Light Intensity Variation of Kelp Gametophytes, *Alaria marginata* and *Nereocystis luetkeana*

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Abstract

Nereocystis luetkeana and *Alaria marginata* were raised from zoospores into young gametophytes under three different light intensities. *A. marginata,* a species only occurring at shallow depths, was collected at a light level measured at 1505µmol $m^{-2}sec^{-1}$. *N. luetkeana,* a species germinating commonly at a depth of 5-30m was collected from 8.17m below the surface with a light level of 2.75 µmol $m^{-2}sec^{-1}$. The study determined that there was no correlation between light intensity and gametophyte growth, and that there was no difference in gametophyte growth between the two-respective species. *A. marginata* was found to have a significantly higher proportion of zoospores germinated.

Introduction

Nereocystis luetkeana and Alaria marginata are two kelp species belonging to the brown algal order Laminariales. These species, along with other kelp, are primary producers that supports intertidal, nearshore, subtidal, and terrestrial food webs, providing nutrients through kelp detritus, dissolved organic matter, and whole plants for herbivore grazing (Mann 1973) (Redmond et al. 2014). It is an essential part of the marine ecosystem (Duggins et al. 1989). Kelp is also incorporated into many common products through its cell wall extract alginate (Draget 2009). Kelp is also a major food source across Asia and around the world (Erlandson et al. 2015). Hence, understanding the biotic and abiotic factors influencing kelp growth are important to quantifying how they perform in providing ecosystem and human services.

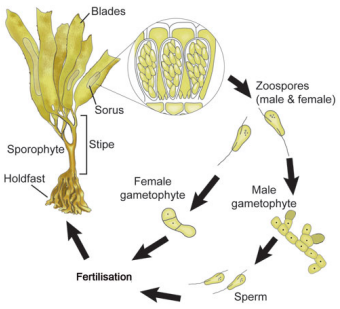

Figure 1. Diagram of the kelp life history that pertains to both *A. marginata* and *N. luetkeana.* This study will be focusing on the release of zoospores from the sorus, leading to germination and gametophyte growth (Pfister 1992).
Graphic: Erika Mackay

We are assuming that where the zoospores settle will be where the gametophytes will grow, leading to where the upright sporophytes will grow. *N. luetkeana* grows between 0 and 30 meters beneath the surface (Springer et al. 2007), meaning the gametophytes are able to growing with less light. *A. marginata* are only found in shallow water and low intertidal, having greater access to light (Swanson and Druehl 2000). Kelp require certain amounts of light and water temperature to survive and grow (Flavin et al. 2013). This study investigates the effects of varying light intensity on the germination of zoospores of two species of kelp that grow in areas with different access to light. Vadas 1972 found reducing access to light severely slows growth of the gametophyte (below approximately 20 µmol m^{-2} sec^{-1}). Light intensity exponentially decreases in the water column, meaning deep water plants receive less light energy for photosynthesis (Ryther 1956). Knowing that *Alaria* is only found where there is strong light intensity, I hypothesized that under high light intensities *Alaria* will grow, but under low light it will not germinate or grow. *Nereocystis* is hypothesized to germinate and grow faster at low light levels since its gametophyte is known to successfully grow at greater depths. *N. luetkeana* also grows on the dock in shallow water next to *A. marginata* sporophytes, so its gametophytes may also germinate and grow in high light intensity, meaning it may have a wider range of conditions for success than *A. marginata.*

It is important to note that male and female gametophytes grow to different sizes, however differentiation does not become visible until roughly the 15th day (Leal et al. 2018). Previous studies (Leal et al. 2018) have measured spores from the beginning of the filamentous tail which extends from the settled zoospore, in order to determine effects of copper pollution on spore germination and gametophyte development. Others have measured the same germ tube length in order to determine impact of UV radiation on early development of kelp gametophytes (Huovinen et al. 2004).

Methods

I collected two different species of fertile kelp, *Alaria marginata* and *Nereocystis luetkeana. Alaria* was collected from the University of Washington Friday Harbor Labs dock from rubber tire substrate (lat. 48.545254, long. -123.011998). The dock floats, meaning that the *Alaria* always remains at a constant 0.5m subtidal. Reproductive *Alaria* is found by looking for side sporophylls off of the stipe, with a dark brown color. This is the sorus and it is where the spores are released from. *Nereocystis* was collected from Turn Rock (lat. 48.5324174, long. -122.9758575). Reproductive *Nereocystis* is found by looking for the dark brown color (sorus) towards the end of the blades. Light intensity was recorded at the surface of the water and bottom depth (8.17m) at Turn Rock using a LICOR LI-25)A light meter, an underwater cable with a 2009S Lowering Frame, and a LiCor LI-192 Underwater Quantum Sensor. Upon returning to lab, using sterile 0.45 micron filtered sea water and a squirt bottle, the sori were sprayed to remove dirt and get the kelp clean. A paper towel was then used to wick away excess water from the sori. Then approximately five by five cm squares were cut out of the sorus and placed in paper towels where they were wrapped up and placed in the refrigerator to cool for 18 hours

(6.3°C). Then they were taken out and placed in individual finger bowls with approximately 100 mL of sterile filtered sea water. The finger bowls were then placed in sea table to keep cool (approximately 10.7 °C water temperature) while the sori released their spores. The water in the finger bowl became murky when the spores have been released. Using a micropipette, I took some of the water out of the fingerbowl and placed it on hemocytometer (Primus Jena 18715). Then looking at the hemocytometer under the microscope, I counted the number of moving spores in each of six grid squares and recorded them. Repeating this two more times to get an accurate estimate, the average was taken for amount of spores per square. This number was then multiplied by 25 to get number of spores on entire grid, and then multiplied by 10,000 to get number of spores per mL. Then the spores were diluted to get 5,000 spores per milliliter in each well (Merrill and Gillingham 1991). This amount was pipetted into each well and filled with 7 milliliters of sterile filtered sea water. Upon looking under the microscope of the spores, it was evident some were dying rather quickly, so I increased concentration by a factor of five to ensure a sufficient number of motile spores were in each well. Well plates (Falcon Polystyrene, Non-pyrogenic, Non-tissue culture treated) were set up as shown in Figure 2.

Figure 2. Gametophyte cultures in well plates containing approximately 5,000 spores per mL of *A. marginata* and *N. luetkeana* wrapped in tin foil and covered by a certain number of light filters (window screen).

Treatments were: one treatment with no light filter, one treatment with one light filter, and one treatment with five light filters. Five light filters were selected because that makes the light reaching the well plate to be 3.4 micromoles, which is comparable to the 2.75 micromoles of light found where *Nereocystis* was collected. Well plates were put into culture box (converted refrigerator), which was set at 10°C, and placed under a Sylvania Warm White 20W fluorescent light. The culture box was on cycle of 16 hours of light followed by eight hours of darkness. Every eight hours of light the plates were rotated 180 degrees and then swapped at random to ensure equal amounts of light intensity under the light. Aluminum foil was wrapped around the bottom and sides of the well plates to keep stray light from reaching the wells. Following 24 hours, well plates were looked at under the inverted microscope (Nikon Eclipse TE2000-U with phase light) set at 300x magnification. While looking at a rectangle of 300x450 micrometers in a random location in the well plate, spores were counted and recorded as either showing signs of growth or not. Then those that were showing signs of growth were scored by measuring the length of the filament (germination tube) growing from the spores using the ocular micrometer. The filamentous growth from the spore is shown in Figure 3.

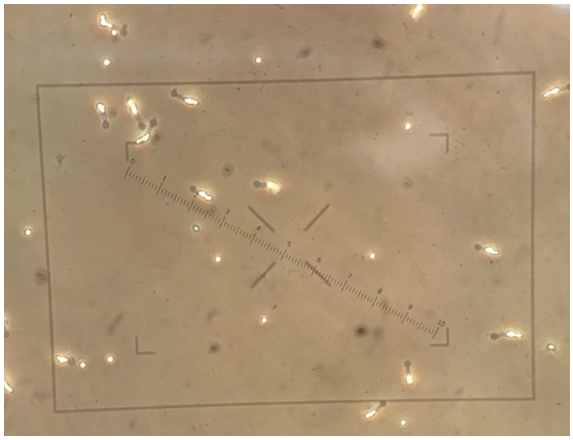

Figure 3. Photo taken of *A. marginata* after 72 hours under no light filters. Showing the original spore (black circle) and the growth measurement is taken of the illuminated germination tube.

Data was then collected again after 72 hours following the same procedure. A two-way ANOVA test was conducted using R v3.511 for significance across species and treatments in terms of proportion growing and growth rate.

Results

Following the 24-hour data collection, a two-way ANOVA was run to test for significance across species and across treatments. The figures below display the data collected.

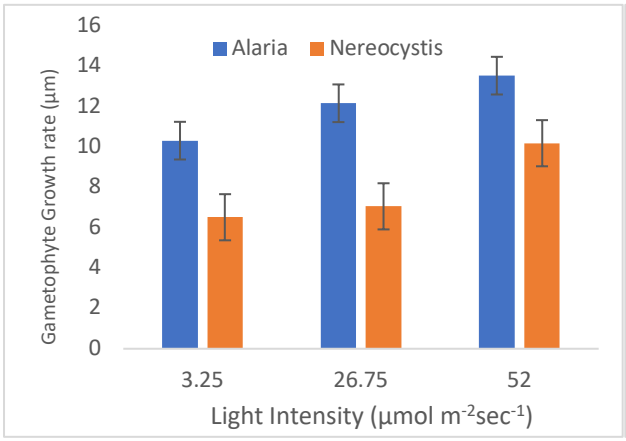

Figure 4. Gametophyte growth rate across the range of light intensity of both species after 24 hours.

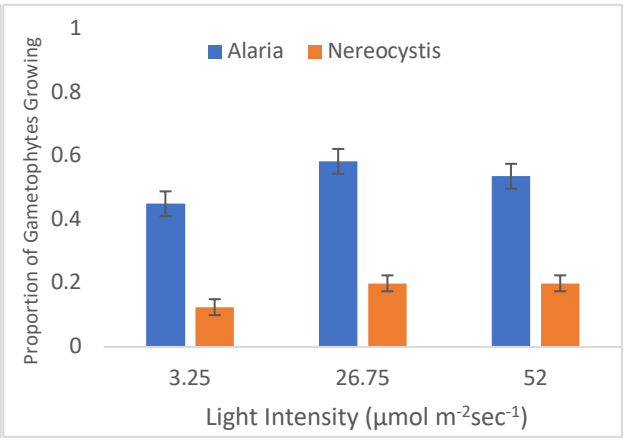

Figure 5. The proportion of gametophytes that showed any growth under the range of light intensity after 24 hours.

The test concluded that there is a significant difference between species and growth rate ($p=0.03$) but no significant difference between treatments ($p=0.12$). This means that *A. marginata* appears to grow at a faster rate than *N. luetkeana* under all light treatments. However, there was no evidence that the light filters had any impact on gametophyte growth. A two-way ANOVA was then run to determine if there was a significant difference in proportion of gametophytes growing. The result was that the difference between species was significant

(p<0.001). It is clear that *A marginata* zoospores germinated at a faster rate than *N. luetkeana* (55.2% growing versus 16.8% when averaged across all treatments).

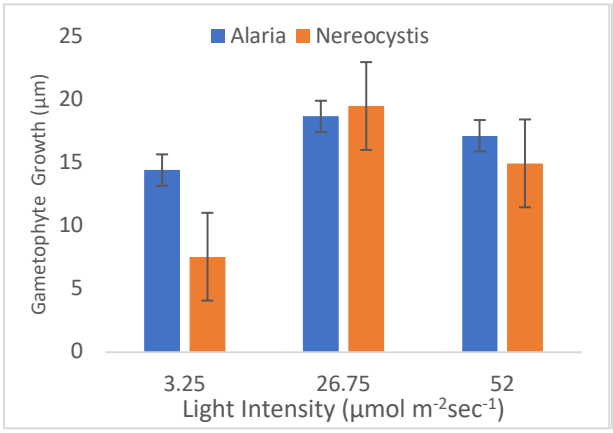

Figure 6. Gametophyte growth rate across the range of light intensity of both species after 72 hours.

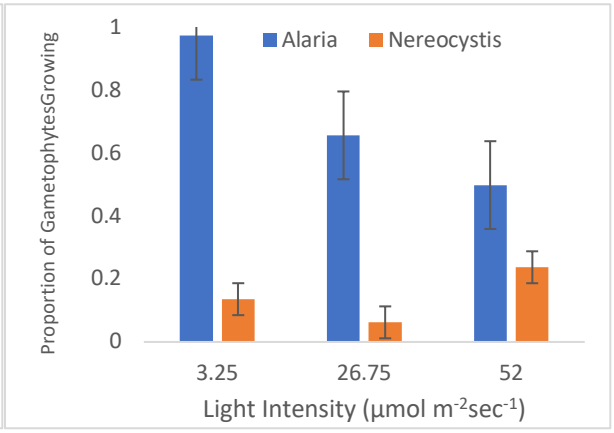

Figure 7. The proportion of gametophytes that showed any growth under the range of light intensity after 72 hours.

Following another two-way ANOVA, it could be concluded that there was no significant difference in gametophyte growth across the light treatments (p>0.05). After 72 hours it was concluded that there was no significant growth difference between the two species of kelp (p>0.05). I repeated with a statistical test on the proportion of gametophytes growing in the field of view, and that remained significant (p=0.001), meaning that there remained a significant gap between the percentage of zoospores growing between the *A. marginata* and *N. luetkeana* (53.7% versus 18.2%).

Discussion

The results of this study reveal little about gametophyte growth rate between the two species. After 24 hours the difference was significant, but it was not after 72 hours. Future research should allow for more time to collect data to see if the difference after 24 hours was true or false. Another method to gather more data would be to add more replicates, or to

record data from multiple positions under each well plate. With non-significant P values, it is inconclusive if altering the light intensity has any effect on gametophyte growth. This contradicts previous research (Vadas 1972) (Neushul 1963) (Tatsumi and Wright 2016) that had stated that decreasing light intensity slows the growing process of kelp gametophytes. Further research is needed to determine if slowed growth is apparent following the first 72 hours period. It is possible the range of light intensities was too narrow, and more treatments were necessary with greater and lesser access to light to see the differences in growth rate across treatments (Xiugeng and Neushul 1984). A previous study (Neushul 1963) used a range from 12µmol to 96µmol and found the young plants show little growth at low intensity as compared to higher light intensity.

There is a significant difference in proportion of developed spores across species. *A. marginata* showed a much higher proportion of gametophyte germination than *N. leutkeana.* Figure 7 is a good example of the difference in proportion between the two species across all light treatments. This could have been because the *N. luetkeana* happened to produce less fit zoospores by coincidence, or it takes longer time period for it to germinate. Being able to track growth for multiple weeks would be beneficial to see full development. Tatsumi and Wright in 2016 tracked their growth over the course of 30 days in their study on effects of light intensity on zoospore density and growth.

My hypothesis was not supported that *N. luetkeana* would have a wider range of germination and growing across the varying light intensity, or that *A. marginata* would be more successful germinating and growing under high light intensity. Subsequently I found that light intensity did not have an impact on germination or growth of either kelp species.

Throughout this process there were small technicalities that could have led to the results that there was no difference in growth rate across treatments. Firstly, the window screen had no way to secure to the top of the well plates. Meaning that stray light could have entered which would lead to them all receiving similar light intensity which would make sense that there was no significant difference in the data. When looking at the *N. luetkeana* zoospores before adding them to the well plates there were a lot of non-motile ones. After looking through many finger bowls of zoospores I found one that had around 70% of spores moving, however it is very possible that most of those died in transport to the culture box, since it seems that they were more susceptible to dying upon release from the sorus. This could explain the major difference in proportion of germinated zoospores between the two species.

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