

# Analysis of Nutrient Enrichment on *Ulva* Growth

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FHL 446 A SU 25: Marine Botany: Diversity and Ecology

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## Abstract

To determine whether *Ulva* is nitrogen or phosphorus limited in San Juan Island oceanographic conditions, samples were cut from a single individual utilizing a cork borer and cultured in a lab environment. In addition to control conditions, five samples each were cultured in four treatments of increased nitrate (5  $\mu\text{M}$ , 25  $\mu\text{M}$ , 50  $\mu\text{M}$ , 100  $\mu\text{M}$ ) and four treatments of increased phosphate (0.3125  $\mu\text{M}$ , 1.5625  $\mu\text{M}$ , 3.125  $\mu\text{M}$ , 6.25  $\mu\text{M}$ ). Temperature of the treatments were kept consistent with field conditions through usage of a sea table circulating water, and light levels were kept constant (125 lx) through suspension of four fluorescent tube lights above the seatable. Samples were cultured over a period of five days, and relative growth of each treatment was measured utilizing a micrometer to determine average change in diameter on the third and fifth day. Increased nitrate treatments had higher average growth than that of increased phosphate, and both displayed significantly higher growth than that of the control. Between the two treatments, statistical significance was determined for nitrate growth conditions having higher average growth ( $p < 0.5$ ). Treatments displayed moderate positive correlation between increasing nitrate levels and growth ( $\sim 0.66$ ), with almost no correlation between increasing phosphate levels and growth ( $\sim -.11$ ). The data supports the hypothesis that *Ulva* growth is nitrogen limited in San Juan Island oceanographic conditions.

## Introduction

In recent years the aquaculture industry has grown globally in economic importance to help support the growing global population. Resultantly, algae specifically have been cultivated for their potential: ranging from functioning as a food source, working to sequester carbon, and the many applications of their biomolecules for production of products such as plastics or cosmetics (Vijayaram et al., 2024). The *Ulva* genus specifically holds interest as it is known for its ability to rapidly increase in biomass over a wide range of conditions throughout its almost global distribution, making it a good candidate for aquaculture (Simon et al. 2022).

In addition, many coastal environments often suffer from eutrophication from nitrogen or phosphorus run-off, and as a result there has been increasing interest to integrate algae into nutrient pollution management (Racine et al., 2021). However, too much algal growth can be detrimental to other local species such as seagrasses, and research has been conducted on limiting *Ulva* growth by regulating nitrogen levels (Han et al. 2021). Sensitivity analysis for nitrogen and phosphorus levels of *Ulva* would be a next step to better inform management decisions about the utilization of *Ulva* for excess nutrient runoff management, and *Ulva* population growth predictions after eutrophication events.

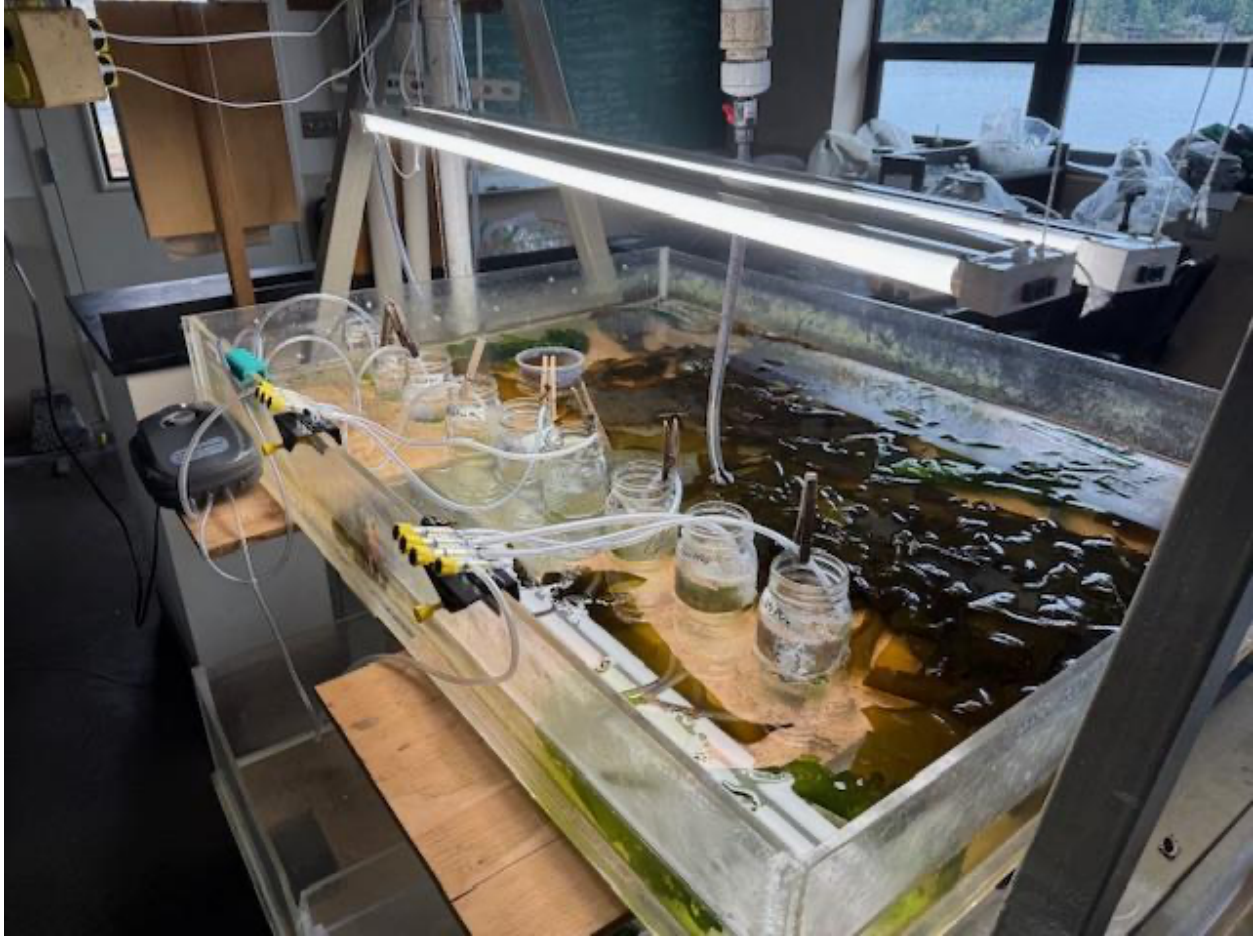
There has been ample research examining what conditions influence the growth rates of *Ulva*. Factors such as nutrient levels, temperature, salinity and photoperiod have been explored (Xiao et al., 2016; Fan et al., 2014; Bao et al., 2022). These conditions have also been explored and weighted through models (Guimaraens et al. 2005), so performing a laboratory experiment and subsequent sensitivity analysis of that experiment will be informative for exploring the accuracy of prior model predictions. This study aims to determine if there is a

substantial difference in nutrient effect on growth rate between nitrogen and phosphorus by the species, and to potentially find the upper bound for growth rate with enriched nutrient conditions. It is anticipated that they will be nitrogen limited as the Pacific Ocean is usually higher in phosphorus than nitrogen (Martiny et al. 2014).

## **Materials and Methods**

### *Experimental Setup*

Circular cutouts of *Ulva* were removed from the blade of a single individual utilizing a 23.15 mm diameter cork borer. The same individual specimen was used for all treatments to ensure prior nutrient conditions would be consistent between treatments. Following that, each treatment consisted of five samples inside a two-liter mason jar that had been cleaned and filled with 300 mL of filtered and tyndalized seawater. An airstone was placed inside of each jar to ensure water circulation and that the samples remained suspended. Four tube lights were suspended above the treatments to maximize growth conditions and ensure that light levels remained consistent ( $125 \mu\text{mol}/\text{m}^2/\text{s}$ ). Temperature was kept consistent with local water by placing the jars inside of a sea table which was actively circulating water from outside of the lab. (temperature? How measured?) did you place the jars randomly?



### Water Chemistry

Seawater was collected and heated at 80°C for two hours inside of an autoclave to kill diatoms in the mixture while preserving the chemical and mineral composition. The control was unaltered from normal water chemistry. For the other experimental treatments, a master mix was created for both nitrate and phosphate. The nitrate master mix consisted of 100 mL of water with 255 mg of sodium nitrate crystal. After which the solution was then mixed to allow the sodium nitrate to disassociate into sodium and nitrate, resulting in a 100 mL solution of 10,000  $\mu\text{M}$  nitrate. The phosphate master mix consisted of 100 mL of water with 71.27 mg trisodium

phosphate dodecahydrate. After which the solution was mixed to allow disassociation into sodium, phosphate and water for a final 100 mL solution of 625  $\mu\text{M}$  phosphate.

The nitrogen treatments increased by measurements of 5  $\mu\text{M}$ / 25  $\mu\text{M}$ / 50  $\mu\text{M}$ / 100  $\mu\text{M}$  above baseline. The phosphorus treatments increased by measurements of 0.3125  $\mu\text{M}$ / 1.5625  $\mu\text{M}$ / 3.125  $\mu\text{M}$ / 6.25  $\mu\text{M}$  above baseline to keep in accordance with the Redfield ratio. For nitrate treatments, using a p1000 and p20 micropipette: 1 mL of the nitrate master mix was added to 300 mL of water to create the 100  $\mu\text{M}$  solution, 500  $\mu\text{m}$  to 300 mL of water for the 50  $\mu\text{M}$  treatment, 250  $\mu\text{m}$  to 300 mL of water for the 25  $\mu\text{M}$  treatment, and 50  $\mu\text{m}$  to 300 mL for the 5  $\mu\text{M}$  treatment. For phosphate treatments, using a p1000 and p20 micropipette: 1 mL of the phosphate master mix was added to 300 mL of water to create the 6.25  $\mu\text{M}$  solution, 500  $\mu\text{m}$  to 300 mL of water for the 3.125  $\mu\text{M}$  treatment, 250  $\mu\text{m}$  to 300 mL of water for the 1.5625  $\mu\text{M}$  treatment, and 50  $\mu\text{m}$  to 300 mL for the 0.3125  $\mu\text{M}$  treatment. Water was changed after each day with freshly enriched water to ensure that the nutrient concentrations remained at the desired levels except for the first day of growth.

A Hanna Instruments™ high range nitrate spectrophotometer kit, and a low range phosphate spectrophotometer kit were utilized to determine respective concentrations in the local water, and to test how much nitrate and phosphate were removed from the water by the *Ulva* over the duration of the growth period.

### *Ulva Collection Data*

*Ulva* utilized in the experiment was from a single specimen collected at the Friday Harbor Laboratories dock on San Juan Island, San Juan County WA (48.5454° N, 123.0141° W). The specimen was collected on 7/8/25 while floating on the surface of the water. Tidal height

at date of collection was reported to be -1.18' MLLW by NOAA (National Oceanographic and Atmospheric Administration). The specimen was collected and identified to genus level by Bailey A. Snodgrass.

### Statistical analysis

To visualize differences in growth, the difference between the initial diameter of the *Ulva* samples (23.15 mm) and the average diameter of each treatment after three days of growth were plotted in logarithmic space using Microsoft Excel. Standard deviation was also plotted while accounting for log space. A two-sample t-test assuming unequal variances was performed between the nitrate growth results and the phosphate growth results to determine whether statistical significance exists between the two treatments using Microsoft Excel. Lastly, a correlation test was performed to determine whether growth rate was correlated with increasing nitrate and phosphate concentrations using Microsoft Excel.

## **Results**

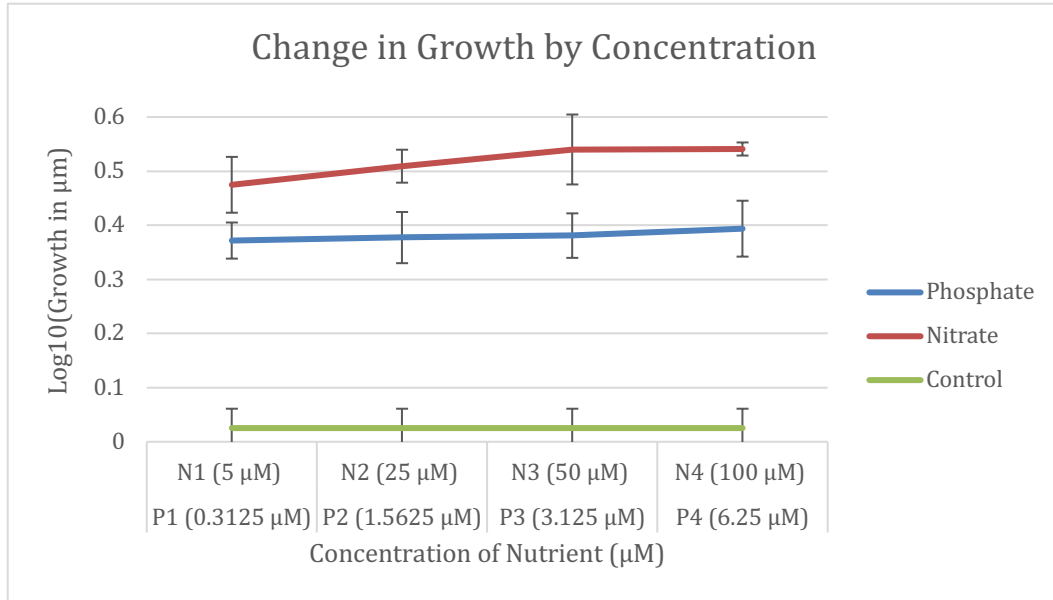


Figure 1). Graph demonstrating differences in *Ulva* growth ( $\mu\text{m}$ ) between increasing nutrient concentrations ( $\mu\text{M}$ ) of phosphate and nitrate in contrast to baseline conditions visualized in logarithmic space.

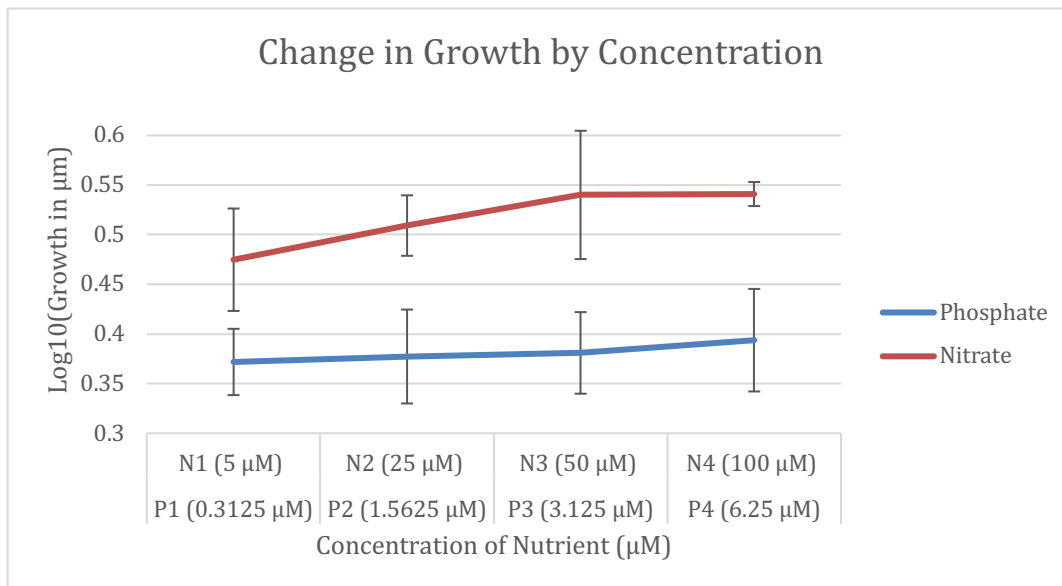


Figure 2). Fig. 1 revisualized without the control values to better emphasize the difference in growth of increased nitrate conditions in comparison to phosphate.

t-Test: Two-Sample Assuming Unequal Variances

	<i>N Values</i>	<i>P Values</i>
Mean	26.422857	1 25.6395
Variance	0.1897296	0.07040
Observations	7	5
Hypothesized Mean Difference	14	20
df	0	
t Stat	20	
P(T<=t) one-tail	5.9953274	
t Critical one-tail	7	
P(T<=t) two-tail	3.6592E-06	
t Critical two-tail	1.7247182	
	4	
	7.3184E-06	
	2.0859634	
	5	

Table 1). Table detailing the results of a t-test between phosphate and nitrate growth rates.

	<i>Relative Nitrate</i>	<i>Relative Phosphate</i>	<i>Growth</i>
Relative Nitrate	1		
Relative Phosphate	-0.376963351	1	
Growth	0.664653757	-0.107738253	1

Table 2). Table showing correlation values between growth rates and nutrient conditions.

Both enriched nitrate and phosphate growth values were substantially greater than control values (Fig. 1). Nitrate growth values were consistently greater than those of phosphate, and while standard deviations were relatively large within treatments, those of nitrate and phosphate had no overlap supporting the accuracy of the conclusion (Fig.1, Fig. 2). The mean of the nitrogen growth values is larger than that of the phosphate, implying that nitrogen enrichment led to greater *Ulva* growth. This observed difference in growth between groups is supported by the two-tail P value ( $>0.5$ ) (Table 1). Relative nitrate has a correlation value with growth of  $\sim 0.66$ , showing moderate positive correlation between increased nitrogen concentration and increased growth. Relative phosphate has a correlation value with growth of  $\sim -0.11$  showing almost no correlation between phosphate concentration and growth (Table 2).

## Discussion

The results support the hypothesis that *Ulva* is limited by nitrogen rather than phosphorus in local waters. This is evidenced by the nitrate having higher growth values than phosphate and the t-test showing significance between groups, indicating that increased nitrogen levels had a greater increase on growth. In addition, there was shown to be a positive correlation between nitrate increases and *Ulva* growth. Coupled with the apparent lack of correlation between increasing phosphate levels and growth, the correlation test helps to demonstrate that the water already possesses adequate amounts of bioavailable phosphorus. The results of this study support similar findings of the *Ulva* genus such as Fan et al. (2014) and Floreto et al. (1996). In addition, the waters being nitrogen limited is in line with the research by Martiny et al. (2014).

The study potentially faces issues regarding the sample size of data due to limitations of techniques. Between day three and day five, a substantial amount of the samples went reproductive, which halted vegetative growth and limited sample size leading to inconclusive sensitivity analysis. As a result, only the growth between days three and five could be used. In addition, the results for nitrogen uptake and phosphorus uptake were inconclusive when measured using the Hanna Instruments™ spectrometers, potentially due to samples going reproductive. In a future study this could be addressed by performing tissue analysis of the samples to determine if proportion of absorbed nitrogen and phosphorus incorporated into biomass changes when the other limit is due to Redfield, building on the work of other studies such as Bews et al. 2021. Lastly, the lack of a water change between day one and two could have impacted growth of the samples.

Moving forward, future studies could be performed on more samples of *Ulva* by altering multiple factors at once to perform Monte Carlo sensitivity analysis to be able to better inform models of wild populations such as those by Guimaraens et al. (2005). Further research could also be dedicated to tissue analysis of samples to better understand how it is incorporated into biomass. In addition, by oversaturating conditions with phosphate availability, luxury uptake of *Ulva* could be studied across different laboratory conditions as prior research has emphasized nitrogen luxury uptake (Pérez-Mayorga et al. 2011).

## **Acknowledgements and Attributions**

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study design and troubleshooting. D. Claar provided assistance with water chemistry and study design. W. Freshwater from UNCW provided assistance with study design and troubleshooting.

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Treatment  
Control

- 1
- 2
- 3
- 4

Equation  
 $\text{Mass (g)} = \text{Concentration (mol/L)} * \text{Volume (L)} * \text{Molecular weight}$

Position

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9



Added Sodium Nitrate (g)	Desired Phosphate Concentration (Micromolar)	
N/A	N/A	
0.12749205		0.3125
0.63746025		1.5625
1.2749205		3.125
2.549841		6.25

Resultant Value (mg)	Desired Phosphate Concentration (molar)	
12.749205		3.125E-07
63.746025		1.5625E-06
127.49205		0.000003125
254.9841		0.00000625
0.2549841		

BaselineP (PPM)	BaselineN (PPM)	
	0.17	0
Added PPM total	Added PPM total	
	0.9	1.4
PPM diff	PPM diff	
	-0.73	6.3

625

Added sodium phosphate (g)	Added sodium phosphate (mg)
N/A	N/A
3.56366E-05	0.035636565
0.000178183	0.178182825
0.000356366	0.35636565
0.000712731	0.7127313

Dilution Amount	Dilution amounts (x1000) mg
x100	3.5636565
x100	17.8182825
x100	35.636565
x100	71.27313

g	0.07127313
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PPM to um Calculator Nitrate	PPM to um Calculator Phosphate
101.6048732	1.790012572
6.3	0.17

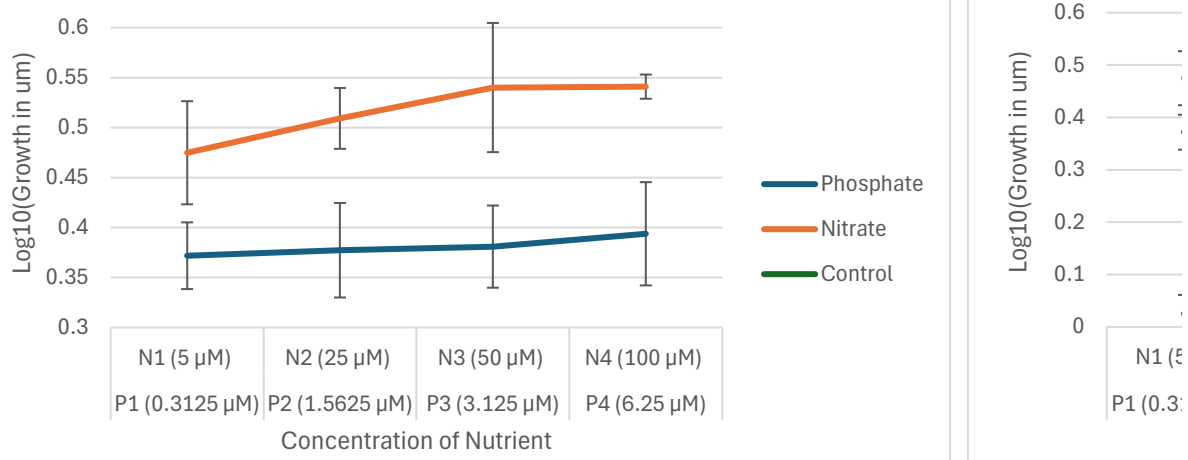
Input PPM above	Input PPM above
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Constants	Units
Molecular weight of sodium nitrate	84.9947 g/mol
Molecular weight of trisodium phosphate	163.94 g/mol
Molecular weight of water	18.01528 g/mol
Ratio of water to trisodium phosphate 12 to 1	N/A
Molecular weight of trisodium phosphate 12-Hydrate	380.12336 g/mol
Volume of water mL	300 mL
Volume of water L	0.3 L

Treatment / Sample	3 day size (mm)	3 day change in size (mm)	5 day size (mm)
Control / 1	24.28	1.13	23.9
Control / 2	24.26	1.11	24.65
Control / 3	24.13	0.98	25.1
Control / 4	24.17	1.02	24.17
Control / 5	24.08	0.93	24.29
P1 (0.3125) / 1	25.54	2.39	
P1 (0.3125) / 2	25.49	2.34	
P1 (0.3125) / 3	25.4	2.25	
P1 (0.3125) / 4	25.3	2.15	
P1 (0.3125) / 5	25.79	2.64	
P2 (1.5625) / 1	25.92	2.77	28.4
P2 (1.5625) / 2	25.93	2.78	26.73
P2 (1.5625) / 3	25.74	2.59	28.16
P2 (1.5625) / 4	25.36	2.21	
P2 (1.5625) / 5	25.42	2.27	
P3 (3.125) / 1	25.45	2.3	27.35
P3 (3.125) / 2	25.61	2.46	27.79
P3 (3.125) / 3	25.67	2.52	27.98
P3 (3.125) / 4	25.23	2.08	28.67
P3 (3.125) / 5	25.81	2.66	27.93
P4 (6.25) / 1	25.88	2.73	28.4
P4 (6.25) / 2	26.28	3.13	
P4 (6.25) / 3	25.85	2.7	
P4 (6.25) / 4	25.72	2.57	
P4 (6.25) / 5	25.4	2.25	
N1 (5) / 1	26.69	3.54	28.46
N1 (5) / 2	25.81	2.66	27.98
N1 (5) / 3	25.93	2.78	
N1 (5) / 4	26.32	3.17	
N1 (5) / 5	25.92	2.77	
N2 (25) / 1	26.54	3.39	
N2 (25) / 2	26.22	3.07	
N2 (25) / 3		N/A	
N2 (25) / 4		N/A	
N2 (25) / 5		N/A	
N3 (50) / 1	26.08	2.93	28.59
N3 (50) / 2	26.35	3.2	29.32
N3 (50) / 3	27.32	4.17	28.74
N3 (50) / 4	26.32	3.17	29.51
N3 (50) / 5	27.02	3.87	

N4 (100) / 1	26.63	3.48
N4 (100) / 2	26.77	3.62
N4 (100) / 3	N/A	
N4 (100) / 4	N/A	
N4 (100) / 5	N/A	

Change in Growth by Concentration



Treatment	Average 3 days
Control	1.034
P1 (0.3125)	2.354
P2 (1.5625)	2.524
P3 (3.125)	2.404
P4 (6.25)	2.676
N1 (5)	2.984
N2 (25)	3.23
N3 (50)	3.468
N4 (100)	3.55

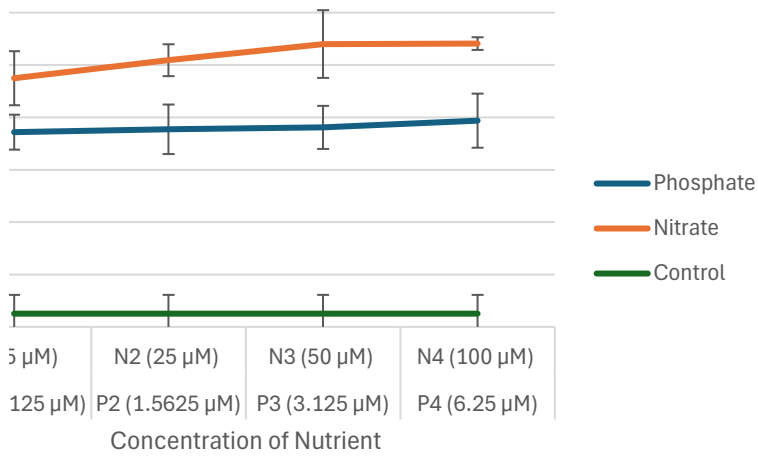
Growth rate

Phosphate

P1 (0.3125)	0.371806459
P2 (1.5625)	0.377306251
P3 (3.125)	0.380934463
P4 (6.25)	0.39375064

P1 (0.3125  $\mu$ M) N1 (5  $\mu$ M)  
P2 (1.5625  $\mu$ M) N2 (25  $\mu$ M)  
P3 (3.125  $\mu$ M) N3 (50  $\mu$ M)  
P4 (6.25  $\mu$ M) N4 (100  $\mu$ M)

### Change in Growth by Concentration



Standard Dev 3 days (Log10)

0.035797002  
0.033389706  
0.047295061  
0.041110077  
0.051650255  
0.051591155  
0.030448953  
0.064623126  
0.012112263

Nitrate

N1 (5)	0.47479882
N2 (25)	0.50920252
N3 (50)	0.54007909
N4 (100)	0.54095481



Treatment	Average 5 days
Control	0.238
P1 (0.3125)	N/A
P2 (1.5625)	2.0893333333
P3 (3.125)	2.39
P4 (6.25)	2.574
N1 (5)	2.086
N2 (25)	N/A
N3 (50)	2.422
N4 (100)	N/A

Initial (mm)

23.15

Observations

Week 3 N2 looks dead or reproductive

means went reproductive

irradiance

125

Means Discarded b/c too many went reproductive to be useful data

Control

Control

0.025305865

0.025305865

0.025305865

0.025305865



Standard Dev 5 days

#DIV/0!

N/A

#DIV/0!

#DIV/0!

#DIV/0!

#DIV/0!

N/A

#DIV/0!

N/A

Control	N Values	P Values
	26.69	25.54
	25.81	25.49
	25.93	25.4
	26.32	25.3
	25.92	25.79
	26.54	25.92
	26.22	25.93
	26.08	25.74
	26.35	25.36
	27.32	25.42
	26.32	25.45
	27.02	25.61
	26.63	25.67
	26.77	25.23
		25.81
		25.88
		26.28
		25.85
		25.72
		25.4

t-Test: Two-Sample Assuming Unequal Variances

	<i>N Values</i>	<i>P Values</i>
Mean	26.4228571	25.6395
Variance	0.18972967	0.070405
Observations	14	20
Hypothesized Mean $\mu$	0	
df	20	
t Stat	5.99532747	
P(T<=t) one-tail	3.6592E-06	
t Critical one-tail	1.72471824	
P(T<=t) two-tail	7.3184E-06	
t Critical two-tail	2.08596345	

P1	P2	P3	P4	N1	N2
25.54	25.92	25.45	25.88	26.69	26.54
25.49	25.93	25.61	26.28	25.81	26.22
25.4	25.74	25.67	25.85	25.93	
25.3	25.36	25.23	25.72	26.32	
25.79	25.42	25.81	25.4	25.92	

	N1	
Growth Relative to Control		2.885880077
Relative concentration		1

2.984  
3.23  
3.468  
3.55

Relative Nitrate

1  
5  
10  
20  
0  
0  
0  
0  
0

N3		N4
	26.08	26.63
	26.35	26.77
	27.32	
	26.32	
	27.02	

Relative Nitrate  
Relative Phosphate  
Growth

N2		N3		N4		P1		P2		P3
	3.123791103		3.353965184	3.43326886	2.27659574	2.4410058	2.32495164			
	5		10	20	1	5	10			

2.354  
2.524  
2.404  
2.676

Relative Phosphate	Growth
0	2.885880077
0	3.123791103
0	3.353965184
0	3.433268859
1	2.276595745
5	2.441005803
10	2.324951644
20	2.588007737
0	1.034

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<i>Relative Nitrate</i>	<i>Relative Phosphate</i>	<i>Growth</i>
1		
-0.376963351	1	
0.664653757	-0.107738253	1

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P4

2.58800774

20