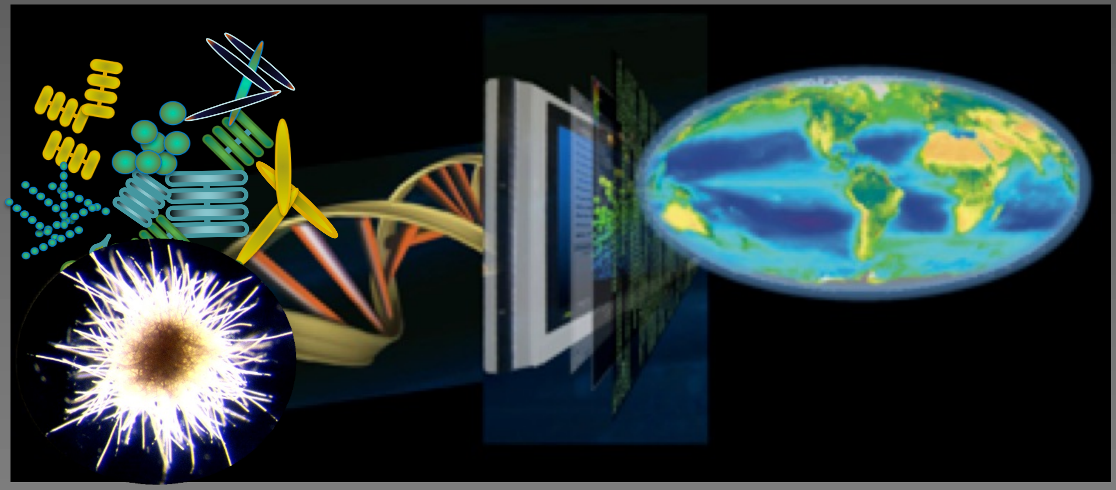
Ocean genomics: phytoplankton physiological ecology across marine ecosystems

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

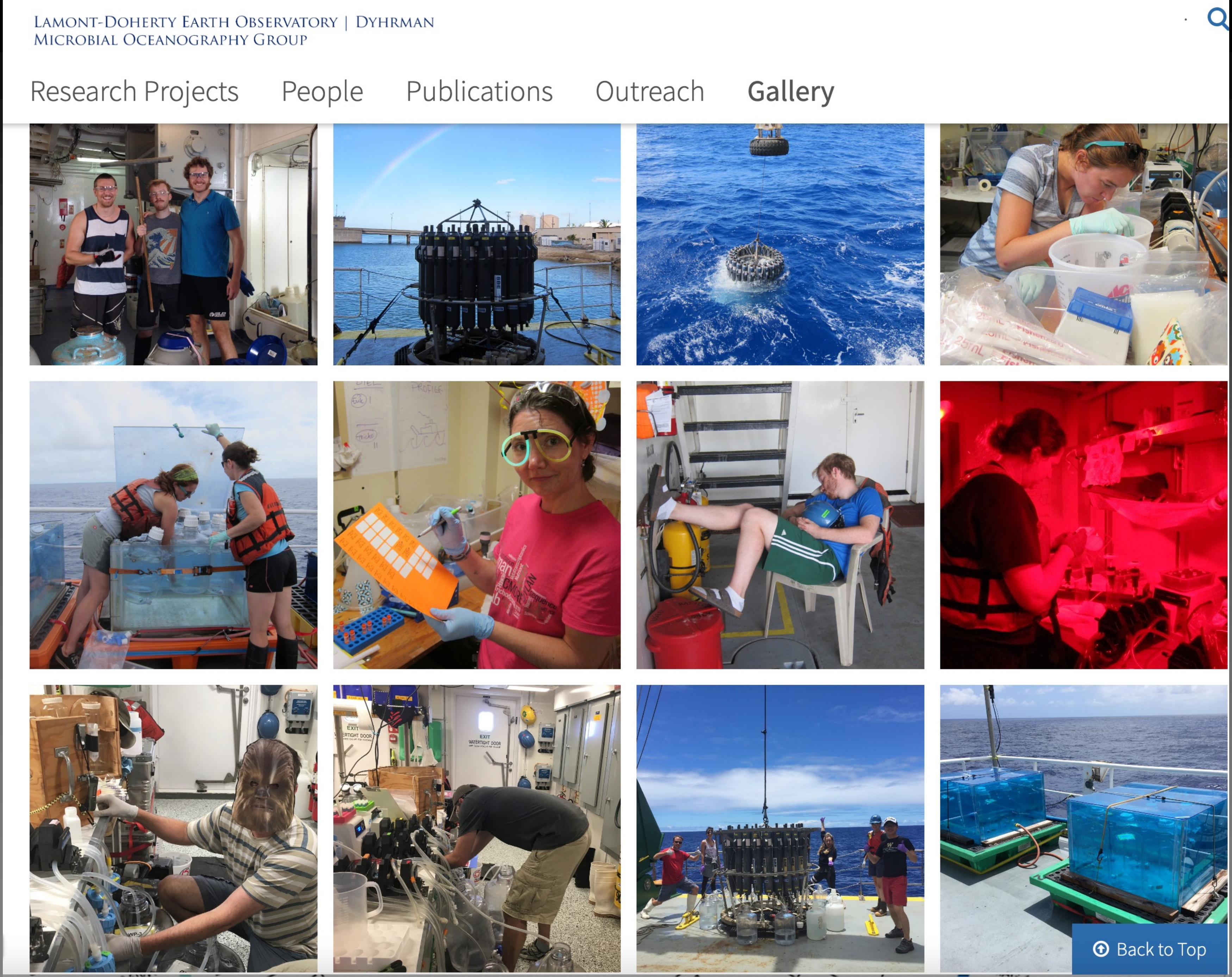
Faculty Columbia University

Columbia runs RV Langseth

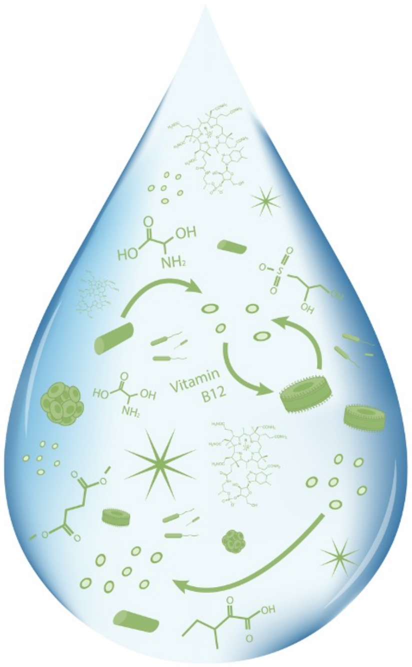
Sonya – random (fun?) facts

[illegible]

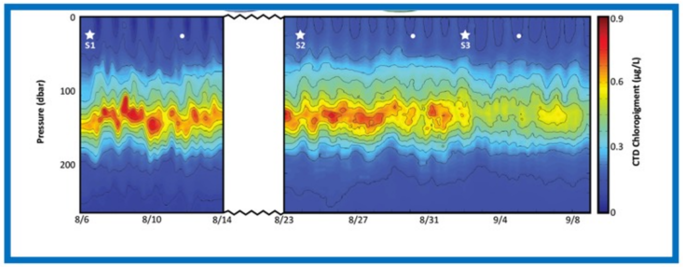
Hanging chads. I don't have secret knowledge. Rick Astley. Guava foam. Water polo. Darva Conger. Creme patisserie. Mahalo. Antarctica. Easter Island. Watch that dewar tumble. Blue filter paper leis. Aloha. A proper pudd. Tres leches cake. You can't make this


Lamont-Doherty Earth Observatory | Dyhrman Microbial Oceanography Group
Research Projects
People
Publications
Outreach
Gallery
Back to Top

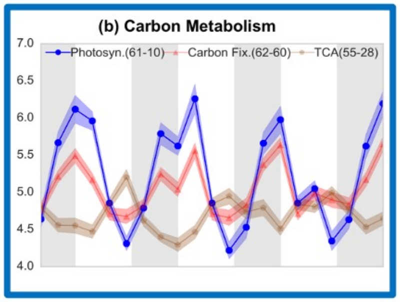
Understanding the vast network of microbes and chemicals in the ocean

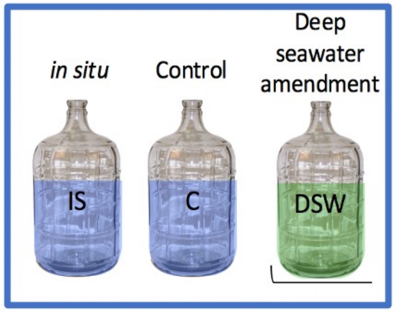


Monthly time-series



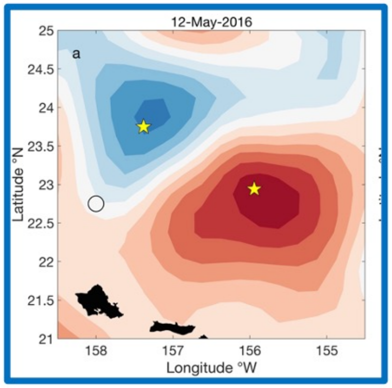
Diel time-series





Incubations

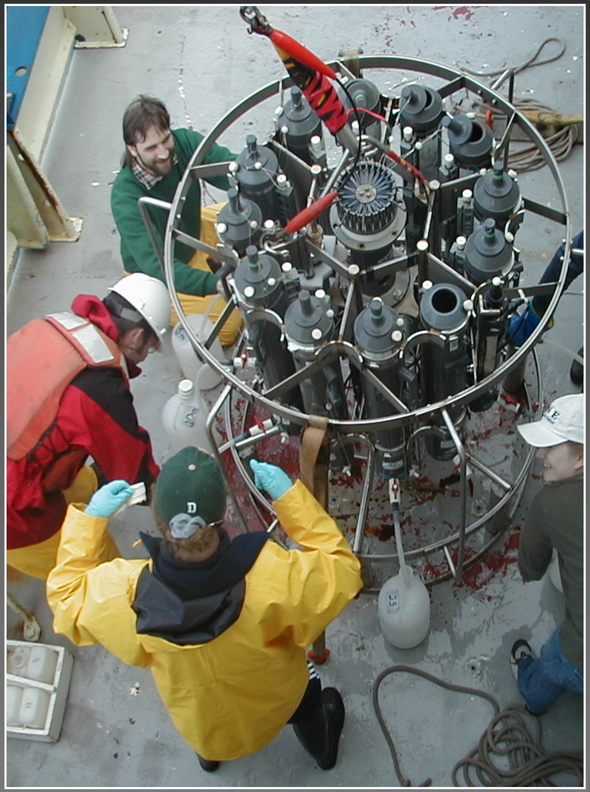
Natural disturbance



Eddy-driven forcing

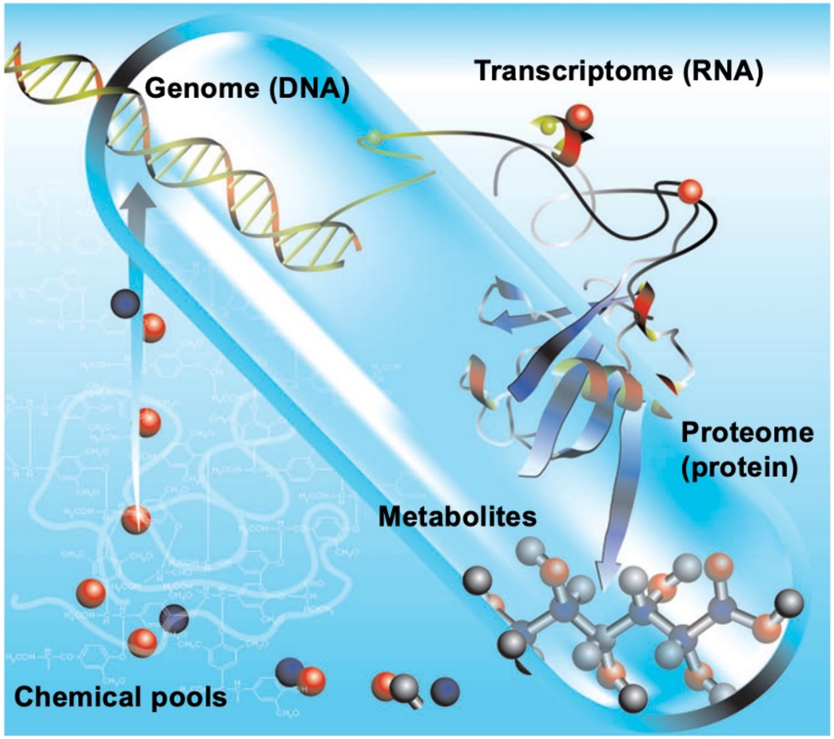
High School

Postdoc

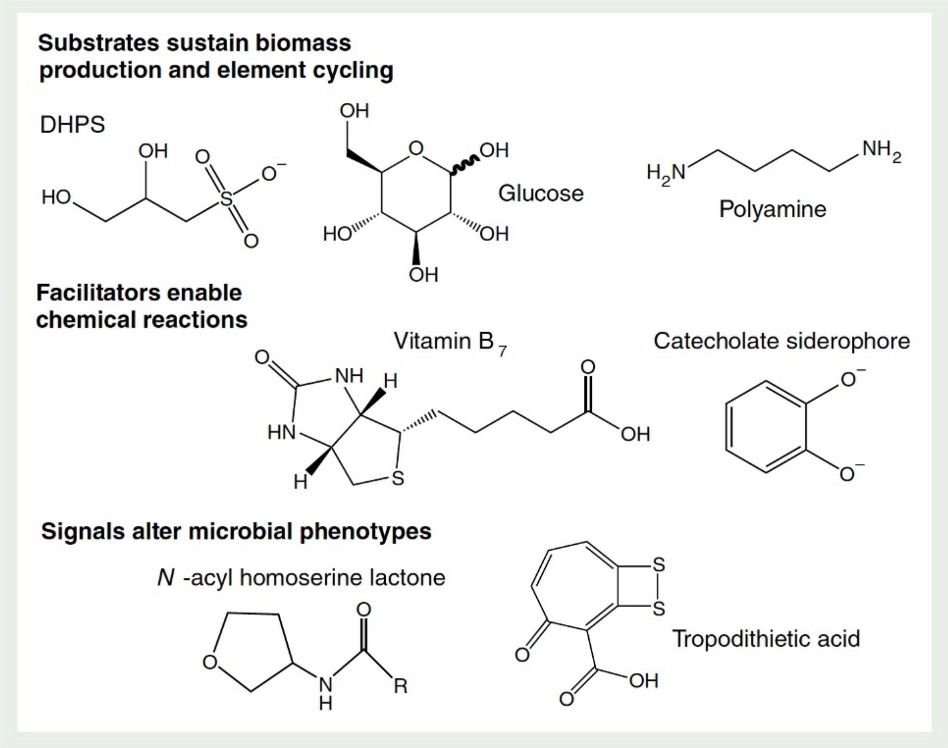


Professor

Evolving ‘omics and chemical methods allow new progress



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



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

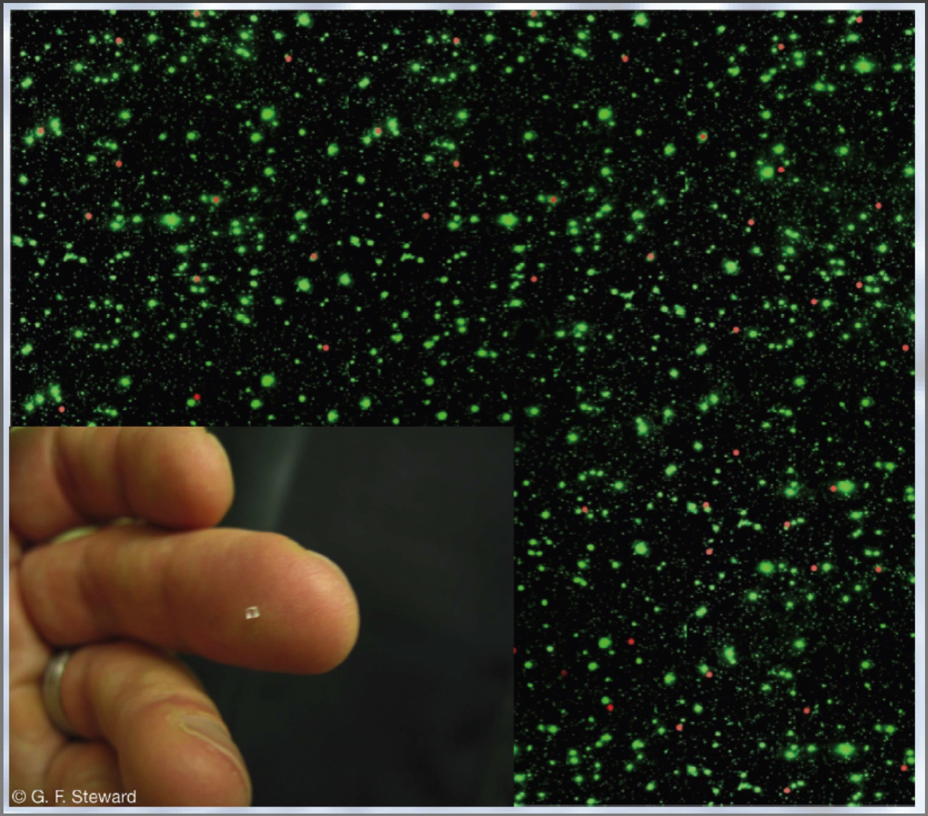
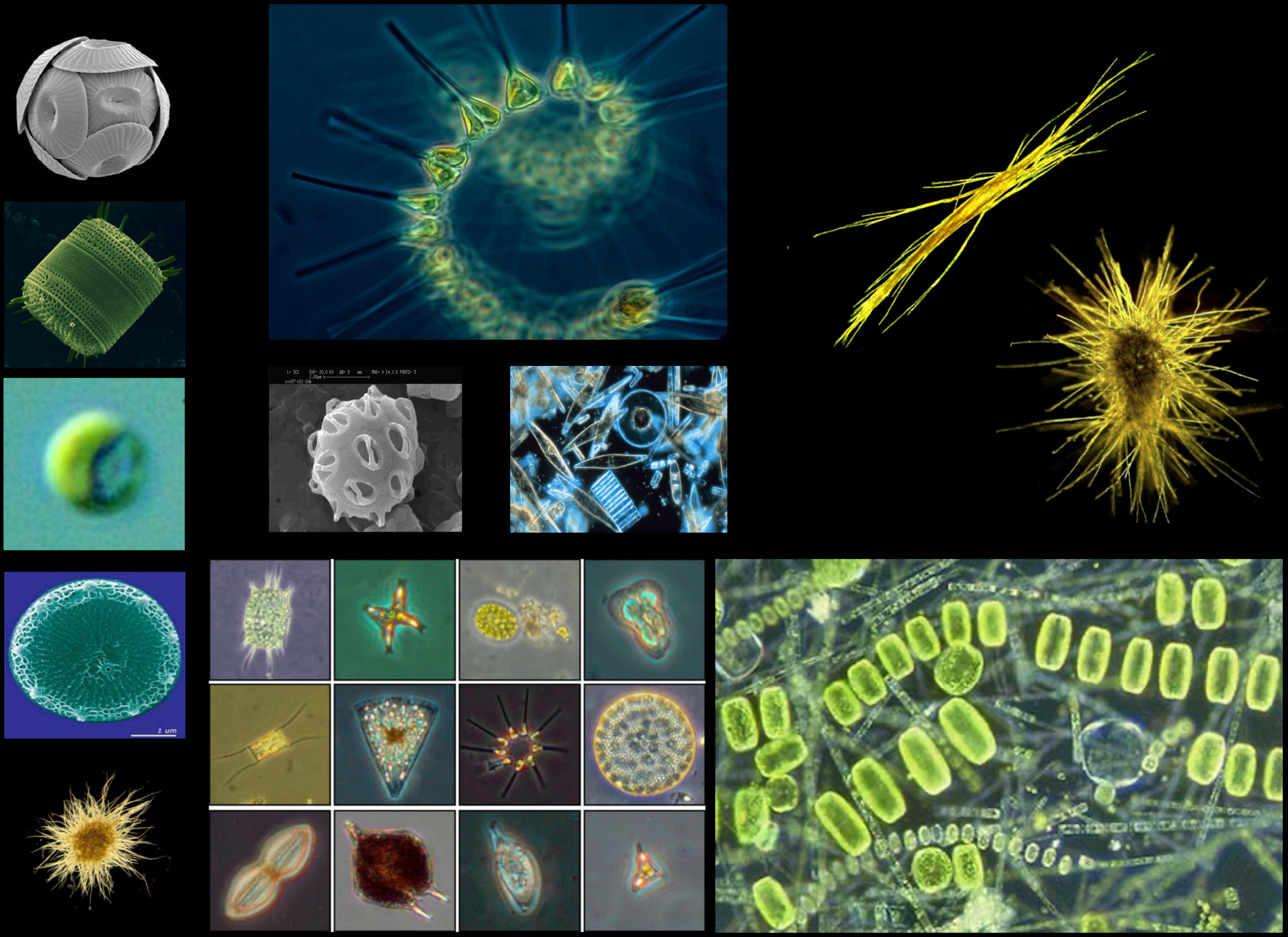
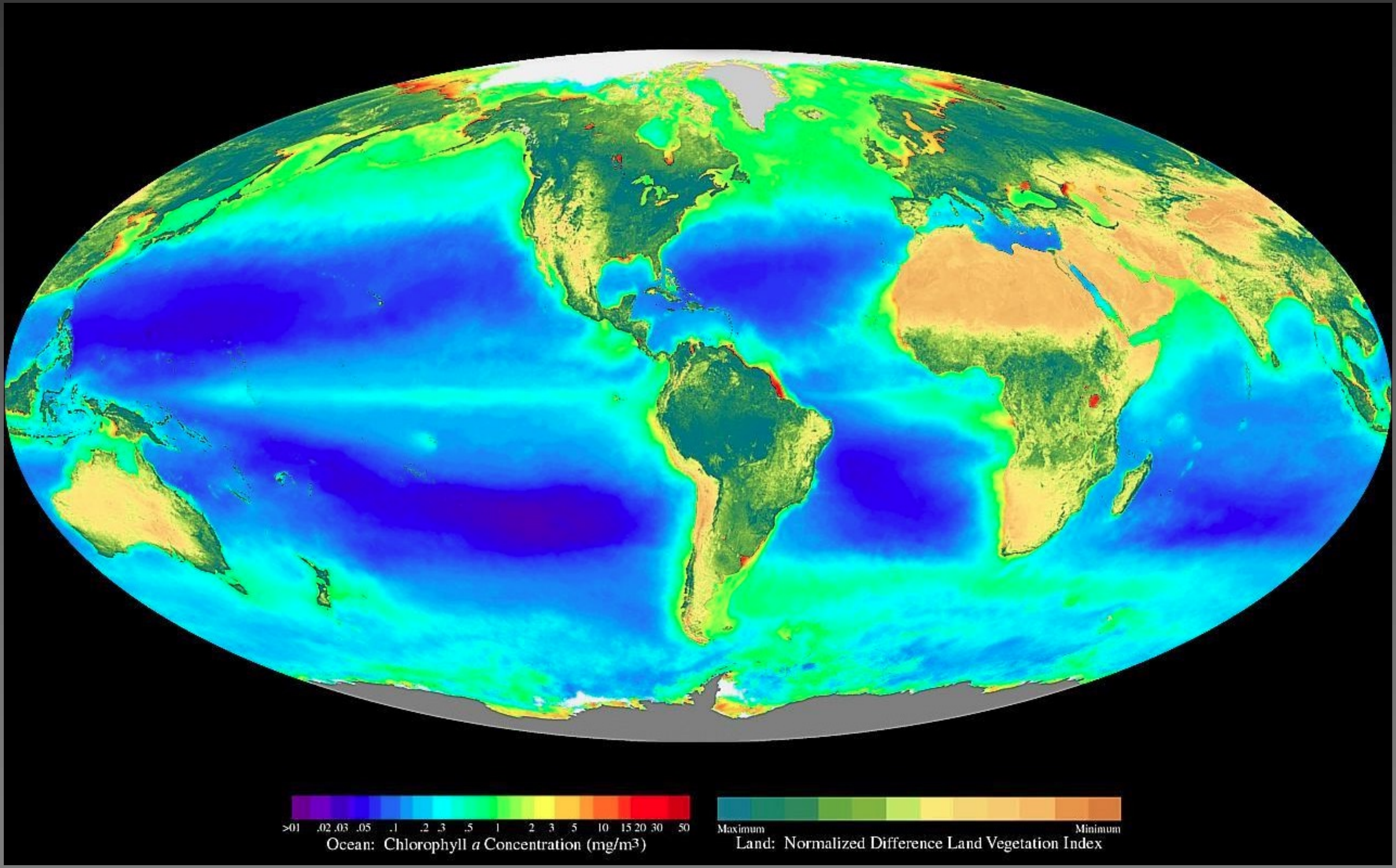


Image courtesy C-MORE

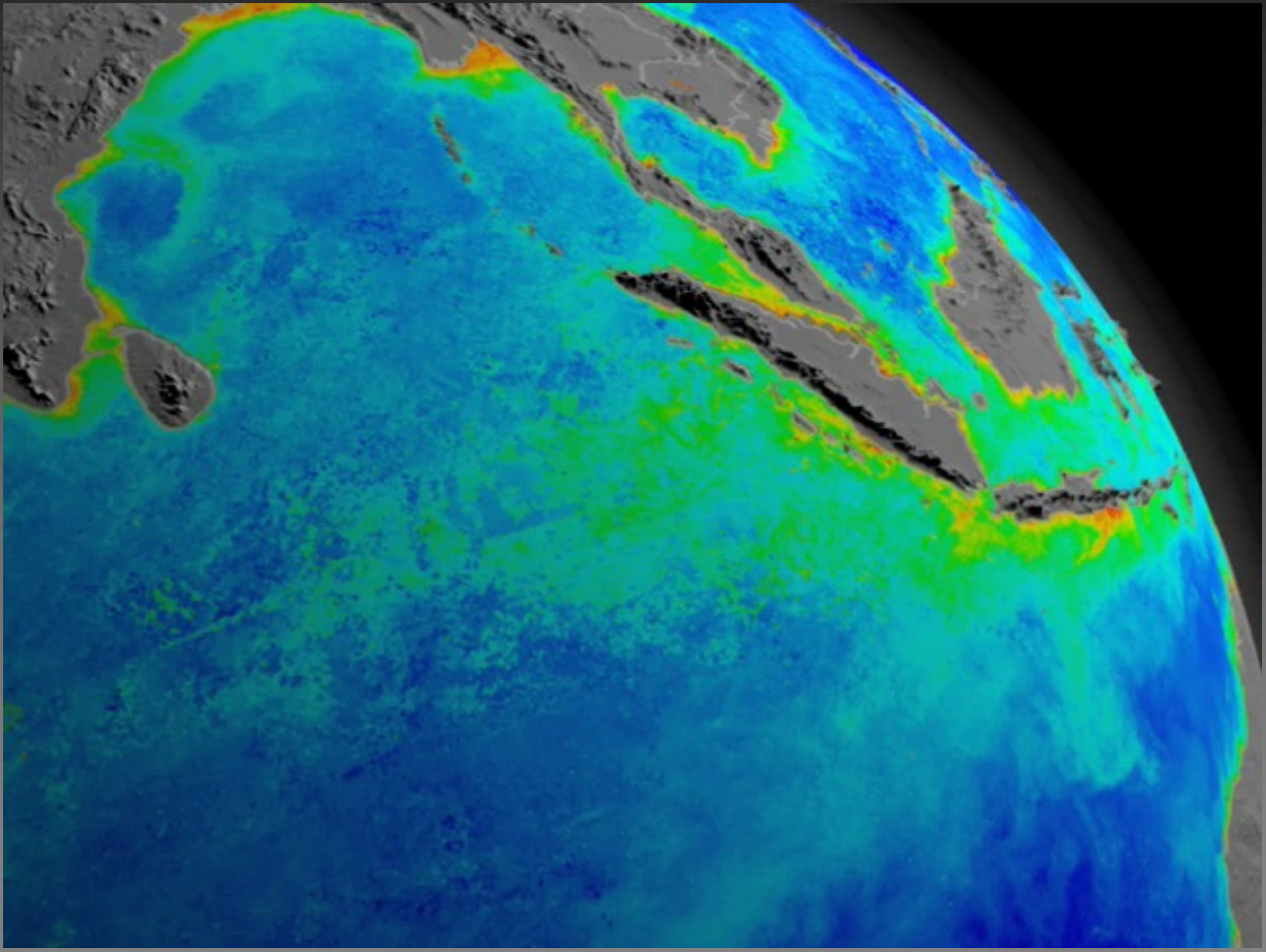

L= SE1 EHT= 20.0 KV WD= 9 mm MAG= X 14.3 K PHOTO= 5
2.00µm
sin97-n11-10m
1 um

Phytoplankton play a profound role in the earth system

Half of global primary production

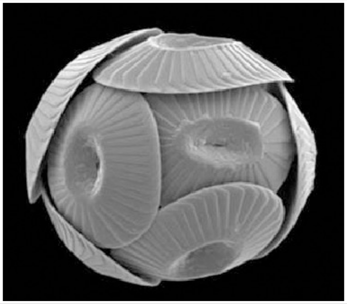


Seasonal chlorophyll distributions in the sea - highlights the global significance of phytoplankton

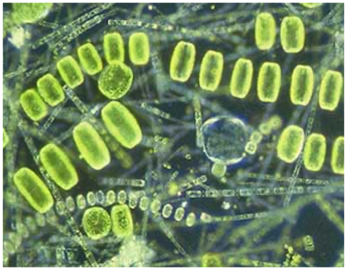


Phytoplankton underpin ocean ecosystem function

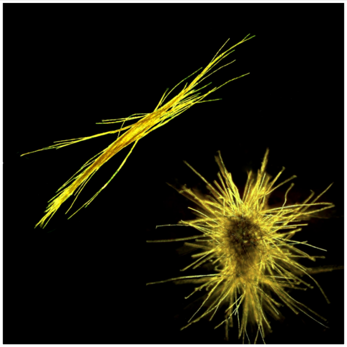
Haptophytes

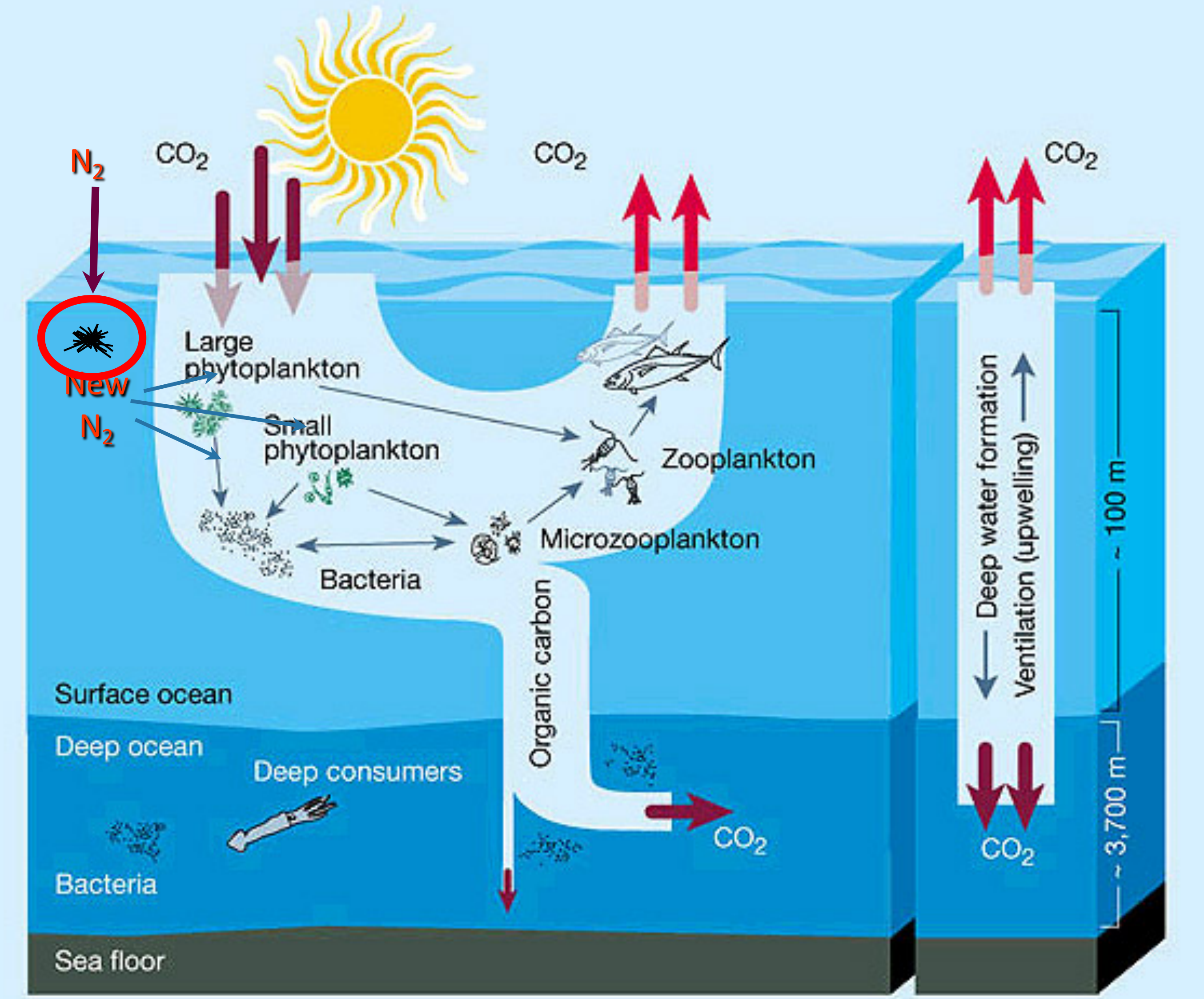


Diatoms

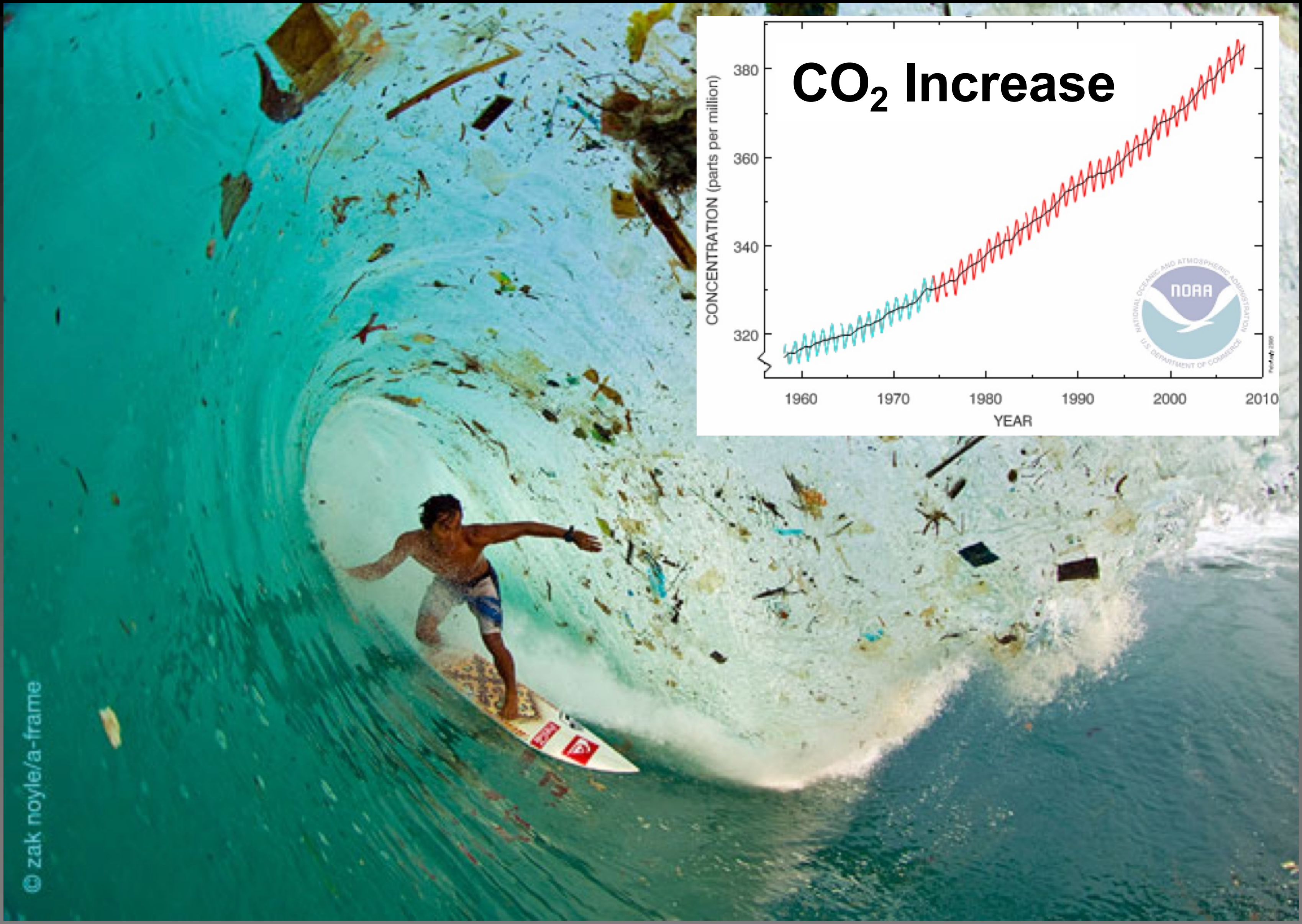


N_2 Fixers



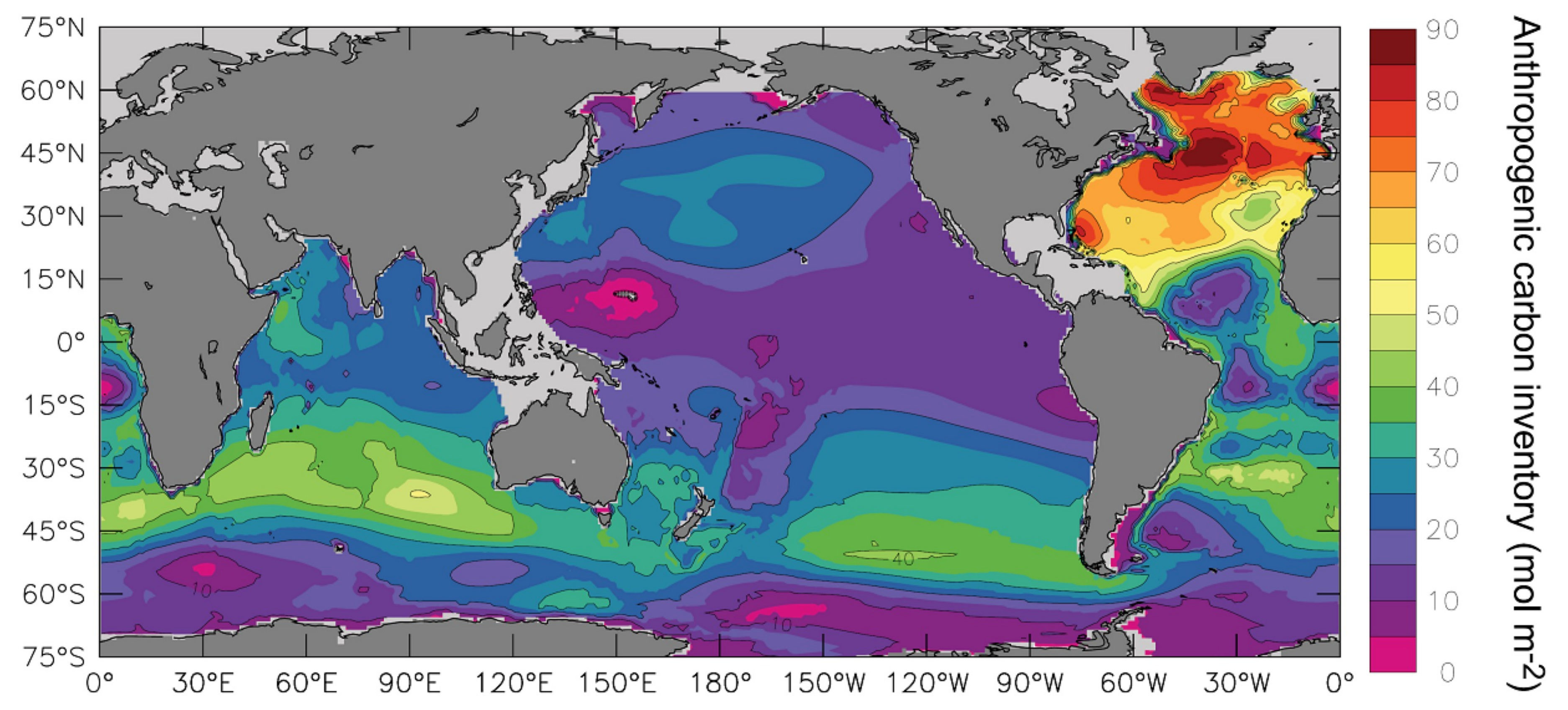


Chisholm 2000 *Nature*



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

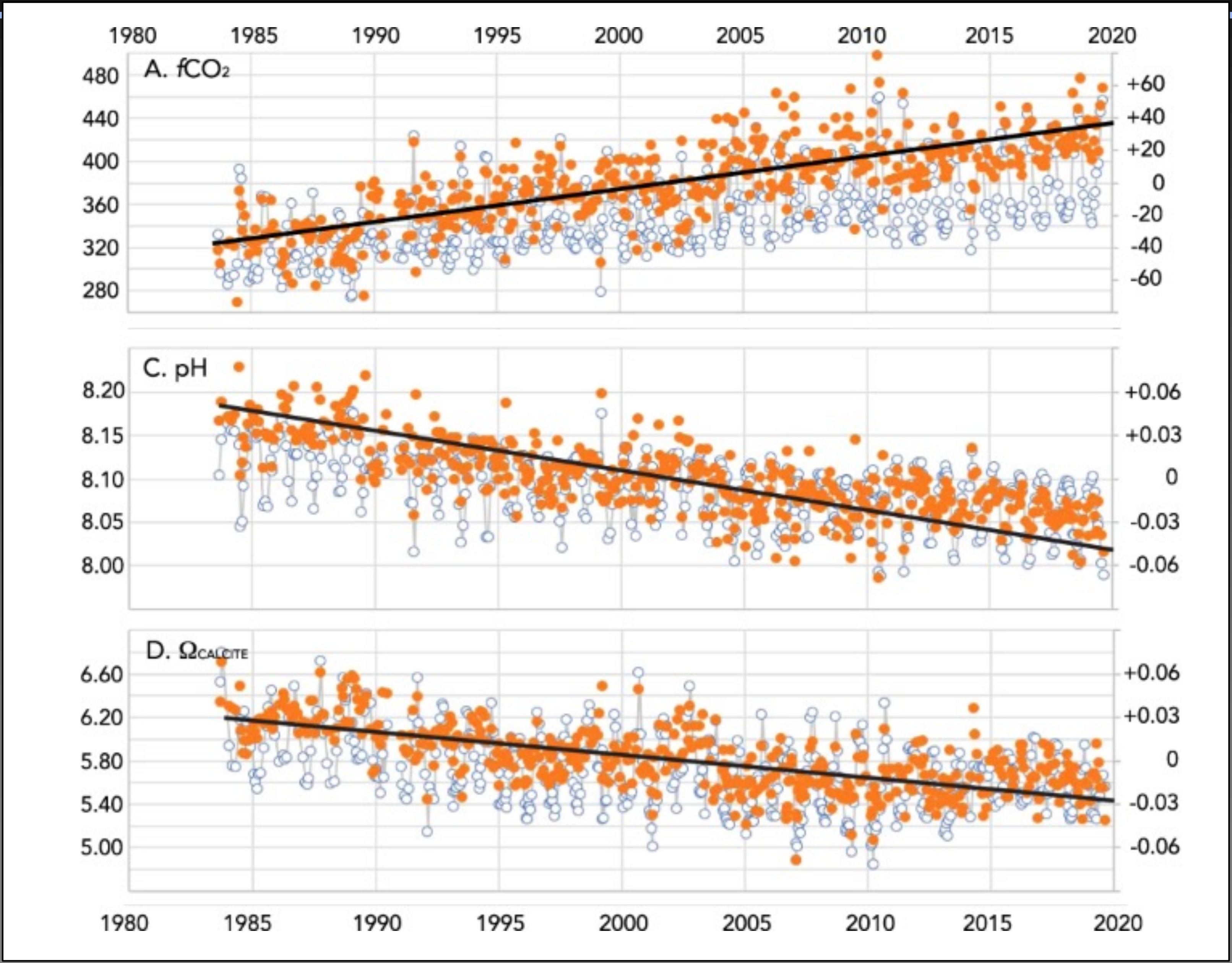
The ocean acts as a buffer for CO_2 in the atmosphere



Sabine et al. (2004) *Science*

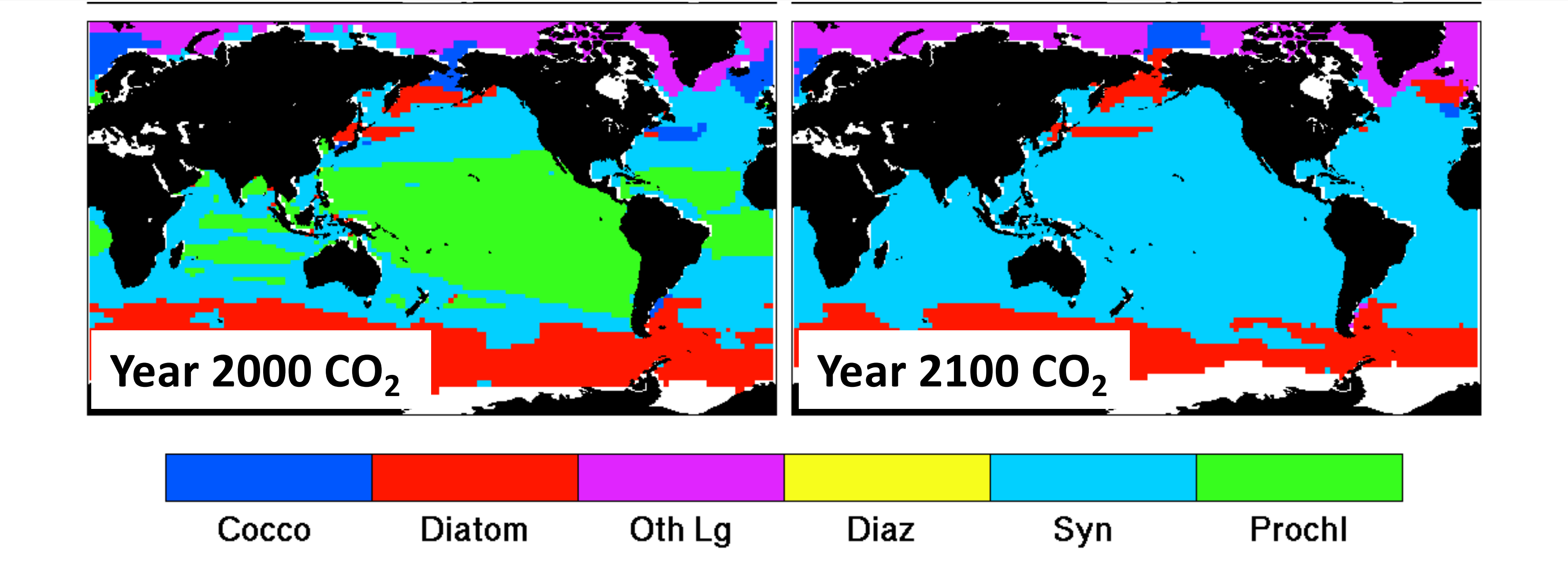
Oceanic reservoir is 38,000 petagrams of carbon

Changes in temperature CO_2, pH, etc.



Bates and Johnson 2020 *Communications Earth Env.*

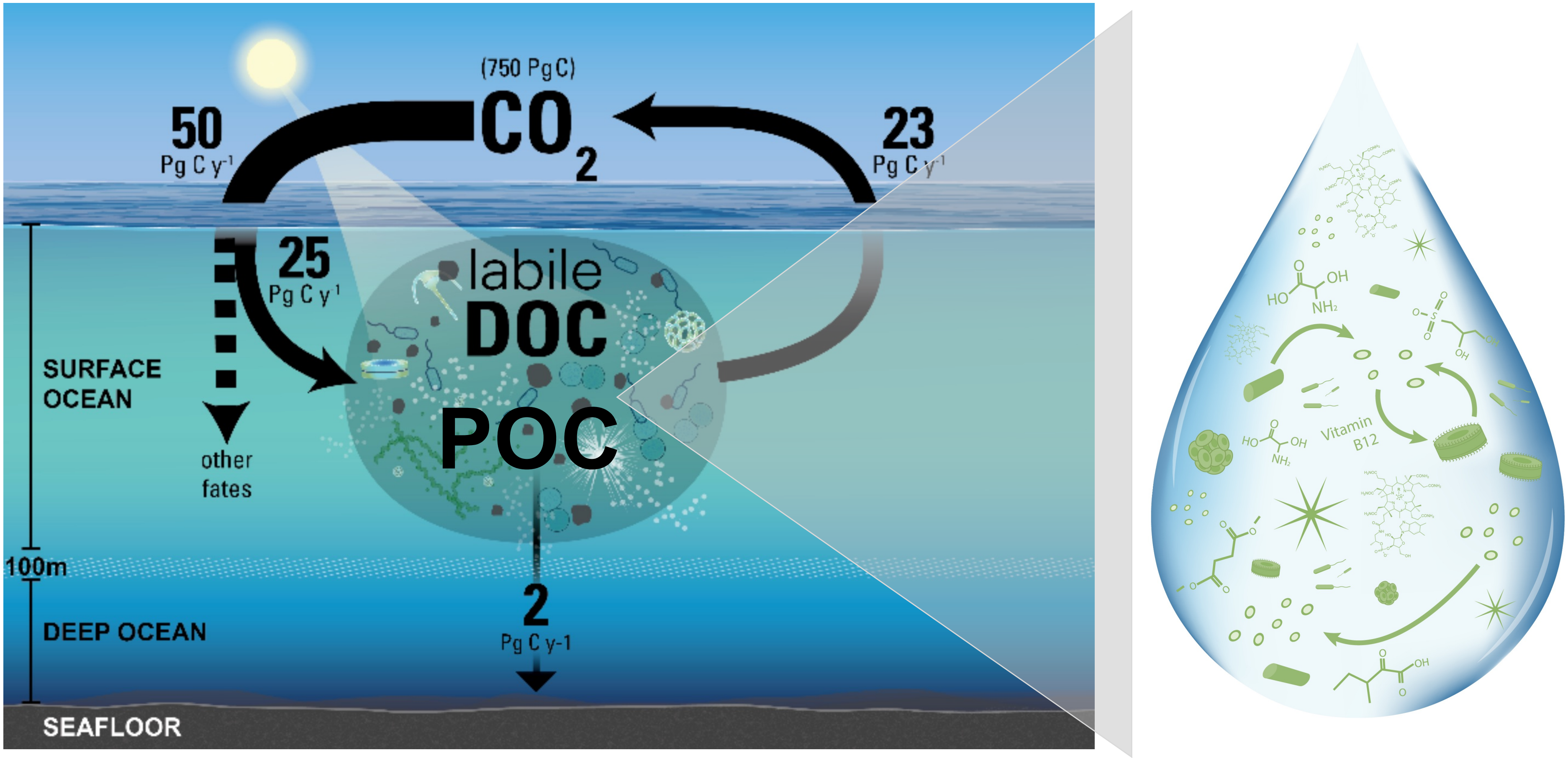
Models predict future shifts in microbial communities


Dominant Functional Group

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

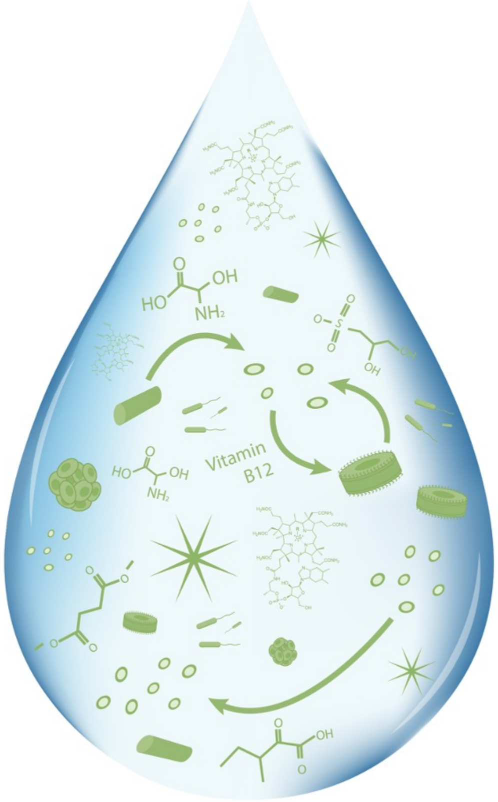
Pressing need to understand the rules that govern the **ocean chemical – microbe network** of interactions

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

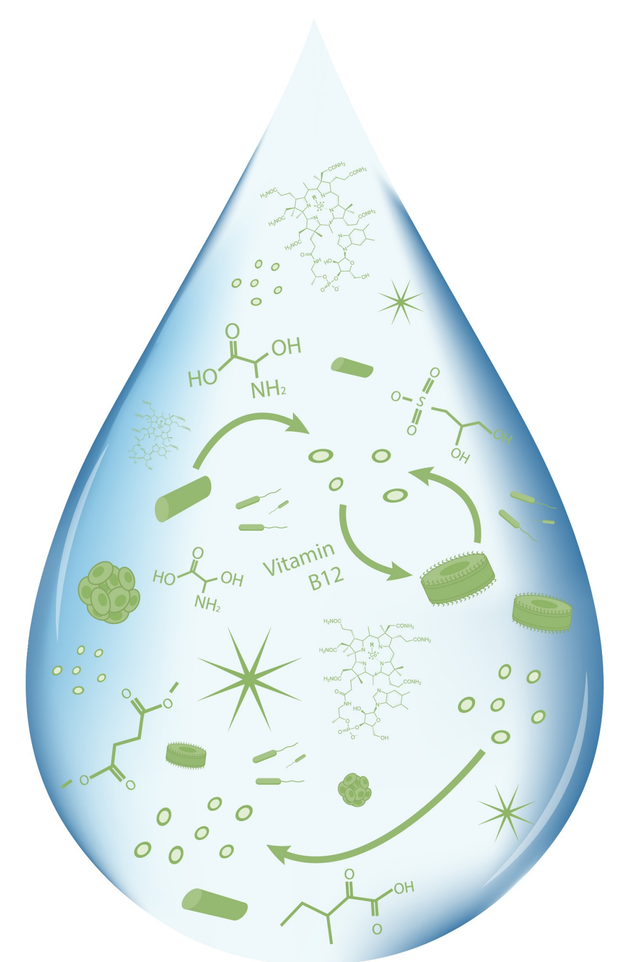


Chemical – microbe network key uncertainties

- 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

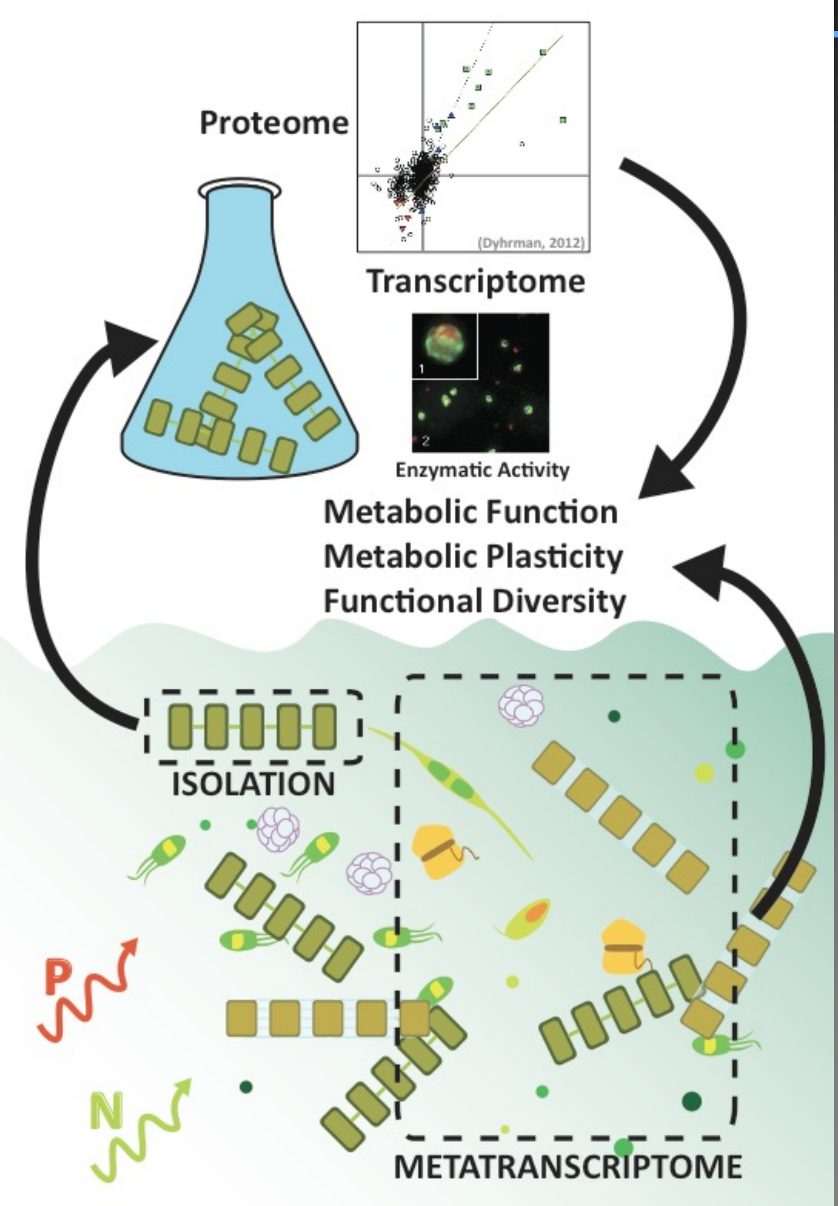
- Long standing challenges:
  - Populations are dilute and they move around!
  - Some keystone groups not in culture
  - Few genomes or transcriptome sequences

- New opportunities
  - Increases in genomes/transcriptomes for eukaryotic taxa
  - *New array of bioinformatics and sequencing tools*

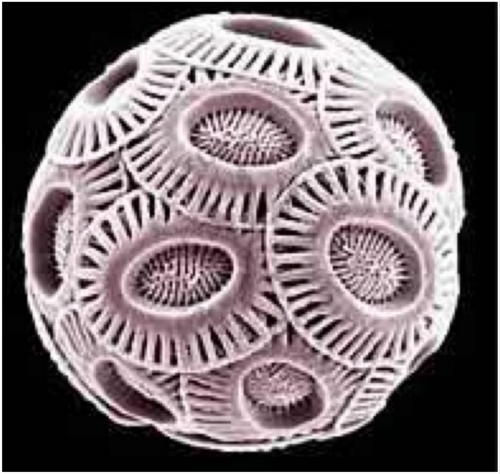
A decade of meta ‘omic and multi ‘omic approaches!

Leveraging metatranscriptome data to study the chemical – microbe network

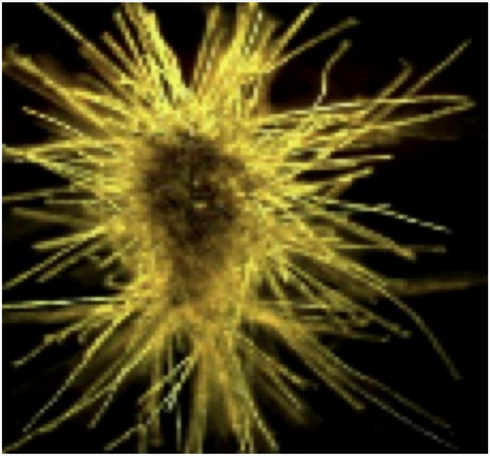
- Cultures offer controlled conditions
- Field samples offer context
- Metatranscriptomes are species-specific
- Metatranscriptomes offer high resolution tracking of finely tuned responses



Vignettes



- Light and nutrients: defining the drivers of physiological ecology and competition in microeukaryotes



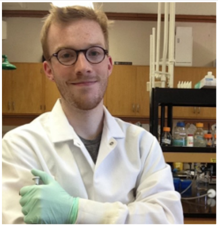
- Microbiome interactions: Tracking the microbiome of a keystone N_2 fixer

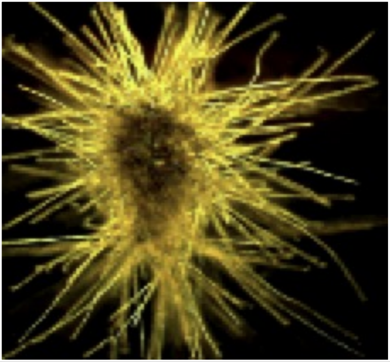
Thank you Dyhrman Lab family past and present


Gwenn Hennon


Mónica Rouco


Sheean Haley


Kyle Frischkorn




John Tracey


Hanna Anderson

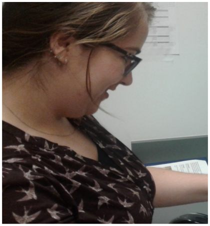

Angela Zoumplis


Erin Maybach

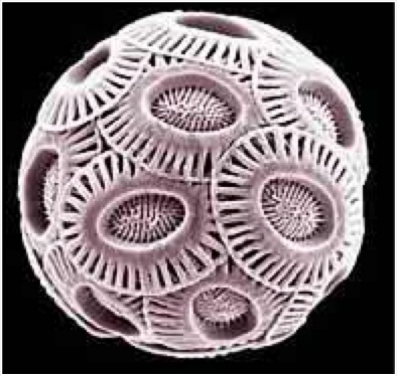

Hannah Goldberg


Sheean Haley


Matt Harke


Maria Hernández-Limón


Harriet Alexander



Microeukaryote metratranscriptomes with RNA-Seq

3x20L

↓

Filter
(>5µm)

↓

Extract RNA

Selected

↓

De novo assembly
Reference mapping

Unselected

↓

De novo assembly
Reference mapping

Quality Control
- Trimmomatic
- FastQC

Assembly/Reference
- Trinity
- TransABYSS
- rnaSPADES
- MEGAHIT

Read Mapping
- Salmon
- BWA
- Bowtie2
- RSEM

Removal of Sequences
- riboPicker
- BBMap

Protein Prediction and Clustering
- TransDecoder
- OrthoFinder

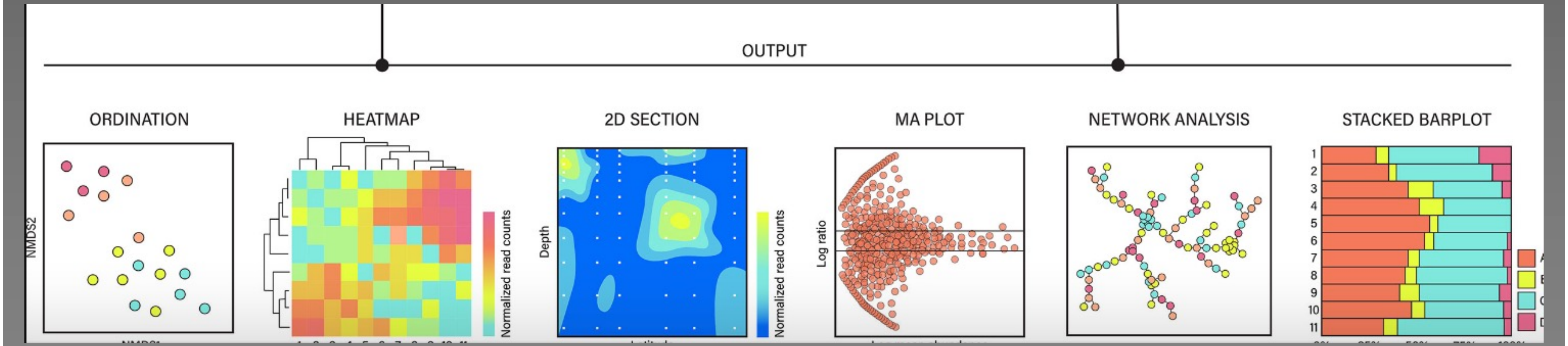
Normalization & DE
- EdgeR
- DESeq2
- TPM
- TMM

Functional Annotation
- BLAST
- DIAMOND
- HMMer

- KEGG
- KOG
- GO
- PFam

Taxonomic Annotation
- BLAST
- DIAMOND
- MMSeq2

- MMETSP
- EukProt
- EukZoo
- PhyloDB



Cohen et al. (2020) Front. Mar. Science

Sequencing and analytical workflows are constantly improving

Harriet Alexander @nekton4plankton · Apr 27

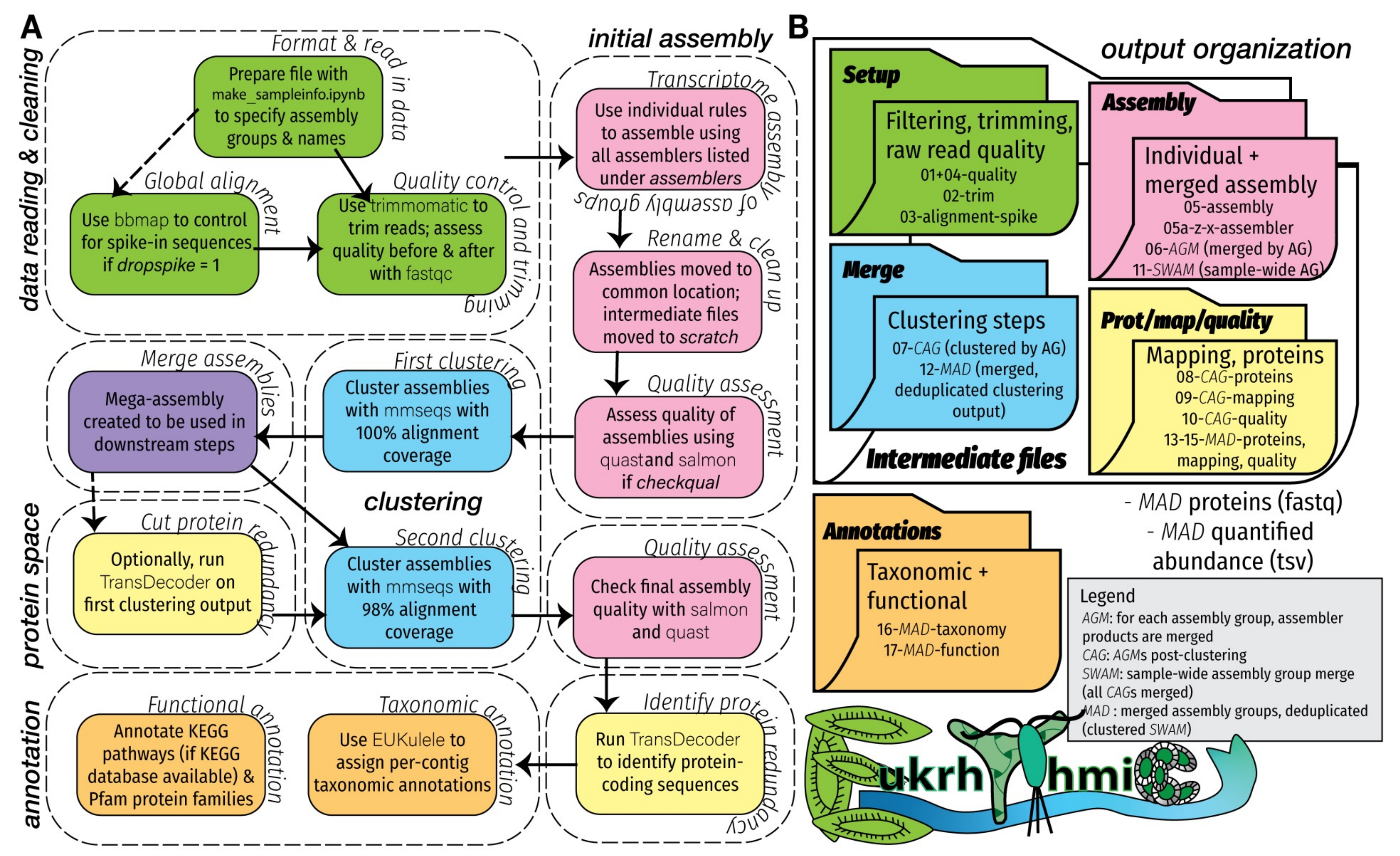
So check it out!

GitHub here: github.com/AlexanderLabWH...
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



Krikinos et al. 2023 *BMC Bioinformatics*

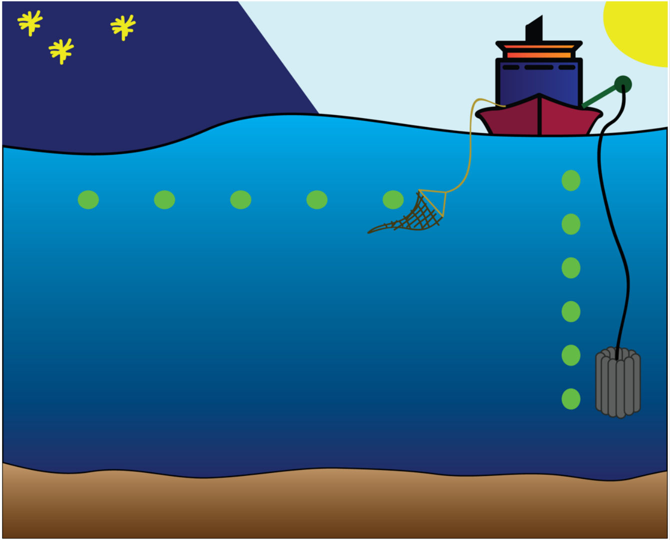
Core questions… adventures in eukaryote metatranscriptomics

HOE:DYLAN

Limitation

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

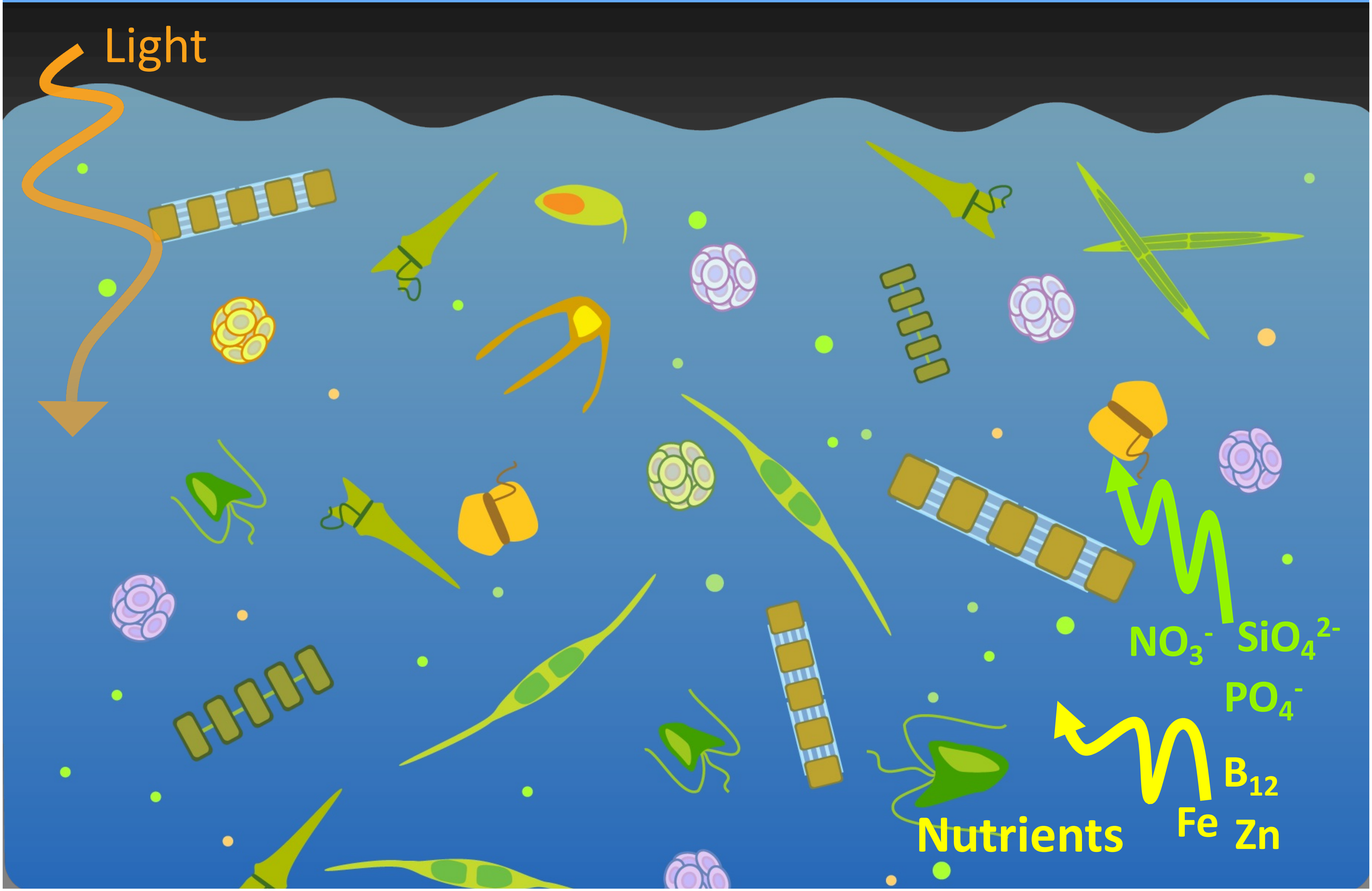
HOE:MAHALO



Competition

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

Complex community dynamics driven in part by resources



Tracking phytoplankton physiological ecology

Ecosystem function and biogeochemistry

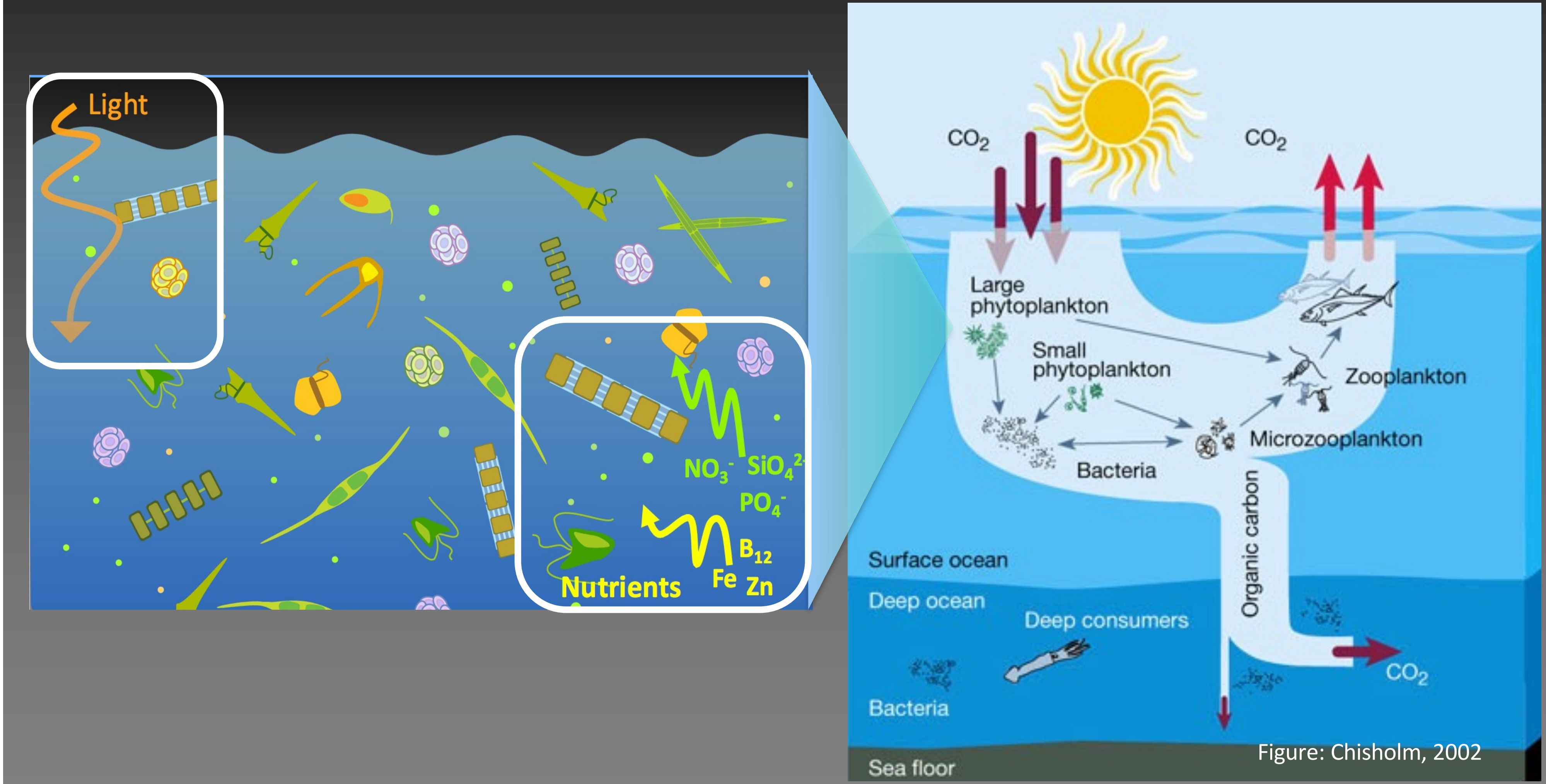
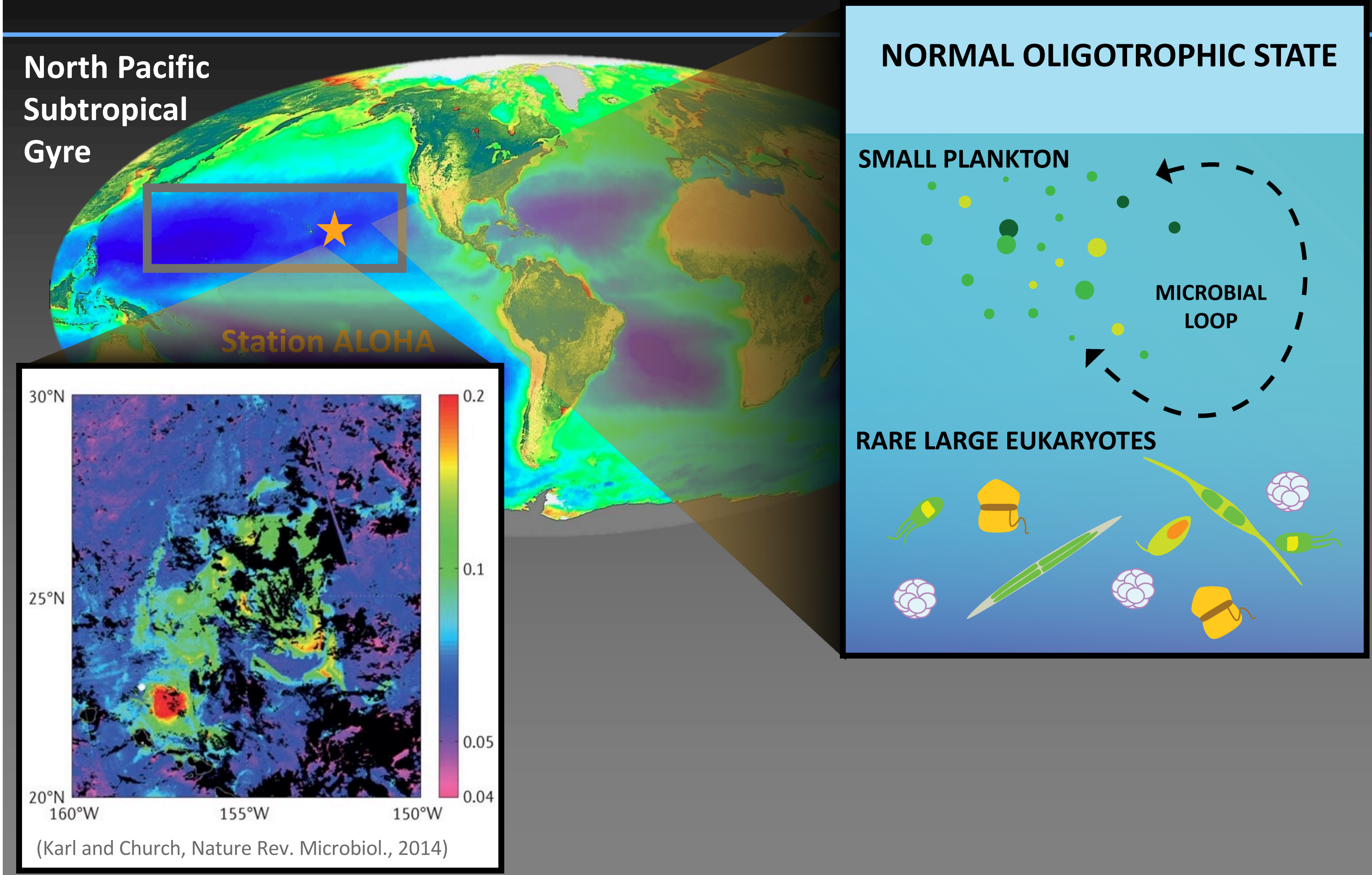


Figure: Chisholm, 2002

How do resources drive phytoplankton distributions and activities?

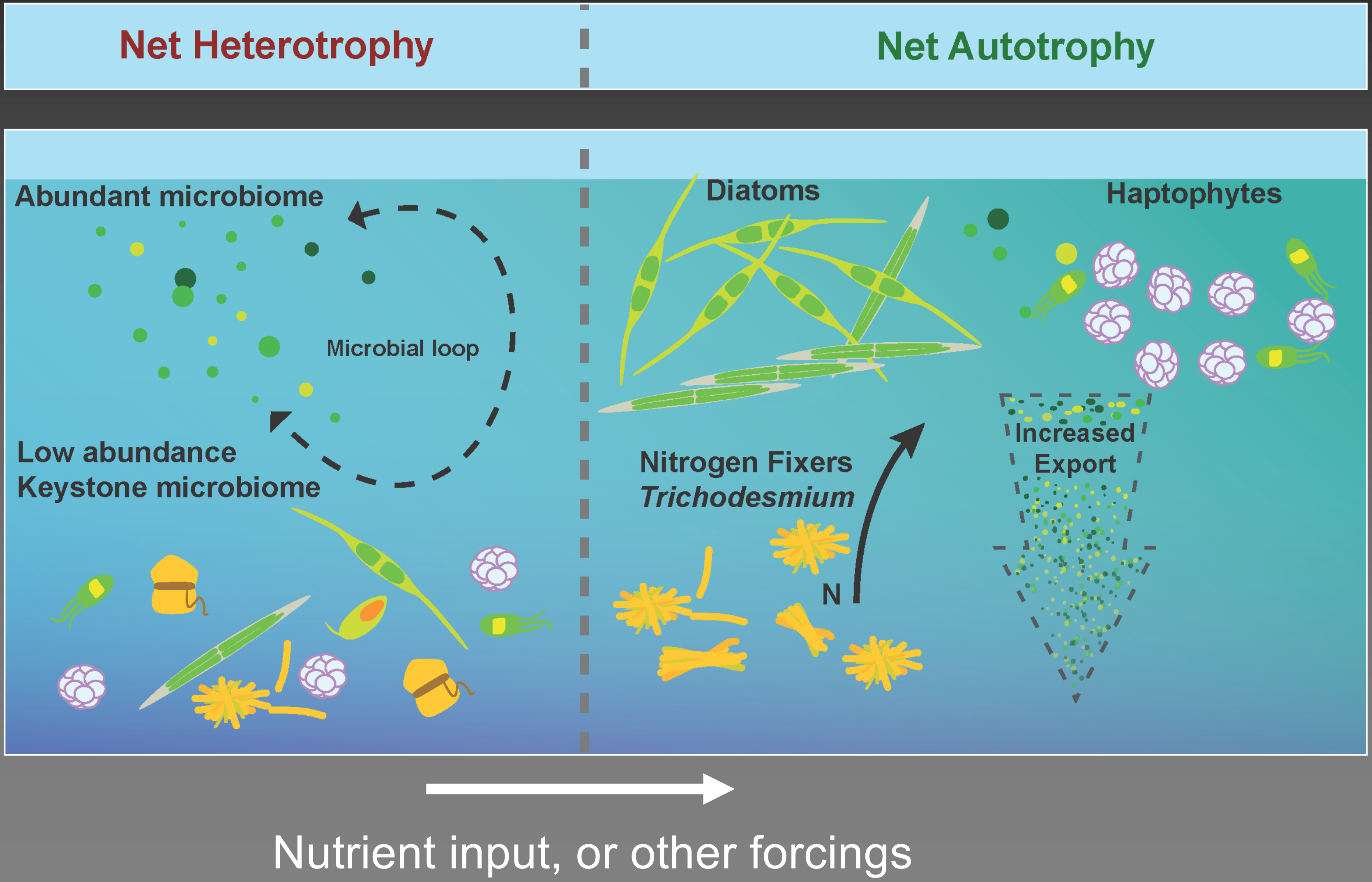
North Pacific Subtropical Gyre



(Karl and Church, Nature Rev. Microbiol., 2014)

Image credits: SeaWIFS Global Chlorophyll

Blooms of keystone species can shift ecosystem state and carbon flow



Emiliania 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

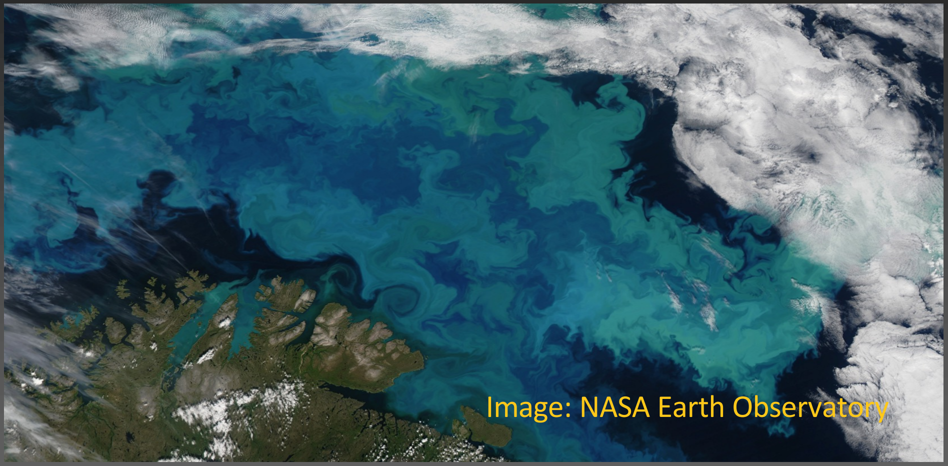
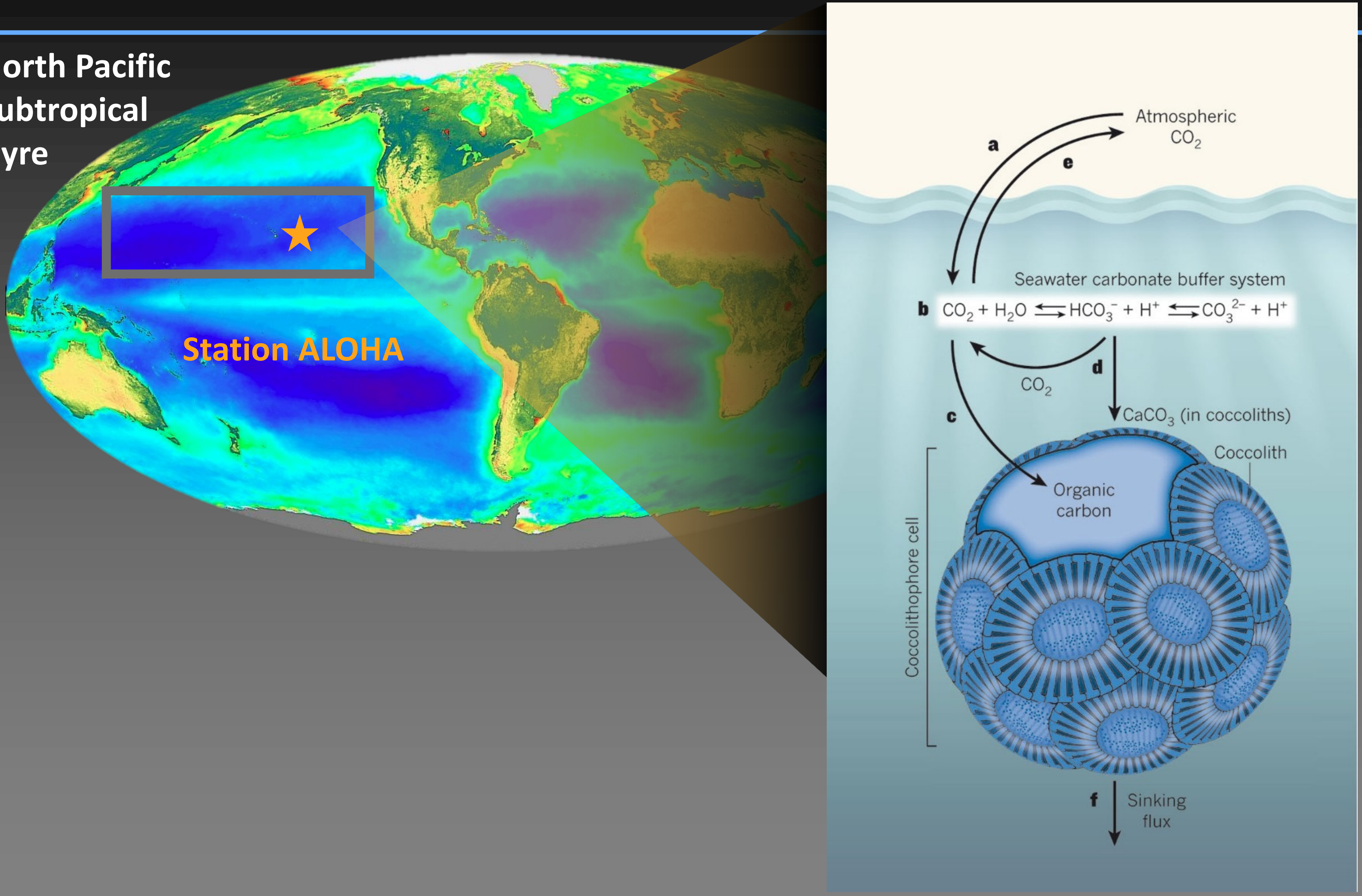


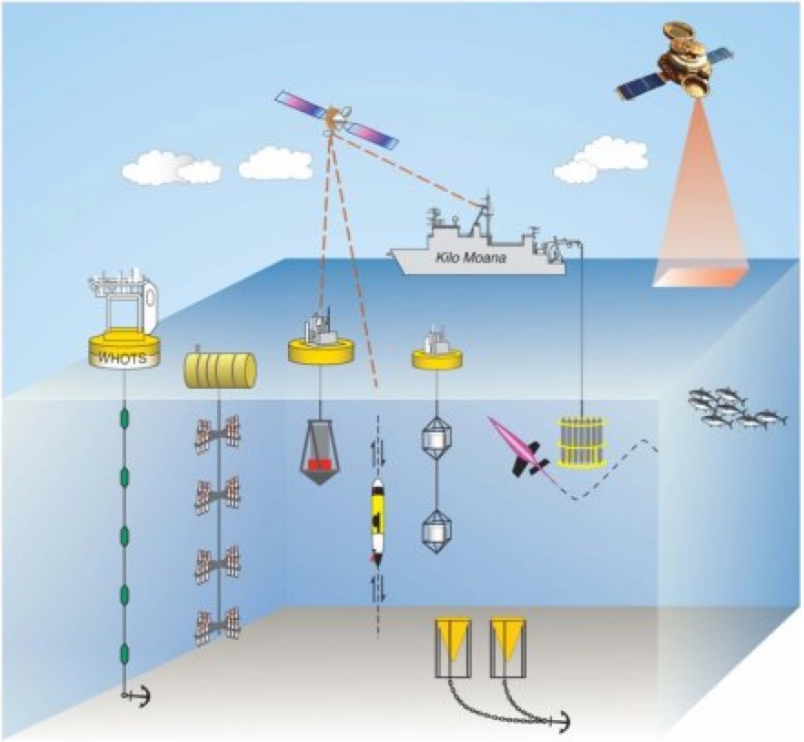
Image: NASA Earth Observatory

Drivers of carbon flow in oligotrophic systems

North Pacific
Subtropical
Gyre



Hawaii Ocean Experiment: Dynamics of Light and Nutrients



Wilson et al. 2015 *GBC*

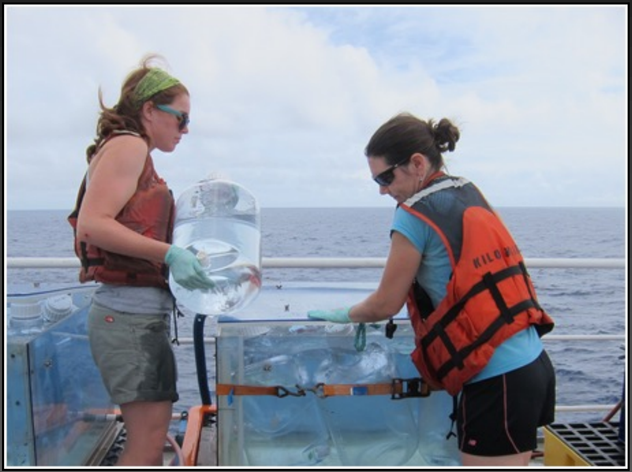
center for microbial oceanography: research and education

c·more *linking genomes to biomes*

(HOE-DYLAN)

HOE:DYLAN: Water sampling

HOE:DYLAN: Semi-factorial resource additions



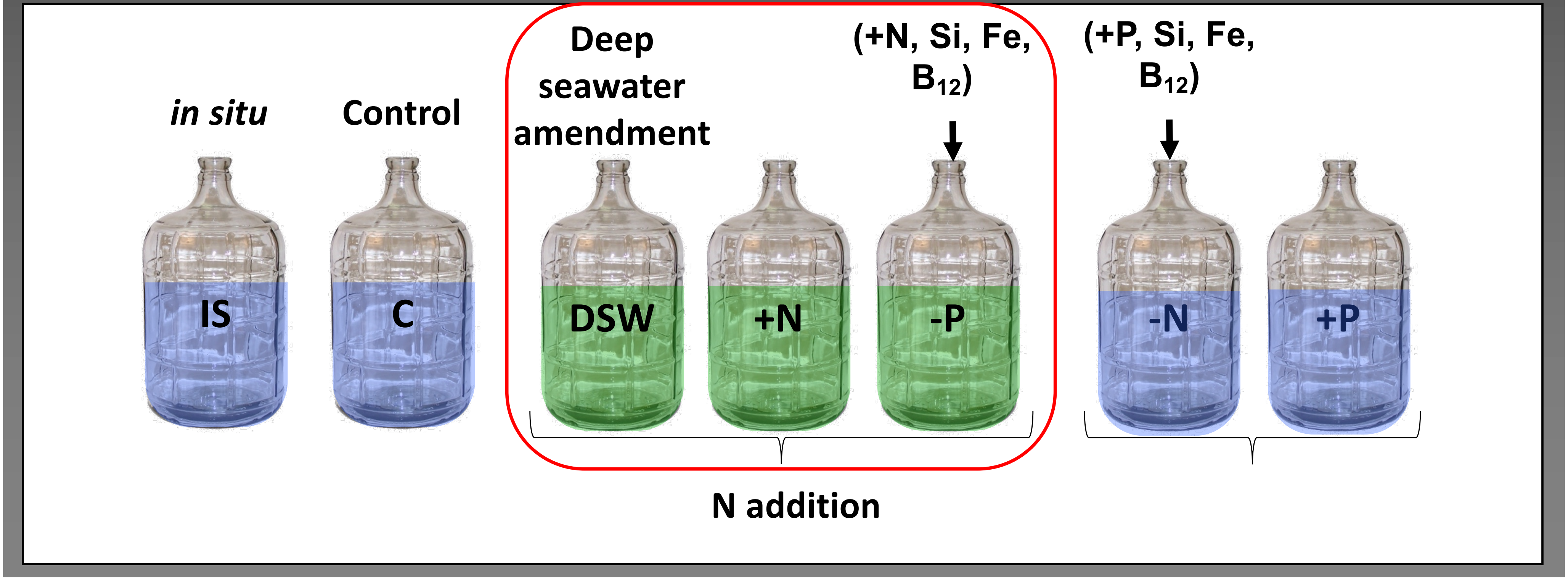
Nitrate/nitrite*: ~40 µM

Phosphate*: ~2.6 µM

Silicate*: ~100 µM

Fe: ~0.6 nmol/kg

B_{12}: <0.1 pM



in situ

Control

Deep seawater amendment

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

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

IS

C

DSW

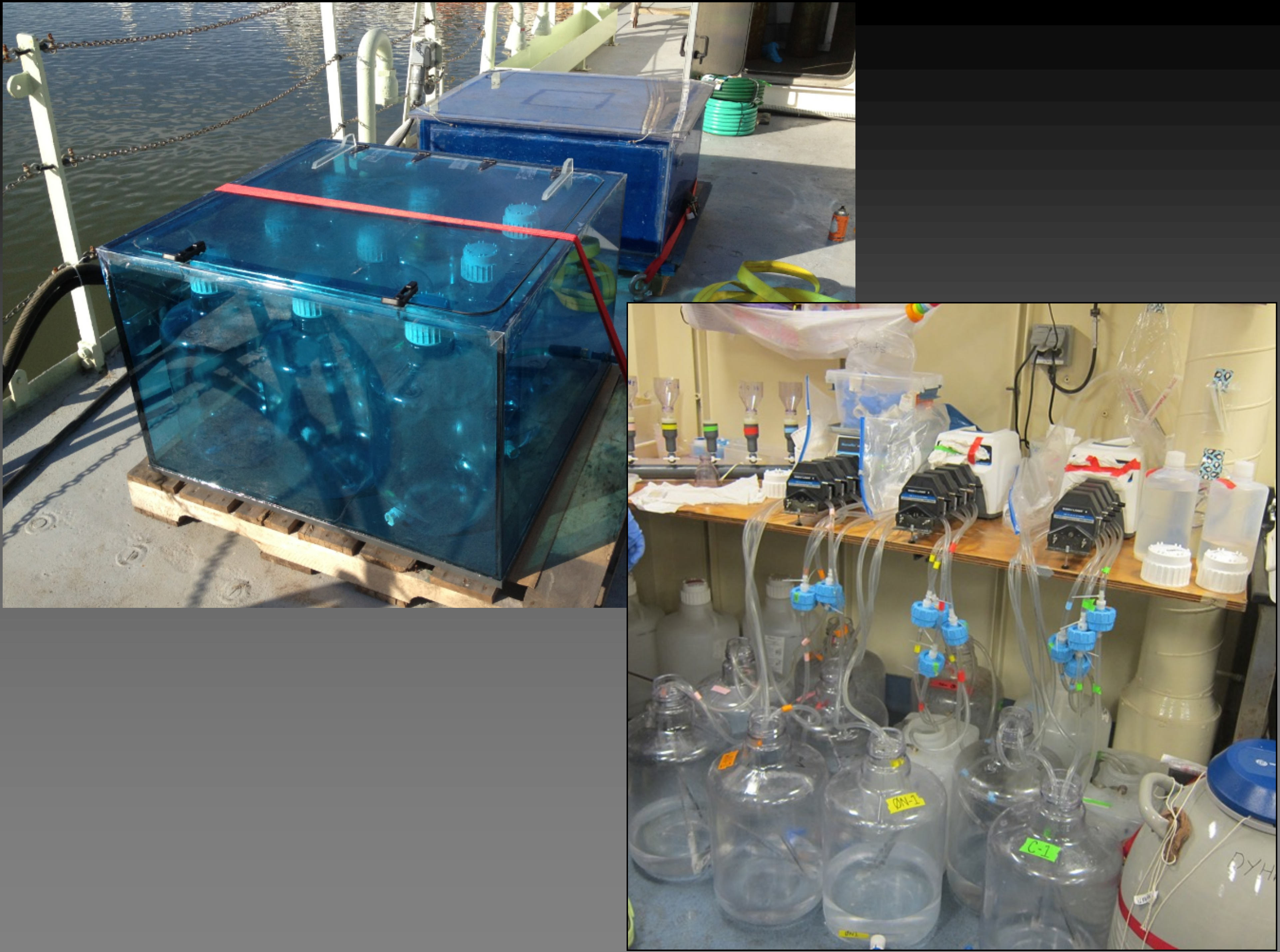
+N

-P

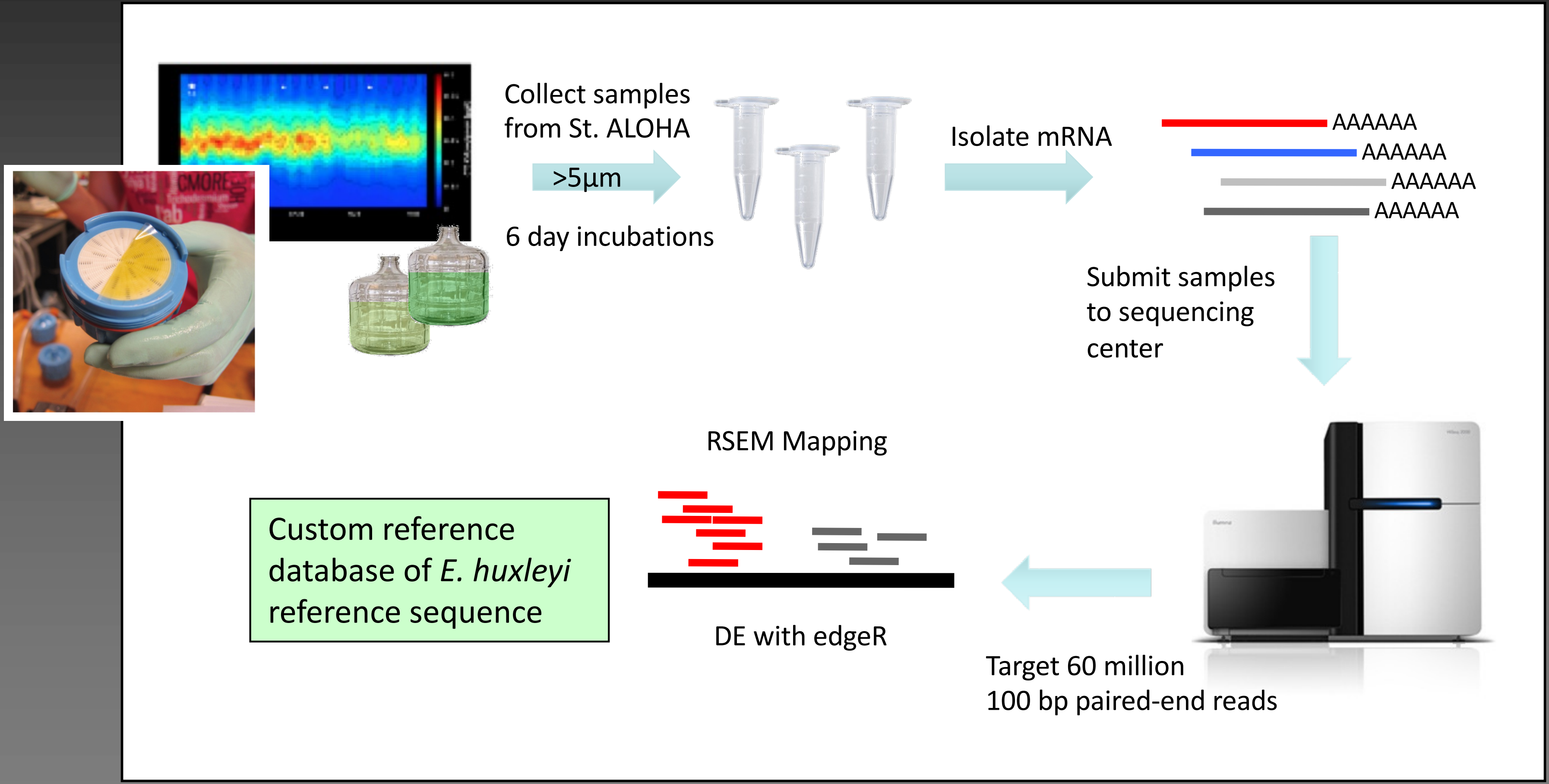
-N

+P

N addition


0N-1
C-1

Sampling and pipeline



Collect samples from St. ALOHA

>5µm

6 day incubations

Isolate mRNA

Submit samples to sequencing center

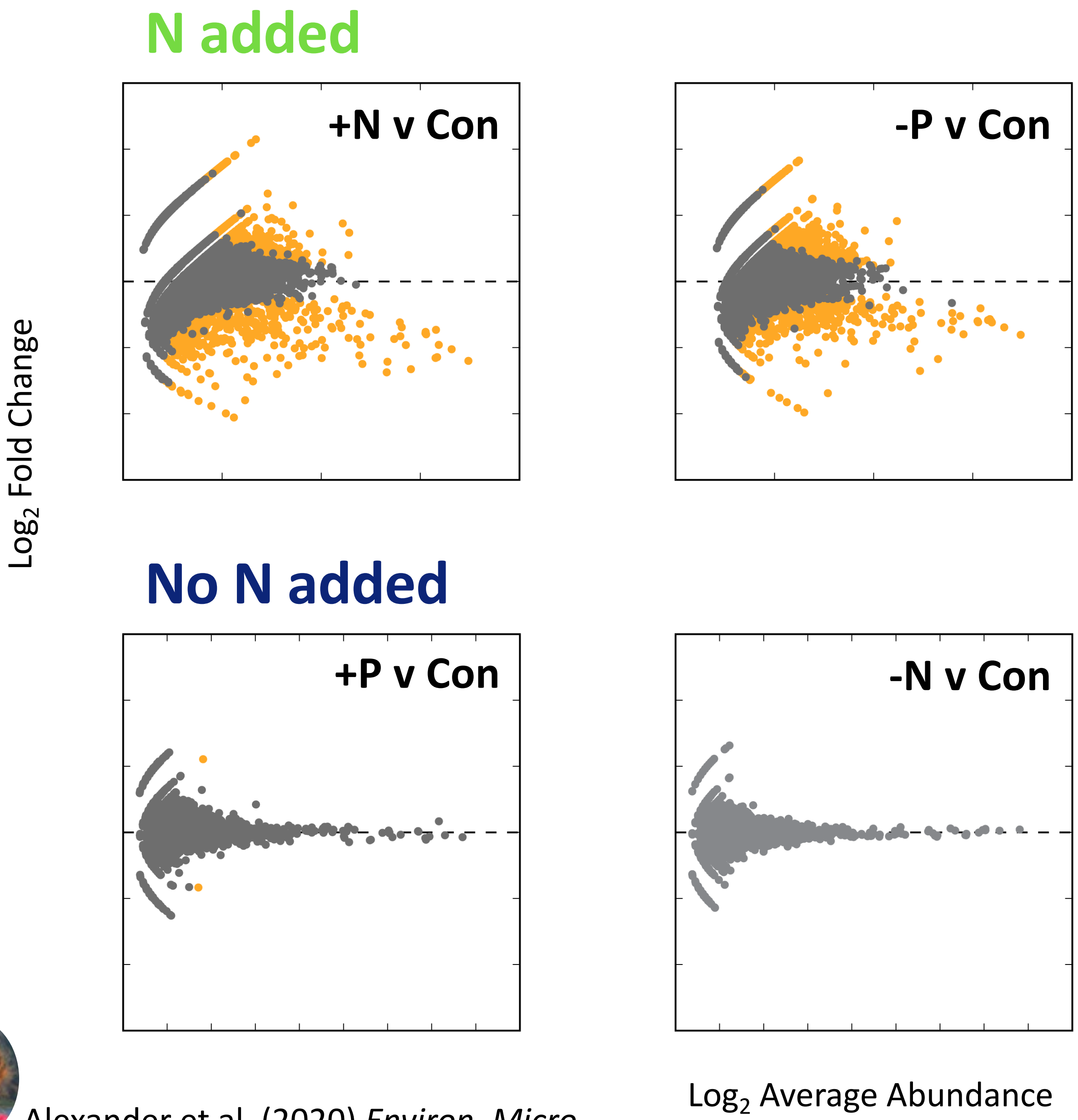
Target 60 million 100 bp paired-end reads

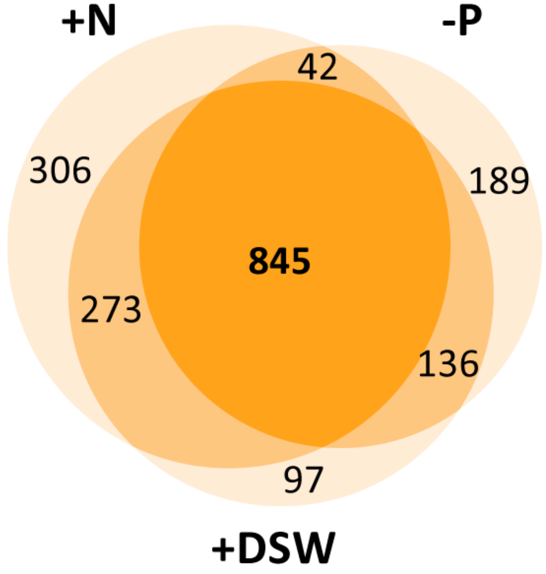
RSEM Mapping

DE with edgeR

Custom reference database of *E. huxleyi* reference sequence

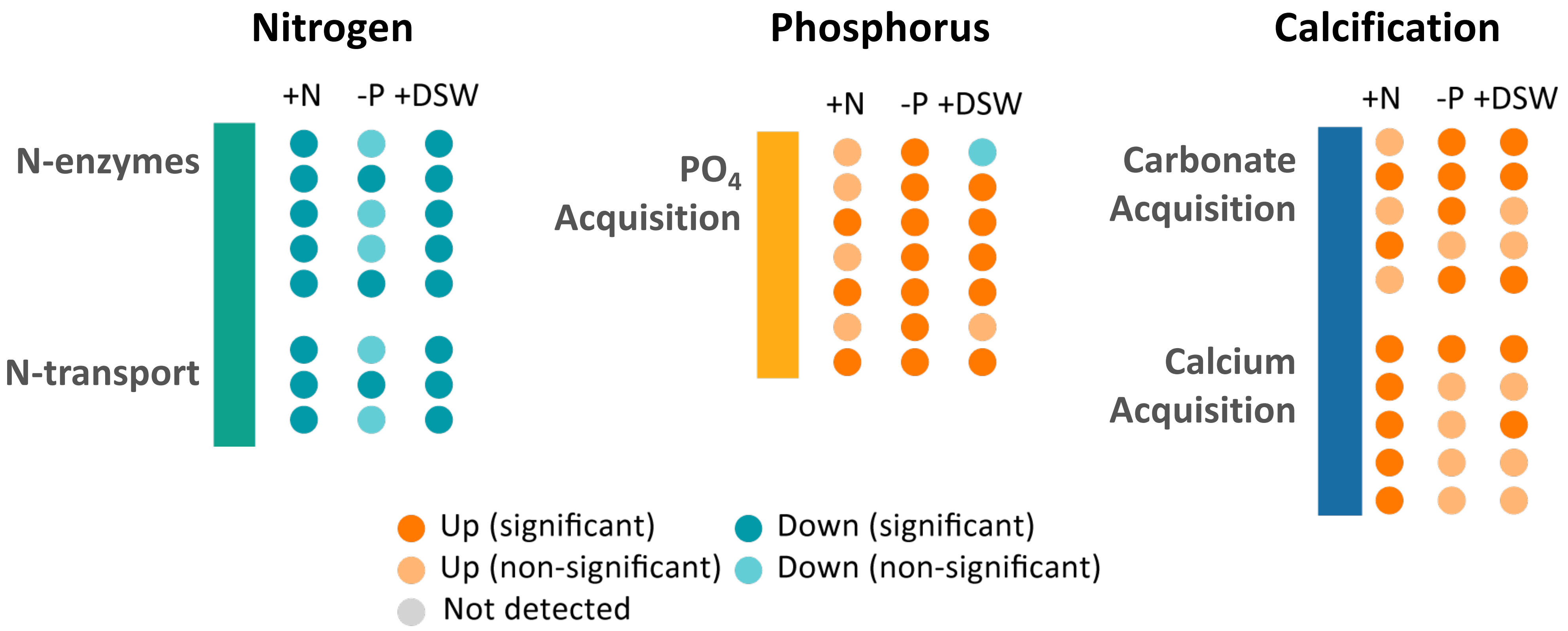
E. huxleyi physiological response suggests N control




Significantly Differentially Abundant Genes

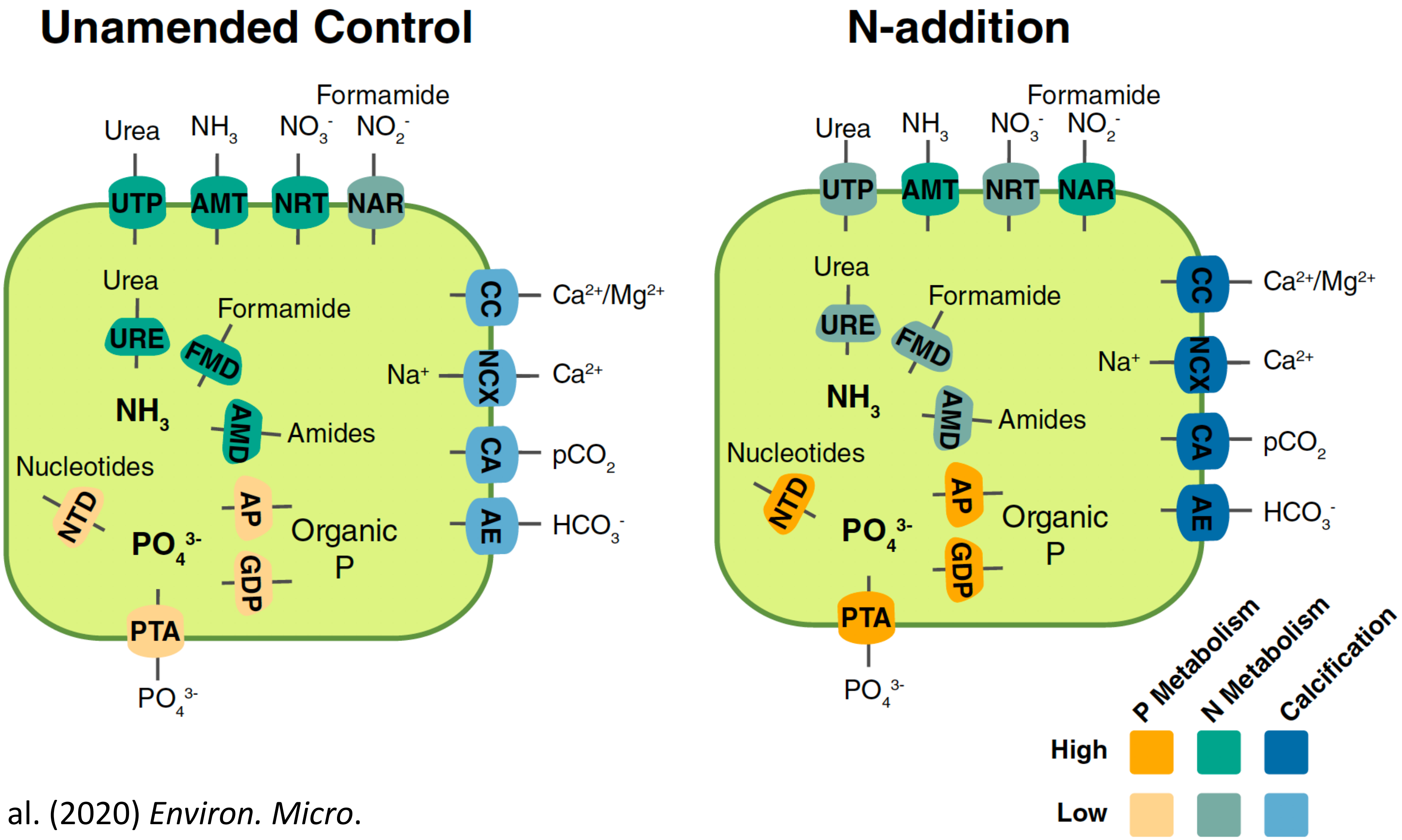
Alexander et al. (2020) *Environ. Micro.*

Transcriptional response



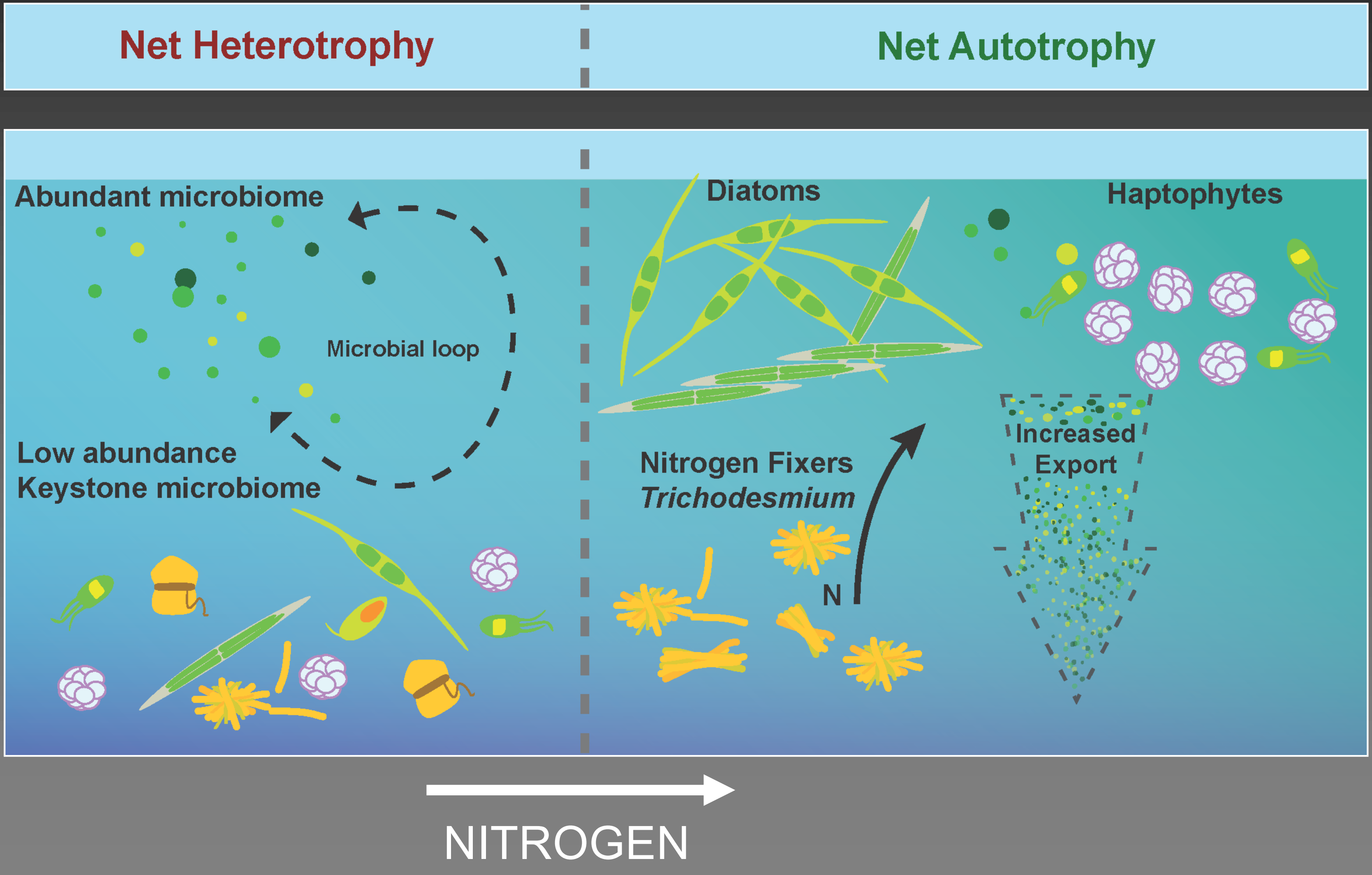
Alexander et al. (2020) *Environ. Micro*.

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

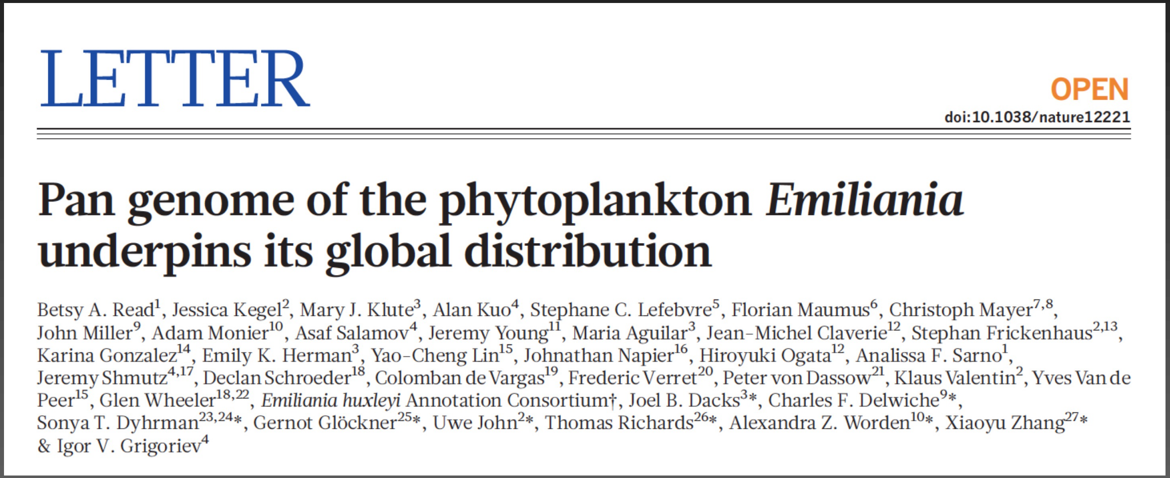


Alexander et al. (2020) *Environ. Micro.*

Nitrogen is a driver of *E. huxleyi* dynamics



Emiliania huxleyi has many diverse isolates



LETTER

OPEN

doi:10.1038/nature12221

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

Betsy A. Read[1], Jessica Kegel[2], Mary J. Klute[3], Alan Kuo[4], Stephane C. Lefebvre[5], Florian Maumus[6], Christoph Mayer[7,8], John Miller[9], Adam Monier[10], Asaf Salamov[4], Jeremy Young[11], Maria Aguilar[3], Jean-Michel Claverie[12], Stephan Frickenhaus[2,13], Karina Gonzalez[14], Emily K. Herman[3], Yao-Cheng Lin[15], Johnathan Napier[16], Hiroyuki Ogata[12], Analissa F. Sarno[1], Jeremy Shmutz[4,17], Declan Schroeder[18], Colomban de Vargas[19], Frederic Verret[20], Peter von Dassow[21], Klaus Valentin[2], Yves Van de Peer[15], Glen Wheeler[18,22], *Emiliania 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. Grigoriev[4]

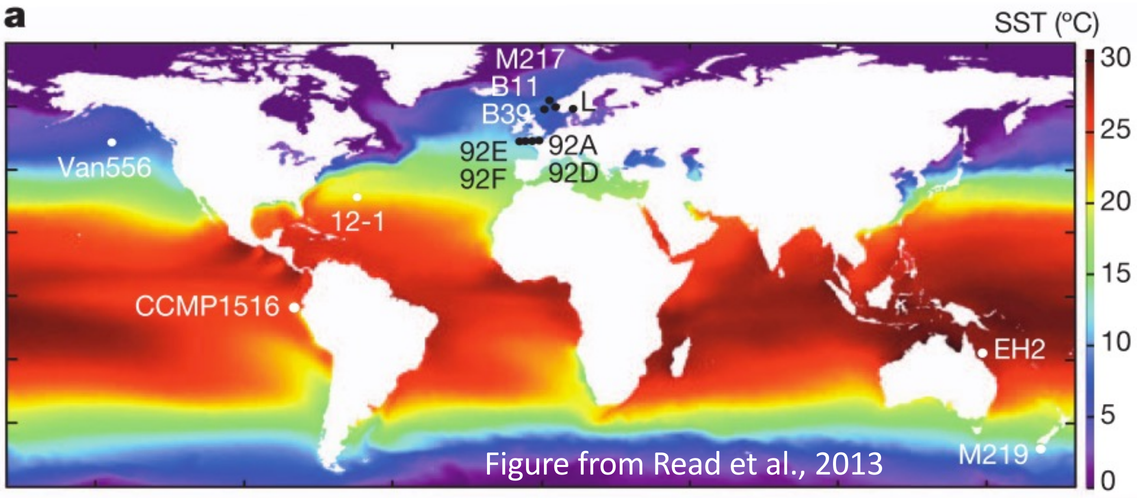


Figure from Read et al., 2013

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

- ● CCMP1516
- ● CCMP379
- ● CCMP374
- ● CCMP370
- ● PLYM219

↓

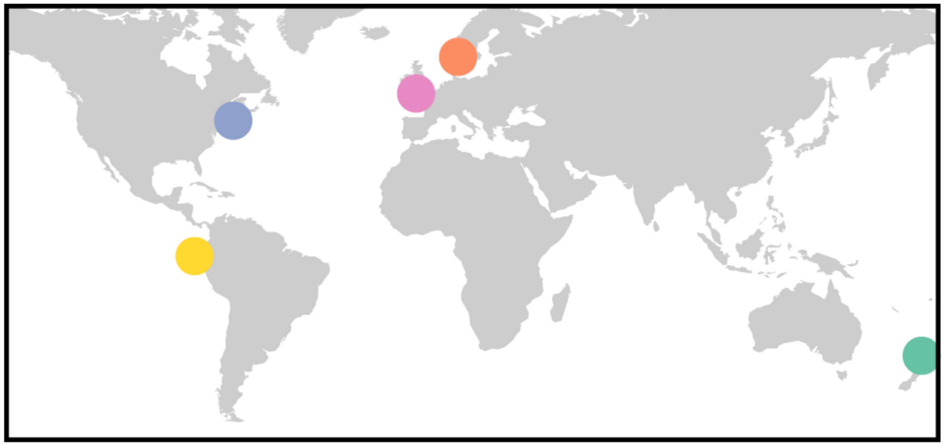
Cluster into orthologous groups to identify core and variable OGs

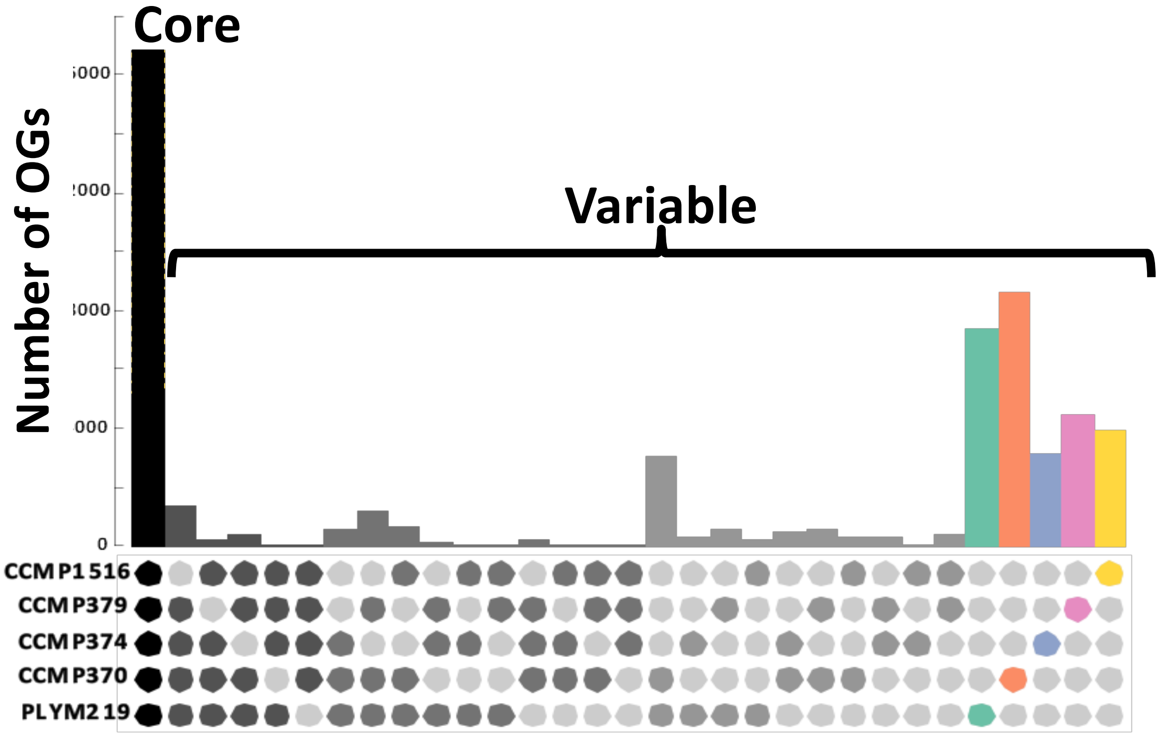
(OrthoMCL)

↓

Map field data and examine shifts in expression

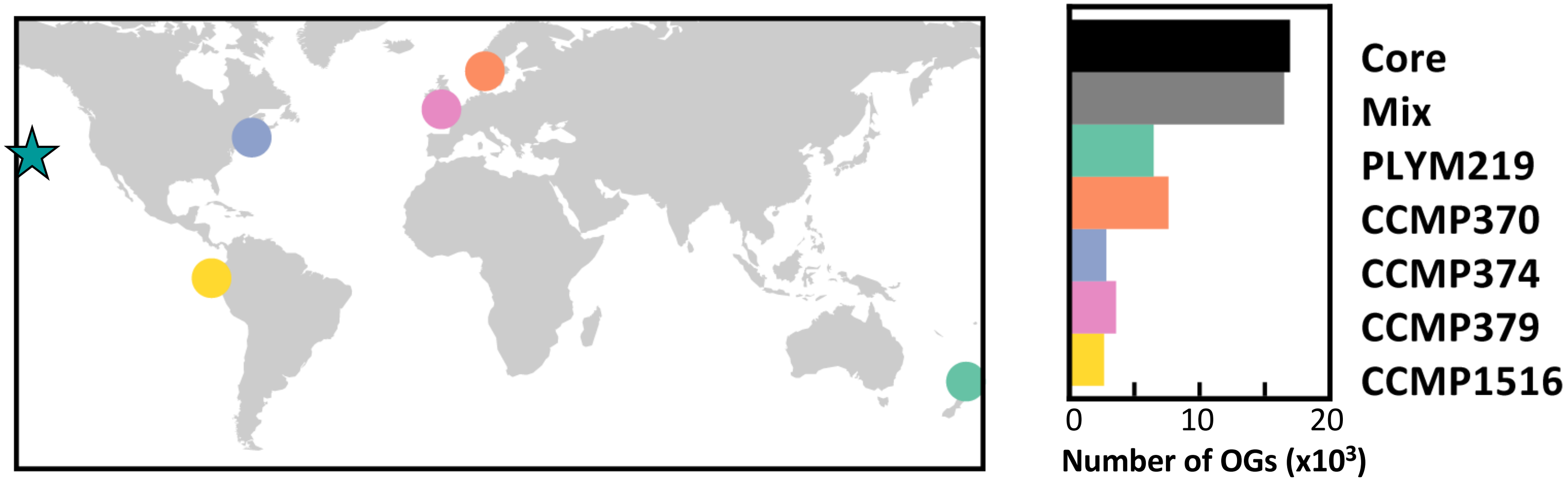
(RSEM, edgeR)





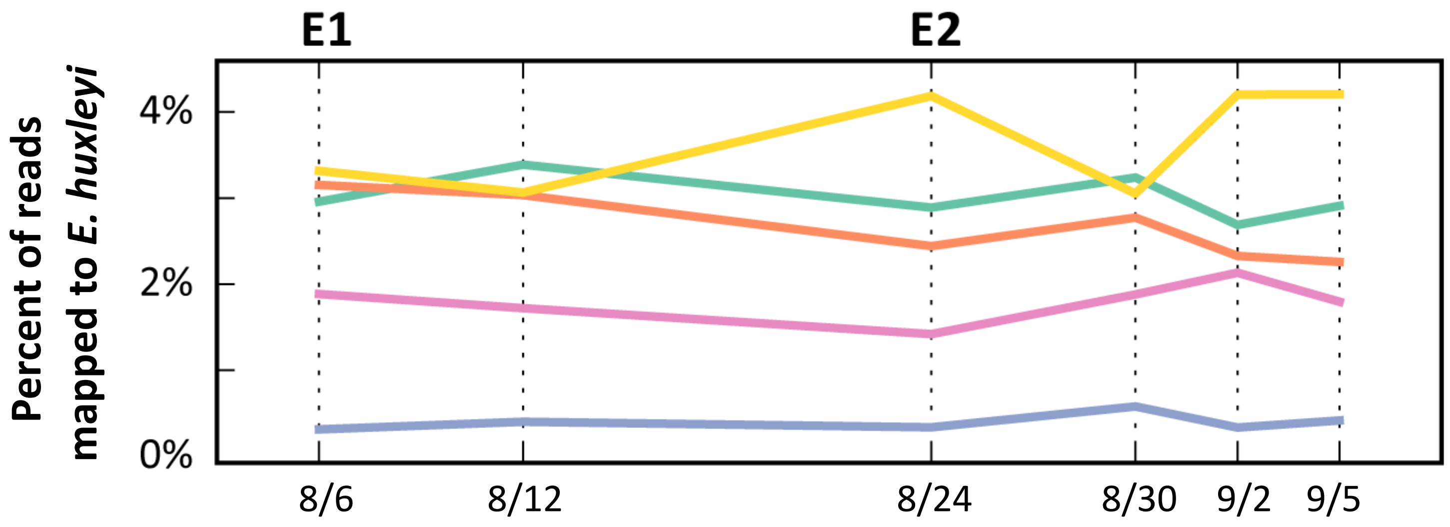
Alexander et al. unpub.

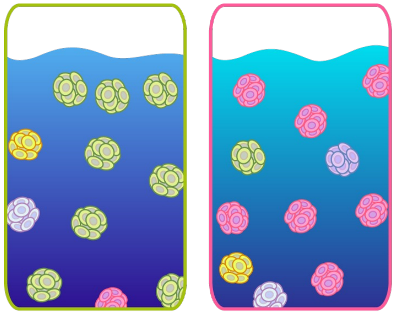
Tracking orthologous groups at St. ALOHA

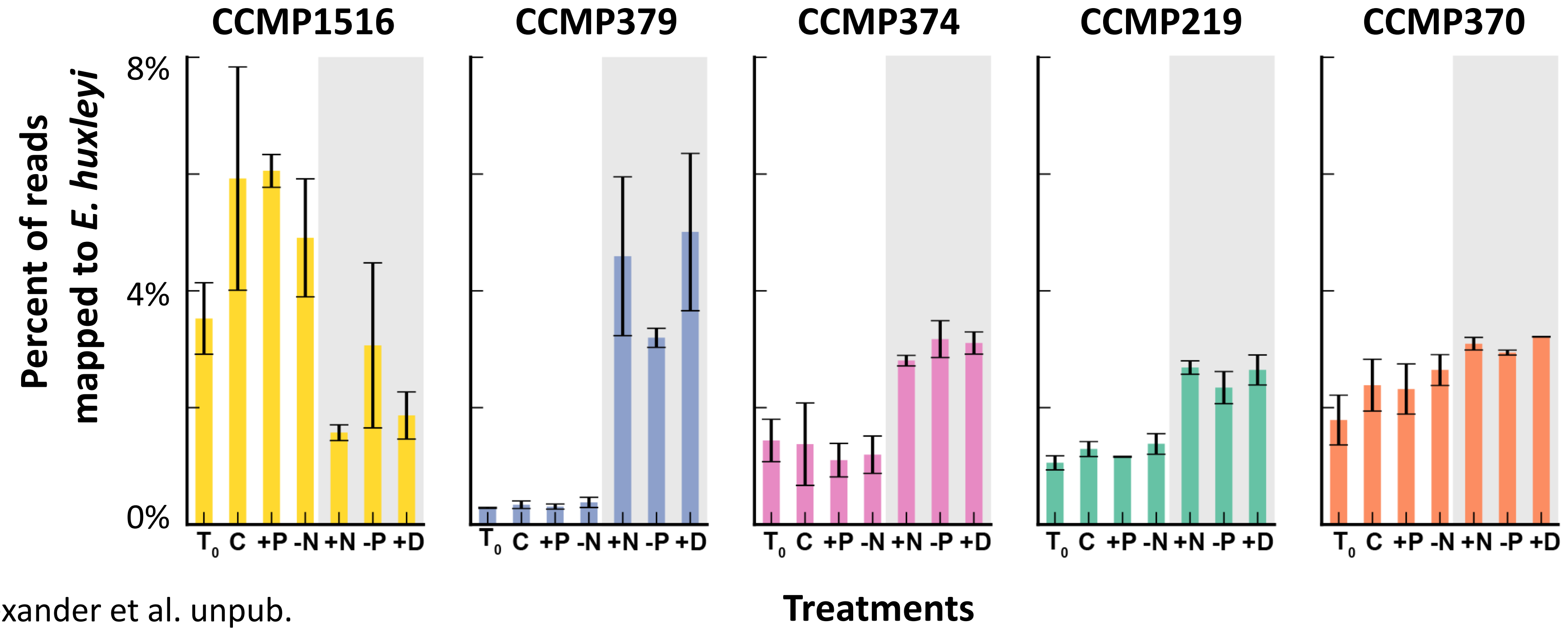


Use diagnostic OGs to track strain distribution

Strain distribution *in situ* and in the incubations

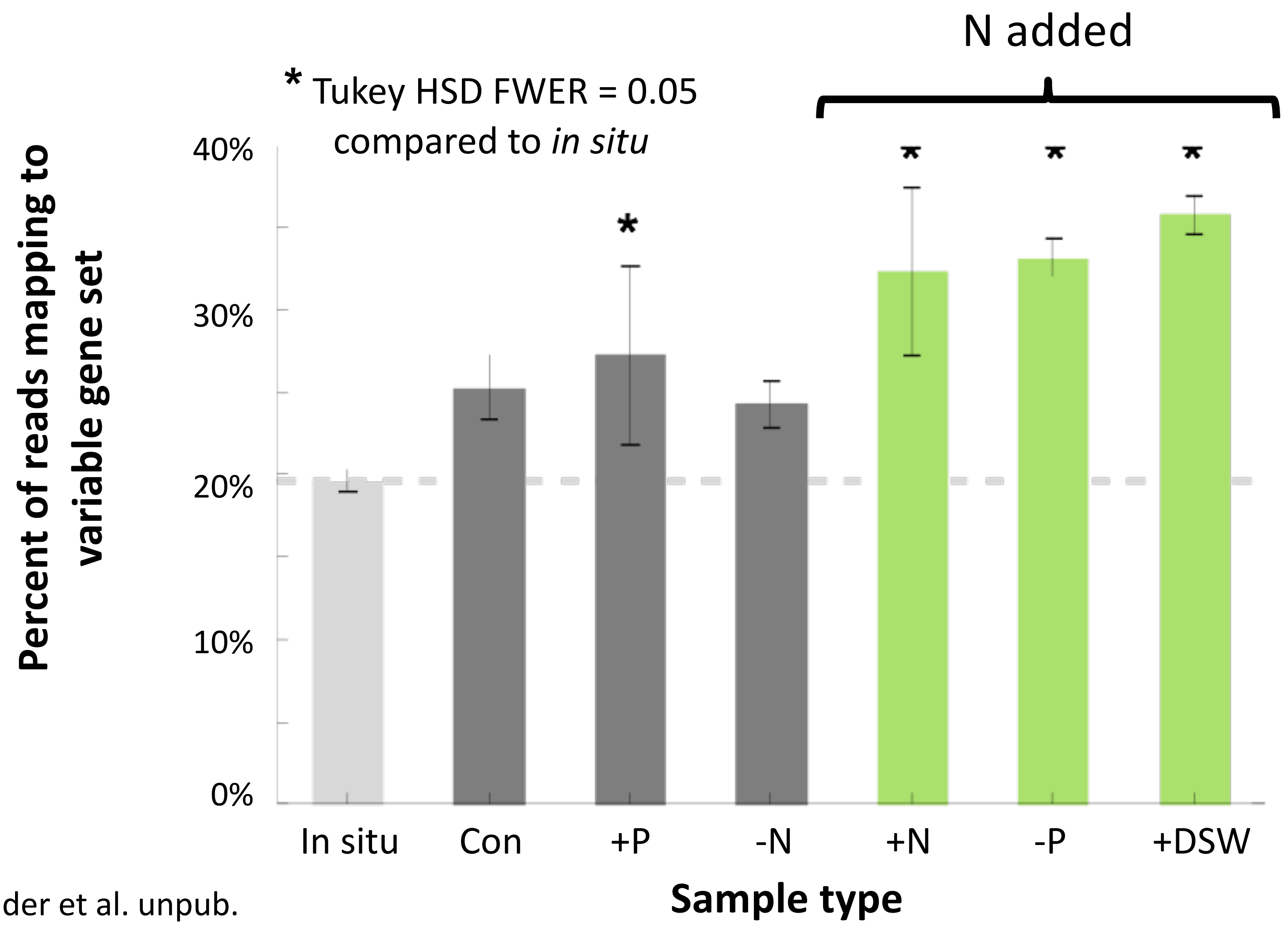






Alexander et al. unpub.

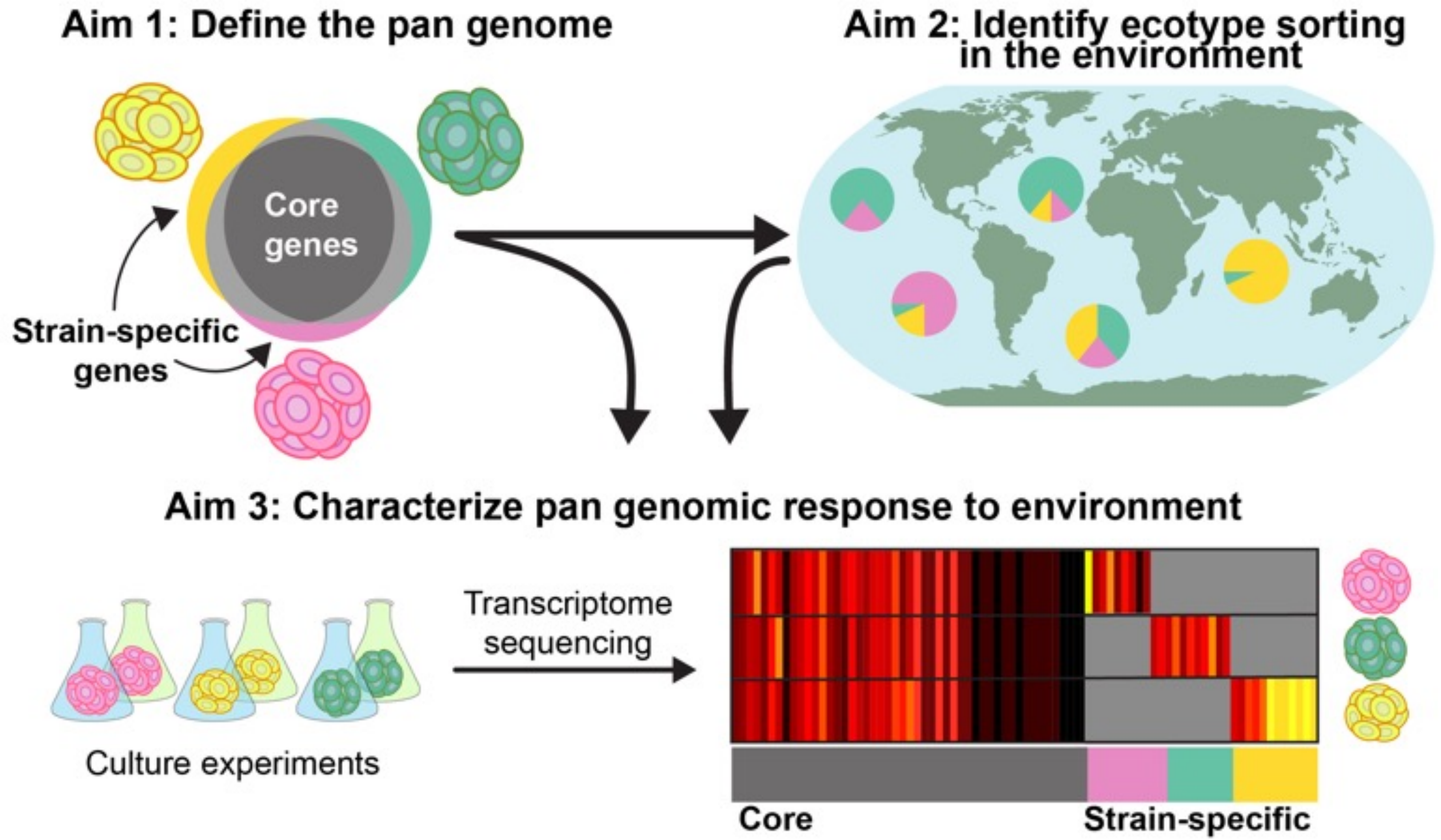
Expression of the variable portion of the pan-genome



Alexander et al. unpub.

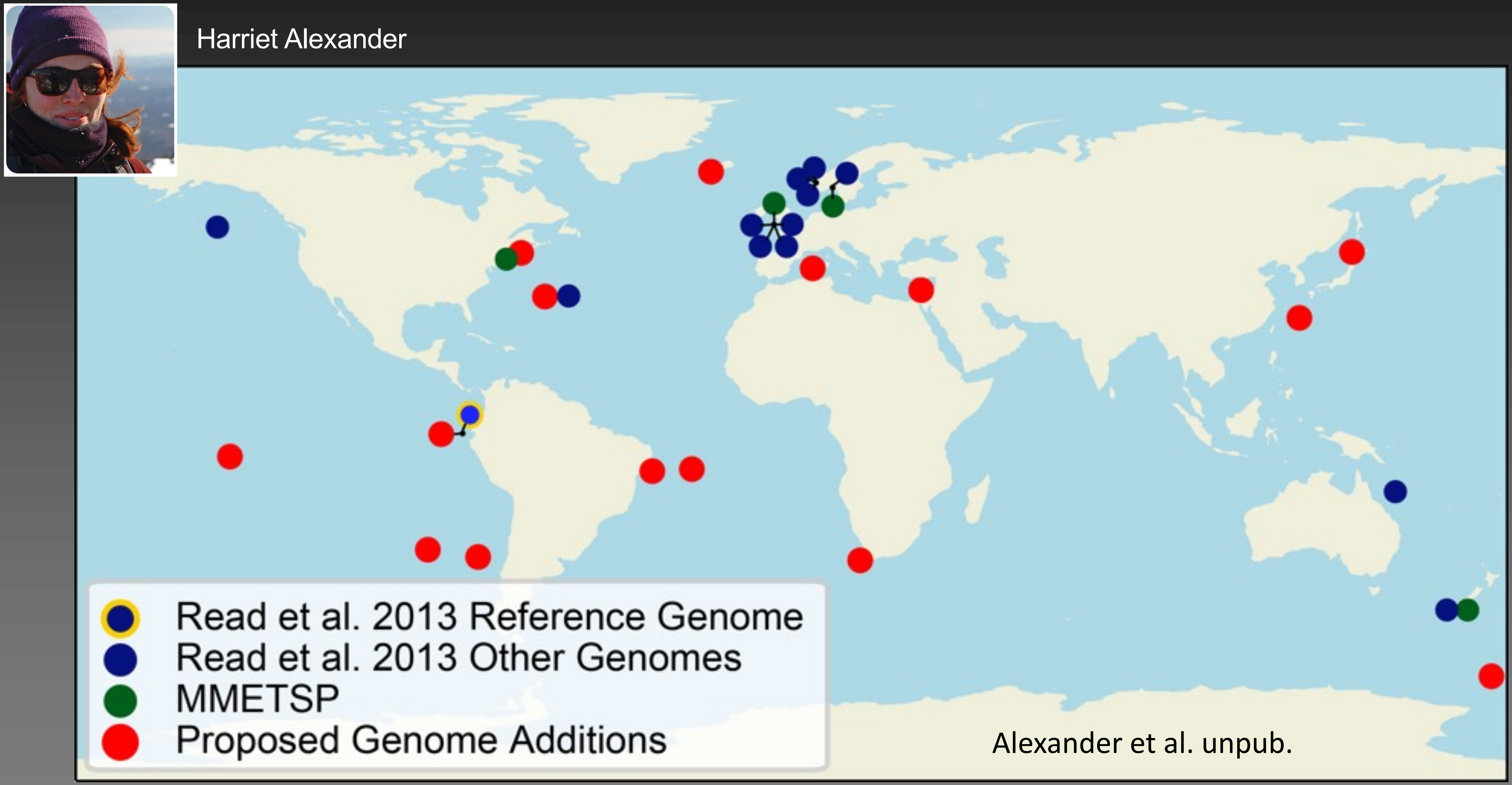
Expression of variable gene set significantly increased following N-addition

Future work to define the pan genome



Alexander et al. unpub.

Expanding the pan genome



Harriet Alexander

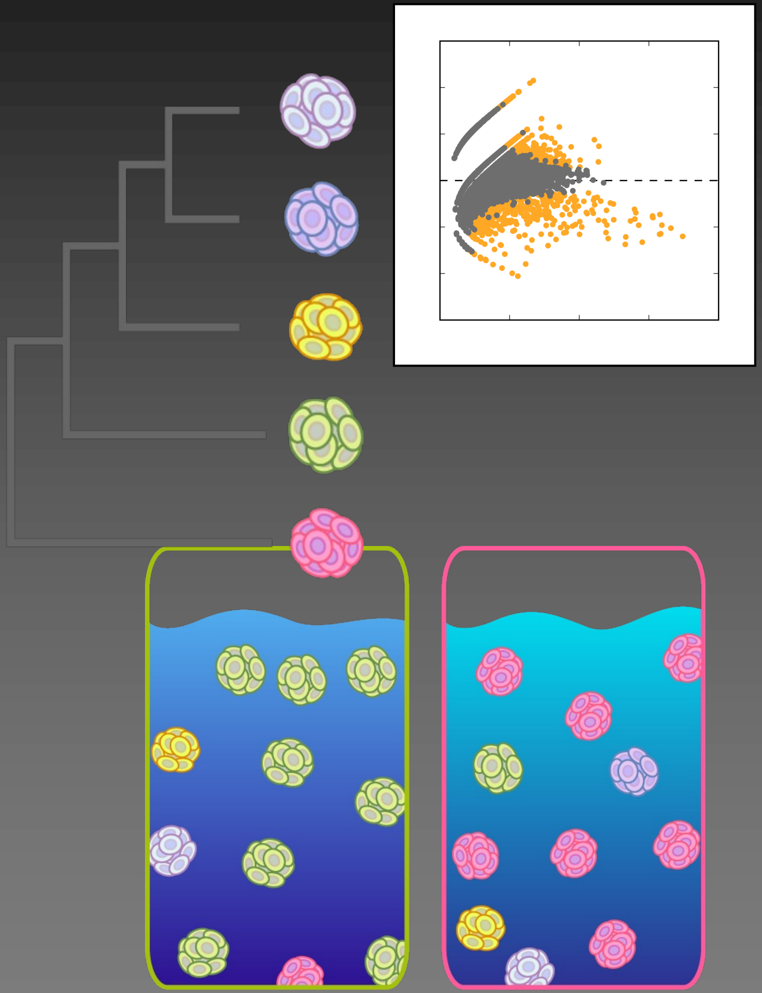
Alexander et al. unpub.

~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



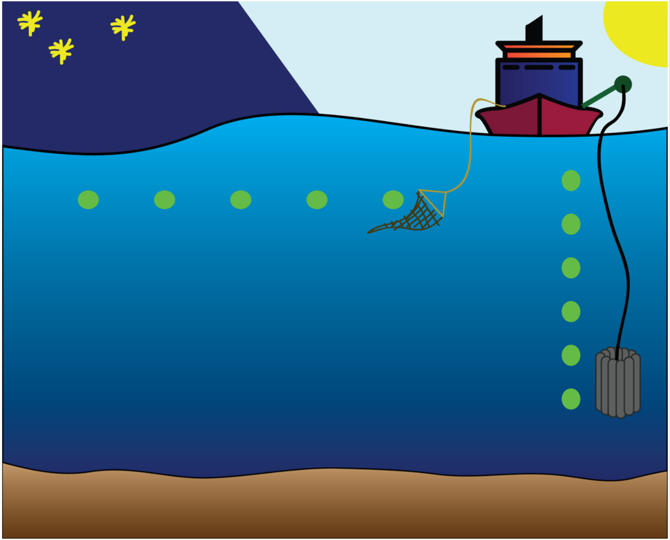
Core questions… adventures in eukaryote metatranscriptomics

HOE:DYLAN

Limitation

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

HOE:MAHALO



Competition

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

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)

SCOPE

SIMONS COLLABORATION ON OCEAN PROCESSES AND ECOLOGY

Inputs

Microbes

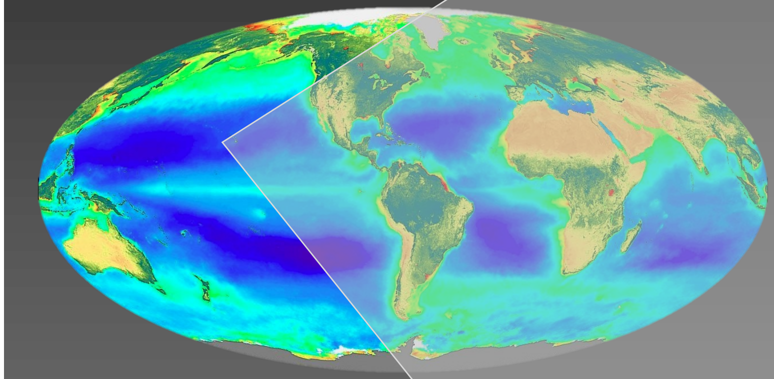
Models

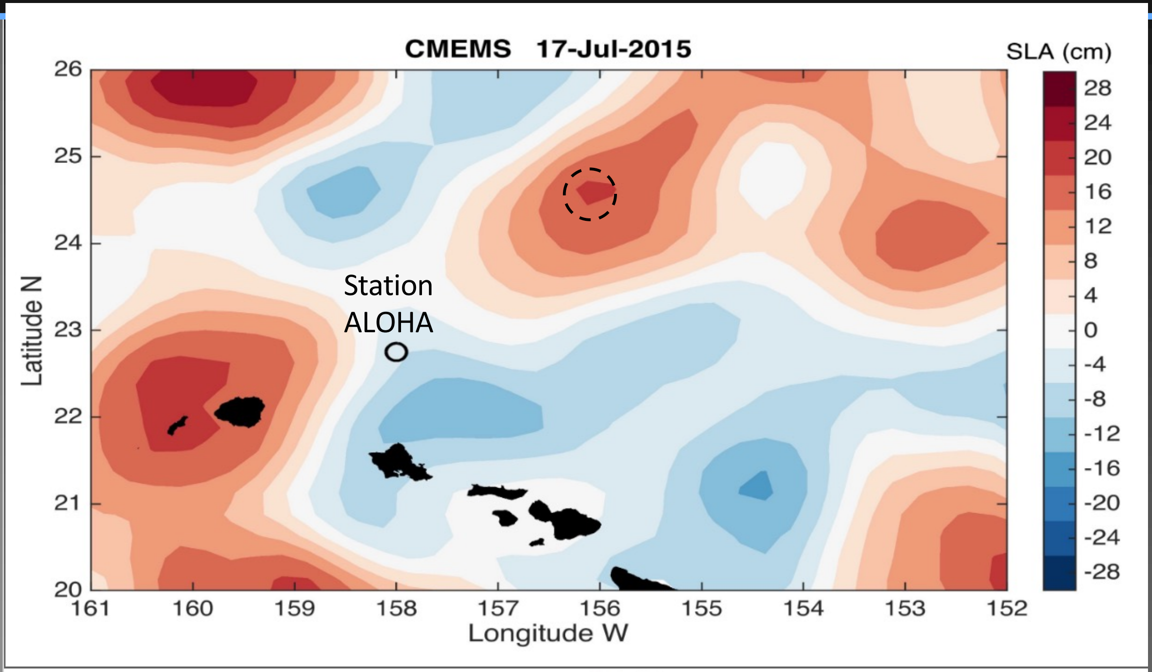
Outputs

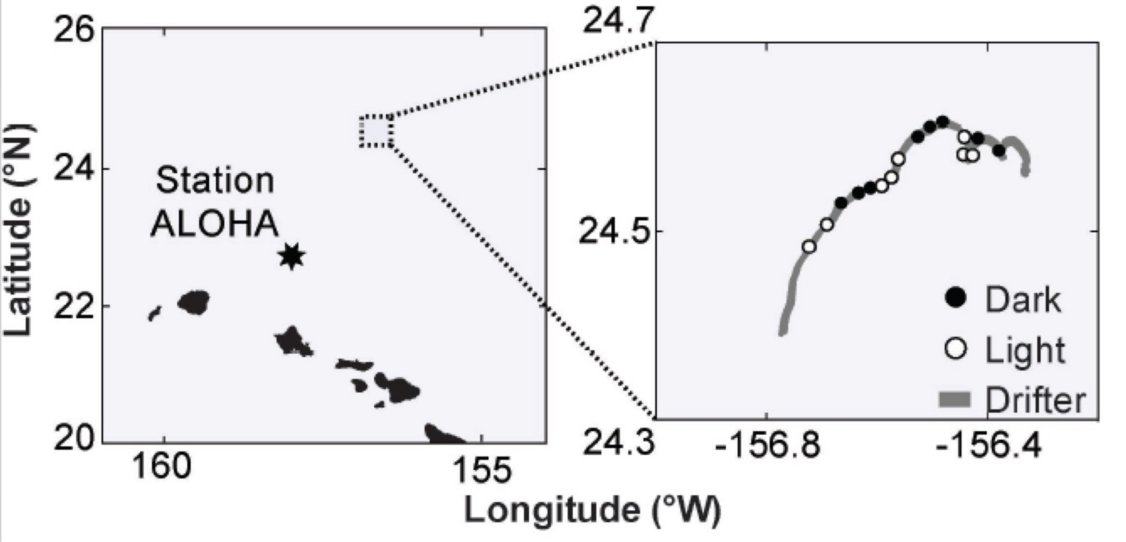
SCOPE.SOEST.HAWAII.EDU

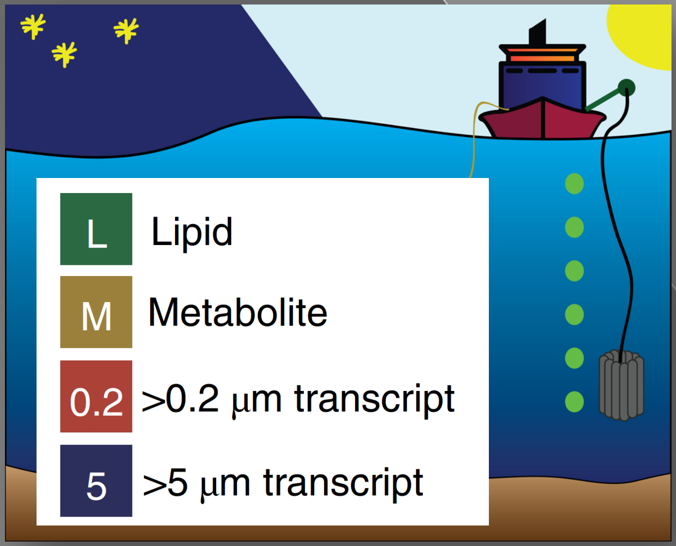
MAHALO: Special thanks to Daniel Muratore and Joshua Weitz for synthesis efforts

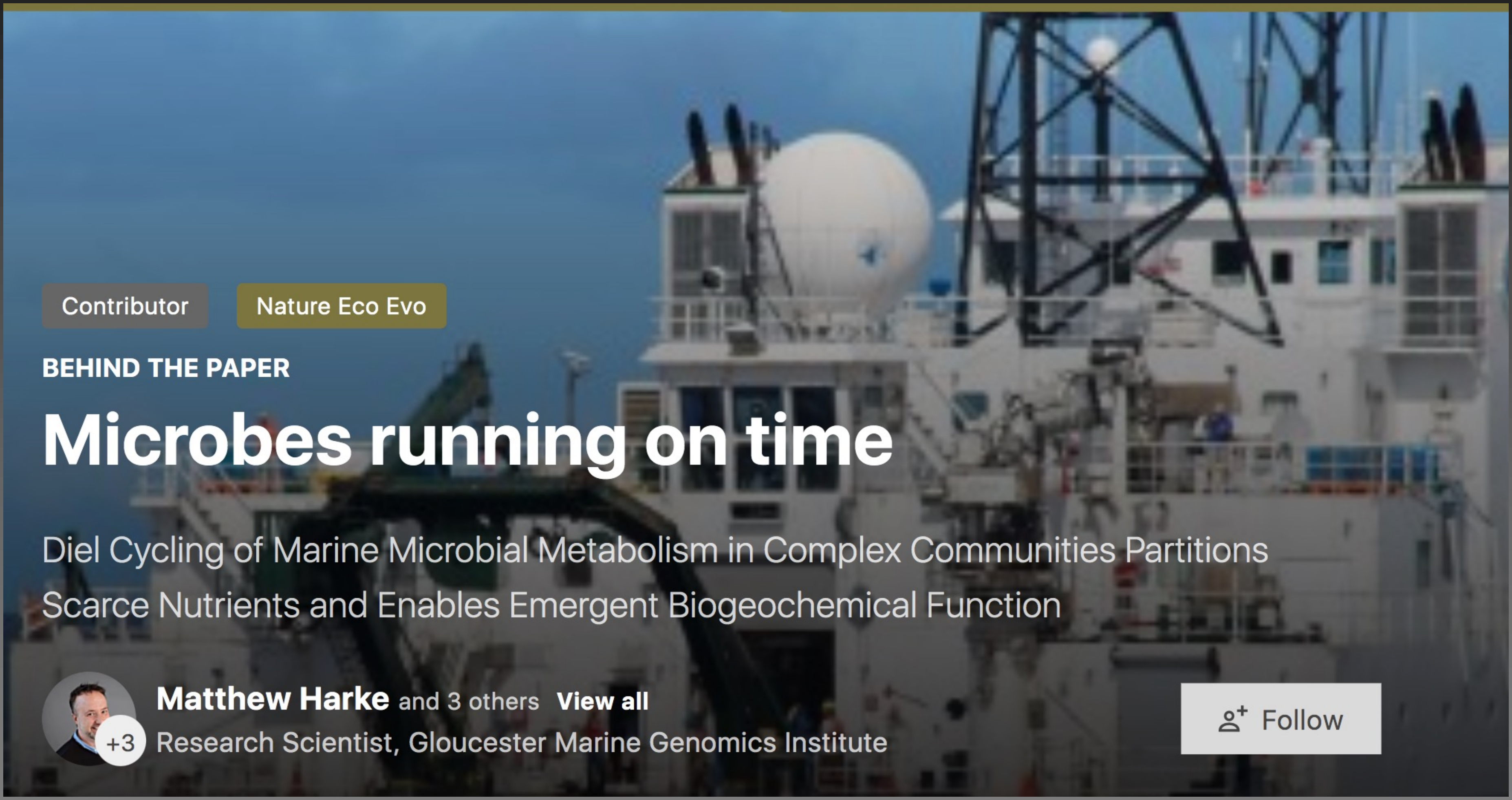
Sampling light driven responses in the NPSG











BEHIND THE PAPER

Microbes running on time

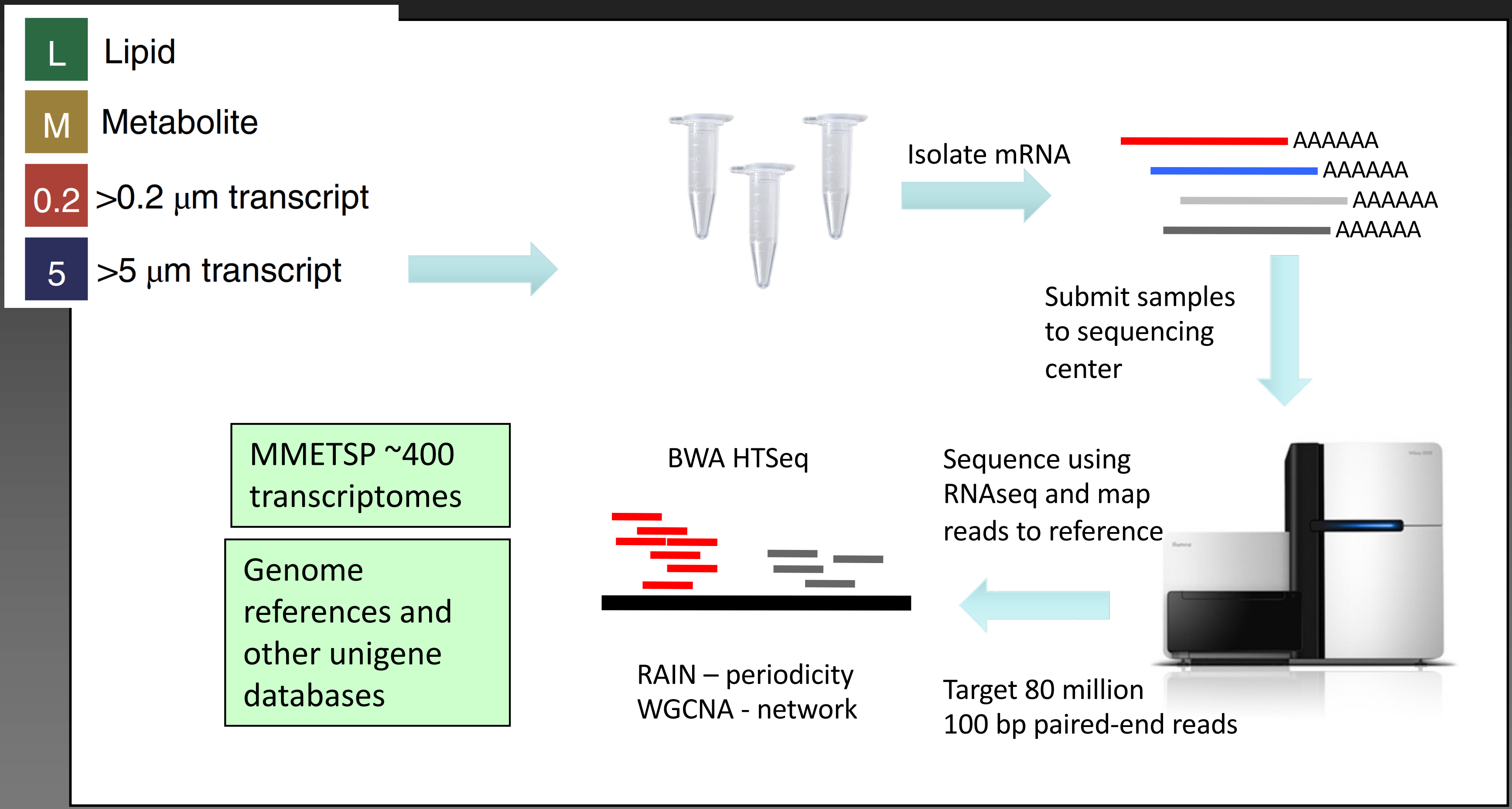
Diel Cycling of Marine Microbial Metabolism in Complex Communities Partitions Scarce Nutrients and Enables Emergent Biogeochemical Function

Matthew Harke and 3 others **View all**
Research Scientist, Gloucester Marine Genomics Institute

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

Sampling every 4hrs for many days ….

Sampling and pipeline



L Lipid

M Metabolite

0.2 >0.2 μm transcript

5 >5 μm transcript

Isolate mRNA

Submit samples to sequencing center

Sequence using RNAseq and map reads to reference

Target 80 million 100 bp paired-end reads

BWA HTSeq

RAIN – periodicity
WGCNA - network

MMETSP ~400 transcriptomes

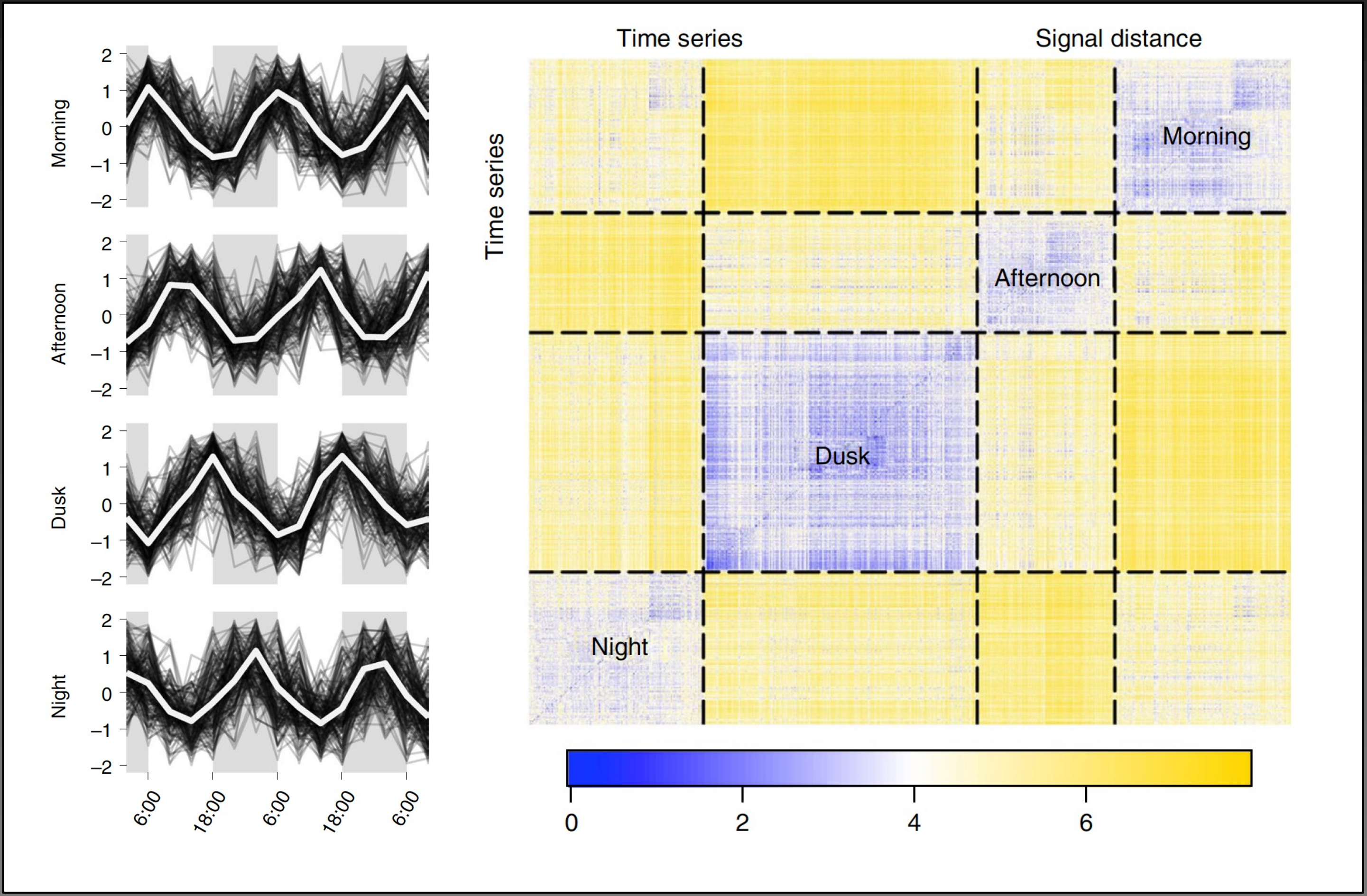
Genome references and other unigene databases

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

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

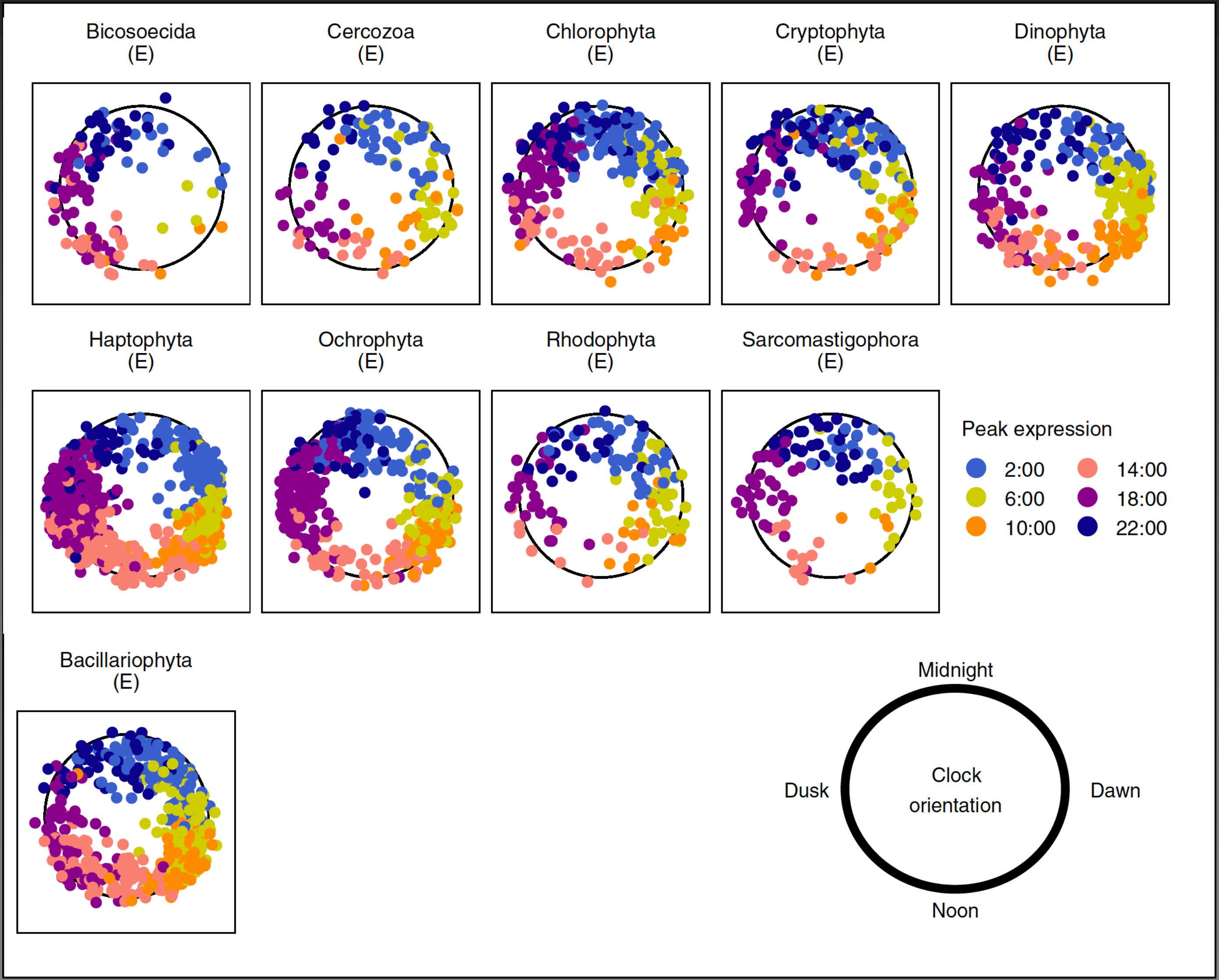
MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Archetypal patterns



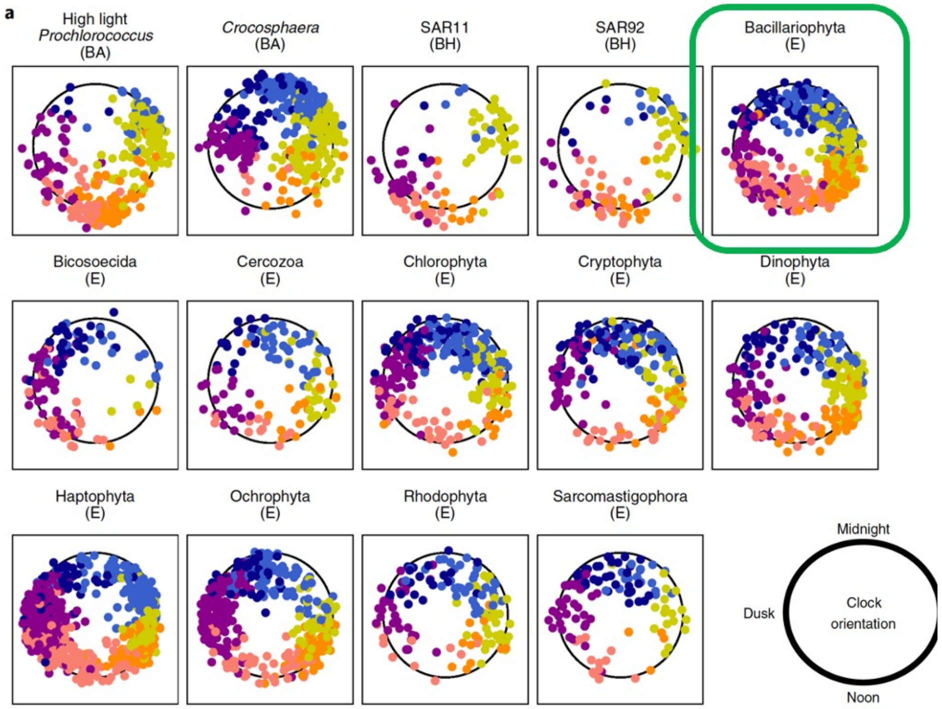
Muratore, Boysen, Harke et al. (2022) *Nature Ecol Evo*.

Different taxa have distinct diel transcription patterns



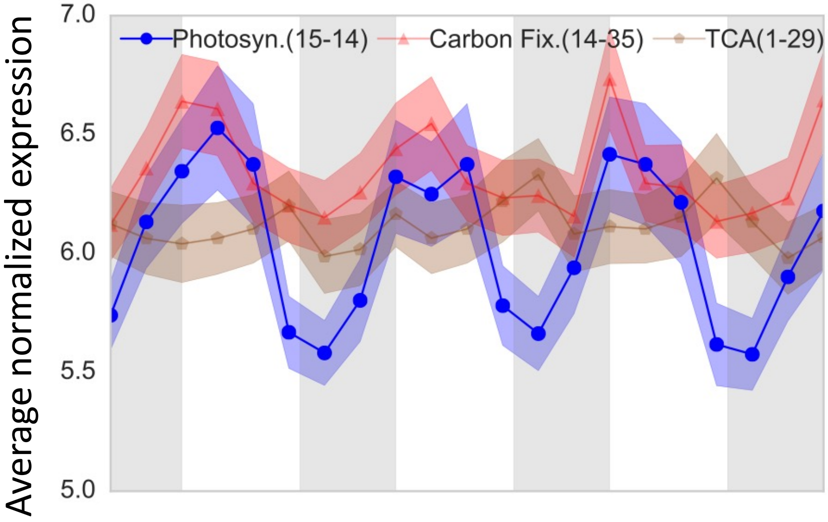
Muratore, Boysen, Harke et al. (2022) *Nature Ecol Evo*.

Carbon fixation and respiration are highly coordinated



Rhizosolenia

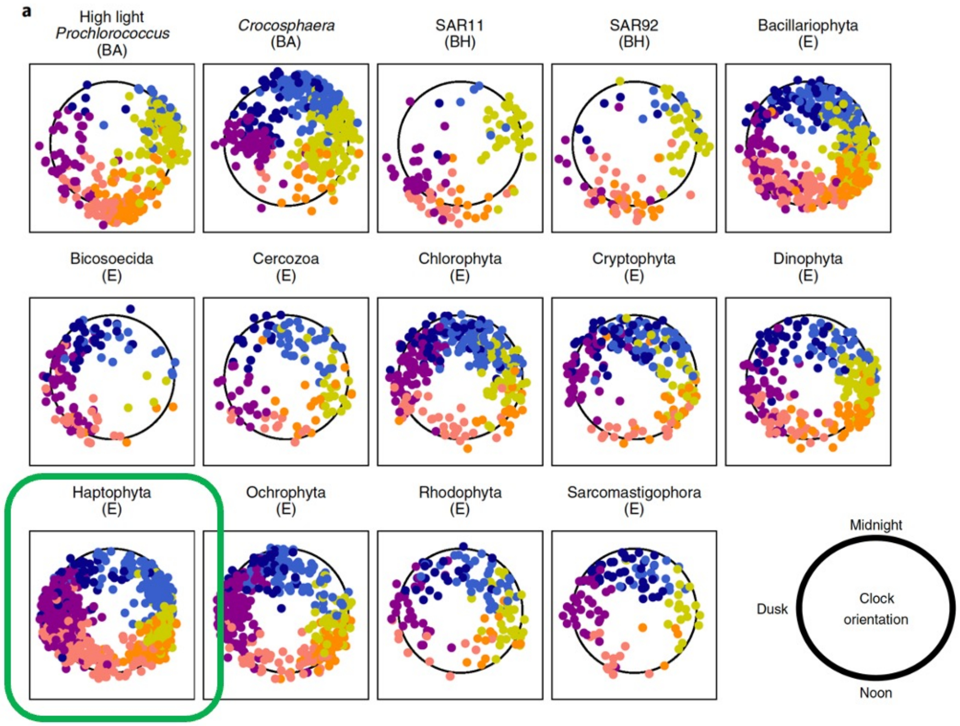
(b) Carbon Metabolism



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

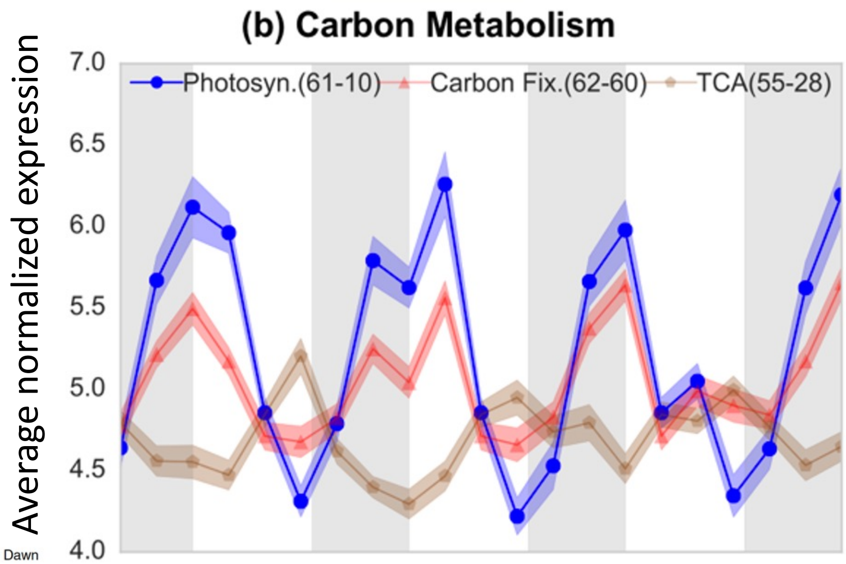
Harke et al. 2019 *ISME J*

Carbon fixation and respiration are highly coordinated



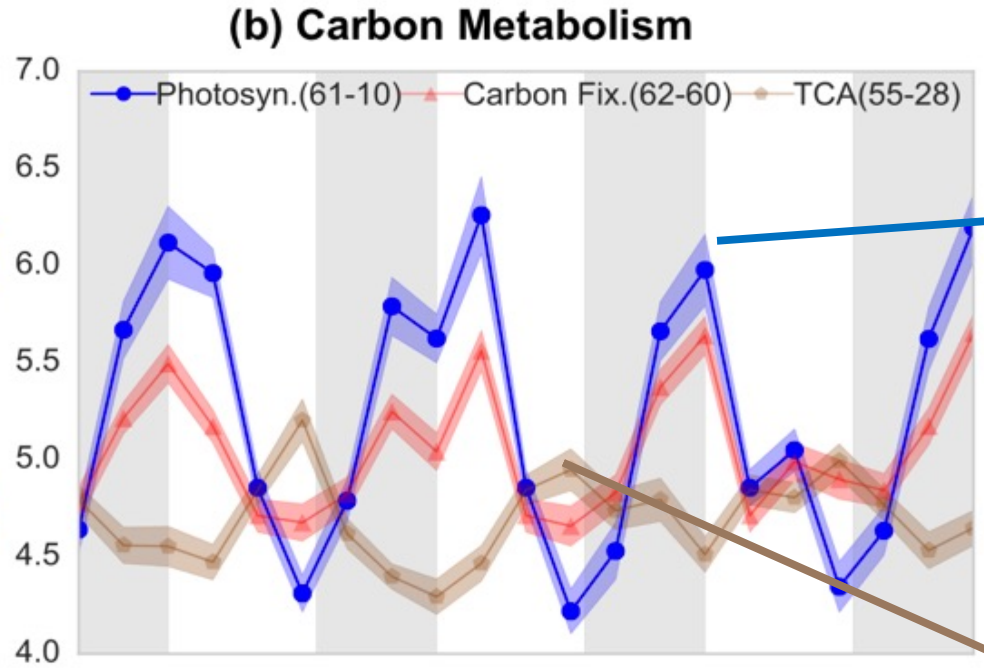
Muratore, Boysen, Harke et al. (2022) *Nature Ecol. Evol.*

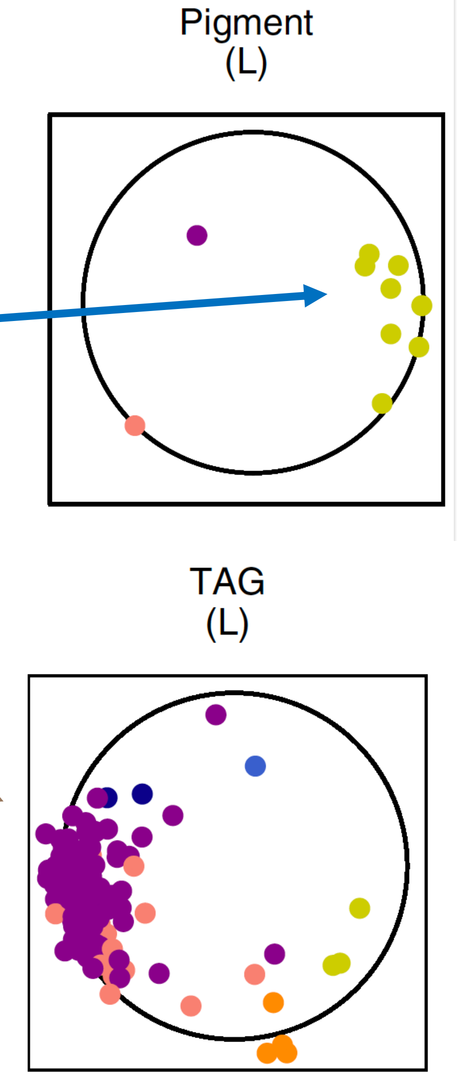
Emiliania

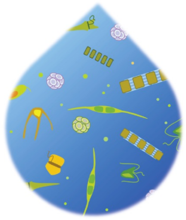


Limón-Hernandez et al. (2020) *Environ. Micro.*

Carbon metabolism synchronized across all phytoplankton







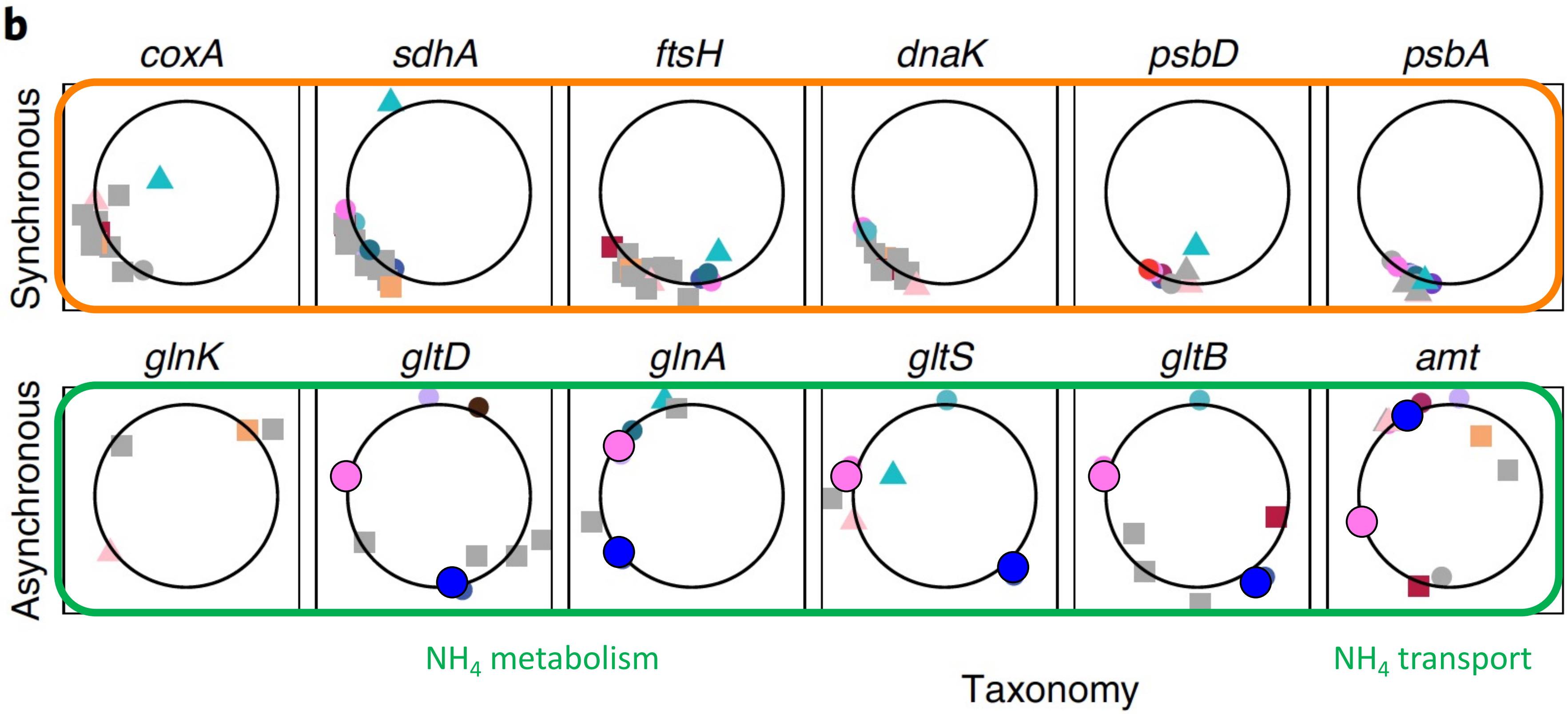
All photosynthesis, carbon fixation, and TCA cycle processes are synchronized across bacteria and eukaryotes!

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

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

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

N metabolism functions are expressed asynchronously



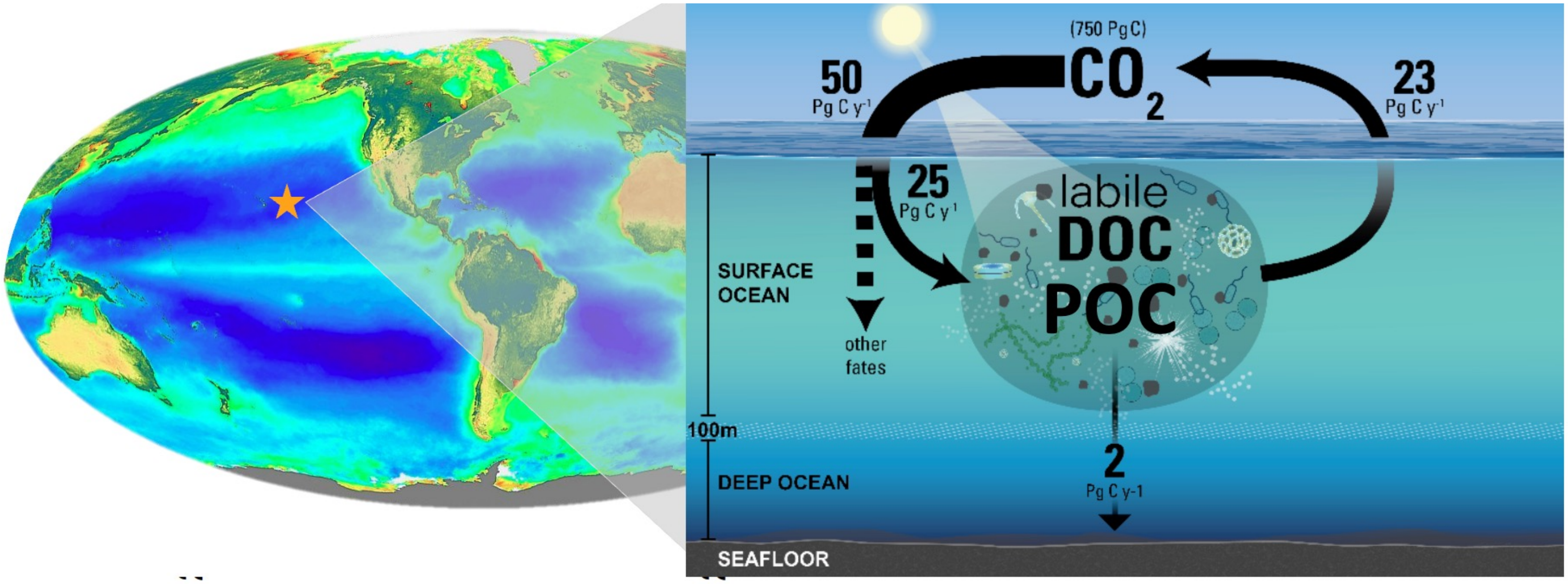
NH_4 metabolism

NH_4 transport

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

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

Tracing carbon flow through the network

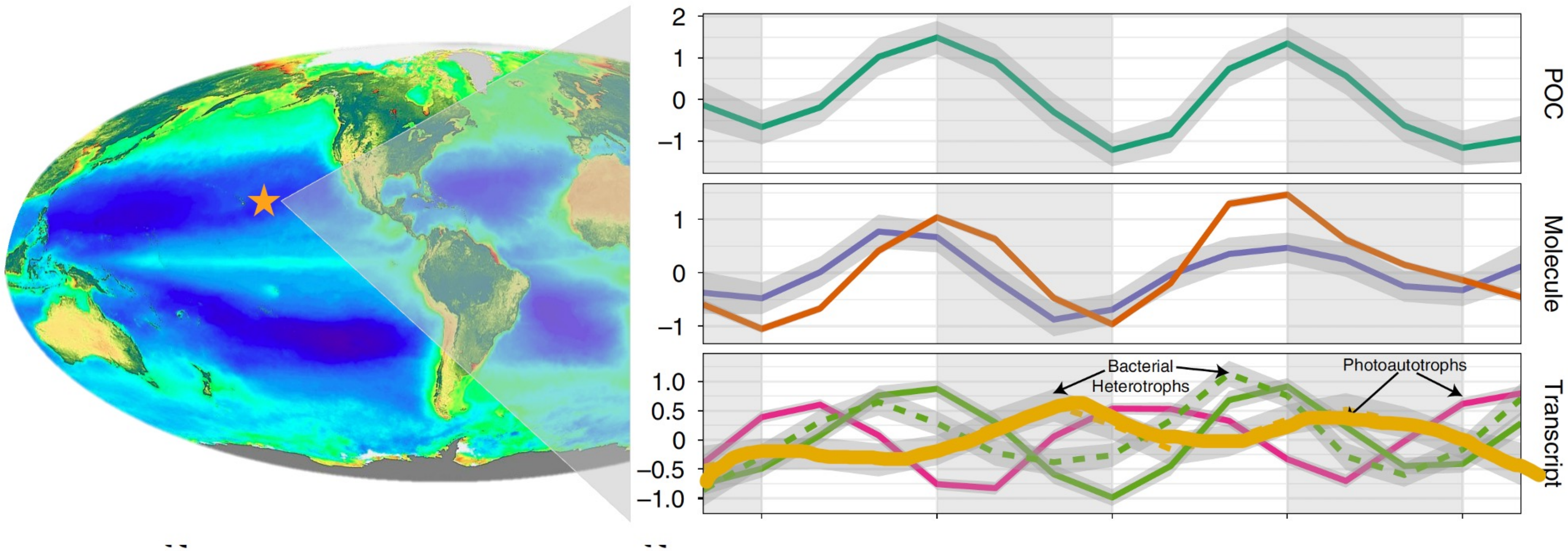


▬ POC
▬ Triacylglycerol
▬ Metabolite

▬ Photosynthesis
▬ TCA cycle
▬ Sugar transporters

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

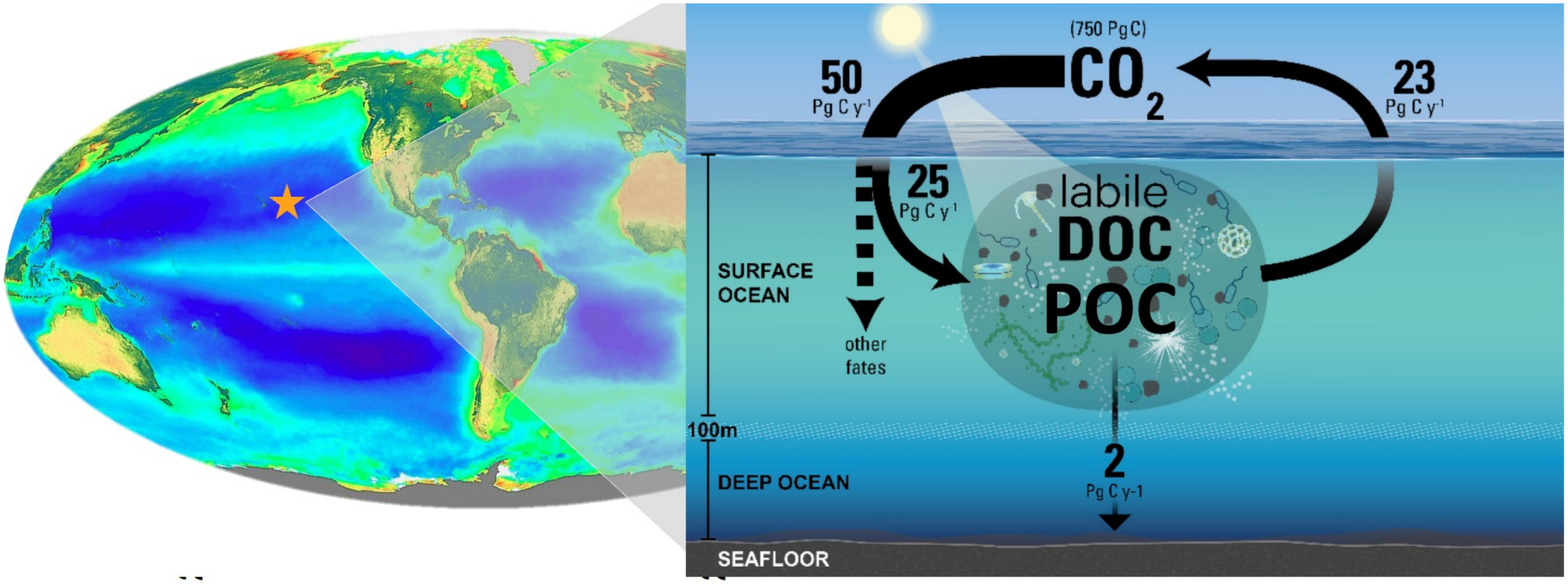
Tracing carbon flow through the network



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

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

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



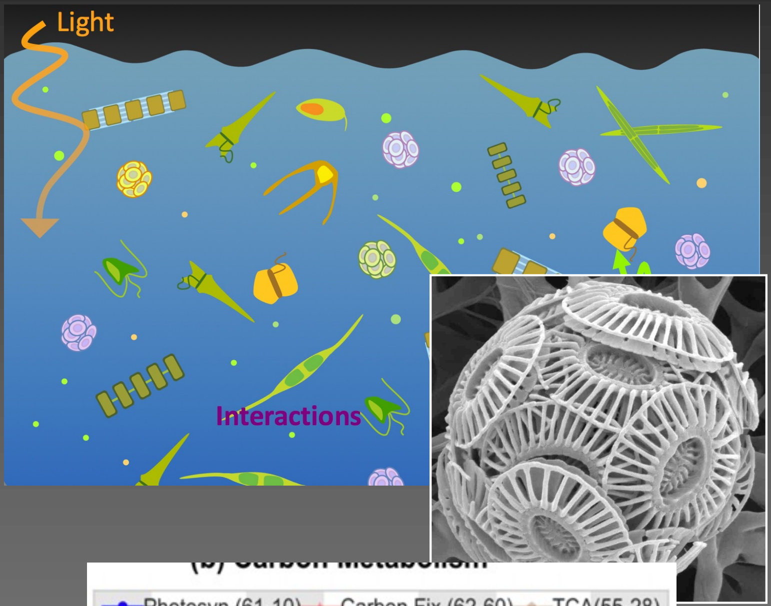
🟩 POC
🟧 Triacylglycerol
🟪 Metabolite

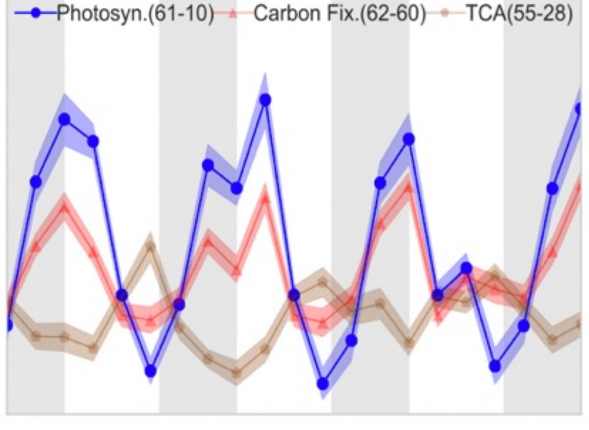
🟥 Photosynthesis
🟩 TCA cycle
🟨 Sugar transporters

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

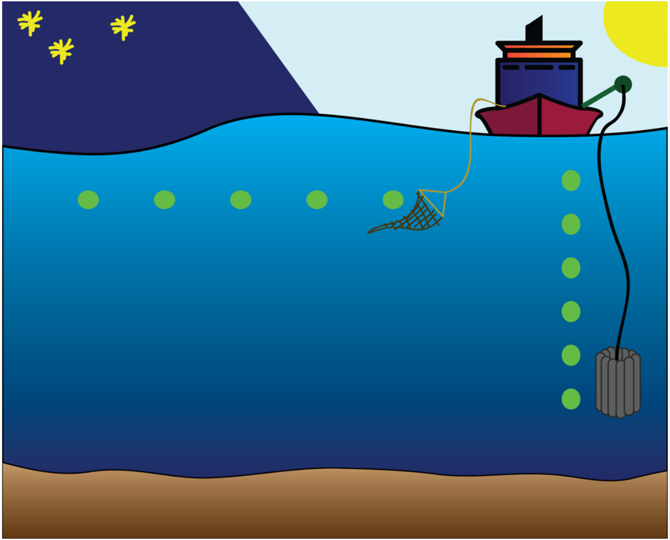
HOE:DYLAN

Limitation

- **Nitrogen limits carbon export and cycling in a keystone hapotophyte**

- **The environment selects**

HOE:MAHALO



Competition

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