

The effect of irradiance on the short-term growth rates of two strains of *Griffithsia pacifica* Kylin

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Abstract

The growth of the warm-water, Mexican strain of the giant-celled marine red alga *Griffithsia pacifica* Kylin has been studied in great detail. However, a comparison of the growth rates of this strain to the local strains located in Friday Harbor, Washington has not been conducted. This study compared the short-term growth rates of the two strains of *Griffithsia* under varying light intensities. Stock cultures from the Botany Department at the University of Washington, Seattle and wild samples collected from the dock at Friday Harbor Laboratories in Friday Harbor, Washington were examined. Wild samples were collected from coordinates at N 48° 32.723' W 123° 00.729'. Prior to cell excision, wild samples were cleaned using sterile razor blades and agar to remove epiphytes and contaminants. All cells were excised and grown in f/2 media for 6 days under LED lights at three intensities: 25, 16 and 8 $\mu\text{mol}/\text{m}^2/\text{s}$. Morphological difference between stock and wild cultures were observed. Half the stock culture subjects and one third the wild culture subjects died. However, others experienced growth, with the lowest light intensity cultures having a higher growth rate than the higher intensities. The low light stock (8 $\mu\text{mol}/\text{m}^2/\text{s}$) cultures had significantly higher growth rates than both the low light wild and ambient light (25 $\mu\text{mol}/\text{m}^2/\text{s}$) stock. The increased growth rate is likely a result of the stock cultures being acclimated to irradiances between 5-10 $\mu\text{mol}/\text{m}^2/\text{s}$. Future studies of longer time periods and with varying light intensities and temperatures are proposed to further investigate perceived differences.

Abbreviations: GPS, global positioning system

Introduction

Griffithsia pacifica Kylin is a filamentous, red marine alga. This species is found in the Pacific Ocean, in the lower intertidal to subtidal ranges, typically no deeper than 20 feet below the surface (Abbott and Hollenberg 1976). The range of the species extends as far north as Alaska and as far south as Peru, with a concentration between southern British Columbia and Baja California (Algaebase.org; Abbott and Hollenberg 1976). The type locality is in Friday Harbor, Washington (Abbott and Hollenberg 1976). As a Florideophyte, this alga experiences a triphasic life cycle with free-living gametophyte and tetrasporophyte phases and a parasitic carposporophyte phase (Graham and Wilcox 2000).

Morphologically, this species has regularly dichotomous branching in lower parts while irregular branching in upper parts. The thalli are tufted, 3-5 cm tall and an orange-pink color (Fig. 1). The unusually large, turgid cylindroconical cells can easily be observed with the naked eye or dissecting microscope (Abbott and Hollenberg 1976). Cells of this species are classified as either rhizoidal or shoot cells; the latter tend to grow at a rate of 1-2 cells/day (30-40 shoot cells/week) under optimal conditions (16L: 8D photoregime, 20-25°C at 60 $\mu\text{mol}/\text{m}^2/\text{s}$) (Duffield et al. 1972; Waaland and Cleland 1972; Graham and Wilcox 2000). Intercalary cells can be induced to divide when adjacent cells are removed. Cell growth is confined to the top and bottom of the cell; this is defined as 'bipolar band growth' (Waaland et al. 1972). Development of an isolated cell starts when, at the apex of the cell, a shoot cell develops, followed by a rhizoidal cell at the base. Branching is signaled by a gathering of pigment at the branch site, then bulging at the nodal cell wall, forming another wall (Duffield et al. 1972). The fast growth rates and

the ability of *G. pacifica* to regenerate whole new plants from single cells make this species ideal for growth studies (Duffield et al. 1972; Graham and Wilcox 2000).

While the growth of the warm-water Mexican strain of the giant-celled marine red alga *Griffithsia pacifica* Kylin has been studied in great detail, a comparison of the growth rates of this strain to the type locality from Friday Harbor, Washington has not been conducted. The objectives of this study were to investigate the survival rates of the Mexican strain of *Griffithsia* in a temperature lower than their optimal and to compare the growth rates of the two strains of *G. pacifica* under varying irradiances. The hypothesis of the study was that there would be differences in growth rates, not only between light levels but between the warm-water, Mexican stock cultures from the University of Washington and the wild samples growing in Friday Harbor, Washington.

Materials and Methods:

Natural Light and Temperature Measurements

Prior to the start of the experiment, light and temperature data were collected at the wild *Griffithsia* sample site. Water temperature was measured in 10 minute intervals over a 24 hour period using two IButton temperature data loggers (Maxim Integrated Products, Inc., San Jose, CA, USA) and irradiance levels were measured on a one time basis using a LI-COR LI 1000 light meter (LI-COR Biosciences, Lincoln, Nebraska, USA) using the Spherical Quantum sensor (LI 1935A) with conversion factor -234.11 at the sample collection site. Sampling coordinates were recorded using a Garmin GPS 76 (Garmin International, Inc., Olathe, Kansas, USA).

Sample Collection

Samples were collected at the Friday Harbor Laboratories dock (Fig. 2) in between the floating dock and rowboats. The wild strain of *Griffithsia pacifica* was found colonizing a nylon boat-securing strap, which was hanging into the water. This strap was located on the 7th tire from the metal walkway between the high dock and rowboat docks at coordinates at N 48° 32.723' W 123° 00.729'. Stock culture samples were obtained from the Botany Department at the University of Washington, Seattle.

Cell Cleaning and Excision

After collection, cell cleaning and processing occurred. Whereas the stock culture alga was clean, the wild plants were cleaned using sterile forceps and razor blade to remove epiphytes or visible growths. Then, they were dipped and pushed through 1% seawater agar to further remove contaminants before proceeding (Duffield et al 1972; Anderson 2005).

Clumps of the cleaned cells were selected and transferred to a slide with a drop of f/2 media. A sterile razor blade was used to excise intercalary shoot cells (4th or 5th cells from the apex of the filament). Cells were then transferred, 3 in each 6 x 1.5cm Petri dish (Fig 3). (Duffield et al 1972; Anderson 2005).

Cell Culturing

Twenty-four 6x 1.5 cm Petri dishes were labeled with name, culture and date and filled with 10mL of sterile f/2 media. Stock *Griffithsia* dishes were labeled 1A-D, 2A-D and 3A-D, whereas wild *Griffithsia* dishes were labeled 1E-H, 2E-H, and 3E-H (Fig 4A).

Cultures were grown on a 16:8 light: dark regime (Duffield et al 1972; Anderson 2005). at 15 °C. A modified Thermoelectric Wine Cooler, model TWR282S (EdgeStar Products, Austin, TX, USA) was used as an incubator with the addition of taped cardboard on the glass door to block external light, and the internal placement of 3x10 inch LED strip lights (Warm White, 3100 K) (LED Wholesalers.com, Inc., California, USA). The lighting was adjusted as follows: “Ambient Light” (AL) was defined as 25 $\mu\text{mol}/\text{m}^2/\text{s}$, “Medium Light”(ML) was defined as 16 $\mu\text{mol}/\text{m}^2/\text{s}$ and ”Low Light” (LL) as 8 $\mu\text{mol}/\text{m}^2/\text{s}$. Layers of cheesecloth were draped over the Petri dishes to reduce the irradiance to for the ML and LL replicates (Fig. 4B). Growth was measured every 24 hours at the same time of day for 6 days, cultures were removed from the incubator, observed with a dissecting scope and the cells counted. Photos were taken using the Micropublisher 3.3 RTV camera (QI Imaging, Inc., Surrey, British Columbia, Canada).

Results were analyzed using a daily average of cell numbers in each dish to calculate growth rate/day when divided by 6, and t-tests for dependent samples ($\alpha=0.05$) to investigate growth differences.

Results

Natural Light and Temperature Measurements

The average temperature over 24 hours was 11.77°C. The irradiance as measured in the afternoon of the sampling date (8/8/12) was 48.79 $\mu\text{mol}/\text{m}^2/\text{s}$ (Table 1).

Morphology and Cell Growth

During the cell excision process, morphological differences were observed between the Mexican and wild strain of *Griffithsia pacifica*. The wild strain had longer

and thicker cells than the stock (Fig 5A). The stock strain was more delicate difficult to cut while the wild strain had a more robust cell construction. The cultures grown at the lowest light intensity tended to be darker in color than the ones grown in the highest light intensity.

Some of the cell in the culture dishes died over the course of the experiment (Table 2). Cell death was usually gradual and accompanied by a fading of pigment and light spots appearing on the sides or center of the cell. The AL treatment subjects 1B and 1C died by day 3, and the 1G by day 5. The ML treatment had the highest occurrence of cell death; subjects 2A and 2D died by day 2, 2B by day 4, and 2E and 2F by day 6. The LL treatment subjects had the fewest culture death; only 3B by day 4 and 3E by day 3, especially in the ML ($16 \mu\text{mol}/\text{m}^2/\text{s}$) treatment. In total, half the stock cultures and one third of the wild cultures died over the course of the experiment.

While some cells experienced no growth, a few in each treatment grew apical cells and/or rhizoids over the course of the experiment. In the AL treatment, subject 1A started to grow a rhizoid and experience some budding by day 6 (Fig. 5B). Subject 1H in the same treatment did not show evidence of a rhizoid but consisted of 3 cells by day 6 (Fig. 5C). In the ML treatment, subject 2H grew a rhizoid by day 4, experienced cell division by day 5 and consisted of 3 cells plus rhizoid by day 6 (Fig. 5D). In the LL treatment, subject 3A experienced the fastest and most prolific growth of all subjects. By day 2, a bud appeared on the side, by day 3, it had grown 2 other cells, by day 4, a rhizoid appeared and by day 6, this subject consisted of 4 cells plus a rhizoid (Fig. 5E).

Growth Rate Differences

The average cell numbers between subjects varied (Fig. 6A-C). In the AL treatment, no growth was observed until day 5 (Fig. 6A). Growth in the ML treatment, started to occur at day 3 for the wild culture with no observed change for the stock culture (Fig.6B). Growth in the LL treatment started to occur at day 2 for the stock cultures and day 5 for the wild cultures (Fig. 6C).

Growth rates between the treatments varied. The stock growth rate in the AL treatment was 0.25 divisions/day while for the wild it was 0.22 divisions/day (Table 3). Growth rates in the ML treatment, varied between 0.17 divisions/day for the stock and 0.42 divisions/day for the wild. Growth rates in the LL treatment varied between 0.42 divisions/day for the stock and 0.31 divisions/day for the wild (Table 3).

There were only three significant differences in growth rates observed (Table 4). The stock cultures in the LL treatment had significantly higher growth rates than the wild cultures ($p = 0.02$). The AL stock cultures had significantly lower growth rates than the LL stock cultures ($p=0.02$), and the AL wild cultures had significantly lower growth rates than the LL stock cultures ($p = 0.02$).

Discussion

Culture Survival

One of the goals of the experiment was to investigate survival of a warm water adapted strain of *Griffithsia* in a temperature that was below their optimal range (15°C as compared to the optimal 20-25° C). Half the stock cultures died over the course of the experiment. This could be a result of several factors. If the issue was temperature, the

expected result would have been that all the stock cultures died, yet that was not the case. If the issue was irradiance, the expected result would be that at the lowest treatment (LL, $8 \mu\text{mol}/\text{m}^2/\text{s}$), most of the cultures would die. That was also not the case, and in fact, more cultures, both wild and stock survived at the highest and lowest light intensities than at the middle light intensity. In addition, it should be noted that the culture temperature was 3.2 degrees higher than the temperature at which the wild *Griffithsia* grows (15°C as compared to the optimal 11.77°C), yet most of the wild cultures still survived.

Maintaining a sterile culture environment is always a concern in studies like these, and it is possible that due to available lab space and setup, some of the cultures, especially in the ML treatment, became overrun with bacteria. Additionally, sterilizing with alcohol runs the risk of providing a carbon source to bacteria. However, that still does not explain why more stock cultures than wild cultures died over the course of the experiment. It is also possible that experimenter error occurred; the stock cultures were harder to excise and manipulate and more cells may have been damaged during preparation. Another issue with sterile conditions occurs when working with a wild population that may be covered with diatoms and epiphytes. The wild *Griffithsia* had a high occurrence of epiphytes when it was sampled. While every effort was made to remove all epiphytes, some still appeared in the culture dishes from time to time and may have affected growth rates.

Morphology and Cell Growth

The observed differences in morphology between the stock and wild cultures could have been a result of differing light intensities. Under long day length with high

light intensity, plants tend to be bushy with shorter, wider cells, whereas the opposite is true with shorter day length or low light intensity (Waaland and Cleland 1972). The stock *Griffithsia* was grown in intensities between 5-10 $\mu\text{mol}/\text{m}^2/\text{s}$ whereas the wild strain was in an area which experienced intensities of $\sim 48.8 \mu\text{mol}/\text{m}^2/\text{s}$ during at least some part of the day. The stock *Griffithsia* was bushier and with cells that appeared shorter than its wild counterpart. Therefore, despite the one time point which is higher, the wild counterpart could be acclimated to an average of lower light intensities than the stock over the course of the day. The wild strain was located several feet down the boat strap and in murky water. The differences in pigmentation between high and low light treatments found in this experiment were described by Waaland and Cleland (1972); cells are darker red in lower light intensities and lighter in higher light intensities.

Due to available incubator conditions, cells were grown in light conditions that were less than optimal. Optimal growth of 1-2 cells/day (30-40 shoot cells/week) occurs under a 16L: 8D photoregime, 20-25°C at 60 $\mu\text{mol}/\text{m}^2/\text{s}$ (Duffield et al. 1972; Waaland and Cleland 1972; Graham and Wilcox 2000). Cultures here were grown at less than half that irradiance, and therefore, growth rates were expected to be slower than demonstrated in the literature. The overall growth rates of the wild cultures were lower than those found in the stock cultures, yet except in a few cases, not significantly so. This result directly contrasts the literature in which higher light intensity grown *Griffithsia* experienced more than 4 times the number of shoot cells. However, it appears that while apical division is not affected by changes in light intensity, the rate of branching goes up with higher intensities (Waaland and Cleland 1972).

Both kinds of cultures, both wild and stock grew faster at the lowest light intensity (60 $\mu\text{mol}/\text{m}^2/\text{s}$) The surprising result of the rapid growth of a stock culture subject (3A) at the lowest light treatment could be the result of light intensity issues. There could have been a thin spot in the cheesecloth covering subject 3A which allowed more light to enter the dish; however a similar response was not observed in any of the other stock cultures that were grown at the higher light intensities. Perhaps two cells fused; a result of being too close together in the dish. However, one reason for the rapid growth, at least in part, was that subject 3A (like the other stock subjects) was acclimated to a light intensity between 5-10 $\mu\text{mol}/\text{m}^2/\text{s}$, and it was easy for those cells to be more metabolically optimized in the 8 $\mu\text{mol}/\text{m}^2/\text{s}$ treatment.

The hypothesis about differing growth rates between the stock vs. wild cultures was proven true, especially with the low light cultures, whereas the idea that the varying light intensities would produce differences in growth rates was not confirmed. Perhaps the differences in light intensities had to be more pronounced or a control culture at the optimal light intensity would have proven useful in terms of comparing growth rates.

Conclusions

This pilot study and comparison of growth of two strains of *Griffithsia pacifica* demonstrated that this species not only tolerates very low light levels, but in some cases, can actually flourish in them. Both strains survived in temperatures outside their optimal range and at least one subject experienced marked growth. Differences in base morphology (length of cells and level of bushiness) may be a result of the acclimation of cultures to differing light intensities.

Future studies using a similar setup but more in depth and over a much longer time period are recommended to see the full effect. An incubator setup with brighter lights and a variety of temperatures and light intensities is recommended. It would be interesting to see if cool white fluorescent lamps would produce the same results as the LED light. Additionally, an experiment with PE (Provasoli's Enriched Seawater) medium should be conducted to see which yields the best result. As the stock cultures are usually grown in f/2, that was the media of choice for this experiment.

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Table 1: Light and Temperature at the Friday Harbor Laboratories Dock on 8/8/12. Water temperature was measured using two IButton temperature data loggers (Maxim Integrated Products, Inc., San Jose, CA, USA) and light intensity measured using a LI-COR LI 1000 light meter (LI-COR Biosciences, Lincoln, Nebraska, USA)

Friday Harbor Dock Ambient Conditions		
Temperature (°C)		Irradiance ($\mu\text{mol}/\text{m}^2/\text{s}$)
Average	11.77	48.79
SD	0.39	n/a

Table 2: Treatment subject deaths and times at AL ($25 \mu\text{mol}/\text{m}^2/\text{s}$), ML ($16 \mu\text{mol}/\text{m}^2/\text{s}$) and LL ($8 \mu\text{mol}/\text{m}^2/\text{s}$).

Subject	Treatment	Type	Day 2	Day 3	Day 4	Day 5	Day 6
1B	AL	Stock		X			
1C	AL	Stock		X			
1G	AL	Wild				X	
2A	ML	Stock	X				
2B	ML	Stock			X		
2D	ML	Stock	X				
2E	ML	Wild					X
2F	ML	Wild					X
3B	LL	Stock			X		
3E	LL	Wild		X			

Table 3: Growth rates of stock and *Griffithsia* cultures over 6 days as measured by end average cell #/6.

Irradiance Level ($\mu\text{mol}/\text{m}^2/\text{s}$)	Cells/Day	
	Stock	Wild
25	0.25	0.22
16	0.17	0.42
8	0.42	0.31

Table 4: Dependent T-Tests ($\alpha = 0.05$) for differences in *Griffithsia pacifica* growth rates between and within treatment groups. “Ambient Light” (AL) was defined as 25 $\mu\text{mol}/\text{m}^2/\text{s}$, “Medium Light” (ML) was defined as 16 $\mu\text{mol}/\text{m}^2/\text{s}$ and “Low Light” (LL) as 8 $\mu\text{mol}/\text{m}^2/\text{s}$.

Test Type		
Within Groups	p Value	Significant?
AL Stock vs. Wild	1.0	No
ML Stock vs. Wild	0.13	No
LL Stock vs. Wild	0.02	Yes
Between Groups	p Value	Significant?
AL Stock vs. ML Stock	0.36	No
AL Stock vs. LL Stock	0.02	Yes
AL Stock vs. ML Wild	0.08	No
AL Stock vs. LL Wild	0.20	No
AL Wild vs. ML Wild	0.13	No
AL Wild vs. LL Wild	0.39	No
Al Wild vs. ML Stock	0.18	No
AL Wild vs. LL Stock	0.02	Yes
ML Wild vs. LL Stock	0.05	No

Figure Legends

Figure 1: Dissecting microscope photograph (4X magnification) of a clump of the wild strain of *Griffithsia pacifica*, obtained from the docks a Friday Harbor Laboratories on 8/8/12.

Figure 2: Photograph of the sampling site for the wild strain of *Griffithsia pacifica*. The site was located on nylon boat straps at the docks at coordinates N 48° 32.723' W 123° 00.729'.

Figure 3: Diagram illustrating sterile culture/cell excision methods for *Griffithsia pacifica* (from Duffield et al 1972; Anderson 2005).

Figure 4A: Diagram illustrating labeling and arrangement of culture Petri dishes for stock and wild *Griffithsia pacifica* at three different light levels.

Figure 4B: Photographs of the incubator/culture chamber used to grow the *Griffithsia*. The incubator used was a modified Thermoelectric Wine Cooler, model TWR282S (EdgeStar Products, Austin, TX, USA) with internal placement of 3x10 inch LED strip lights (Warm White, 3100 K) (LED Wholesalers.com, Inc., California, USA).

Figure 5A: Dissecting microscope photograph (4X magnification) of a clump of the stock strain of *Griffithsia pacifica* (left), obtained from the botany department at University of Washington in Seattle next to a clump of wild strain of *Griffithsia pacifica* (right), obtained at the docks at Friday Harbor Laboratories on 8/8/12.

Figure 5B: Microscope photographs (4x magnification), using the Micropublisher 3.3 RTV camera (QI Imaging, Inc., Surrey, British Columbia, Canada), illustrating the growth progression over 6 days of subject 1A, consisting of stock *Griffithsia pacifica* grown in “Ambient Light” (AL), which was 25 $\mu\text{mol}/\text{m}^2/\text{s}$.

Figure 5C: Microscope photographs (4x magnification), using the Micropublisher 3.3 RTV camera (QI Imaging, Inc., Surrey, British Columbia, Canada), illustrating the growth progression over 6 days of subject 1H, consisting of wild *Griffithsia pacifica* grown in “Ambient Light” (AL), which was 25 $\mu\text{mol}/\text{m}^2/\text{s}$.

Figure 5D: Microscope photographs (4x magnification), using the Micropublisher 3.3 RTV camera (QI Imaging, Inc., Surrey, British Columbia, Canada), illustrating the growth progression over 6 days of subject 2H, consisting of wild *Griffithsia pacifica* grown in “Medium Light” (ML), which was 16 $\mu\text{mol}/\text{m}^2/\text{s}$.

Figure 5E: Microscope photographs (4x magnification, using the Micropublisher 3.3 RTV camera (QI Imaging, Inc., Surrey, British Columbia, Canada), illustrating the growth progression over 6 days of subject 3A, consisting of stock *Griffithsia pacifica* grown in “Low Light” (LL), which was $8 \mu\text{mol}/\text{m}^2/\text{s}$.

Figure 6A: Average cell number over 6 days for stock and wild treatments grown in “Ambient Light” (AL) ($25 \mu\text{mol}/\text{m}^2/\text{s}$).

Figure 6B: Average cell number over 6 days for stock and wild treatments grown in “Medium Light” (ML) ($16 \mu\text{mol}/\text{m}^2/\text{s}$).

Figure 6C: Average cell number over 6 days for stock and wild treatments grown in “Low Light” (LL) ($8 \mu\text{mol}/\text{m}^2/\text{s}$).

Figure 1



Figure 2



Figure 3

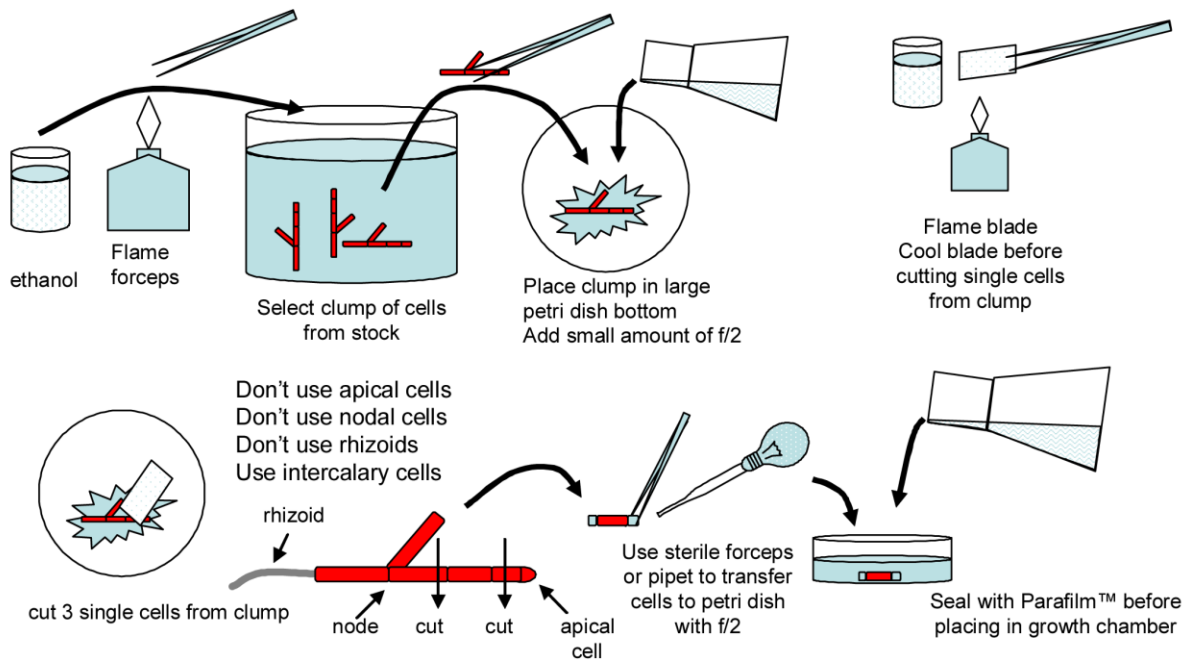
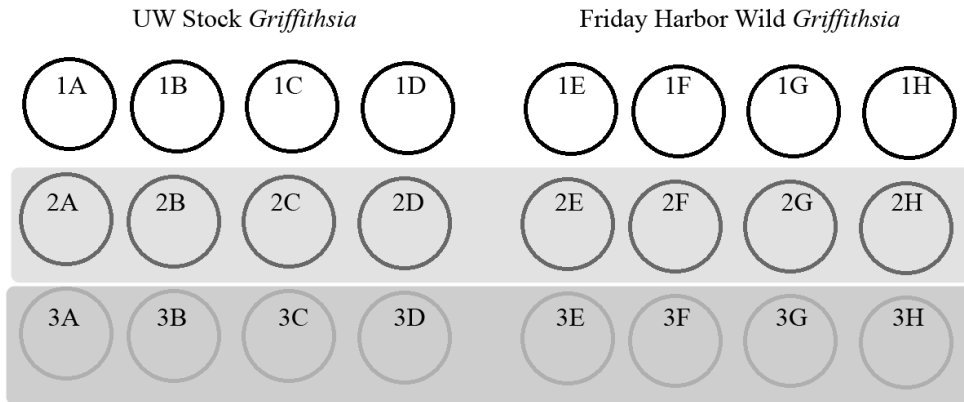


Figure 4A



Key: 1A-1H are at "Ambient Lght" ($\sim 25 \text{ umol/m}^2/\text{s}^2$)
2A-2H are at "Medium Light" $2/3 * \text{Ambient}$ ($\sim 16 \text{ umol/m}^2/\text{s}^2$)
3A-3H are at "Low Light" $1/3 * \text{Ambient}$ ($\sim 8 \text{ umol/m}^2/\text{s}^2$)

Labels:

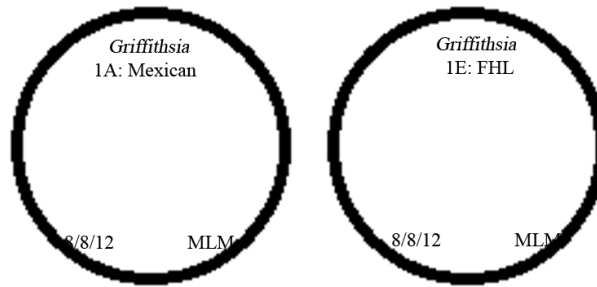


Figure 4B

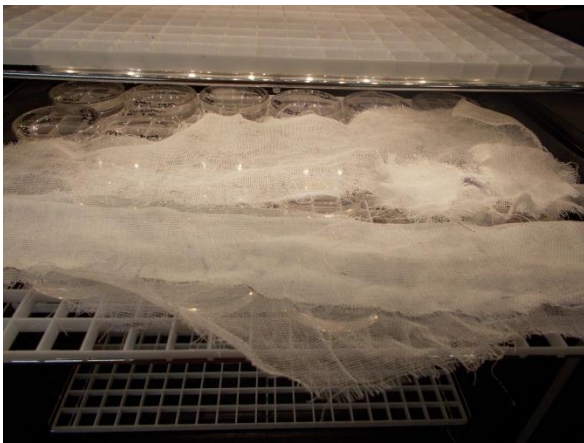


Figure 5A

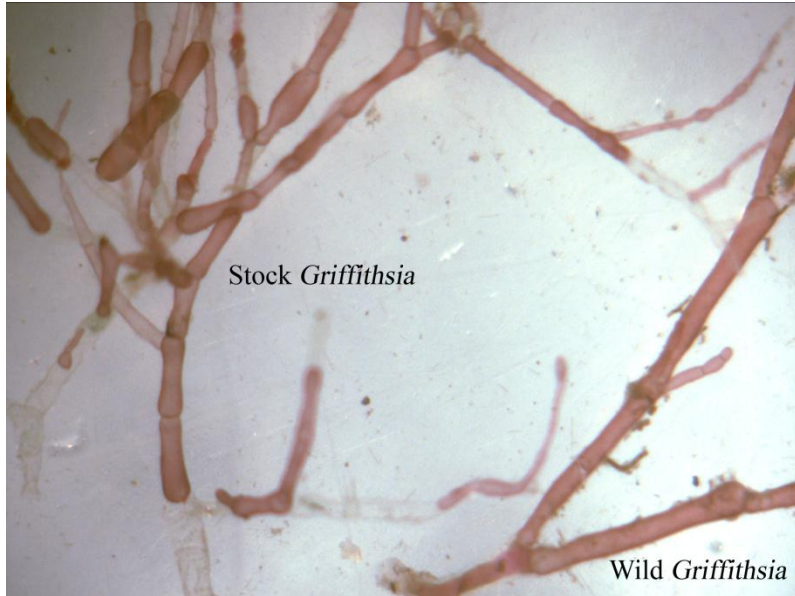


Figure 5B



Figure 5C



Figure 5D



Figure 5E

Subject 3A (Stock) at LL ($8 \mu\text{mol}/\text{m}^2/\text{s}$)

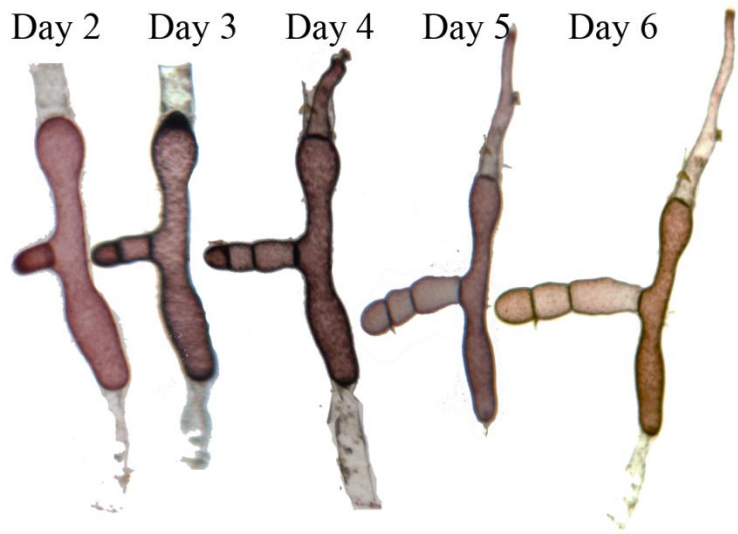


Figure 6A

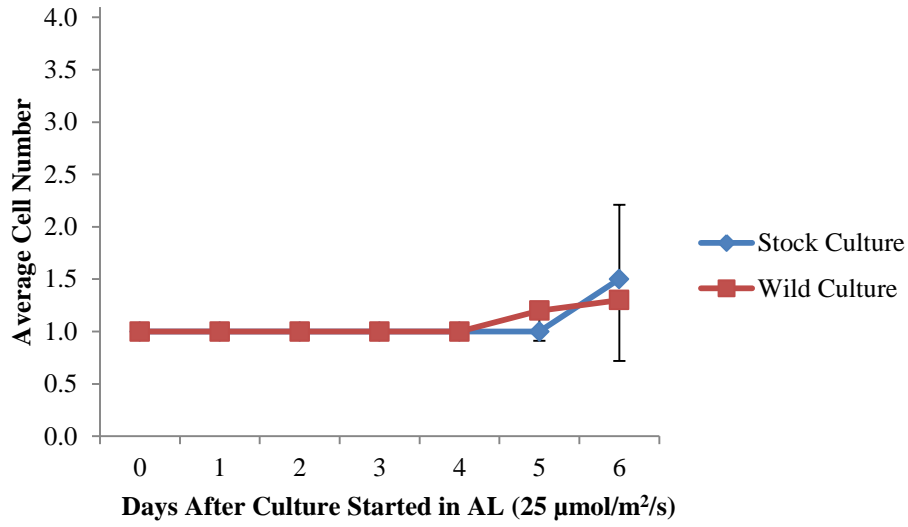


Figure 6B

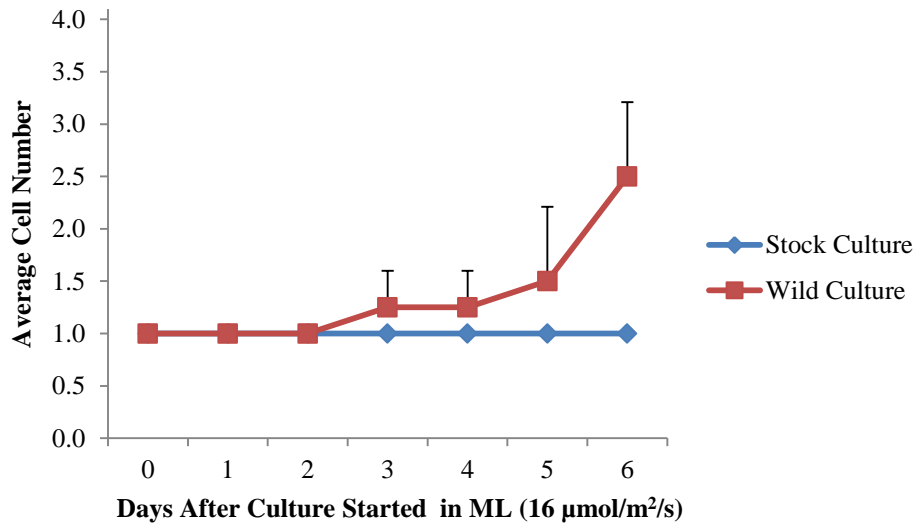


Figure 6C

