Effects of varying pH on the growth rate of the marine diatom *Licmophora abbreviata*

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Abstract

Growth rates of *Licmophora abbreviata* were studied under three different treatments at pH 7.0, 8.0, and 9.0 with each treatment consisting of three replicates. Previous studies have indicated that diatoms prefer a pH of 8.1 for optimal growth and therefore, the pH 8.0 treatment was the control for the experiment. The initial concentration of each replicate was $1.55x10^3$ cells/mL and was determined through the use of a haemocytometer. After a period of 11 days, the maximum growth rates for each treatment was calculated and found to be 2.18×10^4 cells/day for pH 7.0, $1.46x10^4$ cells/day for pH 8.0 and $1.13x10^4$ cells/day for pH 9.0. Additionally, the diatoms grown in pH 8.0 media resulted in the highest concentration after 11 days with pH 7.0 and pH 9.0 following respectively. An ANOVA one-way test was conducted to determine the statistical significance of the data and came back statistically significant, with a calculated p-value of 0.00079, at a confidence level of 95%. Observations of cell shape between the different treatments found that *L. abbreviata* was triangular shaped in pH 7.0 and 8.0 but not in pH 9.0.

Introduction

Licmophora abbreviata (*L. abbreviata)* is a photosynthetic marine diatom isolated from Vancouver and is shaped similar to a slice of pizza. As diatoms are responsible for nearly half of the primary production in oceans, varying environmental conditions resulting from climate change may affect *L. abbreviata* growth and consequently B.C.'s salmon populations.

Salmon are a keystone species in the West Coast of British Columbia and are a crucial part of numerous ecosystems. Previous studies have demonstrated that salmon influence environments through the addition of nutrients from their carcasses, allowing organisms and plants to flourish with the increase of nitrogen in the soil (Stockner, 2003). Salmon also increase nitrogen indirectly through the additional traffic of bears to rivers and streams. This leads to

major inputs of nitrogen-enriched urine and feces which can be further taken up and used by various organisms (Stockner, 2003). However, for salmon to prosper, they rely on a variety of food sources ranging from zooplankton and larvae as juveniles, to small fish as adults in the open ocean (Strickland, 1983). Zooplankton are an important food source for juvenile salmon, therefore their biomass will greatly affect the size and number of salmon runs each year. They are chemotrophic protists and feed by filtering organisms in the environment, such as dinoflagellates and diatoms, to produce energy (Fenchel, 1988). While diatoms consist of a vast array of organisms, *L. abbreviata* is a local diatom species that comprise a portion of the basis of British Columbia's food web.

Climate change causes ocean pH values to fluctuate and decrease rapidly (Turley *et al.,* 2009), which can affect *L. abbreviata's* growth rate. This study will investigate whether rising pH influences the *L. abbreviata's* growth rate, with our null hypothesis being pH does not influence growth rates of *L. abbreviata* and our alternate hypothesis being that pH does influence growth rates of *L. abbreviata*.

While it has been shown that diatoms are able to grow in high pH waters of up to 9.0, we believe that pH will influence the growth rate of *L. abbreviata* (Taraldsvik and Myklestad, 2000). Fluctuations in pH can cause both environmental and physiological changes. For instance, pH can influence dissolved inorganic carbon speciation, as well as the bioavailability of trace metals (Gensemer *et al., 1993)*. Carbon and trace metals are essential to diatom growth, especially the trace metal silicon, as it makes up *L. abbreviata's* frustule (the silicified cell wall of a diatom).

Materials and Methods

Growth curves were created representing three different pH levels: pH = 7.0, pH = 8.0, and pH = 9.0. Each treatment had three corresponding replicates. Samples were counted three times using a haemocytometer, and this number was averaged to obtain a growth rate of number of cells/mL vs. time. The control for this experiment was the pH = 8.0 treatment, as Hinga (1992) suggests a pH of 8.1 is optimal for diatom growth. In this experiment, the initial concentration of cells was $1.55x10^3$ cells/mL for all treatments.

Resuspension of Diatoms

The initial growth media of *L. abbreviata* with unknown concentration of cells at pH 8.0 was provided to us. The diatom media was separated evenly into 3 separate falcon tubes and the tubes were centrifuged for 8 minutes at 4000 RPM. The initial growth media in the tubes was then removed with a disposable pipette and resuspended in 5 mL of pH-adjusted media, then swirled to mix.

Determination of Starting Concentration

To determine the appropriate amount of pH-adjusted media to add to the tubes, the initial concentration of the cells was determined to dilute accordingly. A haemocytometer was used to view and count the diatoms at 160x magnification under an Axiostar compound light microscope. All three treatments were counted three times, and the number of cells counted was averaged between treatments to determine the average cells/mL for the pH. Once the initial concentration was obtained, the appropriate volume of pH-adjusted solution to add in each treatment was determined to reach a final volume of 10 mL and a final concentration of $1.55x10^3$ cells/mL.

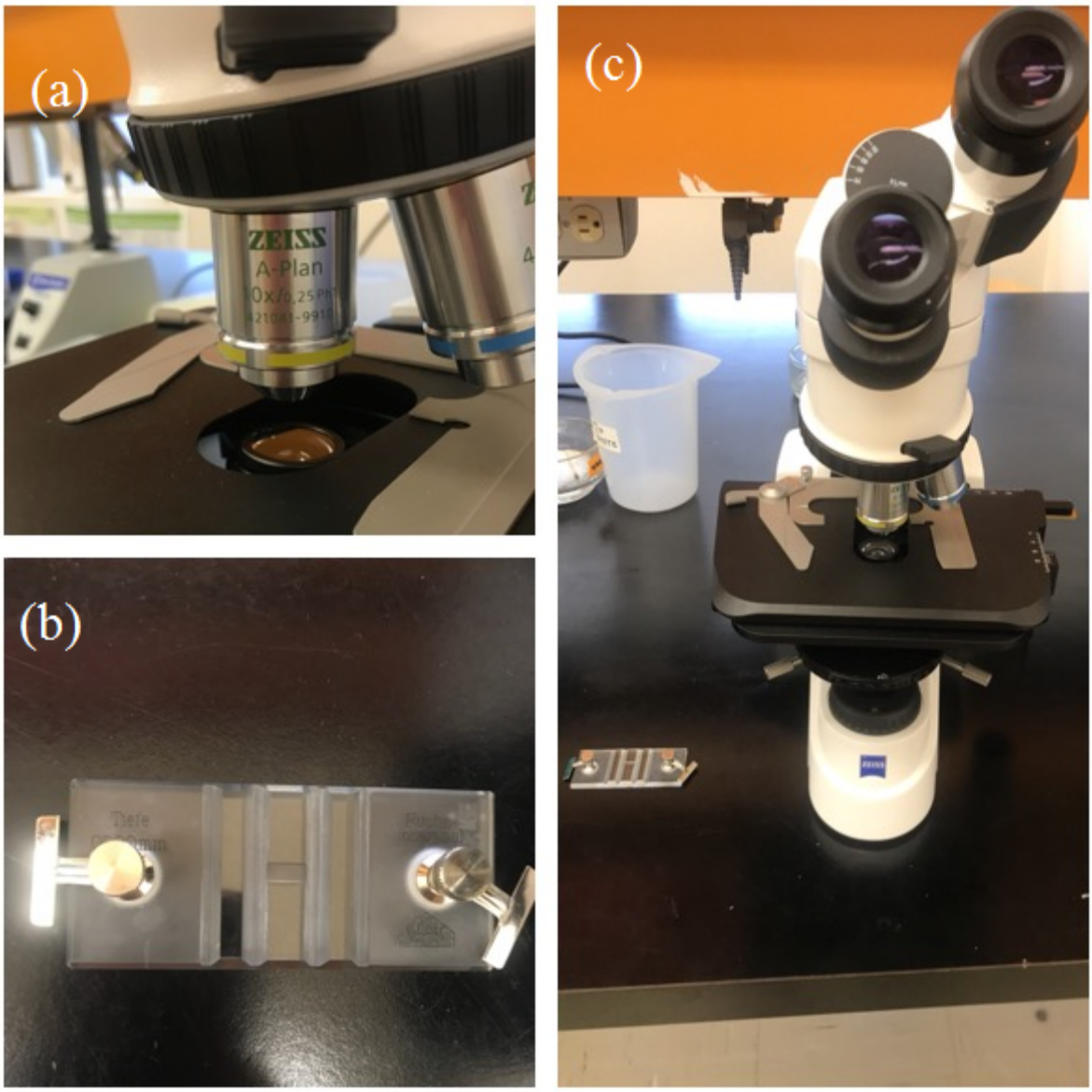

Figure 1. Set up to determine initial concentration of media. (a) is the magnification used to view cells. (b) is the specific haemocytometer (note: labeled) used to count cells. (c) is the Axiostar compound microscope used to view cells.

Preparation of Initial Diatom Cultures

Using a disposable glass pipette, a total of 10 mL of pH-adjusted media was delivered into 9 test tubes (three treatments and three replicates for each treatment). Diatoms were swirled to mix, and set aside in a 20°C incubator to grow.

Incubation and Sub-Sampling of Diatom Cultures

Every Monday, Wednesday, and Friday for a total of 11 days, 100 µL of media was extracted and added to 10 µL of IKI fixative in a separate counting tube. Each of the 9 tubes were vortexed to mix, then set aside in the fridge. This procedure occurred 5 times over a period of 11 days. At the end of 11 days, the 45 samples were counted using a haemocytometer to determine the concentration of cells in each sample. Interesting characteristics such as the shape of cells were recorded and photographed. A growth rate was produced from the concentration of cells/mL vs. time (days) for each pH treatment.

Statistical Analyses

A 1-way ANOVA test was conducted to analyze the data recorded. The dependent variable was the average growth rate while the independent variable was pH.

Results

From our data collection, growth curves were produced for each replicate at pH 7.0, 8.0 and 9.0. Best fit lines were fitted for each treatment with the slope of the line representing the averaged growth rate. Figure 2 shows the average growth rates of each treatment being: 5.33×10^3 cells mL^{-1} d^{-1} for pH 7.0, 7.40×10^3 cells m^{-1} d^{-1} for pH 8.0 and 5.49×10^3 cells mL^{-1} d^{-1} for pH 9.0.

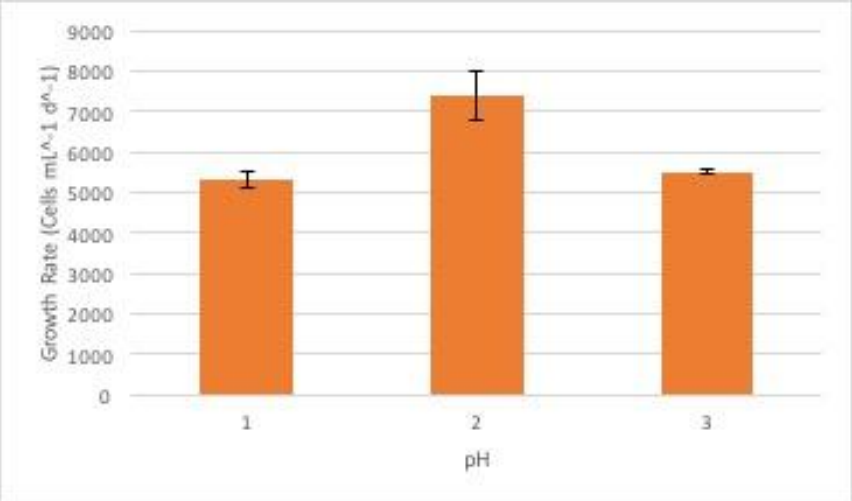

Figure 2. *Licmophora abbreviata* averaged growth rates at pH treatments 7.0, 8.0 and 9.0. Specific *L. abbreviata* strain is a local strain of UBC culture collection #697. HESNW media was used for for all three different treatments. X-axis represent the pH treatments, with 1 = pH 7.0, 2 = pH 8.0 and 3 = pH 9.0. Growth took place over 11 days. Error bars on 1, 2 and 3 are 206.97 cells mL^{-1} d^{-1}, 599.41 cells mL^{-1} d^{-1} and 61.53 cells mL^{-1} d^{-1} respectively.

A one-way ANOVA test was used to compare the average growth rates for each treatment. A p-value of 0.00079 at a 95% confidence level, along with a F value (39.36) that was greater than F critical (5.14) resulted in statistically significant results. It can be seen in Figure 2. that pH 8.0 had an average growth rate of approximately 1.37 times greater than pH 7.0 or 9.0. The standard deviation for pH 9.0 was 61.53 cells mL^{-1} d^{-1}, much smaller than the standard deviations of 206.97 cells mL^{-1} d^{-1} and 599.41 cells mL^{-1} d^{-1} for pH 7.0 and pH 8.0 respectively.

L. abbreviata from the three treatments were photographed and their characteristics were noted to see if pH influences the diatoms' physical appearance. *L. abbreviata* loosely resembles a slice of pizza and were orientated in the direction and adjacent to each other under pH 7.0 and 8.0, as shown in Figure 3. and Figure 4.; however, under a more basic pH, as was the case for pH 9.0 in Figure 5., the diatom shape was cylindrical with many cells clumping together.

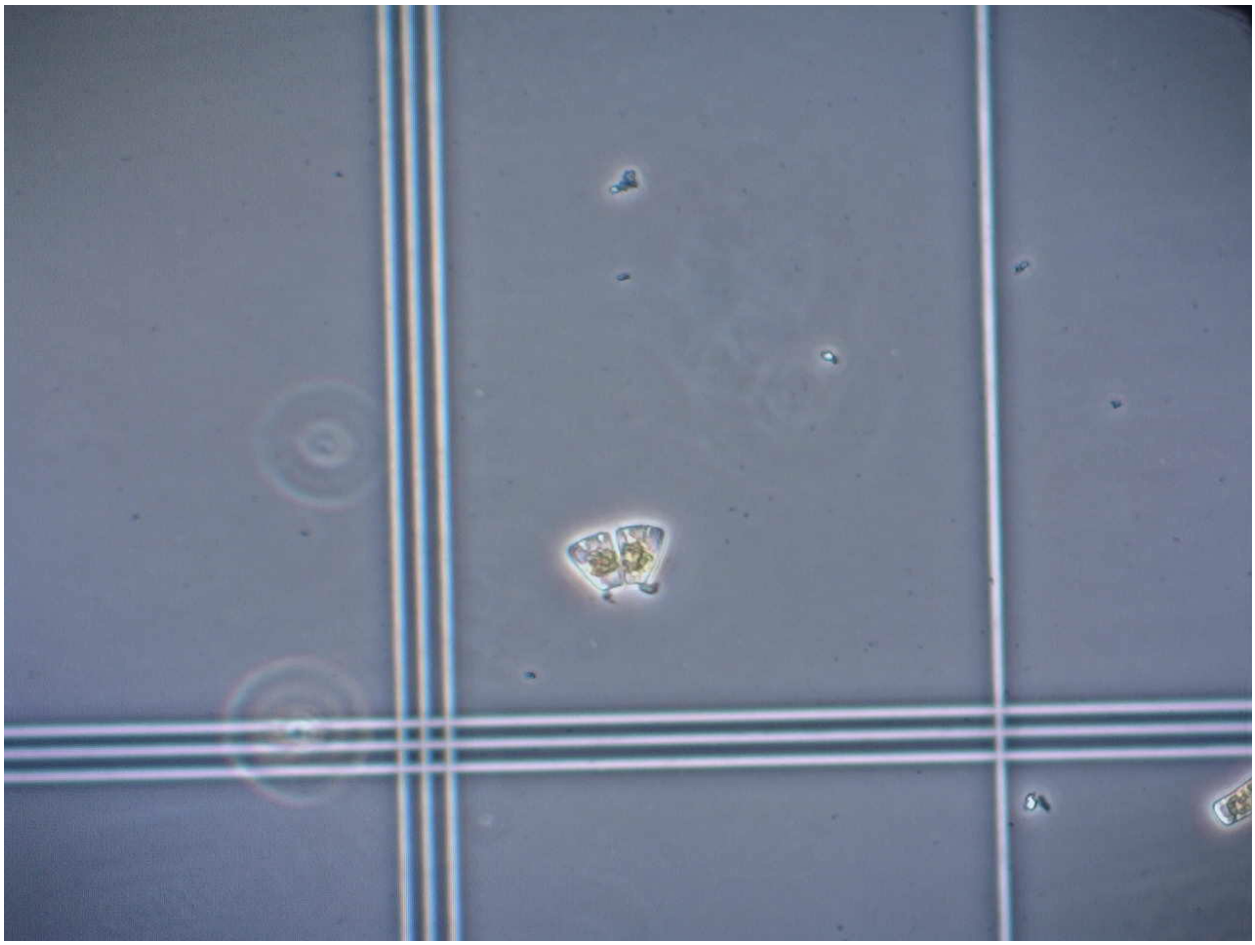

Figure 3. *Licmophora abbreviata* grown in HESNW media at pH 7.0 for 11 days. Spacing between horizontal white lines are 0.25 mm. Individual *L. abbreviata* has an approximate cell height of 21 micrometers. Viewed on Axiostar plus with total magnification of 160x.

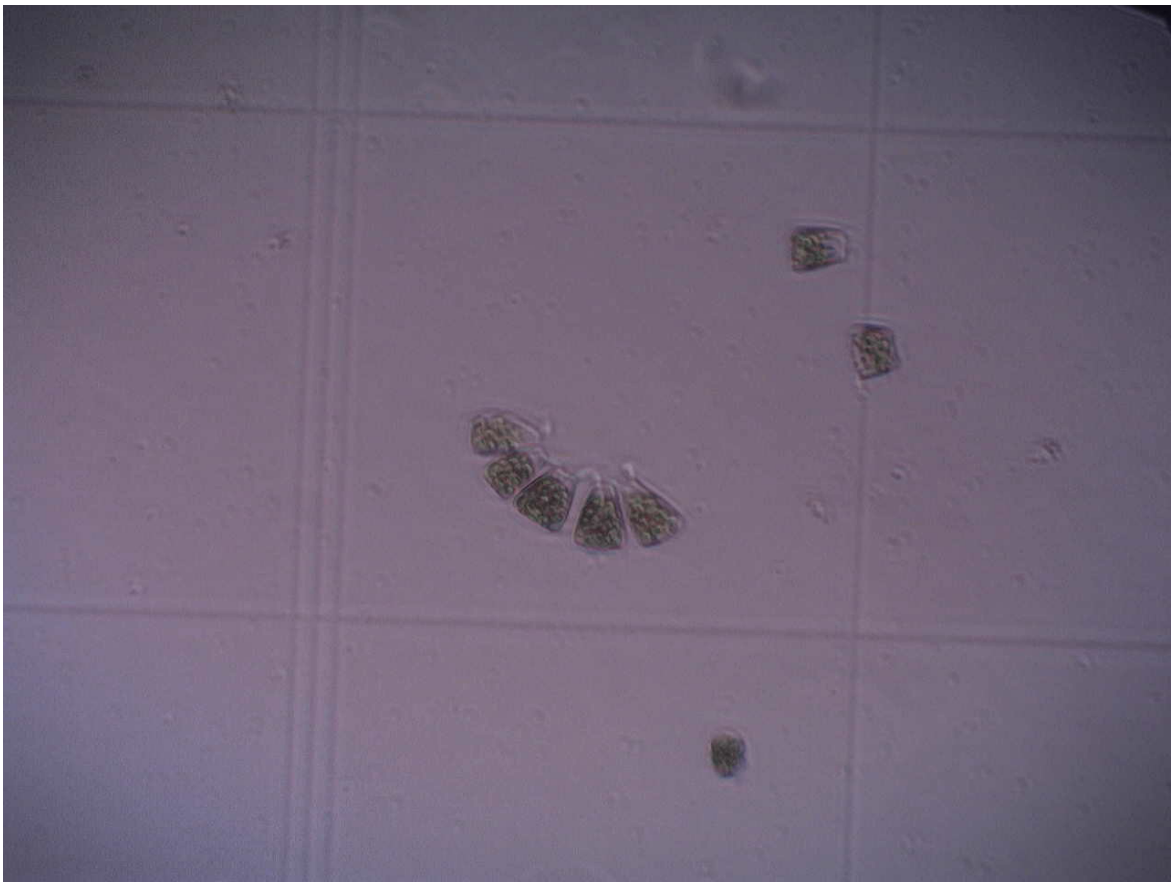

Figure 4. *Licmophora abbreviata* grown in HESNW media at pH 8.0 for 11 days. Spacing between horizontal white lines are 0.25 mm. *L. abbreviata* has an approximate cell height of 29 micrometers. Viewed on Axiostar plus with total magnification of 160x.

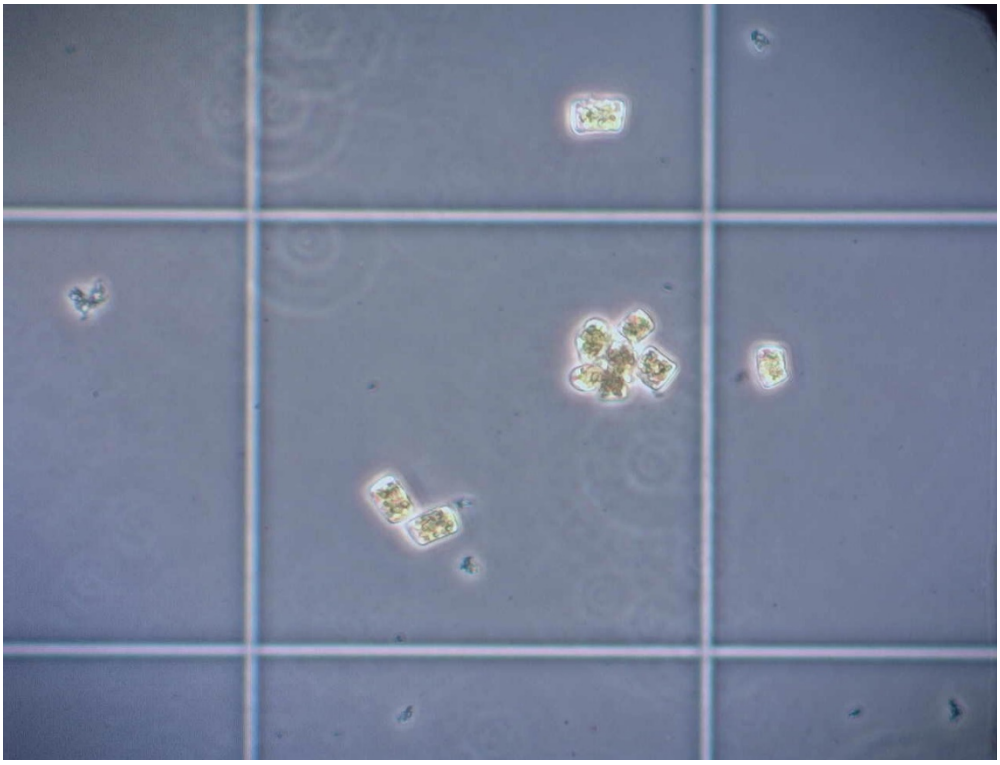

Figure 5. *Licmophora abbreviata* grown in HESNW media at pH 9.0 for 11 days. Spacing between horizontal white lines are 0.25 mm. *L. abbreviata* has an approximate cell height of 20 micrometers. Viewed on Axiostar plus with total magnification of 160x.

Discussion

Based on our statistical analysis, we rejected our null hypothesis that pH does not influence growth rates of *L. abbreviata* and supported the alternate hypothesis that pH does influence growth rates o*f L. abbreviata*. The results matched our initial prediction and Hinga's (1992) study which found that diatom growth is optimal at pH 8.1. A previous study by Taraldsvik and Sverre (2000) had found that growth rates only varied slightly between pH 6.5 to pH 9.5 for the diatom *Skeletonema costatum,* Interestingly, Figure 2 contradicts these findings, showing at a pH of 8.0, *L. abbreviata* had a significantly higher growth rate.

The significance in our results may be due to the lack of a carbon dioxide-carbonate (CO_2-CO_3^{2-}) buffer in our media. As mentioned above, varying pH can affect the dissolved inorganic carbon speciation and the bioavailability of trace metals (Gensemer *et al.,* 1993). Studies by Chen and Durbin (1994) on *Thalassiosira oceanica* revealed that growth rates decreased in higher pH levels due to the decrease in carbon dioxide (CO_2) and bicarbonate (HCO_3^-) concentrations. This change in carbon speciation results from the CO_2-CO_3^{2-} buffer system in marine environments. As pH rises, the carbonate concentration will increase while the concentrations of carbon CO_2/HCO_3^- decreases. Diatoms are photosynthetic, thus the decreased concentration of CO_2 would impact photosynthetic rates and therefore growth, as there is less product available. In our experiment, there was no CO_2-CO_3^{2-} buffer, meaning the CO_2 levels in our experiment was not affected in this manner.

Some unique data was collected from the pH 7.0 solutions. In Figure 2., the growth rate for *L. abbreviata* became negative from days 4 to 7. Additionally, the concentration on day 7 was higher than the concentration on day 9 which likely indicates a fixing error. During the fixing process on either day, the growth solution may not have been properly mixed resulting in an uneven distribution of diatoms in the sample collected. This would result in a decreased growth rate as a large portion of the diatoms were excluded.

Further, it was observed the diatom's shape differed depending on the pH of the growth media. In both pH 7.0 and 8.0, as seen in Figure 2 and Figure 3, the shape of *L. abbreviata* was observed to be triangular shaped. In contrast for pH 9.0, the diatom remained cylindrical (unchanged) since the start of the study. This indicates that the pH of the environment has morphological effects on diatoms. Previous studies on other species of diatom has indicated that

silica cell walls are not sensitive to the pH of the ocean (Gross, 2012); however, based on the results of this study, the change in pH may have influenced the shape of the diatom. Further investigation would be required to infer the significance of changes in shape in *L. abbreviata.*

To improve the accuracy of the study and results obtained, several improvements can be implemented. One of the problems experienced was the amount of time available to grow the cultures, collect the samples and determine the concentrations. In this study, the concentration of the colonies was determined at six different time points over an 11-day span. The first sampling day was during the initial set-up of the cultures and therefore, the concentration was known, while the second day had too low of a concentration to observe under a compound light microscope. As a result, only four data points were collected in the creation of the growth curve. The data collected indicated the colonies just reached the end of the exponential growth phase and the beginning of the stationary phase. Therefore, if more time was available, more samples could have been taken over a longer period of time to improve the accuracy of the growth curves produced.

Additionally, several areas of interest should be studied in order to gain a better understanding of the interactions that pH and temperature have on diatom growth and salmon abundance. According to a study conducted on *Thalassiosira pseudonana (T. pseudonana)*, Javaheri et al. (2015) found that *T. pseudonana* grew the fastest at 23°C initially, in comparison to treatments at 14 degrees Celsius and 18°C. However, at 23°C, the culture also had the lowest population density of the three. This negative relationship with rising temperature vs. population density would be prudent to study in species such as *L. abbreviata,* an important part of the salmon ecosystem in the Lower Mainland. If a negative relationship is demonstrated between

temperature and abundance this would be an area of concern, and another point to bring up in the growing conversation around the ramifications of climate change. Lastly, to determine whether the effects of changing pH are localized to a specific region, studying various populations of *L. abbreviata* from different locations across the world would provide much stronger evidence towards the possibly devastating effects of pH change in ocean water.

Conclusion

As discussed, the 1-way ANOVA conducted on the maximum growth rates for each of the three pH treatments resulted in a calculated p-value of 0.00079. At a confidence level of 95%, this indicates the data was statistically significant and that the null hypothesis that pH does not influence the growth rate of diatoms is rejected. Consequently, the data collected indicates that pH may have an effect on the growth rate of *L. abbrreviata*. Additionally, an interesting characteristic that evolved was the differing shape of *L. abbreviata* grown in different pH conditions. An area of growing concern, and further investigation, is the acidification of oceans due to climate change and how this will affect the growth of diatoms and ultimately salmon as well. Both diatoms and salmon play a key role in the food web and therefore, their decrease in population would have detrimental consequences for the ecosystems in British Columbia.

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Appendix

Raw Data

Table 1. Average number of cells per mL and standard deviation data for the three pH treatment levels, 1 = pH7, 2 = pH 8 and 3 = pH 9.

Date of Sample Collection	pH Treatment (1=7, 2=8, 3=9)	Rep	Number of Diatoms per mL	Average Number of Diatoms per mL	Standard Deviation
2017-10-30	1	1	1,550	1,550	0
		2	1,550		
		3	1,550		
	2	1	1,550	1,550	0
		2	1,550		
		3	1,550		
	3	1	1,550	1,550	0
		2	1,550		
		3	1,550		
2017-11-01	1	1	1,550	1,550	0
		2	1,550		
		3	1,550		
	2	1	1,550	1,550	0
		2	1,550		

		3	1,550		
	3	1	1,550	1721	296.18
		2	1,550		
		3	2,063		
2017-11-03	1	1	48,125	45,104.17	6345.623
		2	49,375		
		3	37,812.5		
	2	1	17,875	21,312.5	17,443.41
		2	40,218.75		
		3	5,843.75		
	3	1	12,031.25	12,031.25	3093.75
		2	15,125		
		3	8,937.5		
2017-11-06	1	1	1,719	26,239.67	22,659.57
		2	30,593.75		
		3	46,406.25		
	2	1	40,218.75	28,177	24,205.53
		2	44,000		

		3	312.25		
	3	1	38,156.25	33,114.58	6,006.615
		2	26,468.75		
		3	34,718.75		
2017-11-08	1	1	53,125	55,520.83	2,127.144
		2	56,250		
		3	57,187.5		
	2	1	60,000	59,375	826.797
		2	59,687.5		
		3	58,437.5		
	3	1	50,312.5	55,625	6,910.42
		2	53,125		
		3	63,437.5		
2017-11-10	1	1	68,750	59,114.58	8,374.087
		2	55,000		
		3	53,593.75		
	2	1	84,328.75	86,890.83	12,403.2
		2	100,375		

		3	75,968.75		
	3	1	51,531.25	53,604.17	2,078.145
		2	55,687.5		
		3	53,593.75		

Figure 6. Growth rate of *L. abbreviata* over entire 11 days of growth. Each pH treatment represents the average of three replicates.

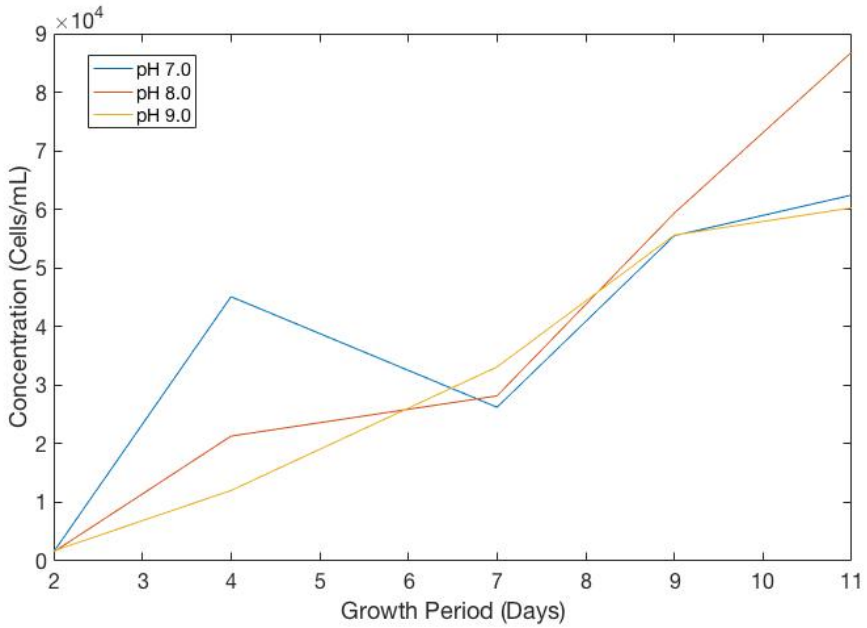

Figure 7. One way ANOVA test statistics.The dependant variable was average growth rate while the independent variable was pH. Group 1 represents pH 7.0, group 2 pH 8.0 and group 3 pH 9.0.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
1	3	15995.8	5331.93333	42839.6133
2	3	22192	7397.33333	359300.853
3	3	16456.3	5485.43333	3786.76333

ANOVA

Source of Variation	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2	3972400.51	29.3579751	0.00079693	5.14325285
Within Groups	6	135309.077			
Total	8				