVC tubes for different light treatments (Fig. 1). The LEDs and fluorescent light were controlled independently to provide either pure primary light or white light. During the experimental period both *S. fusiforme* and *U. pertusa* were cultivated in filtered and sterile natural seawater. The seawater and nutrients (PO_4^{3-} and NO_3^-) were renewed every 2 days, and water was sufficiently aerated by air bumped into cylindrical bottles. The phosphate and nitrate concentration of seawater is $15 \mu\text{mol L}^{-1}$ and $150 \mu\text{mol L}^{-1}$, respectively. Temperature in aquarium was kept at 16°C with circulating filtered seawater.

Growth

Specific growth rate (SGR) representing the increasing fresh weight (FW) biomass per day was calculated as following (Xiao et al., 2015):

$$SGR = \ln\left(\frac{W_t}{W_0}\right) \times t^{-1} \times 100 \quad (1)$$

where W_0 refers to initial algal biomass, and W_t is algal biomass after t days of cultivation. Fresh weights of *S. fusiforme* and *U. pertusa* were measured at the start and every 2 days during the experimental period.

Photosynthetic pigment and soluble protein

Chlorophyll a and carotenoid content were measured as photosynthesis pigment. Chlorophyll a was extracted in acetone (90%) neutralized with sodium carbonate from samples, as described in (Jeffrey & Humphrey, 1975). Carotenoid concentrations were calculated according to Seely's experiments (Seely, Duncan & Vidaver, 1972). All absorbances were measured at the initial day and every 2 days during cultivation by spectrophotometer (Inesa 722S, Shanghai, China). The soluble protein concentrations were determined spectrophotometrically at 595 nm by Coomassie brilliant blue method (Bradford, 1976).

Seaweed growth model

S. fusiforme was chosen as an example to study the differences in the growth of seaweed cultivated under LEDs and fluorescent light sources. The accumulation model of seaweed was conducted according to Xiao (2015) (Xiao et al., 2015), as following:

$$W_t = W_0 \times e^{\frac{SGR \times t}{100}} \quad (2)$$

where W_0 refers to initial algal biomass and set to be 1, and W_t is algal biomass after t days of cultivation, SGR is the specific growth rate mentioned above. The growth of *S. fusiforme* was divided into three stages according to its life cycle and the cultivation behavior of local farmers the early (propagule stage), middle (indoor cultivation) and late stage (raft cultivation). The SGR for these three stages under various light sources were calculated using data either generated from our own experiments or reported in the literature (Luo & Wei, 2002; Qing-Jun et al., 2010; Zou & Gao, 2010; Zhao et al., 2015).

Data analysis

Differences between light treatments were tested for each species separately using one-way ANOVA with a significance level of $p < 0.05$. Results of SGR, photosynthetic pigments and soluble protein concentrations were analyzed by t tests. Statistical tests were performed with SPSS (version 19.0).

Results

Specific growth rate

For both seaweed species (*Ulva pertusa* and *Sargassum fusiforme*), white-LED light is stimulating seaweed growth ($5.25 \% d^{-1}$, Fig 3a and $3.24 \% d^{-1}$, Fig 3b) significantly as compared to traditional fluorescent light ($3.59 \% d^{-1}$ and $1.82 \% d^{-1}$), although these two lights (white-LED light, fluorescent light) were similar in color to naked eye. In general, despite the light color, all the LED lights promoted the growth of both species, as compared to the fluorescent light (Fig. 3). The only exception was for *S. fusiforme* under red LED light. Regarding the light colors of LEDs, white lights were found to accelerate the growth of *U. pertusa* ($5.25 \% d^{-1}$) significantly, as compared to the green light ($4.19 \% d^{-1}$) ($p < 0.05$). As for *S. fusiforme*, the SGR decreased following the sequence of white LED light ($3.24 \% d^{-1}$) > green and blue LED light (2.44 and $2.33 \% d^{-1}$) > red LED light ($1.37 \% d^{-1}$).

Photosynthetic pigments and soluble protein

The biosynthesis of photosynthetic pigments and soluble protein of seaweeds was generally up-regulated when exposed to LED lights, as compared to the fluorescent light (Fig. 4, Fig. 5). The only exception was for the white LED light exposed *U. pertusa* where chlorophyll a (Chl a) concentration was similar to those growing under fluorescent light. Different LED lights induced changes in pigments and soluble protein synthesis in the two species. For instance, the Chl a concentration of the red LED light treated *U. pertusa* ($1.19 mg g^{-1}$, Fig. 4A) was significantly higher than those treated by white and green LED lights ($0.82 mg g^{-1}$ and $0.97 mg g^{-1}$, $p < 0.05$,

Fig. 4A). However, for *S. fusiforme*, Chl a concentration (0.61 mg g^{-1} , Fig. 4C) of the white LED exposed individuals, was slightly lower than seedlings growing under green and blue lights, though no significance was found. As for the carotenoid content, the white LED light resulted in lowest concentrations in *U. pertusa*. Nevertheless, the colors of LED lights showed no significant influence on the carotenoid production of *S. fusiforme*. The concentrations of soluble proteins in *U. pertusa* were higher under irradiation of blue and green LEDs than other colors, though showed no significance. However, for *S. fusiforme*, the treatment of blue and white light effectively motivated the biosynthesis of soluble proteins (Fig. 5).

Seaweed biomass accumulation model

The seaweed biomass accumulation model results showed that LED lights could stimulate seaweed growth for all the three growth phases of *S. fusiforme*, as compared fluorescent light in same light intensity (Fig. 6A). In this way, *S. fusiforme* cultivated under LED lights would accumulate more biomass compared to traditional fluorescent light (Fig. 6B). The final yield of *S. fusiforme* growing under LED light was nearly fivefold of those growing under fluorescent light. In addition, our results also revealed that the difference between *S. fusiforme* growing under LED and fluorescent light became smaller over time.

Discussion

Light driven shifts in seaweed growth

For both seaweed species *U. pertusa* (green algae) and *S. fusiforme* (brown algae), the experimental seedlings achieved highest growth rate under white LED lighting, which is consistent with previous studies (Tovar et al., 2000; Kim et al., 2015). This may be partially explained by the broad light wavelengths of white light, which covers light wavelengths of 430 to 630 nm (Fig. 2). White LED light, with the ability to provide spectrum comparable to the sunlight (Glemser et al., 2016), is capable of supporting C and N metabolism (Figuroa, Aguilera & Niell, 1995; Tsekos et al., 2002; Korb, Figuroa & Aguilera, 2005). White LED light with a broad continuous emission spectrum, is also providing higher luminous efficiency compared to fluorescent white-light source (Pimputkar et al., 2009). Interestingly, other than the white LED light, the blue LED light also simulated the growth of *U. pertusa* (Fig. 3). Spore production of seaweed (i.e. *Petalonia fascia*, *Petalonia zosterifolia*, *Scytosiphon* and *Saccharina japonica*) become fertile only in the presence of blue light (Lüning, 1973; Wang et al., 2010). As shown in numerous studies in microalgae growth, blue light was also found to efficiently promote the growth of marine phytoplankton, including *Cyclotella nana*, *Dunaliella tertiolecta*, *Isochrysis galbana*, *Chaetoceros gracilis* and *Heterocapsa circularisquama* etc. (Wallen & Geen, 1971; Gorai et al., 2014). In fact, the effect of light quality (colors) might be highly species specific (Zhao et al., 2008). For instance, our results on *S. fusiforme* growth agreed with previous observation on other brown algae, where better growth and development of *Sargassum horneri*, *Saccharina japonica* were achieved under blue light, as compared to red light (Wang et al., 2010; Miki et al., 2017). Nevertheless, growth of a red algae *Porphyra umbilicalis* is favored by red

light (Figueroa, Aguilera & Niell, 1995). For another red alga *Gracilaria birdie*, the highest SGR was obtained under green light (Bonomi Barufi, Figueroa & Plastino, 2015).

Light driven shifts in seaweed biosynthesis

The accumulation of photosynthetic pigments and soluble protein in *U. pertusa* and *S. fusiforme* were also influenced by light sources, which could help explain their difference responses in growth. For both seaweed species, the concentrations of Chl a and carotenoid in the individuals grown under LED lights were significantly higher than those grown under fluorescent light. Seaweeds are able to change their light-harvesting pigment system to satisfy the quantity of photons (Ramus, 1983), and this may therefore further influence the seaweed growth rates. Other than the white LED light, our results also indicated that blue LED light could be a promising artificial light source in *S. fusiforme* and *U. pertusa* cultivation, since it stimulated chlorophyll a formation without reducing the productivity of the seaweed (Fig. 3, Fig. 4). Additionally, the production of soluble protein was also **favorable** by blue light for both species, which is consistent with previous finding that the accumulation of N compounds and soluble protein was primarily stimulated by blue light (Figueroa et al., 1995; Luis Godinez-Ortega et al., 2008; Wu, 2016). Similar to our findings for seaweed, light quality was found to directly influence the photosynthesis, pigments and protein production of marine microalgae (Schulze et al., 2014). These findings are promising for light regulation of bioproduct from seaweed origin. Our results were consistent to the previous study that blue light facilitate photosynthesis in *Laminaria saccharina*, *Saccharina japonica*, *Dunaliella salina* (Schmid & Dring, 1993; Fu et al., 2013; Wang et al., 2013). Nevertheless, pigment concentrations of *Gracilaria tikvahiae* grown under fluorescent light were similar to red and blue LED lights (Kim et al., 2015). Again, our results suggested the biochemical response of seaweed to light is species specific.

LED versus fluorescent as artificial light sources

Many species of seaweeds play an important role in worldwide food and feed supply (Makkar et al., 2016). The growth and biochemical composition of seaweed were affected by light quality, indicating the potential for using artificial light to increase the yield and proportion of high value biomolecules in seaweed aquaculture. Although the cost of LEDs is still higher than fluorescent lights, LEDs have longer operating lifespans and better energy efficiency. In addition, fluorescent lamps generate excessive heat that would interfere the in-door cultivation temperature. Our seaweed biomass accumulation model clearly showed the general benefits of LED lighting over fluorescent lighting in supporting seaweed growth. Interestingly, red LED light has been widely applied in the cultivation of microalgae and terrestrial plants (Goins et al., 1997; Poudel, Kataoka & Mochioka, 2008), however, a negative influence of red LED light was found in the growth of *S. fusiforme*. This hinted the importance of further investigations on more seaweed species, since the influence of light quality appears highly species-dependent. It is also notable that juvenile seaweeds are more sensitive to artificial LED lights, and the difference in the SGR and biomass accumulation between various light sources were decreasing over time (Fig. 6). Therefore, it is most cost-efficient to provide LED lights in the initial cultivation phase.

Although, for the moment, the application of LEDs light does not form a certain scale in seaweed cultivation, the LEDs lighting have a bright application prospect in seaweed cultivation indoor and in field.

Conclusions

In summary, this study highlighted the potential of using LED light sources in seaweed cultivation. Results indicate that the effects of artificial light to seaweed, regarding the growth rate, photosynthetic pigments and soluble protein, are highly species-dependent. In the future, it is promising to manipulate the artificial light source for biomolecular production from seaweed.

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Figure 1

Photos of LED light source settings and experimental species.

Up: PVC tubes (height: 25 cm, diameter: 20 cm) with flexible rope LED lights (3 m length for each tube) affixed to the inner walls, the light sources from left to right are white, red, green and blue, respectively. Down: cylindrical bottles (1000 ml) for seaweed cultivation with five individuals placed in each bottle, the experimental species are *S. fusiforme* and *U. pertusa*.



Figure 2

Emission spectral distribution of the white, red, green, blue LEDs and fluorescent light sources.

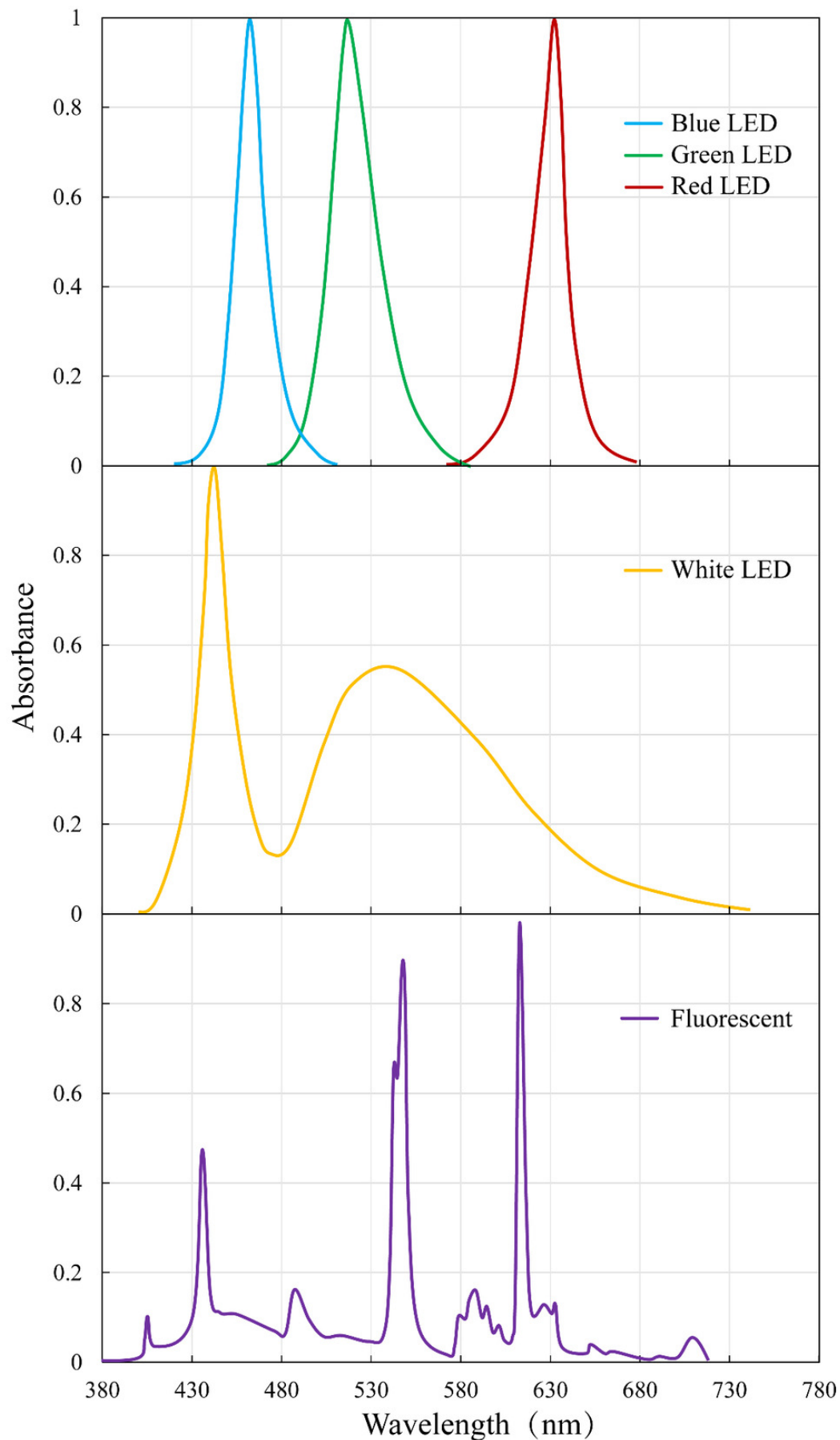



Figure 3

Specific growth rate (SGR) of *U. pertusa* and *S. fusiforme* after 18 days cultivation under various LEDs and fluorescent light sources. 

(A) SGR of *U. pertusa*. (B) SGR of *S. fusiforme*.

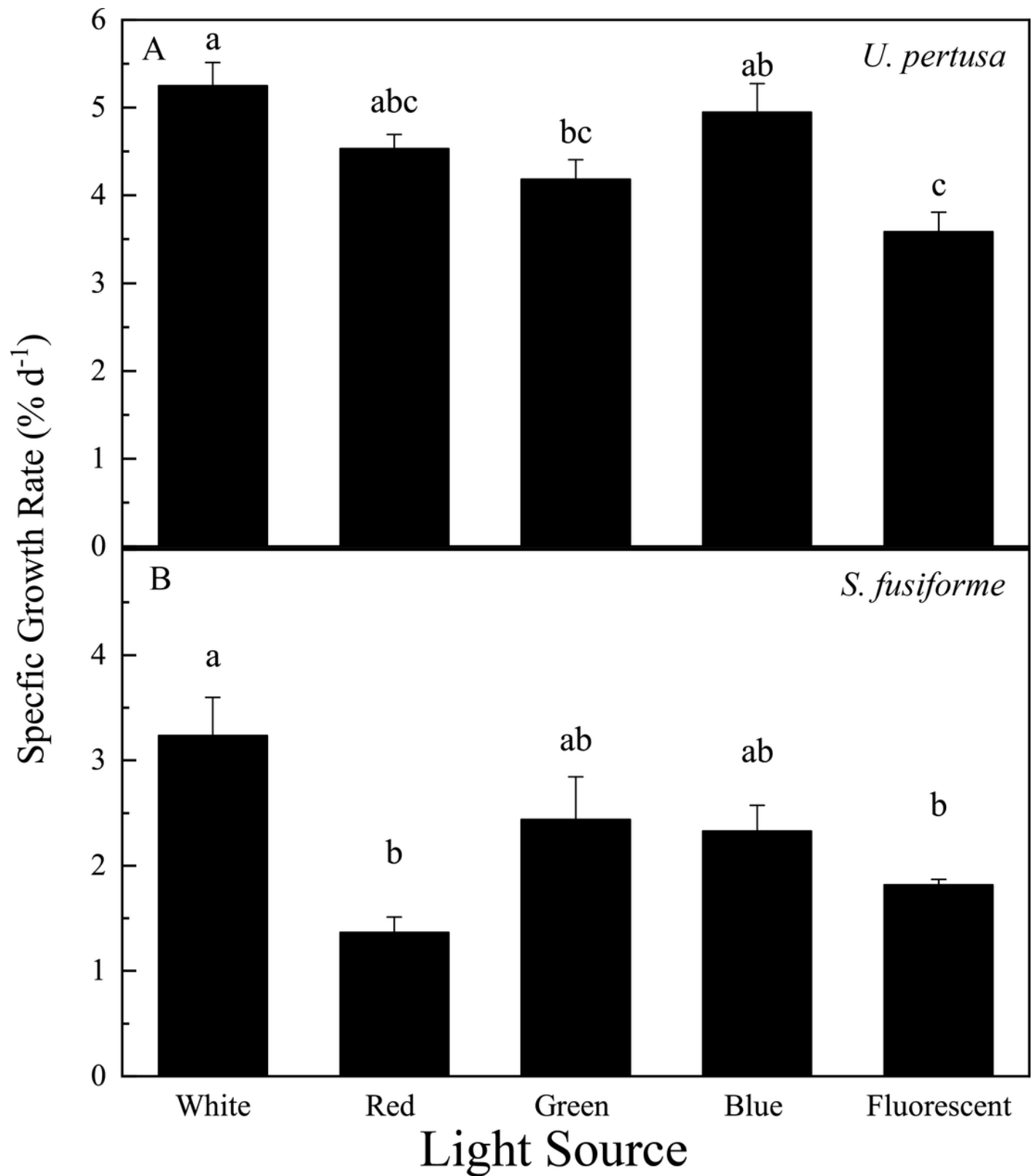


Figure 4

Chlorophyll a and carotenoid concentrations of *U. pertusa* and *S. fusiforme* after 18 days cultivation under various LEDs and fluorescent light sources.

(A&B) Chlorophyll a and carotenoid concentration of *U. pertusa*. (C&D) Chlorophyll a and carotenoid concentration of *S. fusiforme*.

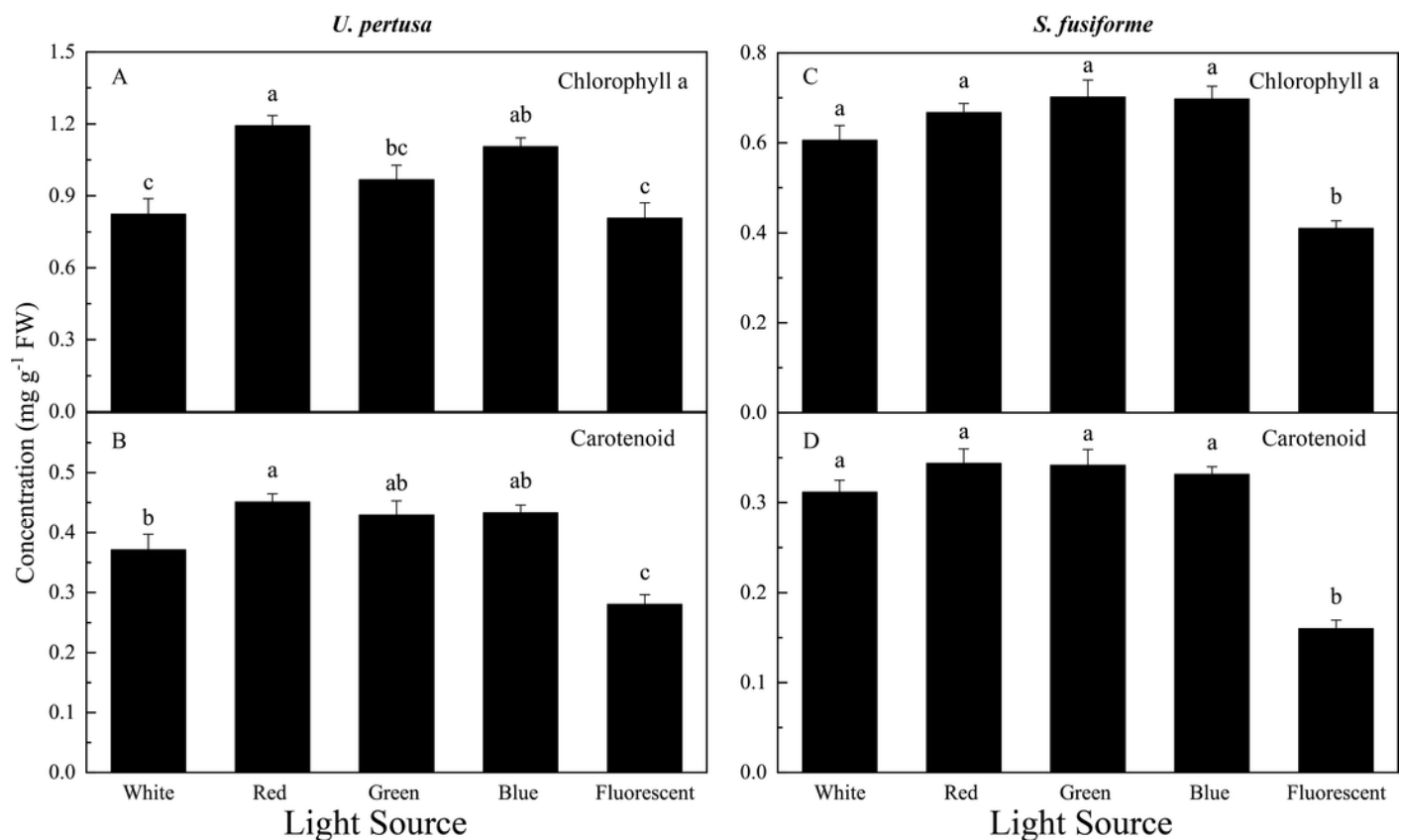


Figure 5

Soluble protein concentrations of *U. pertusa* and after *S. fusiforme* 18 days cultivation under various LEDs and gluorescent light sources.

(A) Soluble protein concentration of *U. pertusa*. (B) Soluble protein concentration of *S. fusiforme*

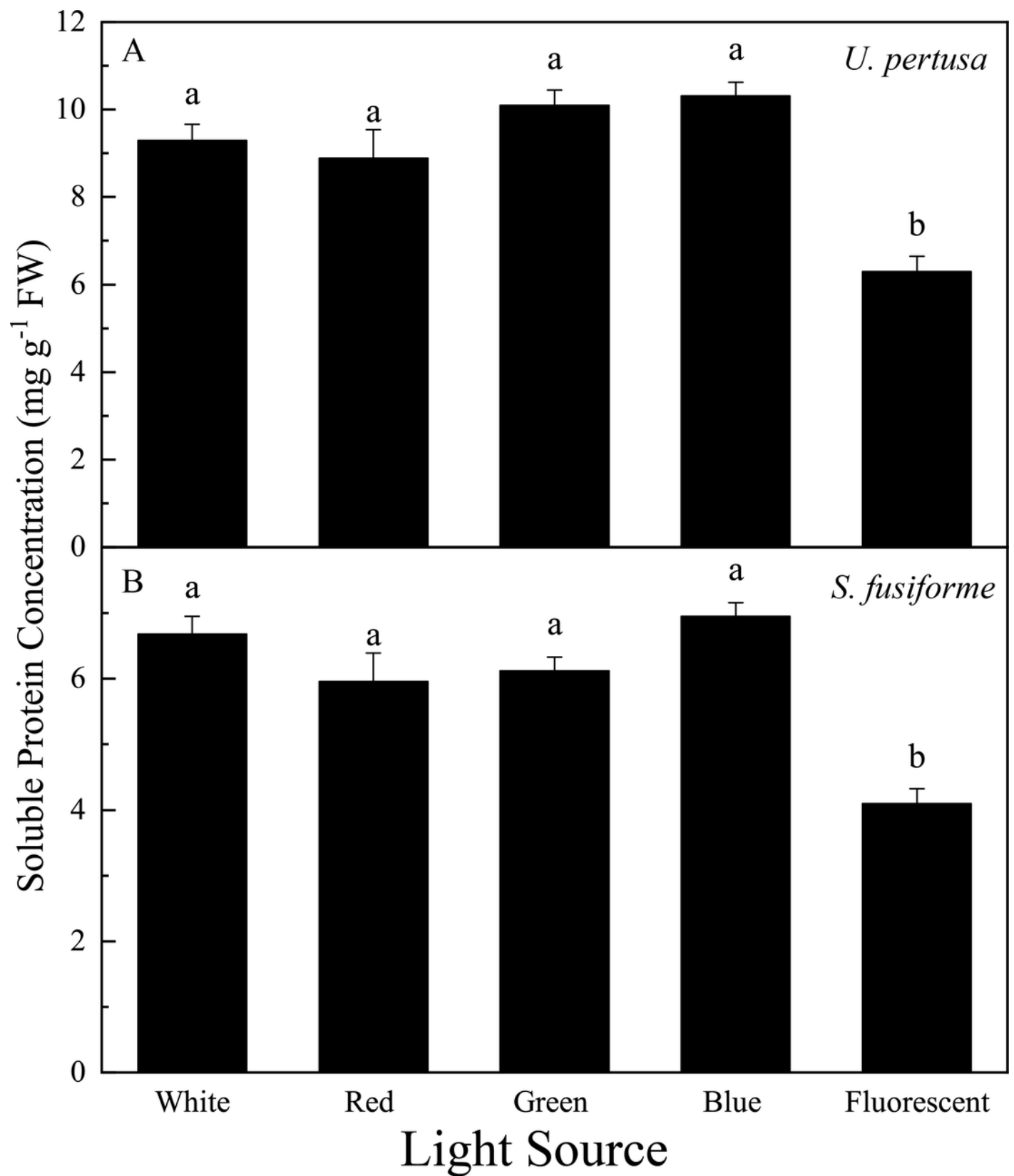


Figure 6

Specific growth rate (SGR) and biomass accumulation of *S. fusiforme* under LEDs (all LED lighting sources averaged) and fluorescent light during three life periods.

(A) SGR values for the early, middle and late stage of *S. fusiforme* cultivation, data collected from this study and publications. (B) biomass accumulation of *S. fusiforme* for the early, middle and late stage of its cultivation. 