Effect of Temperature on the Growth Rate of *Licmophora abbreviata*

Ashley Jung, Parisa Safavi, Anna Tam, and Casper Tsai

Abstract

Licmophora abbreviata is a photosynthetic diatom found in marine euphotic zones. Diatoms are responsible for nearly half of the primary production in oceans, a process that forms the foundation of food webs in aquatic ecosystems. To understand how increasing global temperatures will impact the growth of algae and salmon, we grew *L. abbreviata* at different treatment temperatures. Three incubation temperature treatments were 11°C, 17°C (optimal), and 20°C. Three replicates, containing the HESNW nutrient solution were incubated for 10 days. We counted the number of diatom cells using the hemocytometer at 11 AM every Monday, Wednesday, and Friday. The growth rate for 11°C, 17°C and 20°C over a course of 8 days were 4300, 5800 and -230 cells/mL/day respectively. The highest growth rate occurred in the 17°C treatment. Our one-way ANOVA analysis of the results ($p < 0.05$) indicates that there is a significant difference between the three temperature treatments. Therefore, we rejected our null hypothesis. However, after further analysis, we did not find a significant difference between 11°C and 17°C treatments. We observed no significant growth for the 20°C treatment.

Introduction

Licmophora abbreviata are photosynthetic brown algae which are abundant worldwide in the euphotic zones of ocean waters (Goldman, 2003). This diatom can appear both as a rectangular cell and a triangular one, depending on the strain (Fig. 1,2). *L. abbreviata* are primary producers at the bottom of the food chain and are a major food source that many marine animals depend on for survival (Barker, 2008). Diatoms are a primary food source for small fish and are an indirect food source for larger marine animals higher up in the food chain (Goldman, 2003). *L. abbreviata* also influences the oxygen levels in the benthic zone because they undergo photosynthesis, and account for nearly half of the primary production in the oceans (Barker, 2008). In this study, we want to compare the ideal temperatures for the growth of *L. abbreviata* and salmon. *L. abbreviata* are present in the salmon's habitat, in both freshwater and marine environments (Sabater, 2009). Since *L. abbreviata* is an indirect food

source for salmon and influences the oxygen levels in the environment, the presence of *L. abbreviata* in the salmon habitat likely influences the salmon.

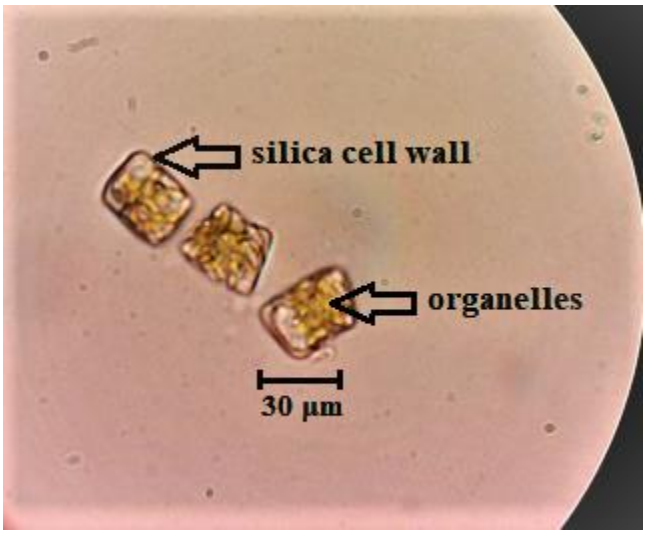

Figure 1. *L. abbreviata* strain from English Bay, B.C., Canada. Cells are rectangular-shaped. Brown, circular organelles are inside the silica cell wall.
Viewed under dissecting microscope. Total Magnification: 1000x.

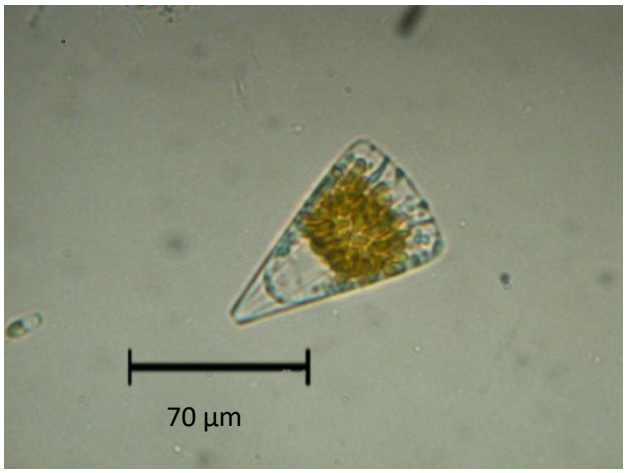

Figure 2. *L. abbreviata* cell in triangular shape and clear silica cell wall, dissecting microscope. Total Magnification: 1000x. Image courtesy of: http://baltazar.izor.hr/roscop/fitop_web_sel?p_grupa=2

L. abbreviata are sensitive to environmental factors such as light, pH, temperature, water velocity, and nutrient concentration (Sabater, 2009). In our study, we examined the influence of temperature on the growth rate of *L. abbreviata.*

A study found that immature Coho salmon had the highest growth rate at 17°C (Carter, 2005). Another study found that the optimum range for growth was between 12.1 - 20.8°C, where the median temperature was 16.5°C (Carter, 2005). Other studies have also found lower optimum temperatures for growth, 10-14°C, while temperatures of 21-26°C inhibited salmon growth, and proved to be detrimental to the salmon's health (Carter, 2005)

On the other hand, a field study on Chinook salmon found that the growth rate of young salmon was greater at 16°C compared to at 20°C (Carter, 2005). They observed that Chinook salmon's growth was fastest between 17 - 20°C, and decreased at 21-24°C (Carter, 2005).

A study by Ohgai et al. found that the range of optimal temperature for growth of *L. abbreviata* was 15-25°C (1984). In this study, we wanted to test a lower temperature range for *L. abbreviata*'s growth and expected growth rates to decrease as the temperature drops below 15°C. To do so, we measured the diatom's growth rate by monitoring the number of cells at three different temperatures: 11°C, 17°C, and 20°C.

Our hypotheses were:

Ho: Temperature does not affect the growth rate of *L. abbreviata.*

H_A: Temperature does affect the growth rate of *L. abbreviata.*

Methods

To observe the effect of temperature on the growth rate of *Licmophora abbreviata*, we first had to grow the diatoms in a medium to obtain a large population of cells. The *L. abbreviata* we used for our experiment is a local strain labeled as #697 from the UBC Culture Collection. The lab technician grew the cells for seven days in a HESNW medium at 17°C in an incubator. The HESNW medium allows *L. abbreviata* to grow in numbers, and it contains seawater, sodium bicarbonate, nutrients, vitamins, and silica stock. The final salt content was 28-30 ppm. We received approximately 150 mL of medium and approximately 100 mL of cell culture in two Erlenmeyer flasks (Fig. 3)

Figure 3. Media and the *L. abbreviata* cell culture in Erlenmeyer flasks. The cell culture flask had a cloudy brown tint, and the medium was clear white.

Initial preparation

On Oct. 30, 2017 we calculated the initial concentration of cells in our culture. We used a haemocytometer slide under a Zeiss Axiostar microscope with 10x objective lens. After loading the culture on the haemocytometer, we counted the number of cells in four red squares (Fig. 4) using a hand-tally, and we divided it by four to get the average. Then, we

repeated this process four times to get the average of 4 samples, which was 17.5 cells/square. We found the initial cell concentration by multiplying 17.5 cells/square by the dilution factor of $5x10^3$. The initial cell concentration was found to be $7.8x10^4$ cells/mL. We then diluted the sample to obtain a final concentration of $1x10^4$ cells/mL with a final volume of 10 mL for each of our replicate in nine sterilized test tubes. After putting on the caps, we stored the nine replicates at 11°C, 17°C and 20°C (three replicates per temperature treatment) in incubators. The incubator had a light cycle of 8hrs starting from 4:30 PM. The light was off for the remaining 16 hours. The pH of all solutions were 8.

Conducting measurements

Over the next few days, we collected a small sample and fixed the cells using iodine fixative (IKI) to count them later. To fix the samples, we first vortexed the test tubes to evenly mix the specimen with the IKI fixative. Then, we collected 100 µL of sample and put it in an Eppendorf tube and added 10 µL of fixative to them. All of the fixed samples were stored in a 4°C fridge. We collected and fixed the cells starting on Nov. 2nd to Nov. 10th, every Monday, Wednesday, and Friday at 11AM. On Friday, Nov. 10th, we counted the diatoms in each sample using a haemocytometer and AXIS microscope with a total magnification of 100x. To do so, we first pipetted the sample up and down a few times to mix them well. We counted all the cells that were inside the large blue square or four red squares, as done in the preparation step (refer to Fig. 4), and then multiplied the cell count by the corresponding dilution factor of 3.125 $x10^2$.

We were looking for an exponential growth curve for all temperature treatments and expected to observe the most growth at 17°C and 20°C. We also planned to conduct a one-way ANOVA to see if the difference in the three treatment populations are statistically significant or not, with a significance level of 95%. We plotted the cell density against time for each treatment, and considered the slope of the graph (cells/mL/day) at the exponential phase to be the growth rate for our diatoms.

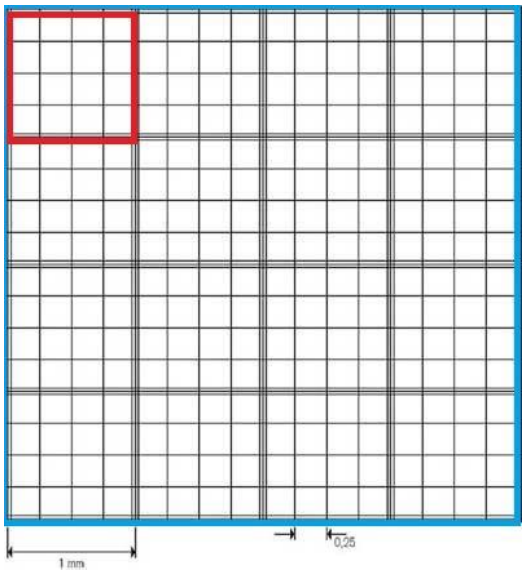

Figure 4. A haemocytometer grid with blue and red squares indicated. Dilution factor for Red Square is 5 x10^2 and for Blue 3.125 x10^2 cells/mL.

Results

After collecting the raw cell counts, we multiplied them by their respective dilution factors from counting the cells through a haemocytometer and from the dilution with the fixative solution. This gave us the *L. abbreviata* cell density over the course of the experiment for all three treatments.

We obtained the average cell densities over the three replicates and plotted the cell densities over the number of days our sample grew. Day 0 was omitted because we were selectively interested in the exponential growth phase and not the initial growth phase.

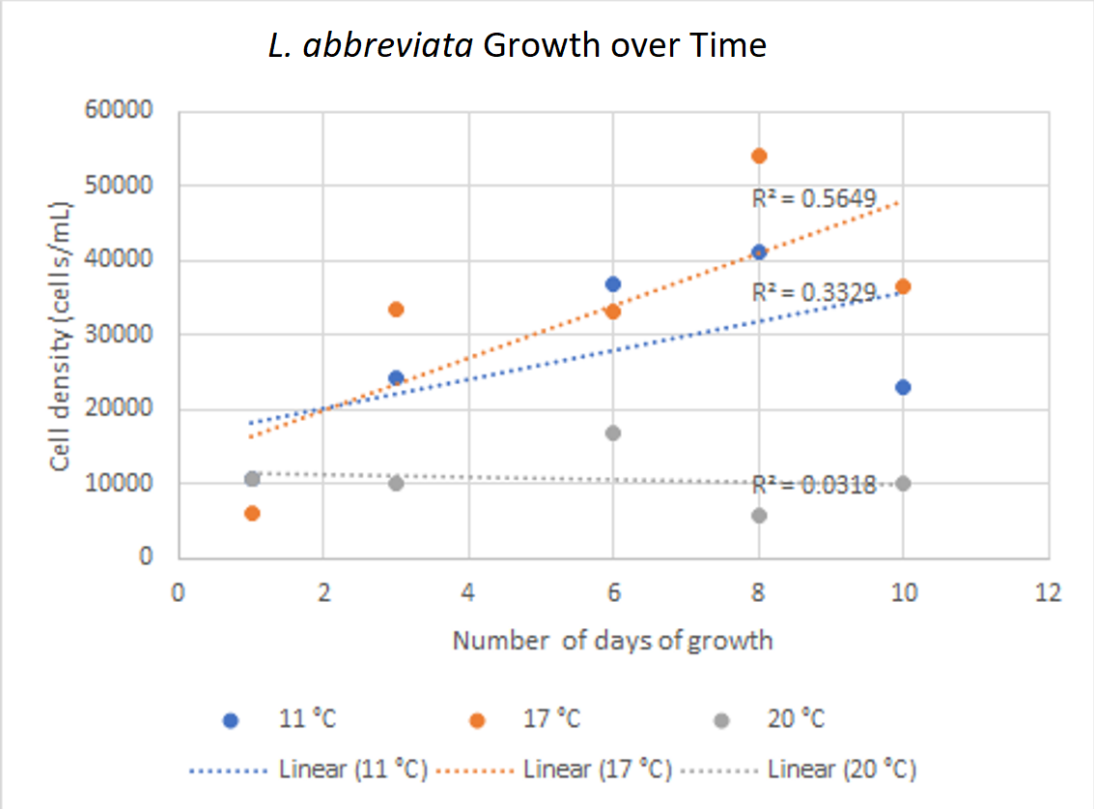

Figure 5. *L. abbreviata* growth over time measured as number of cells per mL for all three temperature treatments. The data point for 11°C and 20°C treatment overlap on day 1, making the point for 11°C not visible on the graph.

Contrary to expectations, we did not see a linear trend as shown by the low correlation coefficient. However, as we can see from Figure 5, the lack of linearity appears to be caused by the data collected on day 10. The figure below shows the *L. abbreviata* growth curve that excludes data points from day 10.

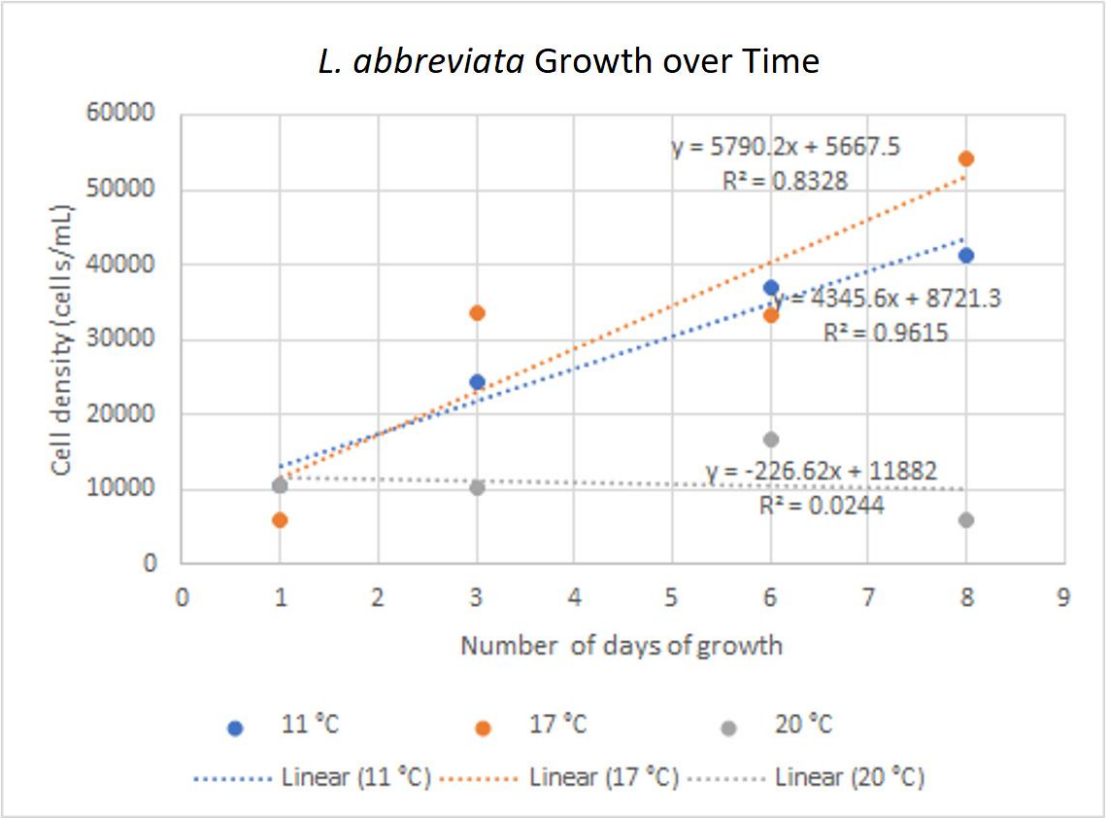

Figure 6. *L. abbreviata* growth over time until day 8 measured as number of cells per mL for all three temperature treatments. The data point for 11°C and 20°C treatment overlap on day 1, making the point for 11°C not visible on the graph.

As shown in Figure 6, there is a much higher correlation coefficient in the 11°C and 17°C temperature treatments when the data from day 10 are omitted. The treatment at 20°C however still showed no correlation. Therefore, we performed our ANOVA test without including the dataset from day 10.

We found the lines of best fit for each replicate set of data and the slopes of these best-fit lines. The growth rate for 11°C, 17°C and 20°C over a course of 8 days were 4300, 5800 and -230 cells/ml/day respectively (Fig.7). The highest growth rate happened at the 17°C treatment. From these slopes, we performed a one-way ANOVA test and found significance between the three treatments (p=0.034). However, as noted before in the growth curve, since there seems

to be little growth in the 20°C treatment, we then performed another one-way ANOVA test, this time only comparing the 11°C treatment and the 17°C treatment. The ANOVA test showed no statistical significance (p = 0.54) between the 11°C treatment and the 17°C treatment.

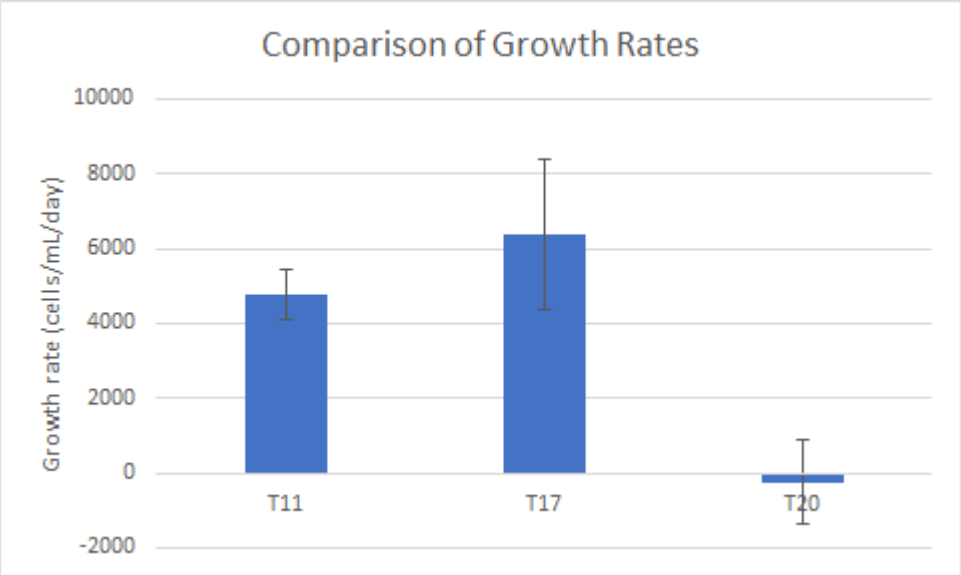

Figure 7. Comparison of *L. abbreviata* growth rate between 11°C, 17°C, and 20°C treatments. Error bars indicate the standard error of the regression.

Discussion

Based on our initial one-way ANOVA test (p=0.034), we can reject the null hypothesis which states that temperature does not have an affect on the growth of *Licmophora abbreviata*. Therefore, we found support for our alternative hypothesis, which states that temperature does have an effect on growth rate of *L. abbreviata*. However, our second one-way ANOVA test showed no significance between our 11 and our 17°C treatment. Therefore, our data support that *L. abbreviata* has a larger growth rate at 11 and 17°C in comparison to 20°C while showing no significant difference in growth rate between growing at 11 and 17°C.

Our findings contradicts Ohgai et al.'s fndings that *L. abbreviata*'s optimal growth temperature range is between 15-25°C (1984). Instead, we observed no significant growth at 20°C over ten days and a non-significant difference between *L. abbreviata* growth at 11 and 17°C. However, this difference in results is unsurprising. Our *L. abbreviata* strain was isolated from a coastal body of water in Vancouver, Canada while Ohgai et al.'s strain was isolated from a farm in Shimonoseki, Japan (1984). The difference in habitat and climate easily supports that these two *L. abbreviata* were very different strains.

Previous studies have shown *L. abbreviata* to be present in salmon's habitat (Sabater, 2009). We also know that while different species of salmon have different optimum temperatures, salmon species, in general, thrive at temperatures below 20°C (Carter 2005). It seems very likely that, with *L. abbreviata* being so prevalent in salmon's habitat, they would thrive under the same conditions as salmon. Our data supports this theory by showing high *L. abbreviata* growth rates at 11°C and 17°C and no growth at 20°C.

The result of Montagnes and Franklin's phytoplankton experiment has indicated that a diatom's cell volume would significantly decrease once the temperature has increased past optimal temperature (2001). Higher temperatures also affected the silicate morphology of the diatom's cell wall, as it dissolves the silicon-meshwork content, and results in thinner cell walls (Javaheri *et al*., 2015). Javaheri *et al.* states that the cell cycle and growth of most diatoms is strictly controlled by the silicon availability (2015). This proves to be detrimental to *L. abbreviata* because silicon is required to perform metabolic processes. Without a substantial amount of silicon in the diatom's cell walls, it will be unable to metabolize nutrients available in the ocean, which inhibits cell reproduction (Javaheri *et al*., 2015). Silicon is a major limiting

nutrient for diatom growth and hence is a controlling factor in primary productivity (López-Fuerte et al., 2010). Therefore, increasing temperatures in the ocean cause diatoms to slow down their cell growth cycle, leading to reduced cell division, and results in decreased cell count (Javaheri et al., 2015).

Sources of error

First possible source of error is in conducting the cell counts. For the initial setup, since we had a large number of cells to count, we counted four red squares (Fig. 4) and divided the number by four to get the average in one red square. However, for the two other counts (Nov 10th and Nov 8th), we counted one big blue square, as the number of cells in the smaller squares were less than what we ideally wanted to observe. For the haemocytometer to be accurate, we want to observe at least 50-100 cells per counting area. Therefore, our way of counting had not been consistent for all the data points. Moreover, it is possible that on Day 1, our initial counting was biased and higher than the actual cell count, so when counted the blue squares on the last days of counting, we got smaller numbers, thus explaining the decline in the cell count on day 10.

Another possible source of error is each individual's subjectivity when doing the counts, which results in variance among the students who conducted the counts. Freund and Carol analyzed variance found in manual counts among technicians and in repeated counts made by the same technician (1965). Half of the variance of the haemocytometer came from the variance among technicians (57.4%) and one-half of that variance was among duplicate haemocytometer counts made by the same technician (42.6%). This study concluded that the

variance associated with the haemocytometer counting is large and should be considered while designing experiments.

Conclusion

In our experiment for testing the factor of temperature on the *L. abbreviata'*s cell growth over ten days, we were able to reject the null hypothesis and provide support for our alternate hypothesis. Cells at 11ºC and 17ºC grew the most, while seeds at 20ºC grew the least. Our results supported the alternate hypothesis and predictions stating that temperatures above *L. abbreviata's* optimal growth temperature affected the cell growth cycle. In our experiment, cell growth was inhibited in temperatures above 17ºC.

Acknowledgements

We would like to express our appreciation towards Dr. Celeste Leander for her assistance with the design and proposal of our experiment, as well as providing us access to the rooms with microscopes and incubators for our use during the open lab hours. We would also like to acknowledge Jordan Hamden for showing us how to set up the hemocytometer on the microscope. We would also like to thank Will Wei for his dedication to ensuring that our group followed sterile, gloved procedures when we transferred our organisms through the micropipettes. We would also like to thank Mindy Chow for providing us with the diatom cultures of *Licmophora abbreviata,* and all the lab equipment we required to perform our experiment. Lastly, we would like to recognize the University of British Columbia for giving us

the opportunity to carry out our scientific experiment through the BIOL 342 Course: Integrative Biology Laboratory.

Literature Cited

Barker, H. A. (1935). Photosynthesis in diatoms. *Archiv für Mikrobiologie, 6*(1-5), 141-156. doi:10.1007/bf00407284

Carter, K. (2005). The Effects of Temperature on Steelhead Trout, Coho Salmon, and Chinook Salmon Biology and Function by Life Stage. *California Regional Water Quality Control Board.* Retrieved November 13, 2017, from https://www.waterboards.ca.gov/northcoast/water_issues/programs/tmdls/shasta_river/060707/28appendixaetheeffectsoftemperatureonsteelheadtroutcohosalmonandchinooksalmonbiologyandfunction.pdf

Freund, M., & Carol, B. (1965). Factors Affecting Haemocytometer Counts of Sperm Concentration In Human Semen. *Obstetrical & Gynecological Survey, 20*(2), 310. doi:10.1097/00006254-196504000-00037.

Goldman, J. C. (1993). Potential role of large oceanic diatoms in new primary production. *Deep Sea Research Part I: Oceanographic Research Papers, 40*(1), 159-168. doi:10.1016/0967-0637(93)90059-c

Javaheri, N., Dries, R., Burson, A., Stal, L. J., Sloot, P. M., & Kaandorp, J. A. (2015). Temperature affects the silicate morphology in a diatom. *Scientific Reports, 5*(1). doi:10.1038/srep11652

López-Fuerte, F. O., Siqueiros-Beltrones, D. A., & Yabur, R. (2015). First record of benthic diatoms (Bacillariophyceae and Fragilariophyceae) from Isla Guadalupe, Baja California, Mexico. *Revista Mexicana de Biodiversidad, 86*(2), 281-292. doi:10.1016/j.rmb.2015.04.016

Montagnes, D. J., & Franklin, M. (2001). Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. *Limnology and Oceanography, 46*(8), 2008-2018. doi:10.4319/lo.2001.46.8.2008

Ohgai, M & Matsui, T & Tsukahara, H & Nakajima, K. (1984). The Effect of the Environmental Factors on the Growth of two Epiphytic Diatoms Licmophora abbreviata Agardh and L. paradoxa (Lyngbye) Agardh in vitro. NIPPON SUISAN GAKKAISHI. 50. 1157-1163. 10.2331/suisan.50.1157.

Sabater, S. (2009). Diatoms. *Encyclopedia of Inland Waters,* 149-156. doi:10.1016/b978-012370626-3.00135-6