Minidiscus trioculatus growth in response to climate change

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ABSTRACT

Nanoplanktonic marine diatoms play a large role in the global carbon cycle, but their photophysiological response to future oceanic conditions is uncertain. This experiment used *Minidiscus trioculatus* as a model species, and tested how the growth rate of an intermediate-scale culture of the species will be affected by changes to temperature and pH/pCO2 as projected by modeling of high-emission scenarios. I subjected four separate cultures to combinations of either 13.5 +/- 1°C or 23.5 +/- 1°C and either 8.1 +/- 0.1 pH or 7.6 +/- 0.1 pH for a 15-day duration, and then quantified growth through change in spectrophotometric absorbance measurements. Changes to temperature and pH, as well as the interaction between the two, resulted in significantly altered growth rate. 23.5°C / 7.6 pH conditions resulted in a mean change in absorbance 0.54 lower than at 13.5°C / 8.1 pH. A resulting model predicted growth rate for combinations of temperature and pH between and outside of those tested. 18.5°C / 7.85 pH conditions were predicted to produce a ~0.19 change in absorbance, whereas 28.5ºC / 7.35 pH were predicted to result in a ~-0.26 change in absorbance. Results are likely explained by temperature and pH-induced inactivation of enzymes critical to the organism's functioning, and suggest a significant change to the photosynthetic base of many food webs and positive feedback to climate change in coming decades.

KEYWORDS

Nanoplanktonic diatom, climate, carbon cycle, temperature, pH

INTRODUCTION

It is imperative to understand the major processes that can change as well as be changed by $[CO_2]_{atm}$ in order to study climate change effects on atmospheric carbon dioxide concentrations. The ocean is certainly one of these processes: it is a sink for approximately 25% of anthropogenic carbon dioxide emissions, which has reduced its average pH from the pre-industrial ~8.17 to today's ~8.07 (Watson et al. 2020). It will drop by only 0.16 +/- 0.002 by the end-of-century under a high-mitigation Shared Socioeconomic Pathway (SSP)1, but is projected to go as low as -0.44 +/- 0.005 under a high-emission SSP5. In addition to acidification, the ocean is also warming: mean end-of-century sea surface temperatures will rise by 1.42 +/- 0.32°C under SSP5 and by 3.48 +/- 0.78°C under SSP5 (Kwiatkowski 2020). Other models stress the importance of the acceleration of warming, projecting that the average rate of warming for the upper 2000 meters of ocean will have increased by more than four times within the next 60 years alone (Cheng et al. 2019). Increased temperature and acidity have the potential to disrupt all life due to the basics of protein function. At high temperatures, excess heat causes breakage of hydrogen bonds, disulfide bridges and other forces that hold a protein's secondary structure to itself. This results in a loss of tertiary structure referred to as denaturation, which decreases enzyme activity nonlinearly. At low pH the excessive charge present can cause electrostatic repulsion between amino acids that, again, disrupt the tertiary structure. (Anson 1945, Anderson et al. 1990, Daniel et al. 1996). These molecular fundamentals hold true for marine organisms; an experiment on gene expression patterns in the pearl oyster *Pinctada fucada* found that future conditions will disrupt the physiology and potential adaptability of the species (Liu et al. 2012).

Diatoms are key to understanding the global carbon cycle, as they account for around 20 percent of total photosynthetic carbon fixation and about 40 percent of both total oceanic primary productivity and carbon export to ocean depths (Leblanc et al. 2018, Benoiston 2017, Cavicchioli et al. 2019). They likely evolved during the Permian and so consequently have survived atmospheric CO_2 concentrations around twice as high as our planet will experience within the next century, so it is tempting to generalize them as resilient to coming changes; however, these miniature silicate marvels have since diversified, with varying sizes and physiological characteristics (Gao & Campbell 2014, Farooqui et al. 2015). Additionally, rate of temperature and pH change has the potential to be faster than anything seen in the diatoms' evolutionary

timespan, further threatening them (Hönisch et al. 2012). Many modern diatom species have been tested under conditions of higher $p(CO_2)$; a review of 19 studies on the topic reports 11 species displaying growth stimulation, 9 being unaffected, and 11 growth-inhibited in response to the treatment. The size trend pointed to growth stimulation of the larger diatoms, but technical experiments to determine the difference that acidification will have on the spectrum of diatom size are still needed (Gao & Campbell 2014). *Minidiscus*, the genus with the smallest centric diatoms of all marine phytoplankton, has not been directly tested under such conditions (Aké-Castillo et al. 2001). Despite the species' average diameters being but a few micrometers, they should not be overlooked. They are in the top 20 most abundant diatom genera and have been located in ocean basins off the coast of every continent (Leblanc et al. 2018). And with the new technology of high-throughput sequencing we can see that they are predominant at many of these locations, at both surface-level and farther down at the Deep Chlorophyll Maximum: the depth that contains the highest [chlorophyll] (Cullen 1982). Their size seems like it would prevent them from sinking to such depths, but they are documented as being able to aggregate during the stationary phase of their growth, enhancing their ability to fall through the water. A massive spring bloom of the genus has been recorded in the northwestern Mediterranean Sea, but they thrive in the southernmost parts of the globe as well: a study found that *Minidiscus chilensis* dominated in both abundance and volume in eastern Bransfield Strait, Antarctica with the species occupying 87% of the total diatom flux into the sediment trap at its highest (Leblanc et al. 2018, Kang et al. 2003).

The sparse literature on nanoplanktonic diatom response to temperature and pH demands replication and further experimentation. Previous experiments indicate that increased temperature and $p(CO_2)$ led to an increased relative abundance of *Minidiscus chilensis* (Cartaxana et al. 2015). While we can infer some success of the smaller diatoms under climate change conditions, there are limitations as to what we can draw from this study. One species of the genus is examined, the conditions are raised to 24°C and lowered to pH 7.4, but we learn about only relative abundance rather than growth: change in biomass is measured, but at only the community level, and *Minidiscus* is not the only genus in the community. A study that quantifies *Minidiscus* change in growth, specifically, is necessary. Diatom abundance, growth rate, and carbon export are not necessarily all positively correlated: the interplay between these factors is heavily dependent on processes such as aggregation, remineralization, and grazing by organisms like copepods (Toullec et al. 2019). Other experiments looking at different species of diatoms also lack in the scope of

their tests: Li et al. 2012 tests for atmospheric CO_2 concentrations only up to 950ppm, which does not even take us to SSP5 projected increases for end-of-century. Further testing must be done to achieve results consistent with projections. Another shows *Minidiscus* having relative success until a pH of 7.4 (Kaczmarska 2009). Pancic et al. 2015 tests diatoms around ten times as large as *Minidiscus* under pH conditions as low as 7.1, and temperature change as high as an 8°C increase. An experiment on *Thallassiosira pseudonana*, another species of centric diatom of slightly larger size, indicated positive growth increases with increasing temperature up to 24°C (Berges et al. 2002). Despite this valuable, the need to quantify a *Minidiscus trioculatus* population-level response to combined temperature and pH/pCO_2 as projected by high-emission scenario end-of-century ocean conditions remains.

This experiment seeks to quantify the change in growth rate that *Minidiscus trioculatus* exhibits under different abiotic conditions. More specifically, 1) how will the growth rate of an intermediate-scale culture of *Minidiscus trioculatus* be affected by changes to temperature and pH/pCO2 as projected by modeling of high-emission scenarios? 2) How will growth rate be affected by a combination of present-day pH and elevated temperature? 3) How will growth rate be affected by a combination of present-day temperature and lowered pH? 4) How will growth rate be affected by a combination of elevated temperature and lowered pH? And finally, 5) what does a least squares predictive model say about growth-rate change for parameter values between and outside of those tested? Diatom growth is a product of respiration and photosynthesis, which uptakes carbon from the surroundings into the cell. Therefore, growth rate will be used as a proxy for carbon uptake rate and subsequently the impact on the global carbon cycle. Changes in absorbance around the carotenoid maximum absorbance wavelength will be used, as carotenoids are the dominant pigments of this diatom species. Because the combination of expressed carotenoids varies between planktonic species, only *Minidiscus trioculatus* will be used in attempt to keep this ratio as similar as possible (Kuczynska et al. 2015). I will perform a two-way Analysis of Variance (ANOVA) statistical test to determine if pH/temperature levels are associated with significantly different absorbance, and if the relationships between temperature and absorbance depend on the pH level. More specifically, the hypotheses that accompany this test hypotheses are that (1) the means of temperature are not equal, (2) the means of pH are not equal, and (3) there is an interaction between temperature and pH. The corresponding null hypotheses are that (1) there

is no difference in the means of temperature, (2) there is no difference in means of pH, and (3) there is no interaction between temperature and pH.

METHODS

Generating diatom cultures

I purchased two 15 mL starter cultures of *Minidiscus trioculatus* from the National Center for Marine Algae and Microbiota's Bigelow Laboratory for Ocean Sciences. The stock culture was cryopreserved on March 14th, 2000 using 10% DMSO as a cryoprotectant. I subcultured the starter cultures per the included instructions: ~13.5°C, alternating between light and dark at a 13:11-hour ratio using a single 43-watt incandescent lightbulb. I created growth media by pasteurizing seawater collected from the ocean at Morro Bay, California at ~80°C for two hours, and then adding concentrated Guillard's f/2 growth media at a 1 mL concentrate per 1 L seawater ratio (Daniel et al. 2012, Guillard & Ryther 1962, Guillard 1975). Additionally, I added a minute volume, below 0.1 mL, of 1.0M NaOH to bring the media to a pH of 8.1. The first subculture contained 5 mL from each starter culture and 20 mL growth media. The second subculture contained the 30 mL first subculture and an additional 90 mL growth media. The third subculture contained the 120 mL second subculture and an additional 600 mL growth media, which is sufficient volume to require aeration (Guillard 1975). I aerated using a filtered-air pump with tubing at an approximate rate of 3 bubbles per second. The time between each subculture transfer was ~72 hours.

Experimental design

I used a two-factor, full factorial design with temperature and pH as the factors. Each factor had a high and low level. These were 23.5 +/- 1°C and 13.5 +/- 1°C for temperature and 8.1 +/- 0.1 and 7.6 +/- 0.1 for pH, respectively. I set the "present-day" temperature value set as the temperature used to maintain the starter culture, and then increased that by 10°C to account for the largest changes in seasonal variability under the CMIP6 SSP5 end-of-century conditions (Kwiatkowski 2020). pH values were decided based on current ocean pH and the SSP5 end-of century projection. Ranges of uncertainty were added to reflect the inaccuracy of the parameter

control and measuring equipment. All possible combinations of temperature and pH were used as treatments, per design.

Experimental proceedings

To perform the experimental treatments, I stirred the third subculture to ensure random diatom distribution and then pipetted out 50 mL of solution. I then diluted said 50 mL with 200 mL of growth media in a beaker, stirred solution, and pipetted 25 mL into the homemade spectrophotometer, described in "Data collection", and recorded transmittance values. To obtain these values I took the difference between control (i.e. pure growth media) and diatom solution values for transmittance of blue light. I then returned the sample diatom solution to the beaker and repeated the process ten times to ensure an accurate mean absorbance value. I used the absorbance by cell pigments as proxy for change in total diatom growth, which has been shown to be comparable to the more traditional method of manual cell counting (Gross & Godhe 2012). I then returned the 25 mL sample to the beaker with the rest of the diluted solution, inserted a tube delivering pure carbon dioxide made from citric acid and baking soda to achieve desired pH, and brought the solution to the approximate desired temperature using a combination of a wine cooler and light bulbs. I alternated between light and dark at a 13:11-hour ratio using a single 43-watt incandescent lightbulb and checked pH and temperature regularly, adding carbon dioxide when necessary. Each treatment was cultivated in this manner for a total of 15 days, which is roughly the time required to accurately simulate an abrupt transition to a new pH and temperature regime (Hennon et al. 2013). I repeated this procedure four times: once for each treatment.

Data collection

I collected raw absorbance data from each diatom solution using a homemade spectrophotometer. This spectrophotometer makes use of an iPhone SE camera with the Color Analyzer app, lamp with a 43-watt incandescent bulb, colored paper with hex code #0000FF (490-450nm wavelength) to complement the dominant orange color of the diatoms, and an 8 cm pathlength in order to enhance spectroscopic sensitivity (Naik & D'Sa 2012). The accuracy of this tool was tested using dilutions of a known concentration of allura red dye and colored paper with hex code #00FF00 (560-520 nm wavelength). A R^2 value of 0.976 was obtained when plotting

absorbance vs. dye concentration. The numbers collected represent dimensionless change in absorbance (Δabsorbance) from the mean of the 1L mass culture samples to the mean of the experimental culture samples.

Analysis

I used a two-way Analysis of Variance (ANOVA) statistical test to determine if the differences in absorbance between treatments and control were significant. ANOVA also requires the results in question to be independent, of equal size, normally distributed, and to display homogeneity of variance. The first two requirements are satisfied by the experimental procedure. The normality of the distribution was tested for using the Jarque-Bera Test for Normality, which determines if the skewness and kurtosis of the data are significantly different from that of a normal distribution. Levene's Test for Homogeneity of Variance determined if the variance across groups is homogenous. All tests were ran using packages "tidyverse," "dplyr," "tseries," "car," and "multcomp" in R (Henry & Wickham 2020, Wickham et al. 2019, Trapletti & Hornik 2020, Fox & Weisberg 2019, Hothorn et al. 2008, R Core Team 2019).

Predictive model

I used a least squares model to predict diatom abundance under a range of temperature and pH conditions. This model is based on equation (1)

$$y = b_0 + b_A x_A + b_B x_B + b_{AB} x_A x_B \quad (1)$$

where y is either the predicted or measured Δabsorbance value, b_0 is a baseline constant, b_A is the main effect of temperature, x_A is the coded temperature value of either -1 or +1, b_B is the main effect of pH, x_B is the coded pH value of either -1 or +1, and b_{AB} is the two-factor interaction term. A -1 indicates ~13.5ºC or ~8.1 pH, whereas a +1 indicates ~23.5ºC of ~7.6 pH. Interaction means that the effect of one factor depends on the value, or level, of another factor. I ran four experimental trials, and so I created four separate equations (2), (3), (4), (5) corresponding to each trial:

$$y_1 = b_0 + b_A(-1) + b_B(-1) + b_{AB}(-1)(-1) \quad (2)$$

$$y_2 = b_0 + b_A(+1) + b_B(-1) + b_{AB}(+1)(-1) \quad (3)$$

$$y_3 = b_0 + b_A(-1) + b_B(+1) + b_{AB}(-1)(+1) \quad (4)$$

$$y_4 = b_0 + b_A(+1) + b_B(+1) + b_{AB}(+1)(+1) \quad (5)$$

I transformed the equations (2) through (5) into matrix format as shown in equation (6):

$$\begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{pmatrix} = \begin{pmatrix} 1 & -1 & -1 & +1 \\ 1 & +1 & -1 & -1 \\ 1 & -1 & +1 & -1 \\ 1 & +1 & +1 & +1 \end{pmatrix} \begin{pmatrix} b_0 \\ b_A \\ b_B \\ b_{AB} \end{pmatrix} \quad (6)$$

Finally, I used the mean Δabsorbance values from the four trials as well as the linear modeling with interaction function in R to find all four b values in equation (1), allowing for prediction of diatom growth under conditions not tested for.

RESULTS

Experimental data

All four experimental trials were able to satisfy the temperature and pH conditions as explained previously in Experimental design. The means and medians of Δabsorbance values for the trials conducted at 23.5ºC are negative. All four interquartile ranges are smaller than 0.3 in width, and none overlap with each other (Figure 1). The non-parallel lines connecting the means of the measured samples indicate possible interaction between factors.

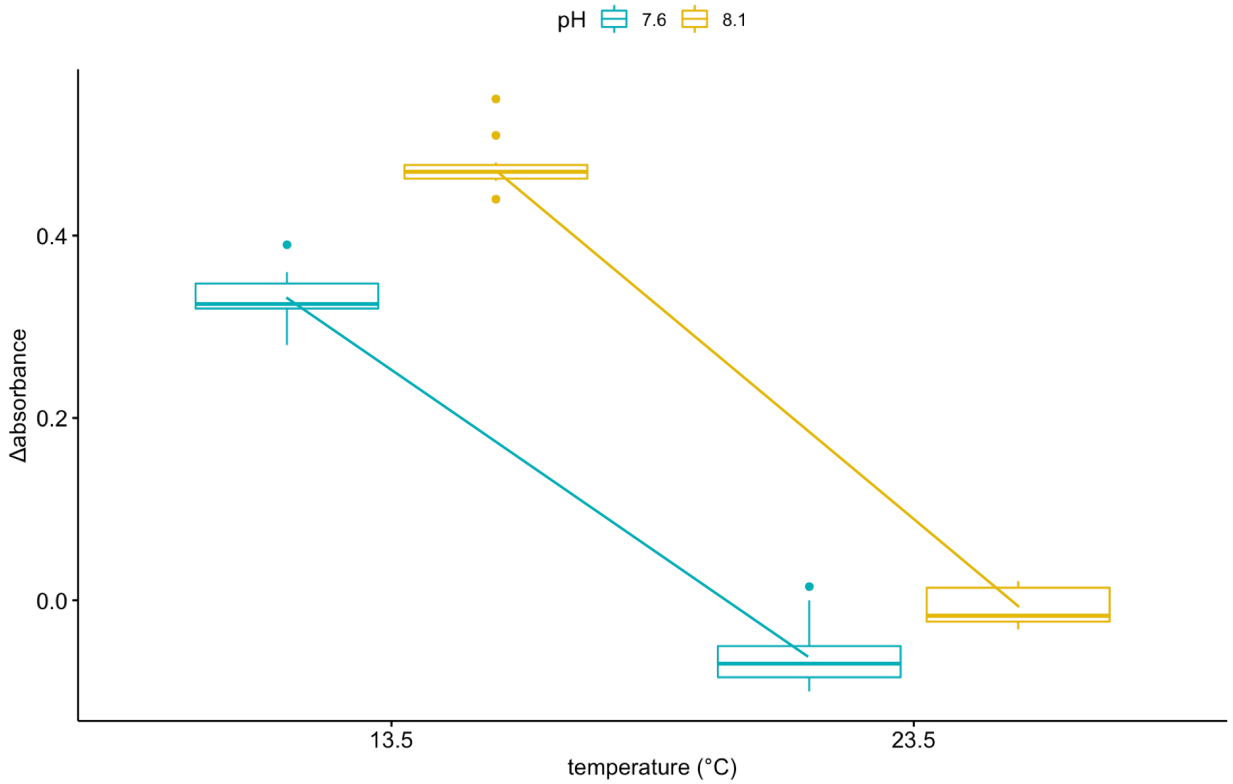

Figure 1. Boxplots of Δabsorbance in different temperature-pH treatments. The sample size of each box is 10, and temperature and pH are associated with significantly different Δabsorbance. The diagonal lines between boxes are non-parallel, and connect the means of the boxes.

Checking assumptions

Two-way ANOVA assumptions were met. Namely, the variance across groups is statistically insignificant, and the skewness and kurtosis of the data are not significantly different from that of a normal distribution.

Analysis of variance

Temperature, pH, and their interaction were all significant drivers ($p < 0.05$) of diatom abundance (Table 2). An increase in water temperature had a negative effect on diatom growth at both levels of pH, exhibiting ~0.49 and ~0.38 decreases in mean Δabsorbance at pH levels of 8.1 and 7.6, respectively. A decrease in pH had a negative effect on diatom growth at both temperatures, exhibiting ~0.16 and ~0.05 decreases in mean Δabsorbance at temperatures of

13.5ºC and 23.5ºC, respectively. Finally, the effect that pH had on Δabsorbance was dependent on the temperature of the diatom solution.

Table 2. Summary of the analysis of variance model. The Pr(>F) column indicates the probability of obtaining a F-value larger than the one resultant from the data if there were a random variable that varies in accordance with the F-curve, so it can be seen as a P-value.

	Degrees freedom	**Sum of squares**	**Mean square**	**F-value**	**Pr(>F)**
pH	1	0.0982	0.0982	102.7	4.36e-12
temp.	1	1.9219	1.9219	2009.6	< 2e-16
pH:temp.	1	0.0220	0.0220	23.0	2.80e-05
residuals	36	0.0344	0.0010		

Predictive model

I used the resultant data to complete the predictive model. I inserted the values for the four b variables into Equation (1), creating the final equation (6):

$$y = 0.18567 - 0.21932x_A - 0.05067x_B + 0.02432x_Ax_B \quad (6)$$

X_A values of -1 and 1 correspond to temperatures of 13.5ºC and 23.5ºC, respectively. X_B values of -1 and 1 correspond to pH values of 7.6 and 8.1, respectively. Additionally, a X_A value of 0 indicates 18.5ºC and a X_B value of 0 indicates 7.85 pH. Therefore, the equation predicts Δabsorbance of ~0.19 at 18.5ºC and a pH of 7.85 (Figure 2), ~0.42 at 8.5ºC and 7.35 pH, ~0.82 at 8.5ºC and 8.35 pH, and ~-0.26 at 28.5ºC and 7.35 pH (Figure 3).

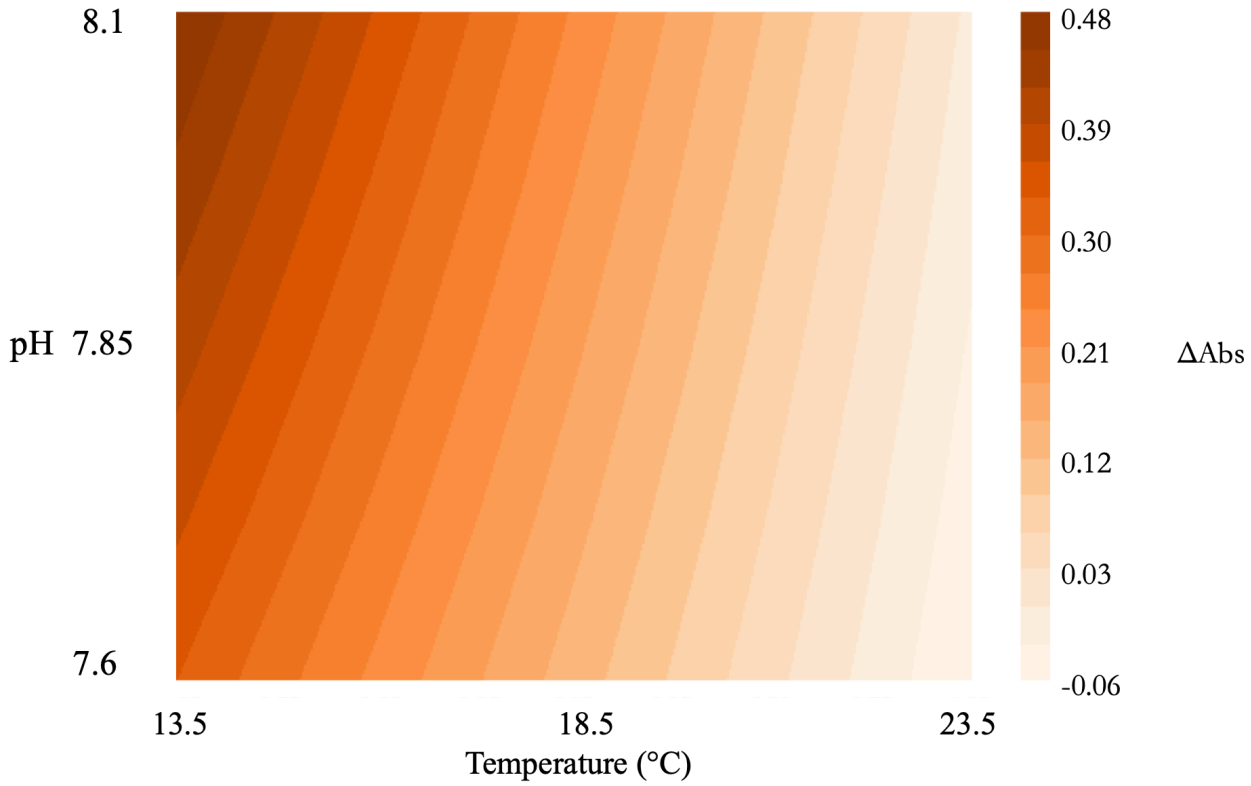

Figure 2. Predicted change in absorbance (ΔAbs) as a function of temperature and pH.

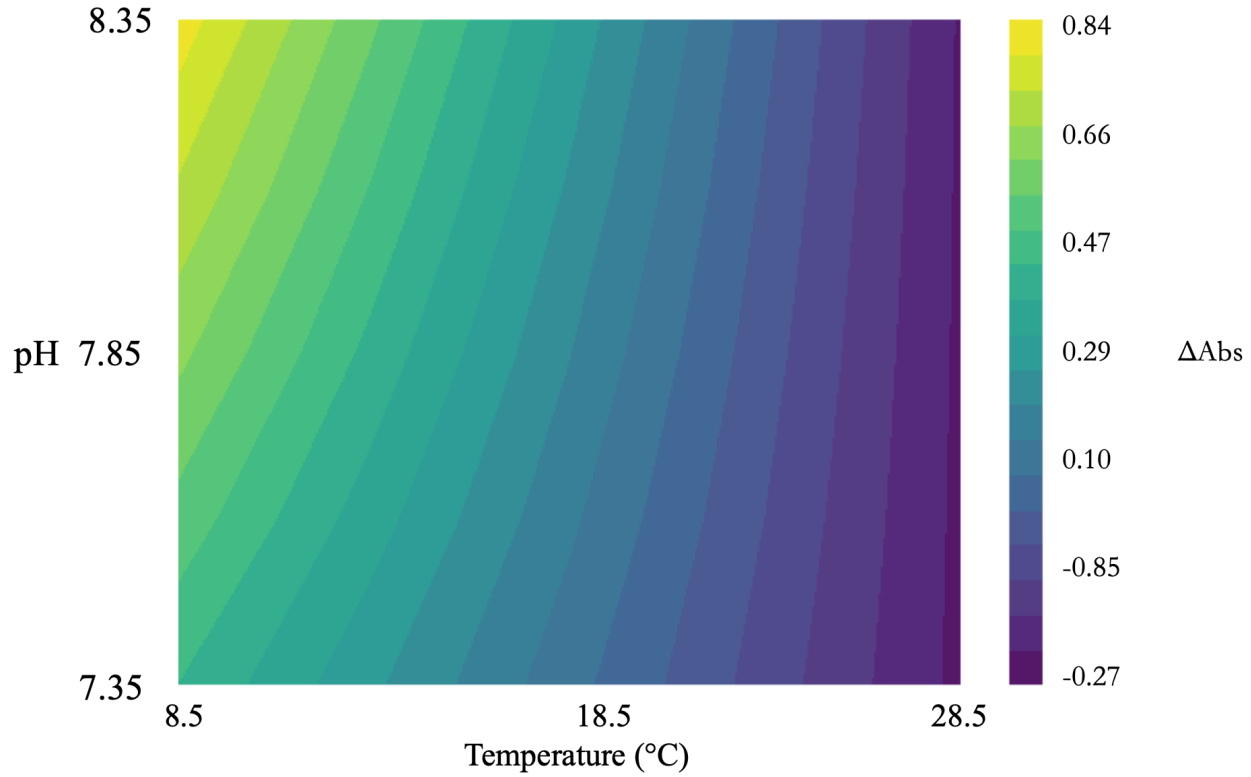

Figure 3. Predicted change in absorbance (ΔAbs) values as a function of temperature and pH.

DISCUSSION

The results from these four experimental trials provide clues to projected changes to the global carbon cycle. Multiple experiments on diatom growth rate in response to parameter variation have followed a full factorial design (de la Peña 2007, Everaert et al. 2016, Nagai & De Schamphelaere 2016, Saros & Fritz 2000). *Minidiscus* has been experimentally subjected to warmer and more acidic conditions before, but this is the first time that the response of an isolated *Minidiscus* population was quantified (Cartaxana et al. 2015). Despite the novel combination of species and experimental design, the outcome was predictable to some extent. Growth rate of the *Minidiscus trioculatus* population, measured by change in absorbance of blue light, was indeed significantly affected by changes to the temperature and pH of the growth media they were cultured in. The obtained results fall in line with fundamental molecular theory and are likely explained by protein denaturation. This is unsurprising, as enzymes have been shown to be dismantled by non-optimal temperature and pH many times over (Eed 2013, Jahromi et al. 2011). Present-day conditions proved to be best for this species, and increases to both the temperature and acidity at the same time stifled growth most effectively of all, with the least squares predictive model producing a negative interaction term between the two parameters.

Present-day pH and elevated temperature

The present-day pH and elevated temperature trial resulted in a slightly negative growth rate, indicating that diatoms fail to grow under these conditions. The difference between this result and the resultant change in absorbance for the trial with positive growth rate two trials suggests that temperature will have a greater effect on growth rate than pH in the future. At a molecular scale, there are very likely key enzymes failing at these parameter values that rely heavily on hydrogen-bonds and disulfide bridges to maintain the structure of their active sites (Xie et al. 2014, Siddiqui et al. 2005). It is very possible that these enzymes are completely inactivated rather than simply having their reaction rates decreased, because the population fails to expand its biomass whatsoever (Daniel et al. 2010). However, if the individual requires the enzymes in question to maintain a certain reaction rate to keep the cell alive, then the enzymes would not necessarily be completely inactivated. These spatially variable temperature increases may result in *Minidiscus*

trioculatus migrating to previously unoccupied areas, as centric diatom species did during the Cretaceous mass extinction (Armbrust 2009, Kwiatkowski 2020).

Present-day temperature and lowered pH

Present-day temperature and lowered pH resulted in growth rate significantly smaller than the trial with present-day temperature and pH, but the rate was still positive indicating that the diatoms were able to grow. This could be explained by enzymes key to the cell's metabolism not becoming completely inactivated by the electrostatic repulsive forces of the increased proton concentration (Yadav & Prakash 2011). It could also be explained by the increased concentration of carbon dioxide, and therefore also bicarbonate, available to these photosynthetic organisms as carbon dioxide is the mechanism used to alter pH; however, experiments on multiple species of centric diatom grown under different partial pressures of carbon dioxide exhibited only limited effects on the photosynthesis-mediating protein complexes, suggesting that the result of this experiment trial is explained by the resilience of enzymes to pH changes (Gao & Campbell 2014, Adamczyk et al. 2009).

Elevated temperature and lowered pH

The combination of both elevated temperature and lowered pH resulted in a growth rate smaller than any other trial. There is a significant interaction between the two factors as shown by the ANOVA results as well as the lines connecting the means of the trials; however, this interaction is mitigative, as the mean Δabsorbance for the trial would have to be smaller for the lines to be parallel (Figure 1). Despite this effect, the result is undoubtedly still bad news for the species, as the future sea surface will definitely change in those directions (Kwiatkowski 2020). The trial further supports the idea that this species might migrate, perhaps colonizing an area with little to no current diatom activity, in attempt to avoid this lethal combination of temperature and pH (Armbrust 2009). If not, the species would probably be quickly replaced by other species that are able to withstand the conditions (Gao & Campbell 2014).

Least squares predictive model

The least squares predictive model generated from the results of the full factorial design provides additional valuable insight as to change in absorbance would be for parameter values not tested for (Aldrich 2007). The resultant equation (6) predicting change in absorbance as a function of temperature and pH has valuable elements, most notably its near-zero, but positive, interaction term. This implies that temperature and pH combine to decrease growth rate less significantly than if both effects subtracted from the Δabsorbance value separately. Equation (6) provides a satisfactory view of roughly where *Minidiscus trioculatus* growth rate might be mid-century, mid-late-century, or otherwise (Figure 2) (Kwiatkowski 2020). Pushing the equation past the parameter bounds set by the experimental design, such as by predicting for 8.5ºC and 7.35 pH conditions, serves as a great reminder of the flaws inherent in the relative simplicity of the model: literature shows *Minidiscus* doubling times to be week-long at ~7 degrees C but the model shows growth rate increasing towards that temperature (Leblanc et al. 2018).

Limitations and Future Directions

My experiment is limited in that it is population-level growth rate that does not account for the community interactions that happen in the real world. For example, marine diatom *Thallasiosira gravida* was outcompeted by *Skeletonema costatum* and *Chaetoceros septentrionalis* when nutrient scarcity was introduced to the community via a chemostat (Mickelson et al. 1979). In the future, studies should sample communities from known geographical areas where *Minidiscus* is located, and test cell counts when exposed to probable nutrient limitations or excesses (Leblanc et al. 2018). Use of a chemostat/turbidostat/auxostat, other more advanced technology, and chemical engineering knowledge could improve upon my experiment by controlling aspects not only relating to nutrients but also parameters such as fugacity of carbon dioxide, forward light scatter, and flow cytometry likely to affect growth rate (Hennon et al. 2013).

Minidiscus trioculatus may make additional contributions to the climate and hydrological system through a different mechanism than simply photosynthesis: by producing dimethylsulfoniopropionate (DMSP) upon death (Stoer 2017). DMSP is frequently produced by diatoms, and when broken down into dimethyl sulphide by bacteria and oxidized into

methanesulphonate it acts as cloud condensation nuclei (CCN) (Dickschat et al. 2015). CCN assist in cloud formation and therefore increase the albedo of the planet, cooling the earth (Charlson et al. 1987). Whether or not *Minidiscus trioculatus* produces this chemical remains unknown, further reducing confidence about the impact this species will have on future climate.

Since my experiment does not consider the effect that aggregation of the diatom on the carbon export of the species, future experiments can determine the effect that climate change will likely have on the aggregation of nanoplanktonic diatoms like *Minidiscus trioculatus*. This research is a key piece of the puzzle, as aggregation is a main mechanism for carbon export to the deeper ocean as well as influencing a specie's capacity for regulation (Benoiston et al. 2017, Toullec et al. 2019, Cavicchioli et al. 2019). *Thalassiosira weissflogii* and *Skeletonema marinoi* both produce a larger aggregate when exposed to higher temperature and therefore could increase the sedimentation rate of carbon, effectively a negative feedback to global warming (Chen et al. 2021). Other research asserts that climate change's effect of increasing ocean stratification and reducing vertical mixing will favor death of diatoms and replacement by smaller phytoplankton, resulting in decrease in primary productivity as well as carbon (Kemp & Villareal 2013). However, they conclude that modeling is insufficient when the complexities of diatoms are considered and that further *in vivo* experimentation needs to happen to provide physiological data and that data then needs to be incorporated into models.

Broader Implications

Nanoplanktonic marine diatoms are important in the carbon cycle and also form a large portion of the base of ocean food webs (Armbrust 2009, Cavicchioli et al. 2019). Therefore, their success is connected to that of the rest of the ecosystem, ranging from other miniature grazing organisms to huge mammals useful for combatting climate change. For example, 160,000 tons per year of carbon could be removed from the atmosphere by restoring whale populations to pre-disturbance numbers (Pershing et al. 2010). Loss of a significant portion of the photosynthetic base of marine ecosystems, in systems where diatoms are significantly grazed, would effectively either collapse or reduce the biomass of the rest of the ecosystem, and therefore reduce the mass of carbon that the hypothetical system can pull out of the atmosphere (Benoiston et al. 2017). Unfortunately,

positive feedback to the loss of the ocean's ability to sequester carbon is a further positive feedback to climate change, with serious implications for humanity's future (Cavicchioli et al. 2019).

ACKNOWLEDGEMENTS

Funding and space to perform this experiment was provided by my kind parents, Susan and Joel Westwood. Kyle Leathers was my mentor and assisted with theory and design of the project, as well as editing of the paper. Additional thanks to Heather McNair, Lewis Westwood, Tina Mendez, Tara Seely, Sasha Mizenin, Zhenshan Guo, Matthew French, Christian Schwarzer, and Jake Peterson. Their varied support, ranging from biomolecular and laboratory knowledge to peer review to controlling experimental conditions during parts of the trial, made this all possible.

REFERENCES

Adamczyk, K., Prémont-Schwarz, M., Pines, D., Pines, E. and E.T.J. Nibbering. 2009. Real-time observation of carbonic acid formation in aqueous solution. Science 326(5960): 1690-1694.

Aké-Castillo, J., Hernández-Becceril, D.U., Meave del Castillo, M.E., and E. Bravo-Sierra. 2001. Species of *Minidiscus* (Baccillariophyceae) in the Mexican Pacific Ocean. Cryptogamie Algologie 22(1): 101-107.

Aldrich, J. 2007. Doing least squares: perspectives from Gauss and Yule. International Statistical Review 66(1):61-81.

Anderson, D.E., Becktel, W.J. and F.W. Dahlquist. 1990. pH-Induced denaturation of proteins: a single salt bridge contributes 3-5 kcal/mol to the free energy of folding of T4 lysozyme. Biochemistry 29(9):2403-2408.

Anson, M.L. 1945. Protein denaturation and the properties of protein groups. Advances in Protein Chemistry 2:361-386.

Armbrust, E.V. 2009. The life of diatoms in the world's oceans. Nature 459:185-192.

Benoiston, A.S, Ibarbalz, F.M., Bittner, L., Guidi, L., Jahn, O., Dutkiewicz, S., and C. Bowler. 2017. The evolution of diatoms and their biogeochemical functions. Philosophical Transactions Royal Society B 372:20160397.

Berges, J. A., Varela, D. E., and P. J. Harrison. 2002. Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). Marine Ecology Progress Series 225:139–146.

Cartaxana, P., Vieira, S., Ribeiro, L. Rui JM Rocha, Cruz, S., Calado, R. and J. Marques da Silva. 2015. Effects of elevated temperature and CO2 on intertidal microphytobenthos. BMC Ecology 15(10).

Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T., Crowther, T.W., Danovaro, R., Foreman, C.M., Huisman, J., Hutchins, D.A., Jansson, J.K., Karl, D.M., Koskella, B., Welch, D.B.M., Martiny, J.B.H., Moran, M.A., Orphan, V.J., Reay, D.S., Remais, J.V., Rich, V.I., Singh, B.K., Stein, L.Y., Stewart, F.J., Sullivan, M.B., van Oppen, M.J.H., Weaver, S.J., Webb, E.A. and N.S. Webster. 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews: Microbiology 17:569-586.

Charlson, R.J., Lovelock, J.E., Andreae, M.O. and S.G. Warren. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326:655-661.

Chen, J., Guo, K., Thornton, D.C. and Y. Wu. 2021. Effect of temperature on the release of transparent exopolymer particles (TEP) and aggregation by marine diatoms (*Thalassiosira weissflogii* and *Skeletonema marinoi*). Journal of Ocean University of China 20:56-66.

Cheng, L., Abraham, J., Hausfather, Z. and K.E. Trenberth. 2019. How fast are the oceans warming? Science 363(6423):128-129.

Cullen, J.J. 1982. The deep chlorophyll maximum: comparing vertical profiles of chlorophyll a. Canadian Journal of Fisheries and Aquatic Sciences 39(5):791-803.

Daniel, A., Kérouel, R. and A. Aminot. 2012. Pasteurization: a reliable method for preservation of nutrient in seawater samples for inter-laboratory and field applications. Marine Chemistry 128-129:57-63.

Daniel, R.M., Dines, M. and Petach, H.M. 1996. The denaturation and degradation of stable enzymes at high temperatures. Biochemical Journal 317(1):1-11.

Daniel, R.M., Peterson, M.E., Danson, M.J., Price, N.C., Kelly, S.M., Monk, C.R., Weinberg, C.S., Oudshoorn, M.L. and C.K. Lee. 2010. The molecular basis of the effect of temperature on enzyme activity. Biochemical Journal 425(2):353-360.

de la Peña, M.R. 2007. Cell growth and nutritive value of the tropical benthic diatom, *Amphora* sp., at varying levels of nutrients and light intensity, and different culture locations. Journal of Applied Phycology 19:647-655.

Dickschat, J.S., Rabe, P. and C.A. Citron. 2015. The Chemical Biology of Dimethylsulfoniopropionate. Organic & Biomolecular Chemistry 7(13).

Eed, J. 2013. Factors affecting enzyme activity. ESSAI 19(10):49-51.

Everaert, G., De Laender, F., Claessens, M., Baert, J., Monteyne, E., Roose, P., Goethals, P.L.M. and C.R. Janssen. 2016. Realistic environmental mixtures of hydrophobic compounds do not alter growth of a marine diatom. Marine Pollution Bulletin 102(1):58-64.

Farooqui, A., Aggarwal, N., Jha, N. and Binita Phartiyal. 2015. Oldest Record of Freshwater Diatom Frustules in Tests of Permian thecamoebians: Faithfulness of Sedimentary Record. International Journal of Current Microbiology and Applied Sciences 4(7):472-485.

Gao, K. and D. A. Campbell. Photophysiological responses of marine diatoms to elevated CO2 and decreased pH: a review. Functional Plant Biology 41:449–459.

Gross, S. and A. Godhe. 2012. Optical density as a tool for measuring diatom growth in vivo. International Diatom Symposium 22:167.

Guillard, R.L. and J.H. Ryther. 1962. Studies on marine planktonic diatoms *I. Cyclotella nana Hustedt* and *Detonula confervacea (Cleve) Gran*. Canadian Journal of Microbiology 8:229-239.

Guillard, R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. Culture of Marine Invertebrate Animals 29-60.

Hennon, G.M., Quay, P., Morales, R.L., Swanson, L.M. and E.V. Armbrust. 2013. Acclimation conditions modify physiological response of the diatom *Thalassiosira pseudonana* to elevated CO2 concentrations in a nitrate-limited chemostat. Journal of Phycology 50:243–253.

Henry, L. and H. Wickham. 2020. Rlang: functions for base types and core rand 'tidyverse' features. R package version 0.4.10.

Hönisch, B., Ridgwell, A., Schmidt, D.N., Thomas, E., Gibbs, S.J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R.C., Greene, S.E., Kiessling, W., Ries, J., Zachos, J.C., Royer, D.L., Barker, S., Marchitto Jr., T.M., Moyer, R., Pelejero, C., Ziveri, P., Foster, G.L. and B. Williams. 2012. The geological record of ocean acidification. Science 335(6072):1058-1063.

Hothorn, T., Bretz, F., and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50(3):346--363.

Hunter, J. D. 2007. Matplotlib: a 2D graphics environment. Computing in Science & Engineering 9(3):90–95.

Jahromi, R.R., Morris, P., Martinez-Torres, R.J., and P.A. Dalby. 2011. Structural stability of E. coli transketolase to temperature and pH denaturation. Journal of Biotechnology 155(2):209-216.

Kaczmarska, I., Lovejoy, C., Potvin, M. and M. Macgillivary. 2009. Morphological and molecular characteristics of selected species of Minidiscus (*Bacillariophyta, Thalassiosiraceae*). European Journal of Phycology 44(4):461-475.

Kassambara, A. 2020. Ggpubr: 'ggplot2' based publication ready plots. R package version 0.4.0.

Kang, J., Kang, S., Kim, D. and D. Kim. 2003. Planktonic centric diatom Minidiscus chilensis dominated sediment trap material in eastern Bransfield Strait, Antarctica. Marine Ecology Progress Series 255:93-99.

Kemp, A.E. and T.A. Villareal. 2013. High diatom production and export in stratified waters – a potential negative feedback to global warming. Progress in Oceanography 119:4–23.

Kuczynska, P., Jemiola-Rzeminska, M., and K. Strzalka. 2015. Photosynthetic pigments in diatoms. Marine Drugs 13:5847-5881.

Leblanc, K., Quéguiner, B., Diaz, F., Cornet, V., Michel-Rodriguez, M., Durrieu de Madron, X., Bowler, C., Malviya, S., Thyssen, M., Grégori, G., Rembauville, M., Grosso, O., Poulain, J., Colomban de Vargas, and M. Pujo-Pay. 2018. Nanoplanktonic diatoms are globally overlooked but play a role in spring blooms and carbon export. Nature Communications 9:953.

Li, W., Gao, K., and J. Beardall. 2012. Interactive effects of ocean acidification and nitrogen-limitation on the diatom *Phaeodactylum tricornutum*. PLoS ONE 7(12): e51590.

Liu, W., Huang, X., Lin, J. and M. He. 2012. Seawater acidification and elevated temperature affect gene expression patterns of the pearl oyster *Pinctada fucata*. PLoS ONE 7(3): e33679.

Mickelson, M.J., Maske, H. and R.C. Dugdale. 1979. Nutrient-determined dominance in multispecies chemostat cultures of diatoms. Limnology and Oceanography 24(2): 298-315.

Nagai, T. and K.A.C De Schamphelaere. 2016. The effect of binary mixtures of zinc, copper, cadmium, and nickel on the growth of the freshwater diatom *Navicula pelliculosa* and comparison with mixture toxicity model predictions. Environmental Toxicology and Chemistry 35(11):2765-2773.

Naik, P., and E. D'Sa. 2012. Phytoplankton light absorption of cultures and natural samples: comparisons using two spectrophotometers. Optics Express 20(5): 4871-4886.

Pancic, M., Hansen, P. J., Tammilehto, A., and N. Lundholm. 2015. Resilience to temperature and pH changes in a future climate change scenario in six strains of the polar diatom *Fragilariopsis*. Biogeosciences, 12(14), 4235-4244.

Pershing, A., L. Christensen, N. Record, G. Sherwood, and P. Stetson. 2010. The impact of whaling on the ocean carbon cycle: why bigger was better. PLoS One 5(10): 1–9.

Saros, J.E. and Fritz, S.C. 2000. Changes in the growth rates of saline-lake diatoms in response to variation in salinity, brine type and nitrogen form. Journal of Plankton Research 22(6):1071-1083.

Siddiqui, K.S., Poljak, A., Guilhaus, M., Feller, G., D'Amico, S., Gerday, C. and R. Cavicchioli. 2005. Role of disulfide bridges in the activity and stability of a cold-active α-amylase. Journal of Bacteriology 187(17):6206-6212.

Toullec, J., Vincent, D., Frohn, L., Miner, P., Le Goff, M., Devesa, J., and B. Moriceau. 2019. Copepod grazing influences diatom aggregation and particle dynamics. Frontiers in Marine Science 6:751.

Trapletti, A. and K. Hornik. 2020. tseries: time series analysis and computational finance. R package version 0.10-48.

Vanderplas, J. T. 2017. Python data science handbook: essential tools for working with data. O'Reilly Media.

Watson, A.J., Schuster, U., Shutler, J.D., Holding, T., Ashton, I.G.C., Landschützer, P., Woolf, D.K. and L. Goddijn-Murphy. 2020. Revised estimates of ocean-atmosphere CO_2 flux are consistent with ocean carbon inventory. Nature Communications 11:4422.

Wickham, H., François, R., Henry, L., and K. Müller. 2019. Dplyr: a grammar of data manipulation. R package version 0.8.3.

Xie, Y., An, J., Yang, G., Wu, G., Zhang, Y., Cui, L. and Y. Feng. 2014. Enhanced enzyme kinetic stability by increasing rigidity within the active site. Journal of Biological Chemistry 289(11):7994-8006.

Yadav, J.K. and V. Prakash. 2011. Stabilization of α-amylase, the key enzyme in carbohydrates properties alterations, at low pH. International Journal of Food Properties 14(6):1182-1196.

APPENDIX

Table 1. Results from the 40 samples from four experimental trials.

Δabsorbance	Temperature (+/- 1°C)	pH (+/- 0.1)
-0.024	23.5	8.1
0.010	23.5	8.1
-0.013	23.5	8.1
-0.032	23.5	8.1
0.021	23.5	8.1
0.019	23.5	8.1
0.015	23.5	8.1
-0.026	23.5	8.1
-0.022	23.5	8.1
-0.021	23.5	8.1
0.47	13.5	8.1
0.55	13.5	8.1
0.47	13.5	8.1
0.47	13.5	8.1
0.44	13.5	8.1
0.48	13.5	8.1
0.46	13.5	8.1
0.47	13.5	8.1
0.51	13.5	8.1
0.46	13.5	8.1
-0.069	23.5	7.6
0.015	23.5	7.6
-0.098	23.5	7.6
0.00	23.5	7.6
-0.10	23.5	7.6
-0.089	23.5	7.6
-0.044	23.5	7.6
-0.071	23.5	7.6
-0.070	23.5	7.6
-0.069	23.5	7.6
0.32	13.5	7.6
0.39	13.5	7.6
0.34	13.5	7.6

0.36	13.5	7.6
0.28	13.5	7.6
0.32	13.5	7.6
0.32	13.5	7.6
0.31	13.5	7.6
0.33	13.5	7.6
0.35	13.5	7.6