Acclimation to Nutrient Limitation in Microalgae

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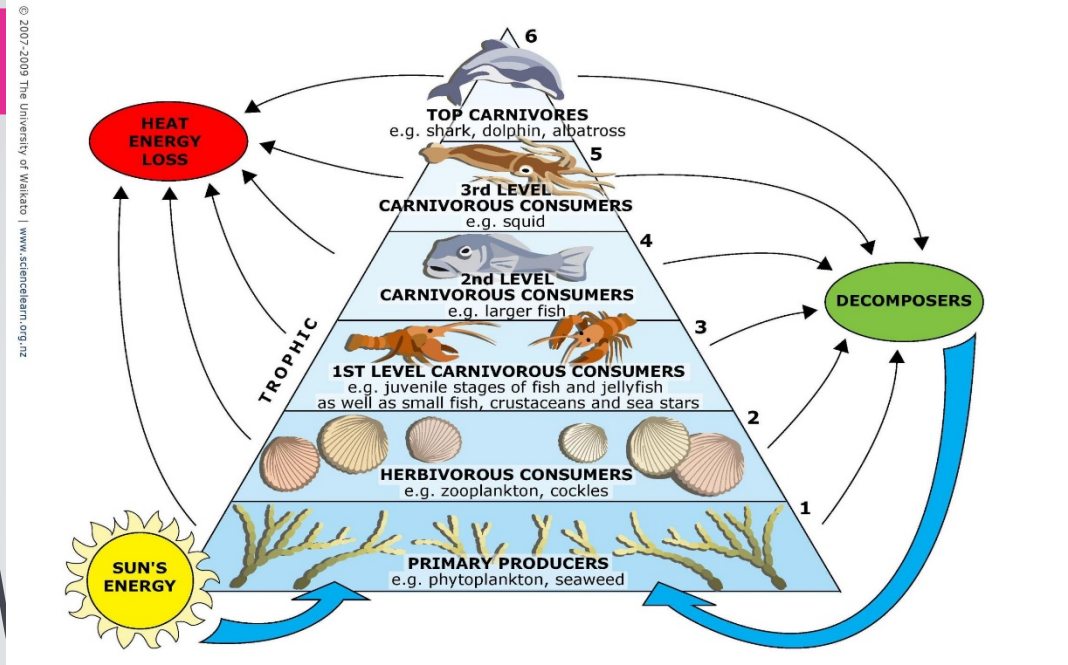
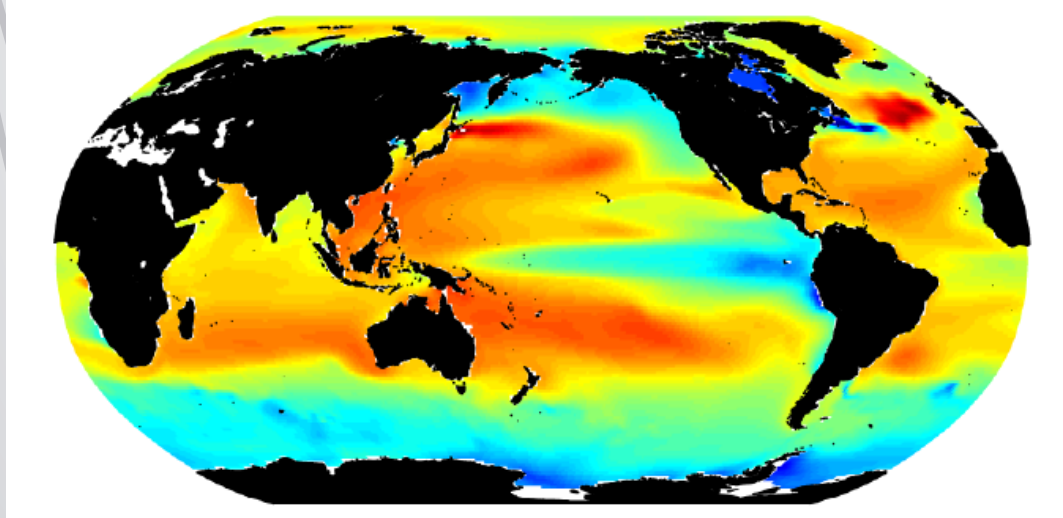
Department of Microbiology

University Honors College Thesis Defense

Oregon State University

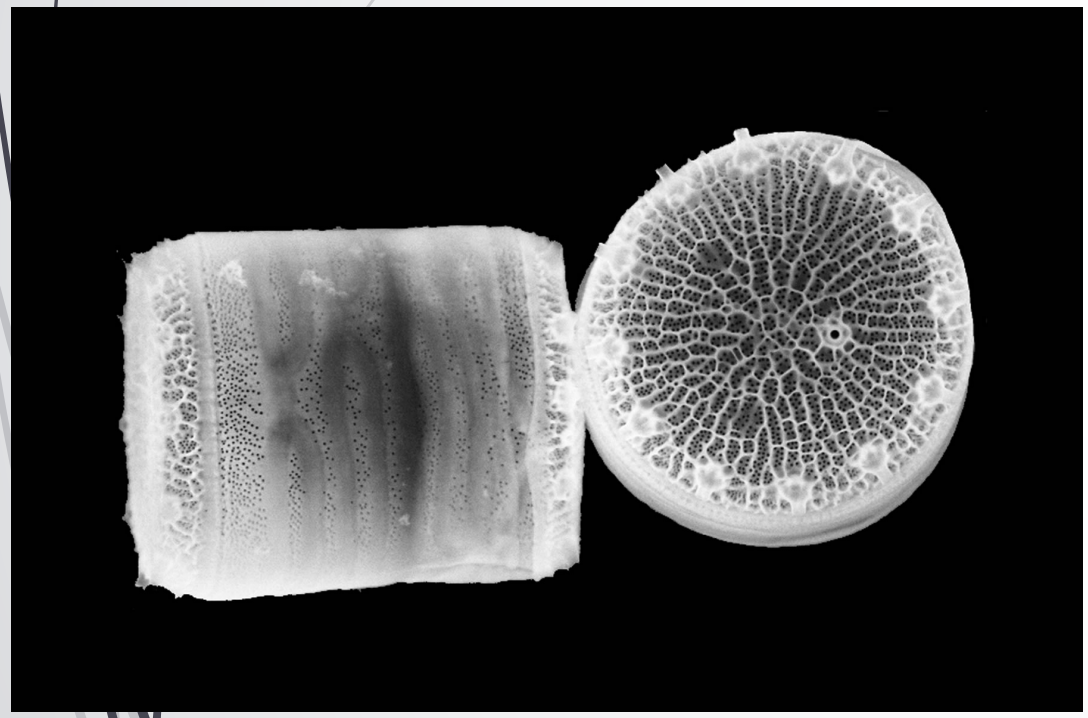




Why is phytoplankton physiology important?

- Phytoplankton (microalgale) form the base of the aquatic food web
  - Primary producers
- Food chain changes
  - Higher trophic levels
    - Zooplankton
  - Energy transfer through trophic levels
- Global carbon cycle
- Affected by seasonality (short term climate changes- temperature influences)

Diatoms (*Thalassiosira pseudonana)*



- Brown algae
- Model organism
- Non motile
- 4-6 µm in diameter
- Glass-like silicon cell wall
- Widely distributed and abundant throughout oceans
- First eukaryotic marine phytoplankton to be sequenced

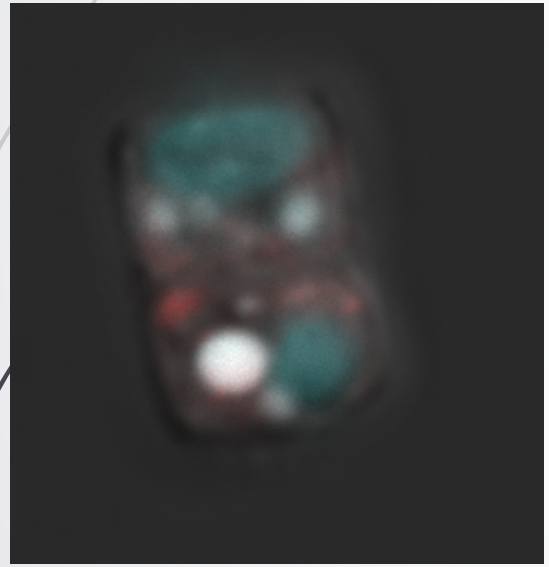
Green Algae (*Dunaliella tertiolecta*)

- Model organism
  - Heavily studied as a biofuel alternative
- motile (two flagella)
- Oval shaped
- 10-12 µm long in diameter
- Greasy
- Not as abundant in natural ecosystems like *T. pseudonana*
- Grows more easily compared to *T. pseudonana*

Hypothesis

- Previous research in Halsey lab has shown that green algae and diatoms will have different acclimation strategies (Halsey and Jones 2015)
- Hypothesis:
  - Microalgae accumulate lipid bodies as they acclimate to nutrient limitation
  - Lipid body accumulation is dependent on the degree of nutrient limitation. Slower growing nutrient limited cells will have higher lipid body content than faster growing nutrient limited cells

Chlorophyll and Lipid Bodies



- Forms of energy
- Both influenced by nutrient availability
- Important, because they provide information about the environmental conditions of their habitat
- Chlorophyll
  - to absorb light energy to fuel photosynthesis.
  - Increase in nutrient availability means an increase in chlorophyll content
- Lipid bodies
  - Highly rich energy and carbon reserves produced by microalgae metabolism

Nutrient limitation types

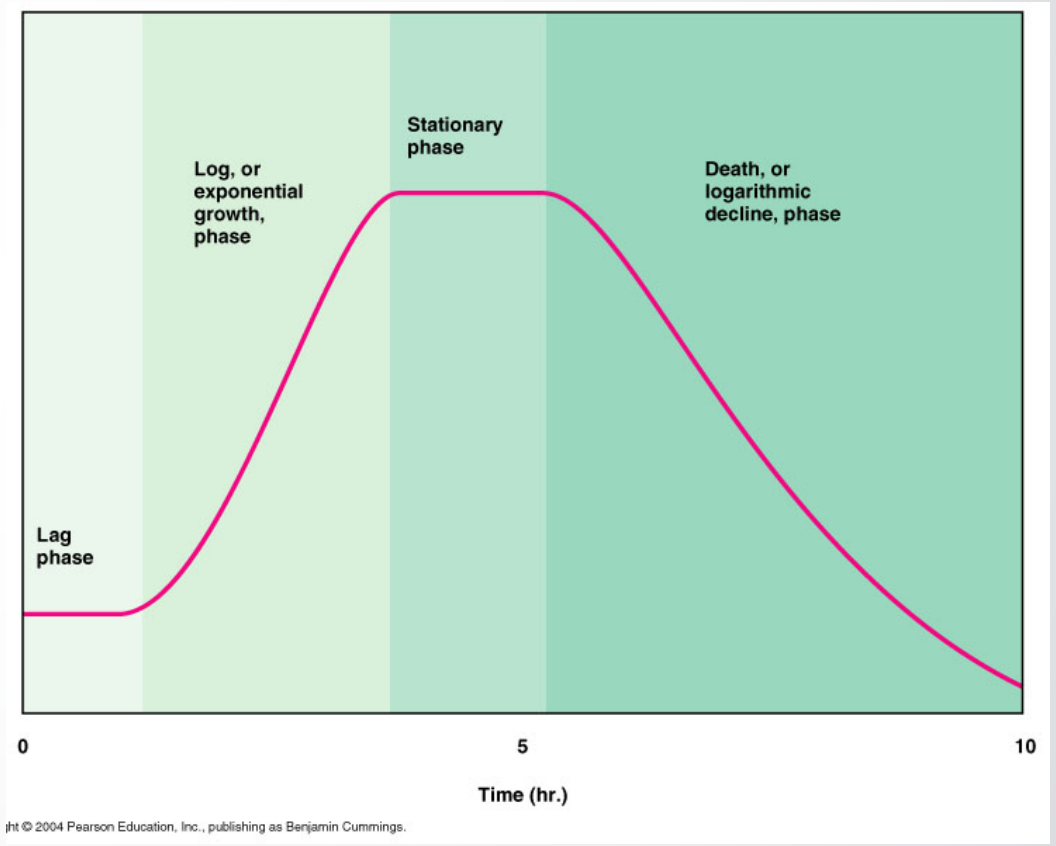
- "Fast growing" and "Slow growing" nutrient limited microalga
- "Fast growing" nutrient limited
  - Bigger diameter silicon tubing (more nutrients)
  - Peristaltic pump set at 300 ml d^{-1}
- "Slow growing" nutrient limited
  - Smaller diameter silicon tubing (less nutrients)
  - Peristaltic pump set at 60 ml d^{-1}

Methods

- Continuous culture chemostat
  - Constant temperature of 18°C
  - Supplied with f/2 + Si media with 100 µm sodium nitrate
  - Full light saturation
  - Chemostat equipment (test tubes and silicon tubing) were acid washed and autoclaved before use.
- Physiology measured every 3 days until steady state is reached

Types of physiology being measured

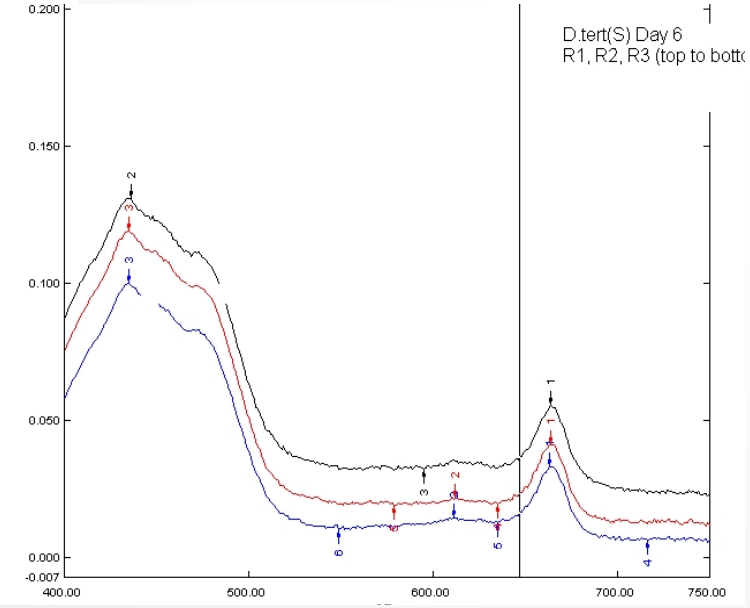
- Cell Density
  - Population
- Chlorophyll content
- Relative lipid:protein content
  - Fourier transform infrared spectroscopy
- Confocal microscopy
  - Physical appearance



Cell Density (Population)

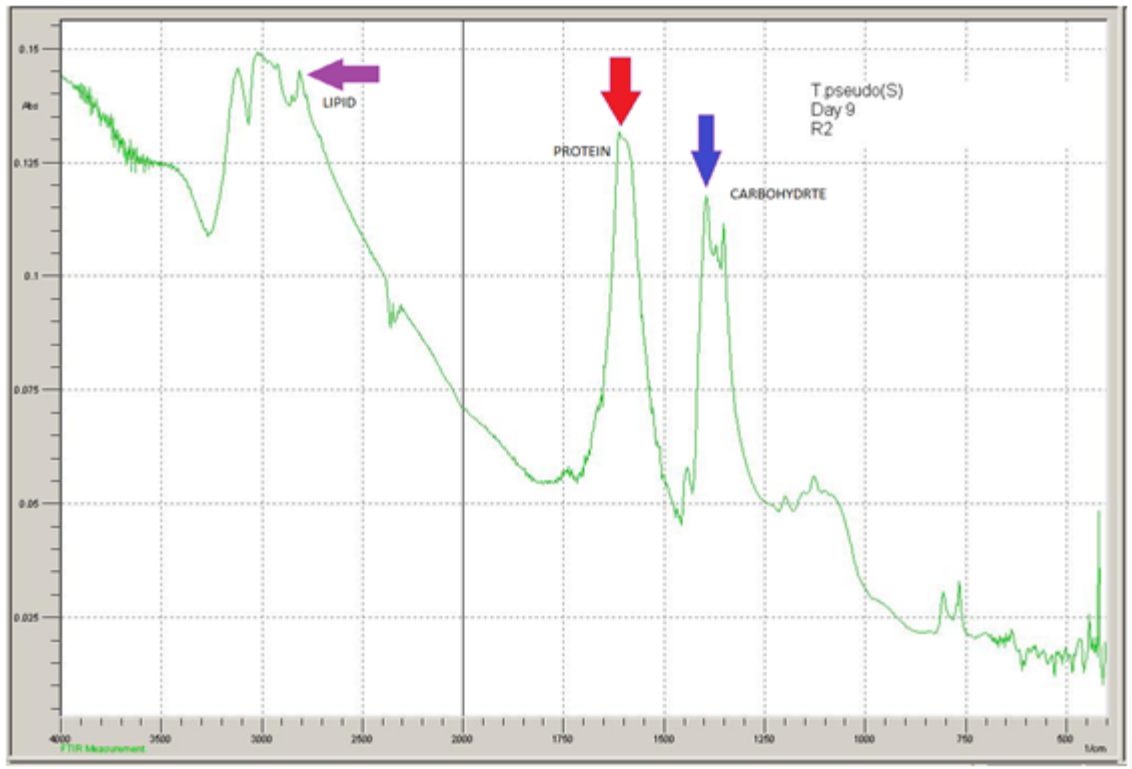
- Coulter particle counter
- Data collected and measured until steady state
  - Cell density fluctuates <7% day-to-day

Chlorophyll Concentration



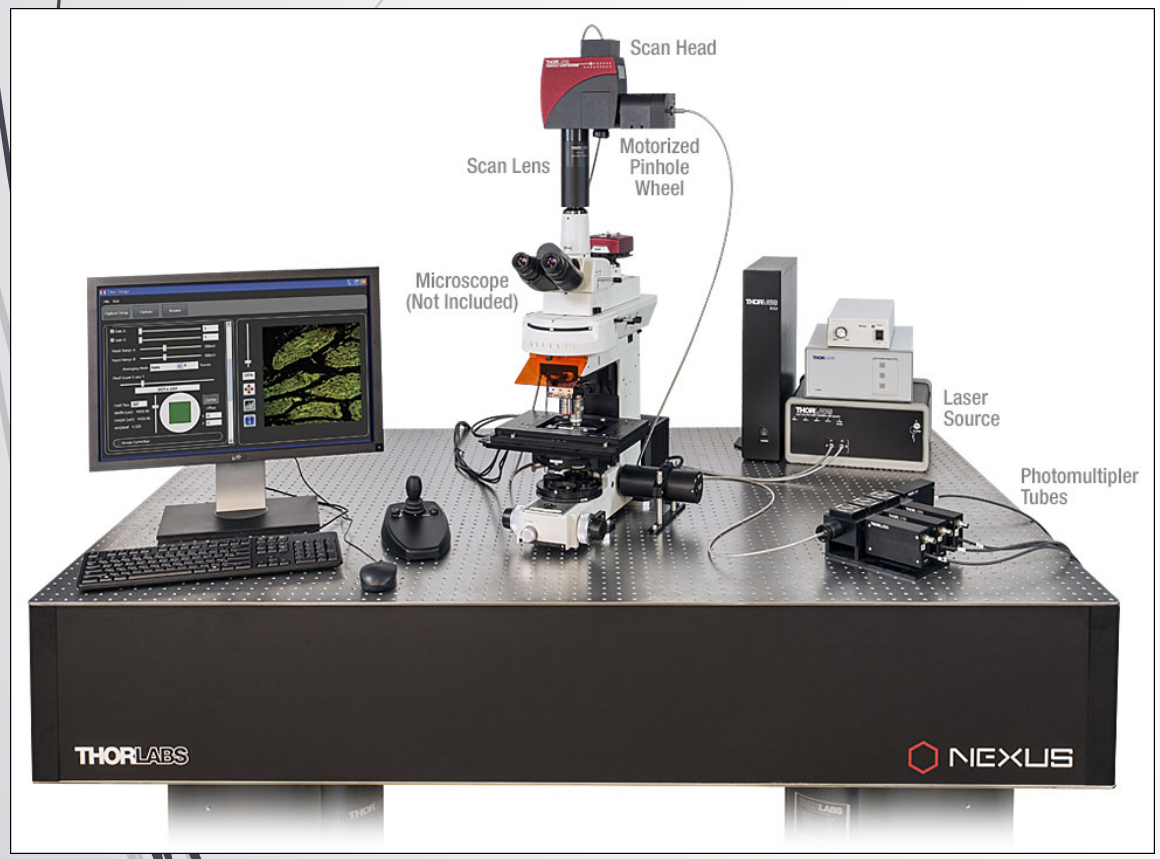
- Measured to aid in determination of steady state growth
- To collect and analyze:
  - GF/F filters filtered through vacuum pump
  - Filters placed in scintillation vials
  - Addition of 2.5 ml 90% acetone
  - Placed in freezer for 24-48 hrs to allow chlorophyll to develop
  - Chlorophyll measured by spectrophotometer
    - Peaks read were where chlorophyll hit the wavelength spectrum

Relative lipid and protein content



- To semi-quantitatively determine carbon composition
- To collect and analyze sample culture:
  - Centrifugation
  - Cell pellet placed Si window
  - Si window placed on hot plate to dry
    - ~2 hours
  - Fourier transform infrared spectroscopy
    - vibrational frequency detection of bonds and molecules
    - Lipid peak: 2800 cm^{-1} (PURPLE)
    - Protein Peak: 1600 cm^{-1} (RED)
    - Carbohydrate Peak: 1410 cm^{-1} (BLUE)

Confocal Microscopy



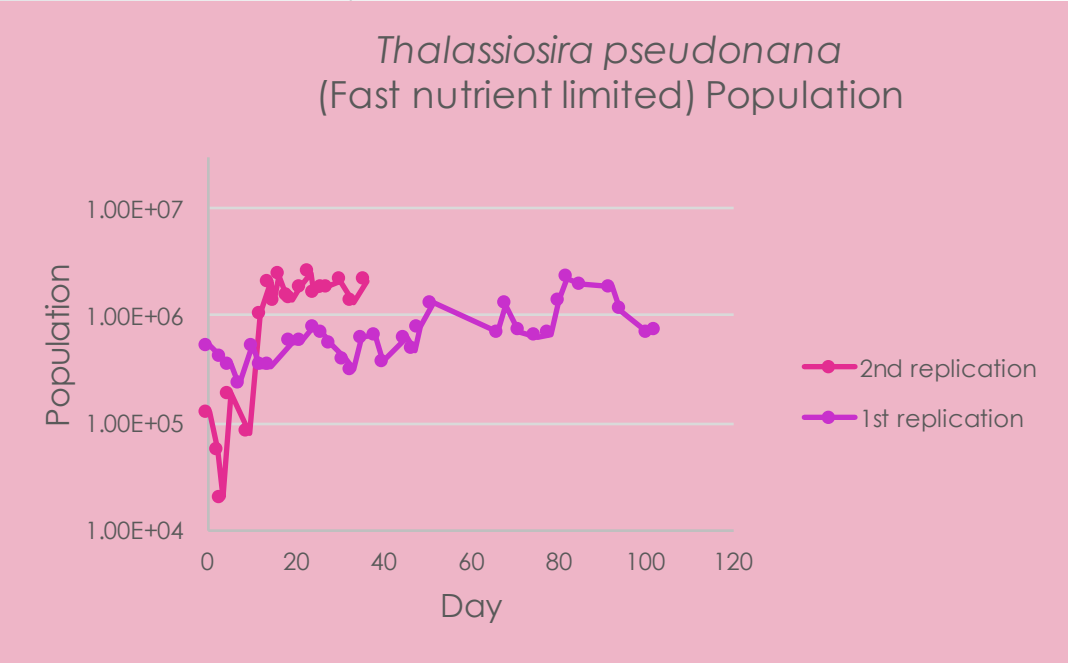
- Time snap shots of microalgale physiology
- To collect and analyze:
  - Fix sample cells with liquid nitrogen
  - Place in freezer until confocal microscopy visitation
  - On day of confocal microscopy visitation:
    - Stain sample with Nile red dye 4 hours prior to microscope observation
  - Lipid bodies appear red and chlorophyll appear greenish-blue

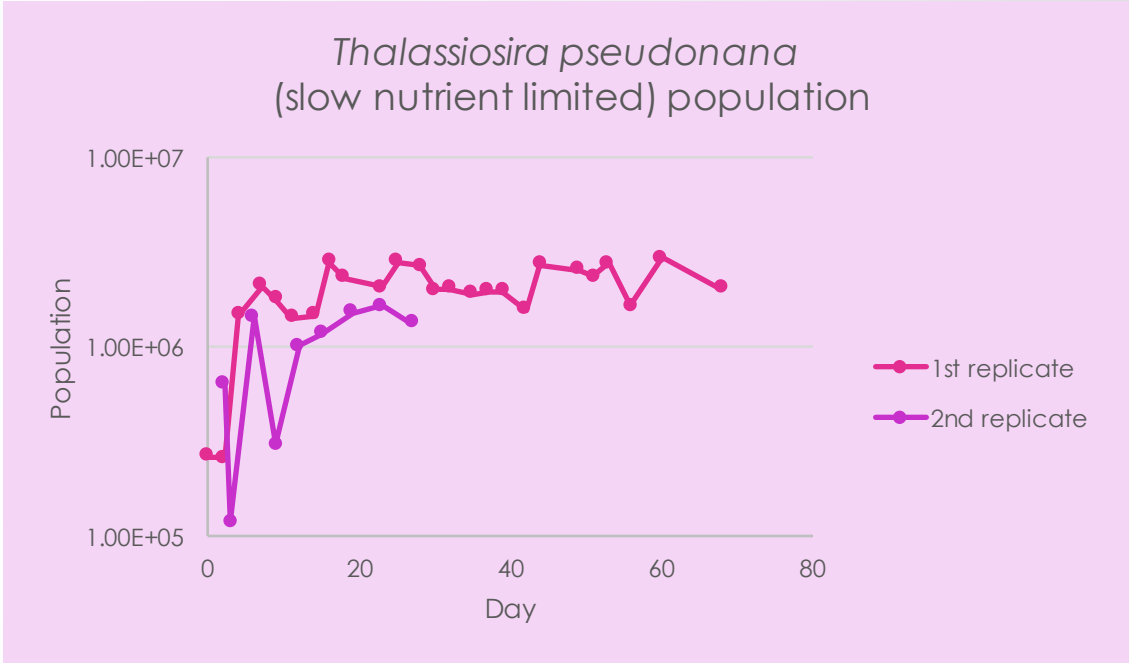
Sampling Day

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Sample Day	Chlorophyll reading		Sample Day	Chlorophyll reading		Sample Day

- Data collection and measurements made every 3 days
- Sample Day
    1. FTIR prep (30 minutes), FTIR drying (~2 hours)
    2. Population cell density counts (10 minutes)
    3. Chlorophyll prep (25 minutes)
    4. Confocal microscopy prep (1 minute)
    5. FTIR readings (30 minutes)
- After sampling day
    1. Chlorophyll readings (30 minutes)
    2. Confocal microscopy images (5 minutes per sample)

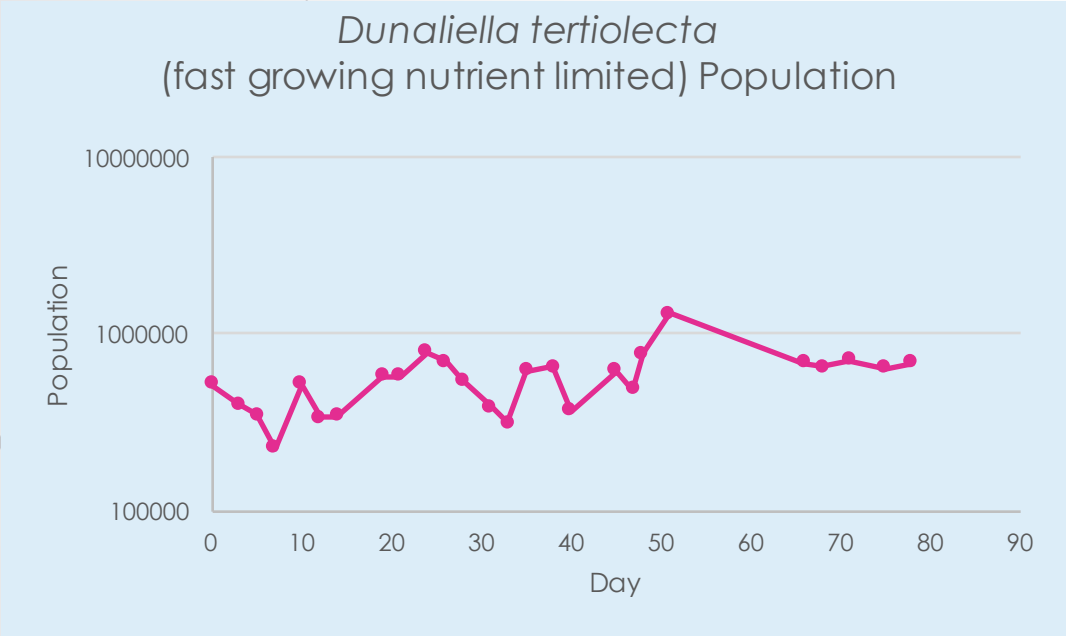
Results: *T. pseudonana* population

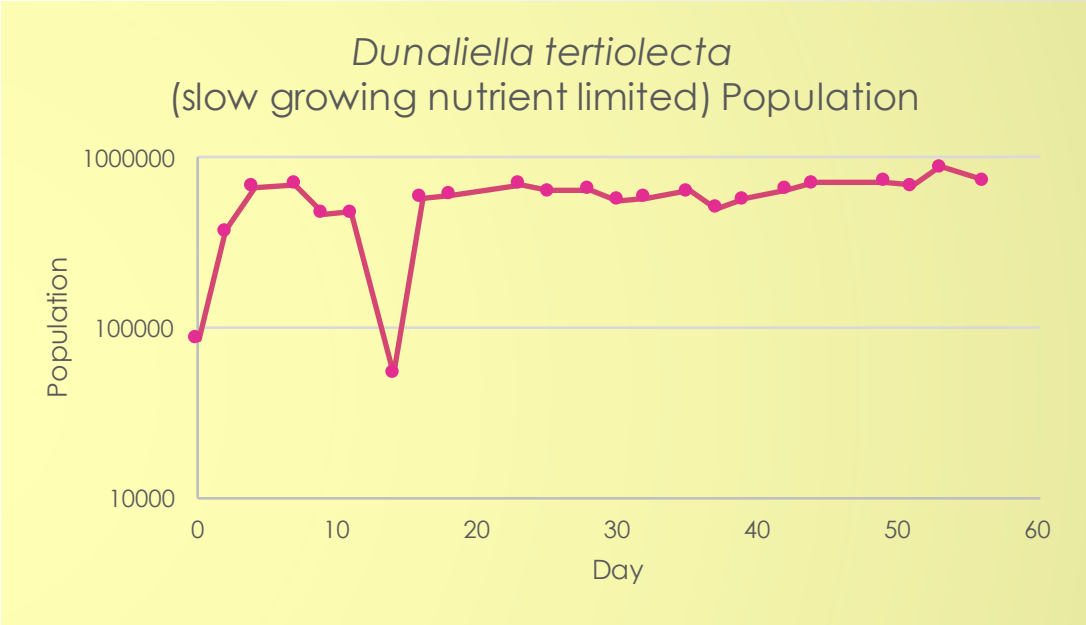




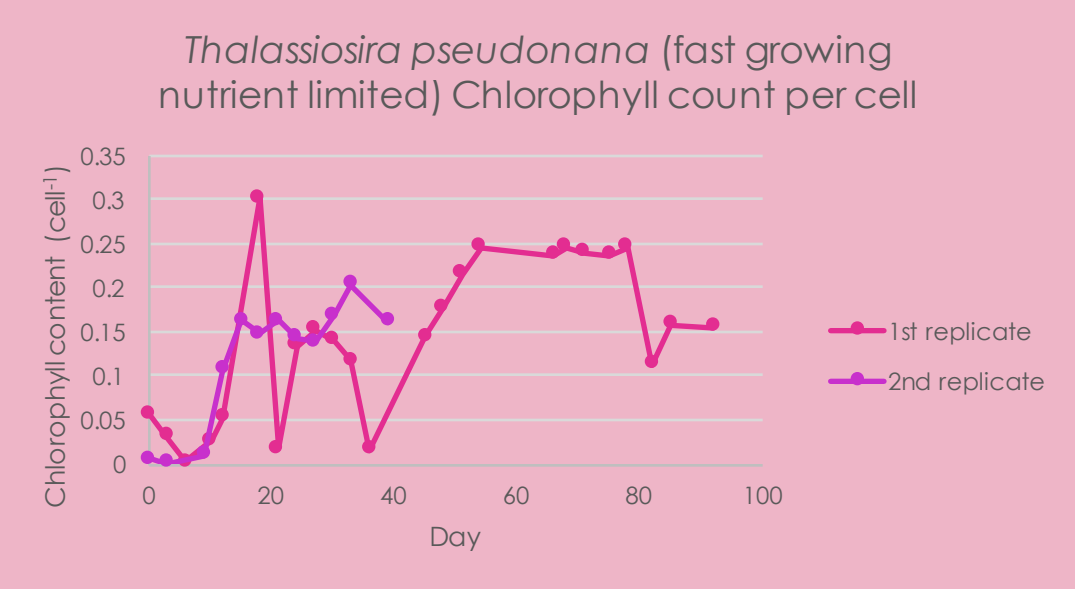
- Difficulty with 1st replicates
- 2nd replicates achieved steady state growth in expected time fram

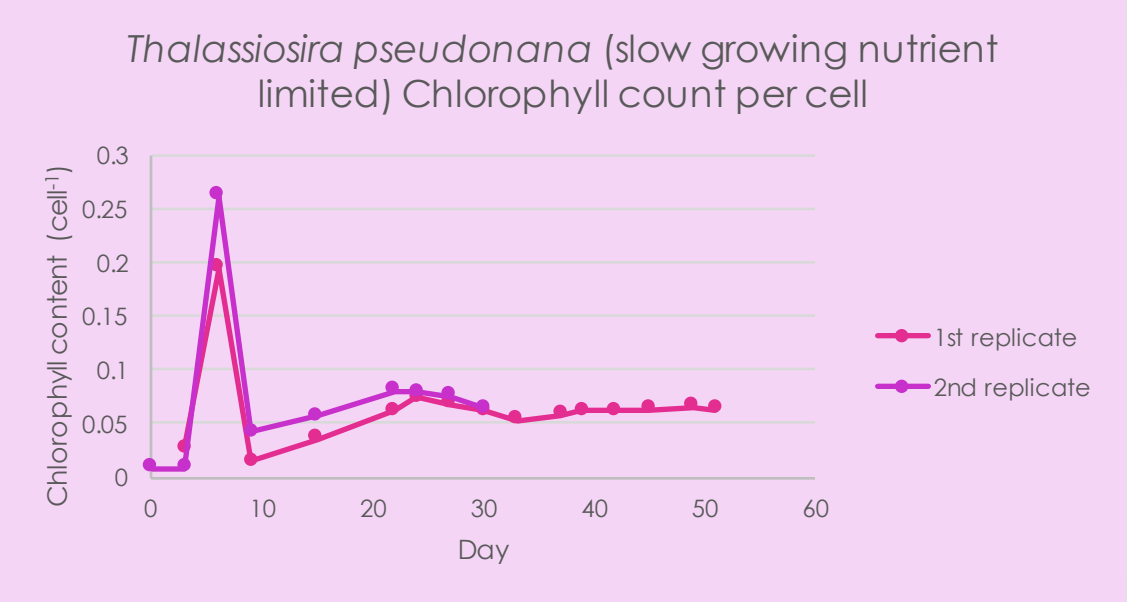
Results: *D. tertiolecta* population





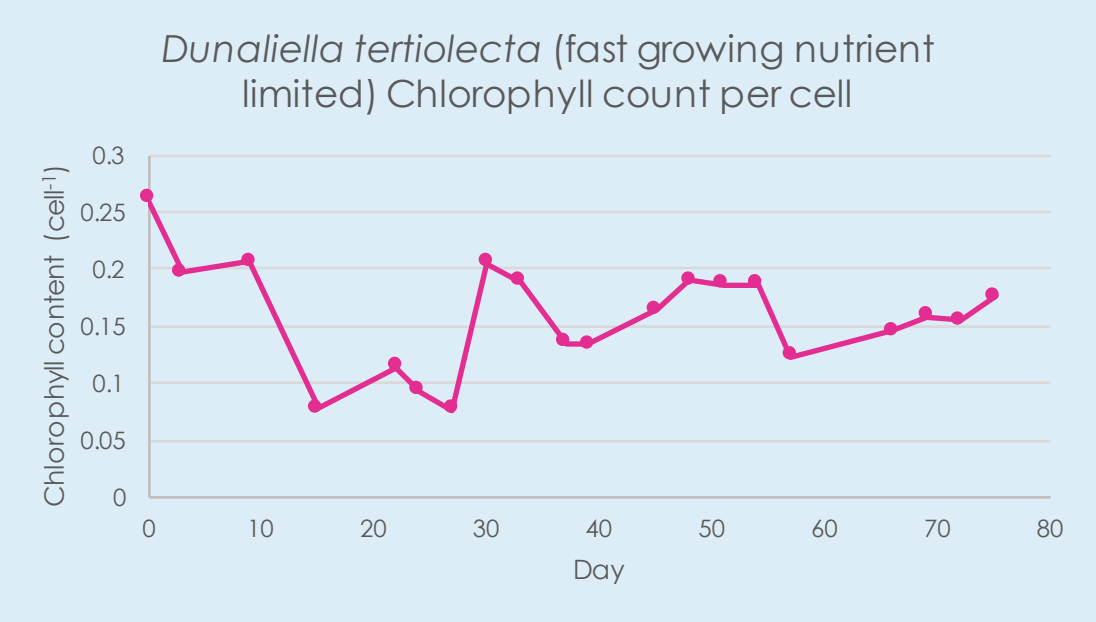
Results: *T. pseudonana* chlorophyll content

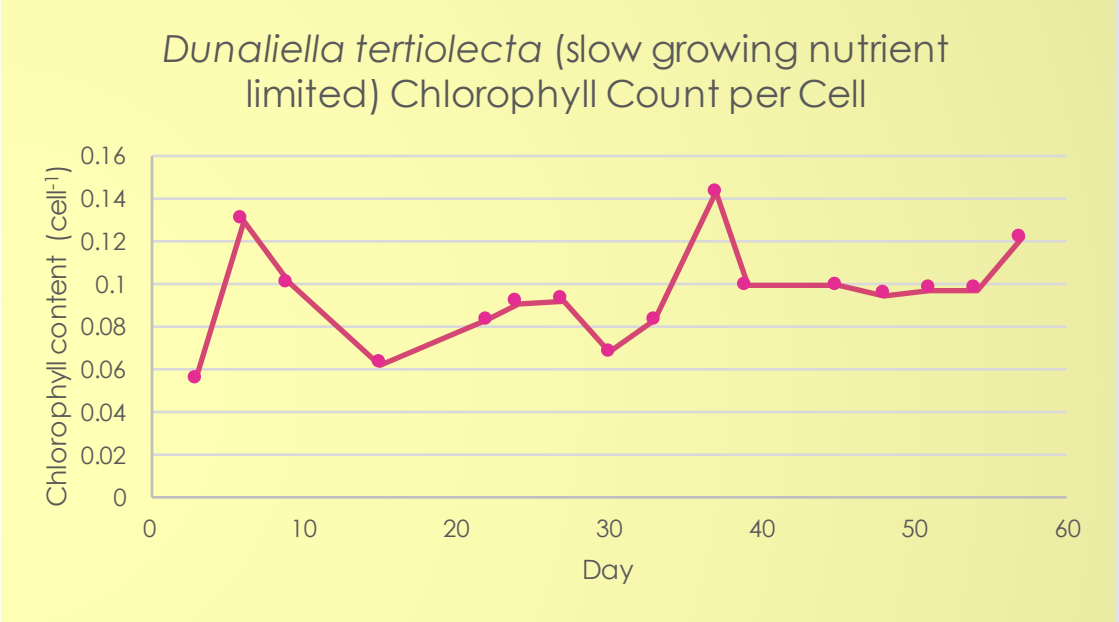




- Increase in nutrients, increase in chlorophyll
  - Different y-axis scales
- "Fast growing" 0.16 pg Chl cell^{-1}
- "Slow growing" 0.06 pg Chl cell^{-1}

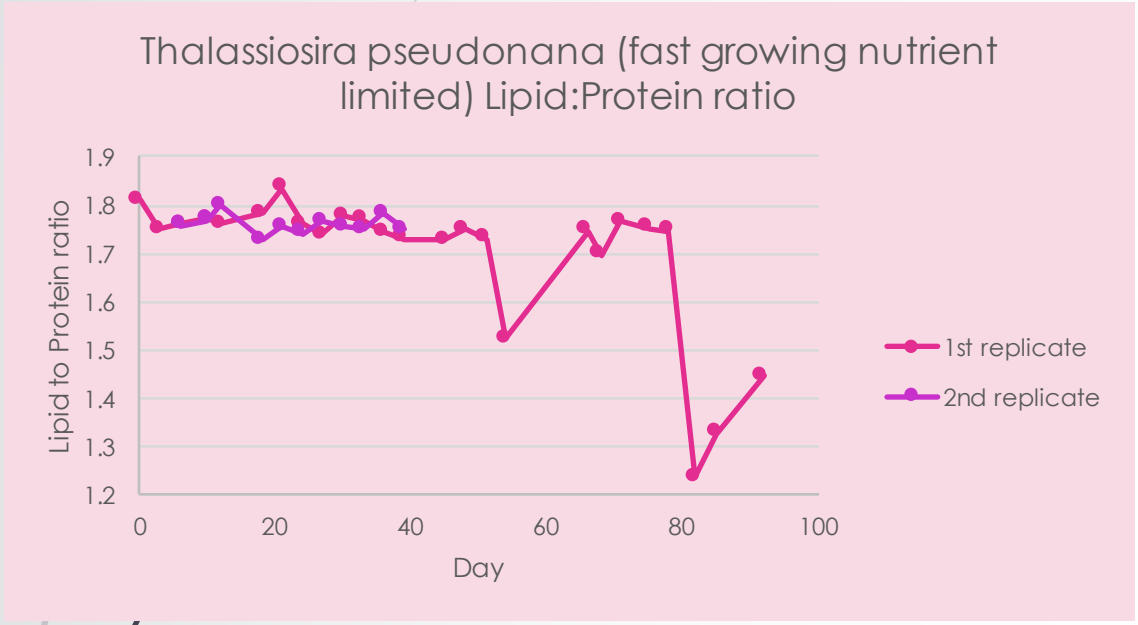
Results: *D. tertiolecta* chlorophyll content

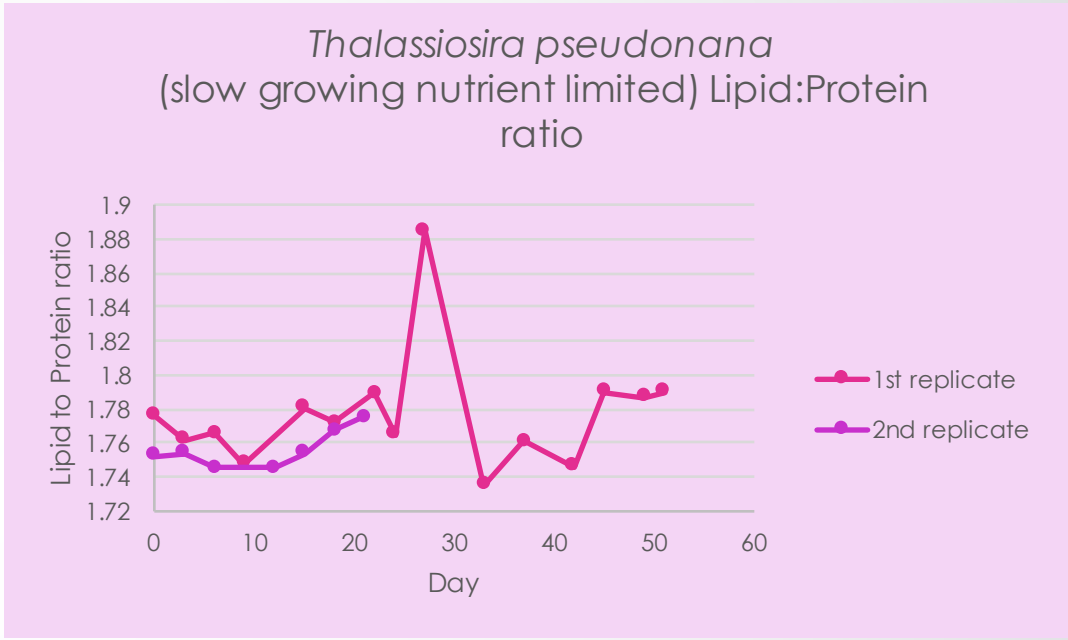




- Increase in nutrients, increase in chlorophyll
  - Different y-axis scales
- "Fast growing" 0.18 pg Chl cell^{-1}
- "Slow growing" 0.12 pg Chl cell^{-1}
- Higher than T. pseudonana

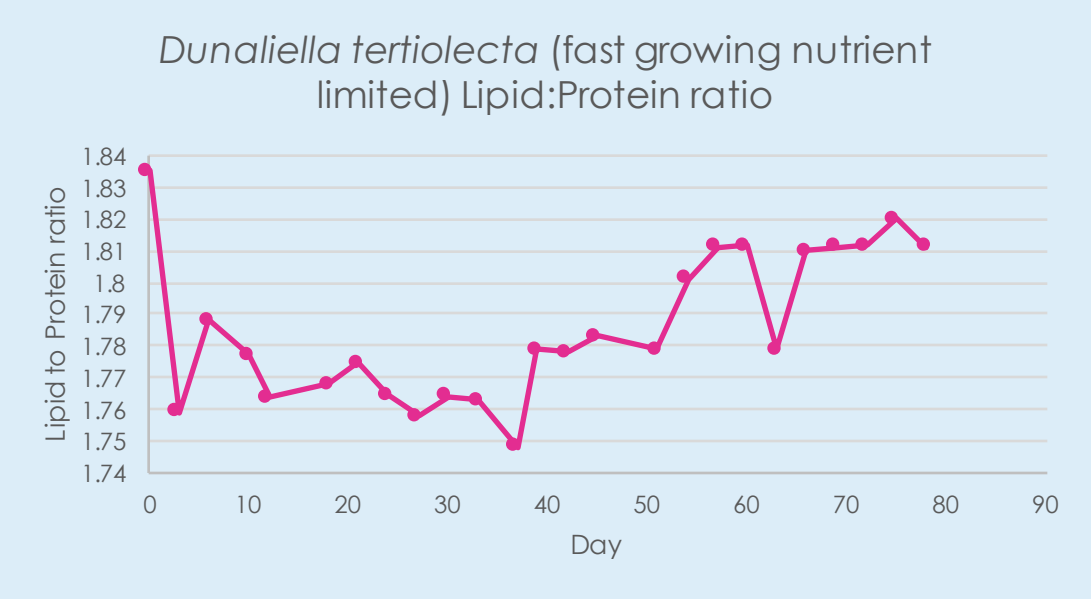
Results: *T. pseudonana* relative lipid:protein ratio

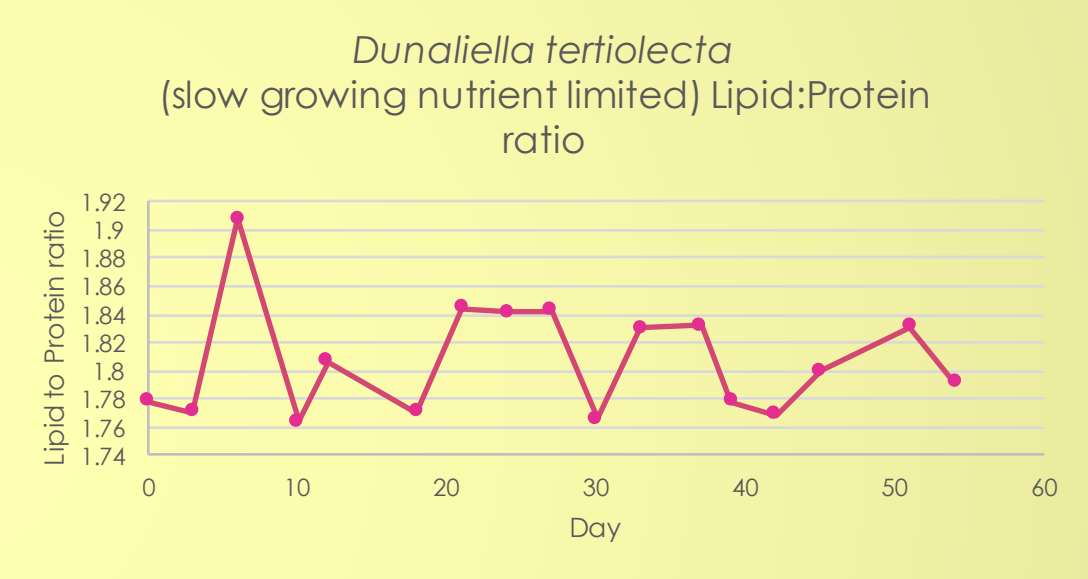




- Lipid:protein ratios higher in "slow growing" than "fast growing"
- 1. 78 vs 1. 75

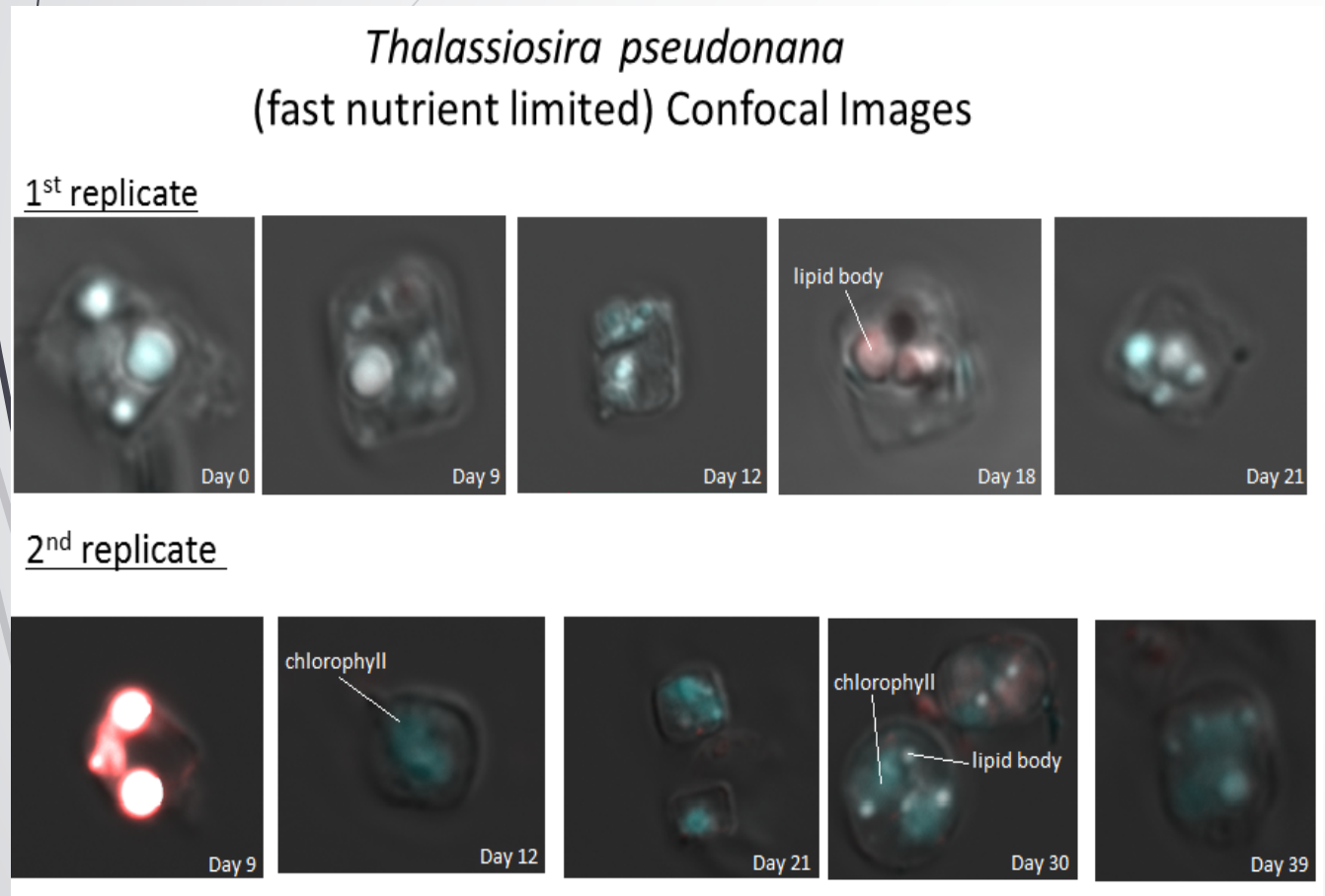
Results: *D. tertiolecta* relative lipid:protein ratio





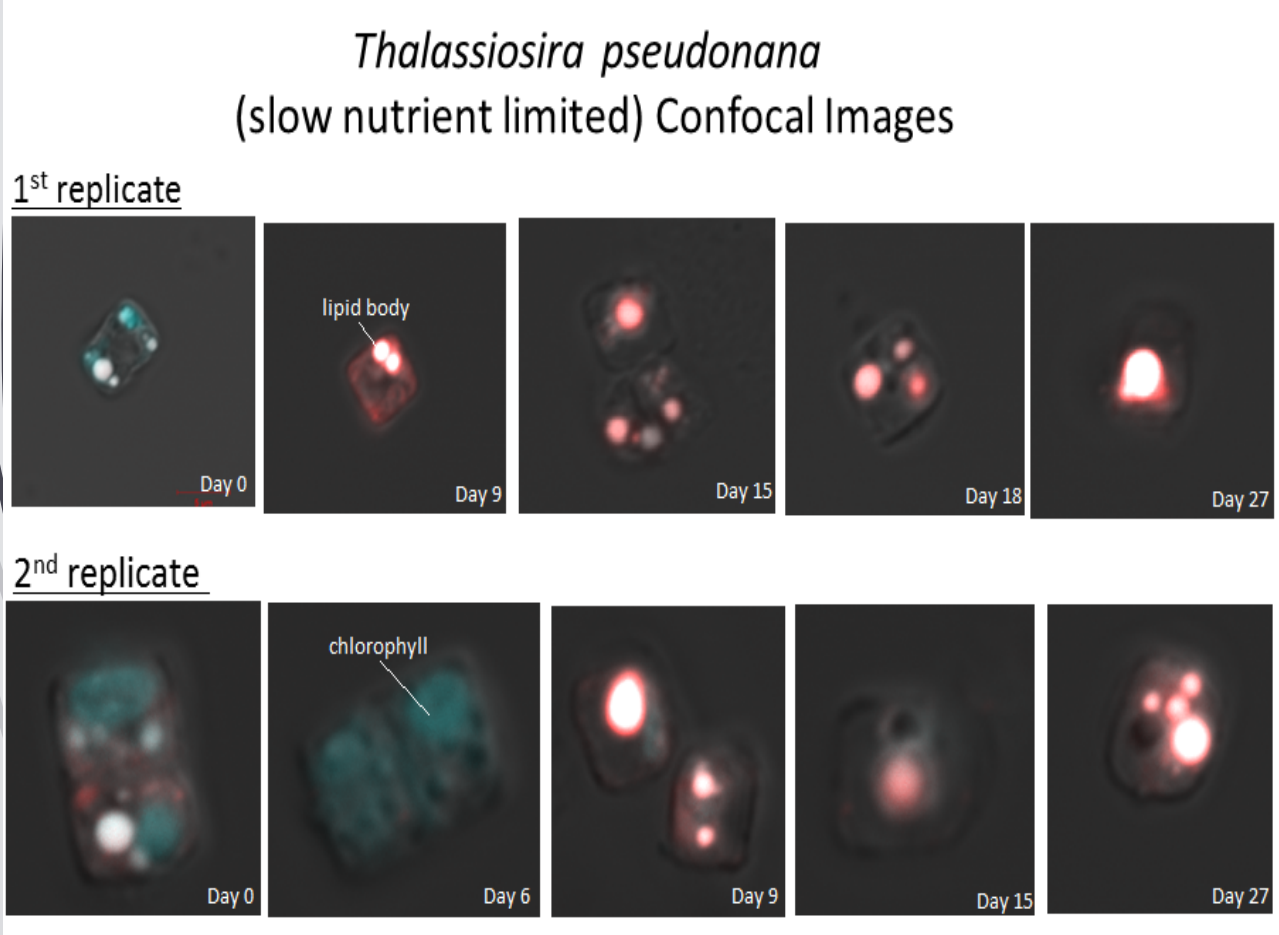
- Lipid:protein ratios higher in "slow growing" than "fast growing"
- 1. 81 vs 1. 79

Results: *T. pseudonana* "fast growing" confocal images



- Lipid body
  - very defined circular shape
  - appear red
- Chlorophyll
  - Does not show defined shape
  - Appears greenish-blue
- Lipid bodies decreased and chlorophyll increased approaching steady state

Results: *T. pseudonana* "slow growing" confocal images



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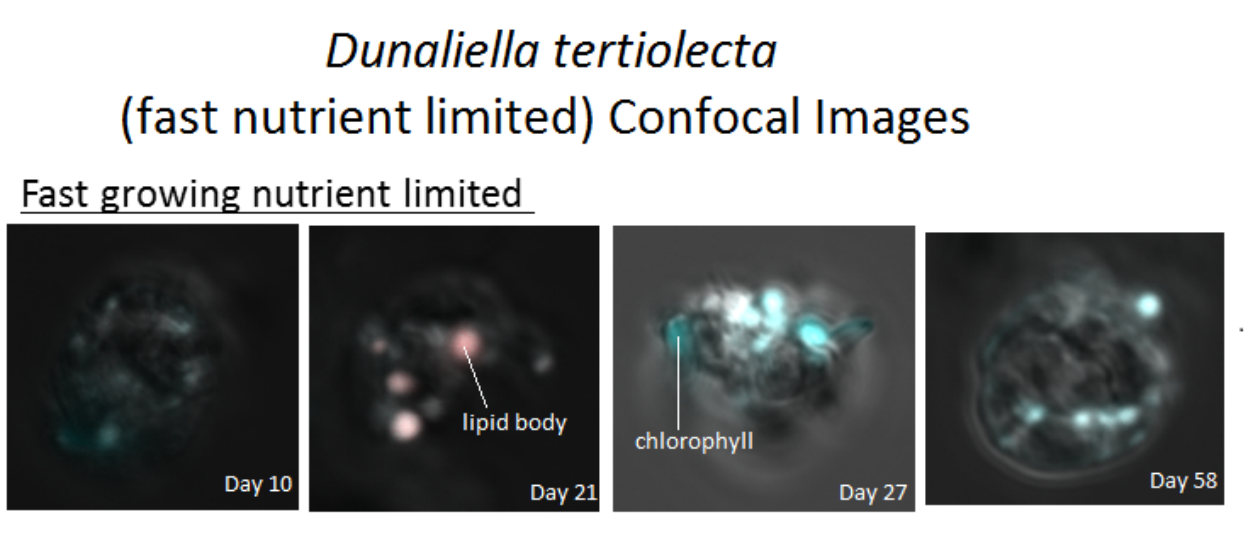

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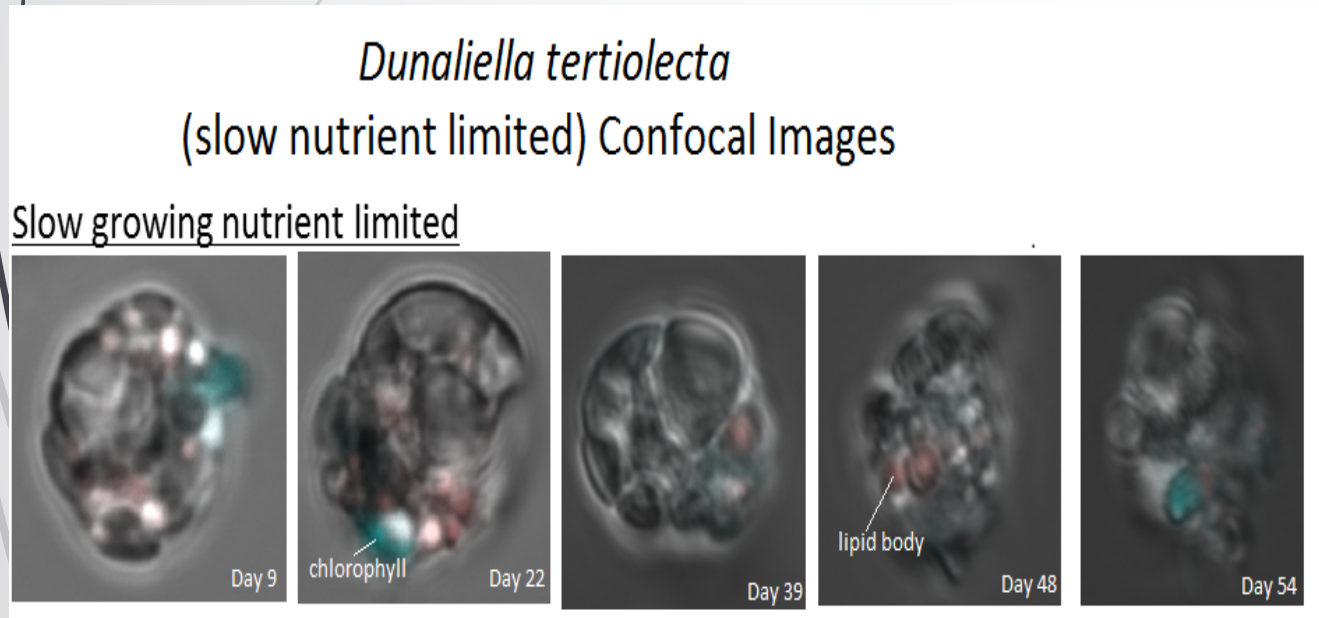
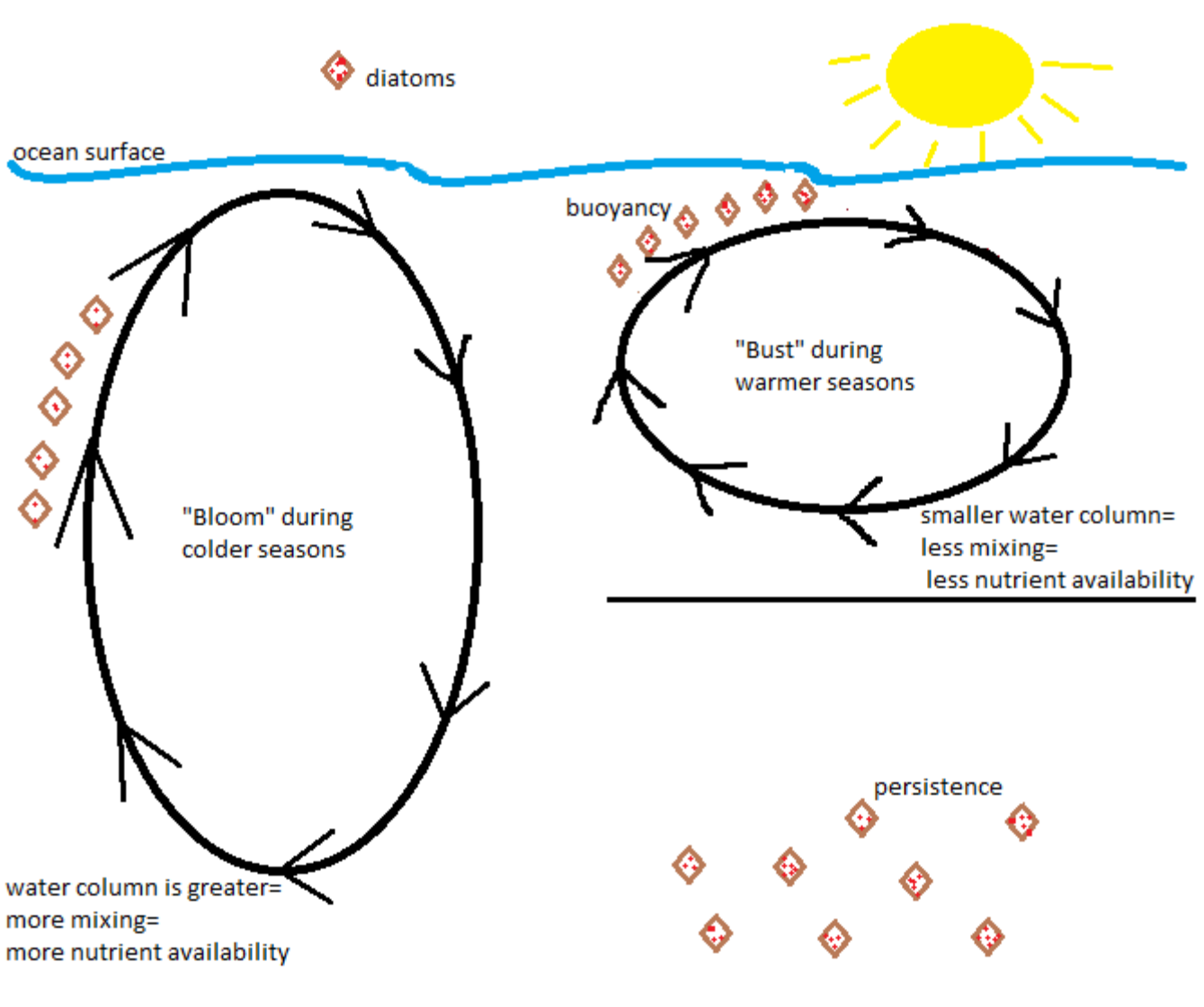


- Lipid body
  - very defined circular shape
  - appear red
- Chlorophyll
  - Does not show defined shape
  - Appears greenish-blue
- Lipid bodies decreased and chlorophyll increased approaching steady state
  - More slow than "fast growing" did

Take away points of Results

- Nutrients play a critical role and can influence physiology
- Chlorophyll increases as nutrients increase
  - Observed and measured in both *T. pseudonana* and *D. tertiolecta*
- Lipid body formation
  - "Fast growing" *T. pseudonana* and *D. tertiolecta* had some lipid body and chlorophyll development
  - "Slow growing" *T. pseudonana* great lipid body production
  - "Slow growing" *D. tertiolecta* few lipid body production

“Bloom and Bust” growth pattern



- Applies to *T. pseudonana* only
- Persistence
- Buoyancy
- *D. tertiolecta*?

Implications of Results

- Help to understand physiological responses of microalgae to nutrient conditions
- Physiological acclimation strategies in response to nutrient limitation are shared across phytoplankton species but other metabolic pathways are regulated very differently
  - chlorophyll regulation
  - lipid body accumulation
- Expected to impact food web dynamics
- Behaviors of *T. pseudonana* and *D. tertiolecta* can influence behaviors of their predators and the ocean environment

What's next?

- Replicates
- Predator behavior influenced by diatom physiology
- Does carbon composition affect microzoplankton?

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