Effects of Light Intensity on the Morphology, Growth Rate and Phenolic Content of *Zygnema*

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Background

Mat-forming algae play an important role in the ecological interactions of shallow freshwater habitats by providing habitat for hundreds of aquatic organisms (Graham et al. 2015). Algae in the Streptophyceae which includes *Zygnema*, are closely related to land plants and should possess pre-adaptations for colonizing the land, including protective mechanisms for light and UVB exposure (deVries and Archibald, 2018).

Freshwater filamentous algae can form thick mats that float at the water surface where algal cells must deal with challenging conditions related to solar radiation (Fig. 1). Exposure to UVB radiation in sunlight is known to be harmful to most organisms due to its ability to break bonds between atoms in biological molecules (Hader et al. 2007). Previous work in this lab showed an increase in phenolic content production in *Zygnema* when exposed to a high light intensity compared to a low light intensity. In addition, this past study produced *Zygnema* cells that had condensed cytoplasm and were significantly shorter in length when exposed to the high light intensity compared to low light intensity.

Figure 1. Floating algal mats at the water surface of a local pond. Algal filaments in the upper portion of the mats are exposed to potentially damaging levels of photosynthetic active radiation and ultraviolet radiation compared to filaments deeper within the mats that may be light limited due to self-shading.

Study Goals

- Determine the light intensity needed to significantly increase phenolic content production in *Zygnema*.
- Determine the light intensity needed to significantly reduce *Zygnema* cell length.
- Identify the relationship between light intensity and *Zygenema* growth rate.

Methods

A *Zygnema* isolate (OK-03) was previously cultured from a freshwater habitat in western Oklahoma. Replicate clumps of filaments standardized by size were grown in cell culture plates containing 10 mL of growth medium under different light intensities (15.6 ± 0.34, 23.9 ± 0.57, 31.0 ± 0.69, 65.8 ± 1.38, and 159.7 ± 2.73 µmoles • m^{-2} • s^{-1} PAR) using gray filters (Edmund Optical) for one week with 4 mL of fresh media transferred to the samples every two days. After seven days of light acclimation, one sample from each culture plate was randomly selected and weighed. The five plates were then exposed to the same PAR levels for an additional week with 4 mL transfers every two days. Light was provided by a bank of 24T12 High Output fluorescent tubes (Philips).

After the seven days of additional light acclimation, algal filaments were photographed using an Olympus BH2 microscope fitted with a SPOT™ IDEA 3.0 MP camera and cell lengths measured using SPOT™ Software version 4.7. Fresh weights of all the samples determined and phenolic compounds were extracted by grinding in 100% methanol. Methanol extracts were scanned using a DR 5000 UV/Vis spectrophotometer (Hach, Loveland, CO, U.S.A.) and the area under the curve from 250-350 nm was measured to determine changes in phenolic content. Content of phenolic compounds was reported absorbance (250-350 nm) per milligram of fresh weight. Growth rate was recorded as doubling per day. Statistical analysis of data was carried out using GraphPad Prizm version 8.0.0 for Mac OSX (GraphPad Software, San Diego, CA, U.S.A.).

Results – Cell Morphology

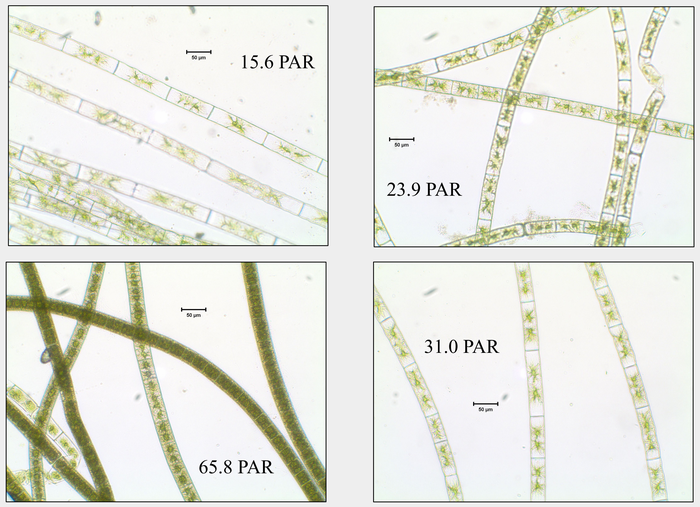
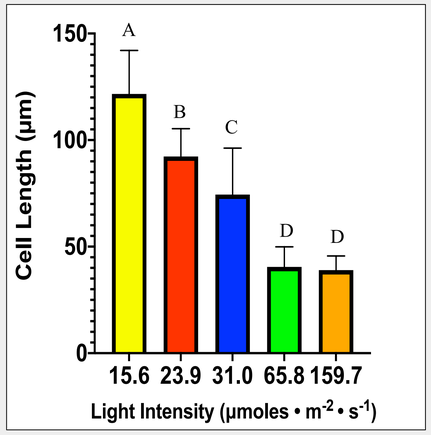


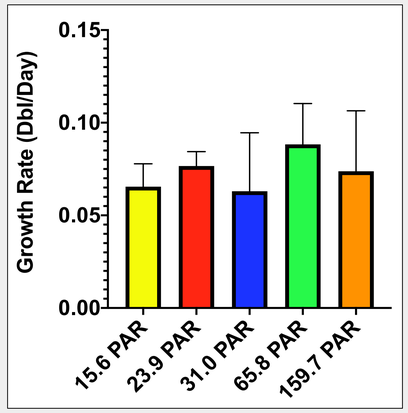
Figure 2. Cell appearances of *Zygnema* cells after seven day exposure to different PAR levels. Note the large clear vacuole spaces in the 15.6 PAR exposed filaments and the shortened cells with dense cytoplasm in the 65.8 PAR exposed filaments. Such cell changes seen here may influence light penetration into thick algal mats and provide protection from high levels of UVB radiation. Note: the cells from the 159.7 PAR exposed filaments are not significantly different from the cells in the 65.8 PAR exposed filaments.

Figure 3. The algal filaments exposed to 15.6 PAR produced longer cells (121.7 µm) than all other treatments and were 212% longer than cells exposed to 159.7 PAR (39.0 µm). The 65.8 PAR and 159.7 PAR treated cells were not significantly different from each other but they were shorter than the 15.6, 23.9, and 31.0 PAR treated cells. At low light intensities, the cells were significantly shortening in smaller intervals (15.6, 23.9, and 31.0 PAR) than at high light intensities (65.8 and 159.7 PAR).



Results – Growth Rate

Figure 4. In this study, there was not a significant increase or decrease in the growth rate of *Zygnema* when exposed to different light intensities. This may suggest that *Zygnema*'s growth rate is not affected by light intensity once the filaments have become acclimated to a certain PAR level.



Results – Phenolic Content

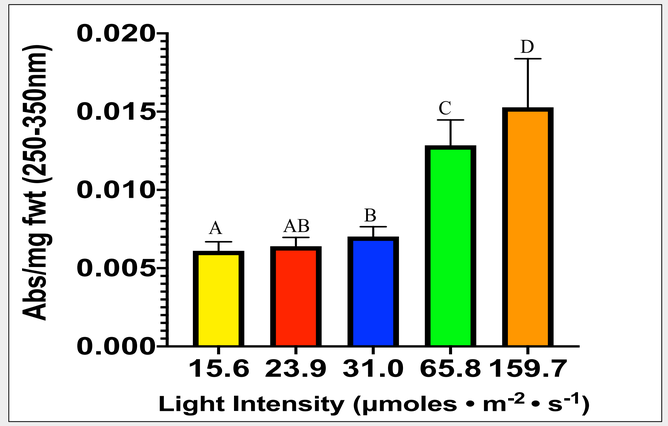


Figure 5. The filaments exposed to the highest intensity (159.7 PAR) produced significantly more phenolic compounds per mg of fresh weight than any other treatment and produced 150% more phenols than the 15.6 PAR treatment. The only treatment not significantly different was the 23.9 PAR compared to the 15.6 PAR and 31.0 PAR treatments.

Conclusions

- A critical light intensity exists between 31 and 66 µmoles/m^2/s at which *Zygnema*'s phenolic content production is significantly increased.
- *Zygnema* cell length is sensitive to increasing light intensity and length increases steadily until a maximum cell length is reached at ~66 µmoles/m^2/s.
- From this experiment, light intensity does not appear to affect *Zygnema*'s growth rate once pre-acclimated.

Future Research

- Determine the spectrum of visible light (red, blue, green) that triggers changes in *Zygnema*'s phenolic content, cell length, and growth rate.
- Identify what phenolic compounds *Zygnema* is producing and compare to the phenolic compounds higher plants produce when responding to increased light intensity and UVB radiation.

Literature Cited

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