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優勝作品專輯

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參展科別　地球與環境科學

作品名稱　Investigating the Effects of Temperature and Carbon Dioxide Levels on Nannochloropsis oceanica Using a Hemocytometer Counting Method

得獎獎項　一等獎

美國國際科技展覽會 ISEF

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關鍵詞　Ocean acidification、Global warming、Phytoplankton

作者簡介

We are Angela Yao and Viki Chiang, ninth-grade students currently enrolled in the International Program at Kang Chiao International School-Hsinchu Campus. We are excited to participate in the 2025 Taiwan International Science Fair, where we can showcase our passion for environmental science to address the challenges that our planet facing. Climate change is an urgent issue that requires immediate action to mitigate its impacts, and the greenhouse effect and ocean acidification are the dominant issues. Our research for this science fair focused on *Nannochloropsisoceanica*, a vital microalgae species. We learned how even the smallest organisms can significantly impact the environment and contribute to an unbalanced ecosystem. This study has also made us realize the profound negative effects of climate change. Our experience has reinforced our understanding of the importance of protecting and preserving our planet.

附件三

研究報告封面

<u>　2025　</u>年臺灣國際科學展覽會

研究報告

區別：

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關鍵詞：<u>Ocean acidification</u>、<u>Global warming</u>、<u>Phytoplankton</u>（最多三個）

編號：
（編號由國立臺灣科學教育館統一填列）

Investigating the Effects of Temperature and Carbon Dioxide Levels on

Nannochloropsis oceanica **Using a Hemocytometer Counting Method**

Abstract

Climate changes that include ocean acidification and global warming are serious problems in the ecosystem, affecting marine phytoplankton, including *Nannochloropsis oceanica*. In the effort to further explore the impact of rising temperature and carbon dioxide (CO_2) concentrations on oceanic ecosystems, the phytoplankton *Nannochloropsis oceanica* was used as a model organism. This study explored the effect of temperature change and CO_2 concentration on the growth of *Nannochloropsis oceanica*, achieving 243 samples that were tested with three different temperatures (24 degrees Celsius (°C), 28°C, 32°C) and CO_2 concentrations (0 milliliter (ml)/min, 0.4 ml/min, 0.6 ml/min), utilizing a hemocytometer counting method. Results indicate that the CO_2 concentration has a significant effect on the population of *Nannochloropsis oceanica*. But the temperature doesn't affect a lot. The *Nannochloropsis oceanica* in the lowest temperature and highest concentration of CO_2 in its environment had the highest population growth, and in the highest temperature and lowest concentration of CO_2, it had the lowest population growth. Results show the serious negative effect of climate change on the ecosystem and the importance of environmental protection. Population blooms due to excess CO_2 taking up ocean resources causing dangerous ecological imbalances.

海洋酸化和全球暖化是引發生態系統變化的重要驅動因素，其中包含浮游植物和此次實驗對象- *Nannochloropsis oceanica*。本次實驗針對*Nannochropsis oceanica* 生長環境的溫度以及二氧化碳濃度 (CO_2) 進行為期一週的環境更動，包含三種溫度 (24 攝氏度 (°C), 28°C, 32°C) 和三種CO_2濃度(0 毫升 (ml)/min, 0.4 ml/min, 0.6 ml/min)，並利用血球計數方法計算總共243個樣本的細胞量。結果顯示，CO_2濃度對於*Nannochloropsis oceanica*的生長有顯著影響，但溫度的影響不大。*Nannochloropsis oceanica* 在生長環境溫度24°C, CO_2 濃度為0.6 ml/min的條件下，有最高的平均生長量與最低的標準差。結果表明，作為氣候變遷的一部分，海洋酸化和全球暖化可能通過浮游植物影響群體，造成生態失衡，對海洋生態系統產生重大負面影響。

1. Introduction

1.1 Motivation

Our interest in CO_2, a key greenhouse gas contributing to the greenhouse effect, began during a science class discussion on ocean acidification. Ocean acidification results from human activities that release extra CO_2 into the atmosphere (National Oceanic and Atmospheric Administration, 2024). The oceans absorb much of this CO_2, disrupting the carbon cycle. This additional CO_2 will change the balance of marine ecosystems by changing essential nutrients and elements that support marine life. As a result, many marine organisms struggle to survive, specifically those that rely on calcium carbonate to form their hard skeletons and shells, as the availability of carbonate decreases (U.S. Environmental Protection Agency, n.d.). Further research focuses on the global carbon system, leading to a critical question: What are the effects of rising CO_2 concentrations and increasing temperatures on marine environments? According to San José State University, this question has focused on phytoplankton, organisms that play a key role in the short-term carbon cycle.

1.2 Background

What is phytoplankton? According to the National Oceanic and Atmospheric Administration, phytoplankton are microscopic marine algae that contain chlorophyll for photosynthesis to produce food and energy. Therefore, it lives on the surface of the ocean for enough sunlight to generate energy (Woods Hole Oceanographic Institution, n.d.). Phytoplankton is the foundation of the food web, and the most important producer of oxygen in the world (Reynolds, 2006). It relies on the nutrients found in it for survival, like phosphorus, nitrate, and calcium (National Geographic, 2023). Phytoplankton provide food for a wide range of species in the ocean. Without phytoplankton, lots of organisms can't survive (U.S. Environmental Protection Agency, n.d.). Therefore, phytoplankton plays a key role in the carbon cycle, significantly contributing to the amount of CO_2 in the atmosphere, and regulating the climate of the Earth (European Environment Agency, n.d.). In our study, we selected *Nannochloropsis oceanica* as the experimental subject. *Nannochloropsis oceanica* is one kind of phytoplankton that can live in salt and freshwater under various conditions.

What is the correlation between CO_2 and marine phytoplankton? When CO_2 meets the ocean water, it reacts with ocean water forming chemicals such as carbonic acid, hydrogen ions, and bicarbonate ions, making the ocean Potential of Hydrogen (pH) more and more acidic (U.S. Environmental Protection Agency, 2023). From 1985 to 2022, the pH value of the ocean decreased by 0.4; this is a massive change for the ocean (U.S. Environmental Protection Agency, 2023). Following François Morel's report "Effects of Ocean Acidification on Marine Phytoplankton", the lowering of pH causes changes in the growth rate of some phytoplankton, resulting in an unbalanced ecosystem - certain species will bloom excessively taking up extra resources that may easily move another species toward extinction.

The ocean absorbs about 90% of the extra heat on Earth. Over the last 100 years, the ocean temperature has risen by 0.74°C, due to ocean warming (Mathez & Smerdon, 2018). Ocean warming damages the structures of proteins and DNA, affecting the survival and growth of embryos (Ocean Acidification and Marine Wildlife, 2021). These environmental factors will reduce the ability of marine organisms to survive due to their inability to tolerate higher temperatures (Freedman, 2011). The combination of ocean acidification and ocean warming has an even stronger effect on the marine ecosystem (Princeton University, 2015).

1.3 General approach

We experimented to examine the effect of CO_2 concentration and rising temperature on *Nannochloropsis oceanica*. Our hypothesis is as follows: The *Nannochloropsis oceanica's* population in the warmer water (28°C and 32°C) and higher CO_2 concentrations (0.4 ml/min and 0.6 ml/min) would increase than the population of *Nannochloropsis oceanica* at temperatures of 24°C and CO_2 concentration of 0 ml/min. From the hypothesis, it is predicted that the population of *Nannochloropsis oceanica* will depend on the pH value and the ocean's temperature. The simulation of the change of the environment and observing for changes was by increasing CO_2 concentration and temperature. First, simulating acidification affects the phytoplanktons. Acidification is caused by the reaction of CO_2 from the atmosphere with water and the formation of carbonic acid. To simulate this environment, a CO_2 pump was used to add the different concentrations of CO_2 to represent the different amounts of acidification. A fish tank heater was used to simulate the rise in water temperature.

2. Materials and Methods

2.1 Enlarged culture experiment

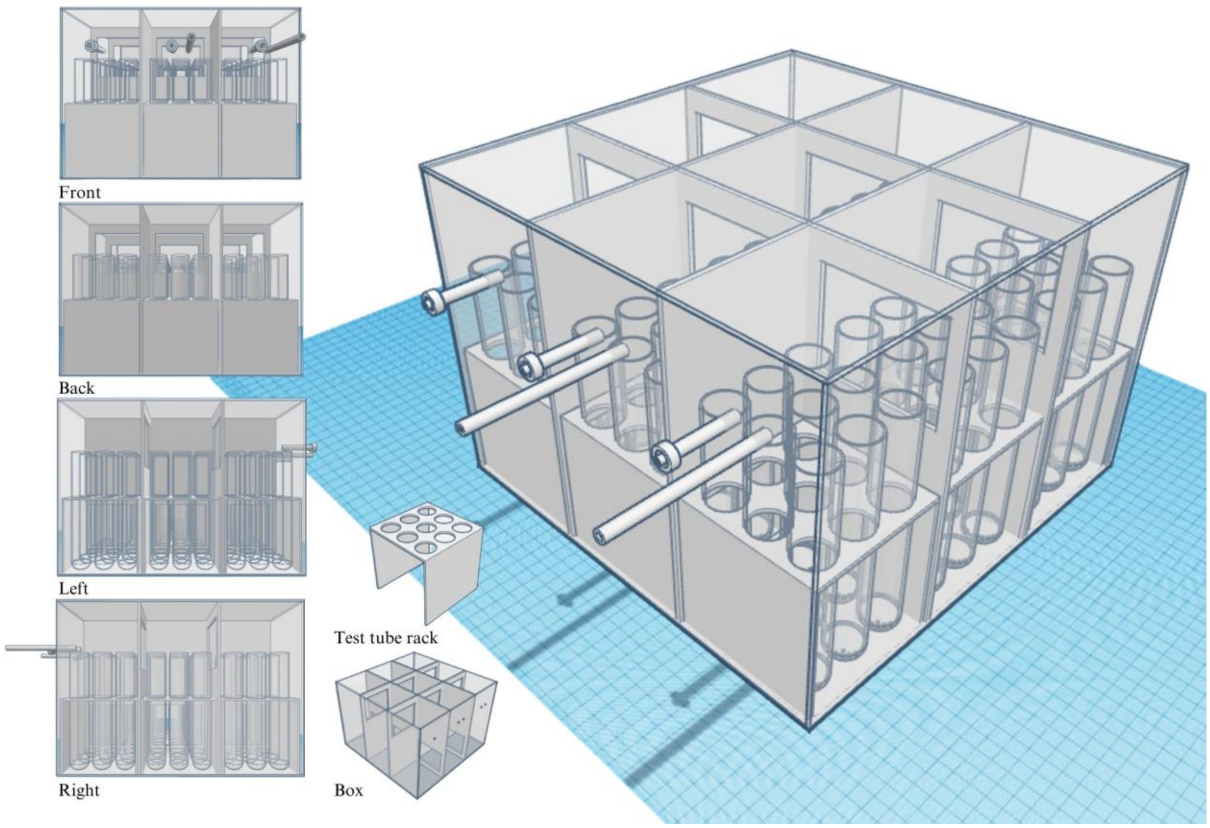
A 12 liter (L) plastic bottle, three air pumps, light, scissors, 30 centimeters (cm) air tube, starting culture, fertilizer, 4L distilled water, 96 grams (g) sea salt, alcohol, and UV light were used in the enlarged culture experiment. Scissors were used to cut a hole in the lid of the plastic bottle for the air tube. Sterilization was important to prevent the growth of unwanted bacteria in all the experimental samples. Therefore each apparatus was sterilized including this container by using clean tap water to wash out all the dust. Next alcohol, which kills bacteria.

Nannochloropsis oceanica dies with alcohol, so purification by distilled water was essential. The proportion of *Nannochloropsis oceanica* and ocean water was a 1:2 ratio, of one part the *Nannochloropsis oceanica*, to two parts seawater. Sea aquarium salt was used to make seawater. The formula for sea salt water was one liter of distilled water and 24g of sea salt needed for one liter of seawater. After measuring, sea salt was added to distilled water in the plastic bottle until the sea salt dissolved completely *(Figure 1)*. To ensure the seawater was the same as natural seawater, salinity, and pH values were checked, 1.024 was the specific gravity with a salinity of 32 parts per thousand, and the pH value was between 8.3 and 8.4 (*Figure 2-3*). After checking, the UV light was turned on for the final sterilization of seawater for 30 minutes (*Figure 4*). Next, the fertilizer was added to the seawater; the fertilizer contained nitrate, phosphate, silicate, calcium, and other nutrients for *Nannochloropsis oceanica* to survive. The original starting culture of *Nannochloropsis oceanica* was poured into the sterilized plastic bottle, and mixed with seawater. The last step was to set up the environment for *Nannochloropsis oceanica* to grow; the light was turned on every 12 hours beside the plastic bottle with the newly added *Nannochloropsis oceanica*. An air tube was put in the plastic bottle and attached to an air pump that was turned on for 24 hours. The culture enlarged every week.

1.  2.  3.  4. 

Figure 1, salt placement into the distilled water (Picture taken by the first author). Figure 2, salinity measurement of seawater (Picture taken by the first author). Figure 3, pH measurement of seawater (Picture taken by the first author). Figure 4, sterilization of test tubes along with use of UV light sterilization (Picture taken by the first author).

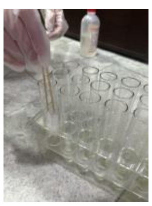
2.2 CO_2 concentration and temperature experiment



Design of the setup for the experiment (Design made on Tinkercad and Canva)

The setup of the experiment was simulated in an acrylic container of nine smaller spaces, connected vertically with space near the top of the partition to control the temperature. At the bottom, 3 fish tank heaters were placed under sponges with fish tank heaters set to temperatures 24°C, 28°C, and 32°C. The horizontal space near the bottom of the partition was used to control the CO_2 concentration. In the front, 5 holes, sized 6.5 millimeters in diameter, with three air tubes and two aquarium CO_2 tubes were inserted. Each air tube was attached to a syringe filter (*Figure 5*). The aquarium CO_2 tubes were attached to the CO_2 cylinder. A CO_2 cylinder adapter (w/22 to G5/8) and CO_2 regulator were attached to the CO_2 cylinder. These

CO_2 regulators were attached to two CO_2 tubes with each connected to two CO_2 flow meters. The CO_2 tubes were then attached to the acrylic box. Flow meters were set to 0.4ml/ min and 0.6 ml/min, with the fish tank heater, working 24 hours for 7 days (*Figure 6*). 81 test tubes all contained 27 ml of *Nannochloropsis oceanica* samples each sealed by filter breathable sealing film *(Figure 7-12)*. The test tubes were grouped by nine, and placed in the corresponding space on the test tube rack. Test tubes were labeled as A1, A2, A3, B1, B2, B3, C1, C2, and C3 for each trial (ABC symbolized CO_2 concentration from low to high; 123 symbolized temperature concentration from low to high). Last but not least, the top of the box was sealed with plastic wrap (*Figure 13-15*). Each day, the plastic wrap was opened to resuspend the samples by shaking.

6.  7.  8.  9.  10.  11. 

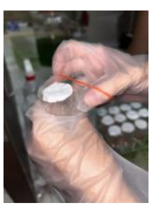
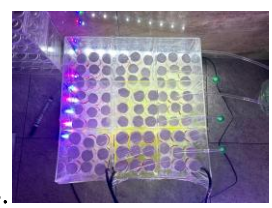
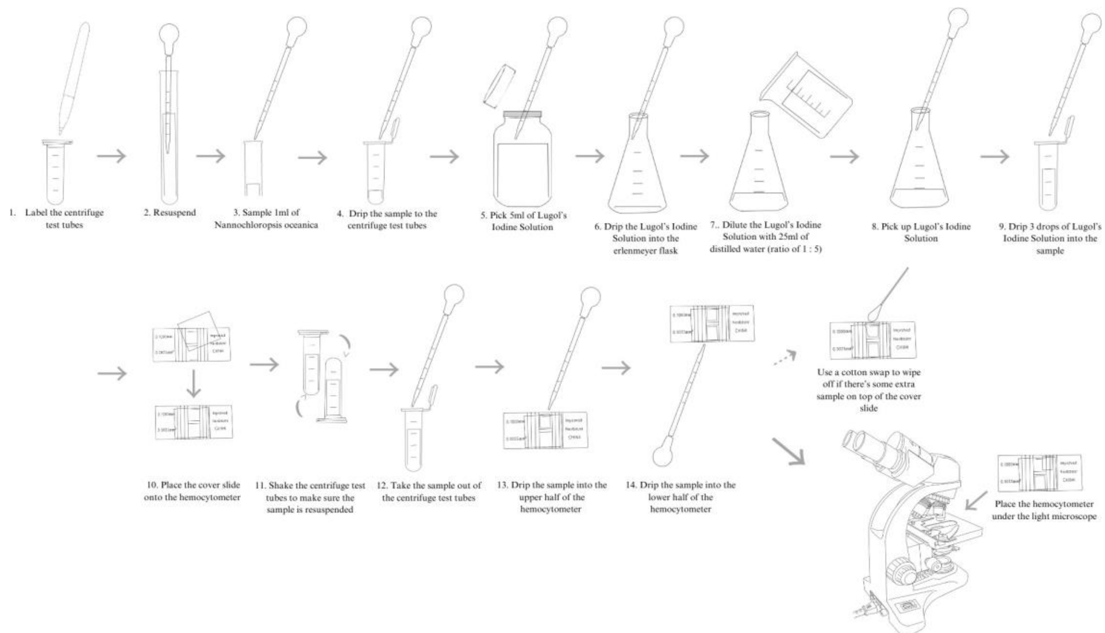
12.  13.  14.  15. 

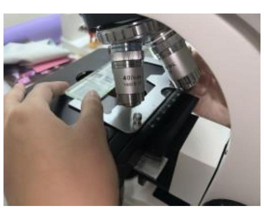
Figure 6, the setup of the CO_2 cylinder attached to regulators, tubes, and flow meters(Picture taken by the first author). Figure 7, alcohol sterilization of test tubes (Picture taken by the first author). Figure 8, rinsing the test tubes with distilled water (Picture taken by the first author). Figure 9, the addition of seawater to the test tubes with a syringe (Picture taken by the first author). Figure 10, enlargement of Nannochloropsis oceanica cultures into test tubes (Picture taken by the first author). Figure 11, application of test tubes filter breathable sealing film. Figure 12, sealing each sample of Nannochloropsis oceanica by filter breathable sealing film (Picture taken by the first author). Figure 13-15, different dimensions of the experiment (Picture taken by the first author).

2.3 Cell counting method



Method of the preparation for hemocytometer (Diagram drawn and made on Procreate and Canva).

Eighty-one centrifuge test tubes were rinsed with distilled water, disinfected by UV lights, and labeled as A1, A2, A3, B1, B2, B3, C1, C2, C3 for each trial (ABC symbolized CO_2 concentration from low to high; 123 symbolized temperature from low to high), were each filled with two ml of distilled water. The samples were once again resuspended by a pipette, where it was rinsed with distilled water each time before resuspension. Then, one ml of sample from each test tube was removed and put into a corresponding centrifuge test tube. This sample was then diluted by a ratio of 1: 2 (algae sample to distilled water). In an Erlenmeyer flask, the Lugol's Iodine Solution is diluted with 25 ml of distilled water (by a ratio of 1:5). Next, 3 drops (approximately 0.3 ml) of the diluted Lugol's Iodine Solution were added to the sample in each centrifuge test tube (*Figure 17*). A sterilized hemocytometer counting slide and cover slip were rinsed again with distilled water and rubbed with Kimwipes to dry after being disinfected by UV light. The cover glass was placed on top of the mounting supports. With an approximately 45-degree angle of holding a 0.2 ml pipette that was rinsed with distilled water, the diluted sample was slowly and gently pushed into the chamber until the chamber was filled. This was repeated for each sample count using another chamber. The excess sample was swabbed with a Q-tip cotton swab. After the hemocytometer slides were prepared, the hemocytometer slide was gently placed on the microscope stage (*Figure 18*). Under a 4x objective lens, the words on two sides of the hemocytometer were focused first. The x-axis knob was turned to the hemocytometer's middle transparent part (*Figure 19*). To find the grids, the condenser focusing knob, coarse focus knob, and fine focus knob were used to bring the grid into view (*Figure 20*). The x and y- axis knobs were moved to find the counting grids (*Figure 19*). Then, a 10x objective lens was used to zoom in, using the fine focus knob to bring it into view. The counter was set to zero. The counting proceeded routinely following this order: Cells were counted up the left, then down the left, next up the right, and then down the right, and the middle of the counting grid was counted (*Figure 21-22*). Note that if the cell overlaps with the grid line, it was counted up and left, whereas it was not counted down and to the right. The two parts of the hemocytometers were counted and recorded.

17.  18.  19.  20.  21. 

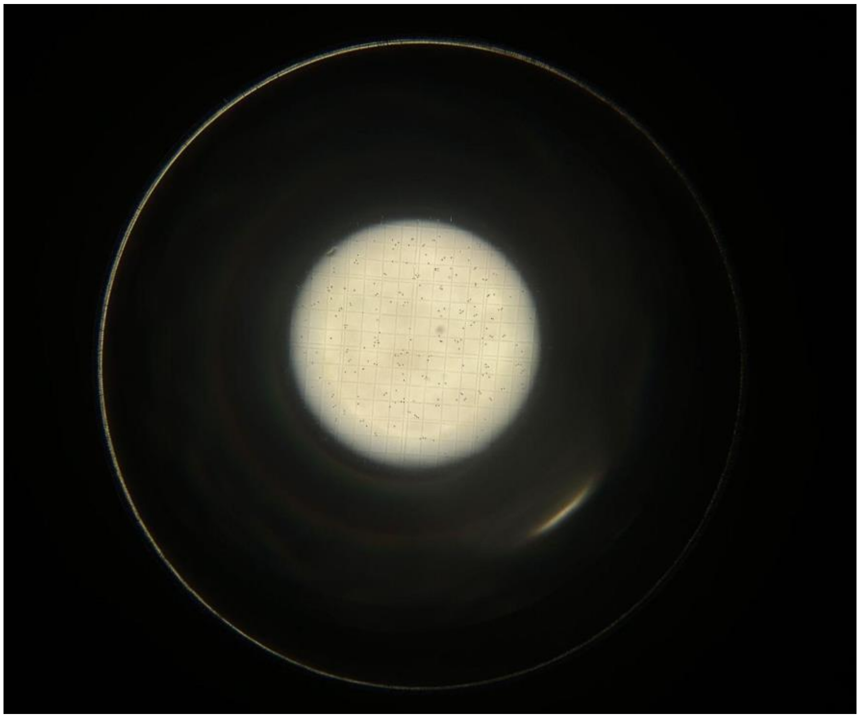
22. 

Figure 17, Nannochloropsis oceanica with Lugol's Iodine Solution *inside the centrifuge test tubes (Picture taken by the first author). Figure 18, slide placement on the microscope stage (Picture taken by the first author). Figure 19, adjusting the x-axis and y-axis knob (Picture taken by the first author). Figure 20, fine focus knob adjustment (Picture taken by the first author). Figure 21, using a counter to count the cells of Nannochloropsis oceanica under the microscope (Picture taken by the first author). Figure 22. Nannochloropsis oceanica under 1000x (Light microscope).*

3. Results

ANOVA

Temperature in celsius

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	110.500	148	.747	1.363	.053
Within Groups	51.500	94	.548		
Total	162.000	242			

ANOVA Effect Sizes[a,b]

		Point Estimate	95% Confidence Interval Lower	95% Confidence Interval Upper
Temperature in celsius	Eta-squared	.682	.000	.358
	Epsilon-squared	.182	-1.574	-.653
	Omega-squared Fixed-effect	.181	-1.558	-.648
	Omega-squared Random-effect	.001	-.004	-.003

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.
b. Negative but less biased estimates are retained, not rounded to zero.

1

ANOVA

Co2 concentrationn ml/min

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	110.767	148	.748	1.373	.048
Within Groups	51.233	94	.545		
Total	162.000	242			

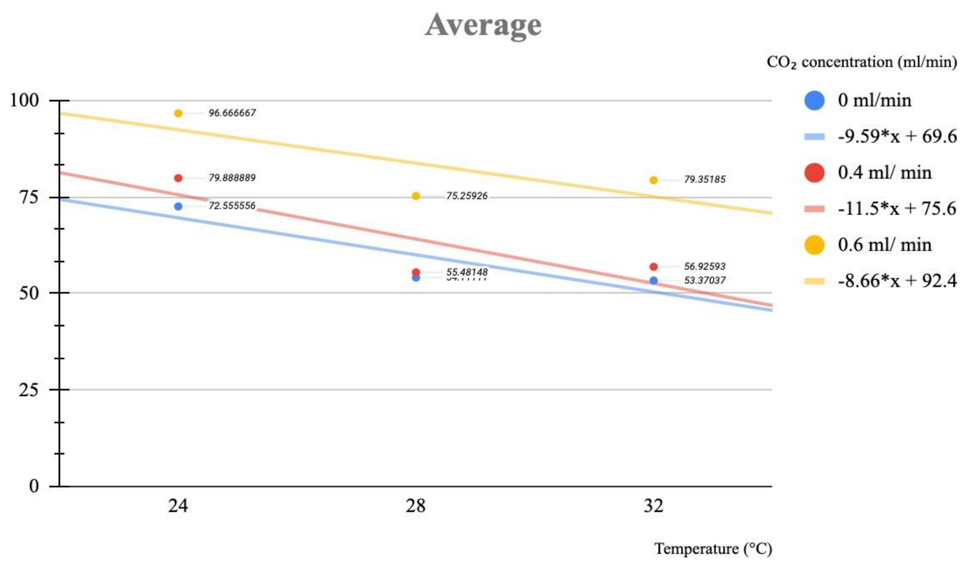
ANOVA Effect Sizes[a,b]

		Point Estimate	95% Confidence Interval Lower	95% Confidence Interval Upper
Co2 concentrationn ml/min	Eta-squared	.684	.000	.362
	Epsilon-squared	.186	-1.574	-.644
	Omega-squared Fixed-effect	.185	-1.558	-.639
	Omega-squared Random-effect	.002	-.004	-.003

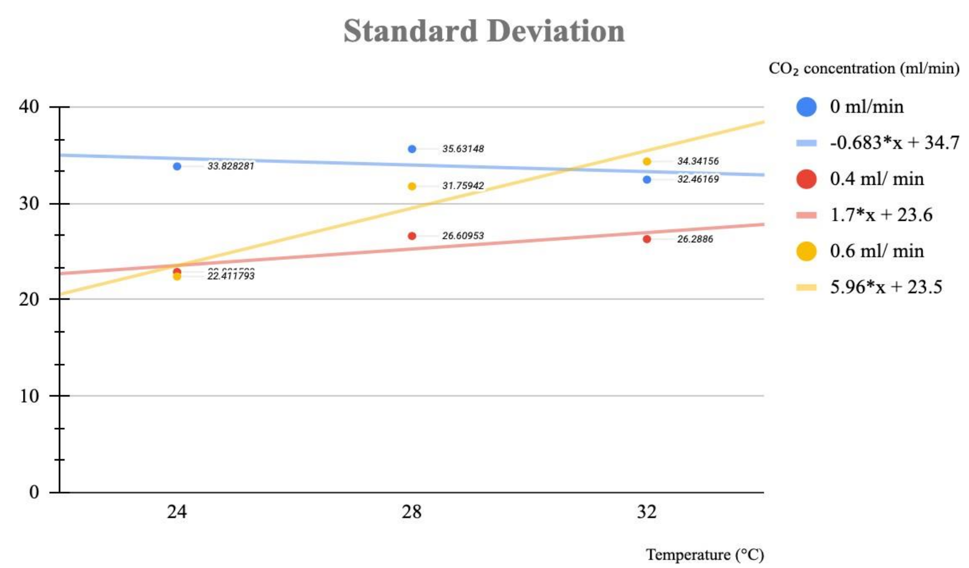
a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.
b. Negative but less biased estimates are retained, not rounded to zero.

The results of the ANOVA test (Table made on SPSS).

The p-value for CO_2 concentration is 0.048, which was less than 0.05, indicating that CO_2 concentration significantly affects *Nannochloropsis oceanica's* growth. The p-value for temperature is 0.053, in contrast, the value is greater than 0.05, suggesting that temperature has no significant effect on *Nannochloropsis oceanica's* growth.



The graph shows the averages of Nannochloropsis oceanica's population (Graph made on Google Sheets).



The graph shows the standard deviations from the averages (Graph made on Google Sheets).

The two graphs illustrate that at a temperature of 24℃ and a CO_2 concentration of 0 ml/min, the population average is 72.55556, with a standard deviation of 33.828281. At the same temperature of 24℃ but a CO_2 concentration of 0.4 ml/min, the population average changed to 79.888889, with a standard deviation of 22.881523. At the highest CO_2 concentration at 24℃, the population becomes 96.6667, with a standard deviation of 22.411793. After the temperature increased to 28℃ and a CO_2 concentration of 0 ml/min, the population average becomes 54.11111, with a standard deviation of 35.63148. At the same temperature of 28℃ but a CO_2 concentration of 0.4 ml/min, the population changed to 55.48148, with a standard deviation of 26.60953. At the highest CO_2 concentration at 28℃, the population becomes 75.25926, with a standard deviation of 31.75942. With the temperature increase to 32℃ and a CO_2 concentration of 0 ml/min, the population average becomes 53.37032, with a standard deviation of 32.46169. At the same high temperature of 32℃ but a CO_2 concentration of 0.4 ml/min, the population average changed to 56.92593, with a standard deviation of 26.2886. Finally, at the highest temperature of 32℃ and highest CO_2 concentration of 0.6 ml/min, the population average becomes 79.35185, with a standard deviation of 34.34156. From the data above, we can conclude that the population has the highest average value with the lowest standard deviation when the temperature is 24℃ and the CO_2 concentration is 0.6 ml/min.

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	325.000[a]	296	.119
Likelihood Ratio	379.009	296	<.001
Linear-by-Linear Association	21.586	1	<.001
N of Valid Cases	243		

a. 447 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	313.800[a]	296	.228
Likelihood Ratio	366.505	296	.003
Linear-by-Linear Association	14.884	1	<.001
N of Valid Cases	243		

a. 447 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

The results of the Chi-Square Tests (Tables made on SPSS).

The values of 0.119 and 0.228 indicate that there is a significant difference between concentration and temperature because the value is less than 0.5.

Correlations

		Temperature in celsius	Co2 concentrationn ml/min
Temperature in celsius	Pearson Correlation	1	-.047
	Sig. (2-tailed)		.477
	N	236	236
Co2 concentrationn ml/min	Pearson Correlation	-.047	1
	Sig. (2-tailed)	.477	
	N	236	243

The results of the correlation test (Table made on SPSS).

The significance value for the correlation to be positive should be exactly 1.0. The table above shows that there is a moderate positive correlation between temperature and CO_2 concentration.

4. Conclusion

The result indicates that the former hypothesis has been partly rejected. It shows that there is no statistically significant effect on *Nannochloropsis oceanica* in temperature but in CO_2 concentration. There is a statistically significant difference between CO_2 concentration and temperature, as well as a moderate positive correlation between temperature and CO_2 concentration. According to our study, *Nannochloropsis oceanica* exhibited the highest average population growth at 24°C with a CO_2 concentration of 0.6 ml/min.

However, here are some suggestions for the future, including how the experiment is set up. First, how we cultured *Nannochloropsis oceanica* was not in a sterile experimental environment, (it was performed at home on the balcony or bathroom) actually led to a failed culture attempt at the beginning of our study thinking that it was contaminated by various kinds of bacteria. As a result, sterilization techniques were revised but still performed at home in a non-laboratory setting. In the future, the culture and the whole experiment should be performed in a sterile experimental environment to prohibit unknown microorganisms that could contaminate the culture and the samples. Second, the container that is sealed by plastic wrap, which is opened every day, means that every day the inner environment could be disrupted by the outer environment. Methods that do not require the opening of the plastic wrap to suspend the *Nannochloropsis oceanica* of the sample can be developed. Lastly, the method of containing the population in the sample could be improved. We counted the population within the sample by human, although each sample was counted twice on the hemocytometer to lower the possibility of error. Still, we suggest using some kind of scientific equipment that could count the samples efficiently and precisely.

Our study indicates the best environment for *Nannochloropsis oceanica* on CO_2 concentration and temperature, however, fluctuations in CO_2 concentration and temperature may adversely affect the ecosystem. With increasing CO_2 levels on earth, this CO_2 will be further absorbed by the ocean, producing ocean acidification. Our results show that this increase in dissolved CO_2 will facilitate significant algae blooms of at least this one species, affecting the food web where the number of nutrients for other organisms will reduce. This will result in a further unbalanced oceanic ecosystem. Hopefully, this study will warn people about climate change globally, and cause the world to work toward achieving Sustainable Development Goal 13, climate action, specifically on ocean acidification and global warming to maintain biodiversity and environmental stability. We call for saving our environment, not just for phytoplanktons, but for all of the organisms on the planet.

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【評語】180010

本研究針對 Nannochropsis oceanica 生長環境的溫度以及二氧化碳濃度（CO_2）進行為期一週的環境更動，包含三種溫度 和三種CO_2濃度，並利用血球計數方法計算總共 243 個樣本的細胞量。結果顯示，CO_2濃度對於 Nannochloropsis oceanica 的生長有顯著影響，但溫度的影響不大。結果表明，作為氣候變遷的一部分，海洋酸化和全球暖化可能通過浮游植物影響群體，造成生態失衡，對海洋生態系統產生重大負面影響。實驗過程之外部干擾與不確定性之討論可以加強。建議可以加入 Ph 值脂測量，以了解大氣二氧化碳融入水中的可能影響。