

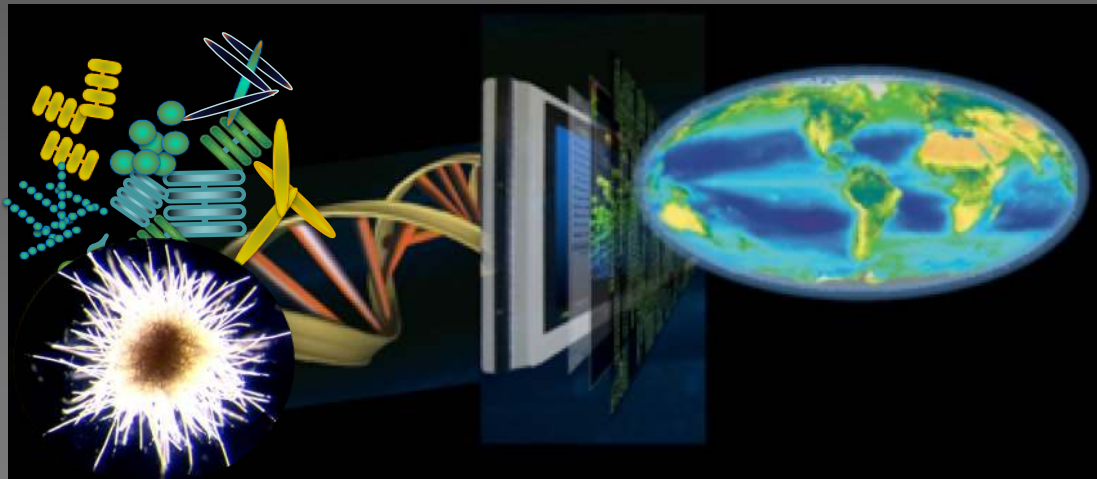
Ocean genomics: phytoplankton physiological ecology across marine ecosystems

Sonya Dyhrman

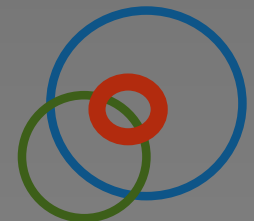
Department of Earth and Environmental Sciences

Lamont-Doherty Earth Observatory

Columbia University



COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK



LAMONT-DOHERTY
EARTH OBSERVATORY

Sonya Dyhrman

Krumlov - 2016

PhD Scripps



Postdoc WHOI



Scientific Staff WHOI

Faculty Columbia University



Columbia runs RV Langseth



Sonya – random (fun?) facts



C-CoMP at COP27

Dr. Sonya Dyhrman

The Key Role of Microbes
in the Surface Ocean

November 16th, 2022

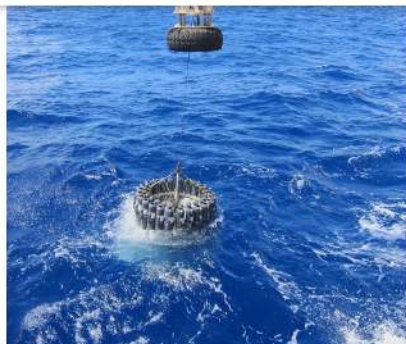
Research Projects

People

Publications

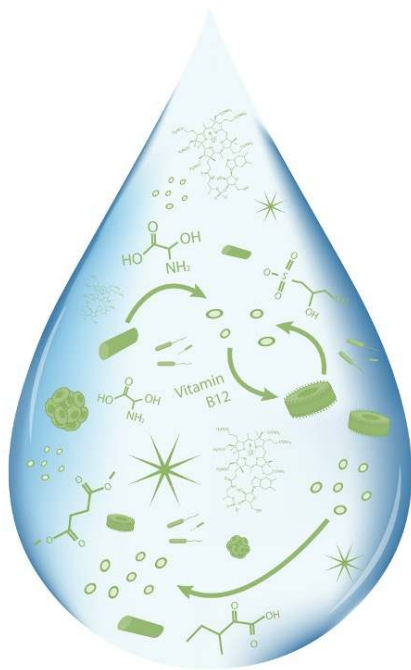
Outreach

Gallery

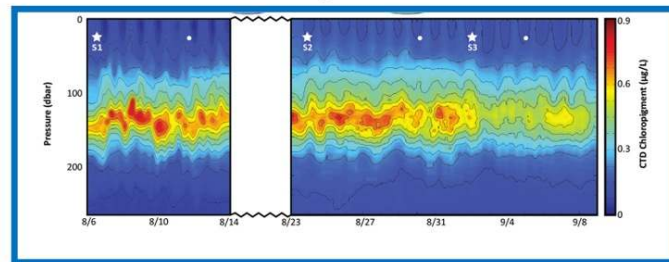


[Back to Top](#)

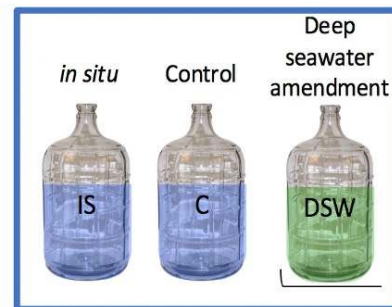
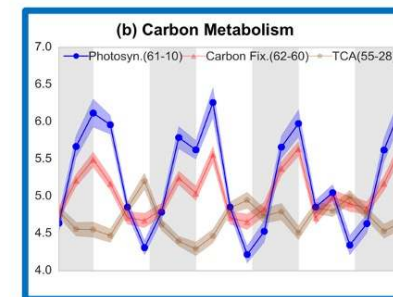
Understanding the vast network of microbes and chemicals in the ocean



Monthly time-series



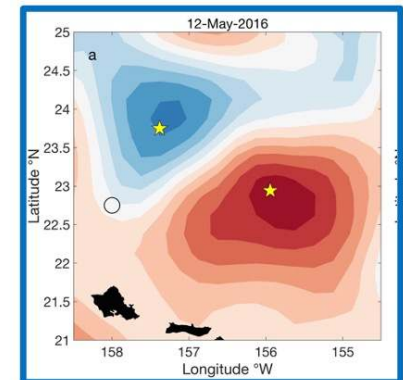
Diel time-series



Incubations



Natural disturbance



Eddy-driven forcing

High School



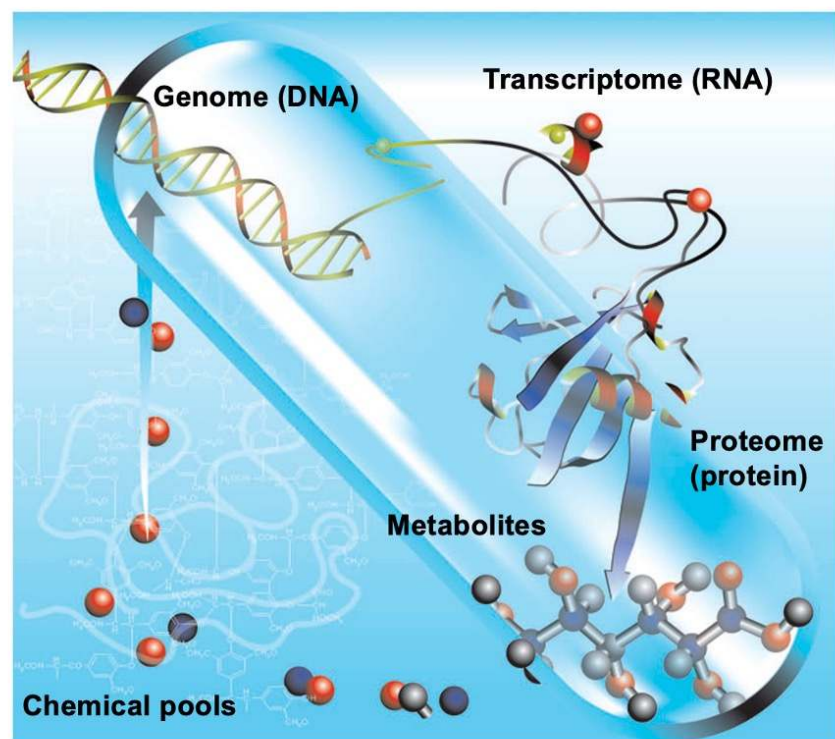
Postdoc



Professor



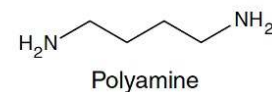
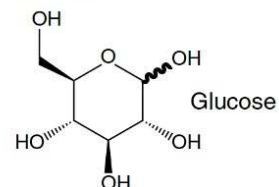
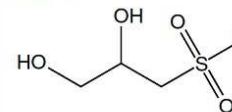
Evolving 'omics and chemical methods allow new progress



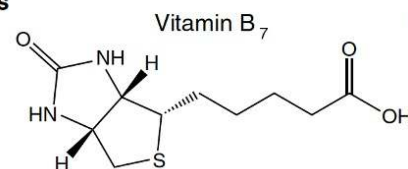
Kujawinski et al. (2011) *Annual Review Marine Science*

Substrates sustain biomass production and element cycling

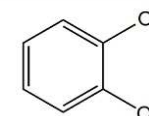
DHPS



Facilitators enable chemical reactions

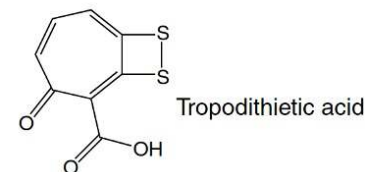
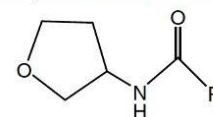


Catecholate siderophore



Signals alter microbial phenotypes

N -acyl homoserine lactone



Moran et al. (2022) *Nature Microbiology*

Key points for today

- Highlight how we ask and answer questions in the ocean with genomic tools
- Eukaryote physiological ecology
 - *Metatranscriptomes*
- Host –Microbiome interactions
 - *16S Amplicons*
 - *Metagenomes*
 - *Metatranscriptomes*
- *Share my enthusiasm for marine microbes and 'omics*



The ocean makes our planet habitable



The vast unseen microbial populations play a critical role in ocean function



Marine Microbes - fundamental to ocean ecosystem function

- Marine microbes...
 - Produce and consume green house gases
 - Supply the marine food web
 - Recycle organic matter
 - Account for roughly half of global primary production
- *make the planet habitable*

phyto- = photosynthetic
-plankton = floating organisms

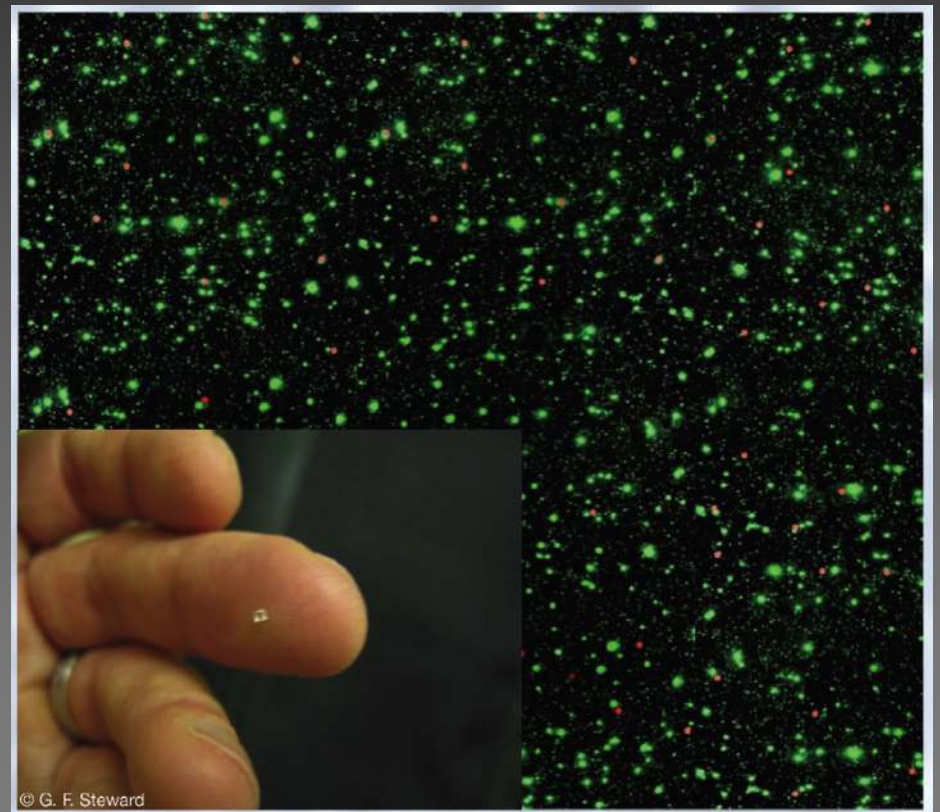
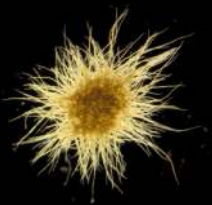
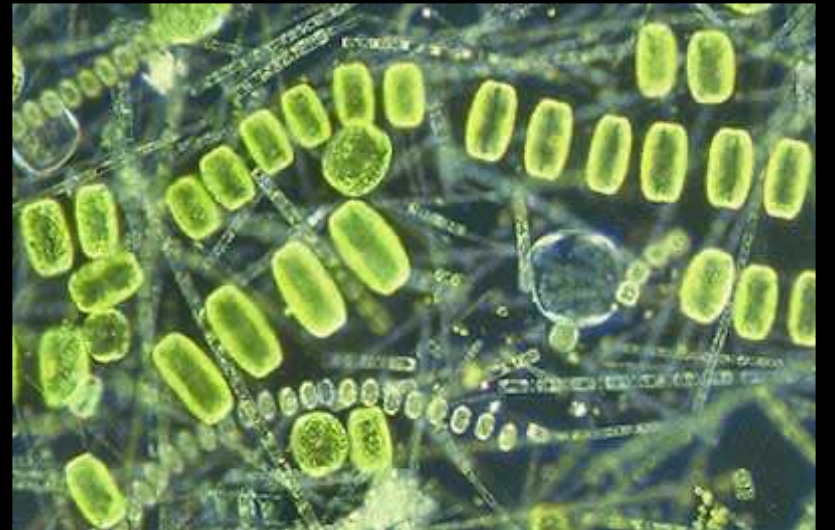
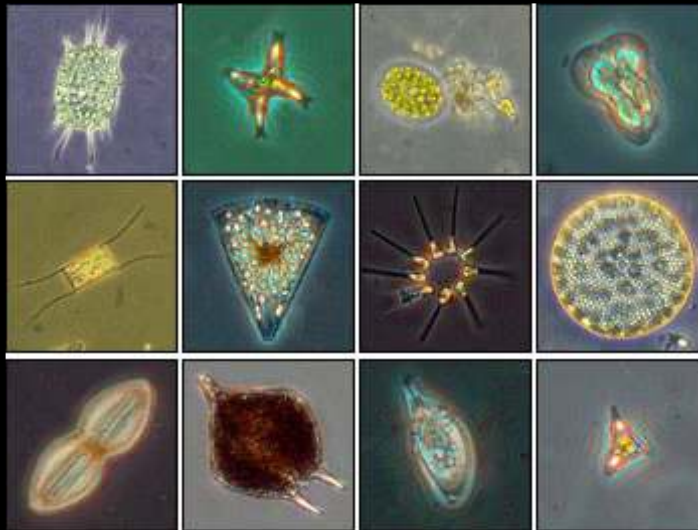
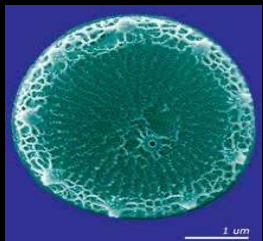
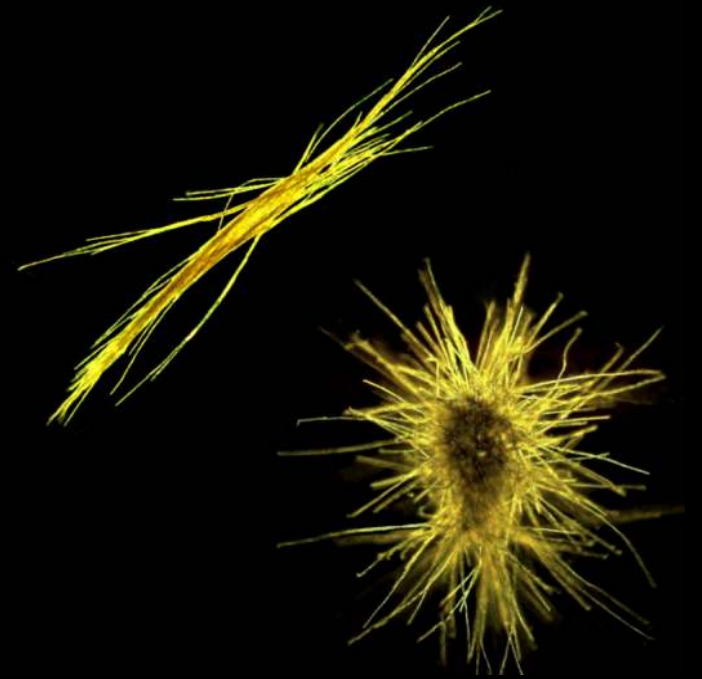
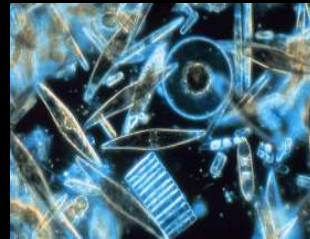
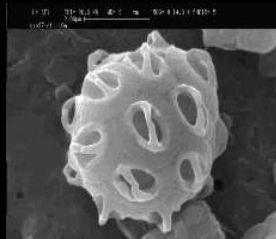
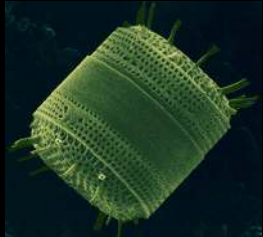
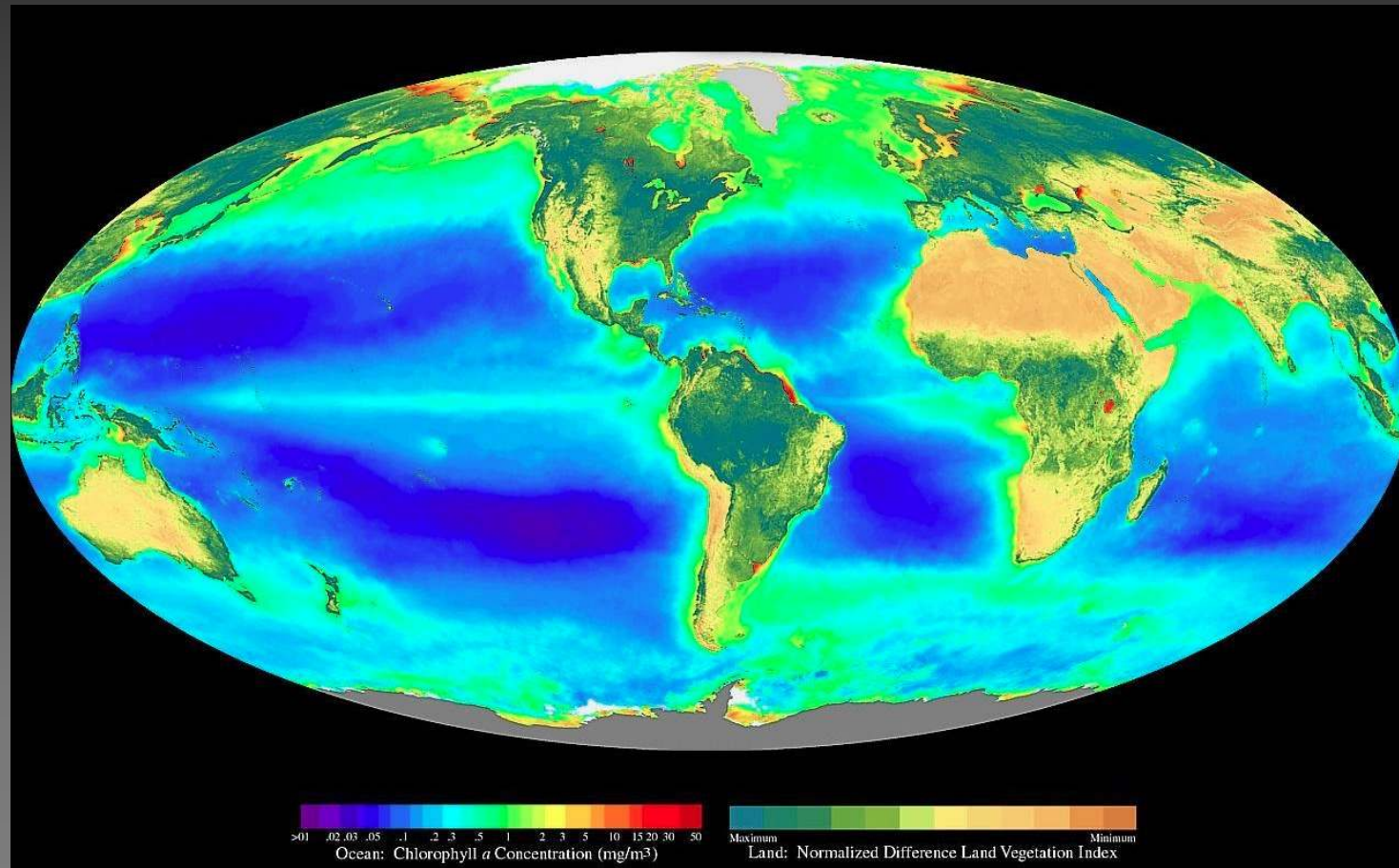


Image courtesy C-MORE

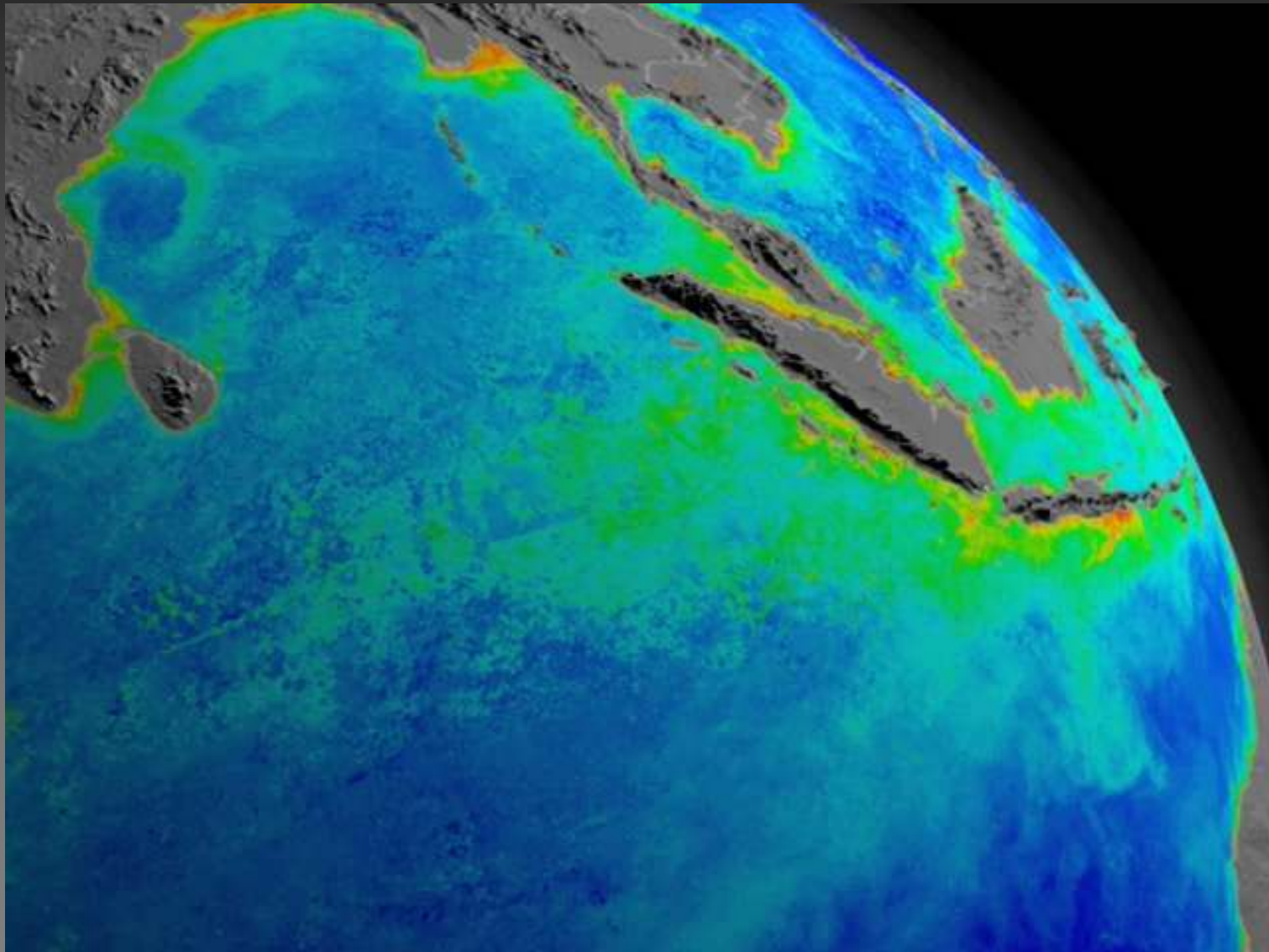


Phytoplankton play a profound role in the earth system

Half of global primary production

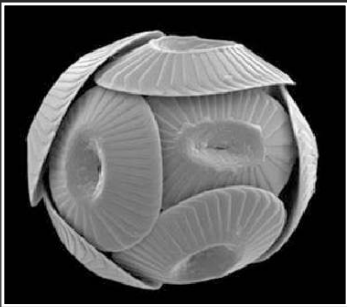


Seasonal chlorophyll distributions in the sea - highlights the global significance of phytoplankton

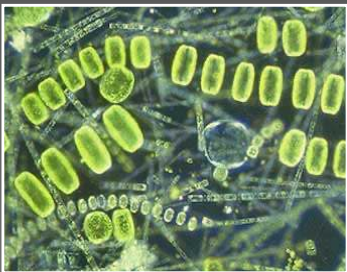


Phytoplankton underpin ocean ecosystem function

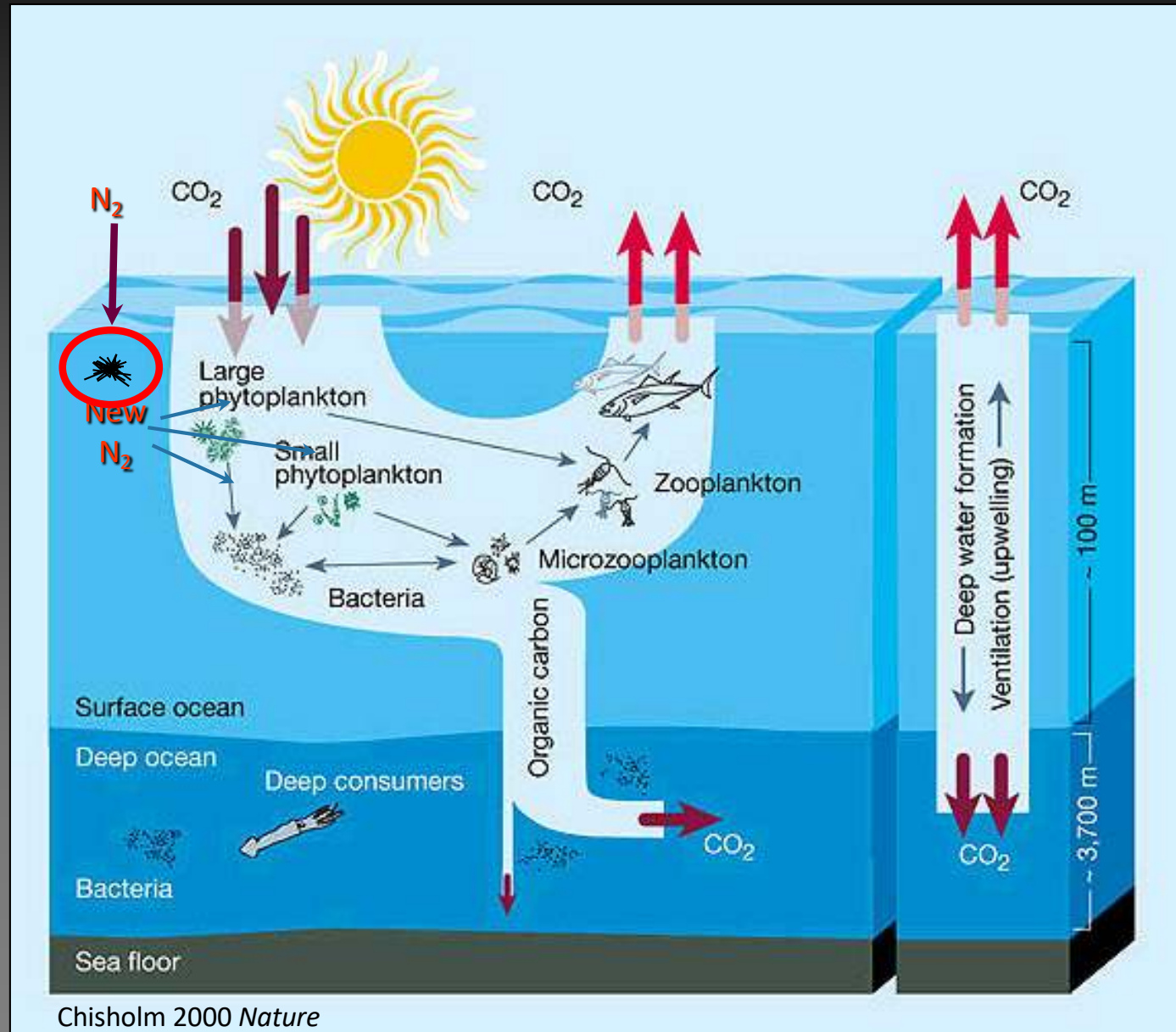
Haptophytes

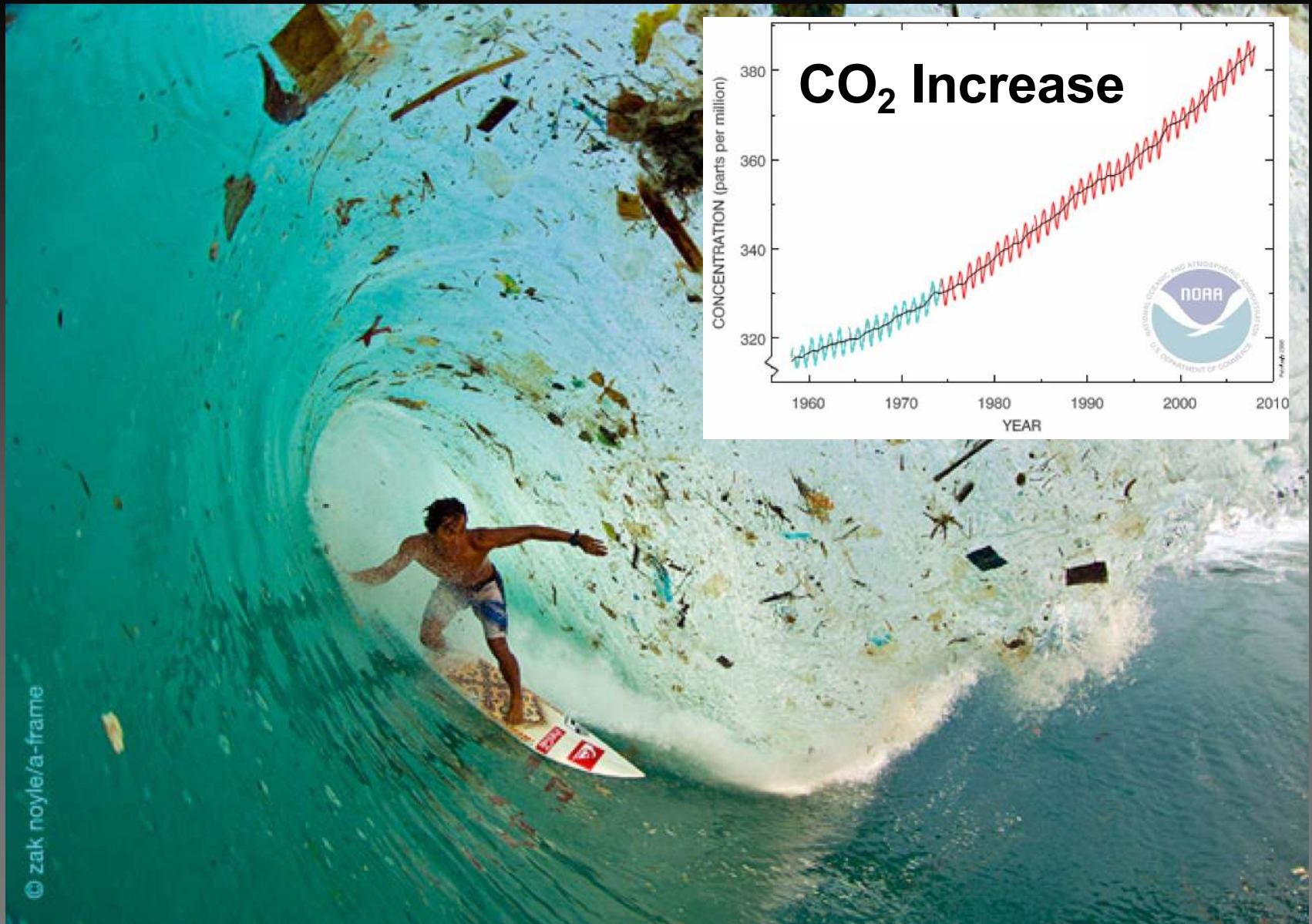


Diatoms



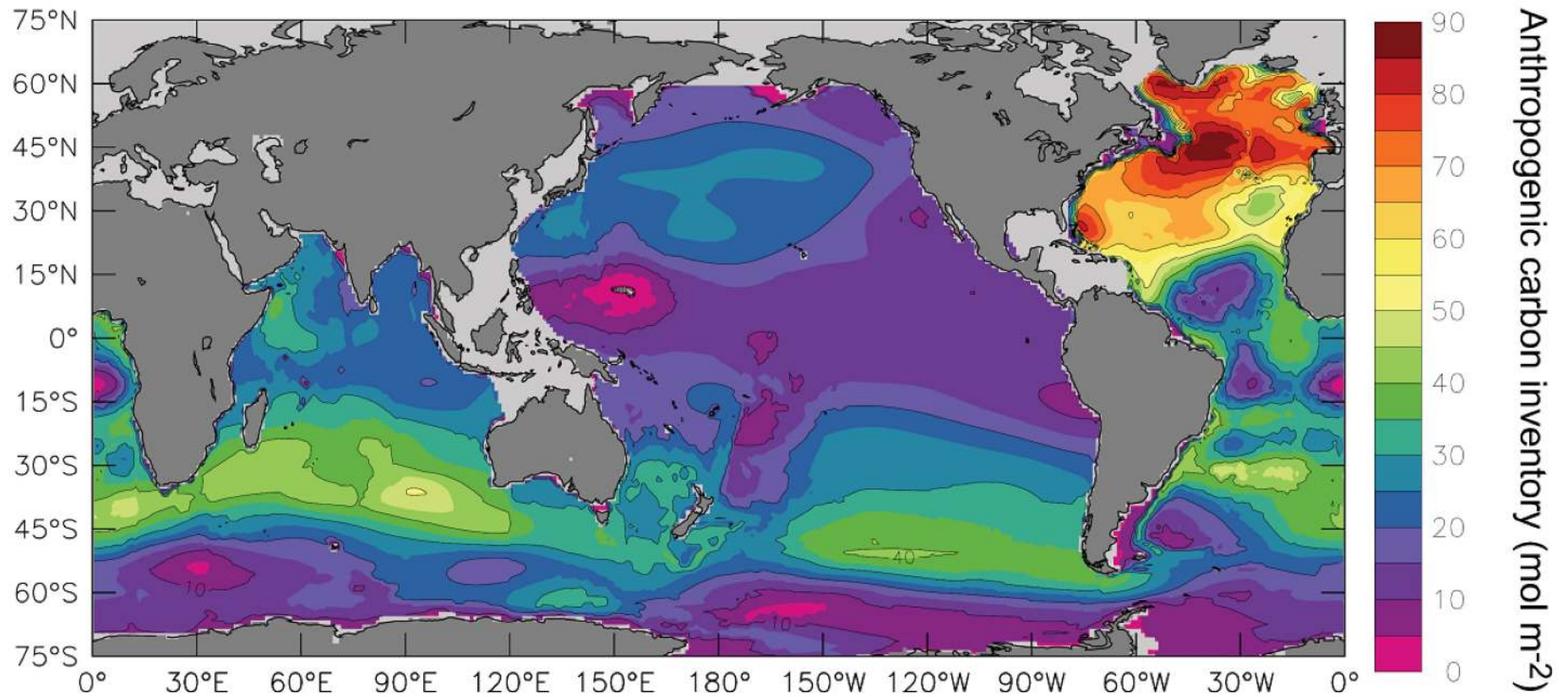
N₂ Fixers





Ocean conditions are changing and this will change the distribution and activities of microbes

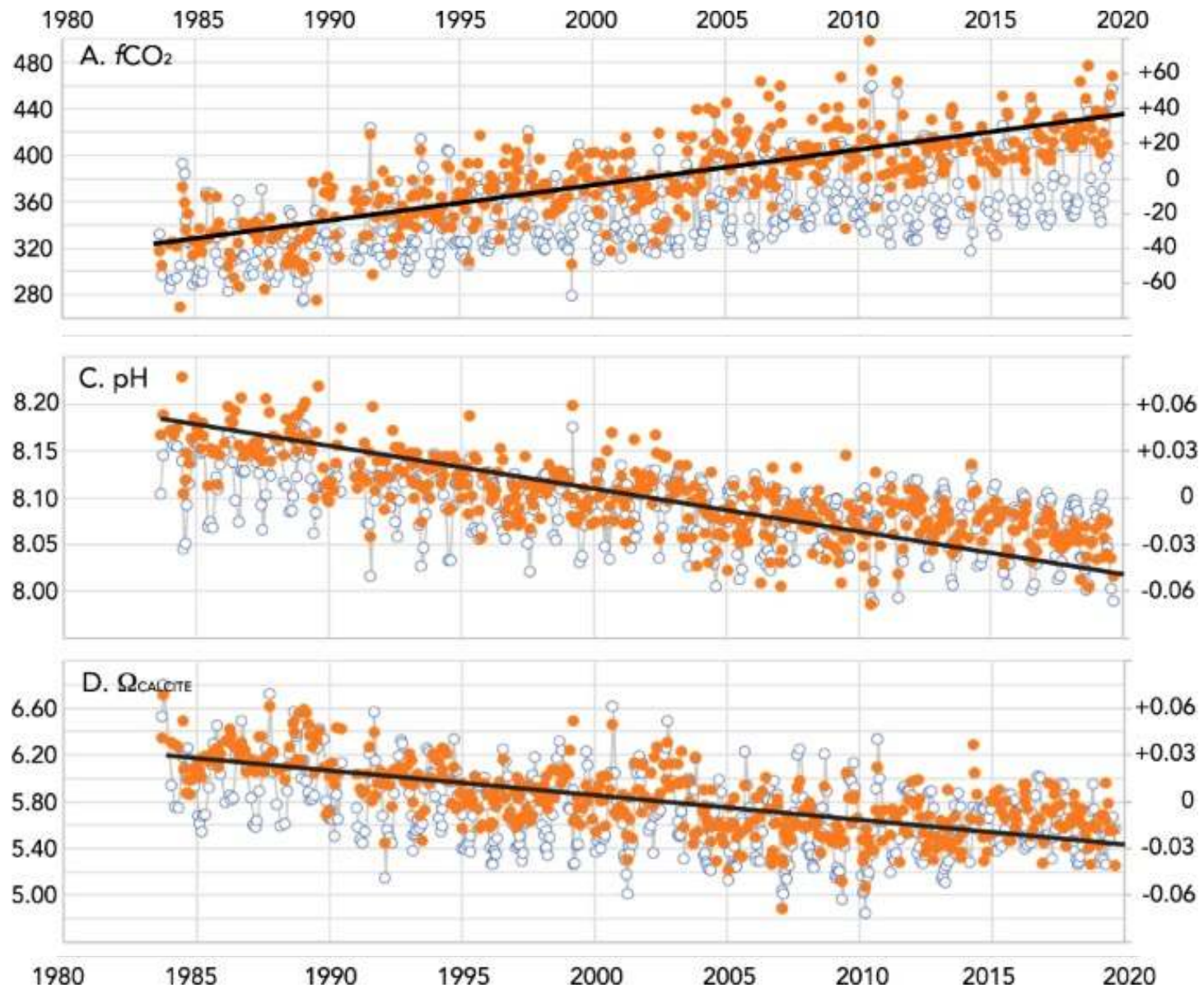
The ocean acts as a buffer for CO₂ in the atmosphere



Sabine et al. (2004) *Science*

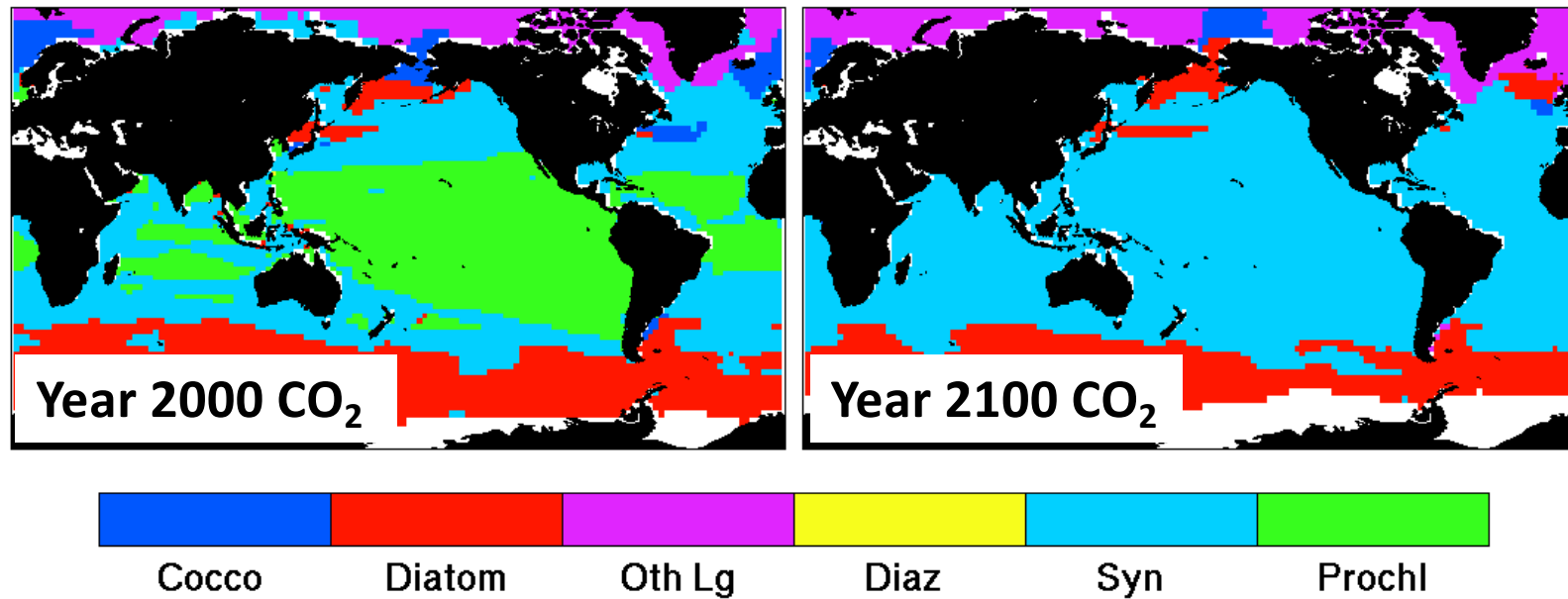
**Oceanic reservoir is 38,000
petagrams of carbon**

Changes in temperature CO₂, pH, etc.



Models predict future shifts in microbial communities

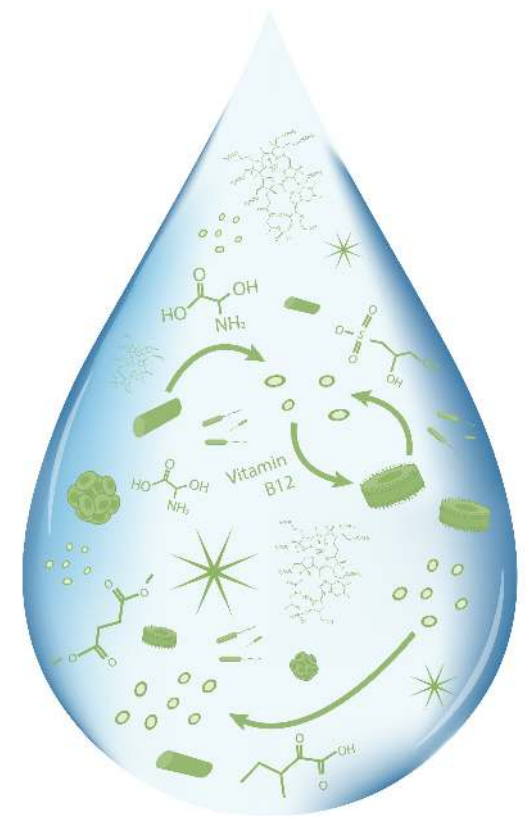
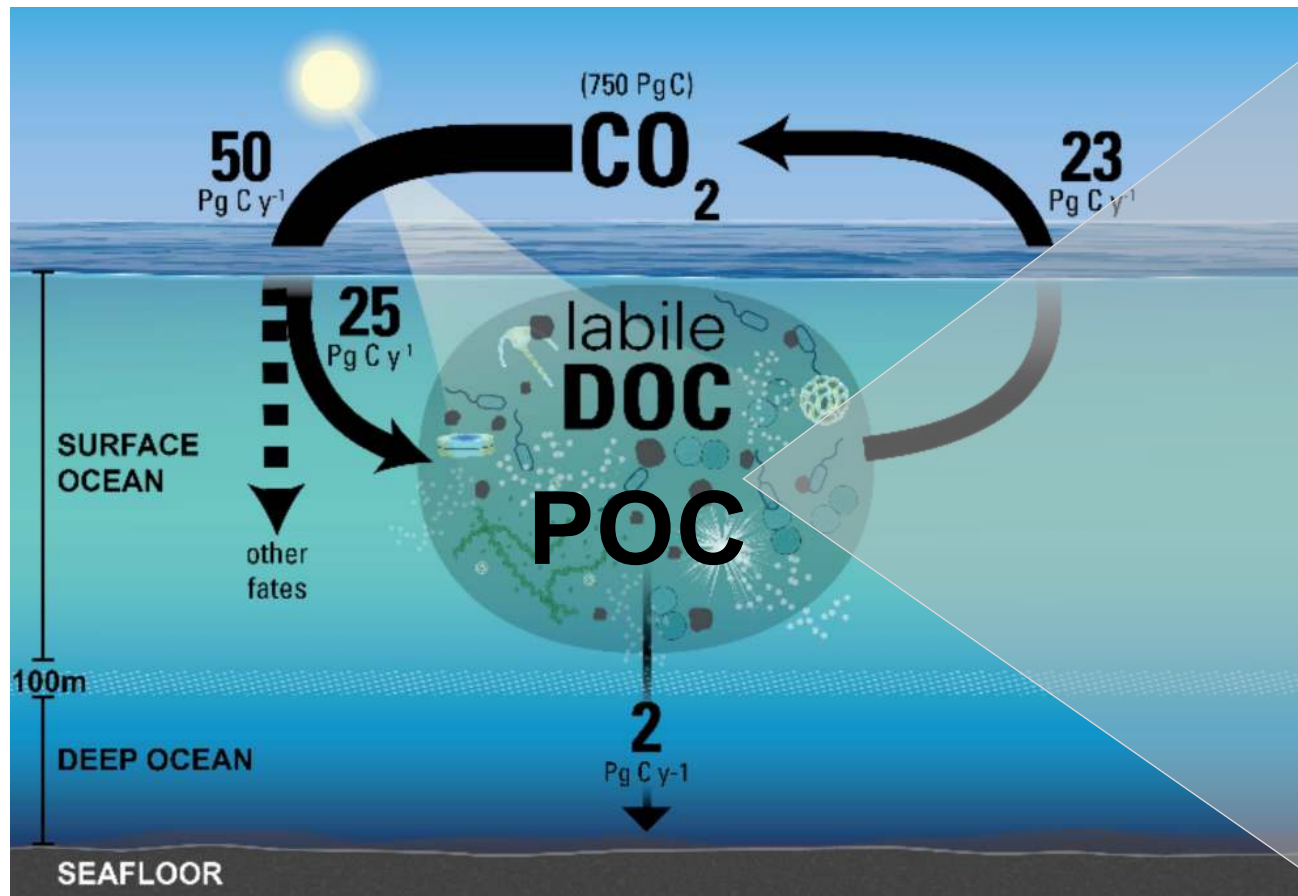
Dominant Functional Group



Dutciewicz et al. (2015) *Nature Clim. Change*.

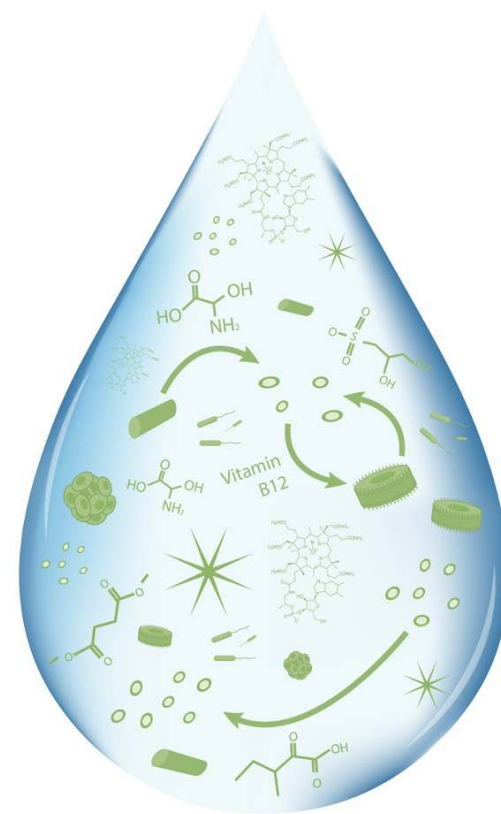
Pressing need to understand the rules that govern the **ocean chemical – microbe network** of interactions

The chemical – microbe network drives the fate of *a lot* of carbon

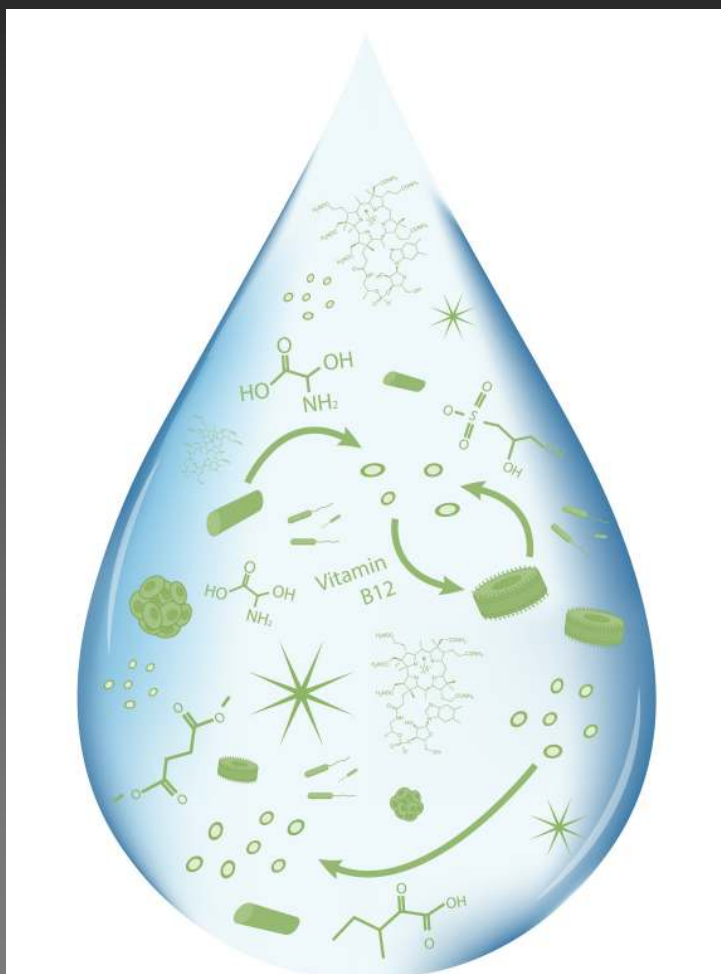


Chemical – microbe network key uncertainties

- What is the network of interacting microbes and chemicals?
- What is the resilience of this network to perturbation?
- Aspects of this network that predict regional and global patterns?



What are the rules that govern the chemical – microbe network?



Who is there?
What are they doing?

If we learn the rules for how
the system operates – then we
have a better chance of
predicting future ocean
responses

‘Omic-enabled advances allowing to query cells in their
environment in a species-specific way

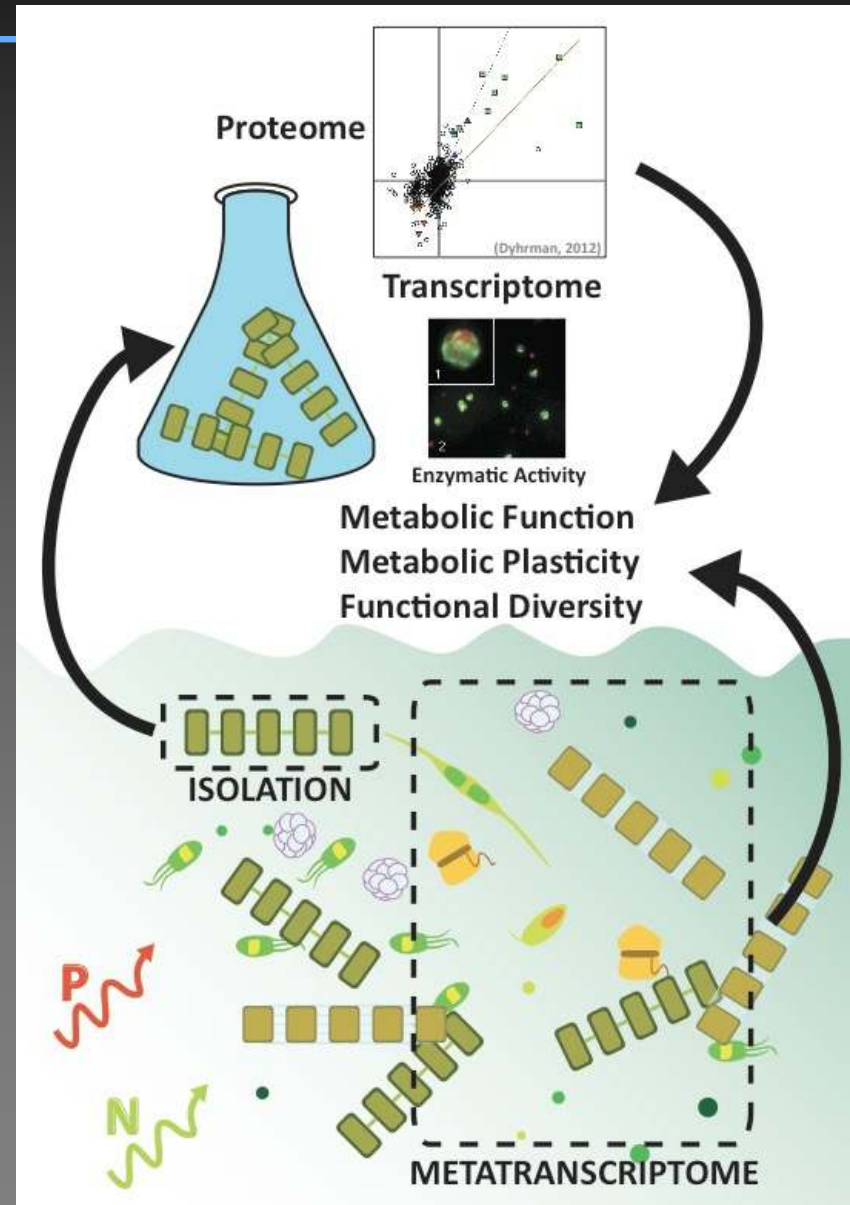
Challenges and opportunities

- Long standing challenges:
 - Populations are dilute and they move around!
 - Some keystone groups not in culture
 - Few genomes or transcriptome sequences
- New opportunities
 - Increases in genomes/transcriptomes for eukaryotic taxa
 - *New array of bioinformatics and sequencing tools*

A decade of meta 'omic and multi 'omic approaches!

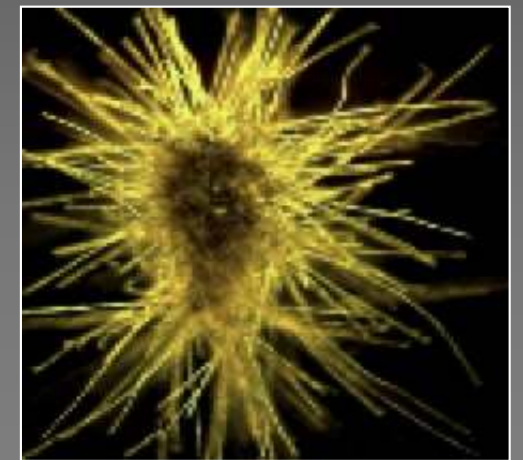
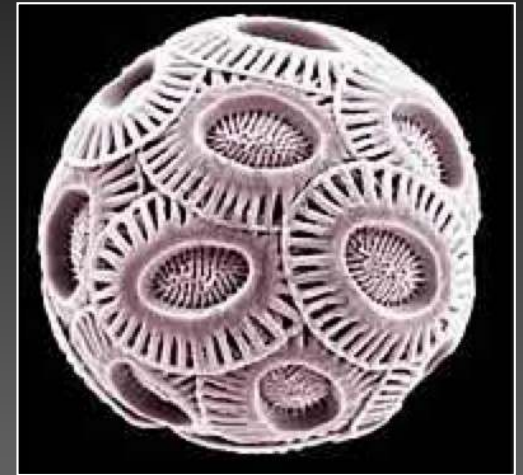
Leveraging metatranscriptome data to study the chemical – microbe network

- Cultures offer controlled conditions
- Field samples offer context
- Metatranscriptomes are species-specific
- Metatranscriptomes offer high resolution tracking of finely tuned responses



Vignettes

- Light and nutrients: defining the drivers of physiological ecology and competition in microeukaryotes
- Microbiome interactions: Tracking the microbiome of a keystone N₂ fixer



Thank you Dyhrman Lab family past and present



Gwenn Hennon



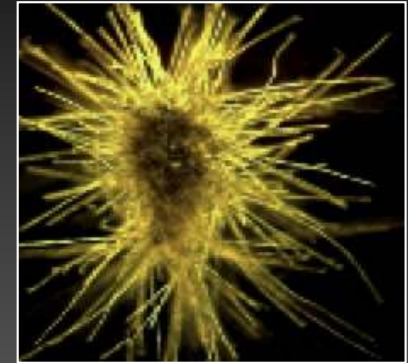
Mónica Rouco



Sheean Haley



Kyle Frischkorn



John Tracey



Hanna Anderson



Angela Zoumplis



Erin Maybach



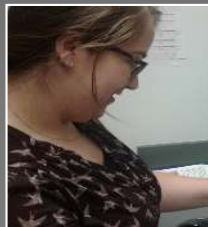
Hannah Goldberg



Sheean Haley



Matt Harke



Maria
Hernández-
Limón



Harriet Alexander



Microeukaryote metatranscriptomes with RNA-Seq



3x20L

Filter
($>5\mu\text{m}$)



Extract RNA

Selected



Unselected



De novo assembly
Reference mapping

De novo assembly
Reference mapping

Quality Control

- Trimmomatic
- FastQC

Removal of Sequences

- riboPicker
- BBDuk

Functional Annotation

- BLAST
- DIAMOND
- HMMer

Assembly/Reference

- Trinity
- TransABYSS
- rnaSPADES
- MEGAHIT

Protein Prediction and Clustering

- TransDecoder
- OrthoFinder

- KEGG
- KOG
- GO
- Pfam

Taxonomic Annotation

- BLAST
- DIAMOND
- MMSeq2

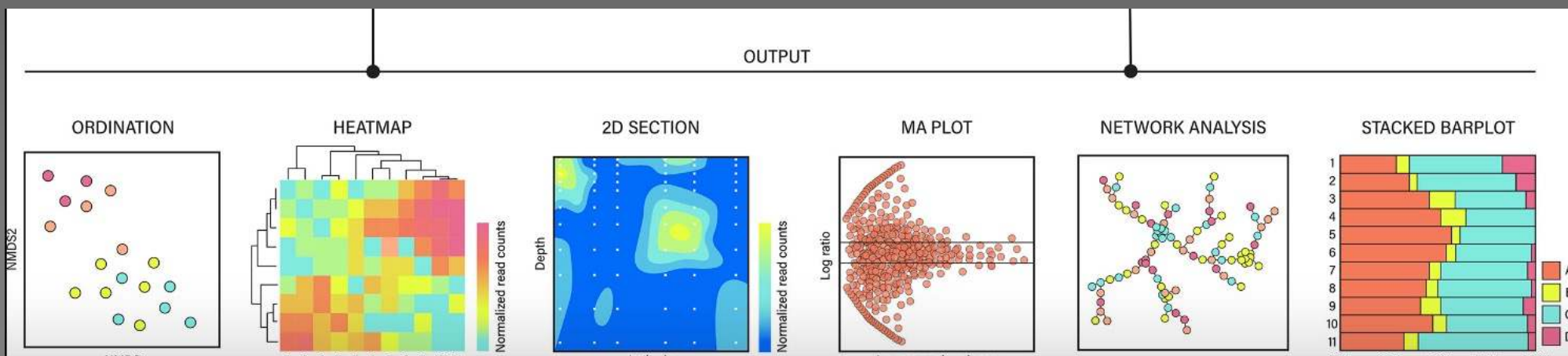
Read Mapping

- Salmon
- BWA
- Bowtie2
- RSEM

Normalization & DE

- EdgeR
- DESeq2
- TPM
- TMM

- MMETSP
- EukProt
- EukZoo
- PhyloDB



Sequencing and analytical workflows are constantly improving



Harriet Alexander @nektion4plankton · Apr 27



So check it out!

GitHub here: [github.com/AlexanderLabWH...](https://github.com/AlexanderLabWHOI/eukrhythmic)

Documentation here: eukrhythmic.readthedocs.io/en/latest/

jEUKbox here: eukrhythmic.readthedocs.io/en/latest/

Feedback / comments welcome!

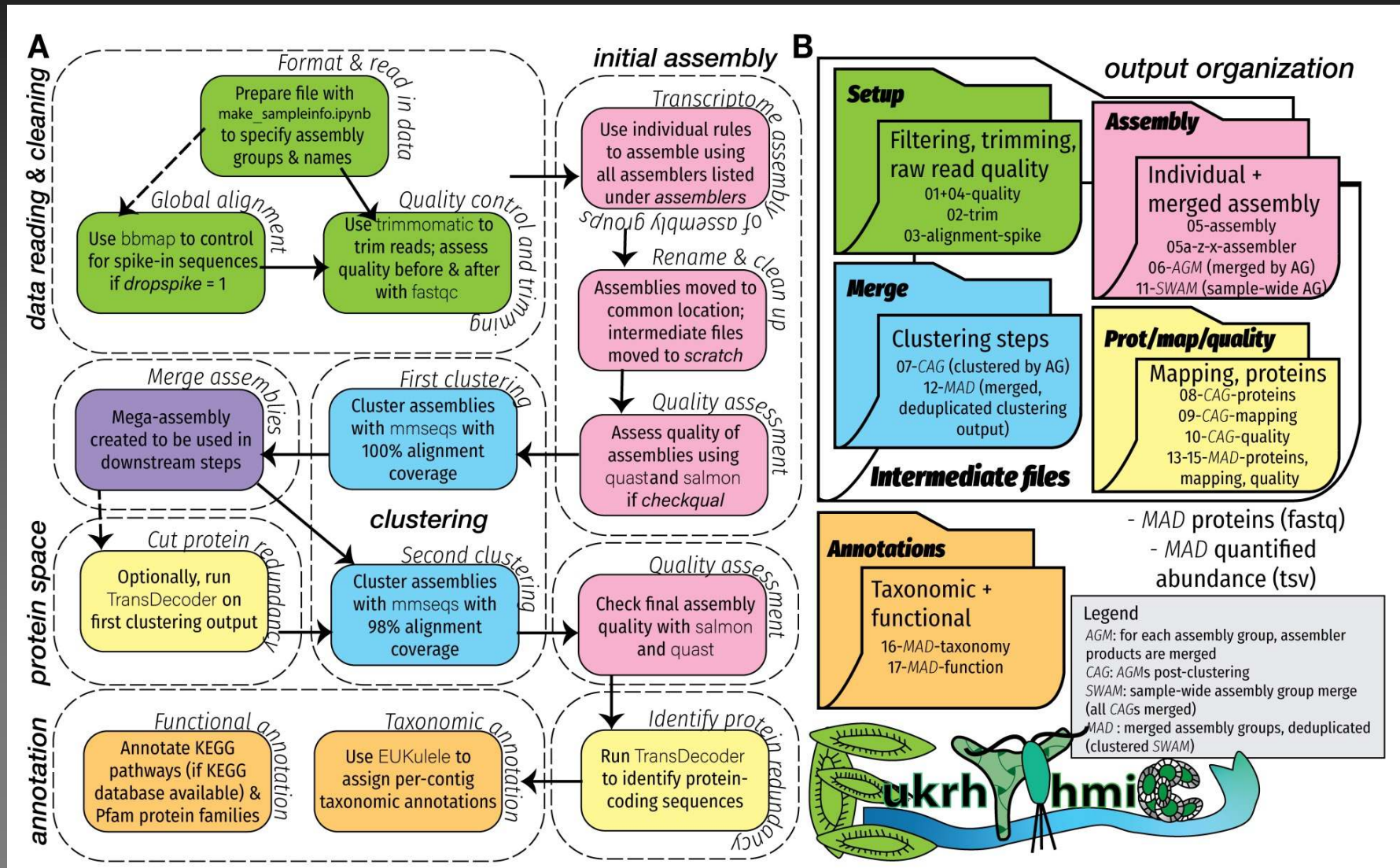


github.com

GitHub - AlexanderLabWHOI/eukrhythmic: A metatranscriptomic pipe...

A metatranscriptomic pipeline optimized for the study of
microeukaryotes. - GitHub - AlexanderLabWHOI/eukrhythmic: A ...

Sequencing and analytical workflows are constantly improving



Core questions... adventures in eukaryote metatranscriptomics

Limitation

- What limits carbon cycling? – tracing physiological ecology in an expanding oligotrophic ocean with metatranscriptomics

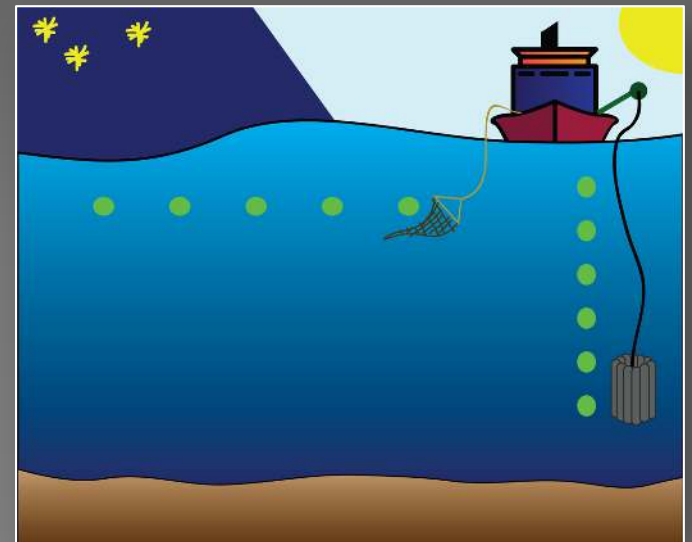
HOE:DYLAN



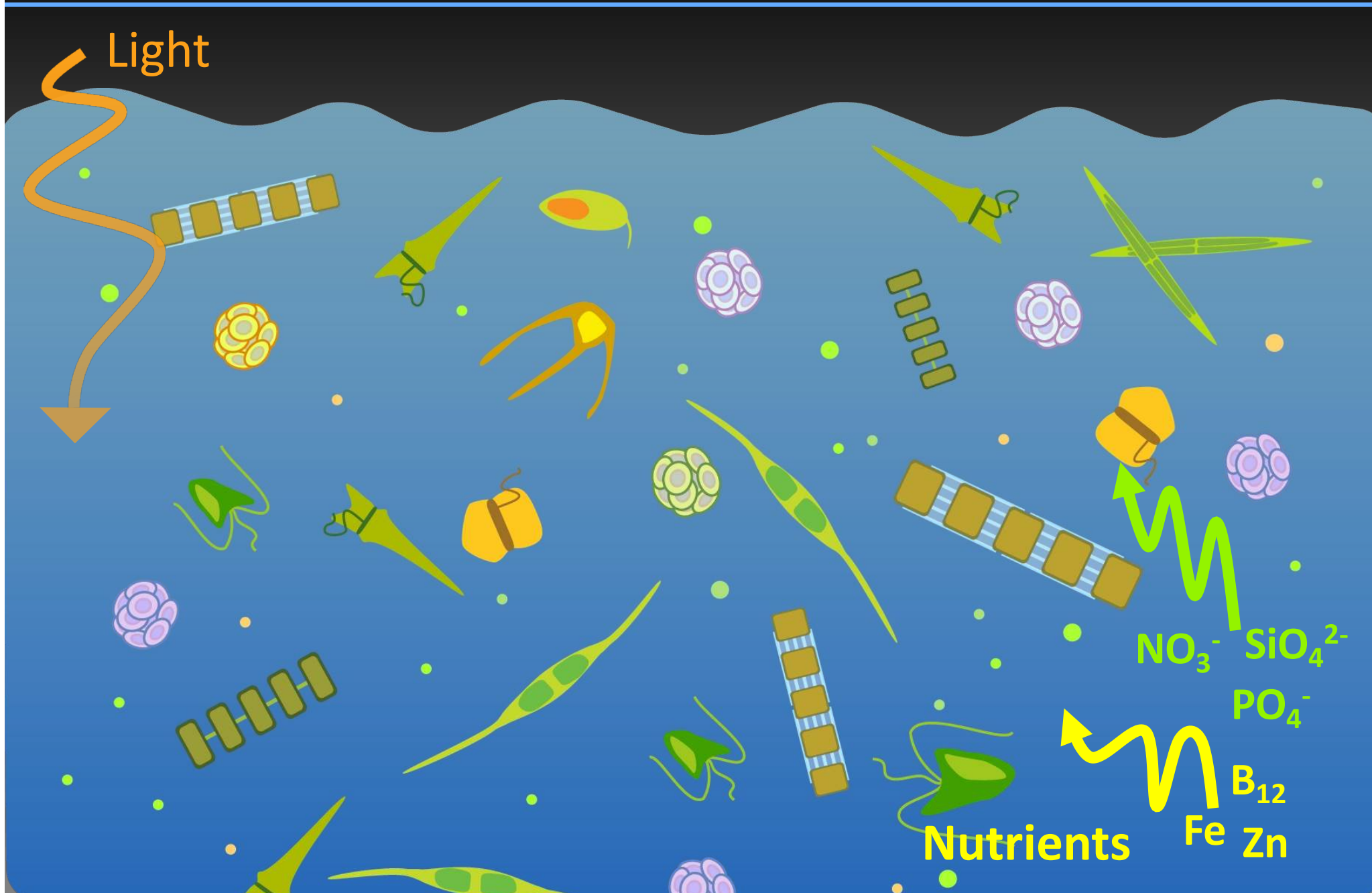
Competition

- A day in the life – tracing competitive interactions with multi-'omics

HOE:MAHALO

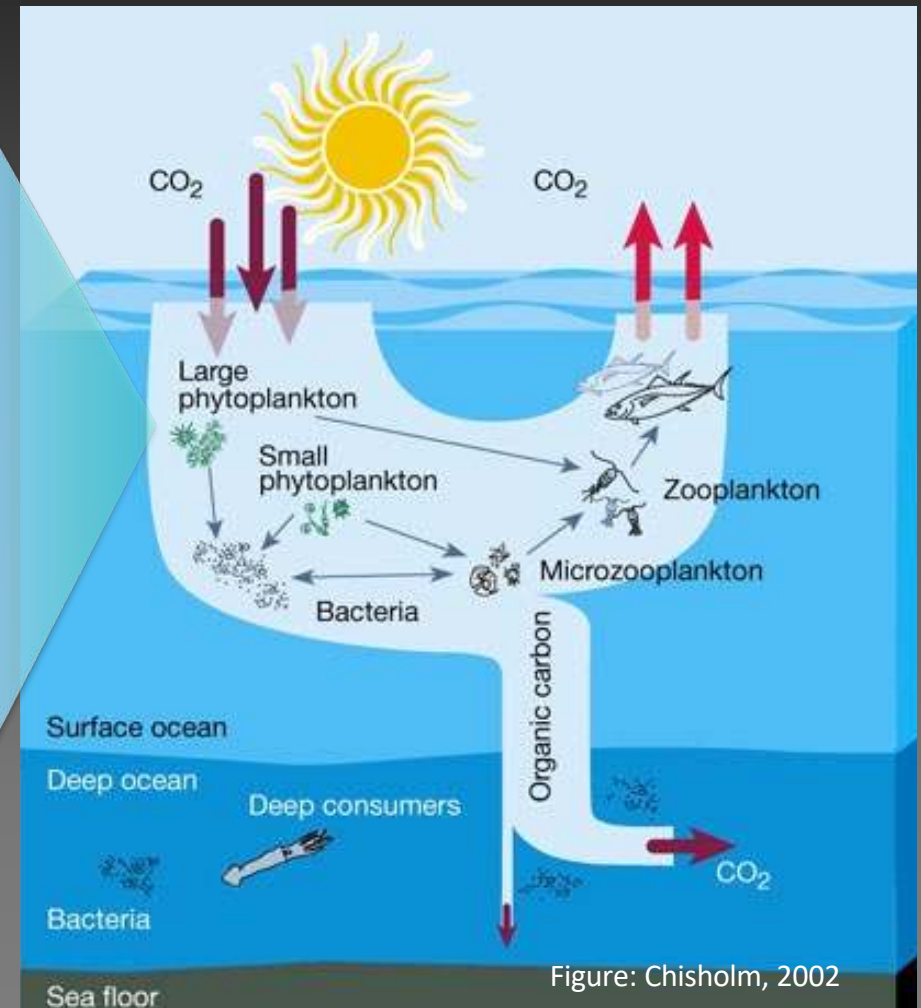


Complex community dynamics driven in part by resources



Tracking phytoplankton physiological ecology

Ecosystem function and biogeochemistry

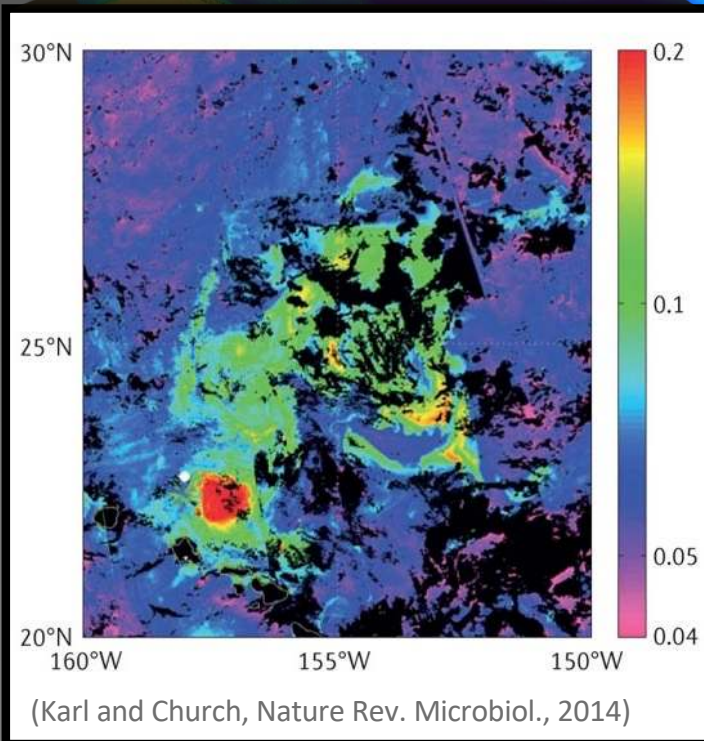


How do resources drive phytoplankton distributions and activities?

North Pacific Subtropical Gyre

North Pacific
Subtropical
Gyre

Station ALOHA

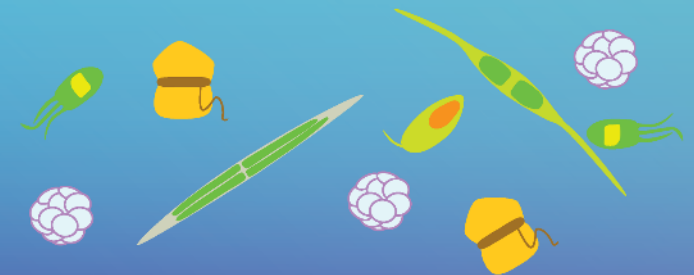


NORMAL OLIGOTROPHIC STATE

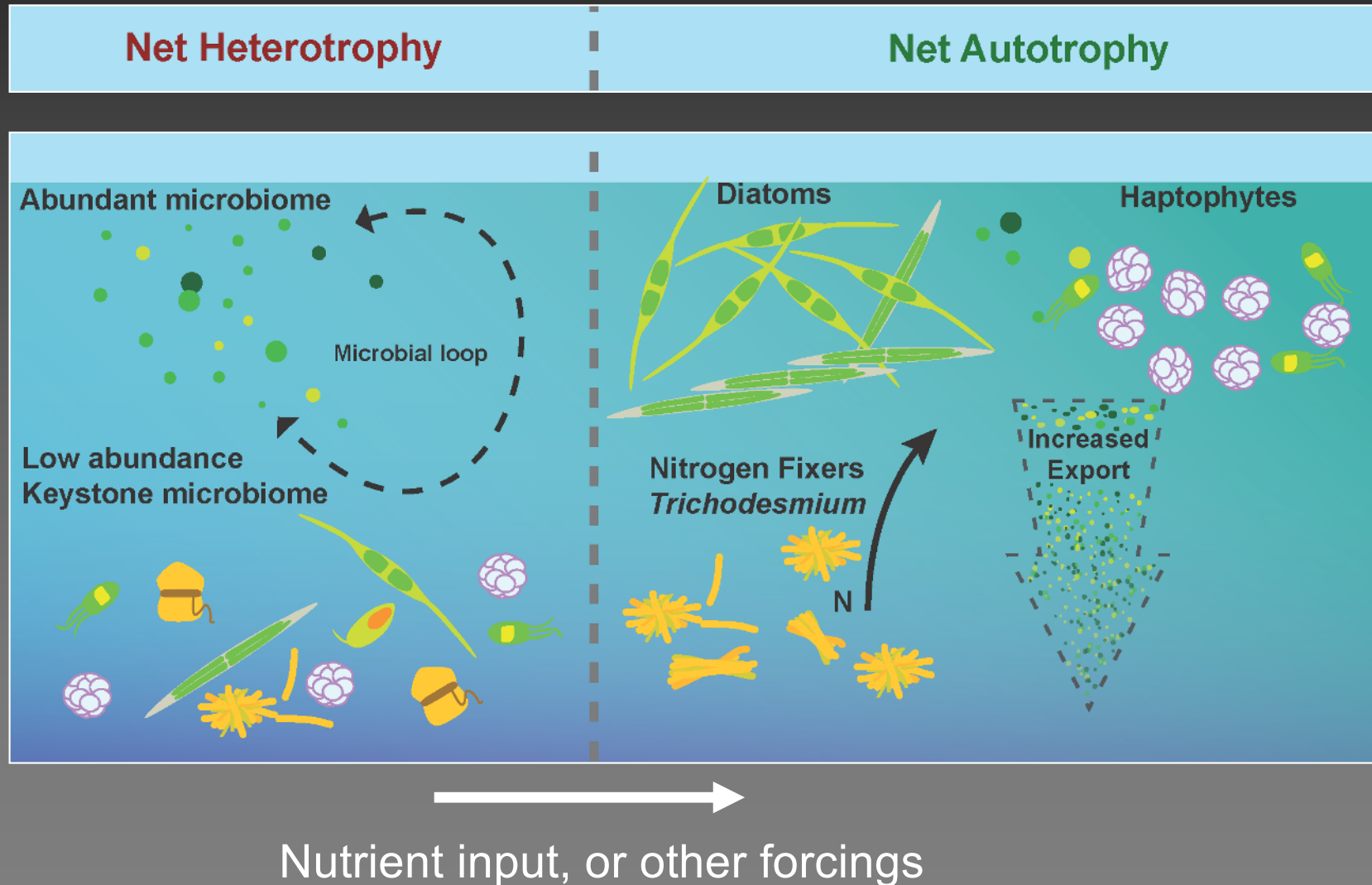
SMALL PLANKTON

MICROBIAL
LOOP

RARE LARGE EUKARYOTES

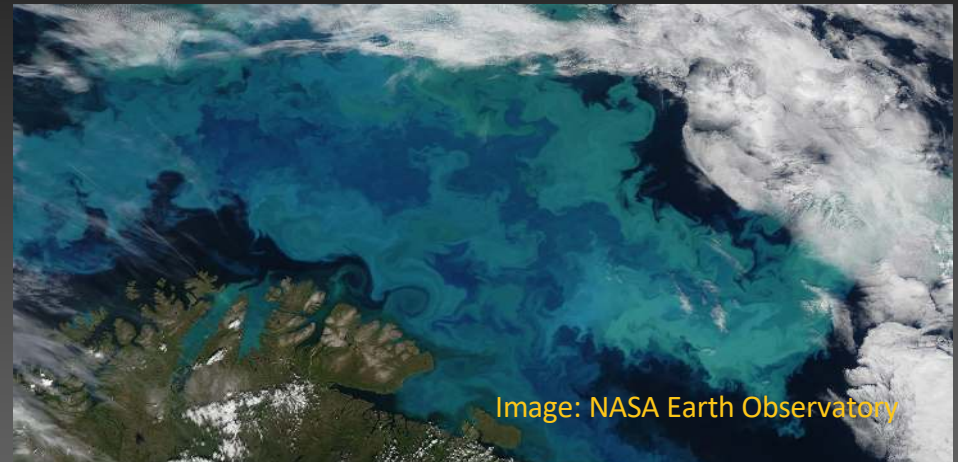


Blooms of keystone species can shift ecosystem state and carbon flow



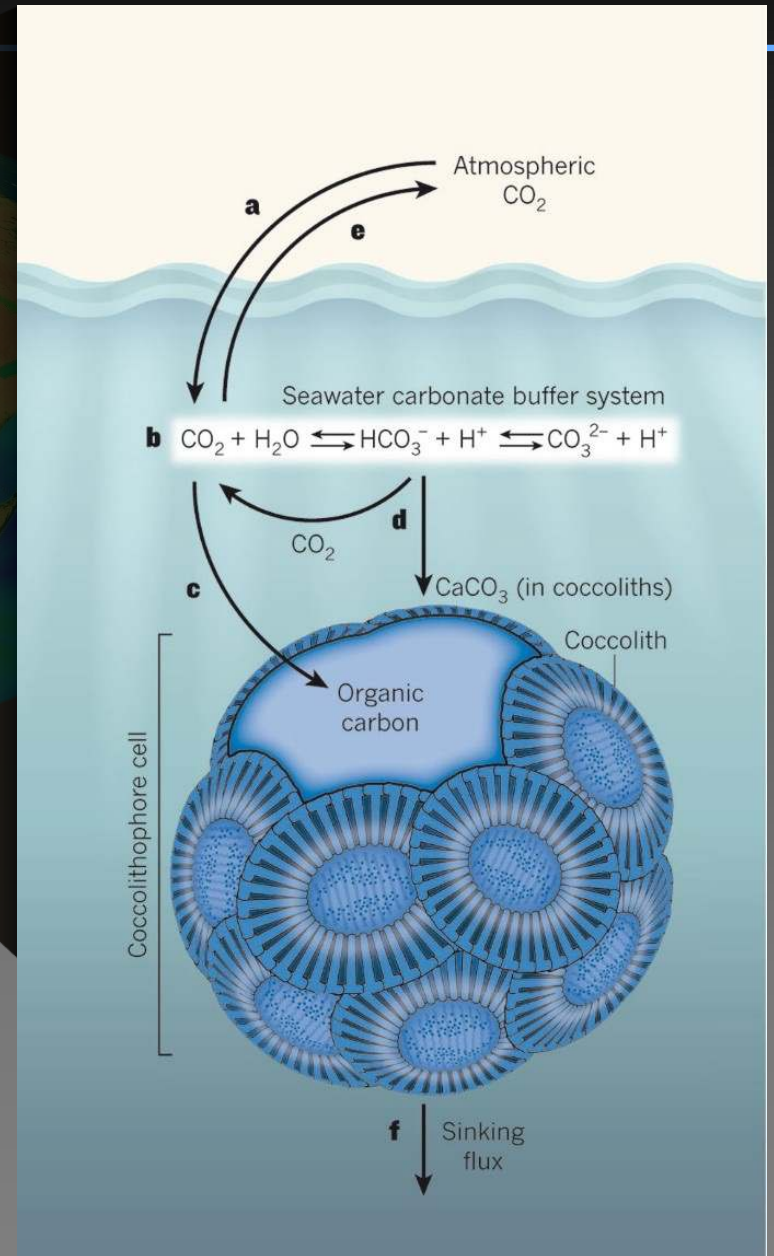
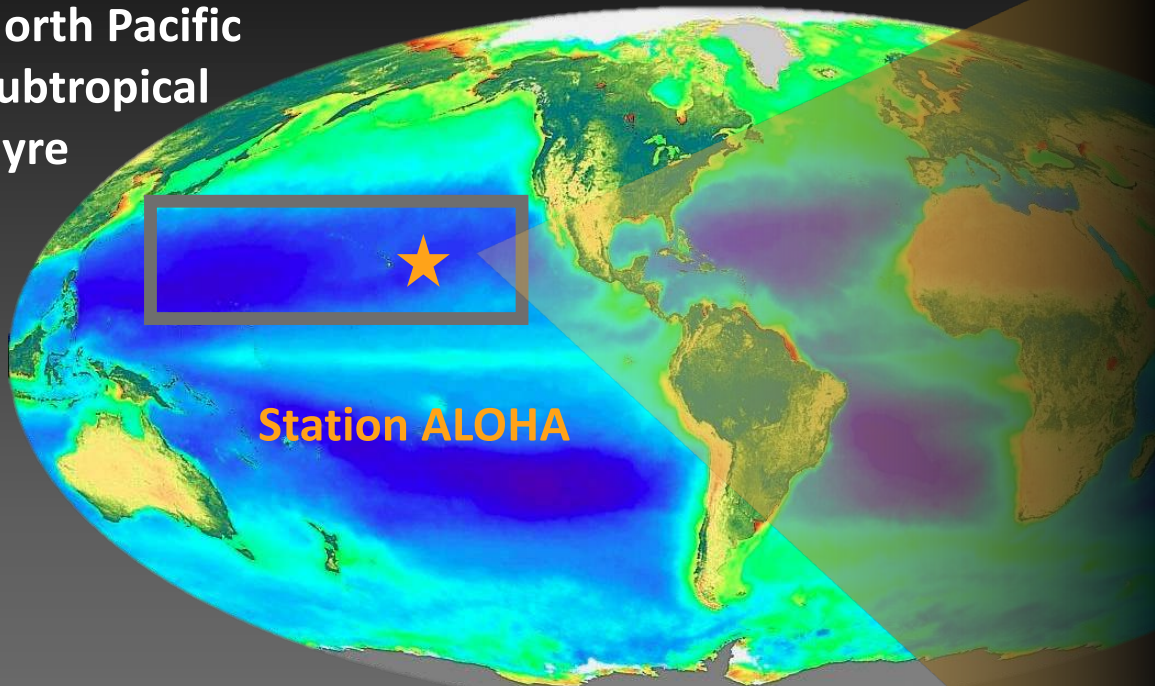
Emiliana huxleyi: a cosmopolitan, globally significant species

- Calcification - critical role in global carbon cycle and strongly linked to climate driven ocean acidification
- Source of paleoproxies for climate reconstructions
- Form dense blooms, drivers largely unknown
- First eukaryotic marine phytoplankton to have multiple strains sequenced, identifying pan genome

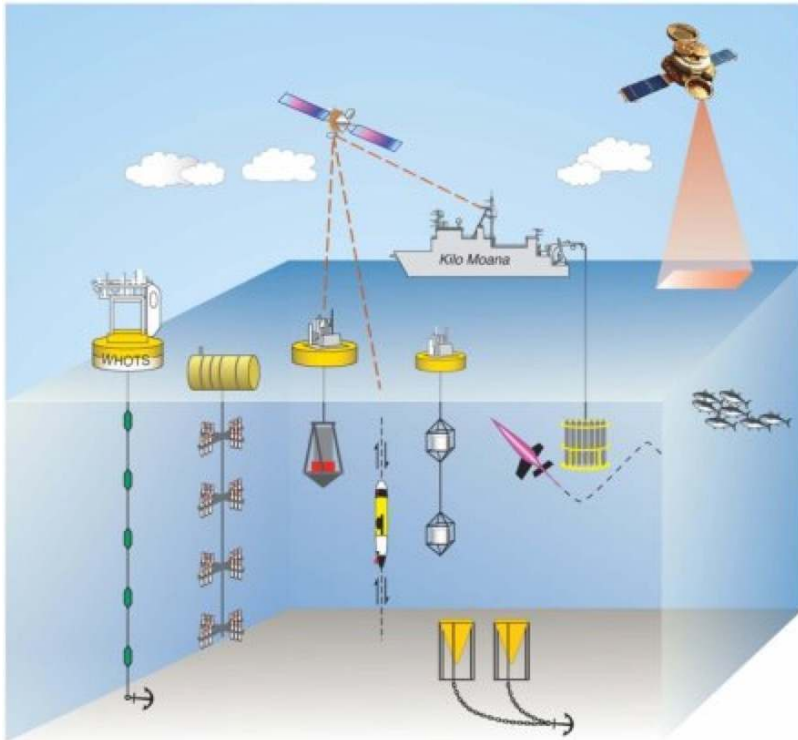
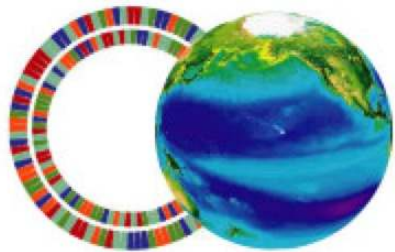


Drivers of carbon flow in oligotrophic systems

North Pacific
Subtropical
Gyre



Hawaii Ocean Experiment: Dynamics of Light and Nutrients

Wilson et al. 2015 *GBC*

center for microbial oceanography: research and education

C·more *linking genomes to biomes*
(HOF BY)

(HOE-DYLAN)



HOE:DYLAN: Water sampling



HOE:DYLAN: Semi-factorial resource additions



Nitrate/nitrite*: $\sim 40 \mu\text{M}$
Phosphate*: $\sim 2.6 \mu\text{M}$
Silicate*: $\sim 100 \mu\text{M}$
Fe: $\sim 0.6 \text{ nmol/kg}$
 B_{12} : $< 0.1 \text{ pM}$

in situ

Control



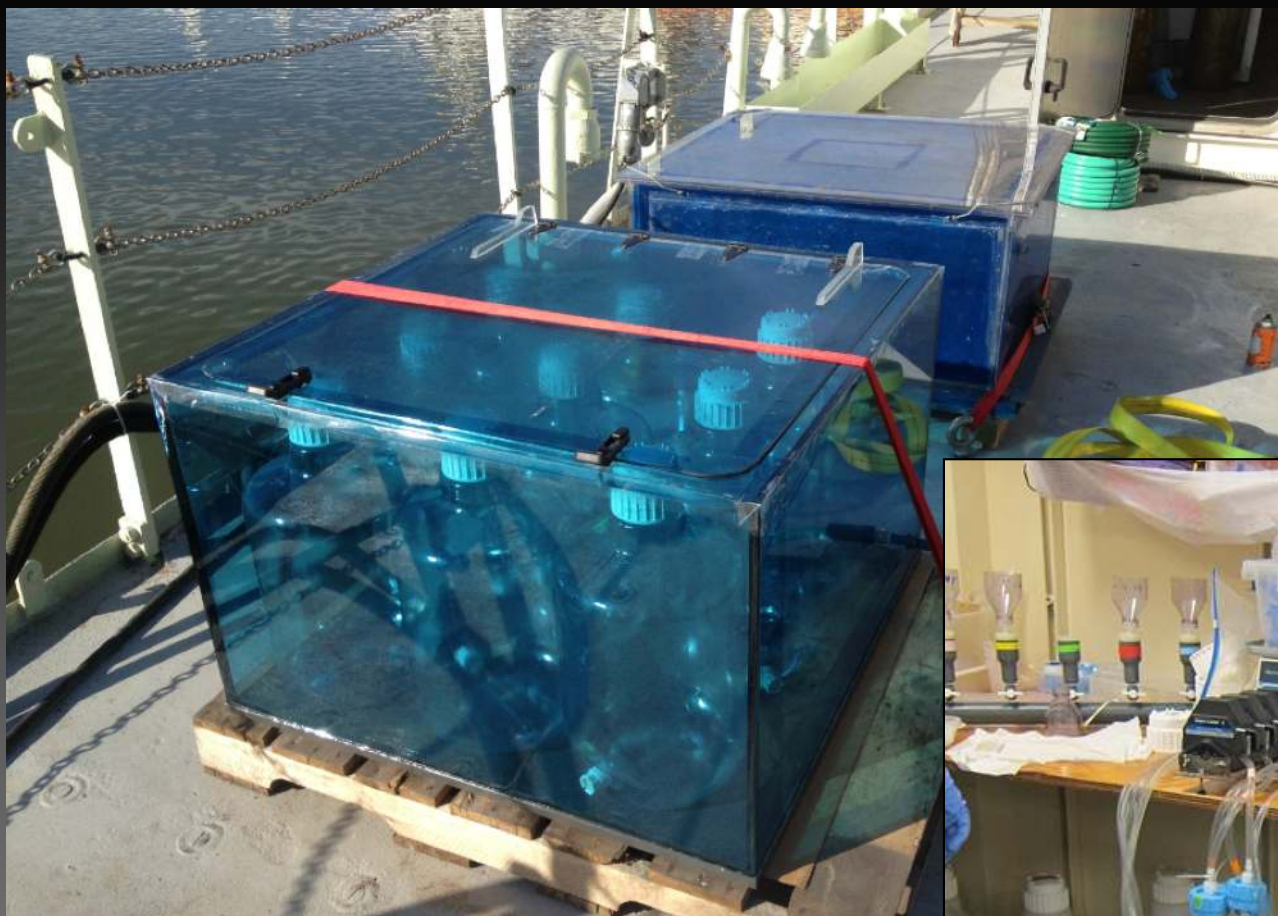
Deep
seawater
amendment

(+N, Si, Fe,
 B_{12})

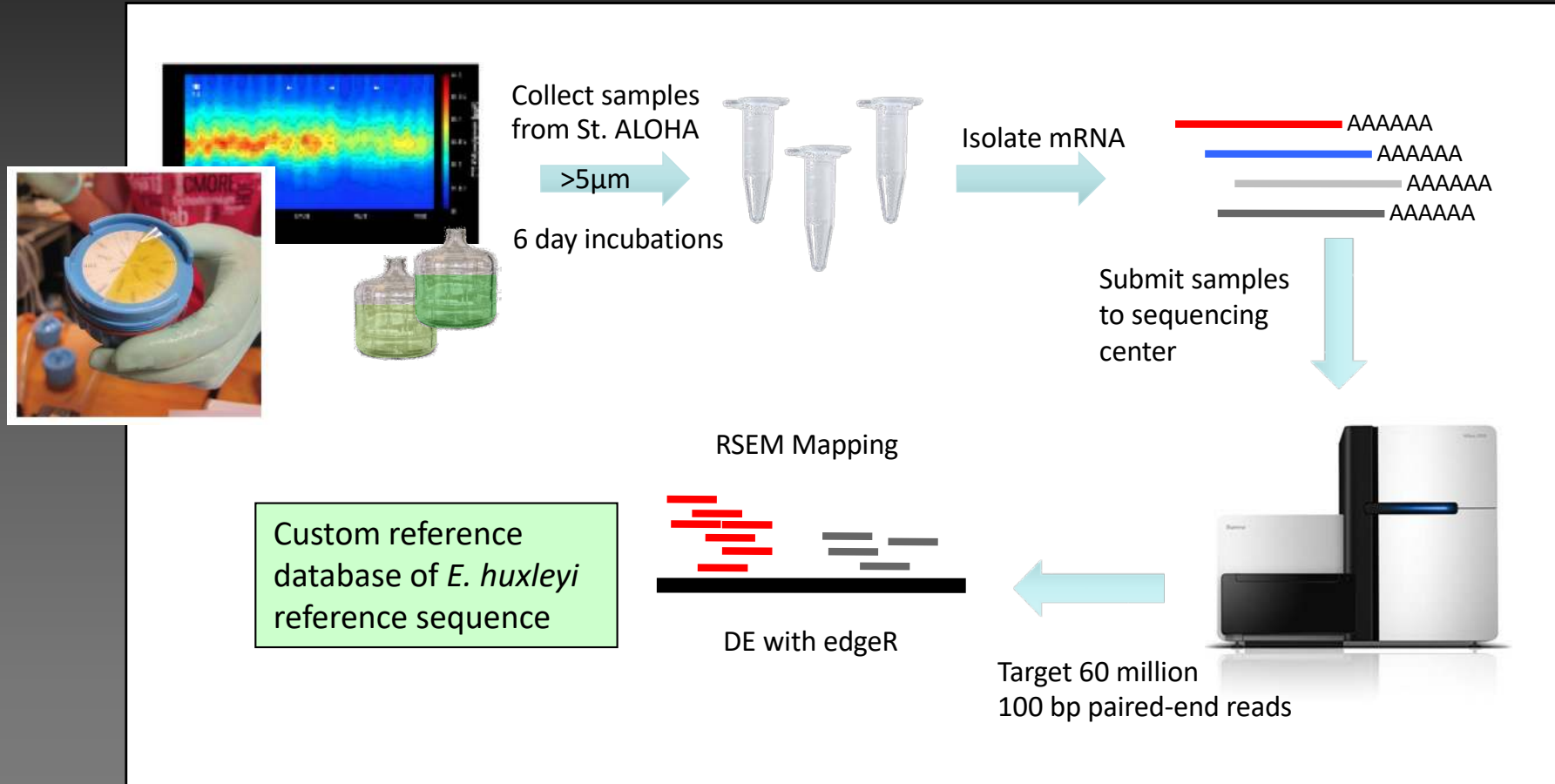
(+P, Si, Fe,
 B_{12})



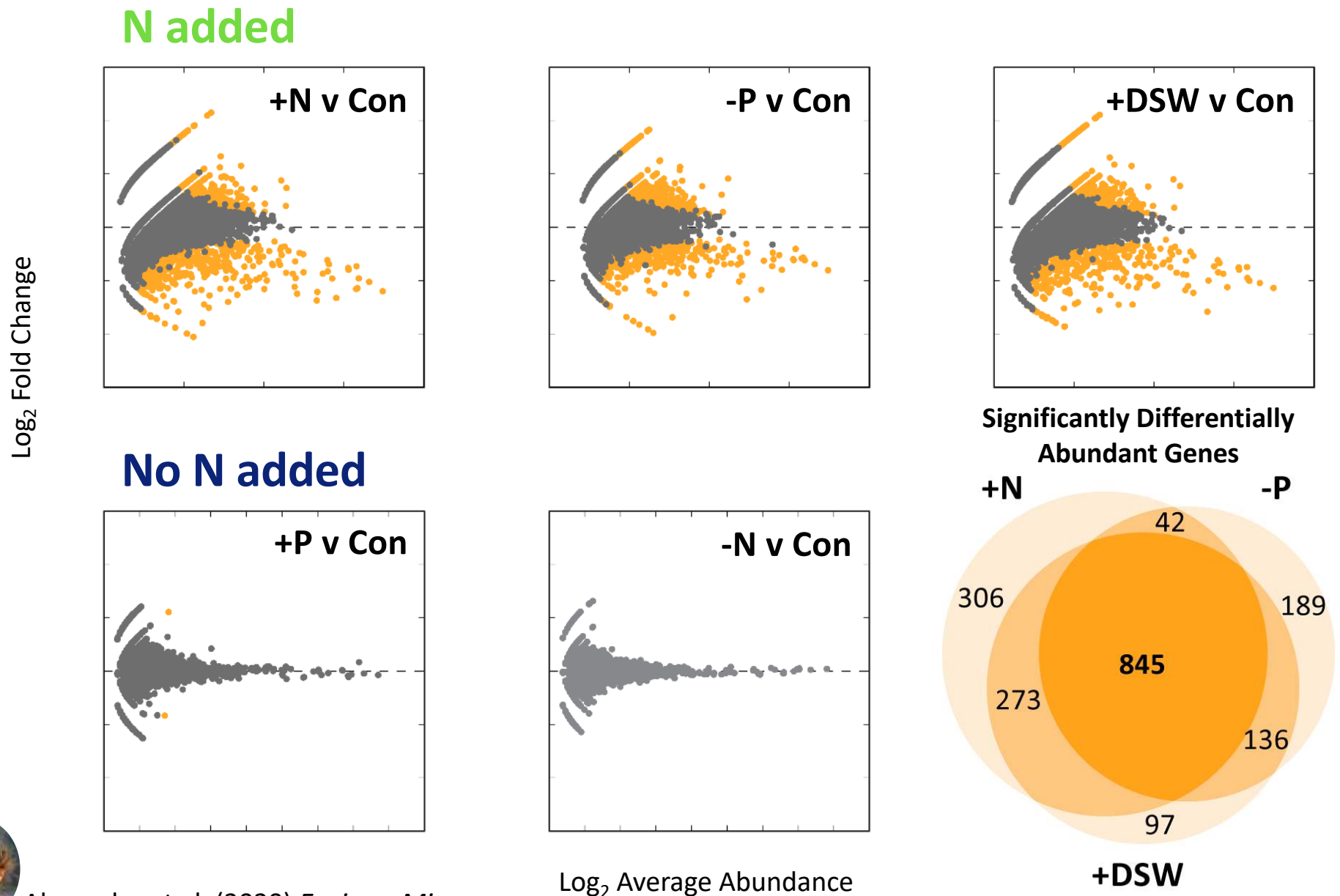
N addition



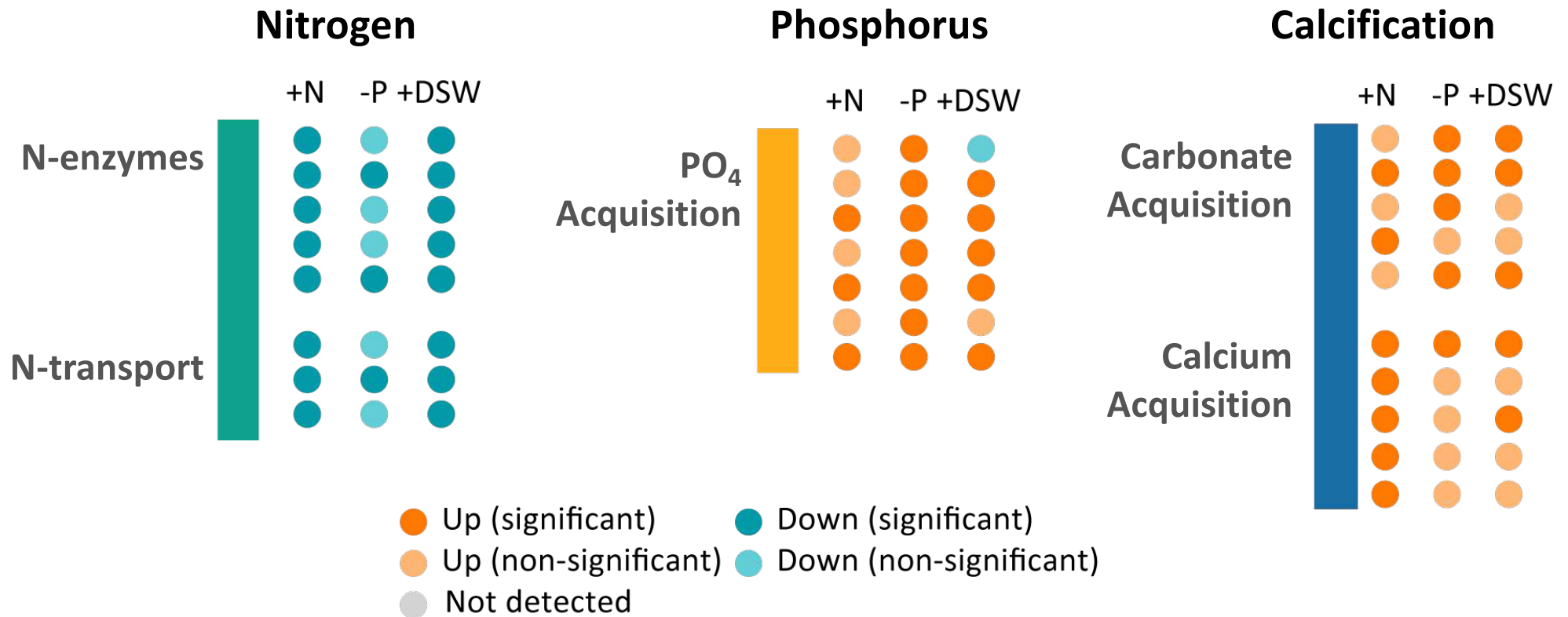
Sampling and pipeline



E. huxleyi physiological response suggests N control



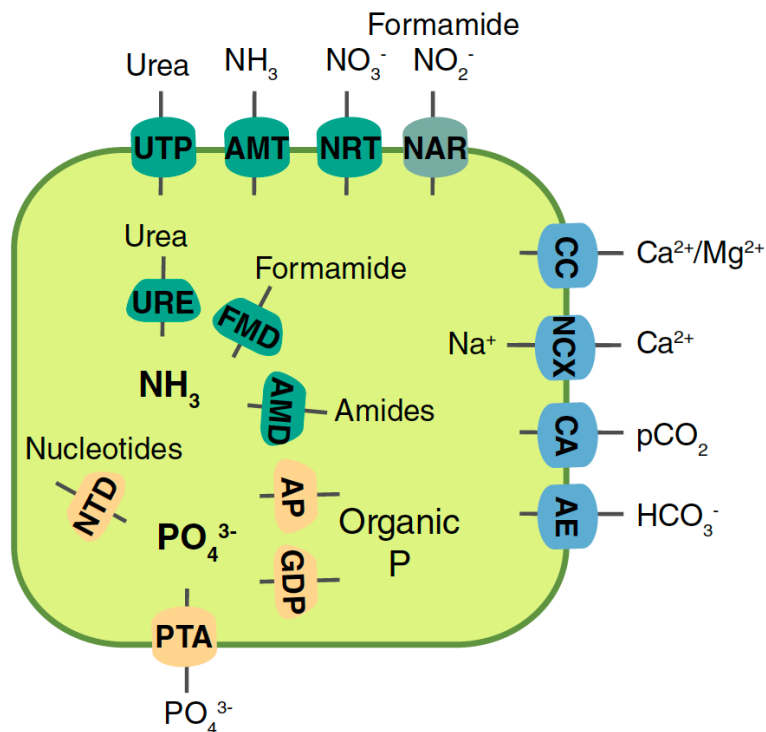
Transcriptional response



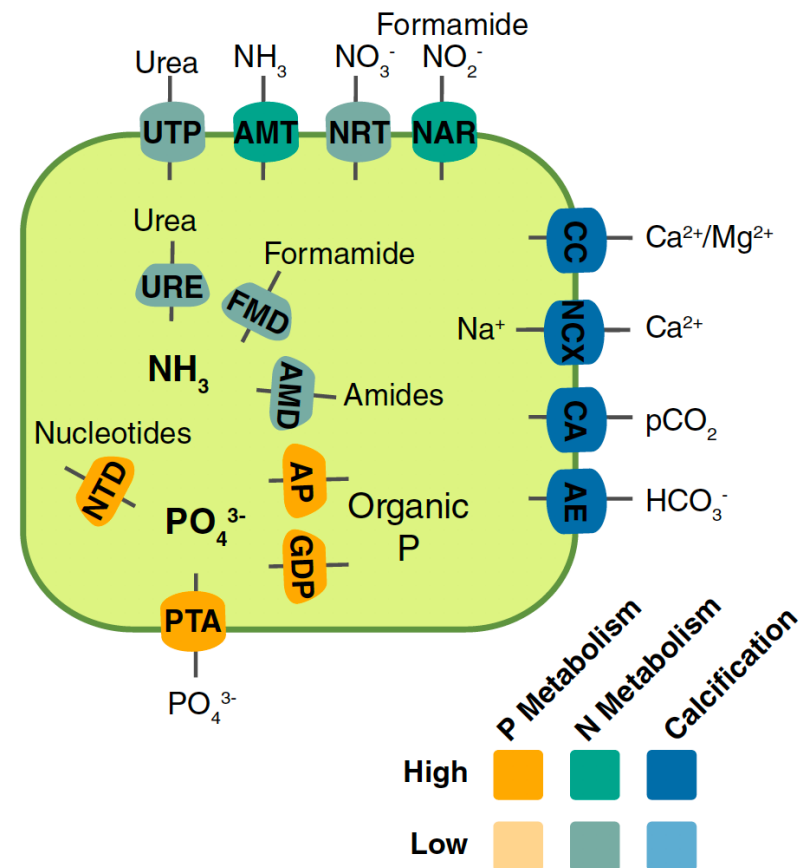
Alexander et al. (2020) *Environ. Micro.*

Nitrogen limits *E. huxleyi* growth and calcification in NPSG

Unamended Control

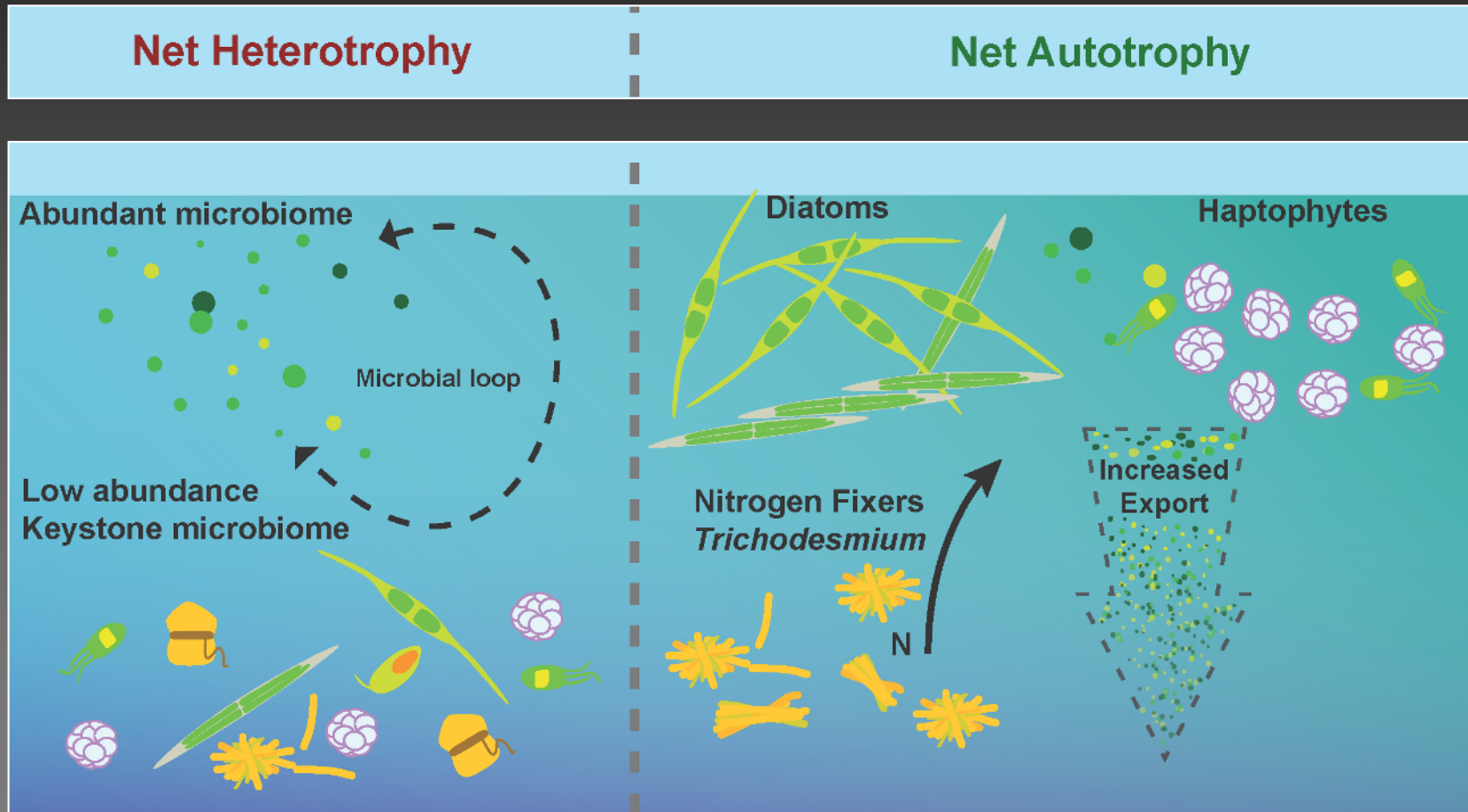


N-addition



Alexander et al. (2020) *Environ. Micro.*

Nitrogen is a driver of *E. huxleyi* dynamics



→
NITROGEN

Emiliana huxleyi has many diverse isolates

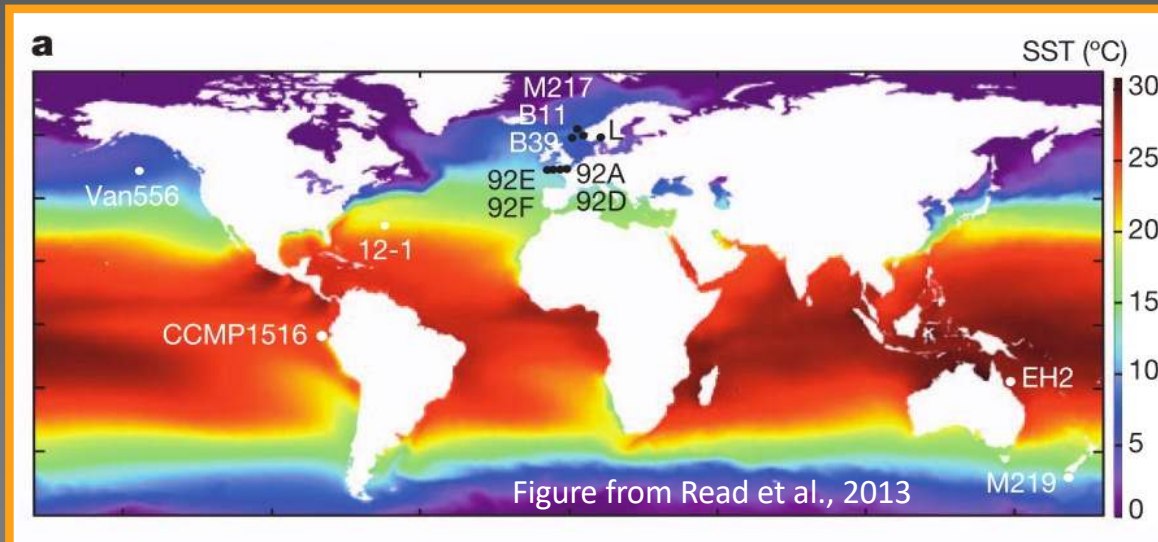
LETTER

OPEN

doi:10.1038/nature12221

Pan genome of the phytoplankton *Emiliana* underpins its global distribution

Betsy A. Read¹, Jessica Kegel², Mary J. Klute³, Alan Kuo⁴, Stephane C. Lefebvre⁵, Florian Maumus⁶, Christoph Mayer^{7,8}, John Miller⁹, Adam Monier¹⁰, Asaf Salamov⁴, Jeremy Young¹¹, Maria Aguilar³, Jean-Michel Claverie¹², Stephan Frickenhaus^{2,13}, Karina Gonzalez¹⁴, Emily K. Herman³, Yao-Cheng Lin¹⁵, Johnathan Napier¹⁶, Hiroyuki Ogata¹², Analissa F. Sarno¹, Jeremy Shmutz^{4,17}, Declan Schroeder¹⁸, Colomban de Vargas¹⁹, Frederic Verret²⁰, Peter von Dassow²¹, Klaus Valentin², Yves Van de Peer¹⁵, Glen Wheeler^{18,22}, *Emiliana huxleyi* Annotation Consortium†, Joel B. Dacks^{3*}, Charles F. Delwiche^{9*}, Sonya T. Dyhrman^{23,24*}, Gernot Glöckner^{25*}, Uwe John^{2*}, Thomas Richards^{26*}, Alexandra Z. Worden^{10*}, Xiaoyu Zhang^{27*} & Igor V. Grigoriev⁴



Cultured strains are highly diverse - isolated from a broad temperature range and displaying considerable physiological diversity

Baas Becking hypothesis

“Everything is every where, but
the environment selects”

Does the variable component of the pan-genome underpin
which strain dominates a given environment?

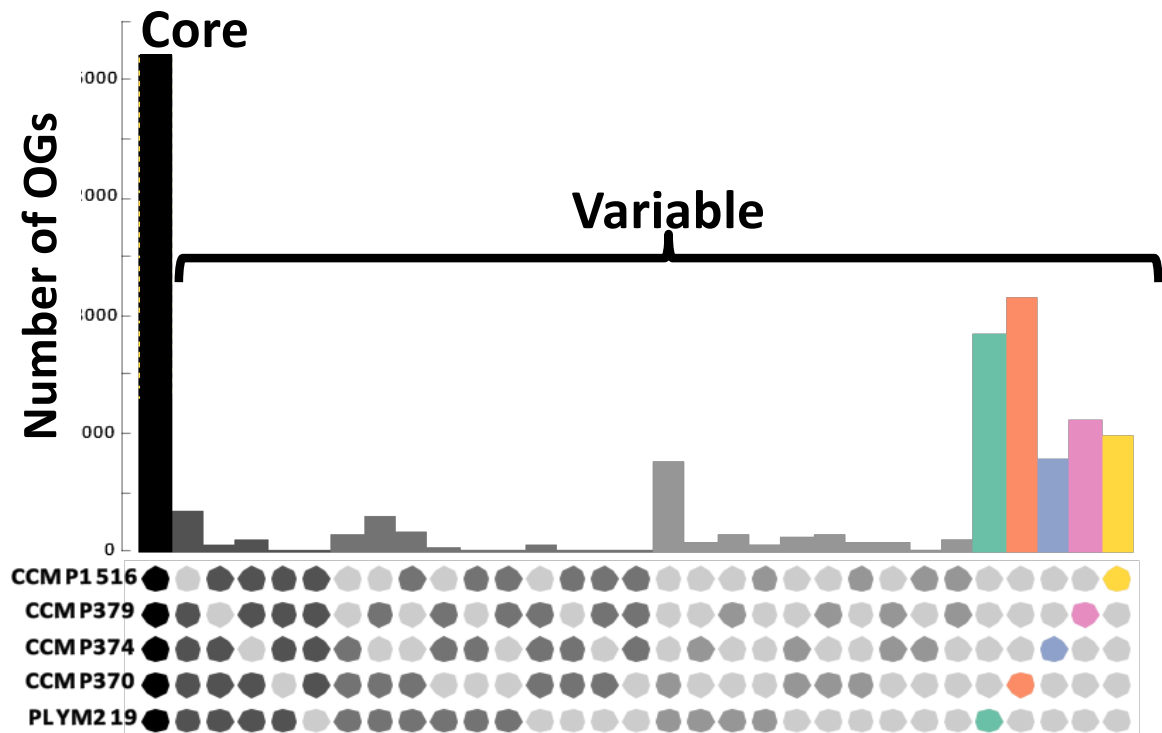
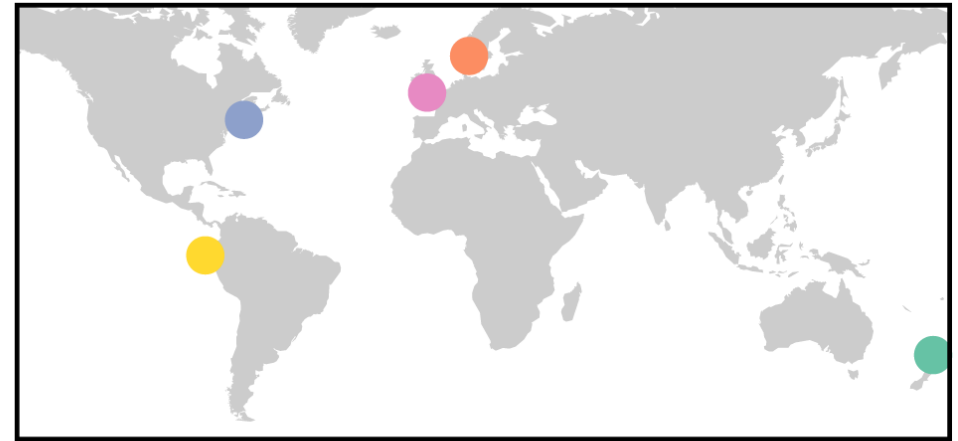
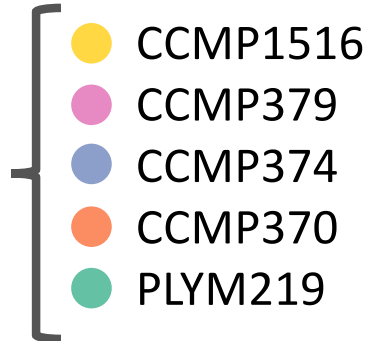
Sequences from
E. huxleyi strains



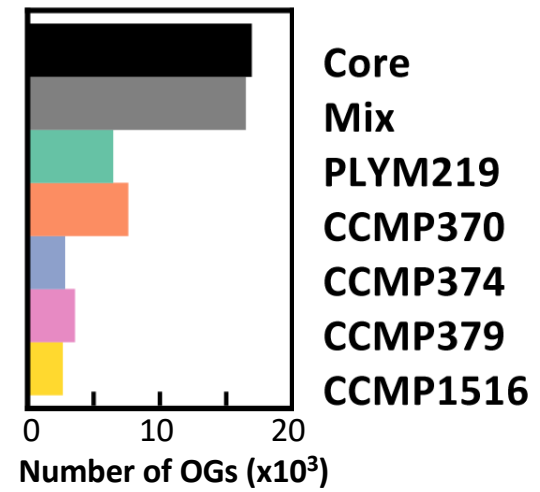
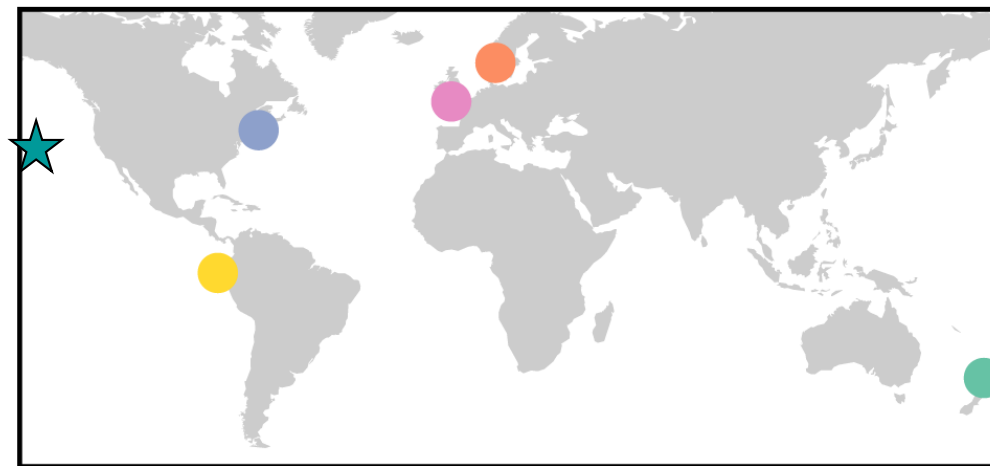
Cluster into
orthologous groups to
identify core and
variable OGs
(OrthoMCL)



Map field data and
examine shifts in
expression
(RSEM, edgeR)

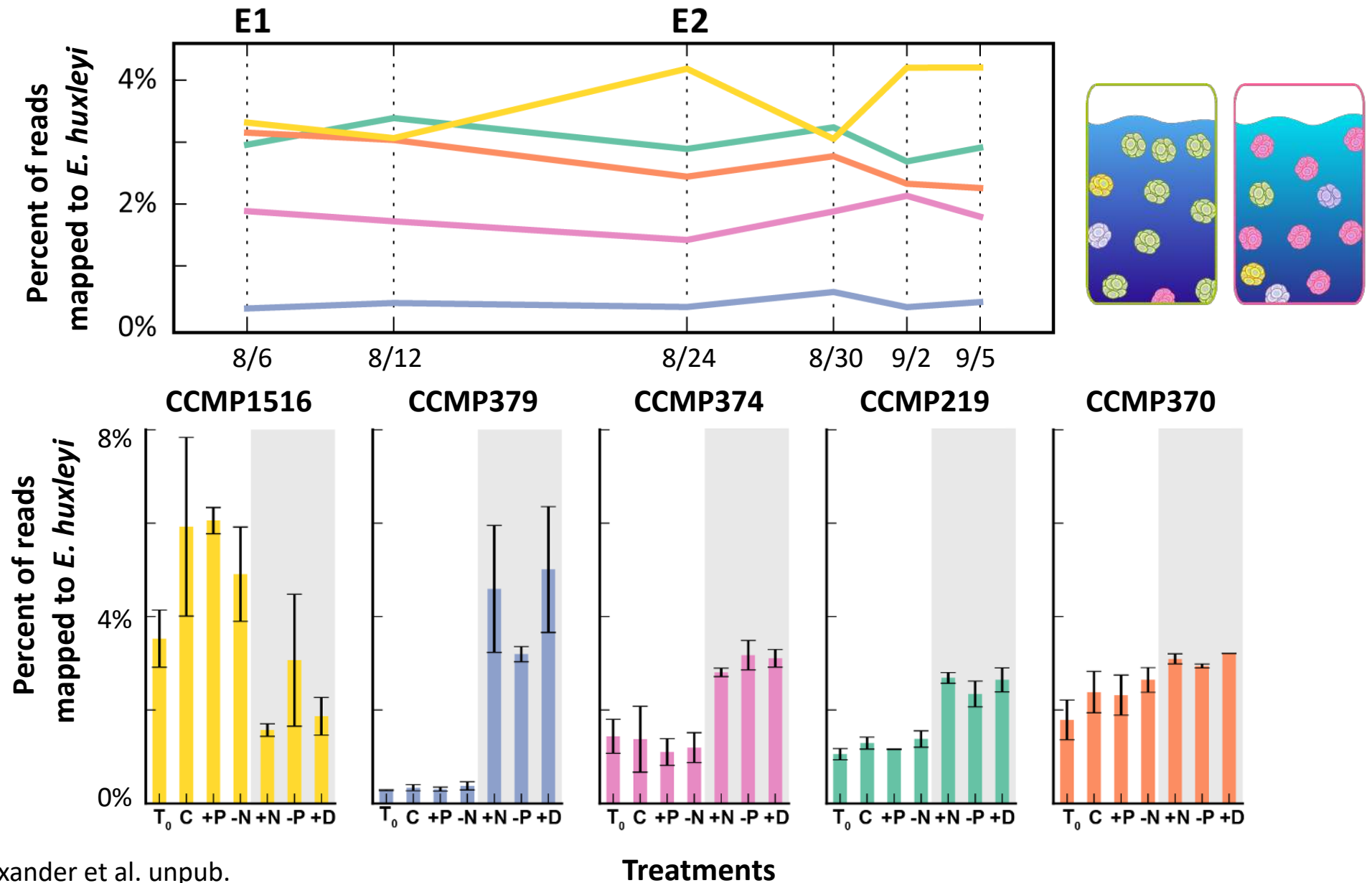


Tracking orthologous groups at St. ALOHA

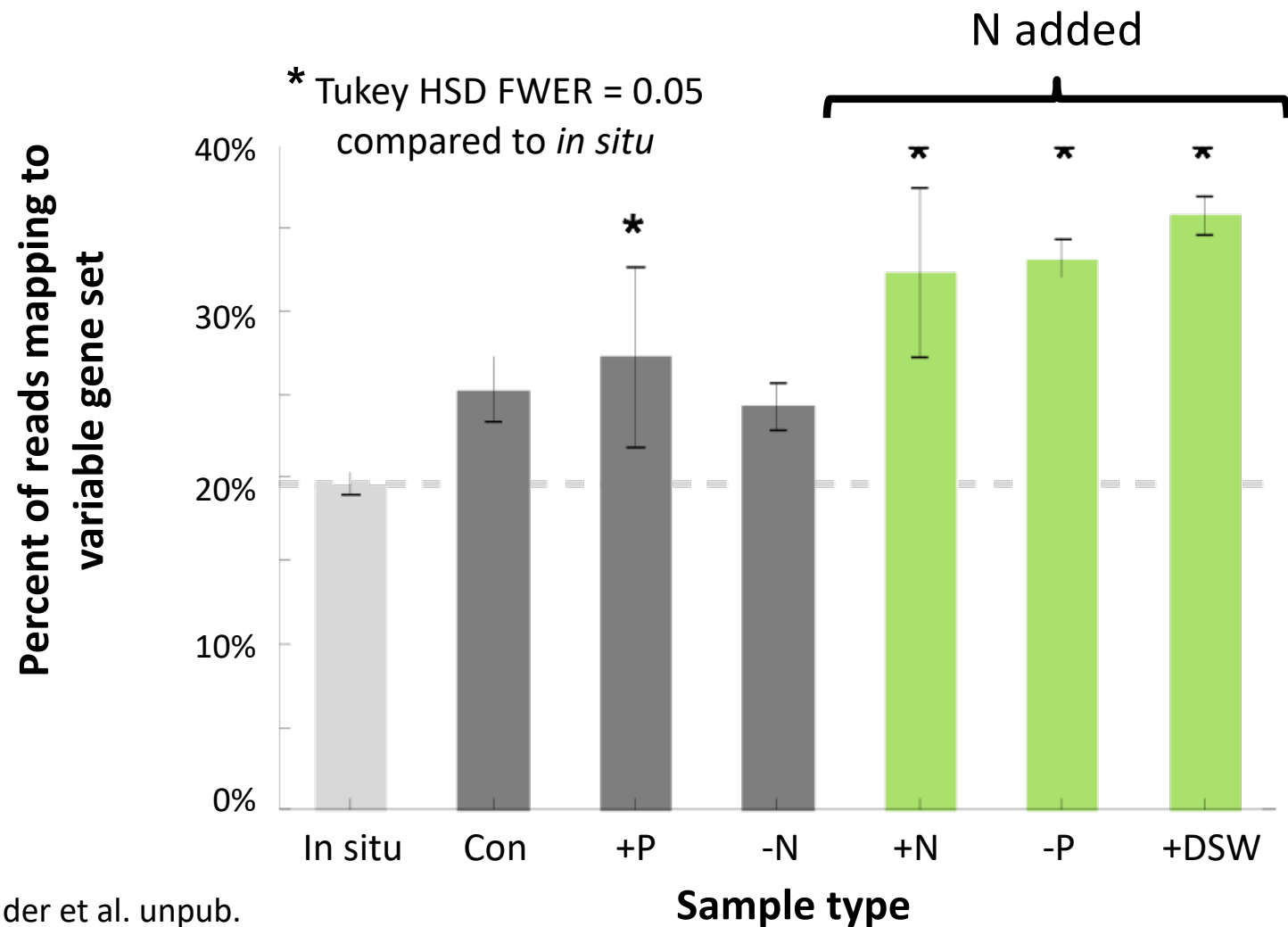


Use diagnostic OGs to track strain distribution

Strain distribution *in situ* and in the incubations

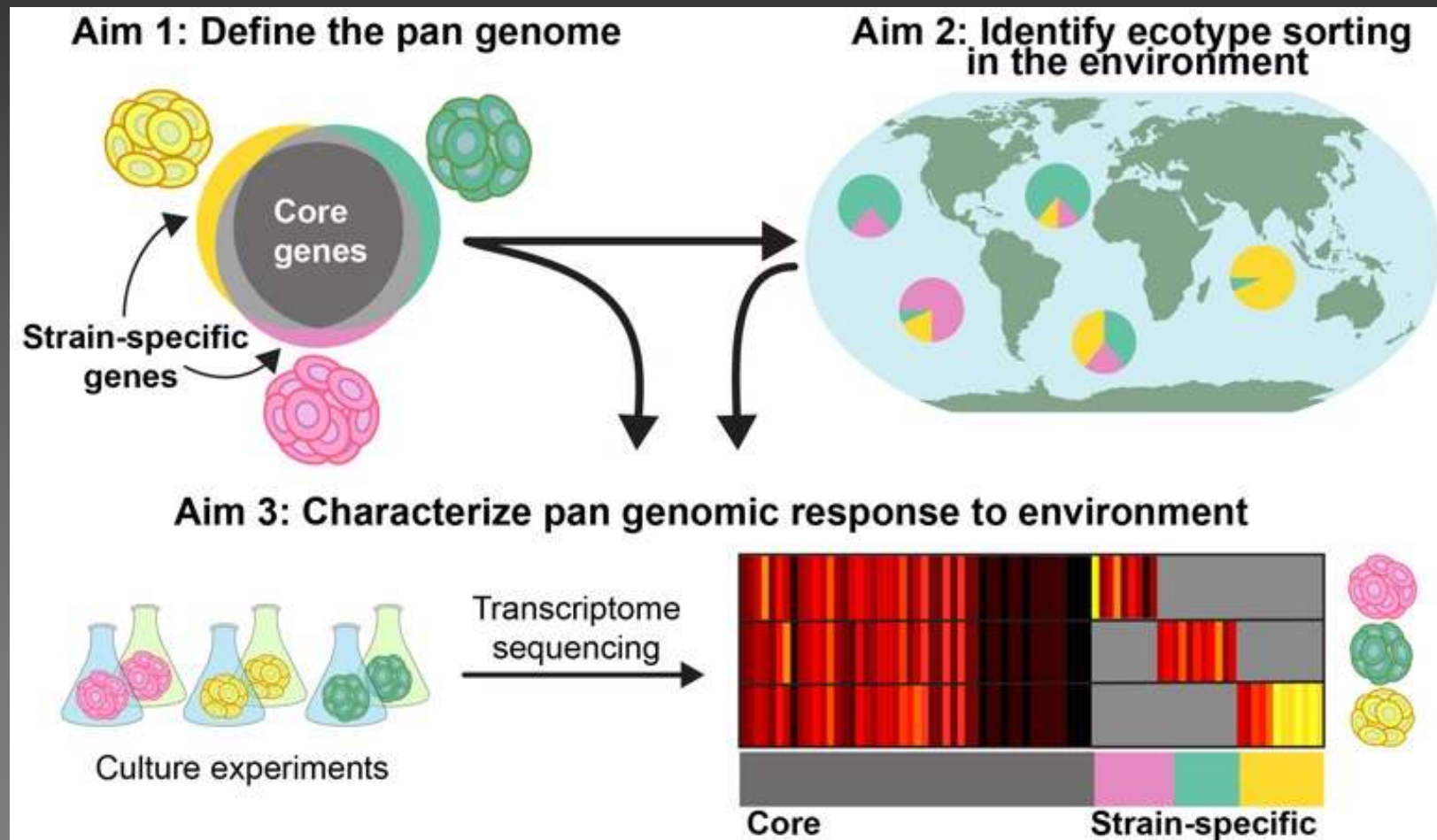


Expression of the variable portion of the pan-genome



Expression of variable gene set significantly increased following N-addition

Future work to define the pan genome

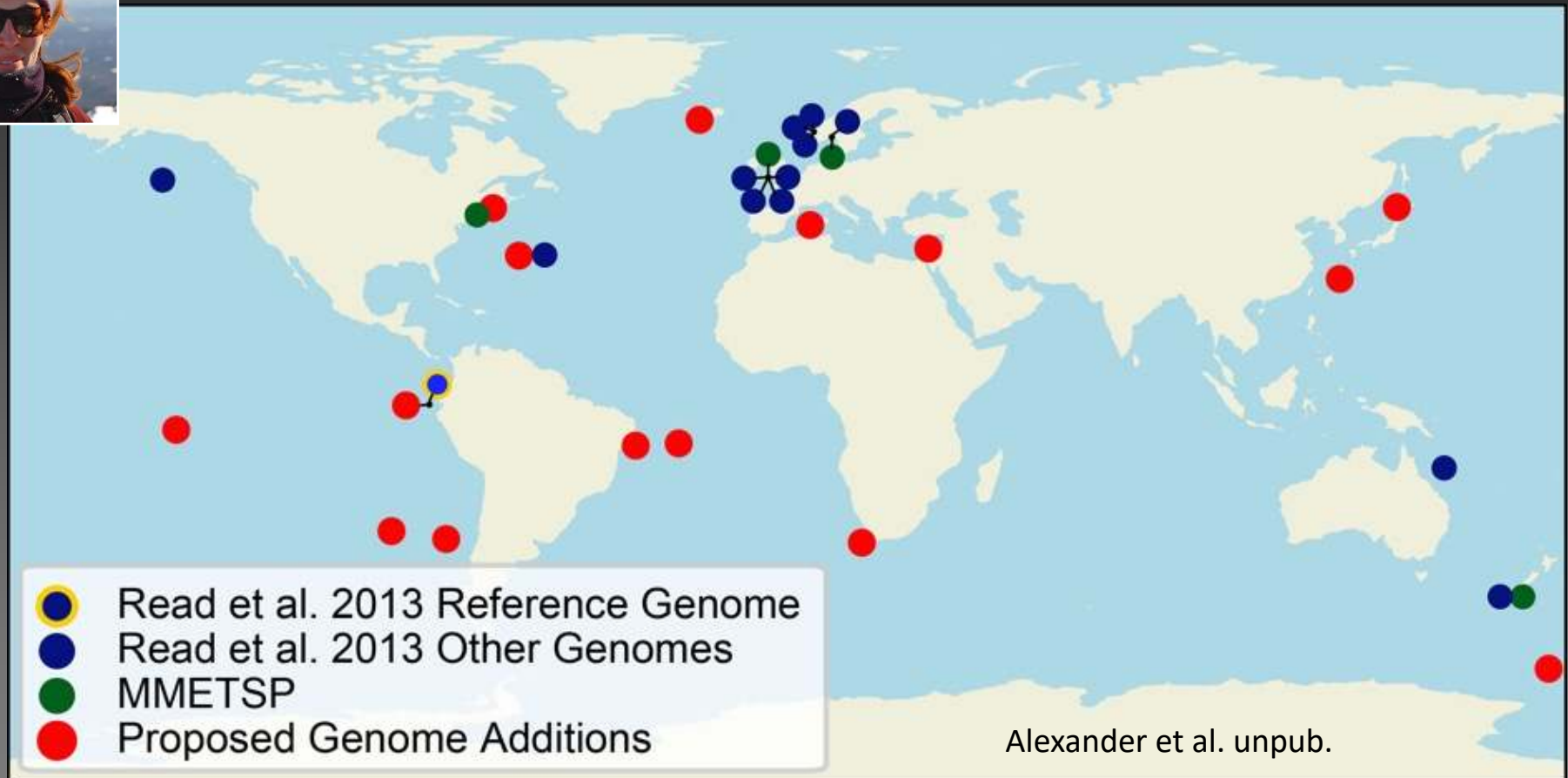


Alexander et al. unpub.

Expanding the pan genome



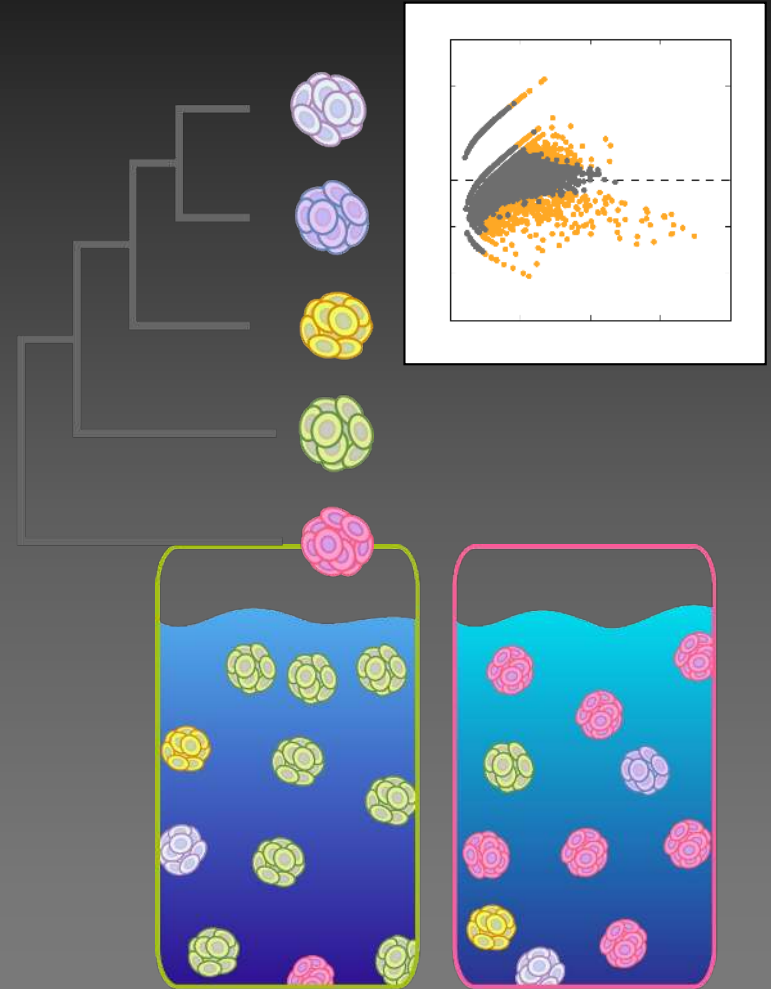
Harriet Alexander



~15 new whole genome sequences underway (Nanopore/Illumina)
RNASeq libraries to support gene prediction

Summary

- **Metatranscriptome RNA-Seq:**
- Species-specific analyses are possible even for low abundance keystone populations.
- Nitrogen limits *E. huxleyi* activity, and influences calcification
- Variable genes in the pan-genome may influence ecotype strain sorting.
- Move towards predictive responses of the chemical –microbe network in a changing ocean



Core questions... adventures in eukaryote metatranscriptomics

Limitation

- **Nitrogen limits carbon export and cycling in a keystone haptophyte**
- **The environment selects**

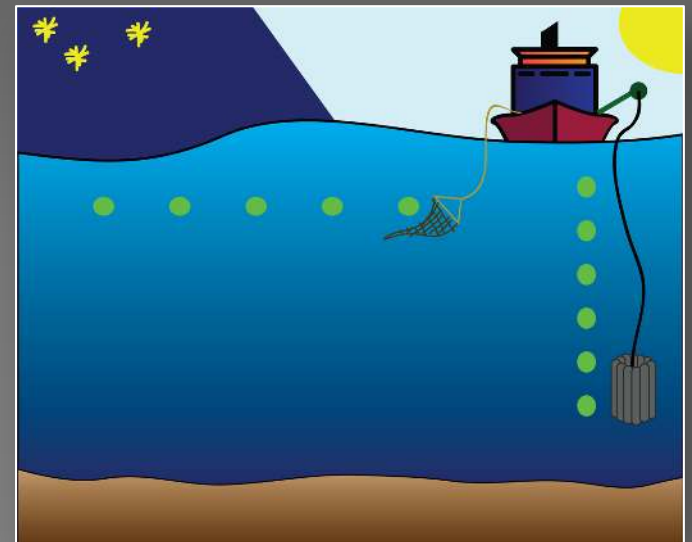
Competition

- **A day in the life – tracing competitive interactions with multi-'omics**

HOE:DYLAN



HOE:MAHALO



Hawaii Ocean Experiment: Dynamics of Light (MAHALO)



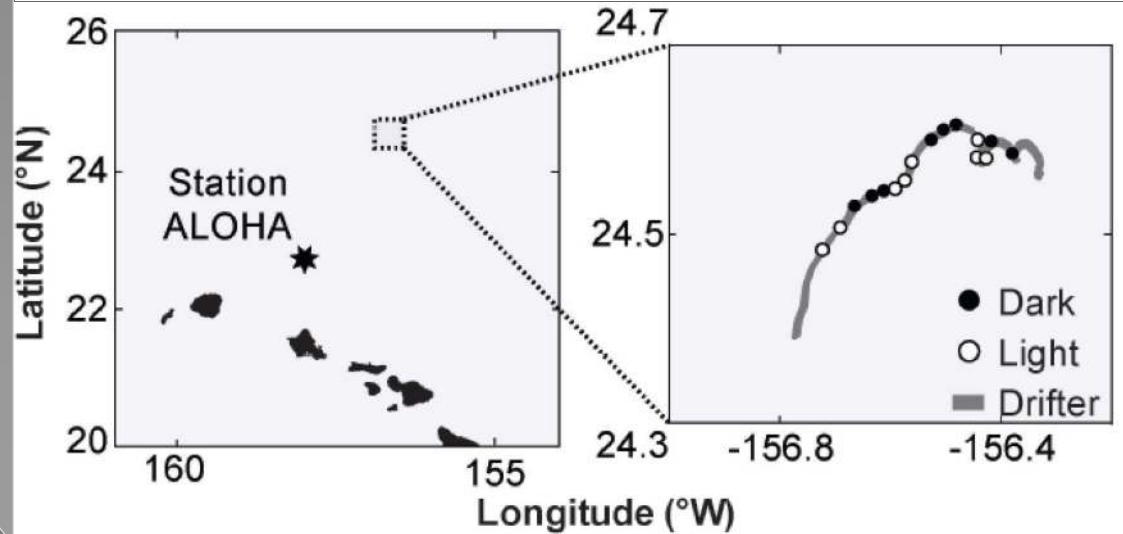
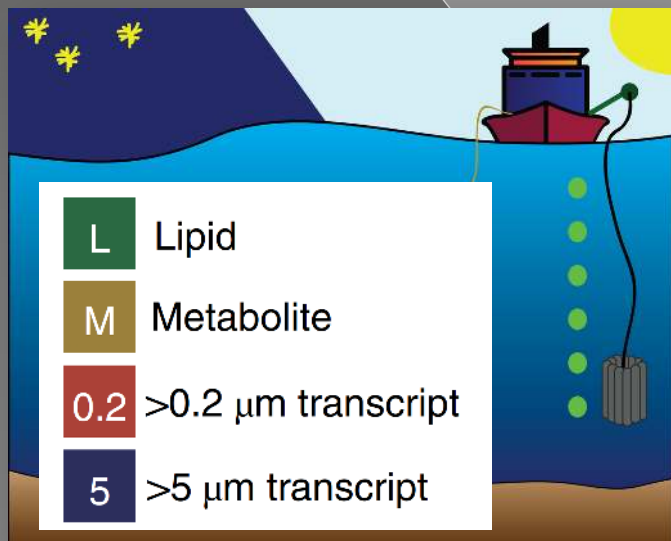
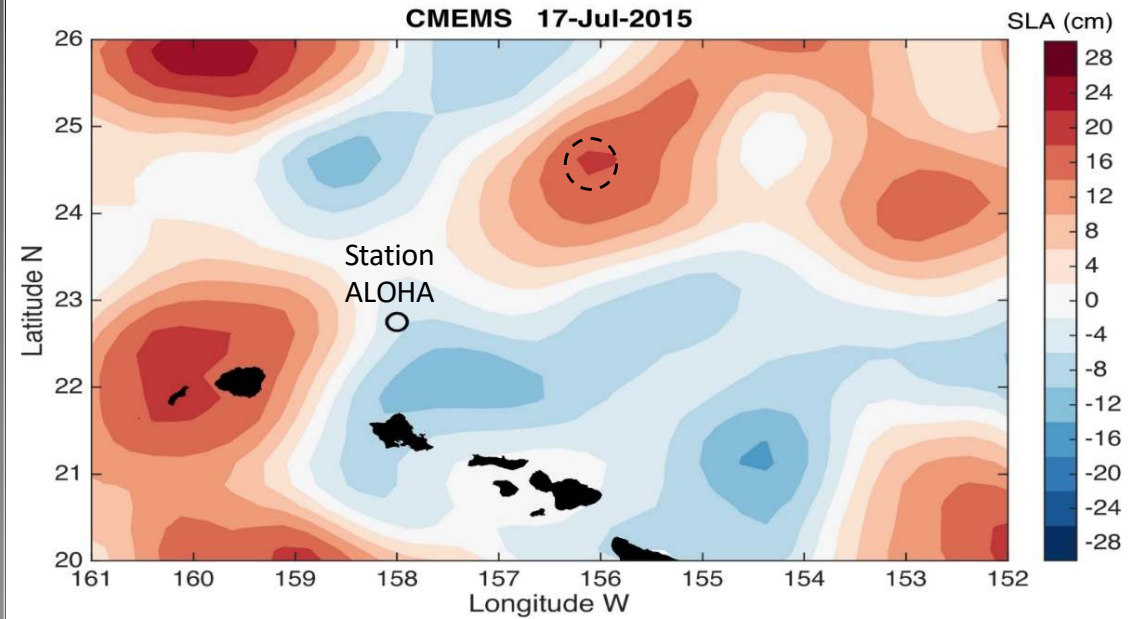
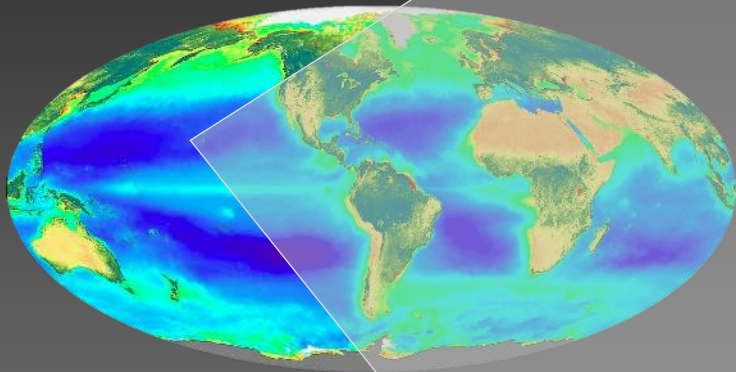
Figure 1 R/V Kilo Moana conducting one of the diel study CTD casts in 2015 (photo: Tara Clemente, taken from the R/V Ka'imikai-O-Kanaloa)



MAHALO: Special thanks to Daniel Muratore and Joshua Weitz for synthesis efforts



Sampling light driven responses in the NPSG



Contributor

Nature Eco Evo

BEHIND THE PAPER

Microbes running on time

Diel Cycling of Marine Microbial Metabolism in Complex Communities Partitions
Scarce Nutrients and Enables Emergent Biogeochemical Function



Matthew Harke and 3 others [View all](#)

+3

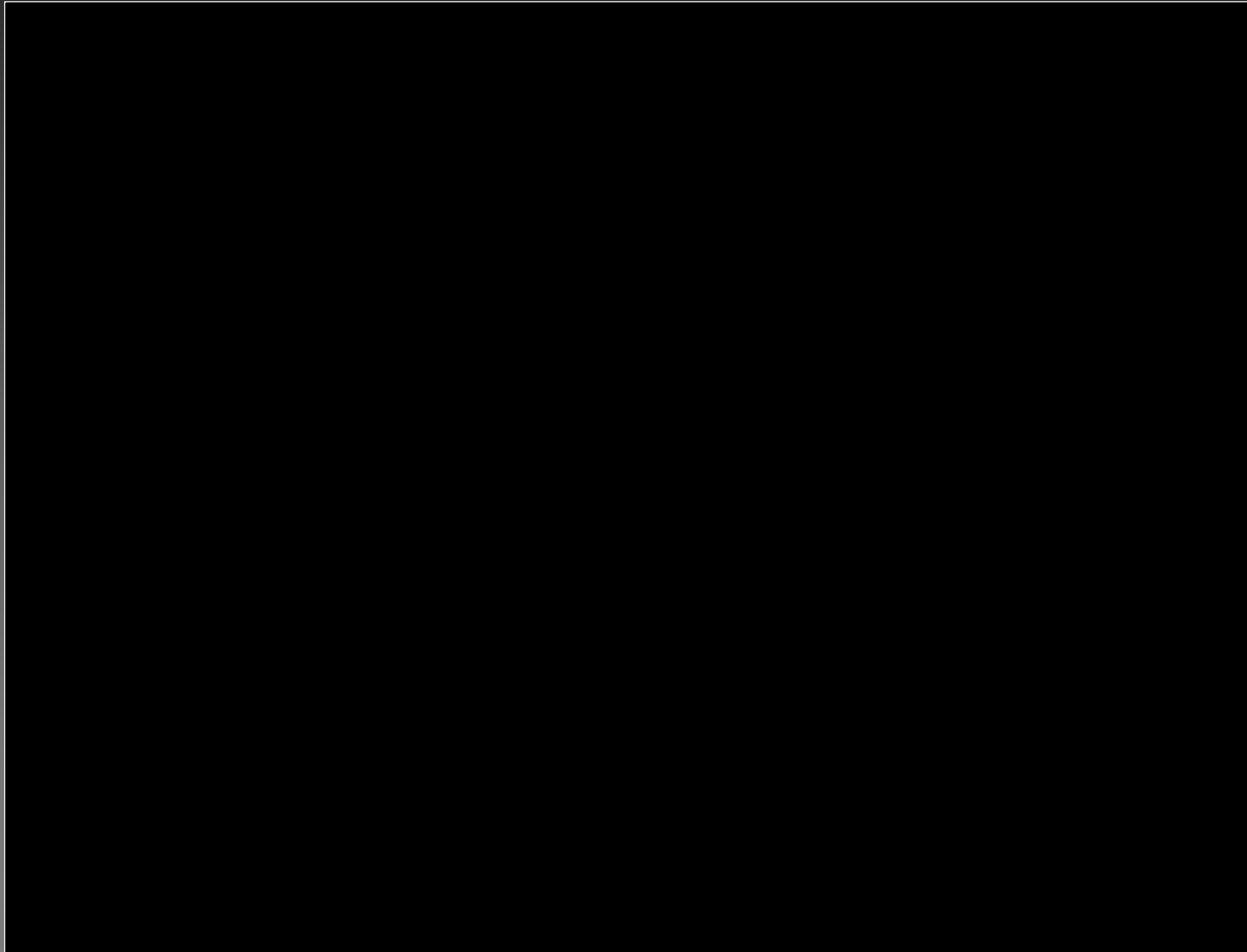
Research Scientist, Gloucester Marine Genomics Institute

 Follow

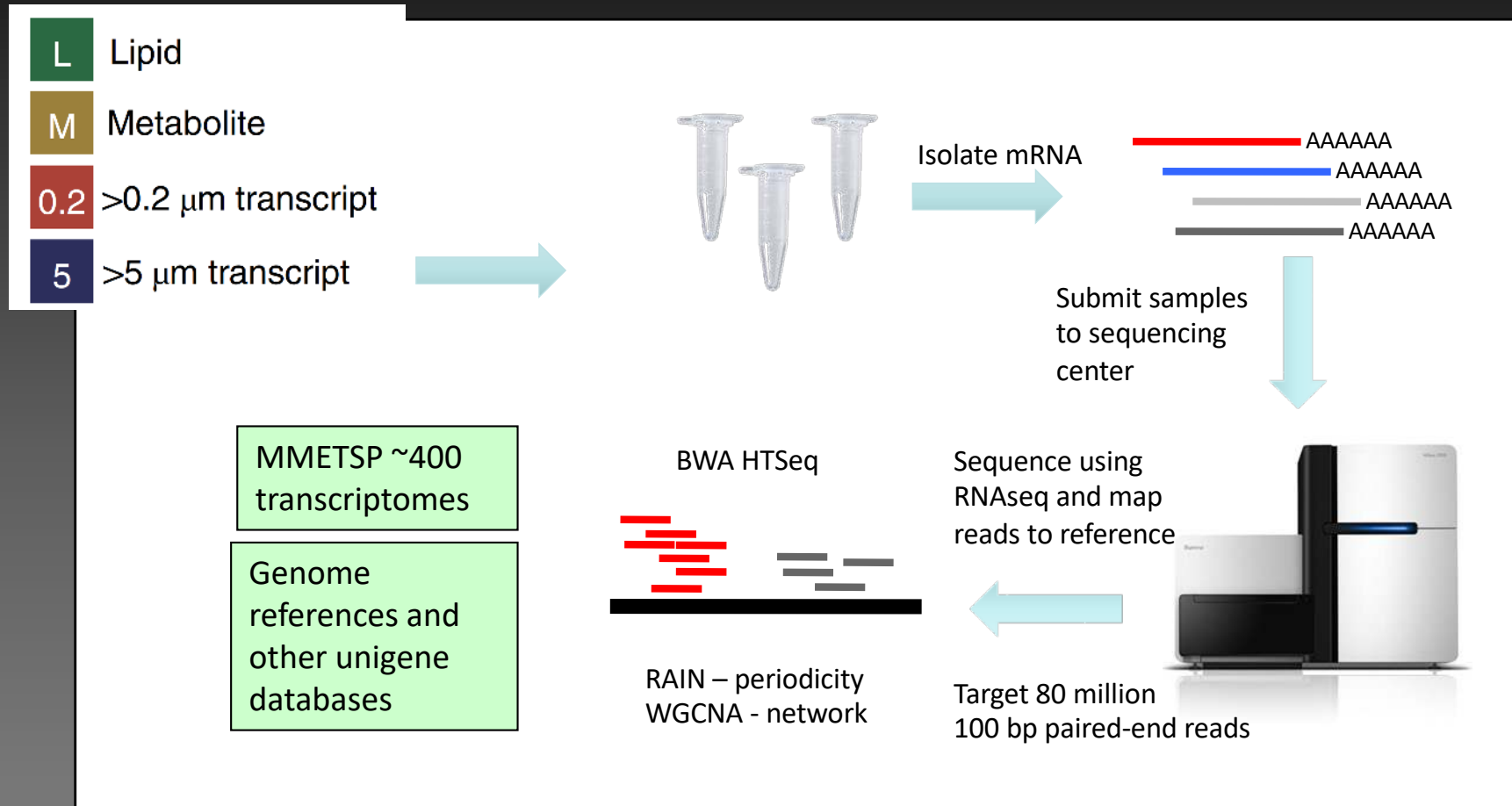


Muratore, Boysen, Harke et al. (2022) *Nature Ecol Evo*.

Sampling every 4hrs for many days



Sampling and pipeline

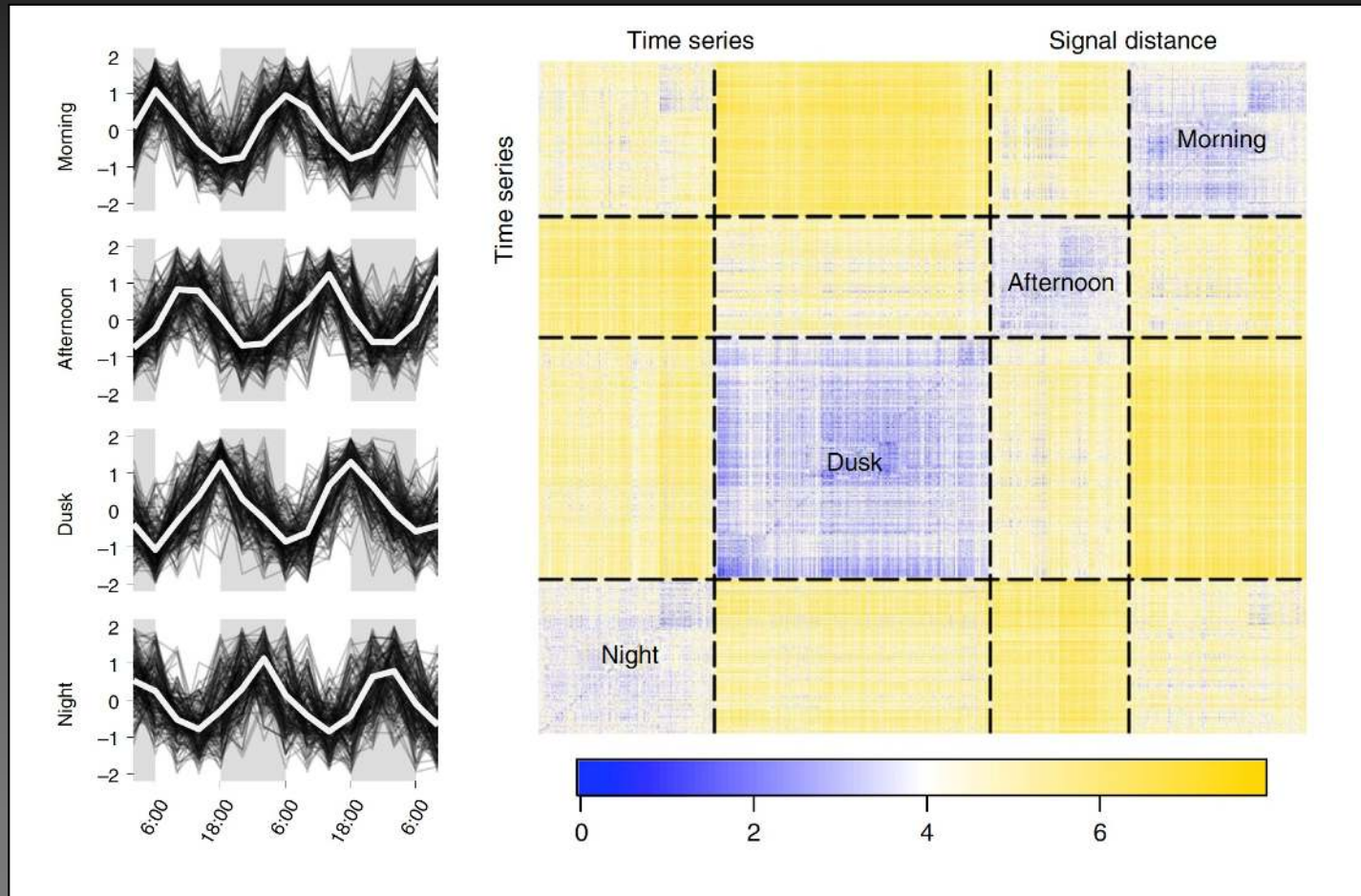


<https://omictools.com/rhythmicity-analysis-incorporating-nonparametric-methods-tool>

<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-9-559>

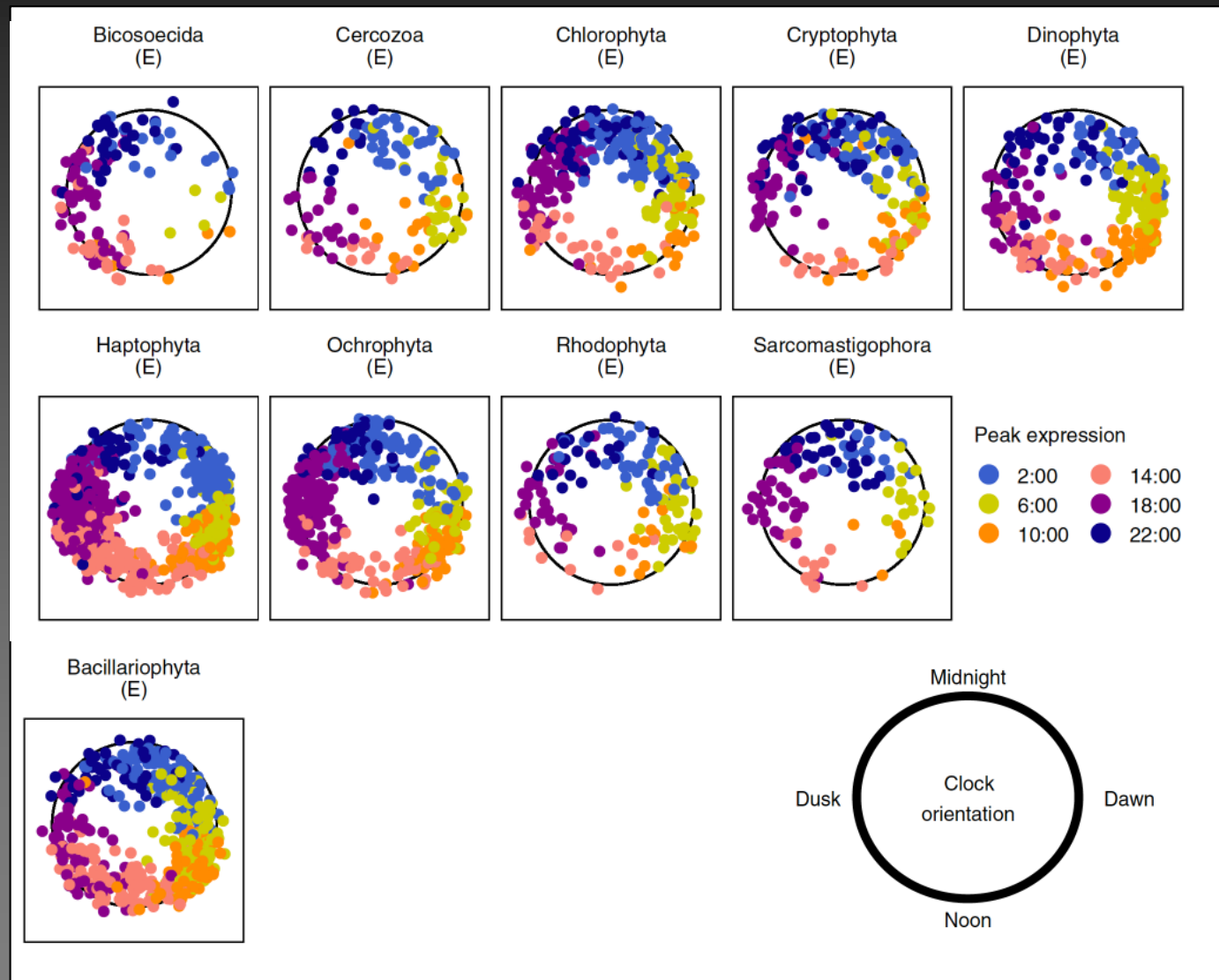
MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Archetypal patterns



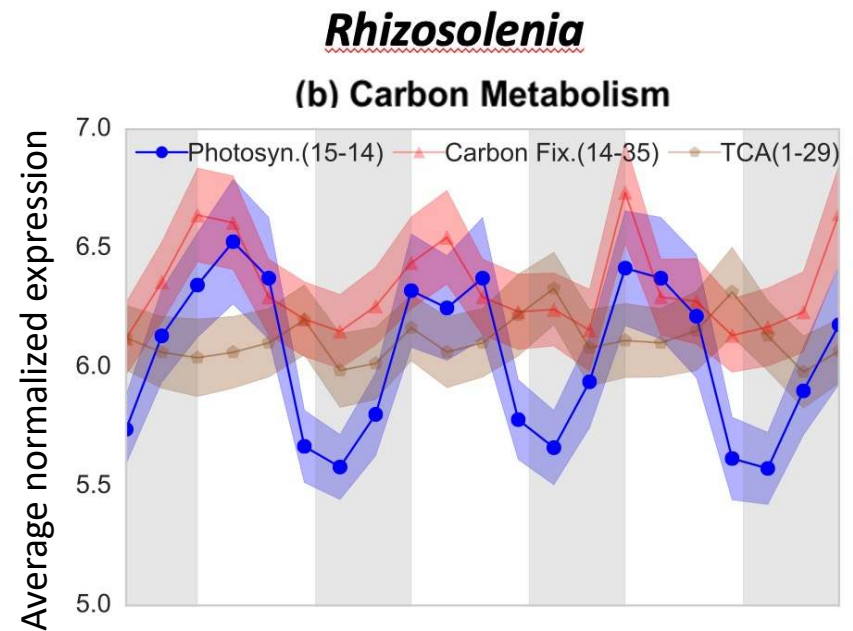
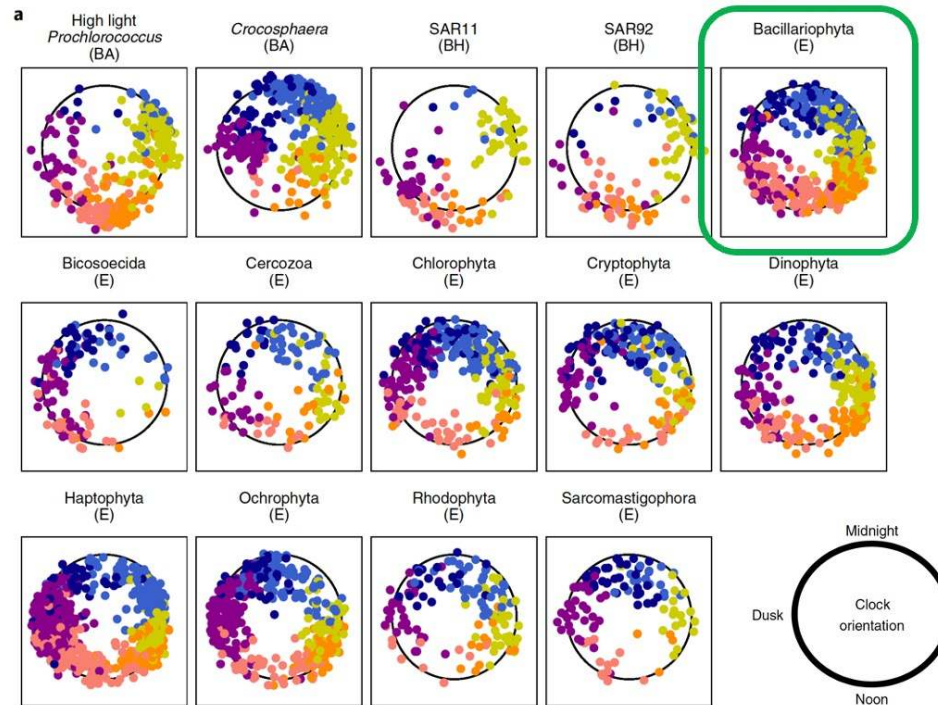
Muratore, Boysen, Harke et al. (2022) *Nature Ecol Evo.*

Different taxa have distinct diel transcription patterns



Muratore, Boysen, Harke et al. (2022) *Nature Ecol Evo.*

Carbon fixation and respiration are highly coordinated

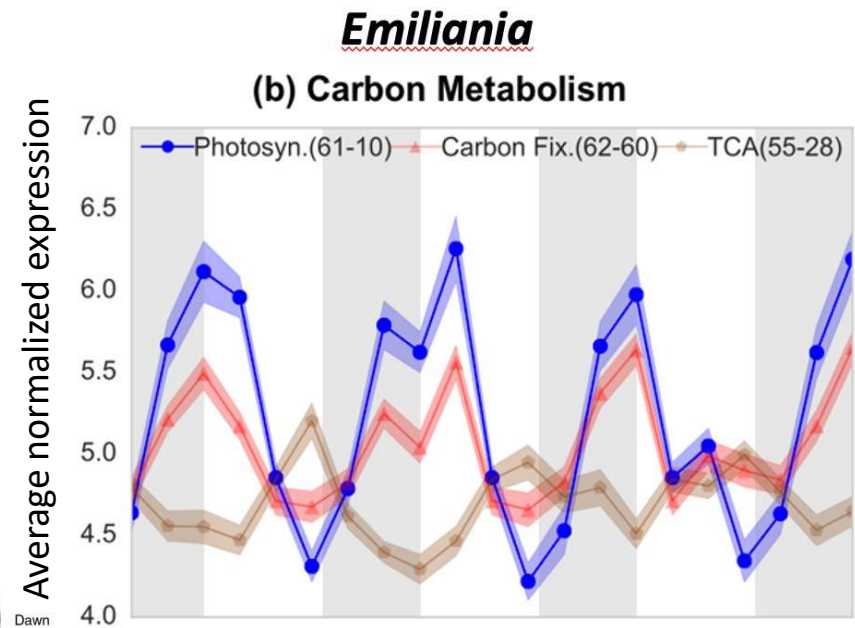
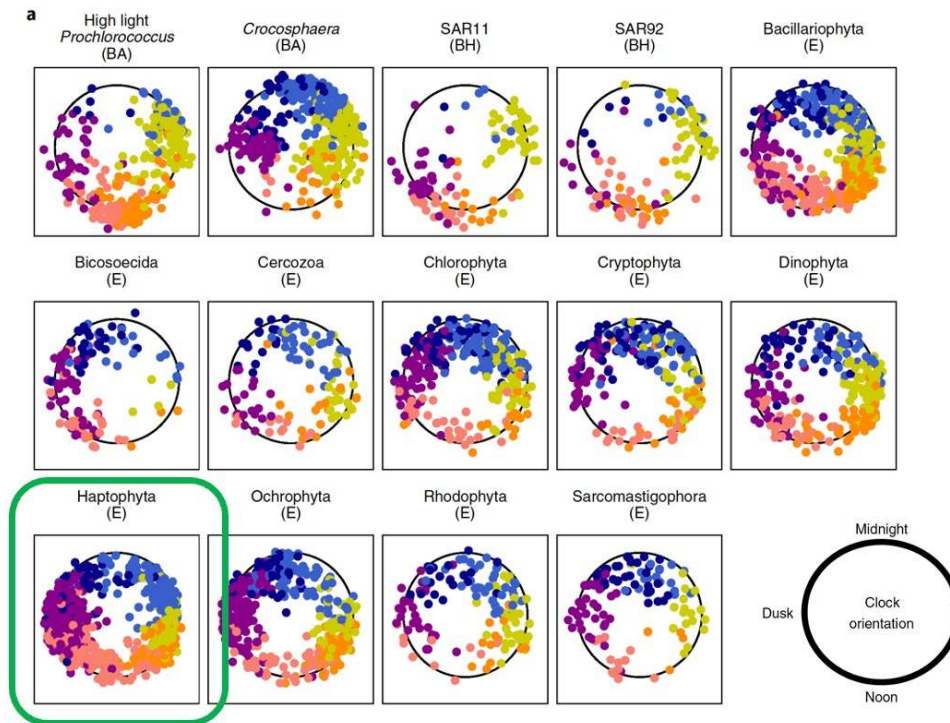


Muratore, Boysen, Harke et al. (2022) *Nature Ecol. Evol.*



Harke et al. 2019 *ISME J*

Carbon fixation and respiration are highly coordinated

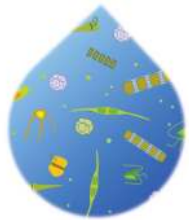
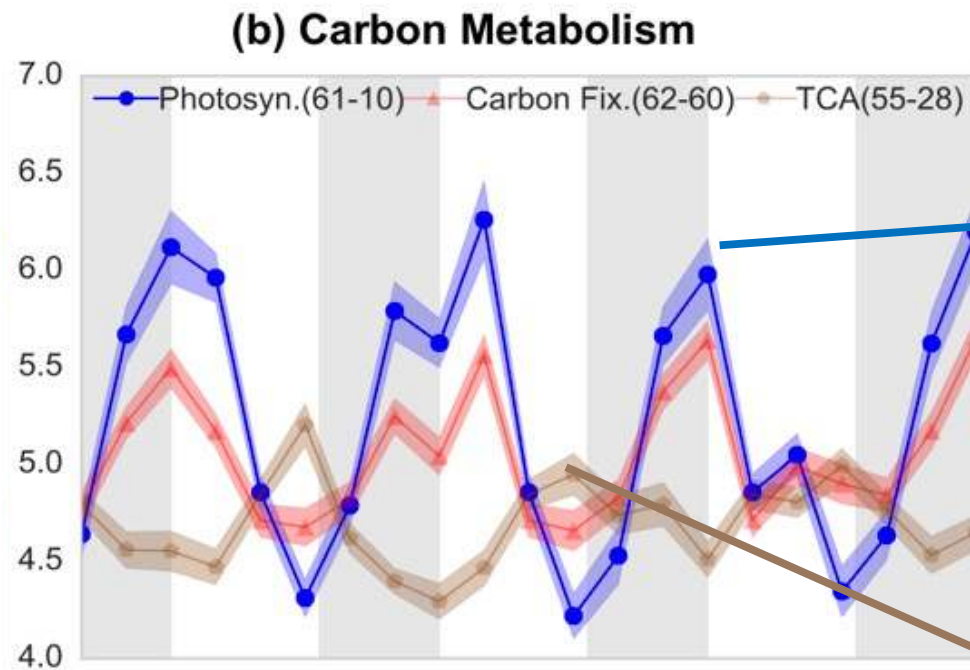


Muratore, Boysen, Harke et al. (2022) Nature Ecol. Evol.



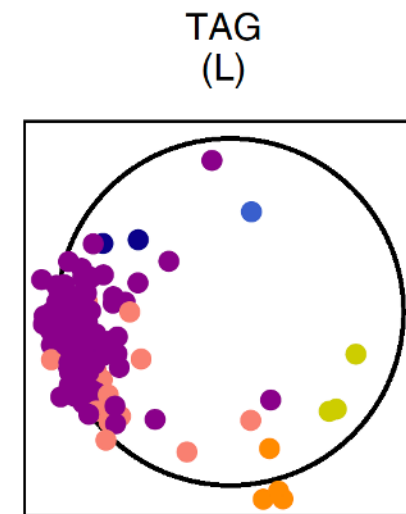
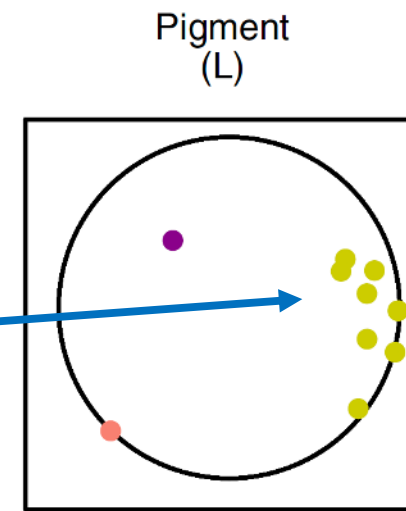
Limón-Hernandez et al. (2020) *Environ. Micro.*

Carbon metabolism synchronized across all phytoplankton

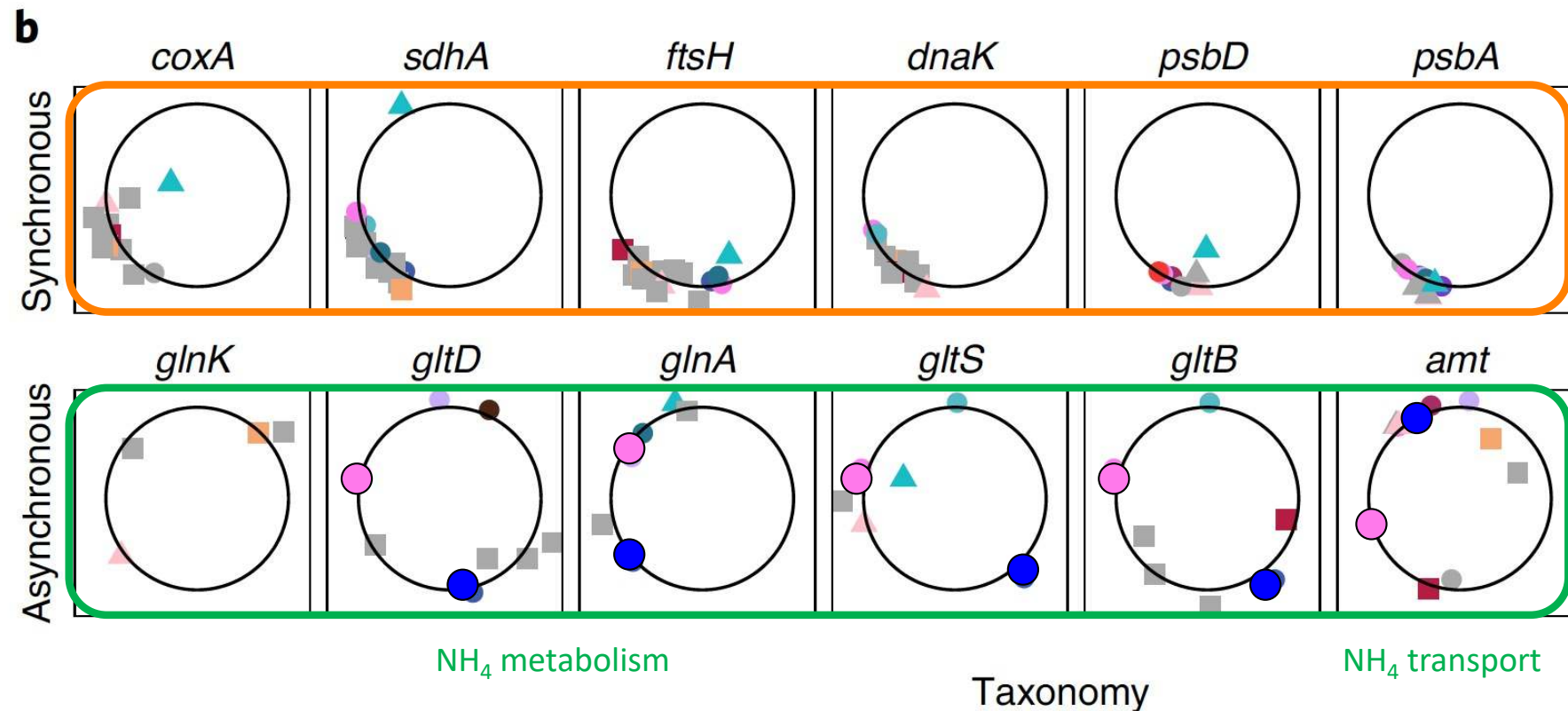


All photosynthesis, carbon fixation, and TCA cycle processes are synchronized across bacteria and eukaryotes!

Becker, Harke et al. (2021) *ISME J*

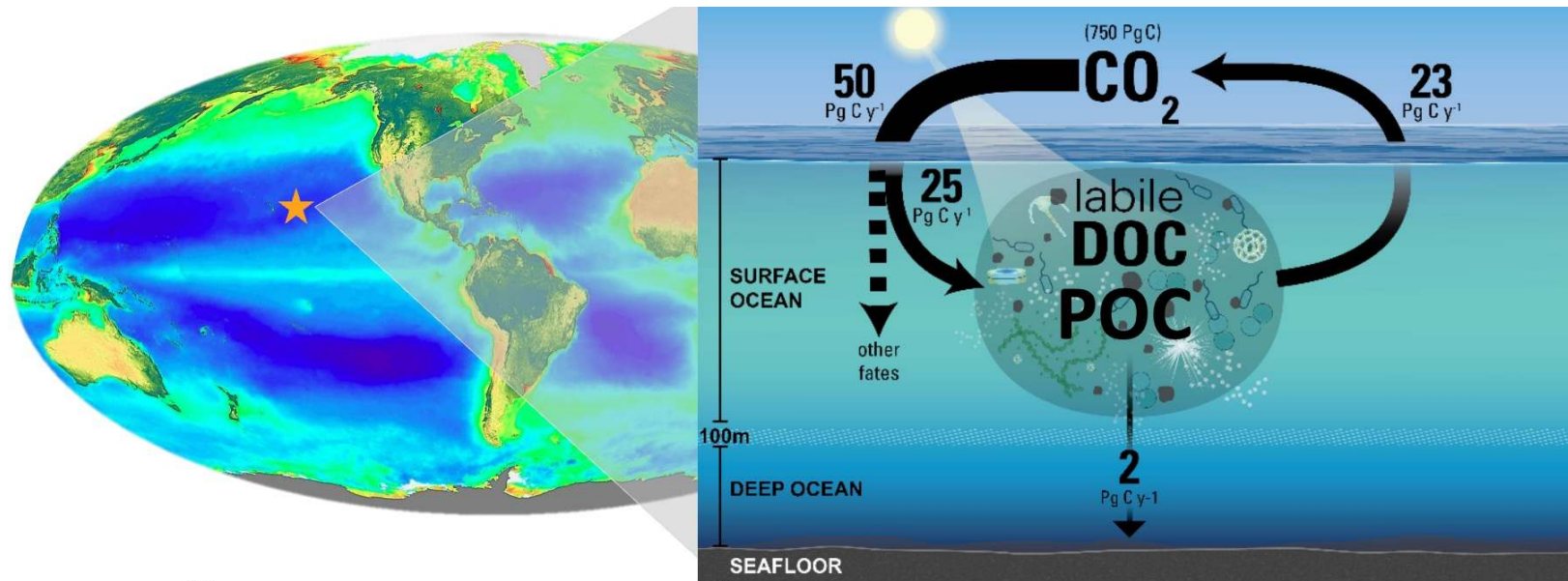


N metabolism functions are expressed asynchronously



Asynchronous transcription of N uptake and metabolism between microeukaryotes and between taxonomic domains suggest competition for limiting resource is driving temporal niche partitioning

Tracing carbon flow through the network

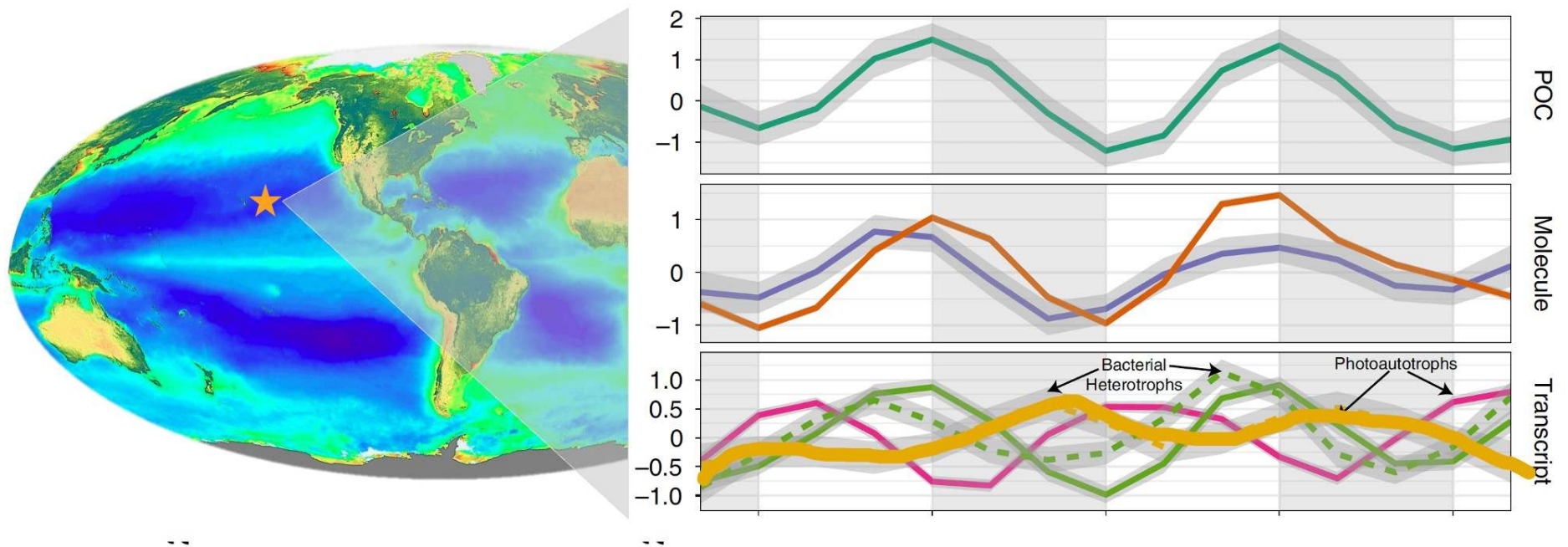


- POC
- Triacylglycerol
- Metabolite
- Photosynthesis
- TCA cycle
- Sugar transporters



Muratore, Boysen, Harke et al. (2022) *Nature Ecol. Evol.*

Tracing carbon flow through the network

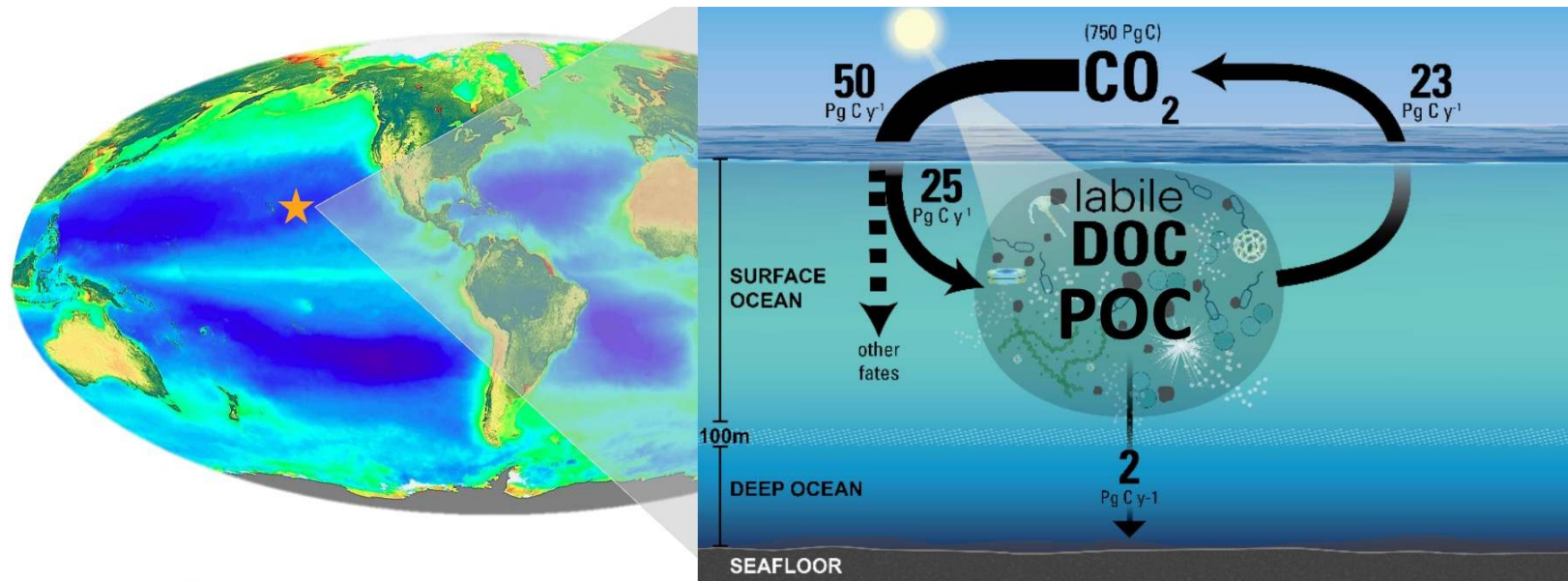


- POC
- Triacylglycerol
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Muratore, Boysen, Harke et al. (2022) *Nature Ecol. Evol.*

Multi-omics in the field: progress in resolving the microbial-chemical network



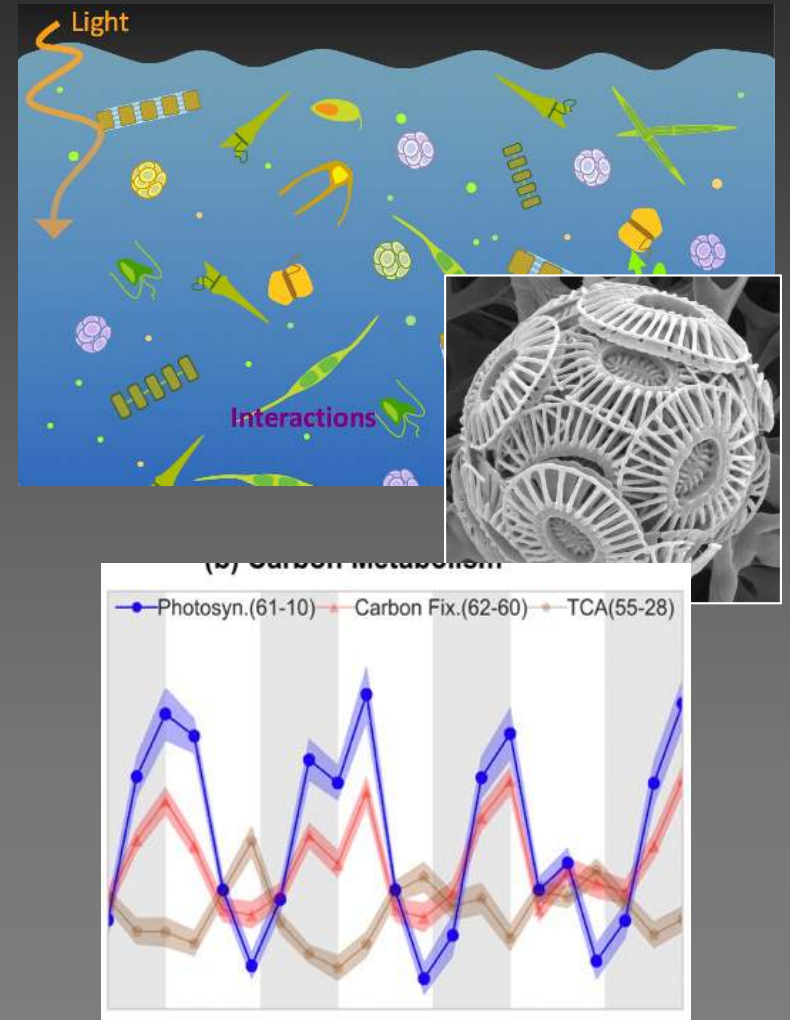
- POC
- Triacylglycerol
- Metabolite
- Photosynthesis
- TCA cycle
- Sugar transporters



Muratore, Boysen, Harke et al. (2022) *Nature Ecol. Evol.*

Summary

- **Metatranscriptomes:**
- Light drives synchronous metabolic cascades which underpin carbon cycling in the NPSG
- Light drives asynchronous metabolism of nitrogen – a limiting resource in the NPSG
- Temporal niche partitioning of N uptake and metabolism may decrease competition for limiting resource



Core questions... adventures in eukaryote metatranscriptomics

Limitation

- **Nitrogen limits carbon export and cycling in a keystone haptophyte**
- **The environment selects**

Competition

- **Temporal niche partitioning supports a complex community in a resource poor environment**

HOE:DYLAN



HOE:MAHALO

