

Effects of Light Wavelength on the Growth Rates of *Microcystis* and *Scenedesmus*

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Abstract

Light quality can influence the growth of photosynthetic microorganisms because different organisms contain different pigments and light-harvesting systems. This study investigated how white, red, green, and blue LED light affected the growth of *Microcystis* and *Scenedesmus* over a three-week experimental period. *Microcystis* is a photosynthetic cyanobacterium that contains chlorophyll a and phycobiliproteins rather than chlorophyll b, whereas *Scenedesmus* is a green microalga that primarily uses chlorophylls and carotenoids for light capture. Cultures were exposed to the four light-color treatments, and changes in absorbance were used as an indicator of changes in culture density. The mean growth values were compared using one-way ANOVAs within each species and independent comparisons between species under each light treatment. Neither one-way ANOVA showed a statistically significant effect of light treatment for *Microcystis*, $F(3, 12) = 1.636$, $p = 0.233$, or *Scenedesmus*, $F(3, 12) = 1.561$, $p = 0.250$. The original species comparisons were significant under green light ($p = 0.004$) and blue light ($p = 0.013$); however, after Bonferroni correction for four comparisons, only the green-light comparison remained statistically significant because the adjusted significance level was $\alpha = 0.0125$. An estimated species-by-light interaction based on the reported means, standard deviations, and sample sizes was not statistically significant, $F(3, 24) \approx 1.67$, $p \approx 0.20$. Therefore, the results do not provide sufficient statistical evidence that the two organisms responded differently to wavelength. The findings suggest that light color may influence growth patterns, but the effects were not statistically significant within either species under the conditions tested.

Keywords

Photosynthesis, Light Wavelength, *Microcystis*, *Scenedesmus*,
Cyanobacteria, Green Algae, Absorbance, Growth

1. Introduction

Photosynthesis allows photosynthetic organisms to convert light energy into chemical energy that can support cellular growth and metabolism. However, photosynthetic organisms do not all capture light in the same way. Their responses to different wavelengths depend on their pigments, cellular structures, and physiological characteristics.

Microcystis is a cyanobacterium rather than a eukaryotic green alga. Cyanobacteria do not contain chloroplasts, and *Microcystis* should not be described as containing chlorophyll b. Instead, cyanobacteria contain chlorophyll a and phycobiliproteins, including phycocyanin, that participate in light harvesting. This pigment system allows cyanobacteria to use portions of the visible spectrum differently from green algae. Recent research on *Microcystis aeruginosa* has demonstrated that changes in light quality can affect cell growth, biomass accumulation, pigment production, and photosynthetic performance. For example, Chen *et al.* found that red light promoted biomass accumulation and increased chlorophyll content in *M. aeruginosa*, whereas blue light reduced growth under their experimental conditions [1].

Scenedesmus, in contrast, is a green microalgal genus whose photosynthetic apparatus contains chlorophylls and carotenoids. Research has shown that the growth response of *Scenedesmus* can depend on wavelength, light intensity, and photoperiod. Fettah *et al.*, for example, found that *Scenedesmus quadricauda* responded differently to combinations of wavelength and light intensity and that excessive light could inhibit growth [2]. More recent research has also demonstrated that changes in light quality can affect the biochemical composition of *Scenedesmus* without necessarily producing a statistically significant difference in growth rate.

Light wavelength is therefore an important experimental variable, but wavelength cannot be considered independently from other characteristics of the light source. Light intensity and photoperiod can also affect growth. In addition, different LED sources can produce different spectral distributions even when they are described using the same color. Because the present experiment used white, red, green, and blue LED treatments but did not provide measured wavelength ranges, photon intensity, or photoperiod in the laboratory protocol, the treatments are interpreted here as light-color conditions rather than precisely defined spectral wavelengths.

Previous research has not shown one universal light color that produces the greatest growth for all photosynthetic microorganisms. For example, recent studies have reported enhanced growth under red light in some microalgae, while other studies have found similar growth across several light treatments. Helamieh *et al.* found that white and red light produced high biomass in *Monoraphidium braunii*, while blue-green wavelengths strongly influenced pigment and fatty-acid composition [3]. These differences demonstrate that responses to light quality can be species-specific and dependent on experimental conditions.

The purpose of this experiment was to compare the growth of *Microcystis* and *Scenedesmus* under white, red, green, and blue light over a three-week period. The original hypothesis predicted that red and blue light would produce the highest growth and that green light would produce the lowest growth. Because the two organisms have different pigment systems, differences in their growth patterns were also expected.

2. Methods

2.1. Experimental Design

Two photosynthetic microorganisms, *Microcystis* and *Scenedesmus*, were used to investigate the relationship between light color and culture growth. Each organism was exposed to four light treatments: white, red, green, and blue LED light. The exposure period lasted three weeks.

The cultures were maintained under the laboratory conditions provided for the experiment. Cultures were assigned to the available light-color treatments, with four independent cultures represented for each species-by-light combination ($n = 4$ per treatment). The experiment therefore included eight species-by-light treatment groups and 32 culture observations in total.

The experimental protocol did not provide measured spectral wavelength ranges, photon flux density/light intensity, or a specific light-dark photoperiod for the LED treatments. These variables therefore cannot be reported as exact numerical values. Because light intensity and photoperiod can influence algal growth independently of wavelength, their absence represents an important limitation when comparing the results with studies that report these parameters.

2.2. Growth Measurement

Culture growth was evaluated using absorbance measurements collected before and after the experimental period. Absorbance was used as an indirect indicator of changes in culture density or biomass because an increase in the amount of suspended photosynthetic material can increase the amount of light absorbed by the culture.

Absorbance was not treated as a direct measurement of photosynthetic activity. Photosynthetic activity would require a measurement such as oxygen evolution, carbon fixation, chlorophyll fluorescence, or another physiological measurement. The present experiment instead used changes in absorbance as an indicator of changes in culture density.

The reported growth values were calculated from the absorbance measurements in the laboratory dataset. Because the exact calculation formula and measurement interval used to convert the raw absorbance values into the reported growth-rate values were not included in the paper, the numerical values are referred to as growth values rather than being interpreted as direct measurements of photosynthetic rate.

2.3. Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted separately for *Microcystis* and *Scenedesmus* to determine whether mean growth differed among the four light-color treatments. Independent two-sample comparisons were also originally performed between the two species within each light treatment.

Because four species comparisons were performed, a Bonferroni correction was applied to control the familywise error rate. The corrected significance threshold was $\alpha = 0.05/4 = 0.0125$.

The large difference in variability among the *Microcystis* groups was also considered when interpreting the statistical results. The standard deviation under white light was 0.471, compared with 0.019, 0.035, and 0.036 under red, green, and blue light, respectively. This difference makes the equal-variance assumption questionable and should be formally evaluated using the raw observations. Welch's t-tests or other variance-robust procedures would be preferable if the variance assumptions are violated.

Finally, a two-factor analysis considering species and light treatment was used to evaluate whether a species-by-light interaction was present. An interaction is necessary to determine whether the effect of light differs statistically between species; separate t-tests within each color are not sufficient to establish such an interaction.

3. Results

3.1. Growth Patterns

The mean growth values differed among the light treatments, although the differences were not statistically significant within either species.

For *Microcystis*, the mean growth values were approximately 0.476 under white light, 0.306 under red light, 0.128 under green light, and 0.198 under blue light. White light produced the highest observed mean, while green light produced the lowest.

For *Scenedesmus*, the mean growth values were approximately 0.245 under white light, 0.274 under red light, 0.233 under green light, and 0.288 under blue light. Blue light produced the highest observed mean, while green light produced the lowest.

Thus, the descriptive pattern was not identical between the two organisms. *Microcystis* had its highest observed mean under white light, whereas *Scenedesmus* had its highest observed mean under blue light. However, descriptive differences in means do not by themselves demonstrate that the organisms responded statistically differently to light.

3.2. ANOVA Results

The one-way ANOVA for *Microcystis* did not show a statistically significant difference among the four light treatments, $F(3, 12) = 1.636$, $p = 0.233$.

The one-way ANOVA for *Scenedesmus* also did not show a statistically signif-

icant difference among the four light treatments, $F(3, 12) = 1.561$, $p = 0.250$.

Because both p-values were greater than 0.05, the experiment did not provide sufficient statistical evidence that light color produced a significant difference in growth within either species.

3.2.1. Species Comparisons

The original independent two-sample tests showed no statistically significant difference between the species under white light, $t(6) = 0.976$, $p = 0.367$, or red light, $t(6) = 1.263$, $p = 0.254$.

Under green light, the difference was statistically significant before correction, $t(6) = -4.491$, $p = 0.004$. Under blue light, the original analysis produced $t(6) = -3.516$, $p = 0.013$.

Because four species comparisons were performed, the Bonferroni-adjusted significance threshold was 0.0125. The green-light comparison remained statistically significant because $p = 0.004 < 0.0125$. The blue-light comparison was no longer statistically significant because $p = 0.013 > 0.0125$.

Therefore, after correction, the data provide evidence of a difference between the species under green light but not under blue light.

3.2.2. Species-by-Light Interaction

A species-by-light interaction was evaluated to determine whether the effect of light treatment depended on the organism. Based on the reported means, standard deviations, and equal sample sizes of four cultures per treatment, the estimated two-way interaction was $F(3, 24) \approx 1.67$, $p \approx 0.20$.

This interaction was not statistically significant. Therefore, the data do not provide sufficient evidence that *Microcystis* and *Scenedesmus* had statistically different responses to the light treatments.

This result is important because the separate species comparisons under individual colors cannot independently establish an interaction. The observed differences in means can be described, but they should not be interpreted as proof that wavelength affected the two species differently.

3.3. Variation within Treatments

The variability of the *Microcystis* groups differed substantially. The white-light treatment had a standard deviation of approximately 0.471, whereas the red, green, and blue treatments had standard deviations of approximately 0.019, 0.035, and 0.036, respectively. The white-light variation was therefore much larger than the variation observed in the other *Microcystis* treatments.

This large difference raises concerns about the assumption of equal variances underlying standard ANOVA and pooled-variance t-tests. Formal tests of variance assumptions should be conducted using the original observations. Because the current paper contains summary statistics rather than the raw observations, a definitive variance-assumption test could not be performed from the reported values alone.

4. Discussion

The purpose of this experiment was to determine whether different light colors affected the growth of *Microcystis* and *Scenedesmus*. The results showed different descriptive growth patterns among the light treatments, but neither organism demonstrated a statistically significant overall effect of light color in the one-way ANOVA.

The original hypothesis predicted that red and blue light would produce the greatest growth and that green light would produce the lowest growth. The results provided partial descriptive support for this prediction. Green light produced the lowest mean growth value for both organisms. However, *Microcystis* had its highest mean under white light rather than red or blue light, while *Scenedesmus* had its highest mean under blue light. Because neither ANOVA was significant, these differences should be interpreted as observed patterns rather than statistically confirmed effects.

The relatively low growth values under green light are biologically plausible, but they should not be explained simply by saying that green light is “reflected” and therefore cannot be used by photosynthetic microorganisms. Light absorption and utilization are more complicated than this simplified explanation suggests. Pigment composition, light intensity, wavelength bandwidth, acclimation, and the structure of the photosynthetic apparatus can all affect how efficiently an organism uses a particular light environment.

The response of *Microcystis* is particularly important because it has a different light-harvesting system from green algae. As a cyanobacterium, *Microcystis* contains chlorophyll a and phycobiliproteins rather than chlorophyll b. Recent work on *M. aeruginosa* demonstrates that light quality can affect both biomass accumulation and photosynthetic physiology. Chen *et al.*, for example, found that red light increased biomass content and chlorophyll levels, whereas blue light reduced growth under their experimental conditions [1]. These findings show that cyanobacterial responses to light can differ substantially depending on the spectral environment and physiological outcome being measured.

The *Microcystis* results in the present experiment do not exactly match those findings because white light produced the highest observed mean and red light produced a lower mean. However, the difference is not necessarily contradictory. The experiments differed in light conditions, experimental duration, culture conditions, and measurement methods. Most importantly, the current experiment did not document the exact wavelengths or photon intensities of the LED treatments. Consequently, it is not possible to determine whether the light treatments in this experiment were comparable to those used in previous studies.

The *Scenedesmus* results also demonstrate why species responses should not be generalized. Research on green microalgae has shown that wavelength, light intensity, and photoperiod can interact to affect growth. Fettah *et al.* found that *Scenedesmus quadricauda* showed different growth responses depending on both wavelength and intensity [2]. More recent work with *Scenedesmus obliquus* found

that changing light color affected biochemical characteristics such as protein and pigment production while producing little difference in maximum growth rate. These findings support the idea that a change in light quality does not necessarily produce a large change in growth.

The present results also agree with recent research showing that different light colors can produce different physiological outcomes without necessarily producing significant differences in growth [4]. Helamieh *et al.* found that white and red light produced high biomass in *Monoraphidium braunii*, while blue-green light had strong effects on pigment and fatty-acid composition [3]. Similarly, Esteves *et al.* reported that red light increased growth in *Chlorella vulgaris*, while blue light produced lower growth-related values under their experimental conditions. These studies illustrate that light quality can influence photosynthetic organisms in species-specific ways and that growth is only one possible response variable [5].

An important statistical consideration is the difference in variability among the *Microcystis* treatments. The white-light standard deviation was 0.471, which was considerably larger than the standard deviations of the red, green, and blue groups. Such unequal variability can affect the reliability of statistical tests that assume equal variances. The original t-tests used pooled variance, so the raw data should be examined before treating those tests as definitive. Future analysis should use formal variance tests and, if necessary, Welch's t-test or another variance-robust approach.

The multiple-comparison correction also changed the interpretation of the species comparisons. The original analysis identified significant differences under both green and blue light. After Bonferroni correction, the green-light comparison remained significant at $p = 0.004$, but the blue-light comparison did not because $p = 0.013$ was slightly above the corrected threshold of 0.0125. Therefore, the blue-light result should no longer be described as statistically significant.

The interaction analysis provides an additional reason for caution. Although the mean responses appeared different between species, the estimated species-by-light interaction was not significant. Therefore, the experiment does not provide sufficient evidence to conclude that the species responded statistically differently to wavelength. The appropriate conclusion is that the two organisms showed different descriptive patterns, while the statistical evidence for a differential wavelength response was insufficient.

Several limitations affect the interpretation of this experiment. First, the exact wavelength ranges of the LED treatments were not provided. Second, light intensity was not documented. Third, the photoperiod was not documented. These factors are important because two lights described simply as "red" or "blue" can have different spectral distributions and intensities. Research on *Scenedesmus* has demonstrated that intensity and photoperiod can substantially affect growth, meaning that these variables should be reported in future experiments.

Another limitation is the use of absorbance as an indirect measure of growth. Absorbance can provide useful information about changes in culture density, but it is not a direct measurement of photosynthetic activity. Future experiments

could strengthen the conclusions by measuring cell density, dry biomass, chlorophyll concentration, oxygen production, or chlorophyll fluorescence in addition to absorbance.

Finally, the experiment used a three-week exposure period, but only before-and-after measurements were used in the reported analysis. More frequent measurements would allow growth curves to be generated and would make it possible to calculate a clearly defined specific growth rate during the exponential phase. This would provide more information about how growth changed throughout the experiment rather than only describing the difference between the beginning and end.

5. Conclusions

This experiment investigated the effects of white, red, green, and blue light on the growth of *Microcystis* and *Scenedesmus* over three weeks. Green light produced the lowest observed mean growth for both organisms, while the highest observed mean occurred under white light for *Microcystis* and blue light for *Scenedesmus*. However, one-way ANOVA results indicated that the differences among light treatments were not statistically significant for either species.

The species comparisons also required correction for multiple testing. After Bonferroni correction, the difference between *Microcystis* and *Scenedesmus* under green light remained significant, whereas the blue-light comparison did not. In addition, the estimated species-by-light interaction was not significant, so the data do not establish that the two organisms responded differently to wavelength.

Overall, the findings suggest that light color may influence the observed growth patterns of photosynthetic microorganisms, but the evidence from this experiment is insufficient to conclude that light color significantly changed growth or that the two species had significantly different wavelength responses. Differences in pigment systems, light intensity, photoperiod, and other culture conditions may contribute to the observed patterns. Future studies should report exact wavelength ranges, photon intensity, and photoperiod; use more frequent growth measurements; include direct measurements of photosynthetic activity; and analyze the species-by-light interaction using the complete raw dataset.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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