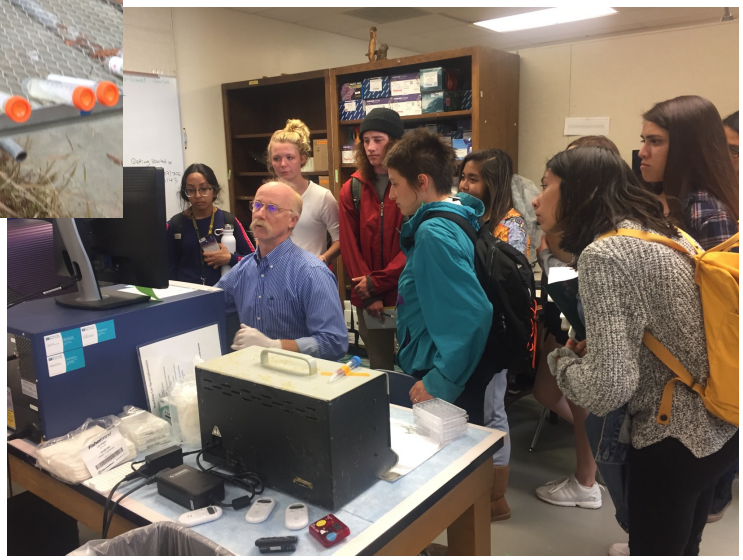
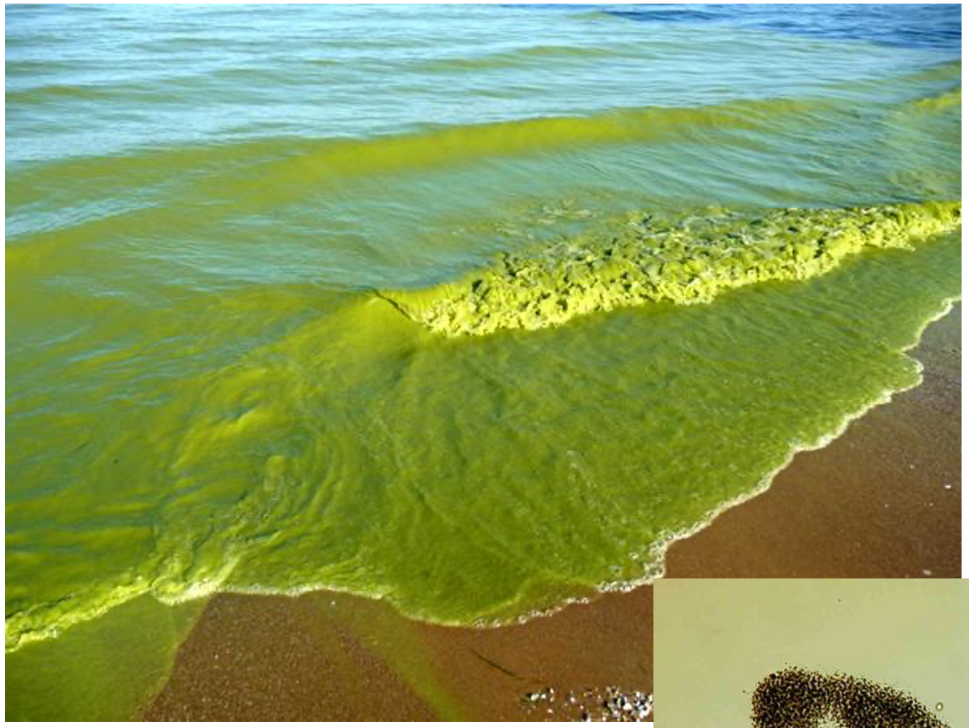
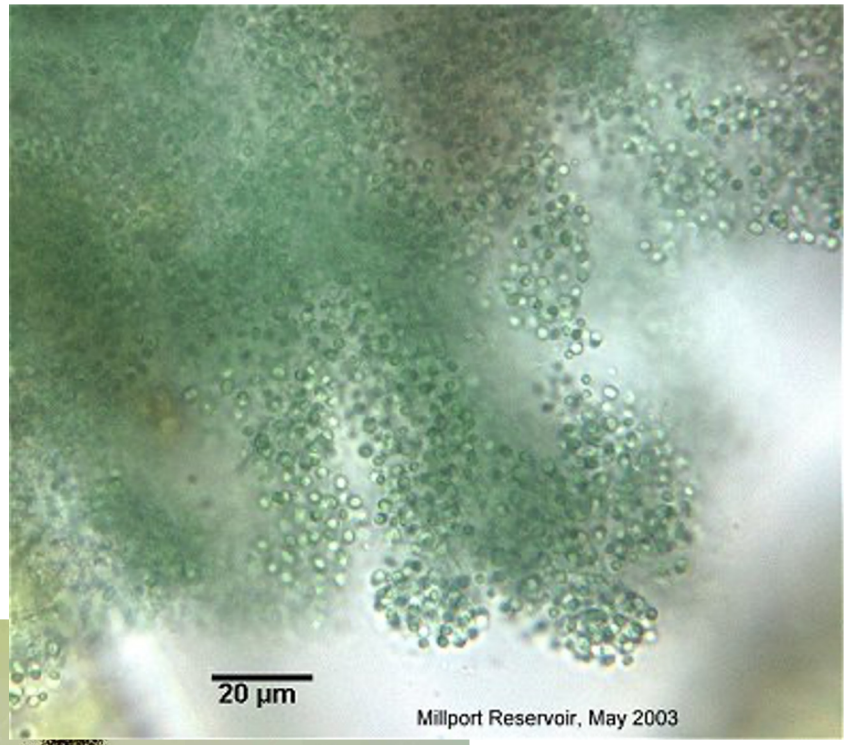
Revisiting the Eutrophication Experiment

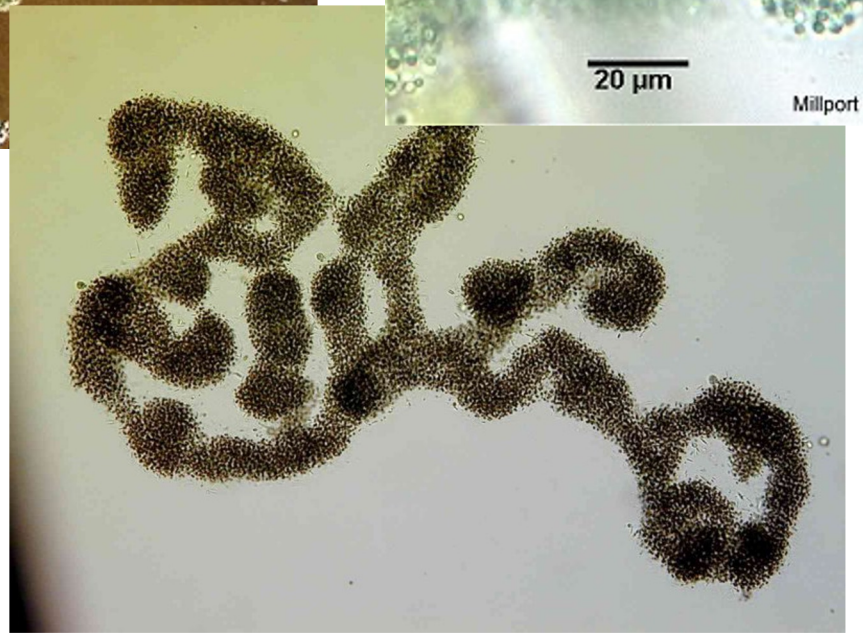
Through the Lenses of Botany, Math, Biology and Critical Thinking



Microcystis – one of several cyanobacteria (blue green algae) that may form Harmful Algal Blooms (HABs)





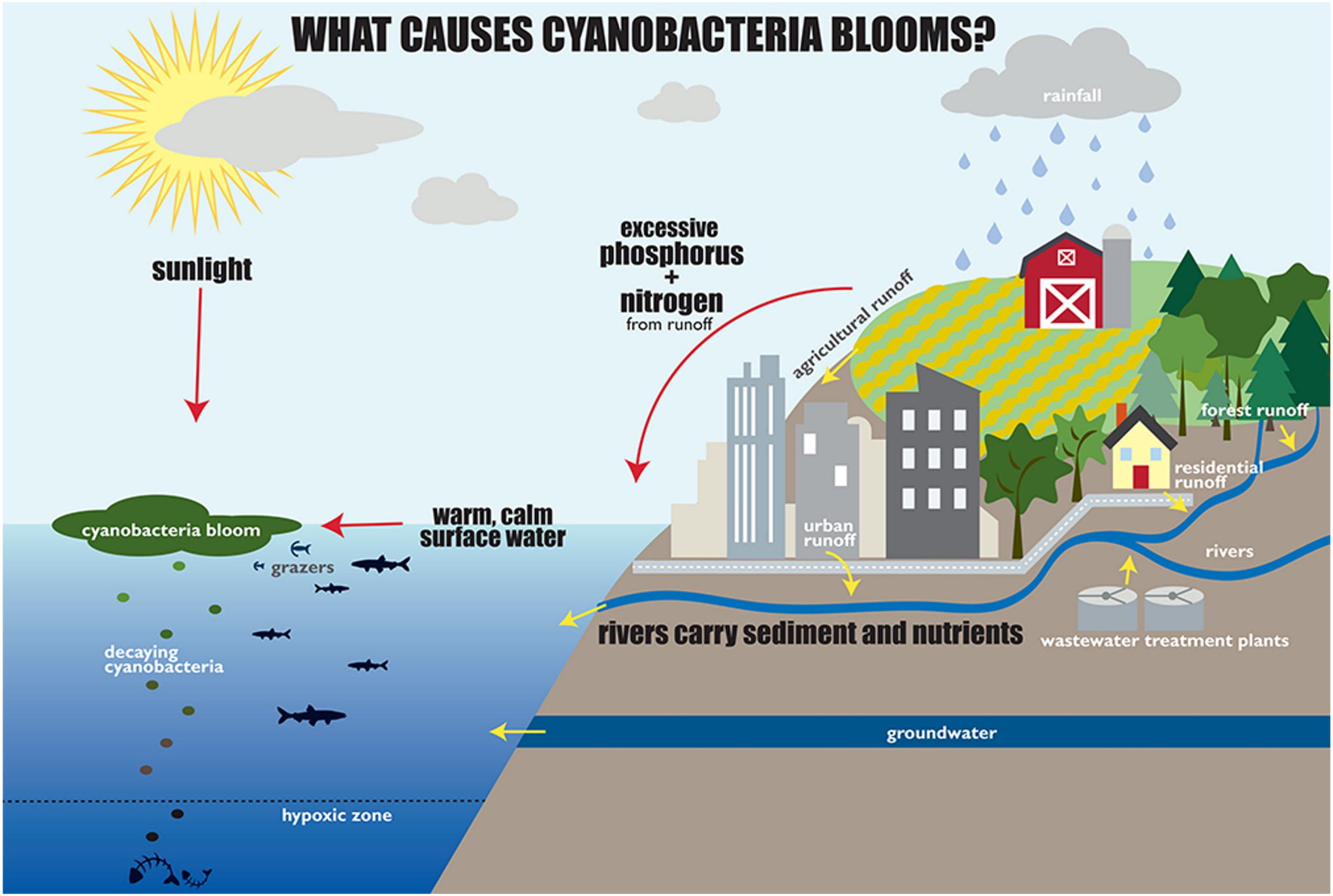


http://cfb.unh.edu/phycokey/Choices/Cyanobacteria

Why is Nitrogen the *Limiting Factor* ?

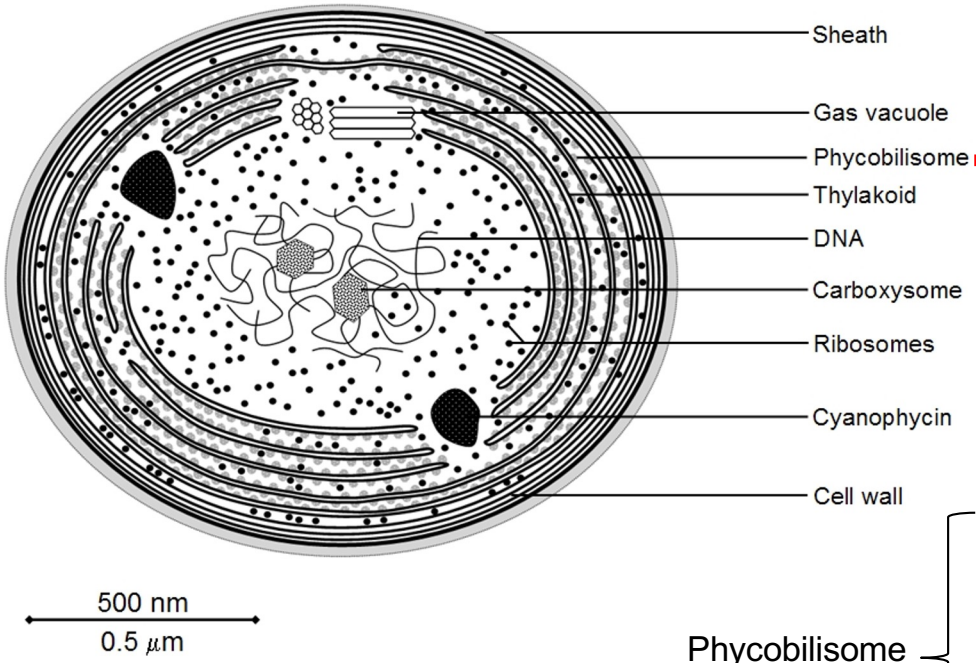
http://blog.healthkismet.com/wp-content/uploads/2012/04/klamath_lake_blue_algae.jpg

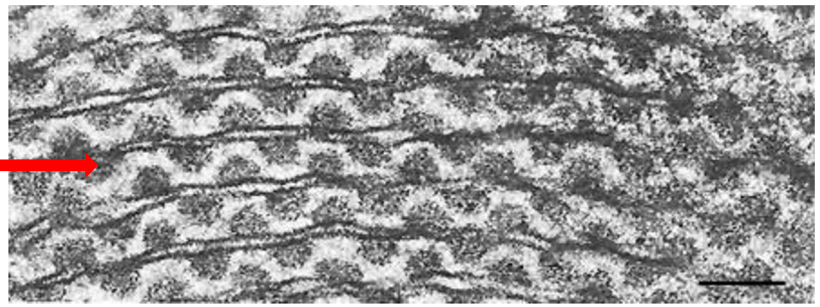
Klamath Lake 2012


WHAT CAUSES CYANOBACTERIA BLOOMS?
rainfall
sunlight
excessive
phosphorus
+
nitrogen
from runoff
agricultural runoff
forest runoff
residential
runoff
urban
runoff
rivers
cyanobacteria bloom
warm, calm
surface water
grazers
decaying
cyanobacteria
rivers carry sediment and nutrients
wastewater treatment plants
groundwater
hypoxic zone

Method for Measuring Microalgal Growth: The Pigment Approach (Proxy for Biomass and/or Cell #)

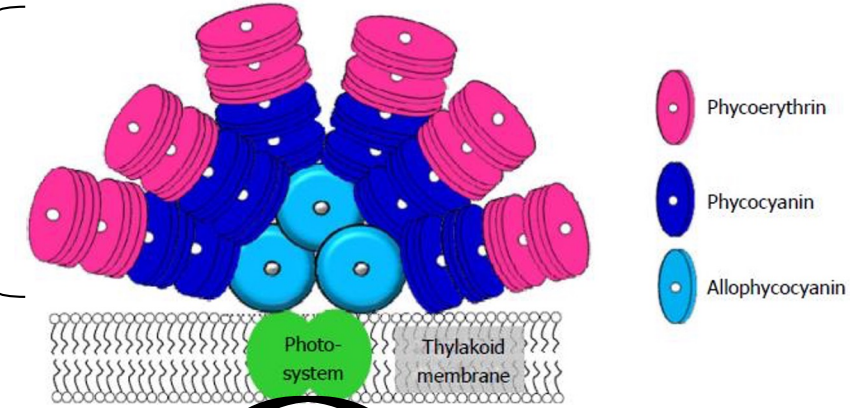
Cross-section through a cyanobacterial cell





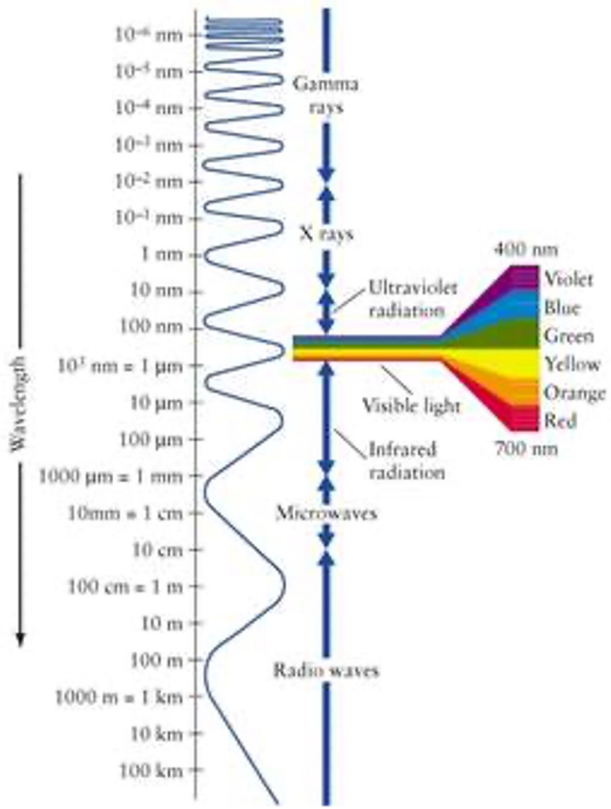
0.05 μm

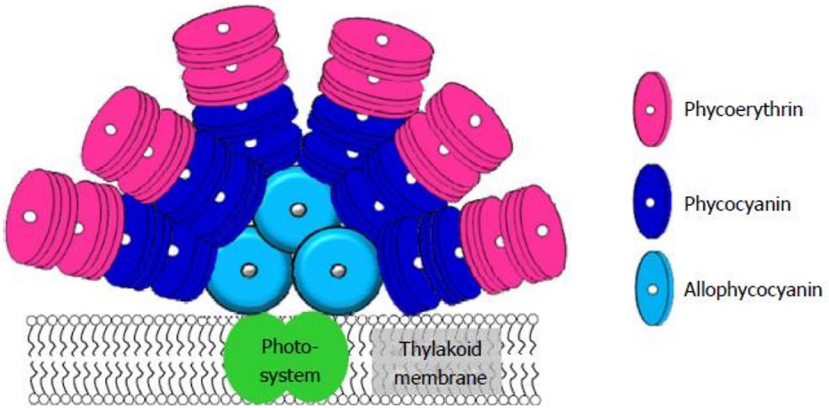
Phycobilisome



$2H_2O$ $O_2 + 4H^+$

The Pigment Approach Continued: Relative Fluorescence Units (RFUs)



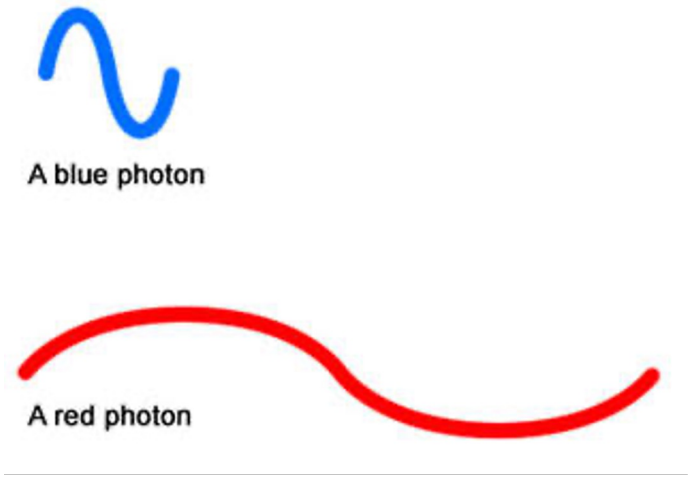


Upon reaching a plant, 4 things can happen to light energy:

1. Reflected rather than absorbed.
2. Not absorbed by a pigment, becomes heat
3. Absorbed by a pigment and runs through the electron transport reactions (= light reactions) of photosynthesis to make high energy molecules used for carbon fixation – good!
4. Absorbed by a pigment but fails to move through electron transport; returns to a lower energy state via giving off energy as light - as fluorescence.

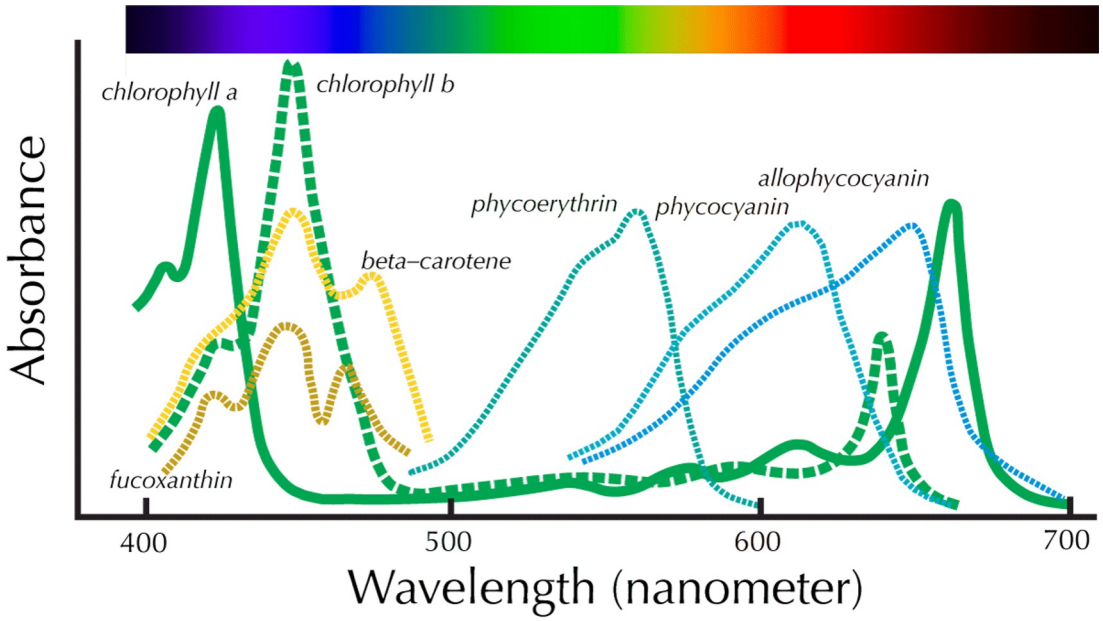
Your Experiment Takes Advantage of Fluorescence to Quantify the Amount of Algal Cells Present in a Sample

Assumption: More Fluorescence (RFUs) = More Algal Cells



Fluorescence occurs at a longer wavelength than the wavelength of the original photon. RFUs are measured at the new, longer wavelength.

The RFU Pigment Approach can Discriminate between General Algal Groups



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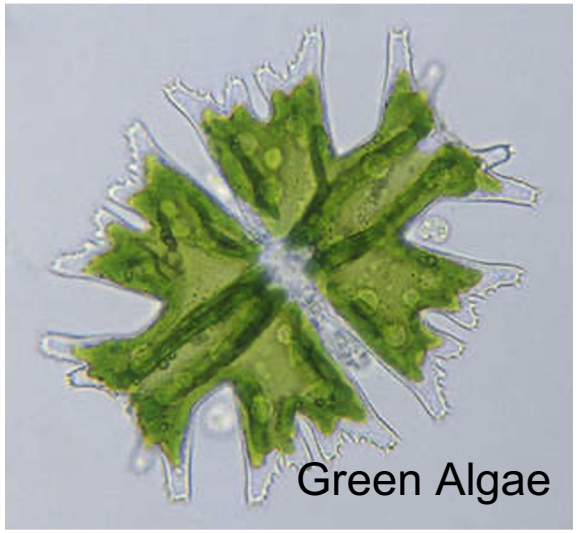


H-D method

In the case of the Klamath River samples, we posed the following hypotheses and predictions:

H_1: N is limiting algae growth in Klamath River water samples. *If N is limiting, then we predict that RFUs will increase more in flasks with added N than in control flasks.*

H_0: N is not limiting algae growth in Klamath River water samples. *If N is not limiting, then we predict that RFUs will be as high as or higher in controls than in flasks with added N.*

1. For H_1, do you think the prediction is logically necessary? Why or why not? What "word trick" can you use to examine a prediction's logical necessity?

H-D method

In the case of the Klamath River samples, we posed the following hypotheses and predictions:

H_1: N is limiting algae growth in Klamath River water samples. *If N is limiting, then we predict that RFUs will increase more in flasks with added N than in control flasks.*

H_0: N is not limiting algae growth in Klamath River water samples. *If N is not limiting, then we predict that RFUs will be as high as or higher in controls than in flasks with added N.*

2. Do you think the prediction for H_1 is logically sufficient? Why or why not? What "word trick" can you use to examine a prediction's logical sufficiency?

H-D method

In the case of the Klamath River samples, we posed the following hypotheses and predictions:

H_1: N is limiting algae growth in Klamath River water samples. *If N is limiting, then we predict that RFUs will increase more in flasks with added N than in control flasks.*

H_0: N is not limiting algae growth in Klamath River water samples. *If N is not limiting, then we predict that RFUs will be as high as or higher in controls than in flasks with added N.*

3. Suppose the data are consistent with the prediction that *RFUs will increase more in flasks with added N than in control flasks* and we conclude that N limits algae growth in lower Klamath River Water samples. Is this conclusion deductively valid? Why or why not? Is it strong?

Collect data & test prediction

Prediction of H_1:

If N is limiting, then we predict that RFUs will increase more in flasks with added N than in control flasks.

If *Polly is a bird*, then *Polly has feathers*.

Data:

RFUs increased more in flasks with added N than in control flasks.

Polly has feathers.

Conclusion:

N is limiting algae growth in Klamath River water samples.

Polly is a bird.

Is this conclusion reached by valid deduction?

1.If H, then P
2.P
3.So, H

NO! Affirming the consequent.

Not technically deductively valid, but provides strong presumptive (inductive) evidence for H_1. Why?

Collect data & test prediction

Prediction of H_0:

If N is not limiting, then we predict that RFUs will be as high as or higher in controls than in flasks with added N.

Data:

RFUs increased more in flasks with added N than in control flasks.

Conclusion:

We can reject the null hypothesis that N is no limiting algal growth in Klamath River samples.

Is this conclusion reached by valid deduction?

1.If H, then P
2.Not P
3.So, not H

Yes! Modus Tollens, falsifying the consequent.
Deductively valid.

H-D method

So…what did the data look like?

It is all in the numbers!

- We looked at the amounts of Chl A and Phycocyanin for treated (nitrogen added) and untreated samples as measured in RFUs at time 0 and seven days later.
- Samples from Terwer were analyzed.
- We performed linear regression and compared rates of change for treated and untreated samples.

The Big Spread Sheet

Chlorophyll Fluorescence 470/700

RED= Plastic RFU

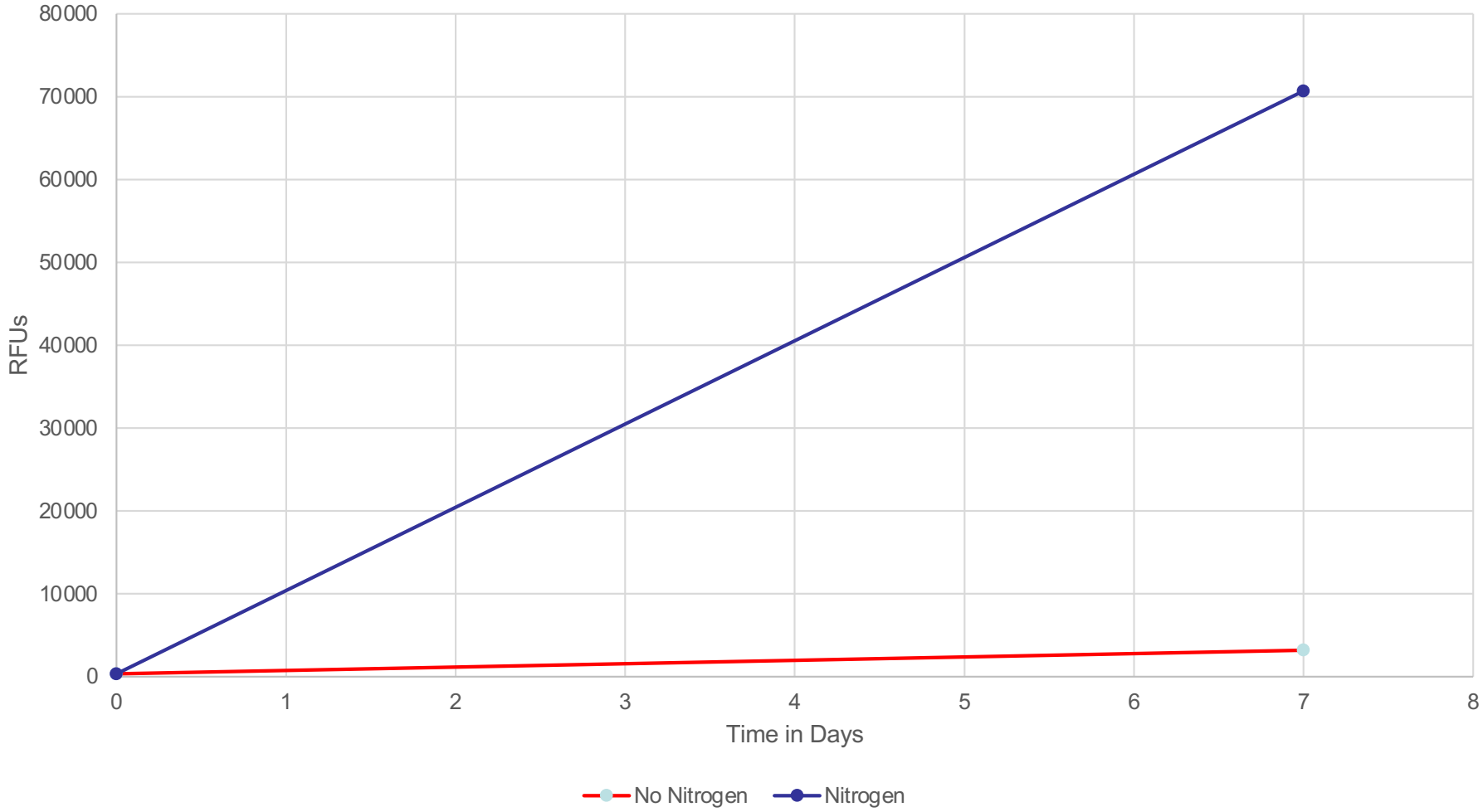
	1	2	3	4	5	6	7	8
A	9144	5827	71836	1304	1420	287641	35905	23917
B	17500	8998	4332	65730	15274	5869	1937	1423
C	1879	2166	1710	199738	1768	1882	1026	1881
D	1711	1369	1939	10950	1996	1938	103206	162055
E	1140	1939	1882	1426	1312	1312	41058	97566
F	1483	1997	1483	1483	2282	1825	23781	176734

Phcocyanin 590/670

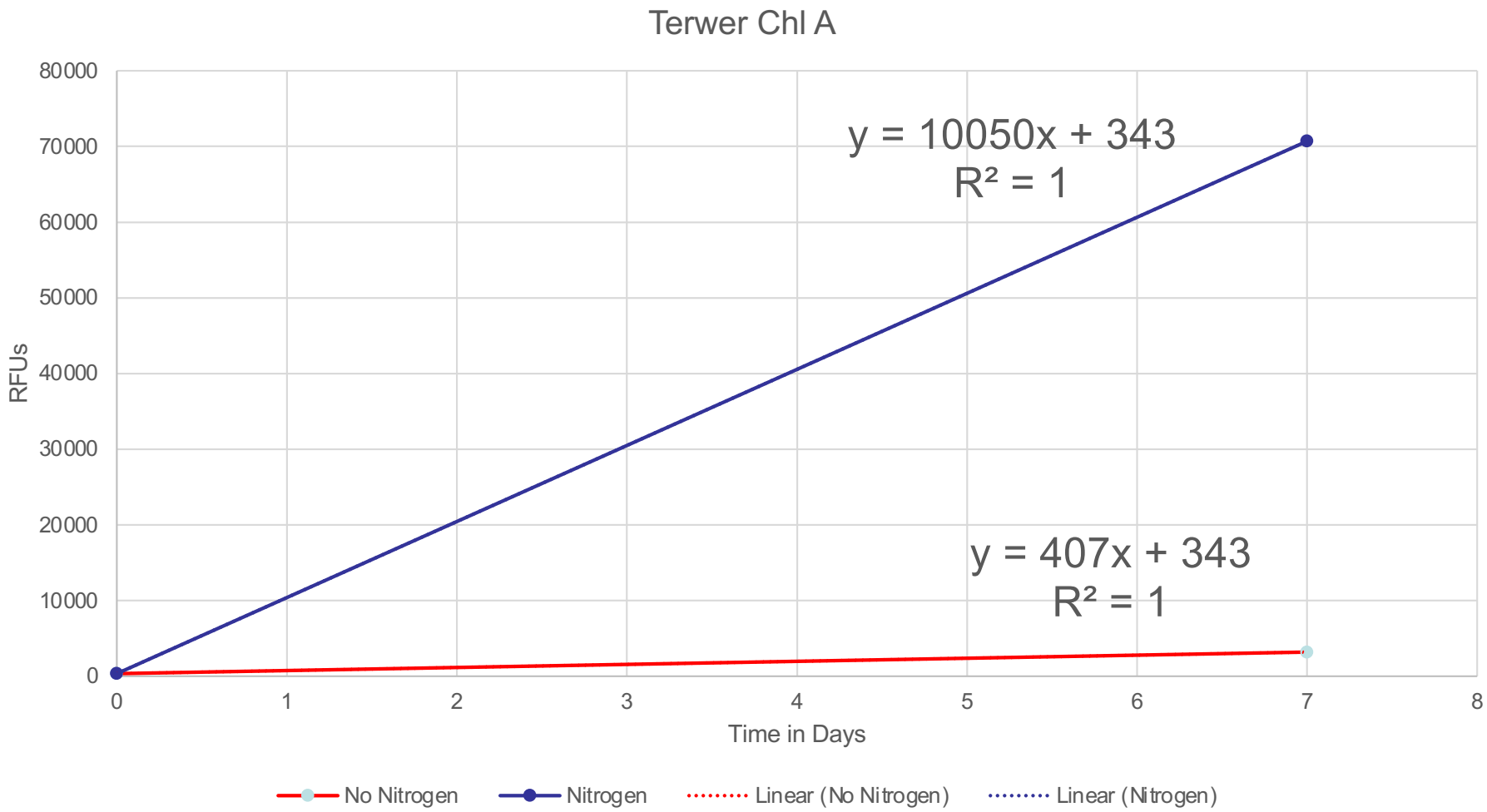
RED= Plastic RFU

	1	2	3	4	5	6	7	8
A	289677	5827	30974	6738	4494	92153	16185	10793
B	11249	8998	7199	32843	10347	8546	7196	3597
C	3599	3150	4050	58951	5400	3150	4051	4500
D	5402	4501	2701	9002	7651	4950	36905	57140
E	3150	4951	4501	3151	5402	4051	18006	31063
F	5403	4502	5402	7203	4502	6302	12605	55357

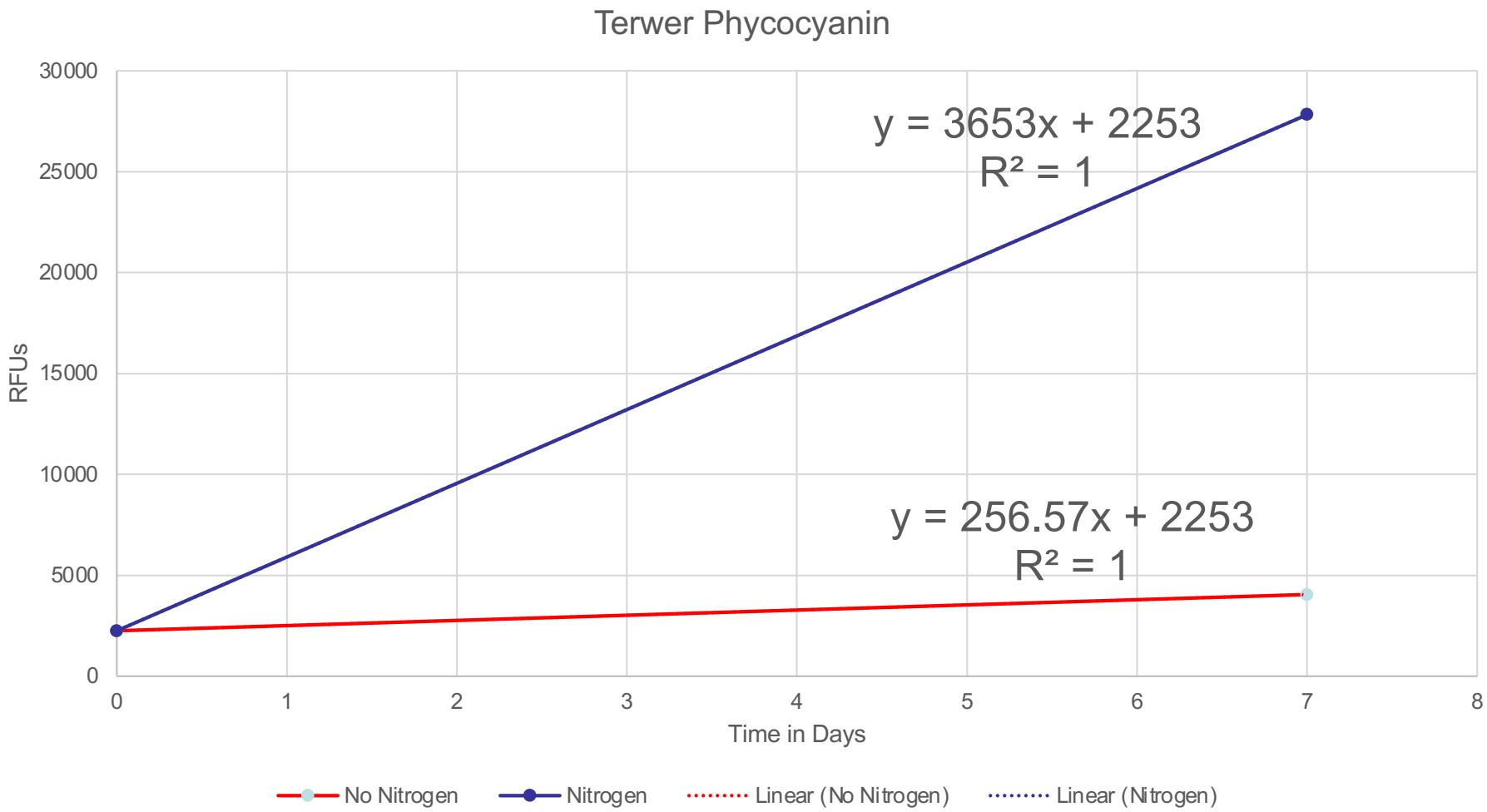
Terwer-Chl A


80000
70000
60000
50000
40000
30000
20000
10000
0
RFUs
0
1
2
3
4
5
6
7
8
Time in Days
No Nitrogen
Nitrogen

Terwer-Chl A


Terwer Chl A
80000
70000
60000
50000
40000
30000
20000
10000
0
RFUs
y = 10050x + 343
R² = 1
y = 407x + 343
R² = 1
0
1
2
3
4
5
6
7
8
Time in Days
No Nitrogen
Nitrogen
Linear (No Nitrogen)
Linear (Nitrogen)

Phycocyanin



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Conclusions

- Are the RFUs for the controls the same as the treated samples?
- The rate of change of RFUs over time was greatest for water samples treated with nitrogen.
- The rate of change of Chl A was larger than the rate of change of phycocyanin measured in RFUs.

First look was very limited!

SUMMARY DATA

ORLEANS

Chlorophyll a

Date	Time	Nitrogen added Mean	No N added Mean
18-Aug	0	4709	4709
20-Aug	2	15192	10542
22-Aug	4	22948	6567
25-Aug	7	685835	2428

TERWER

Chlorophyll a

Date		Nitrogen added Mean	No N added Mean
18-Aug	0	5612	5612
20-Aug	2	20665	20243
22-Aug	4	36855	10874
25-Aug	7	667697	17485

SITES POOLED

Chlorophyll a

Date		Nitrogen added Mean	No N added Mean
18-Aug	0.00	5161	5161
20-Aug	2.00	17929	15392
22-Aug	4.00	29901	8721
25-Aug	7.00	676766	9957

ORLEANS

Phycocyanin

Date		Nitrogen added Mean	No N added Mean
18-Aug	0	4420	4420
20-Aug	2	14694	11261
22-Aug	4	43234	4623
25-Aug	7	186122	1706

TERWER

Phycocyanin

Date		Nitrogen added Mean	No N added Mean
18-Aug	0	4202	4202
20-Aug	2	8737	8944
22-Aug	4	9300	4655
25-Aug	7	152194	6799

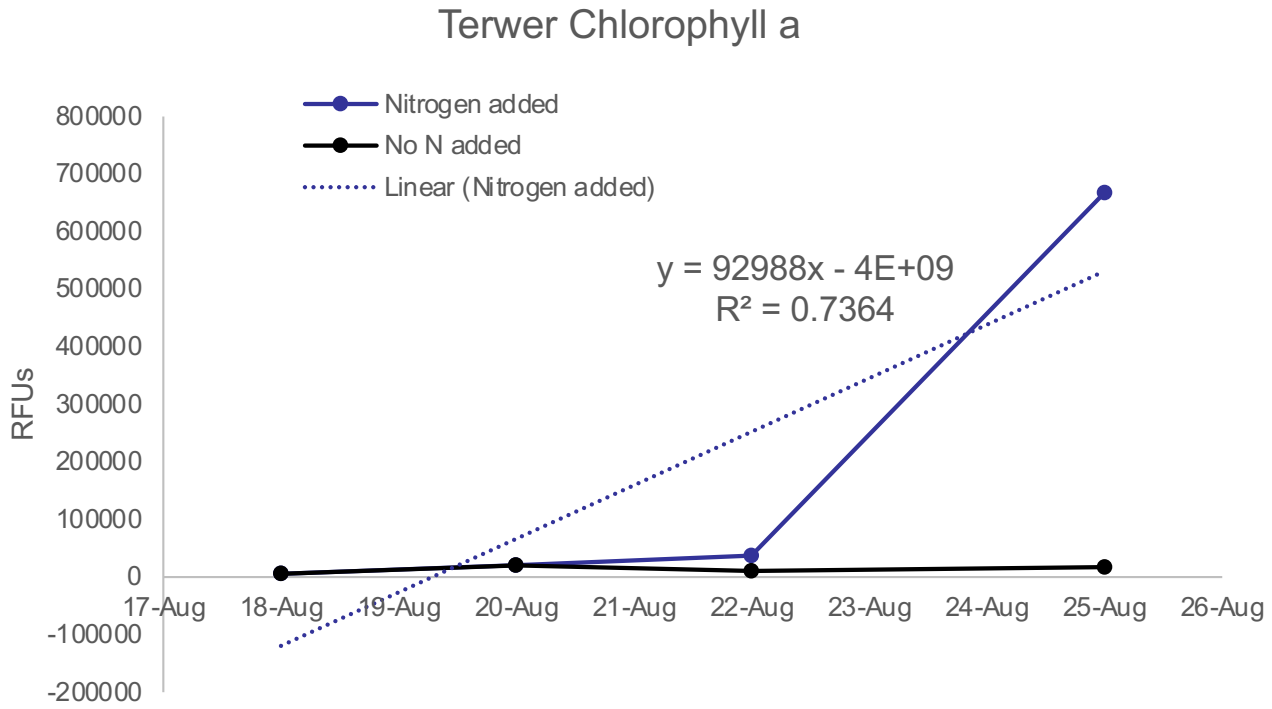
SITES POOLED

Phycocyanin

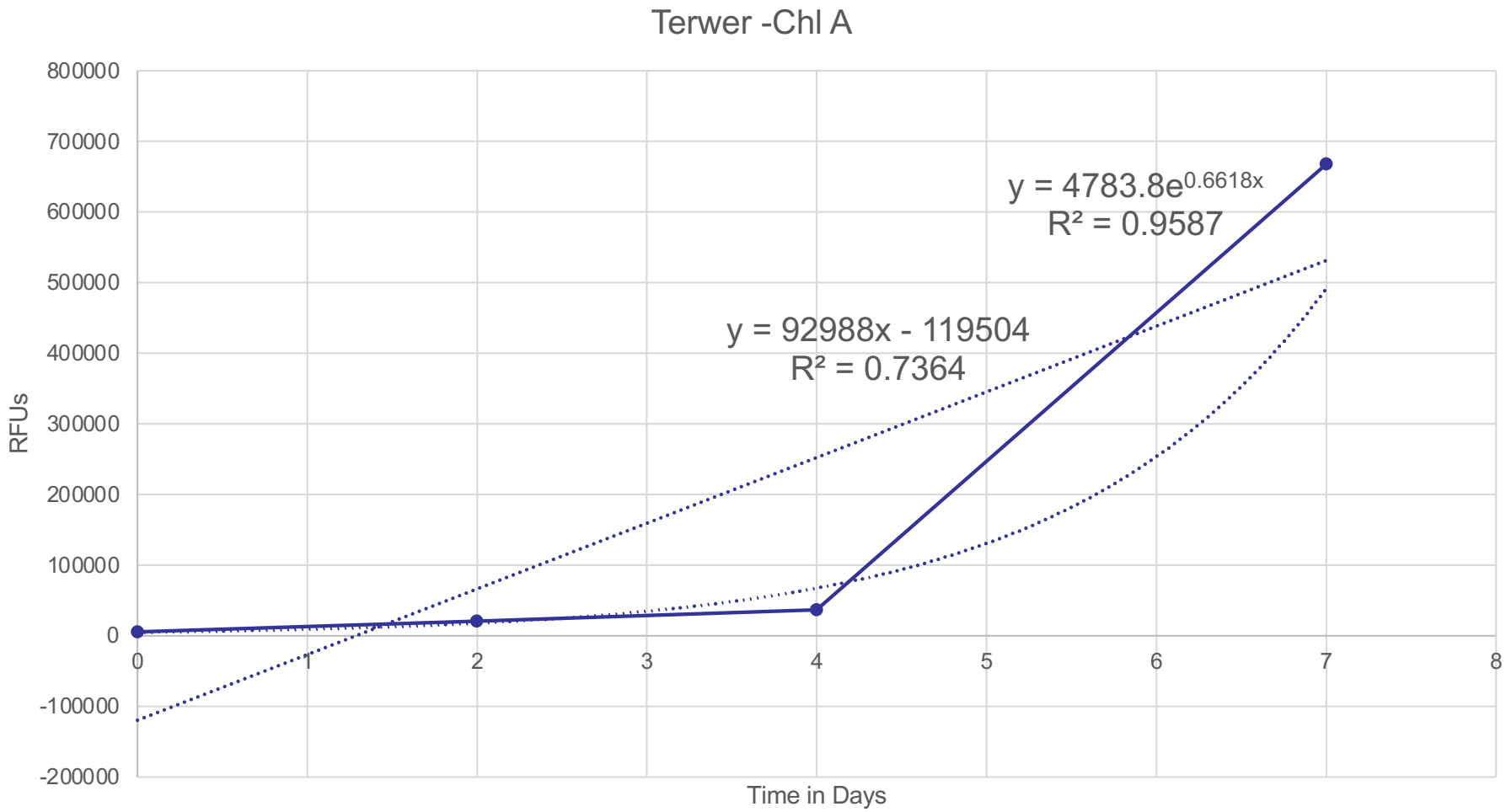
Date		Nitrogen added Mean	No N added Mean
18-Aug	0.00	4311	4311
20-Aug	2.00	11716	10102
22-Aug	4.00	26267	4639
25-Aug	7.00	169158	4253

Is the Data really Linear?

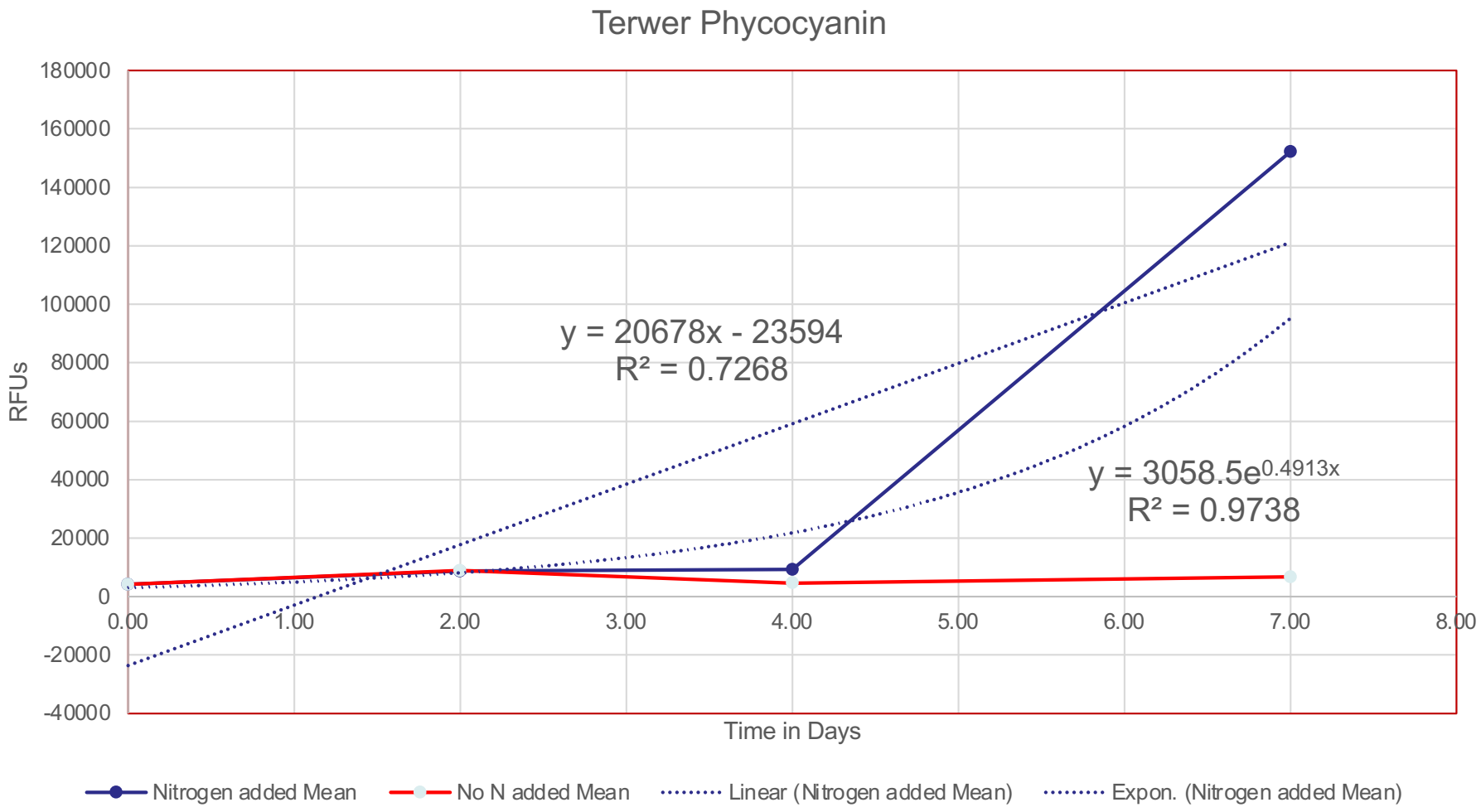
- When we consider data from 4 points in time, things change.



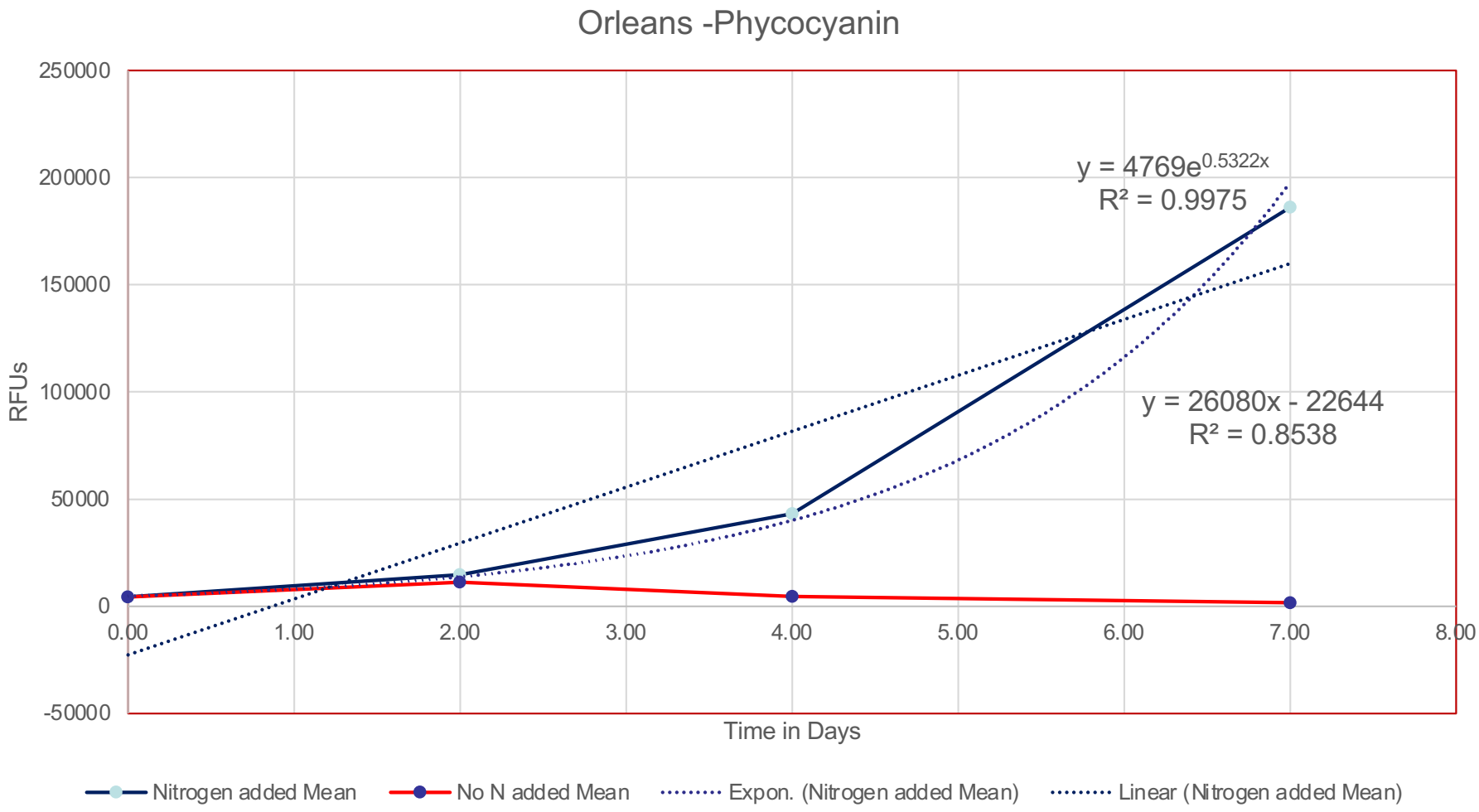
Exponential Equation fits Better



Terwer-Phycocyanin



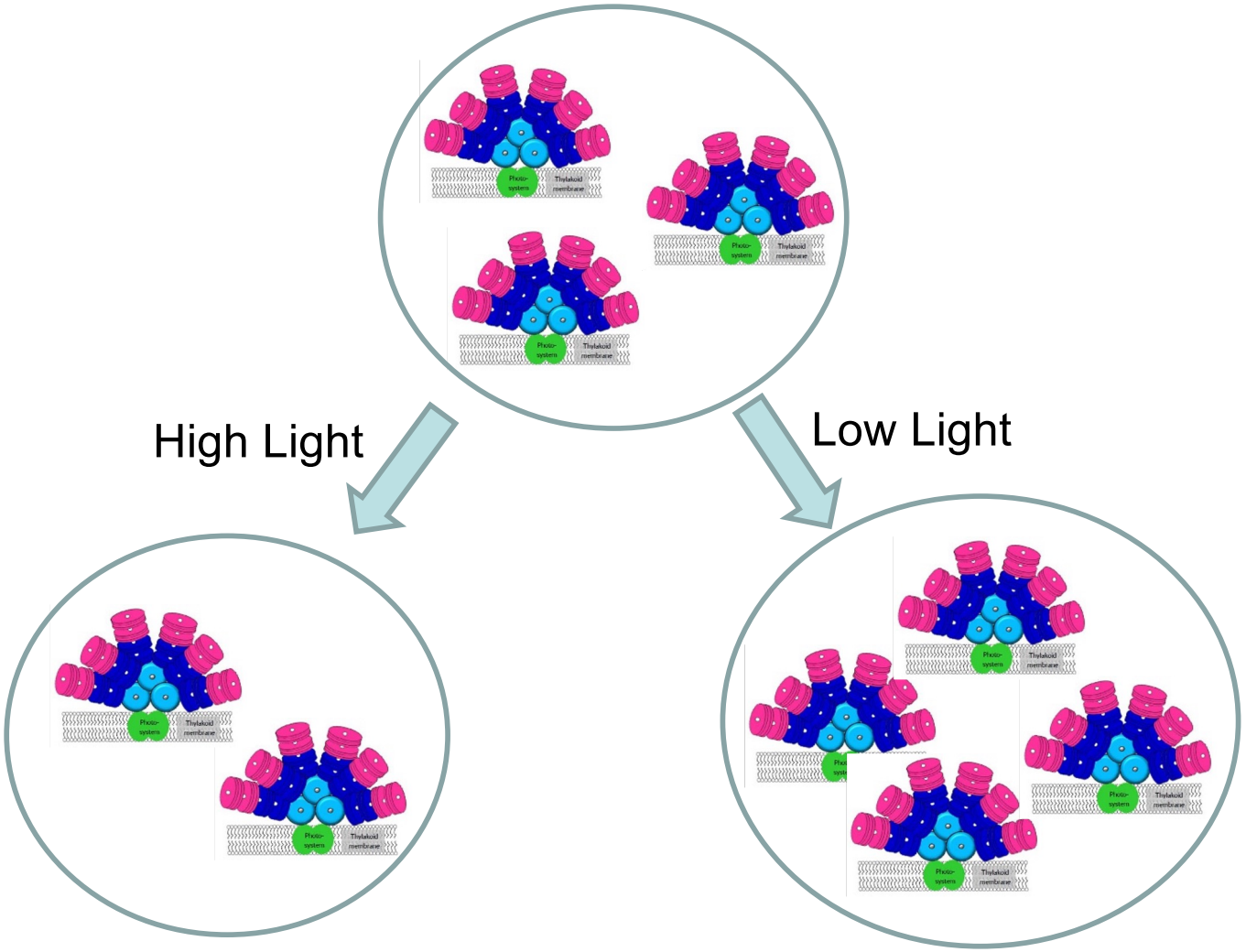
Orleans-Phycocyanin



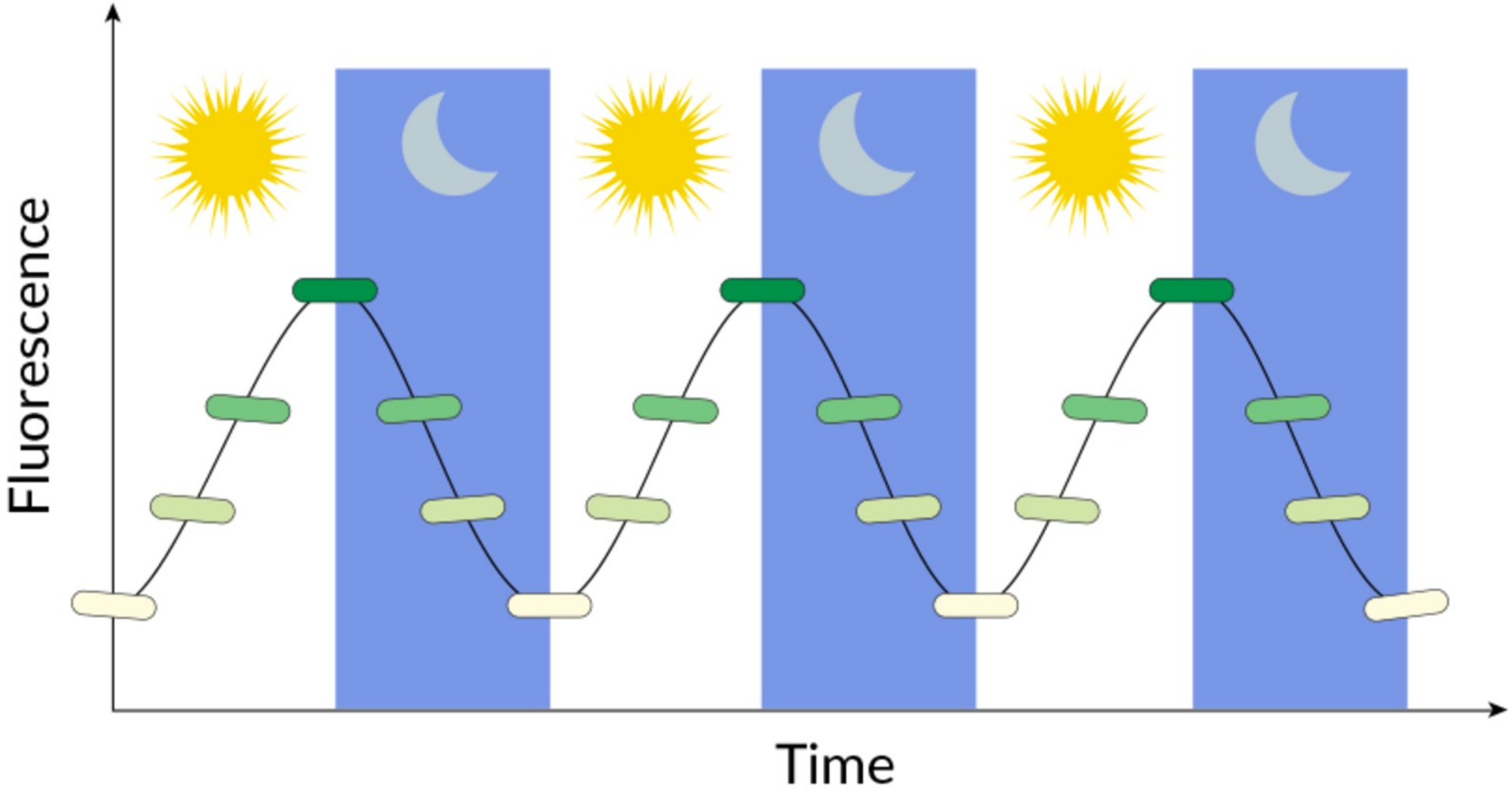
Is there an alternative explanation for the data?

The ‘Problem’ with the RFU Pigment Method

RFUs Could Change Even Though Cell # or Biomass has not Changed



If increased ChlA or Phycocyanin is Due to light, RFUs would fluctuate on 12 hour cycles



Conclusion

- “Overall, in both the Orleans and Terwer samples, the water treated with nitrogen experienced much greater exponential growth of both phycocyanin and chlorophyll a than the untreated water.” ~Math 113 student

- “I believe that the growth of Chl a and Phycocyanin is more exponential than linear. I do not think that the algae will be able to grow exponentially forever.” ~Math 113 student

More Conclusions

- “Based on my graphs I can conclude that nitrogen is a limiting factor in the growth of Chlorophyll a and Phycocyanin in both Orleans and in Terwer.” ~Math 113 student

- “In the long run, I feel the growth of Chl a and Phycocyanin will perform in a logistic growth pattern more than an exponential or linear pattern.” ~Math 113 student

Can we reject $\mathbf{H_0}$?

We can reject H_0,
the data support H_1:

N is limiting algae growth in Klamath River water samples.

Why is Nitrogen the *Limiting Factor* ?

http://blog.healthkismet.com/wp-content/uploads/2012/04/klamath_lake_blue_algae.jpg

Klamath Lake 2012

Phases of Mitosis

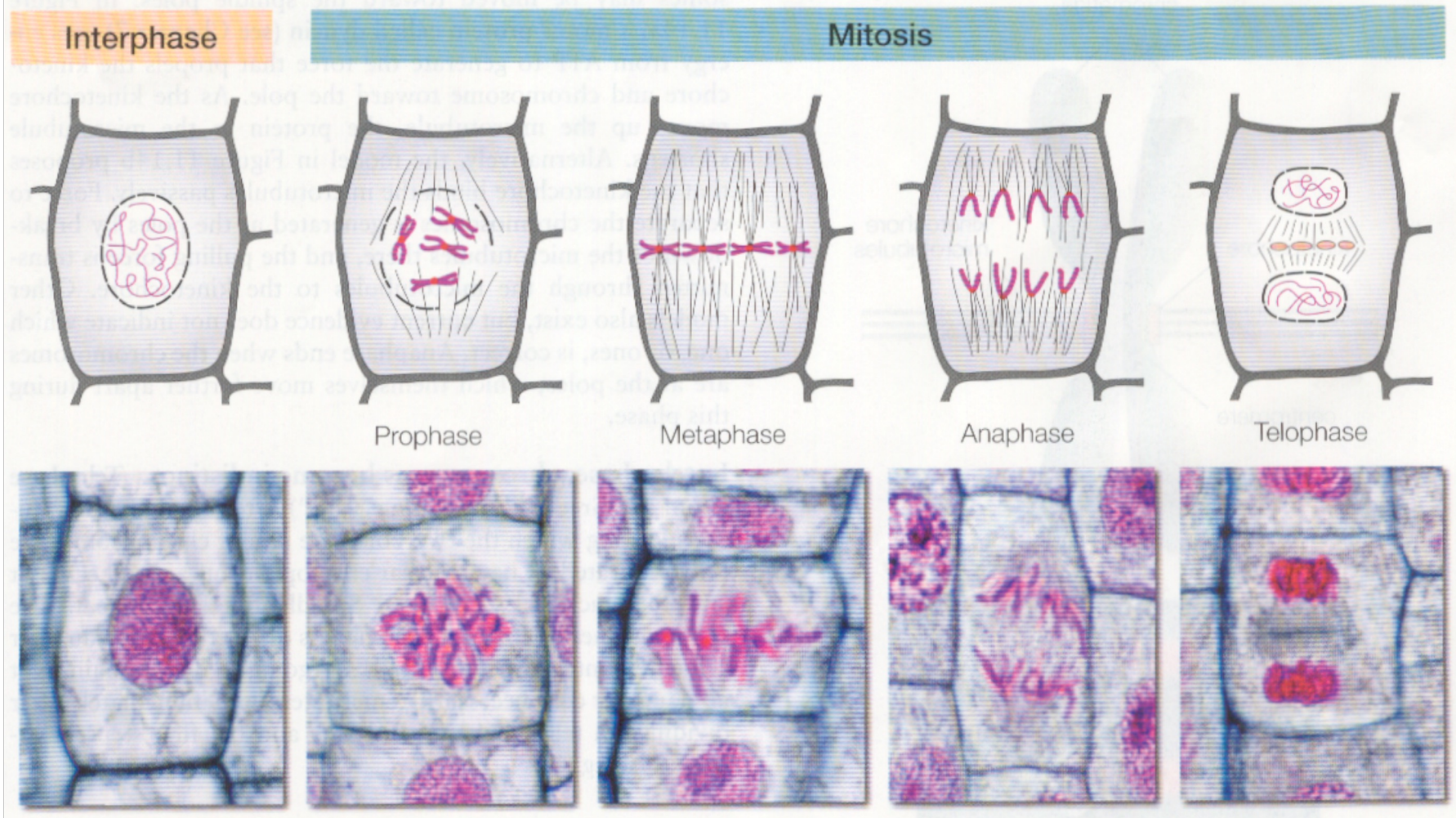
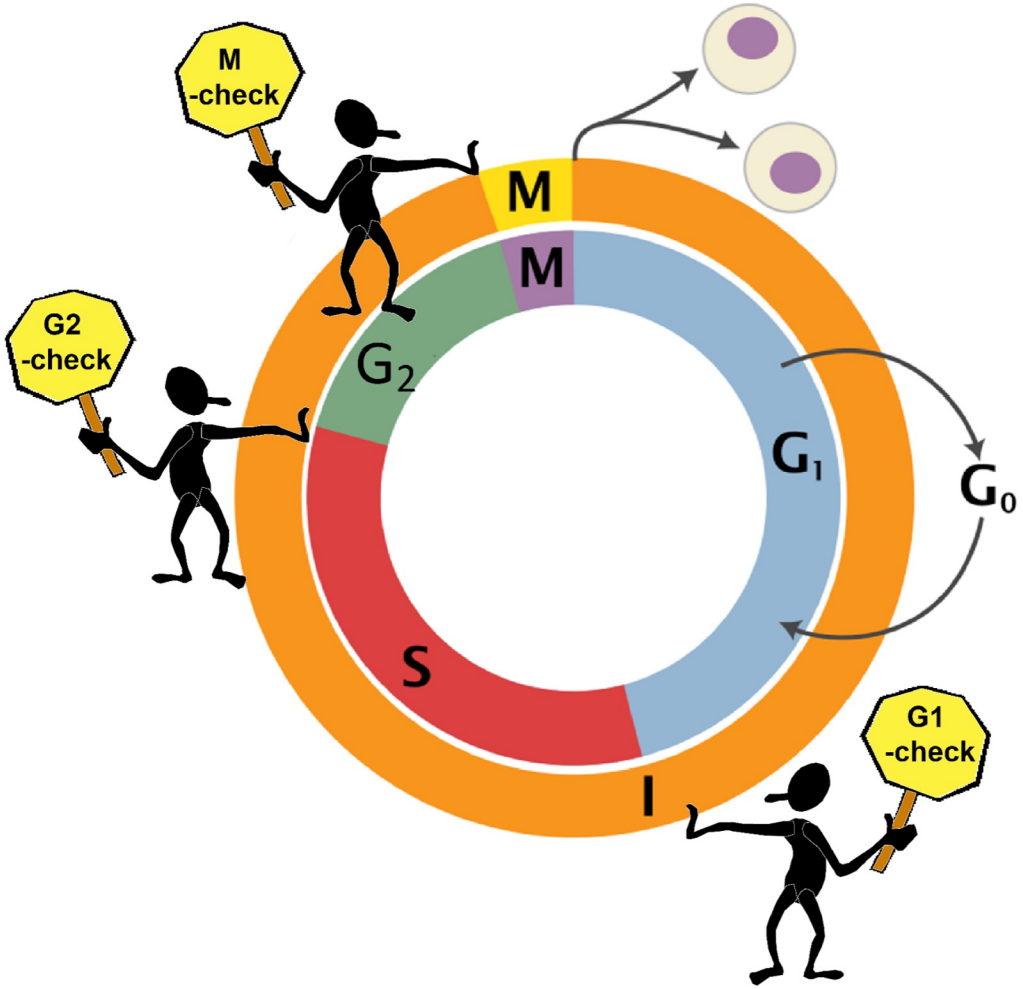
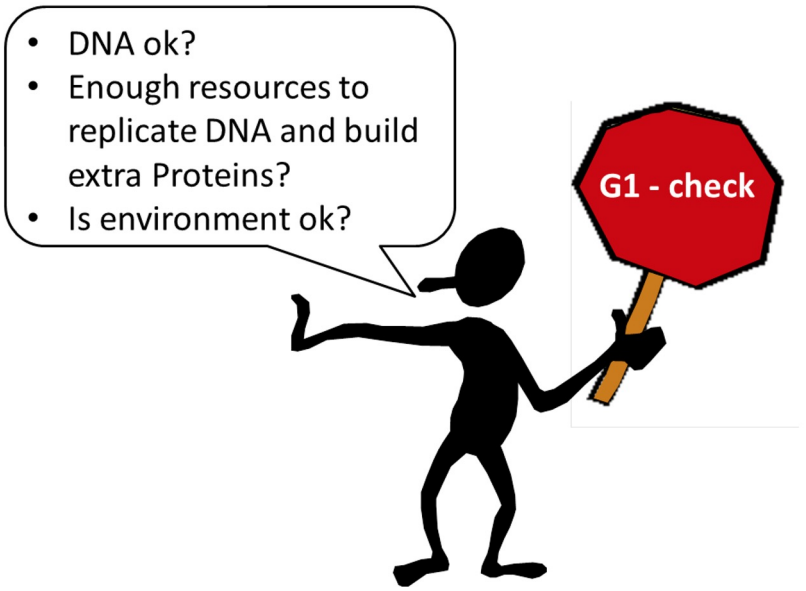


Figure 11.10 **The phases of mitosis** In interphase, the chromosomes are not visible with the light microscope and the nuclear envelope is intact. The nucleolus (or nucleoli) is apparent (though not in the photograph shown here). In prophase, the DNA condenses and chromosomes become apparent. The mitotic spindle begins to form and the nuclear envelope breaks down. Chromosomes align themselves in the center of the cell at metaphase. Sister chromatids separate in anaphase and move to the poles. In telophase, the sister chromatids have completed their poleward migration and the chromosomes become diffuse. The nuclear envelope re-forms and the nucleolus reappears. Cytokinesis begins during telophase with a phragmoplast and cell plate being visible. The photographs shown here are of mitosis in an onion root tip. Because of its simple structure, the growing root is an excellent place to observe cell division in plants.

Graham et al. 2003

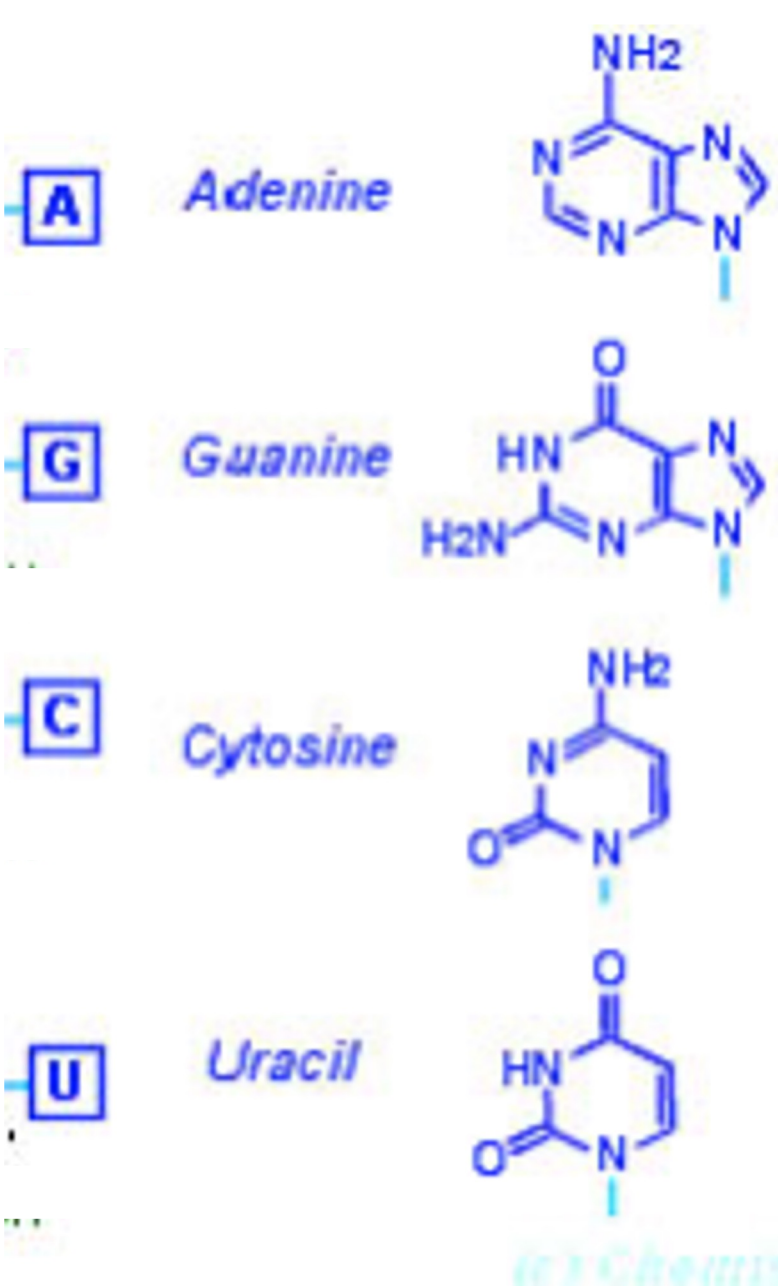
Cell Division Cannot Happen Without DNA Replication

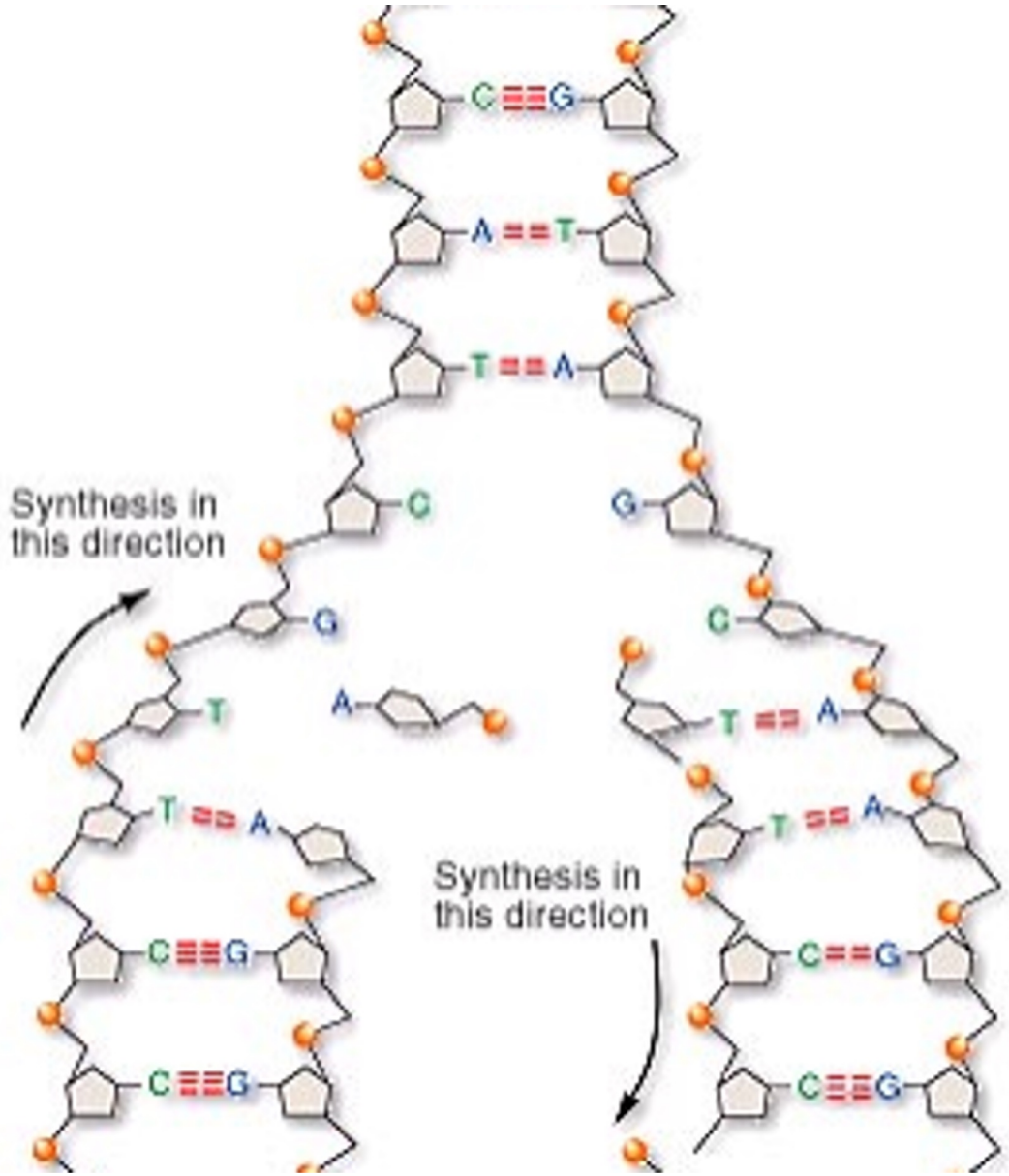




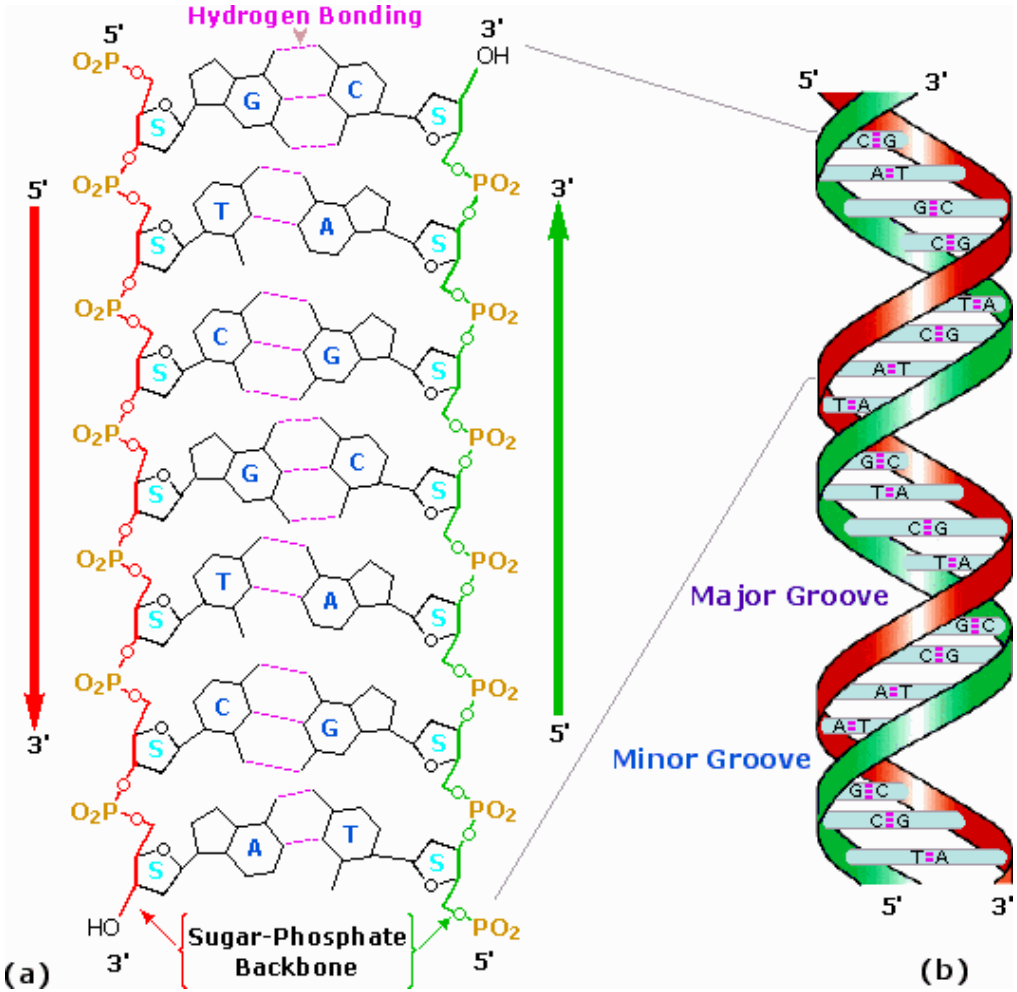
https://adapaproject.org/images/biobook_images/Checkpoints1.jpg

Nitrogen is required for DNA Synthesis





Phosphorous is Required, too!

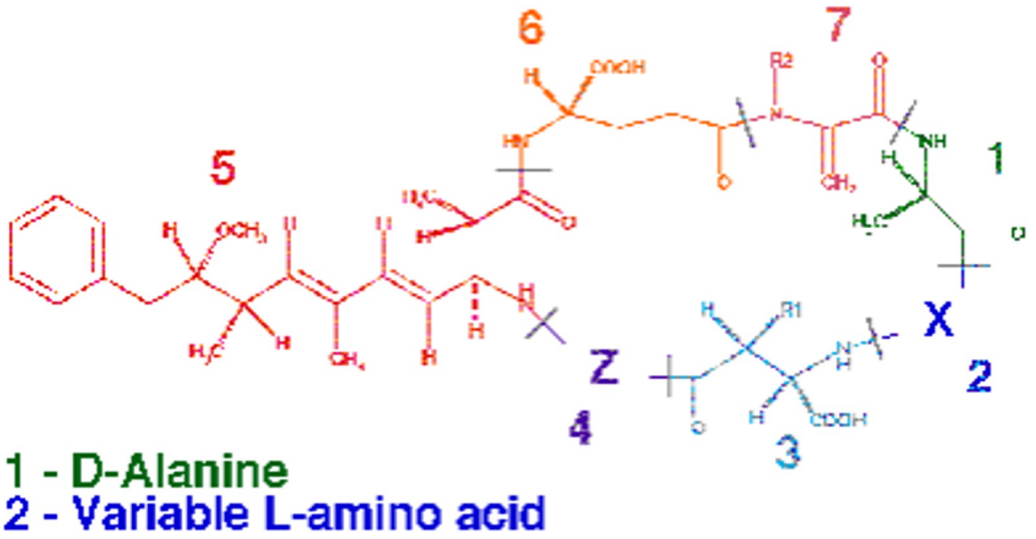


As You'll Study In Chemistry Next Semester!

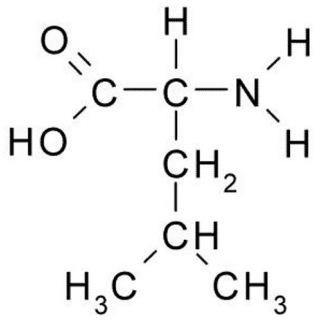
Why do we care?

The Microcystin Peptide is Toxic

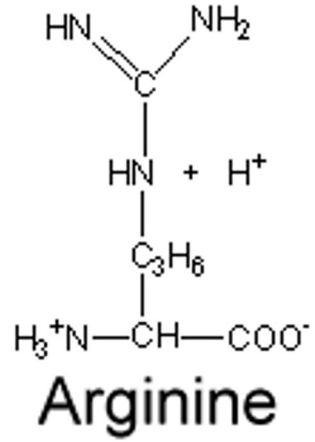
- Rashes
- Blistering
- Nausea
- Vomiting
- Fever
- Liver Failure



X= Leucine



R= Arginine



The World Health Organization has set the following thresholds for both cell and toxin concentrations in recreational waters

	cells/ml	toxin µg/L
Low Risk:	20,000	4
Moderate Risk:	100,000	20
CA Posting	40,000	8
Copco 7/13/05	11 x 10^6	667*
2005 Maximum	163 x 10^6	1994

>92x the Tolerable Daily Intake!
TDI= 0.04 µg/kg bw^{-1}

Copco shoreline standing on shoreline 7-13-05

Klamath Reflection #3

Due Thursday at 11:59pm

Now that you have completed the eutrophication experiment, think about your own intellectual growth over the semester. What did you think of the process back in the Summer Immersion? Do you think about it differently now, and how? What do you think about the role of math, botany, and chemistry in understanding complext environmental and social problems? Have those thoughts changed since you've arrive at HSU?