Inferring physiology from omic data



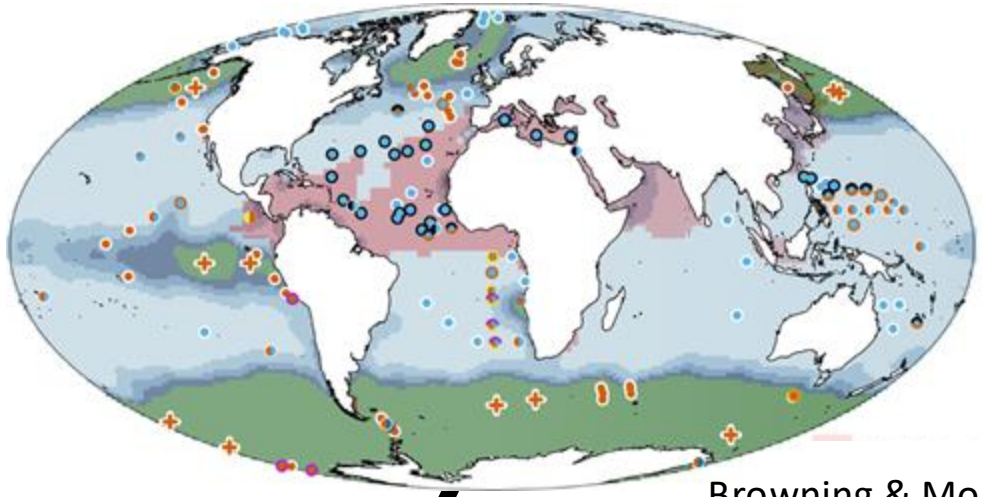


NATALIE COHEN

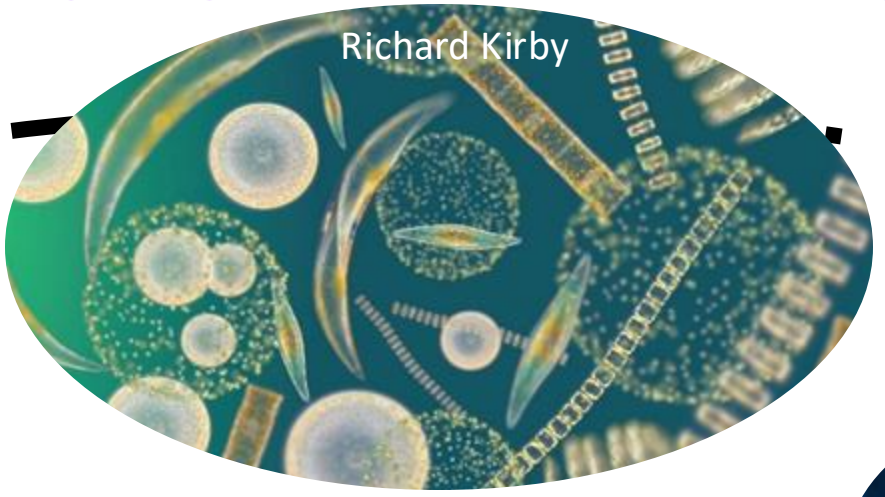
CROUCHER SUMMER COURSE 2025

Microeukaryotes (protists) play key roles in ocean biogeochemistry

Micronutrient co-limitation

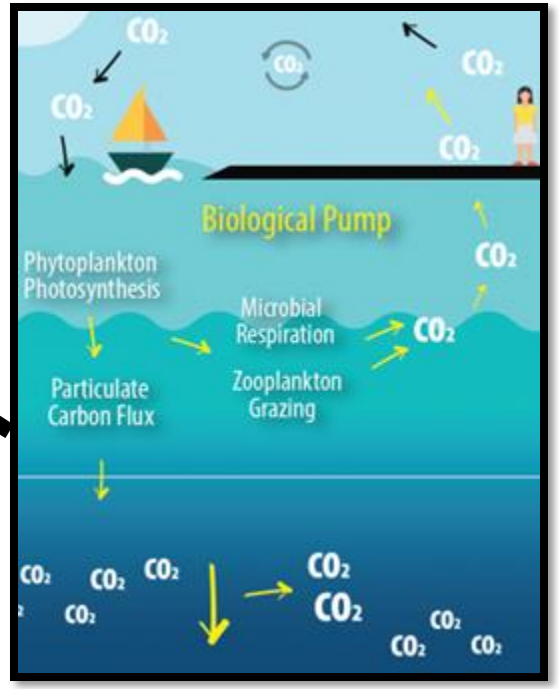


Browning & Moore 2023



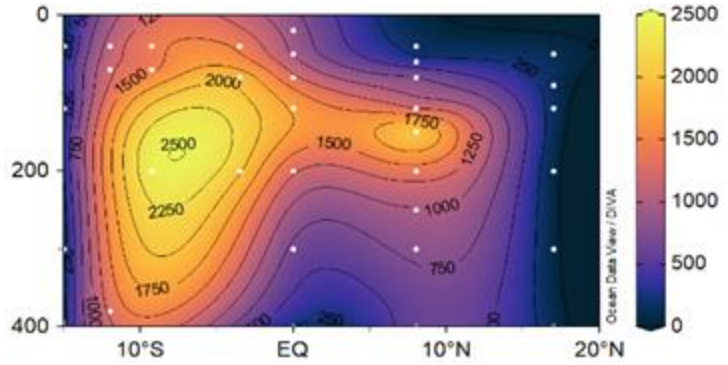
Transcripts/proteins



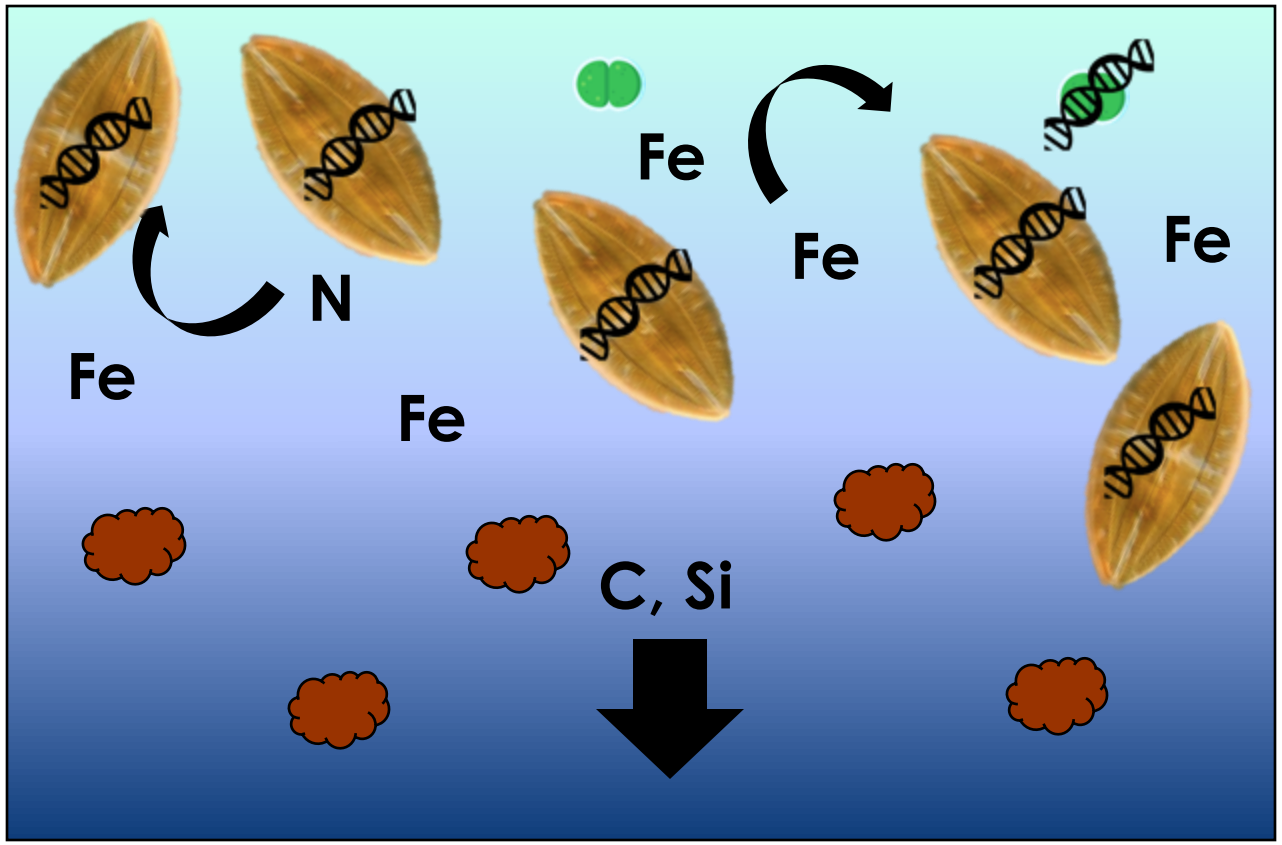


How are they contributing to ocean biogeochemistry?

Nutrient biomarkers



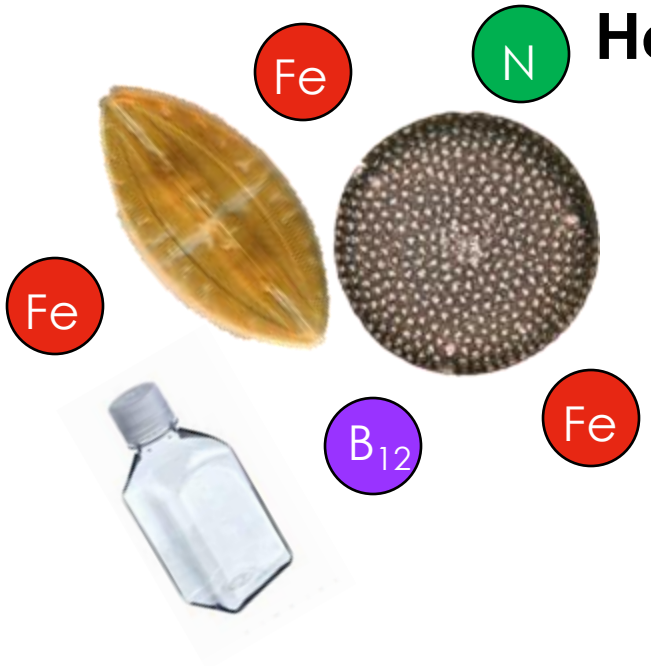
Microeukaryotes can impact biogeochemical cycling of elements



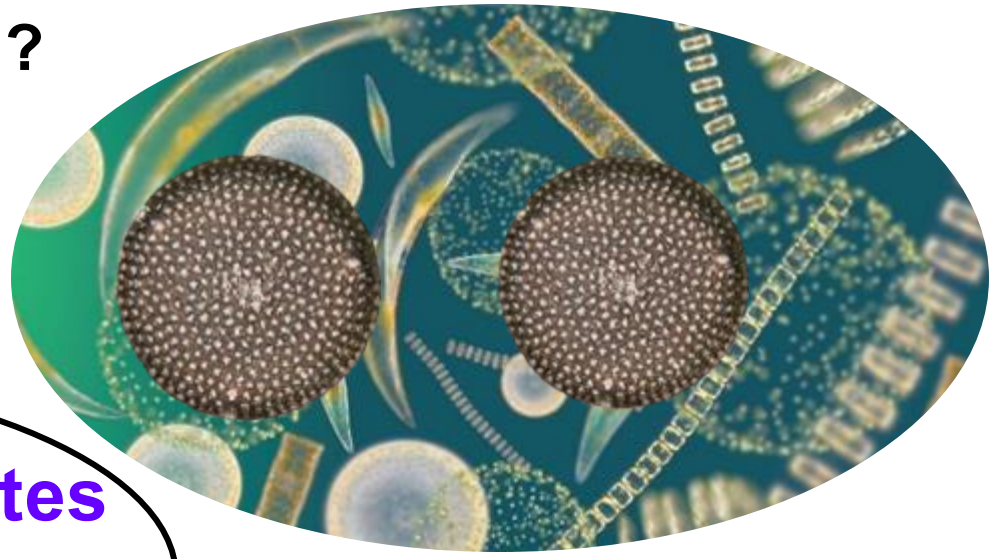
Diverse phytoplankton contain distinct physiologies

Ecological advantage

Growth dynamics influence biogeochemical cycling



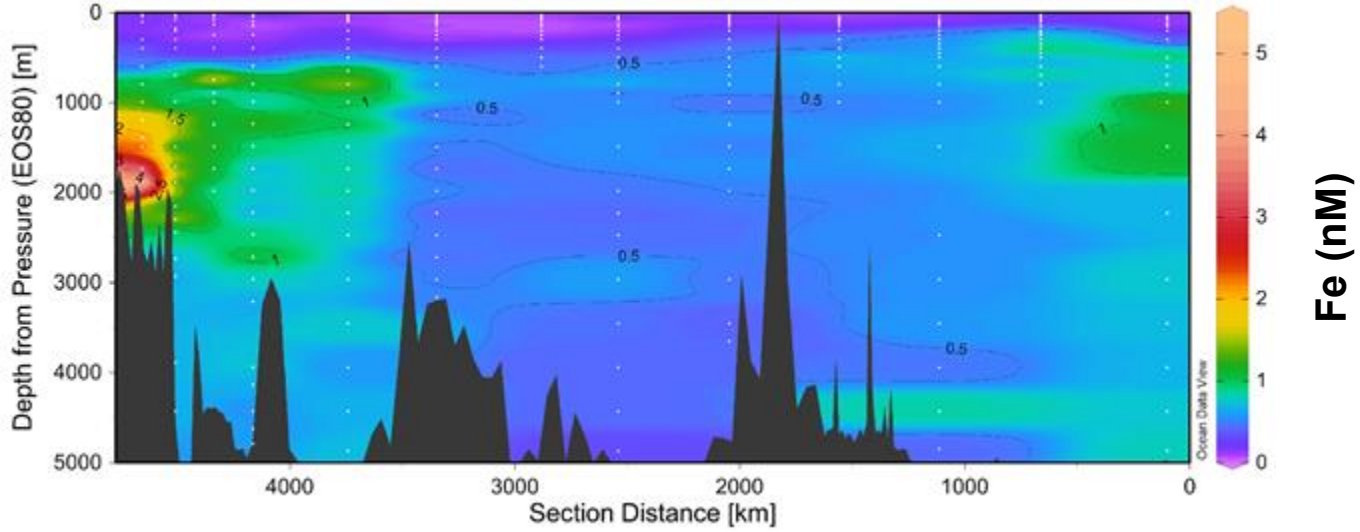
How is cell metabolism altered?



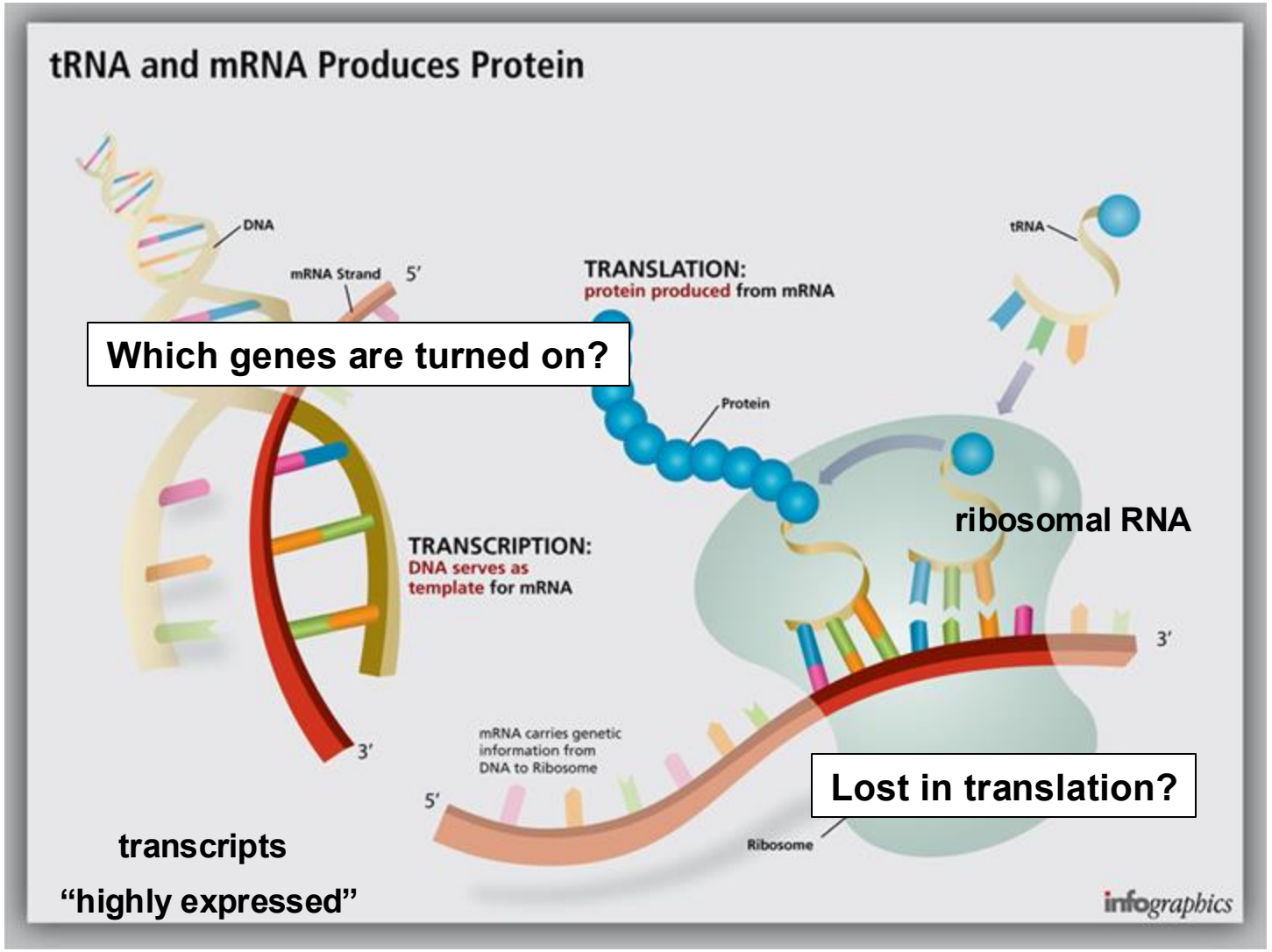
How do microeukaryotes respond to shifts in chemical resources?

Which taxonomic groups and physiologies are favored?

How are biogeochemical cycles impacted?



Central dogma of molecular biology



Which genes are turned on?

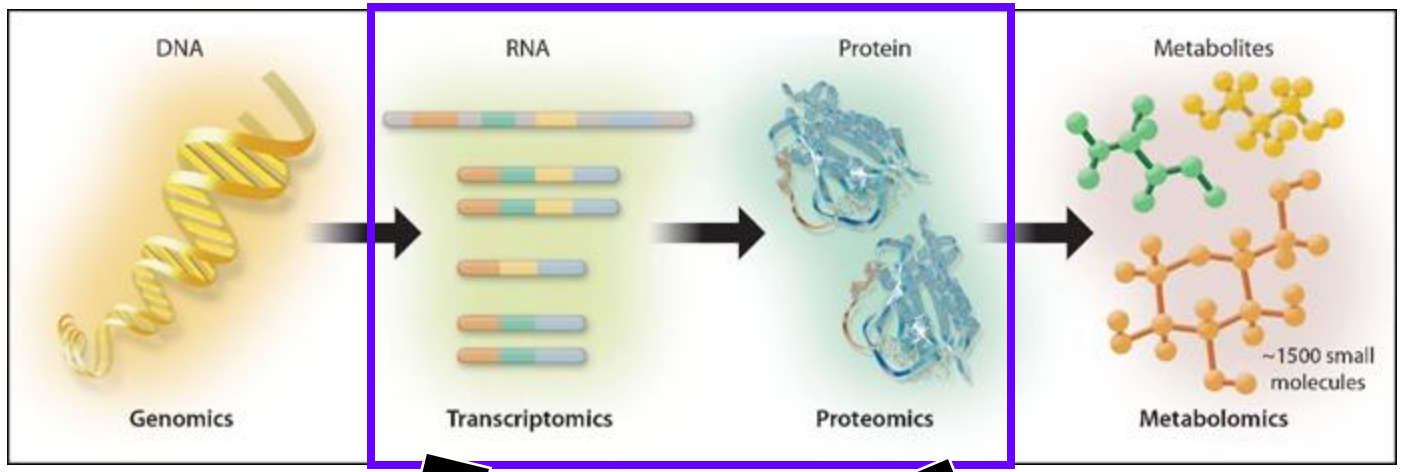
ribosomal RNA

Lost in translation?

transcripts

"highly expressed"

Probing marine metabolism using 'omics

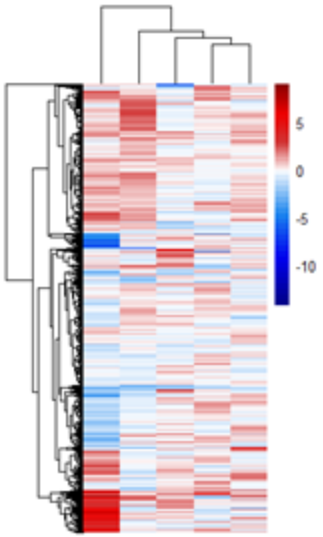


US DOE 2009



OCB metaT intercomparison

Bioinformatics & visualization





Sequencing



Analytical chemistry

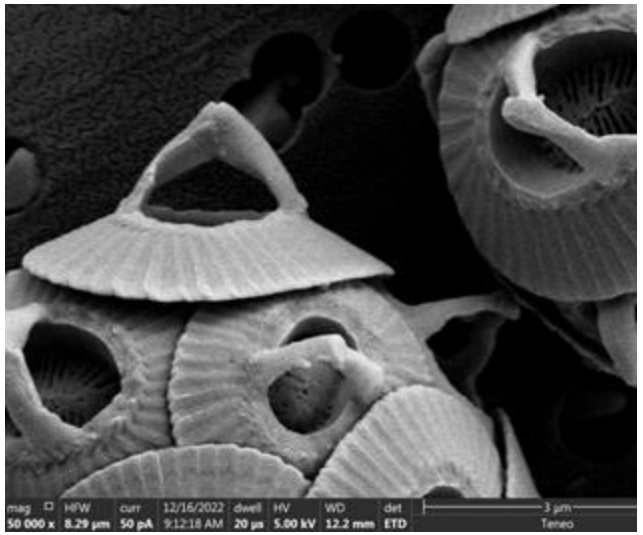




Collect samples

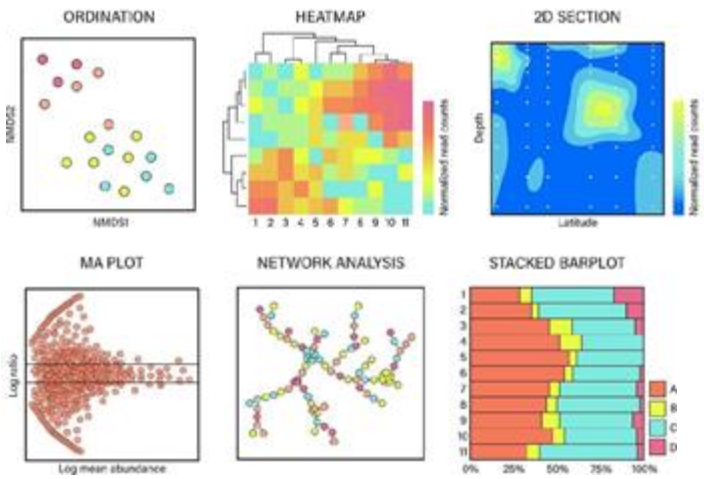
Leverage the power of 'omics to address how diverse eukaryotes interact with their chemical environment

Outline



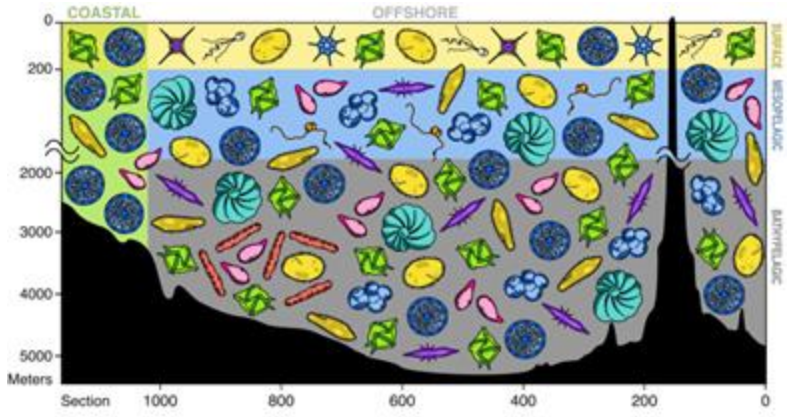
Inferring physiology from omics

Primary production, nutrient biomarkers, links to ocean biogeochemistry



Best practices in metatranscriptomics

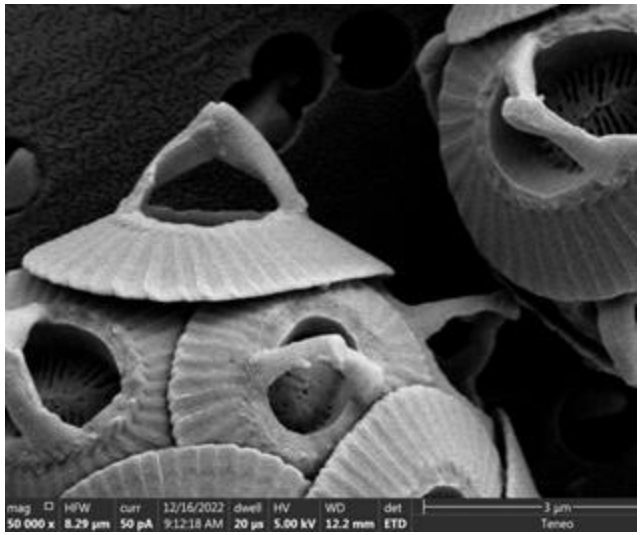
From sample collection to data visualization



Microeukaryote metabolism across the ocean

Biogeography & ocean gradients

Outline



Inferring physiology from omics

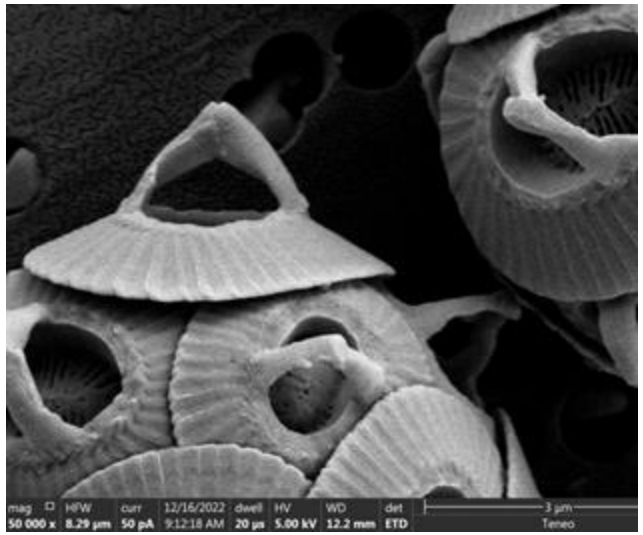
Primary production, nutrient biomarkers, links to ocean biogeochemistry

Best practices in metatranscriptomics

From sample collection to data visualization

Microeukaryote metabolism across the ocean

Biogeography & ocean gradients



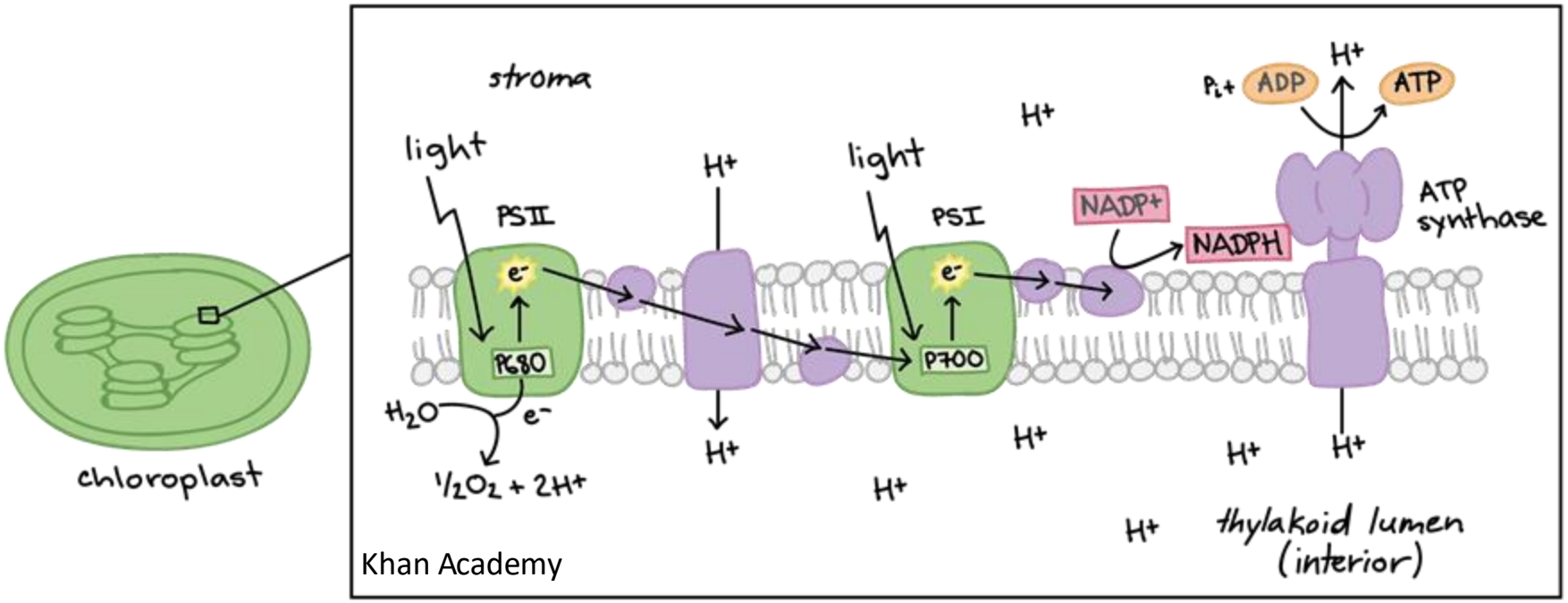
Inferring physiology from omics

Primary production, nutrient biomarkers, links to ocean biogeochemistry

- Photosynthesis
- Biomarkers of nutrient limitation
- Changes in trophic modes (mixotrophy)
- Limitations

Photosynthesis

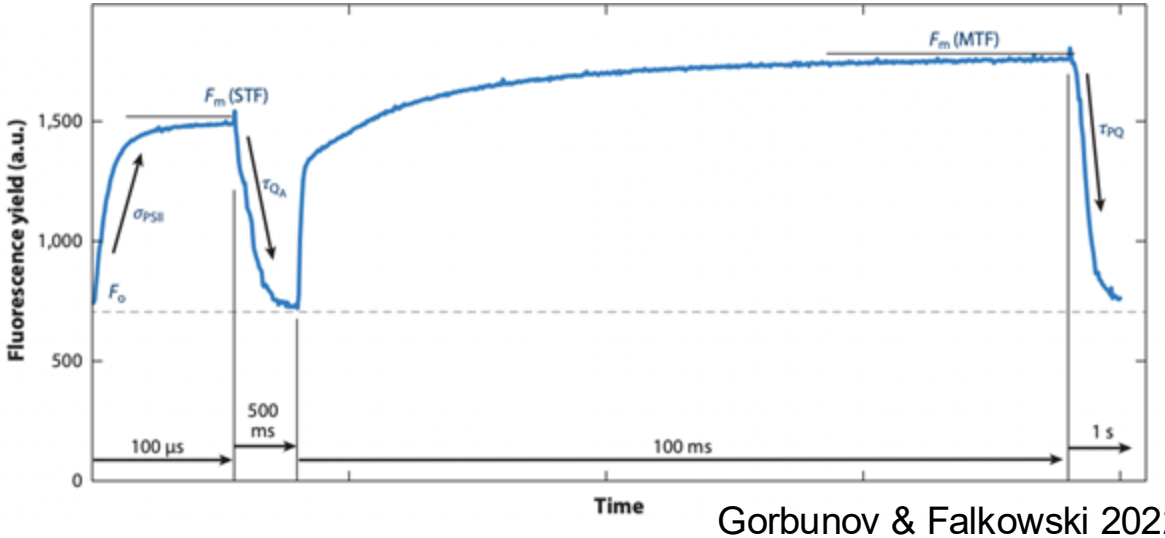
$$106\,CO_2 + 16\,HNO_3 + H_3PO_4 + 122\,H_2O \xrightarrow{\text{light}} C_{106}H_{263}O_{110}N_{16}P + 138\,O_2$$



Primary production = rate of **photosynthesis**, mainly performed by photosynthetic autotrophic organisms in the surface ocean

Tools for measuring autotrophic growth, photosynthetic efficiency & primary production

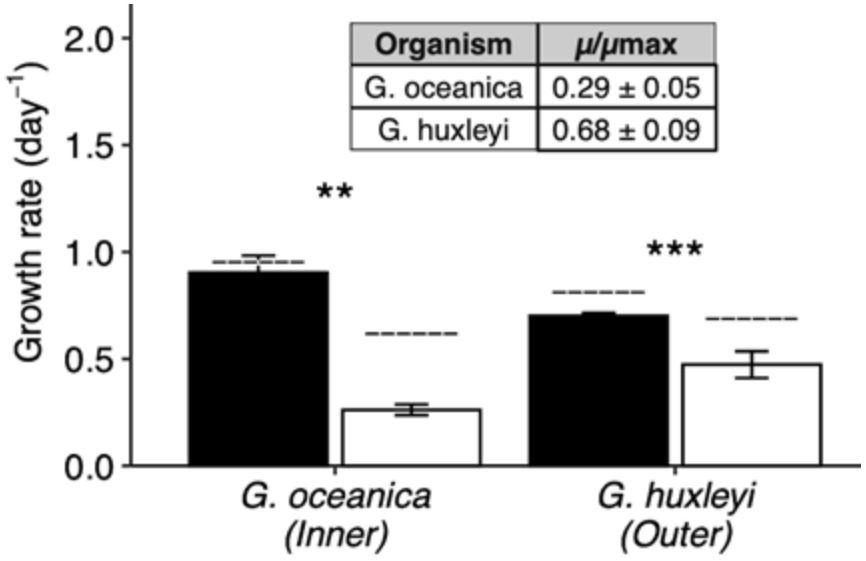
Fluorescence induction and relaxation system (FIRe)



Gorbunov & Falkowski 2022 *ARMS*

In vivo chlorophyll measurements to derive growth rates





Quirk et al. in revision

C^{13} or C^{14} assimilation (as bicarbonate)

-Add to bottles in the light

-Filter, count radioactivity/stable isotope to determine C incorporated

If incubated over 24 hours, can give you net primary production

C^{13} or C^{14}



Tools for measuring autotrophic growth, photosynthetic efficiency & primary production

O_2 light-dark bottle experiments

-Measures changes in O_2 discrete samples

-light bottle = Net primary production

-dark bottle = Respiration



Measure O_2 using titrations



Light incubations



Dark incubations

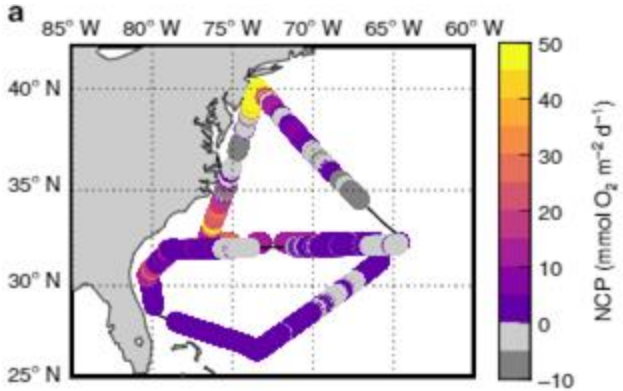
Tools for measuring autotrophic growth, photosynthetic efficiency & primary production

<u>Estimate variations in oxygen, nutrients, carbon or chlorophyll</u>

-Get seasonal average rates, will miss small scale features



Measure O_2 + chlorophyll *a* using sensors



Tang et al. 2019



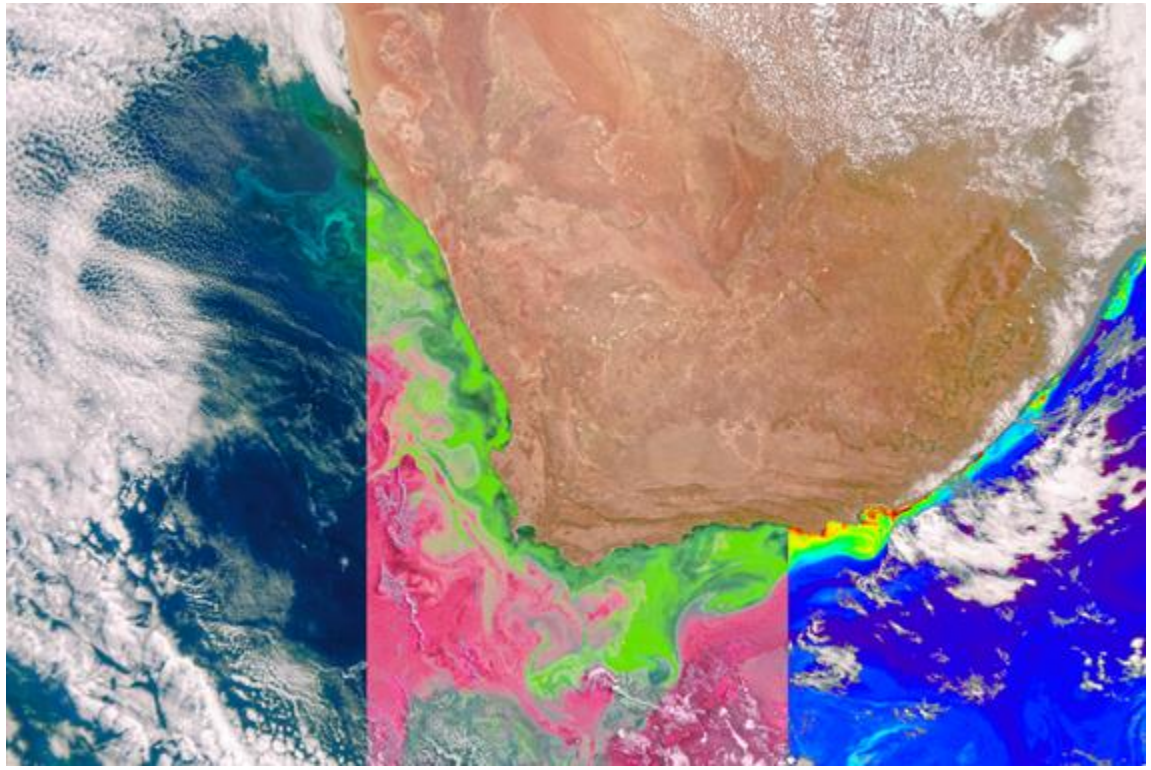
In situ – measure directly in sea

Tools for measuring autotrophic growth, photosynthetic efficiency & primary production

Satellite remote sensing – "ocean color" ***surface***

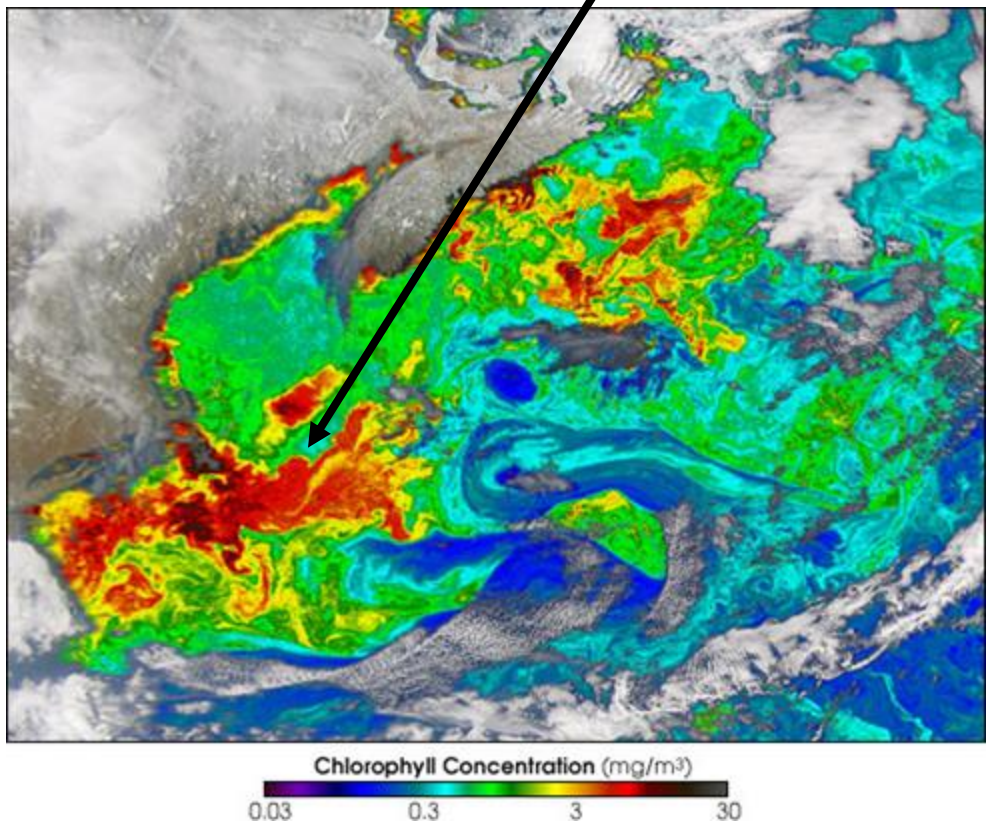
-Temperature, light, chlorophyll can be measured. Need models and conversion factors to relate these to primary production

NASA PACE



North Atlantic bloom

NASA/SeaWiFS

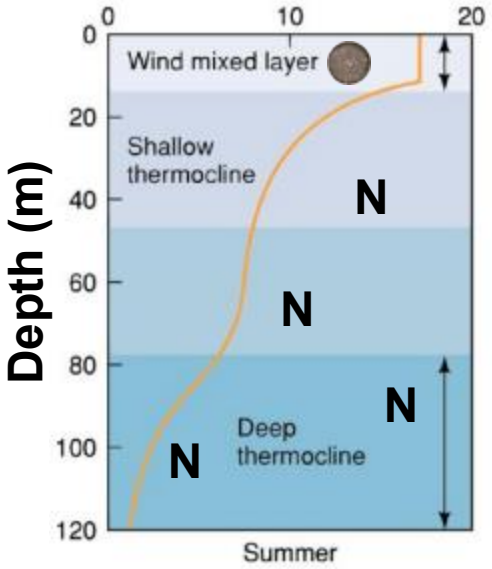


So what can omics offer?

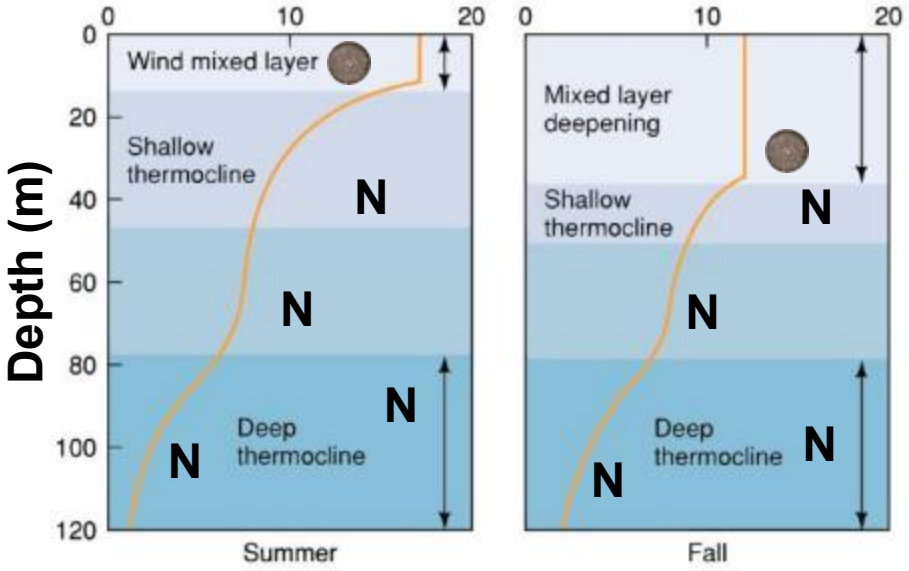
- Investigate mechanisms underlying trends
- How do individual taxa contribute to a system?
- Which processes are responsible for observed in situ measurements?
- How is photosynthesis sensitive to external stressors?
- How does photosynthesis change over short time scales? (Diel, warming, etc)

Systems level understanding of ocean processes

Mixed layer depth effects on phytoplankton

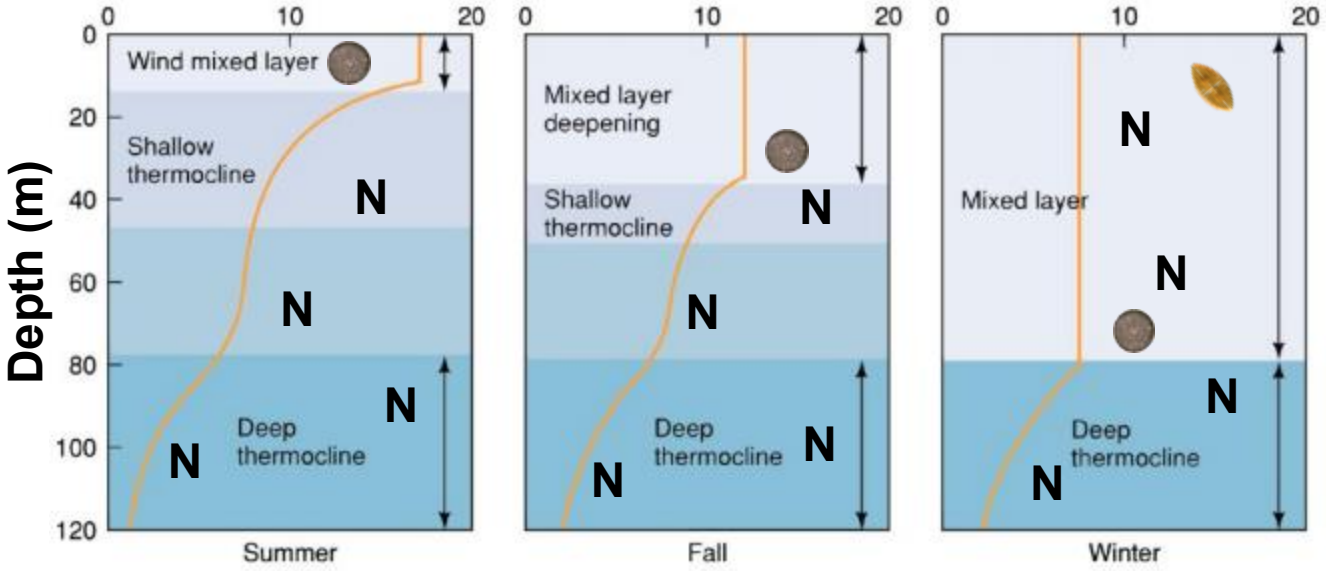


Mixed layer depth effects on phytoplankton



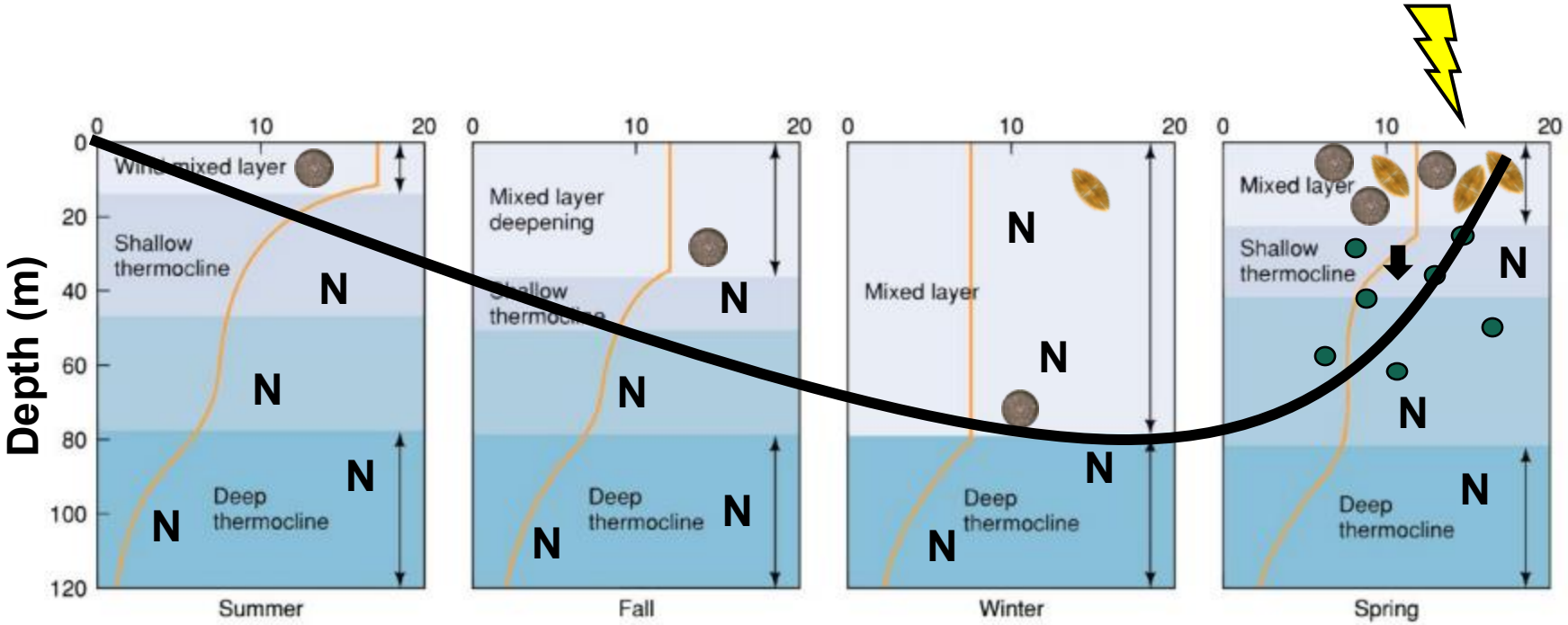
mixed layer depth = depth at which a rapid density change is observed

Mixed layer depth effects on phytoplankton



mixed layer depth = depth at which a rapid density change is observed

Mixed layer depth effects on phytoplankton



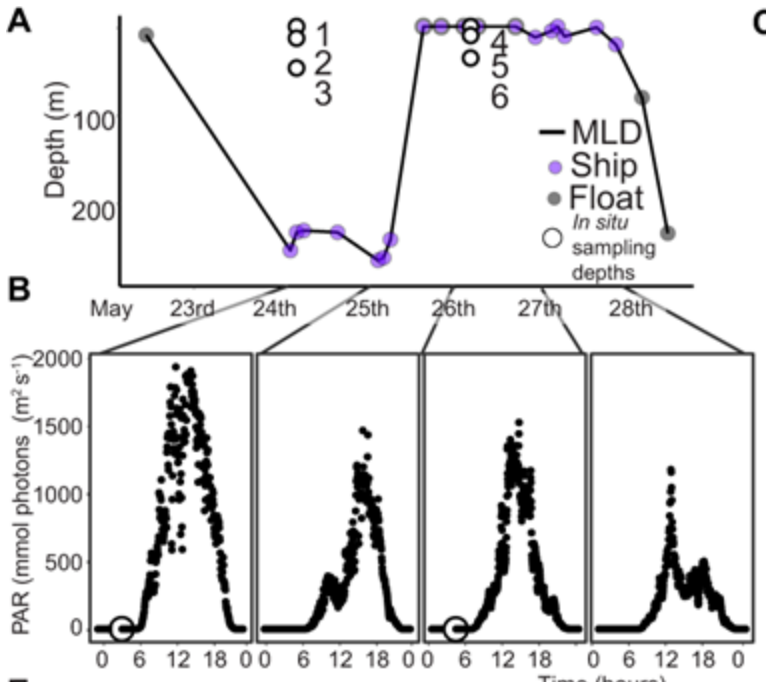
mixed layer depth = depth at which a rapid density change is observed

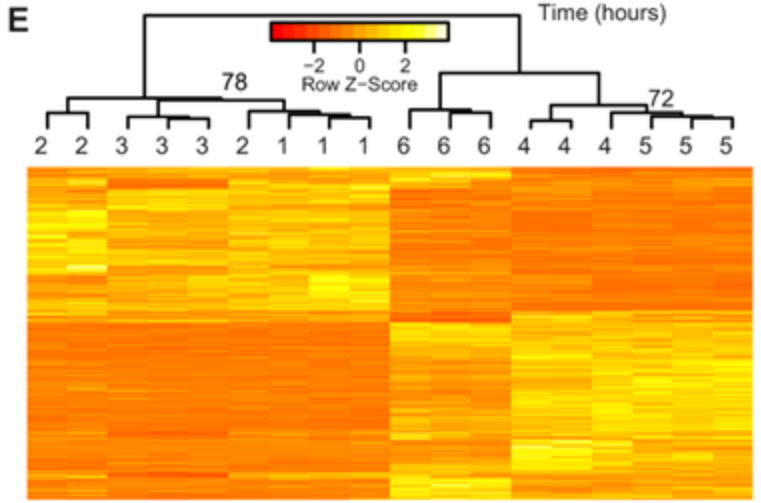
mixed layer depth fluctuates seasonally….

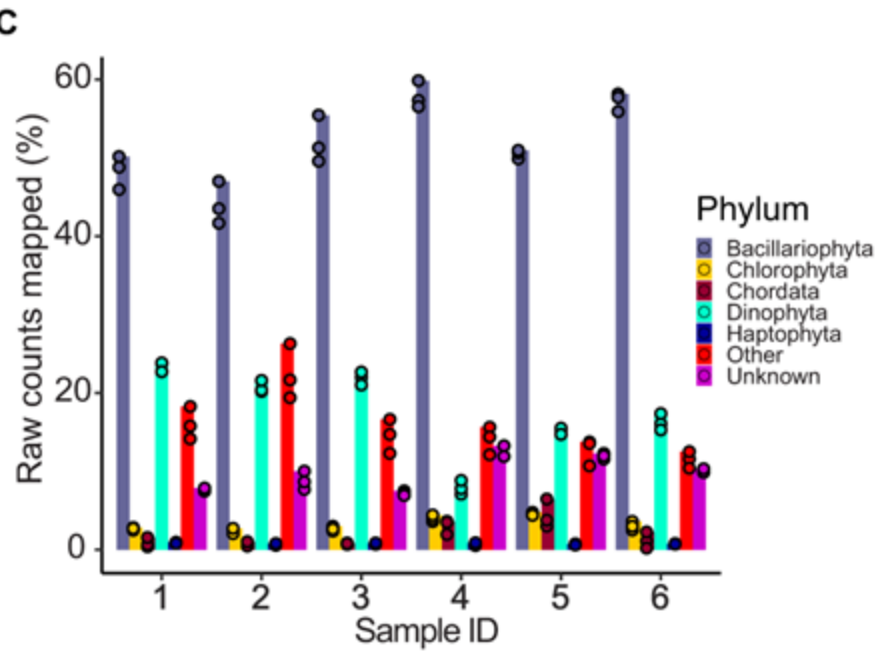
…or even daily/weekly depending on disturbances

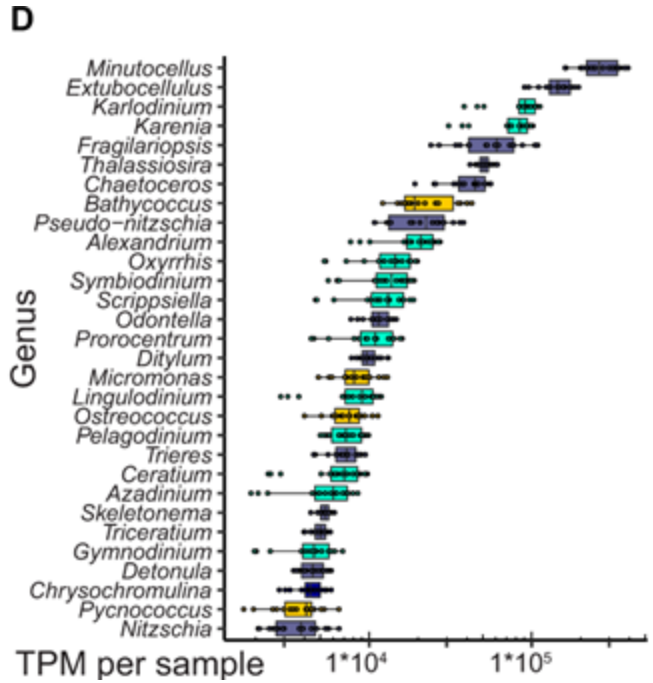
How is photosynthesis impacted?

Rapid stratification event in the North Atlantic







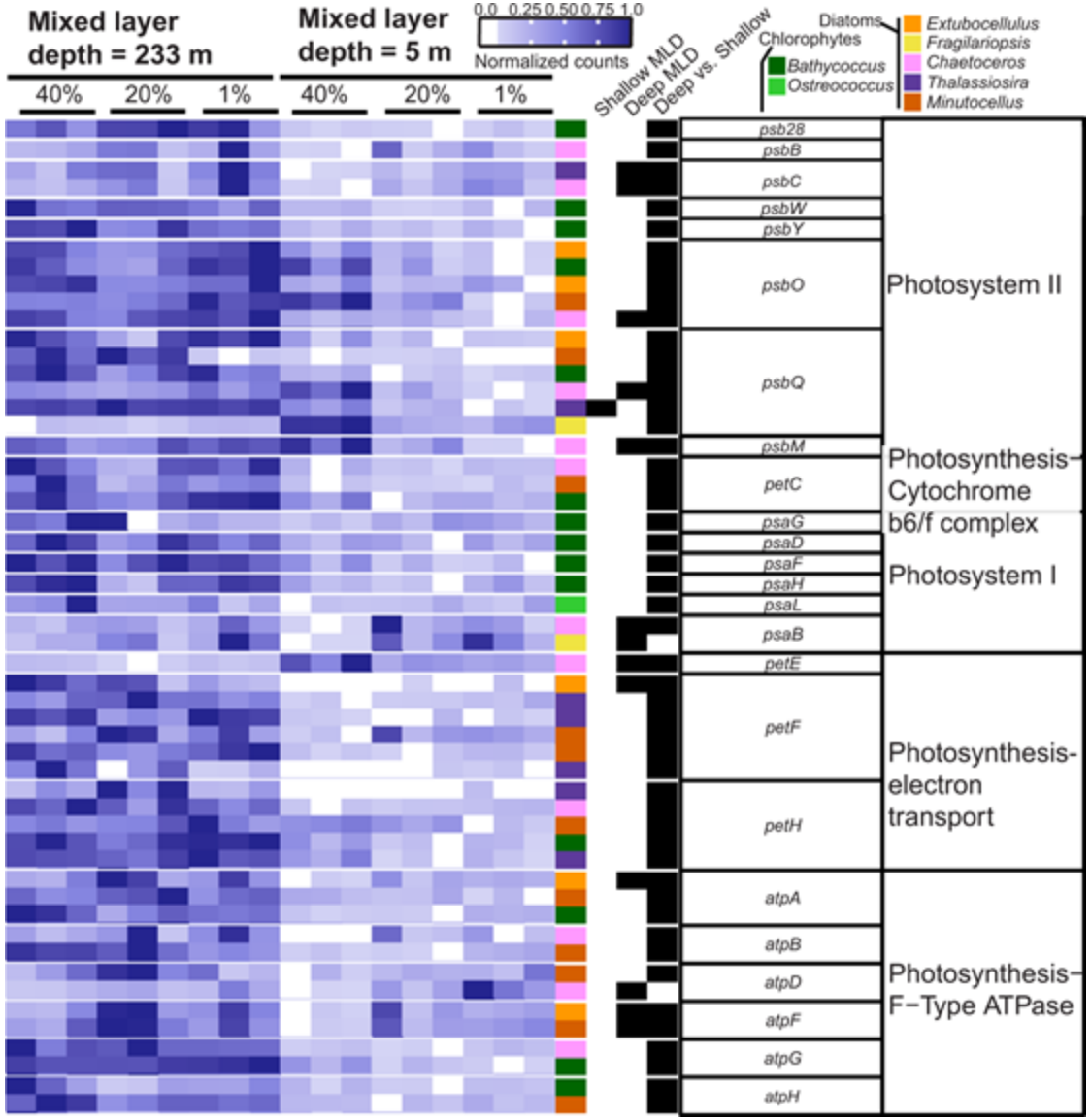


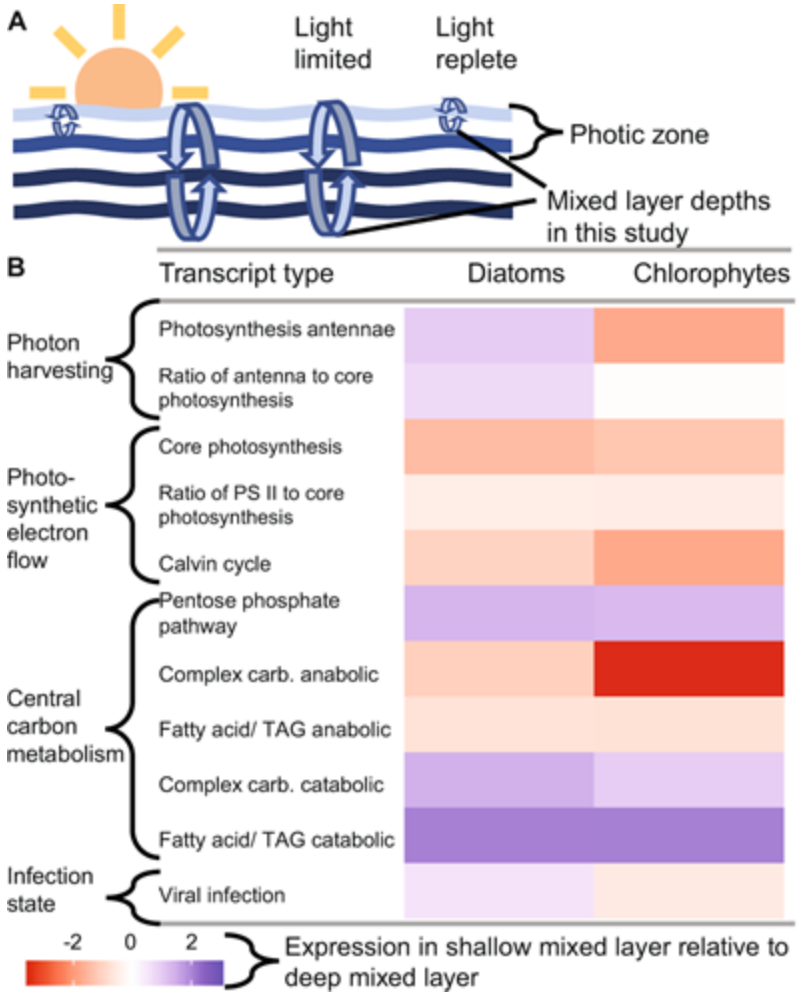
- Shoaling from ~230m to 5m
- Reduction in PAR following event
- Clear transcriptomic signature associated with before/after
- Communities composed of diatoms, chlorophytes, and dinoflagellates

Diaz et al. 2023 *ISME*

Downregulation of photosynthesis during shoaling

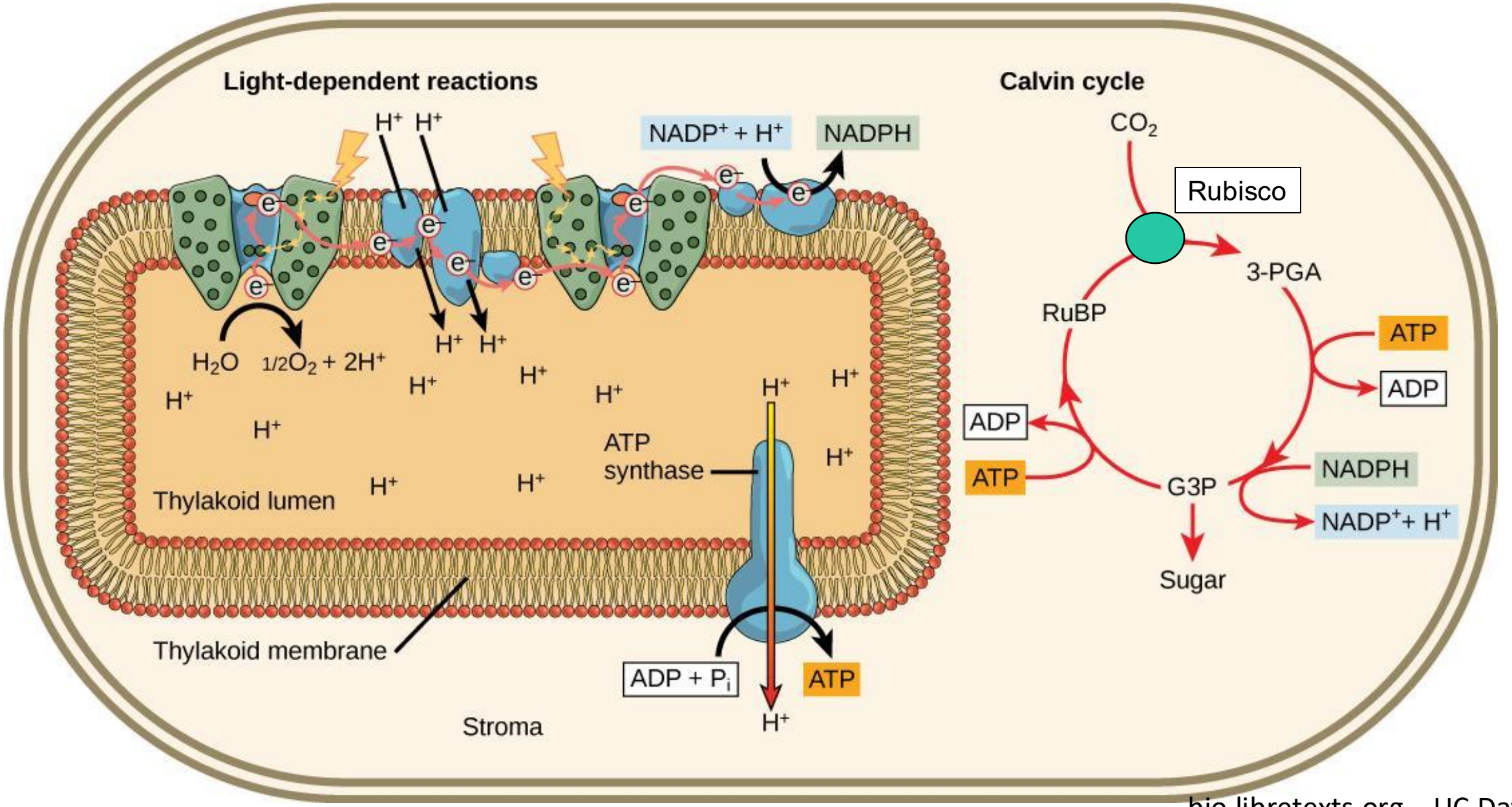
Diaz et al. 2023 *ISME*





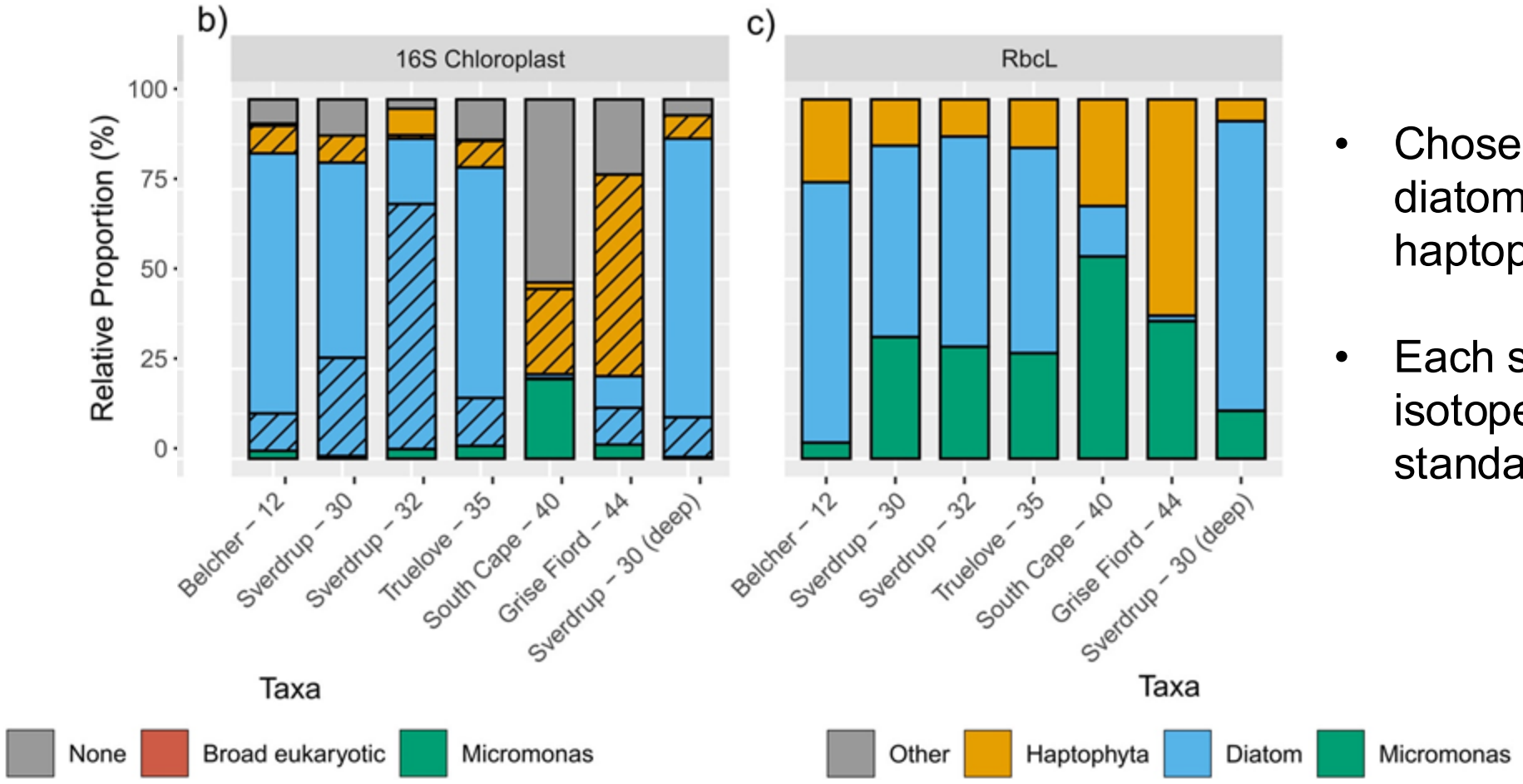
Shallow transition induced decrease in PS and carbon fixation, but increased carbon catabolism genes, potentially to support cell division

Carbon fixation



bio.libretexts.org – UC Davis

How is carbon fixation impacted by glaciers in the Arctic?

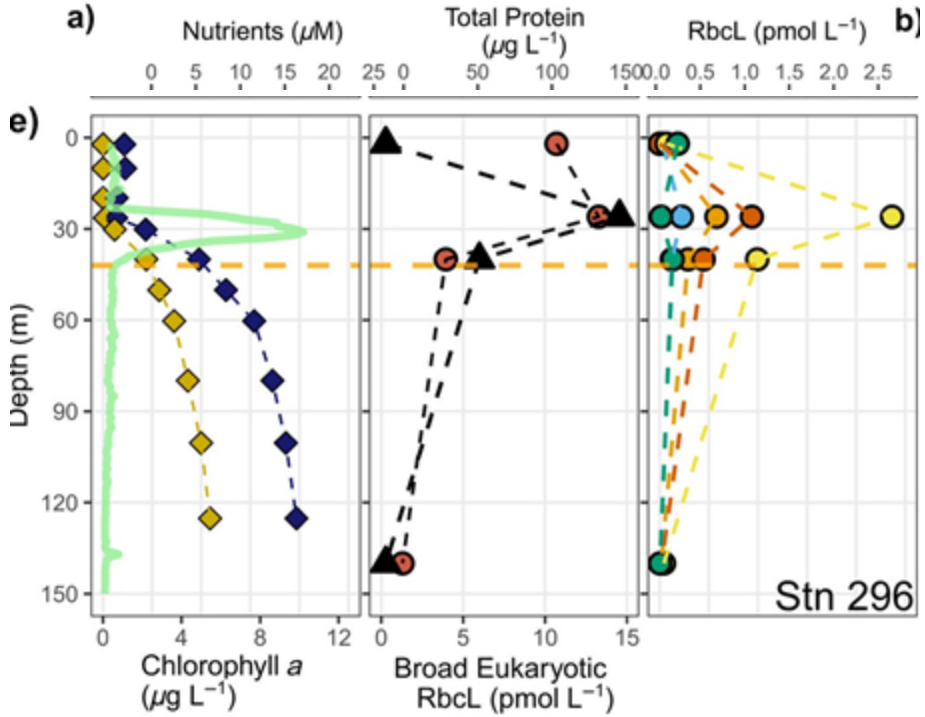


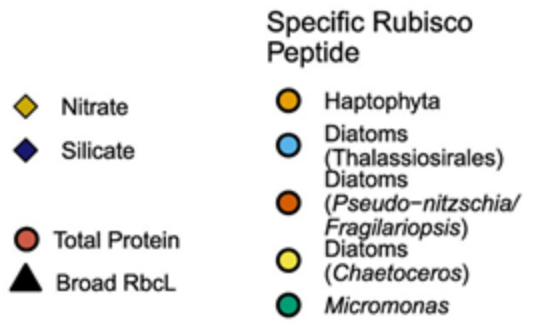
- Chose peptides in ~80% of diatoms (n=91) & 50% of haptophytes (n=12)
- Each sample spiked with heavy isotope-labeled internal standard for each peptide

Quantitative metaproteomics of Rubisco

Roberts et al. 2024 *Limnology & Oceanography*

How is carbon fixation impacted by glaciers in the Arctic?





- One diatom contributing largely to protein and chlorophyll pool
- Subsurface peak
- Enables carbon fixation rate estimates

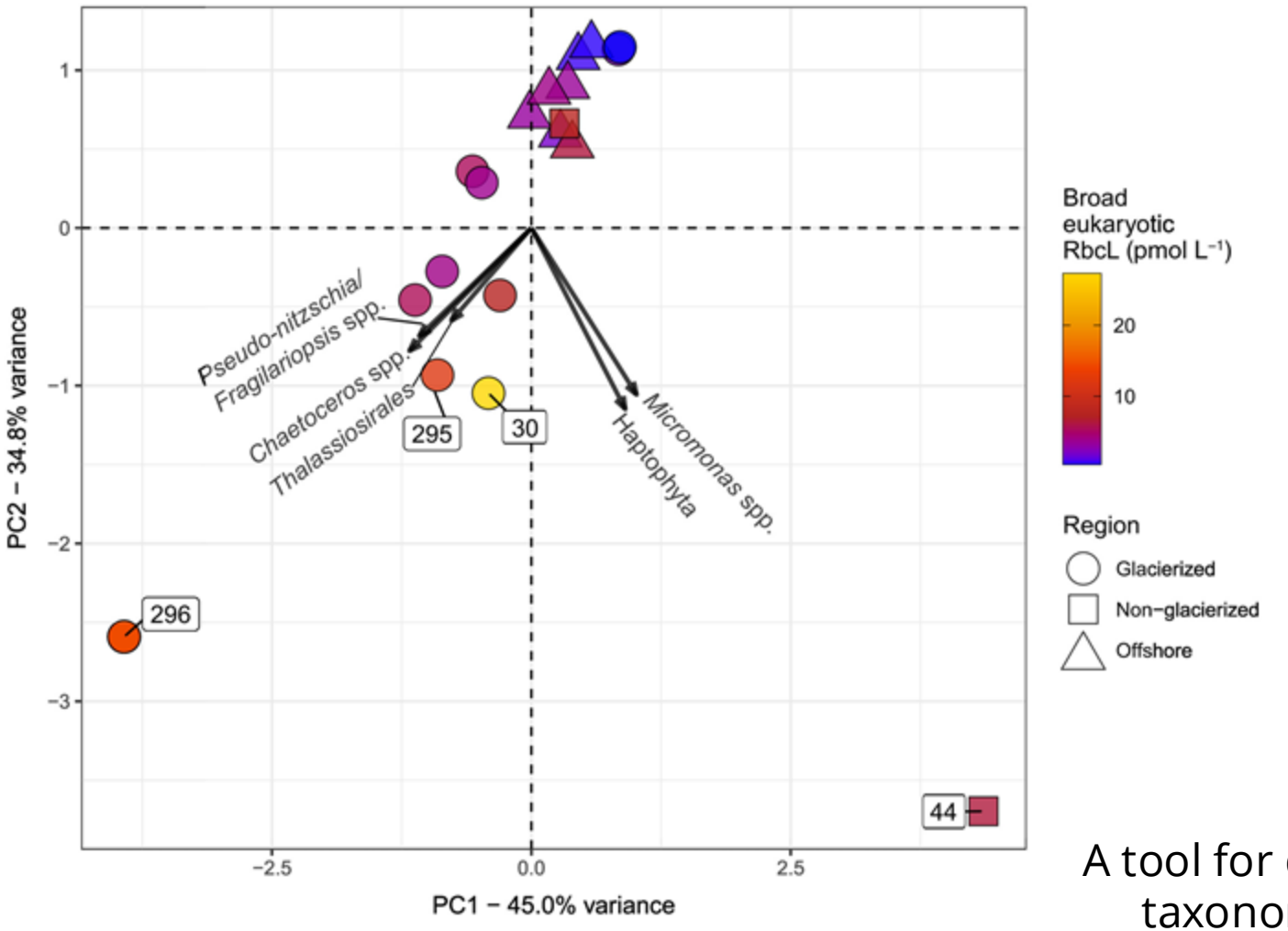
$$\text{Carbon fixation rate}\left(\text{mg C m}^{-3}\,\text{d}^{-1}\right) = \text{RbcL}\left(\text{mol L}^{-1}\right) \times k_{\text{cat}}^{c}\ \left(\text{mol C active sites}^{-1}\,\text{s}^{-1}\right) \quad (6)$$

Turnover rate

Carbon fixation rates from targeted proteomics

Roberts et al. 2024 *Limnology & Oceanography*

How is carbon fixation impacted by glaciers in the Arctic?



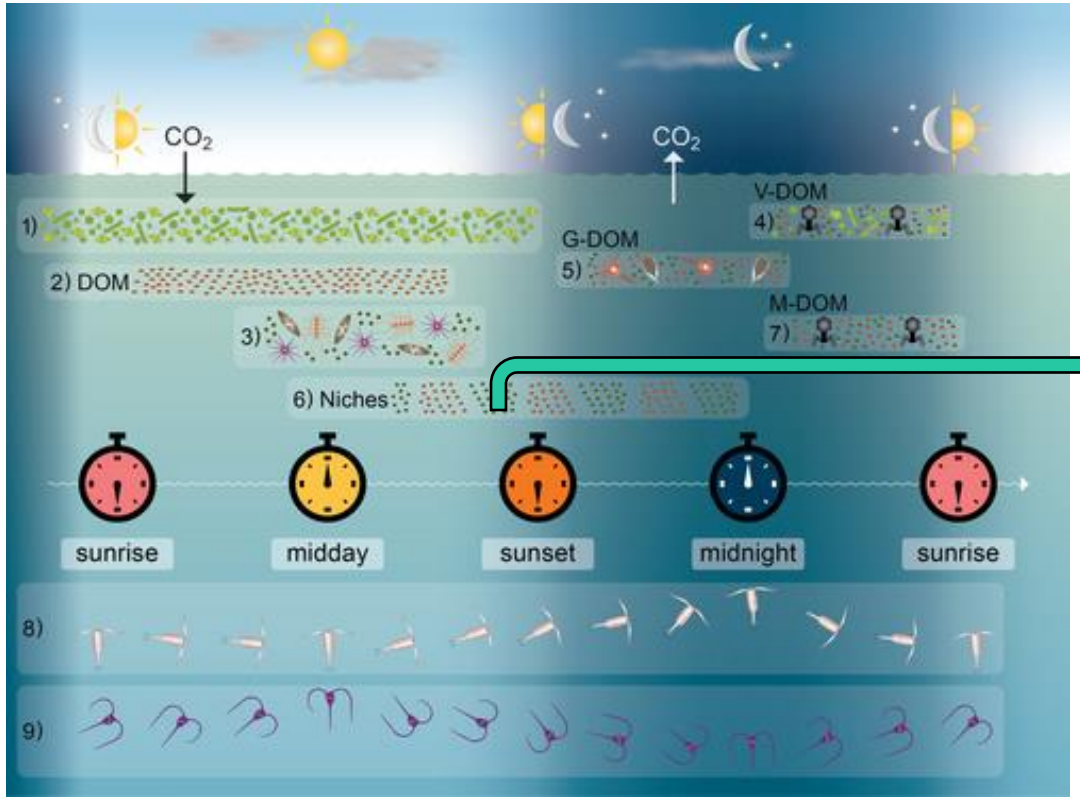
- Diatom Rubisco was higher in glacier influenced regions
- Rubisco from chlorophyte was greater in non-glacierized regions

Future climate scenarios may favor smaller phytoplankton groups (chlorophytes), with implications for food web structure

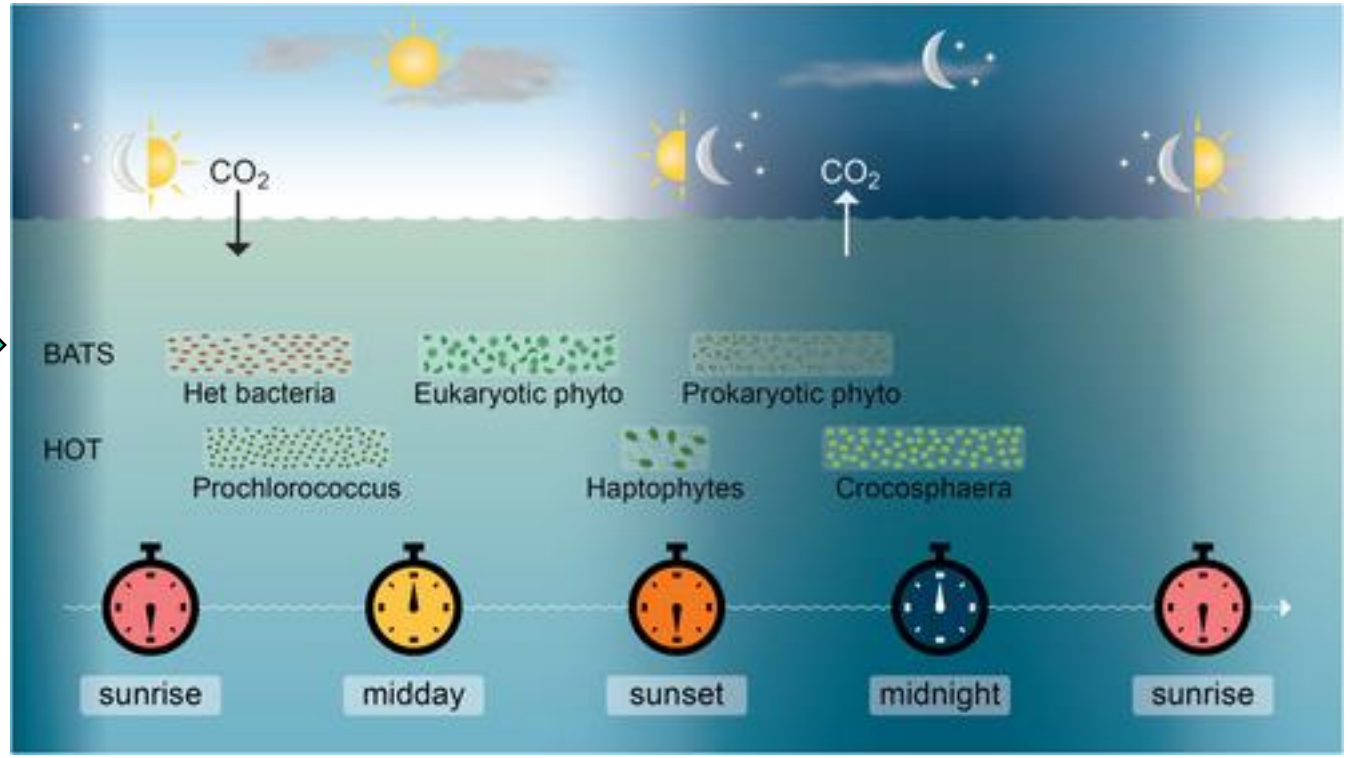
A tool for quantifying carbon fixation potential across taxonomic groups and contributions to primary production

Roberts et al. 2024 *Limnology & Oceanography*

The Diel Cycle: biological and chemical changes driven by light/dark cycle



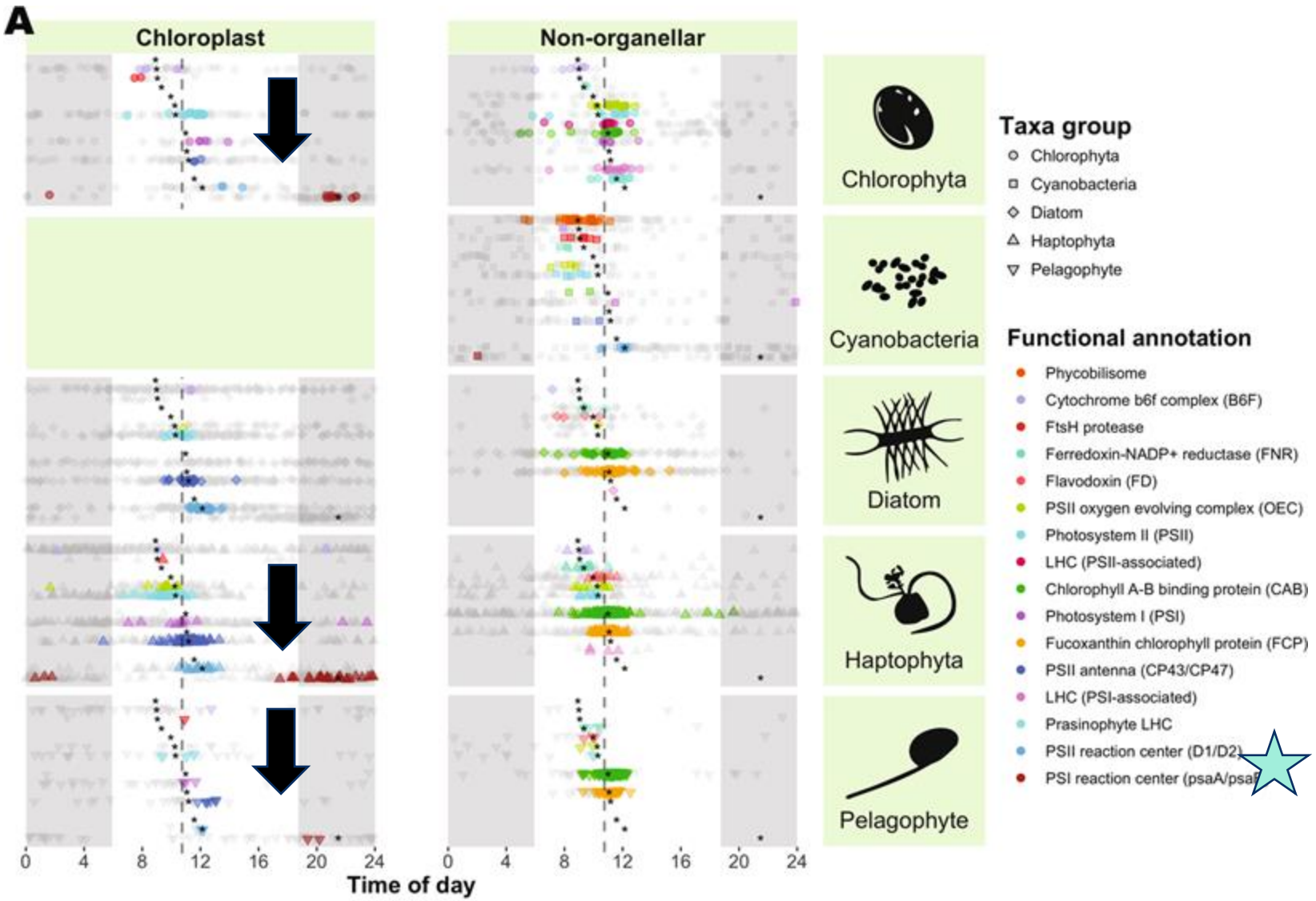
Community diel rhythm



Temporal niches

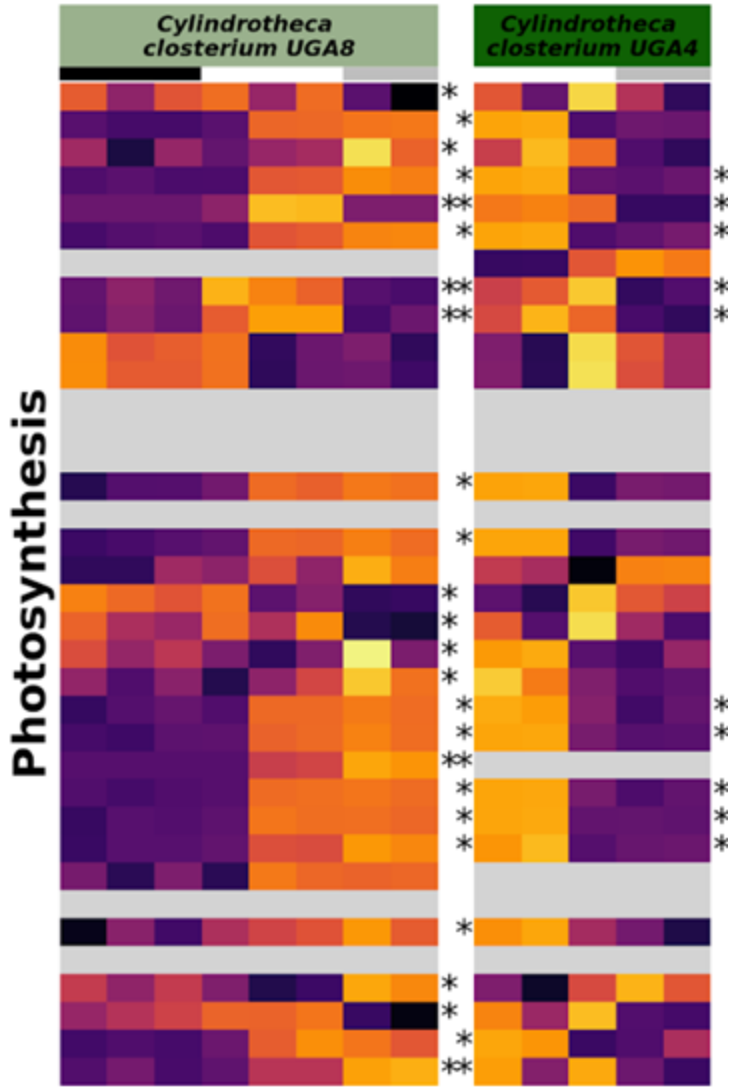
Boyd & Van Mooy 2025 *Limnology & Oceanography*

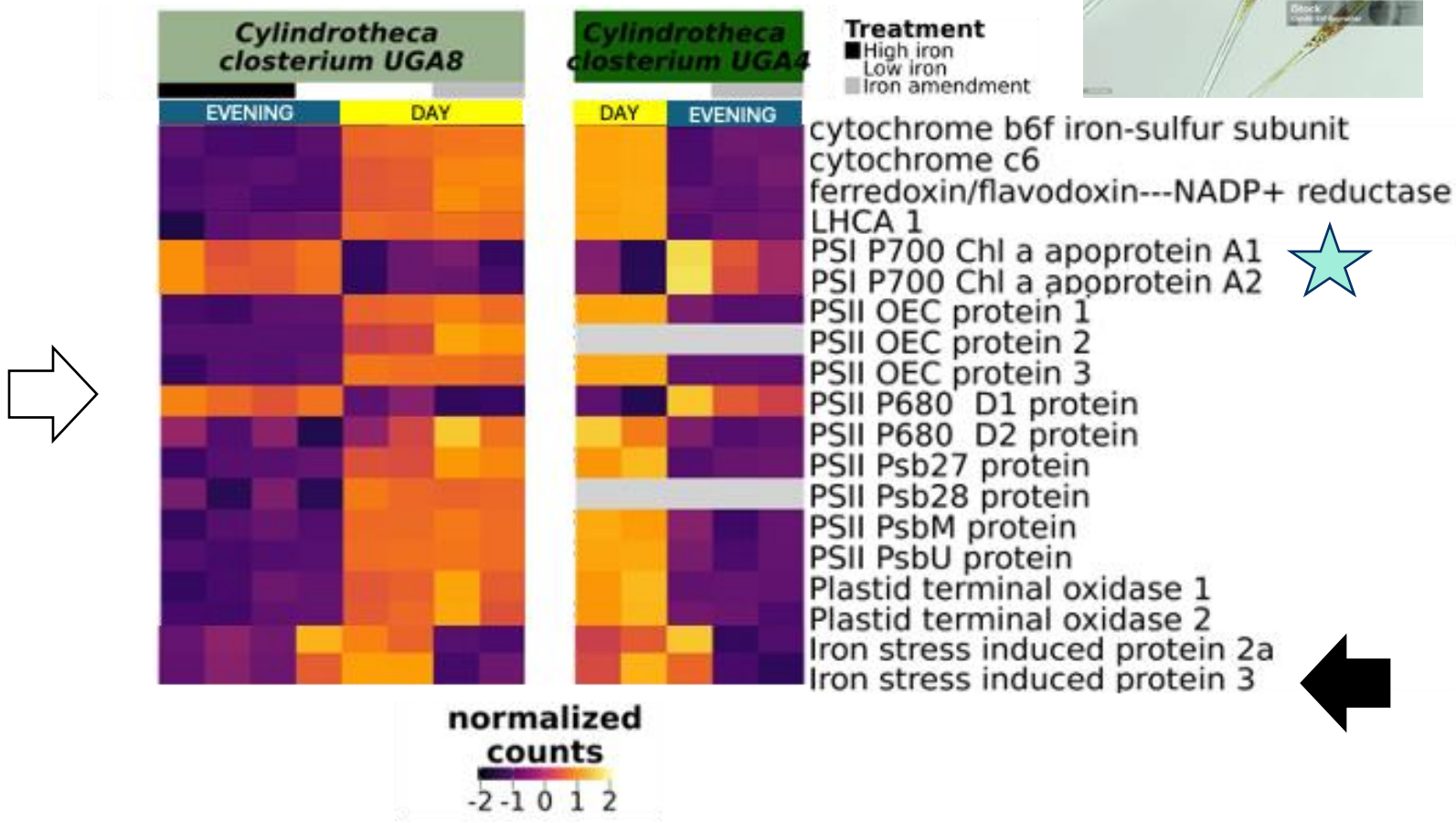
Diel transcriptional responses in the California Current



Kolody et al. 2019 *ISME*

Circadian rhythm detected via transcriptomics



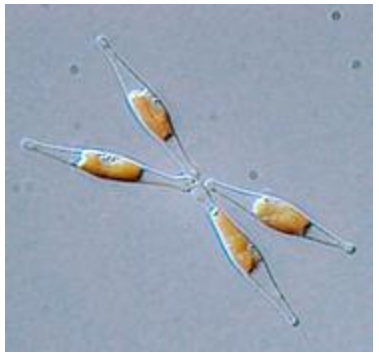




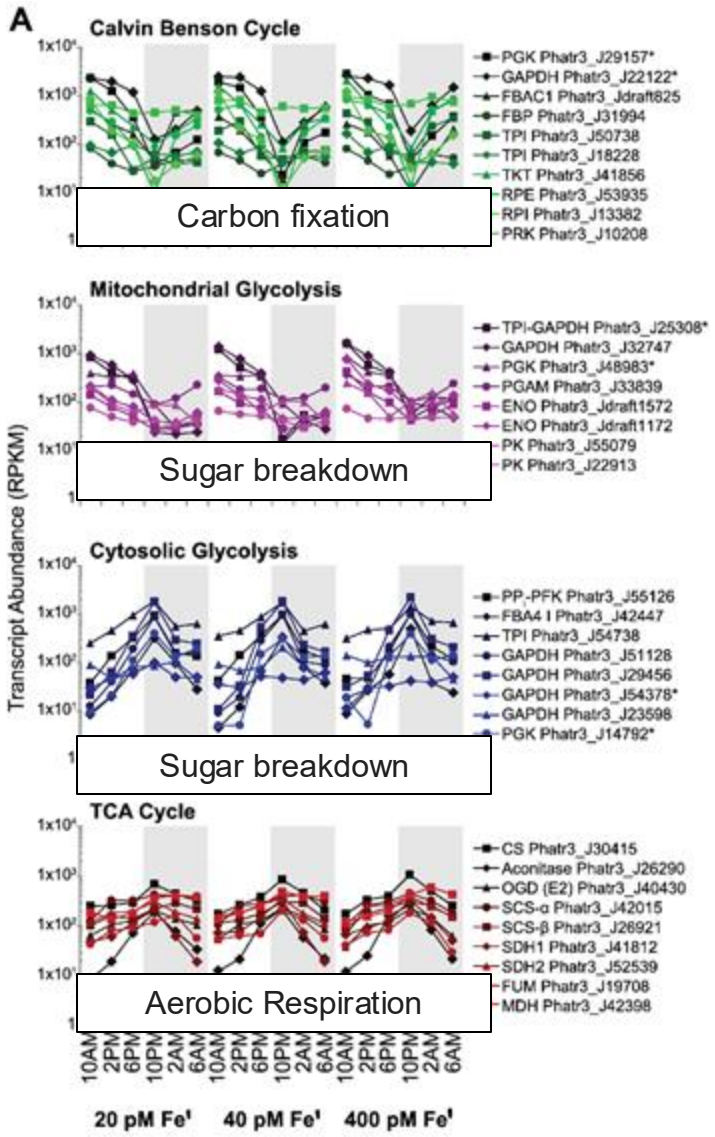
"Discovery based metatranscriptomics"

Quirk et al. in revision

Diel effects on central carbon metabolism



Evaluated transcriptome of *P. tricornutum* under low Fe and as a function of the light/dark cycle

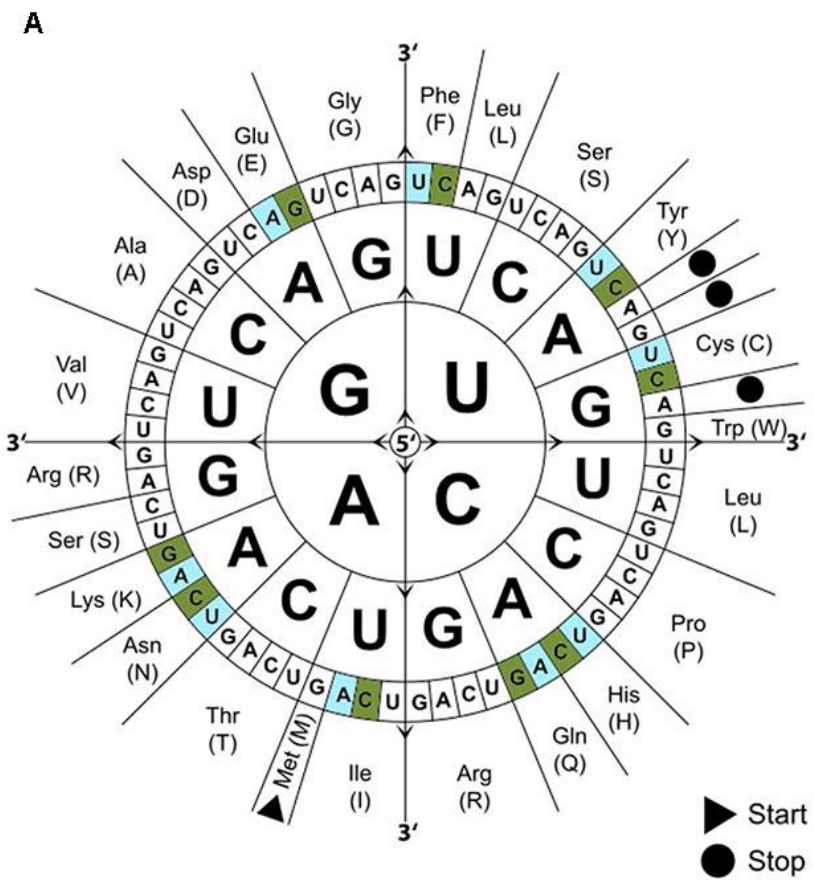


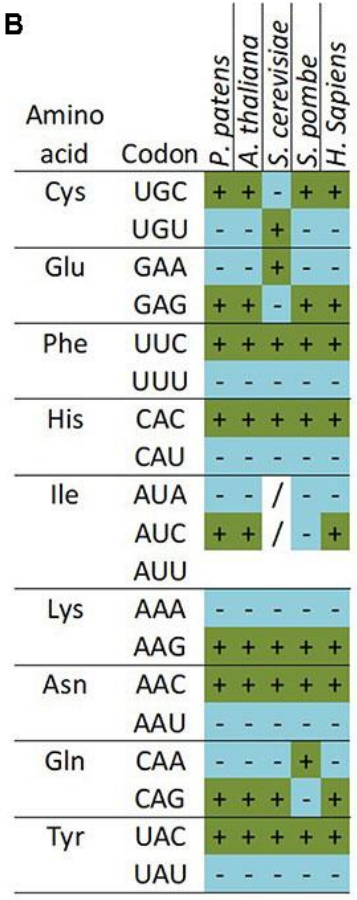
One form of sugar breakdown was more active at night, along with the TCA cycle - likely related to nocturnal respiration

Major coordinated shifts in transcription of primary and intermediate metabolism

Smith et al. 2016 PLOS Genetics

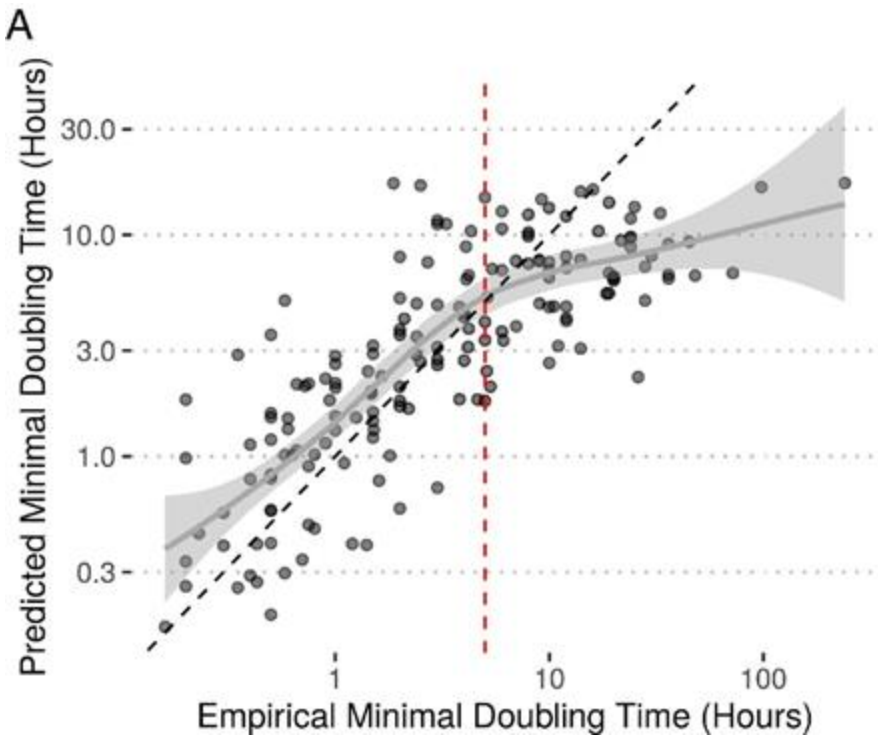
Predicting growth rates from genomes





genome-wide codon usage statistics

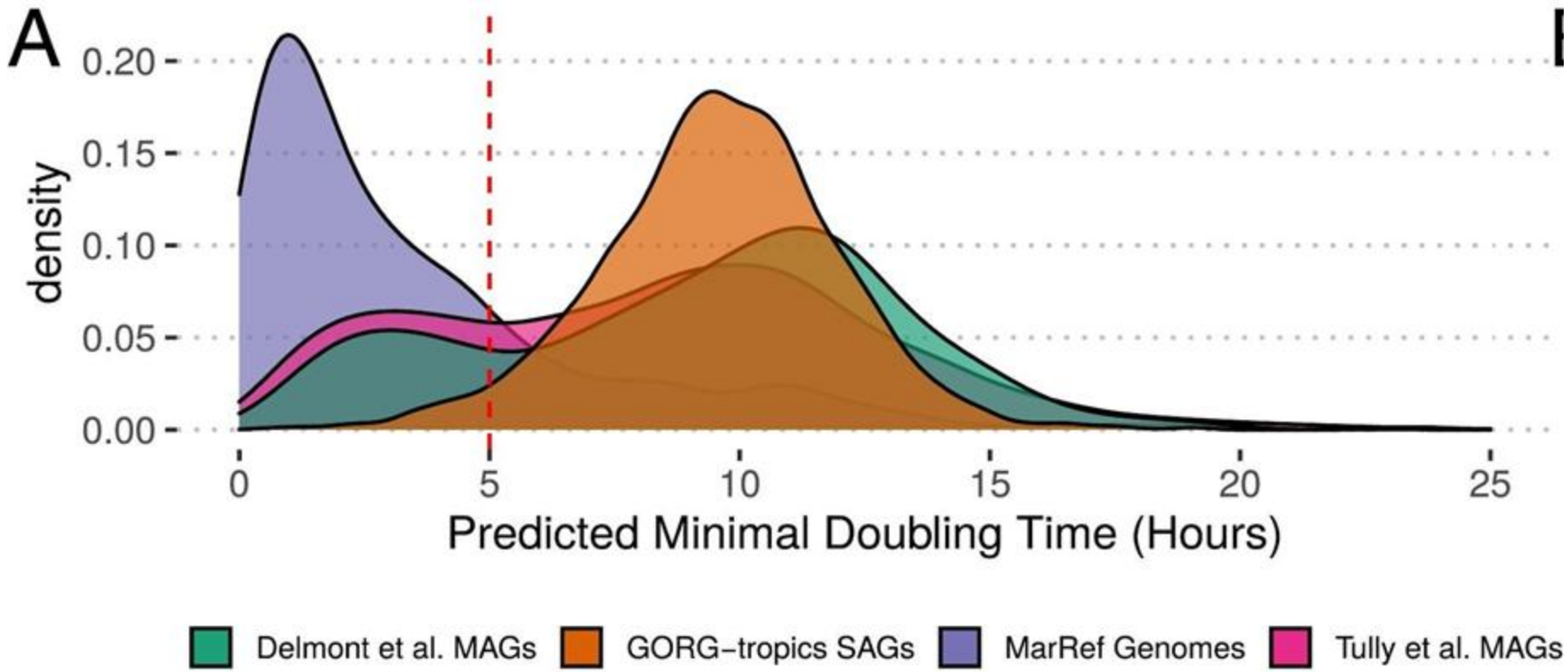
Fast-growing organisms need to make proteins quickly



- Most microbes are not cultured, and growth rates vary over orders of magnitude
- Developed an estimation from predicted growth rates from over 200,000 genomes, metagenome-assembled genomes, and single-cell amplified genomes

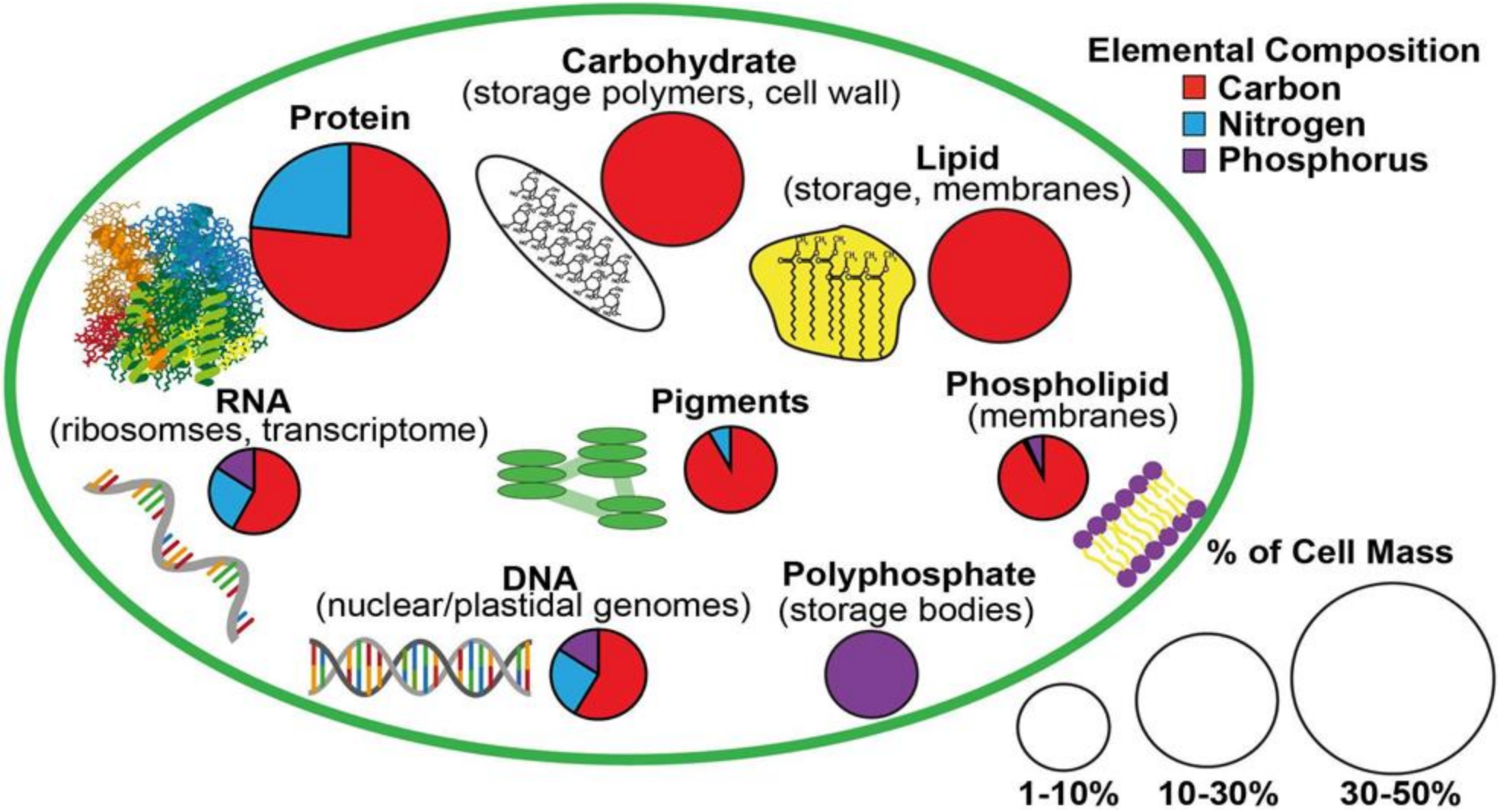
Weissman, Hou, Furman 2021 PNAS

Predicting growth rates from genomes



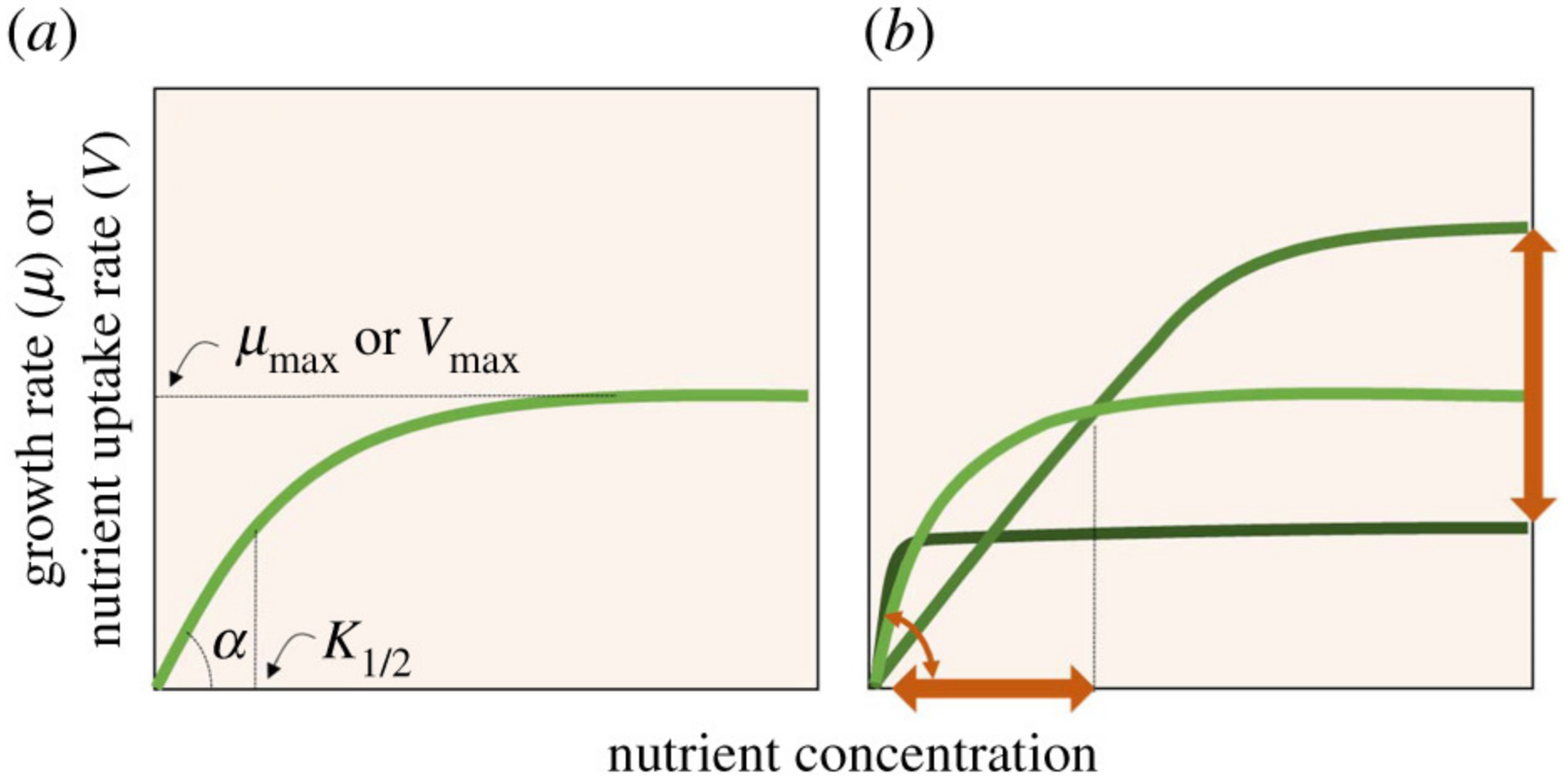
Weissman, Hou, Furman 2021 PNAS

Nutrients are required for growth



Liefer et al. 2019

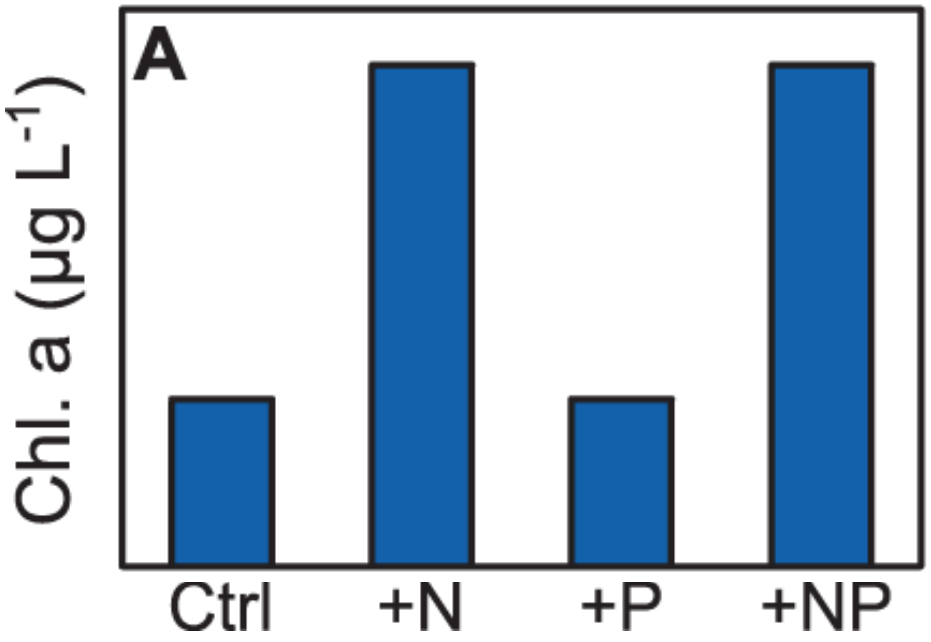
Phytoplankton have various nutrient requirements
Traits vary by phytoplankton type

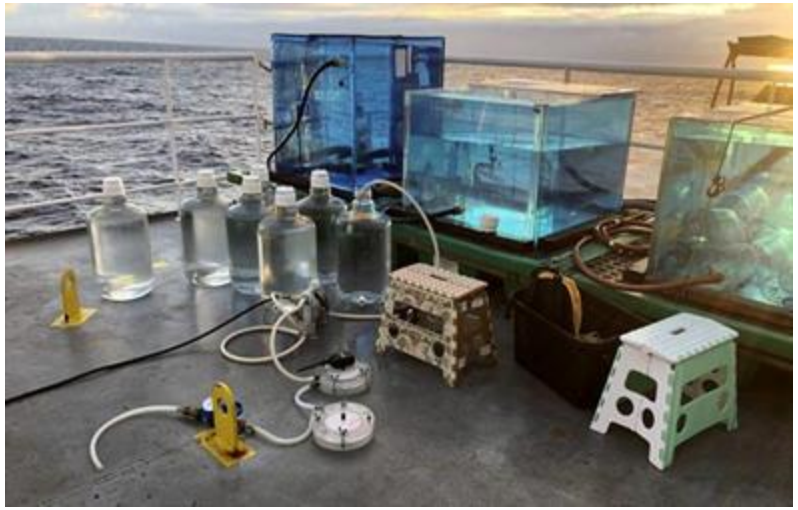


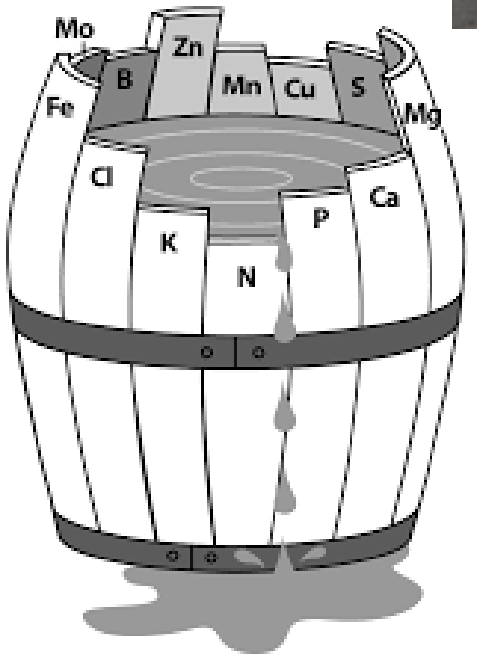
Michaelis-Menten enzyme kinetics

Van de Waal & Litchman 2020

Which nutrient is limiting?

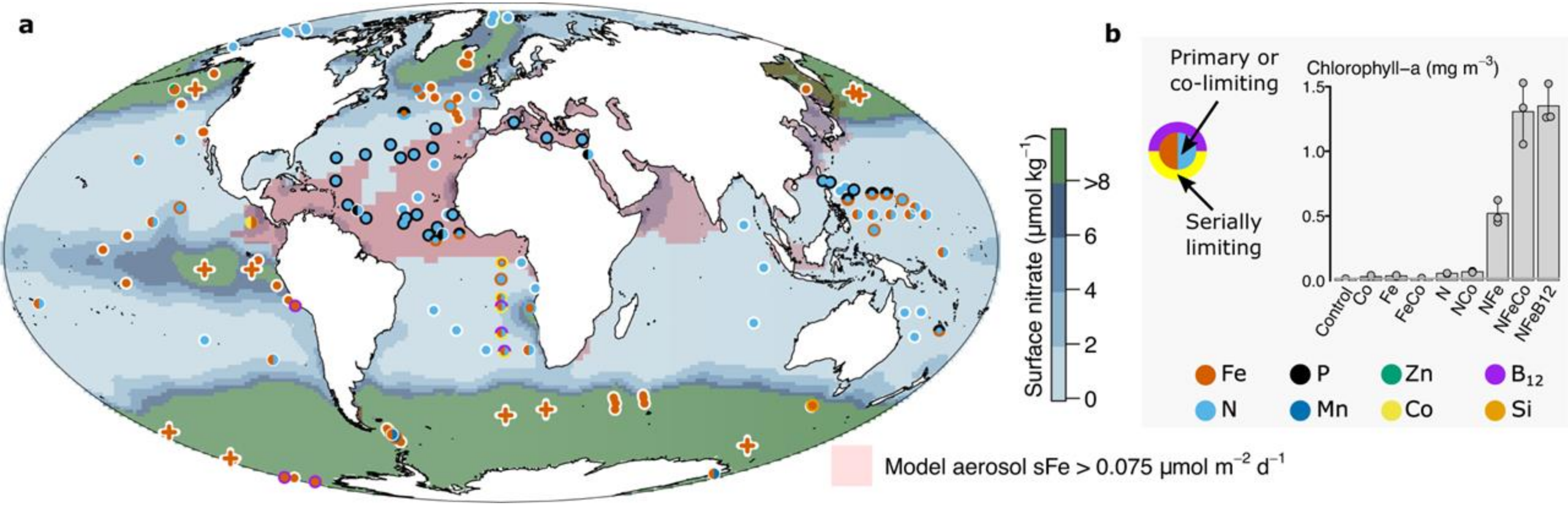






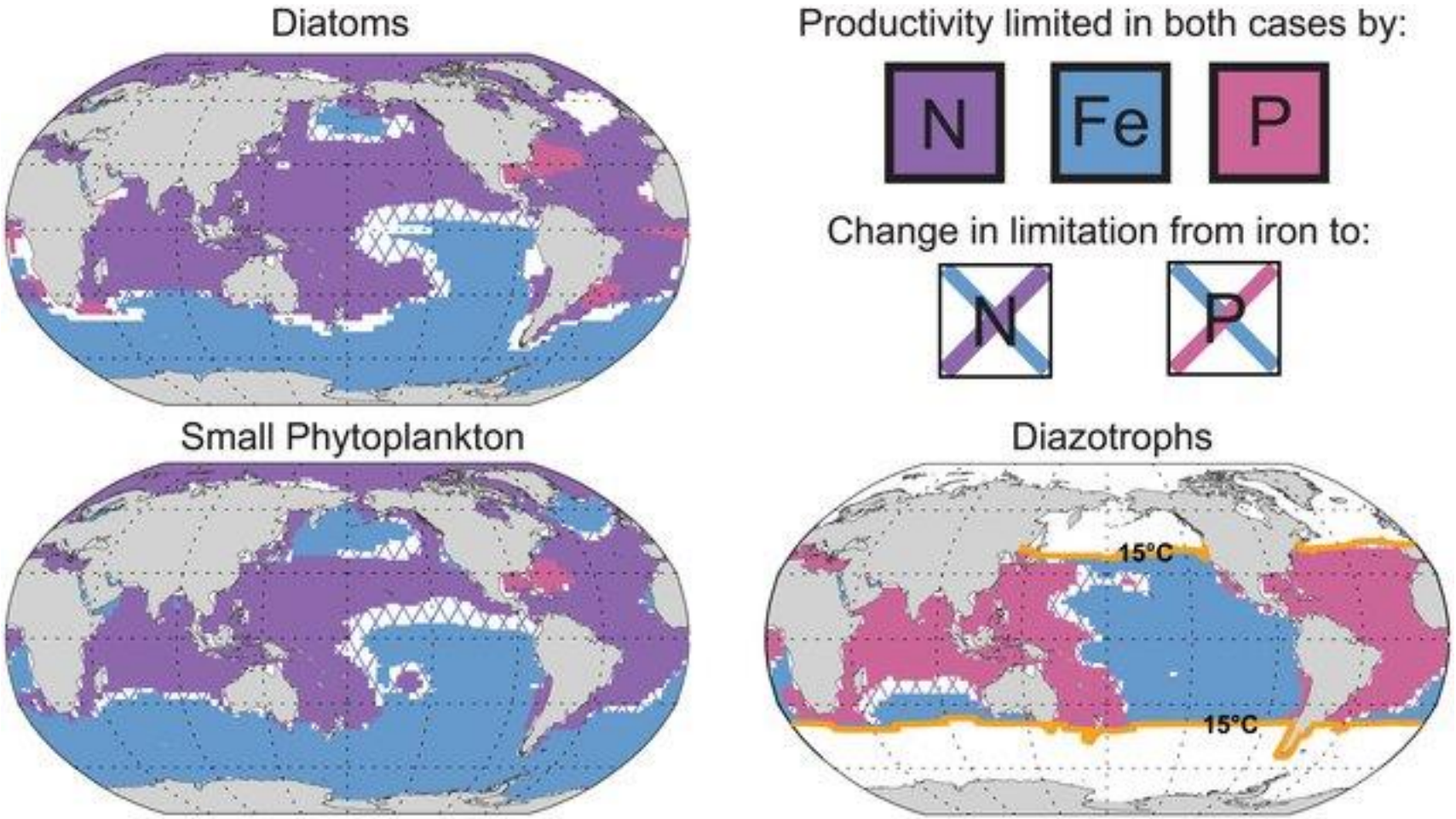
Nutrient composition influences community composition

Nutrient co-limitation observed in global ocean



Browning & Moore 2023

Nutrients limit phytoplankton growth

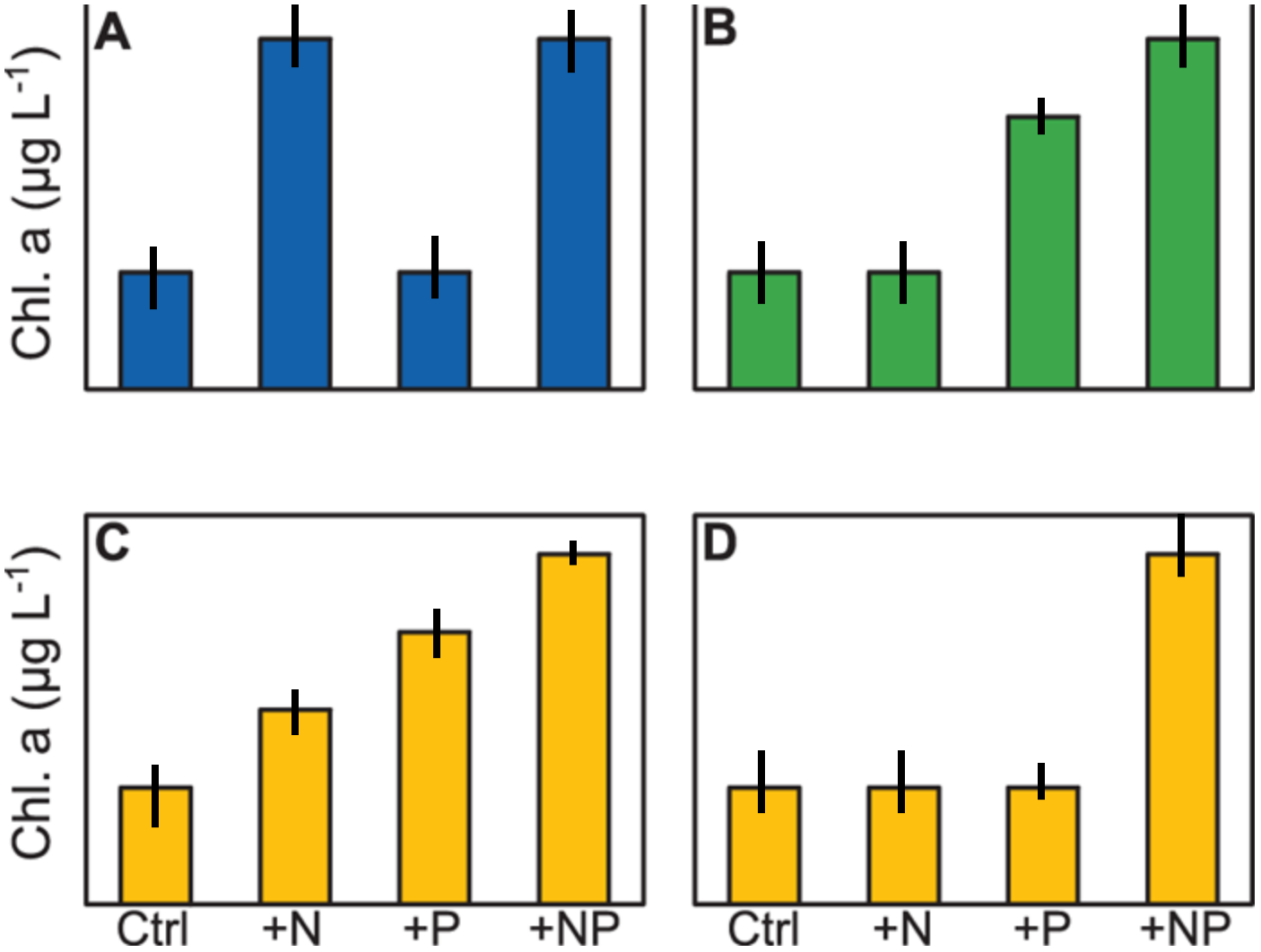


Change in nutrient limitation from PIBASE to FUBASE cases

Douglas et al. 2020 *Global Biogeochemical Cycles*

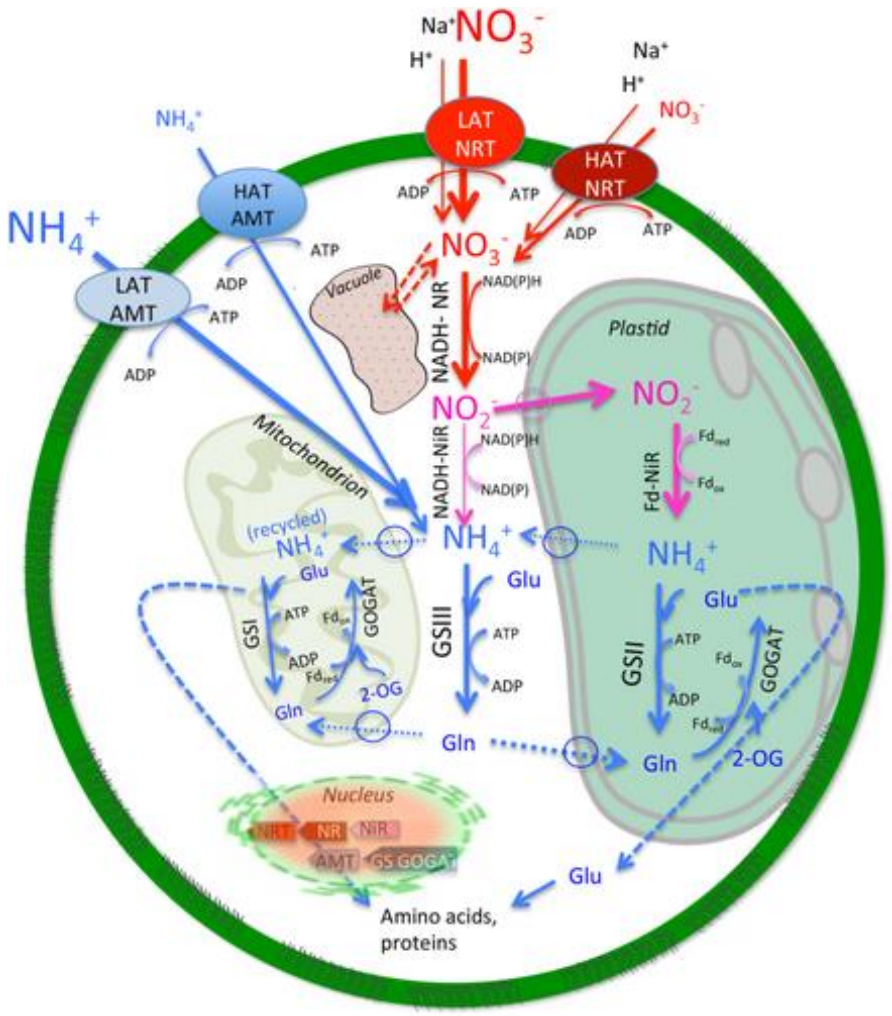
Which nutrient is limiting? Growth assays (incubations)



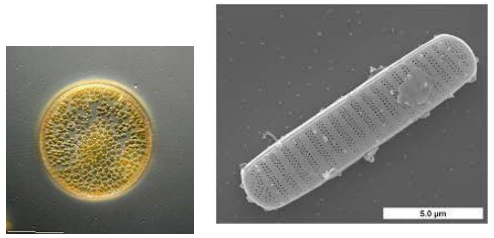


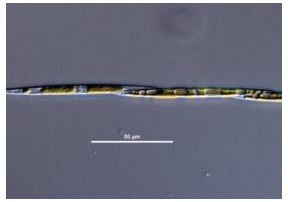
Kolzau et al. 2014

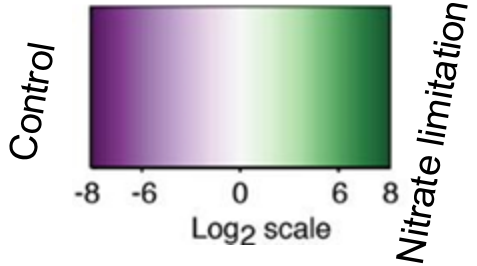
Nitrate stress biomarkers

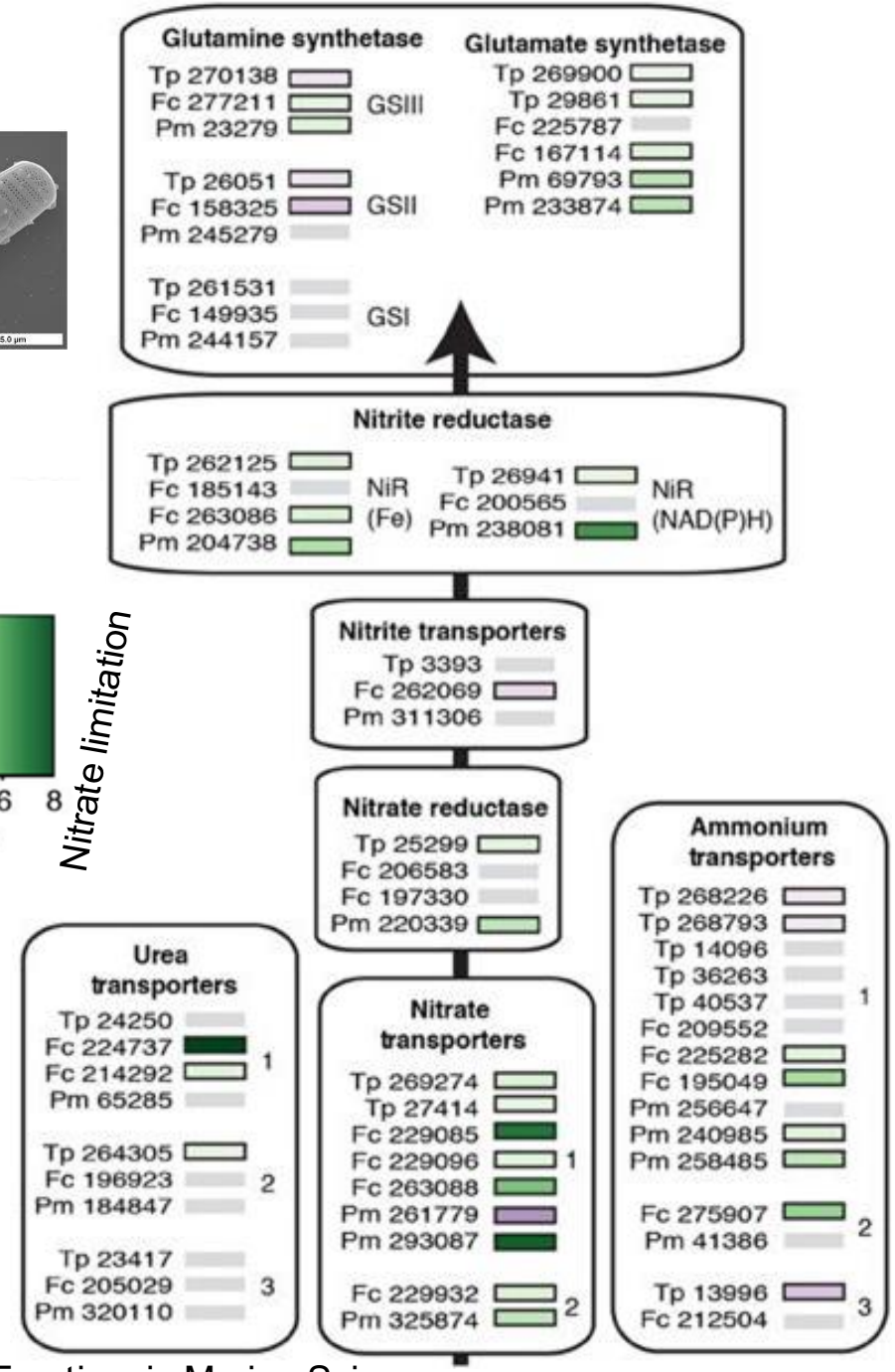


Glibert et al. 2015 *Limnology & Oceanography*



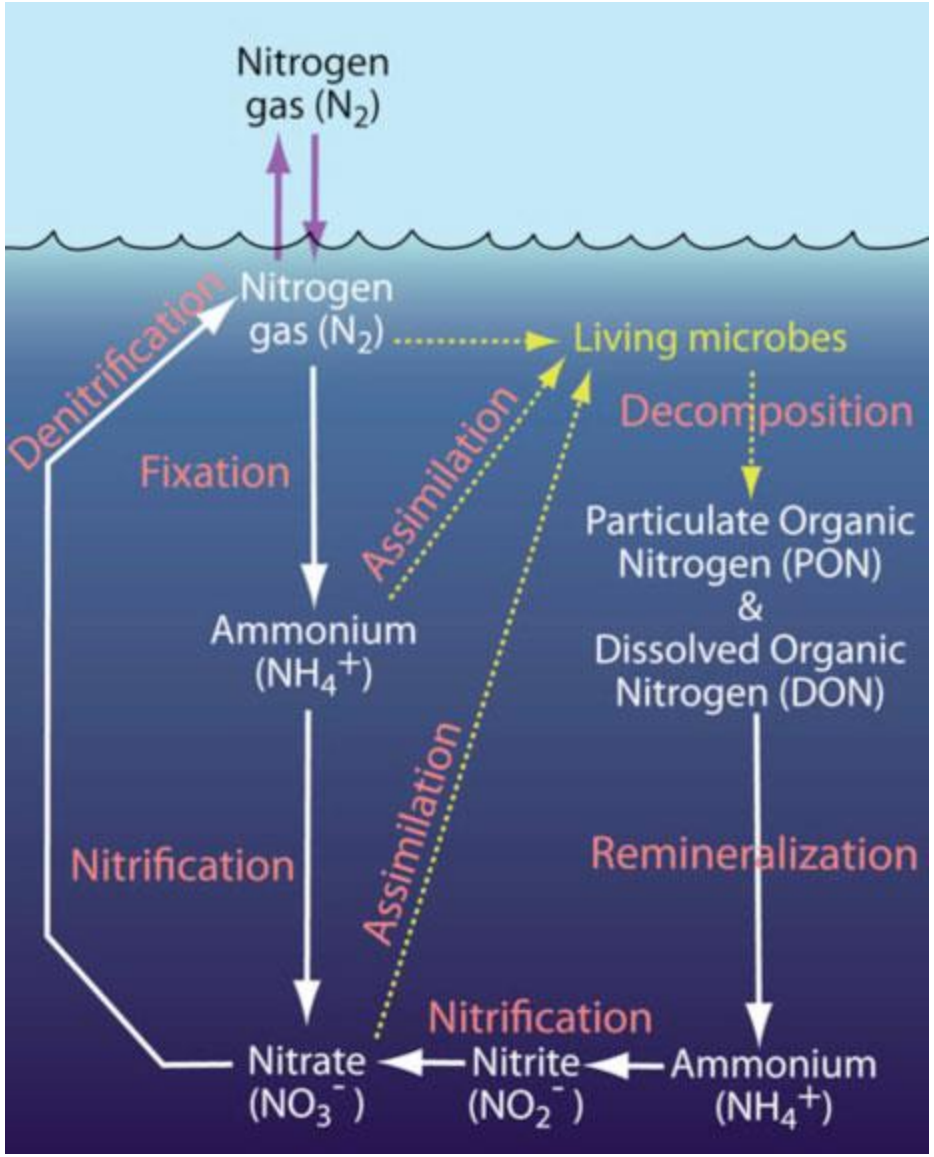






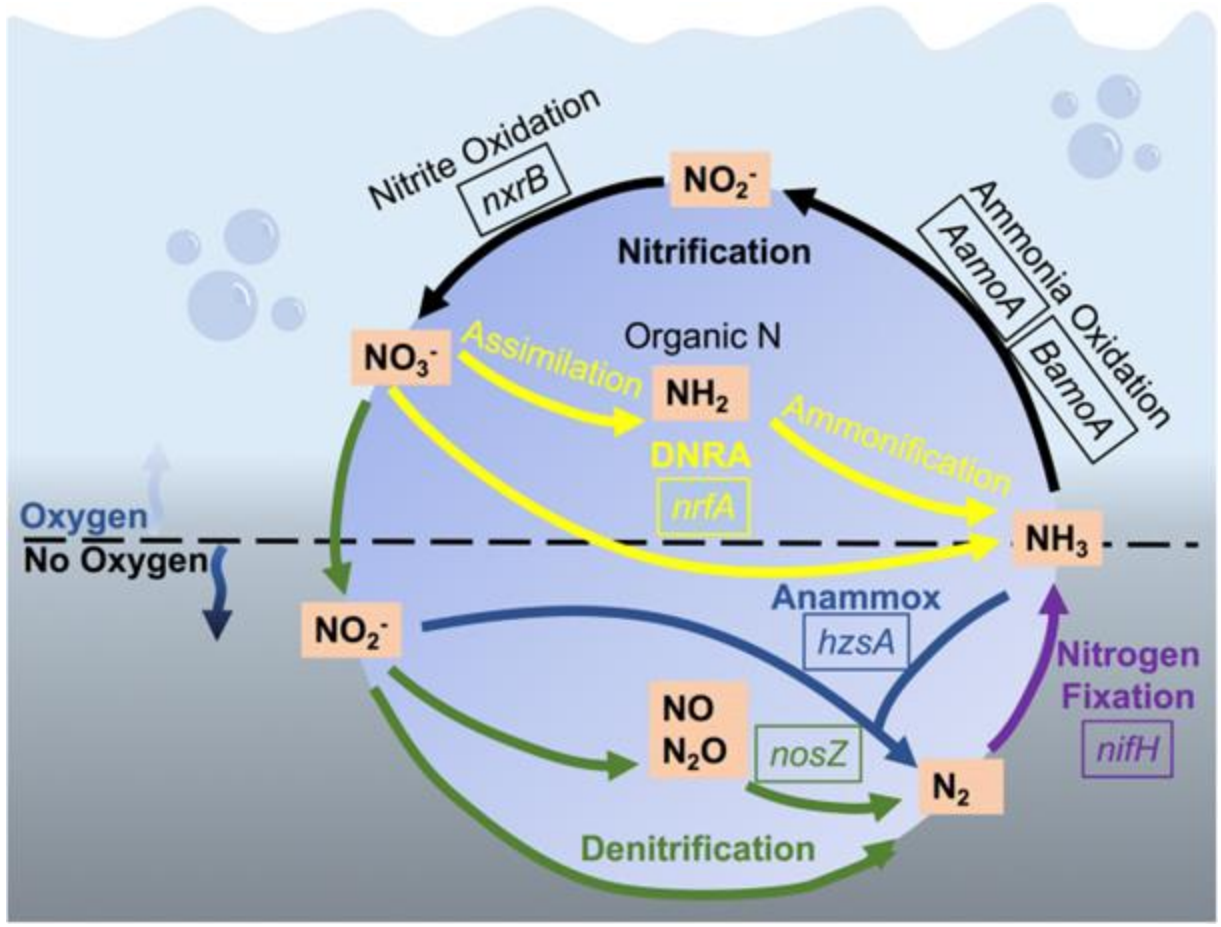
Bender et al. 2014 Frontiers in Marine Science

Nitrogen cycling is mediated by microbes



Forms of N used by microbes: N_2, NO_3^-, NO_2^-,NH_4^+, urea

Nitrification = chemoautotrophy
Photosynthesis = photoautotrophy

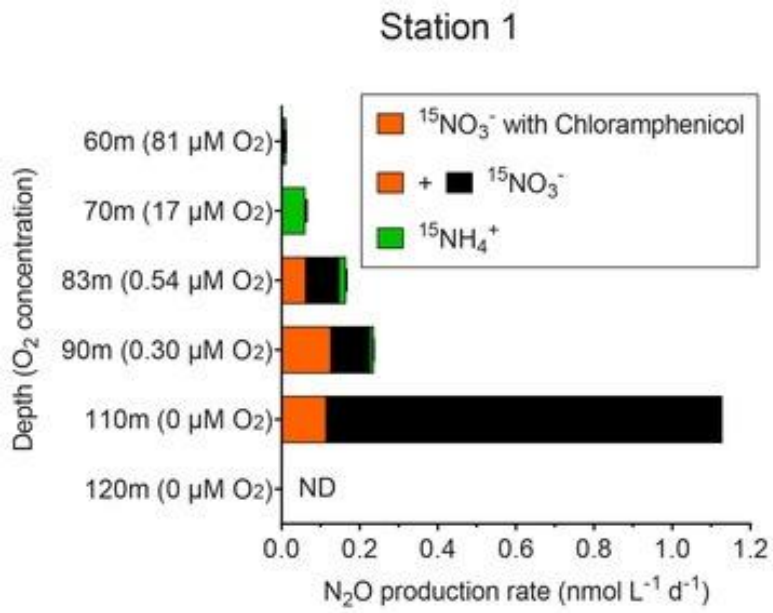


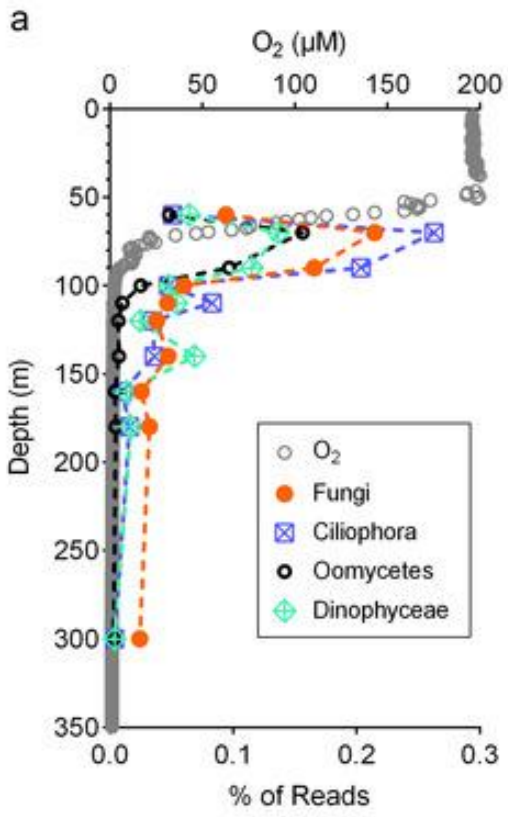
Microeukaryotes contribute to denitrification

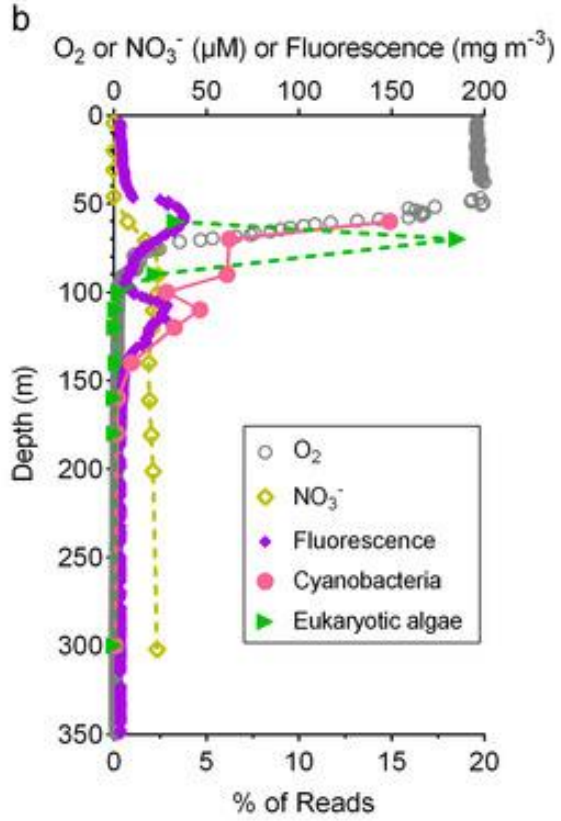
18-22% of the total N_2O production from fungi

Fungi are significant contributors to denitrification in oxic-anoxic transition waters

N_2O available to be taken up by bacteria





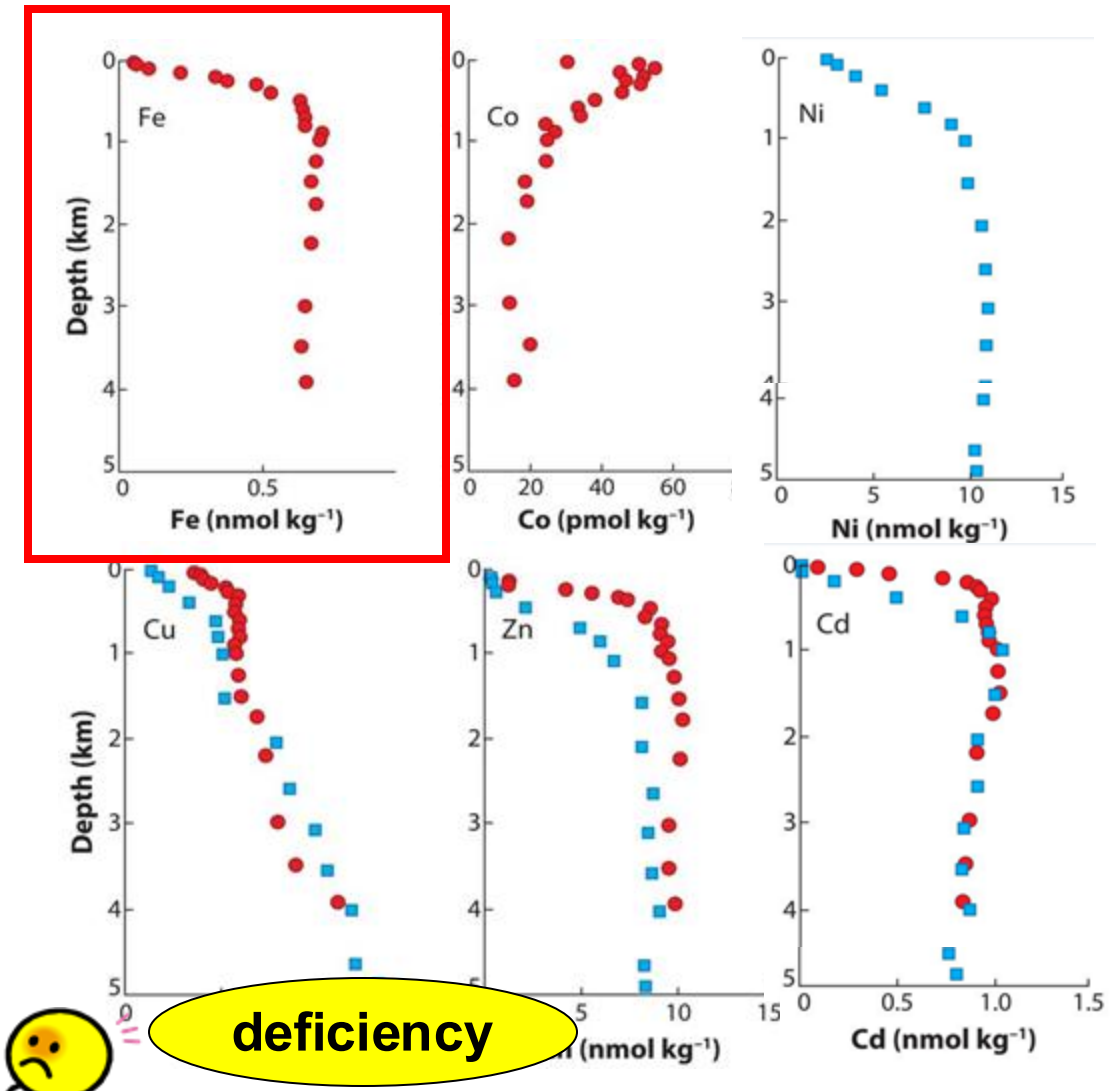


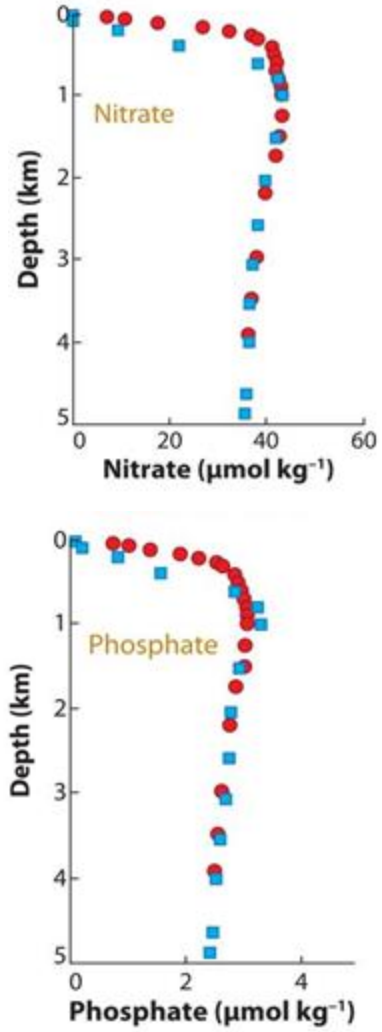
Peng & Valentine 2021 *Journal of Fungi*

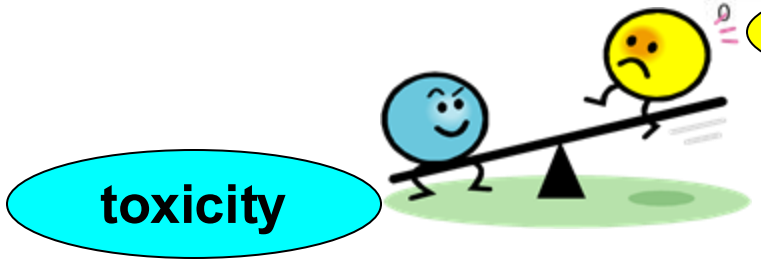
Trace metals are critical for life

Photosynthesis
Respiration
Vitamins
Defense against toxins
Carbon concentration
Nutrient uptake

Different sources & sinks

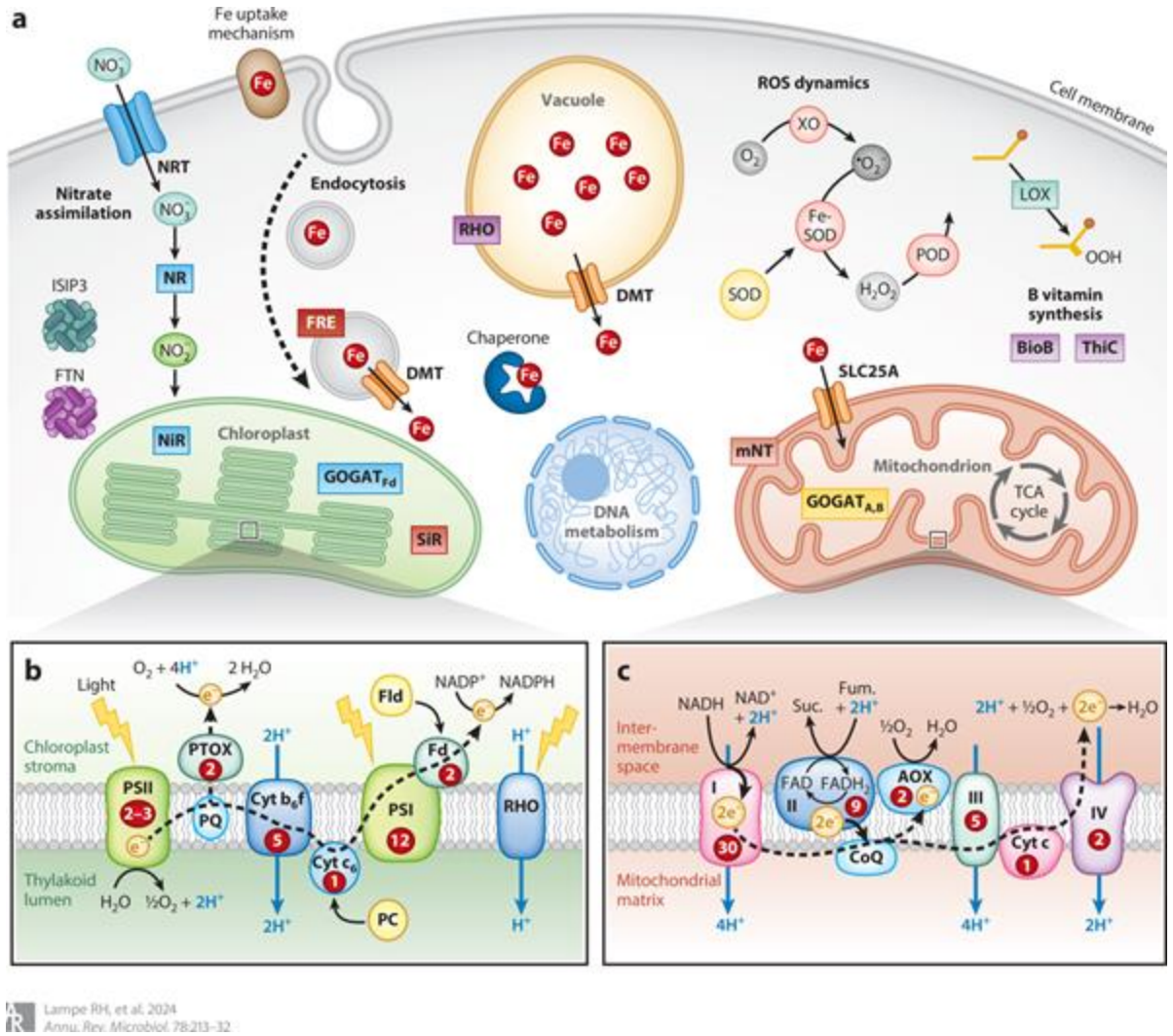






Vraspir & Butler 2009

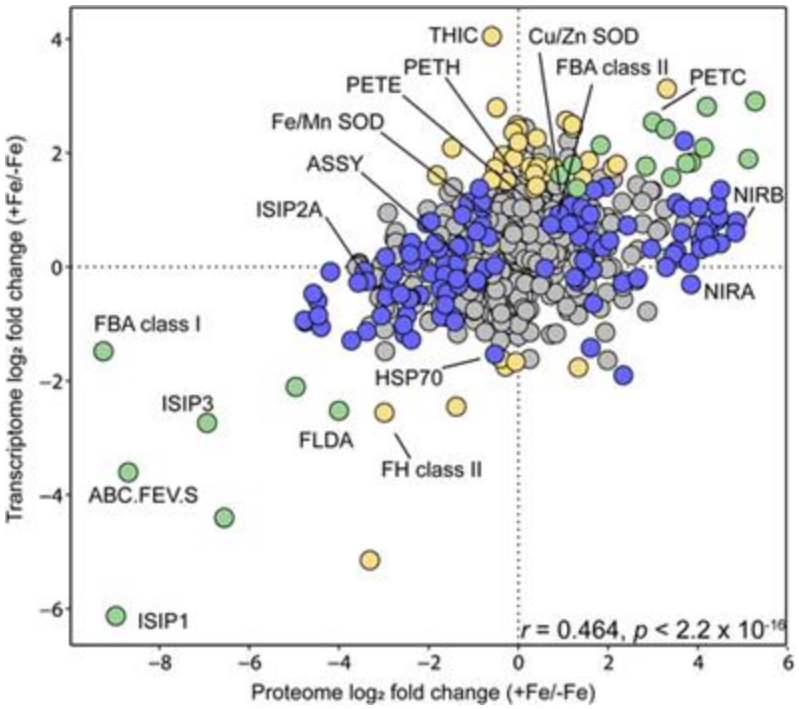
Iron requirements in cell



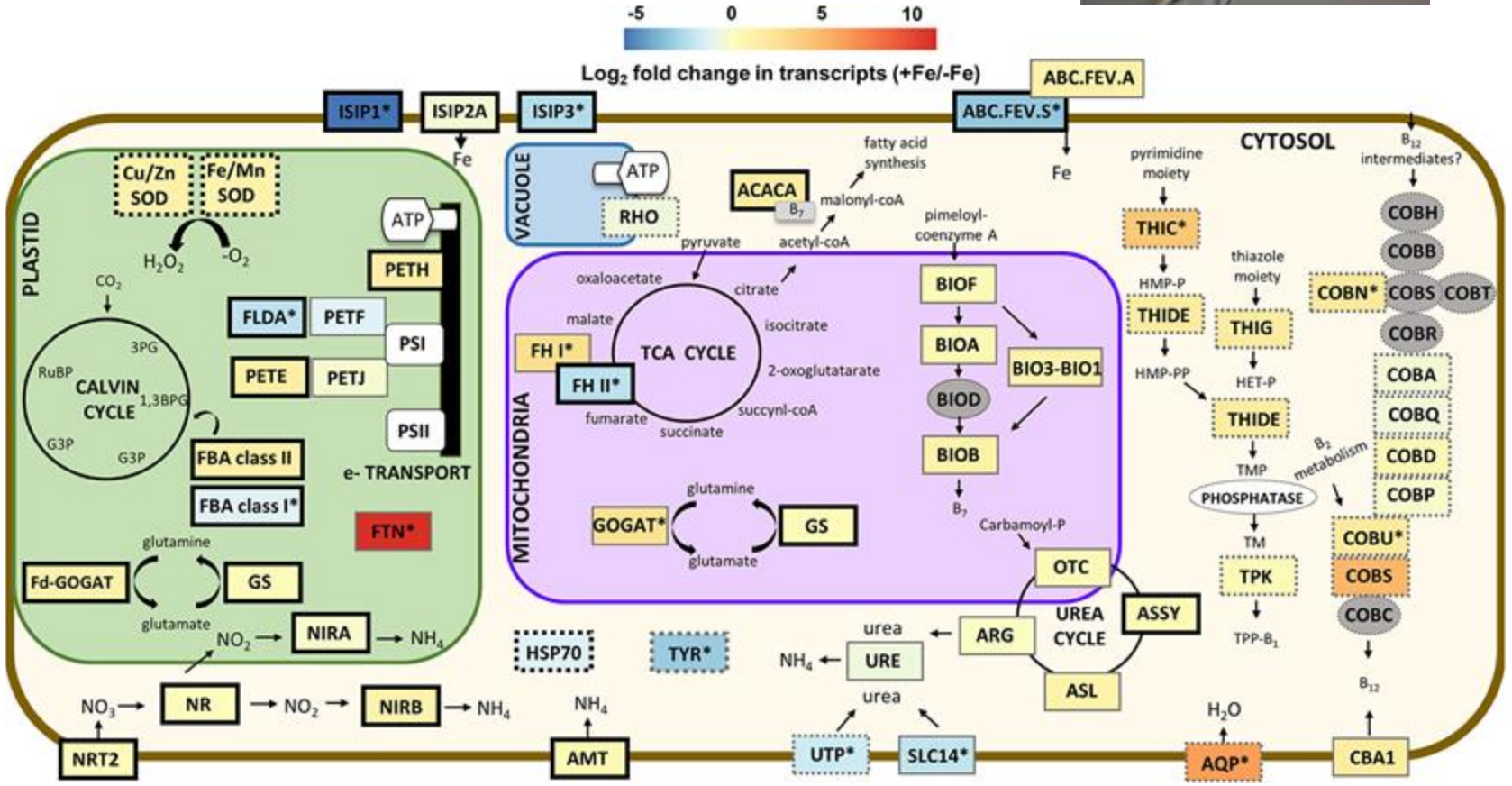
Lampe RH, et al. 2024
Annu. Rev. Microbiol. 78:213–32

Lampe et al. 2024 Annual Reviews in Marine Science

Iron limitation inferred from multi-omics



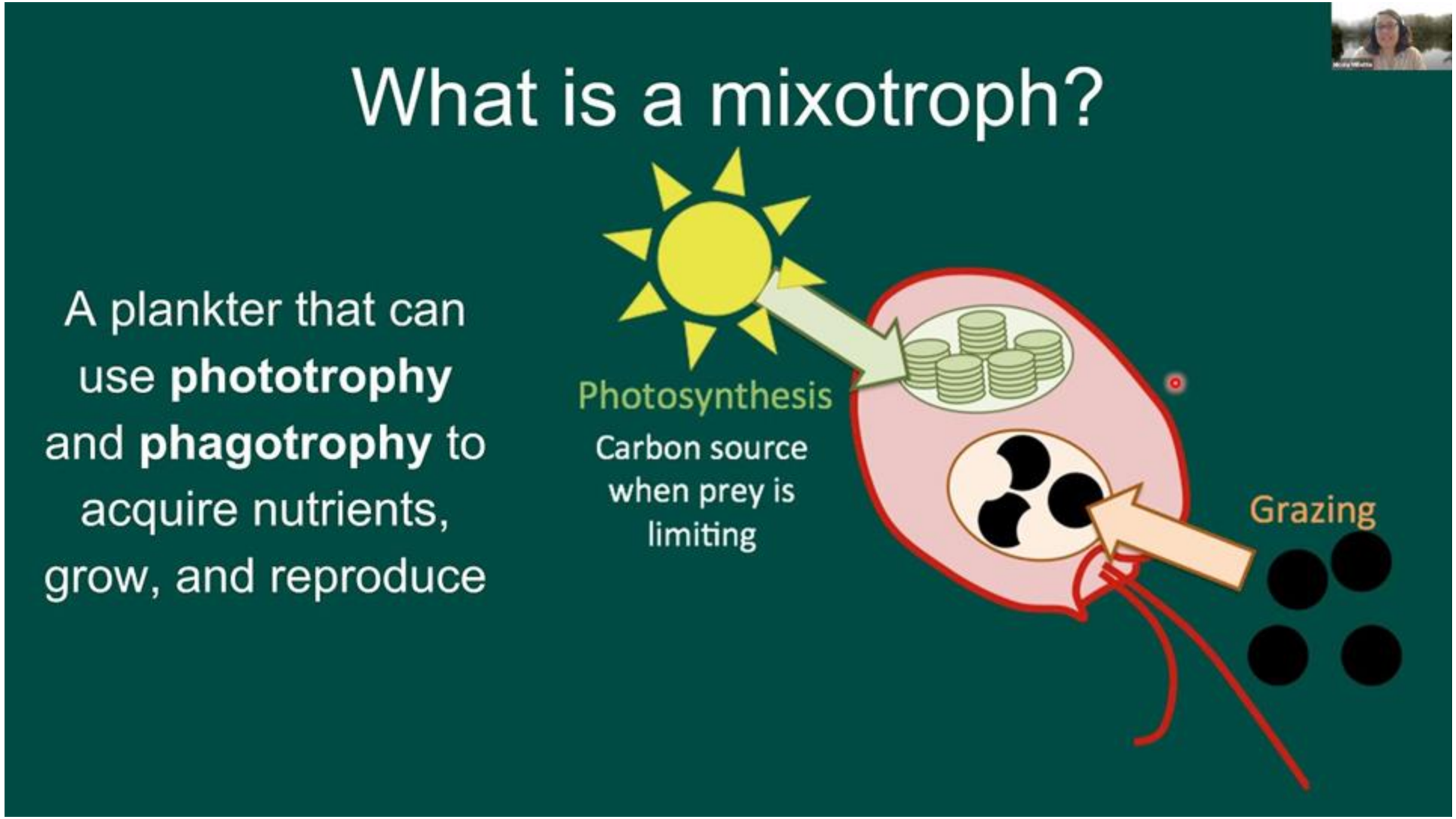




Cohen et al. 2018 Environmental Microbiology

Tracking trophic modes

Nicole Millette (VIMS)

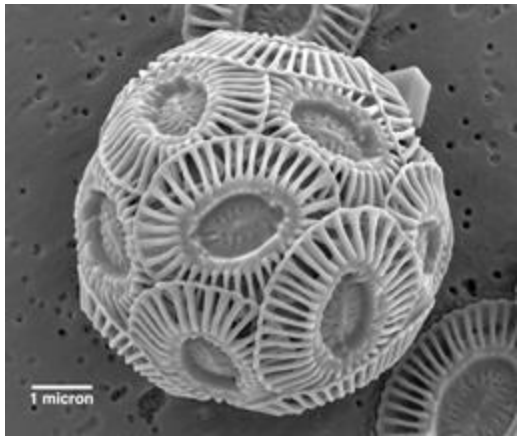


Carbon or nutrient source when light/nutrients are not available in seawater

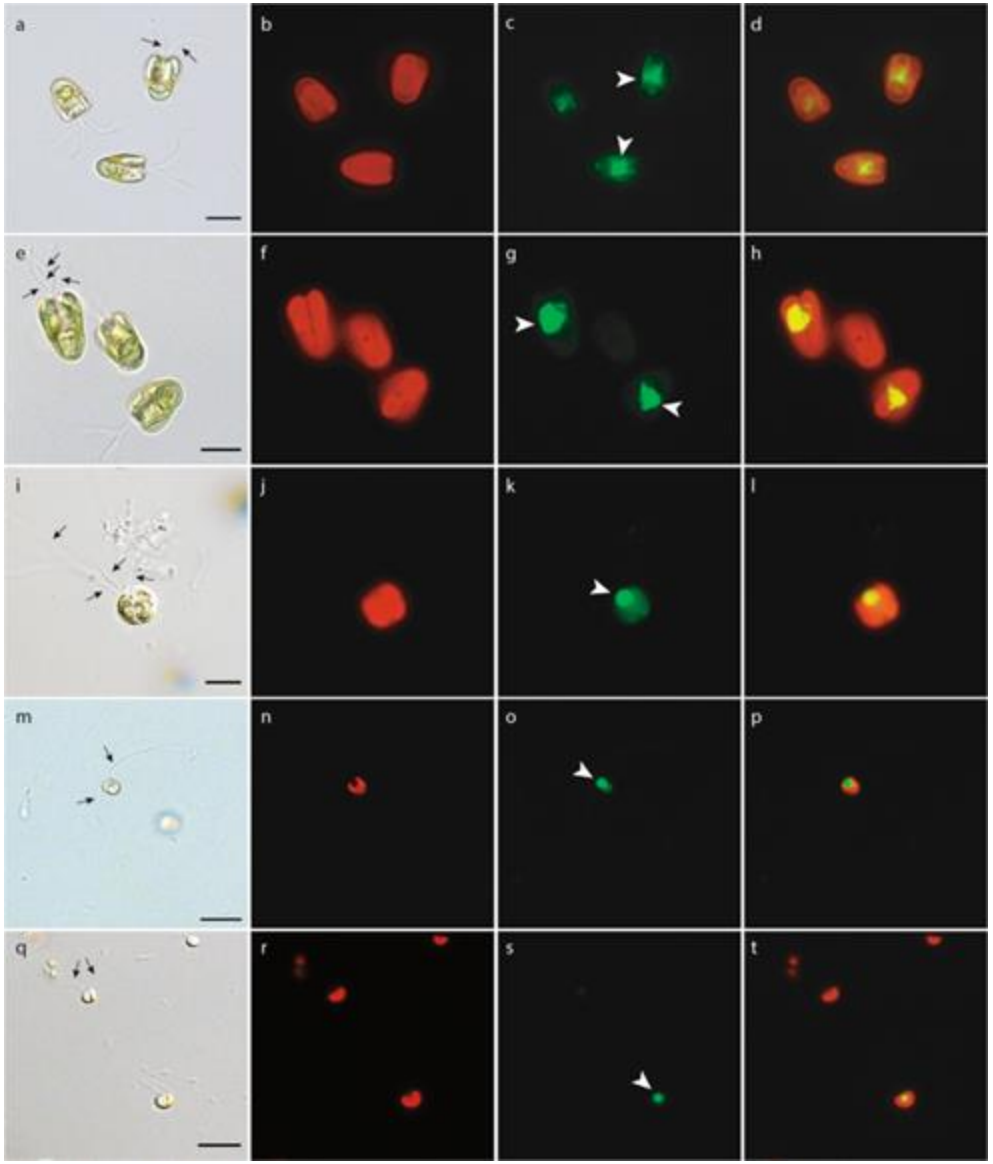
Autotrophs (?)



Some:
Chlorophytes/prasinophytes:
Smallest eukaryotes. Can be single cells, or colonies, or flagellated (can swim). More common in freshwater.
Found even in the Southern Ocean!
<2-3 µm in size



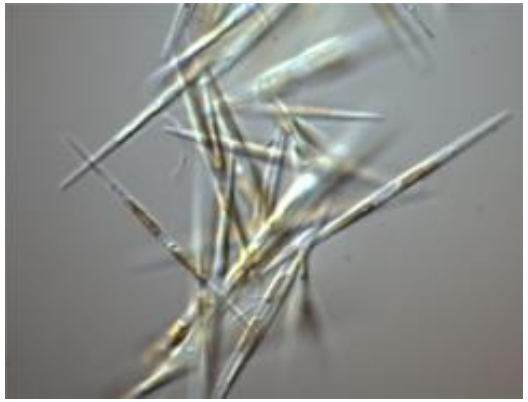
Some: Haptophytes: Include coccolithophores, made out of calcite ($CaCO_3$). Form massive blooms and coccoliths shed to bottom of ocean. Useful in the geological record. Includes *Phaeocystis*, also bloom-forming, and produces DMSP which influences cloud formation
2-5 µm in size



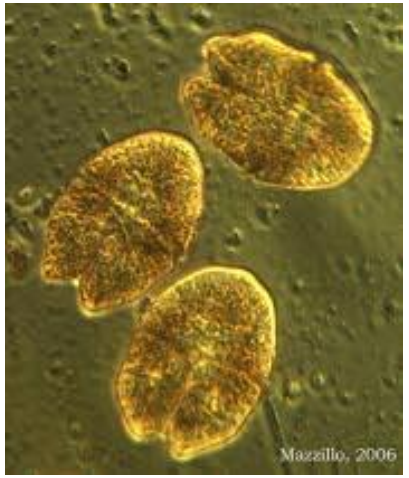
Anderson et al. 2018, Unrein et al. 2013

Bock et al. 2021, ISME

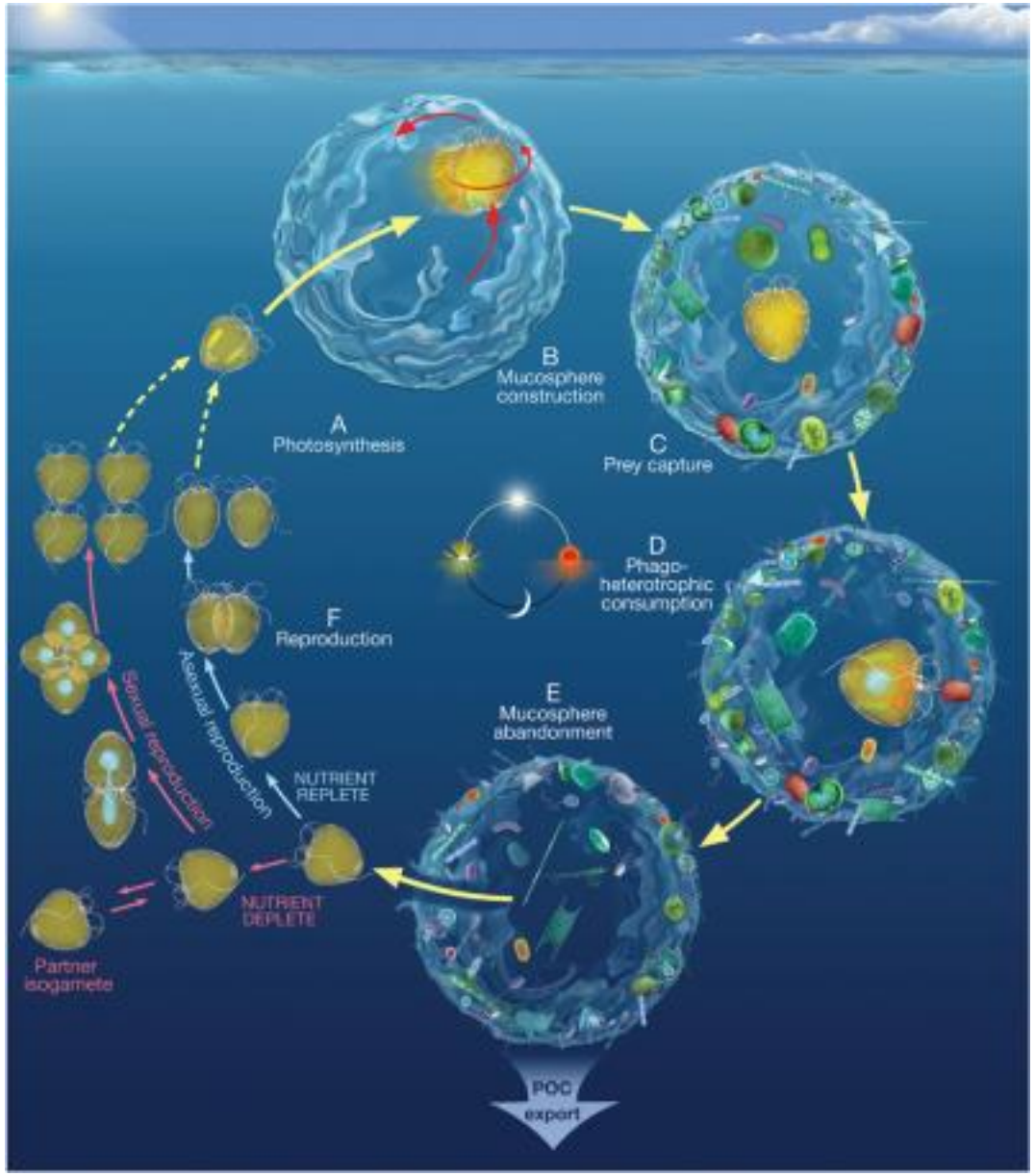
Autotrophs (?)



YES: Diatoms: Responsible for 20% of global production
Dominant in marine, coastal & freshwater environments
Silica cell walls
Quick growth rates
Generally 20-200 µm in size

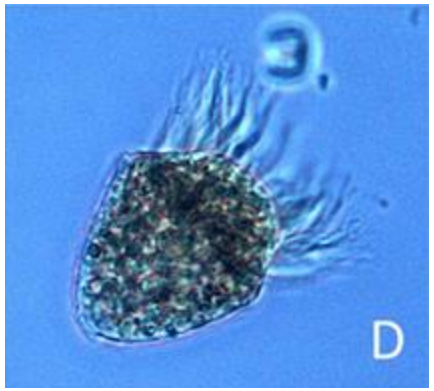


FEW: Dinoflagellates:
Successful in estuaries, coastal environments, and open ocean
Can be autotrophic, heterotrophic, or mixotrophic (both)
Have flagella, are capable of movement
Many are toxin producers
Generally 20-200 µm in size

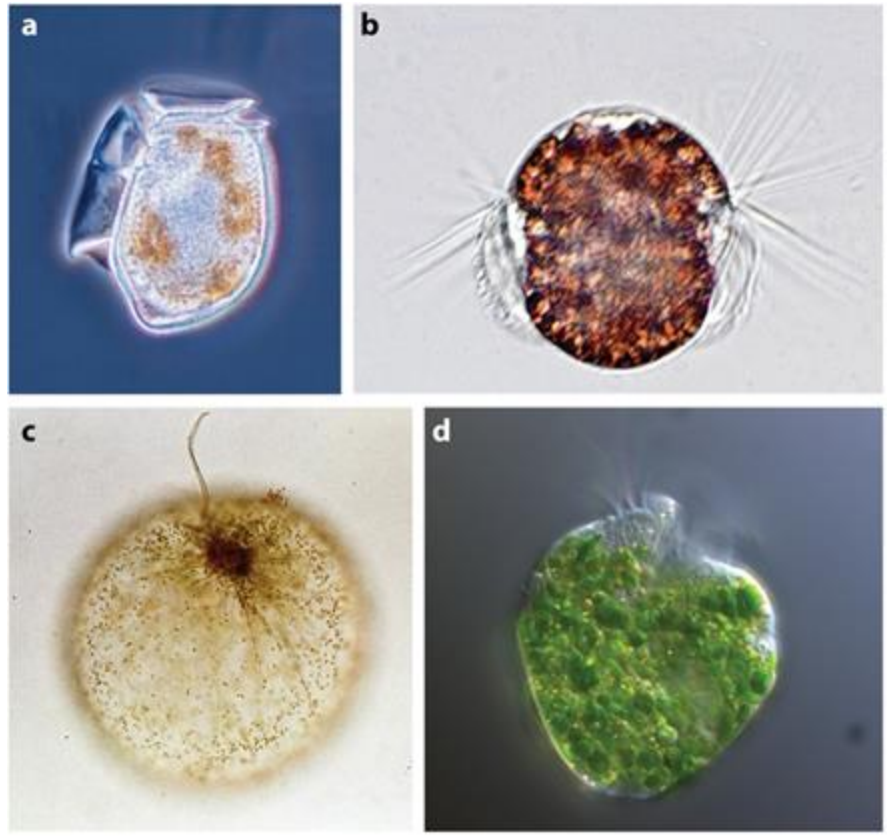


Larsson et al. 2022, *Nature Communications*

Autotrophs (?)



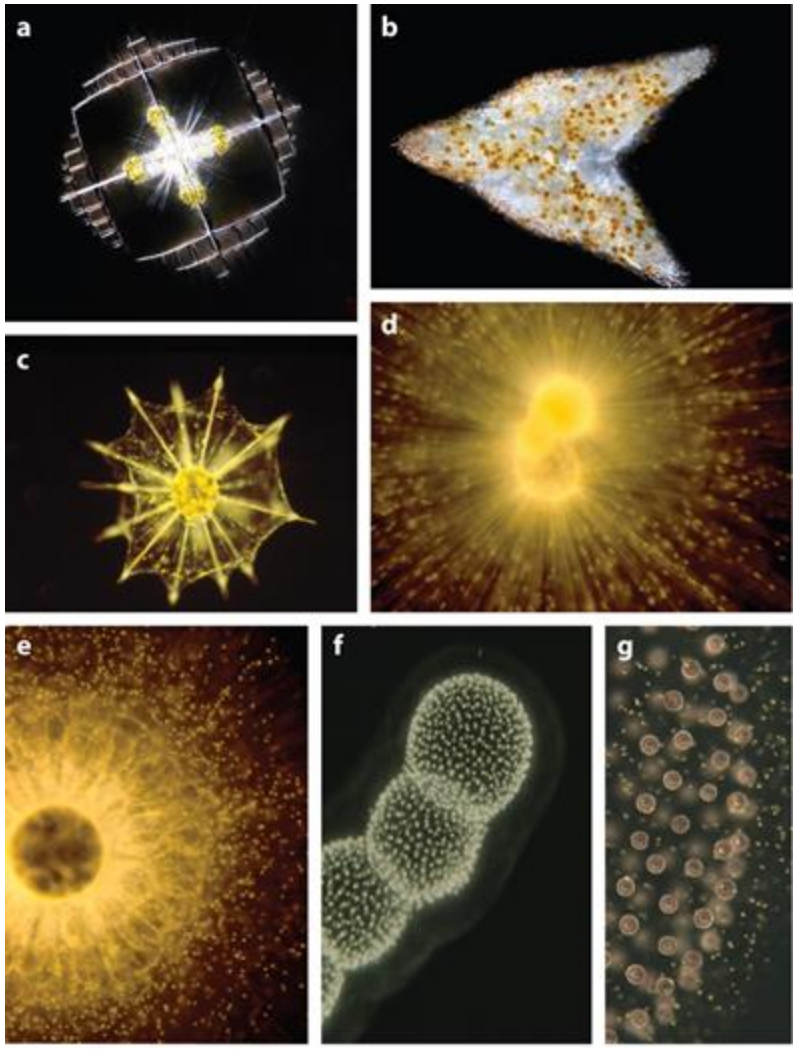
SOME Ciliates: Single celled heterotrophs
Dominant in marine & coastal environments
Covered with cilia used to move
Engulfs phytoplankton prey via phagotrophy
Can use chloroplasts from phytoplankton (**kleptoplasty**)
Generally >100 µm in size



Stoecker DK, et al. 2017.
Annu. Rev. Mar. Sci. 9:311–35

Stolen plastids: “kleptoplasty”

Autotrophs (?)



Stoecker DK, et al. 2017.
Annu. Rev. Mar. Sci. 9:311–35

Some. **Rhizaria:** include radiolarians and foraminifera, which are heterotrophic but can harbor phytoplankton as endosymbionts. ~10 um -1 mm

How to measure mixotrophs?

Microscopy

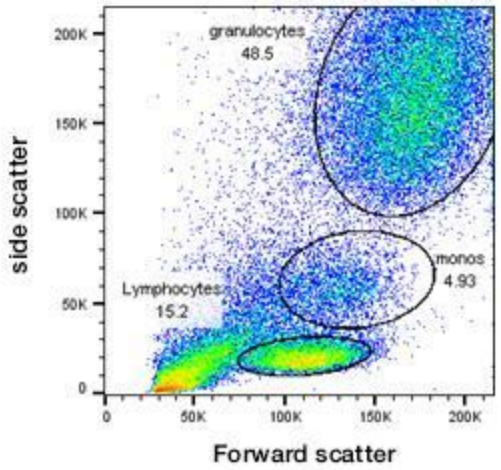


Flow cytometry



Fluid imaging

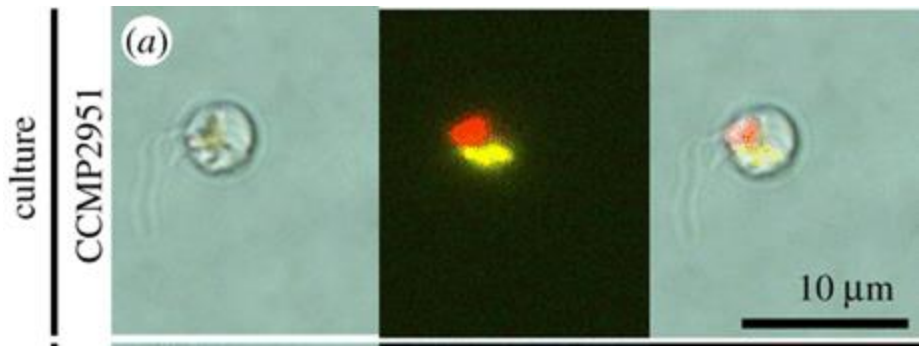




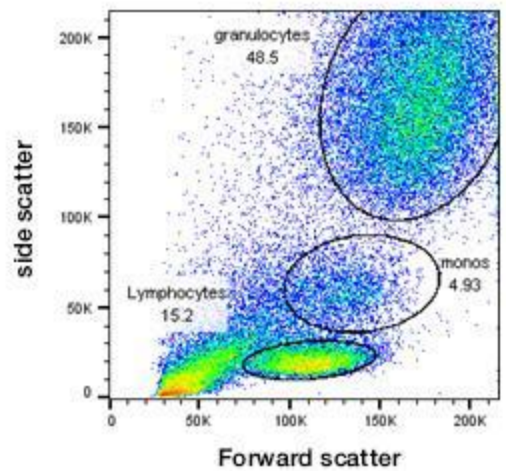
Nucleotides/proteins



Tracking mixotrophs: acidotropic dyes

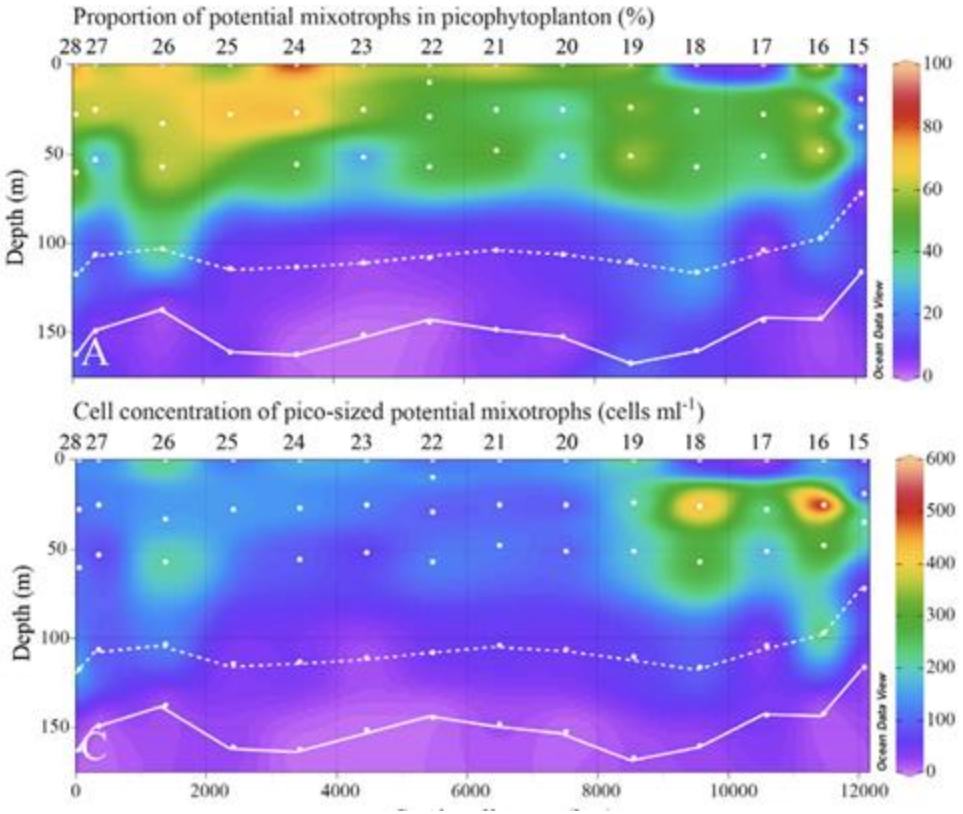




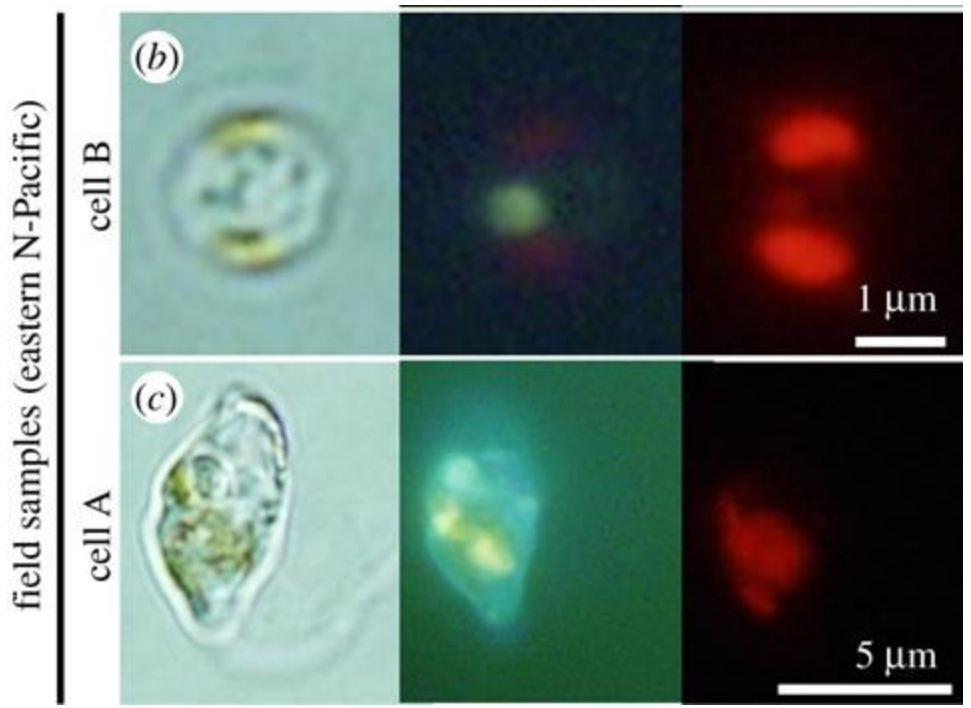


Wilken et al. 2019

Is it reliable for tracking mixotrophy in the field?



Sato & Hashihama, 2019



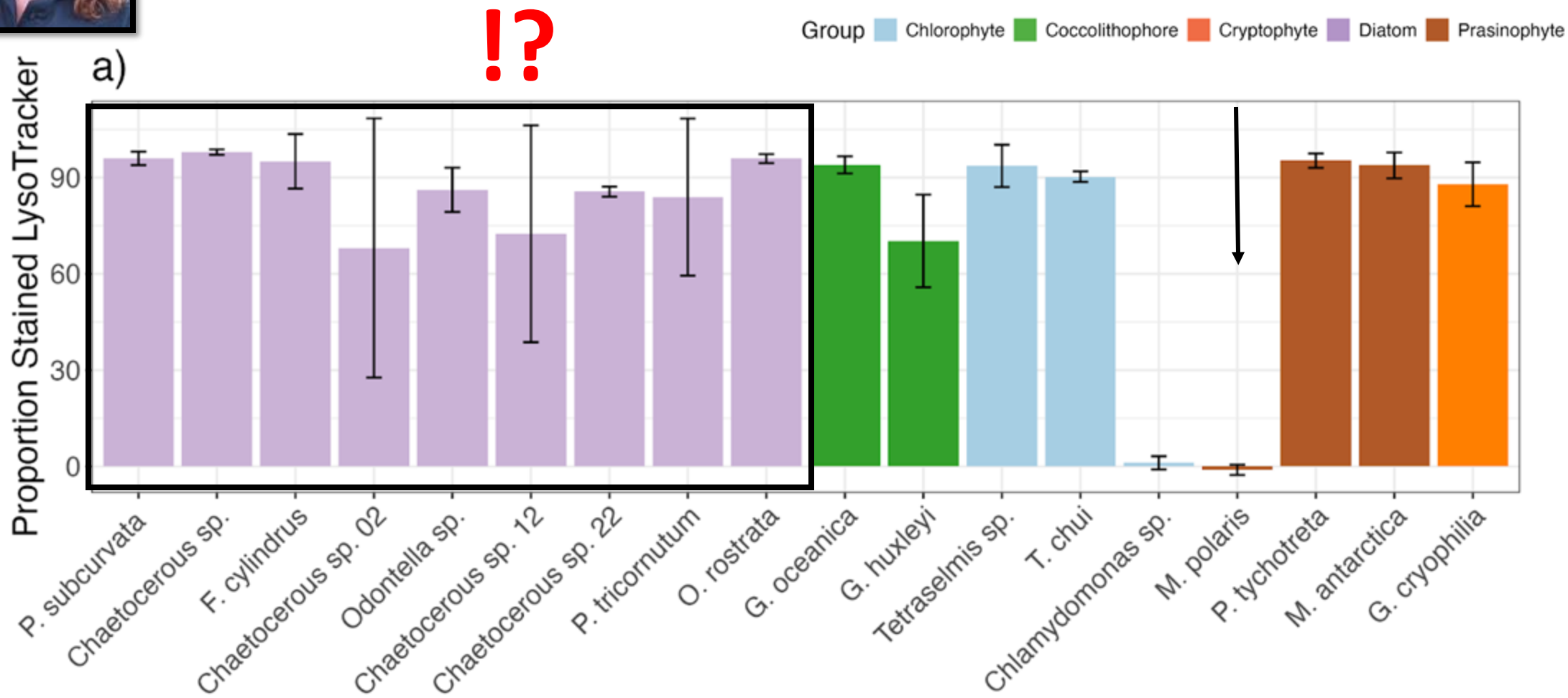
Wilken et al. 2019 *Philos. Trans. R. Soc. B*



Surveying a diverse suite of microeukaryotes with LysoTracker

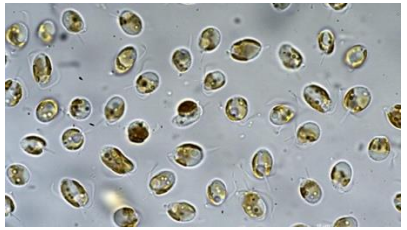
ALFRED P. SLOAN FOUNDATION

!?



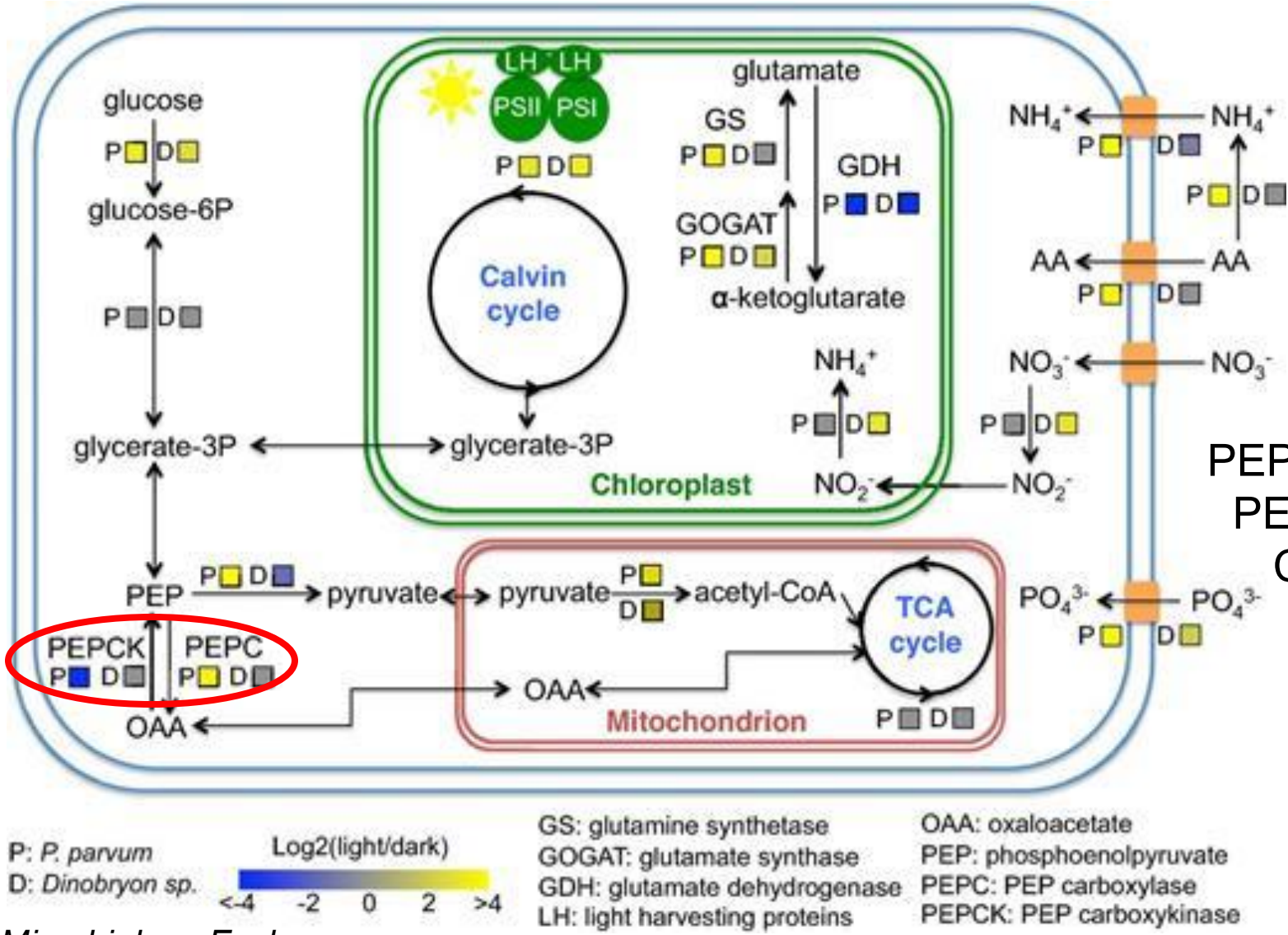
Claire Cook, PhD student

Tracking trophic mode with gene expression





Grew mixotrophs in the light and dark in presence of prey



PEP carboxykinase (dark)
PEP carboxylase (light)
Changing C source

Differences in genes involved in central carbon metabolism and phagotrophy in the dark

Liu et al. 2016 *FEMS Microbiology Ecology*

How can omics inform physiology and ocean biogeochemistry?

- Short scale disturbances influence productivity
- Quantitative estimates carbon fixation by taxa
- Diel cycle controls primary production and respiration
- Growth rates may be predicted from genomes
- Nutrient stress biomarkers indicate growth state
- Carbon metabolism expression signatures may be indicative of trophic status