FINAL REPORT

The 2016 Undergraduate Research Opportunities Program (UROP) Research Project

Title of the Project: Evaluating how rising sea surface temperature alters estuarine phytoplankton growth

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Credit taken for the project: OCS 3999 Undergraduate Independent Research– 3 credits

Introduction

Natural and anthropogenic stressors can affect ecosystem functioning through changes in biodiversity (Vinebrooke et al. 2003). Changes in aquatic ecosystems will likely lead to alterations in both primary producer community composition and the food webs they support. The growth and diversity of phytoplankton is known to be controlled be environmental conditions such as temperature, pH, nutrient availability, and pollution (Brand 1984, Murrel and Lores 2004, Petersen et al. 2008).

Phytoplankton are the most abundant organisms in the ocean. Not only do they form the base of the food web, but also they are also great indicators of climate change. Because they are scarcely used for commercial purposes, have a short life- span, and are sensitive to environmental change, we can study them to get quick, long-term, and reliable results on how they are affected by environmental changes (Hays et al. 2005.)

An increase in atmospheric carbon dioxide leads to an increase in ocean acidification. Ocean acidification is when dissolved carbon dioxide increases and the pH of the water decreases (Sommer et al. 2015). Ocean acidification can also affect phytoplankton populations. Some species of phytoplankton are able to tolerate the lower pH than other species. In turn, this

could affect higher trophic levels because different animals rely on multiple species of phytoplankton for food and nutrients (Hays et al. 2005).

Phytoplankton growth may also be affected by exposure to contaminants or pollutants (Errera, R. Personal Communication. 2015). As a result of their large surface area, phytoplankton are able to absorb contaminants easily and effectively (Gerofke et al. 2005). Methyl mercury (MeHg) is a well-known organic contaminant of concern that biomagnifies up estuarine food webs (Errera, R. Personal Communication. 2015). Phytoplankton can be an entry point for toxins into the food web. Once they are present in the food web, toxins can bioaccumulate and biomagnify. Biomagnification is a serious concern as it can affect the health of many wildlife and humans (Gerofke et al. 2005). However, very few studies have considered the role of climate change on the bioaccumulation and biomagnification of contaminants at the base of the estuarine food web (Errera, R. Personal Communication. 2015).

The alteration of phytoplankton community size structure and taxonomic composition due to eutrophication in addition to/or related to climatic changes will result in large differences in the amount of energy (e.g., nitrogen and carbon) and possibility contaminant bioaccumulation at the base of the marine food web. Smaller phytoplankton have a high surface area to volume ratio. This means that smaller phytoplankton will uptake contaminants more efficiently than larger phytoplankton. If the contaminant is fat soluble, it will stay in the tissue of living organisms. As the trophic level increases, the fat-soluble containment will become more and more abundant, this process is called biomagnification. If smaller phytoplankton become more abundant in the community, another trophic level might be added. A few consequences of an addition of another trophic level are that there will be less energy passed along into higher

trophic levels but on the other hand there will be more contaminants that will be accumulated in the tissues of those organisms as well.

The overall study objective is to investigate very important yet poorly understood processes—impacts or potential shifts in phytoplankton communities and the bioaccumulation and biomagnification of persistent organic pollutants (POPs) in costal or estuarine food web systems to changing climate regimes. In order to fulfill the objective, phytoplankton species growth responses were quantified under current and predicted sea surface temperature and pCO_2 conditions, as well as zooplankton grazing preferences and the biomagnification of toxic organic contaminants (e.g., methylmercury) at the base of the estuarine food web.

Materials and Methods

Phytoplankton growth responses to current and predicted sea surface temperature and pCO_2 *conditions*

We assessed phytoplankton growth rates to current and predicted pCO_2 conditions using two different size estuarine phytoplankton that are commonly found in Louisiana estuaries: the small diatom *Thalassiosira oceanica* and larger diatom *Ditylum brightwellii*. The average diameter of *Thalassiosira oceanica* is about 3-12µm. and the average diameter of *Ditylum brightwellii* is about 80-130µm (Hasle et al. 1997). Two different cell sizes were used to determine if cell surface area influences their growth response and increases potential for contaminant accumulation. The media used was f/2+Silica. Three experiments were conducted in total: the phytoplankton growth experiment, the phytoplankton uptake of methyl mercury (MeHg) experiment (parts A and B), and the zooplankton uptake of MeHg experiment. All of the cultures were semi-continuous batch cultures and were held at a temperature of 24.0ºC. The light

source was cool-white, fluorescent lights with an irradiance of 85 μE m^{-2} s^{-1} kept on a 12:12-h light/dark cycle (Ozhan et al. 2015).

Treatments included a ~current pCO_2 of 400 ppm (~ pH of 7.75-7.86) and a predicted pCO_2 of 1000 ppm (~ pH of 7.29 - 7.37) for salinities observed in Louisiana estuaries, based on the IPCC scenario A1 (2007). Experimental flasks were equipped with stopper and ports to allow for controlled gas flow in and out of the vessel, to evaluate the impact of pCO_2 on the phytoplankton community. An outflow tube were placed at the top of the vessel, which releases air into a vessel of MilliQ water as a visual indicator of positive airflow and a means of preventing ambient air from entering the vessel. Biological replication of each experiment was conducted with triplicate bottles and each experimental treatment was repeated at least three times. The setup of the growth experiment is shown in Figure 1. Compressed zero air tanks with added CO_2 were prepared gravimetrically and certified to the specified CO_2 concentrations. Air were gently bubbled into the enriched seawater through a fine glass frit. The enriched seawater media were bubbled with the proper pCO_2 and agitated with a stir bar for at least a 3-day period to ensure proper dissolution of CO_2. Prior to the addition of cells, DIC and alkalinity measurements were taken to confirm that pCO_2 is at equilibrium.

Each of the species at each of the pCO_2 levels was acclimated up to the correct pCO_2 for two months prior to the start of the experiment. Three hundred mL total of media and phytoplankton were placed in 500mL autoclaved flasks. During the growth experiment, there were three replicates of each species at pCO_2 [400] and pCO_2 [1,000]. All of the replicates were constantly bubbling at the indicated pCO_2level. The experiment was run for ten days total, starting on day zero and ending on day nine. Subsamples were taken from each flask throughout

the experiment for determining *daily changes on phytoplankton abundance* (both chl *a* and microscopy).

On day zero and day nine, dissolved inorganic carbon (DIC) and alkalinity (ALK) samples were taken from each replicate to ensure that the media was at the pCO_2that was targeted. Forty-five mL of media was vacuum filtered through a combusted 25µm GF/F filter into a 50mL centrifuge tube for ALK measurements. 45mL of media was vacuum filtered through a combusted 25µm GF/F filter into a 50mL centrifuge tube for DIC measurements. The ALK samples were refrigerated until they were sent off to be analyzed and the DIC samples were placed in the freezer until they were sent off to be analyzed.

Every day of the experiment, cell count samples were taken. Five mL of sample was taken from each replicate and placed into a 7mL scintillation vial. The samples were fixed by adding 50% a glutaraldehyde solution. The final concentration of the solution was 2% glutaraldehyde. The cell count samples were then placed in the refrigerator until they were analyzed. Duplicate cell counts were done by taking 1mL of the fixed sample and placing it on a gridded Sedgewick Rafter Counting Chamber. Only compact cells were counted and the empty frustules were not included in the total count.

Cells were counted up to 200 cells and the number of boxes that contained 200 cells was recorded. In order to calculate the amount of cells in 1mL, the following equation was used: number of cells in 1mL = (number of cells/number of boxes)(1,000). In order to calculate how many cells were in 1L, the number of cells in 1mL was multiplied by 1,000.

On days one, three, five, seven, and nine, chlorophyll *a* samples were taken. Ten ml of subsamples were vacuum filtered through a 25µm GF/F filters. The filters were kept and placed

in a 25mL centrifuge tubes that were wrapped in aluminum foil and placed in the freezer until it was analyzed.

In order to analyze the chlorophyll a samples, 10mL of a 90% acetone solution were added to the centrifuge tubes containing the filters. The tubes were vortexed for thirty seconds then covered in aluminum foil. The samples were placed in a -20ºC freezer for twenty-four hours. The tubes were taken out of the freezer, brought to room temperature in dark, and placed in a centrifuge for fifteen minutes on setting six. After the tubes were taken out of the centrifuge, the supernatant was poured into a clear test tube and analyzed for chlorophyll a using a Turner fluorometer (Model 10-AU) (Parsons et al. 1984).

During the phytoplankton growth experiment, additional 1.5L of media and phytoplankton were bubbling at each pCO_2 in autoclaved 2.0L culture flasks. These cultures were used for the phytoplankton uptake of MeHg experiment at the end of the growth experiment.

Dissolved inorganic carbon and alkalinity samples were sent off to be analyzed. The DIC was originally measured in mg/L. This was then converted to µmole/L. ALK was originally measured in mg/L of $CaCO_3$. ALK measurements were converted to µmoles/L. Given DIC and ALK measurements, pCO_2 levels could be calculated. The average ALK of seawater was 2,200 µmoles/L. The ALK measurements for both species at both pCO_2 levels on both days were significantly lower than what they should have been. The only exceptions were replicates two and three of *Thalassiosira oceanica* on day nine at pCO_2 [400] and replicate one of *Thalassiosira oceanica* on day nine at pCO_2 [1,000], all of which were much higher than average. Seawater inorganic carbon chemistry was verified, calculated and predicted using measured DIC and ALK

values with the seacarb package in R 2.13.1 (H. Lavigne, et al. 2010. R Core Development Team, 2012).

Phytoplankton uptake of MeHg—Part A

Figure 2 shows the setup of the phytoplankton uptake of MeHg experiment—part A. The goal of this experiment was to determine if different sized phytoplankton accumulate different concentrations of a known contaminant of concern for the marine environment, methyl mercury (MeHg). A working stock of MeHg solution was prepared by combining 1.8mL of filtered seawater and 0.2mL of MeHg (1ppm and CH_3Hg-Cl), and kept in the refrigerator for twenty-four hours before it could be used.

One hundred and fifty mL of media and phytoplankton were placed into autoclaved 250mL flasks. There was one control and two replicates for each species at each pCO_2level. Control for each species were not treated with MeHg and grown under similar conditions as the treatment groups. In order to obtain initial cell count samples, 2mL of sample were taken from each flask and placed into 2mL centrifuge tubes that contained 50% glutaraldehyde solution in order to create a 2% glutaraldehyde solution. 60µL of the MeHg were placed into each flask except the control flasks. The phytoplankton were exposed to the MeHg for six hours. After six hours, final cell count samples were taken using the same methods and concentrations as the initial cell count samples. Then, the rest of the samples were filtered through acid-washed 1.2µm polycarbonate filters. The filters were then placed in 7mL acid-washed scintillation vials and placed in the freezer until they were sent off to be analyzed for MeHg. Three additional acid-washed 1.2µm polycarbonate filters were placed in acid-washed 7mL scintillation vials and frozen. The additional filters were used as blanks for MeHg analyses.

Phytoplankton uptake of MeHg—Part B

The second goal of this part of the study was to determine how MeHg biomagnifies from phytoplankton to zooplankton using two different sizes of zooplankton, rotifer and copepod that have been fed contaminated phytoplankton. The first of this experiment was to contaminate different sized phytoplankton with MeHg.

Figure 2 shows the setup of the phytoplankton uptake of MeHg experiment—part B. Three hundred mL of media and phytoplankton were placed into autoclaved 500mL flasks. There was one control and two replicates for each species at each pCO_2level. Control for each species were not treated with MeHg and grown under similar conditions as the treatment groups. These cultures were used to feed to the zooplankton in zooplankton uptake of MeHg experiment.
In order to obtain initial cell count samples, 2mL of sample were taken from each flask and placed into 2mL centrifuge tubes that contained 50% glutaraldehyde solution, in order to create 2% final glutaraldehyde solution. 30µL of the stock MeHg were placed into each of the flasks, except for the control flasks. The phytoplankton were exposed to the MeHg for six hours. After six hours, final cell count samples were taken using the same methods and concentrations that were used to take initial cell count samples. Then, the phytoplankton cultures were either filtered and resuspended or placed in 50mL centrifuge tubes and centrifuged in order to create a pellet of phytoplankton. The contaminated phytoplankton were transferred into the flasks containing the zooplankton.

Zooplankton MeHg uptake

This study was to determine how MeHg biomagnifies from phytoplankton to zooplankton using two different sizes of zooplankton, rotifer and copepod that have been fed contaminated

phytoplankton. Figure 3 shows the setup of the zooplankton MeHg uptake experiment. Copepods are a larger sized zooplankton and rotifers are a smaller sized zooplankton. The average size of a copepod is about 0.5-5mm. The average size of a rotifer is less than 400µm (Johnson and Allen. 2005). Two different sized zooplankton species were chosen to correspond with the two different sizes of phytoplankton. Zooplankton were transferred to large containers for acclimation and fed with non-contaminated food. At the end of the acclimation (2 days), animals were starved for 12 hours and placed in 500-ml bottles (1000 rotifer/L and 30 copepods/L) with freshly prepared growth media for the experiments. Two different sizes of phytoplankton were initially incubated with MeHg for 6 hours (see above). The small size phytoplankton were filtered through 0.2 micron filter and resuspended in bottles containing rotifers. Larger size phytoplankton were filtered through 0.2 micron filter and resuspended in bottles containing copepods.

At pCO_2 [400], there were two control flasks that contained rotifers and *Thalassiosira oceanica* and two control flasks that contained *Ditylum brightwellii* and copepods. The control flasks contained non-contaminated phytoplankton and zooplankton. At the same pCO_2, there were also two contaminated flasks that contained *Thalassiosira oceanica* and rotifers and two contaminated flasks that contained *Ditylum brightwellii* and copepods. At pCO_2 [1,000], there were two control flasks that contained rotifers and *Thalassiosira oceanica* and two control flasks that contained *Ditylum brightwellii* and copepods. The control flasks contained non-contaminated phytoplankton and zooplankton. At the same pCO_2, there were also two contaminated flasks that contained *Thalassiosira oceanica* and rotifers and two contaminated flasks that contained *Ditylum brightwellii* and copepods.

Initial cell count samples were taken by placing 2mL of sample into a 2mL centrifuge tube that contained 50% glutaraldehyde to create a 2% solution. The initial cell count samples were then placed into the refrigerator until they were analyzed. After the zooplankton were exposed to the phytoplankton for nine hours, final cell count samples were taken using the same methods and concentrations as the initial cell count samples. The zooplankton were separated from the phytoplankton by vacuum filtering the cultures through acid-washed 20µm polycarbonate filters. The filters that contained the zooplankton were placed in acid-washed 7mL scintillation vials. The vials were placed in the freezer until they were sent off to be analyzed. Three additional acid-washed 1.2µm polycarbonate filters were placed in acid-washed 7mL scintillation vials and frozen. The additional filters were used as blanks during analyses.

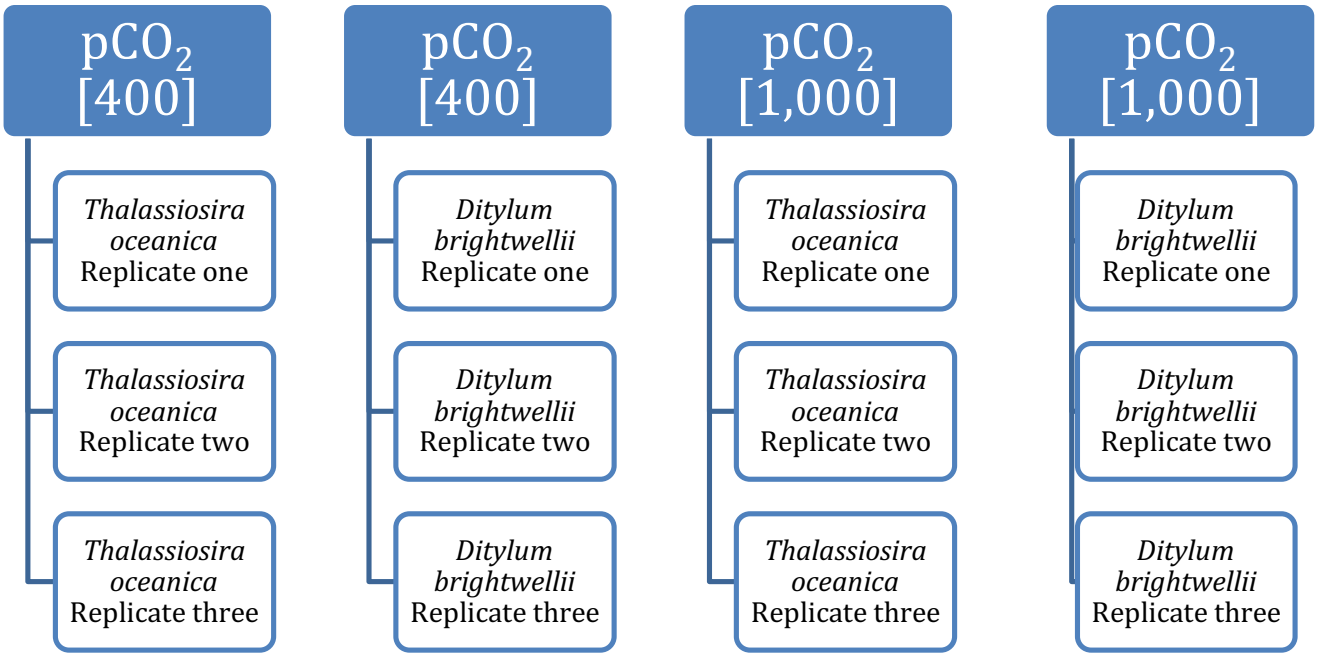

Figure 1: Figure one shows the set up of the growth experiment

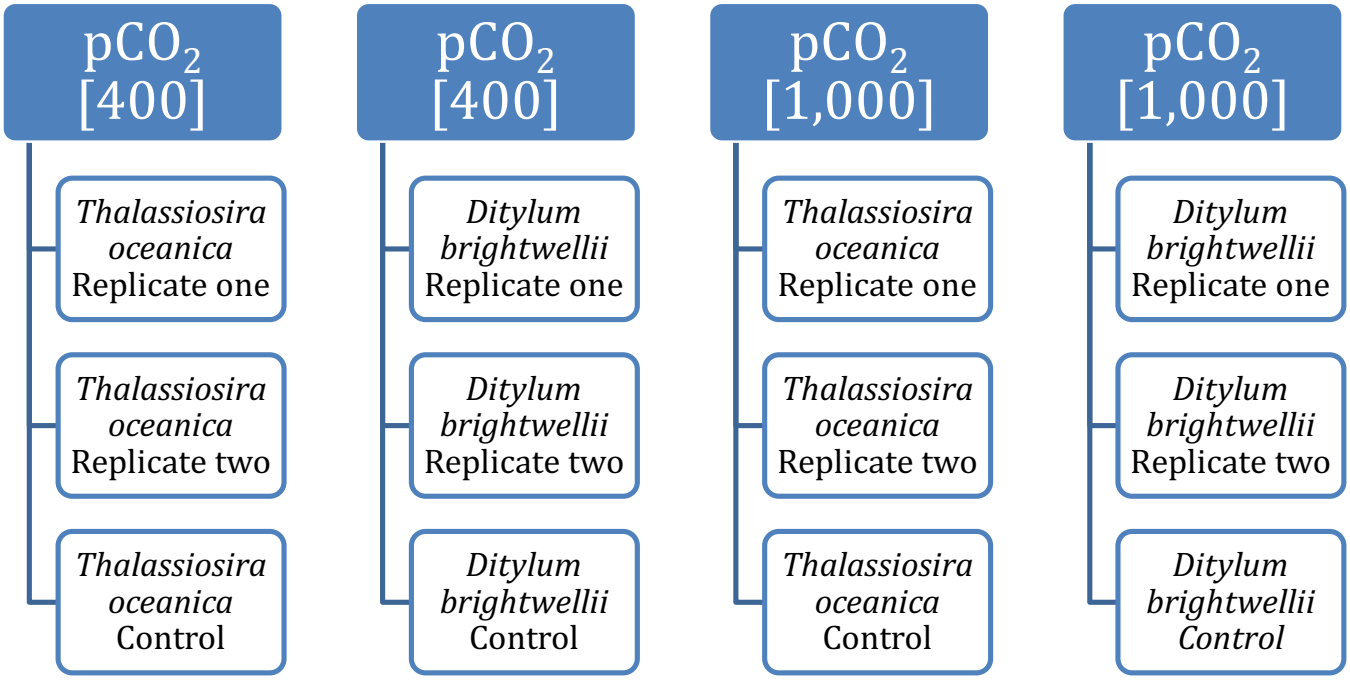

Figure 2: Figure two shows the experimental set up for the phytoplankton uptake of MeHg—part A and B.

Results

Phytoplankton growth under pCO_2 [400] and [1,000]

The cell count data for *Ditylum brightwellii* at pCO_2 [400] is shown in figure 4A. From day zero to day four, the cell abundance in all three replicates was very low. Cell abundance remained low for replicates one and three. However, cell abundance was very high for replicate two by day nine. The cell count data for *Ditylum brightwellii* at pCO_2 [1,000] is shown in figure 4B. All of the replicates had similar cell abundances to each other over all ten days. The average amount of cells per mL of all three replicates of *Ditylum brightwellii* at both pCO_2 [400] and [1,000] is shown by figure 4C.

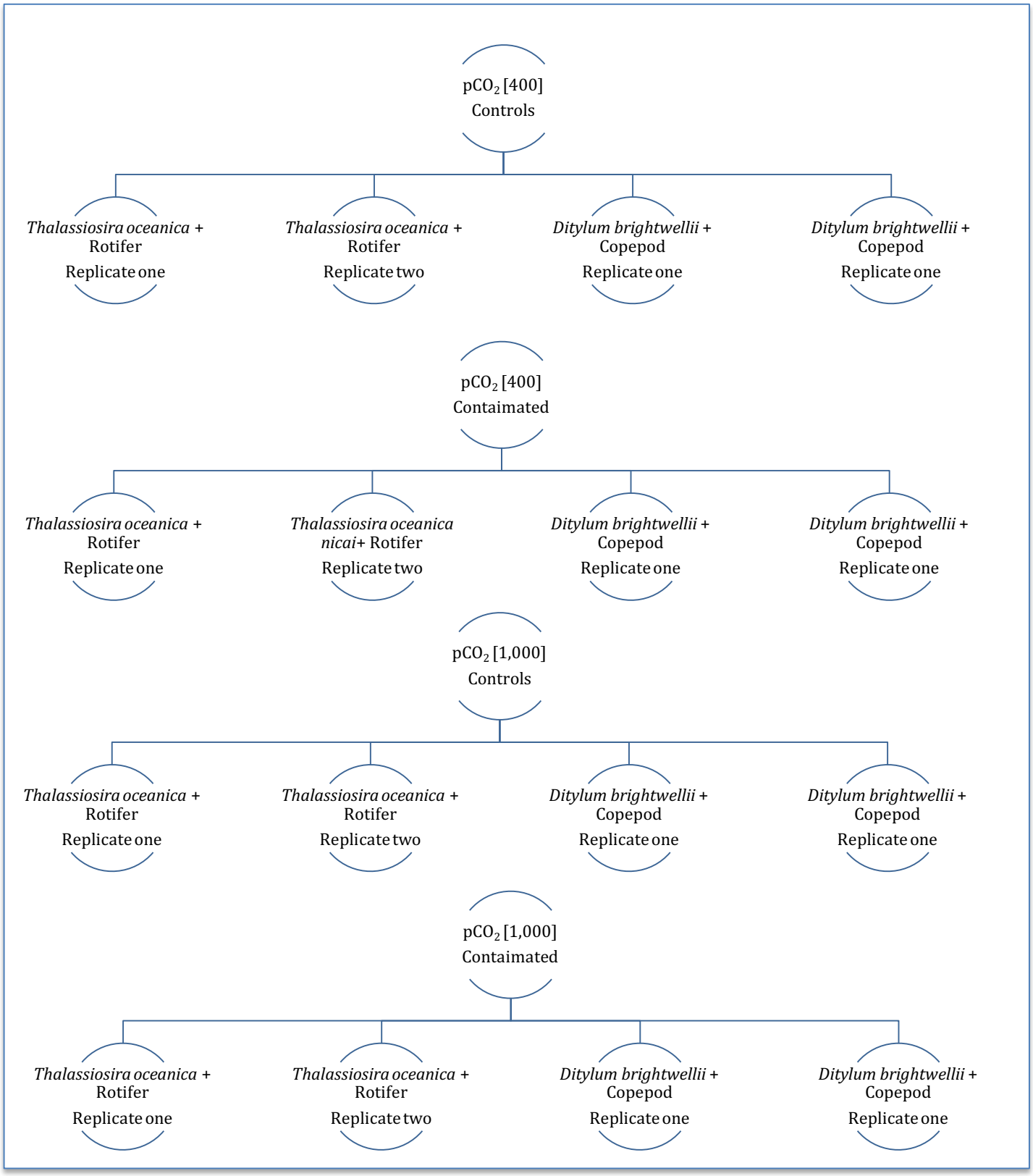

Figure 3: Figure three shows the experimental set up of the zooplankton uptake experiment.

From day zero to about day four, the cell abundance was very low in both treatments. However, after day five, *Ditylum brightwellii* at pCO_2 [400] had a significantly higher cell abundance than *Ditylum brightwellii* at pCO_2 [1,000]. The average maximum amount of *Ditylum brightwellii* at pCO_2 [400] cells reached 2,404 cells per mL. The average maximum amount of *Ditylum brightwellii* at pCO_2 [1,000] cells reached 282 cells per mL.

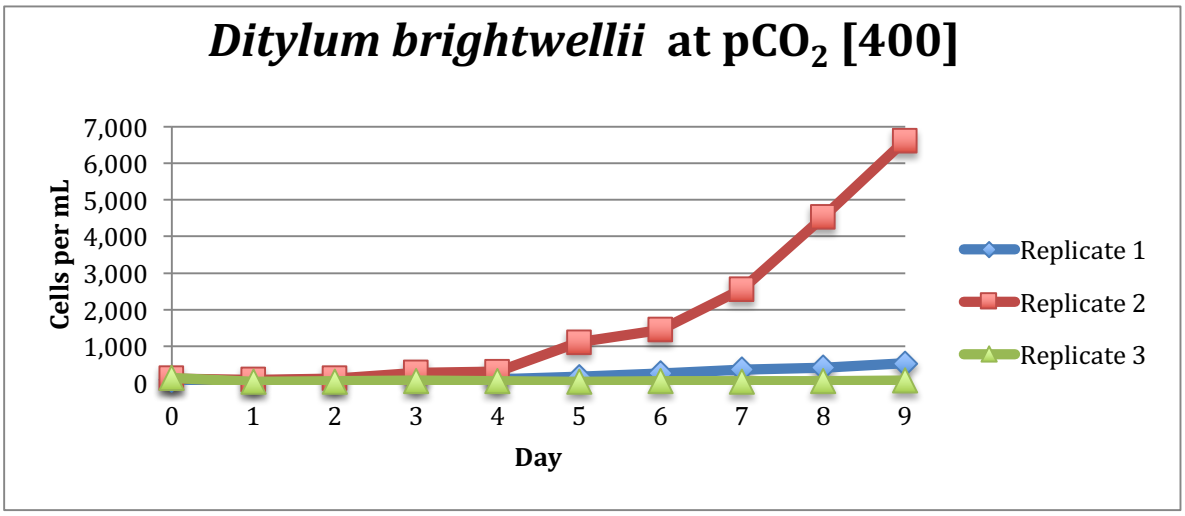

Figure 4A: The figure shows the amount of *Ditylum brightwellii* cells per mL from day zero to day nine at pCO_2 [400].

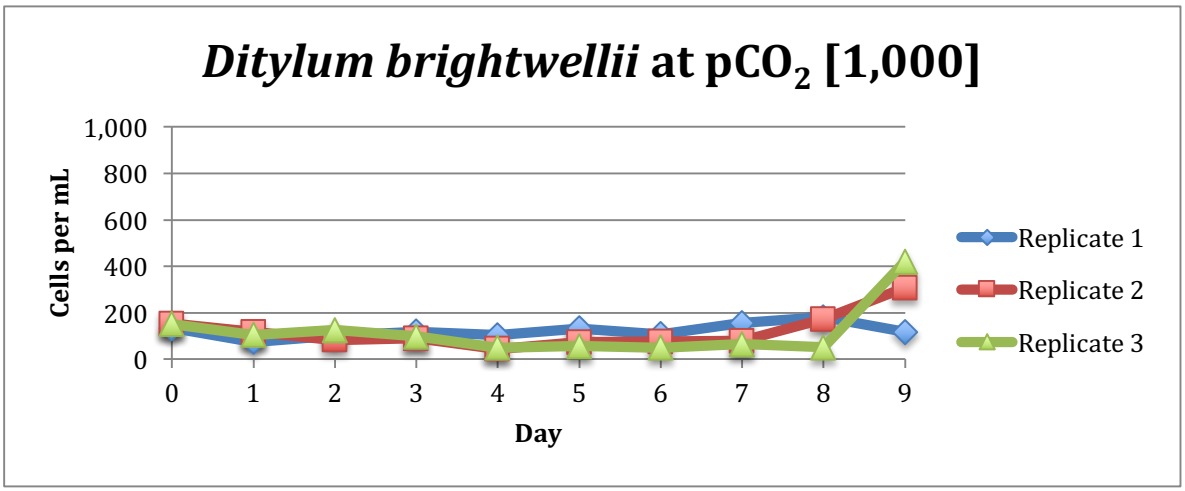

Figure 4B: The figure shows the amount of *Ditylum brightwellii* cells per mL from day zero to day nine at pCO_2 [1,000].

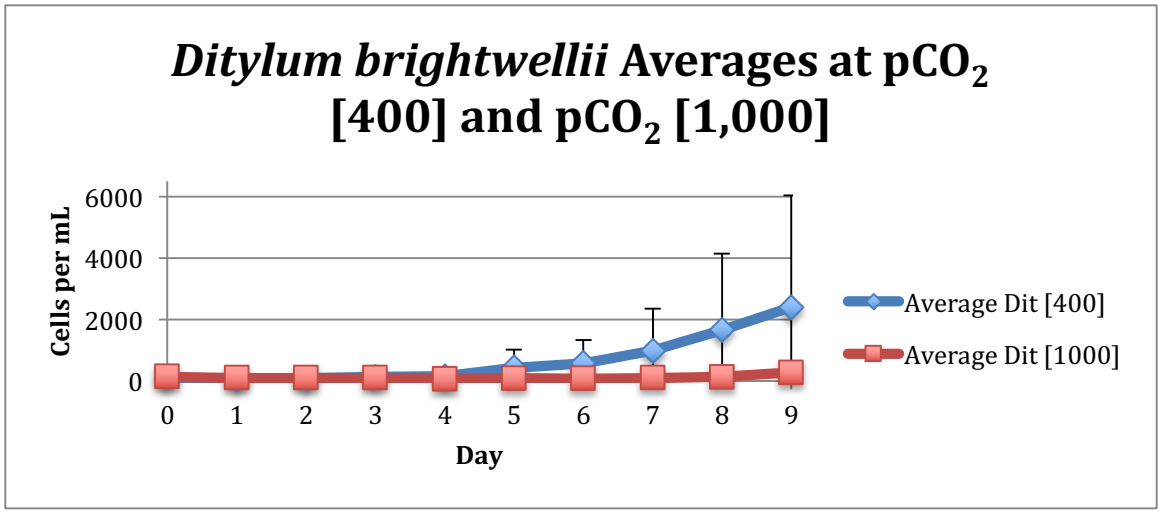

Figure 4C: The figure shows the average amount of *Ditylum brightwellii* cells per mL of the three replicates at both pCO_2levels.

Interestingly, It was also observed that *Ditylum brightwellii* formed resting cells at pCO_2 [1,000]. Figure 5 shows the *Ditylum brightwellii* cell count averages at pCO_2 [400] and pCO_2 [1,000]. Over all nine days, the average amount of resting cells is higher at pCO_2 [1,000] than at pCO_2 [400]. The average highest amount of resting cells for pCO_2 [400] was six. Whereas, the average highest amount of resting cells at pCO_2 [1,000] was nineteen.

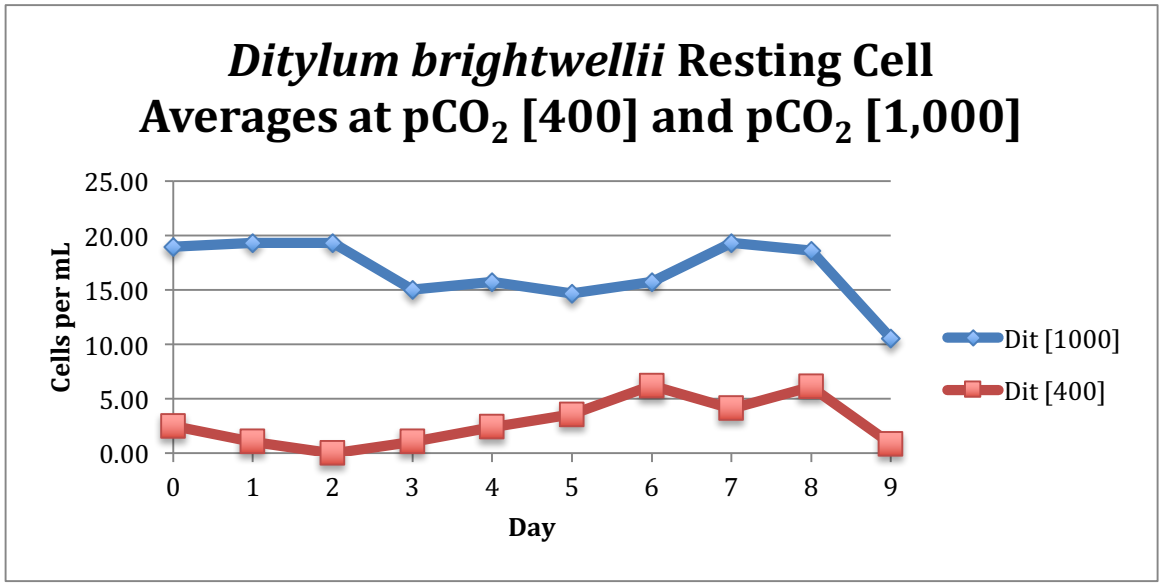

Figure 5: This figure shows the *Ditylum brightwellii* cell count averages at pCO_2 [400] and pCO_2 [1,000]. Over all nine days, the average amount of resting cells is higher at pCO_2 [1,000] than at pCO_2 [400].

The cell count data for *Thalassiosira oceanica* at pCO_2 [400] is shown in figure 6A. For the first eight days, all three replicates had a really low cell abundance. After day eight, replicate three had a really sharp increase in cell abundance. Cell abundance remained low in replicates one and two. The cell count data for *Thalassiosira oceanica* at pCO_2 [1,000] is shown in figure 6B. From day zero to day six, all replicates had a significantly low cell abundance. After day six, cell abundance started to increase in replicate two. However, cell abundance remained low in replicates one and two. The average amount of cells per mL of all three replicates of *Thalassiosira oceanica* at both pCO_2 [400] and [1,000] is shown by figure 6C. The cell abundance from day zero to day six was very low. At day six, the average cell abundance for *Thalassiosira oceanica* at pCO_2 [400] and [1,000] started to increase. At day eight, the average amount of cells per mL of *Thalassiosira oceanica* at pCO_2 [1,000] exceeded the average amount of cells mL at pCO_2 [400]. The maximum average amount of *Thalassiosira oceanica* at pCO_2

[400] cells was 95,679,430 cells per mL. The maximum average amount of *Thalassiosira oceanica* at pCO_2 [1,000] cells was 57,499,666 cells per mL.

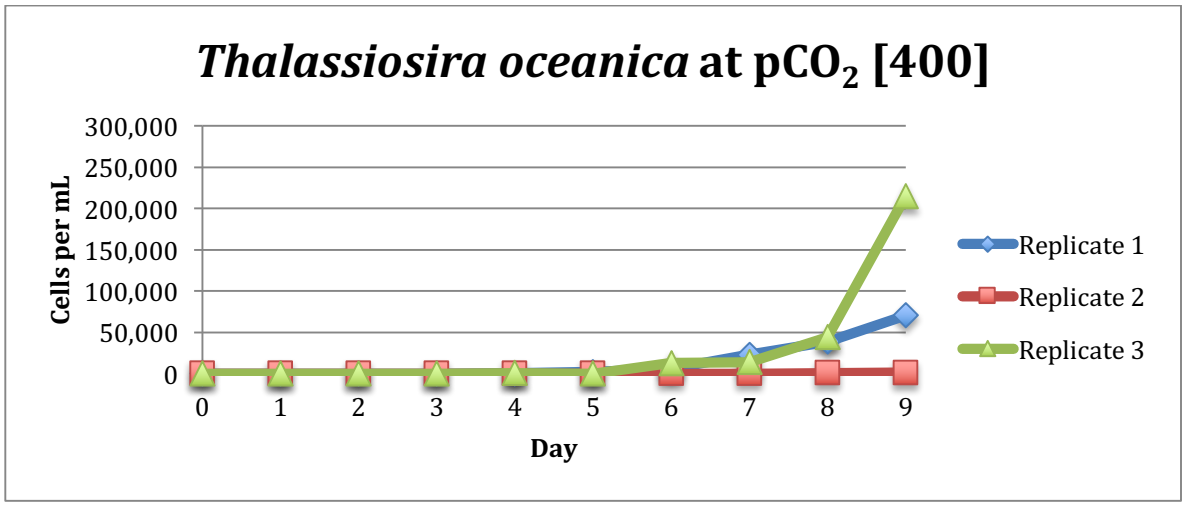

Figure 6A: The figure shows the amount of *Thalassiosira oceanica* cells per mL from day zero to day nine at pCO_2 [400].

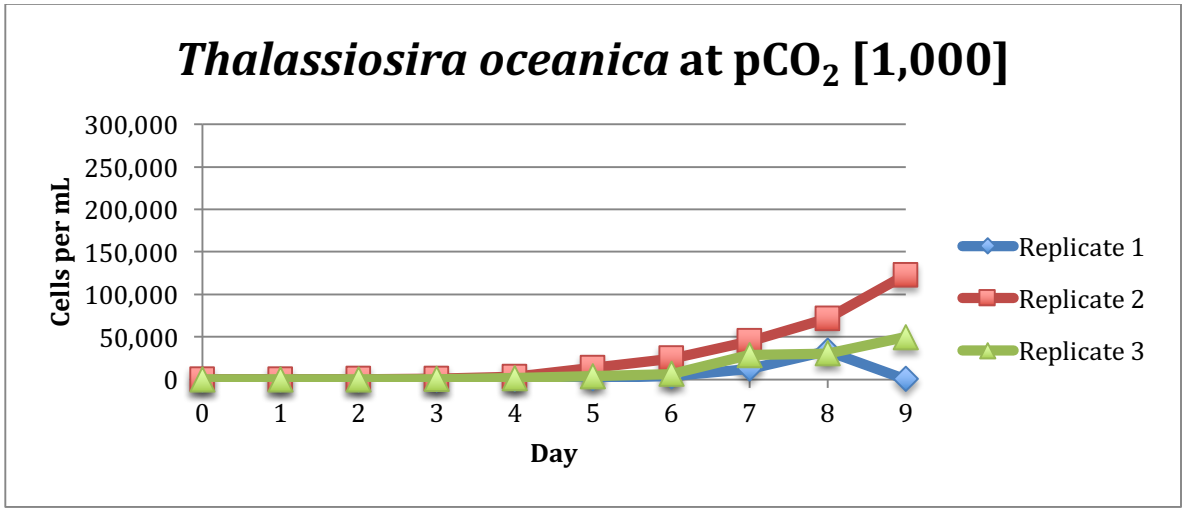

Figure 6B: The figure shows the amount of *Thalassiosira oceanica* cells per mL from day zero to day nine at pCO_2 [1,000].

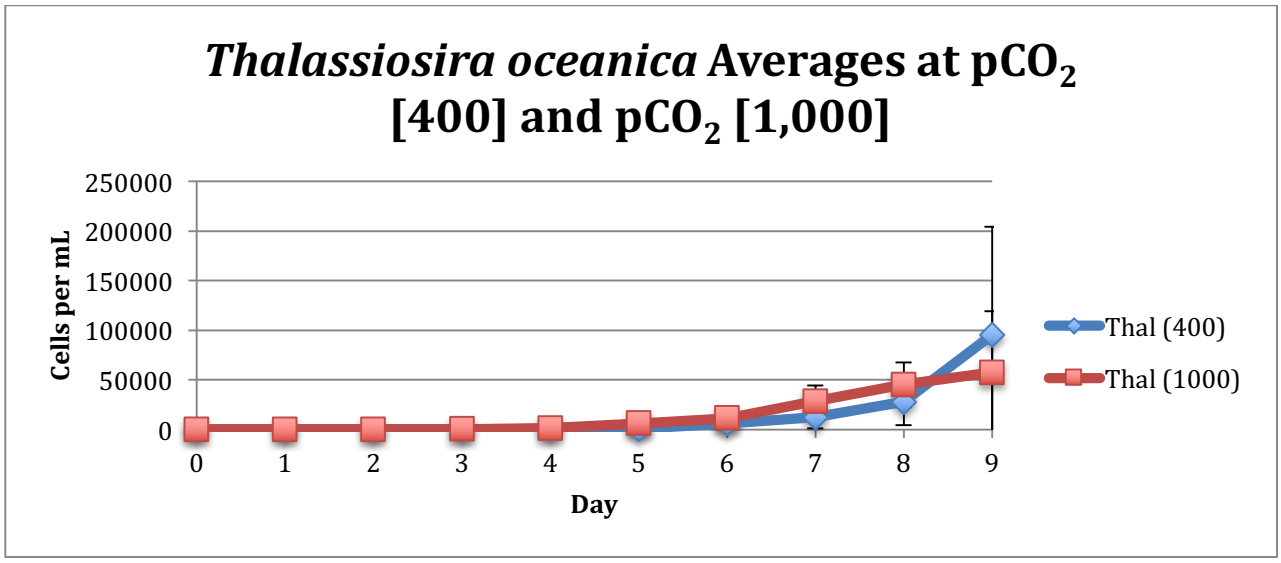

Figure 6C: The figure shows the average amount of *Thalassiosira oceanica* cells per mL of all three replicates at each pCO_2level.

In addition to cell counts, *chlorophyll a* concentrations were also analyzed. The chlorophyll *a* data for *Ditylum brightwellii* at pCO_2 [400] is shown in figure 7A. The chlorophyll *a* data corresponds nicely with cell count data at pCO_2 [400]. The chlorophyll *a* data for *Ditylum brightwellii* at pCO_2 [1,000] is shown in figure 7B. The chlorophyll *a* data for pCO_2 [1,000] also corresponds with the cell count data.

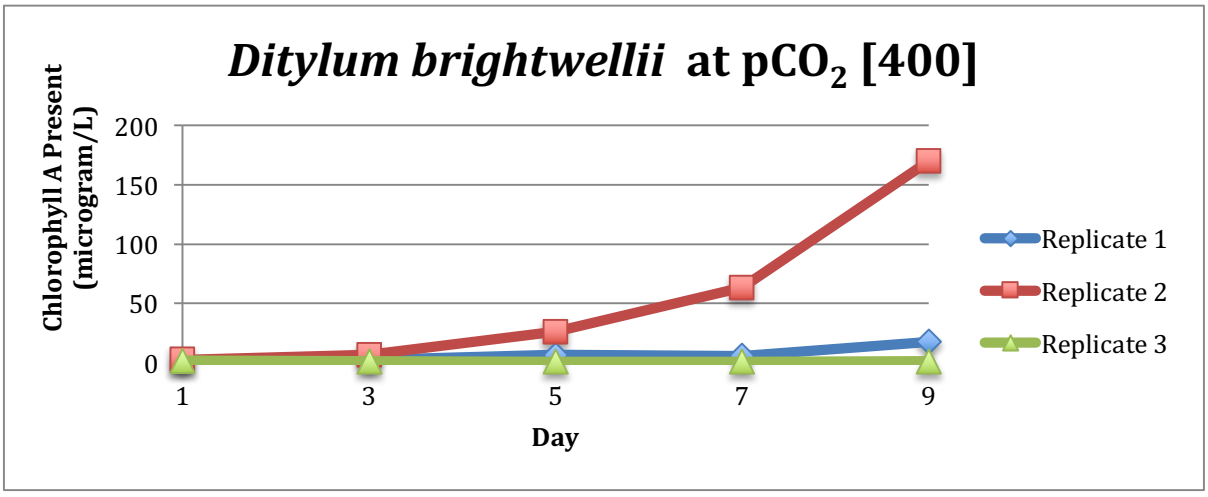

Figure 7A: The figure shows the amount of chlorophyll *a* present in each replicate of *Ditylum brightwellii* at pCO_2 [400].

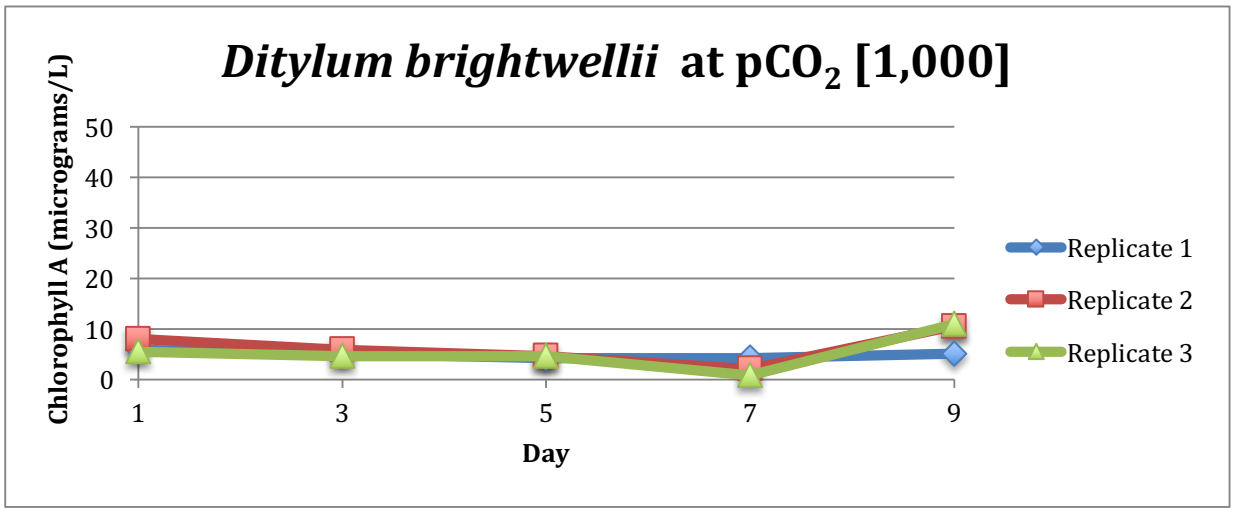

Figure 76B: The figure shows the amount of chlorophyll *a* present in each replicate of *Ditylum brightwellii* at pCO_2 [1,000].

The chlorophyll *a* data for *Thalassiosira oceanica* at pCO_2 [400] is shown in figure 8A. The data for chlorophyll *a Thalassiosira oceanica* at pCO_2 [400] shows the same trends as the data for the cell counts. The data for chlorophyll *a* in *Thalassiosira oceanica* at pCO_2 [1,000] is shown in figure 8B. The chlorophyll *a* amounts do not correlate well with the cell count data for *Thalassiosira oceanica* at pCO_2 [1,000]. Figure 8C shows the average chlorophyll *a* amounts of all three replicates of both species at both pCO_2levels. The chlorophyll *a* averages for *Thalassiosira oceanica* at both pCO_2levels differ from the cell count average. However, the *Ditylum brightwellii* chlorophyll *a* averages are very similar to the average cell count numbers at both pCO_2levels.

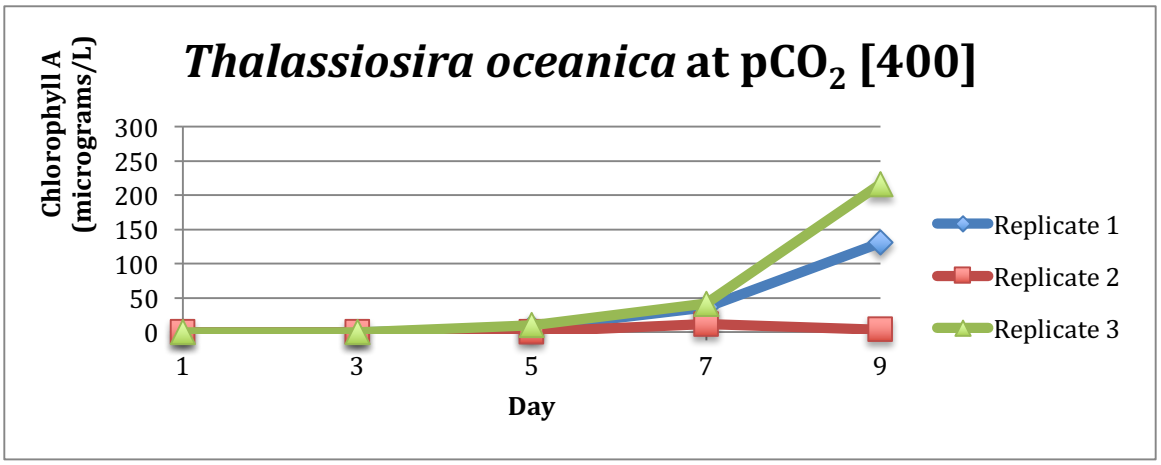

Figure 8A: The figure shows the amount chlorophyll *a* present in each replicate of *Thalassiosira oceanica* at pCO_2 [400].

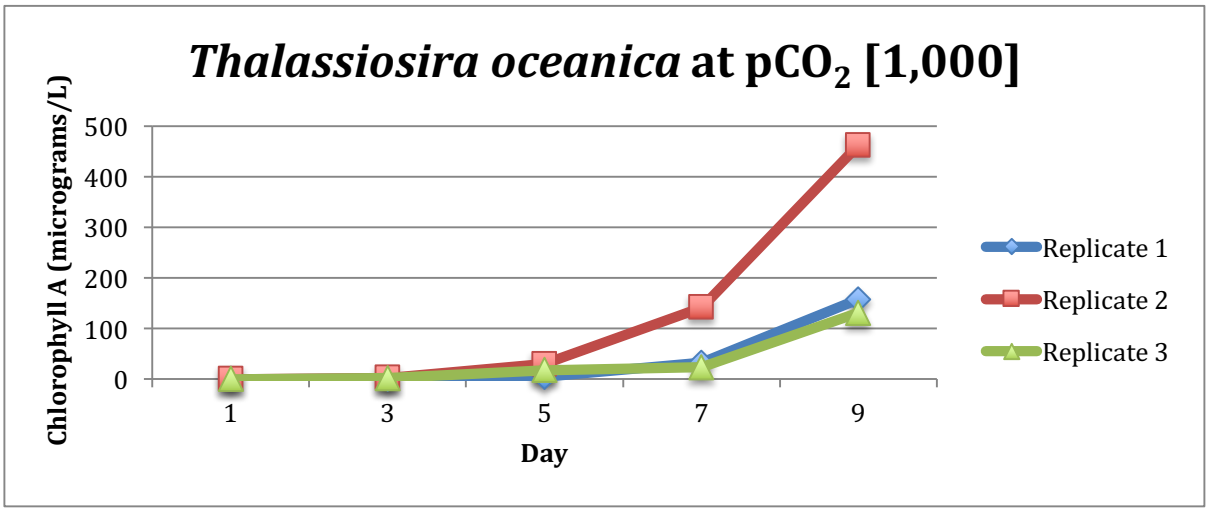

Figure 8B: The figure shows the amount chlorophyll *a* present in each replicate of *Thalassiosira oceanica* at pCO_2 [1,000].

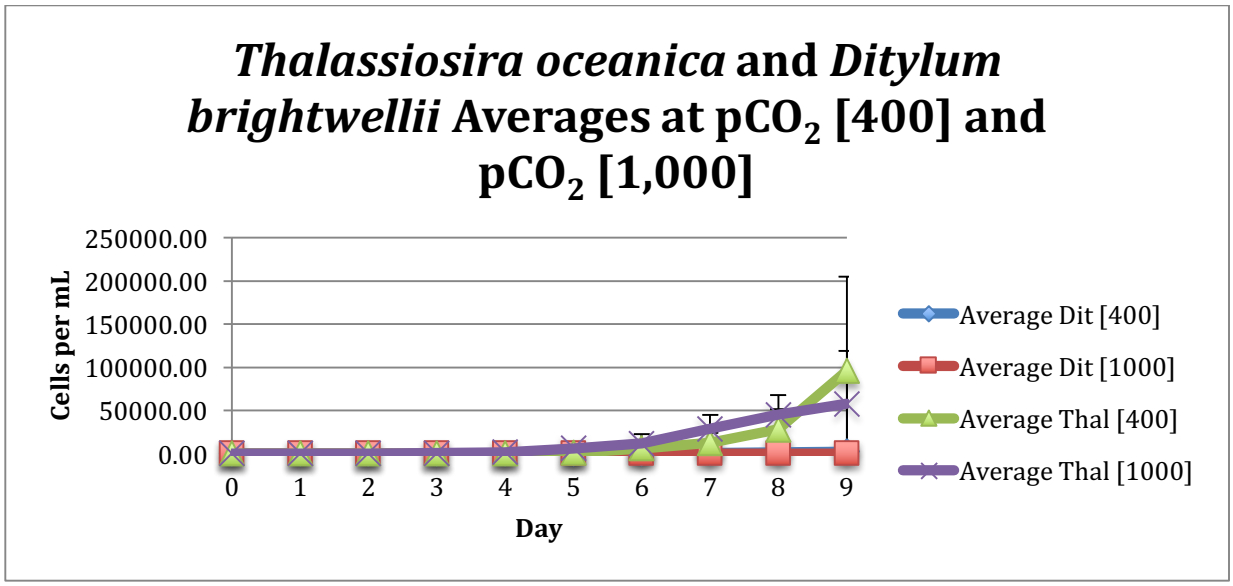

Figure 8C: The figure shows the average amount of chlorophyll *a* present of each species at each pCO_2level.

Growth of phytoplankton DIC & ALK data

Alkalinity and DIC data have yet to be processed.

3.3 Phytoplankton uptake of MeHg—part A & B & zooplankton uptake of MeHg data

Methyl mercury samples were sent off for analysis, and data has not been returned yet.

Discussion

Our results indicated that each of the diatom species, the large celled *Ditylum brightwellii* and small cell-sized *Thalassiosira oceanica,* had a distinct growth rate response when exposed to two different levels of pCO_2. Large cell-sized *D. brightwellii* growth was significantly reduced when exposed to higher pCO_2 concentration (0.06 ± 0.02 d^{-1}) compared to the growth rate at the lower pCO_2 of 400 (0.33 ± 0.13 d^{-1}). More interestingly, this species also formed significant amount of resting cells under high pCO_2 concentration. This suggests that *D. brightwellii* cells were stressed under high pCO_2 exposure and less likely to survive in an acidic water system and

would have a decline in fitness under future climate scenarios. On the contrary, small cell-sized diatom *Thalassiosira oceanica* did not show a significant difference in growth under the two pCO_2 treatments and appeared healthy throughout the experiment period.

Since the findings were drastic between the species and resting formation of *Ditylum* was unexpected, we repeated the experiments to ensure reproducible results. This replication will conclude in February, 2016. Primarily results indicate that the *D. brightwellii* was stressed under enhanced pCO_2 conditions, with cultures grown at a pCO_2 concentration of 1000 having a lower chl. a and increased resting cells. The additional data will assist in finalizing our analysis, and provide a more complete dataset that can help us evaluate the findings in more detail. Of particular interest are the results of the *T. oceanica* replication, another similar species, *Thalassiosira pseudonana*, showed a down-regulation in the carbon concentrating mechanism (CCM) when grown under elevated pCO_2 conditions similar to our experiments (Yang and Goa, 2012). But as in our experiments, Yang and Goa (2012) reported no significant difference in the growth rate or chl. a concentrations, and was suggested that a difference in growth and CCM activity might be stimulated when light levels were limiting, during our experiments light was not a limiting factor. In addition, MeHg accumulation analysis is still being processed by an outside vendor, once completed we can begin processing the data, therefore that part of the discussion will be developed at a later time.

Overall, our project provides critical information regarding the impact of increased pCO_2 influence on individual phytoplankton species and suggests that a possible shift in phytoplankton species may alter the trophic transfer of POPs in an estuarine system. In addition, we will provide information on the uptake and accumulation of organic contaminants at the base of the

marine food web, for which little is known, especially for a highly productive ecosystem. Ultimately, our results will further our understanding of the impact of pCO_2 on contaminant accumulation in marine predators as well as humans that rely on an estuarine-based diet.

The main anticipated outcome of this study is a comparative assessment of predicted phytoplankton species shifts due to climate change and the effect of these shifts on contaminant bioaccumulation, marine trophic structure, and the trophic transfer of contaminants. As phytoplankton are at the base of the food web, the results can provide critical information that can be used in models of higher trophic level species to examine potential impacts on ecosystem productivity. Furthermore, the results can inform managers, scientists and modelers to help predict impacts of future scenarios of ocean acidification on these vulnerable systems.

Problems Encountered and Proposed Solutions

Over both phytoplankton growth experiments, no stationary phase was observed. This means that over the ten days, none of the replicates of either species at either pCO_2 level reached carrying capacity. However, in the next rendition of the experiment, after the initial ten days of sampling are complete, chlorophyll *a* samples will continue to be taken every other day in an effort to observe a stationary phase.

Since the DIC samples that were taken were vacuum filtered, which allowed for more air to get in contact with the samples, the DIC measurements are lower than what they should have been. In order to correct this error in the next rendition of the experiment, water samples will be taken and placed into combusted 125mL glass containers and vacuum-sealed using grease and glass stopper until they are sent off to be analyzed. This methodology would ensure that the DIC

measurements are more accurate. The ALK values are also somewhat inaccurate. The instrument that was used to measure the pH was calibrated by using freshwater instead of seawater. That is why the ALK measurements are lower than anticipated.

During the final step of the phytoplankton uptake of MeHg—part A, while trying to filter and re-suspend the phytoplankton into the zooplankton cultures, it was found that the phytoplankton were not going to come off of the filters. Because of this, contaminated *Thalassiosira oceanica* replicate one at pCO_2 [400] was unable to be re-suspended into the rotifer culture. This resulted in a complete loss of a contaminated *Thalassiosira oceanica* and rotifer replicate at pCO_2 [400].

In order to avoid losing all cultures and replicates to that method, the total volume of each of the replicates were divided into four 50mL centrifuge tubes. The cultures were centrifuged in order to create a concentrated phytoplankton pellet that was then added to the zooplankton cultures. In the next rendition of the experiment, all re-suspension will be done by centrifuging the phytoplankton cultures into pellets.

When filtering the zooplankton out of the phytoplankton for MeHg analysis, it was found that *Thalassiosira oceanica* chains were being caught in the filter with the rotifers. This impacted our findings in such a way that the amount of MeHg that was present in the sample on the filter would be higher than what the rotifers actually did uptake. It was also realized that *Ditylum brightwellii* was not filtered out correctly. Because of this, the copepods were carefully removed from the media and then rinsed with filtered seawater and placed onto a clean acid-washed polycarbonate filter. In order to correct these errors in the next rendition of the experiments, only one type of zooplankton, copepods, will be used.

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