Acclimation to Nutrient Limitation in Microalgae

by
Bethany K. Moua

A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in BioHealth Sciences
(Honors Associate)

Presented May 26, 2016
Commencement June 2016

AN ABSTRACT OF THE THESIS OF

Bethany K. Moua for the degree of Honors Baccalaureate of Science in BioHealth Sciences presented on May 26, 2016. Title: Acclimation to Nutrient Limitations in Microalgae .

Abstract approved:

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Kimberly Halsey

Microalgae play an important role in the marine ecosystem. They are primary producers and form the base of the aquatic food web. The effects and changes that occur to microalgae are expected to have influences on the higher tropic levels. One major factor that influences growth of microalgae is the availability of nutrients. Availability of nutrients is highly affected by seasonality- short term climate changes. Previous research suggested that green algae and diatoms appear to have different acclimation strategies to nutrient limitations. This study focused on determining what some of those different acclimation strategies are. The diatom *Thalassiosira pseudonana* and the green algae *Dunaliella tertiolecta* were grown at different nutrient limited rates in continuous culture chemostats and observed from inoculation to steady state. At steady state, both "fast growing" *T. pseudonana* and *D. tertiolecta* showed similar acclimation strategies for chlorophyll and lipid body production; specifically, an increase in nutrient availability caused an increase in chlorophyll and lipid body production. "Slow growing" *T. pseudonana* and *D. tertiolecta* showed the same

acclimation strategy for chlorophyll; namely, less nutrient availability decreased chlorophyll production. However, “slow growing” *T. pseudonana* and *D. tertiolecta* showed different acclimation strategies for lipid body production. In “slow growing” *D. tertiolecta*, lipid body content was very low while lipid body content was significantly greater than any of the other cells and treatments in *T. pseudonana*. These results may reflect the phenomenon known as the “Bloom and bust” growth pattern of *T. pseudonana*. These results also help better understand physiological response of microalgae to nutrient conditions. Some physiological acclimation strategies are shared while other metabolic pathways are regulated very differently between microalgal species.

Key Words: Microalgae, phytoplankton, diatoms, nutrient limitation, green algae

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Bethany Moua, Author

Acknowledgements

This research was conducted at Oregon State University in the Department of Microbiology in Dr. Kimberly Halsey's laboratory.

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Introduction

Phytoplankton, also known as microalgae, are microscopic aquatic organisms that live in fresh and/or marine environments. Phytoplankton are primary producers and are the base of the aquatic food web. Zooplankton feed on phytoplankton and higher trophic levels feed on zooplankton. When phytoplankton are disturbed, those physiological effects can influence the flow of energy and carbon through the higher trophic levels. Not only do phytoplankton influence food web dynamics, but phytoplankton also contribute to the global carbon cycle. Phytoplankton capture carbon through photosynthesis. Photosynthesis is the process by which light energy is converted into chemical energy. This chemical energy is used to "fix" atmospheric carbon dioxide into sugars that the cell can use to fuel their growth and division. The carbon rich phytoplankton cells then influence the predators that will consume them by potentially altering predator growth and fitness.

The two microalgae being studied in this research are *Thalassiosira pseudonana* and *Dunaliella tertiolecta*. *T. pseudonana* is a diatom, a brown algae. It is a model organism and can be found easily and abundantly throughout the oceans. A model organism is an organism that is highly studied and for which a lot of data and information is already known and readily available. Thus, *T. pseudonana* was the first eukaryotic phytoplankton to be fully sequenced. They are responsible for roughly 20% of the global carbon fixation (Armbrust et al. 2004). *T. pseudonana* are non-motile, roughly 4-6 µm in diameter, and have a glass like cell wall composed of silicon. *D.*

tertiolecta is a green algae and also a model organism. While *D. tertiolecta* is not quite as abundant in natural ecosystems as *T. pseudonana*, they have been heavily studied as a biofuel alternative. *D. tertiolecta* is motile, oval shaped, 10-12 µm long, and have two flagella.

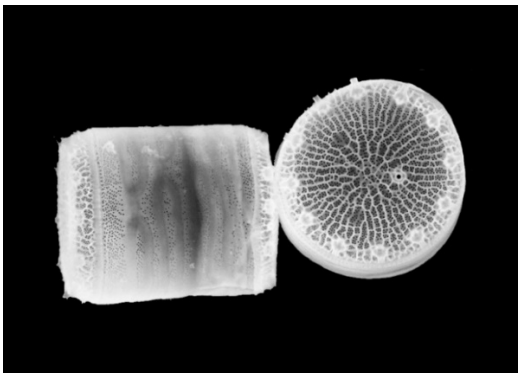

Figure 1: Image of *Thalassiosira pseudonana*[8]

Figure 2: Image of *Dunaliella tertiolecta* [9]

Nitrogen availability is well known to influence the growth of phytoplankton; one of the key physiological changes phytoplankton make in response to nitrogen availability is that they alter their chlorophyll content. Chlorophyll is used by phytoplankton to absorb light energy to fuel photosynthesis. In ocean ecosystems, nitrogen is naturally a limiting factor. Without nitrogen, chlorophyll is an expensive

investment for the cell (Halsey et al. 2015). Usually, with an increase in nitrogen availability, there is an increase in chlorophyll production.

Lipid bodies are intracellular carbon pools found in many microbes. They are highly rich energy and carbon reserves that are produced by the metabolism of the microalga. Lipid body accumulation is known to be influenced by nutrients and availability of these nutrients. Because there is no nitrogen in lipid bodies used for long term storage, some cells are thought to accumulate lipids in response to N-starvation. Thus, cells may be able to endure long periods of nitrogen starvation by replying only on carbon stores to fuel (albeit very slow) metabolism. Ecologically, lipid bodies are important, because they provide information about the environmental conditions of their habitat, and they may influence the upstream food web through the transfer of carbon and energy.

Previous research showed that *T. pseudonana* and *D. tertiolecta* displayed different acclimation strategies to steady state nutrient limitation (Halsey et al. 2015). The research conducted in this study was aimed to specifically observe and determine the chlorophyll and lipid body acclimation strategies in *T. pseudonana* and *D. tertiolecta* in response to their nutrient limiting environments. *T. pseudonana* and *D. tertiolecta* were grown in continuous culture chemostats and exposed to two different nutrient availabilities. I will refer to those different nutrient availabilities as: “fast growing” and “slow growing.” “Fast growing” nutrient limited cells received a nutrient supply at a fast rate (300 ml d^{-1}/300 ml culture) as such, they eventually grew at a specific growth rate of 1.0 d^{-1}. “Slow growing” nutrient limited cells, received nutrients at a slow rate (60 ml min^{-1}/300 ml culture), as such, they eventually grew at a specific

growth rate of 0.2 d^{-1}. Nutrients supplied to cultures were provided by nitrate limited f/2 + Si media which was an artificial seawater mixture. Microalgae cultures were grown until they reached the steady state growth rates described above. In steady state, microalgale physiology has fully acclimated and does not change from day to day. Determination of steady state was done through population counts using a Coulter particle counter. When population counts fluctuated less than 7% day to day, the culture had been determined to have reached steady state.

My hypotheses were (1) microalgae would accumulate lipid bodies as they acclimated to nutrient limitation, and (2) lipid body accumulation was dependent on nutrient limitation with "slow growing" nutrient limited cells expected to have a higher lipid body content than "fast growing" nutrient limited cells by steady state.

I found that "fast growing" nutrient limited cells ultimately accumulate a lot of chlorophyll and some lipid bodies by steady state. However, it was the "slow growing" nutrient limited cells that showed surprising data. "Slow growing" nutrient limited *D. tertiolecta* had very few lipid body and chlorophyll content by the time they reached steady state growth. In contrast, "slow growing" nutrient limited *T. pseudonana* had a high lipid body content and very little chlorophyll content. Two very different acclimation styles were observed in these two different microalgae. These results show that some physiological acclimation strategies in response to nutrient limitation are shared across phytoplankton species (i.e., chlorophyll regulation), but other metabolic pathways are regulated very differently (i.e., lipid body accumulation). These results impact how we understand the carbon and energy content of different microalgae

depending on the degree of nutrient limitation, and could provide important information for researchers wanting to improve oil production using microalgae.

Materials and Methods

Culture Conditions

All cultures were grown at a constant temperature of 18°C in continuous culture chemostats. The chemostats allowed for a continuous influx and outflux of culture medium. Chemostats were supplied with f/2 + Si media with 100 µm sodium nitrate and full light saturation (200 µmol photons $m^{-1}s^{-2}$). There were two treatment types for each microalgae: "slow growing" and "fast growing." "Fast growing" nutrient limited cells received nutrient at a fast rate (300 ml d^{-1}/300 ml culture), as such, they eventually grew at a specific growth rate of 1.0 d^{-1}. "Slow growing" nutrient limited cells, received nutrients at a slow rate (60 ml min^{-1}/300 ml culture), as such, they eventually grew at a specific growth rate of only 0.2 d^{-1}. Each "fast growing" nutrient limited chemostat was inoculated with 295 ml of medium and 5 ml of microalga culture. Each "slow growing" nutrient limited chemostat tube was inoculated with 50 ml of medium and 1 ml of microalga culture. Each chemostat tube was bubbled with 0.2 µm filtered air to ensure that CO^2 was non-limiting and that the chemostat tubes were fully mixed. The chemostat tubes were attached to the culture medium source through silicon tubes. These tube diameters were different to facilitate different medium flow rates through the culture vessels. "Fast growing" nutrient limited chemostats had tubing with a larger diameter and were attached to a digitally controlled peristaltic pump set at 300 ml d^{1} which allowed the microalgae to grow at a specific growth rate of 1.0 d^{-1}. The "slow growing" nutrient chemostats had a smaller diameter and were attached to a digitally

controlled peristaltic pump set at 60 ml d^{-1} which allowed the microalgae to grow at a specific growth rate of 0.2 d^{-1}. This method ensures that the nutrient environment of the microalgae, and thus, their growth rate is controlled by the flow rate of the medium. Roughly, every five days the media vessel would be replaced and the waste collection vessel was emptied every 2-3 days. Measurements for data collection occurred every three days beginning on day zero (the day of inoculation) until the cells reached steady state. Cultures are considered in steady state when chlorophyll concentration and cell density fluctuates <7% day-to-day. Measurements of microalgae physiology included cell density, chlorophyll concentration, relative lipid and protein content, and confocal microscopy. Each replication consisted of a "fast growing" and "slow growing" nutrient limited culture. After one complete replication round for one microalga, a new chemostat culture would be inoculated for that microalga. Replications were not conducted at the same time. Two replicates were completed for *T. pseudonana*. Due to time constraints, only one replication was completed for *D. tertiolecta*.

Figure 3: Image of continuous culture chemostat set up.

Image of the light set up was altered by the camera. All lights were set at the same color and intensity.

A: chemostat tubes containing the microalgae cultures.

B: Media vessel containing f/2 + Si media with 100 µm sodium nitrate suppling the nutrients to the microalgae in the chemostat test tubes.

C: Waste collecting vessel.

D: Peristaltic pump.

Microalagal species

Thalassiosira pseudonana and *Dunaliella tertiolecta* were obtained from the National Center for Marine Algae (NCM). Stock cultures were maintained in batch

cultures in f/2 + Si medium with 100 µM sodium nitrate. Cultures were transferred to a new and clean metal capped flask every 2-3 weeks to maintain viability. Chemostat equipment including test tubes and silicon tubing were acid washed and autoclaved before use.

Cell density

Population counts were measured for control and determination of steady state. Once cells are in steady state, the culture has acclimated to the growth environment. During acclimation, cells adjust their physiology in response to the environmental conditions. Once acclimated, the population stays constant. Cell density was measured using a Coulter particle counter. Oscillations in cell density are expected to decrease as cells approach steady state growth.

Chlorophyll concentration

Chlorophyll content was measured as a secondary property that indicates steady state growth. Photosynthetic cells are known to alter their chlorophyll content depending on nutrient limited growth rate. Culture samples were collected onto GF/F filters using a vacuum pump. Ten ml of culture was filtered, and the filter was placed into a scintillation vial along with 2.5 ml 90% acetone for chlorophyll extraction. The sample was left to extract in the freezer for 24-48 hours, and then chlorophyll was measured by a spectrophotometer. Chlorophyll peaks were read at 630 nm, 664 nm, and 750 nm for *T. pseudonana*. Chlorophyll peaks were read at 647 nm, 664 nm, and

750 nm for *D. tertiolecta*. Chlorophyll content was calculated using the following equations and averaged for the duplicate samples (Jeffrey et al. 1975).

Chlorophyll equations for *T. pseudonana:*

Chlorophyll A:

$$[((11.49) \text{ x } (A664 - A750)) + ((-0.45) \text{ x } (A630 - A750))] \text{ x } \frac{acetone\ ml\ amount}{sample\ ml\ amount}$$

Chlorophyll C:

$$[((22.68) \text{ x } (A630 - A750)) + ((-3.4) \text{ x } (A664 - A750))] \text{ x } \frac{acetone\ ml\ amount}{sample\ ml\ amount}$$

Total Chlorophyll:

Total Chlorophyll= Chlorophyll A + Chlorophyll C

Chlorophyll equations for *D. tertiolecta:*

Chlorophyll A:

$$[((11.867) \text{ x } (A664 - A750)) + ((-1.786) \text{ x } (A647 - A750))] \text{ x } \frac{acetone\ ml\ amount}{sample\ ml\ amount}$$

Chlorophyll B:

$$[((18.978) \text{ x } (A647 - A750)) + ((-4.895) \text{ x } (A664 - A750))] \text{ x } \frac{acetone\ ml\ amount}{sample\ ml\ amount}$$

Total Chlorophyll:

Total Chlorophyll= Chlorophyll A + Chlorophyll C

Relative lipid and protein content

Lipid and protein were measured by Fourier transform infrared spectroscopy (FTIR) to semi-quantitatively determine carbon composition. For this analysis, 1 ml of

each sample was centrifuged for 10 min at 15,000 rpm, rinsed with ammonium formate, and then re-centrifuged as before. The cell pellet was then resuspended, dropped in a 10 µl circle onto a Si window and dried for 1-2 hours on a hot plate at 60°C. Using Fourier transform infrared spectroscopy, lipid and protein peaks were detected. Peaks for lipids were read at 2800 cm^{-1} and peaks for protein were read at 1600 cm^{-1} (Jebsen et al. 2012).

Confocal Microscopy

Confocal microscopy was used to semi-quantitatively measure lipid bodies of the microalgae during acclimation to their nutrient limited growth rates. Confocal microscopy allowed for snapshots of the microalgae physiology. One ml of the microalga cell sample was fixed with liquid nitrogen. Fixed cells were stored at -20°C until use of the confocal microscope was available. On the day of the appointment, fixed cells were stained with Nile red dye at least four hours prior to the observation by microscope. The four hours allowed sufficient time for the dye to penetrate through the cell wall and membrane of the microalgae. Nile red stains lipid bodies so that they will appear red, and chlorophyll appears greenish-blue under the microscope.

Results

Thalassiosira pseudonana “fast growing” nutrient limited and “slow growing” nutrient limited cells were grown in duplicate chemostats at full light saturation at a constant temperature of 18°C.

Dunaliella tertiolecta “fast growing” nutrient limited and “slow growing” nutrient limited cells were grown in single replicate chemostats at full light saturation at a constant temperature of 18°C.

For both species, “fast growing” nutrient limited cells achieved a steady state growth rate of 1.0 d^{-1} which is equivalent to a generation time of 1.4 divisions d^{-1}. For both species, “slow growing” nutrient limited cells achieved a steady state growth rate of 0.2 d^{-1}, which is equivalent to a generation time of 0.3 divisions d^{-1}. Following inoculation, cell density was measured until the cultures reached steady state growth (Figs 4-7).

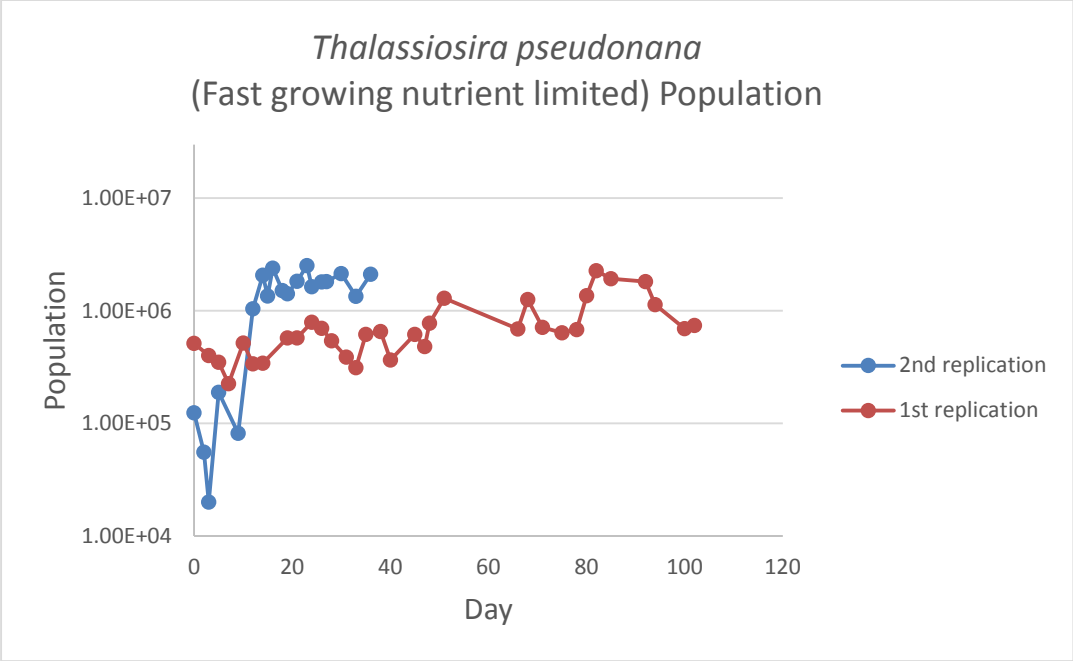

Figure 4: Graph showing *Thalassiosira pseudonana* "fast growing" nutrient limited cell density changes during acclimation to growth at 1.0 d^{-1}.

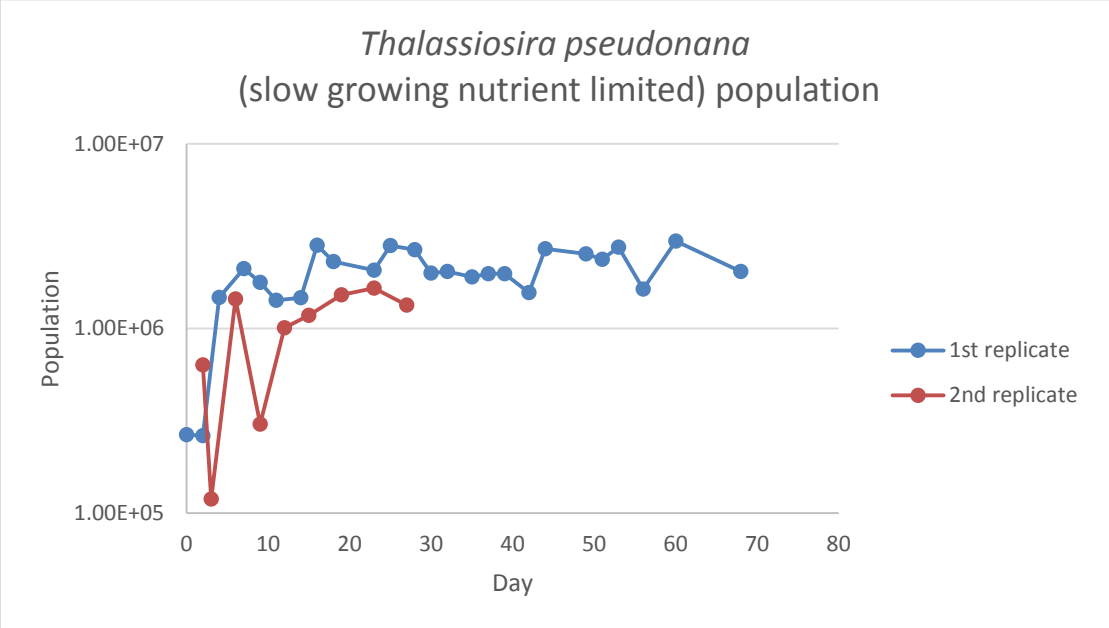

Figure 5: Graph showing *Thalassiosira pseudonana* "slow growing" nutrient limited cell density changes during acclimation to growth at 0.2 d^{-1}.

The data in Figures 4-7 show how the overall population is acclimating to the available nutrient supply of the environment. The cell densities in Figure 4 show that the first replicate varied considerably over the three month period of culture maintenance. The second replicate (Fig 4) achieved steady state growth in the time-frame expected which was just over one month (Halsey et al. 2015). Cultures were considered in steady state growth when the cell density fluctuated less than 7%. Final cell densities for the two replicates were 7.42×10^5 and 2.12×10^6, averaging 1.43×10^6. Figure 5 shows that the cell density of the first replicate varied considerably over the 2.5 month period of culture maintenance. The second replicate achieved steady state growth in the time-frame expected, which was roughly one month (Halsey et al. 2015). Final cell densities for the two replicates were 2.04×10^6 and 1.34×10^6. The cell densities for these "slow growing" nutrient limited replicates averaged 1.69×10^6 which was very similar to the average cell densities of the "fast growing" nutrient limited *T. pseudonana* cultures.

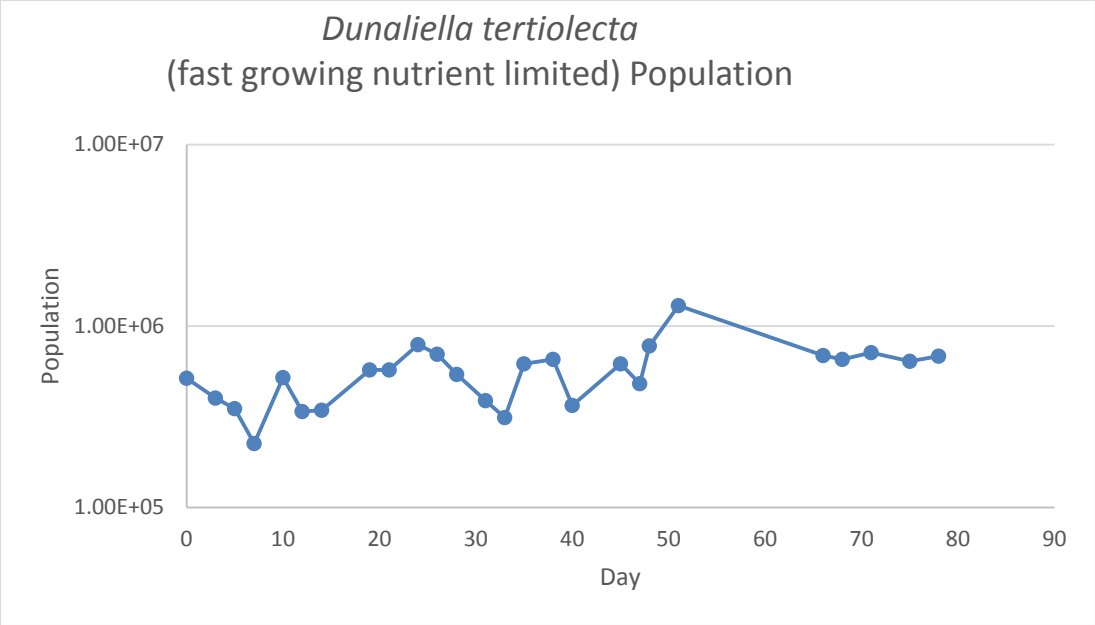

Figure 6: Graph showing *Dunaliella tertiolecta* "fast growing" nutrient limited cell density changes during acclimation to growth at 1.0 d^{-1}.

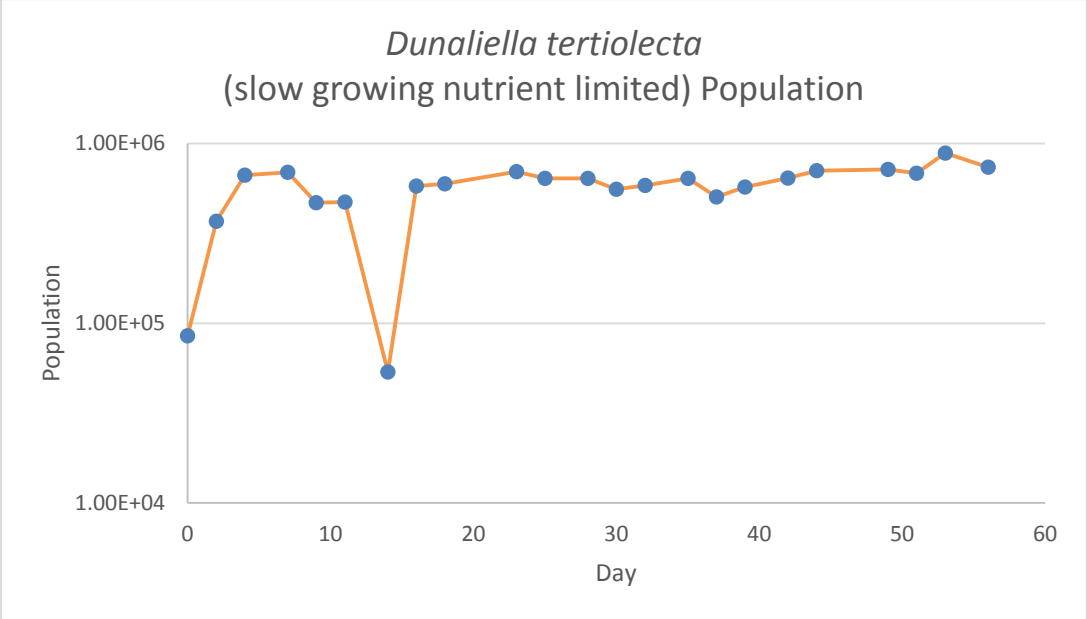

Figure 7: Graph showing *Dunaliella tertiolecta* "slow growing" nutrient limited cell density changes during acclimation to growth at 0.2 d^{-1}.

The cell density of “fast growing” nutrient limited *D. tertiolecta* varied considerably over the 2.5 month period of culture maintenance. However, it achieved steady state growth after two months. Final cell density for the “fast growing” single replicate was 6.83×10^5. “Slow growing” *D. tertiolecta* achieved steady state growth within one month and maintained steady state throughout the 2.5 month period of culture maintenance. Final cell density for the “slow growing” single replicate was 7.393×10^5 (Fig 7). The “slow growing” nutrient limited *D. tertiolecta* had a slightly higher cell density than the “fast growing” nutrient limited *D. tertiolecta*. This is similar to the cell density pattern observed between the “fast growing” and “slow growing” nutrient limited *T. pseudonana* (Figs 4 and 5).

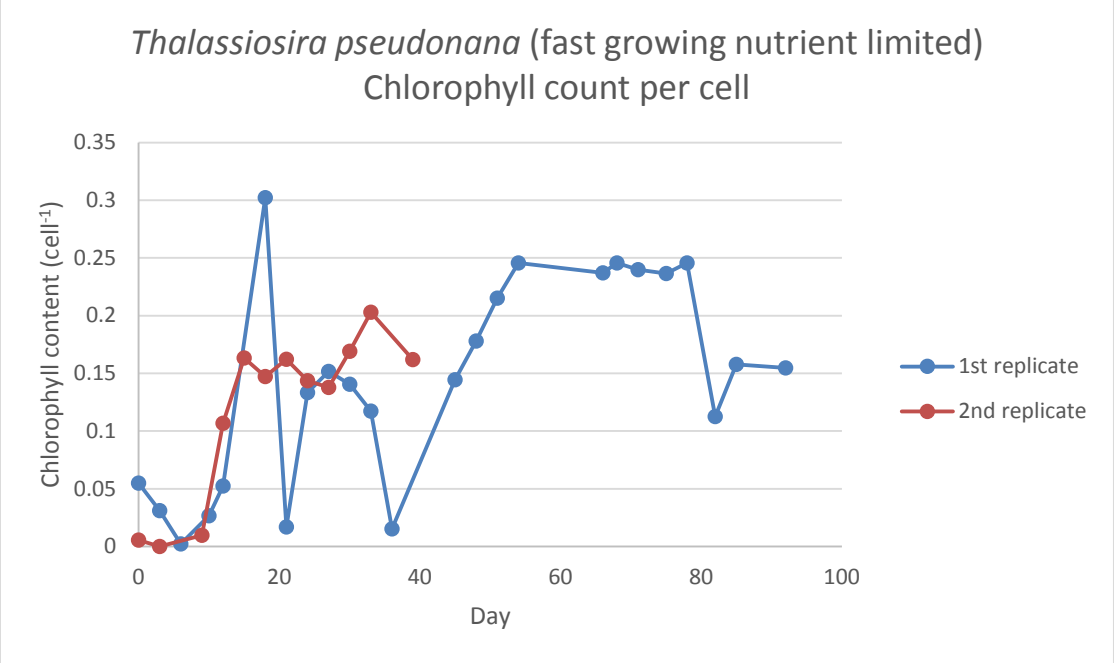

Figure 8: Graph showing *Thalassiosira pseudonana* "fast growing" nutrient limited chlorophyll content normalized per cell

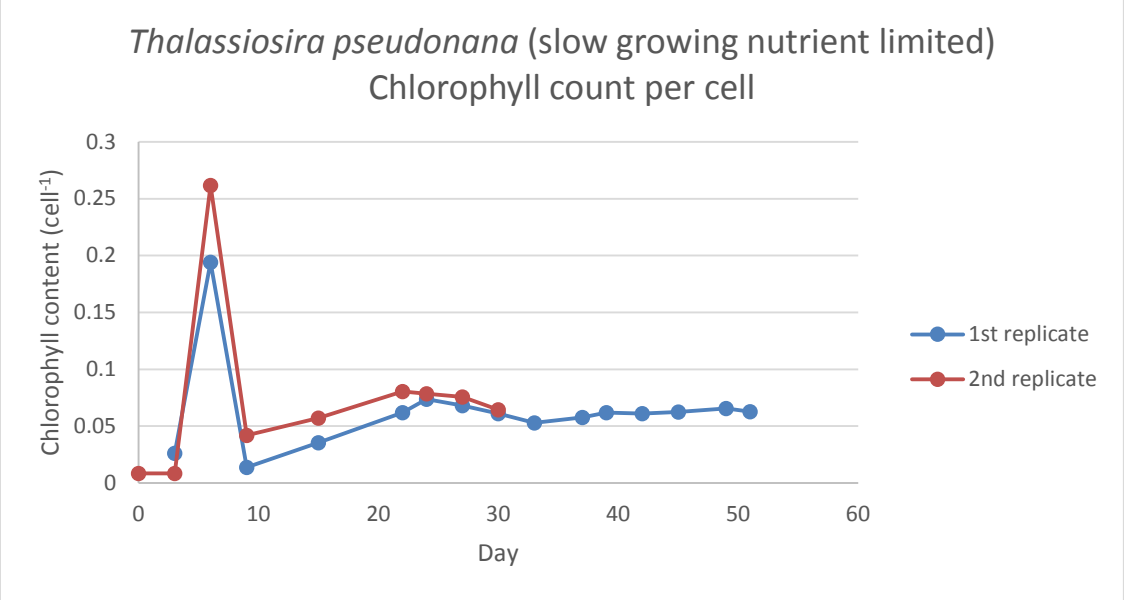

Figure 9: Graph showing *Thalassiosira pseudonana* "slow growing" nutrient limited chlorophyll content normalized per cell

In both species, as nutrient availability increases, chlorophyll content increases (Figs 8-11) and lipid body content also changes, although the direction of change is opposite for the different species (Figs 12-19). Chlorophyll content is a good indicator of the nutrition difference between the "fast growing" and "slow growing" nutrient limited cultures. As the species grew, they acclimated to the nutrient abundance and physiologically responded by increasing chlorophyll content. Although the chlorophyll content varied considerably throughout culture maintenance, once cultures reached steady state, replicates for "fast growing" nutrient limited *T. pseudonana* cells attained the same final chlorophyll content (0.16 pg Chl $cell^{-1}$) (Fig 8). The two replicates for "slow growing" nutrient limited *T. pseudonana* cultures also attained the same final chlorophyll content (0.06 pg Chl $cell^{-1}$) (Fig 9). In similar fashion, *D. tertiolecta* "fast growing" and "slow growing" had the same final chlorophyll measurement patterns. The "fast growing" nutrient limited cells increased in chlorophyll content and the "slow growing" nutrient limited cells decreased in chlorophyll content. The "slow growing" nutrient limited *D. tertiolecta* had less chlorophyll (0.12 pg Chl $cell^{-1}$) (Fig 10) compared to the "fast growing" nutrient limited *D. tertiolecta* (0.18 pg Chl $cell^{-1}$) (Fig 11). There was a greater final chlorophyll content in *D. tertiolecta*, regardless of whether the cells were "fast growing" or "slow growing," (probably because of the larger cell size of *D. tertiolecta* compared to *T. pseudonana*) and a smaller range difference in *D. tertiolecta* compared to *T. pseudonana*.

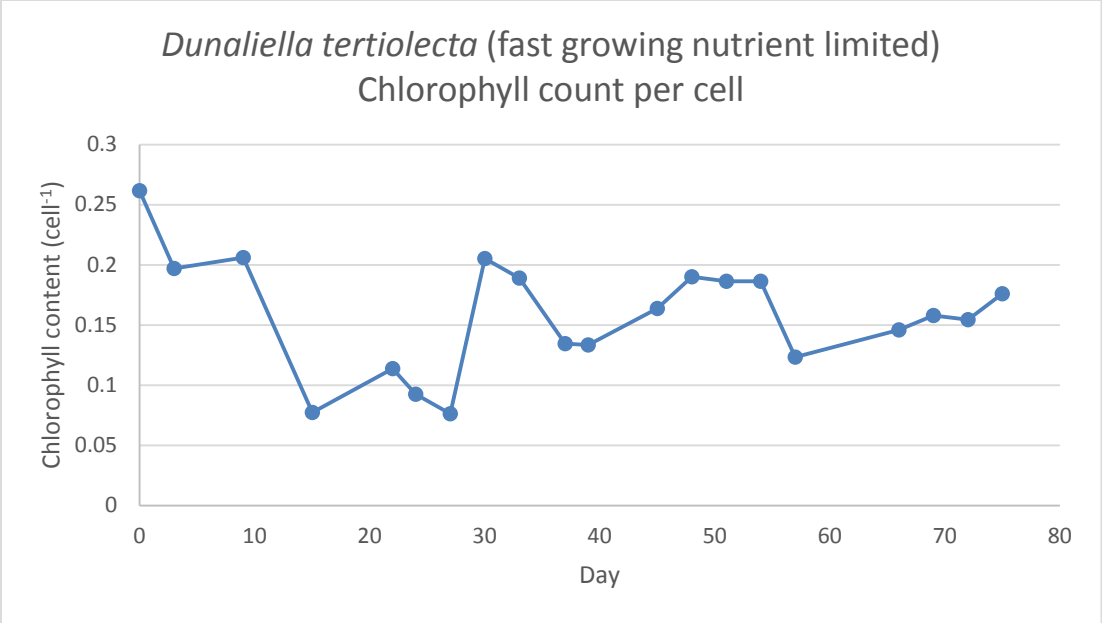

Figure 10: Graph showing *Dunaliella tertiolecta* "fast growing" nutrient limited chlorophyll count per cell measurements.

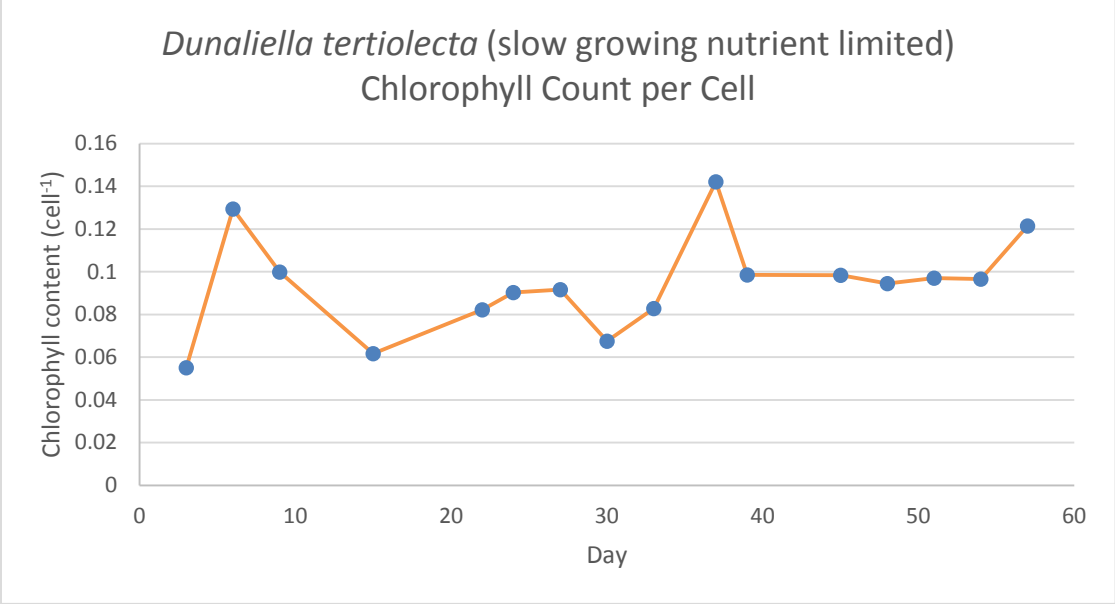

Figure 11: Graph showing *Dunaliella tertiolecta* "slow growing" nutrient limited chlorophyll count per cell measurements.

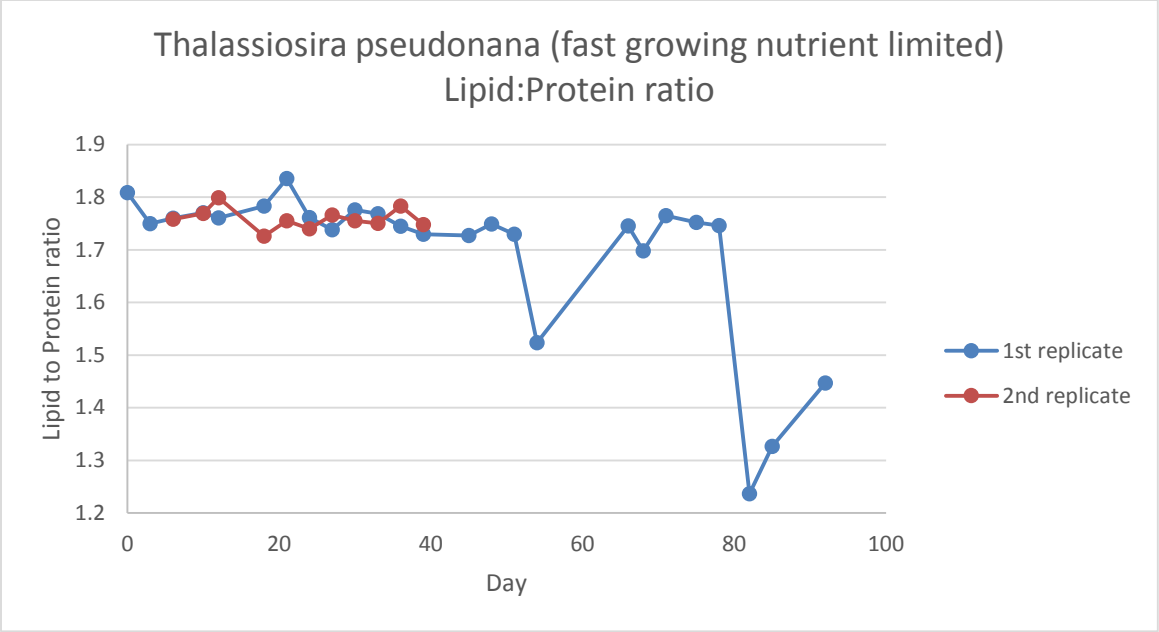

Figure 12: Graph showing *Thalassiosira pseudonana* "fast growing" nutrient limited lipid to protein ratio assessed by FTIR.

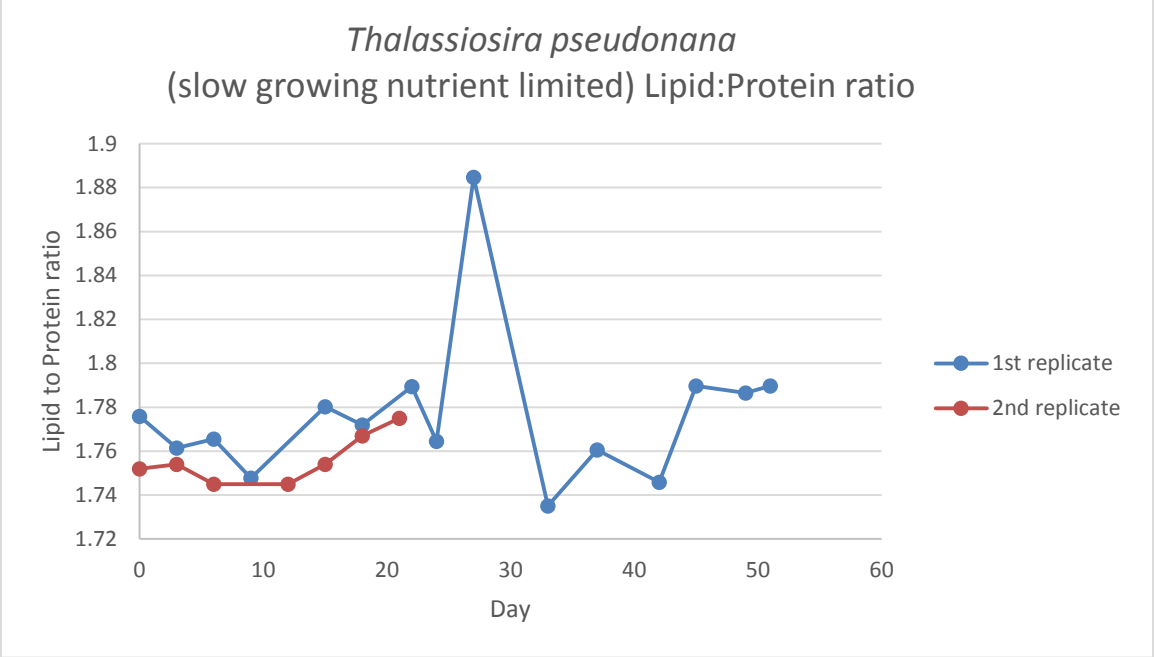

Figure 13: Graph showing *Thalassiosira pseudonana* "slow growing" nutrient limited lipid to protein ratio assessed by FTIR.

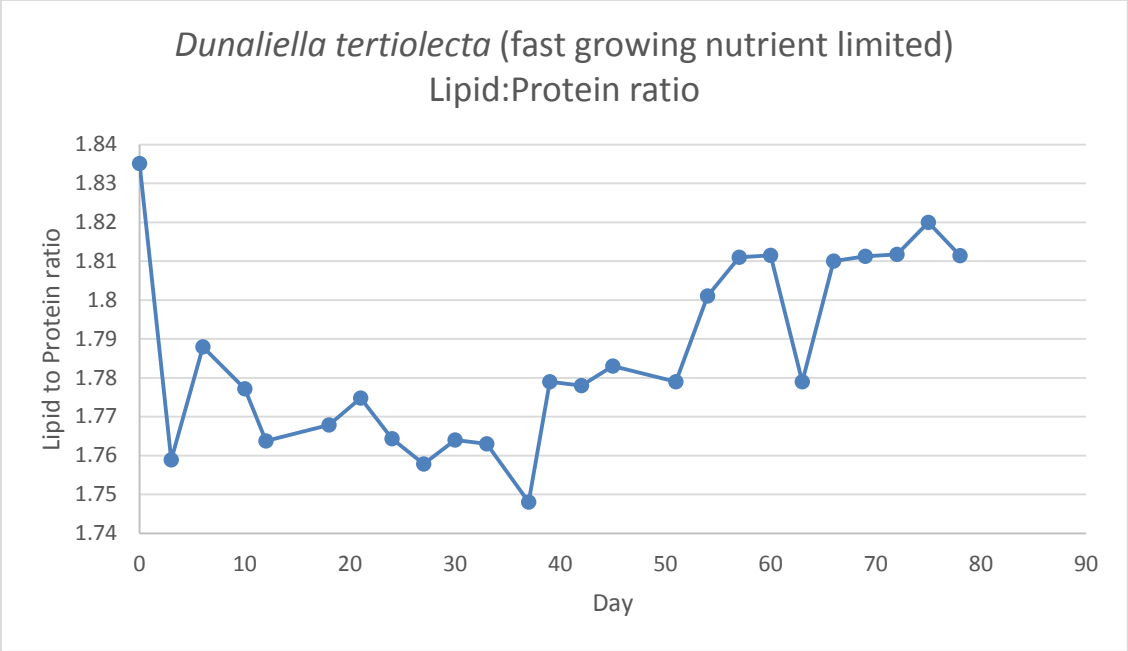

Figure 14: Graph showing *Dunaliella tertiolecta* "fast growing" nutrient limited lipid to protein ratio assessed by FTIR.

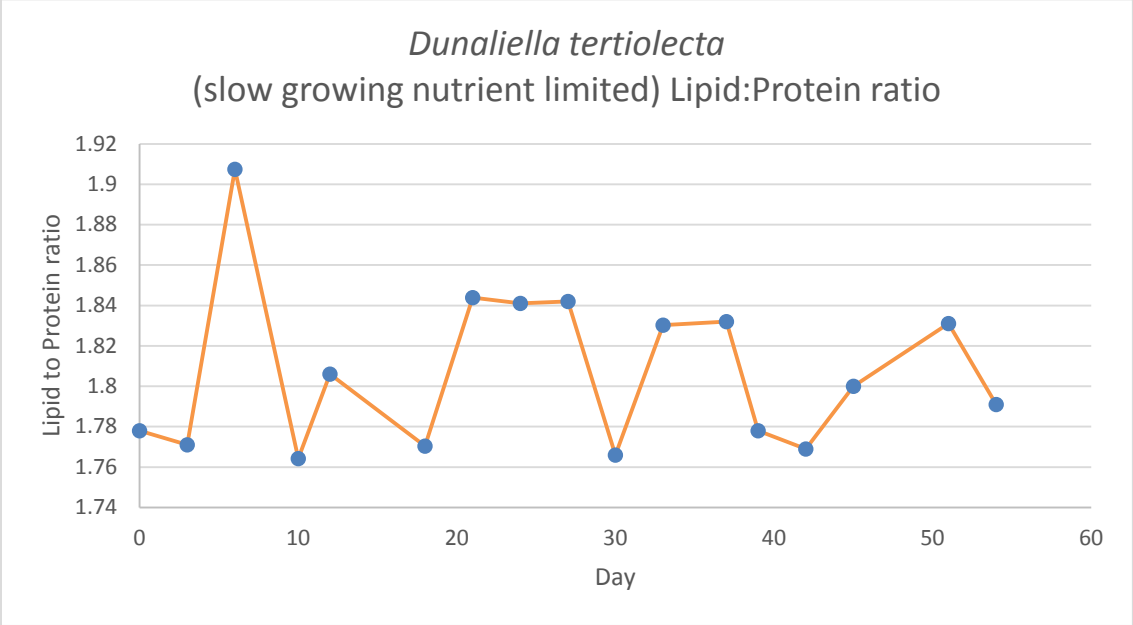

Figure 15: Graph showing *Dunaliella tertiolecta* "slow growing" nutrient limited lipid to protein ratio assessed by FTIR.

The lipid to protein ratio was quite constant in “fast growing” *T. pseudonana* for the two replicates for the first 40 days. However, a steep decline in lipid:protein was observed in the first replicate after 80 days (Fig 12). “Slow growing” nutrient limited *T. pseudonana* replicates also had very similar lipid:protein values for the first 20 days of the experiment (Fig 13). “Slow growing” nutrient limited *T. pseudonana* lipid:protein ratios were slightly higher than the ratios in “fast growing” cells: 1.78 vs 1.75. In contrast, the “fast growing” nutrient limited *D.tertiolecta* lipid:protein ratio was slightly higher than the “slow growing” nutrient limited *D.tertiolecta* lipid:protein ratio 1.81 vs 1.79 (Figs 14 and 15).

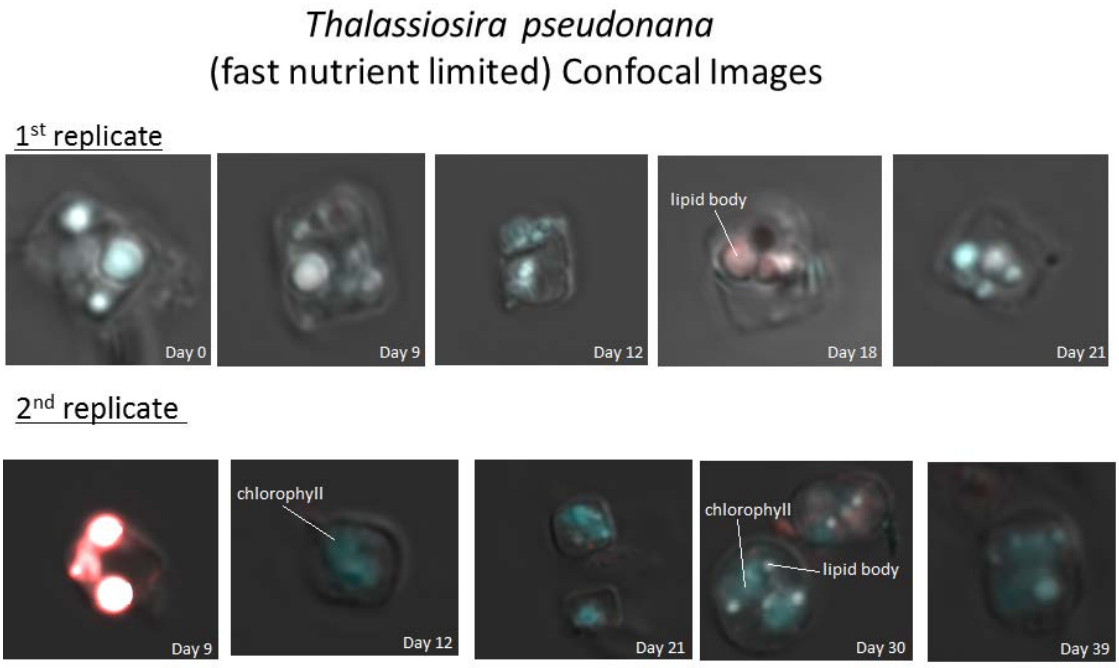

Figure 16: *Thalassiosira pseudonana* "fast growing" nutrient limited microscopy confocal images

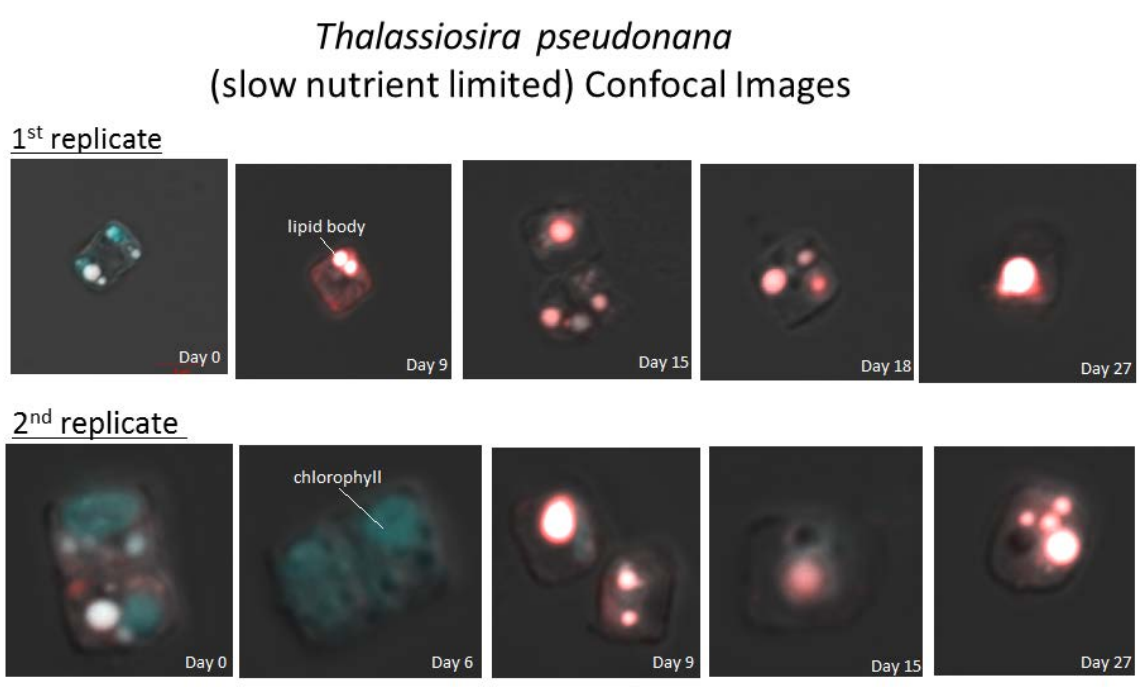

Figure 17: *Thalassiosira pseudonana* "slow growing" nutrient limited microscopy confocal images

Lipid bodies have a very defined circular shape and appear red, however due to oversaturation, may also appear white. Chlorophyll does not show as a defined shape, rather it appears like a smear of greenish-blue within the cell. For both replicates of "fast growing" nutrient limited *T. pseudonana*, from day 0 to steady state, lipid body content decreased and chlorophyll content increased (Fig 16). However, as "slow growing" nutrient limited *T. pseudonana* approaches steady state, lipid body content increased and chlorophyll content decreased (Fig 17). Consistent with results above, little chlorophyll was detected as *T. pseudonana* approached steady state and it appeared that the lipid bodies increased in size, too. Due to very low nutrient levels, it appears "slow growing" nutrient limited *T. pseudonana* accumulated lipid bodies in a stock-piling fashion.

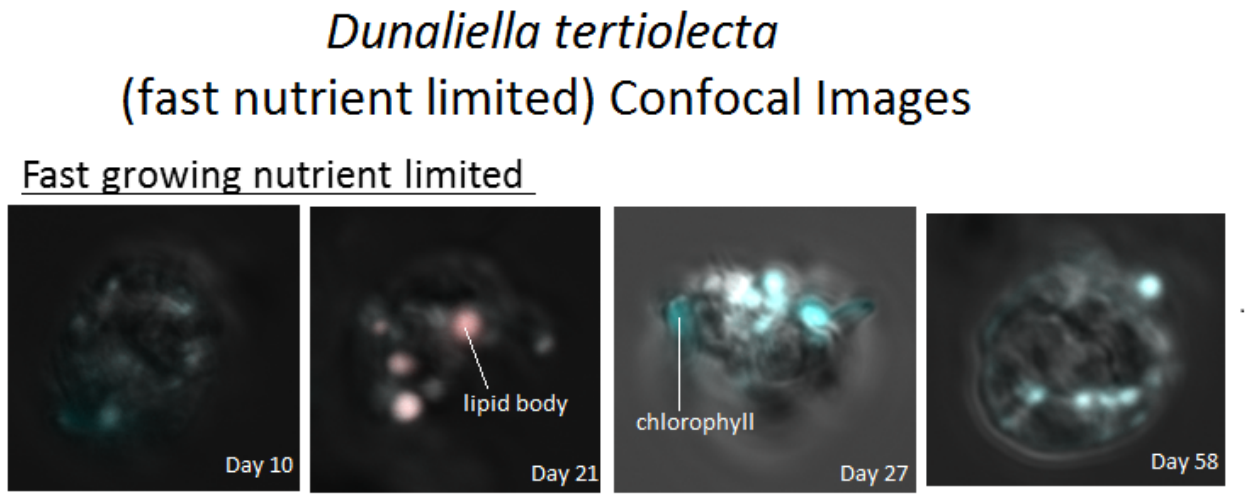

Figure 18: *Dunaliella tertiolecta* "fast growing" nutrient limited microscopy confocal images

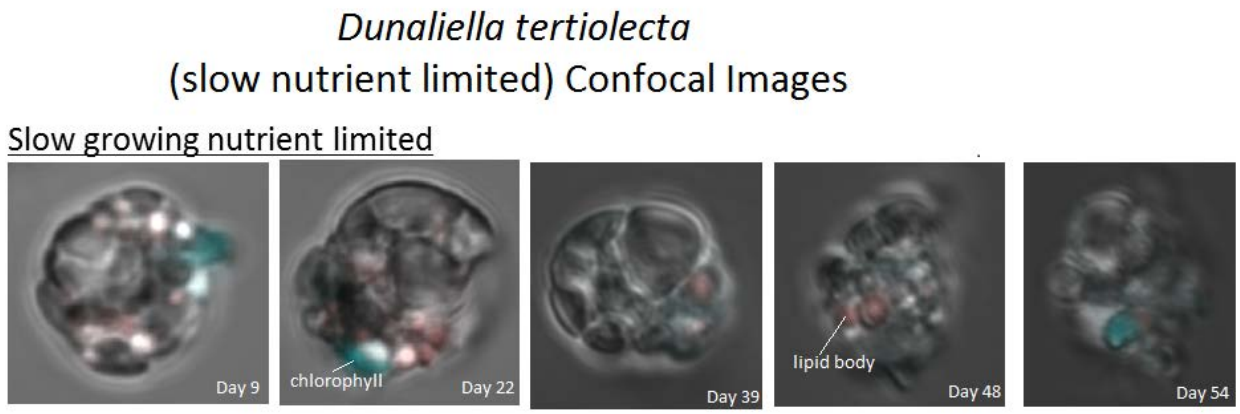

Figure 19: *Dunaliella tertiolecta* "slow growing" nutrient limited microscopy confocal images

In contrast to the results above for *T. pseudonana*, lipid body accumulation showed an opposite response in *D. tertiolecta*. "Fast growing" nutrient limited *D. tertiolecta* increased in chlorophyll content overtime while lipid bodies appeared to accumulate (Fig 18). Some lipid bodies were apparent in "slow growing" nutrient limited *D. tertiolecta* early in the acclimation process (day 9), but by the end of the experiment cells maintained very little chlorophyll or lipid bodies (Fig 19).

Discussion

The purpose of this research project was to observe and measure the acclimation strategies to varying rates of nutrient limitations in two phytoplankton, a diatom, *Thalassiosira pseudonana* and a green alga, *Dunaliella tertiolecta*. These species differ with respect to their morphology, evolutionary relationships, and photophysiology. Nutrient limitation is known to significantly affect algal physiology. For both species, the behaviors of chlorophyll concentration are well established with respect to nutrient availability during steady state growth (Halsey et al 2010, Halsey et al 2013). We were interested in determining whether physiological strategies for acclimation to nutrient limitation would be the same or different in these two algal species. The two questions we sought to answer were:

(1) How does chlorophyll content change during acclimation to steady state growth?

(2) How does lipid body content change during acclimation to steady state growth?

Chlorophyll is a good indicator of the nutritional environment for the "fast growing" and "slow growing" cultures. Microalgal species can continuously adjust their chlorophyll content as they respond to the nutritional changes in the environment. For both microalgae species, as nutrient availability increased, chlorophyll content increased and as nutrient availability decreased, chlorophyll content decreased. For "fast growing" nutrient limited *T. pseudonana*, chlorophyll initially dropped, then

oscillated for about 20 days, and leveled out, reaching steady state. For “slow growing” nutrient limited *T. pseudonana*, chlorophyll dramatically increased within six days, dropped on the tenth day, and eventually leveled out. These are more highly resolved data than has been collected previously during an acclimation period.

Similar to “fast growing” nutrient limited *T. pseudonana*, “fast growing” nutrient limited *D. tertiolecta*, chlorophyll content initially dropped, then chlorophyll oscillated for about 30 days, and finally appeared to level out. For “slow growing” nutrient limited *D. tertiolecta*, their chlorophyll increased in the beginning, dropped on the fifteenth day, and leveled out by the twenty-fourth day.

For both microalgae and both nutrient limitation types, chlorophyll initially varies during acclimation and then becomes steady. One reason why chlorophyll varies, and the reason for the initial drop or spike depending on if the culture is “fast growing” or “slow growing,” is probably because of the initial transfer of the microalga culture from inoculation flasks to chemostat tubes. The nutritional change caused by addition of new nutrients or relative starvation for nutrients causes the strong increase or decrease in chlorophyll. However, once the microalgae sense and acclimate to new nutrient status, they adjust their chlorophyll content accordingly. For both algae, and for both nutrient conditions, chlorophyll content fluctuated most dramatically for the first 10-20 days, but after a month became steady.

Lipids are high in caloric (energy) content, and they are therefore expected to provide energy for cells during periods of darkness or deep mixing, when photosynthesis cannot occur. Cells containing higher lipid content may also be expected to promote the growth of algal grazers (zooplankton). We found that when

nutrient availability is scarce, lipid body accumulation in *T. pseudonana* and *D. tertiolecta* responded very differently. As the cells acclimated to very nutrient deficient growth conditions, “slow growing” cells of *T. pseudonana* accumulated lipid bodies and these lipid bodies also appeared to increase in size. In comparison, there were very few lipid bodies in “fast growing” nutrient limited *T. pseudonana*. In *T. pseudonana* “fast growing” nutrient limited cells, lipid bodies decreased over time. It appears that on the twelfth day, lipid bodies significantly decreased in size and stayed constant throughout the population’s growth to steady state. In *T. pseudonana* “slow growing” nutrient limited cells, lipid bodies significantly increased over time. Initially, the lipid bodies do not take up much space in the cell, however by day 20, it appears that the lipid bodies take up a significant portion of the cell size. By steady state, there is a greater lipid body content in “slow growing” nutrient limited compared to “fast growing” nutrient limited *T. pseudonana*.

In contrast, during acclimation to “slow growing” nutrient limited conditions, the lipid bodies in *D. tertiolecta* dissipated and decreased relative to their body size. In “slow growing” nutrient limited *D. tertiolecta*, lipid bodies decreased so much that they are barely distinguishable in the confocal images. However, “fast growing” nutrient limited *D. tertiolecta* accumulated some lipid bodies by steady state compared to their initial start. By day 20, in the “fast growing” nutrient limited *D. tertiolecta*, lipid bodies appear to be at their biggest. “Fast growing” nutrient limited *D. tertiolecta* appear to be able to collect some lipid bodies due to the higher rate of nutrients they received overall in comparison to the “slow growing” nutrient limited cells.

When cells are first moved into chemostat tubes for culturing, the new nutrient environments and cells are being mixed and cells sense that they are inhabiting a changing nutrient environment. However, over time, as cells acclimate to their environment, cell population grows and the cells physiologically acclimate to their limited nutrient environment. In *T. pseudonana*, it takes about 20 days for the cells to acclimate and adjust their physiology accordingly. The overall population adjustment takes roughly about 1.5-2 more weeks to adjust and acclimate before reaching steady state. In "fast growing" nutrient limited *D. tertiolecta*, it takes about twenty days to adjust their physiology and in "slow growing" nutrient limited *D. tertiolecta*, acclimation takes about thirty days. Astonishingly, it is not the acclimation of the "fast growing" nutrient limited species that are bewildering, but the "slow growing" nutrient limited species. We were surprised that there was such a striking difference in lipid body accumulation in the two "slow growing" species. "Slow growing" nutrient limited *T. pseudonana* seemed to sense the scarce availability of nutrients and ultimately stock piled all the energy they can get. Their lipid bodies increased dramatically relative to their body size.

The behavior through which *T. pseudonana* (and diatoms in general) grows in natural ecosystems, and can collect lipid bodies and store them, may be discussed in context of a phenomenon known as the "bloom and bust" growth pattern (Teeling et al. 2012). My results may help to more fully understand the "bloom and bust" growth pattern by describing the survival mechanisms of phytoplankton species as seasonal changes disrupt their nutrient environment (Fig 20). Bloom is in reference to when the cells have adequate nutrient availability and can grow with high productivity. Bust is

in reference to when the cells do not have adequate nutrient availability and grow with low productivity. During colder seasons, the water column depth that diatoms grow in is much greater than the warmer seasons. Since the water column is deeper, there is a greater biological mixing which also brings up nutrients to a greater nutrient concentration. During warmer seasons, as temperature of water and other physical changes occur in the water column, the water column that diatoms grows in is much shallower and as a result, there are less nutrients available. My results suggests that the decreased nutrient availability is sensed by the diatoms and signals them to stock pile the energy they can obtain.

Lipid bodies are an energy rich reserve and can nourish and support cells when there is an inadequate supply of nutrients. Accumulation of lipid bodies can contribute to two survival behaviors: persistence and buoyancy. Persistence helps keep the cells alive until physical properties of water change again to deepen the mixing depth and bring back higher nutrient concentration levels. Persistence in diatoms is a similar concept to hibernation in certain animal species. Many animals fatten-up during the late fall and early winter before hibernating, slowing down their metabolism, through the winter. During hibernation, they rely on their fat reserves for survival. Similarly, the lipid bodies can provide a source of energy for the diatoms to metabolize very slowly in the deep, dark ocean until they are brought back to the surface through mixing.

The accumulation of lipid bodies may also help for buoyancy. Since lipid bodies are lighter than water, lipid bodies may help the cells stay afloat. Buoyancy helps keep the cells near the top of the ocean surface, closest to the sun for chlorophyll production

through photosynthesis. Buoyancy is like a more refined adjustment with respect to light and nutrients. “Bloom and bust” is a very efficient survival strategy that diatoms have developed.

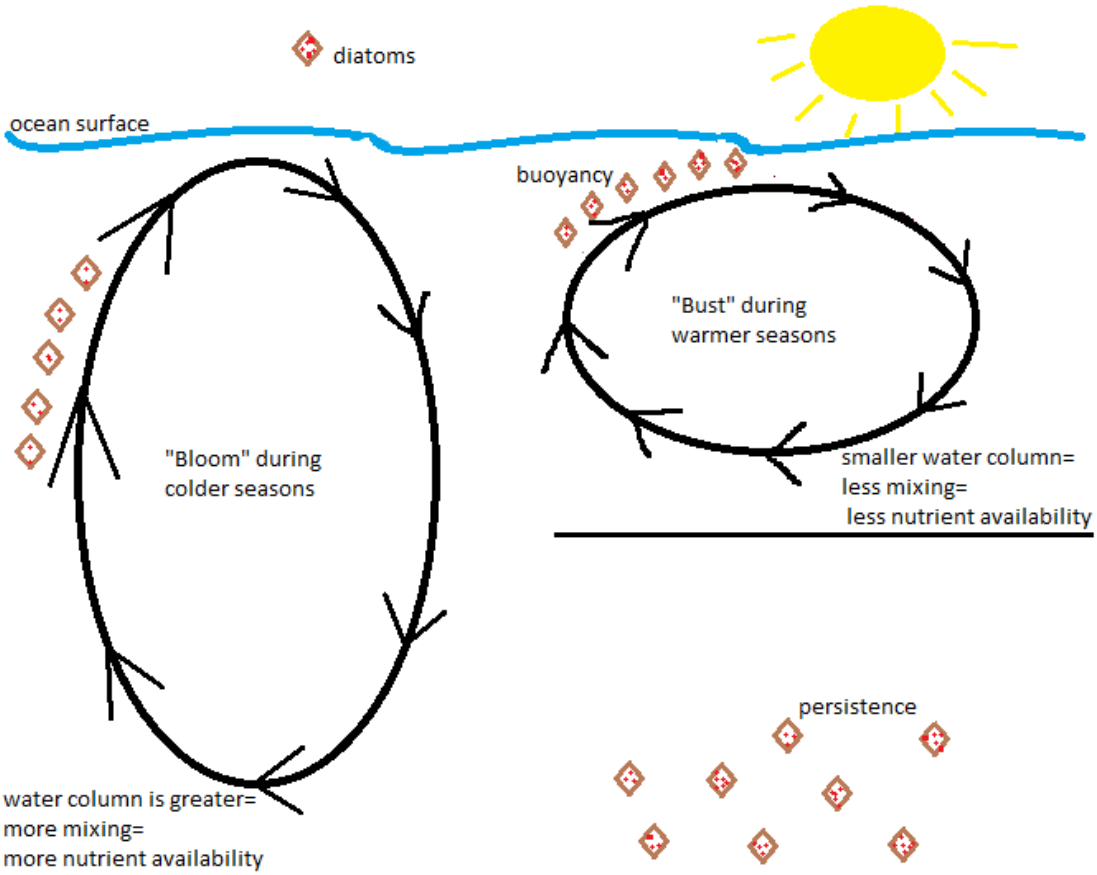

Figure 20: Conceptual image depicting the “bloom and bust” growth pattern

It is interesting then, to observe that *D. tertiolecta* does not display similar survival mechanisms. Why did “slow growing” nutrient limited *T. pseudonana* accumulate lipid bodies and not “slow growing” nutrient limited *D. tertiolecta*? One key difference, in regards to the “bloom and bust” growth pattern, is that *D. tertiolecta* aren’t found as deep in the water columns of the ocean and are closer to the surface of

the oceans in comparison to *T. pseudonana*. Therefore, the idea of persistence does not relate to *D. tertiolecta* and therefore can help explain the differences in lipid body accumulation. In addition, *D. tertiolecta* is motile, and so can actively change its position in the water column and would not need to rely on buoyancy strategies. The different acclimation strategies reveal new ideas about how cells adjust to nutrient limitation.

Finally, these data will help us understand physiological responses of algae to nutrient limited conditions. These results show two very different strategies for acclimation that are expected to impact food web dynamics, and may also help predict growth responses. Climate change may result in more short-lived storm events that could result in a patchy ocean environment where some populations must acclimate quickly to changing nutrient environments. The behaviors of *T. pseudonana* and *D. tertiolecta* can go on to influence the behaviors of their predators and the ocean environment. Similar to *T. pseudonana* and *D. tertiolecta* needs for nutrients, predators need high concentrations of *T. pseudonana* and *D. tertiolecta.* Whether the carbon composition of these microalgae effects the zooplankton is a question that is currently being studied in the Halsey lab.

References

1. Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH, Zhou S, Allen AE, Apt KE, Bechner M, Brzezinski MA, Chaal BK, Chiovitti A, Davis AK, Demarest MS, Detter JC, Glavina T, Goodstein D, Hadi MZ, Hellsten U, Hildebrand M, Jenkins BD, Jurka J, Kapitonov VV, Kröger N, Lau WW, Lane TW,Larimer FW, Lippmeier JC, Lucas S, Medina M, Montsant A, Obornik M, Parker MS, Palenik B, Pazour GJ, Richardson PM, Rynearson TA, Saito MA,Schwartz DC, Thamatrakoln K, Valentin K, Vardi A, Wilkerson FP, Rokhsar DS. 2004. The Genome of the Diatiom Thalassiosira Pseudonana: Ecology, Evolution, and Metabolism. *Science* **306** (5693):79-86.

2. Halsey, K.H. and Jones, B.M. 2015. Phytoplankton Strategies for Photosynthetic Energy Allocation. *Annual Review of Marine Science* **7**:265-297.

3. Halsey, KH; Milligan, AJ; Behrenfeld, MJ. 2010. Physiological Optimization Underlies Growth Rate-Independent Chlorophyll-specific Gross and Net Primary Production. *Photosynthesis Research* **103** (2): 125-137.

4. Halsey, KH; O'Malley, RT; Graff, JR; Milligan, AJ; Behrenfeld, MJ. 2013. A Common Partitioning Strategy for Photosynthetic Products in Evolutionarily Distinct Phytoplankton Species. *New Phytologist* **198**:1030-1038. doi 10.1111/nph, 12209

5. Jebsen C, Norici A, Wagner H, Palmucci M, Giordano M, Wilhelm C. 2012. FTIR Spectra of Algal Species can be used as Physiological Fingerprints to Assess their Actual Growth Potential. *Physiol. Plant*. 146:427–38

6. Jeffrey, S.W.; Humphrey, G.F. New Spectrophotometric Equations for Determining Chlorophylls a, b, c1 and c2 in Higher Plants and Natural Phytoplankton. *Biochemie und Physiologie der Pflanzen* 1975, 191–194.

7. Teeling, Hanno; Fuchs, Bernhard M.; Becher, Dorte; Klockow, Christine; Gardebrecht, Antje; Bennke, Christin M.; Kassabgy, Mariette; Huang, Sixing; Mann, Alexander J.; Waldmass, Jost; Weber, Marc; Klindworth, Anna; Otto, Andreas; Lange, Jana; Bernhardt, Jorg; Reinsche, Christine; Hecker, Michael; Peplies, Jorg; Bockelmann, Frank D.; Callies, Ulrich; Gerdts, Gunnar; Wichels, Antje; Wiltshire, Karen H.; Glockner, Frank Oliver; Schweder, Thomas; Amann, Rudolf. 2012. Substrate-Controled Succession of Marien Bacterioplankton Populations Induced by a Phytoplankton Bloom. *Science* **336** (6081): 608-611.

8. Hines, Sandra. 2004. Diatom Thalassiosira pseudonana. University of Washington Marine Science Portal. http://www.eurekalert.org/multimedia/msp/pub/6615.php

9. Baker, A.L. et al. 2012. Phycokey - an image based key to Algae (PS Protista), Cyanobacteria, and other aquatic objects. University of New Hampshire Center for Freshwater Biology. http://cfb.unh.edu/phycokey/phycokey.htm