

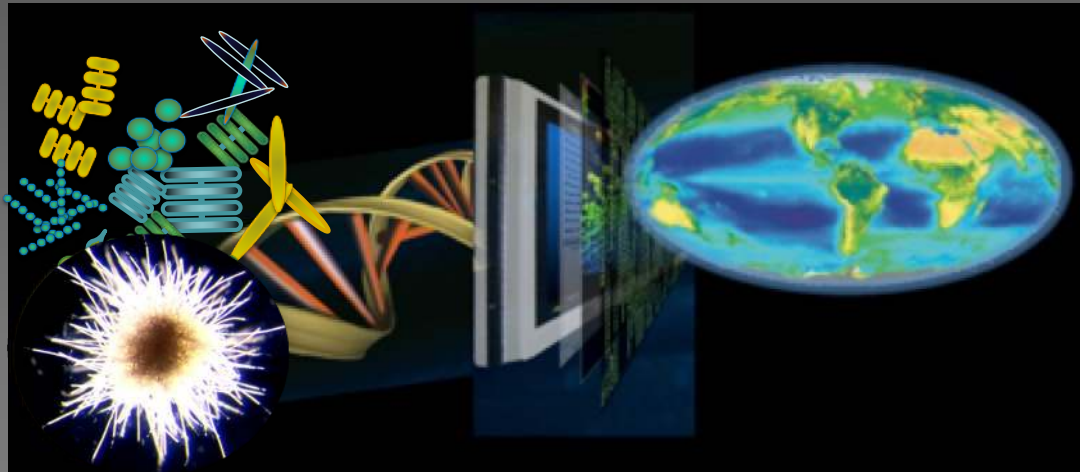
Ocean genomics: microbial physiological ecology across marine ecosystems

Sonya Dyhrman

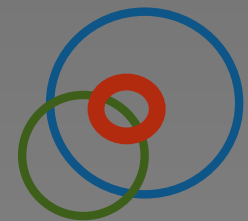
Professor, Department of Earth and Environmental Sciences

Senior Staff, Lamont-Doherty Earth Observatory

Columbia University



COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK



LAMONT-DOHERTY
EARTH OBSERVATORY

Sonya Dyhrman

High School



Grad school/Postdoc



Professor



I have my dream job

Sonya Dyhrman

California
PhD Scripps



Krumlov - 2016



Massachusetts
Postdoc WHOI



Scientific Staff WHOI

New York: Faculty Columbia University



Columbia runs RV Langseth





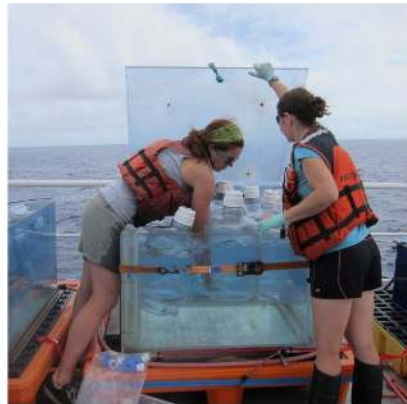
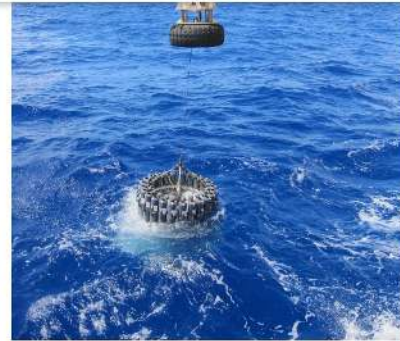
Research Projects

People

Publications

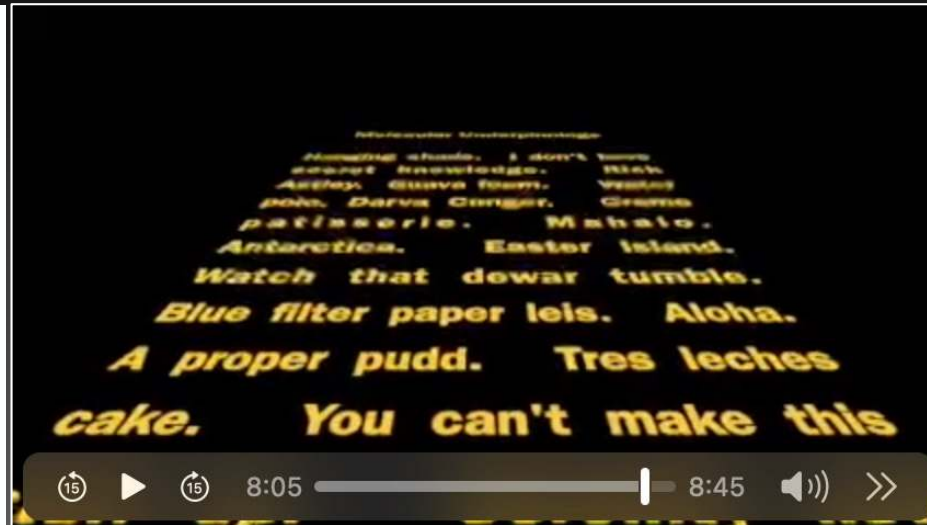
Outreach

Gallery



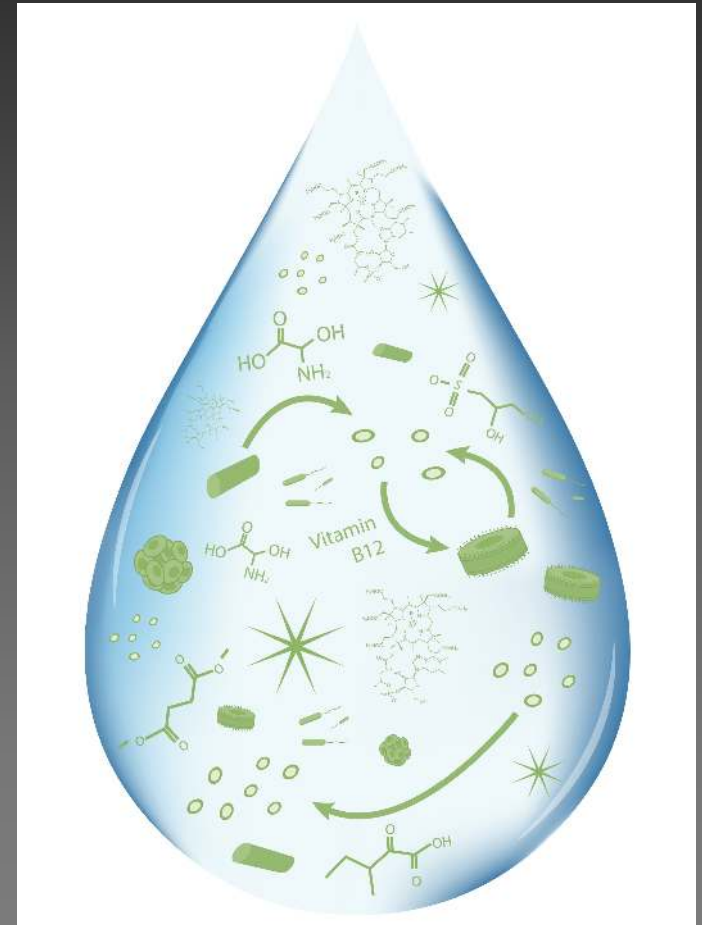
[⬆ Back to Top](#)

Sonya – random (fun?) facts



Key points for today

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



The ocean makes our planet habitable



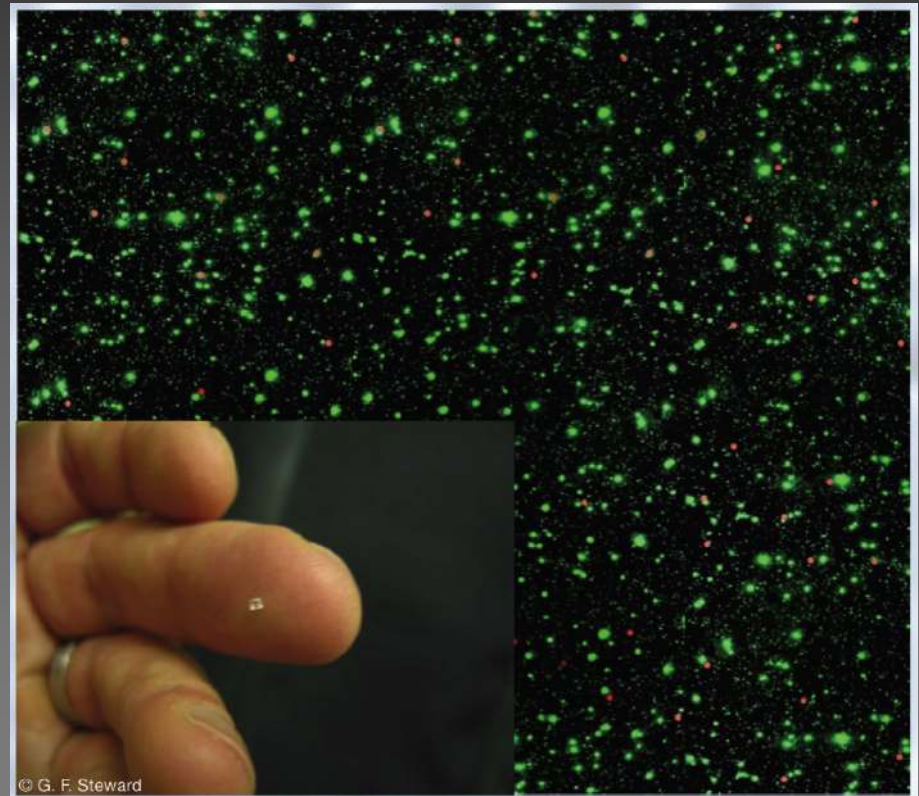
The vast unseen microbial populations play a critical role in ocean function



Marine Microbes - fundamental to ocean ecosystem function

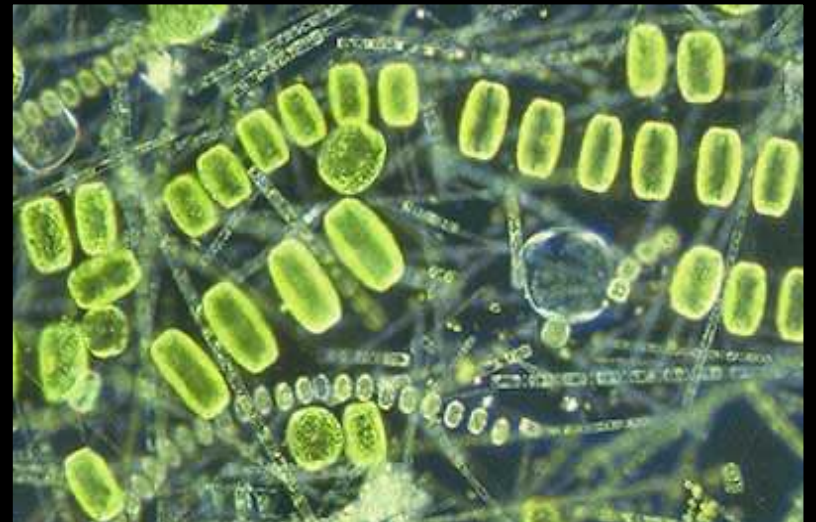
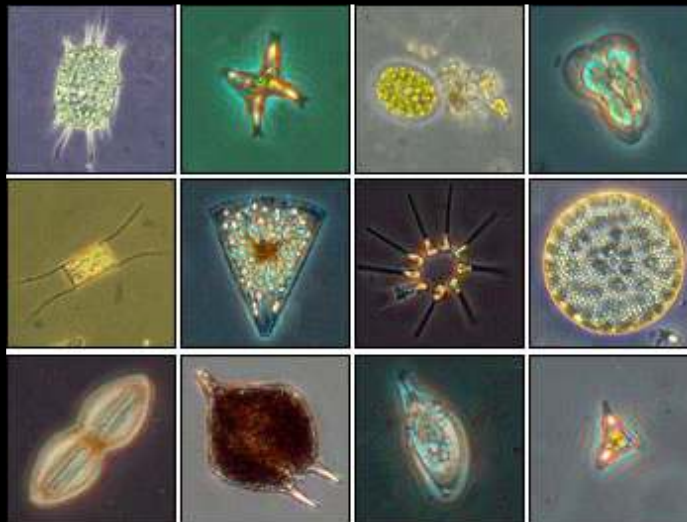
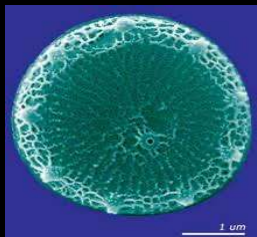
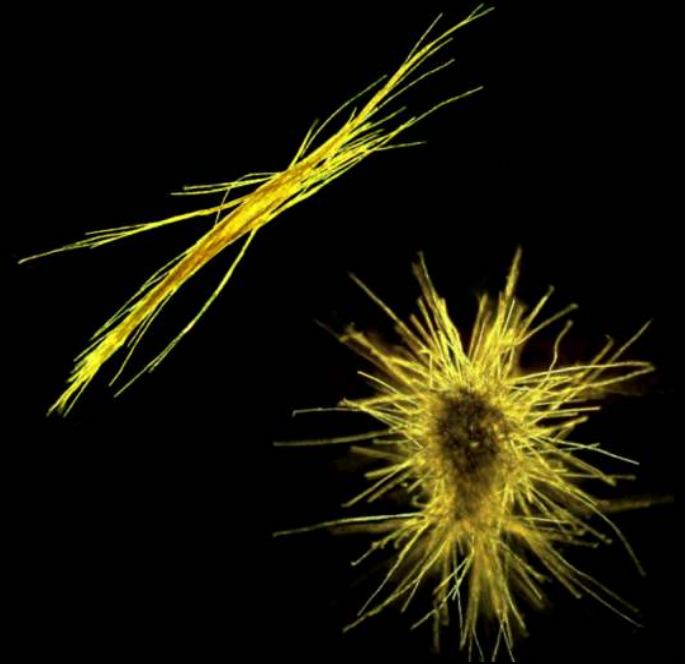
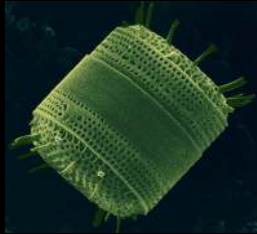
- Marine microbes...
 - Produce and consume green house gases
 - Supply the marine food web
 - Recycle organic matter
- *make the planet habitable*

phyto- = photosynthetic
-plankton = floating organisms



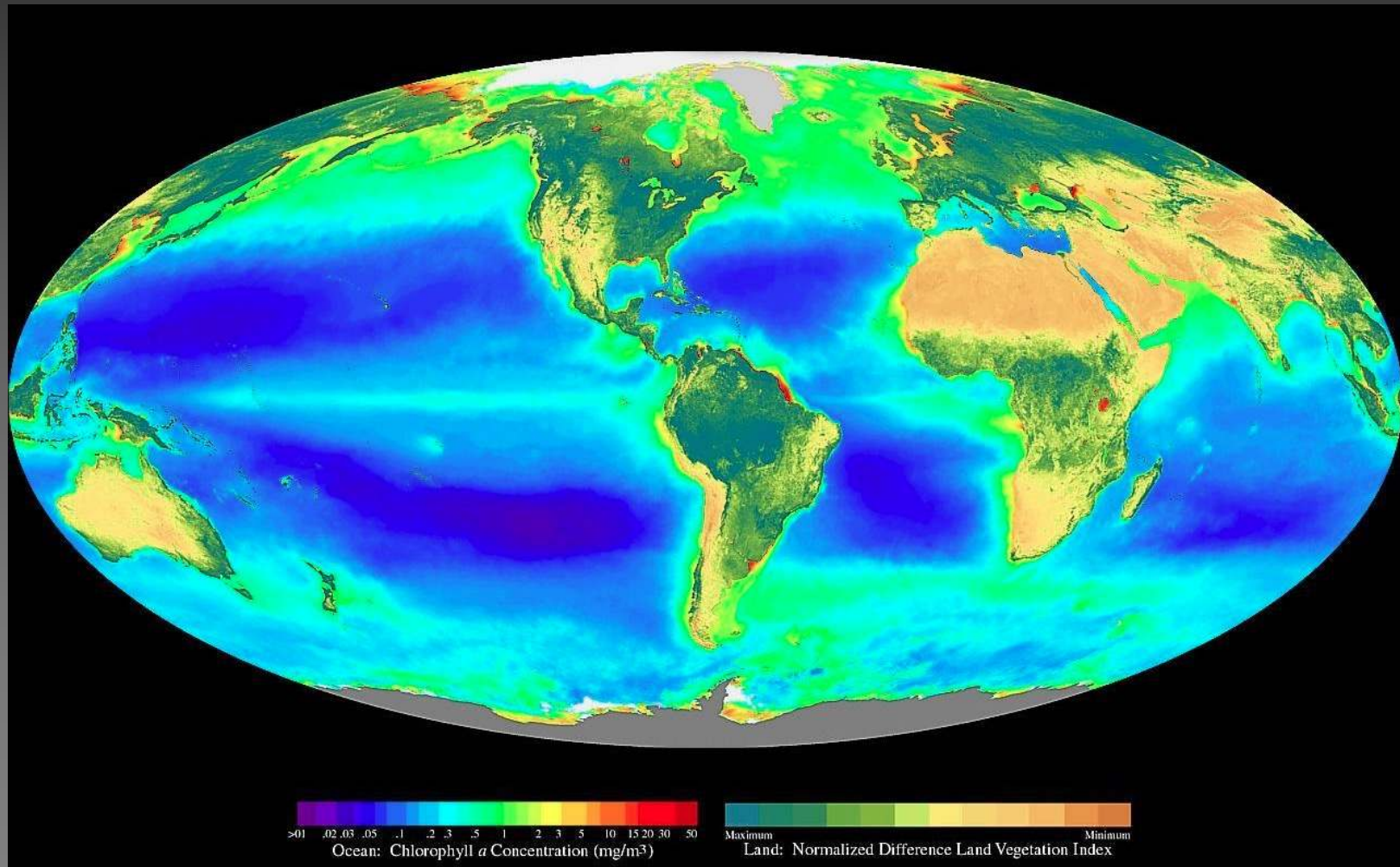
© G. F. Steward

Image courtesy C-MORE

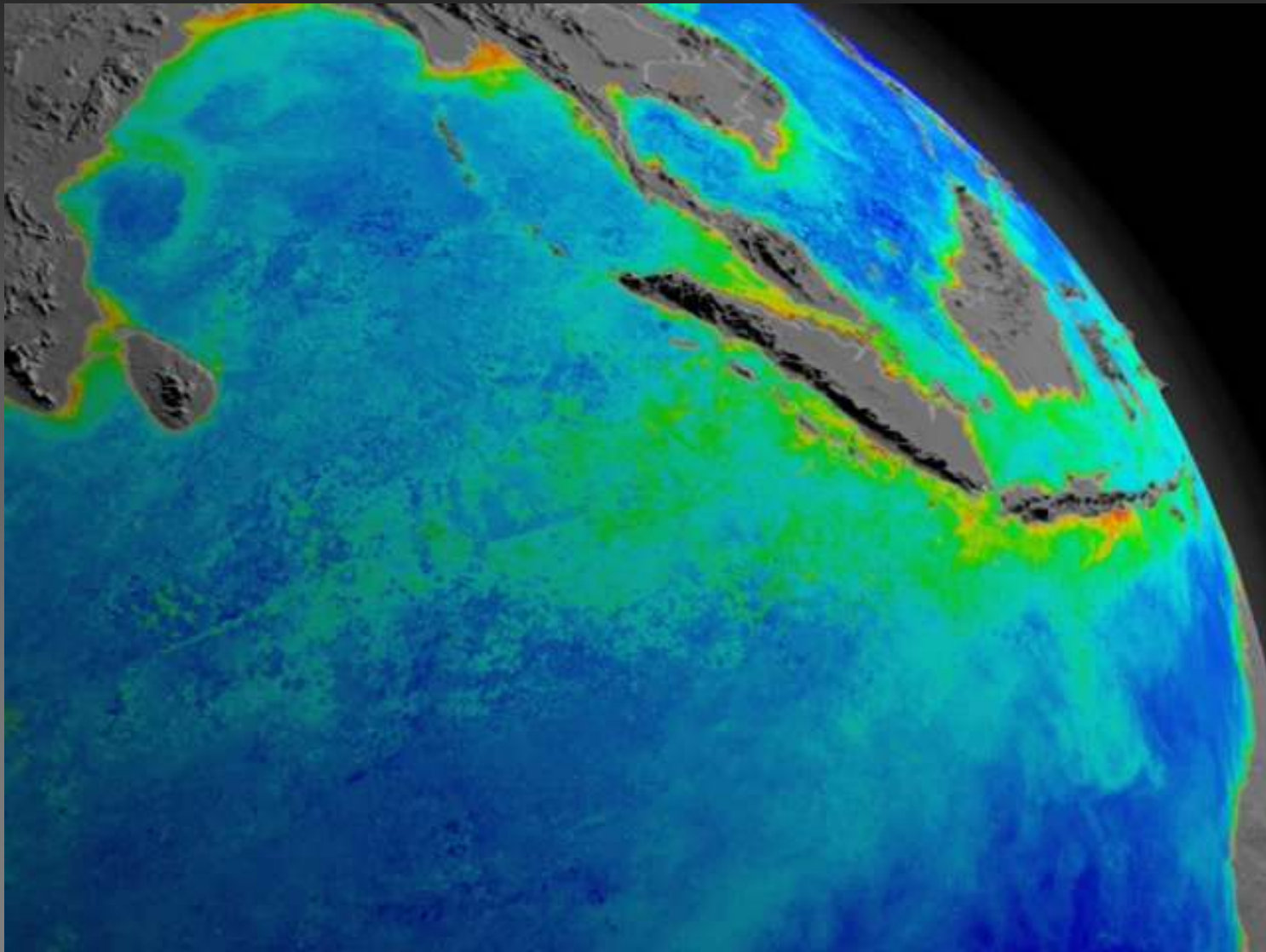


Phytoplankton play a profound role in the earth system

Phytoplankton drive half of global primary production

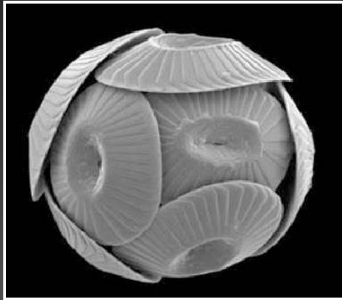


Seasonal chlorophyll distributions in the sea - highlights the global significance of phytoplankton

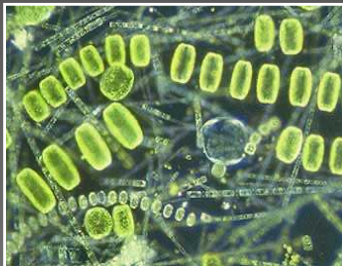


Phytoplankton underpin ocean ecosystem function

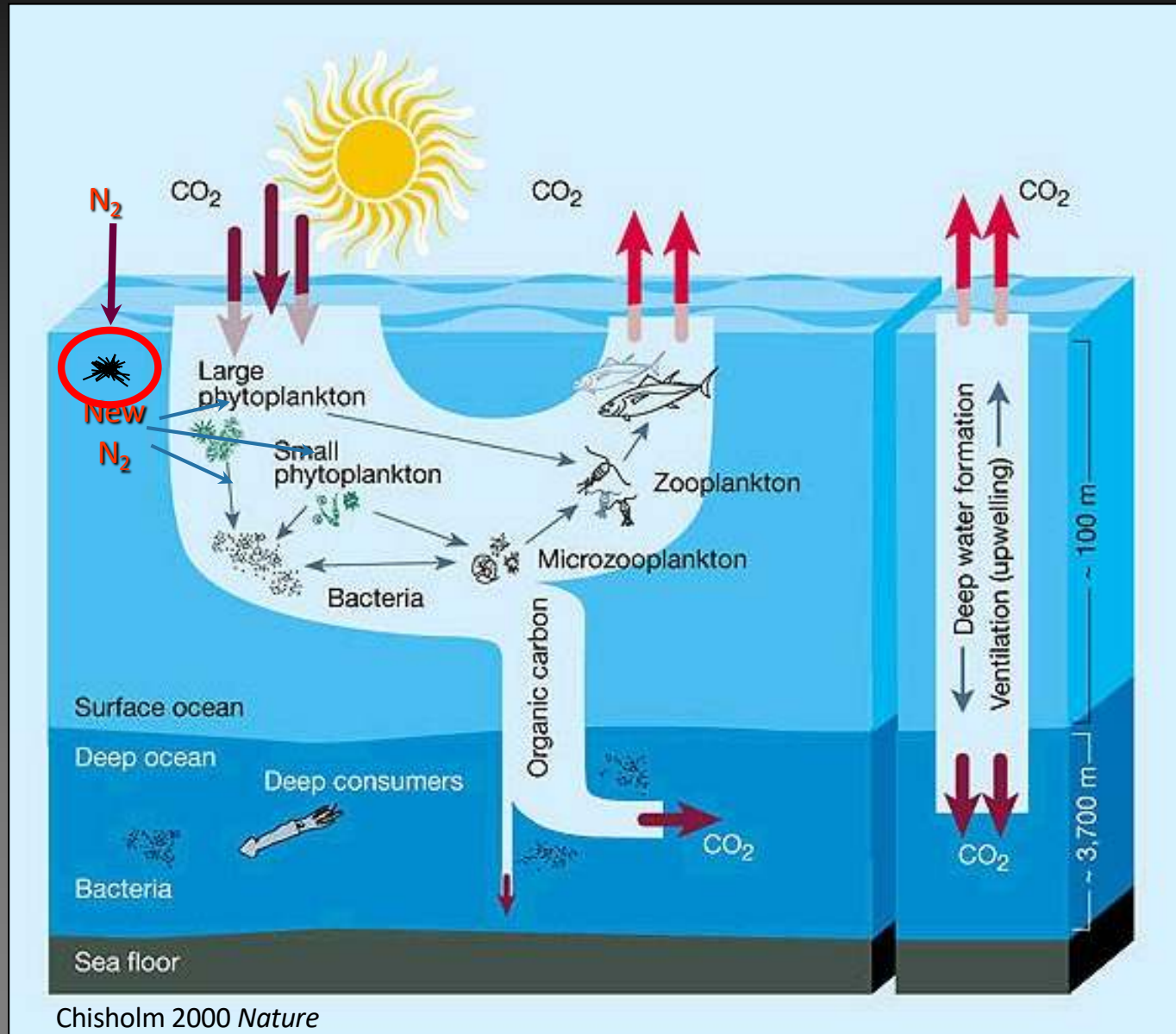
Haptophytes



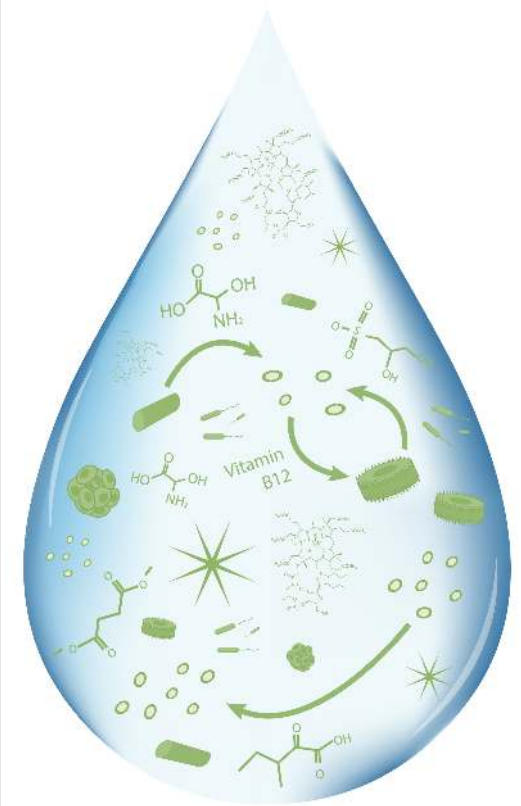
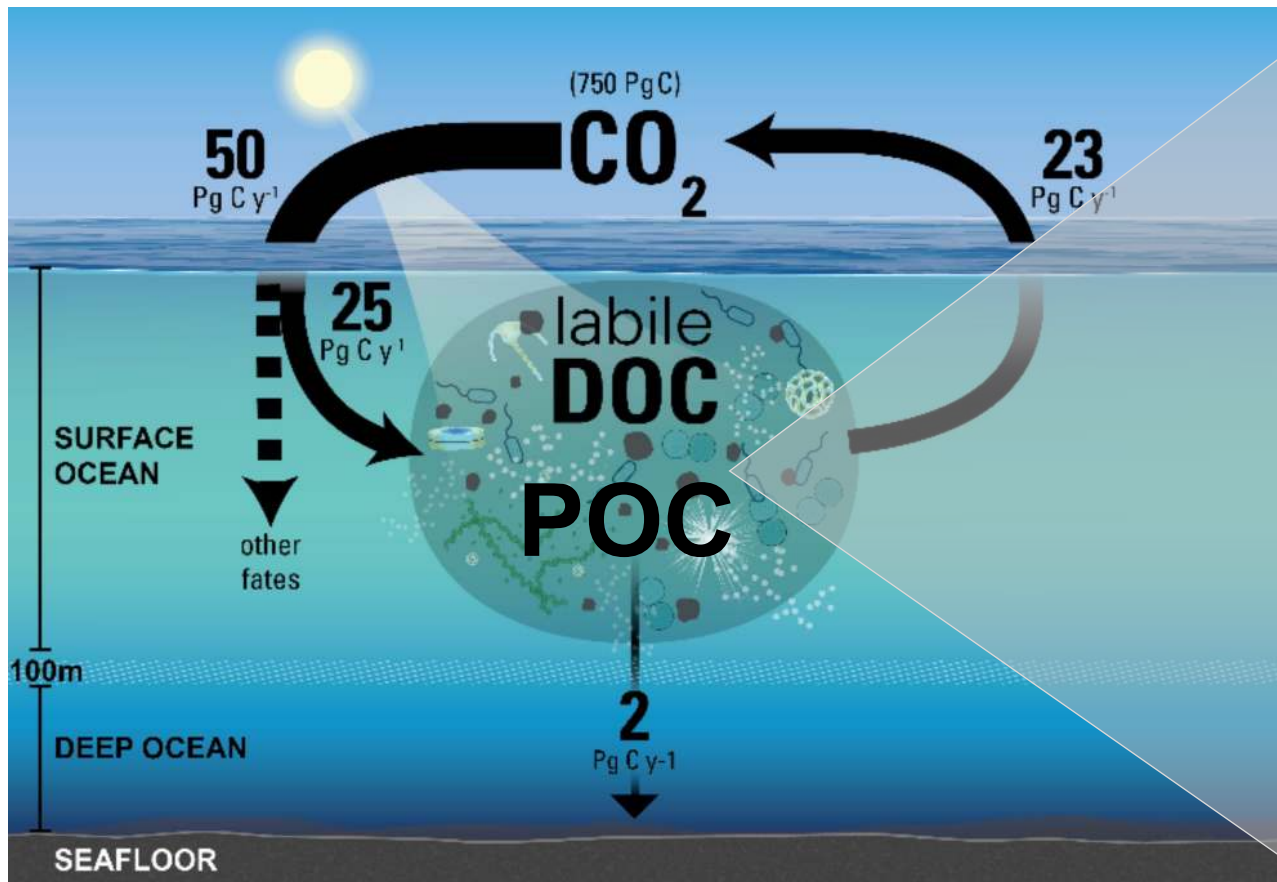
Diatoms

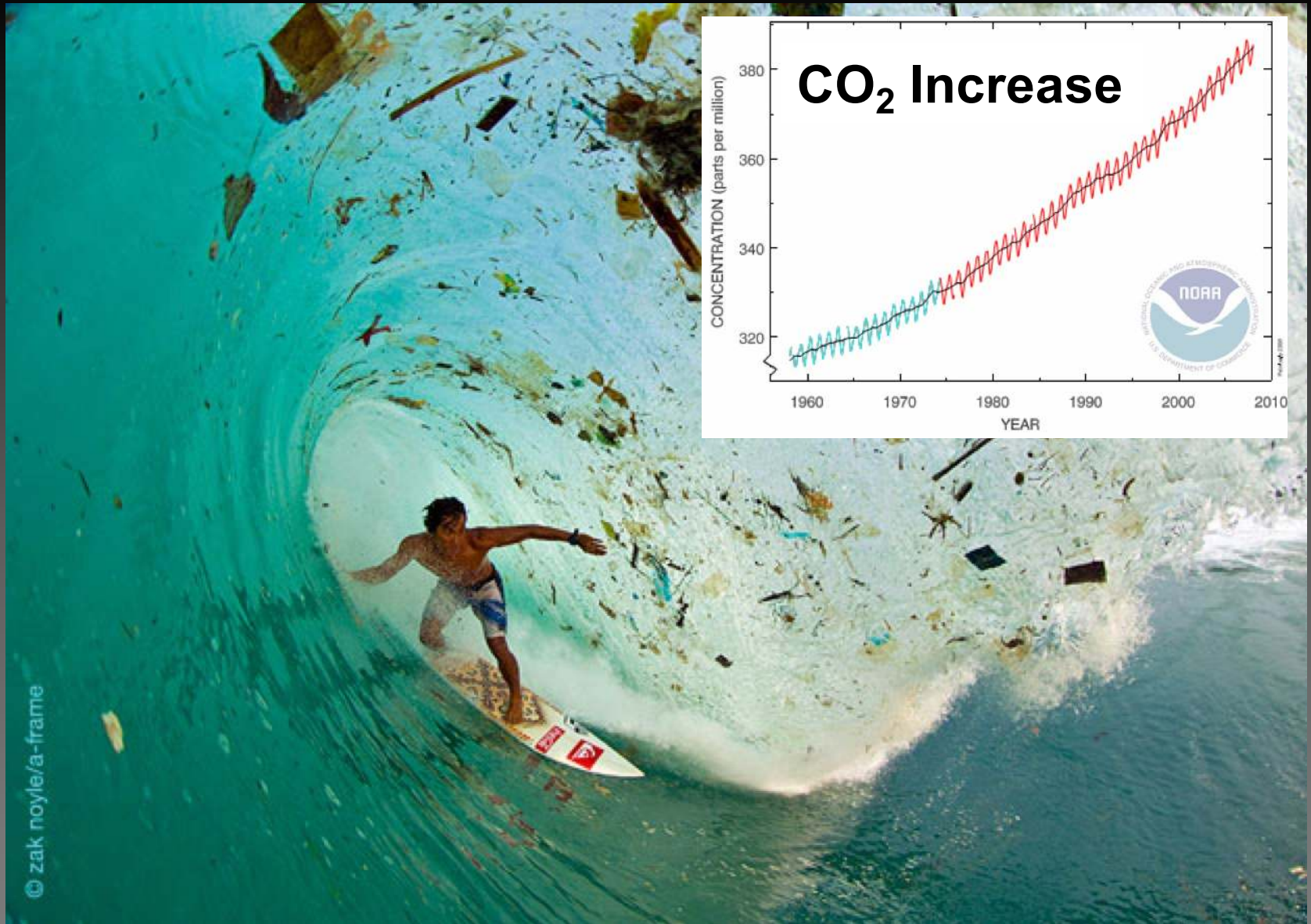


N₂ Fixers



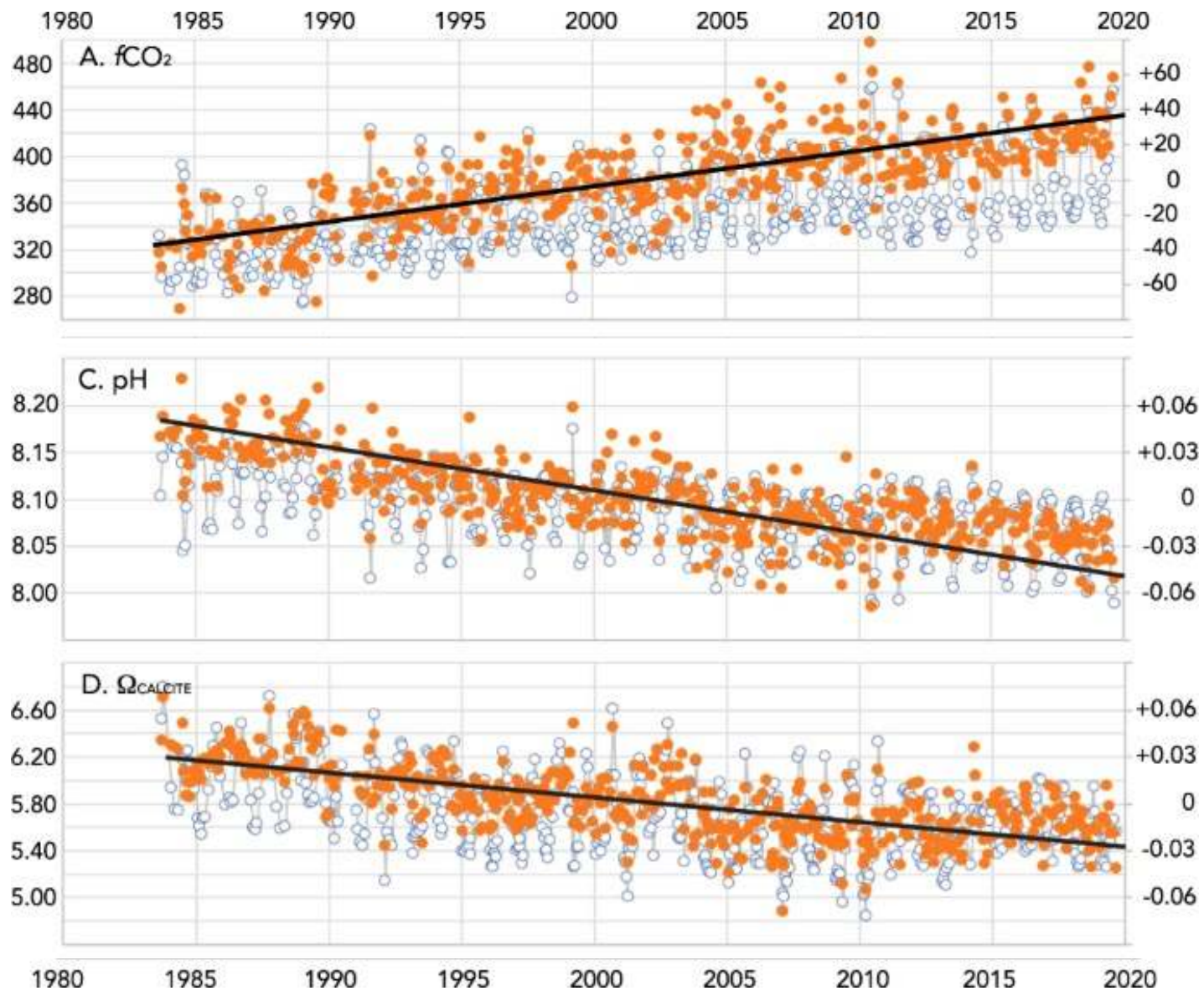
The chemical – microbe network drives the fate of *a lot* of carbon





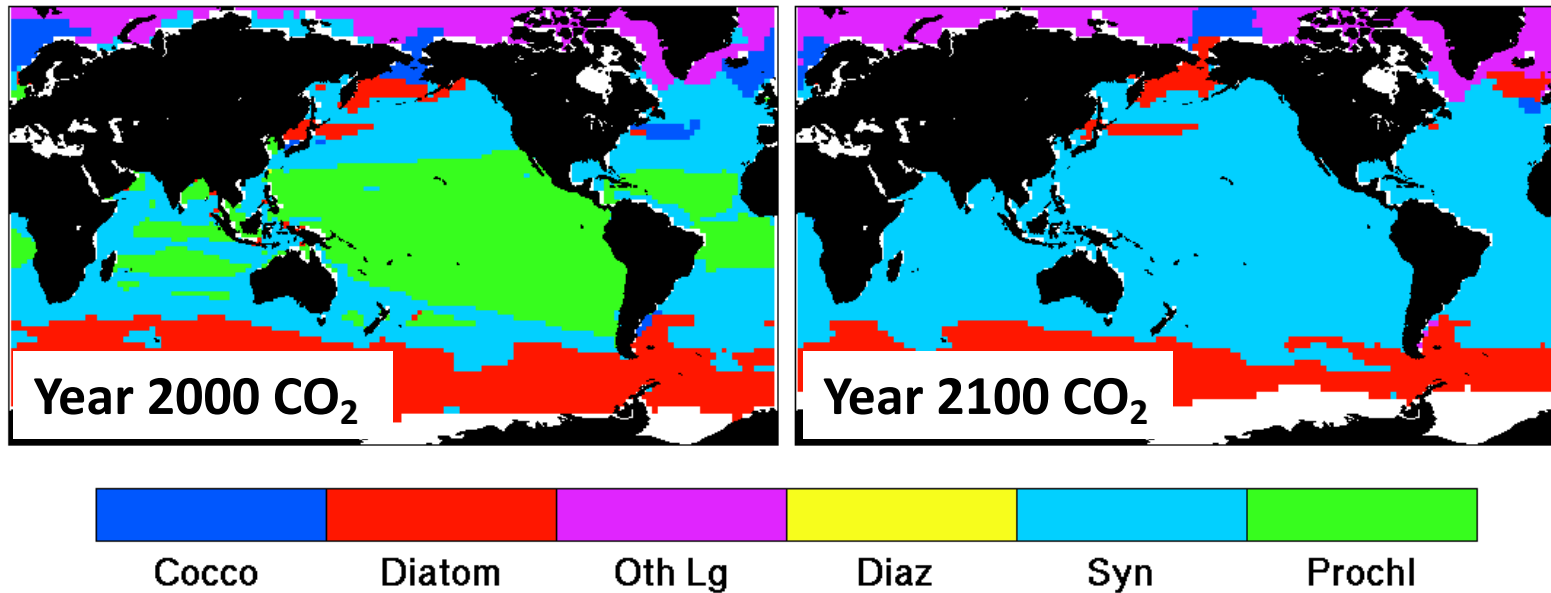
Ocean conditions are changing and this will change the distribution and activities of microbes

Changes in temperature CO₂, pH, etc.



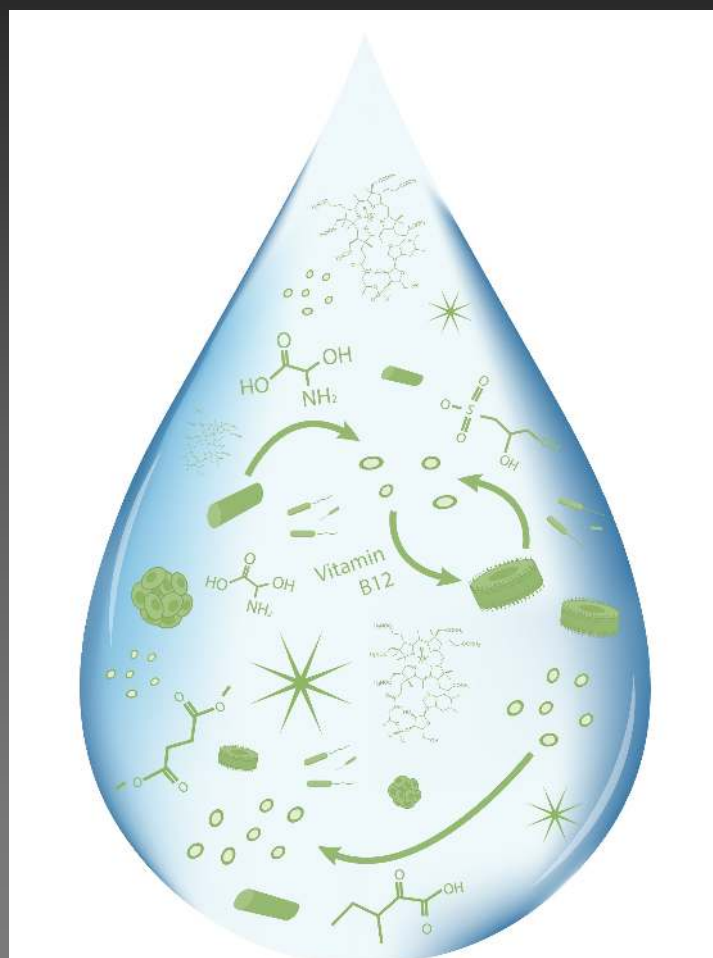
Models predict future shifts in microbial communities

Dominant Functional Group



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

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!
 - Salt!
 - Some keystone groups not in culture
 - Few genomes or transcriptome sequences (particularly for the eukaryotes)
- 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!

Our Projects

[Home](#) > [CSP Plans](#) > 100 Diatom Genomes

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[Secondary Metabolite-Producing Bacteria in Tiny Earth](#)

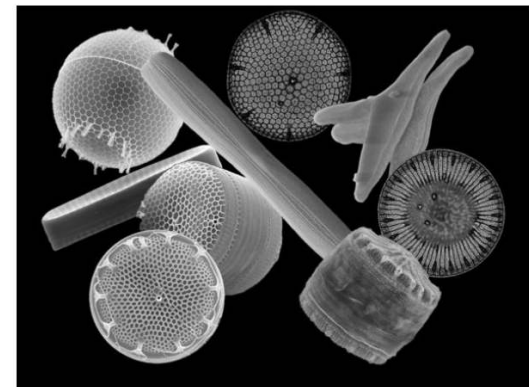
[Enabling Reverse Genetics in *Camelina sativa*](#)

100 Diatom Genomes

Diatoms are the most species-rich algal class with estimated $\geq 100,000$ species. They contribute to 20% of global carbon fixation and oxygen production. However, with genomic information from only about 10 diatoms, our ability to harness their unique biology is very limited. Consequently, this project will sequence 100 diatom species for providing unique insights into their roles as key players for capturing carbon dioxide and as the foundation of diverse aquatic food webs. These insights will also be critical for advancing diatom-based biotechnology and synthetic biology platforms.

Proposer: Thomas Mock, University of East Anglia (UK)
Proposal: 100 Diatom Genomes Project

Share this:



Scanning electron micrographs of diverse diatoms.
(Credits: Diana Sarno, Marina Montresor, Nicole Poulsen, Gerhard Dieckmann)

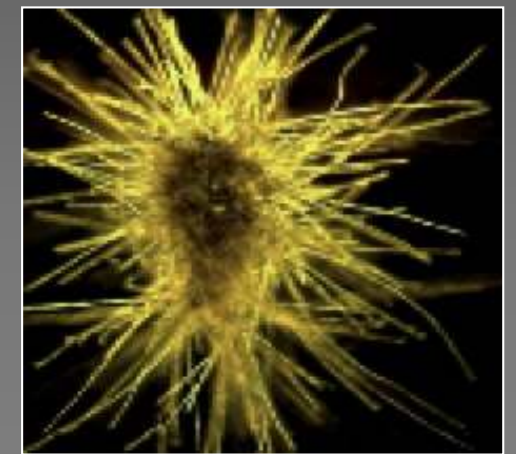
Challenges and opportunities

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

Vignettes

- Co-existing in a sea of competition: tracing drivers of phytoplankton physiological ecology in the open ocean
- Microbiome interactions: Tracking the microbiome of a keystone N₂ fixer



Thank you Dyhrman Lab family past and present



Gwenn Hennon



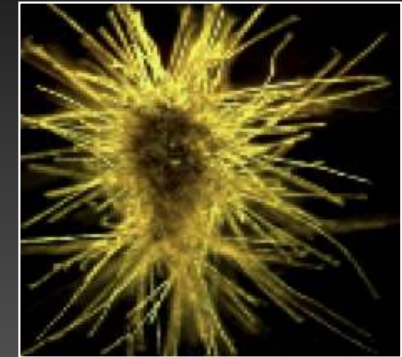
Mónica Rouco



Sheean Haley



Kyle Frischkorn



Sheean Haley



Hanna Anderson



Angela Zoumplis



Erin Maybach



Hannah Goldberg



Maxfield Palmer



Sheean Haley



Matt Harke



Maria
Hernández-
Limón



Harriet Alexander



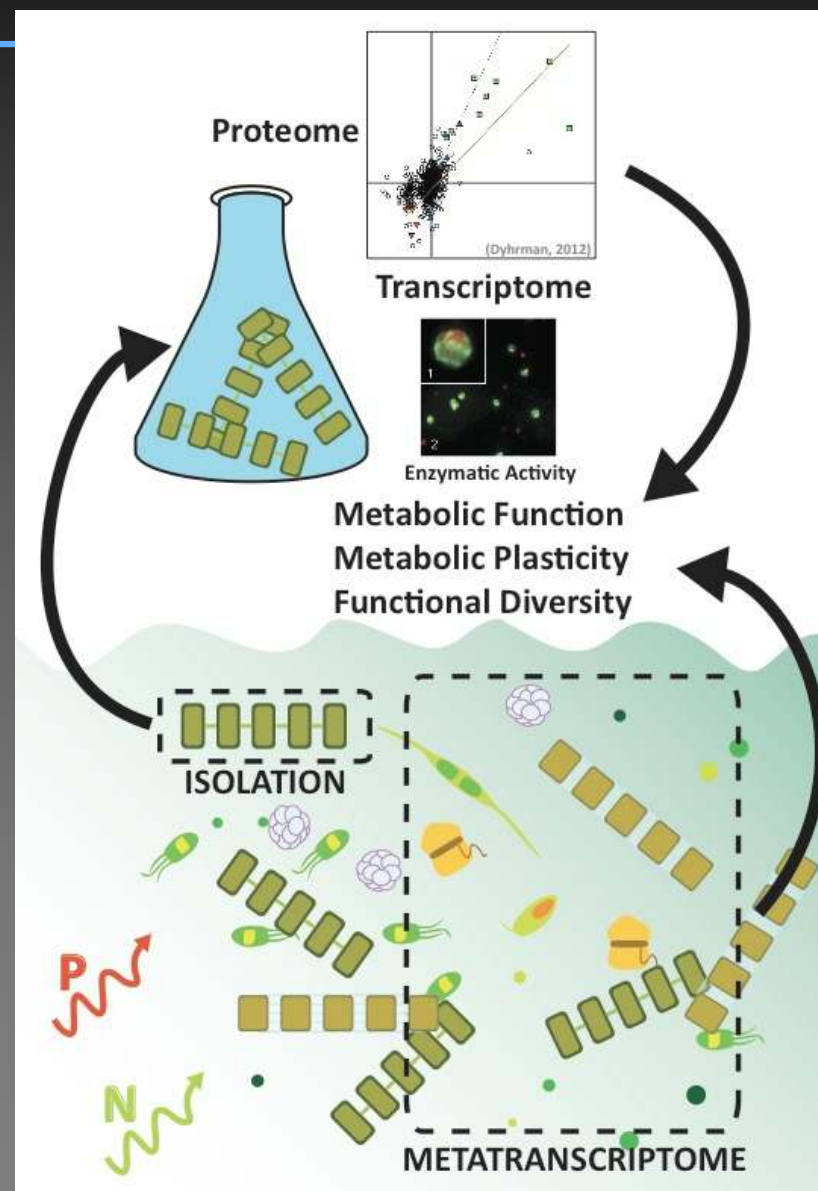
Acknowledgements

- Dyhrman Lab and many extraordinary collaborators
- NSF
- DOE
- Simons Foundation
- World Surf League
- Angell Family Foundation



Leveraging metatranscriptome data to study the chemical – microbe network

- Cultures offer controlled conditions – link genes to functions and study their regulation
- Field samples offer context
- Metatranscriptomes are species-specific
- Metatranscriptomes offer high resolution tracking of finely tuned responses



Graphic: H. Alexander

Microeukaryote metatranscriptomes with RNA-Seq



3x20L



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



Extract RNA
(and standards)



Selected



De novo assembly
Reference mapping

Unselected



De novo assembly
Reference mapping

rRNA
depletion

Quality Control

- Trimmomatic
- FastQC

Removal of Sequences

- riboPicker
- BBDMap

Functional Annotation

- BLAST
- DIAMOND
- HMMer

Assembly/Reference

- **Trinity**
- TransABYSS
- rnaSPADES
- MEGAHIT

Protein Prediction and Clustering

- TransDecoder
- OrthoFinder

Taxonomic Annotation

- KEGG
- KOG
- GO
- PFam
- BLAST
- DIAMOND
- MMSeq2

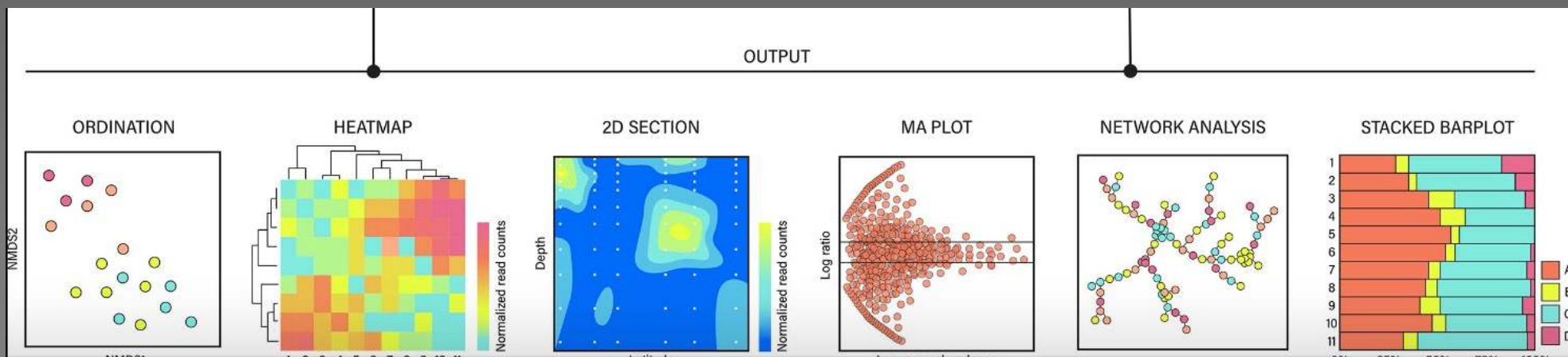
Read Mapping

- Salmon
- Kallisto
- Bowtie2
- RSEM

Normalization & DE

- EdgeR
- DESeq2
- TPM
- Variance stabilization

- MMETSP
- EukProt
- EukZoo
- PhyloDB
- MarFERReT (2024)



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!

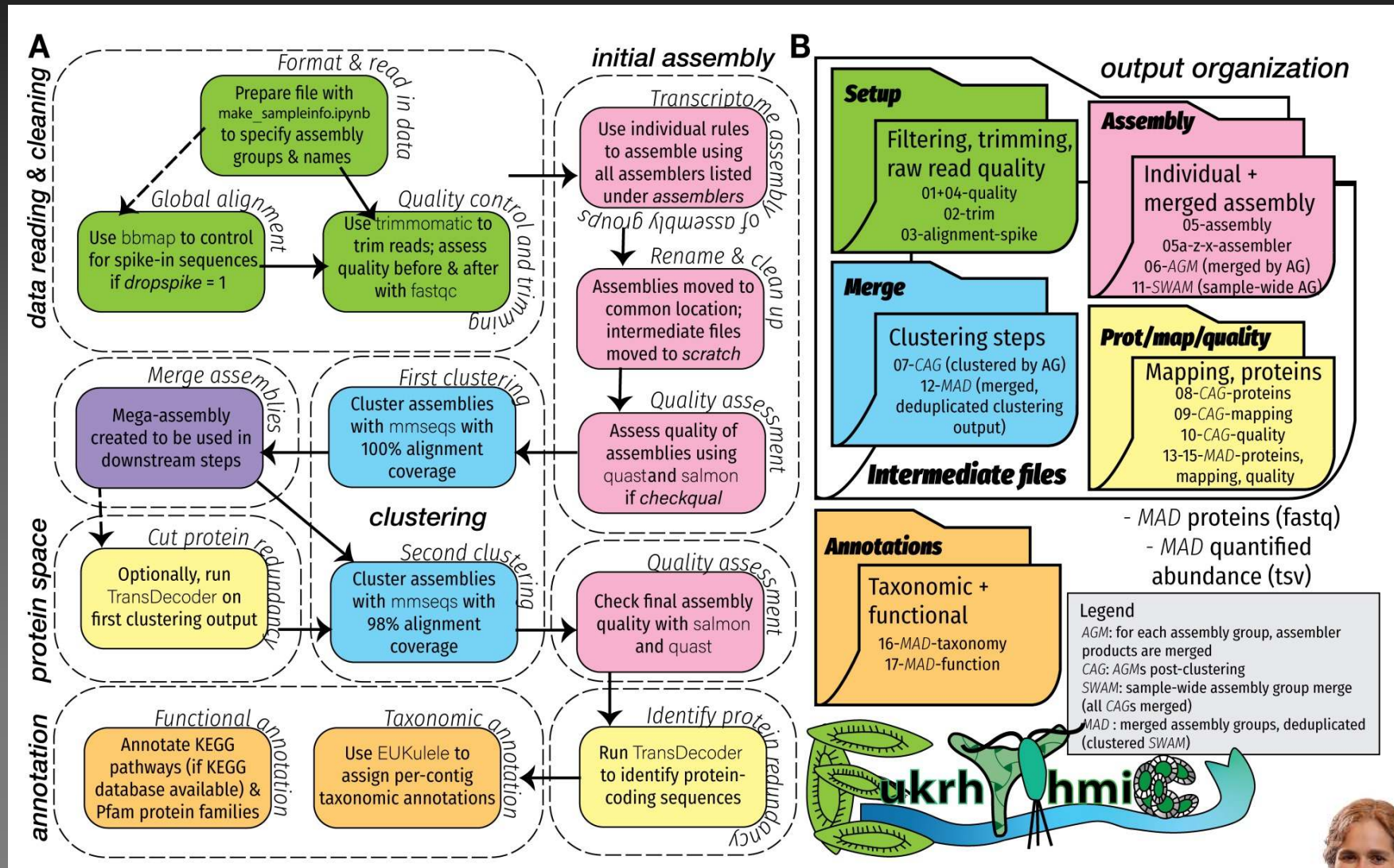


github.com

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

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

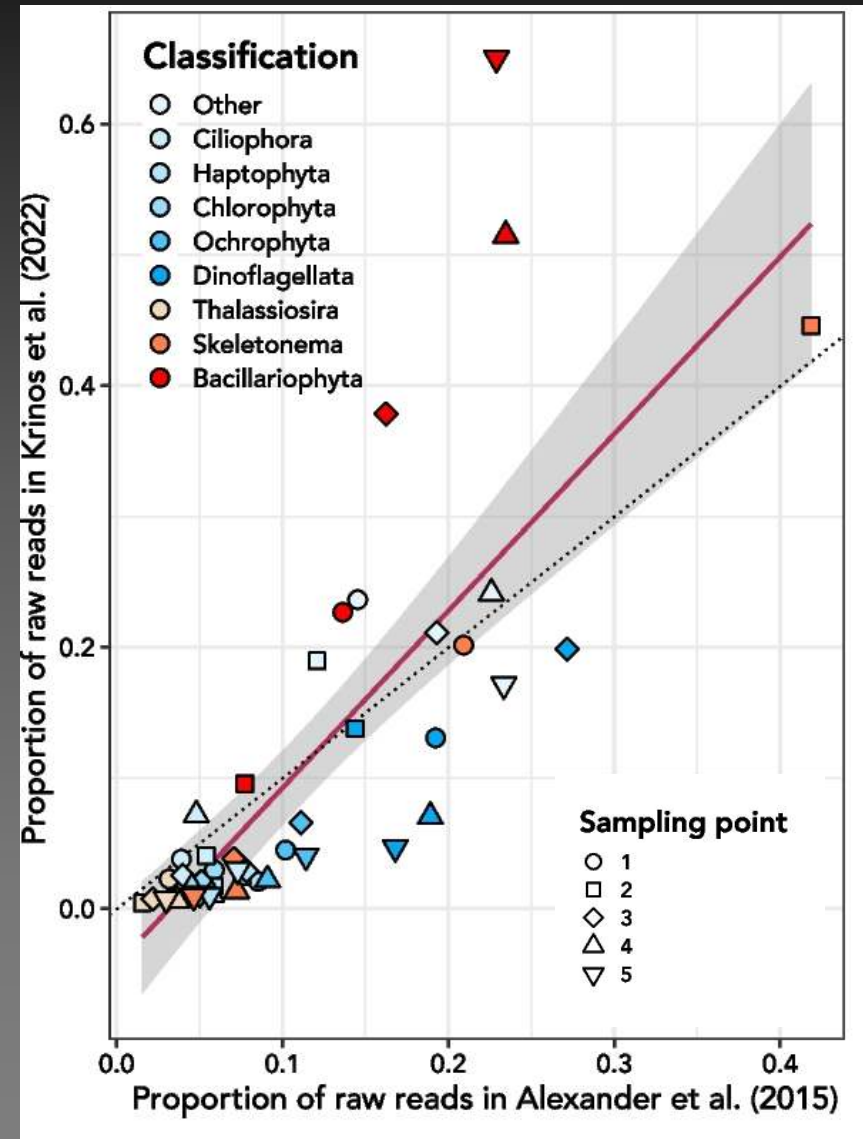
Sequencing and analytical workflows are constantly improving



Metatranscriptome workflow comparison effort

- Assembly captures a swath of reads in high abundance – highly diverse groups (red)
- Reference mapping captures a swath of reads in low abundance groups (blue)
- Generally equally good at recapitulating patterns when references are from local isolates (orange and yellow)

De novo Assembly



Reference mapping

Metatranscriptome intercalibration effort – phase 1

- Large diameter filter sampled with CLIO (June 2023)
- 2x filter pieces to ~ 7 groups
- Evaluate:
 - Extraction
 - Library prep
- Workflow intercomparison
- Will not answer all questions regarding intercalibration – but it will be a valuable starting point for building intercomparable datasets.



Thank you N. Cohen and H. Alexander
for leadership and M. Saito for samples

Core questions... adventures in eukaryote metatranscriptomics

Limitation

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

HOE:DYLAN

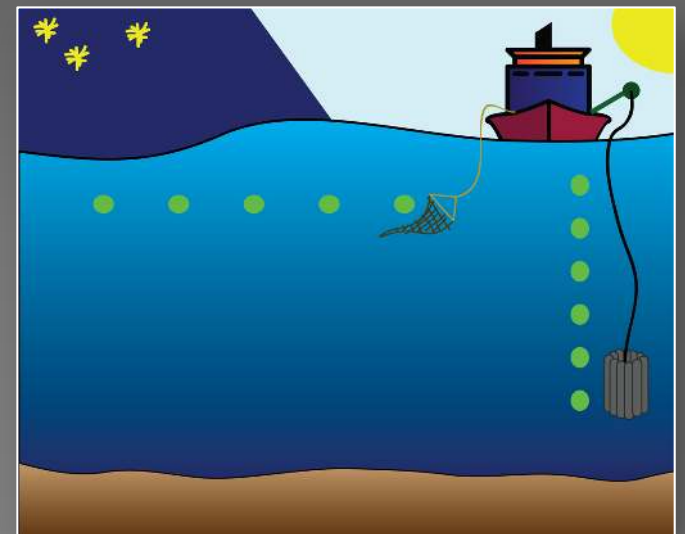


H. Alexander

Competition

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

HOE:MAHALO

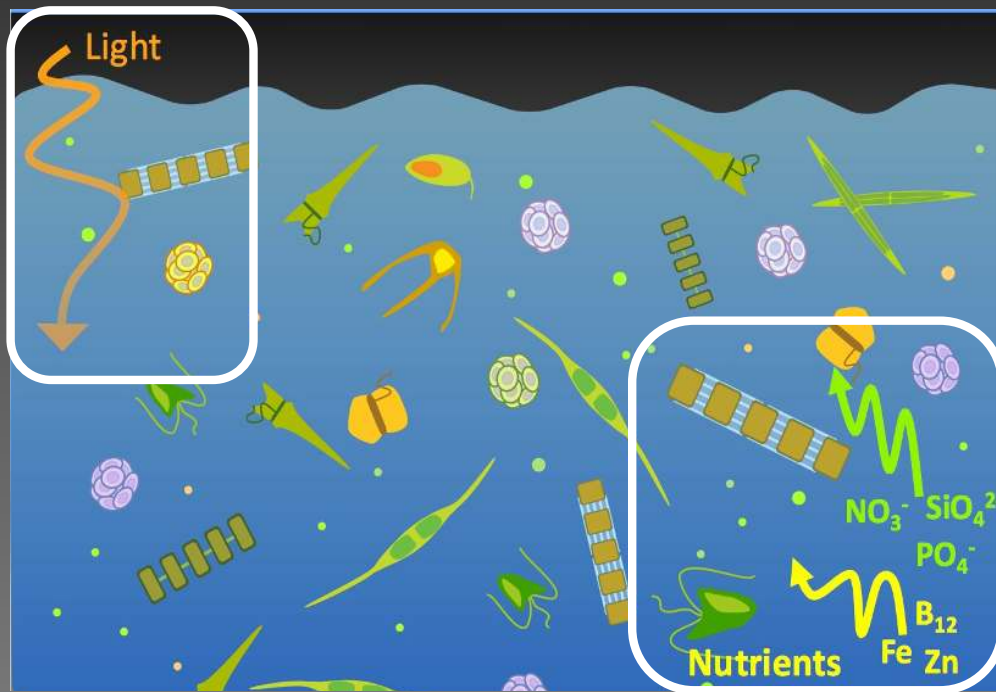


Complex community dynamics driven in part by resources



Tracking phytoplankton physiological ecology

Ecosystem function and biogeochemistry



Graphic: H. Alexander

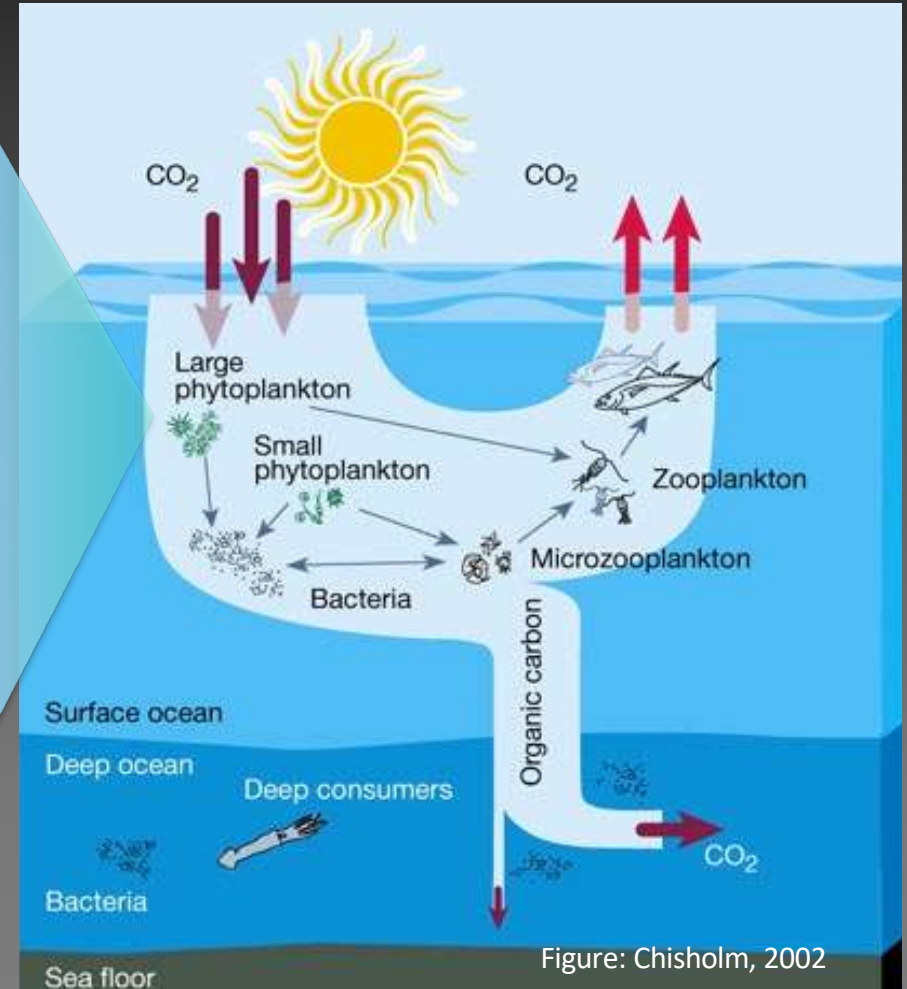


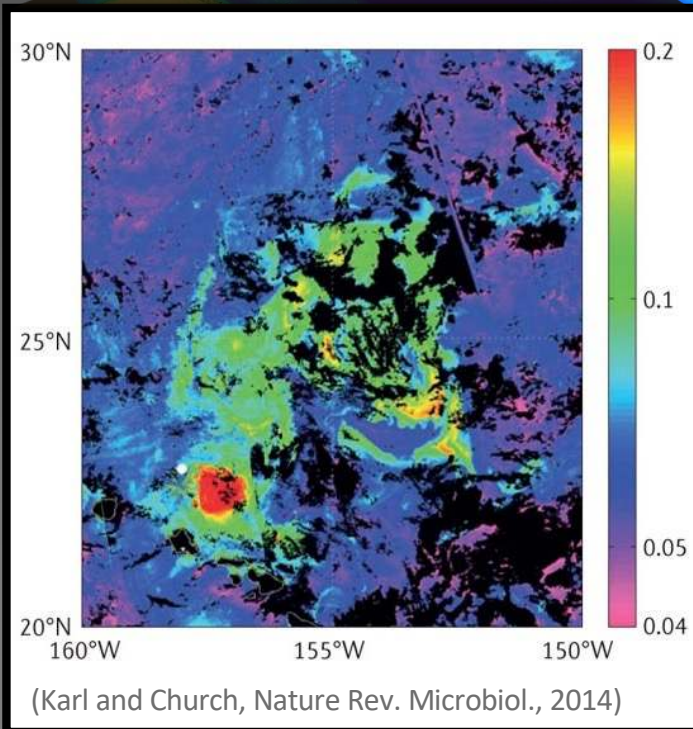
Figure: Chisholm, 2002

How do resources drive phytoplankton distributions and activities?

North Pacific Subtropical Gyre

North Pacific
Subtropical
Gyre

Station ALOHA



NORMAL OLIGOTROPHIC STATE

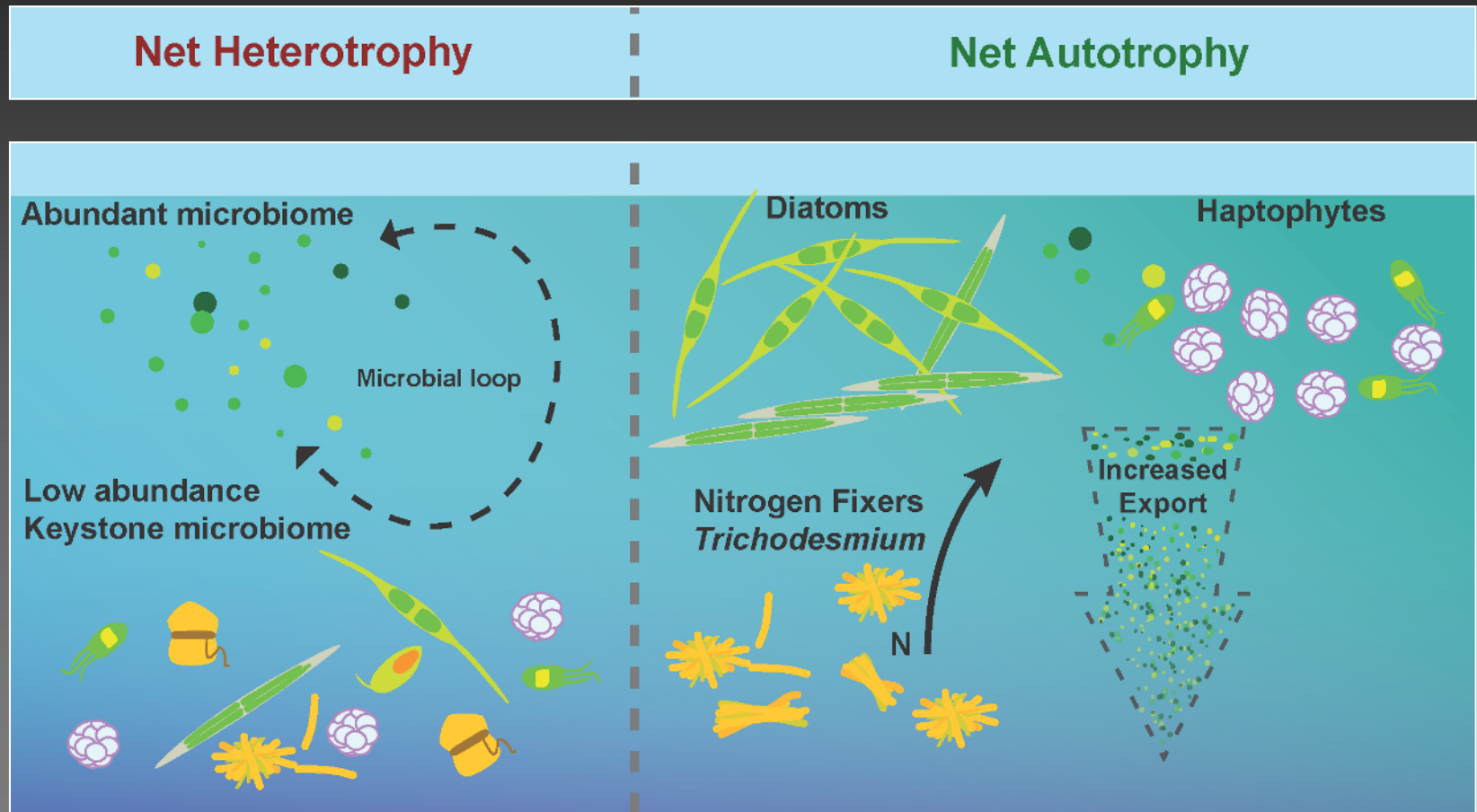
SMALL PLANKTON

MICROBIAL
LOOP

RARE LARGE EUKARYOTES

This resource poor (oligotrophic) region is arguably the largest biome on the planet

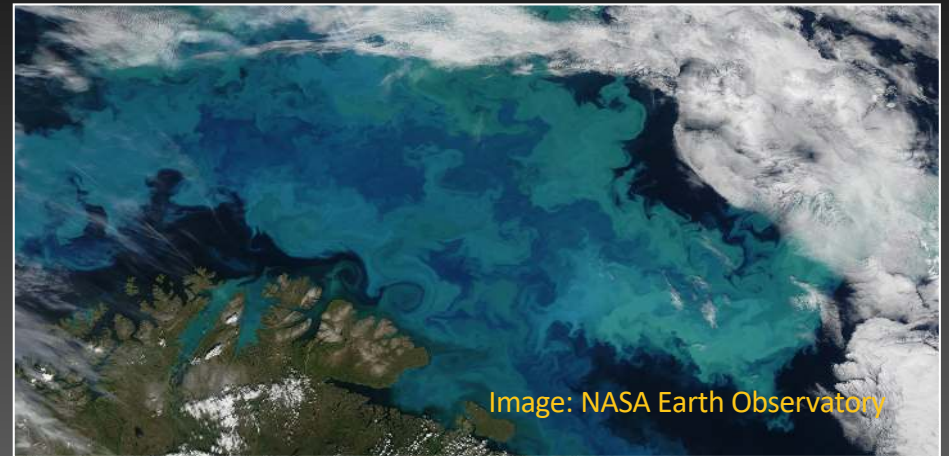
Blooms of keystone species can shift ecosystem state and carbon flow



Graphic: H. Alexander

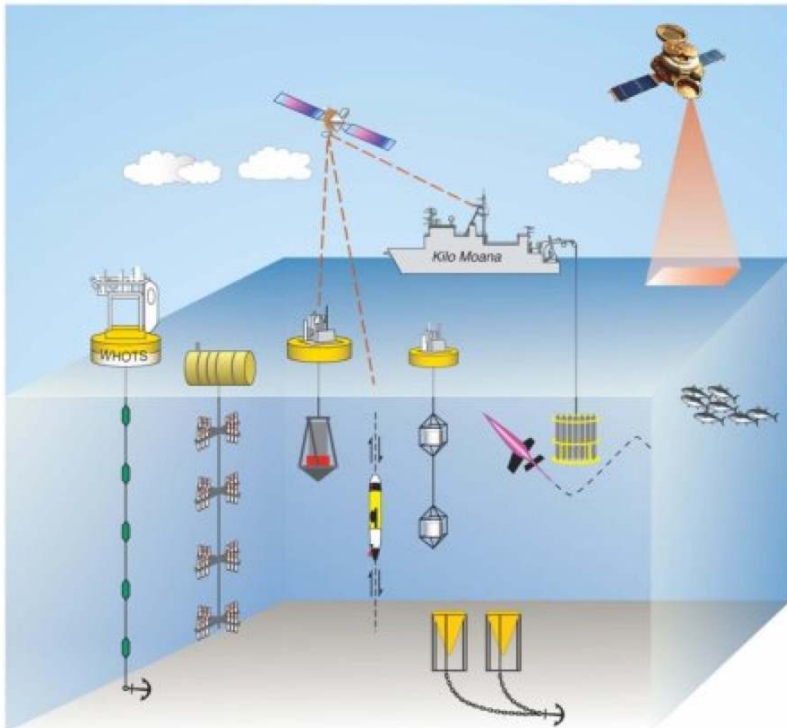
* *Emiliana huxleyi*: a cosmopolitan, globally significant species

- 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

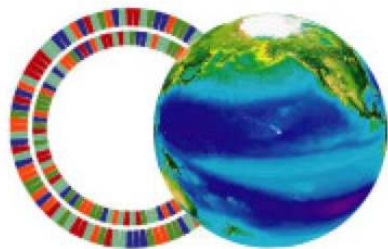


**Gephyrocapsa* (Bendif et al., 2023; Filatov et al., 2021)

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*: $\sim 40 \mu\text{M}$
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



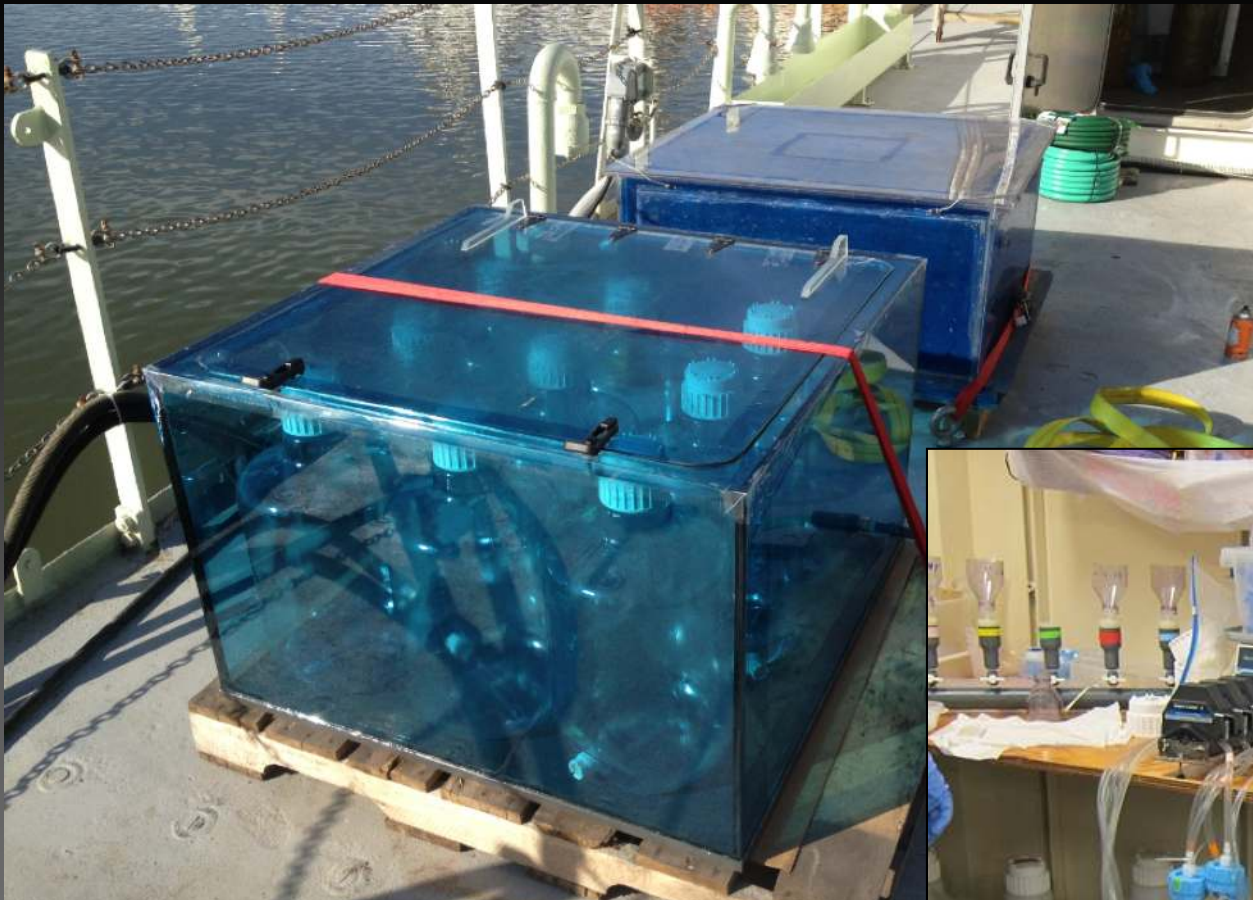
(+N, Si, Fe,
 B_{12})



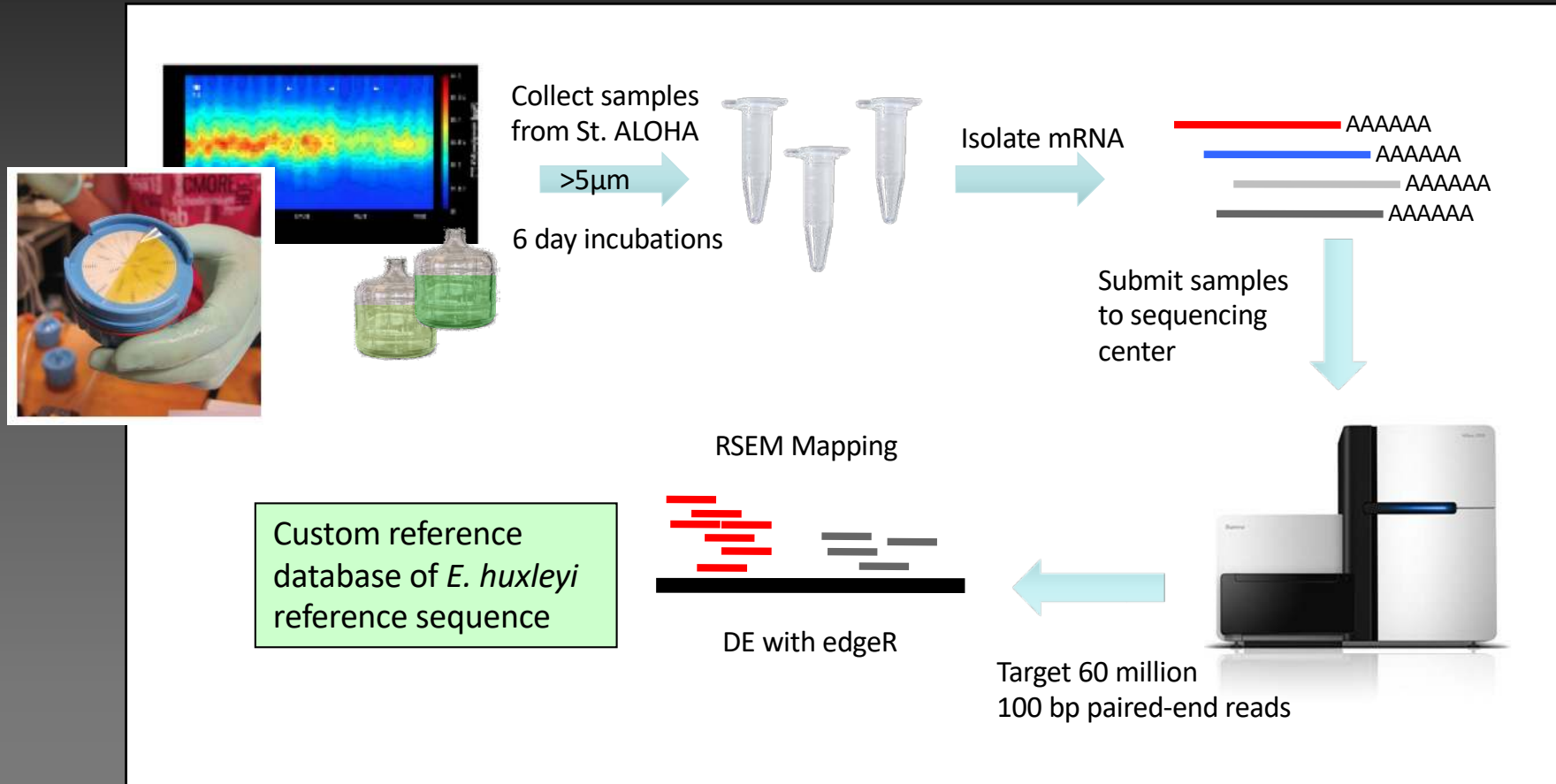
(+P, Si, Fe,
 B_{12})



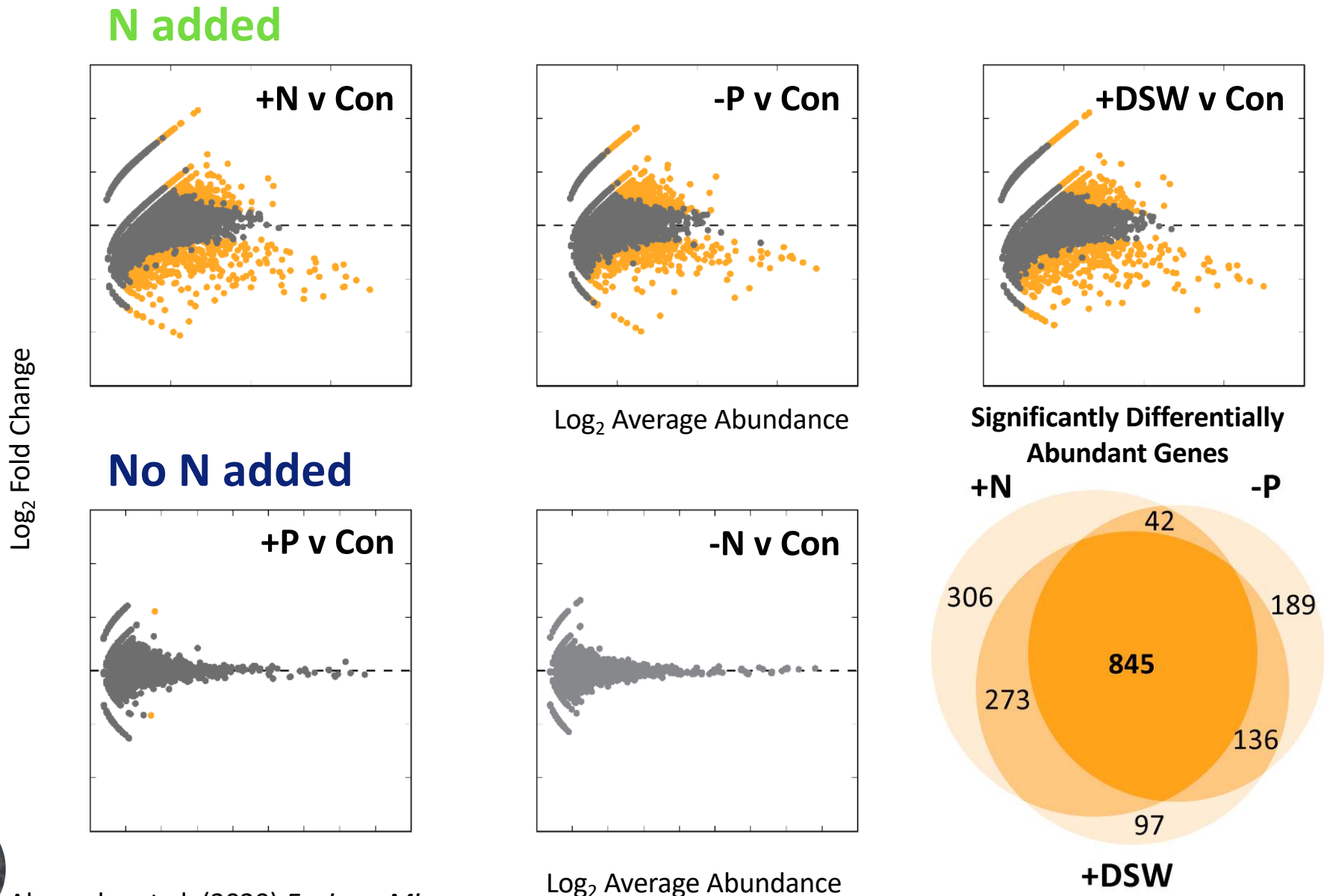
N addition



Sampling and pipeline

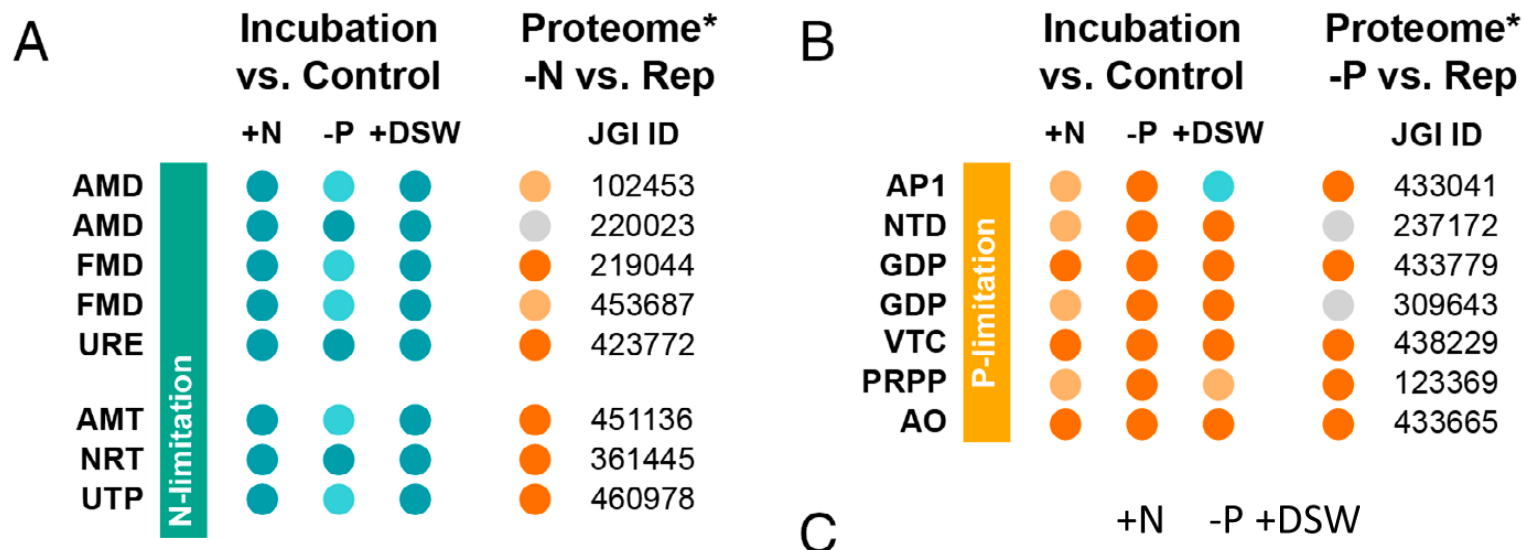


E. huxleyi physiological response suggests N control



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

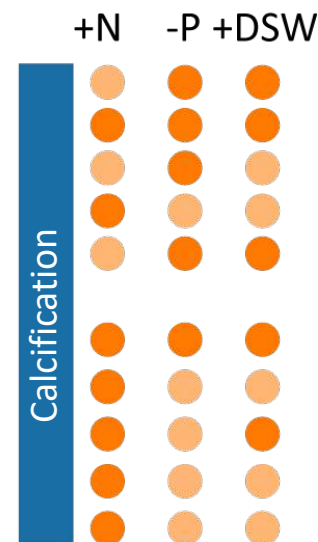
In situ transcriptome responses track with culture proteome



● Up (significant) ● Down (significant)
 ● Up (non-significant) ● Down (non-significant)
 ● Not detected

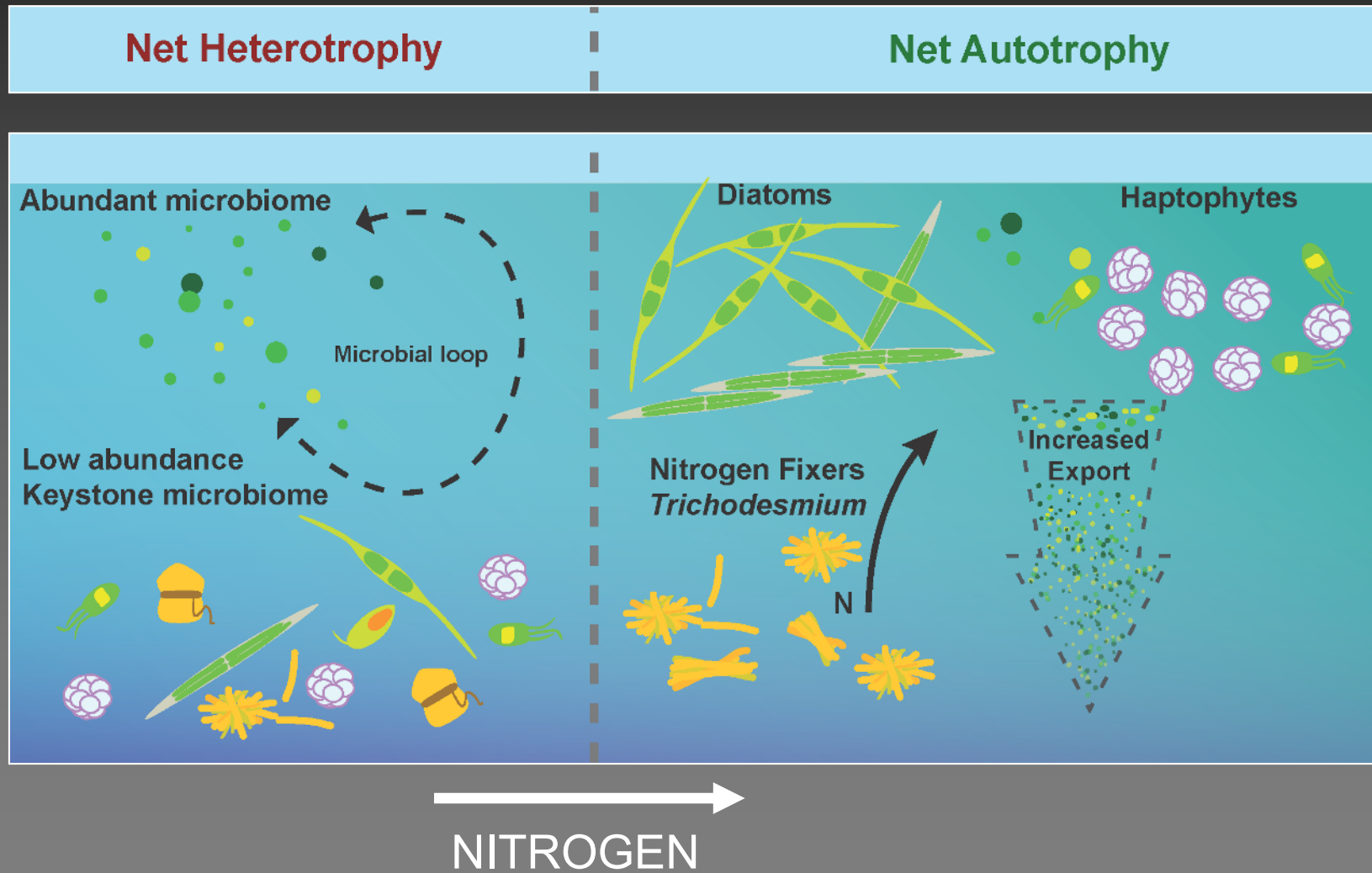
Nitrogen limits *E. huxleyi* growth and calcification in NPSG

C



* Data from McKew et al. (2015) *Environ Microbiol* 17:4050–62.

Nitrogen is a driver of *E. huxleyi* dynamics



Emiliana huxleyi has many diverse isolates

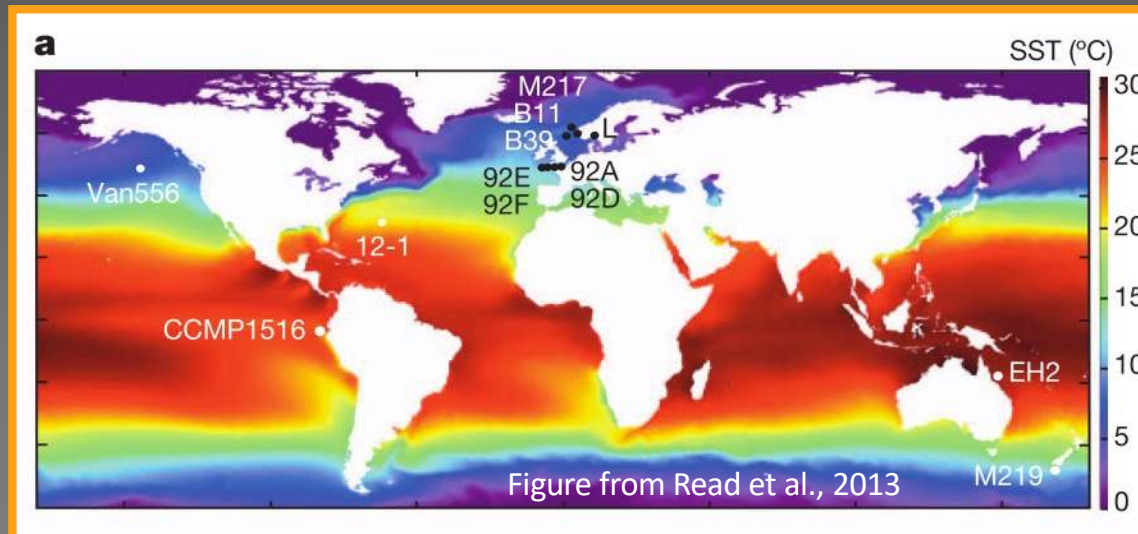
LETTER

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?

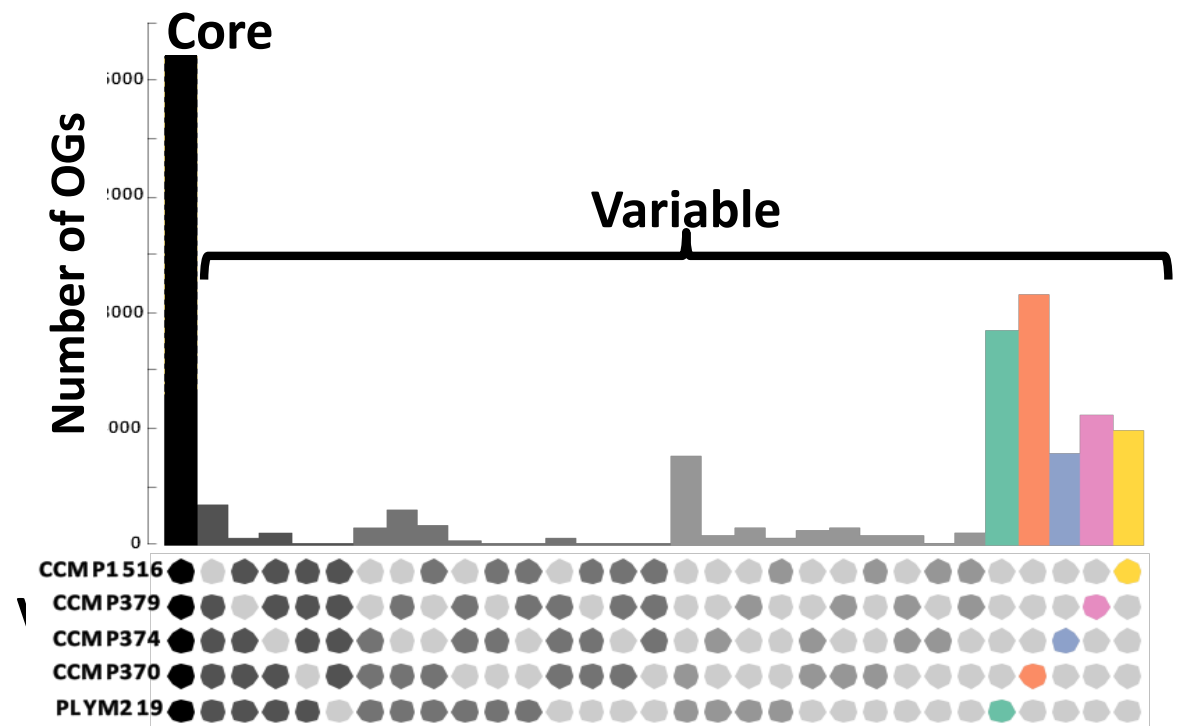
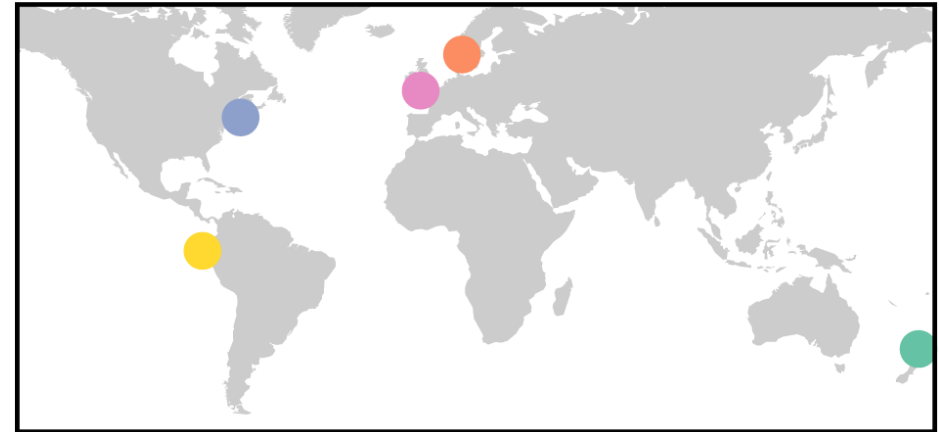
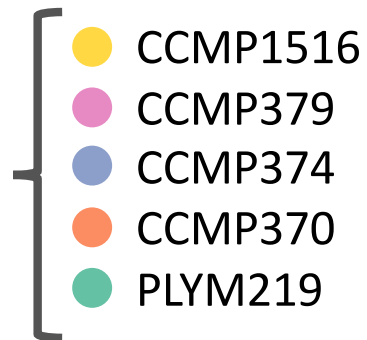
Sequences from
E. huxleyi strains



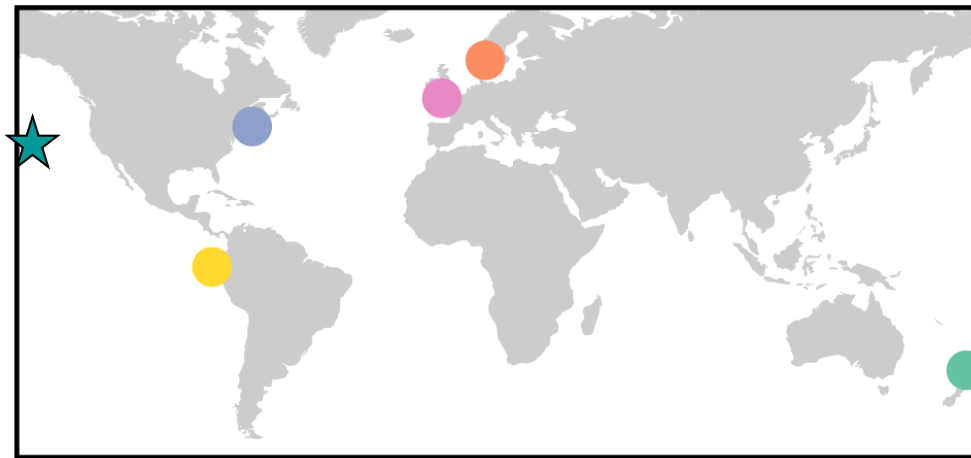
Cluster into
orthologous groups to
identify core and
variable OGs
(OrthoMCL)



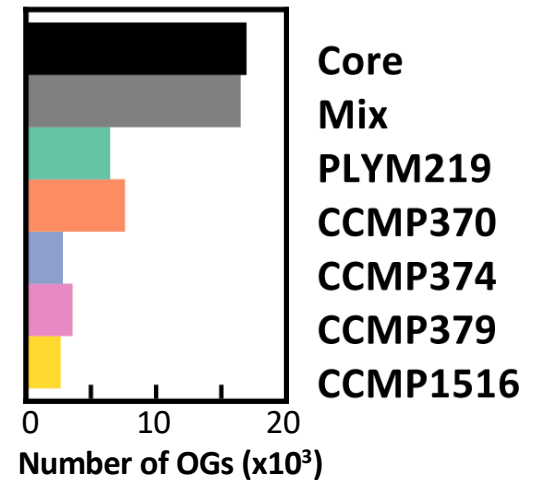
Map field data and
examine shifts in
expression
(RSEM, edgeR)



Tracking orthologous groups at St. ALOHA

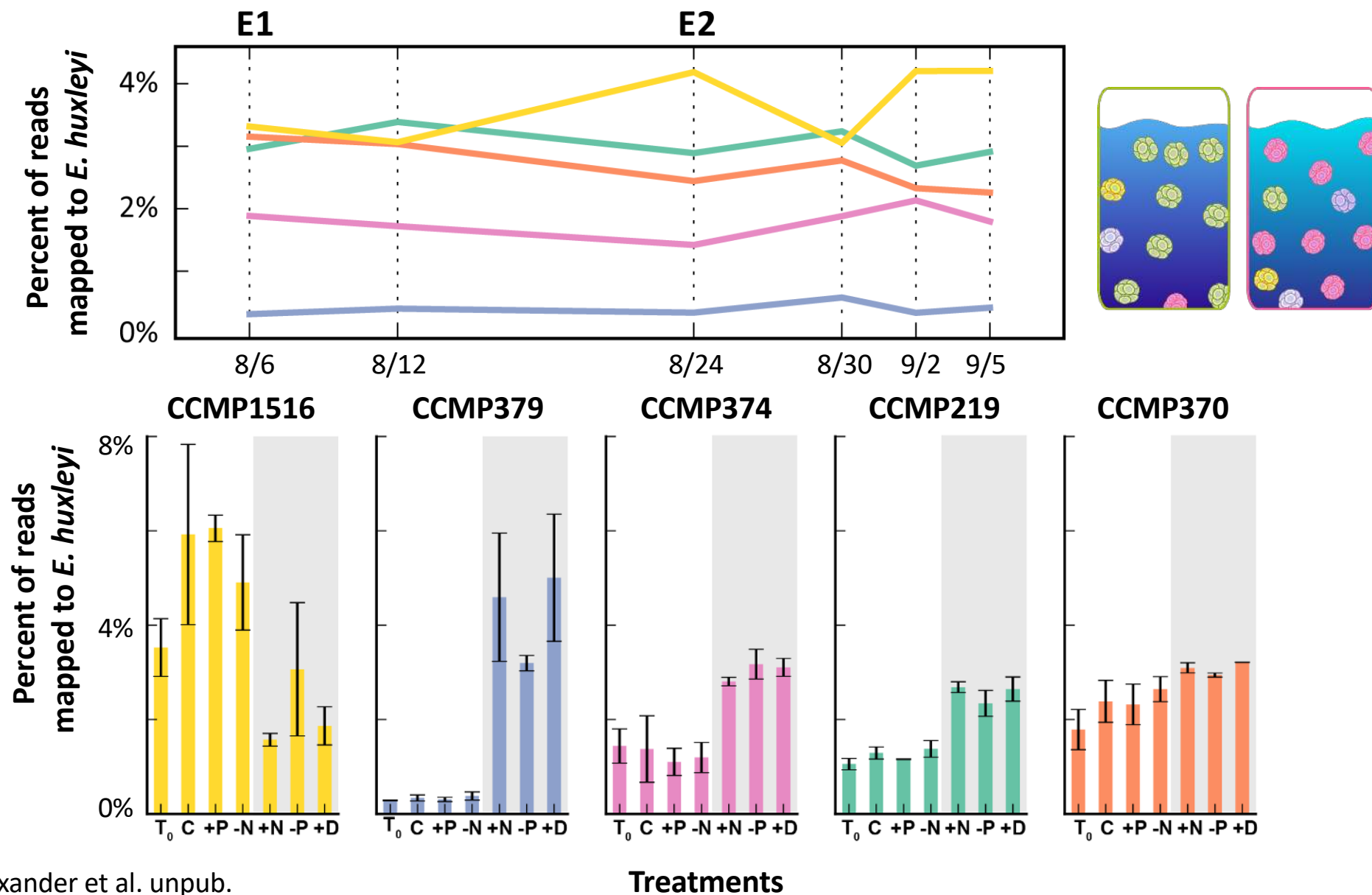


Alexander et al. unpub.

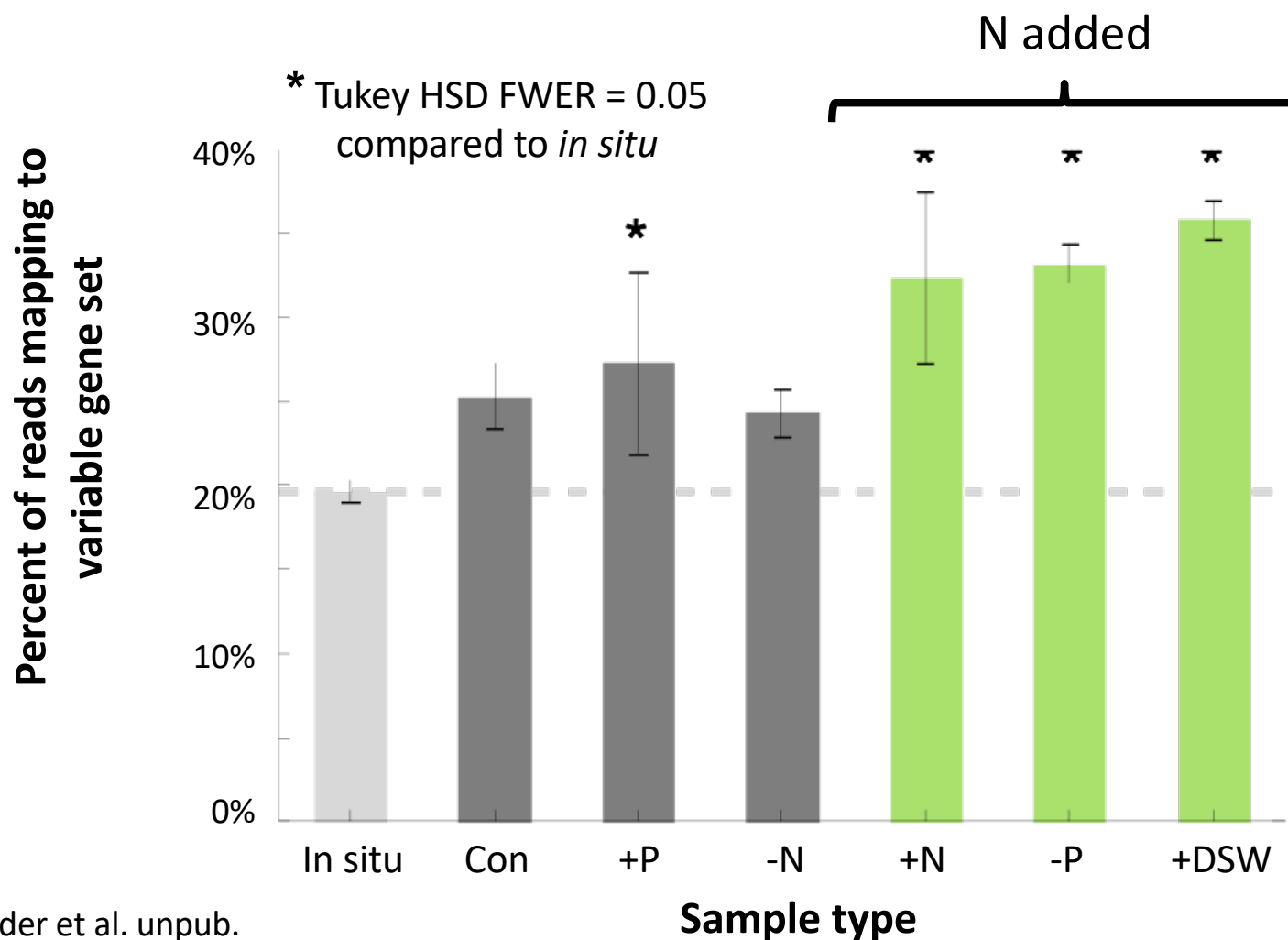


Use diagnostic OGs to track strain distribution

Strain distribution *in situ* and in the incubations

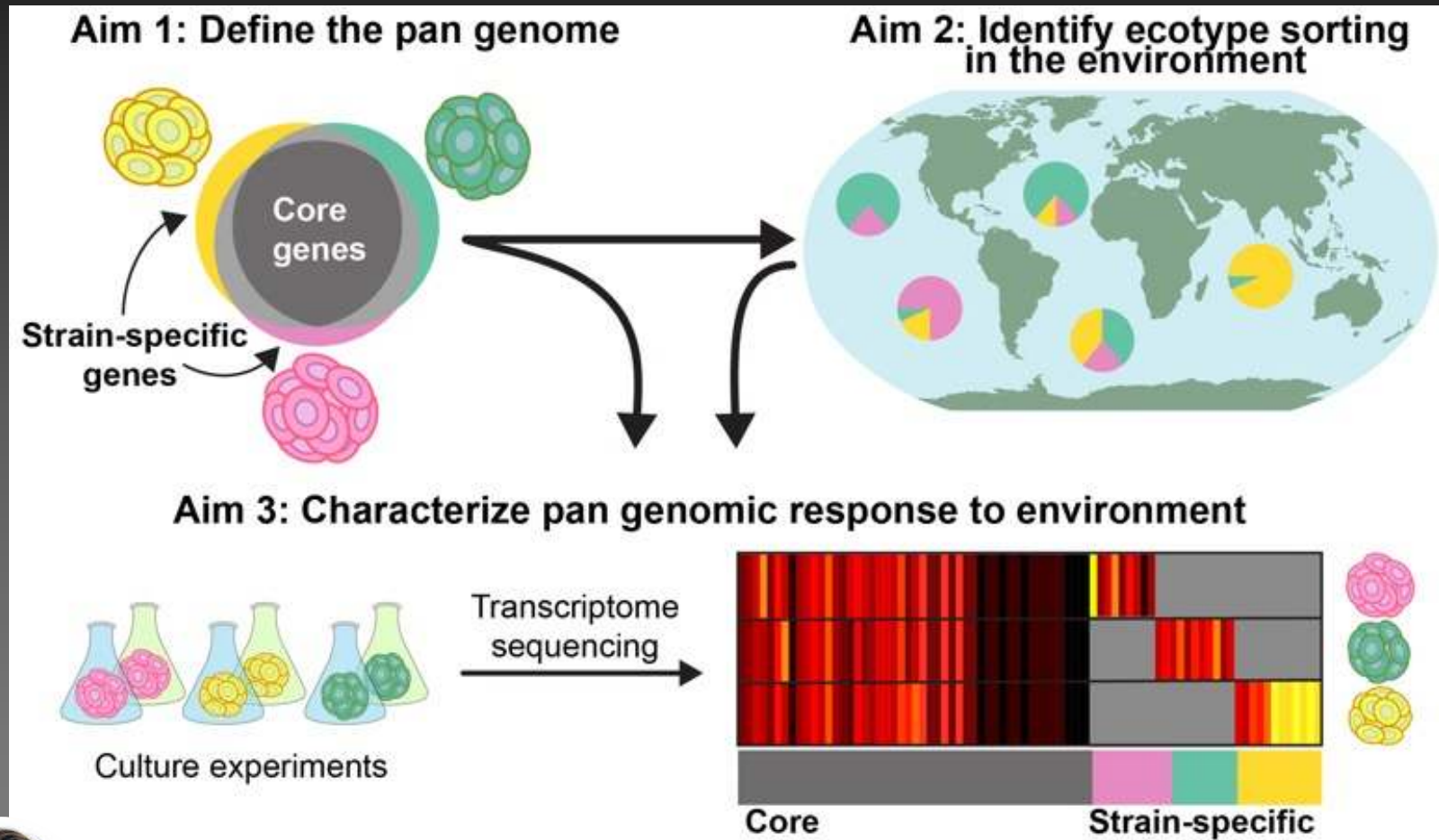


Expression of the variable portion of the pan-genome



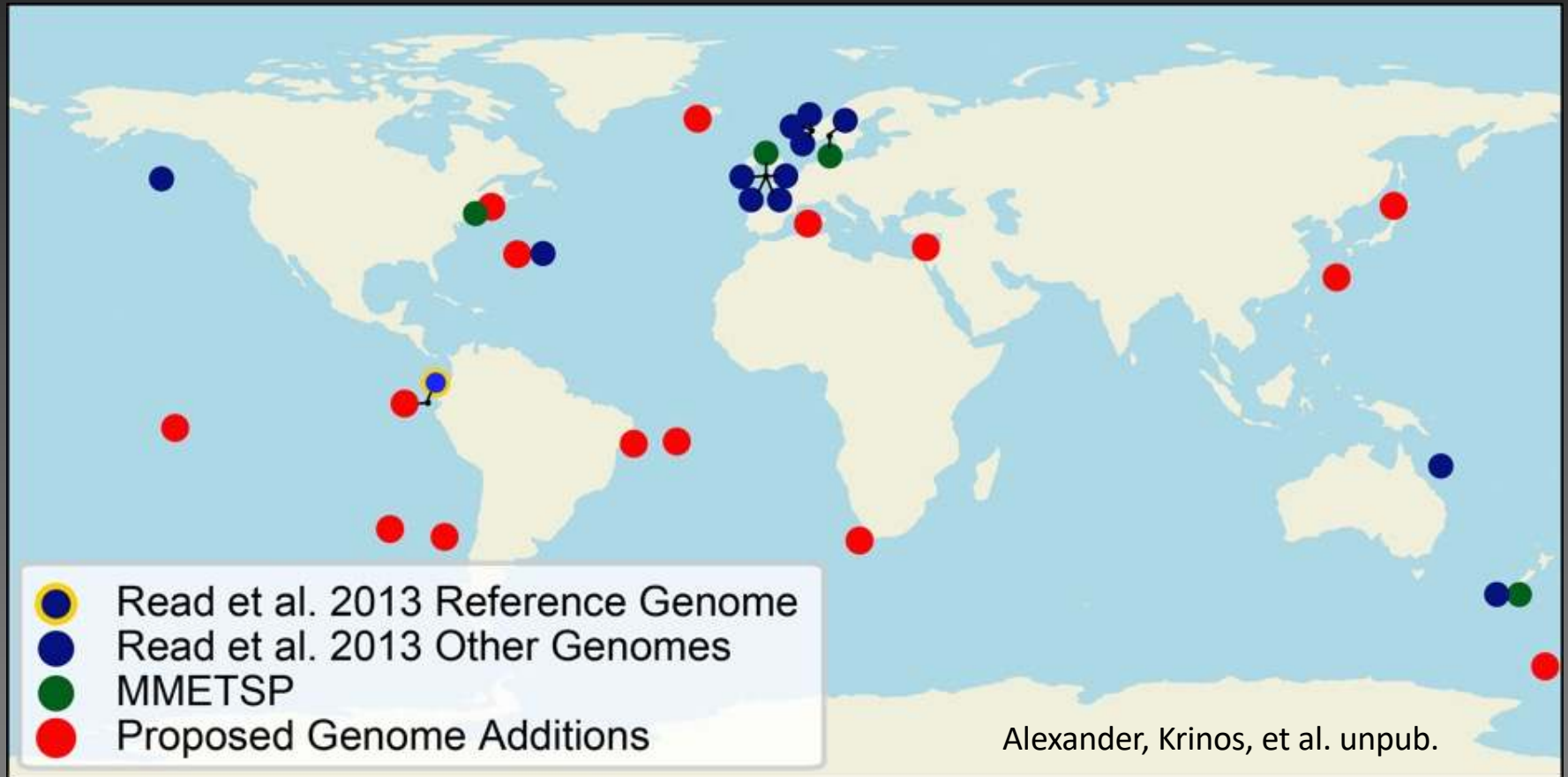
Expression of variable gene set significantly increased following N-addition

Ongoing work to define the pan genome



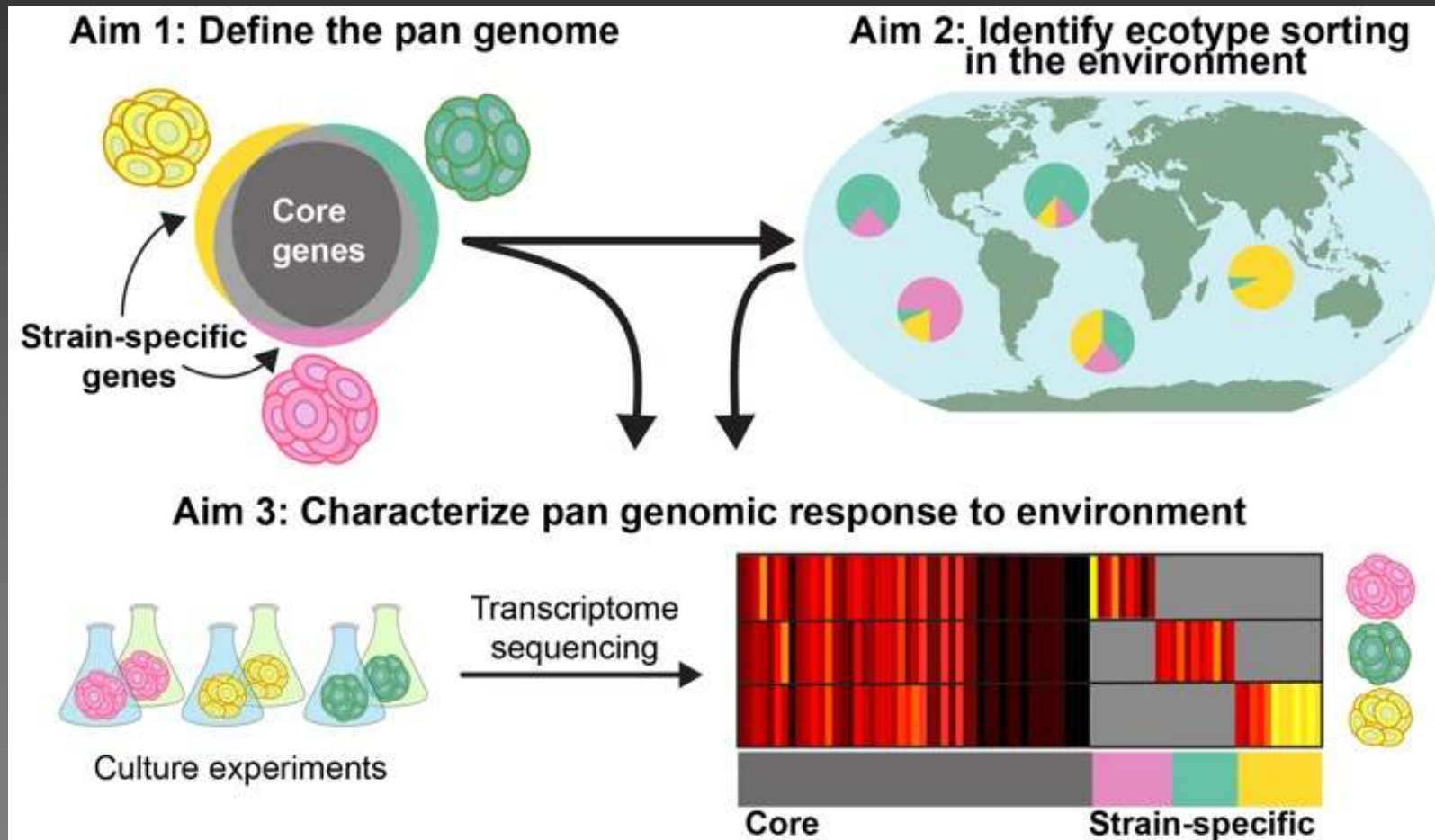
Harriet Alexander Lead PI
<https://alexanderlabwho.github.io/>

Expanding the pan genome



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

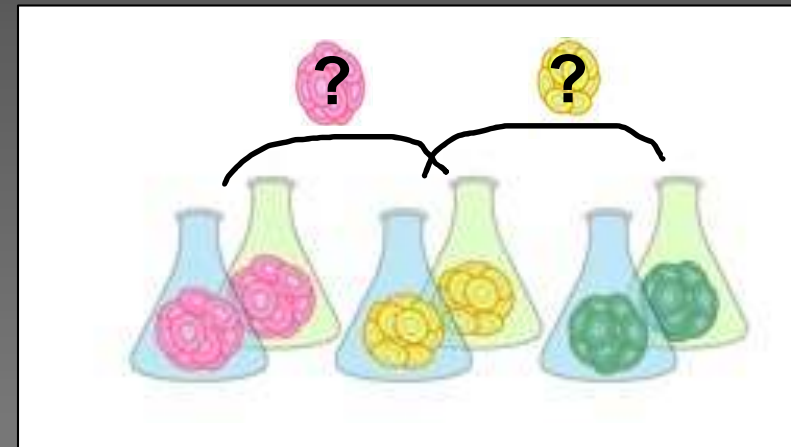
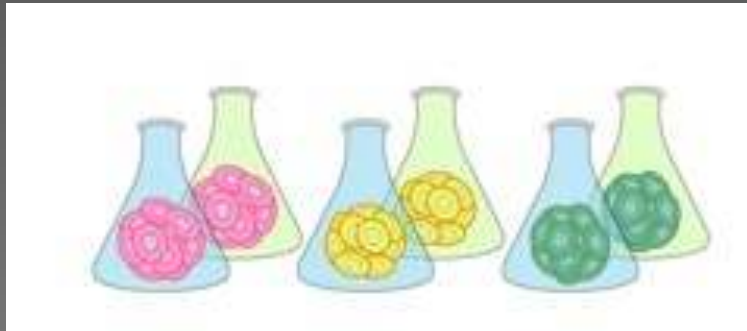
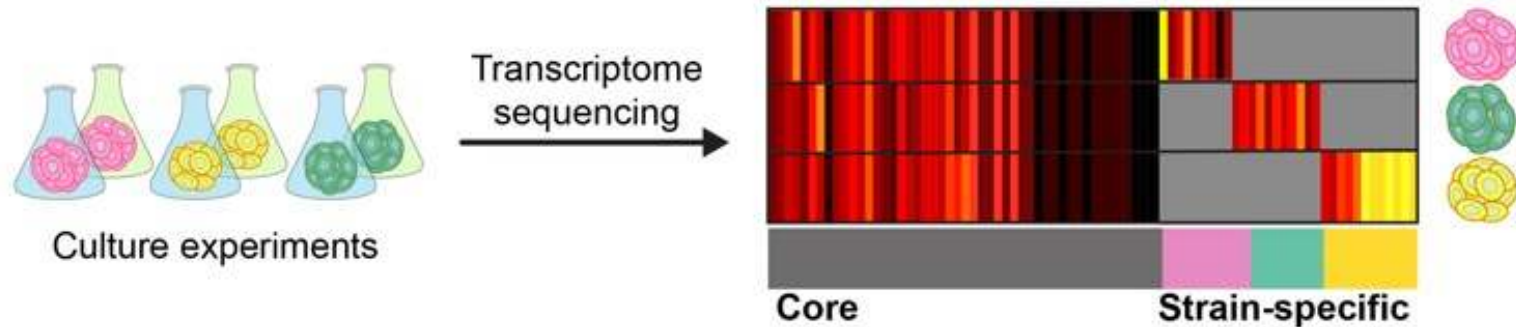
Future work to define the pan genome



Alexander et al. unpub.

How do genomic traits underpin competition outcomes?

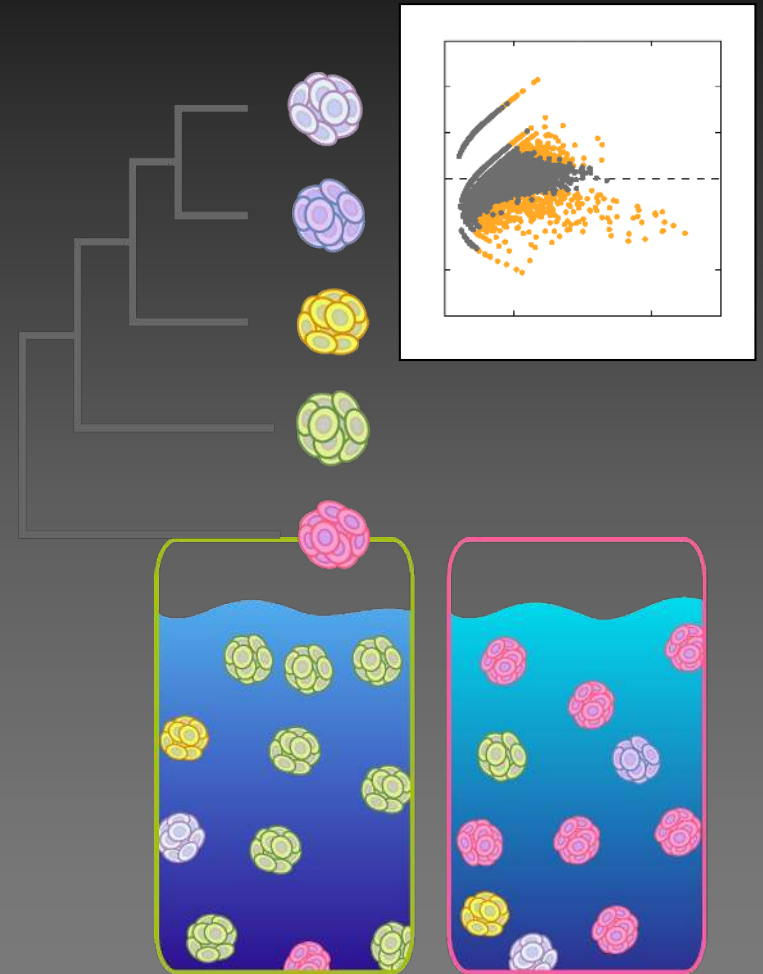
Aim 3: Characterize pan genomic response to environment



Haley, Palmer et al. unpub.

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

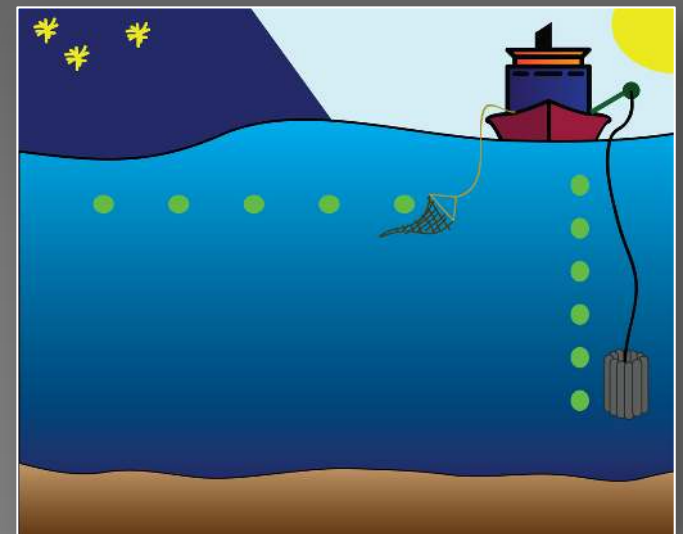
- **A day in the life – tracing competitive interactions with multi-'omics**

HOE:DYLAN



H. Alexander

HOE:MAHALO



Hawaii Ocean Experiment: Dynamics of Light (MAHALO)



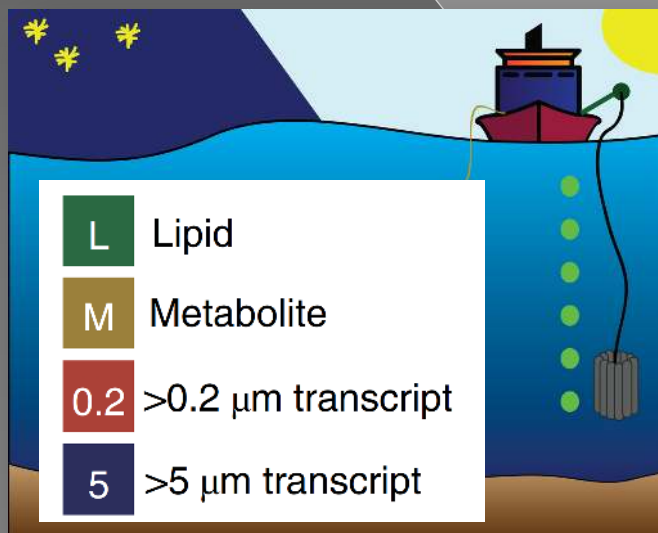
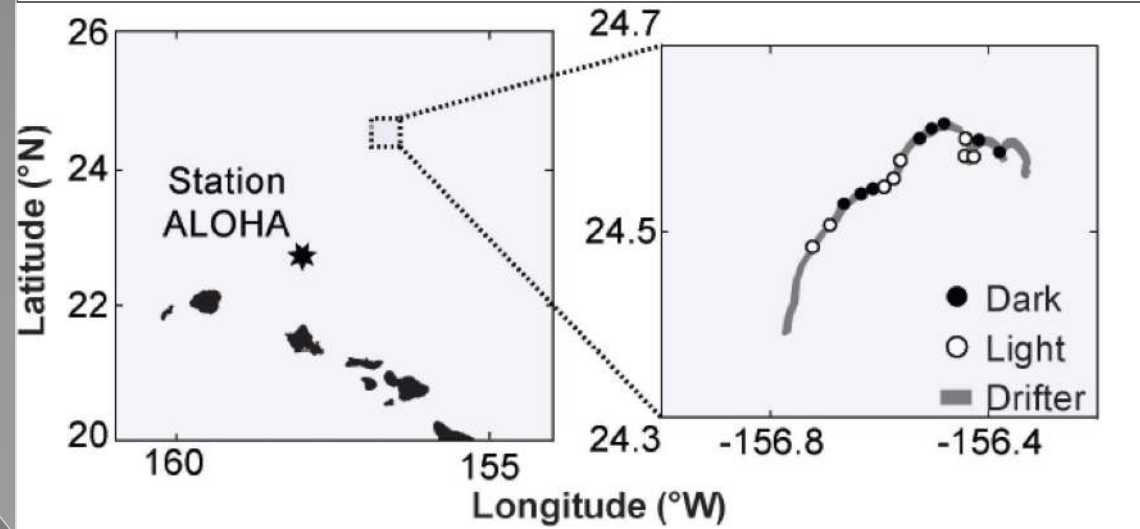
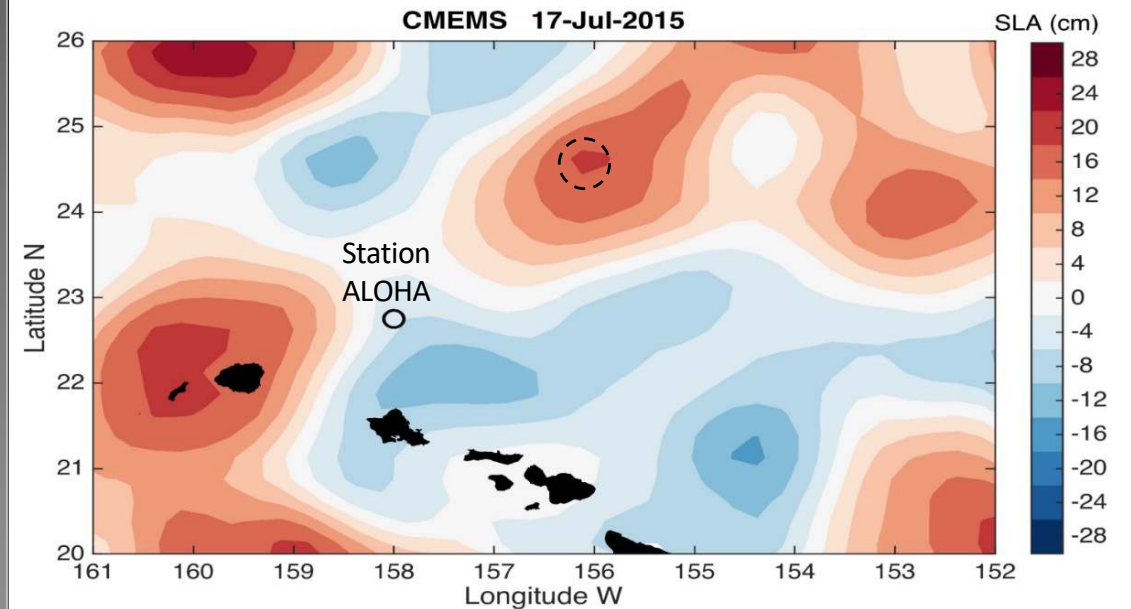
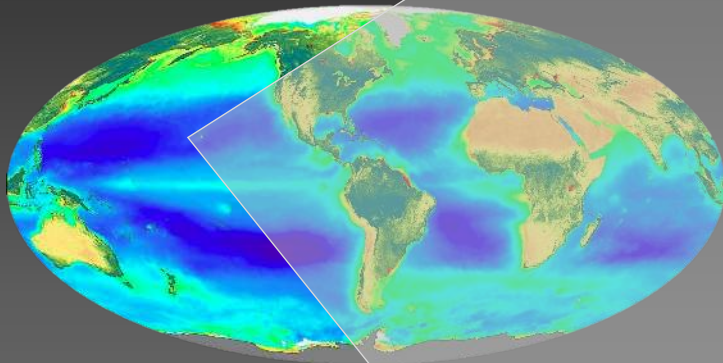
Figure 1 R/V Kilo Moana conducting one of the diel study CTD casts in 2015 (photo: Tara Clemente, taken from the R/V Ka'imikai-O-Kanaloa)



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](#)

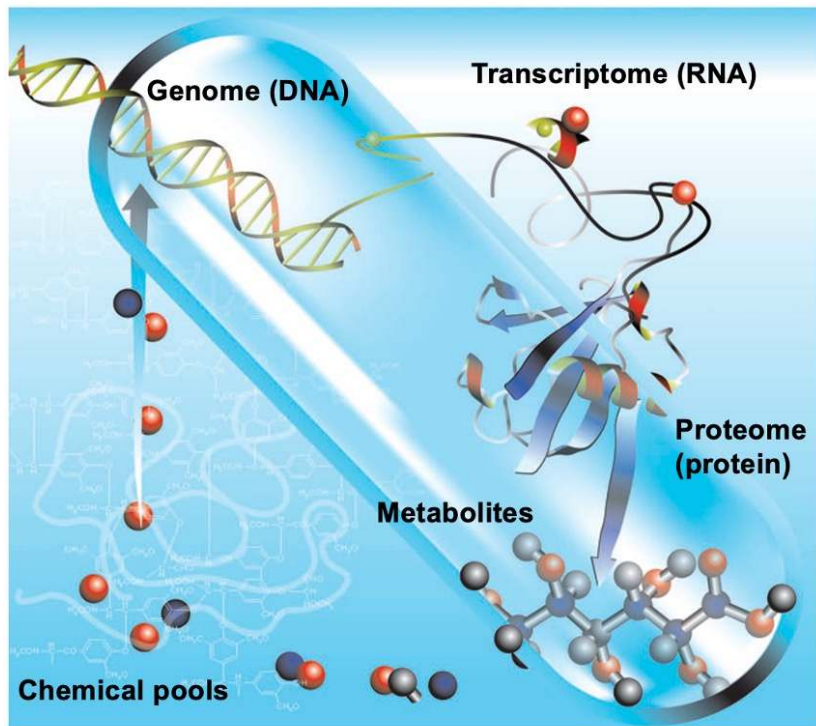
Research Scientist, Gloucester Marine Genomics Institute

 Follow



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

Jointly applying new multi 'omic techniques



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

L

Lipid

M

Metabolite

0.2

>0.2 μm transcript

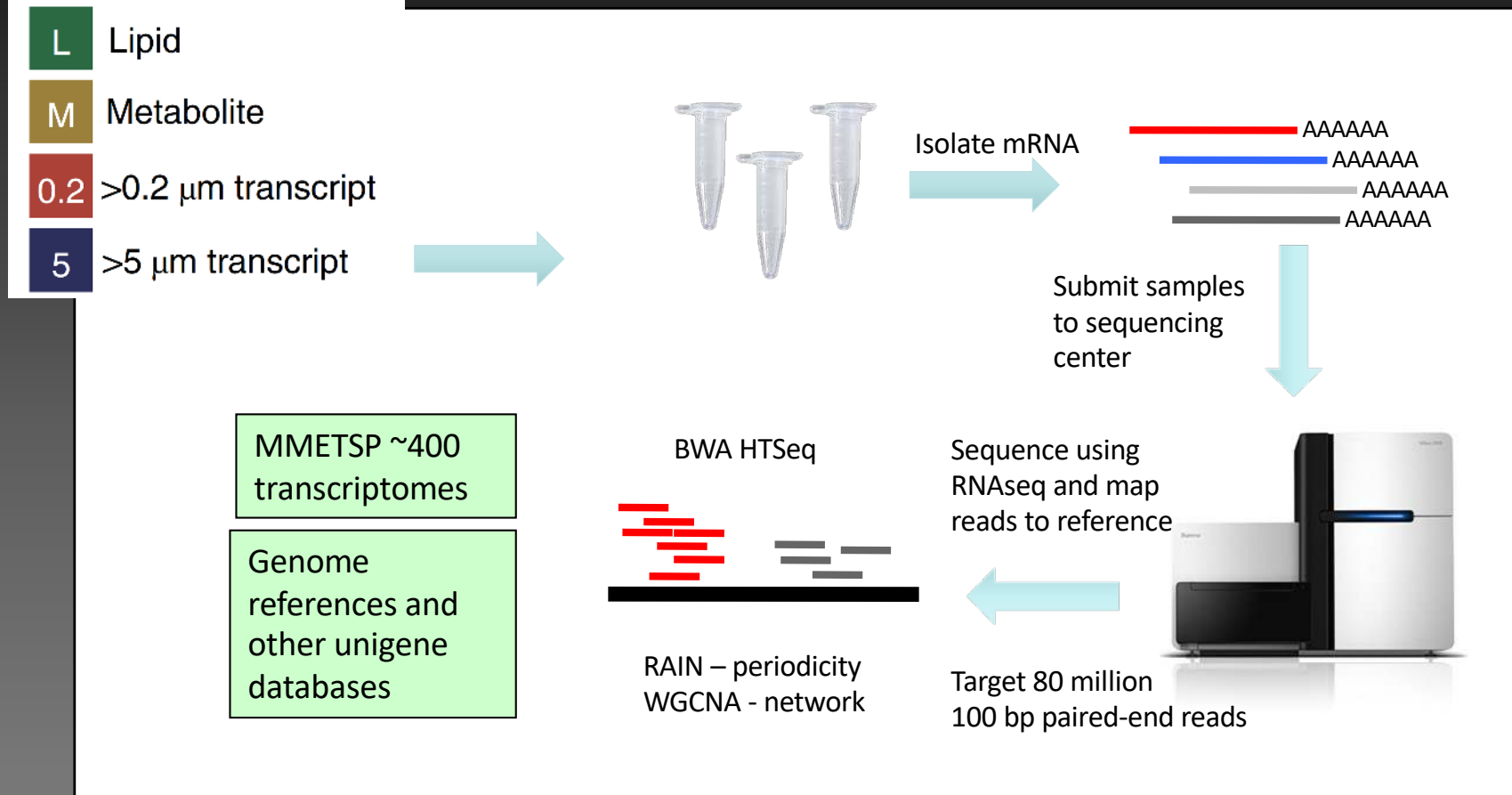
5

>5 μm transcript

Sampling every 4hrs for many days



Sampling and pipeline



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

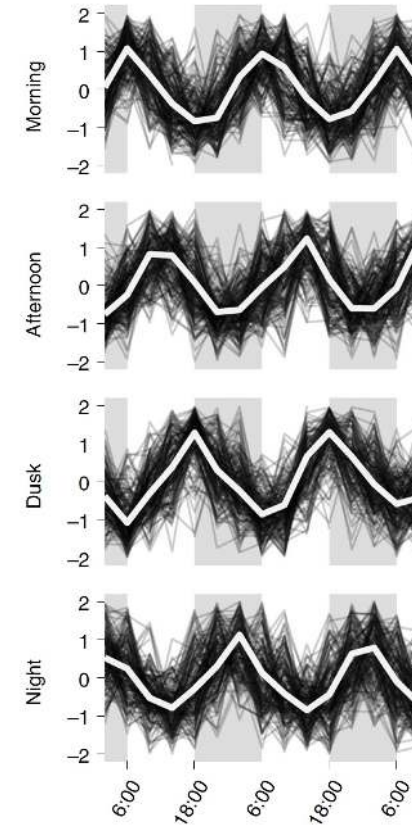
<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-9-559>

MMETSP = Marine Microbial Eukaryotic Transcriptome Project

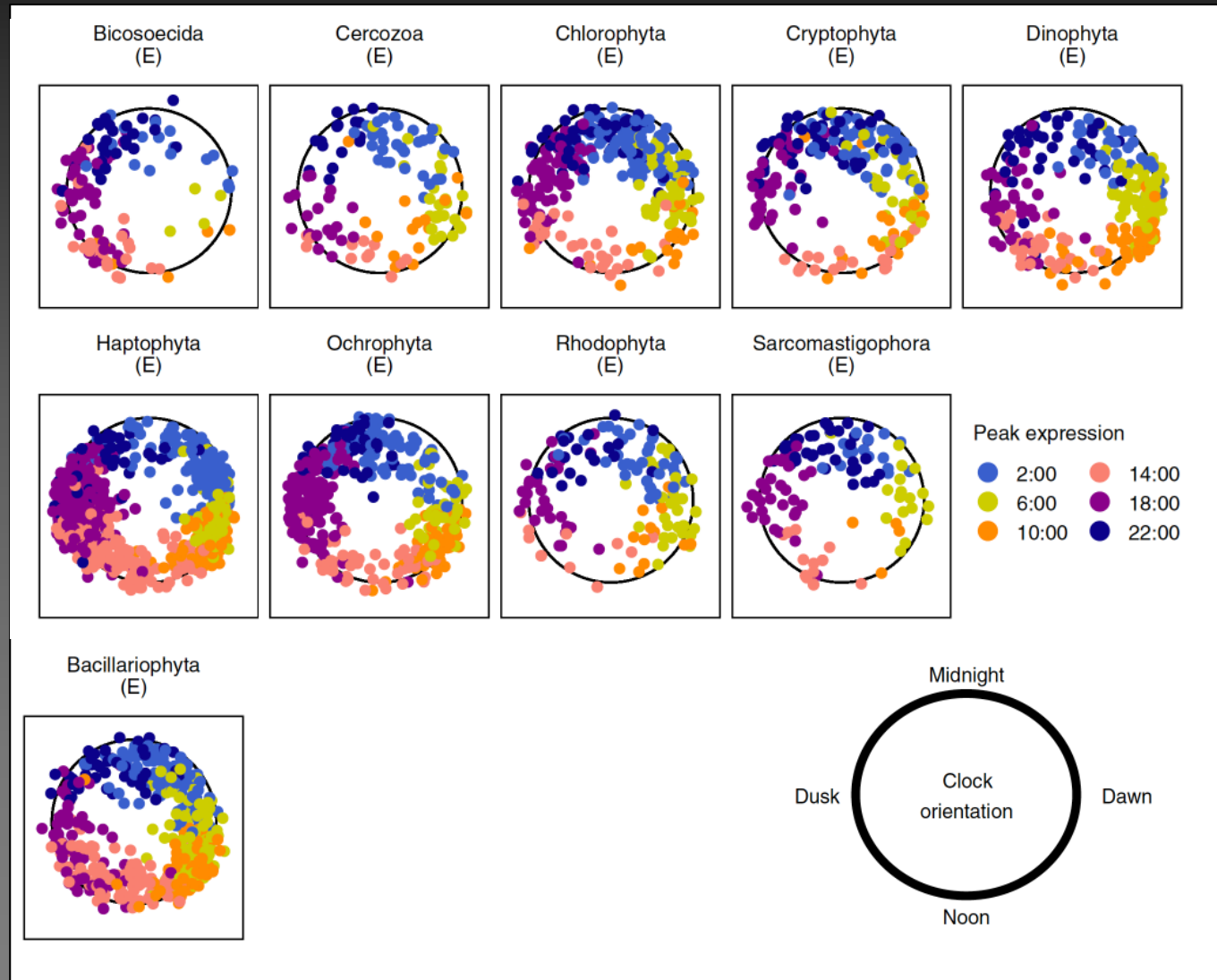
Visualizing diel patterns

- L** Lipid
- M** Metabolite
- 0.2** >0.2 μm transcript
- 5** >5 μm transcript

~65,000 analyte
time series

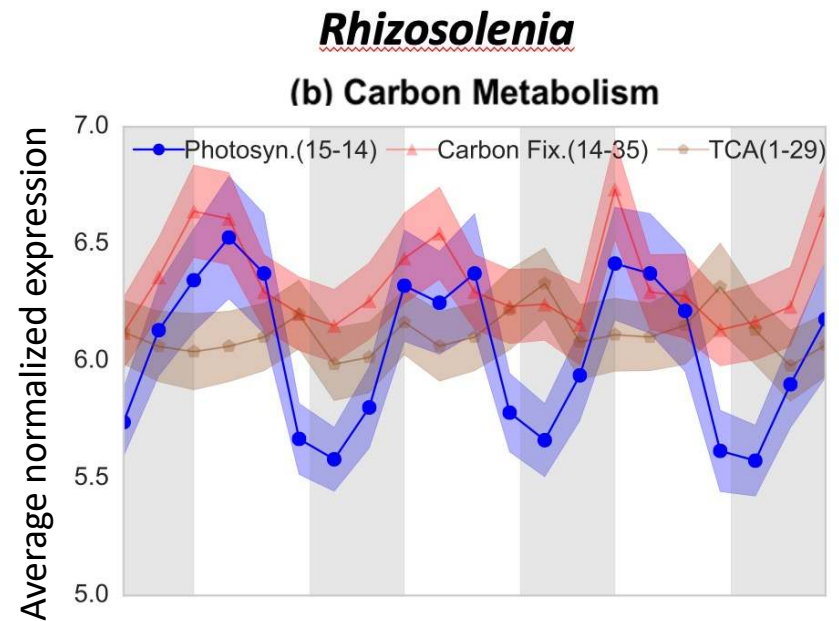
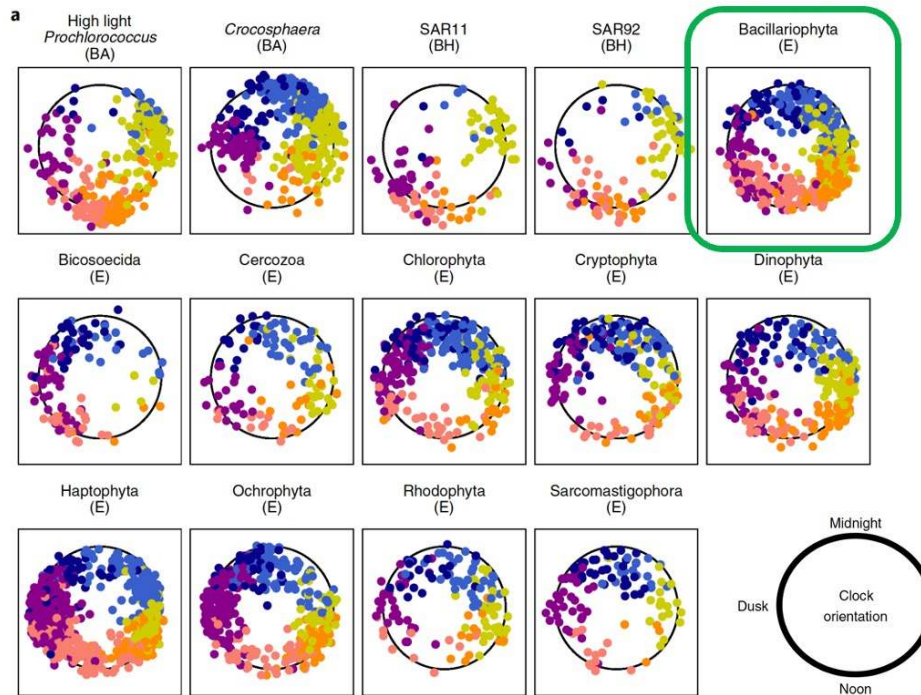


Different taxa have distinct diel transcription patterns



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

Carbon fixation and respiration are highly coordinated

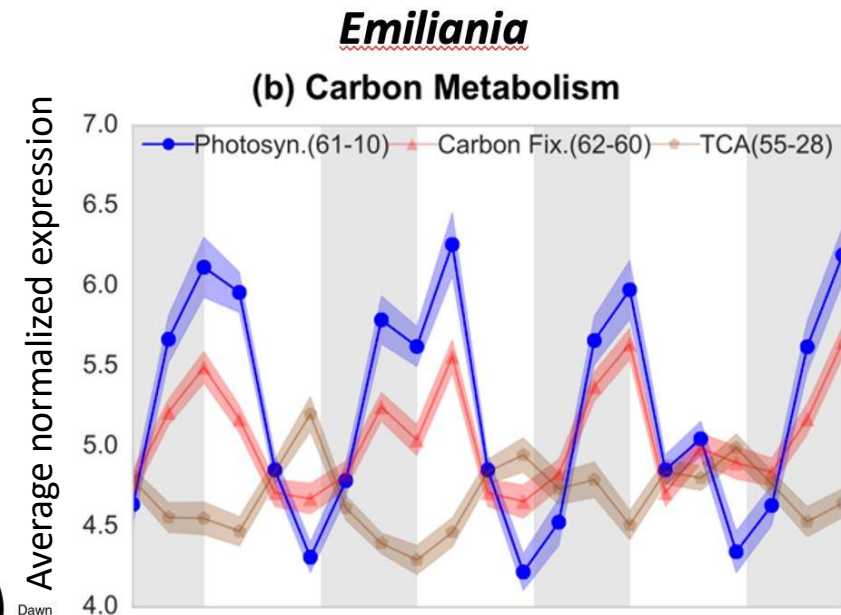
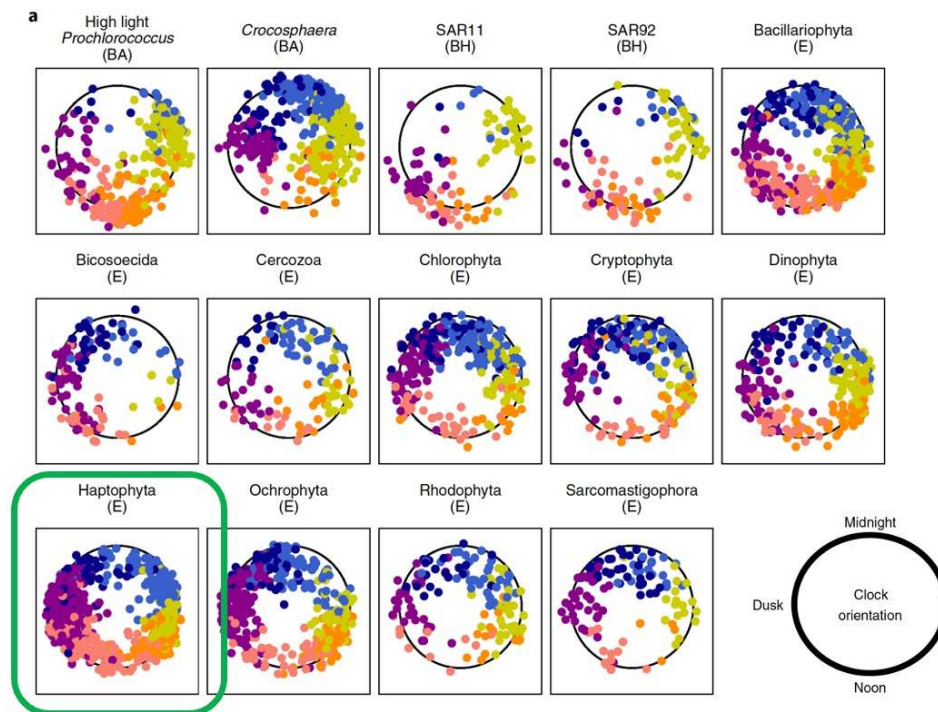


Muratore, Boysen, Harke et al. (2022) *Nature Ecol. Evol.*



Harke et al. 2019 *ISME J*

Carbon fixation and respiration are highly coordinated

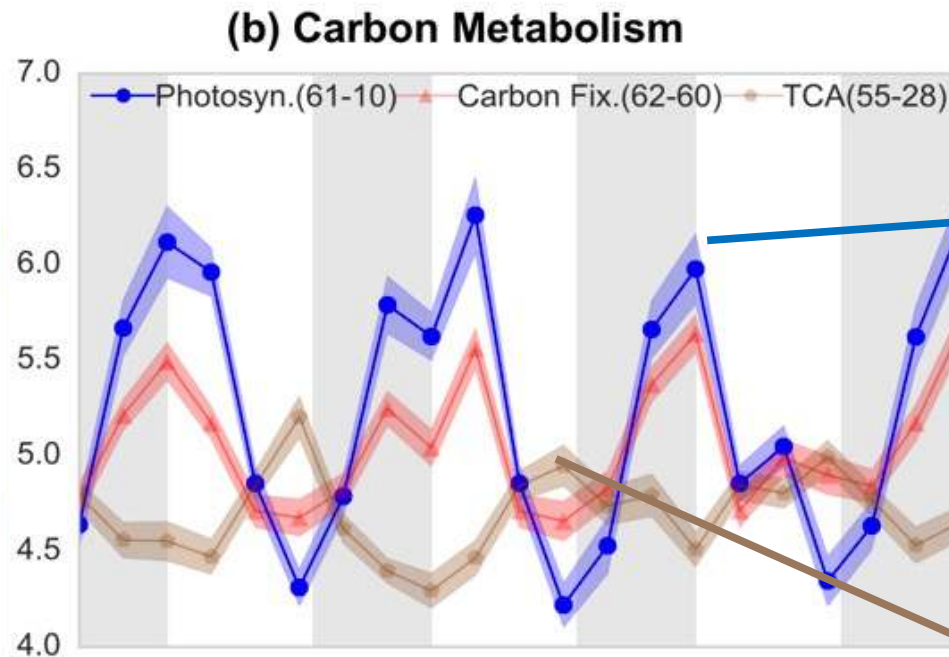


Muratore, Boysen, Harke et al. (2022) *Nature Ecol. Evol.*



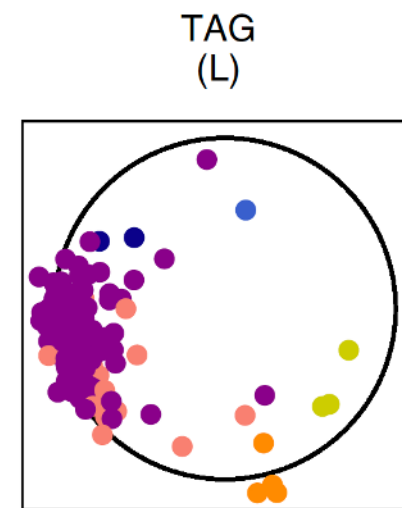
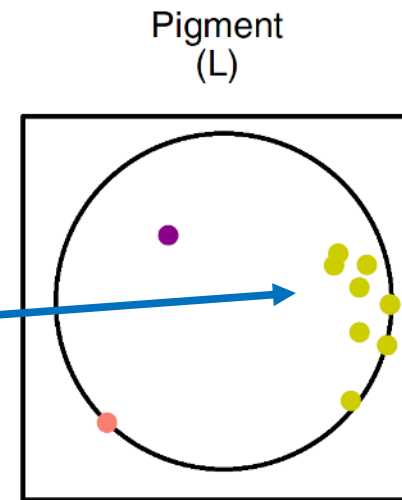
Limón-Hernandez et al. (2020) *Environ. Micro.*

Carbon metabolism synchronized across all phytoplankton

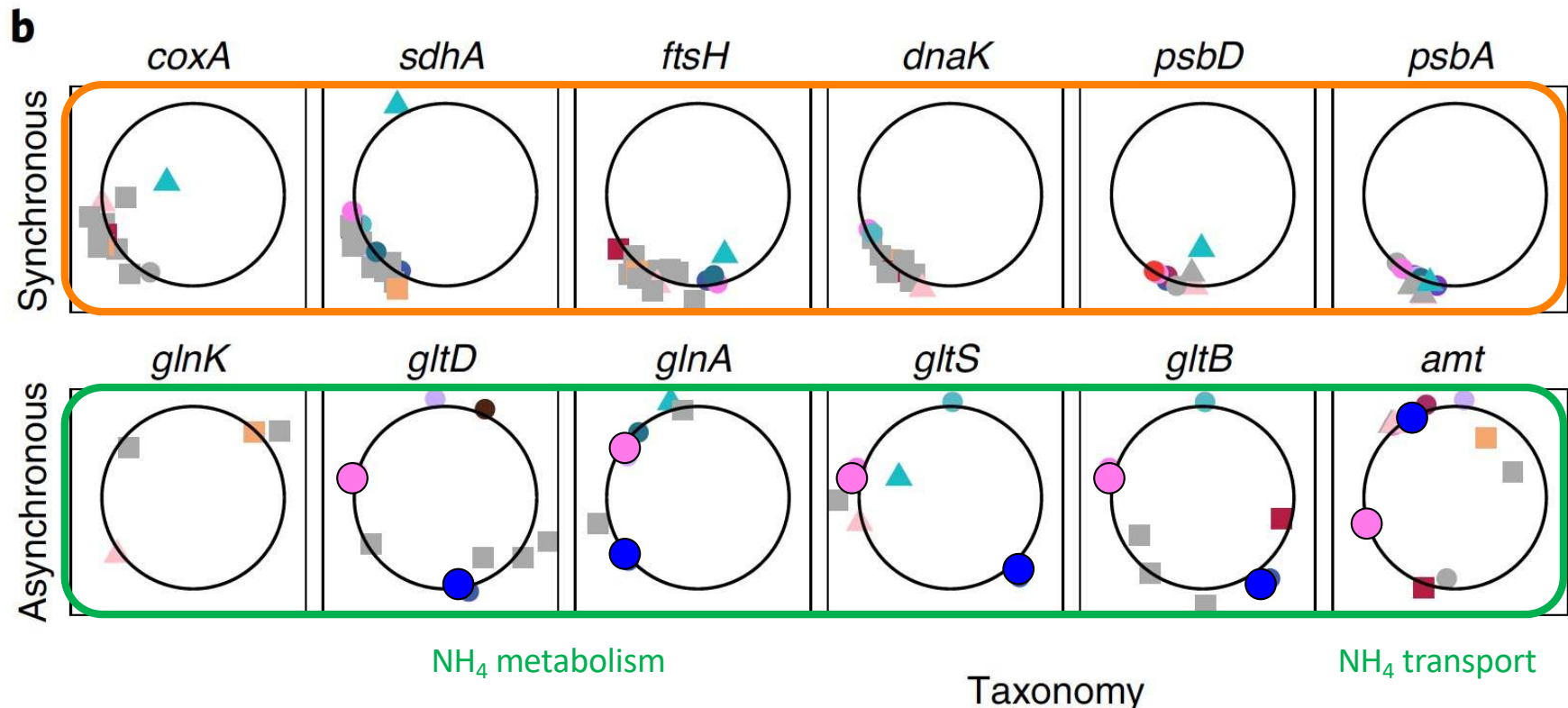


All photosynthesis, carbon fixation, and TCA cycle processes are synchronized across bacteria and eukaryotes!

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



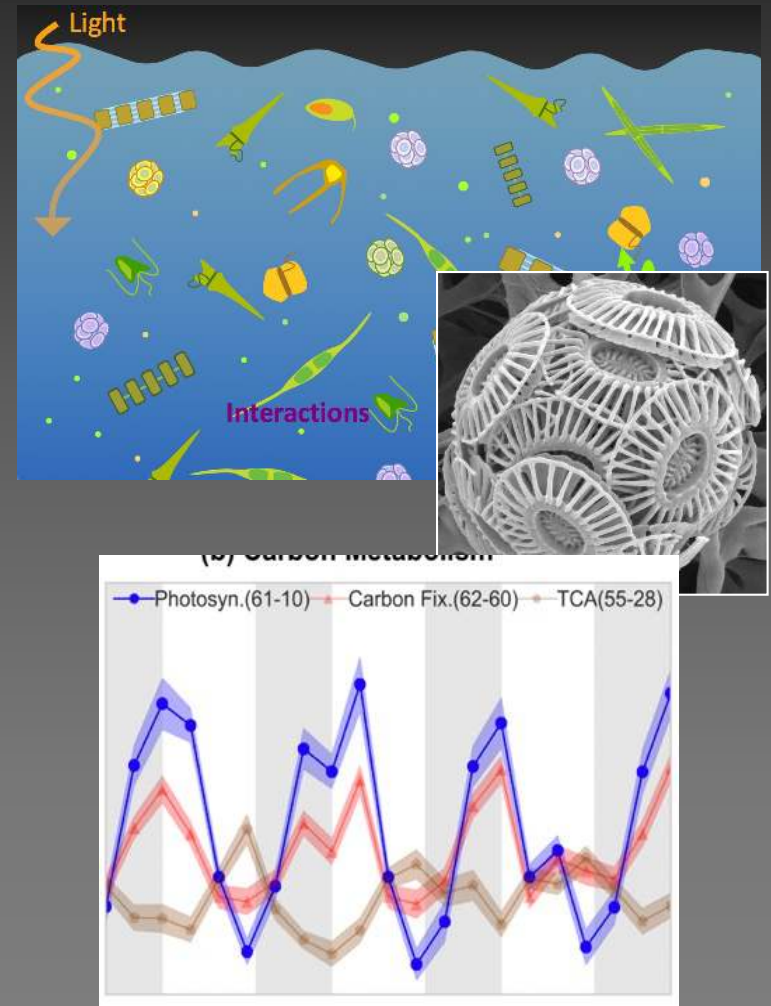
N metabolism functions are expressed asynchronously



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

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 resources



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

