Water on the rise: Bull Kelp Gametophyte survival in rising temperatures

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Abstract

As anthropogenic climate change progresses, increasing seawater temperatures play a large role in shaping changes in the range of benthic species. Predominate cold temperate kelp species are such an impacted organism; they grow at temperature below 18°C and are now seeing a decrease in available habit as they are threatened by increasing water temperature (Lind and Konar 2017). *Nereocystis luetkeana,* a kelp with a floating canopy in the sporophyte phase is found in the eastern Pacific Ocean from central California to the Aleutian Islands (Druehl 1969). As in all kelp species, it has a heteromorphic life history, which leaves two phases susceptible to increasing water temperatures. In this study *N. luetkeana* zoospores were grown in culture to determine how the young gametophyte phase reacted to elevated temperatures. To determine the upper temperature range of gametophytes, three temperature treatments were used to cultivate *N. luetkeana* zoospores found in the San Juan Islands, WA. A significant decrease in the number of gametophytes and their length were observed at temperatures exceeding 15°C. These findings indicate that the decrease in bull kelp beds could be highly correlated to the increase in temperature of the intertidal.

Introduction

Bull kelp, *Nereocystis luetkeana,* is vital to the ecosystem of the Salish Sea as it is one of the main primary producers. Along with production, it provides habitat for larval and juvenile organisms and is an essential part of the marine food web by providing nutrients in the form of detritus (Harrold et al. 1998; Vetter 1995; Kokita and Omori 1998). In recent years, there has been a noticeable difference in kelp beds found along the intertidal, a 30% decline was reported by the Bull Kelp Monitoring Project in the San Juan Islands alone (*A Decade of Disappearance* 2019). Another study recorded a 93% decline in North California, where a 3.5°C increase in average water temperature caused a 97% mortality (Schiltroth 2018). This loss of *Nereocystis* beds has caused a decrease in commercial alginate extractions (Oppliger et al. 2012), habitat for

multiple species, biomass (Koenigs et al 2015, Ramshaw et al 2017), and a decline in urchin and abalone fisheries dependent on kelp as a food source (Tegner, M 2000).

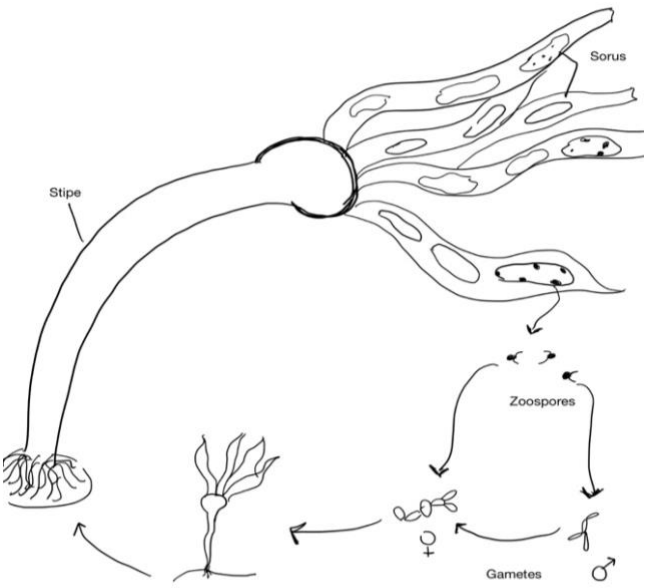

Figure 1. The heteromorphic life cycle of N. luetkeana is depicted. Zoospores are released from fertile sorus, which develop into gametes, which will then go through fertilization.

The life cycle of *N. luetkeana* (Figure 1) has two phases, the sporophyte and gametophyte phase. Zoospores are released from the sorus found at the end of the kelp blades which will then develop into male and female gametophytes. The gametophyte phase is also the stage that is most susceptible to environmental changes (Schiltroth et al. 2018). Recent studies have started looking into how temperature affects the development of gametophytes in the life cycle, due to the recent increase in ambient water temperatures. In 1982 the Diablo Canyon power plant did a 44 day study on the effects of temperature on *N. luetkeana*, the results of this study noted that at temperatures exceeding 11.8°C mechanical damage and tissue abrasions became evident.(TERA Corp, 1982). At temperatures exceeding 18°C zoospores could not survive (Pacific Gas and Electric Company 1981). With a 3.5°C increase, the abundance and density of bull kelp were critically reduced (Springer et al. 2007).

In this experiment, I hypothesized that the temperature zoospores are cultivated in affects their growth and that temperatures exceeding 18°C are fatal. To test this, growth and survival of gametophytes were tracked at varying temperatures over the course of three days. By investigating the early-life stage, this research can help provide details concerning how *N. luetkeana* is impacted by increasing temperatures, why they're becoming less prevalent, and aid predictions regarding how kelp beds will be shaped in the future.

Materials and Methods

24 hours previous to the start of the experiment, three separate 95L water baths were set up in a walk-in refrigerator. The temperatures of the water baths were regulated, but fluctuated by 1-2 decrees, at 10.5°C, 15°C, and 20°C, temperatures were measured by a TITAN® Infrared Thermometer 55010 (temperature range: 14.44°C ~ 450°C). 1.2 meter LED white lights were placed above the water baths and emitted a light intensity averaging 11.4 µmol/$m^{-2}s^{-1}$, an "active eye" quantum PAR meter (www.Hydrofarm.com) was used to measure intensity. 5L of 0.45micron filtered sea water was sterilized by bringing it to a gentle boil, sealing the top with tin foil, and cooling it in the sea table (11°C) overnight.

N. luetkeana blades were collected on May 22, 2019 at 10:46am at the kelp beds on the shore of Turn Rock, San Juan Channel, San Juan County, Washington (48.5324, -122.9758). The blades were then examined in order to decide if they were fertile based of their sorus. Fertile sori can be determined based off the sori having perforations around the edge and having a spotted, chocolate brown pigmentation. Chosen blades were placed into a bucket and kept submerged in sea water. A LICOR LI-25 light meter, an underwater cable with a 2009S Lowering Frame, and a LiCor LI-192 Underwater Quantum Sensor was used to measure the PAR of the kelp bed at the surface (0.5m, 1507.00 µmol/$m^{-2}s^{-1}$), middle (4.09m, 7.38 µmol/$m^{-2}s^{-1}$), and bottom (8.17m, 2.75 µmol/$m^{-2}s^{-1}$.).

All sori were sprayed off with the sterile 0.45micron filtered sea water and rubbed down with a Kimwipe® to remove impurities. 20 - 5 x 5cm squares were then cut out of the cleaned sori and placed in a single row on a paper towel. The paper towel was then wrapped in another paper towel and placed in the fridge (6.3°C). After 18 hours in the fridge, 20 finger bowls were filled with 100ml of sterile 0.45micron filtered sea water and the cut squares were added individually to each bowl. Spores were released when the water turned murky, which took approximately one to two hours. A randomly spawning sori bowl was chosen to use in this experiment. A Primus Jena hemocytometer (0.100mm depth, $\frac{1}{400}$ sq. mm) was used to measure the initial spore count; however, it was then diluted to a concentration of 25,000 spores/mL. Three Falcon non-tissue culture treated plates, 6 well, flat bottom with a low evaporation lid well-plates were used to cultivate zoospores. Each well was filled with 7ml of the 0.45micron filtered sea water and 333.5µl of the spore concentration. Once filled, the well-plates were

transported to the walk-in refrigerator while being covered to avoid direct sunlight and added to each treatment water bath.

Every 12 hours the well-plates were rotated 180° to control for light intensity and exposure. The overhead lights remained on throughout the entire experiment. At the end of day three, an inverted scope was used to capture images of each well. (Figure 1) A 300 x 450µm grid was calibrated on the scope to regulate spore counts. Everything inside the grid was counted and each spore was measured using ImageJ (https://imagej.net/). Excel® was used to record the data and construct one-way ANOVA tables for both length and number of individual spores against the temperature treatment. Once a significant result was noted based off the ANOVA table in each test, the $F_{critical}$ was corrected and a Scheffe Post Hoc test was performed to determine which groups were significantly different from one another.

Results

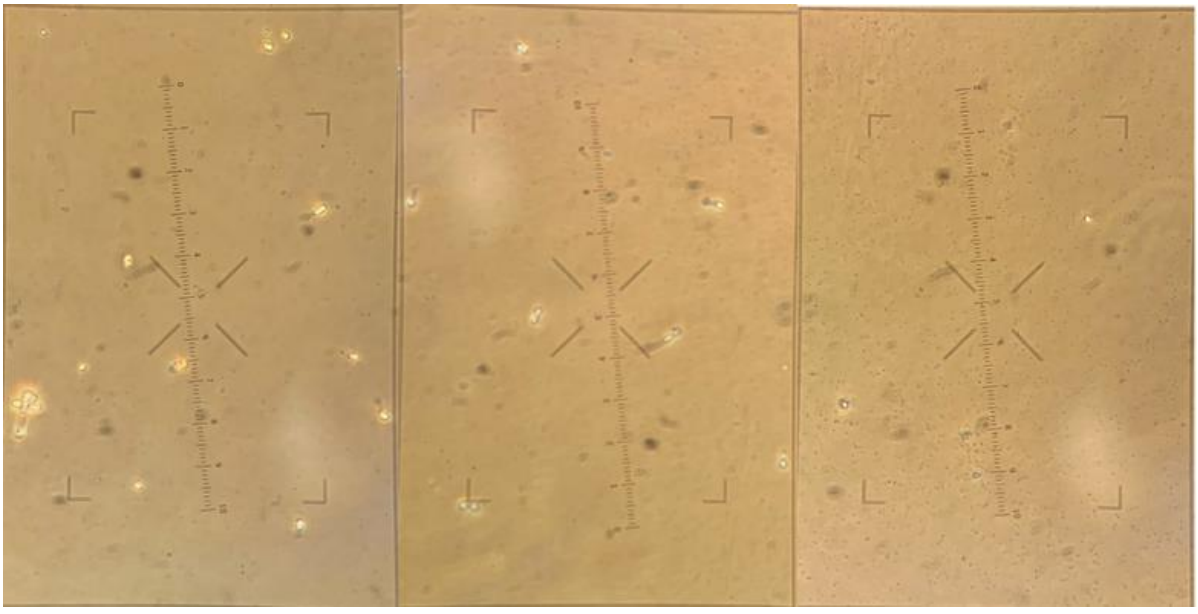

Figure 2.: Images from each treatment captured on day three. Left (10°C), middle (15°C), right (20°C). Abundance and length of spores decreased as temperatures increased. (eyepiece micrometer scale is 1mm in length)

The ambient temperature, 10°C, served as the baseline in scoring how the zoospores handled increasing temperatures. After three days of growth, the abundance was evident in the visual amount of spores in the 300 x 450µm grid (Figure 2). On average the 10°c replicates had 16 spores, that were each about 6.65µm long. Both of these values decreased as temperature increased. At 15°c spore count decreased to 12, averaging each 5.88µm long. Finally, at 20°c, spores averaged at 7 spores in the grid and were only 2.42µm long.

While there was no significant result between 10.5°C and 15°C in regard to spore length ($F_{critical}$= 6.08, $F_{calculated}$=5.14), there was a significant difference between both 10.5°C and 15°C when compared to 20°c length ($F_{calculated}$ =71.28, $F_{calculated}$ =38.85) (Figure 3). These differences are consistent with the hypothesis that zoospore growth is dependent on the temperatures that they are cultivated in.

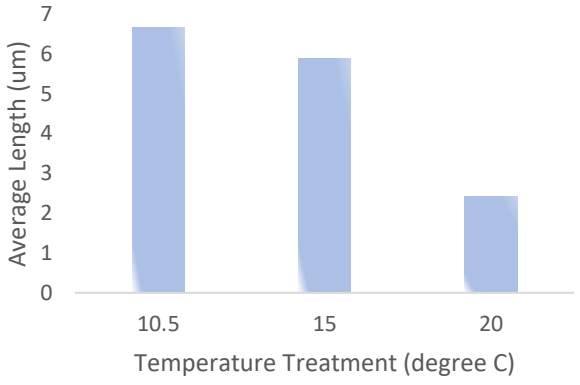

Figure 3: Average length (µm) and temperature treatment (°C) were plotted on a bar graph. Using an f-critical of 6.08, there was no significant difference between 10.5 and 15 ($F_{calculated}$= 5.14). There was significant different between 10.5 and 20 ($F_{calculated}$= 71.28) and between 15 and 20 ($F_{calculated}$=38.85).

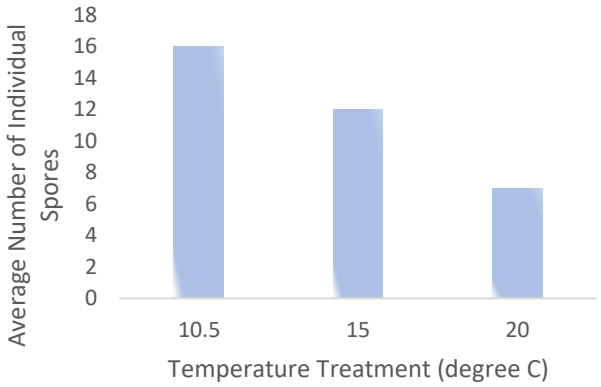

Figure 4: Average number of individual spores in a 300 x 450µm grid and temperature treatment (°C) were plotted on a bar graph. Using an $F_{critical}$ of 7.36, there was no significant difference between the 10.5 and 15 group. There was a significant different between 10.5 and 20 ($F_{calculated}$= 29.06) and between 15 and 20 ($F_{calculated}$= 9.31).

Between 10.5°C and 20°C, the average number of spores in the 300 x 450µm grid was significantly different ($F_{critical}$= 7.36, $F_{calculated}$=29.06) and decreased by over half (figure 4). These results are consistent with the previous length results. However, the number of spores still alive and sprouting at 20°C contradicts the claim that zoospores cannot survive at temperatures above 18°C.

Discussion

One abnormality that occurred during this experiment was during the spawning of zoospores. At that time, the zoospores were dying off extremely fast upon release. In some bowls half of the spores would no longer be moving after just 5 minutes upon release. Due to this issue, the concentration of spores used was increased to compensate the lack of healthy spores. For future studies, 90% of the spores should be moving and a lower concentration used.

N. luetkeana gametophyte survival at elevated temperatures was the emphasis of this study. The length of spore tube after 72 hours was significantly different between 10°C and 15°C when compared to 20°C but not between 10°C and 15°C. This result shows that it's at temperatures exceeding 15°C that the growth of spores is inhibited. Bull kelp on average grow 25cm per a day (Arthur 1919), which allows them to reach the water's surface before releasing their zoospores. By introducing them at the highest point, the zoospores will be carried into the wave column and distributed around the area. However due to the reduction in kelp beds, the question regarding if zoospores are actually being distributed and maturing remains. The decrease in *Nereocystis* beds could also correlate to the second finding in this study: temperatures exceeding 15°C decrease the sheer number of spores. Previous studies have determined that 17°C is when germination decreases and that at 20°C spores are killed off (Schiltroth et al. 2018); however, the results found in the study showed, although decreased, spores were still present and growing, ever so slightly, at 20°C. Both results provide possible explanations for why the bull kelp is disappearing and how temperature is playing a key role.

Due to the divide between if gametophytes were surviving at higher temperatures (20°C) a possible future study could be collecting *N. luetkeana* from a variety of different locations with a range of intertidal temperatures and comparing growth of spores at the temperature range used in this study. By doing so, this could help determine the evolutionary patterns and if fitness has changed between separated intertidal zones. The results of this future study could then be used later on to have possible hybridization with bull kelp surviving in warmer regions that would increase temperature resistance.

Intertidal temperatures have extreme variation which can range anywhere between -7°C to 48°C (Blair 2007). The results from this study gives evidence to the plausible cause of bull kelp decreases and provides future research that could help with the engineering of preserving the kelp beds that are key species in the marine and commercial food web.

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