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Growth and pigment content of *Gracilaria tikvahiae* McLachlan under fluorescent and LED lighting

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ABSTRACT

Light emitting diode (LED) technology has significant potential advantages over other light sources in algal aquaculture. This study investigated LEDs as light sources for the culture of *Gracilaria tikvahiae*. We cultured a wild-type and a green mutant strain of *G. tikvahiae*, comparing growth rate and tissue chlorophyll *a*, total carotenoids, and phycobiliprotein concentrations under high output cool white fluorescent, pure primary color LED, and mixed LED lighting. Under monochromatic light, the growth rates under high output cool white fluorescent lighting were significantly higher than rates under pure LED light (all three colors for wild strain and green and blue for green mutant). However, when pure color LED lighting was mixed (50%/50%), the red + green (wild-type strain and green mutant) and the green + blue LED combinations (wild-type only) showed growth rates similar to those under high output cool white fluorescent lighting. In the trichromatic experiment, growth of the wild-type strain under mixed three-color (40%/40%/20%) LED light was indistinguishable from those of the fluorescent control lighting. Chlorophyll *a* and carotenoid concentrations of *Gracilaria* grown in the dichromatic light experiment were 55% and 74% higher, respectively, under red + blue LED lighting than under the other light treatments. The wild-type strain of *G. tikvahiae* possessed significantly greater concentrations of chlorophyll *a*, and phycoerythrin than did the green mutant, while green mutant thalli had higher phycocyanin levels. With rising LED efficiency and energy savings, LEDs will be an increasingly better choice for indoor seaweed cultivation, especially if control of pigment production and morphogenesis by selective use of particular wavelengths is desirable.

1. Introduction

Light-emitting diodes (LEDs) have become the latest energy-efficient light source (e.g., Bourget, 2008; Pinho et al., 2012). The technology is not new; the first LED was produced in the 1920s, red, yellow, and green LEDs appeared in the 1970s, and blue LEDs in 1993 (Bourget, 2008). NASA's Advanced Life Support System program first investigated the use of LEDs to power plant growth for food during extended space travel (Barta et al., 1992; Massa et al., 2008). More recently, almost all non-industrial applications have centered on the horticulture industry (e.g., Goins et al., 1997; Yorio et al., 2001; Kim et al., 2004). The use of LED light sources in algal research has been limited (Schmid and Dring, 1993a). Lee and Palsson (1994) developed a LED-based system for growing *Chlorella vulgaris* in a microalgal biotechnology context. Pulse amplitude modulated (PAM) fluorometry employs LEDs as a light source in the measurement of photosynthetic efficiency of intact thalli (e.g., Cabello-Pasini et al., 2000; Kühl et al., 2001). Wang et al. (2010) reported that blue LED lighting drove earlier and more reliable egg production by *Saccharina japonica* gametophytes, and larger sporelings than did white light. Xiaolei et al. (2008) reported that red light stimulated division of *Pyropia yezoensis* conchospores. Blue light stimulates photosynthesis in most brown algae when already saturated with red light (Dring, 1988; Schmid and Dring, 1992, 1993b).

The application of LED technology to algal aquaculture has significant potential advantages over other light sources. Past research into the effects of light quality on algal physiology have used colored films or filters to modify incoming light (e.g., Lechowski and Białczyk, 1988; Schmid and Dring, 1992, 1993b; Xiaolei et al., 2008). These approaches have the drawbacks of large bandwidth (films) and small size and expense (filters). In addition, incandescent or fluorescent light sources generate a significant amount of heat, an indication of both energy inefficiency and potential alteration of the growth environment. In contrast, LEDs have the advantage of cool emitting temperature (i.e., heat is directed to the ballast, away from the light), as well as small size, durability, and long lifetime. In addition, the performance (lumens per device) of LEDs has increased ca. 30-fold per decade since 1970, while operating costs (US $ per lumen) have decreased by a factor of 10 per

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decade (Tamulaitis et al., 2005; Bourget, 2008). Currently, LEDs are available in a choice of many emission colors. Each LED device has a narrow emission spectrum (20–30 nm at half peak height), allowing for the precise control of light required by studies of photomorphogenesis and other plant light-based responses. Fixed wavelength LEDs may eventually be supplanted by tunable color LED devices that allow control of the emission spectrum.

Although completing the entire life cycle is an important focus of aquaculture research, growth rate maximization, generation of biomass-based end products, and tissue composition are often of paramount concern (Kim and Yarish, 2014). Research involving terrestrial food plants has repeatedly demonstrated the importance of a combination of red and green wavelengths to maximize yield (Bula et al., 1991; Goins et al., 1997; Yorio et al., 1997; Hogewoning et al., 2010). Several important aquacultured macroalgae are found within the genus *Gracilaria*. The genus includes species that produce agar, some that are edible and others that are animal feeds. Total annual average production of *Gracilaria is* 700,000 tons (2010–2012) worth an estimated USD $170 million per year (FAO, 2013; Johnson et al., 2014). In addition, this genus has utility as a biological nutrient scrubber, removing dissolved nutrients from eutrophic coastal waters (Troell et al., 1997, 1999; Chow et al., 2001; Mao et al., 2009; Yarish et al., 2012; Kim et al., 2014). In this latter application, rapid production of tissue in seaweed nursery operations is required for farm outplanting to initiate the growing season, and to replace senescent tissue and material lost to severe weather events (Kim and Yarish, 2014).

We used the economically valuable species *Gracilaria tikvahiae* McLachlan to examine the suitability of LEDs as light sources for the culture of macroalgae for biomass and pigments. In particular, we cultured a wild and a green mutant strain of *G. tikvahiae*, with the objectives of comparing (i) growth and (ii) tissue pigment concentration of both strains under high output fluorescent, pure primary color LED, and mixed LED lighting.

2. Materials and methods

Tissue for the experiments was obtained from cultures of *G. tikvahiae* maintained at the University of Connecticut Stamford Seaweed Marine Biotechnology laboratory. Two strains were tested; a wild-type (G-RI-ST1) and a green mutant strain (G-RI-ST3). Life stage of the wild strain was a tetrasporophyte but failed to produce any tetrasporangia. It was derived from a carpospores collected April 20, 2010, Potter Pond, South Kingston, RI (41°22′56″; 71°32′04″). The green mutant was also determined to be a tetrasporophyte. It was isolated from an apical segment of a tetrasporophytic from the same collection as the wild-type. Both strains were grown in nutrient-replete von Stosch's enrichment (VSE; Ott, 1965) culture medium (500 μM NO_3, 30 μM PO_4) under 12:12 L:D photoperiod at 100 μmoles photons m^{-2} s^{-1}, at a temperature of 20 °C.

Experiments were run for 16 (green mutant) or 21 (wild-type strain) days. Approximately 0.2 g FW tissue was initially placed into 100 mL cylindrical glass jars (55 mm in diameter) covered with Parafilm, for a starting biomass density of 2 g FW L^{-1}. For the LED treatments, three replicate jars were placed around the center axis of a PVC cylinder, while the three fluorescent treatment replicates were placed under banks of tubes. All jars were supplied with forced air through spargers to produce small scale tumble cultures of seaweed. The growth medium was changed every 3–4 days, and tissue was reduced to the initial biomass (0.2 g FW per jar) every 4–7 days. The air-driven tumble culture ensured that all thalli rotated throughout the light field characterized by a maximum of 100 μmoles photons m^{-2} s^{-1} (measured using a cosine sensor oriented perpendicular to the culture container), to minimize the difference in directionality (downward for fluorescent lighting, lateral for LED) of light sources for LED and fluorescent lighting. For the fluorescent control treatment, illumination came from overhead T12 high output tubes (800 mA). For LED lighting, diodes were placed in circular rows around the inside of a PVC pipe (diameter = 18 cm, height = 16 cm) (Fig. 1; device provided by Metrocrops LLC (model # CL-3-P, Norwalk, CT)). The three primary colors were controlled independently to provide pure primary light (monochromatic light experiment), or blended light sources to produce combinations of the 50%:50% color mixes (dichromatic experiment) or 40%:40%:20% mixes (trichromatic experiment; Table 1). These proportions in the blended LED experiments were chosen to address the low photosynthetic quantum yield of rhodophyte algae in blue light (Dring and Lüning, 1985), but recognizing that blue light is required for N metabolism. LED spectra were 625–630 nm for red, 515–520 nm for green and 465–470 nm for blue. Wet weights of thalli were measured at the start and every 4 days (green mutant) or 7 days (wild-type) during the experimental period. Growth rates were estimated using the equation:

$$\text{Growth Rate}\left(\%\, d^{-1}\right) = \frac{(\ln(\text{end biomass}) - \ln(\text{start biomass}))}{\text{elapsed time}} x100$$

Pigment concentrations were measured in tissue collected after the final growth rate measurements using standard spectrophotometric methods (Lichtenthaler and Buschmann, 2001; Seely et al., 1972; Beer and Eshel, 1985; Kim et al., 2007).

Each light treatment employed three replicate aerated wide-mouth jars. Statistical tests using Sigmaplot 12.5 software (Systat, San Jose, CA) were performed on data collected on the last day of each experiment. Tests for light treatment effects on growth rate and pigment content were conducted via ANOVA. Comparison of growth rates and pigment concentrations in wild type and green mutant tissue used ***t*** tests. All data sets passed the test for normality (Shapiro–Wilk test). Most passed the test for homogeneity of variances (F test). Those which did not were ln-transformed to meet the assumption of homogeneity of variance. In the one case where transformation did not satisfy this assumption, we used the equivalent non-parametric test. When the *t* test or ANOVA did not reject the null hypothesis (no difference among mean values), the power of the test was reported to evaluate the likelihood of falsely concluding no difference when, in fact, one exists. The power of all tests exceeded 0.80, except where noted. Post-hoc Holm–Sidak multiple comparison tests were conducted if the ANOVA revealed significant treatment effects.

3. Results

Both the wild and green mutant strains grew well under experimental conditions. In the monochromatic experiment, exposure of the wild-type strain of *G. tikvahiae* to fluorescent lighting resulted in growth rates (17.1% d^{-1}) that were significantly higher than growth under pure red and green LED light (13.7, 13.3% d^{-1}; Fig. 2), all of which were significantly higher than growth under blue LED light (5.8% d^{-1}). When pure color LED lighting was mixed in the dichromatic experiment, light color significantly influenced growth rate, with the R + B LED producing growth rates that averaged 38% lower than the other, statistically indistinguishable treatments (including the fluorescent control). In the trichromatic experiment, growth of the wild strain under mixed three-color LED light and fluorescent control lighting was statistically indistinguishable ($F = 0.70$, $p = 0.54$), averaging 14.2% d^{-1} over all treatments (Fig. 2). However, the power of this ANOVA test was quite low (0.05), indicating caution in accepting the null hypothesis. Light source explained only 23% of the variation in the growth rate among treatments. Growth rates of the wild strain under fluorescent lighting were equivalent in the monochromatic and trichromatic experiments (average = 16.1% d^{-1}). Measured rates from the dichromatic experiment averaged only 10.0% d^{-1}, a significant difference.

Under fluorescent lighting, growth rates of the green mutant strains (15.3% d^{-1}) were similar to those of the wild type in the monochromatic and trichromatic experiments, but significantly higher than those of the wild dichromatic experiment ($p = 0.007$). Growth of the green mutant

Fig. 1. Photographs of LED illumination cylinder. A *Gracilaria* cultivation system from the trichromatic experiment (right). A PVC tube has three 0.5 m circles of LED strips inside. Each white "square" in the tube is an LED package. Each package contains one red chip, one blue chip, and one green chip (left). From right to left: RGb, RgB and rGB.

strain of *G. tikvahiae* under fluorescent and pure LED light sources produced a pattern similar to that of the wild-type strain (Fig. 3). Growth rates of tissue cultured under pure blue LED light were significantly lower than rates under the other LED and fluorescent lighting. Again, when the pure primary LED lighting was mixed 50%:50%, differences in growth were reduced, with fluorescent and R + G averaging 15.8% d^{-1} and R + B and G + B treatments averaging 12.1% d^{-1}.

Tissue chlorophyll *a* concentrations from the monochromatic light source experiment were influenced by light source (Fig. 4). Chlorophyll *a* concentrations of green LED-grown tissue were 29% greater than average concentrations in tissue grown under fluorescent, red, and green lighting (latter three treatments statistically indistinguishable). Phycoerythrin levels were significantly affected by light source, with concentrations in blue LED-grown thalli 74% higher than concentrations in samples from other light sources, which were statistically indistinguishable (Fig. 4). Neither tissue carotenoid nor phycocyanin concentrations were affected by light source (Fig. 4), though low power (0.28, 0.29, respectively) suggests caution in this conclusion.

Chlorophyll *a* and carotenoid concentrations of thalli grown in the dichromatic light experiment were influenced significantly by light color. Chlorophyll *a* and carotenoid concentrations were 55% and 74% higher, respectively, under R + B LED lighting than under the other light treatments (Fig. 4). Neither phycoerythrin nor phycocyanin concentrations were significantly affected by light source in the dichromatic experiment. *G. tikvahiae* thalli grown under fluorescent light and trichromatic mixtures of LED lights showed no differences in any pigment concentrations (Fig. 4), though the power of ANOVAs for each pigment were low (ca. 0.10).

When grown under fluorescent lighting, the wild strain of *G. tikvahiae* possessed significantly greater concentrations of chlorophyll *a*, and phycoerythrin than did the green mutant, while green mutant thalli had higher phycocyanin levels (Fig. 5). The difference in carotenoid concentration was marginally non-significant. Here, again, low power (0.41) suggests caution in concluding a lack of difference in carotenoid production between the strains. The wild and green mutant strains differed mostly for phycoerythrin concentration ($PE_{wild}/PE_{green} = 12.2$), though large differences were also measured for chlorophyll *a* and phycocyanin ($PE_{wild}/PE_{green} = 1.8$, 0.40, respectively).

4. Discussion

The experiments conducted with the wild-type strain of *G. tikvahiae* revealed that fluorescent lighting was more effective in driving the production of new tissue than pure primary color light. Red or green LED devices alone produced tissue growth rates that were only 79% of the fluorescent rates, while blue LED produced growth rates 34% of the fluorescent rates. These differences in growth rate are amplified when one considers yield; over three weeks, the wild-type strain will have produced twice the tissue under fluorescent light than under red or green LED, and 11-times more tissue if grown under blue LED. The negative effect of blue light on growth by the green mutant of *G. tikvahiae* is even more pronounced. Cool white fluorescent light yields more than 12-times the biomass than blue LED after 21 d of growth. The inability of blue light to power growth in both strains is not a surprise. Dring and Lüning (1985) reported that red algae have a low photosynthetic quantum yield under blue light, compared with green or red light. If the operational costs of the light sources are considered, the value of LED devices is enhanced. Cool white fluorescent lighting requires twice the energy expenditure as LED devices (Khan and Abas, 2011). In the present study, all three 3-color treatments and two dichromatic conditions

Table 1
Experimental lighting conditions.

	Lighting treatments		
Experiment	Code	Lighting details	Strains tested
Monochromatic	Fluorescent	Fluorescent	Wild-type, green mutant
	R	red LED	
	G	green LED	
	B	blue LED	
Dichromatic	Fluorescent	Fluorescent	Wild-type, green mutant
	R + G	50% red + 50% green LED	
	R + B	50% red + 50% blue LED	
	G + B	50% green + 50% blue LED	
Trichromatic	Fluorescent	Fluorescent	Wild-type
	RGb	40% red + 40% green + 20% blue LED	
	RgB	40% red + 20% green + 40% blue LED	
	rGB	20% red + 40% green + 40% blue LED	

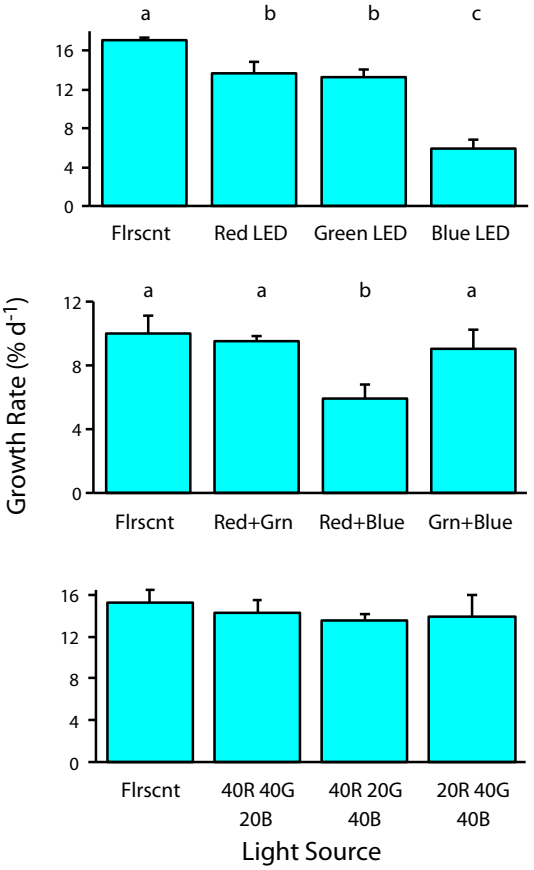

Fig. 2. Comparison via ANOVA of growth rates of *Gracilaria tikvahiae* wild strain as a function of light source during Experiments 1, 2, and 3 (mono LED, 50/50 mixed LED, 40/40/20 mixed LED, respectively; see Table 1 for abbreviations). Letters indicate significant difference within each experiment at $p = 0.05$. Light source explained 97% and 83% of the variation in thallus growth rate in Experiments 1 and 2, respectively. Error bars are standard deviations.

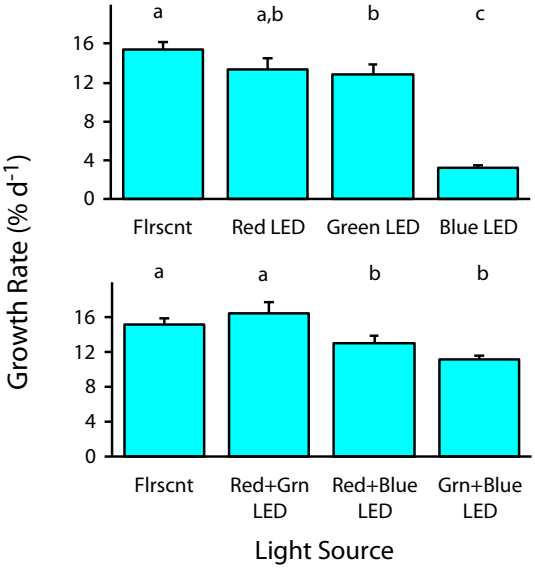

Fig. 3. Comparison via ANOVA of growth rates of *Gracilaria tikvahiae* green mutant strain as a function of light source during Experiments 1 and 2 (mono LED, 50/50 mixed LED, respectively; see Table 1 for abbreviations). Letters indicate significant difference within each experiment at $p = 0.05$. Light source explained 98% and 88% of the variation in thallus growth rate in Experiments 1 and 2, respectively. Error bars are standard deviations.

(i.e. R + B & G + B) provided as good growth as in cool white fluorescent lighting. These results suggest that dichromatic and trichromatic arrangements may yield more biomass (55–157%) than cool white fluorescent lighting, while at an energy cost per biomass that is only 50–60% of that for cool white fluorescent.

Comparison of the results of the dichromatic experiment with those of the monochoromatic and trichromatic experiments, and examination of the statistical power of the analyses suggested additional unidentified influence(s) on tissue production, at least of the wild-type strain. Growth rates under fluorescent lighting in the dichromatic experiment (10.2% d^{-1}) were only 62% of the pooled monochromatic and trichromatic growth rates (16.1% d^{-1}), a significant difference. The source of this variability is unclear. However, with the exception of this comparison of growth rate, our statistical tests were conducted on data sets within each experiment.

While light color has significant effects on the overall biomass accumulation, the effects of light on the photomorphogenesis (e.g., Talarico and Maranzan, 2000) of *G. tikvahiae* are not yet known. However, they are likely to exist; the branching and new meristem development in the rhodophyte *Asparagopsis armata* (Monro and Poore, 2005) are influenced by LED light quality. Blue light controls many metabolic and developmental outcomes in algae, including chlorophyll synthesis and chloroplast formation (Senger, 1987), enzyme synthesis (Roscher and Zetsche, 1986), cell division (Carroll et al., 1970), formation of hairs (Dring and Lüning, 1975), gamete or spore release (Lüning, 1981). Green light influences spore germination (Charnofsky et al., 1982), while red/far-red controls rhizoid formation (Nagata, 1973). In sum, these prior studies suggest that the life history of economically valuable aquacultured species, such as *G. tikvahiae*, might be manipulated in culture to emphasize particular characteristics, and to delay or prevent the onset of reproduction, a process that results in cessation of growth and senescence of vegetative tissue as reproductive propagules form.

The effects of light on the chlorophyll content of *G. tikvahiae* were not as marked as those on growth rate. While the ratio of maximum growth rate under cool white fluorescent:blue LED equaled 2.9 and 4.8 for the wild and green mutant strains, respectively, the same ratio for chlorophyll equaled 1.1 and 0.6. Chlorophyll has limited industrial use as a salable product, though a small market as a health food supplement exists. In general, a larger market exists for carotenoid pigments, the main sources being the cyanobacterium *Spirulina* and alfalfa. While microalgae are currently the non-terrestrial carotenoid source (Guedes et al., 2011), extraction of pigments from macroalgae, cultured as part of bioremediatory integrated multi-trophic aquaculture (IMTA) operations, could form part of a sequential process that would capture maximum value from the produced biomass. Thus, growing seaweeds in conjunction with fish and shell fish would generate algal biomass that might be used first as a human and/or animal food or food supplement, a source of pigments, proteins, polyunsaturated fatty acids, and phycocolloids, with the surplus biomass funneled into the production of biofuels such as bioethanol or biobutanol (e.g., Harun et al., 2010; Kraan, 2013).

If pigment production is most important, then selection of light source and/or strain is important. Unlike the above examples of some green algae (Senger, 1987), blue light didn't stimulate the chlorophyll formation in either wild-type or green mutant *of G. tikvahiae*. Chlorophyll and carotenoid content were less influenced by light source than by strain (Fig. 4 vs. Fig. 5). The wild-type strain produces 78% more chlorophyll than the green mutant strain, and 44% more carotenoids (all light sources pooled). Phycoerythrin levels were elevated under the blue LED treatment, as expected if the plants respond to reduced photon flux density in the phycoerythrin absorption spectrum (Figueroa et al., 1995). Additionally, the wild-type strain produced >12-times more phycoerythrin than did the green mutant strain, the pigment for which the largest difference exists between the two strains. Phycocyanin production may be augmented under blue LED, but this pigment is over-produced in

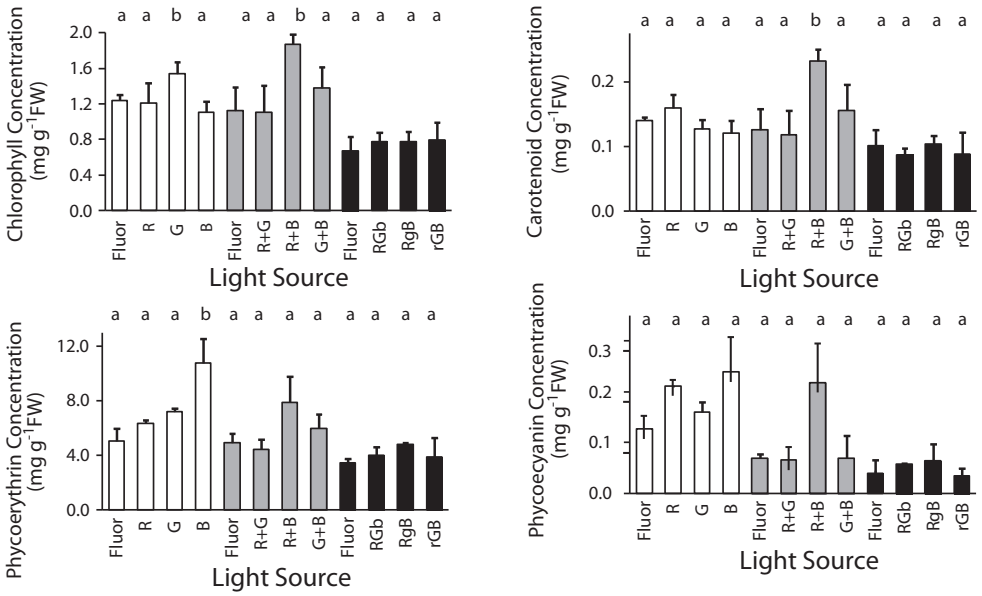

Fig. 4. Pigment concentrations of the wild strain of *Gracilaria tikvahiae* as a function of the light source (comparison via ANOVA). Different letters indicate statistical significance ($p < 0.05$). Comparisons on which letters rely were made within each experiment (monochromatic (white), dichromatic (gray), and trichromatic (black) light LED sources, each with a separate fluorescent control). Error bars are standard deviations.

the green mutant strain, with tissue concentrations 2.5-times higher than levels in the wild strain.

From the standpoint of cost, LED lighting is equal to or superior to the alternatives. Considering only energy (electricity) costs, LEDs consume an estimated 17% less than tube fluorescent lights, and 85% less than incandescent bulbs (Khan and Abas, 2011). When the total cost of LED lamps is examined (i.e., purchase plus operational costs), the differential between LED and other light sources is currently ca. zero for tube fluorescents (though still 73% less than incandescent bulbs). However, increasing efficiency of LEDs, as well as energy costs, will make LEDs the increasingly smarter choice into the future.

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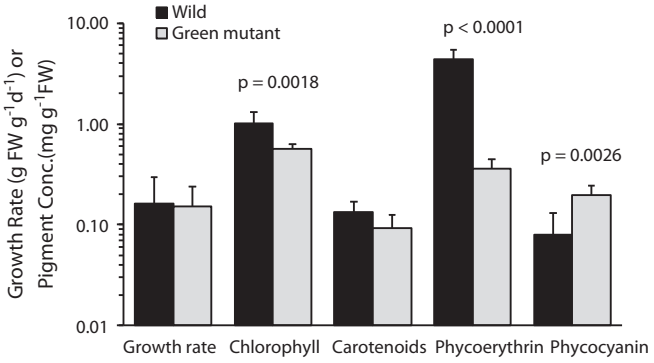

Fig. 5. Growth rates and tissue pigment concentrations of *Gracilaria tikvahiae* wild-type and green mutant strains grown under fluorescent light source (all experiments pooled; results of significant ***t***-tests shown). Pigment concentrations differed significantly between the two strains, except for carotenoids ($p = 0.060$). Thallus type (wild vs. green mutant) explained 55%, 97%, and 62% of the variation in pigment concentration for chlorophyll ***a***, phycoerythrin, and phycocyanin, respectively. Error bars are standard deviations.

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