

The effects of ocean acidification and dissolved nitrogen sources on the growth and elemental stoichiometry of the marine diatoms *Thalassiosira pseudonana* and *Thalassiosira weissflogii*

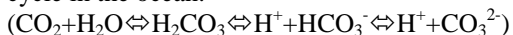
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Abstract:

The effects of ocean acidification to marine organisms are not well understood. Phytoplankton are an important component to the oceanic food chain and the effects of ocean acidification can have potential impact on the rest of the marine food web. Not only could the increase in carbon dioxide alone affect how phytoplankton grow, but increased carbon dioxide in the oceans can also have a direct effect on other chemicals in the ocean (i.e, nitrogen, phosphate, copper, zinc). Most of these chemicals are either major or minor nutrients needed by phytoplankton for growth. This experiment will investigate the effects of different carbon dioxide (pCO₂) exposure levels and different nitrogen compound treatments upon the diatoms *Thalassiosira weissflogii* and *Thalassiosira pseudonana*. Results of this research show that for both diatoms there was no significant difference in growth rate or the stoichiometry under different pCO₂ conditions; however, there was a significant difference for both species when the nitrogen compound was changed. Furthermore, there was a strong interaction between carbon dioxide and the nitrogen compound available for phytoplankton utilization for both species.

Introduction:

Ocean acidification is an issue that is not only directly influenced by our human global carbon dioxide (CO₂) output, is but also irreversible in our lifetime (The Royal Society, 2005). Dissolved CO₂ chemically responds in the ocean as an addition reaction to form carbonic acid (H₂CO₃). Hydrogen ions dissociate from this compound, lowering the ocean's pH, thus causing ocean acidification (Potera, 2010) The following equation demonstrates the carbon cycle in the ocean.



Marine ecosystems are put at risk of complete collapse by even the slightest change in pH scale (Wootten, 2008). Considering that the ocean's chemistry has recently been changing 100 times more rapidly than it has for 650,000 years (Feely, 2006), most, if not all marine organisms have been familiarized with a narrow ranged pH level for thousands of millennia and may react to the present changes occurring (The Royal Society, 2005). Our increasing CO₂ output has caused a ~0.1 decline of surface ocean pH from 8.2-8.1 since the Industrial Revolution (ESF, 2009). An estimated additional drop of 0.3- 0.4 pH is hypothesized by the year 2100 (Wootten, 2008). With pCO₂ levels steadily increasing, organisms will have to face an decrease in pH at rates historical not observed (Feely, 2006).

Ocean acidification is an obstacle that may prevent optimal nitrogen intake for the growth and well being of some marine phytoplankton species. The oxidation of ammonium to nitrate is decreased 3-44% by ocean acidification, in turn causing marine primary producers to depend upon ammonium in high pCO₂ low pH stress (Beman, 2010). Nitrification is a key process that will be affected by ocean acidification (Beman, 2010) and has not been brought to attention in comparison to dissolution of calcium carbonate of marine organisms. Nitrogen is necessary for building proteins that are used for cell function and structure (Bolin, 1977). Both ammonium and nitrate are fixed through the nitrogen cycle in the ocean and are necessary for the cell growth of numerous organisms (Bolin, 1977). Some marine algae favor ammonium and its easy pre-reduced state (Hernandez-Leon, 2008), while others have faster uptake rates with nitrate (Blankenship, 1998). Furthermore, for various phytoplankton species, the presence of both ammonium and nitrate can cause a negative inhibition or positive preference effects on nitrogen utilization rates (Dortch, 1990). To utilize nitrate, phytoplankton must transport it across the gradient of the cell and reduce it to ammonium through a series of reactions (Falkowski, 1975). This process and preferences of nitrogen compound are variable factors upon species and environmental conditions (Dortch, 1990).

Diatom algae play a vital role as a major primary producer, accounting for most of the total primary production in today's world oceans (Brzezinski, 1998). The chosen diatom algae for this research assessment upon their growth with

the presence of nitrogen treatments in different pCO₂ levels were *Thalassiosira pseudonana* and *Thalassiosira weissflogii*, belonging to the class Bacillariophyceae. Both species are centric diatoms (referring to cylindrical shape) that can be found locally amidst the surface of the Long Island Sound. *T. pseudonana* is a coastal and fresh water (Harris, 1995) species with a valve diameter of 2.5-15um (Siuda, 2010; Muylaert and Sabbe 1996; Peters, 2006; Harris, 1995) that gives the experiment the aspect of an organism that has shoreline adaptability to flocculating environments caused by coastal pollution and salinity levels. *T. weissflogii* is an estuarine species with a diameter of 11.7-32 um (Siuda, 2010; Kleppel, 1998; Johansen, 1987) that serves results of being physically larger and differently environmentally adapted than that of *T. pseudonana*.

This paper examines the effects of increased atmospheric pCO₂ combined with different nitrogen species availability for two marine diatoms *T. pseudonana* and *T. weissflogii*. Three null hypotheses were tested in this experiment. They are as follows: (1) Increased pCO₂ will not alter division rates and/or the elemental stoichiometry of the marine diatoms *T. pseudonana* and *T. weissflogii*. (2) Different nitrogen sources will not alter division rates and/or the elemental stoichiometry of the marine diatoms *T. pseudonana* and *T. weissflogii*.

(3) There will be no interaction between pCO₂ and different nitrogen sources for either *T. pseudonana* or *T. weissflogii*.

Materials and Methods:

Small but concentrated pre-acclimated cultures with Guillard's F/2 media were kept for both species in the three nitrogen treatments (Fig. 1). Media was sterile filtered (0.22 μm) with 400ml in each of the 1000 mL Corning® flasks. The original cap was replaced with an autoclaved cap that was assembled with silicon tubing and an air stone to allow CO₂ to be bubbled into the containers. The three sets of twelve for difference of nitrogen treatment were split again into four sets of three for each of the targeted pCO₂ exposure levels (Fig. 1). The media was pre-acclimated to each of the pCO₂ levels before the diatom species was added to the containers.

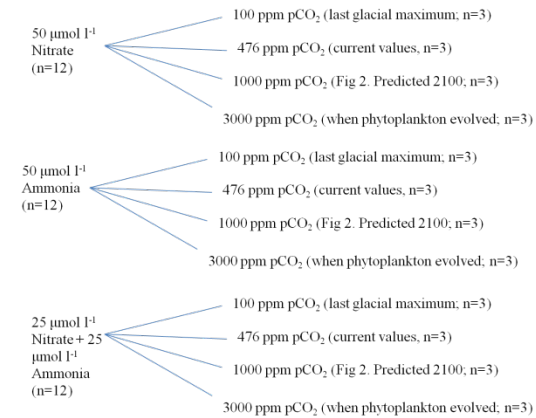


Figure 1. Schematic of the experimental design

Table 1. This table shows the initial measurements of cell counts, pH, DIC, nutrients, and nitrogen compounds along with calculated alkalinity and pCO₂ from CO₂SYN. The nitrate and ammonia values reflect the three nitrogen treatments and are ordered just as Figure 1. Four 50ml samples representing each pCO₂ exposure level were analyzed for this information

Initial Samples	cell counts	DIC (uM)	pH	Calculated Alkalinity	Calculated pCO ₂	Silicate	Phosphate	Nitrate	Ammonia
Species	cells/ml	(umol kg ⁻¹)		(umol kg ⁻¹)*	(ppm)*	(umol kg ⁻¹)	(umol kg ⁻¹)	(umol kg ⁻¹)	(umol kg ⁻¹)
<i>Thalassiosira weissflogii</i>			Mean value ± S. E.						
	737 ± 24	1655	8.41	2028	132	67.73 ± 3.53	8.13 ± 1.34	19.17	0.21
		1859	8.01	2033	408			0.00	109.07
		1958	7.78	2054	746			38.30	66.07
		2139	7.21	2073	3004				
<i>Thalassiosira pseudonana</i>									
	923 ± 44	1654	8.38	2016	143	28.56 ± 0.67	18.47 ± 0.07	62.43	0.83
		1839	7.98	2010	434			0.00	149.15
		1936	7.74	2028	811			34.62	83.56
		2108	7.24	2092	2811				

Inoculation of the diatoms species was 734 ± 24 cell ml⁻¹ for *T. weissflogii* and 923 ± 44 cell ml⁻¹ for *T. pseudonana*. Each species was grown for five-days at 18°C and a light intensity 180

$\mu\text{Einst. m}^{-2}\text{s}^{-1}$, with cell counts conducted each day. Final samples were collected for pH, DIC (dissolved inorganic carbon), and cell counts. Four pre-combusted GF/F filters for nutrient analysis, biogenic silica, particulate organic phosphorous, and C:N ratios were taken from each culture for future analysis. Data from nutrient filters was obtained through with a Quattro (Seal Analytical, WI), particulate organic phosphorous and biogenic silica filters were recorded by spectrophotometer readings, and C:N filters were analyzed through flash combustion with an elemental analyzer (Costech ECS 4010, Velencia, CA).

Results:

With respect to pCO_2 for *T. weissflogii*, there was no significant difference in division rate and the stoichiometry of C:N and N:Si; however there was a significant difference in P:Si (Table 3). The amount of carbon, nitrogen, and silica per cell was not significant, but the amount of phosphorous fmol/cell contained a significant decrease 124.52-75.76 fmol/cell with increasing 63-3507 ppm pCO_2 (Table 3,5). Table 4 shows that P:Si decreased 0.07-0.04 fmol/cell with the increase in pCO_2 exposure 63-3507 ppm (Fig 5). Unlike pCO_2 , there were more significant differences with respect to nitrogen treatment for *T. weissflogii*. As with the pCO_2 treatment, division rate was not affected with the nitrogen

source, however the stoichiometry was affected especially with respect to nitrogen (Table 3). Ammonia as the source of nitrogen caused the nitrogen pmol/cell to be significantly less than nitrate with a 1.04 pmol/cell difference (Table 4). This ammonia reaction also influenced significantly lower values for N:Si and N:P along with a highest value for C:N.

Unlike *T. weissflogii*, *T. pseudonana* did have significance in division rate. The presence of ammonia for uptake increased division rate 0.15 - 0.18 d^{-1} more than nitrate (Table 5). The amount of silica, phosphorous, nitrogen, and carbon per cell all contained significantly higher values from the nitrate and low values from the ammonia treatment for uptake (Table 5). N:Si and N:P ratios were significant for ammonium treatment as the highest yielding value (Table 4). Nitrogen pmol/cell and C:N both contained a significant interaction for *T. pseudonana* (Table 3). Nitrogen pmol/cell was significant for 267ppm pCO_2 and ammonia treatments, both yielding the least nitrogen pmol/cell (Table 5, Fig 2). The C:N interaction for *T. pseudonana* displays significance of ammonia yielding the least ratio (Table 4) with 267ppm pCO_2 following the same trend (Fig 3). C:N was enriched by nitrate presence (Table 4), however, increased pCO_2 decreased C:N under the nitrate system (Fig 3).

	<i>Thalassiosira pseudonana</i>			<i>Thalassiosira weissflogii</i>		
	pCO_2	N trmt	int.	pCO_2	N. trmt	int.
C cell ⁻¹	0.03	0.02	0.83	0.91	0.10	0.58
N cell ⁻¹	0.02	<0.01	0.03	0.62	<0.01	0.41
POP cell ⁻¹	0.54	0.04	0.38	0.01	0.26	0.04
Si cell ⁻¹	0.06	<0.01	0.35	0.61	0.60	0.13
Division Rate (d ⁻¹)	0.13	<0.01	0.09	0.68	0.94	0.55
C:N	0.80	0.03	0.03	0.62	<0.01	0.08
N:Si	0.62	0.03	0.77	0.66	<0.01	0.53
P:Si	0.50	0.96	0.75	<0.01	0.44	0.04
C:Si	0.87	0.45	0.54	0.88	0.15	0.32
C:P	0.59	0.59	0.39	0.08	0.54	0.47
N:P	0.61	0.04	0.97	0.29	<0.01	0.80

Table 3. (Left). A MANOVA using pCO_2 and nitrogen treatment (N trmt) was run on all the dependent variables (left side). Color differences indicated a significant difference. They are as follows: nitrogen treatment blue; both pCO_2 and nitrogen treatment, and/or an interaction yellow.

Species	pH	DIC	Calculated pCO_2	Calculated Alkalinity
		($\mu\text{mol kg}^{-1}$)	(ppm)*	($\mu\text{mol kg}^{-1}$)*
<i>Thalassiosira weissflogii</i>				
Mean value \pm S.E.				
	8.64 \pm 0.02 ^a	1493 \pm 12 ^a	63 \pm 3	1683 \pm 7
	8.04 \pm 0.01 ^b	1869 \pm 14 ^b	382 \pm 16	1949 \pm 13
	7.75 \pm 0.01 ^c	1973 \pm 10 ^c	807 \pm 7	2024 \pm 10
	7.15 \pm 0.02 ^d	2185 \pm 9 ^d	3507 \pm 129	2206 \pm 10
<i>Thalassiosira pseudonana</i>				
	8.88 \pm 0.05 ^a	1283 \pm 40 ^a	29 \pm 5	1532 \pm 29
	8.49 \pm 0.01 ^b	1572 \pm 76 ^b	131 \pm 33	1733 \pm 56
	8.18 \pm 0.06 ^c	1740 \pm 45 ^c	267 \pm 32	1842 \pm 37
	7.30 \pm 0.10 ^d	2128 \pm 19 ^d	2724 \pm 317	2163 \pm 24

Table 2. (Right). Measured pH and DIC values from the experiment, with calculated pCO_2 and alkalinity from CO2SYS. An ANOVA test found that there was a significant difference between treatments ($p < 0.01$).

	C:N	C:P	C:Si	N:P	N:Si	P:Si
pCO ₂ level (ppm)						
<i>T. weissflogii</i>						
63						0.07 ± 0.01 ^a
382	15.05 ± 0.94	226.08 ± 14.60	10.81 ± 0.66	16.48 ± 1.13	0.83 ± 0.08	0.05 ± 0.01 ^b
807						0.04 ± 0.01 ^{bc}
3507						0.04 ± 0.01 ^c
<i>T. pseudonana</i>						
131						
267	7.27 ± 0.15	175.3 ± 5.05	2.71 ± 0.07	24.18 ± 0.65	0.37 ± 0.01	0.02 ± 0.01
2724						
Nitrogen Treatment						
<i>T. weissflogii</i>						
nitrate	12.7 ± 1.66 ^a			20.02 ± 2.00 ^a	1.11 ± 0.20 ^a	
ammonium	20.4 ± 1.23 ^b	226.08 ± 14.60	10.81 ± 0.66	10.66 ± 0.96 ^b	0.48 ± 0.04 ^b	0.05 ± 0.003
both	12.05 ± 0.67 ^a			18.78 ± 1.68 ^b	0.89 ± 0.10 ^a	
<i>T. pseudonana</i>						
nitrate	7.31 ± 0.014 ^{ab}			23.1 ± 0.82 ^a	0.35 ± 0.01 ^a	
ammonium	6.86 ± 0.18 ^a	226.08 ± 14.60	10.81 ± 0.66	26.62 ± 0.94 ^b	0.41 ± 0.01 ^b	0.05 ± 0.01
both	7.65 ± 0.26 ^b			22.81 ± 0.80 ^a	0.37 ± 0.02 ^a	

Table 4.(Left) Elemental stoichiometry ratios for both species *T. weissflogii* and *T. pseudonana*.

Discussion

Thalassiosira pseudonana:

From the ammonium and both treatments resulting in the highest division rates (Table 5), it is reasonable to say that *Thalassiosira pseudonana* prefers the presence of ammonium for growth. There was a 0.15-0.18d⁻¹ difference between the nitrate induced low rate and the highest rates from the other two ammonia-involved treatments (Table 5). The pCO₂ level along with nitrogen treatments played a role with a significance in nitrogen and carbon pmol/cell elemental composition leading to an interaction for C:N (Fig. 5). It can be suggested that the division rates have an impact upon the results of the C:N increase for the nitrate treatment. Increase carbon and nitrogen in the cell could be oriented to enzymatic pathways (Amy, 1974) or morphology adjustments (Armburst, 2004) as a response to an unfavorable condition.

In terms of elemental composition, nitrogen, phosphorous (influencing N:P, Table 4), carbon, and silica (influencing N:Si, Table 4) all concluded with the NH₄ and 'both' treatments with lower values than NO₃ (Table 5). The relationship between these significant data points and the division rate is evident. Low concentrations of carbon, nitrogen, phosphorous, and silica are tied with the high division rates from NH₄ presence. From this data it is concluded that *T. pseudonana* does not

	Division Rate (d ⁻¹)	C (nmol cell ⁻¹)	N (nmol cell ⁻¹)	Si (fmol cell ⁻¹)	P (fmol cell ⁻¹)
pCO ₂ Treatment					
<i>T. weissflogii</i>					
63					124.52 ± 14.65 ^a
382	1.12 ± 0.03	20.63 ± 0.98	1.55 ± 0.13	1991.58 ± 76.99	117.83 ± 23.58 ^b
807					82.50 ± 6.03 ^b
3507					75.76 ± 5.73 ^b
<i>T. pseudonana</i>					
131		1.33 ± 0.04 ^a	0.18 ± 0.01 ^a		
267	1.54 ± 0.02	1.17 ± 0.06 ^b	0.16 ± 0.01 ^b	426.36 ± 15.27	7.22 ± 0.28
2724		1.23 ± 0.05 ^{ab}	0.17 ± 0.01 ^a		
Nitrogen Treatment					
<i>T. weissflogii</i>					
nitrate	1.12 ± 0.03	20.63 ± 0.98	2.06 ± 0.30 ^a	1991.58 ± 76.99	100.16 ± 7.56
ammonium			1.02 ± 0.07 ^b		
both			1.57 ± 0.13 ^a		
<i>T. pseudonana</i>					
nitrate	1.43 ± 0.01 ^a	1.36 ± 0.04 ^a	0.19 ± 0.01 ^a	485.40 ± 19.00 ^a	8.14 ± 0.37 ^a
ammonium	1.58 ± 0.02 ^b	1.17 ± 0.05 ^b	0.17 ± 0.01 ^b	382.34 ± 8.64 ^b	6.47 ± 0.30 ^b
both	1.61 ± 0.02 ^b	1.20 ± 0.05 ^b	0.16 ± 0.01 ^b	411.32 ± 30.81 ^b	7.04 ± 0.40 ^{ab}

Table 5. (Right) Division rate and Elemental stoichiometry per cell for both species *T. weissflogii* and *T. pseudonana*.

efficiently grow or utilize elements for growth with exclusive NO₃ presence for nitrogen compound uptake. The NO₃ presence influences more C, N, P, and Si per cell. This suggests that the NO₃ treatment pressures *T. pseudonana* to readily store elemental components then promptly metabolizing them for division rates as the cultures did for NH₄. C, N, Si, and P per cell concentrations could have been related to the fact that *Thalassiosira* species contain high silica and nitrogen chitin fibers from their frustule that assist floatation to improve nutrient intake in unfavorable conditions (Armburst, 2004). This is a supporting factor that demonstrates silica and nitrogen per cell in higher quantities as a natural attempt to manipulate structural properties to acquire more nutrients in the NO₃ treated cultures. In a chemical aspect, *T. pseudonana* utilizes an enzyme pathway containing nitrate reductase (NR) for assimilating NO₃ across the gradient membrane and oxidizing it to usable NH₄ (Amy, 1974). It is safe to say that in the exclusive NO₃ treatments, *T. pseudonana* would produce more nitrate reductase than for the NH₄ or 'both' treatments. Considering the division rate and the elemental components, the already oxidized state of NH₄ along with current pCO₂ levels are favored by *T. pseudonana*.

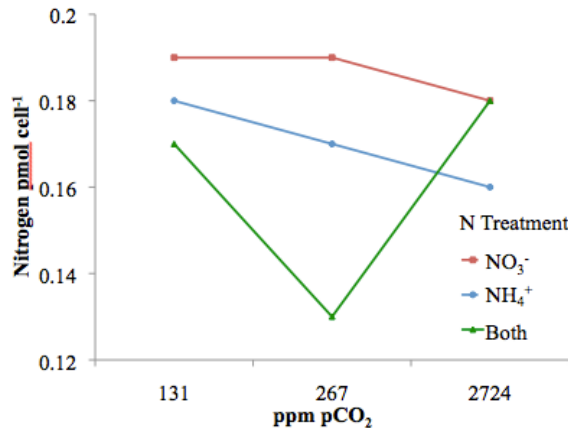


Figure 2. (Left) Interaction plot for the resulting nitrogen pmol/cell-1 values of *Thalassiosira pseudonana*.

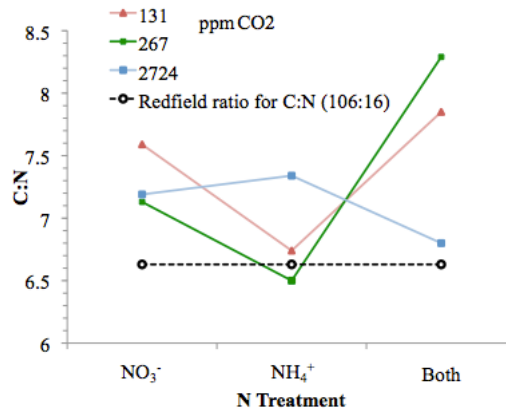


Figure 3. (Right) Interaction plot for the resulting C:N values of *Thalassiosira pseudonana*.

Thalassiosira weissflogii:

Thalassiosira weissflogii did not contain a change in division rate for either of the pCO₂ or nitrogen treatment stresses. Elemental composition, on the other hand, was impacted. Unlike *T. pseudonana*, *T. weissflogii* had a significant interaction with nitrogen treatments and pCO₂ exposure levels on its elemental stoichiometry. The interaction shows that as pCO₂ increased, the phosphorous in the cell decreased in all nitrogen treatments. This data can be reviewed in Figures 4 and 5 for particulate inorganic phosphorous fmol/cell and P:Si ratio. It can be suggested from this data that the mechanisms of the *T. weissflogii* cells for intake of phosphorous were altered by the increase in pCO₂.

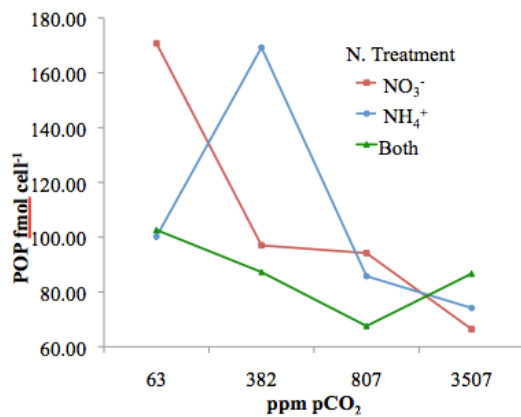


Figure 4.(Left) Interaction plot for the particulate inorganic phosphorous values of *Thalassiosira weissflogii*.

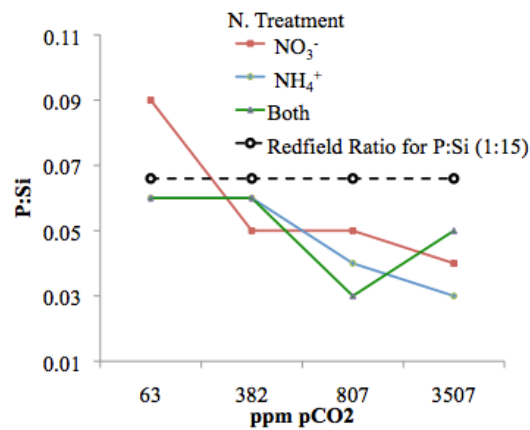


Figure 5.(Right) Interaction plot for the P:Si values of *Thalassiosira weissflogii*.

Like *T. pseudonana*, *T. weissflogii* contained trends of elemental stoichiometry with nitrogen treatments. Nitrogen per cell resulted with lowest values at the NH₄ treatment (Table 5) which influenced the outcomes of N:Si, N:P, and C:N (Table 4). Nitrogen content can be potentially influenced by the use of either nitrate reductase and/or urease utilized in assimilating the nitrogen compounds differently. From past research *T. weissflogii* has shown behaviors of significantly increased nitrate reductase concentrations in NO₃ treatments over NH₄ to allow the cell to take NO₃ across the cell gradient (Lomas, 2004). This reinforces the concluded data for NO₃ yielding the highest nitrogen per cell values for *T. weissflogii*.

Conclusion:

Ocean acidification aspects do introduce impacts to the marine diatoms *T. pseudonana* and *T. weissflogii*. It is evident from this research that diatoms do contain a preference of the ammonium nitrogen compound for assimilation and are acclimated to the contemporary ppm pCO₂ levels. Considering the natural effects of dissolved carbon dioxide and nitrogen compound availability for diatoms (Beman, 2010), the resulting data from this experiment provides a foundation for future research upon potential risks to diatoms if the conditions we see today worsen. Further research can explore the data behind the elemental

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stoichiometry recorded in this research (Tables 4 and 5) to sources of physiological and morphological traits of diatoms as an explanation behind the recorded variations of this study.

	Division Rate pCO ₂	Division rate N Source	Stoichiometry pCO ₂	Stoichiometry N source	Interaction between pCO ₂ and N
<i>T. weissflogii</i>	Null Hypothesis 1 is accepted	Null Hypothesis 2 accepted	Null Hypothesis 1 rejected (P:Si ratio)	Null Hypothesis 2 rejected (C:N, N:P, N:Si)	Null Hypothesis 3 Rejected (P:Si)
<i>T. pseudonana</i>	Null Hypothesis 1 accepted	Null Hypothesis 2 rejected	Null Hypothesis 1 accepted	Null Hypothesis 2 rejected (C:N, N:P, N:Si)	Null Hypothesis 3 Rejected (C:N)

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Biography:

Sam Gurr is currently a junior at the University of New Haven majoring in Marine Biology with the intent on a minor in Chemistry. This is Sam's first experience with a scientific research project and he hopes to continue conducting applied research in his career future in order to improve

knowledge and awareness of the growing issues of today's ocean. Sam's interest in marine biology was ignited at a young age from the influence of the rocky shorelines and lush low tide communities of New Haven's Long Island Sound. He enjoys exploring the same local beaches of his hometown that sparked his curiosity in marine science.

