The Effects of Light Wavelengths on *V. hendryi* Development

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Abstract

Primary producers break down light into different categories, allowing them to respond to light in multiple different ways. This paper answers the questions for *Vertebrata hendryi*, a filamentous red alga, which are: do sensory photoreceptor cells in *V. hendryi* play a role in developmental events by response to wavelength, and does *V. hendryi* grow better under a specific wavelength? Pieces of *V. hendryi* were placed in f/2 medium-filled petri dishes within containers of different colored LED starboards to measure dimensions of cystocarps/pericarps before and after the treatment, along with the number of cystocarps/pericarps and branches. The results showed significant difference between the green and red/far-red wavelength for the number of branches, suggesting increased vegetative growth for the alga under green light wavelength.

Introduction

Light is a necessity in all aspects of life, most importantly, in photosynthesis -- which in turn results in the growth -- of primary producers. Light allows algae and plants to produce carbohydrate and oxygen, as well as playing a key part in consuming CO_2, all things that are essential for life on Earth. Additionally, light is essential not only to the growth of certain algae, but also triggers developmental events independent of overall growth via sensory photoreceptors like the phytochrome (Röhrig et al., 2013). Phytochromes are known to respond to red and far-red light wavelengths and there is much literature written on the subject for the phylum Chlorophyta. Cells such as phytochromes play a key part in circadian rhythms, allowing developmental events based on photoperiodism to occur (Hegemann, 2008). One example that

displays the role of phytochromes is a filamentous green alga *Mougeotia*, in which chloroplast movement was found to be reversibly influenced by red and far-red wavelength (Dring, 1988). However, there is little evidence suggesting the role of photoreceptor cells in any other phyla of algae.

The main pigment cell in red algae (phylum Rhodophyta) is phycoerythrin, which has a peak absorbance at around 550 nm and overshadows chlorophyll *a*, generally the major pigment cell in other primary producers (Barsanti & Gualtieri, 2014). This is explained by their habitat, as it is mostly in shallow subtidal areas (~ 13 m) where efficient usage of blue and green light is crucial to having adequate productivity (Graham et al., 2009). Although sensory photoreceptor cells have yet to be sufficiently studied for Rhodophyta, there has been instances where cryptochrome was found, regulating circadian rhythms and growth (Hurd et al., 2014). With further studies, culturing red algae for carrageenan and agar extraction may become more efficient, as scientists would be able to manipulate wavelengths of light in order to amplify production. Both carrageenan and agar are commercially important, as they contribute to the medical and food industries in the form of gelatin (Armisen & Galatas, 1987; Necas & Bartosikova, 2013).

The topic of focus in this paper is reproductive structure development in *Vertebrata hendryi*, previously known as *Polysiphonia hendryi* and a member of the class Florideophyceae in phylum Rhodophyta. The reproductive structure termed cystocarp (post-fertilization) and pericarp (pre-fertilization) stick out of the female gametophyte and house carpospores and the carposporophyte. The carpospore is a product of the parasitic carposporophyte on the female gametophyte form of the life history (Figure 1). In the experiment described herein, the effect

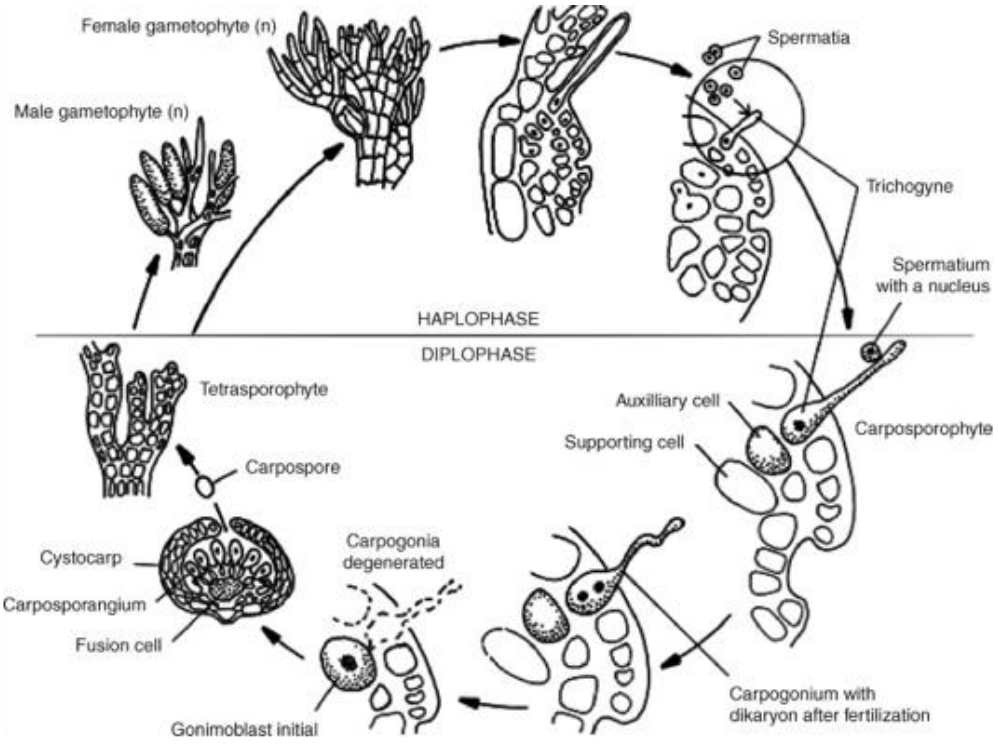

Figure 1: Diagram adapted from Margulis & Chapman (2010) showing developmental stages in *Polysiphonia* sp.

of different wavelengths of light on cystocarp/pericarp growth of *Vertebrata hendryi* was measured, which would correlate to carpospore production due to the storage structure needing to grow in size as the internal structures increase in quantity and size. The potential presence of sensory photoreceptor cell roles influenced by specific light wavelength was also measured. I predicted that growth will be most significant around the blue and green light spectrum regions due to *V. hendryi* habitat mainly being in the shallow subtidal areas. I also predicted that there would be an absence in role of sensory photoreceptor cells under specific wavelengths for cystocarp/pericarp development, as *V. hendryi* seems to show signs of successful reproduction both in the shallow subtidal and intertidal areas (~1 m). In the shallow subtidal, the most abundant wavelengths of light are green and blue light wavelengths and in the intertidal, all types of wavelengths are almost equally abundant due to direct sunlight being available.

Materials & Methods

A female gametophyte, *Vertebrata hendryi*, was collected off the docks of Friday Harbor Laboratories on May 23rd of 2019. The alga was kept in a plastic container on a sea table with some parts of its sides replaced with mesh to allow water flow, as the alga is easily spoiled.

Five out of six 10.7 cm x 10.7 cm x 9.2 cm (l x w x h) culture boxes were spray painted with RUST-OLEUM® PAINTER'S TOUCH MULTI-PURPOSE semi-gloss paint, dried, then re-sprayed with RUST-OLEUM® PAINTER'S Touch® ULTRA COVER PAINT+PRIMER satin dark walnut for a secondary coating. These boxes were next hot glued down to a 47 cm x 31 cm wooden board in an effort to keep it fastened throughout the experiment. The boxes were oriented in a fashion so that two air distributors may be fastened on the sides of the board with clamps and to allow two air pumps to be seated on one side of the board (Figure 2). Then, the boxes had two holes drilled in on the sides -- each on opposite walls -- in order to allow air flow with tubes. 10 out of 14 clear 3/16 in. x 5/16 in. VWR® PVC tubes (one for incurrent and the other for excurrent, clear box does not need the tubes painted and two extra tubes for the air pumper → air distributor connection) were then spray painted only at one tip -- the tip that will eventually be inside the box -- to further prevent external light from penetrating the boxes. Next, the tubes were connected going from the air pumpers to the air distributors and all the incurrent tubes to the two air distributors and culture boxes, and each of the excurrent tubes to the culture boxes.

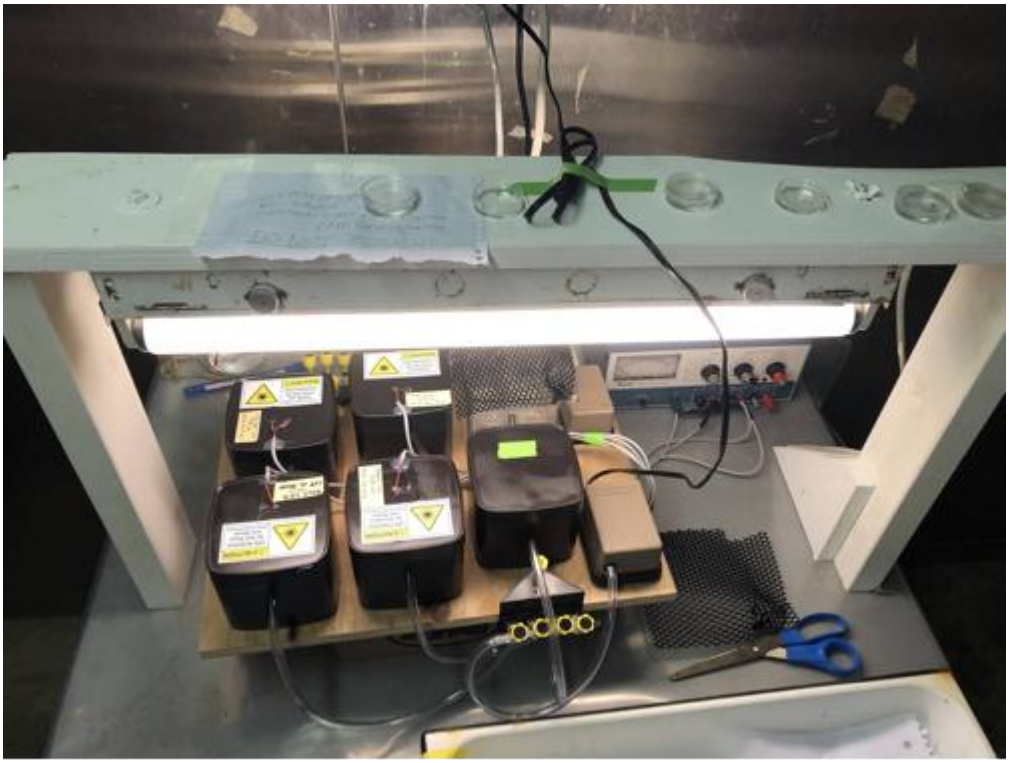

Figure 2: Picture of the experimental setup

Four Opulent Americas Cree Horticulture LED Starboards lights -- royal blue (455 nm) part number XPGDRY-L1-0000-00601-SB01, green (530 nm) part number XPEBGR-L1-0000-00F03-SB01, photo red (655 nm) part number XPEPHR-L1-0000-00901-SB01, and far-red (735 nm) part number XPEFAR-L1-0000- 00601-SB01 -- were then hot glued to their corresponding box lids with hot glue and two small holes, enough to allow the cords through, were drilled on each (except for clear and dark box) lid to allow the light wires to go through the top of the lids.

The whole setup was then placed on top of a box in a cold storage to accommodate the need for increase in height due to the air distributors being clamped on the sides of the wooden stage. The temperature of the storage room was measured by a TITAN® Infrared Thermometer 55010 (temperature range: -58℉ ~ 842℉) and was found to be between 11~13.5℃ at any given time. Next, a HeathKit Tai-Power Supply (model 1P-2718) was connected to an outlet and the wires for the LED lights were then connected to 5V direct current power outlet along with

resistors -- 109 Ω (royal blue), 90 Ω (green), 145 Ω (photo red), and 155 Ω (far-red) (Figure 3).

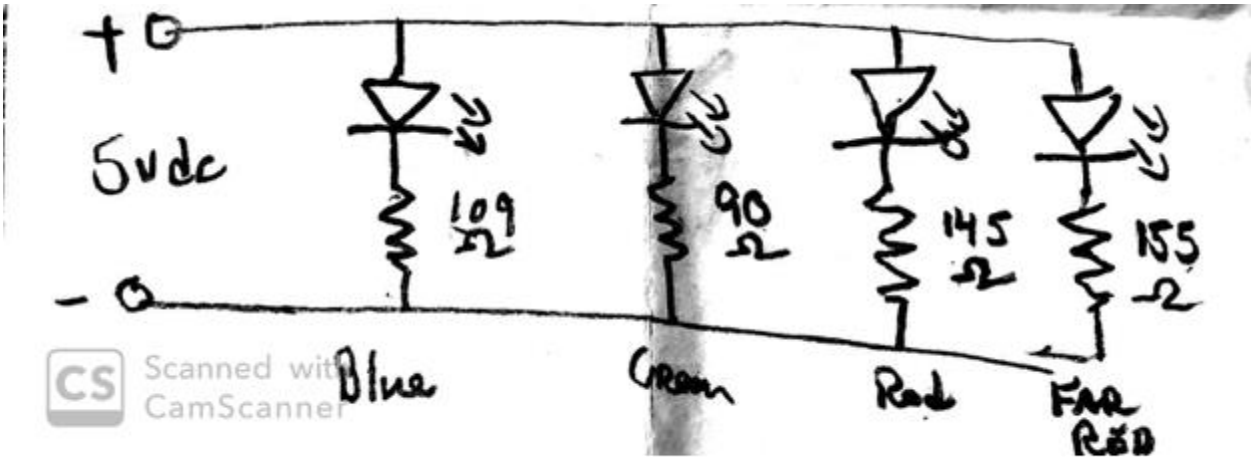

Figure 3: Diagram of current setup, illustrated by T. Mumford

The light source for the clear box was supplied by a GE F20T12-KB-ECO SP30 20W Hg lighting apparatus placed at a height of 17 cm away from the light treatment box, which required a 0.5 cm x 0.7 cm opening black plastic mesh with covering on top of the lid in order to decrease the light intensity to match the other light sources. Each light intensity was measured by placing a LI-COR® QUANTUM 09586 light sensor, connected to a LI-COR® LI-250A Light Meter, inside the culture boxes with the lids closed. The readings gave: 0.55 $\mu E\ m^{-2}\ s^{-1}$ (far-red), 4.99 $\mu E\ m^{-2}\ s^{-1}$ (red), 12.01 $\mu E\ m^{-2}\ s^{-1}$ (blue), 7.76 $\mu E\ m^{-2}\ s^{-1}$ (green), and 4.70 $\mu E\ m^{-2}\ s^{-1}$ (Hg light). The reading for the far-red light was most likely close to zero due to the light meter not being able to detect wavelengths beyond the visible light spectrum. The cultures were exposed to light 24/7 for the duration of the experiment, in an attempt to eliminate responses of sensory photoreceptors via photoperiodism, isolating responses caused by wavelengths rather than the length of darkness.

Next, 24 ‘main’ (longest in length, largest in width, and have the most number of cystocarps/pericarps) branches of a female gametophyte of *Vertebrata hendryi* were cut with a target length of 1.5 cm each. Each ‘main’ branch had three randomly chosen

cystocarps/pericarps stemming off from either the ‘main’ branch or a ‘sub’-branch (any branches that stem off from the ‘main’ branch), and the width and length of these were measured with nomenclature assigned in order for dimensions to be collected from the same cystocarps/pericarps at the end of the treatments. Then, the number of branches and cystocarps/pericarps stemming off of the last specific branch (i.e. the last roman numeral used) were noted in order to determine growth trend over the next four days under treatment conditions (Figure 4). Each piece was then placed in a FALCON® REF 351008 Easy Grip 35 x 10 mm Style Polystyrene Petri Dish, which was filled with 5 mL of f/2 medium prior to the placement of the specimen (McLachlan, 1973). Groups of four petri dishes were next placed into each culture box and had the lids closed with all the lights turned on. The medium was not changed for the duration of the experiment.

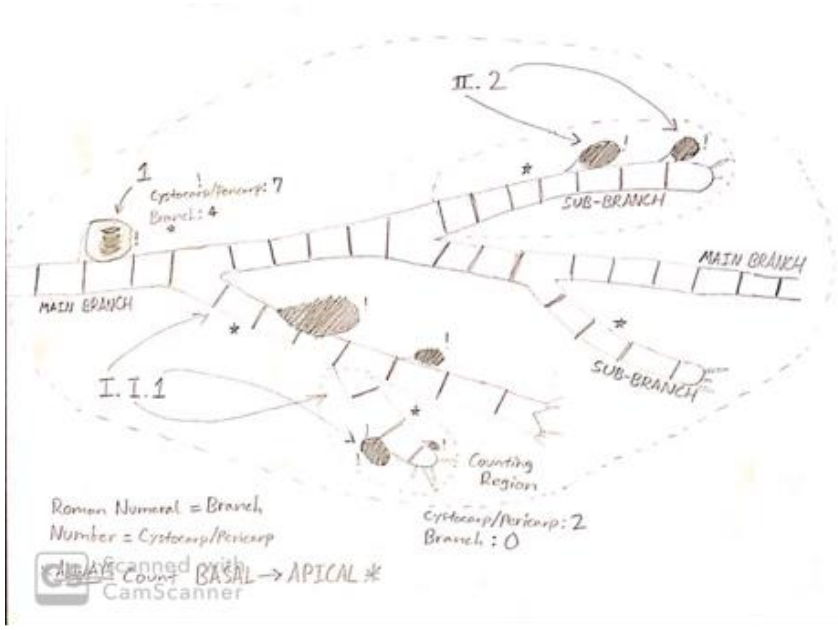

Figure 4: Illustration showing the identification scheme used in the experiment.

Data collection was then done again at the conclusion of the experiment, which was four days after the experiment start date. The final dimensions of the cystocarps/pericarps were then compared to the initial measurements, then assessed. Measurements were collected by taking

each specimen out of the f/2 medium-filled petri dishes and placing them on a concavity slide to look under a compound microscope. Following the identification system made at the beginning of the experiment, the same cystocarps/pericarps were measured again, as well as the number of branches and cystocarps/pericarps. Finally, all of the specimen were disposed of in the ocean.

Results

The length and width collected of the cystocarps/pericarps were all calculated to equal area, which was then run through R version 3.5.2 to show the differences from beginning to end in area, given different light treatments (R Core Team, 2014). The results yielded a p-value of 0.429, which fails to reject the null hypothesis, meaning that the difference in light treatments did not reveal any significance in data. Next, the final number of cystocarps/pericarps were all subtracted by the initial count and put through a one-way anova. Similarly, the results failed to show significance in data, with a p-value of 0.239. However, the number of branches (run in the same manner as the number of cystocarp/pericarp test) showed a significance in data with a p-value of 0.0292. The results were then run through HSD and revealed similarities between

certain wavelengths (Figure 5).

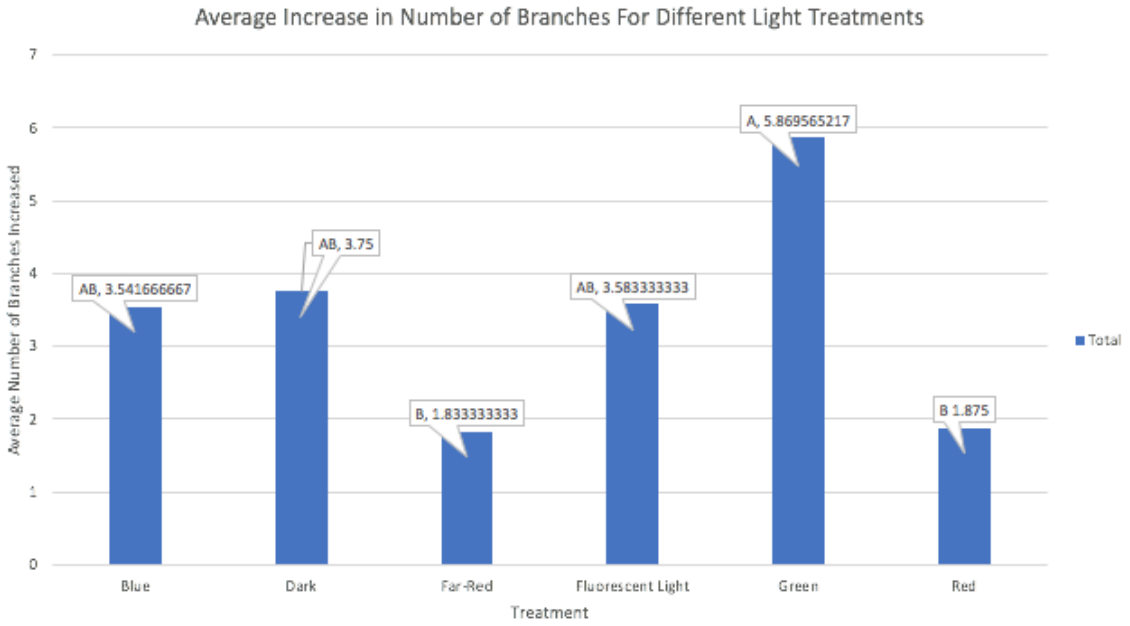

Figure 5: Graph showing the relationships in significance between the different treatments for the average increase in number of branches over the course of the experiment. A's are significantly different than B's, AB's are insignificantly different between each other and A & B.

Observing the specimens, a week after the conclusion of the experiment, it was clear that the success of carpospore settlement was not affected by the different light treatments except in the dark treatment, where the specimens were all found to be dead. However, the observation seemed to suggest a higher sample of settled carpospores under the green wavelength.

Discussion

The questions this experiment was designed to answer were: will *V. hendryi* grow better in certain wavelengths and do sensory photoreceptor cells play a role in developmental events influenced by wavelengths of light? The questions asked in this paper helps in understanding photoperiodism in the genus *Vertebrata*, which is important in helping us understand more about the very complicated life histories of the many genera of the phylum Rhodophyta.

Phototropism is interesting in that, despite the terminology, is influenced by the period in which the organisms lack light, as opposed to the period in which the organisms absorb light.

This phenomenon has been observed many times in terrestrial plants but it has only been seen in certain algae species (Dring, 1970). Instead, many algae responses to light are not enacted by photoperiodism, but by detection of a specific wavelength of light. For instance, *Scytosiphon*, a genus of brown algae, displays a short-day response triggered by a blue-absorbing pigment and not phytochrome (Dring & West, 1983). So, the possibility of pinning down what exactly causes certain responses to occur in a species of algae could help in the understanding of both the evolutionary history and the life history (mainly habitat and habitat selection) significantly better for possibly the whole genus or family.

Going through the results, the insignificance in data between the start and end area in different light conditions ($p=0.429$) may be interpreted as there being an absence in the role of sensory photoreceptor cells in the growth of reproductive structures (e.g. cystocarp/pericarp and carpospore). Had there been an active role played by the cells, the results would have shown significance in a few treatments and not the rest, suggesting responses to those specific wavelengths. The results gathered from the number of branches ($p=0.0292$) also suggests an interesting point. The fact that there was significant difference between the green light treatment and red/far-red light treatment suggests that *V. hendryi* grows considerably better under green light than red or far-red light. One thing to note is that this growth is only vegetative growth, perhaps shedding light that the growth of reproductive structures *is* triggered by sensory photoreceptor cells, but via photoperiodism.

There were many errors made in this experiment, which may have contributed to most of the data resulting in a lack of significance, but one of the bigger glaring problems was the identification system made. Creating a simpler method to keep track of specific cystocarps/pericarps would have been wiser, as it would have avoided many issues in the

collection of data. The experiment may have also run better if the orientations of the specimen could have been kept the same when data were collected at the start and the end. It may have been possible that some cystocarps/pericarps and branches were hidden at either data collection, making the identification of the same cystocarp/pericarp impossible if it were not accounted for.

Another problem with the experiment was the number of specimen that were decaying and/or being fed upon by many microscopic organisms throughout the test. I don't know how this problem can be avoided, but observing parts of the specimen covered in gunk made it difficult to identify certain things properly. Also, having a better definition of what constituted a cystocarp/pericarp and what constituted a branch would have made the experiment more successful. Throughout the data collection, there were many instances in which it was hard to distinguish the tips of branches as two different branches or just one single branch. Similarly, identifying whether a carpogonial branch was a pericarp or not proved to be challenging.

Lastly, a future experiment that can be done in relation to this one is making the light:dark cycle the variable and keeping light (wavelength and intensity) constant. This way, the presence of photoreceptor cell roles through photoperiodism may be detected.

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