From flask to field: tracking the drivers of phytoplankton physiological ecology across marine ecosystems

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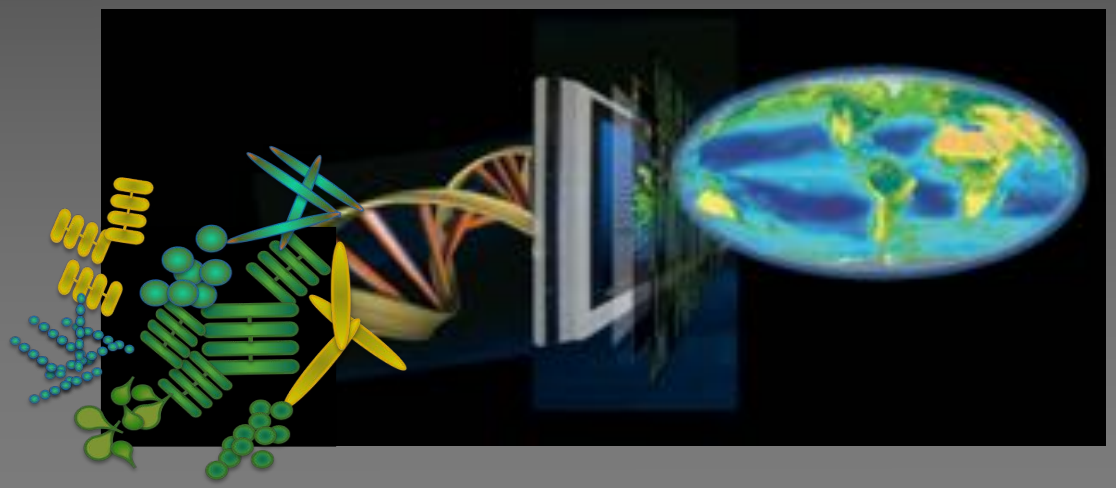

COLUMBIA UNIVERSITY
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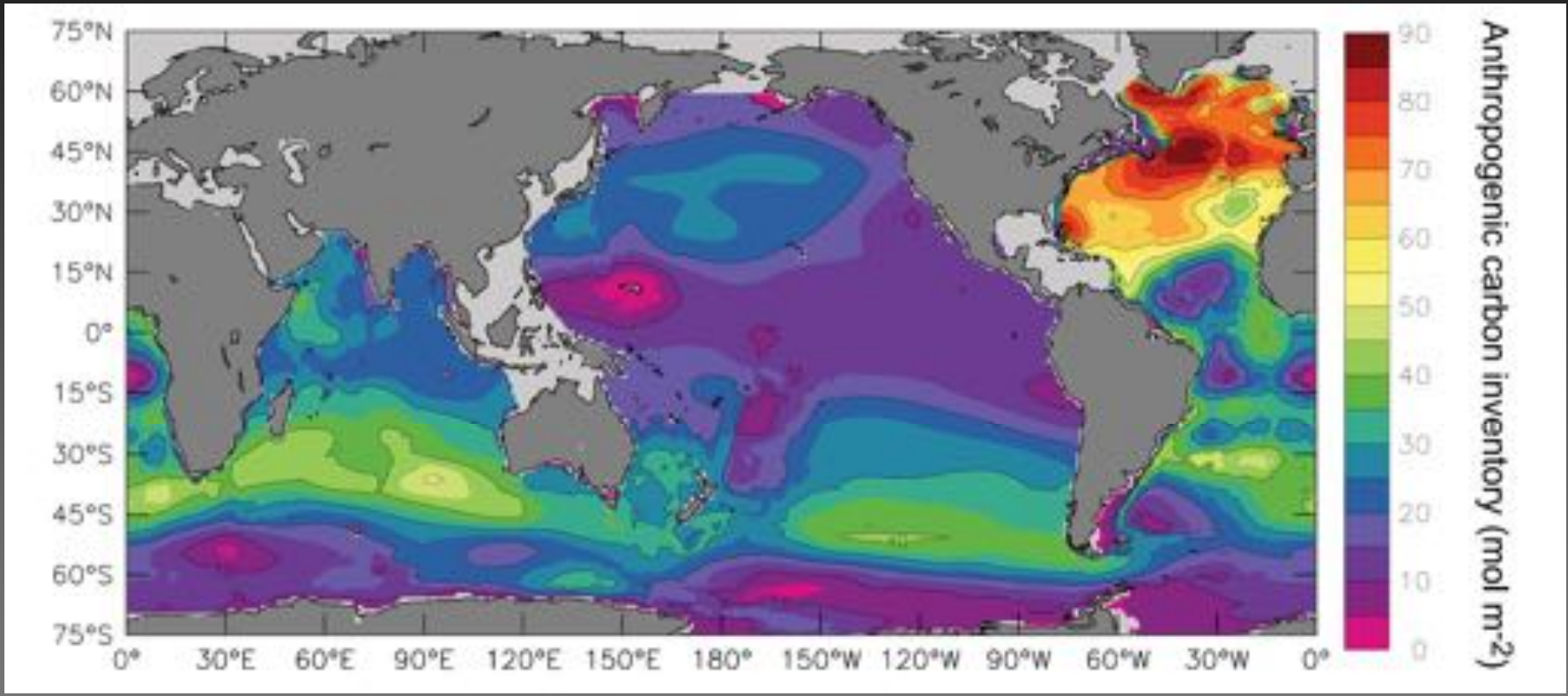
LAMONT-DOHERTY
EARTH OBSERVATORY

The ocean makes our planet livable

The ocean acts as a buffer for CO_2 in the atmosphere



Sabine et al. (2004) *Science*

Between 1800 - 1994, ocean has absorbed ~120 petagrams of CO_2
Oceanic sink accounts for ~48% of fossil-fuel emissions

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

Microbial biogeochemistry - 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*

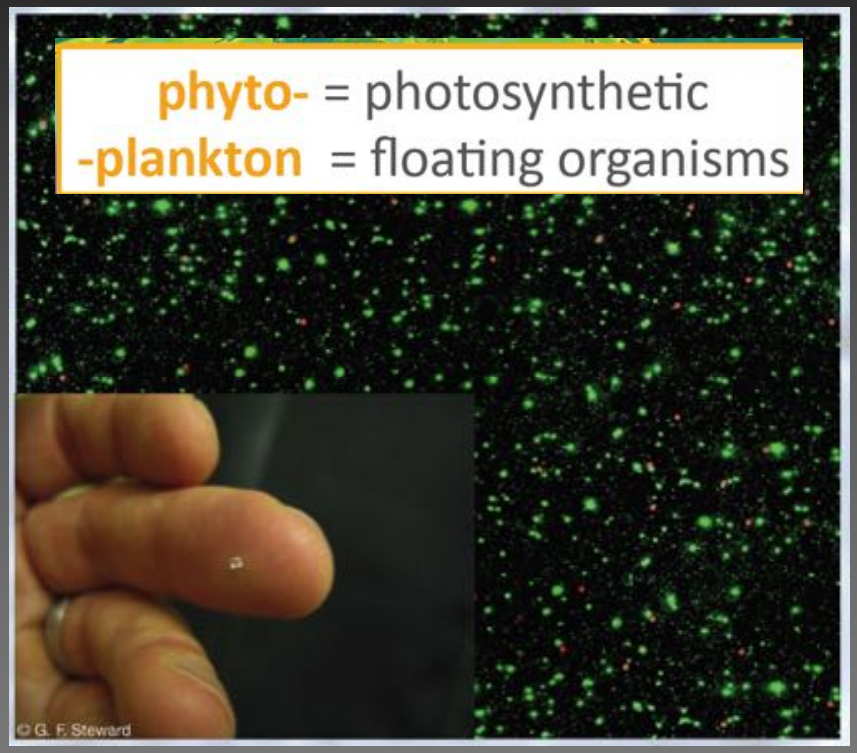
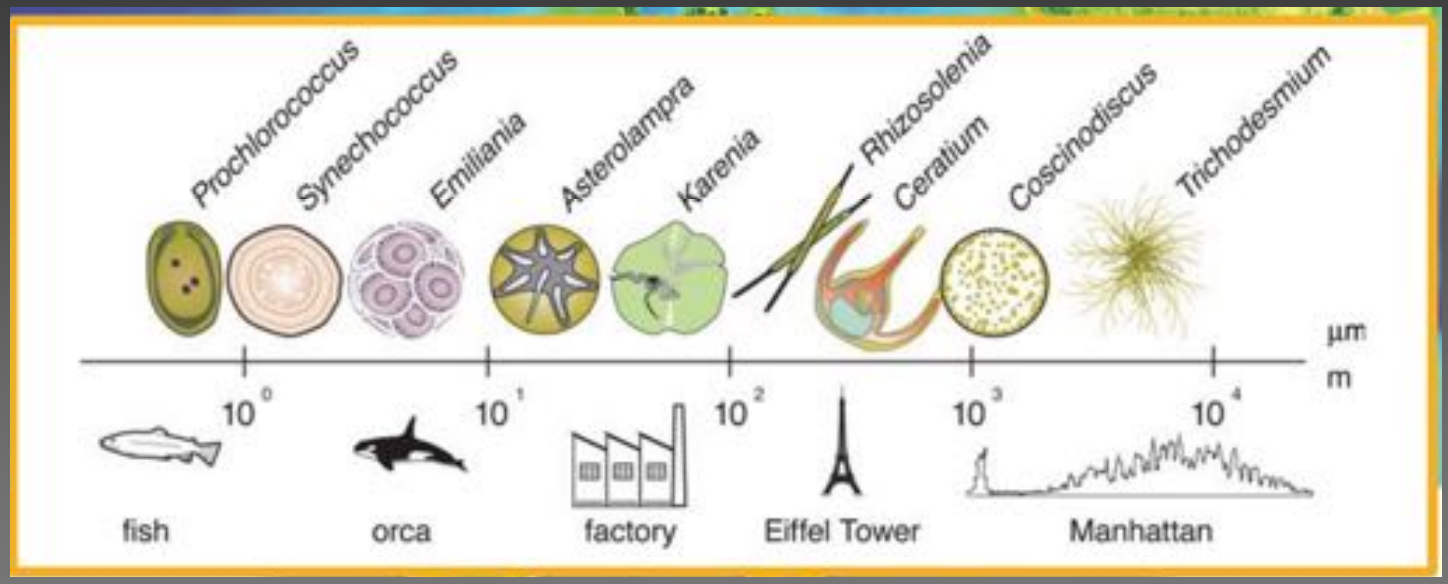
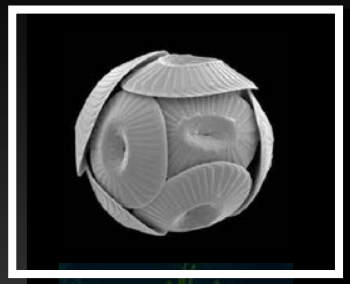
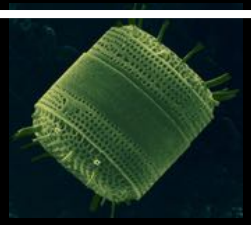


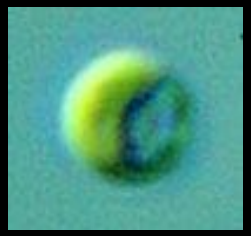
Image courtesy C-MORE

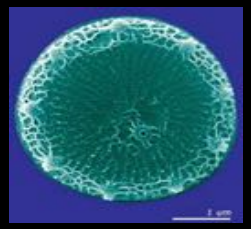
Marine phytoplankton are highly diverse

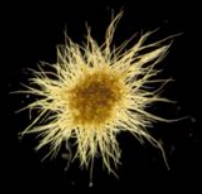


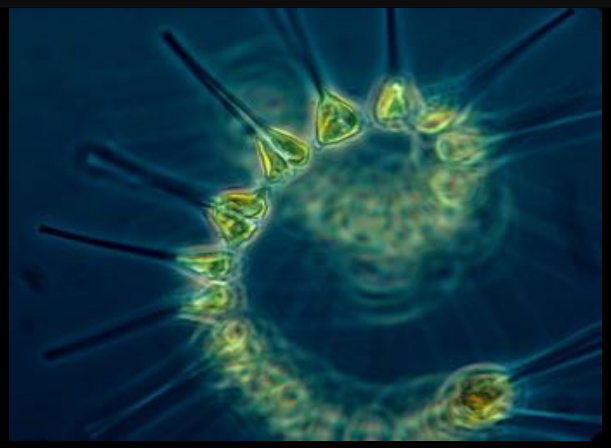


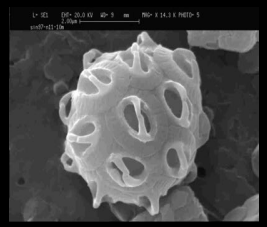


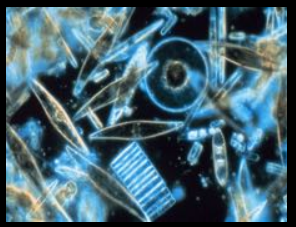


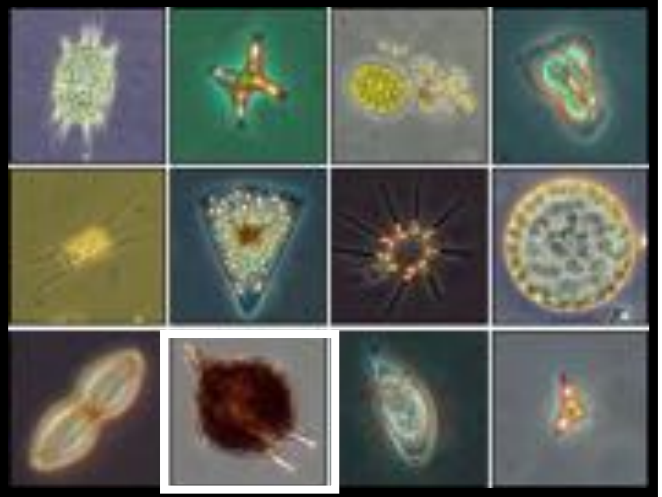


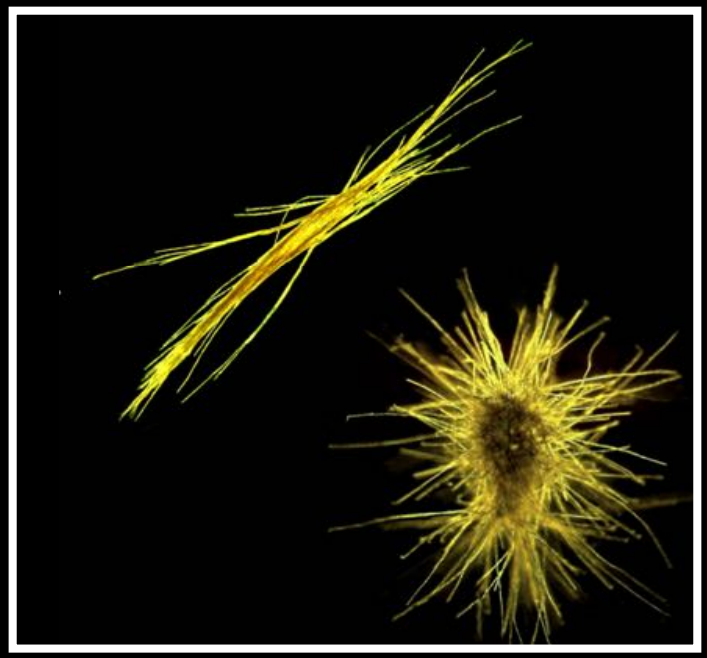


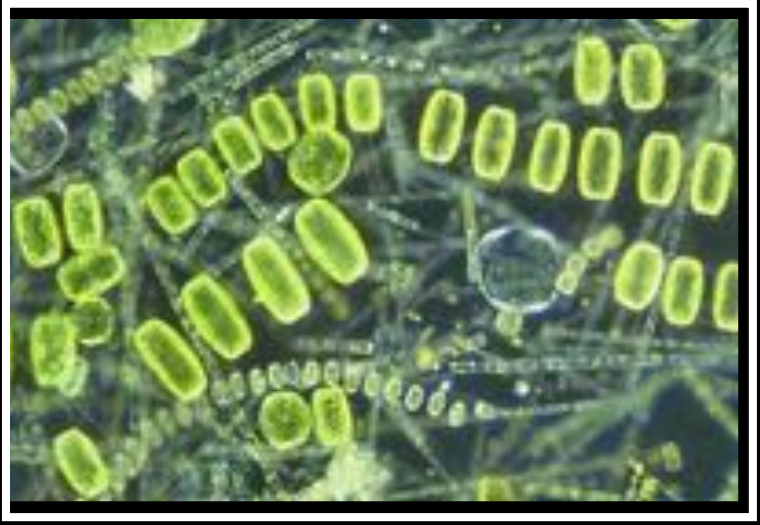










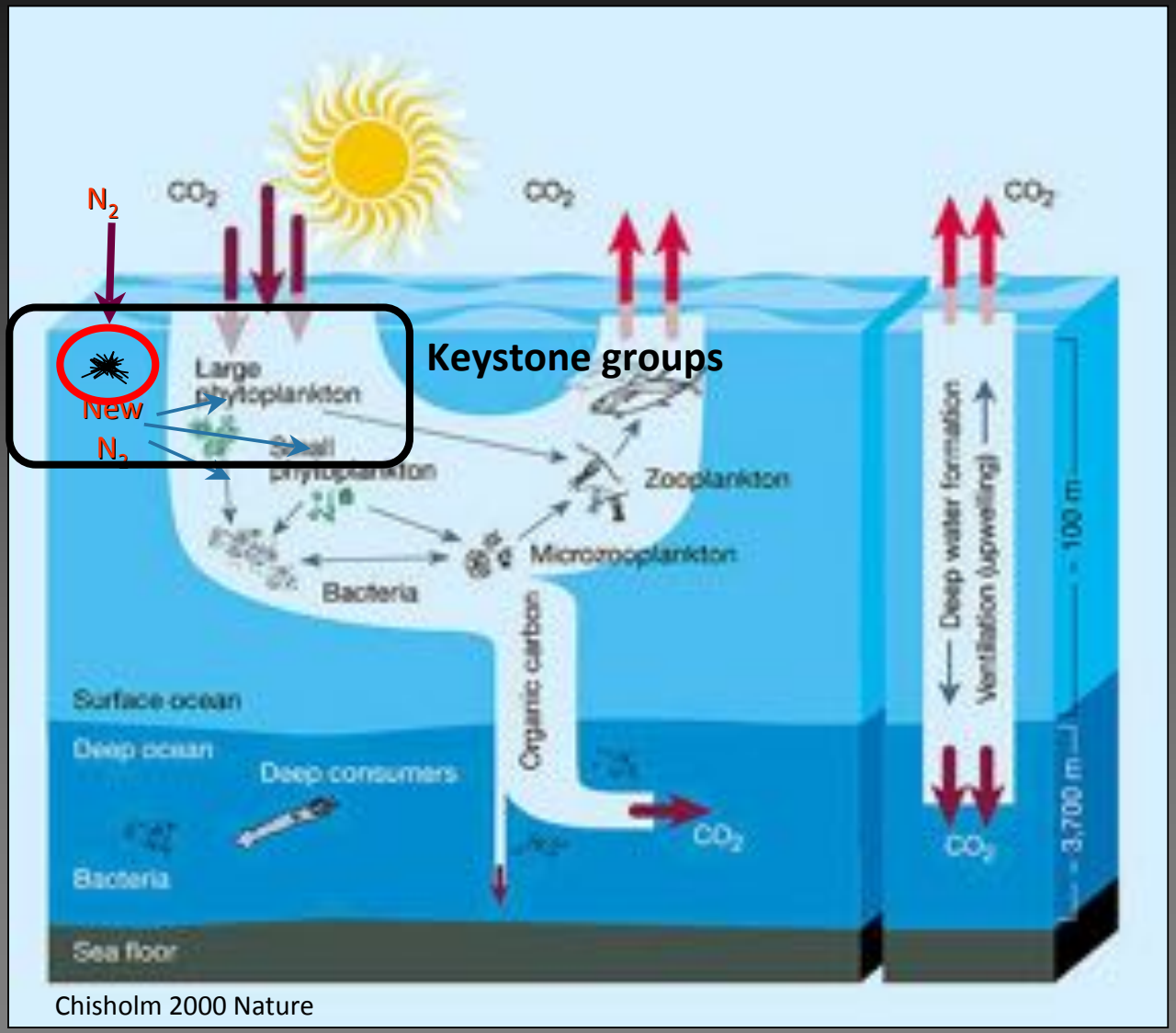


Focus on keystone groups

Haptophytes

Diatoms

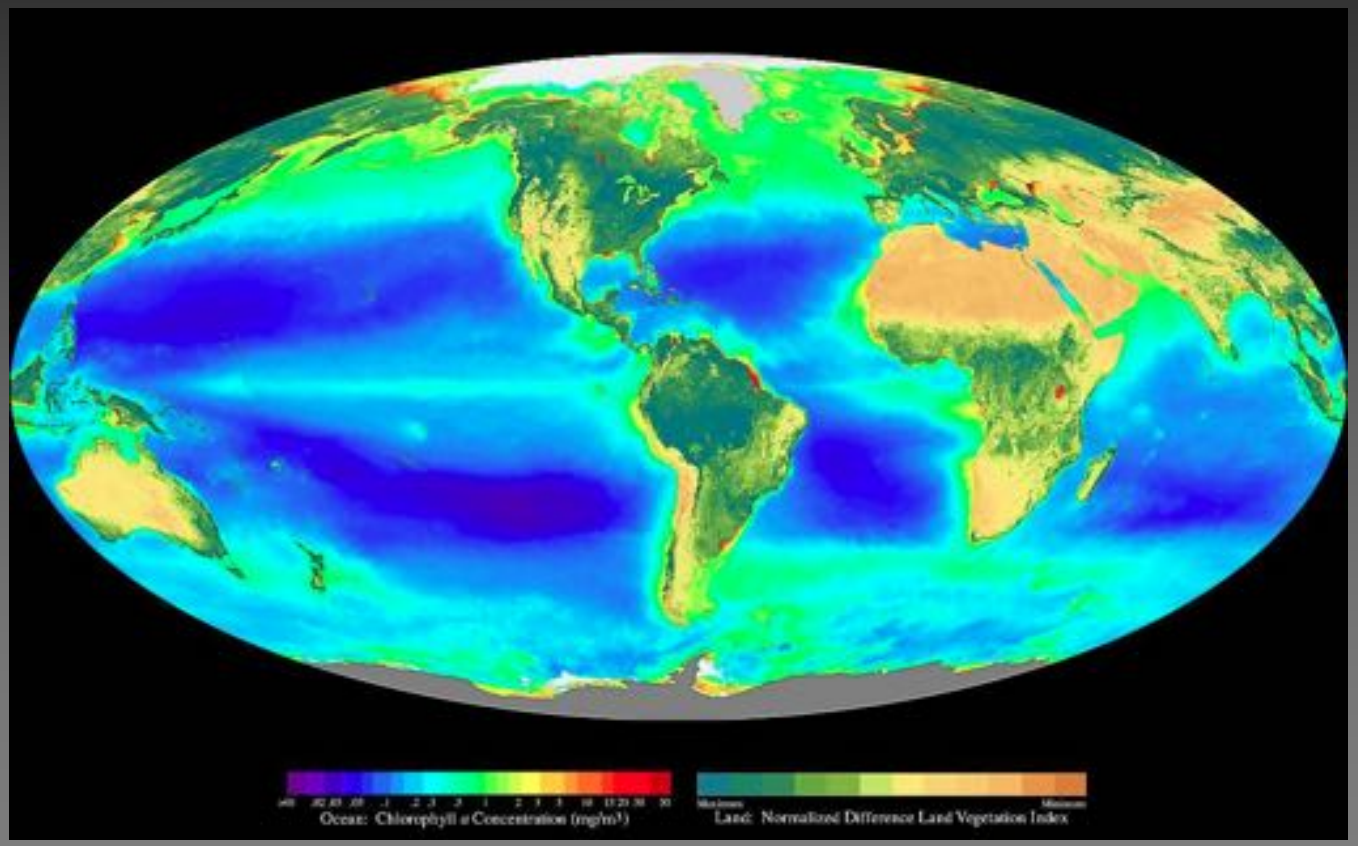
N_2 Fixers



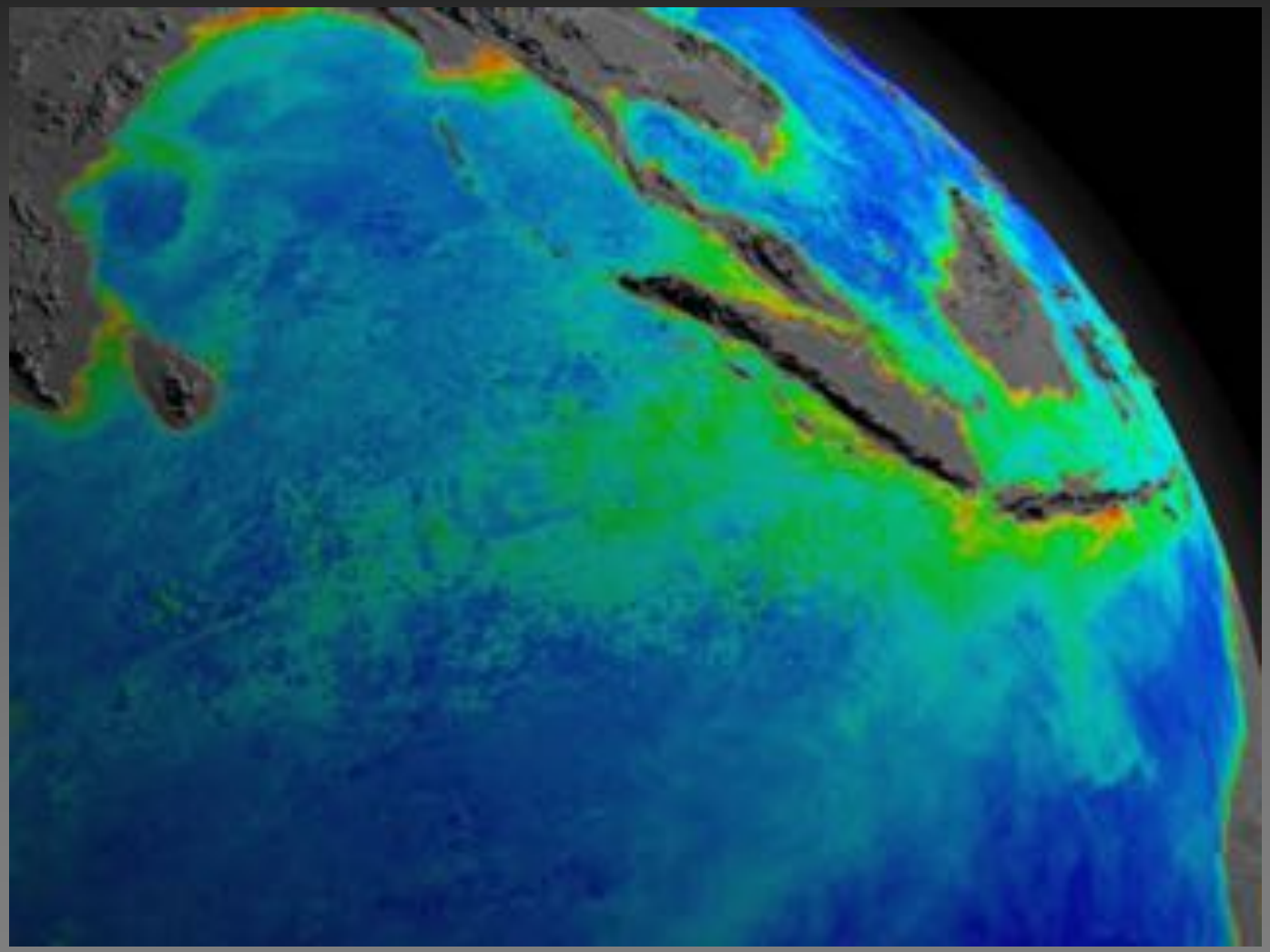
Chisholm 2000 Nature

Phytoplankton play a profound role in the earth system

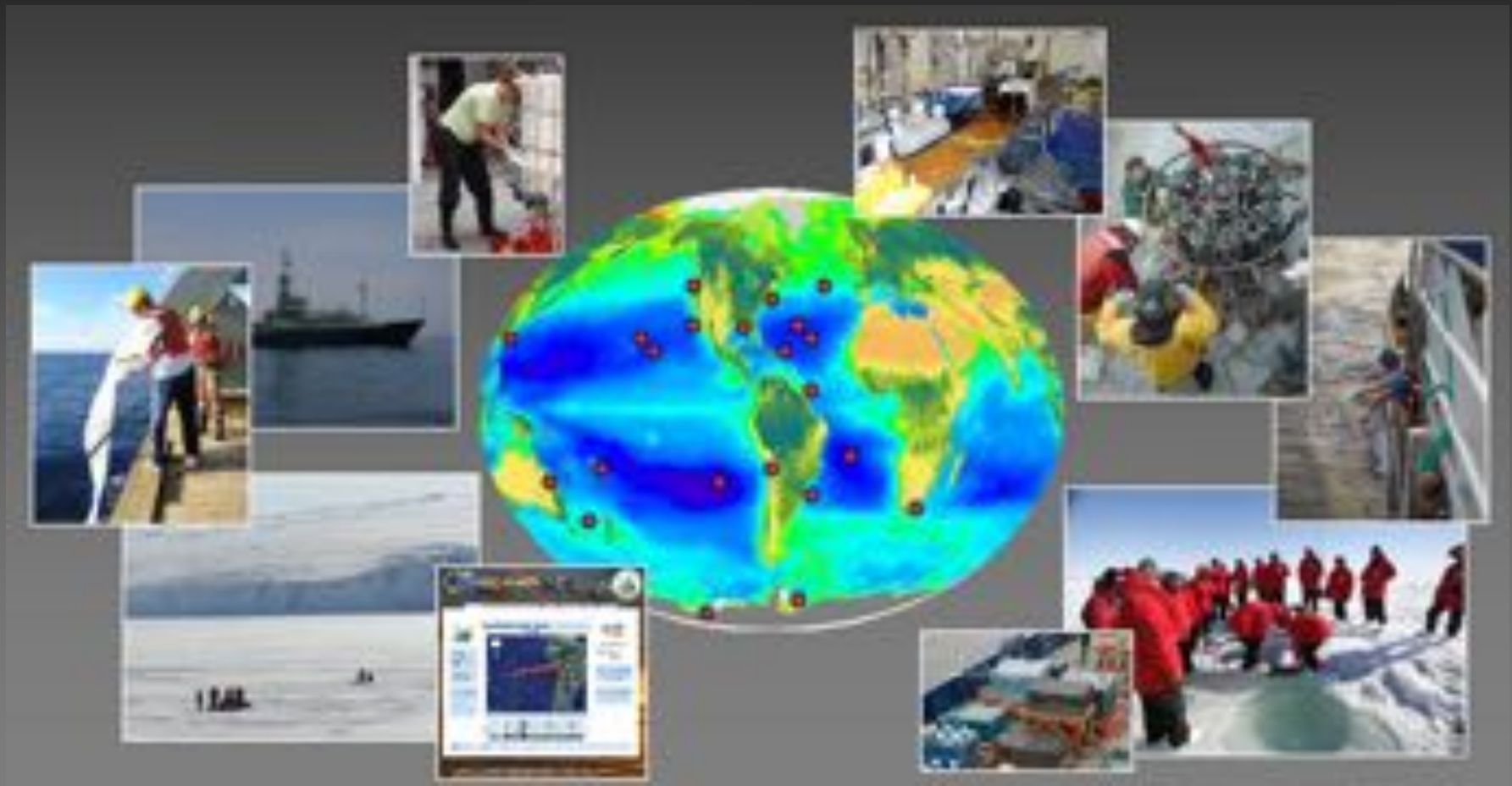
Half of global primary production



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



Sampling microbes across marine ecosystems



Tracking physiological ecology: from the flask to the field

Culture-based experiments

Species-specific responses to well-controlled environment

Limitations:

- Species must be in culture
- Time consuming
- Extrapolations to the field

Field-based studies

Assess whole community dynamics in a natural environment

Limitations:

- Not species-specific

Micro/Mesocosm

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

Challenges and opportunities in microbial oceanography

- Long standing challenges:
  - Populations are dilute
  - Few species-specific assays
  - Few genome or transcriptome sequences
- New opportunities
  - Novel concentration and detection strategies
  - Increases in whole genome sequences
  - Increases in transcriptomes for eukaryotic taxa

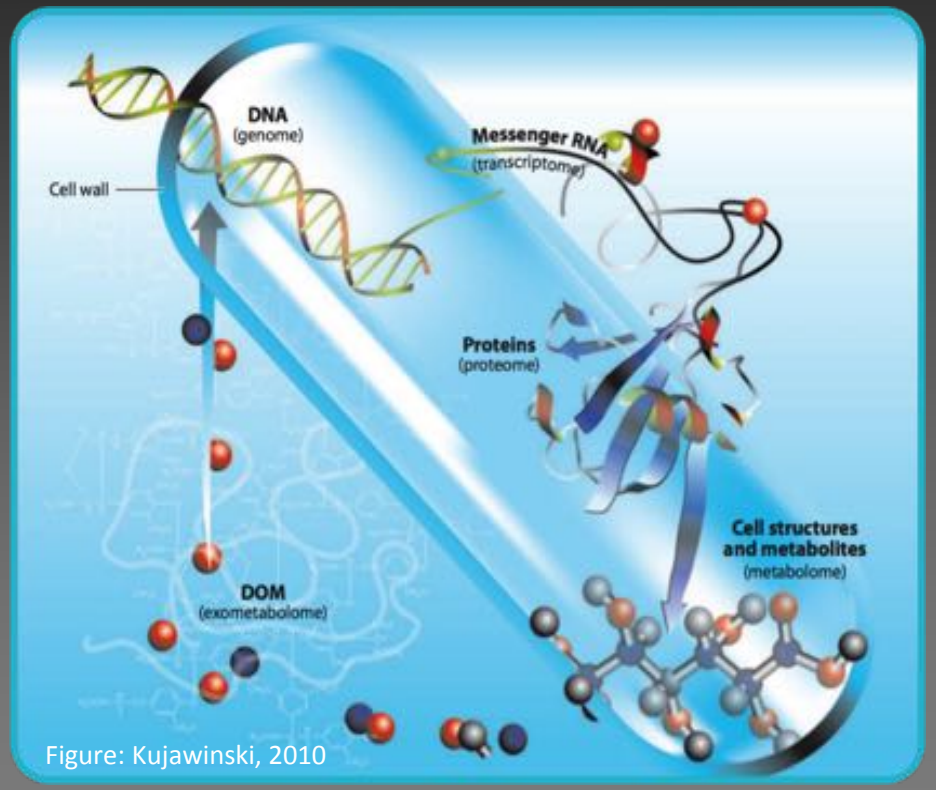


Figure: Kujawinski, 2010

Increasingly able to use 'omic and 'metaomic approaches!

Leveraging 'omic data to study marine microbes

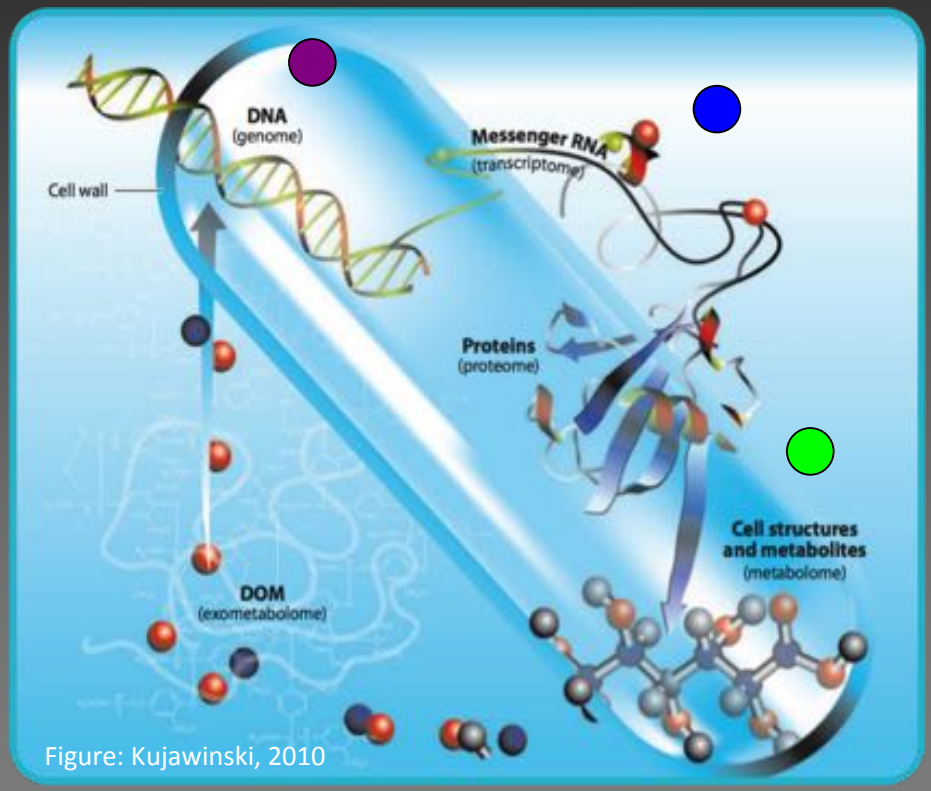


Figure: Kujawinski, 2010

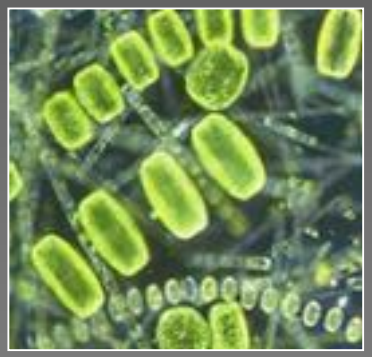
- **Taxonomic Diversity:** Who is there?
- **Metabolic capacity:** What are the molecular underpinnings of resource metabolism?
- **Metabolic plasticity:** How are those pathways regulated?
- **Functional diversity:** How are the pathways expressed *in situ*?
- **Niche space**: How are resources partitioned over time and between species?

Vignettes

- From genome to biome: Tracking the metabolism and microbiome of a keystone N_2 fixer

Genome - enabled

- Co-existing in a sea of competition: Leveraging transcriptome data to identify the physiological ecology of phytoplankton from key groups



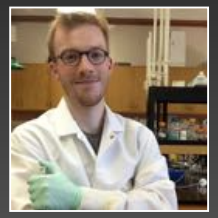
Transcriptome - enabled

Thank you


Liz Orchard


Mónica Rouco

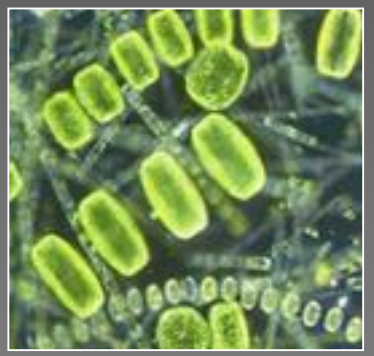

Sheean Haley


Kyle Frischkorn


Matt Harke


Sheean Haley


Harriet Alexander



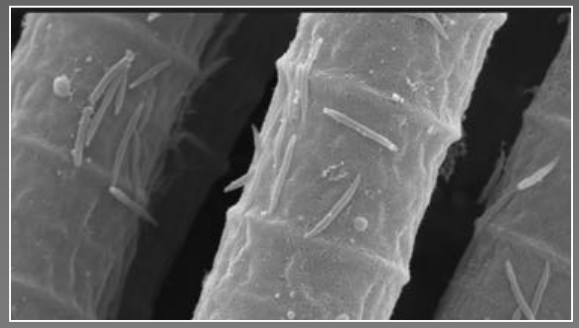
Two themes

Metabolic traits and trade-offs

- What phosphorus is bioavailable?
- What are the biogeochemical constraints on N_2 fixation?

Host microbiome interactions

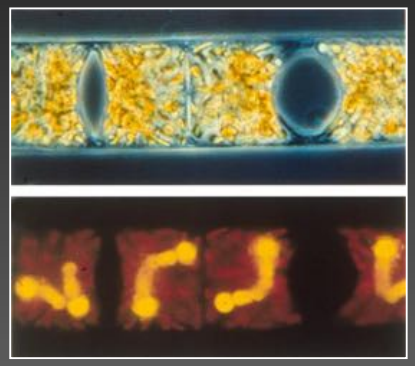
- Who is there? Microbiome diversity
- What are they doing? Microbiome functional diversity and interactions



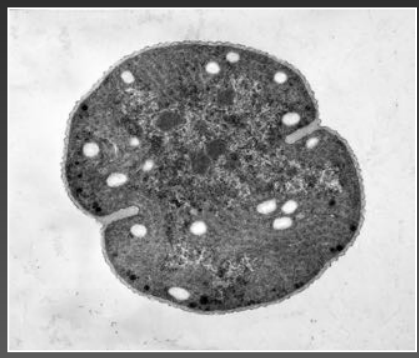
Nitrogen-fixing marine cyanobacteria

- Symbionts
  - UNCYN-A
  - *Richelia*
- Free-living
  - *Crococosphaera*
  - *Trichodesmium*

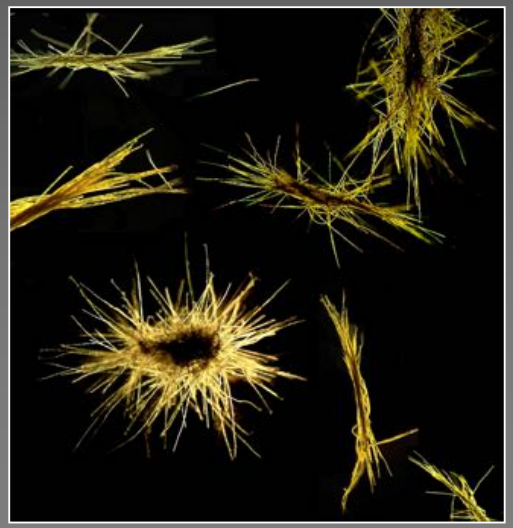
Richelia



Crocosphaera



Trichodesmium



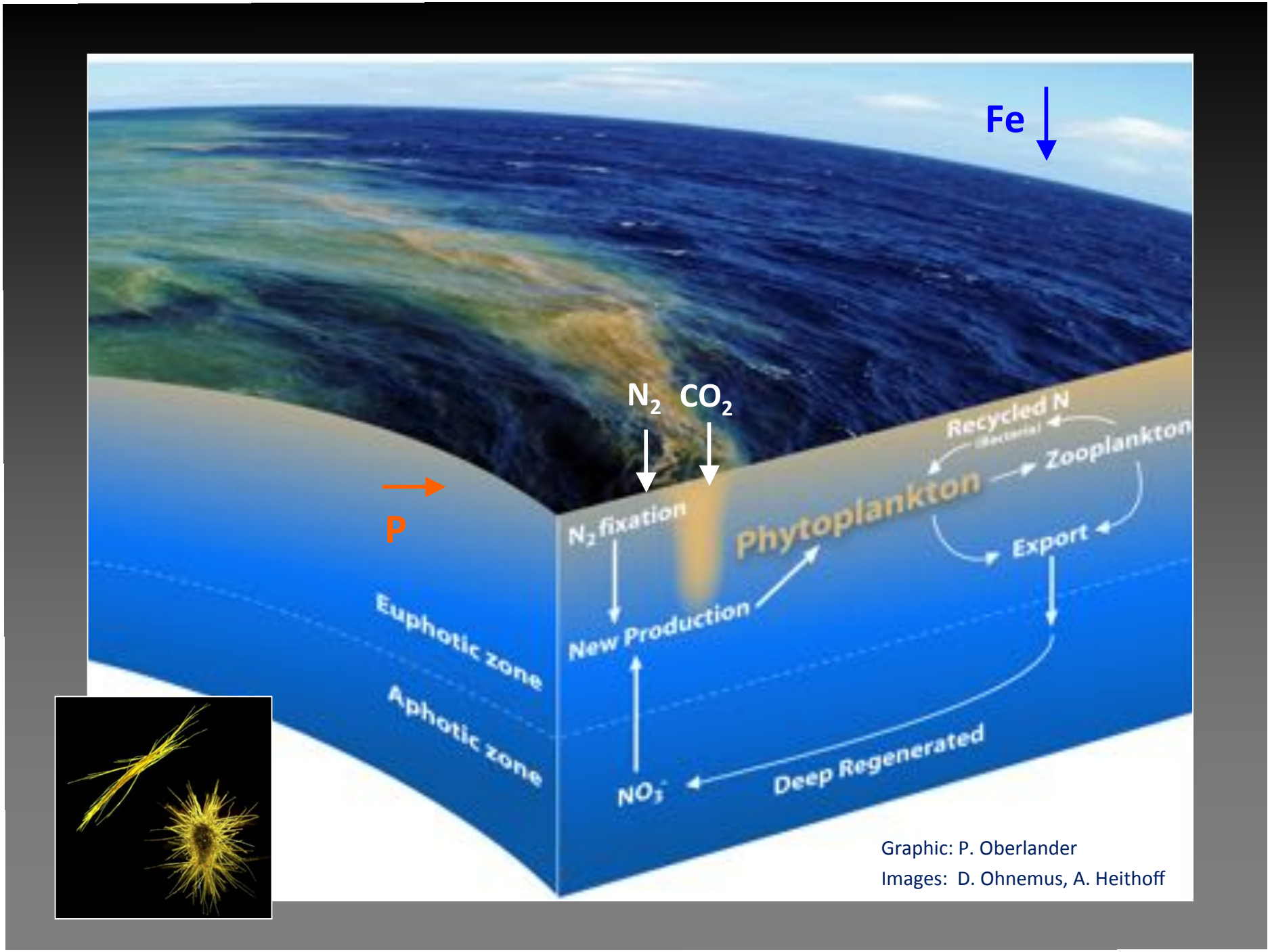
-Trichodesmium contortum
-Trichodesmium erythraeum

} *T. erythraeum* - like Clade

-Trichodesmium tenue
-Trichodesmium thiebautii

} *T. theibautii* - like Clade

-Trichodesmium spiralis
-Trichodesmium hildebrandtii

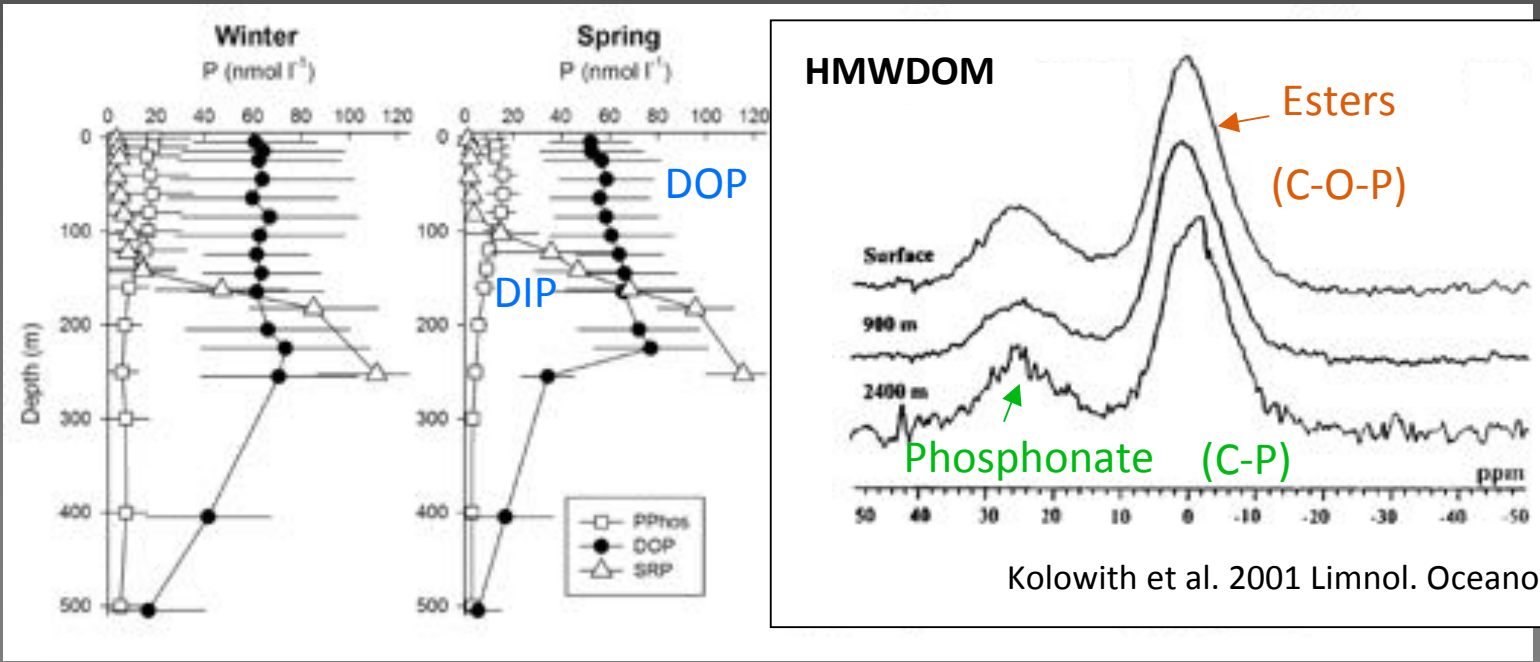

Fe
N_2 CO_2
P
N_2 fixation
Phytoplankton
Recycled N
Zooplankton
Export
New Production
Euphotic zone
Aphotic zone
NO_3^-
Deep Regenerated
Graphic: P. Oberlander
Images: D. Ohnemus, A. Heithoff

Phosphorus pools in the western North Atlantic

Particulate phosphorus

PPhos

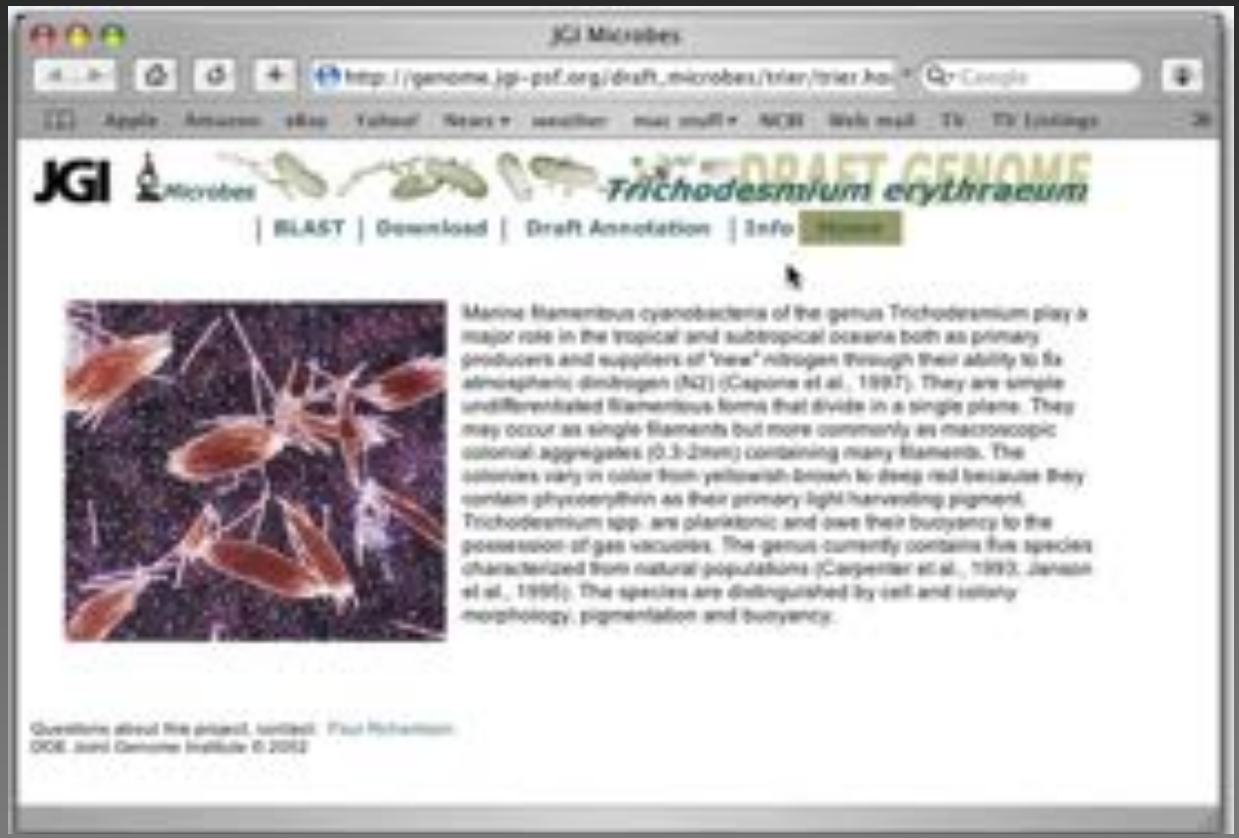
DIP Dissolved inorganic phosphorus ⟷ Dissolved organic phosphorus **DOP**



Kolowith et al. 2001 Limnol. Oceanogr.

Lomas et al. (2010) *Biogeosciences*

Trichodesmium erythraeum IMS101 genome page



www.jgi.doe.gov

Phosphorus metabolic traits and trade-offs

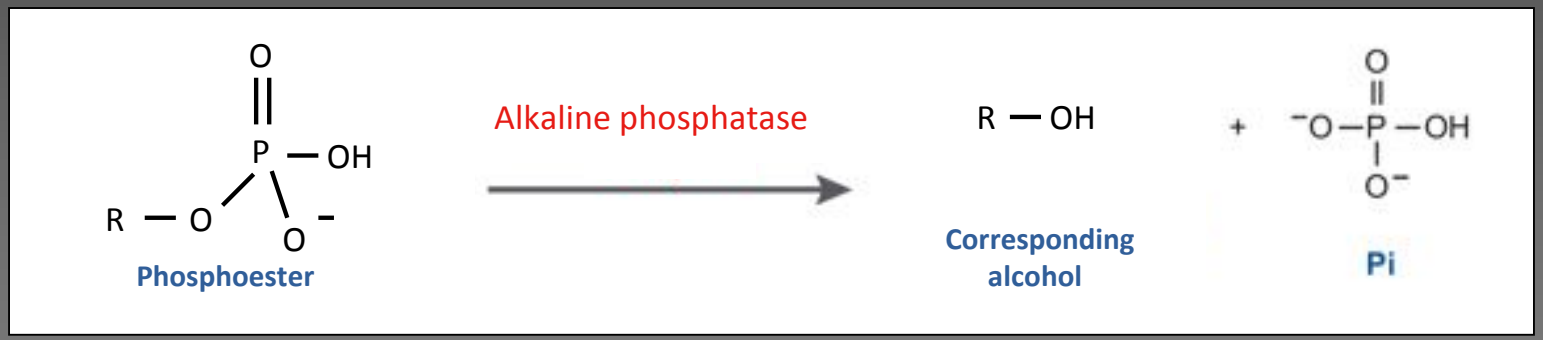
- Phosphonate
  - C-P Lyase (Fe co-factor)

C-P

- Ester
  - *phoX* type alkaline phosphatase (Ca Fe co-factor)
  - *phoA* type alkaline phosphatase (Zn co-factor)

COP

COP

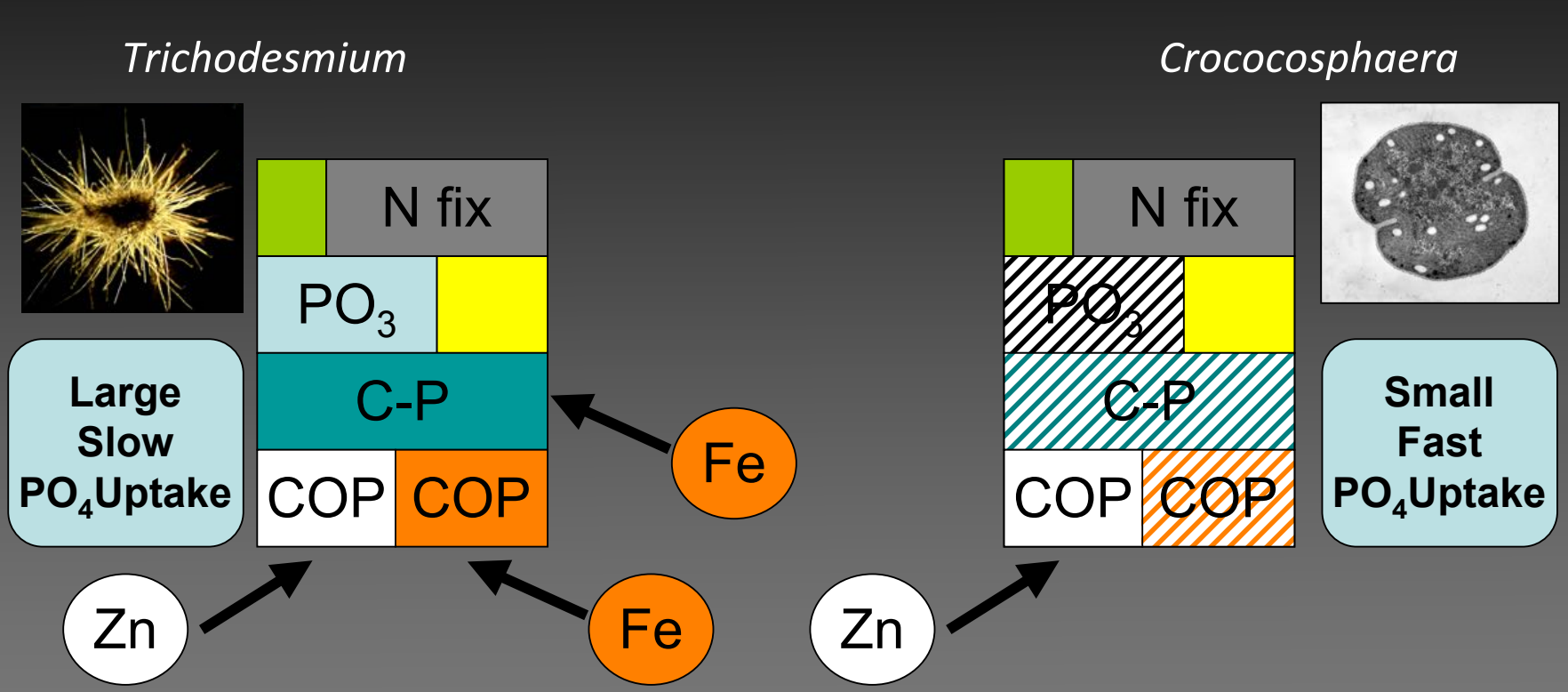


- Phosphite
  - *ptxD* gene cluster

PO_3

Dyhrman et al. 2006 *Nature*, Dyhrman and Haley 2010 *AEM*, Orchard et al. 2010 *L&O*, Van Mooy et al. 2015 *Science*

Comparative genomics: phosphorus traits and trade offs

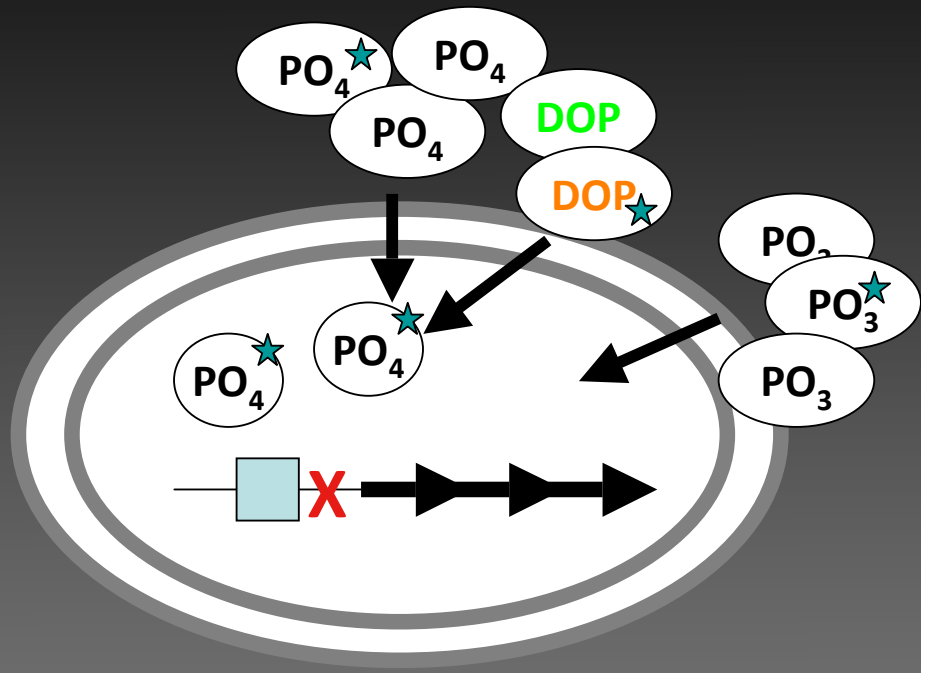


Other N_2 fixing cyanobacteria genomes do not encode the same pathways for phosphorus metabolism - less available substrates, but less metal requirement

Dyhrman et al. 2006 *Nature*, Dyhrman and Haley 2010 *AEM*, Orchard et al. 2010 *L&O*, Van Mooy et al. 2015 *Science*

Assaying P supply with isotope tracers

- Rates are difficult to constrain with tracer studies
  - No tracers for DOP
  - Dynamic P requirement



Trichodesmium:

~ 0.5 nmol $PO_4 h^{-1} ugChla^{-1}$

~ 29 - 80 % ester DOP

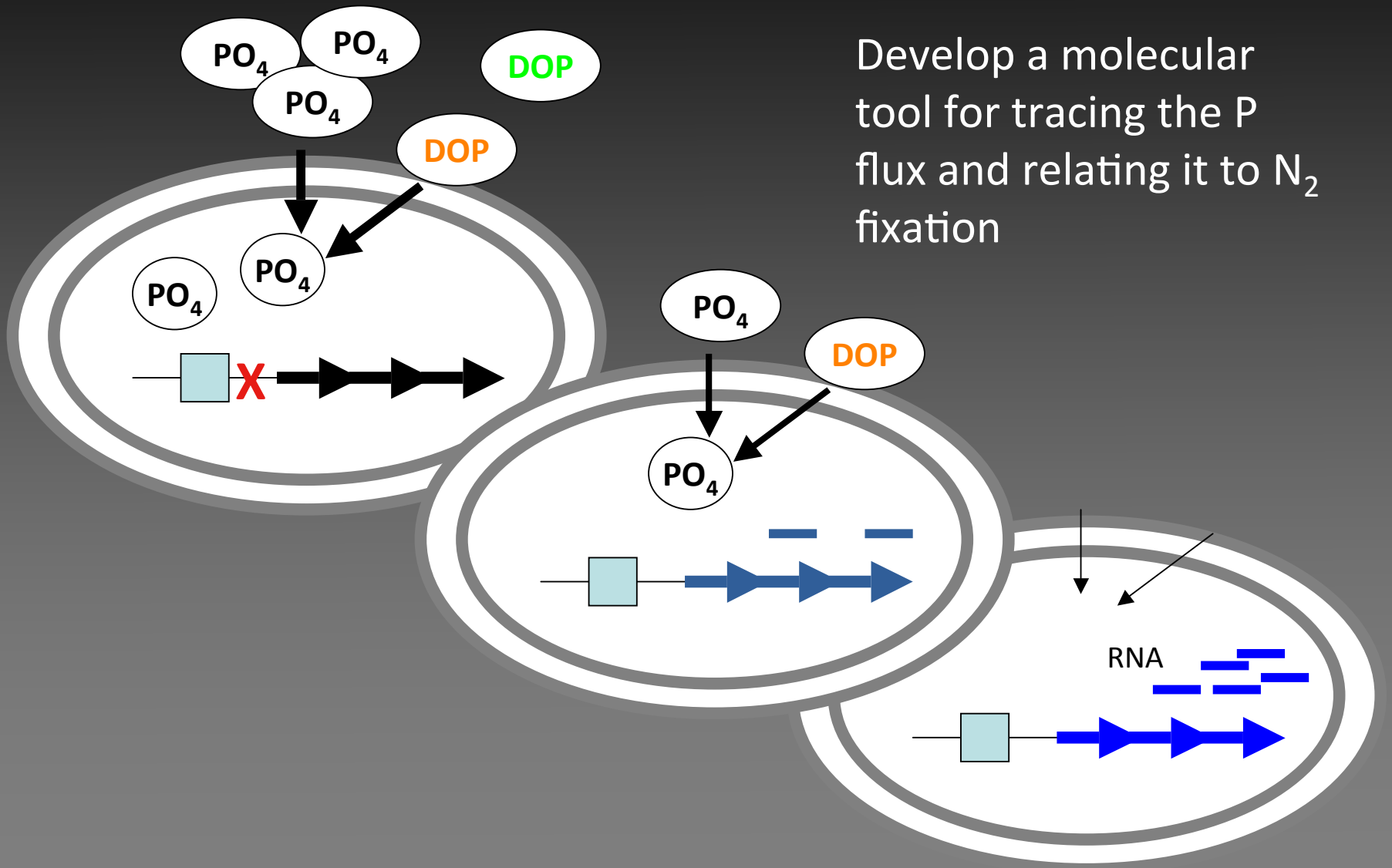
~ ?% phosphonate DOP

~ ?% phosphite (PO_3)

Orchard et al. 2010 *Limnol and Oceanogr*

Van Mooy et al. 2015 *Science*

Assaying P supply with a genome-enabled tool



Develop a molecular tool for tracing the P flux and relating it to N_2 fixation

Tracking genomic potential with expression studies

Culture cells

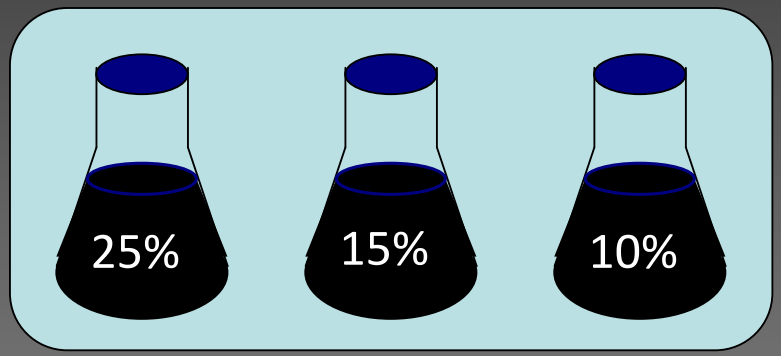
↓

Harvest and preserve samples

↓

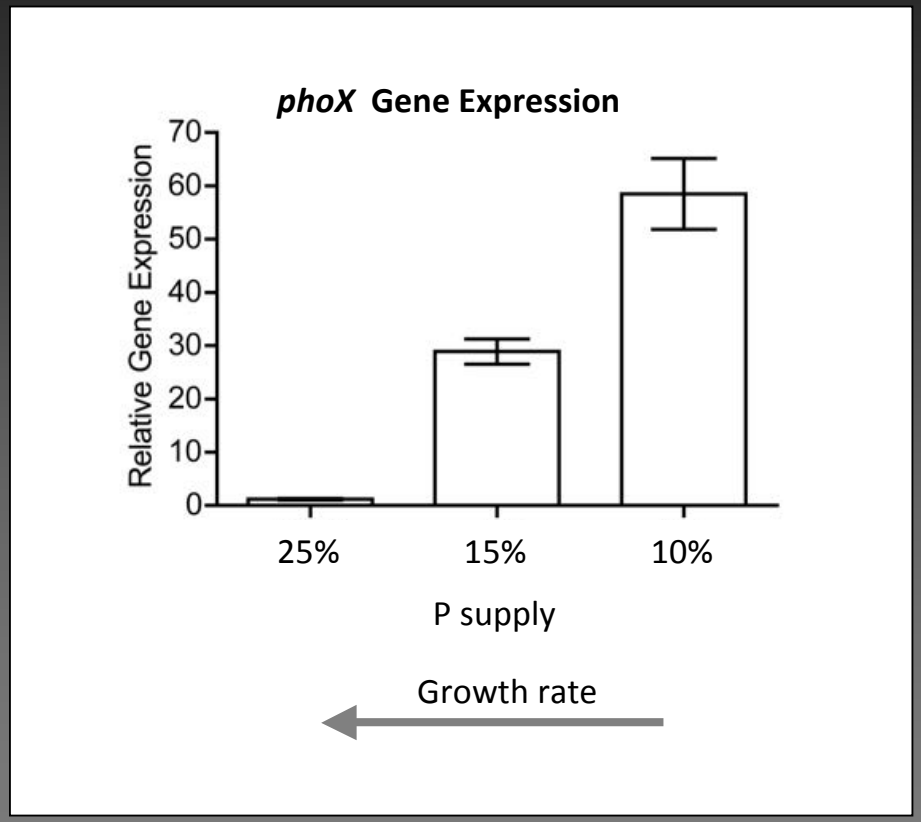
qRT-PCR of *phoX* Activity

P supply



Growth rate

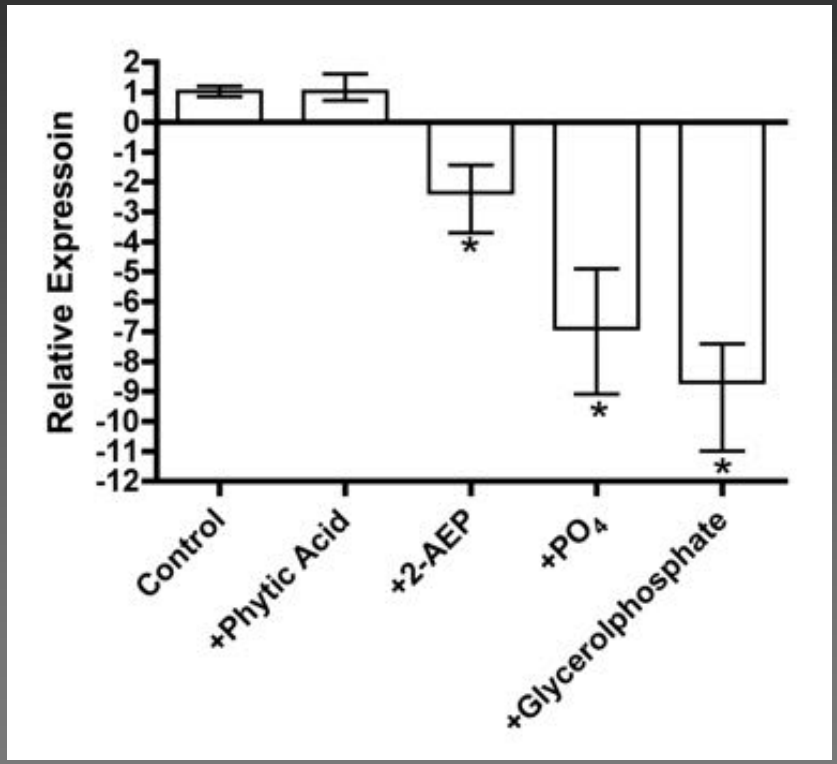
Calibrating gene expression to growth and N_2 fixation



Orchard and Dyhrman unpublished

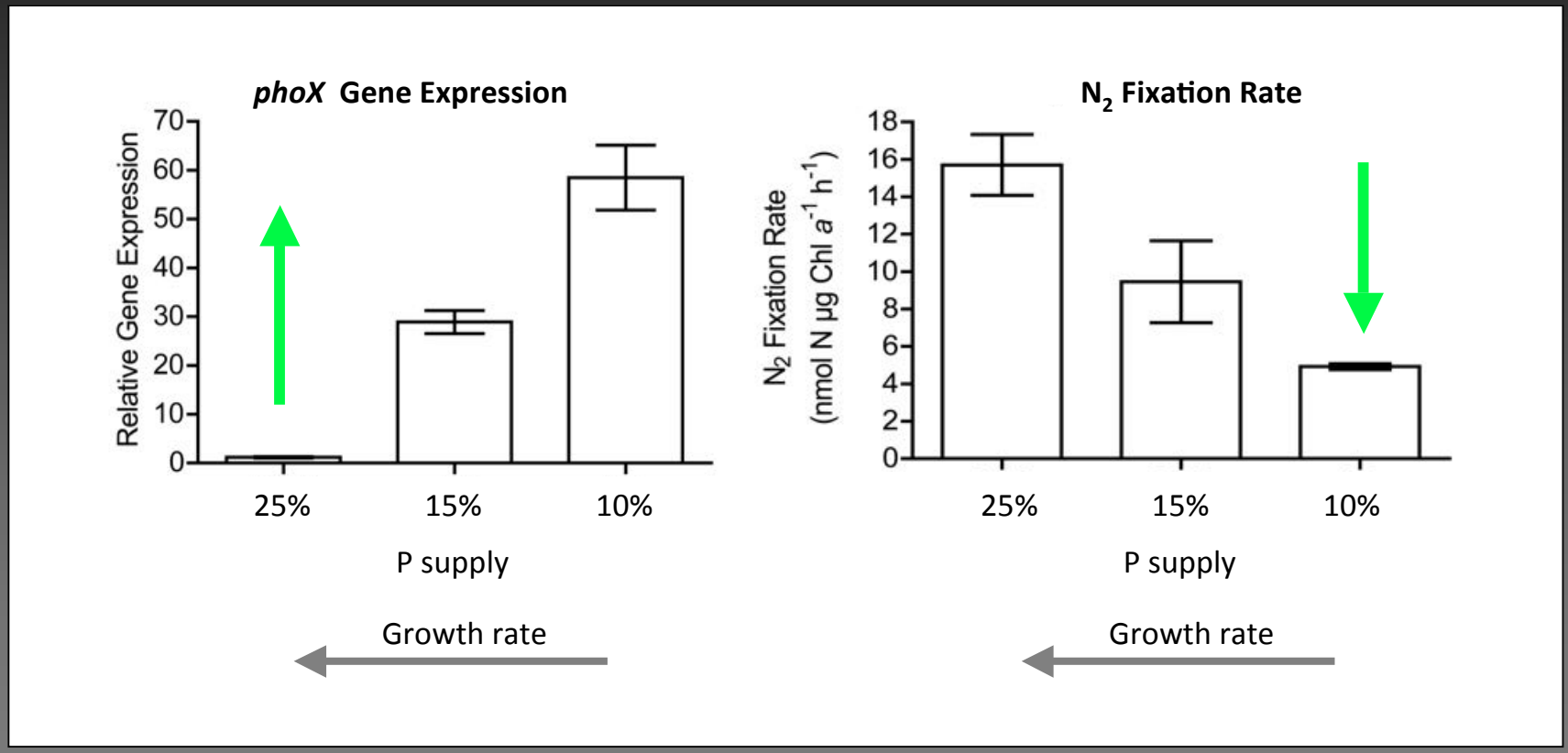
Calibrating gene expression to P supply

- *phoX* expression is responsive to cellular P regardless of exogenous source.
- Transcripts are rapidly turned over.
- Response is similar in culture experiments with both clades



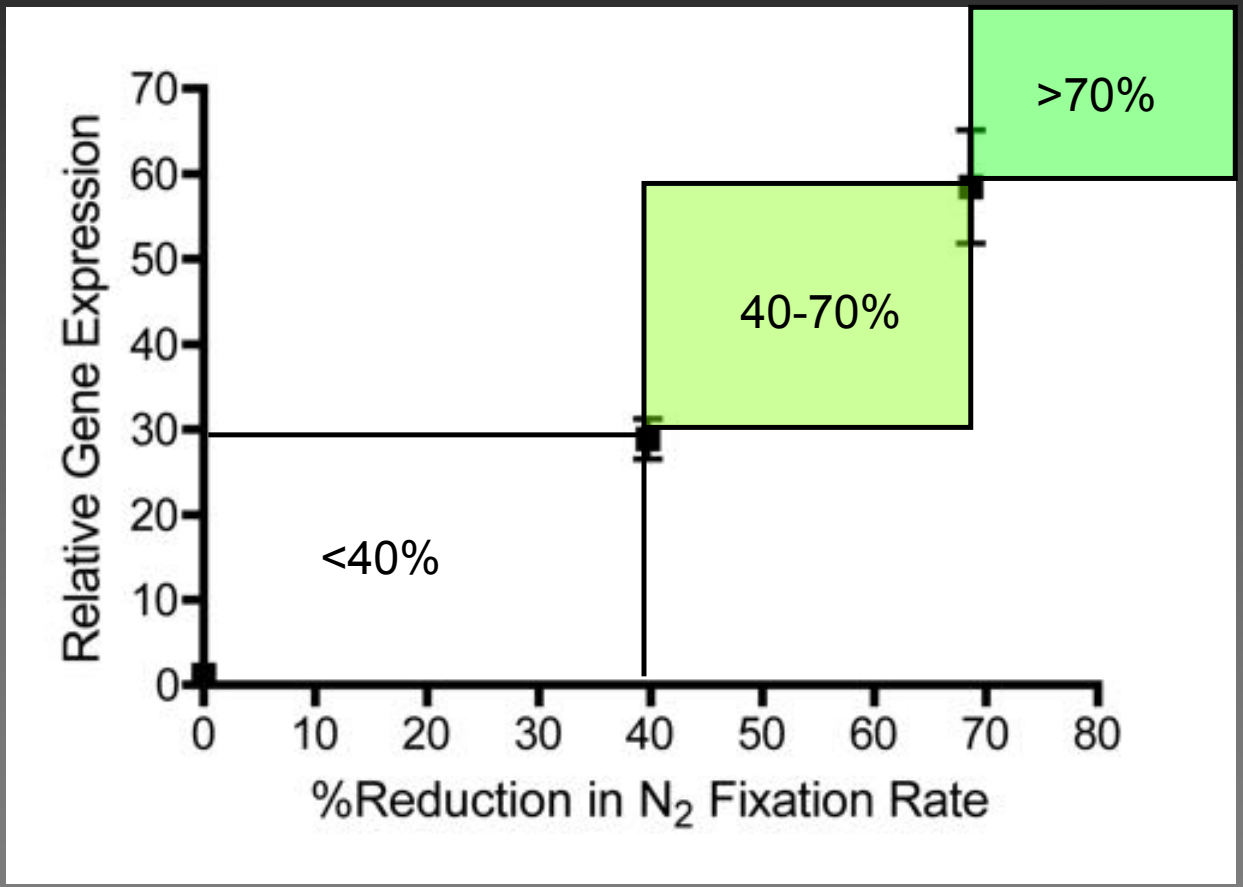
Orchard and Dyhrman unpublished

Calibrating gene expression to growth and N_2 fixation



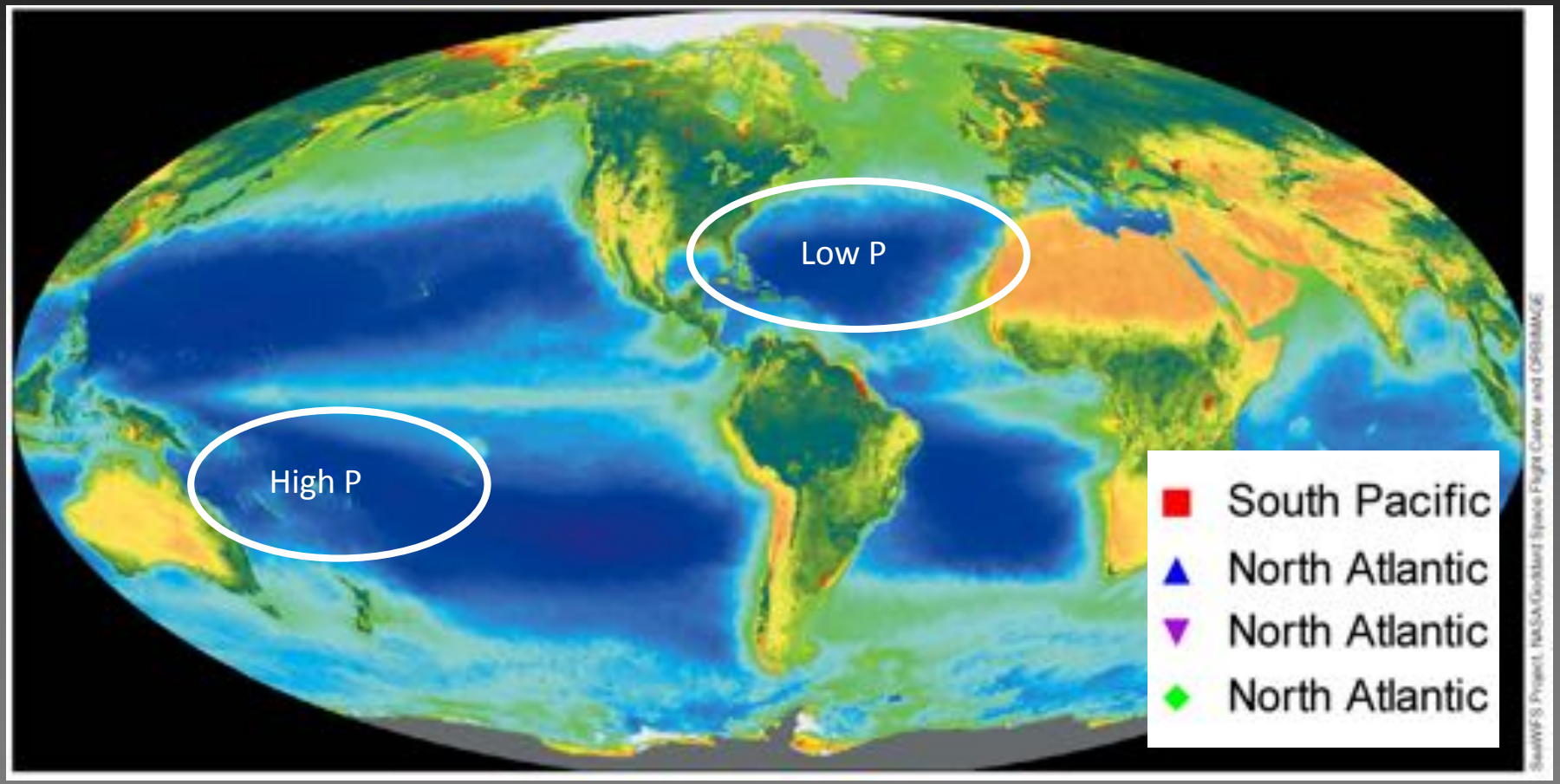
Orchard and Dyhrman unpublished

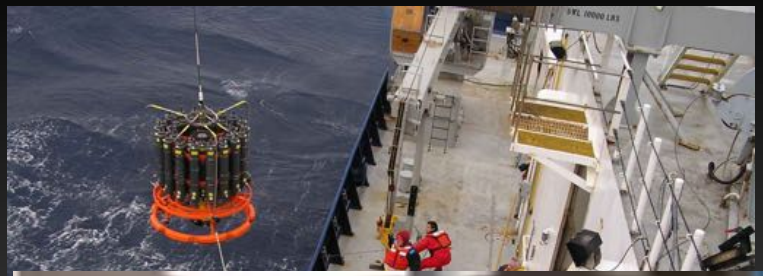
Calibrating gene expression to N_2 fixation

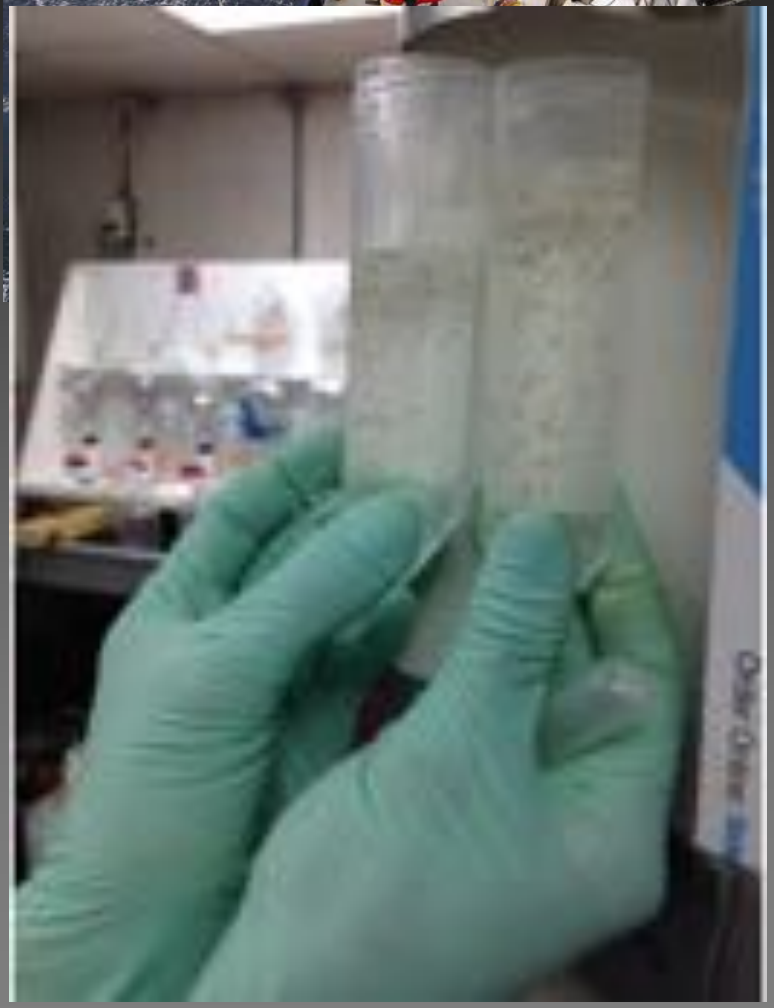


Orchard et al. in prep

Sampling different P regimes



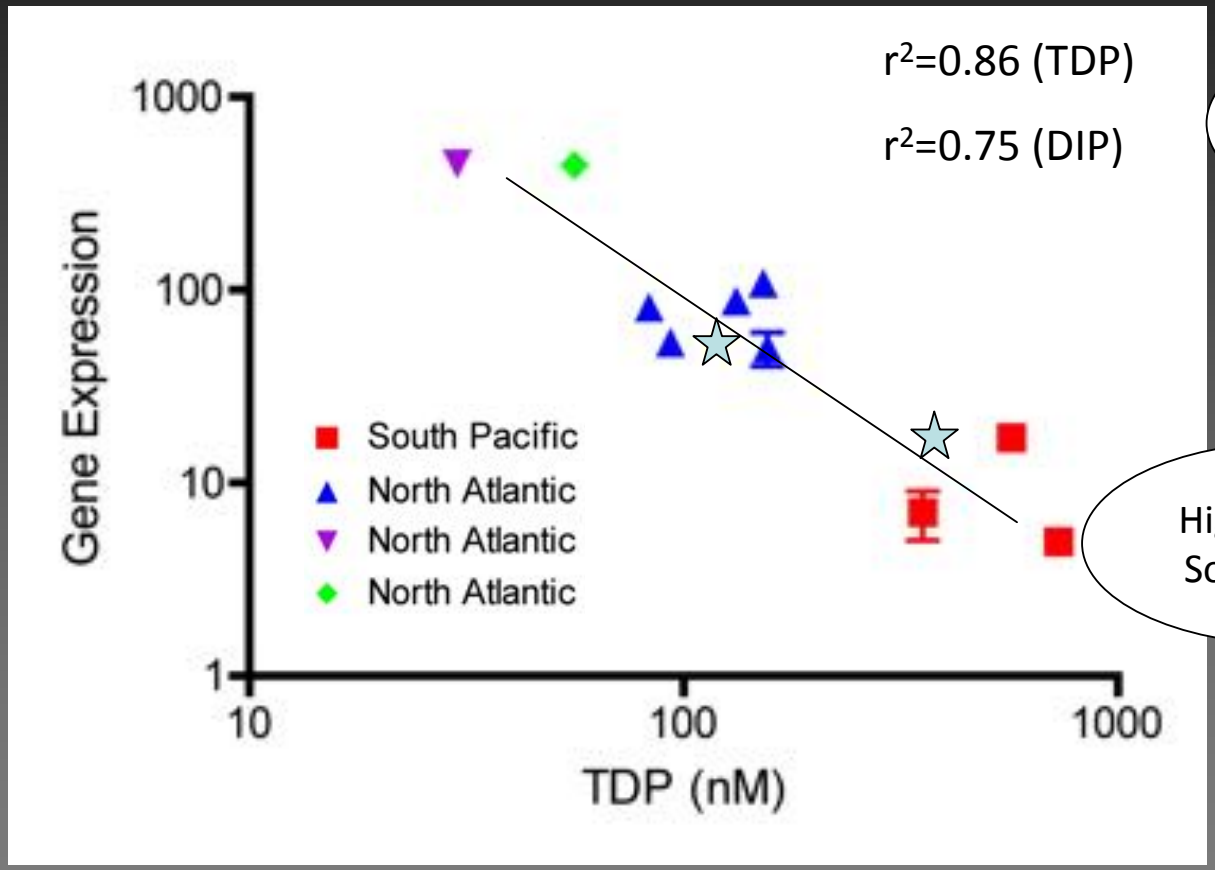




DIP, TDP Measurements

Measurements of quantitative gene expression for *Trichodesmium sp.*

Gene expression increases at low phosphorus

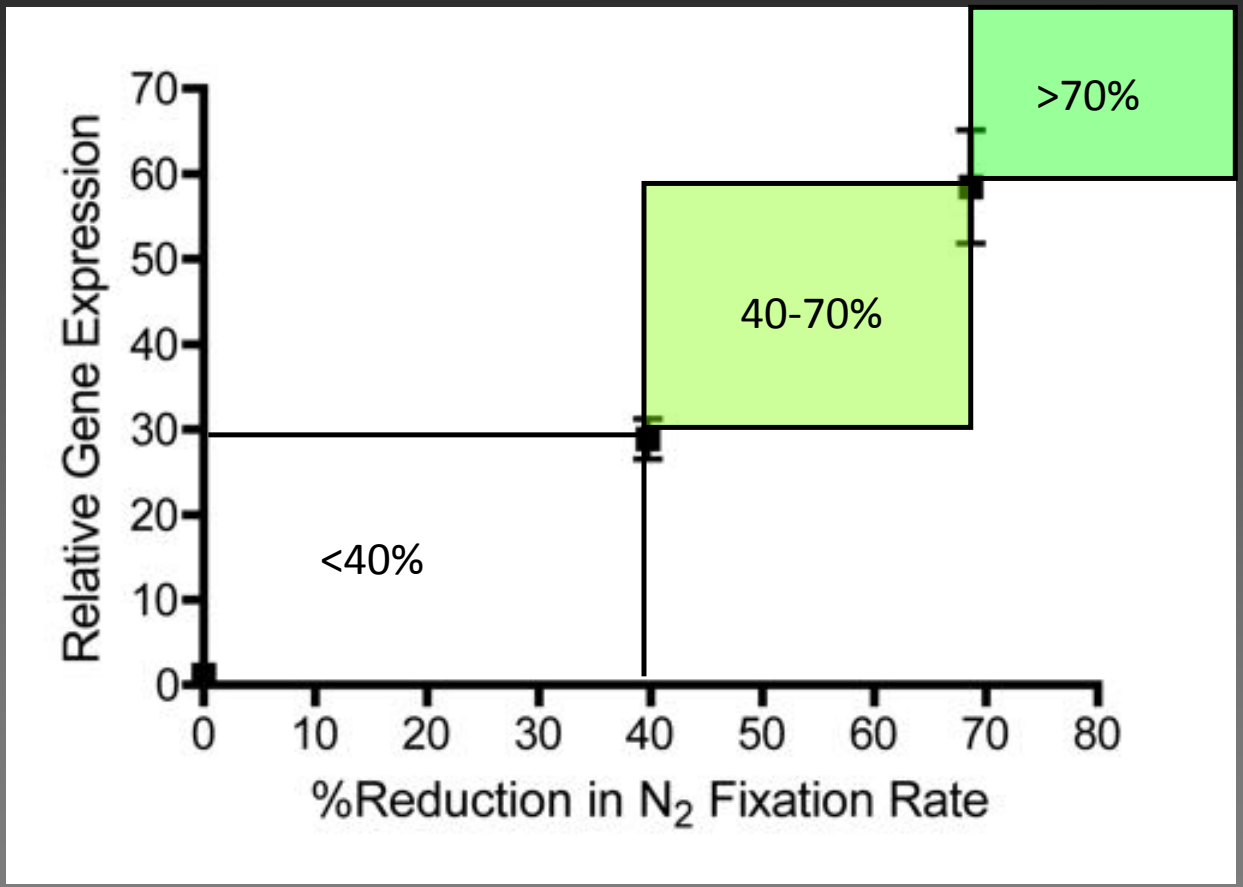


Low P supply
North Atlantic

High P supply
South Pacific

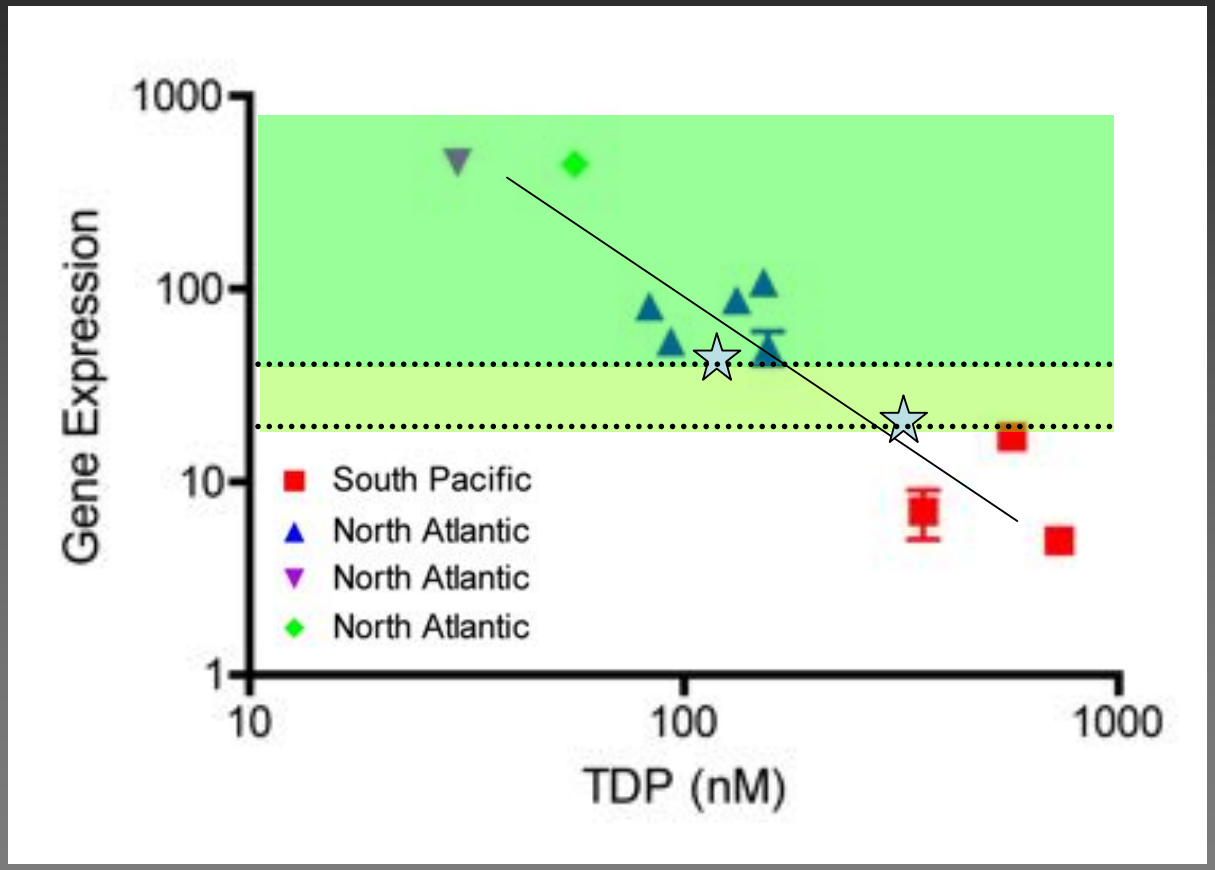
Orchard and Dyhrman unpublished

Calibrating gene expression to N_2 fixation



Orchard and Dyhrman unpublished

Data predicts that P supply limits N_2 fixation in the western N. Atlantic



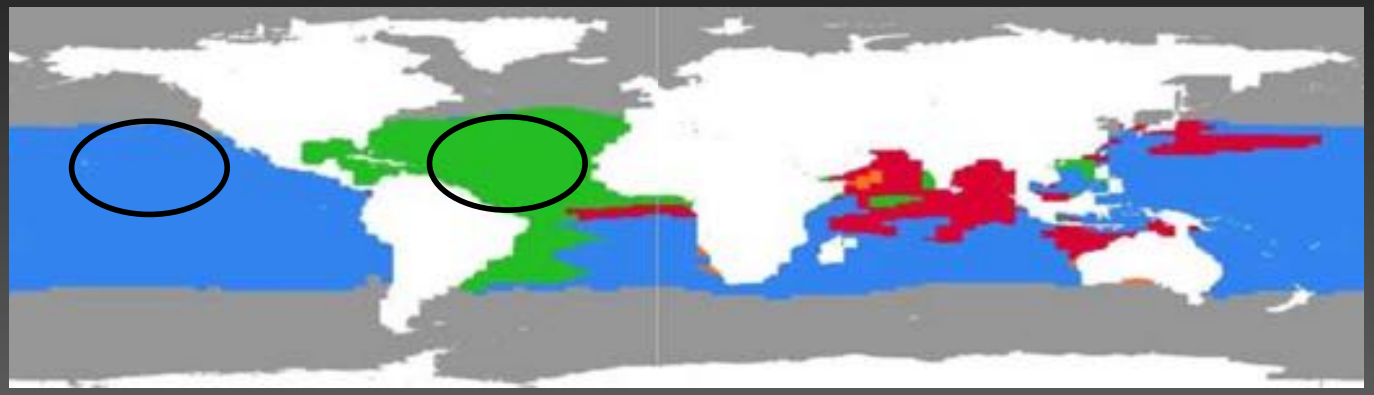
>70%

40-70%

<40%

Orchard and Dyhrman unpublished

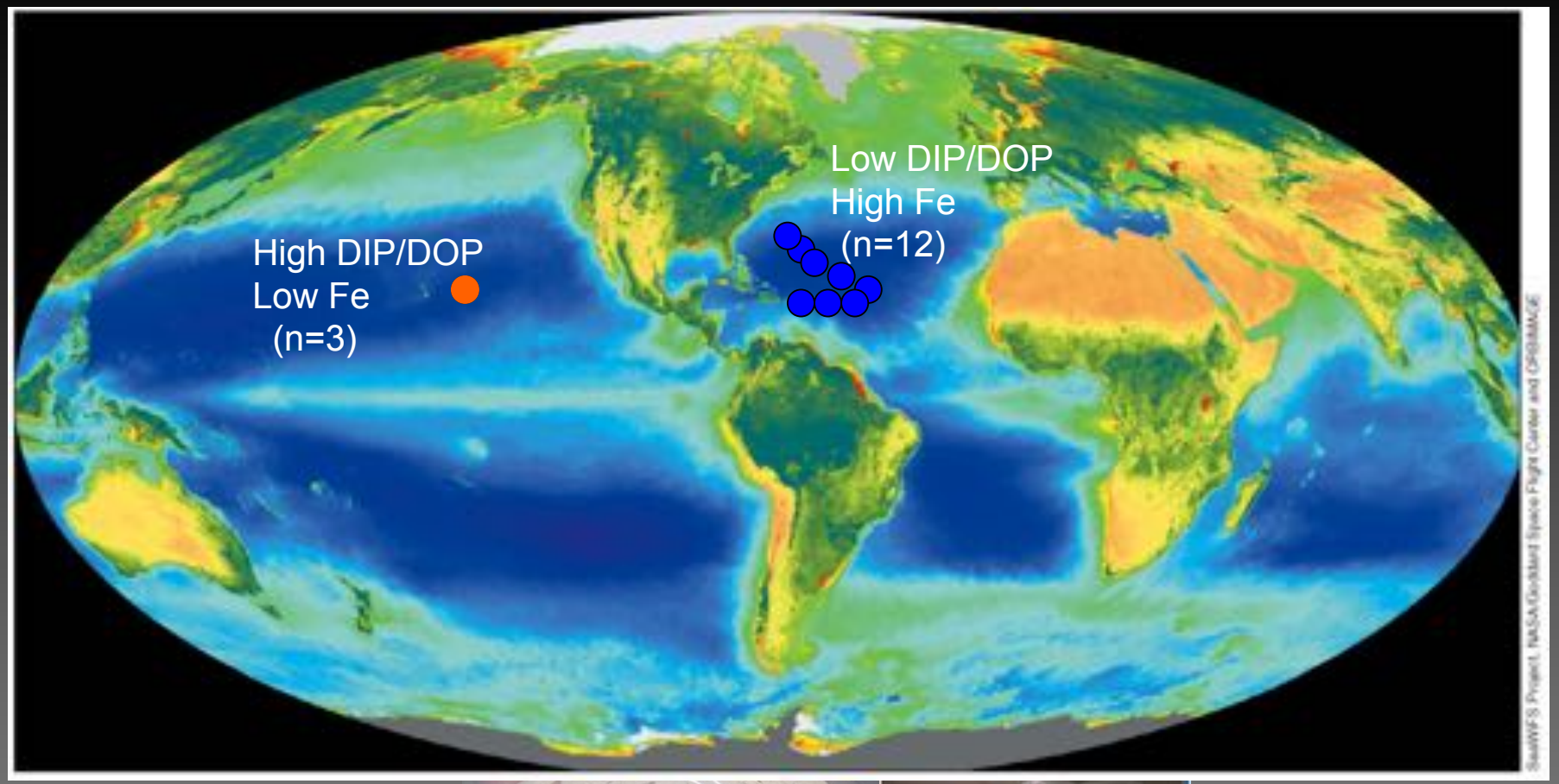
Constraints on *Trichodesmium* N_2 fixation



(Moore et al. 2004)

Molecular patterns corroborate predictive models in the western north Atlantic

phoX - P regulated ester metabolism (Orchard et al. 2009 *Environ. Micro.*)
idiA - Fe regulated iron metabolism (Chappell et al. 2013 *ISME J.*)
rnpB - reference gene
nifH - N_2 - fixation


High DIP/DOP
Low Fe
(n=3)
Low DIP/DOP
High Fe
(n=12)

Analysis pipeline derived from metagenome analyses

Collect samples

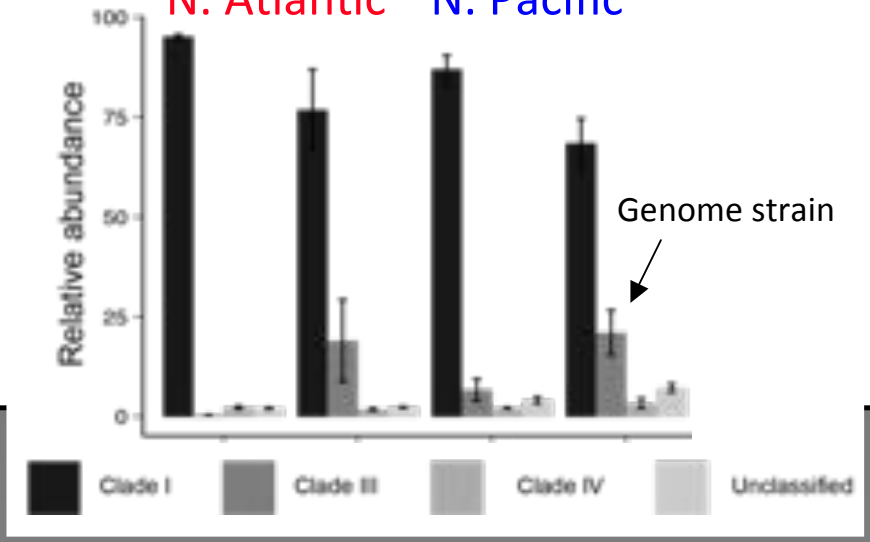
Remove rRNA

Submit samples to sequencing center

Sequence using RNAseq and map reads to genomes

Target 30 - 60 million 100 bp paired-end reads

Frischkorn et al. (in review)

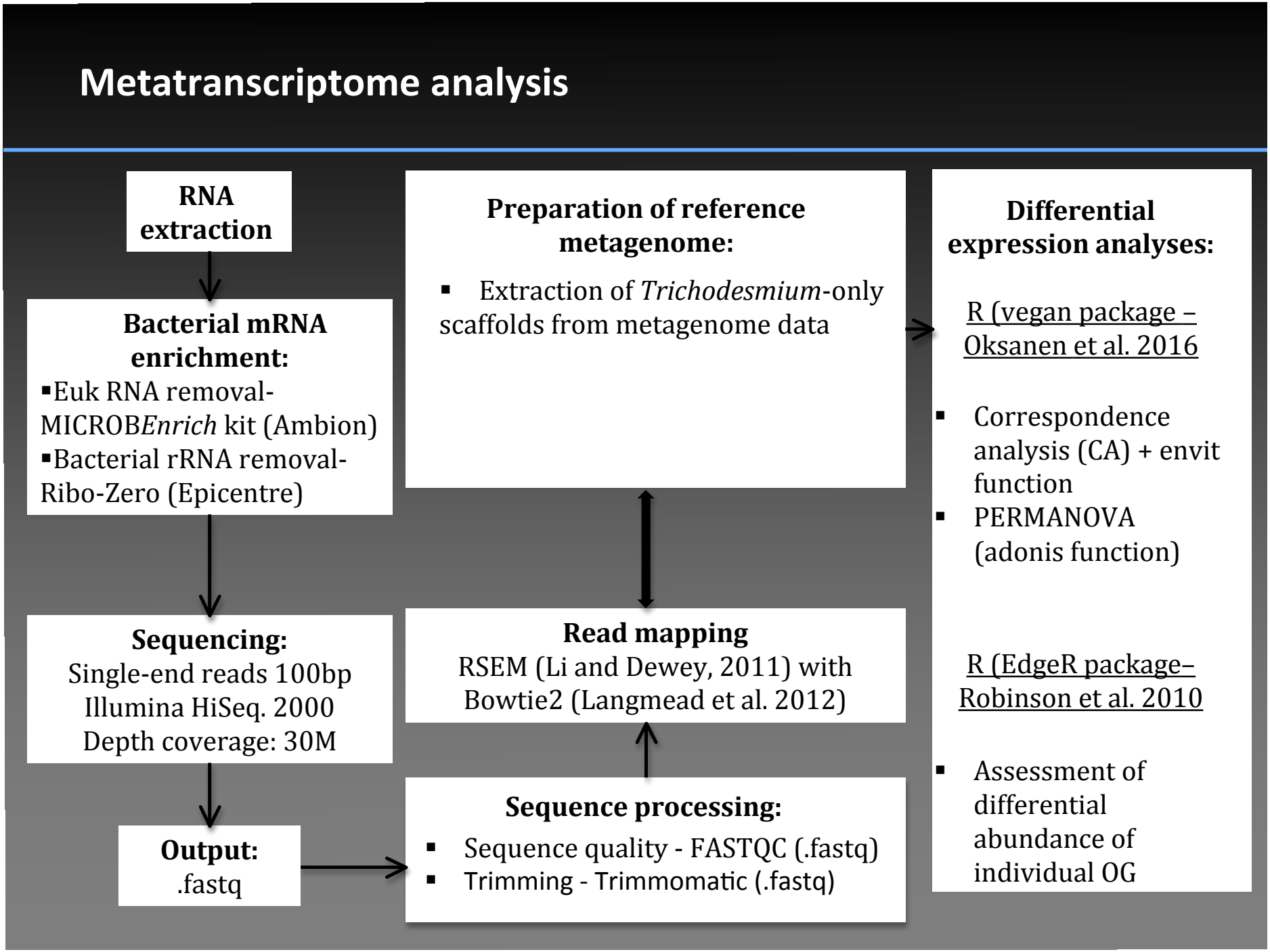


Rouco et al. (2016) *Environ. Micro.*

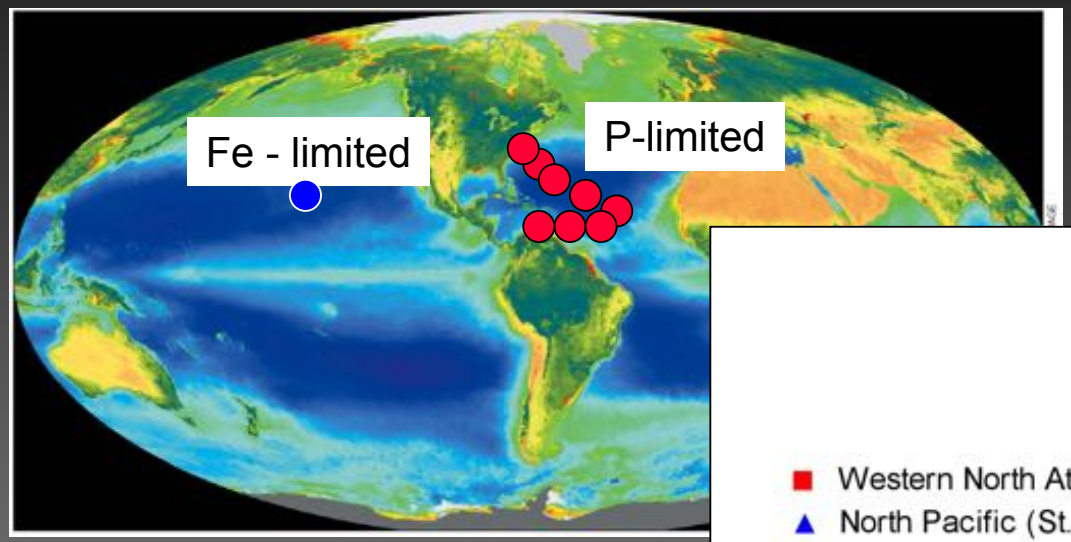
The reality…..


CAACAACATCATACCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
+DHT4KXP1:1:1101:5930:2353#0/1
@DHT4KXP1:1:1101:5904:2385#0/1
CTACCATTTTCATTTTCCATGTCTCTCCCCATCTTGACACATTATTTTTTCT
ACCTCCATCACATCATGATCACGTTATACGATCTCTACAGTAGCCCCCA
+DHT4KXP1:1:1101:5904:2385#0/1
@DHT4KXP1:1:1101:5781:2386#0/1
CTTTCAGATACAGTAGGATTTATTCAGGATCTTCCGACGACATTGATTGCTG

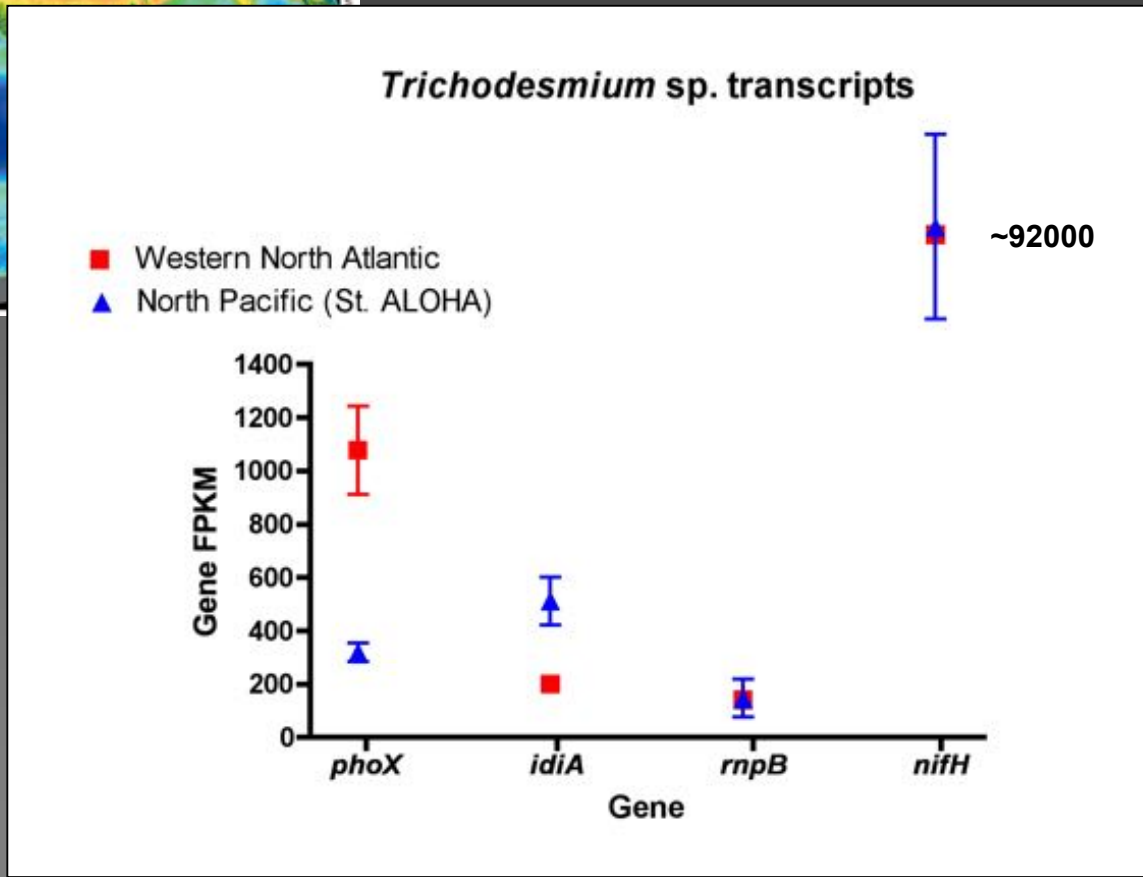
Metatranscriptome analysis



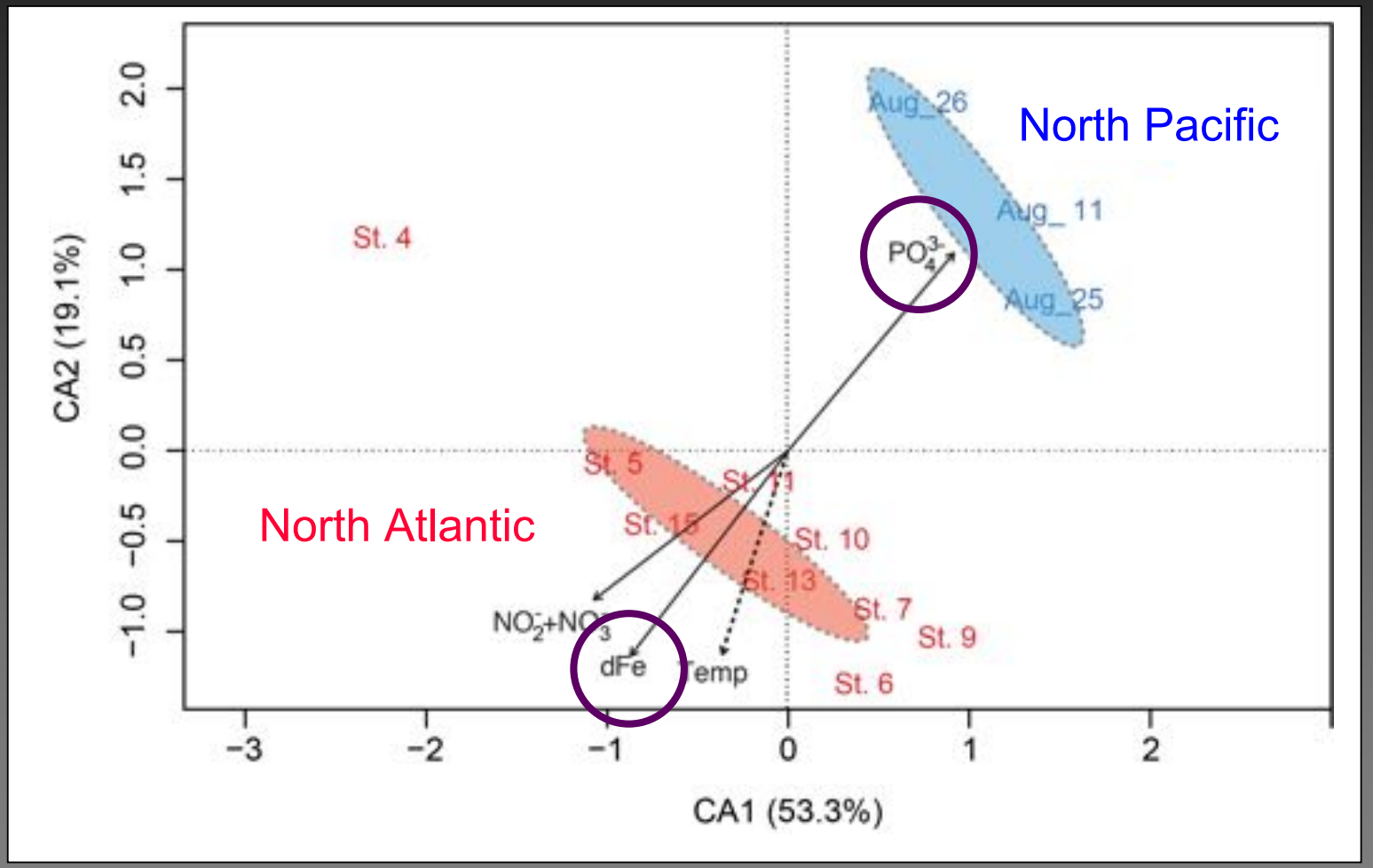
Biomarkers consistent with model prediction



Biomarkers confirm model predictions.

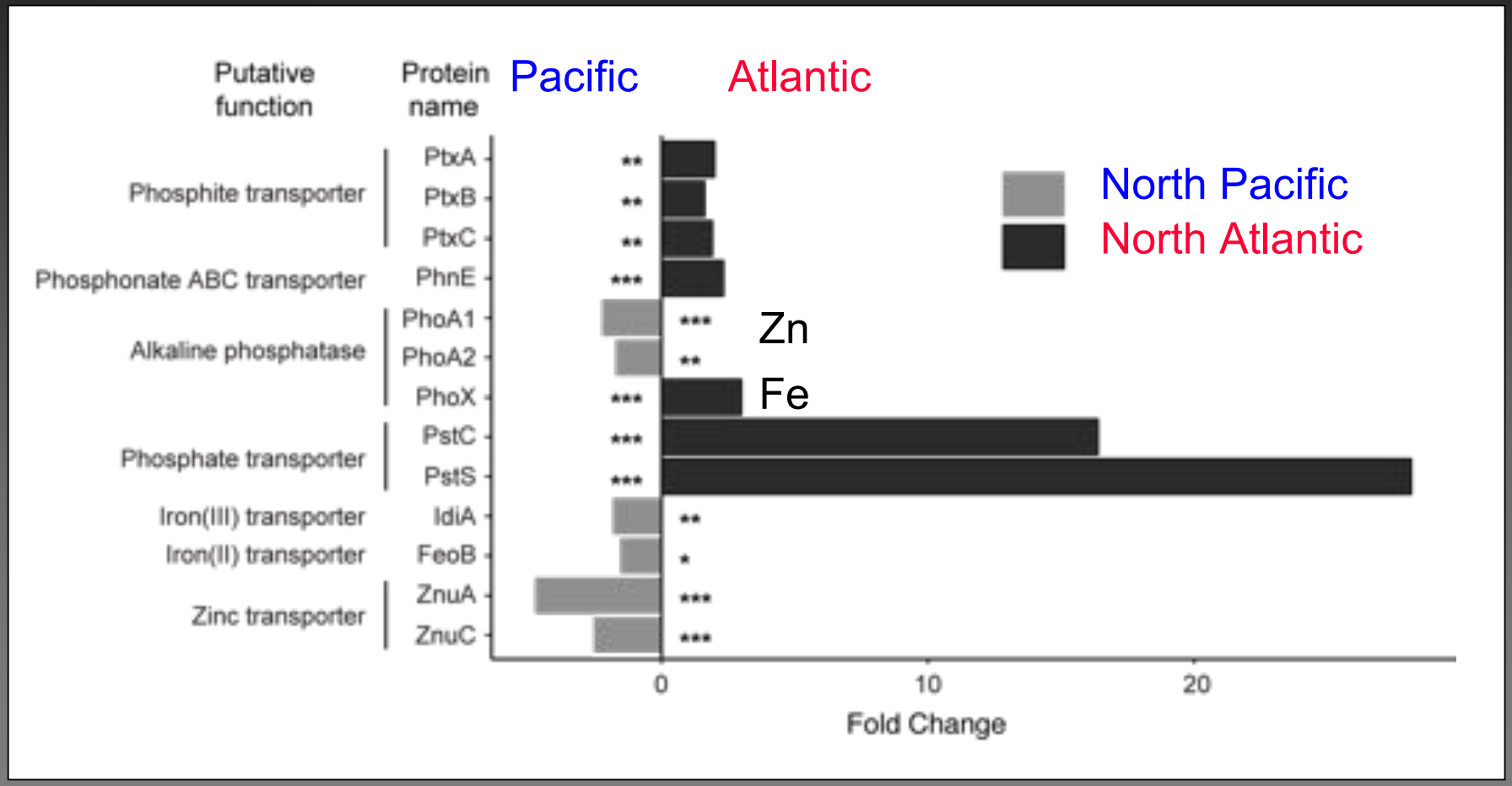


Significant differences in global transcription between the North Pacific and the North Atlantic



Ruoco et al. (in prep)

Transcription patterns indicate metalloenzyme trade-offs and geochemical controls



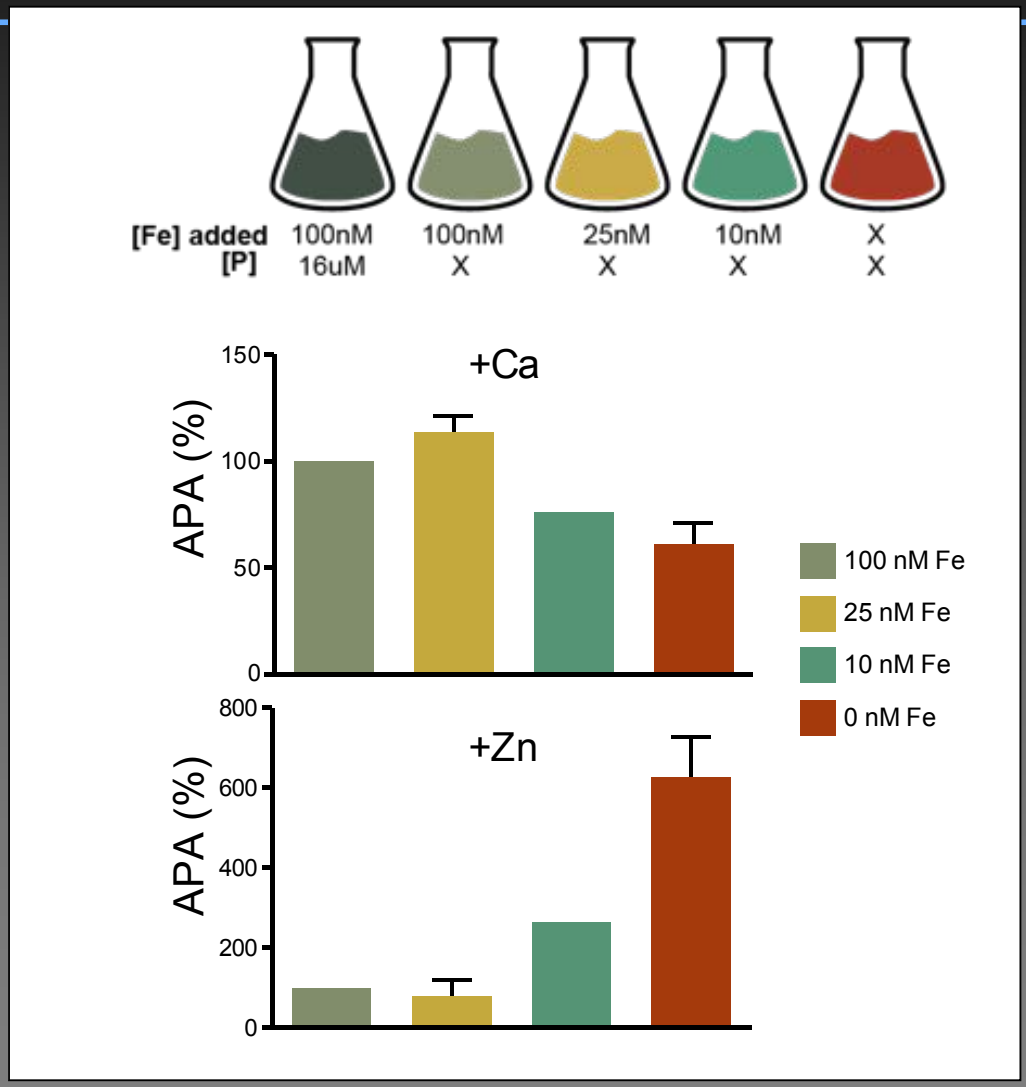
Rouco et al. (in prep)

Contextualizing metatranscriptome data with culture experiments

Enzyme Type	Metal Cofactor
PhoA	Zn, Mg
PhoX	Fe, Ca

Proportion of Ca dependent activity (PhoX) decreases with increasing Fe-stress

Proportion of Zn dependent activity (PhoA) increases with increasing Fe-stress



Frischkorn et al. (in prep)

Summary - Metabolic traits and trade-offs

What phosphorus forms are bioavailable?
What are the biogeochemical constraints on N_2 fixation?

- *Trichodesmium* genome suggests bioavailabiliy of phosphonate, ester, and phosphite
- *Trichodesmium phoX* expression levels suggests that supply of bioavailable P is low in the western N. Atlantic, which could constraint N_2 fixation
- Predicted biogeochemical drivers of N_2 fixation are reflected in *Trichodesmium* transcriptional signals including likely metalloenzyme switching

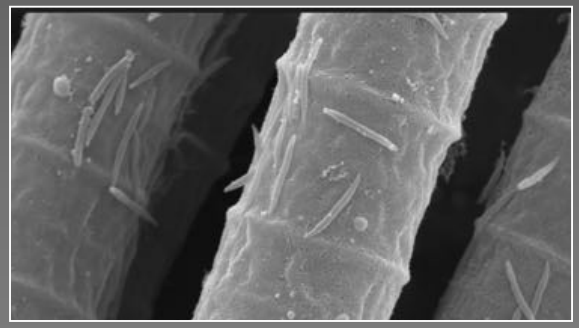
Two themes

Metabolic traits and trade-offs

- What phosphorus is bioavailable?
- What are the biogeochemical constraints on N_2 fixation?

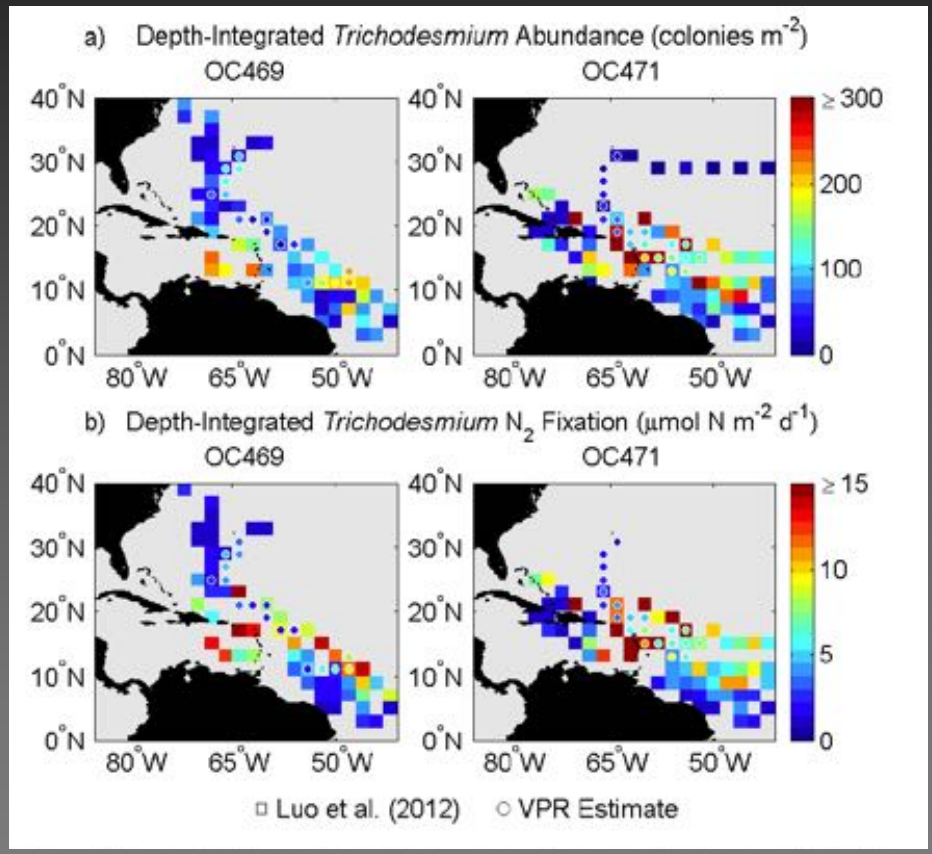
Host microbiome interactions

- Who is there? Microbiome diversity
- What are they doing? Microbiome functional diversity and interactions



Modeling N_2 fixation is still a challenge

- Models do not balance the N cycle in the ocean or recapitulate patterns well
- Assays of nitrogen fixation are technically difficult = variability
- Information on distribution over time and with depth is still patchy
- Geochemistry is not necessarily a good predictor of distribution or N_2 fixation



Olson et al. 2015 *DSR* II

Host microbiome interactions

- *Trichodesmium* colonies harbor epibionts in cultures and field populations (Ruoco et al. 2016 *EM*)
- Quorum sensing communication molecules (acylated homoserine lactones - AHL) detected in colonies (Van Mooy et al. 2012 *ISME J*)
- Addition of AHLs to field colonies changes activity independent of geochemistry (Van Mooy et al. 2012 *ISME J*)

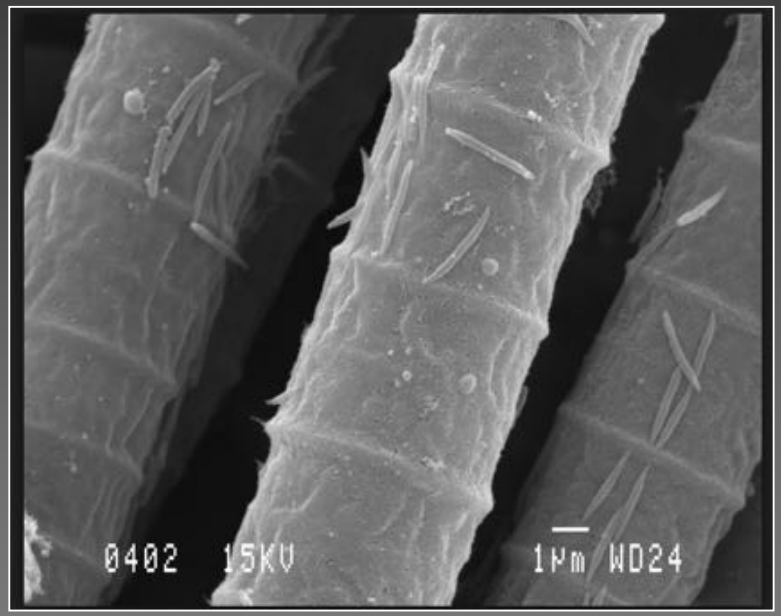
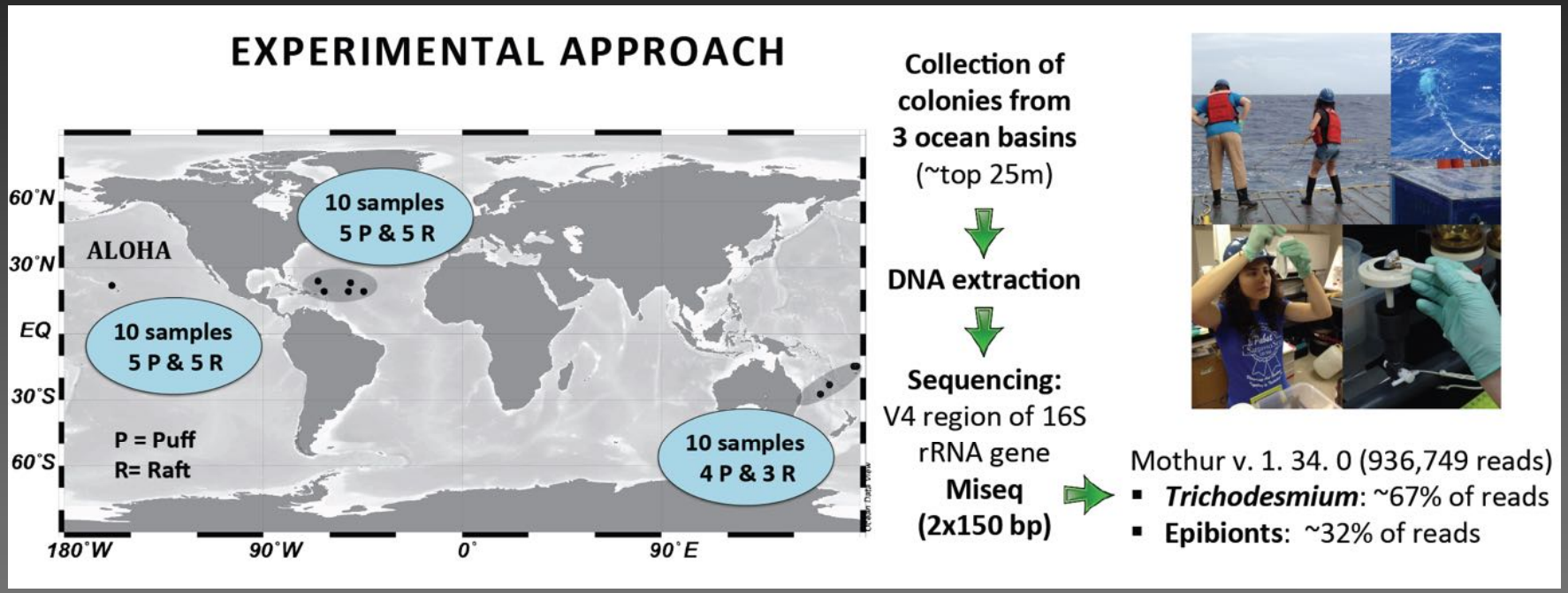


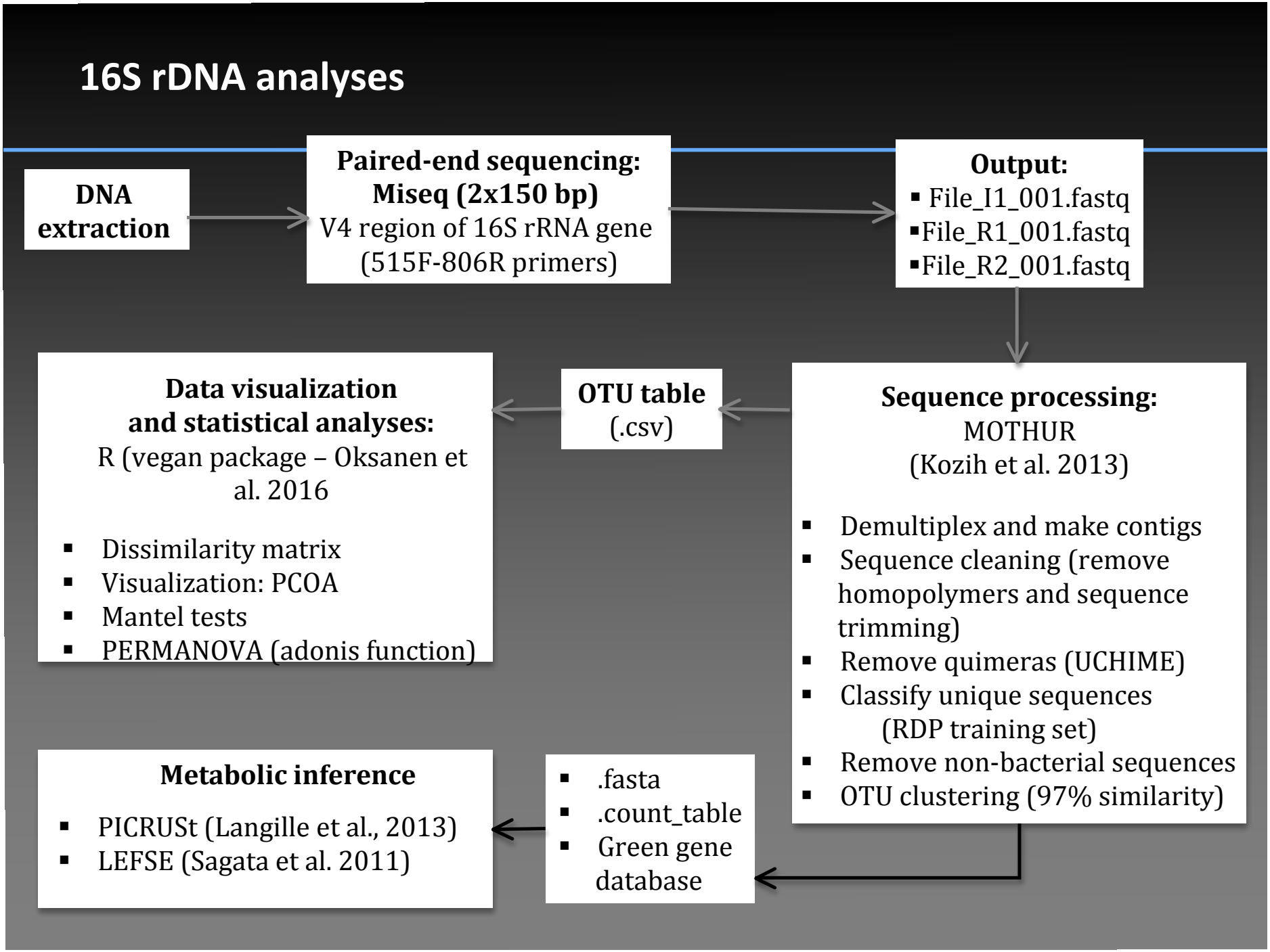
Image courtesy Tracy Mincer

Epibiont diversity



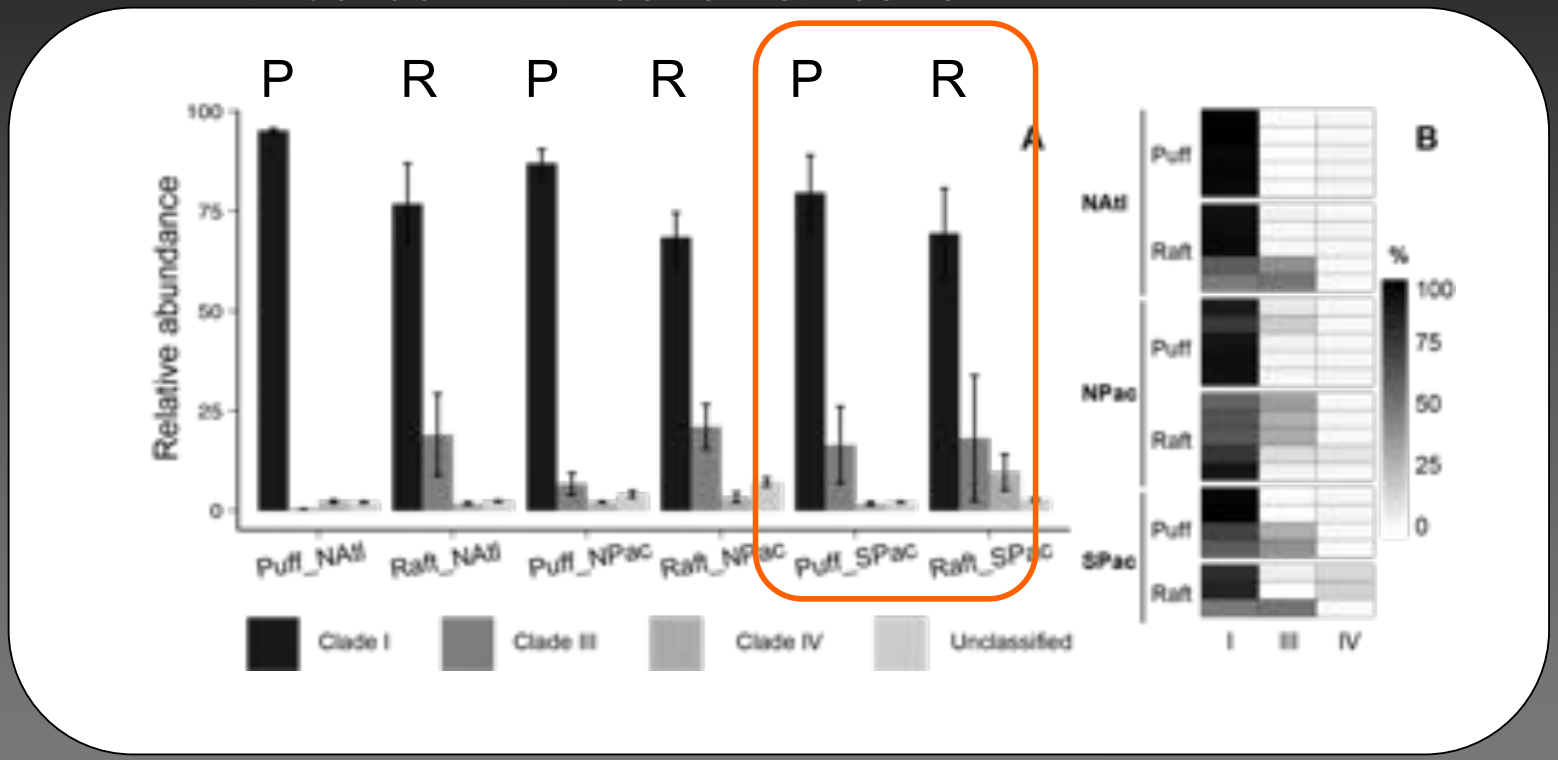
Are epibiont communities distinct as a function of colony morphology or environment?

Mónica Rouco


16S rDNA analyses
DNA extraction
Paired-end sequencing: Miseq (2x150 bp)
V4 region of 16S rRNA gene (515F-806R primers)
Output:
File_I1_001.fastq
File_R1_001.fastq
File_R2_001.fastq
Sequence processing:
MOTHUR
(Kozih et al. 2013)
Demultiplex and make contigs
Sequence cleaning (remove homopolymers and sequence trimming)
Remove quimeras (UCHIME)
Classify unique sequences (RDP training set)
Remove non-bacterial sequences
OTU clustering (97% similarity)
OTU table
(.csv)
Data visualization and statistical analyses:
R (vegan package – Oksanen et al. 2016
Dissimilarity matrix
Visualization: PCOA
Mantel tests
PERMANOVA (adonis function)
.fasta
.count_table
Green gene database
Metabolic inference
PICRUSt (Langille et al., 2013)
LEFSE (Sagata et al. 2011)

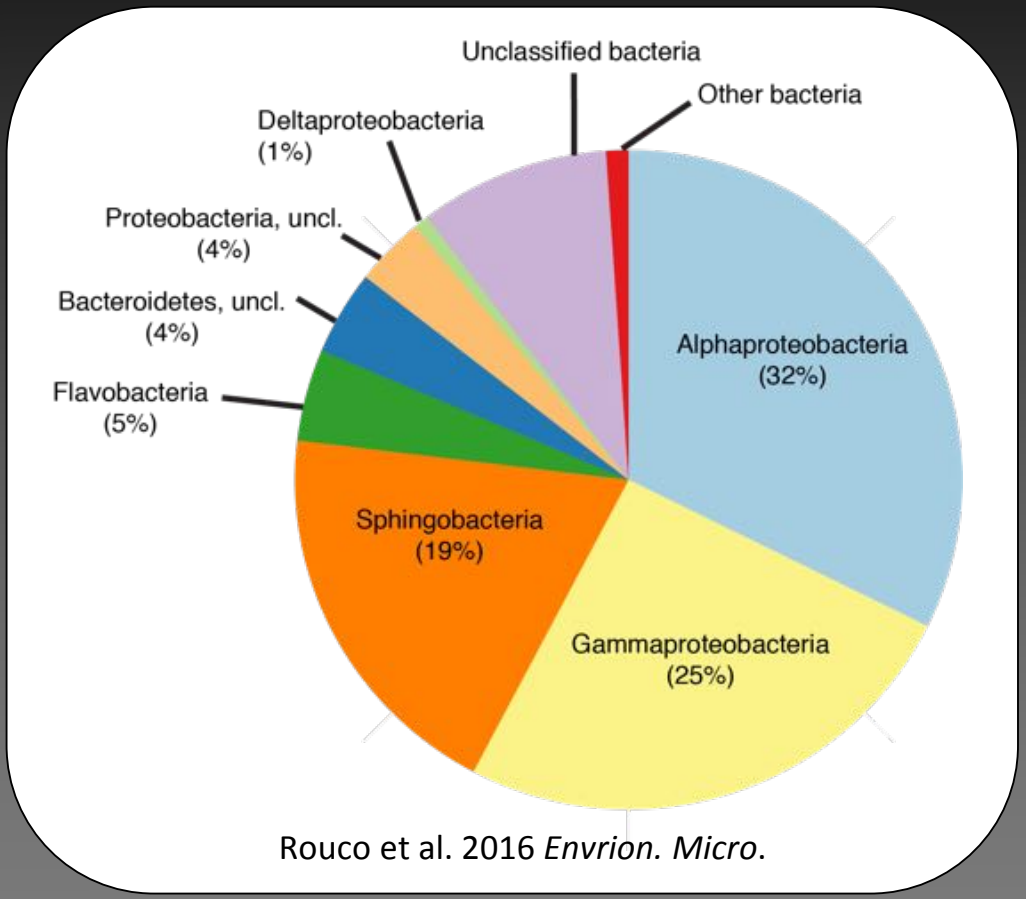
Colony composition by region

N. Atlantic N. Pacific S. Pacific



Colonies are not likely species specific, the raft morphology is more diverse except in the S. Pacific

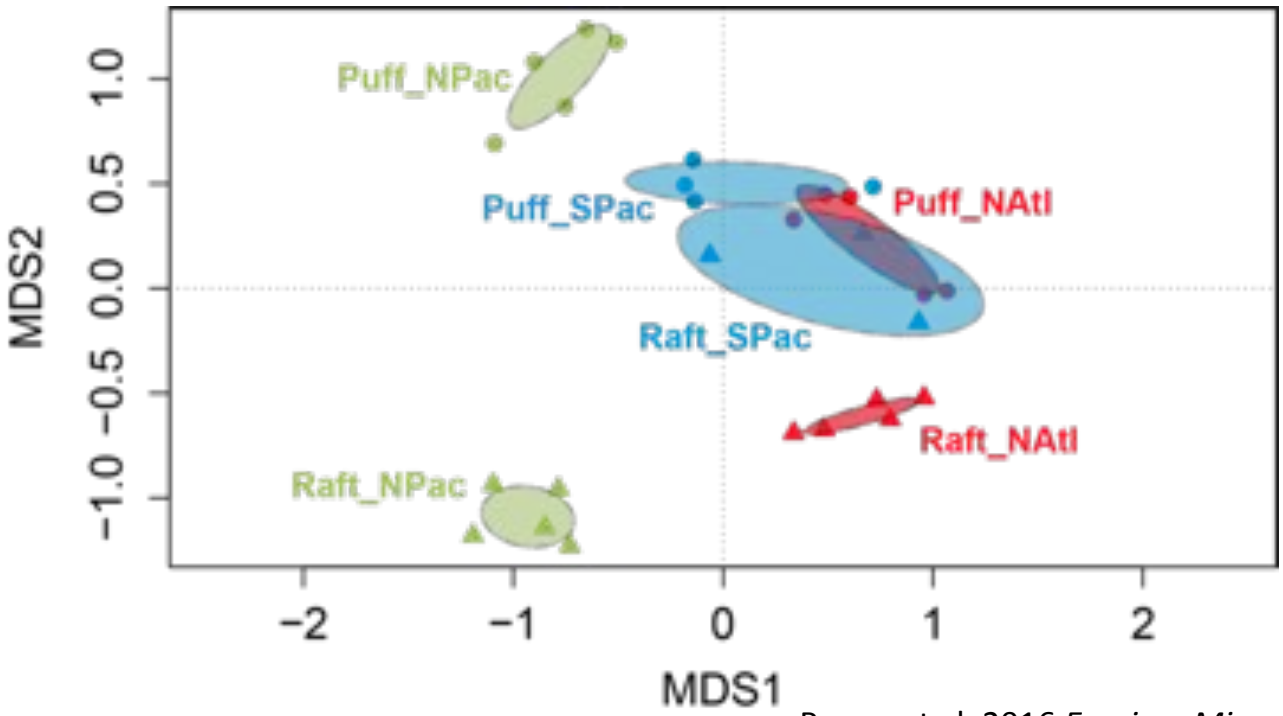
Average epibiont community



Rouco et al. 2016 *Envrion. Micro*.

16S amplicon sequencing indicates that *Trichodesmium* colonies harbor diverse epibionts distinct from common water column bacteria, and those found on sinking particles.

Epibiont community diversity



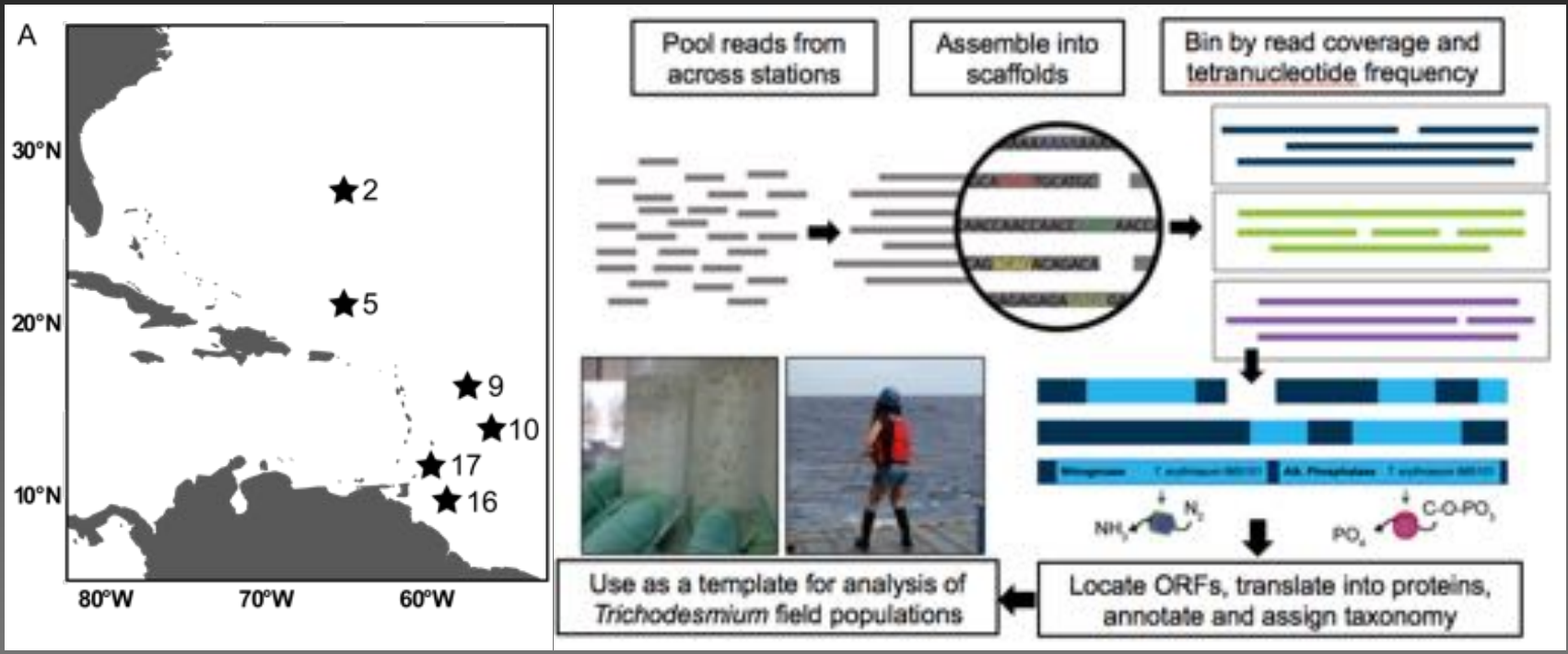
Rouco et al. 2016 *Envrion. Micro*.

Epibiont communities significantly differ by ocean basin, and with colony morphology except for the S. Pacific where the *Trichodesmium* composition of rafts and puffs were not significantly different.

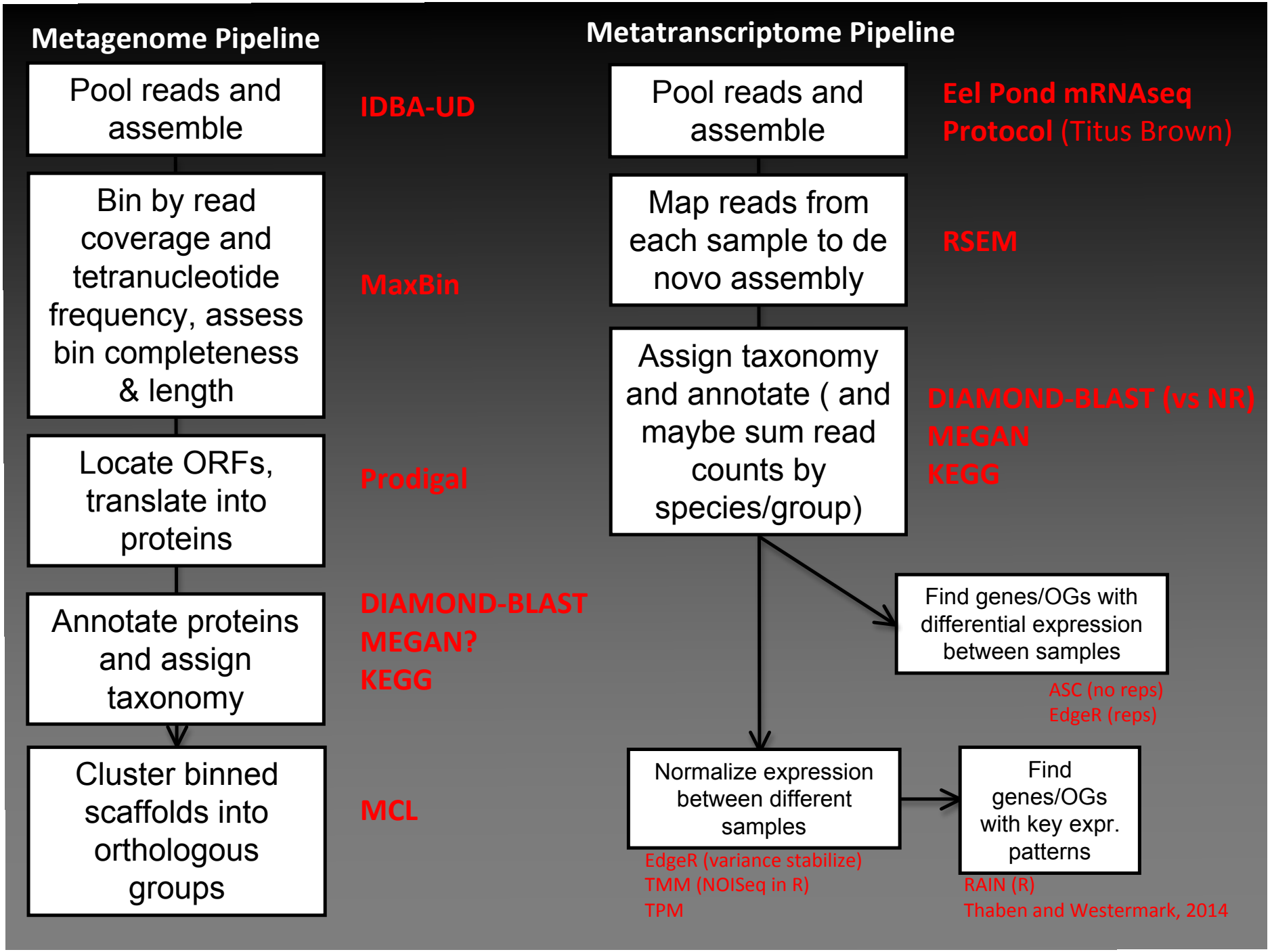
What drives community assembly?

- *Niche?* What type of *Trichodesmium*, physiological ecology in the colony, environment..
- *Lottery?* Random selection of potential copiotrophs, role of taxonomic v. functional group uncertain…
- Working to examine the *Trichodesmium* holobiont with metagenomics/metatranscriptomics.

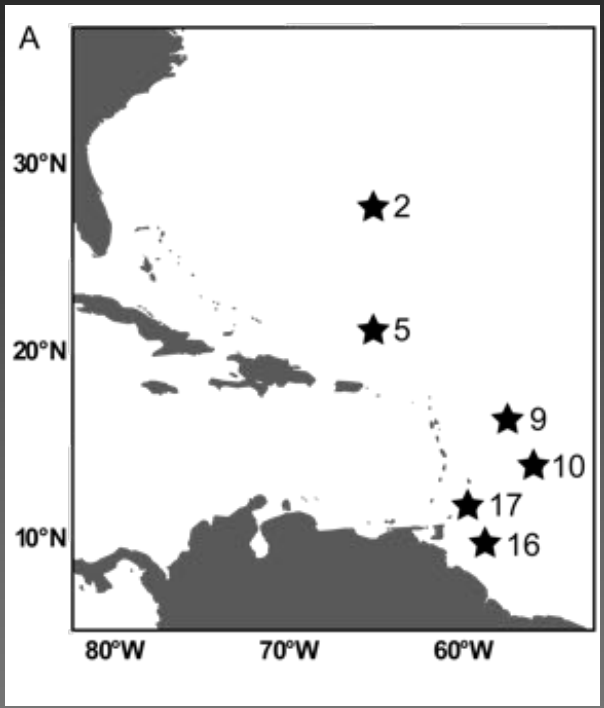
Metabolic potential in the *Trichodesmium* holobiont

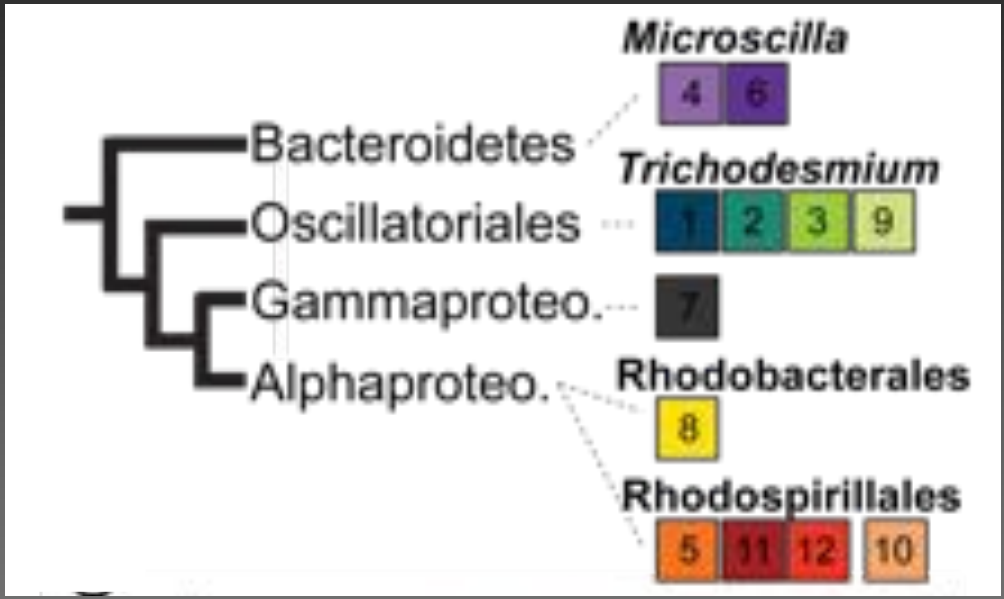


Trichodesmium colonies were isolated for metagenome and metatranscriptome sequencing along a phosphorus gradient in the western north Atlantic.


Metagenome Pipeline
Pool reads and assemble
IDBA-UD
Bin by read coverage and tetranucleotide frequency, assess bin completeness & length
MaxBin
Locate ORFs, translate into proteins
Prodigal
Annotate proteins and assign taxonomy
DIAMOND-BLAST
MEGAN?
KEGG
Cluster binned scaffolds into orthologous groups
MCL
Metatranscriptome Pipeline
Pool reads and assemble
Eel Pond mRNAseq Protocol (Titus Brown)
Map reads from each sample to de novo assembly
RSEM
Assign taxonomy and annotate (and maybe sum read counts by species/group)
DIAMOND-BLAST (vs NR)
MEGAN
KEGG
Find genes/OGs with differential expression between samples
ASC (no reps)
EdgeR (reps)
Normalize expression between different samples
EdgeR (variance stabilize)
TMM (NOISeq in R)
TPM
Find genes/OGs with key expr. patterns
RAIN (R)
Thaben and Westermark, 2014

Composition of the holobiont

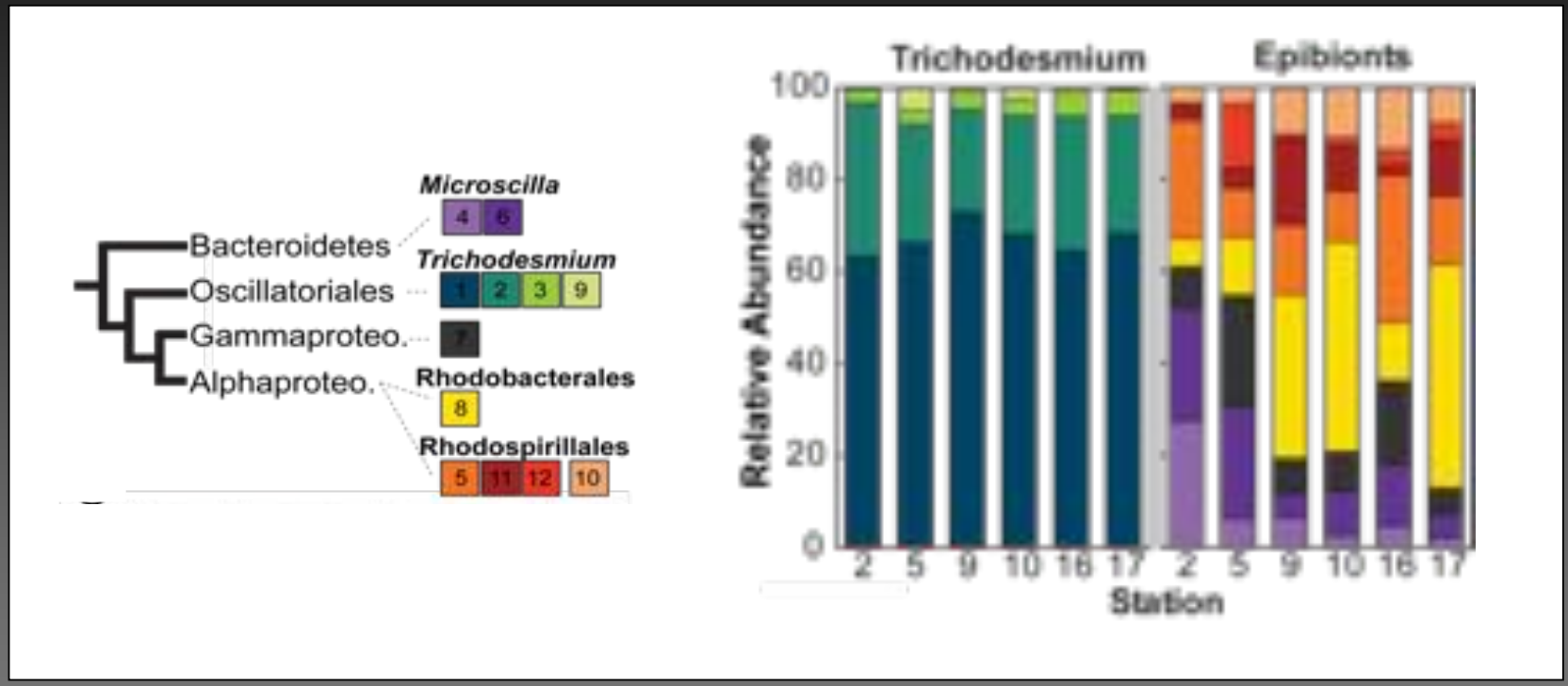




Frischkorn et al. (in review)

Nearly complete (65-90%) genome bins were reconstructed from a merged assembly and results are consistent with 16S data

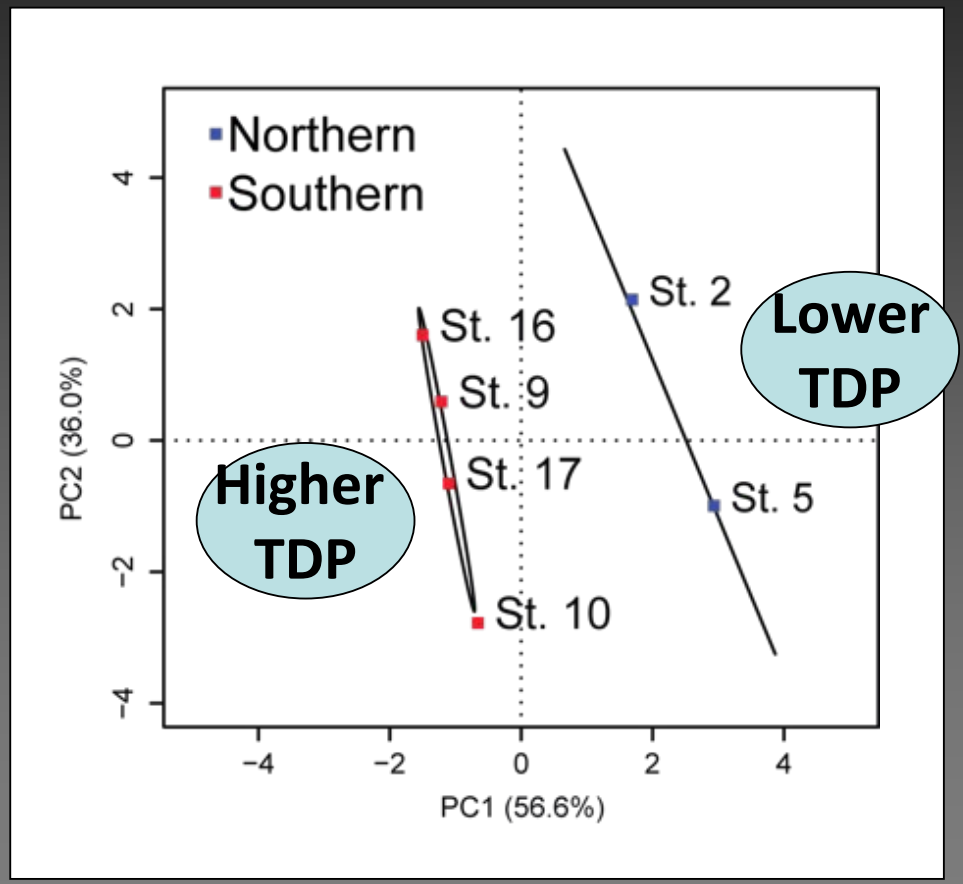
Distribution of holobiont



Frischkorn et al. (in review)

Epibiont genome bins are detected at all stations, but the relative abundance varies

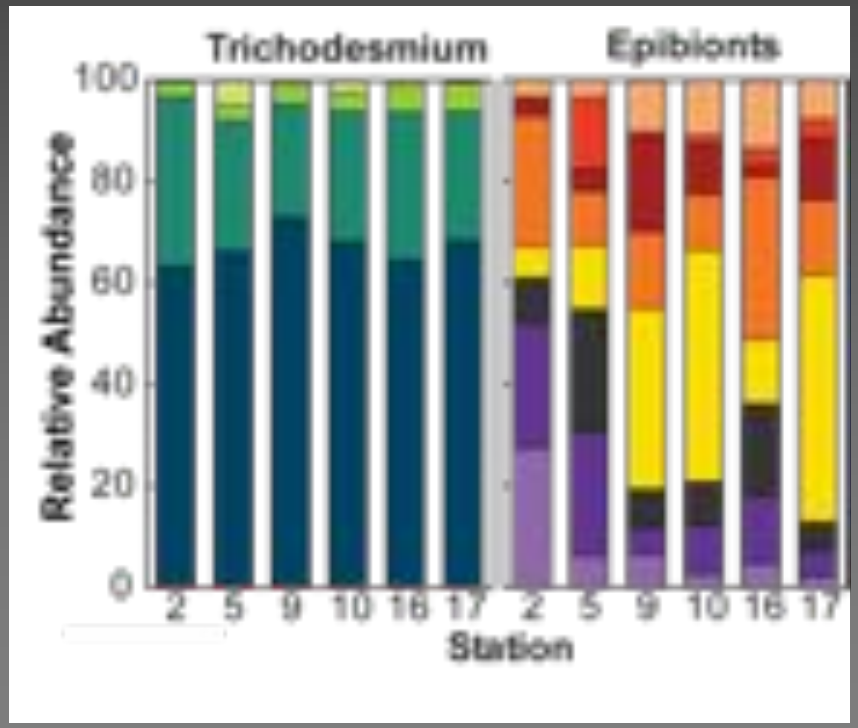
Microbiome diversity differs significantly between regions

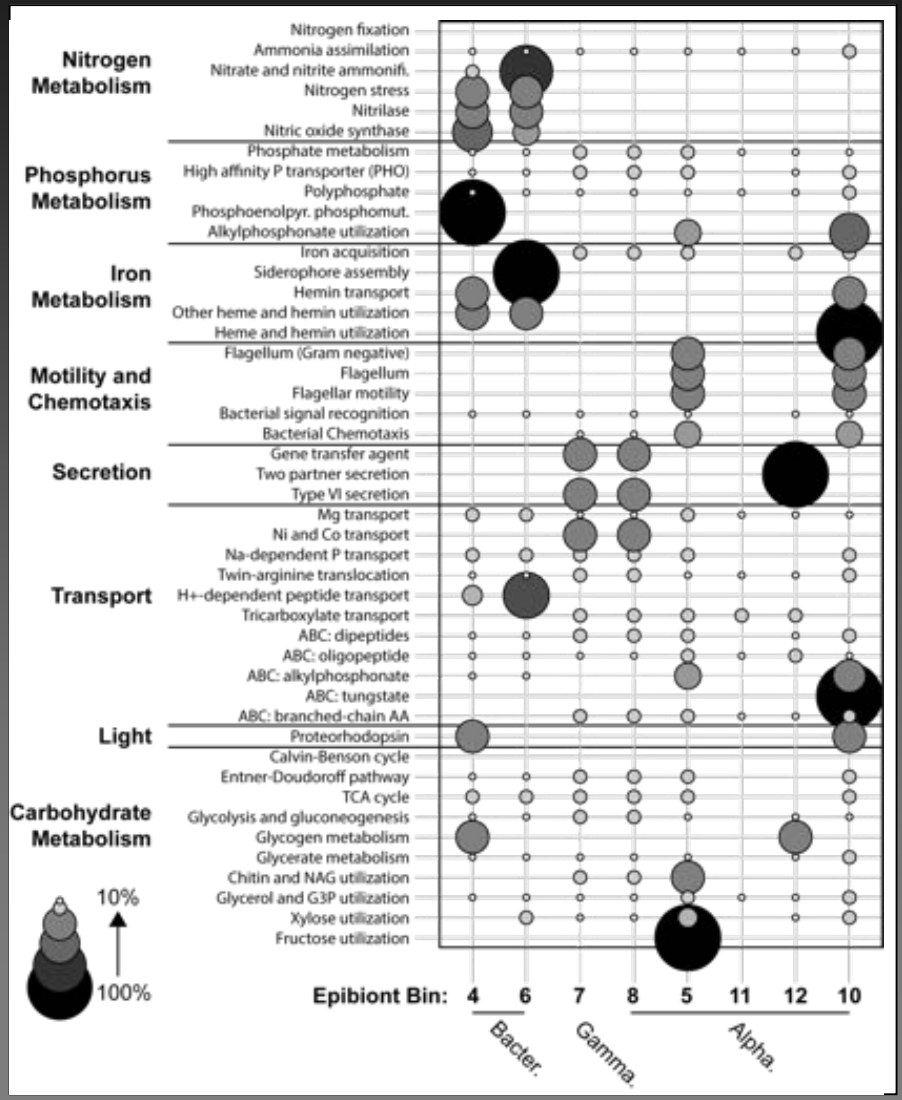


Frischkorn et al. (in review)

Variable distribution of functional pathways among epibionts

- Differential pathway enrichment consistent with a microbiome that is modulated as a function of environment



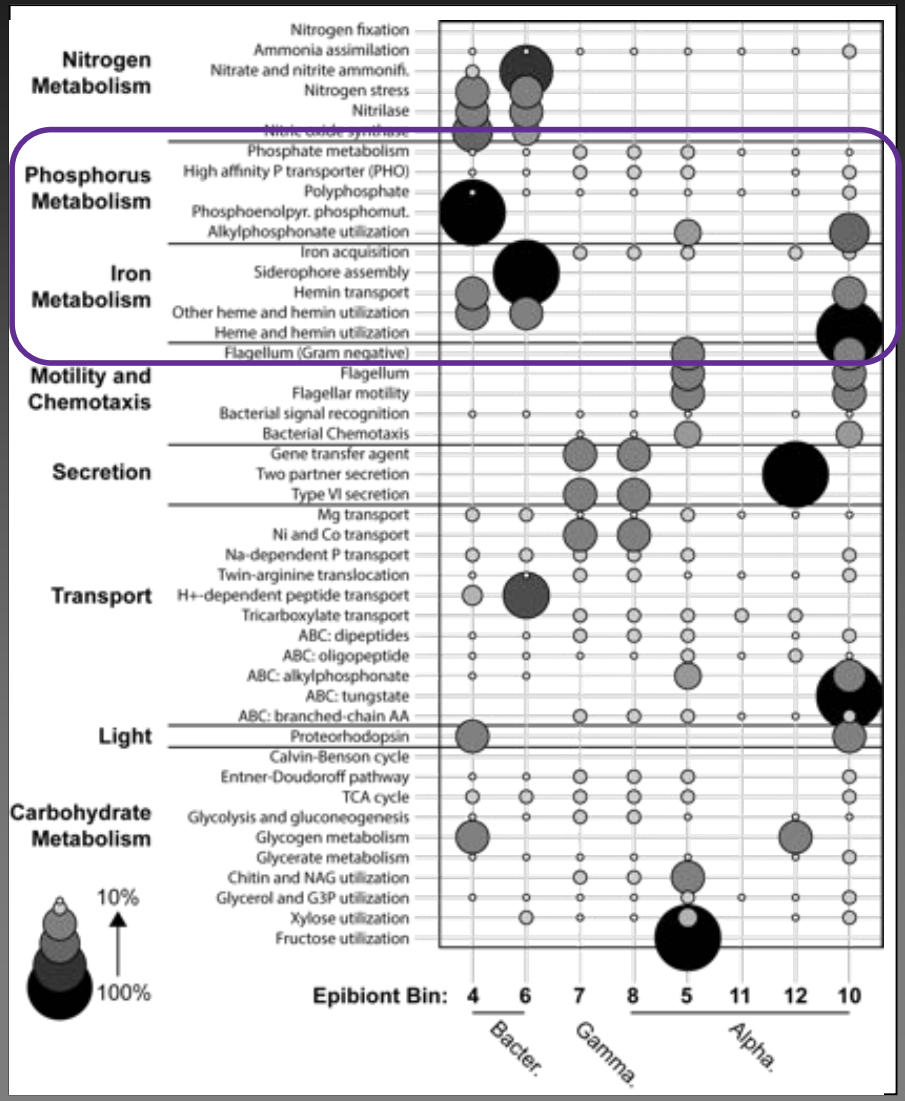


Frischkorn et al. (in review)

Variable distribution of functional pathways among epibionts

- Differential pathway enrichment consistent with a microbiome that is modulated as a function of environment

- Phosphonate, heme and siderophore functions are enriched relative to water column microbes in the Sargasso Sea.



Frischkorn et al. (in review)

Comparing metabolic potential in the holobiont

Metagenomes

Orthologous group analysis

Epibionts v. *Trichodesmium*

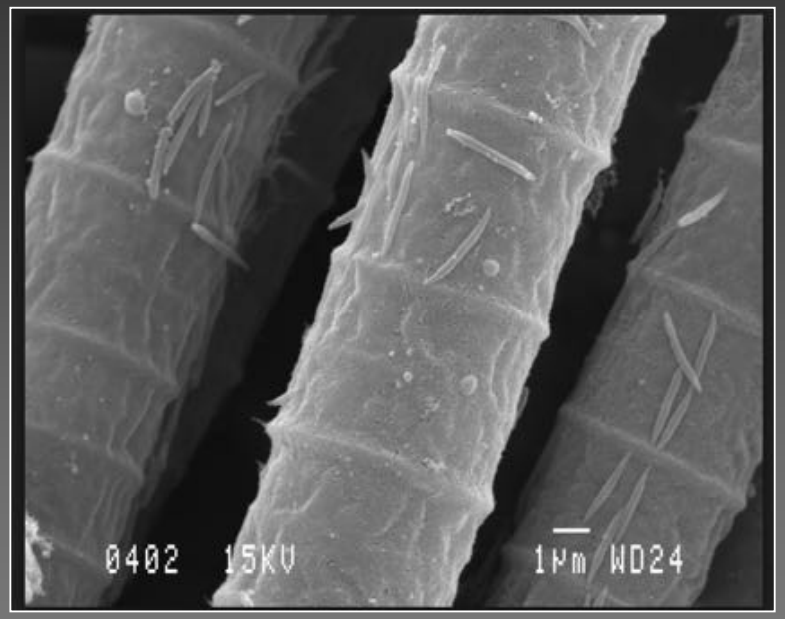
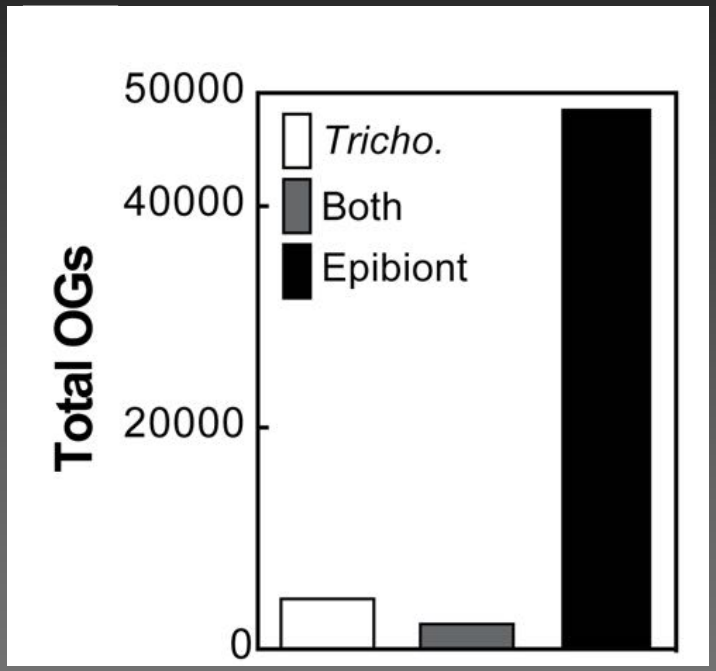


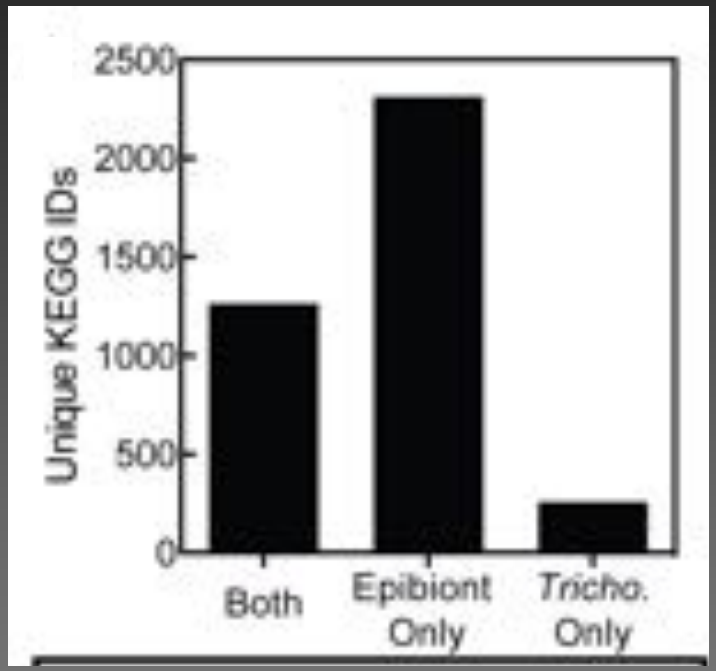
Image courtesy Tracy Mincer

Epibionts confer the majority of the metabolic potential

>90% OGs



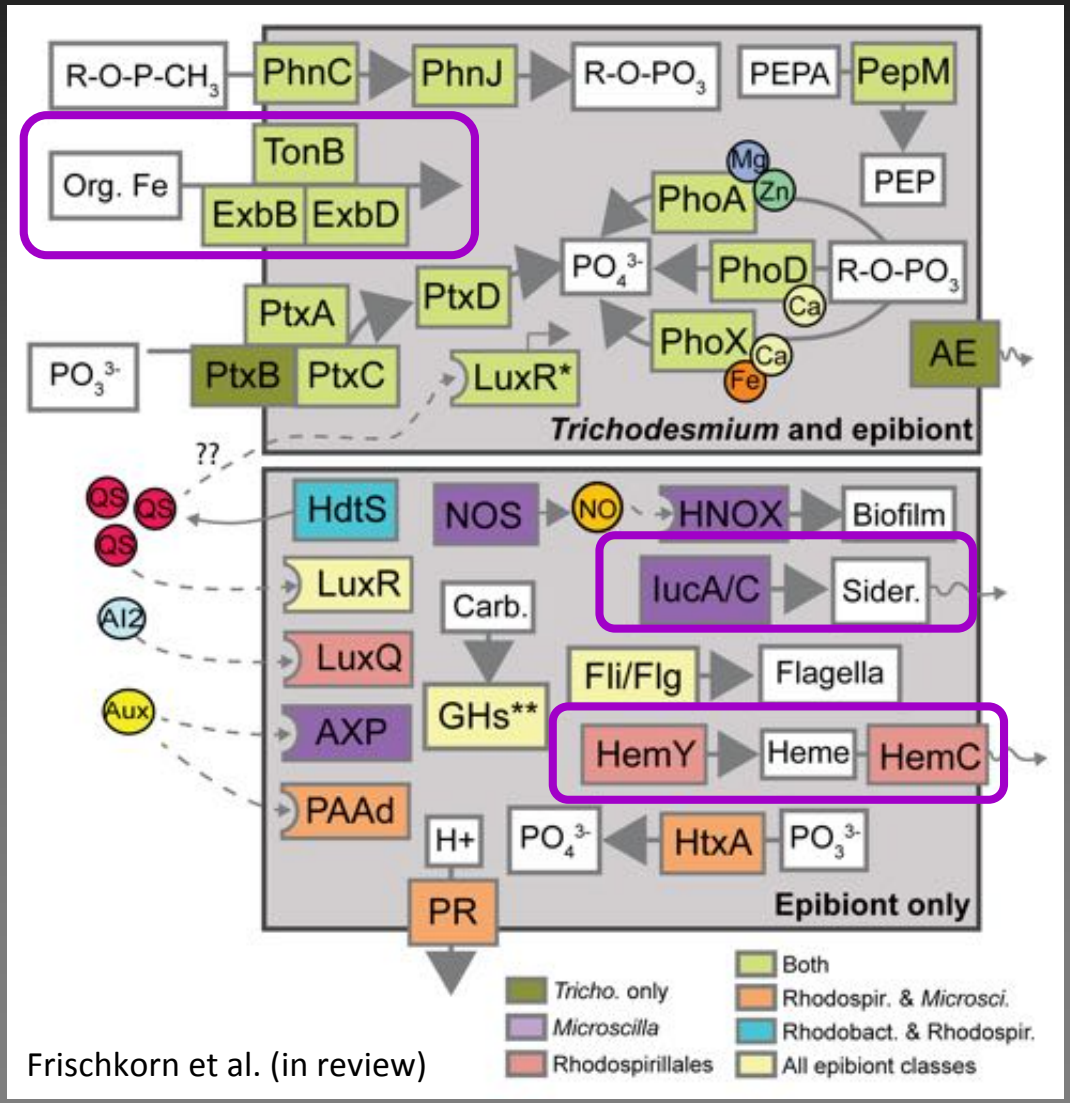
>10x Functions



Frischkorn et al. (in review)

Orthologous (OG) group analysis suggests that epibionts confer the vast majority of metabolic functions to the holobiont.

Metabolic partitioning within the *Trichodesmium* holobiont

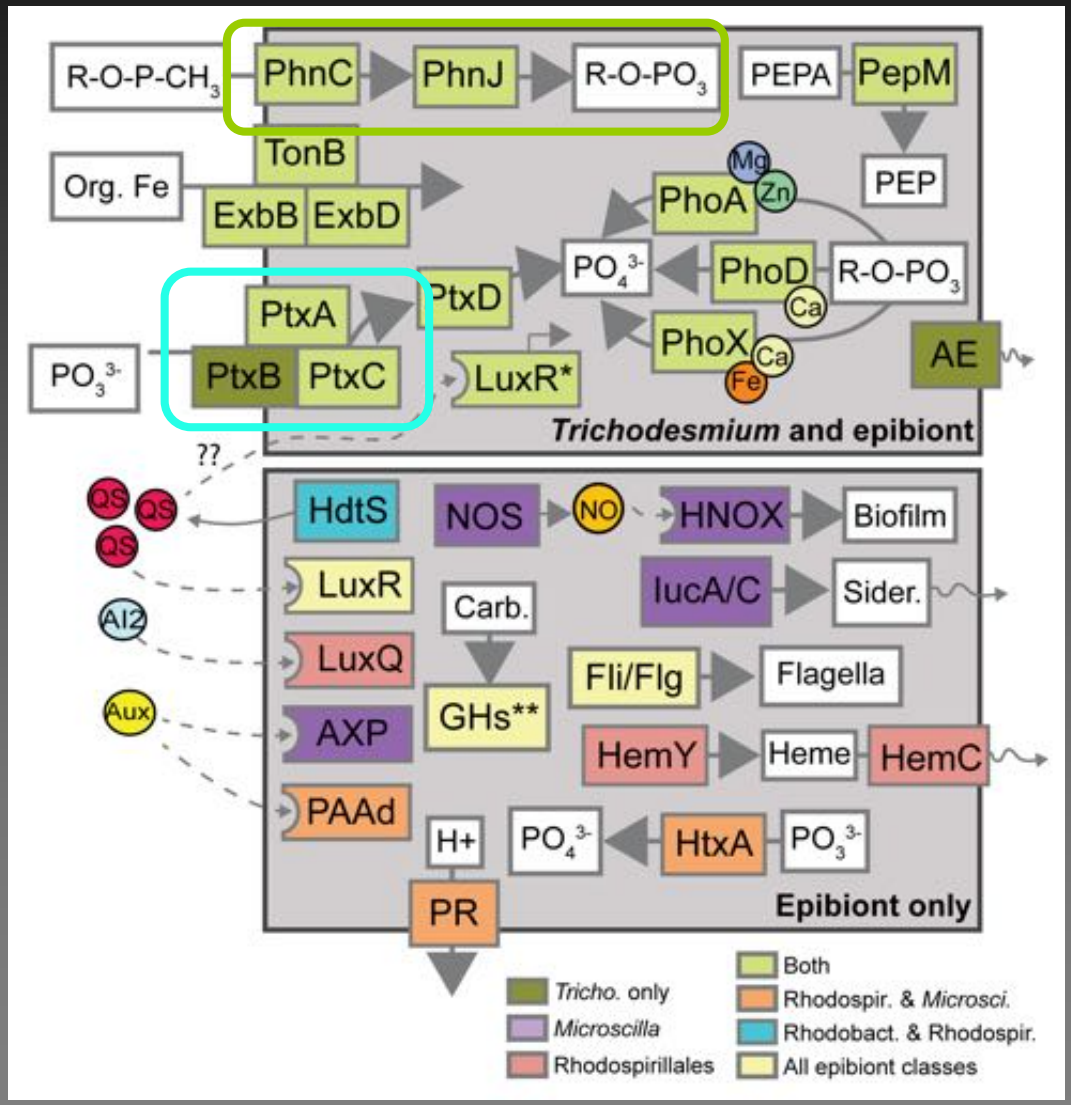


Frischkorn et al. (in review)

Organic Iron

Epibionts can produce organic iron complexes that likely modulate iron in the holobiont microenvironment

Uptake and metabolism of reduced phosphorus forms



C-P Lyase
PhoX
PhoA

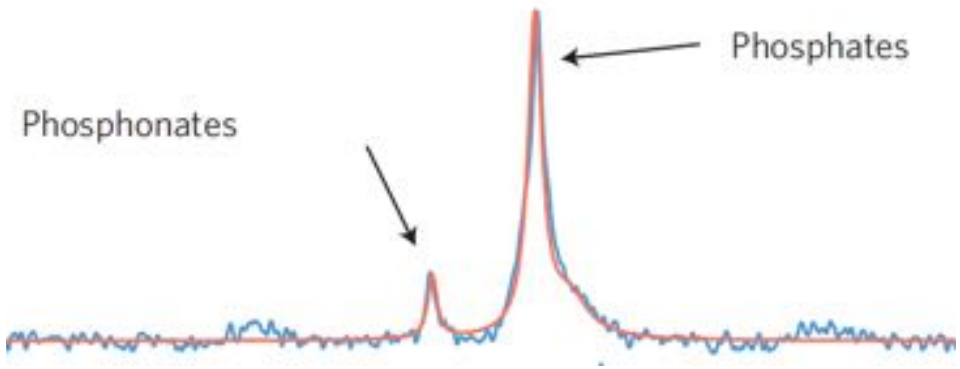
Phosphite uptake

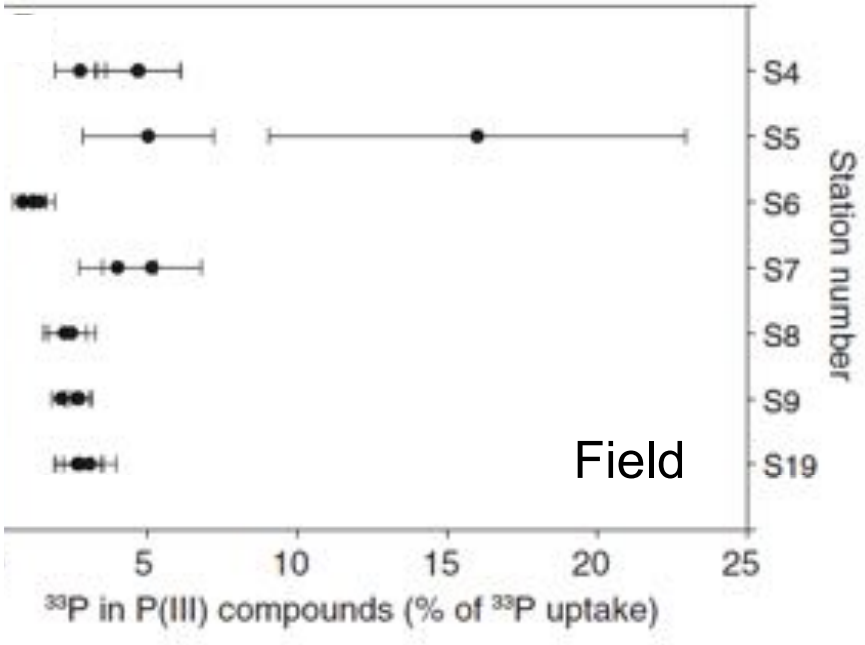
Phosphorus metabolisms are present in both *Trichodesmium* and the microbiome.

Answers to enduring mysteries… who makes C-P compounds?

Phosphonate (C-P) biosynthesis

Culture



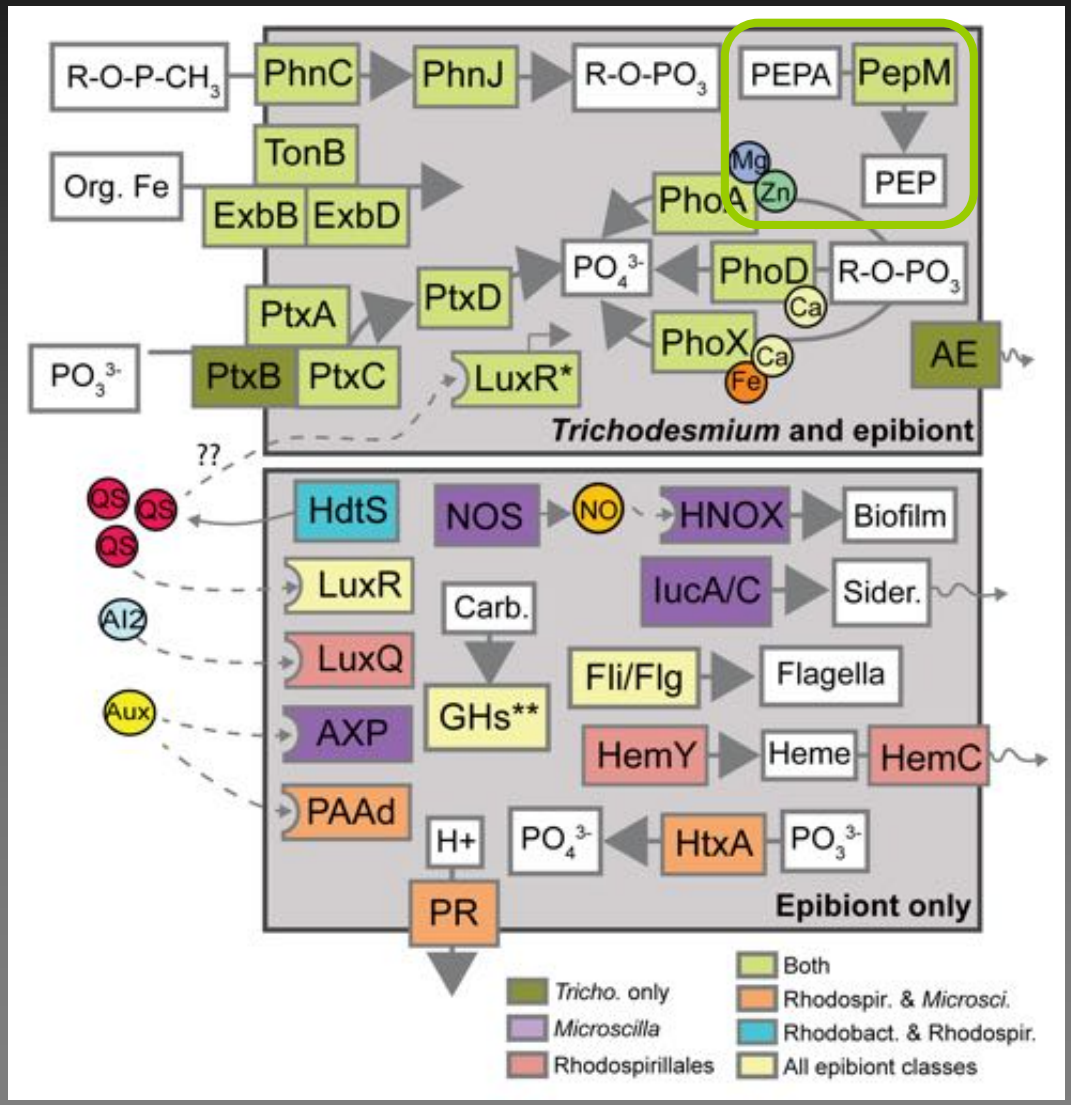


Dyhrman et al. (2009) *Nature Geo.*

Van Mooy et al. (2015) *Science*

Phosphonates are produced at high rates in the holobiont - hot spot for reduced phosphorus cycling. Is it *Trichodesmium* or the epibionts?

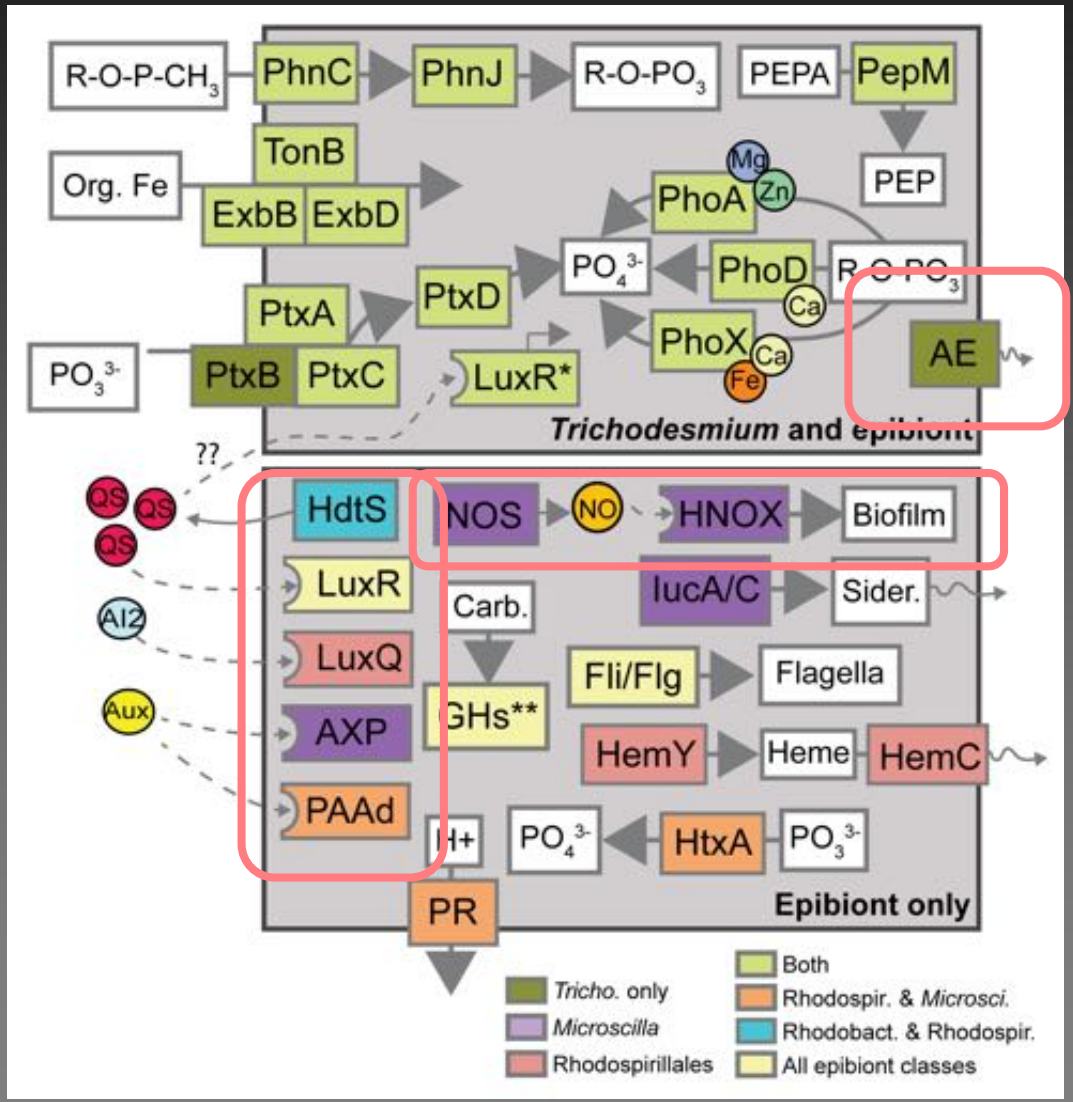
Phosphonate production is a shared metabolism



Phosphonate biosynthesis

Phosphonate produced by both *Trichodesmium* and the epibionts

Microbial cross talk within the *Trichodesmium* holobiont



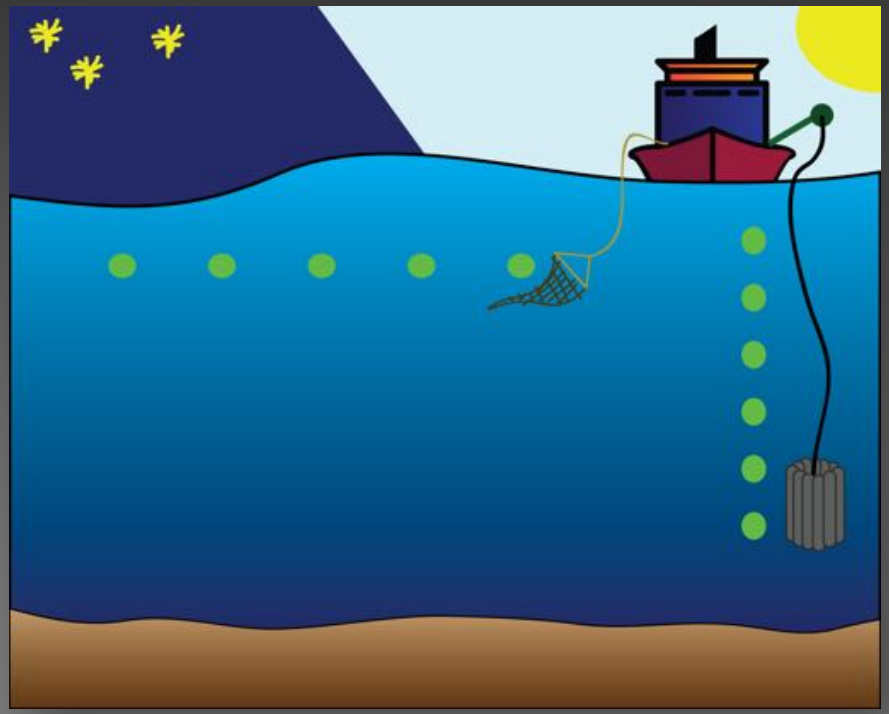
Tracking the interactome… with metatranscriptome profiling after addition of NO and QS molecules

QS and cell signaling

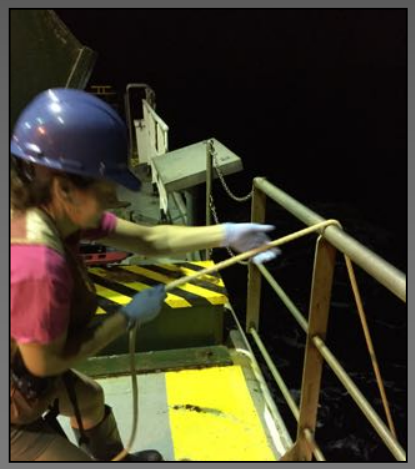
Trichodesmium holobiont interactome…

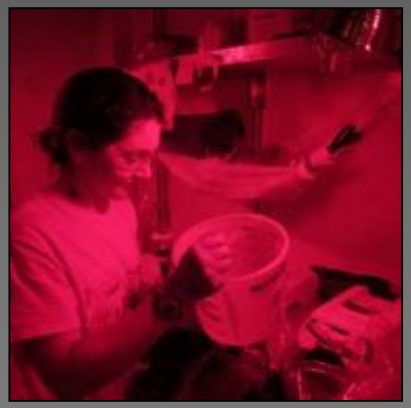
Using diel metatranscriptome sampling to tease apart possible interactions between *Trichodesmium* and the epiboints.

Are there diel signatures to epibiont genes?



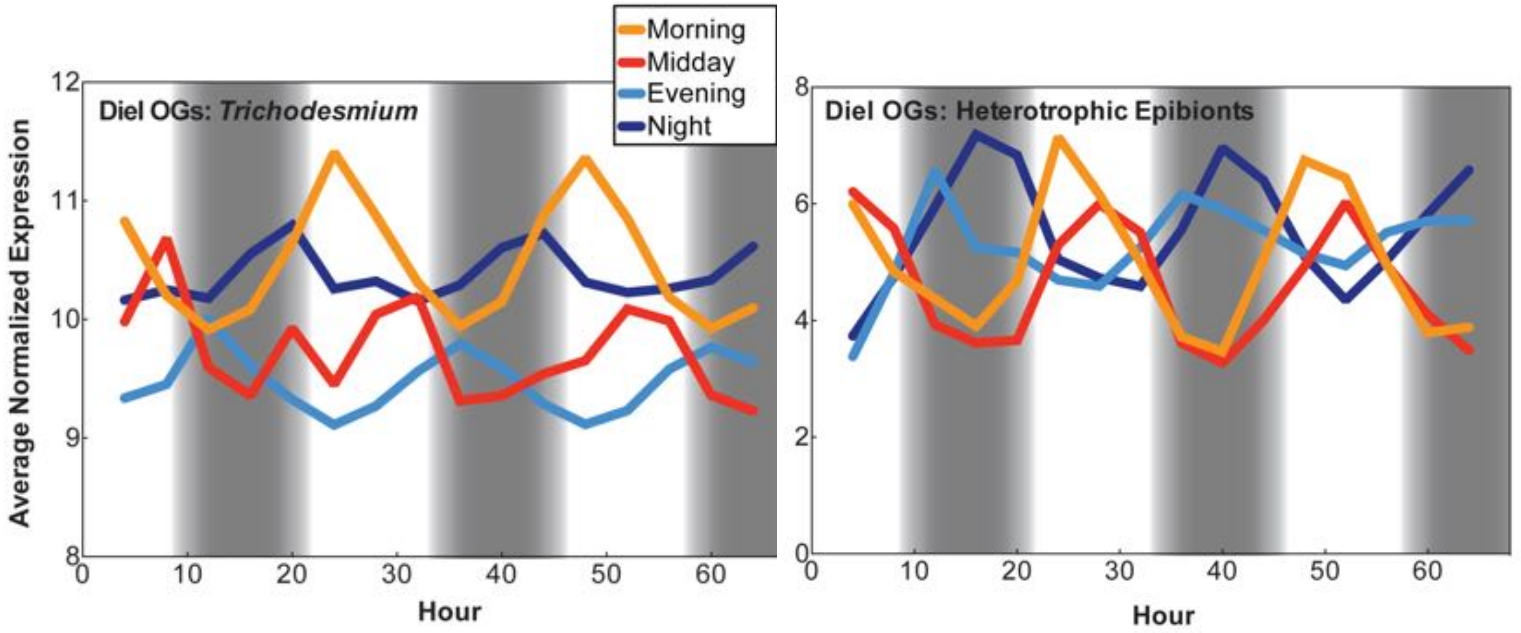
Sampling the interactome…



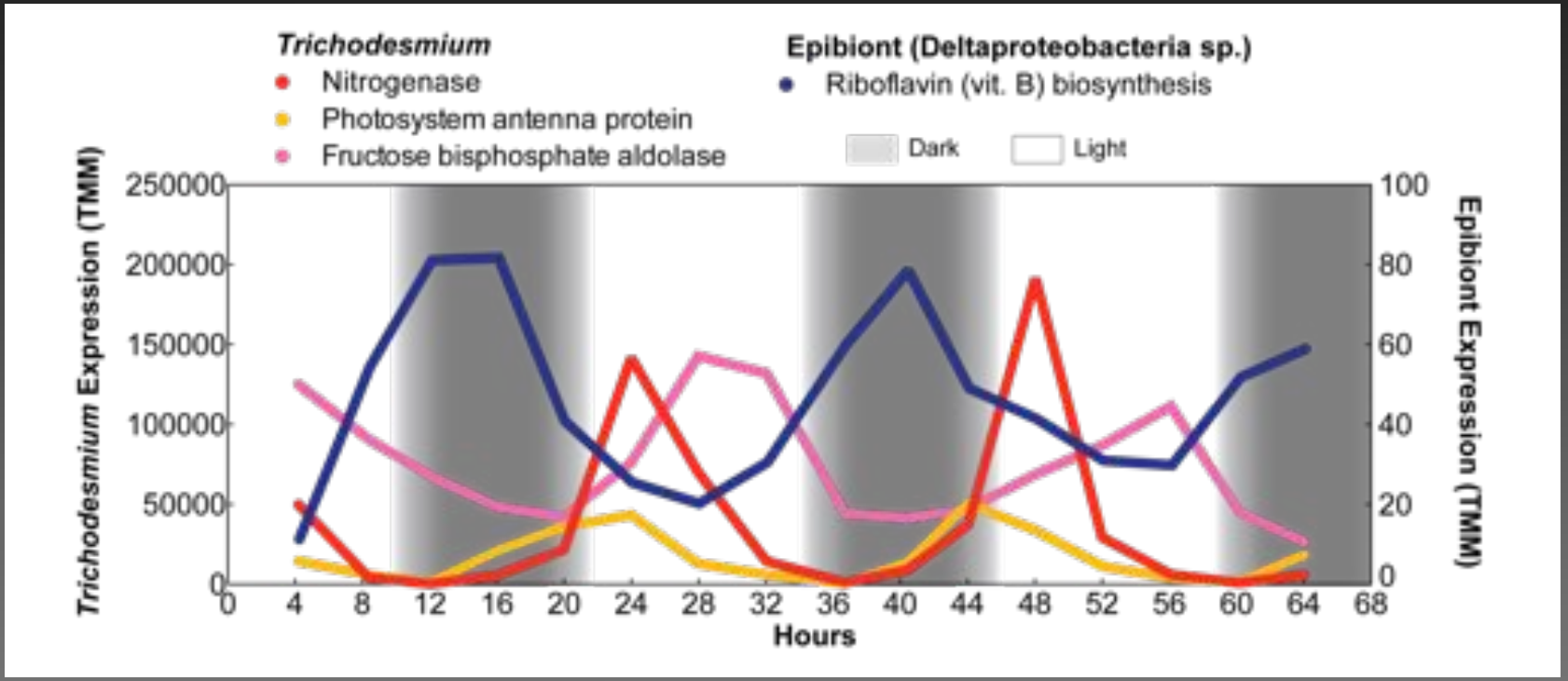


Colony samples were taken every 4 hours for 68 hours, twice!

Diel modulation of transcripts in *Trichodesmium*



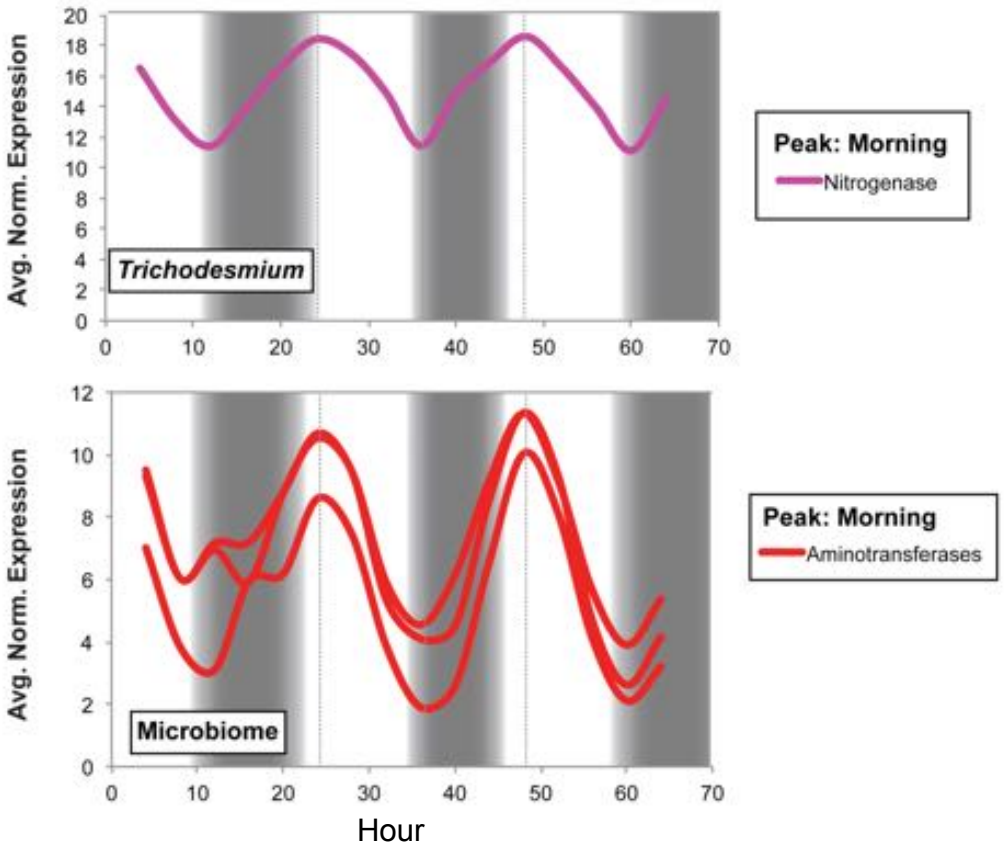
Diel modulation of transcripts within the microbiome

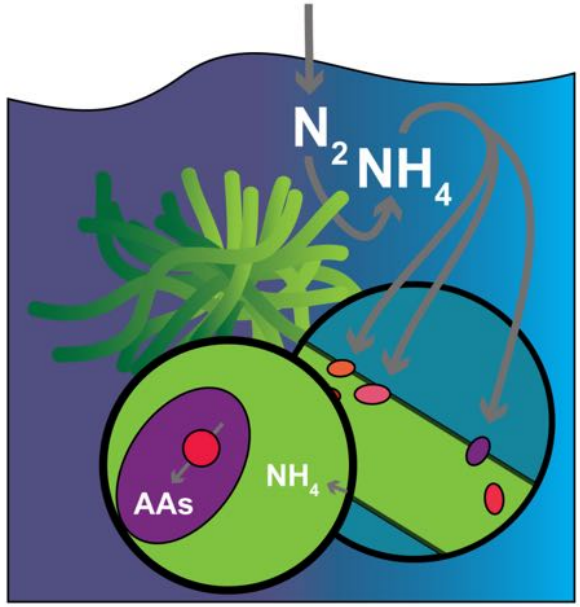


Epibiont riboflavin biosynthesis has a diel pattern oscillating with *Trichodesmium* photosynthesis, carbon fixation and nitrogen fixation.

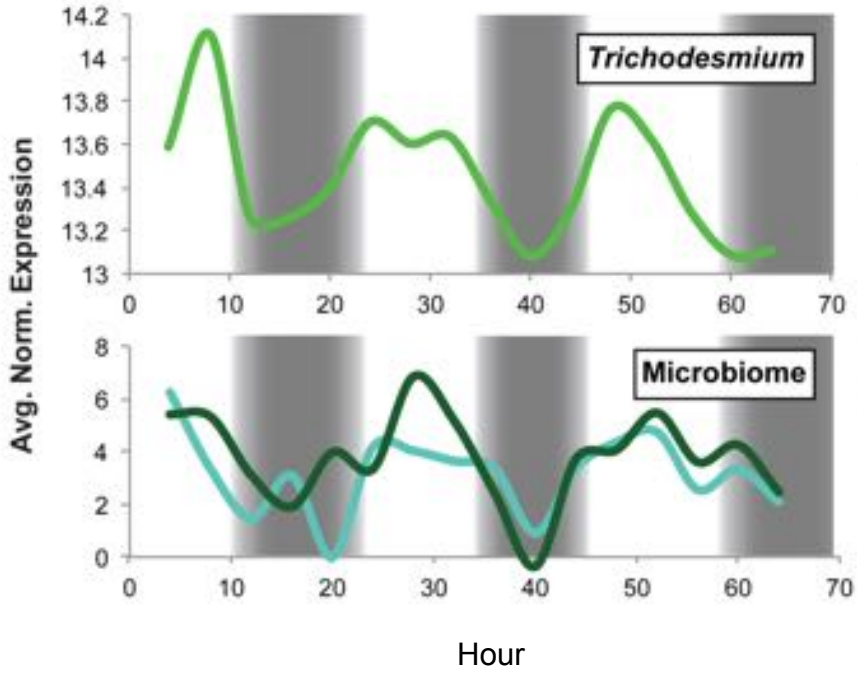
This is suggestive of the dynamic interplay between members of the holobiont.

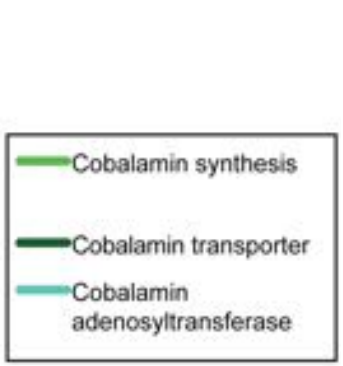
Diel modulation of transcripts suggest coordination: Nitrogen

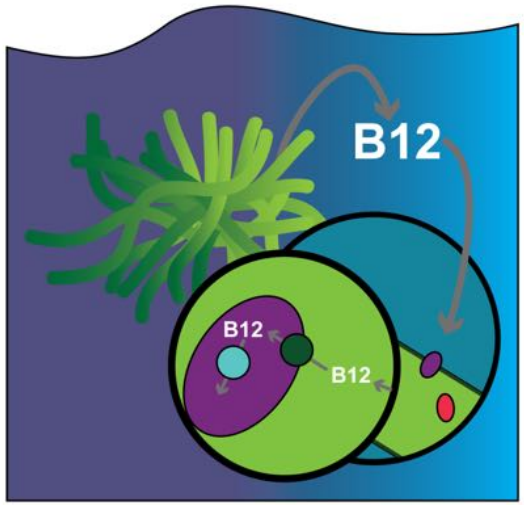




Diel modulation of transcripts suggest coordination: Vitamins



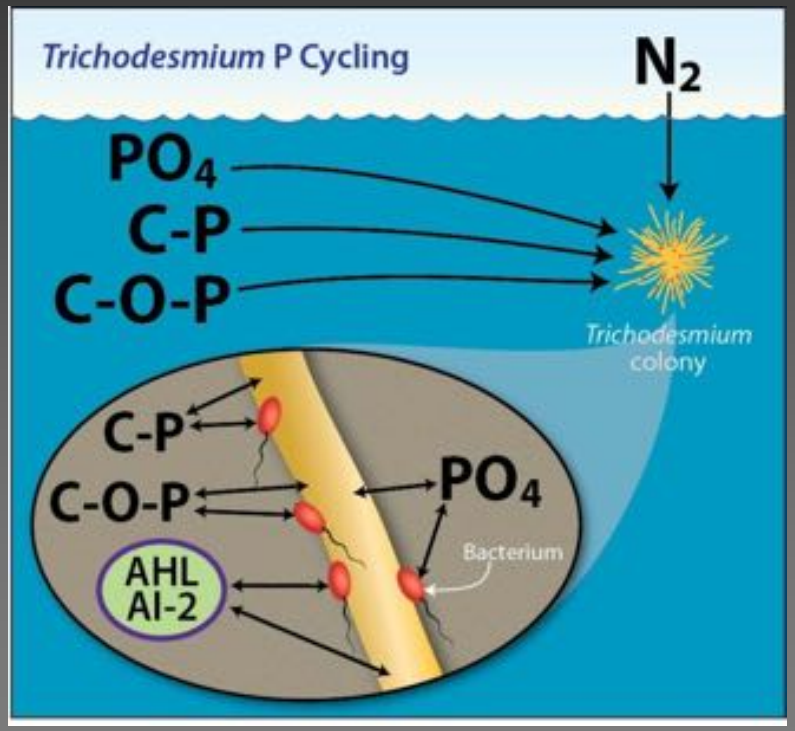




Summary - Host microbiome interactions

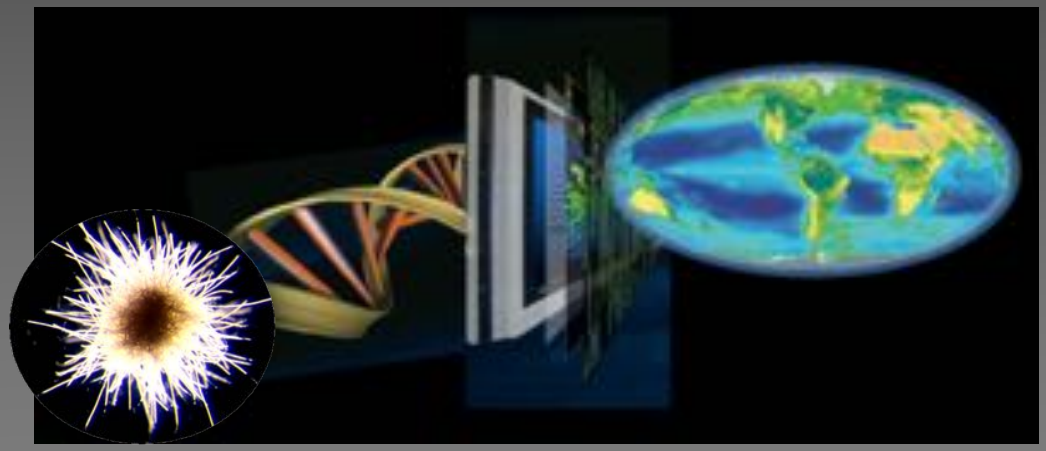
What is the role of the microbiome on *Trichodesmium* physiological ecology?

- Colonies harbor diverse epibionts distinct from water column
- Epibionts are dynamically curated across gradients in the environment
- Epibionts confer substantial metabolic potential which likely underpins *Trichodesmium* fitness
- There is early evidence of interactions between *Trichodesmium* and its microbiome



Conclusions

Genome-enabled approaches are providing new tools to trace the activities and physiological ecology of *Trichodesmium* and its microbiome. Future studies should examine geochemical controls on N_2 fixation in the context of the holobiont.

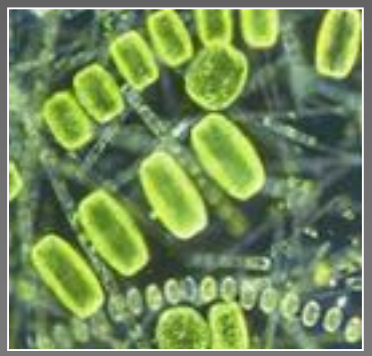


Vignettes

- From genome to biome: Tracking the metabolism and microbiome of a keystone N_2 fixer

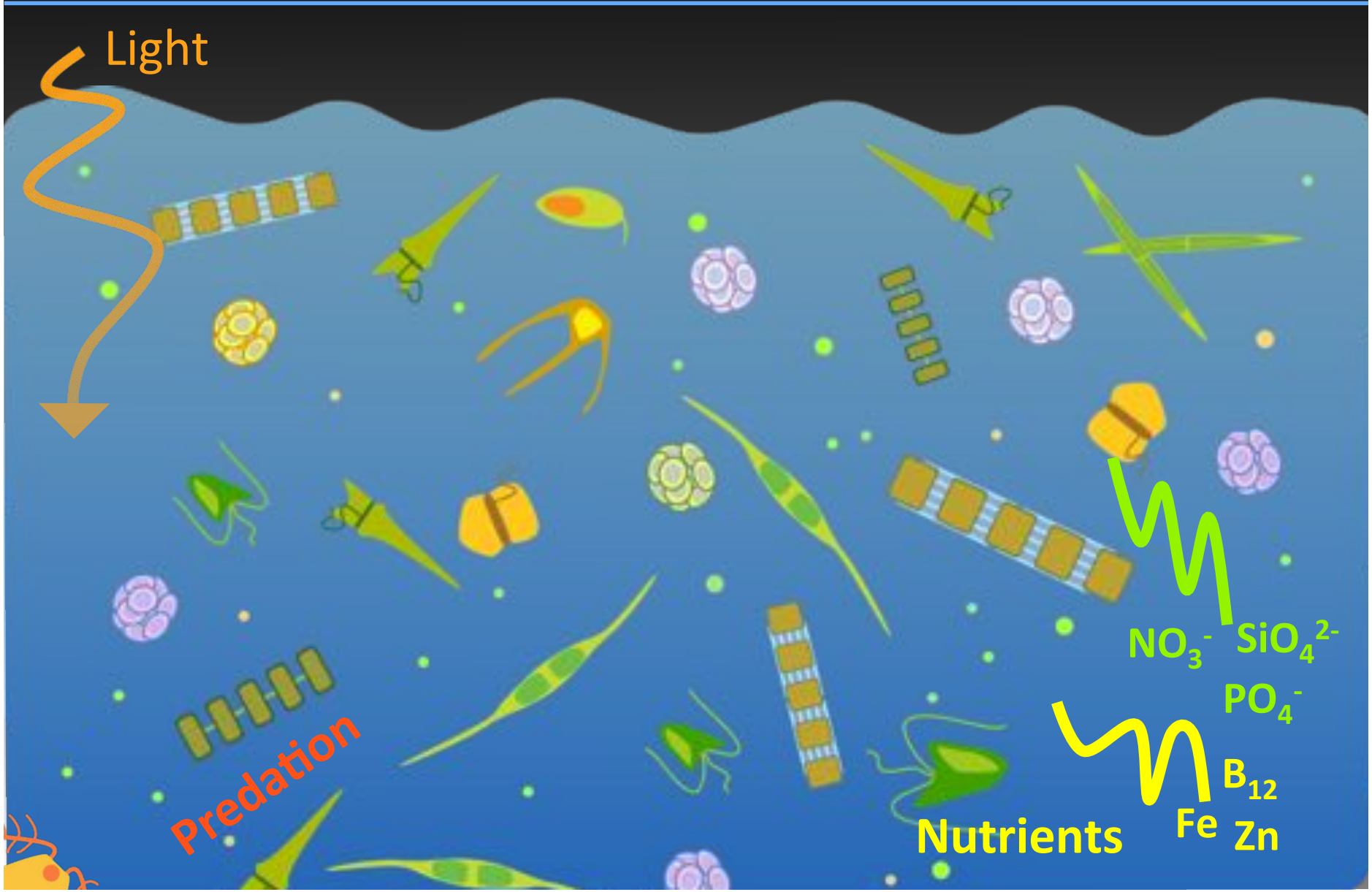
Genome - enabled

- Co-existing in a sea of competition: Leveraging transcriptome data to identify the physiological ecology of phytoplankton from key groups



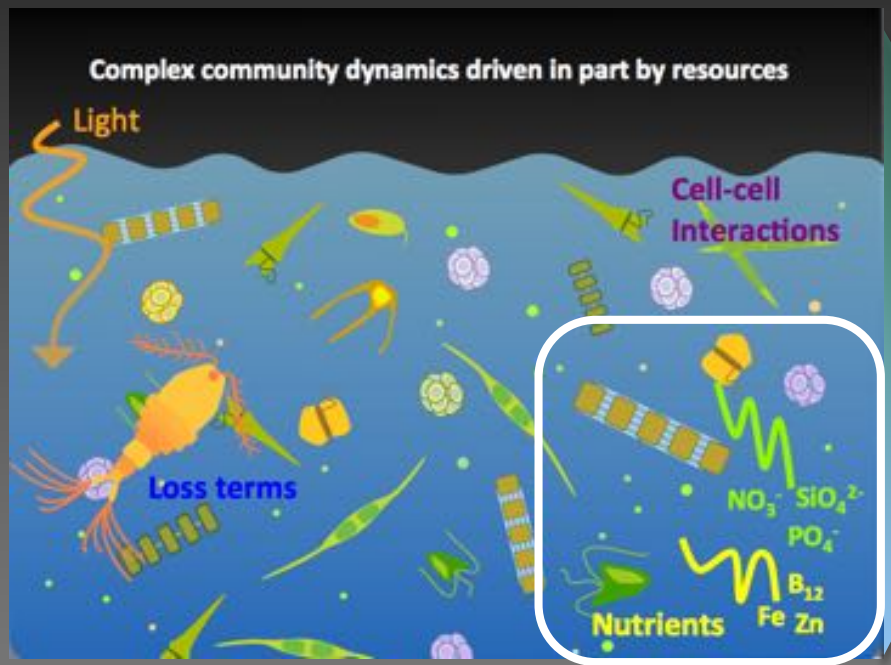
Transcriptome - enabled

Complex community dynamics driven in part by resources



Tracking phytoplankton physiological ecology

Ecosystem function and biogeochemistry



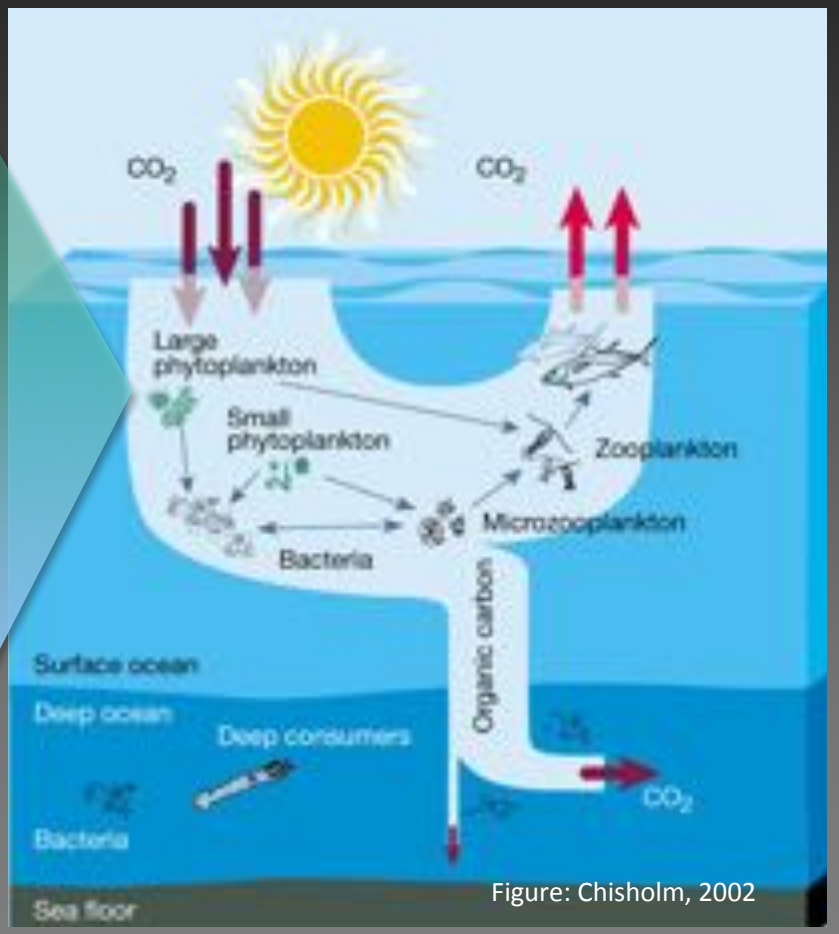


Figure: Chisholm, 2002

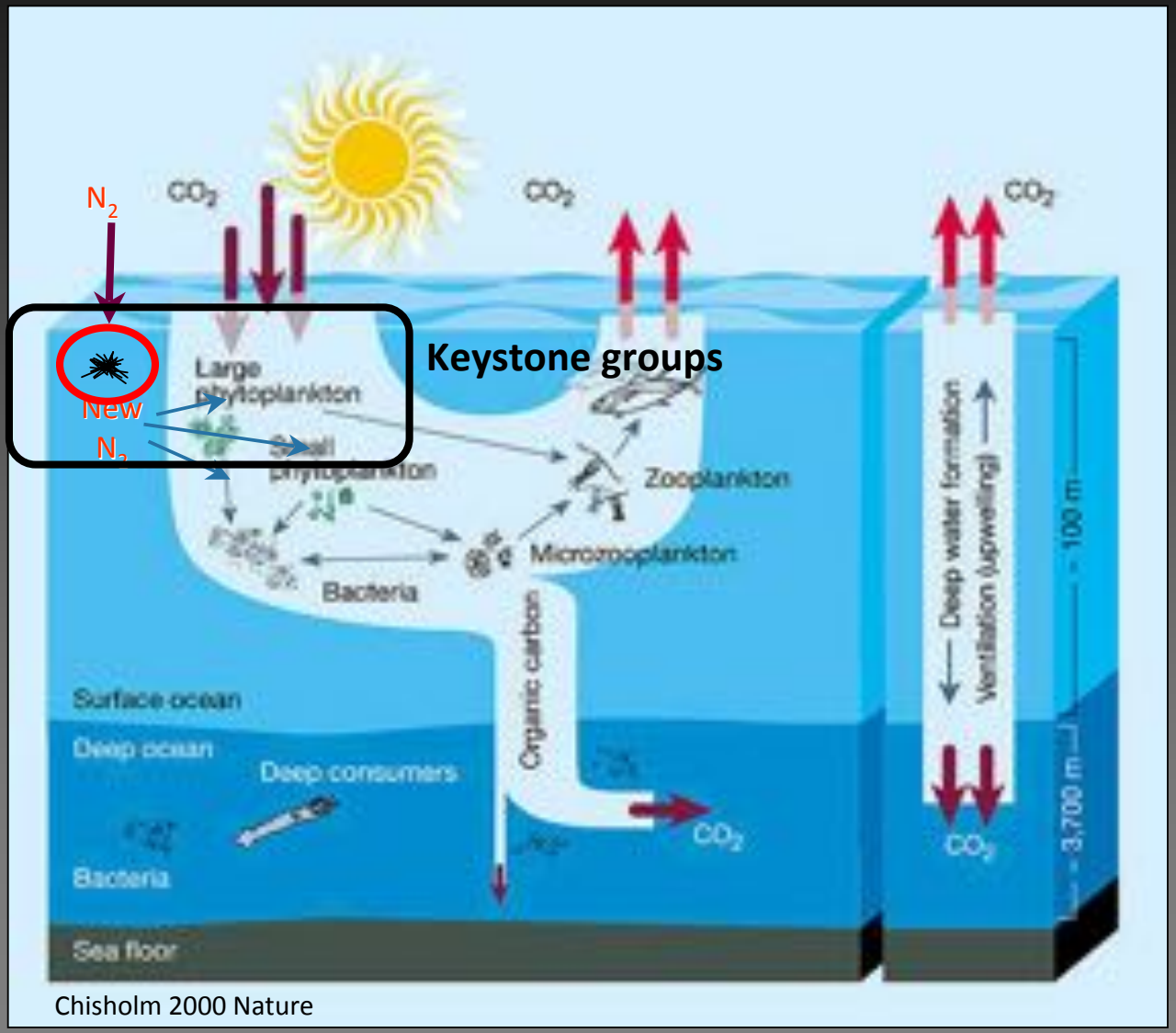
How do resources drive phytoplankton distributions and activities?

Focus on keystone groups

Haptophytes

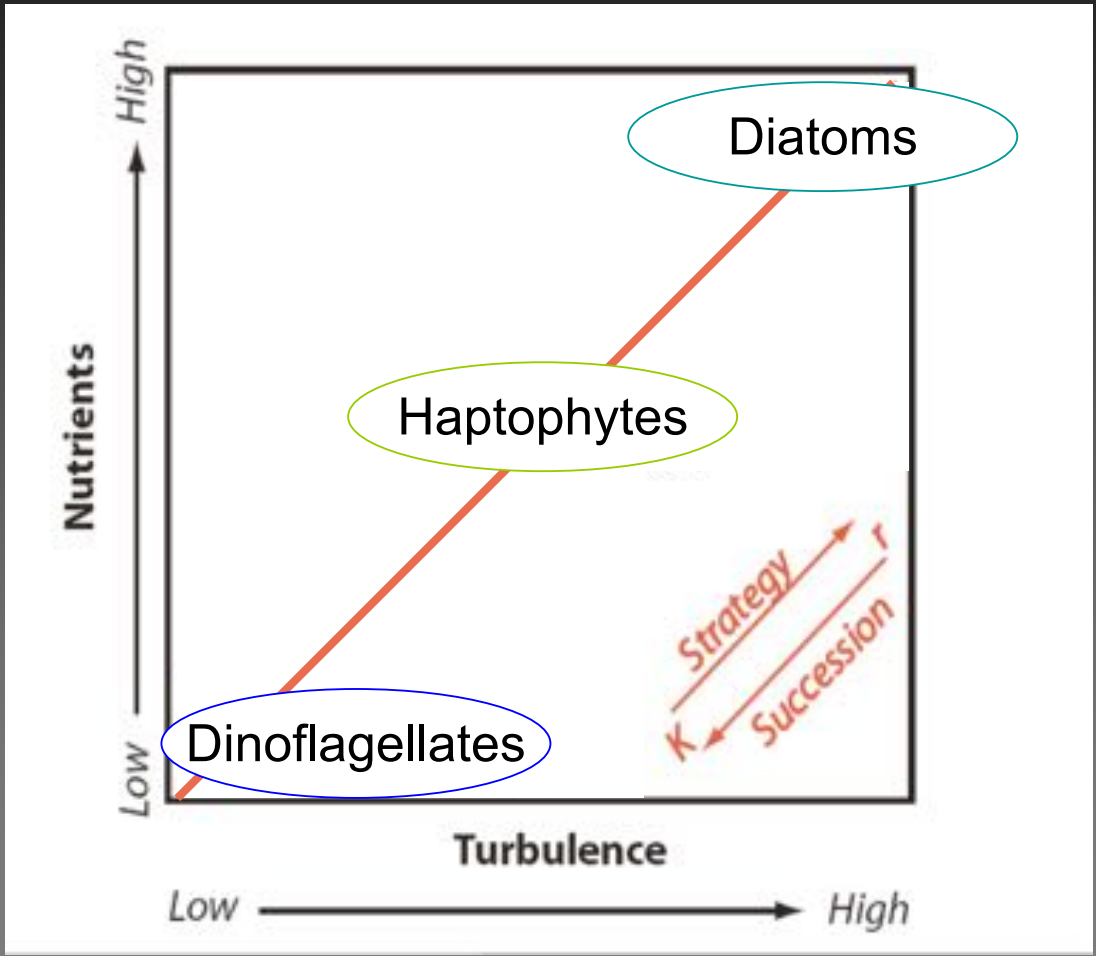
Diatoms

Dinoflagellates



Chisholm 2000 Nature

Margalef's Mandala - succession and ecological traits



Adapted from Margalef 1978

Two themes

Eco-evolutionary traits

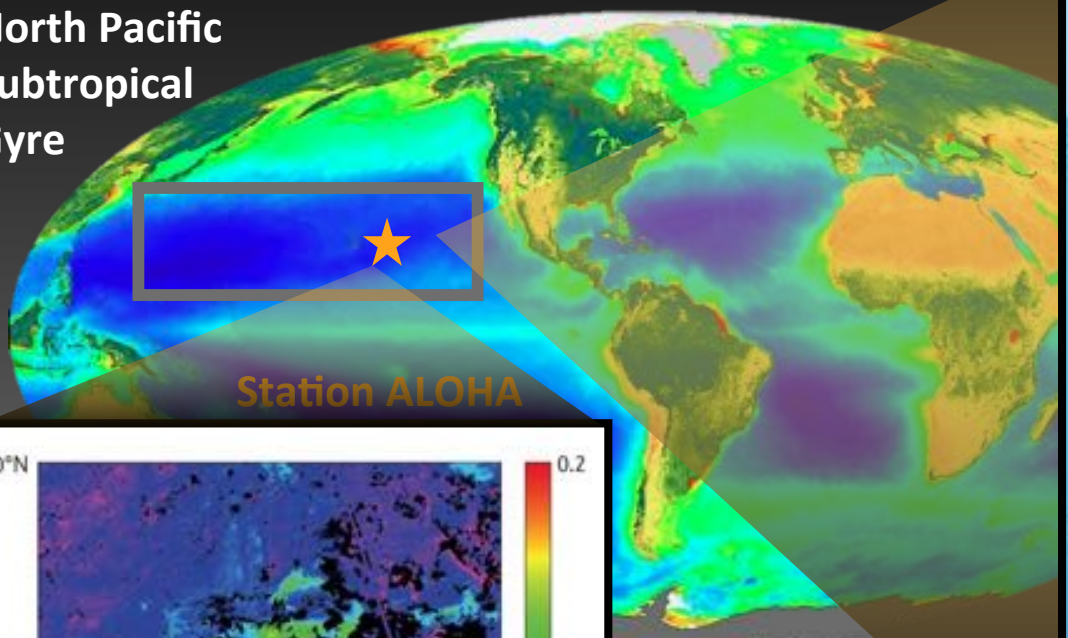
- Are there functional group traits that drive the bloom dynamics that structure the low nutrient North Pacific Subtropical Gyre?

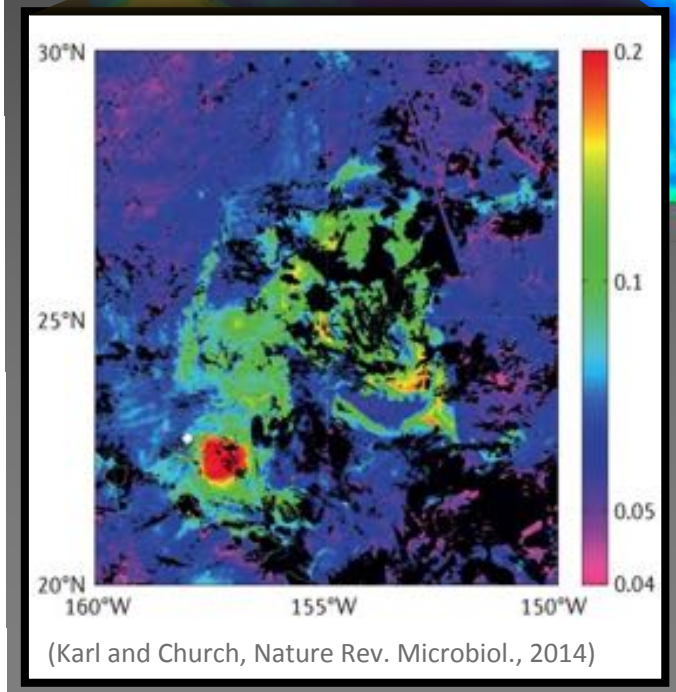
Metabolic plasticity and diversity

- How do diversity and metabolism influence the physiological ecology of a keystone group?

The significance of the NPSG

North Pacific Subtropical Gyre





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

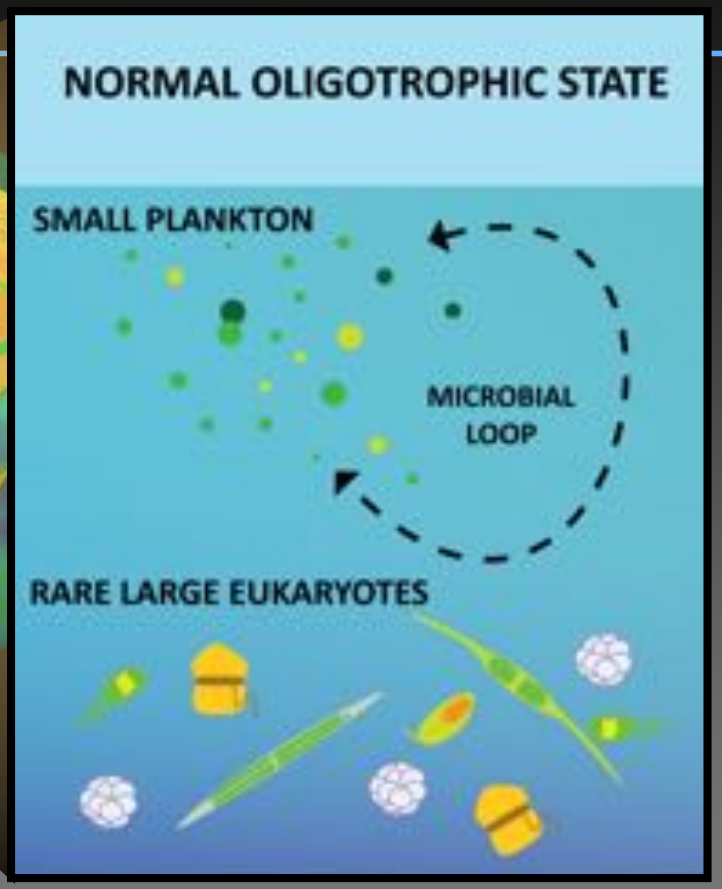
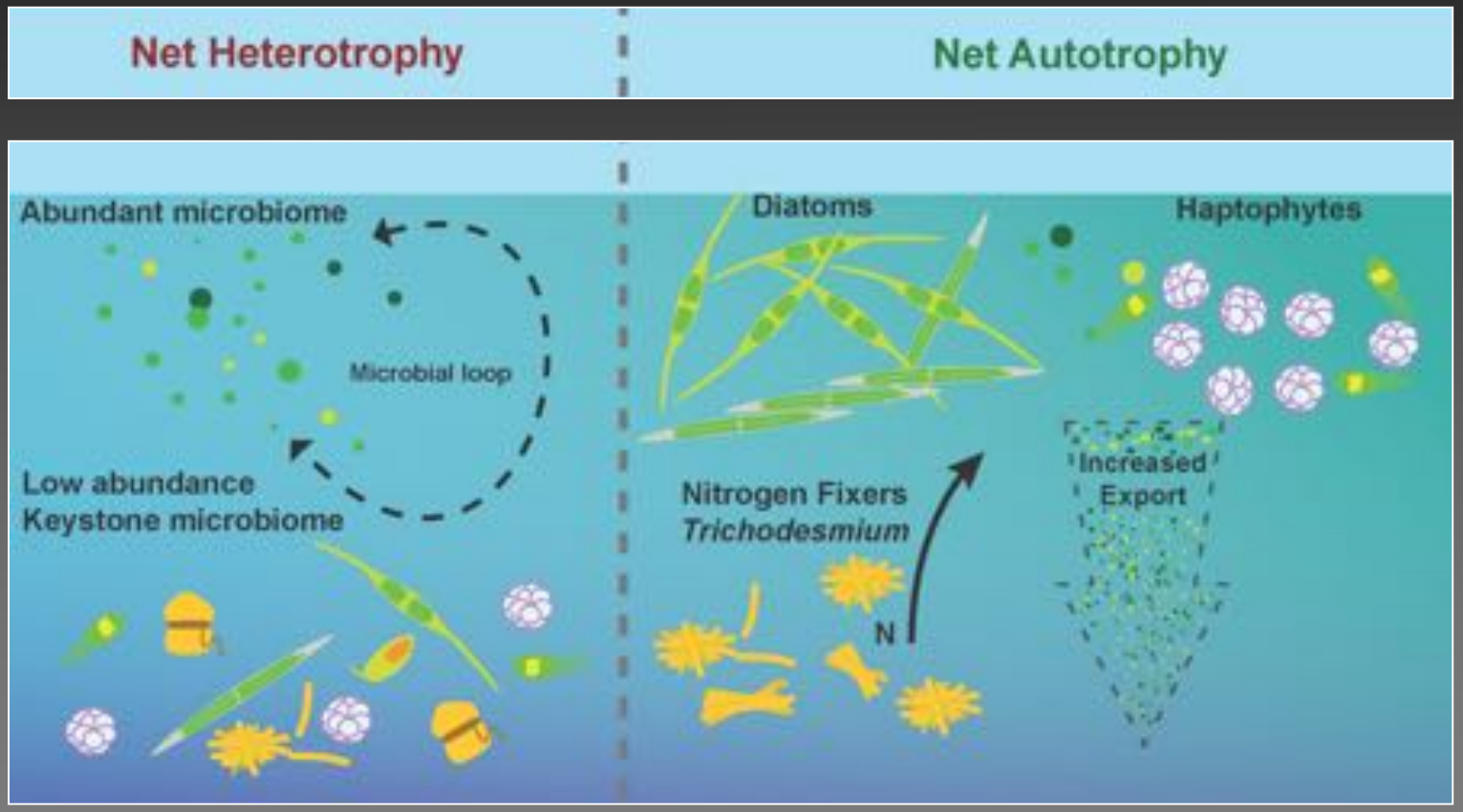


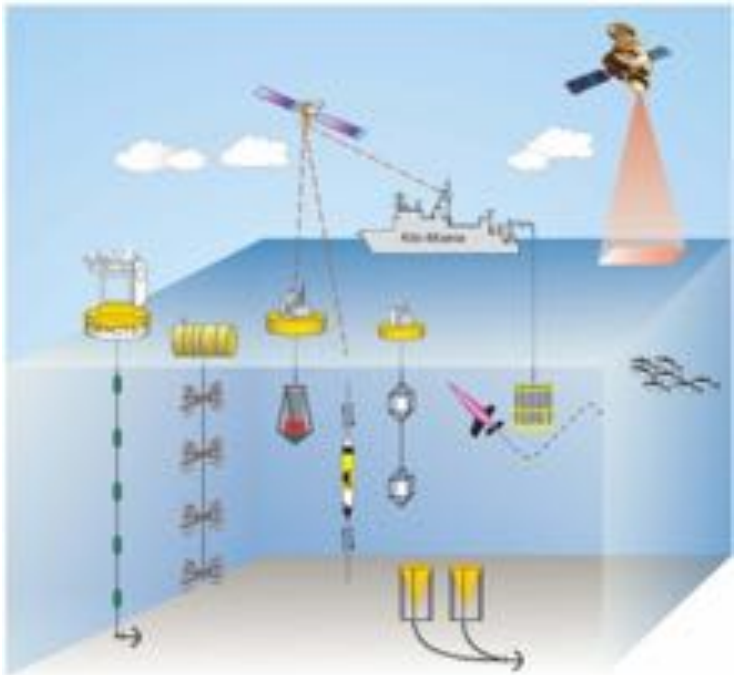
Image credits: SeaWIFS Global Chlorophyll

Blooms of keystone species can shift ecosystem state



Nutrient input,or other forcings

Hawaii Ocean Experiment: Dynamics of Light and Nutrients



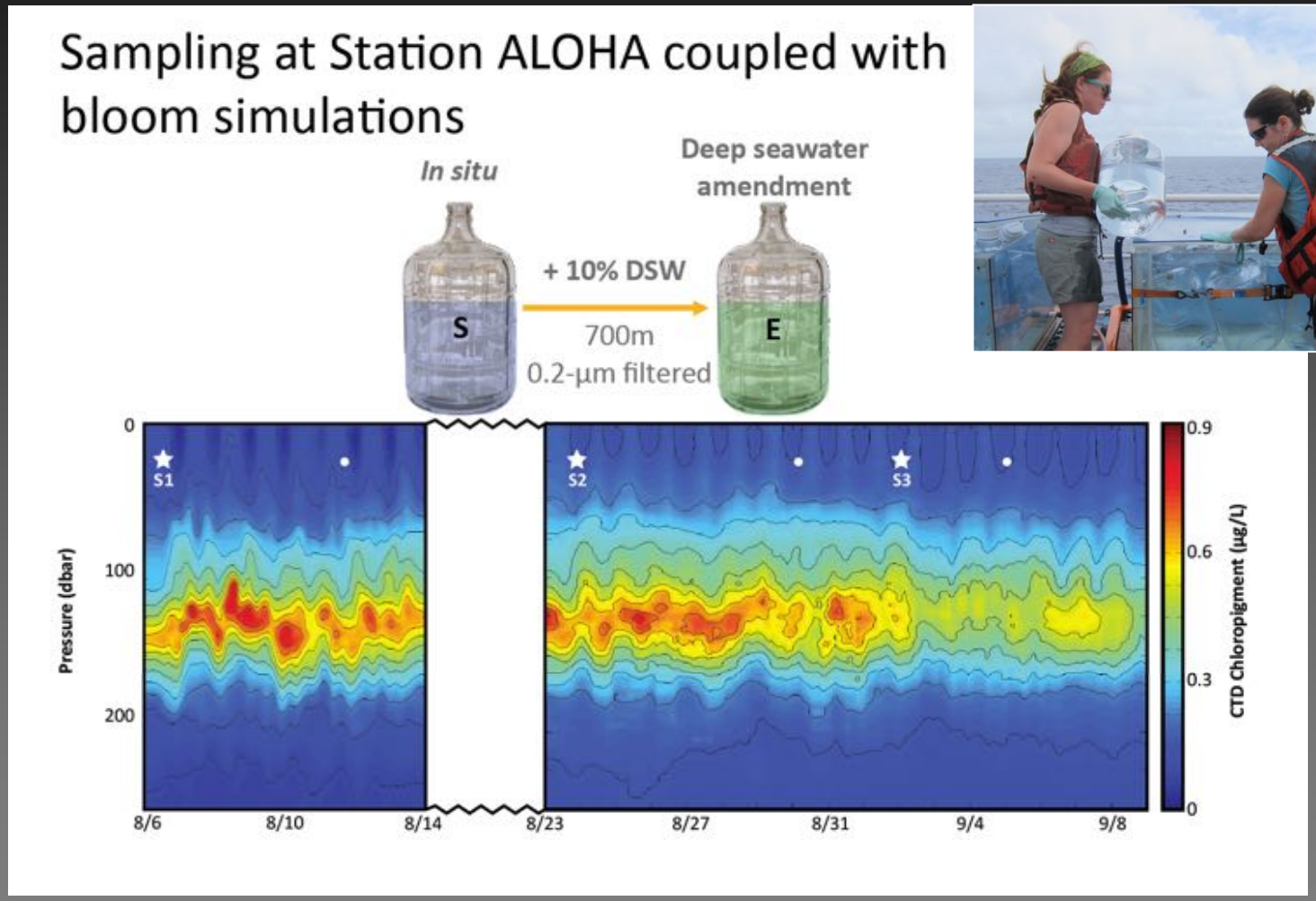
Wilson et al. 2015 *GBC*

center for microbial oceanography: research and education

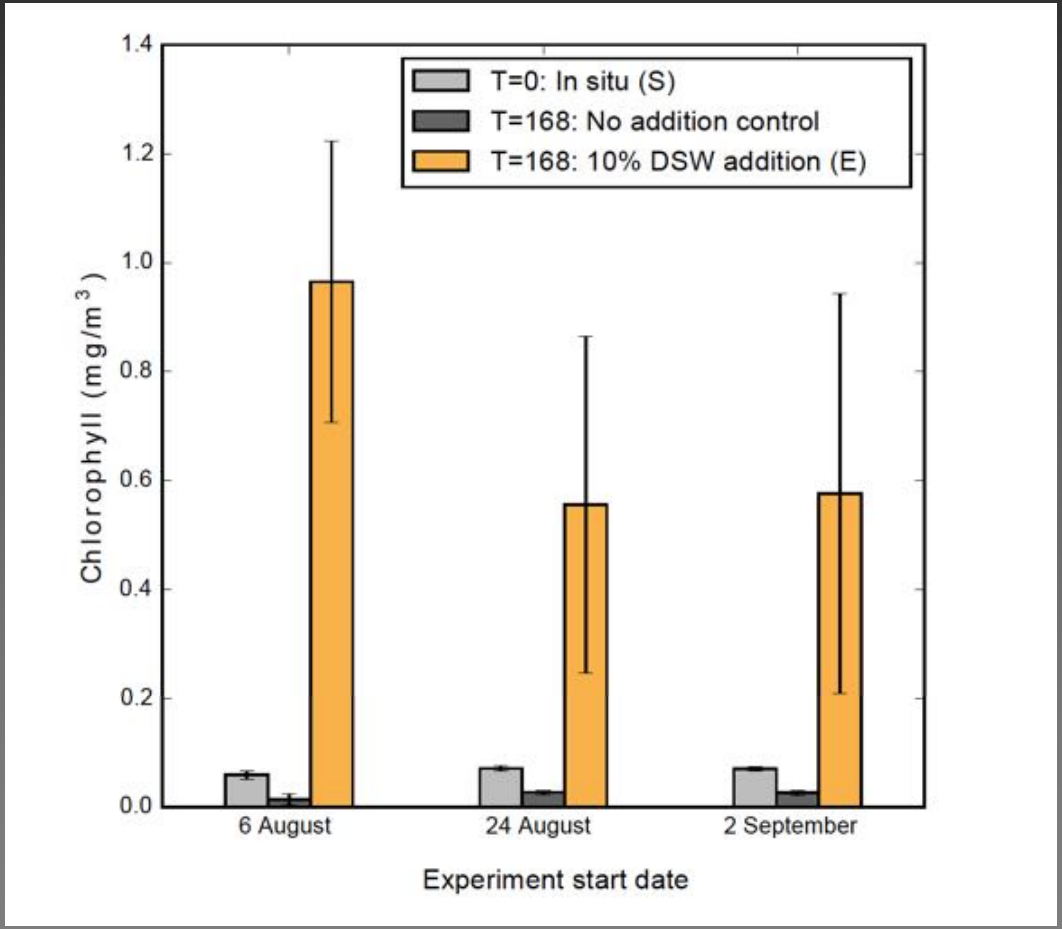
C·more *linking genomes to biomes*

(HOE-DYLAN)

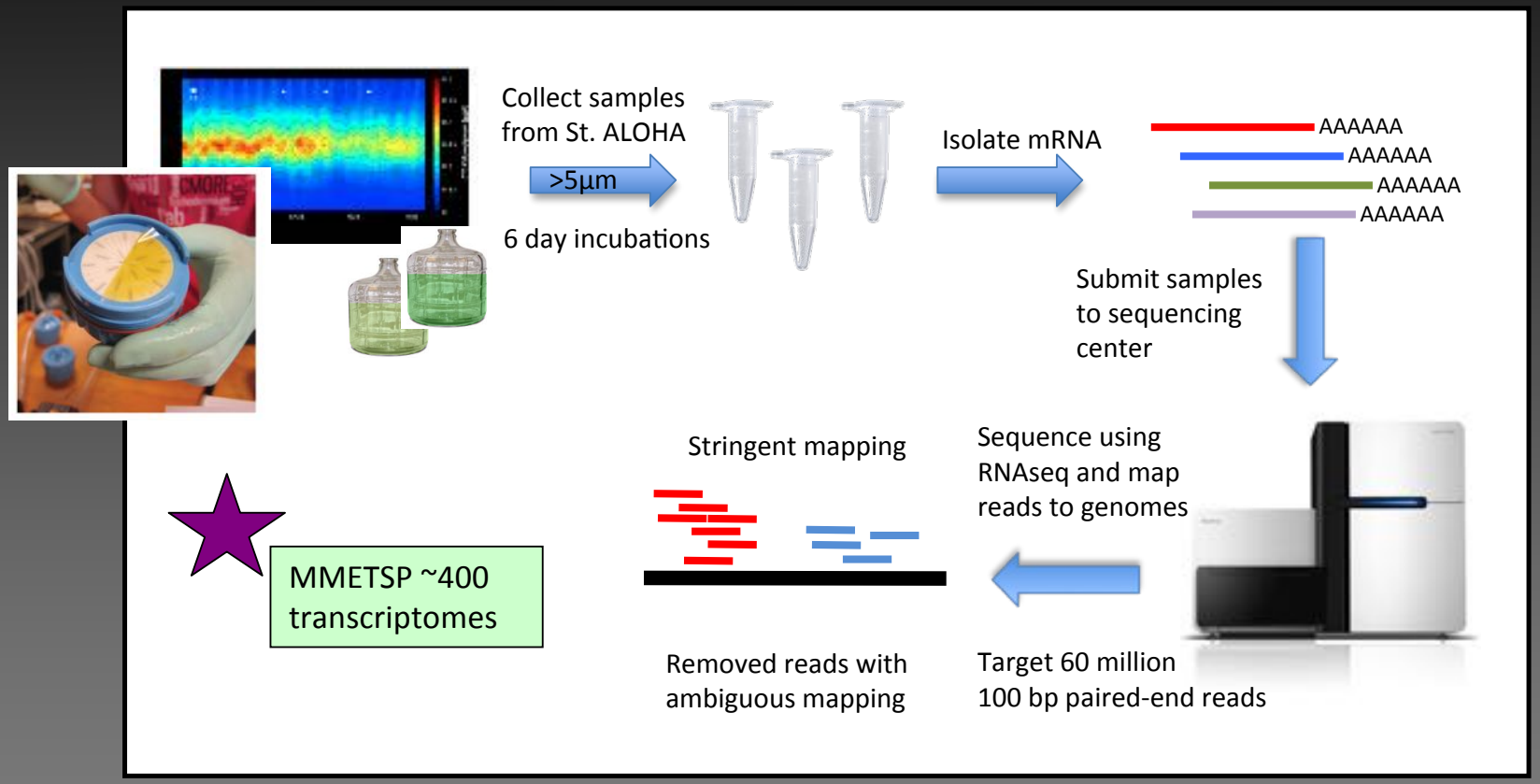
Sampling a bloom



Deep water addition led to simulated “bloom”

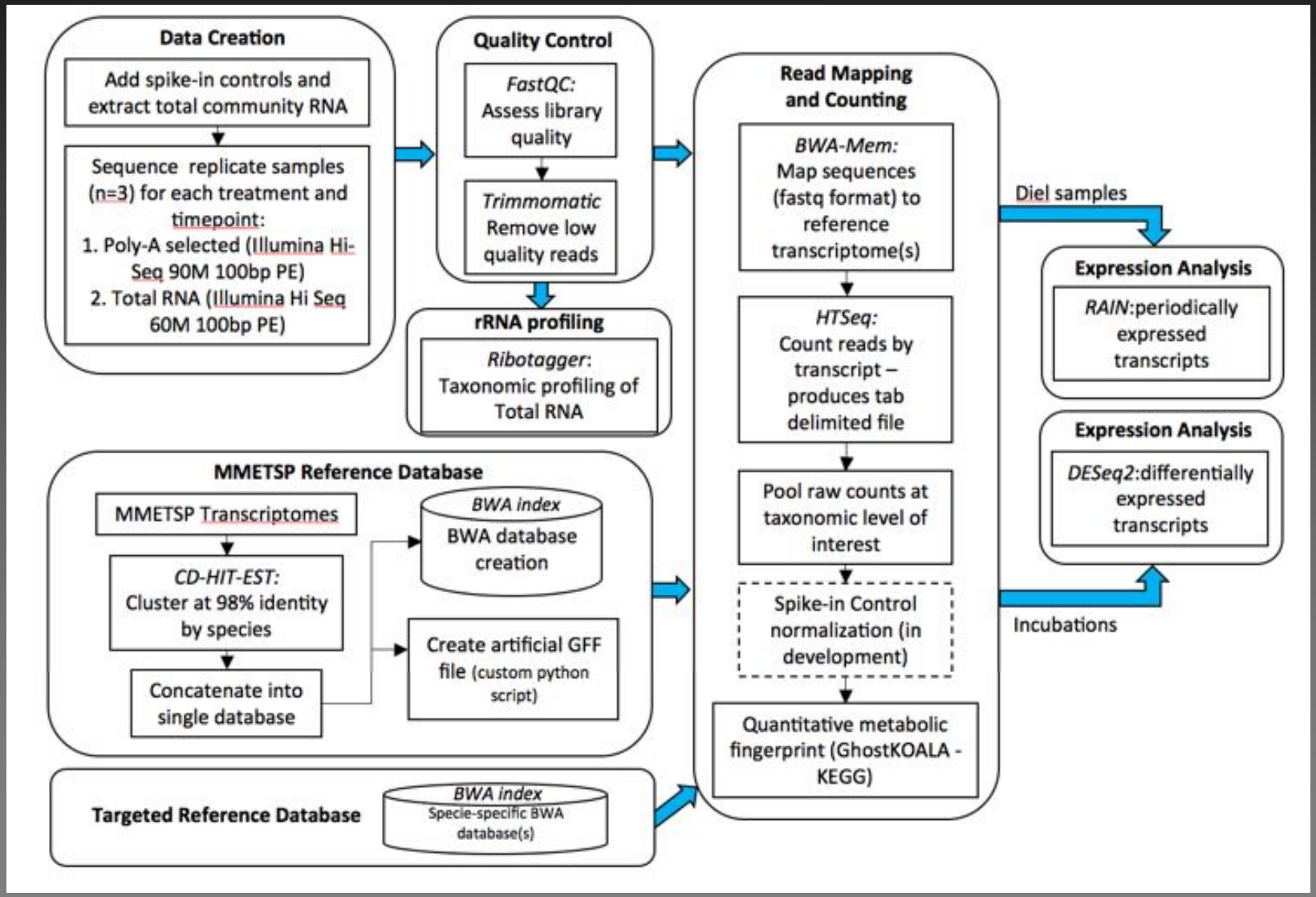


Sampling and pipeline

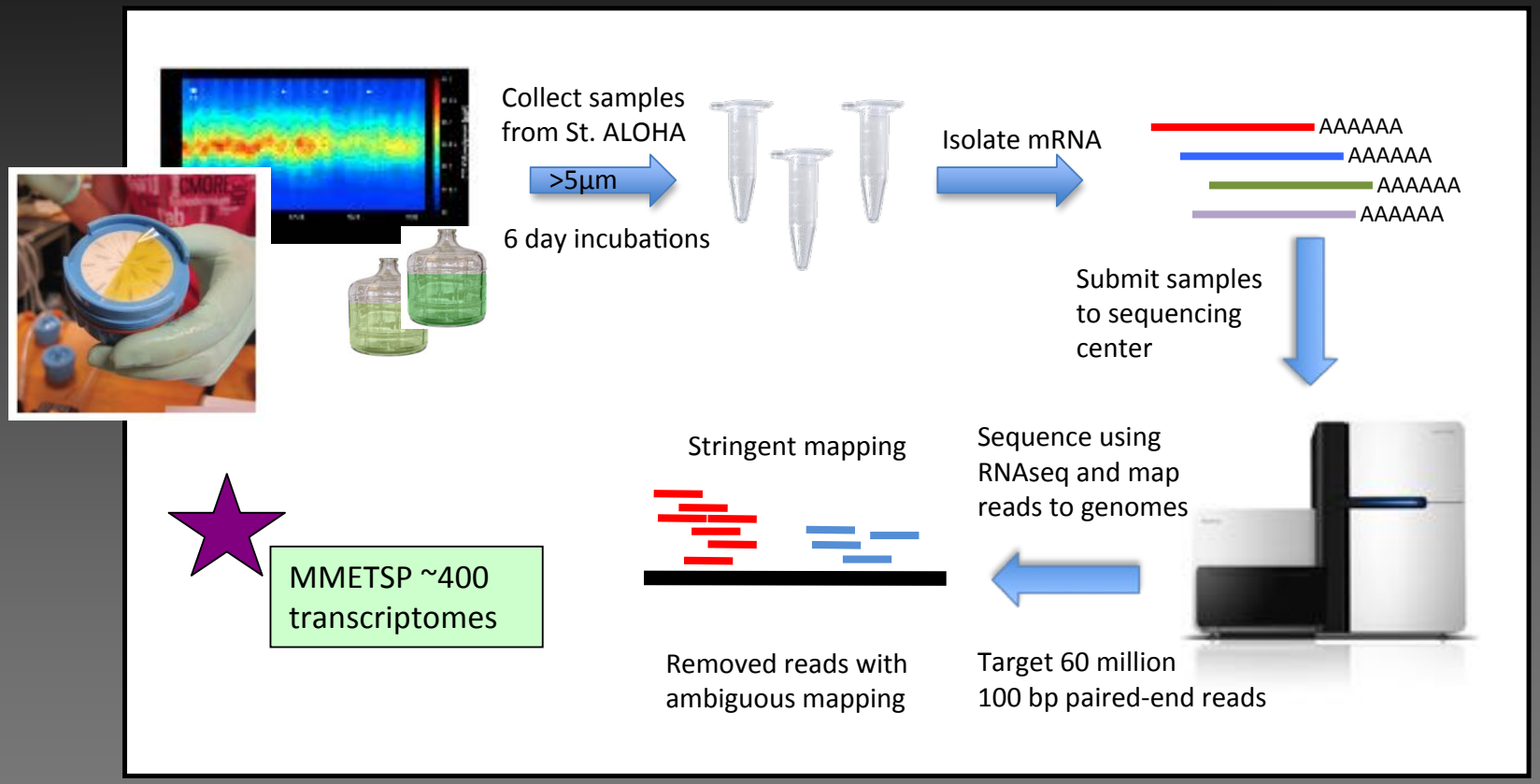


MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Sequencing and analysis strategy



Sampling and pipeline



MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Marine Microbial Eukaryote Transcriptome Sequencing Project

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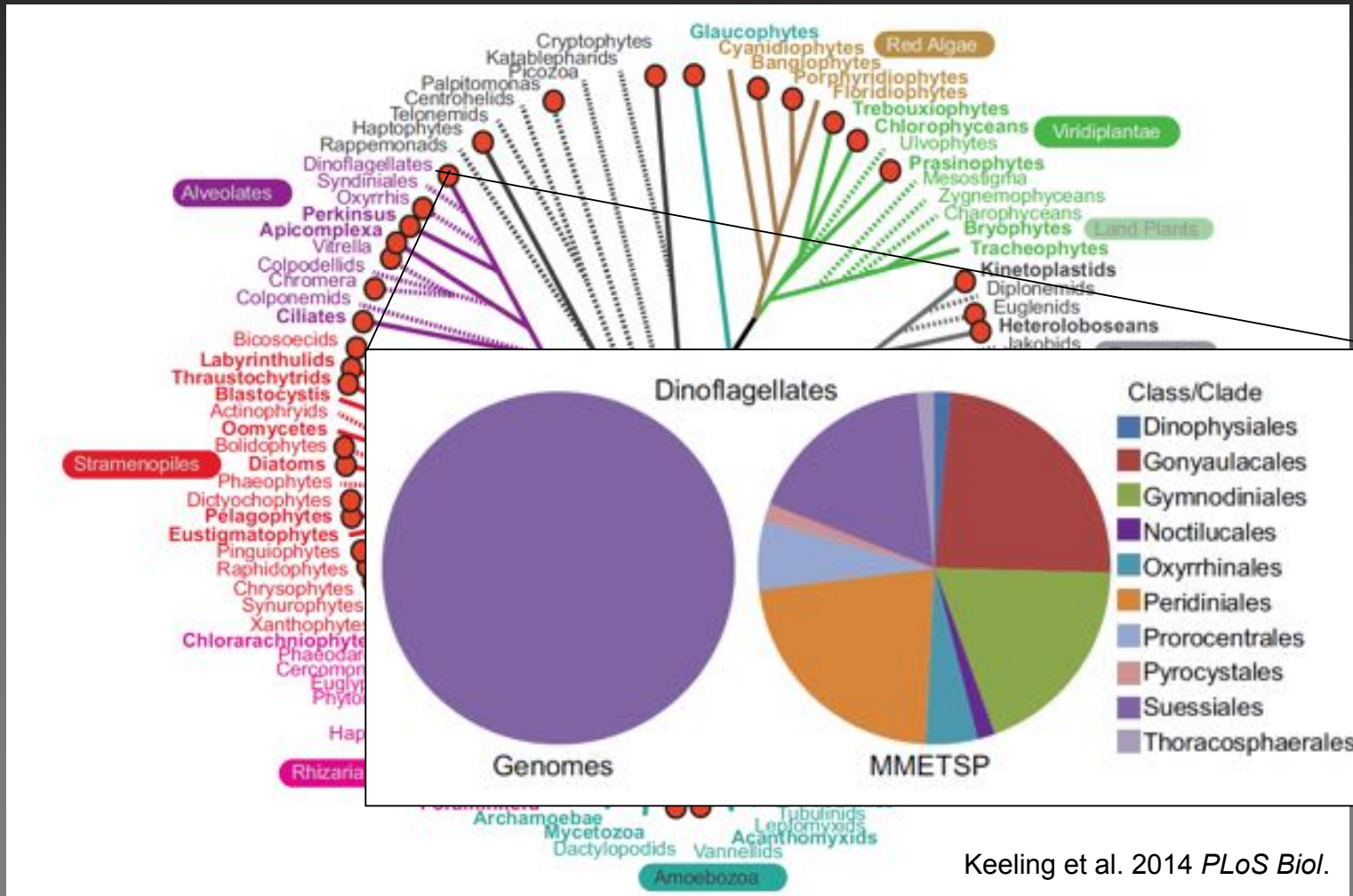
Community Page

The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): Illuminating the Functional Diversity of Eukaryotic Life in the Oceans through Transcriptome Sequencing

Patrick J. Keeling[1,2*], Fabien Burki[1], Heather M. Wilcox[3], Bassem Allam[4], Eric E. Allen[5], Linda A. Amaral-Zettler[6,7], E. Virginia Armbrust[8], John M. Archibald[2,9], Arvind K. Bharti[10], Callum J. Bell[10], Bank Beszteri[11], Kay D. Bidle[12], Connor T. Cameron[10], Lisa Campbell[13], David A. Caron[14], Rose Ann Cattolico[15], Jackie L. Collier[4], Kathryn Coyne[16], Simon K. Davy[17], Phillipe Deschamps[18], Sonya T. Dyhrman[19], Bente Edvardsen[20], Ruth D. Gates[21], Christopher J. Gobler[4], Spencer J. Greenwood[22], Stephanie M. Guida[10], Jennifer L. Jacobi[10], Kjetill S. Jakobsen[20], Erick R. James[1], Bethany Jenkins[23,24], Uwe John[11], Matthew D. Johnson[25], Andrew R. Juhl[19], Anja Kamp[26,27], Laura A. Katz[28], Ronald Kiene[29], Alexander Kudryavtsev[30,31], Brian S. Leander[1], Senjie Lin[32], Connie Lovejoy[33], Denis Lynn[34,35], Adrian Marchetti[36], George McManus[32], Aurora M. Nedelcu[37], Susanne Menden-Deuer[24], Cristina Miceli[38], Thomas Mock[39], Marina Montresor[40], Mary Ann Moran[41], Shauna Murray[42], Govind Nadathur[43], Satoshi Nagai[44], Peter B. Ngam[10], Brian Palenik[5], Jan Pawlowski[31], Giulio Petroni[45], Gwenael Piganeau[46,47], Matthew C. Posewitz[48], Karin Rengefors[49], Giovanna Romano[40], Mary E. Rumpho[50], Tatiana Rynearson[24], Kelly B. Schilling[10], Declan C. Schroeder[51], Alastair G. B. Simpson[2,52], Claudio H. Slamovits[2,9], David R. Smith[53], G. Jason Smith[54], Sarah R. Smith[5], Heidi M. Sosik[25], Peter Stief[26], Edward Theriot[55], Scott N. Twary[56], Pooja E. Umale[10], Daniel Vaulot[57], Boris Wawrik[58], Glen L. Wheeler[51,59], William H. Wilson[60], Yan Xu[61], Adriana Zingone[40], Alexandra Z. Worden[2,3*]

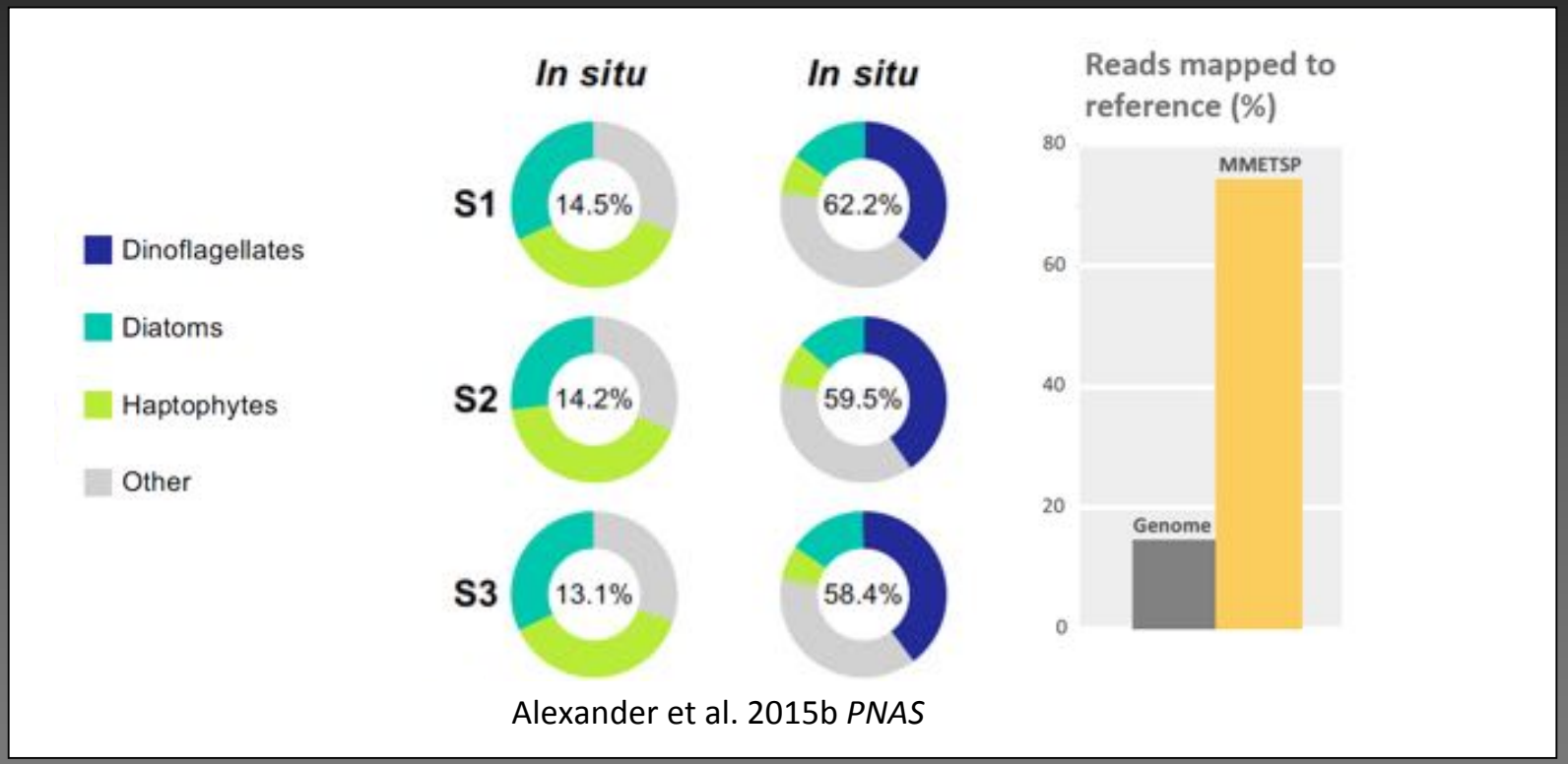
Greatly expanded reference sequences in the tree of life



Keeling et al. 2014 *PLoS Biol*.

MMETSP improves read identification

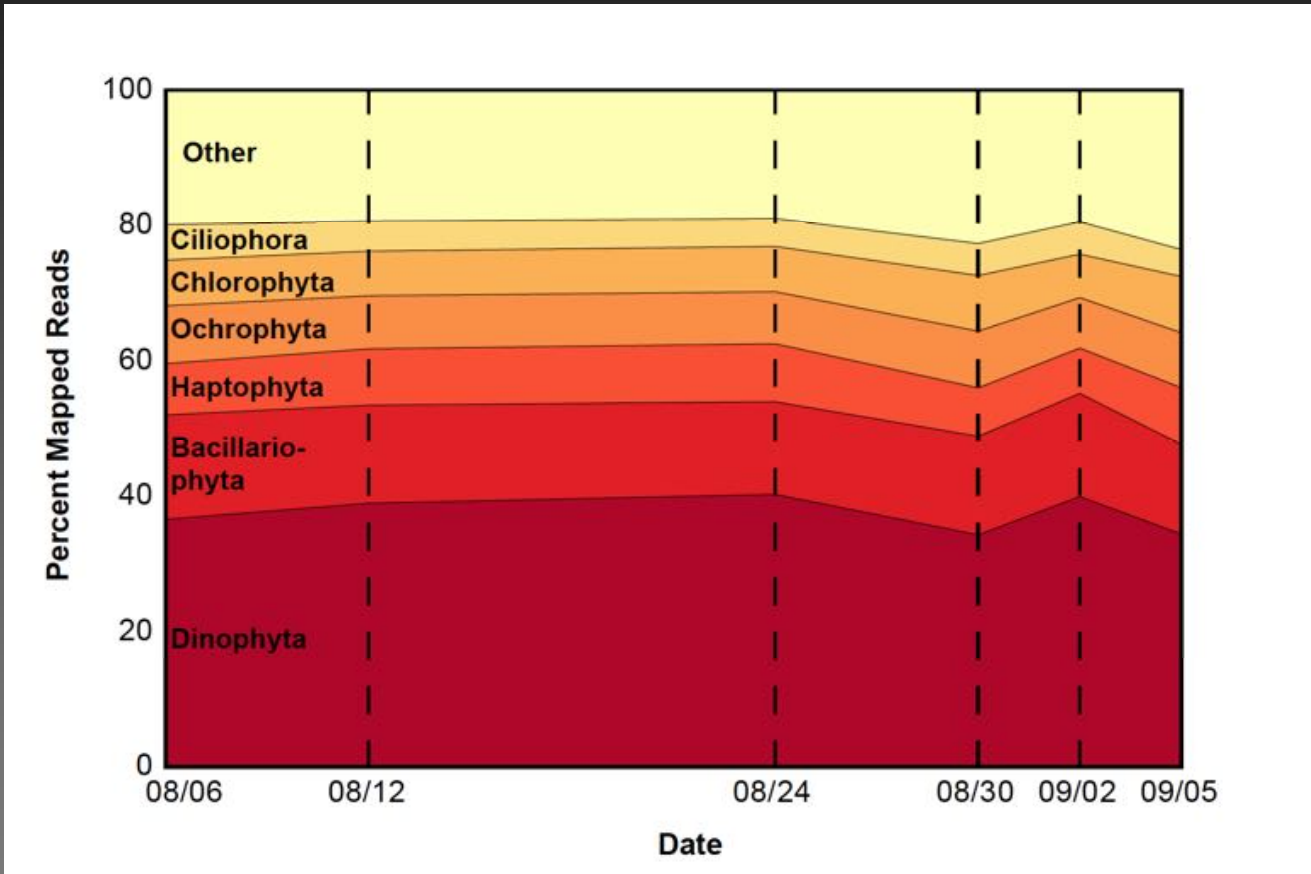
Read mapping against genomes v. MMETSP at St. ALHOA



Alexander et al. 2015b *PNAS*

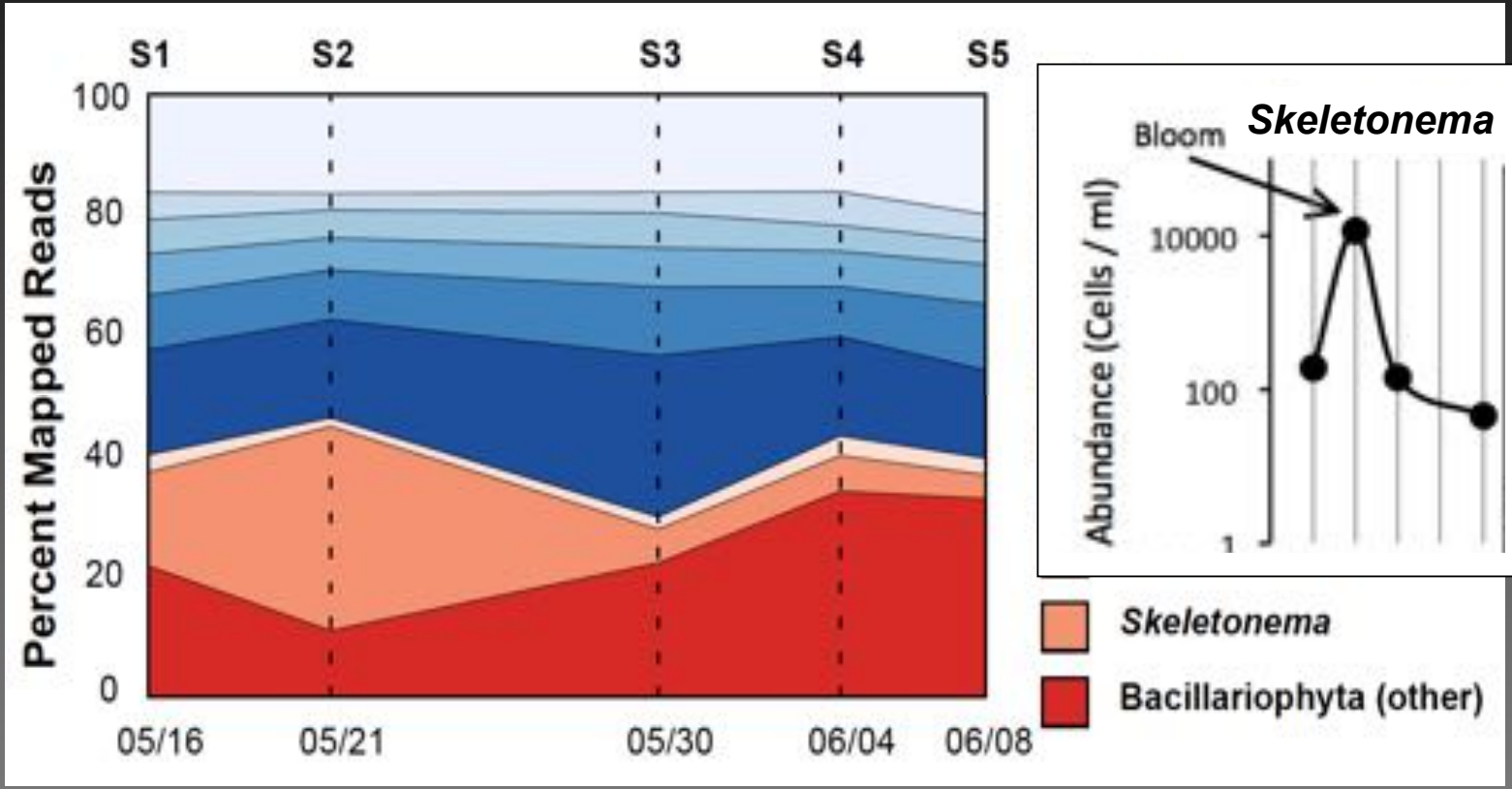
Sequence read identification is substantially improved over using phytoplankton genomes which do not capture the same diversity.

Taxonomic distribution of reads



Distributions is highly stable, much more so than what was observed in coastal system.

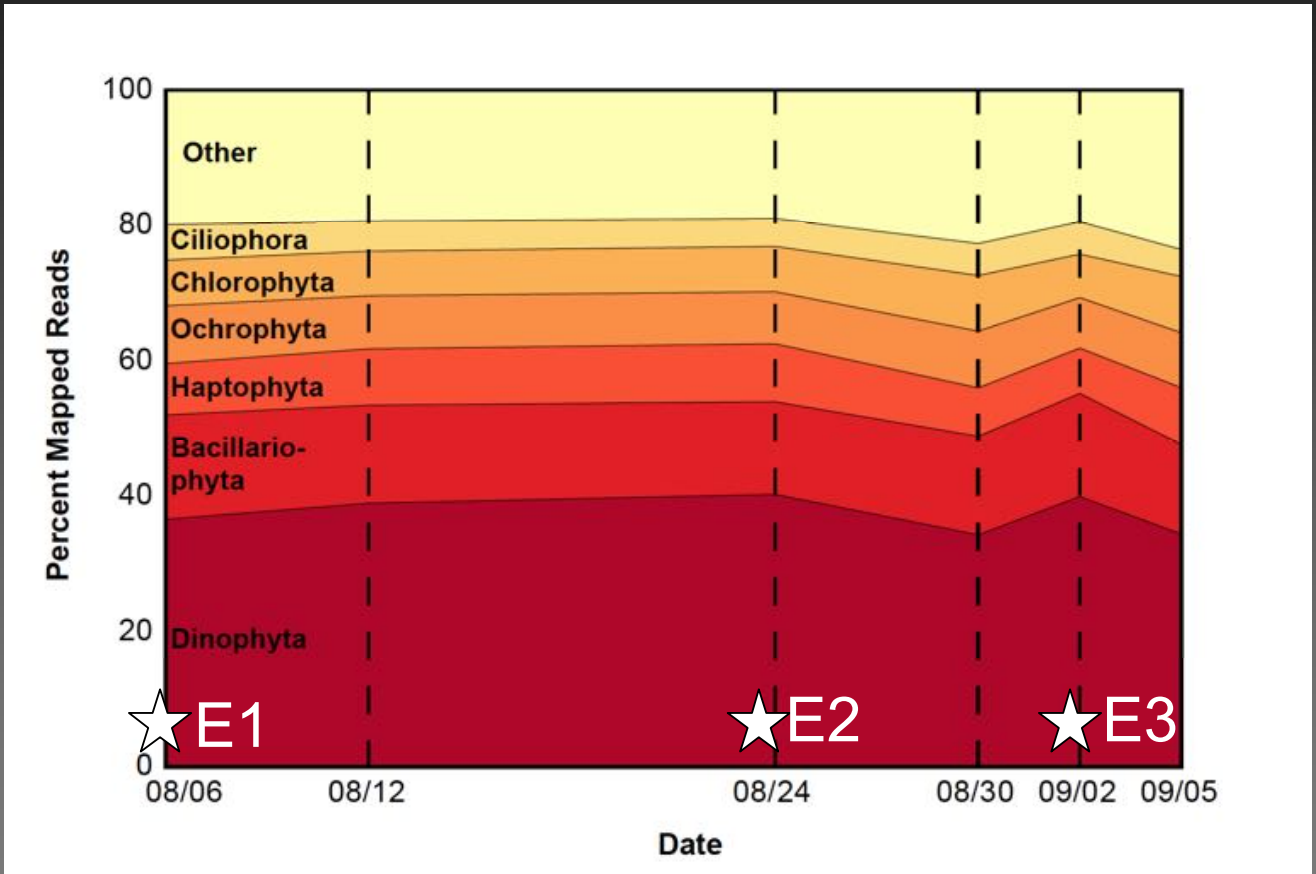
Taxonomic distribution of reads - coastal bloom



Alexander et al. 2015 *PNAS*

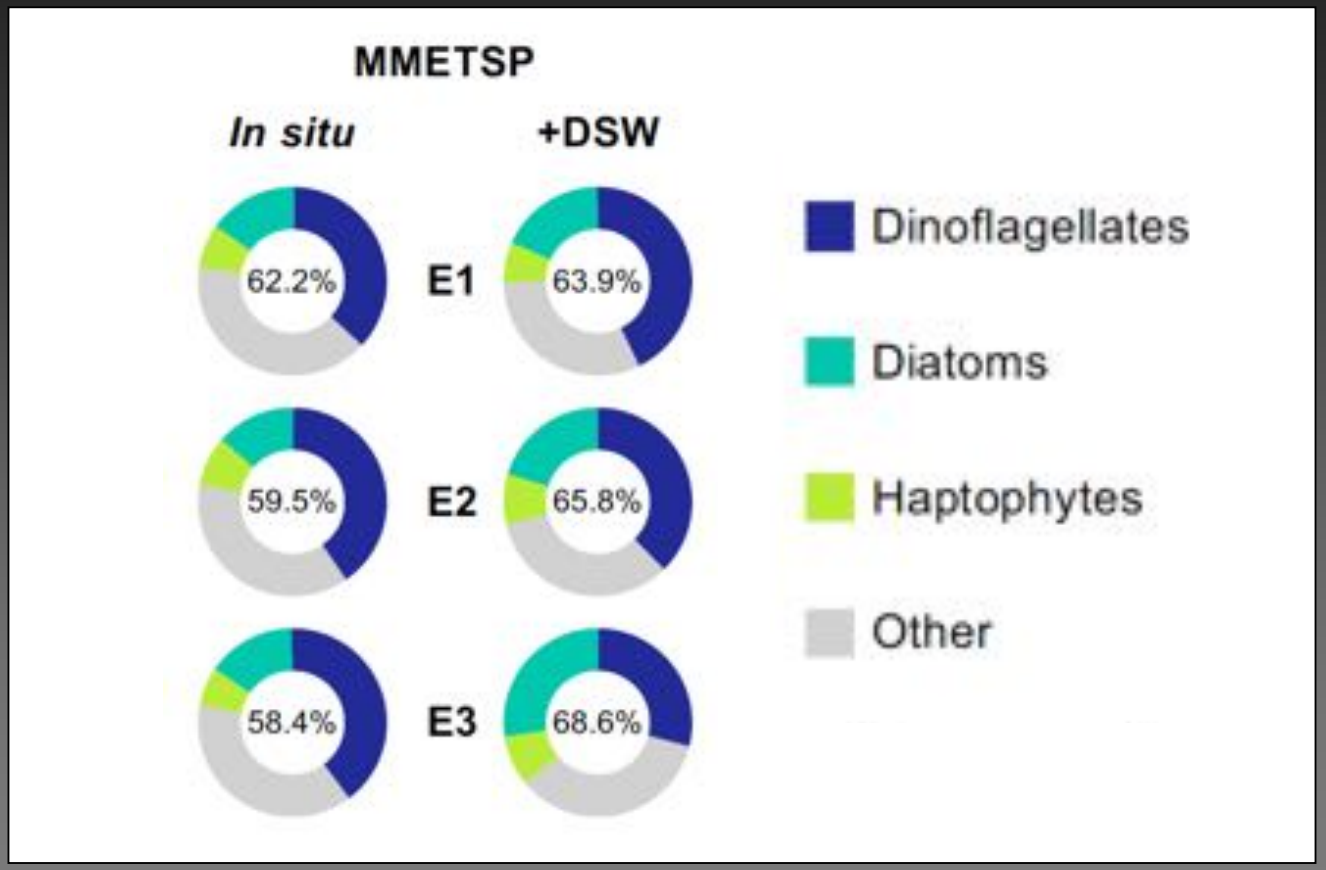
Taxonomic distribution of reads is more variable in a coastal system

Taxonomic distribution of reads



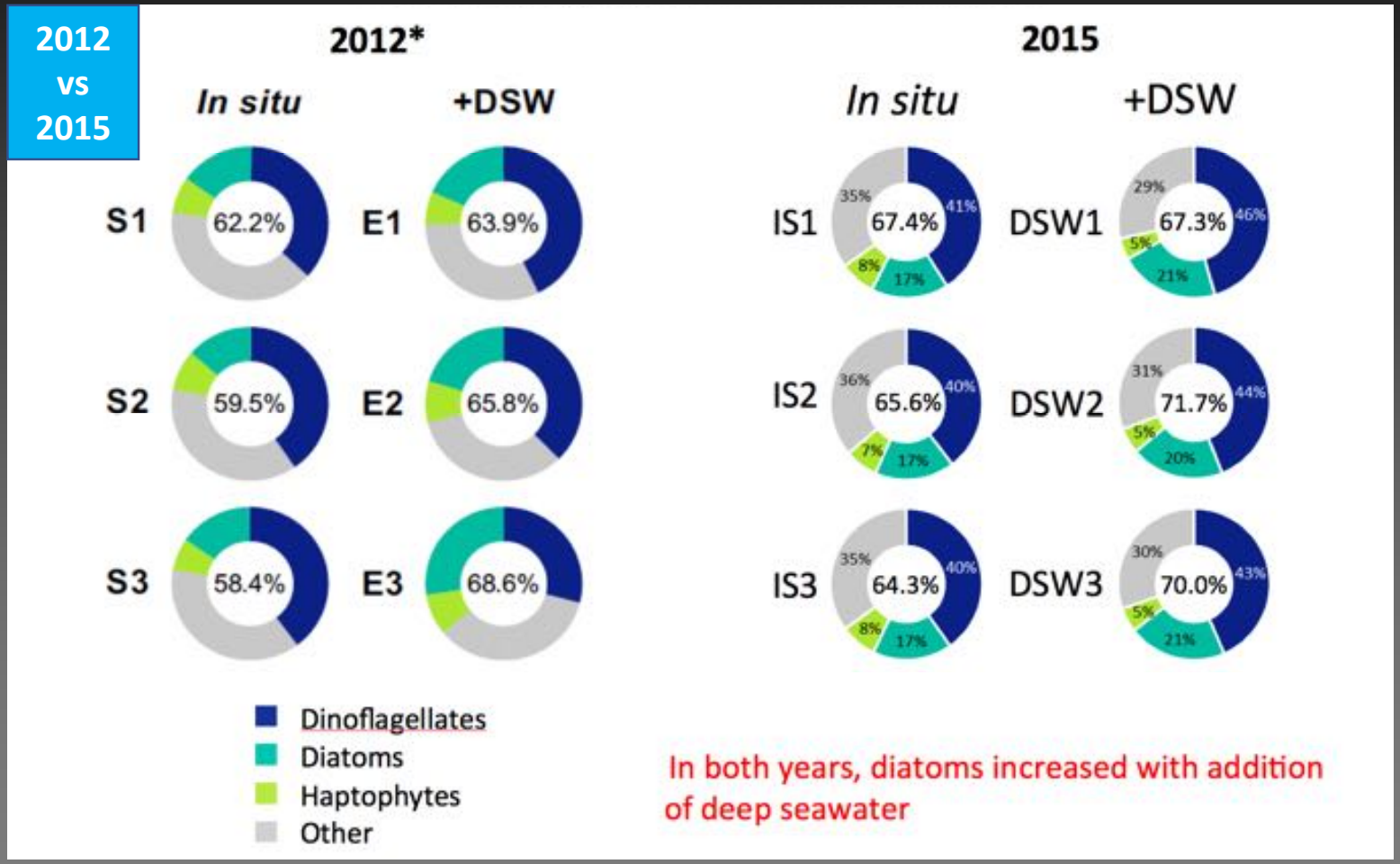
Alexander et al. 2015b *PNAS*

Increase in diatoms during simulated blooms



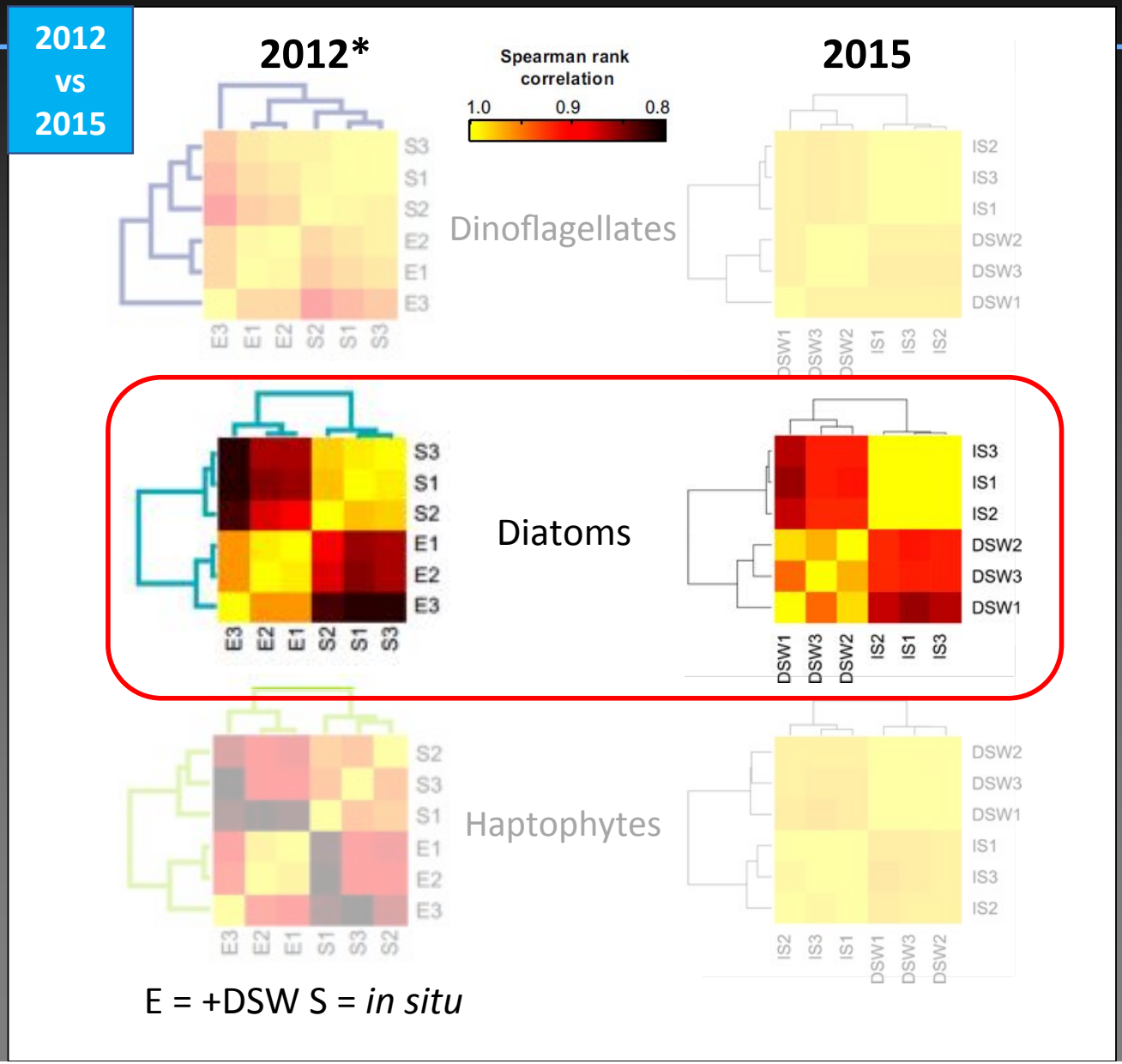
Read identification is robust, most are dinoflagellates, but diatoms increase in simulated blooms (+DSW)

Simulated bloom response was reproducible 3 years later

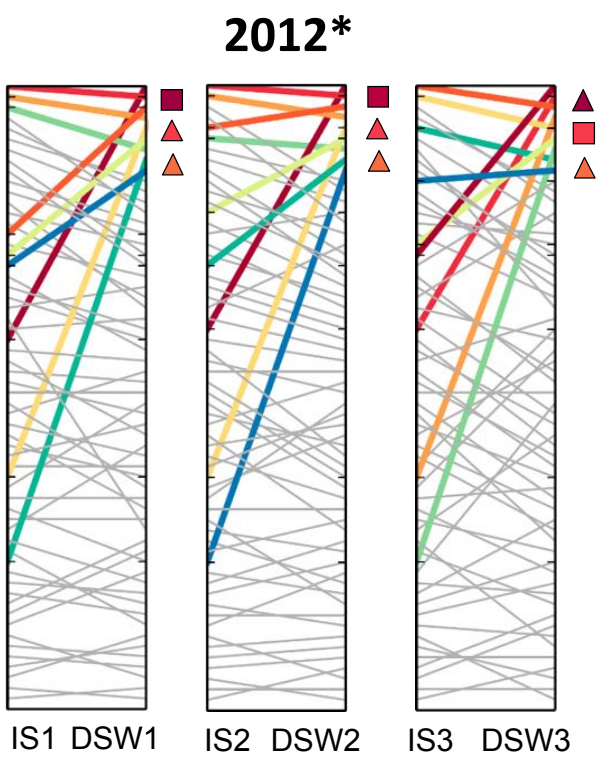


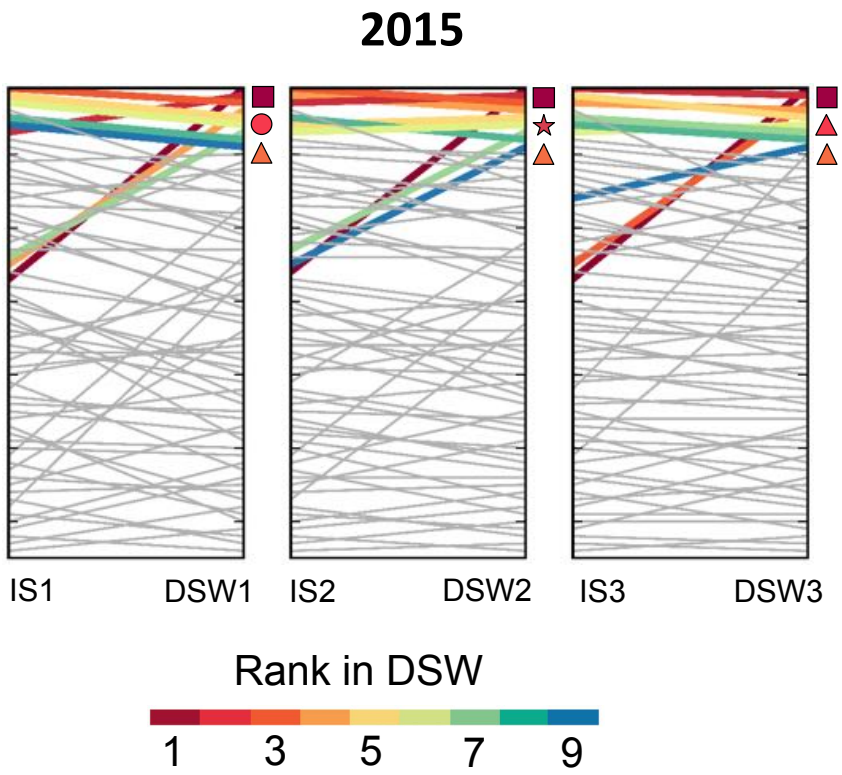
Read identification increases with larger database, remarkable similarity in response between years

Community composition shifts between years



Its even the same species changing…

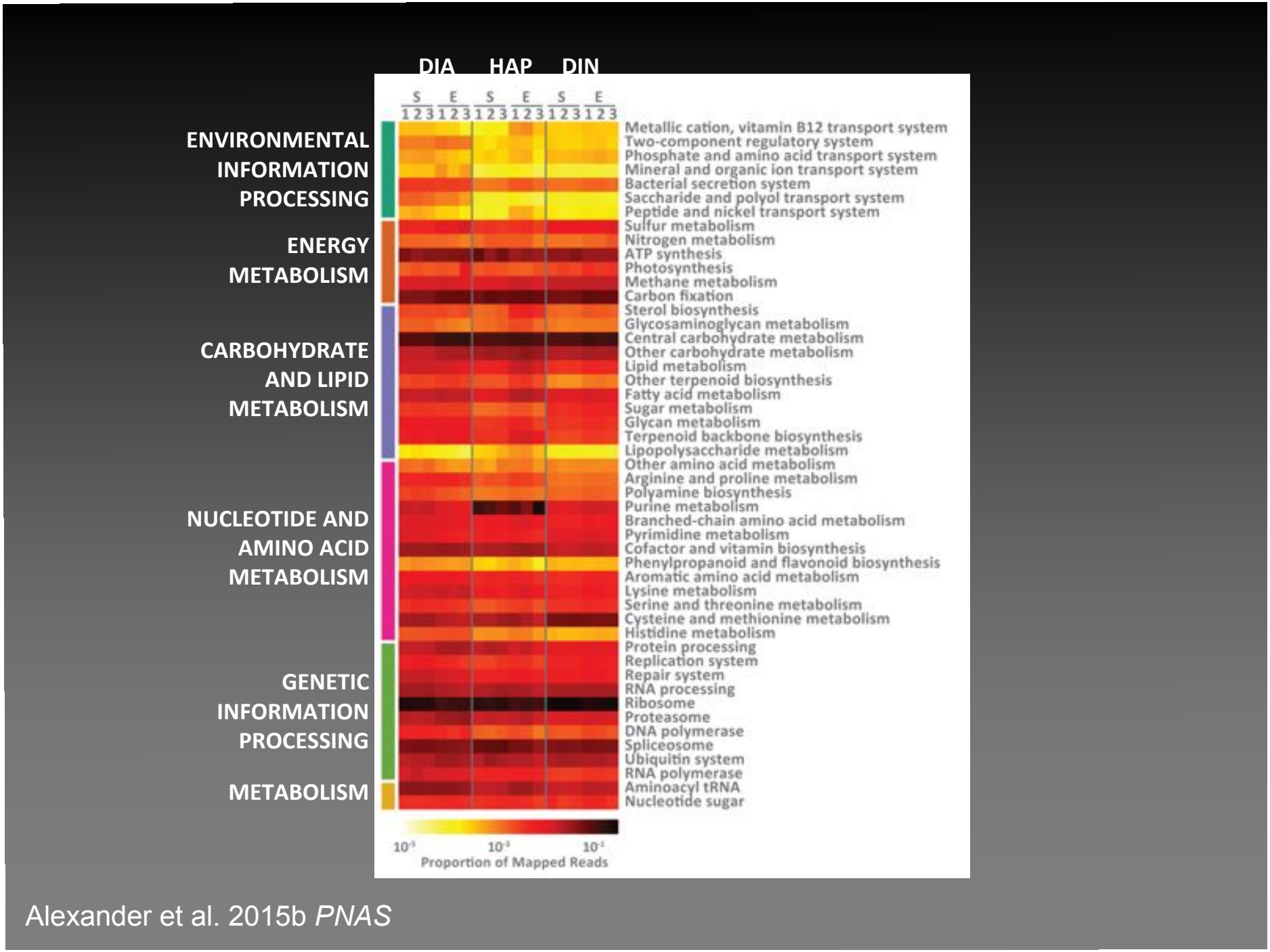




2012 vs 2015

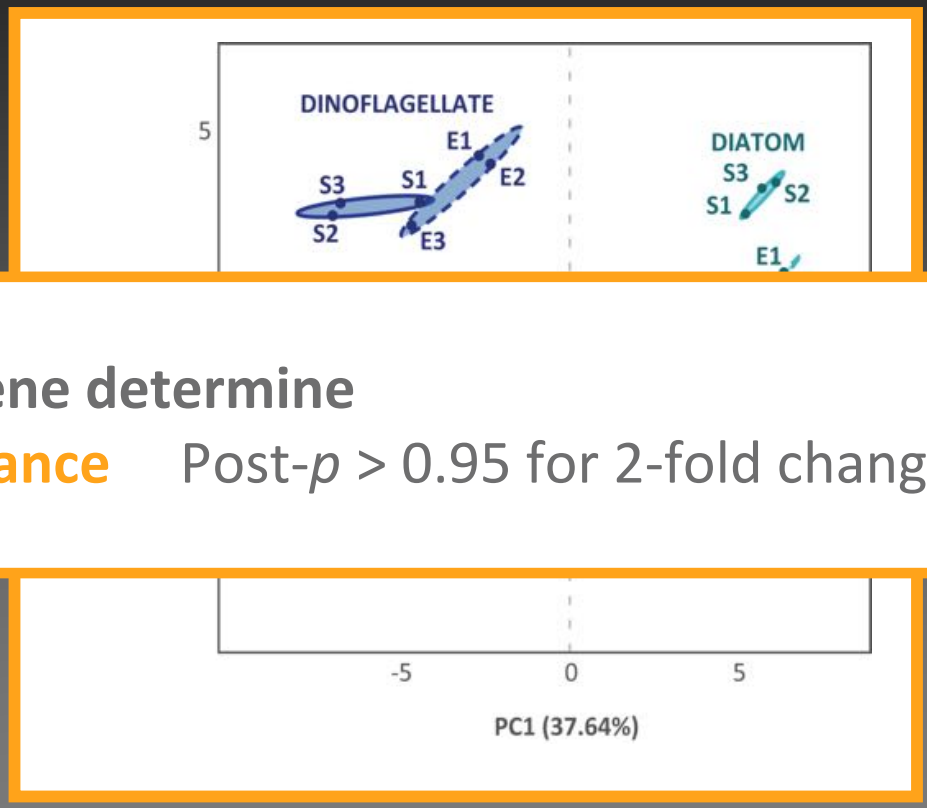
□ *Cylindrotheca sp.*
△ *Pseudo-nitzschia sp.*
○ *Chaetoceros sp.*
☆ *Ditylum sp.*

In both years, *Cylindrotheca* sp. and *Pseudo-nitzschia* sp. dominate with DSW addition



Alexander et al. 2015b *PNAS*

Quantitative metabolic fingerprint

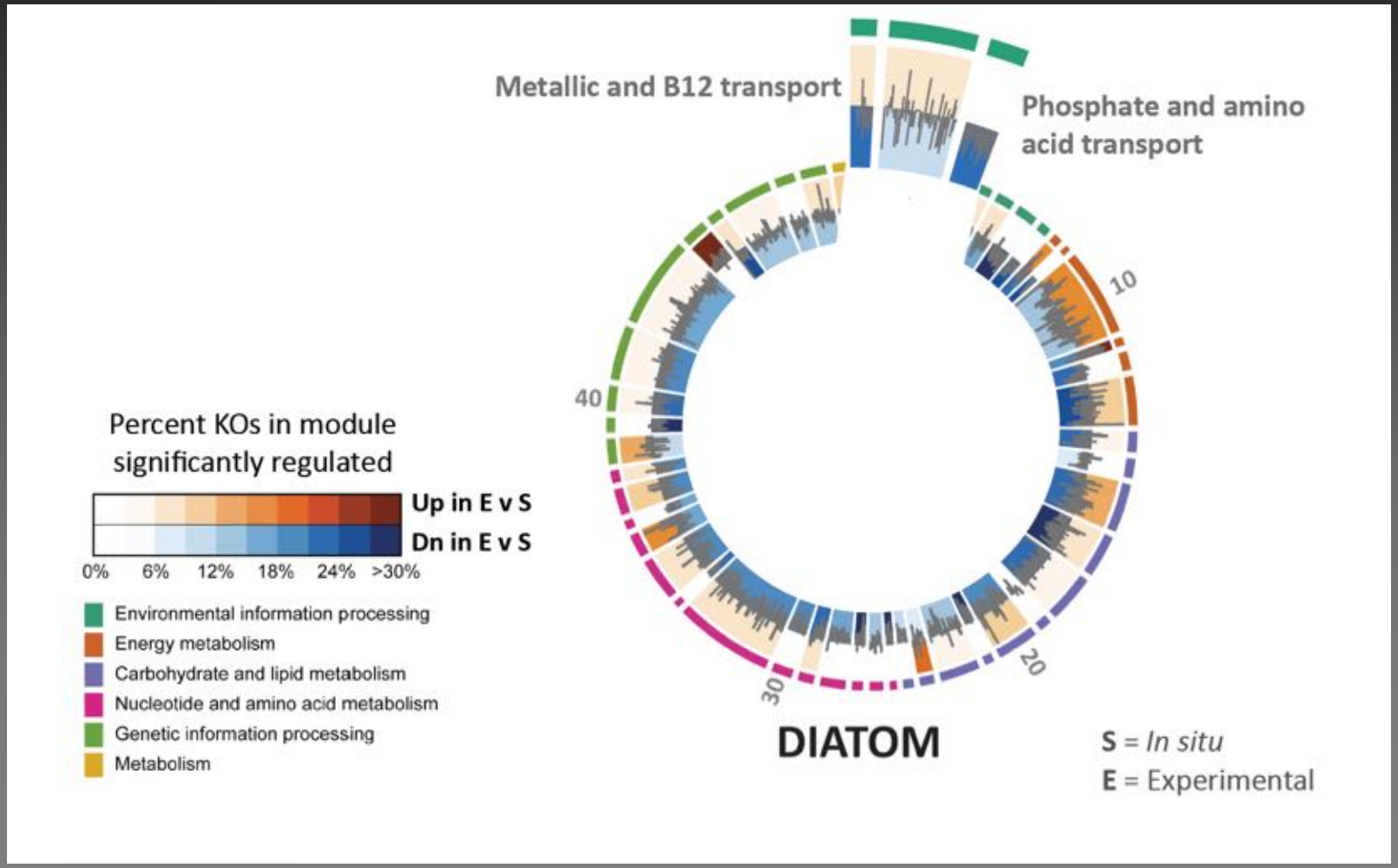


For each gene determine

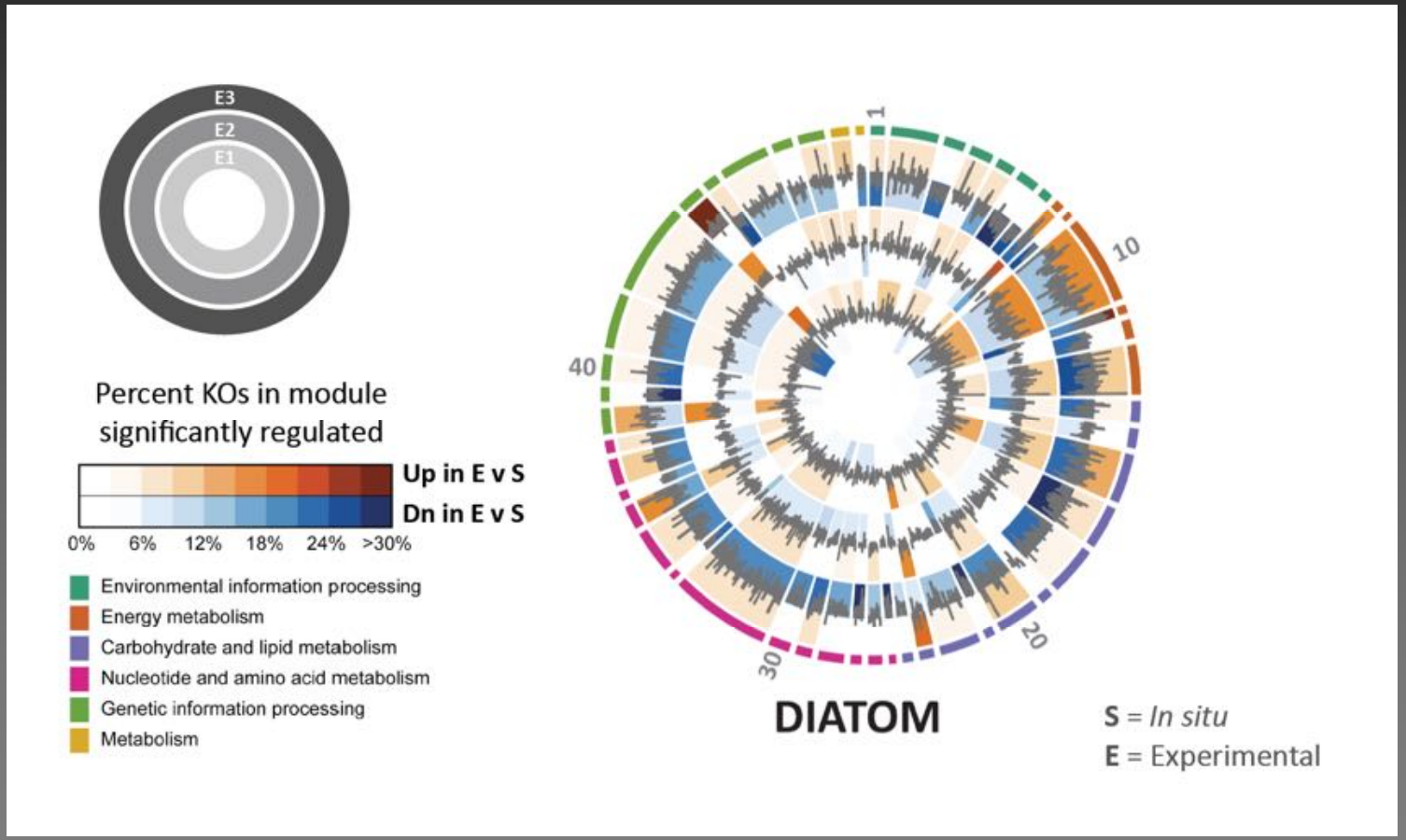
Significance Post-p > 0.95 for 2-fold change

Species are distinct from each other with 95% confidence, and all but dinoflagellates significantly shift in the simulated blooms

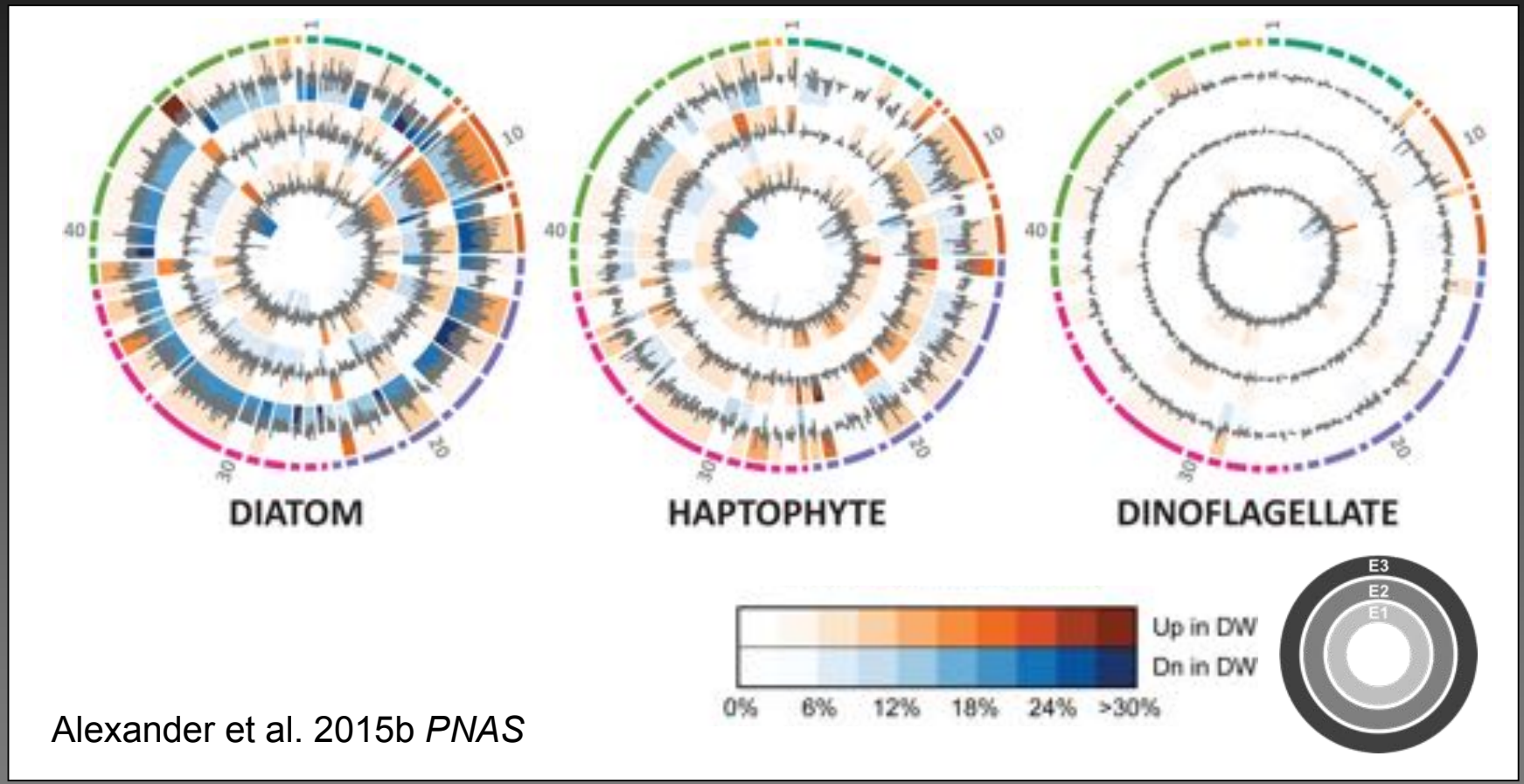
Gene shifts in diatoms



Gene shifts in diatoms



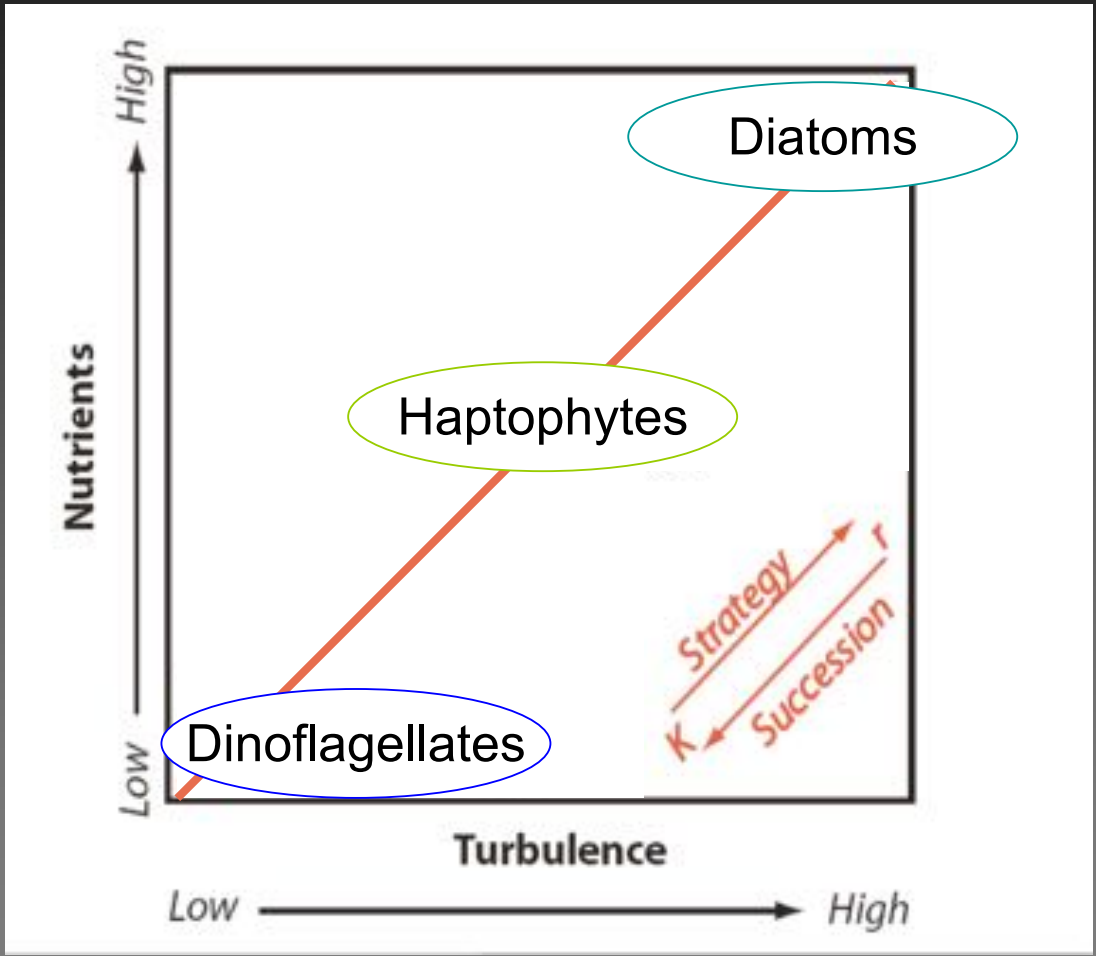
Transcriptional response during simulated blooms



Alexander et al. 2015b *PNAS*

Functional groups have distinct and reproducible transcriptional response during blooms driven by nutrient input

Margalef's Mandala - succession and ecological traits

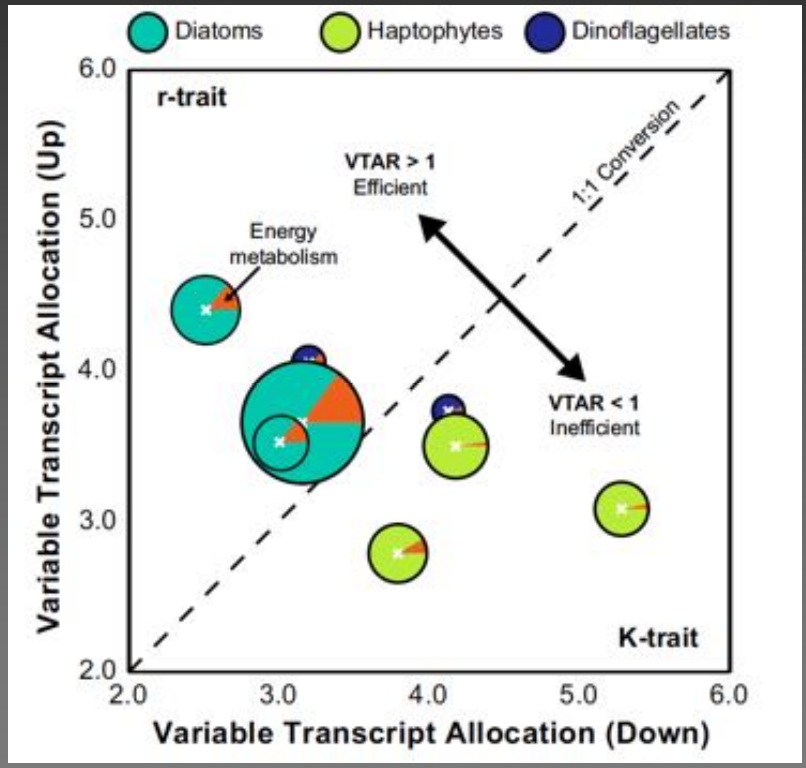


Adapted from Margalef 1978

Variable transcript allocation underpins traits

- Modeling transcript allocation following nutrient addition highlights differences in diatom and haptophyte traits
- Diatoms are more efficient than haptophytes

Variable Transcript Allocation Ratio (VTAR)



Haptophytes: "the survivalists"; **VTAR<1**
Diatoms: "the scavengers"; **VTAR>1**

Alexander et al. 2015b *PNAS*

Transcriptional responses underscore functional group traits

Dinoflagellates

FEW significant shifts → Alternative trophic modes?

Diatoms

BROAD transcript decreases
TARGETED transcript increase → "the scavengers" **r-selected**

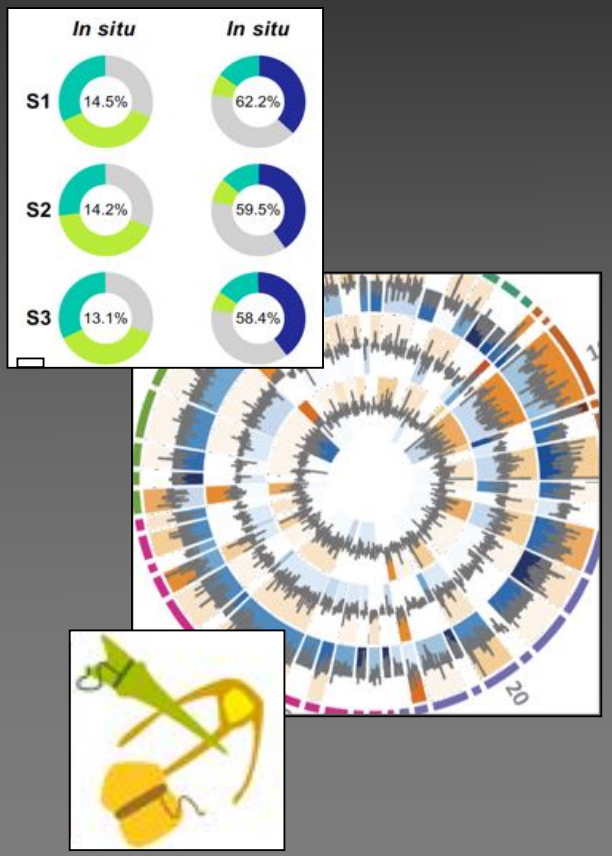
Haptophytes

MUTED increase and decreases → "the survivalists" **K-selected**

Summary - Eco-evolutionary traits

Are there functional group traits that drive the structure and function of the NPSG?

- The better the reference database (MMETSP) the better resolution
- Phytoplankton groups appear to be limited by resource availability in the NPSG.
- Remarkable year to year consistency
- Dinoflagellates have little transcriptional response to nutrient input.
- Diatom are particularly responsive (VTAR>1) to nutrient input which likely underpins their dominance in blooms



Two themes

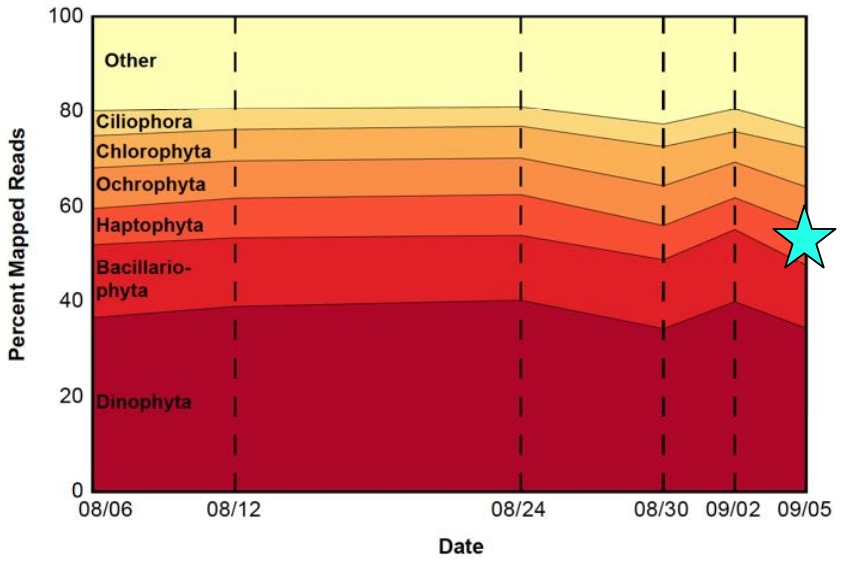
Eco-evolutionary traits

- Are there functional group traits that drive the bloom dynamics that structure the low nutrient North Pacific Subtropical Gyre?

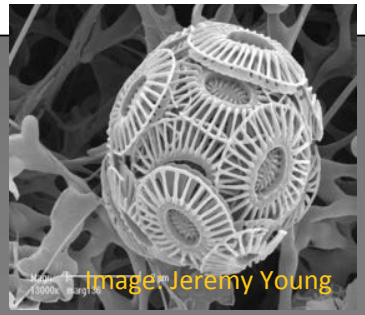
Metabolic plasticity and diversity

- How do diversity and metabolism influence the physiological ecology of a keystone group?

Diving beyond the functional group…

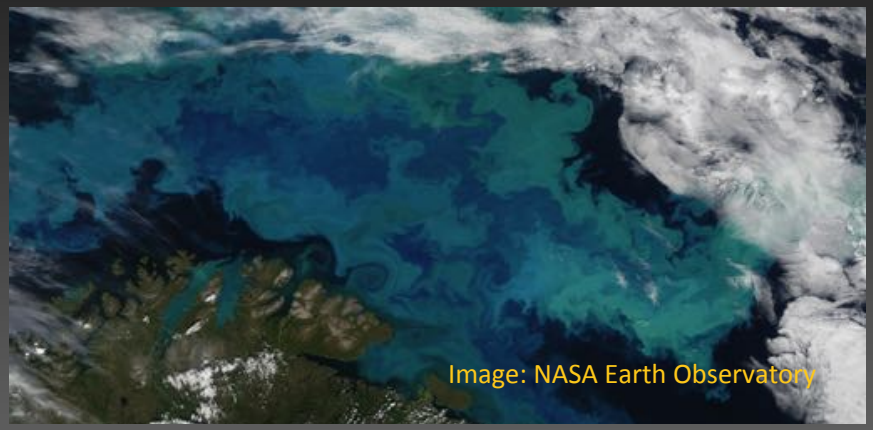


Tracking strain specific responses in the calcifying haptophyte, *Emiliania huxleyi* (Ehux)

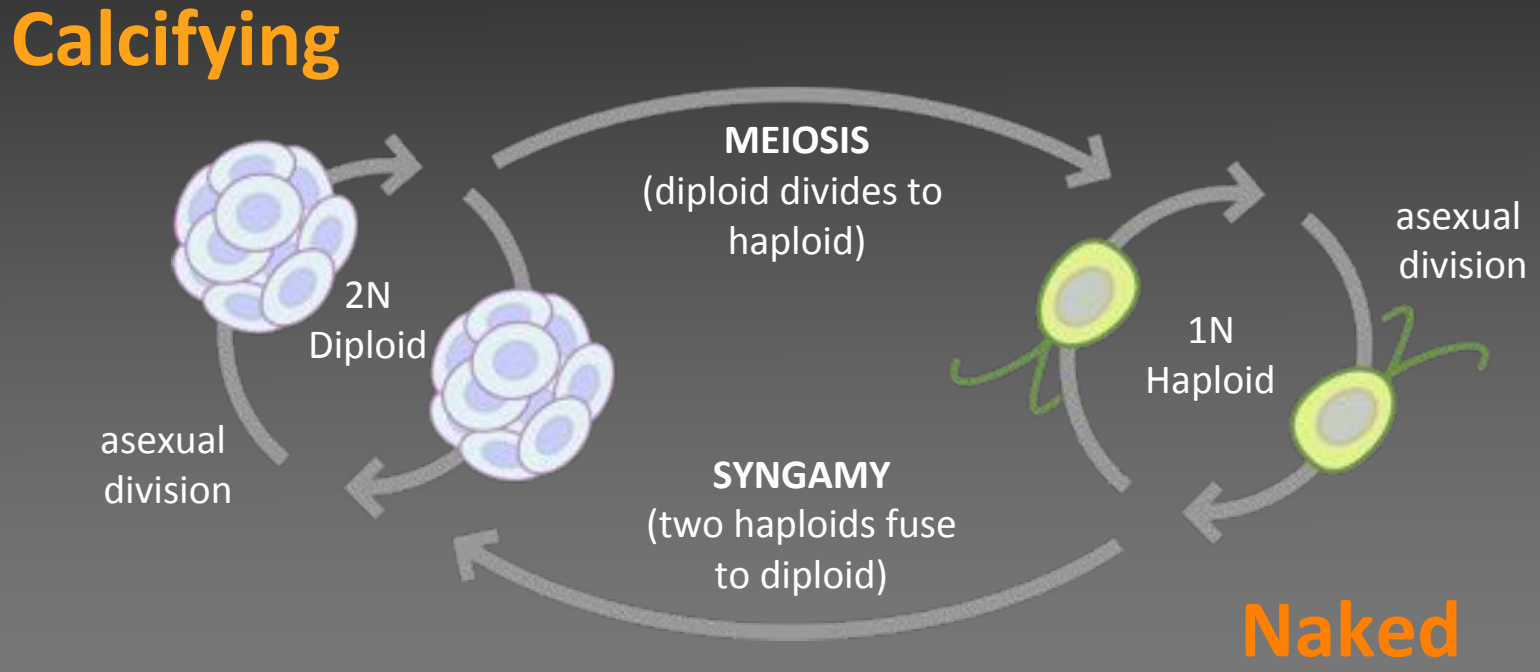


Emiliania huxleyi: a cosmopolitan, globally significant species

- 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 marine phytoplankton to have multiple strains sequenced, identifying pan genome



E. huxleyi life cycle



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]

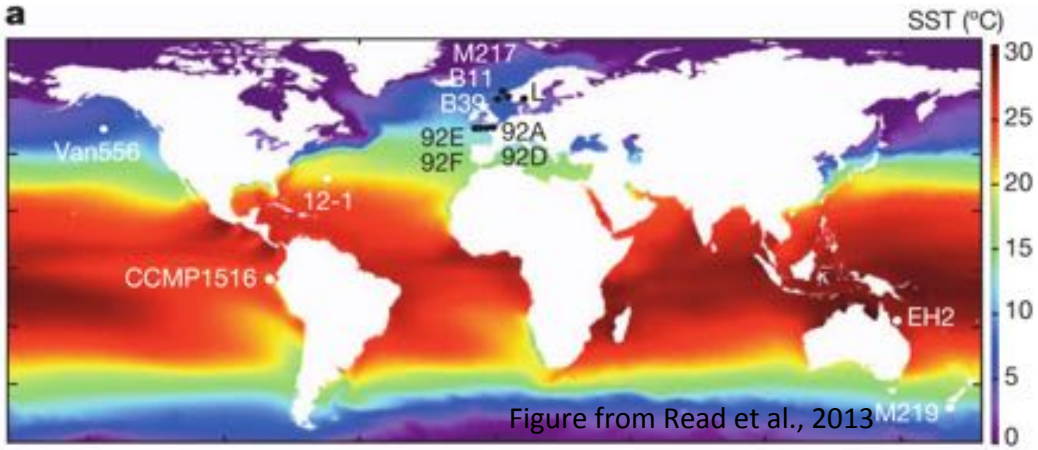
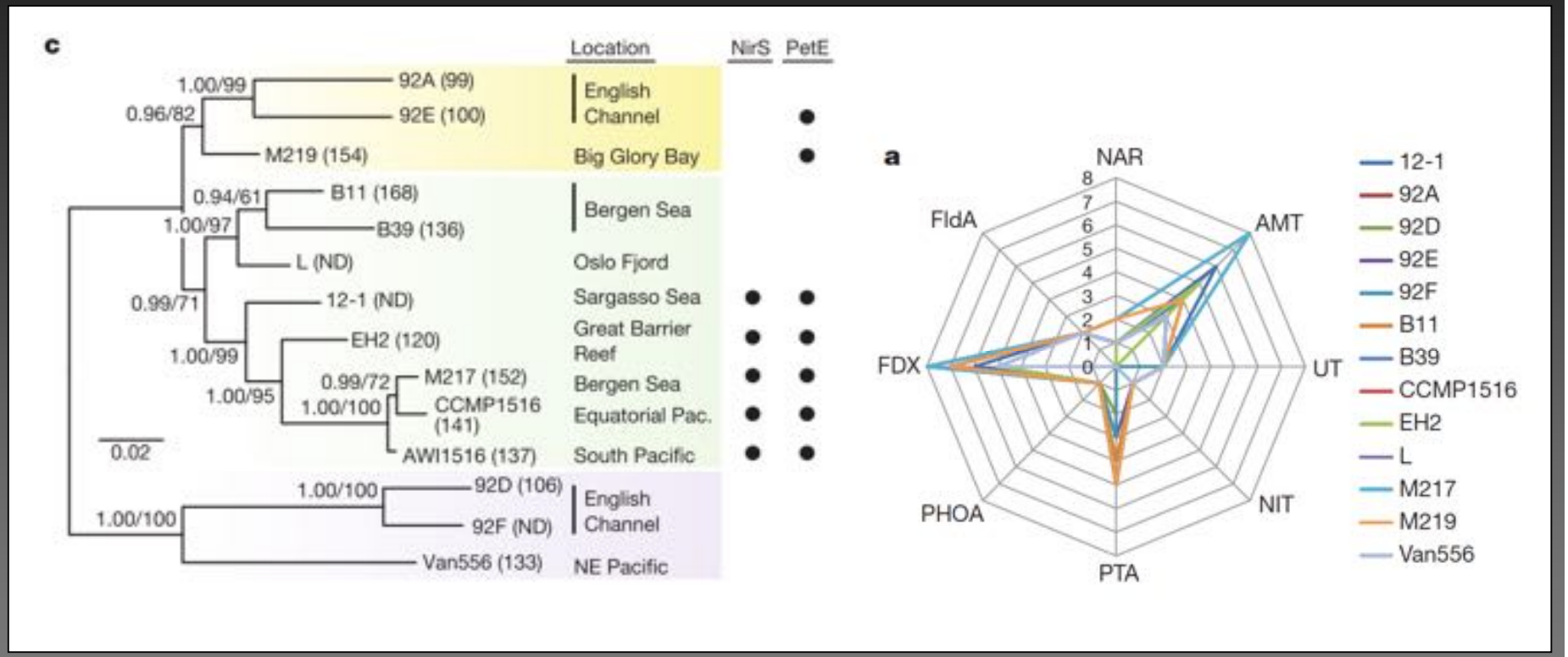


Figure from Read et al., 2013

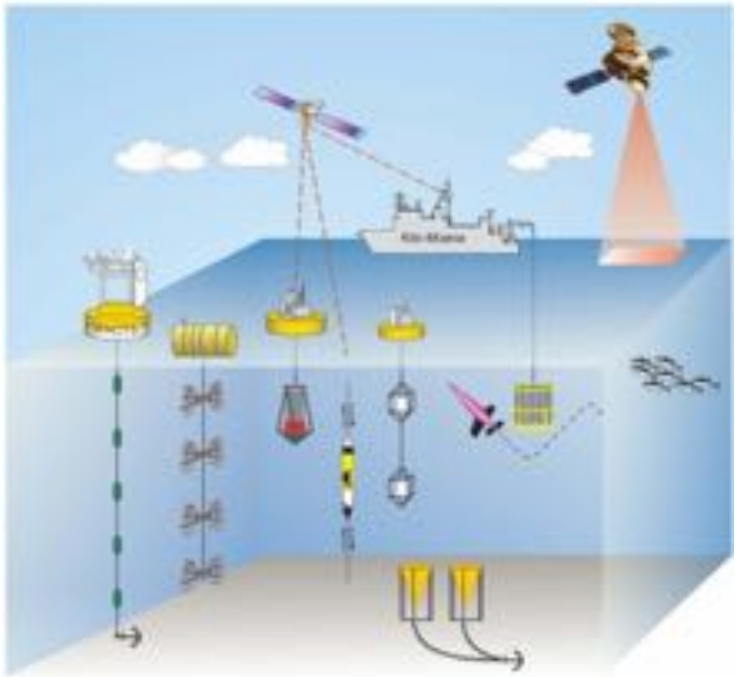
Cultured strains are highly diverse - isolated from a broad temperature range and displaying considerable physiological diversity

E. huxleyi has a pan genome, with core and variable gene content



Key functional genes are variably distributed across cultured isolates.

Hawaii Ocean Experiment: Dynamics of Light and Nutrients



Wilson et al. 2015 *GBC*

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Research Article

Short-term variability in euphotic zone biogeochemistry and primary productivity at Station ALOHA: A case study of summer 2012

Samuel T. Wilson ✉ Benedetto Barone, Francois Ascani, Robert R. Bidigare, Matthew J. Church, Daniela A. del Valle, Sonya T. Dyhrman, Sara Ferrón, Jessica N. Fitzsimmons, Laurie W. Juranek, Zbigniew S. Kolber, Ricardo M. Letelier, Sandra Martínez-García, David P. Nicholson, Kelvin J. Richards, Yoshimi M. Rii, Mónica Rouco, Donn A. Viviani, Angelicque E. White, Jonathan P. Zehr, David M. Karl

View issue TOC
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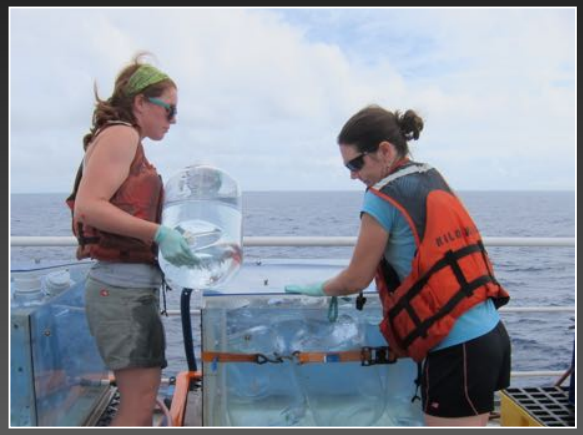
center for microbial oceanography: research and education

C·more *linking genomes to biomes*

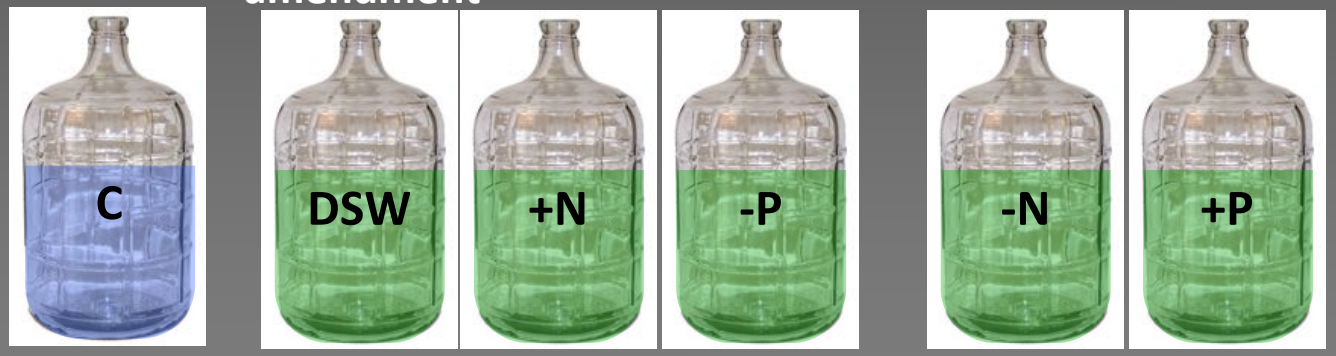
(HOE-DYLAN)

Responses to shifts in resource ratios



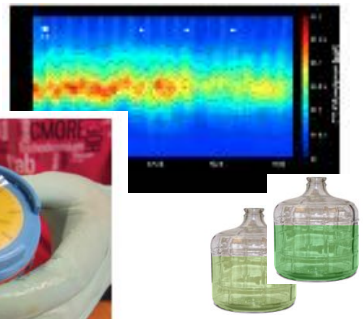
Control

Deep seawater amendment



Treatments with added N

Sampling and pipeline

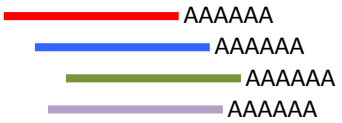


Collect samples from St. ALOHA

>5µm

6 day incubations

Isolate mRNA



Submit samples to sequencing center

Sequence using RNAseq and map reads to genomes

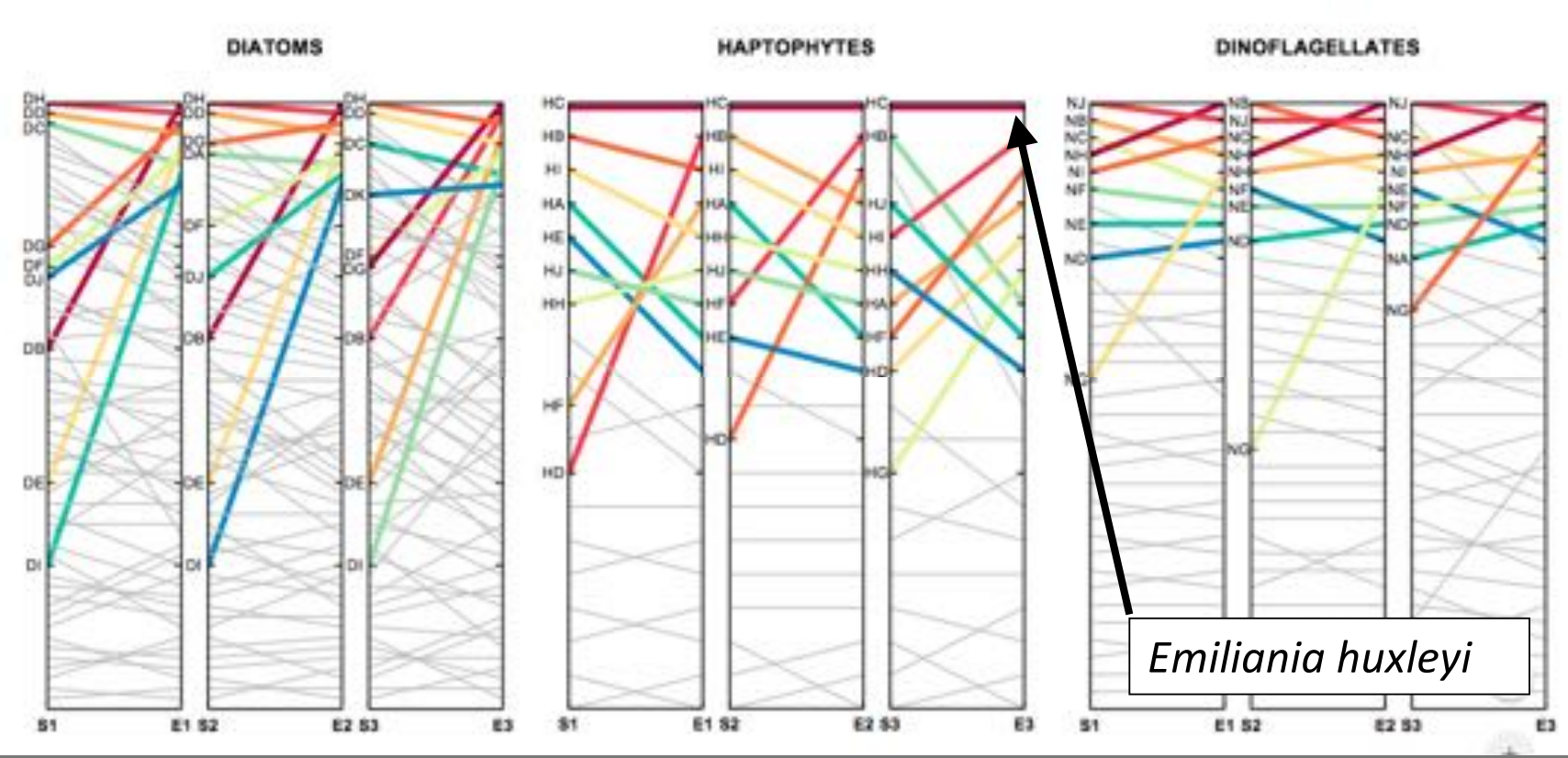
Target 60 million 100 bp paired-end reads

Stringent mapping

Removed reads with ambiguous mapping

Custom database of strains, and the *E. huxleyi* genomes

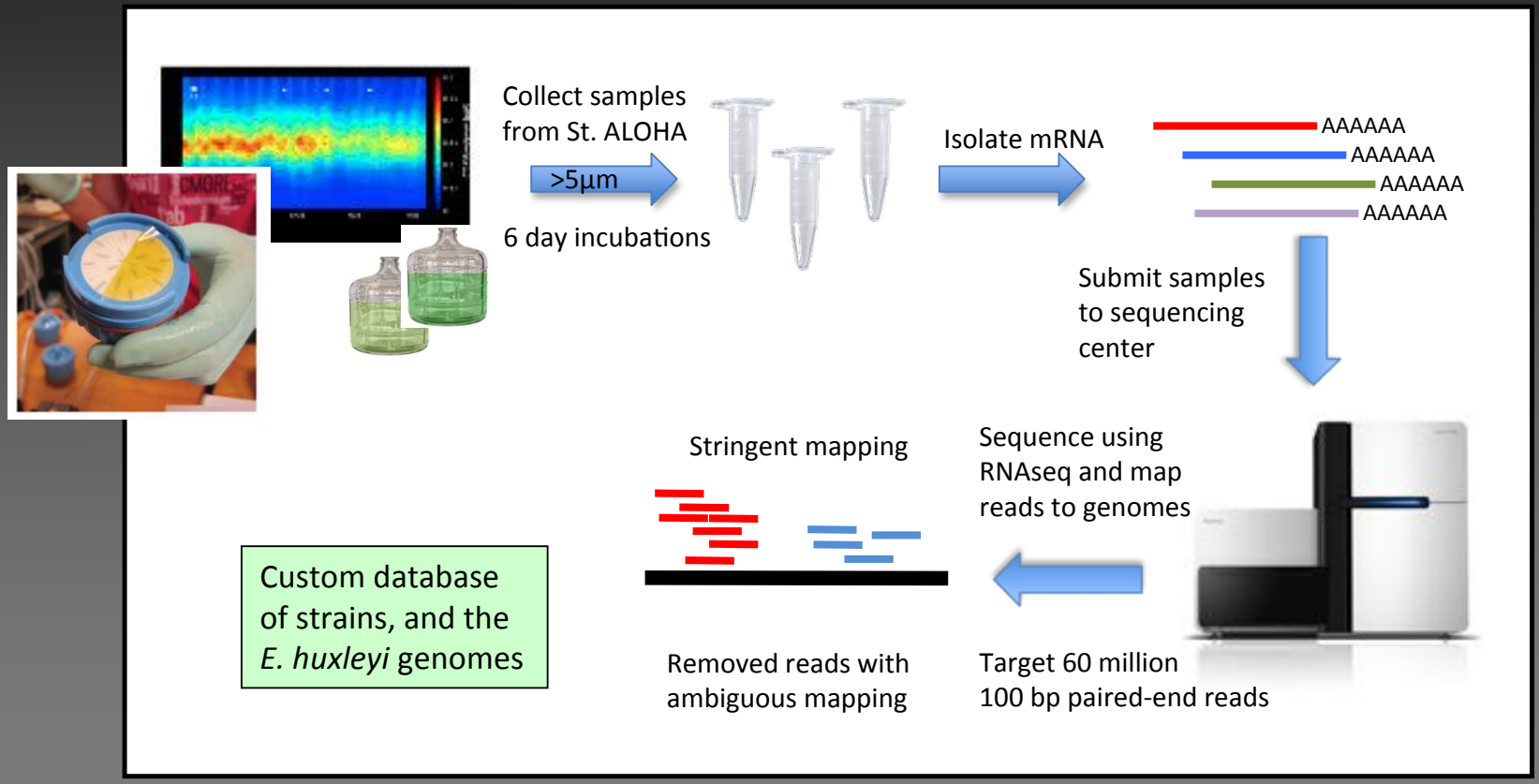
E. huxleyi consistently the dominant haptophyte



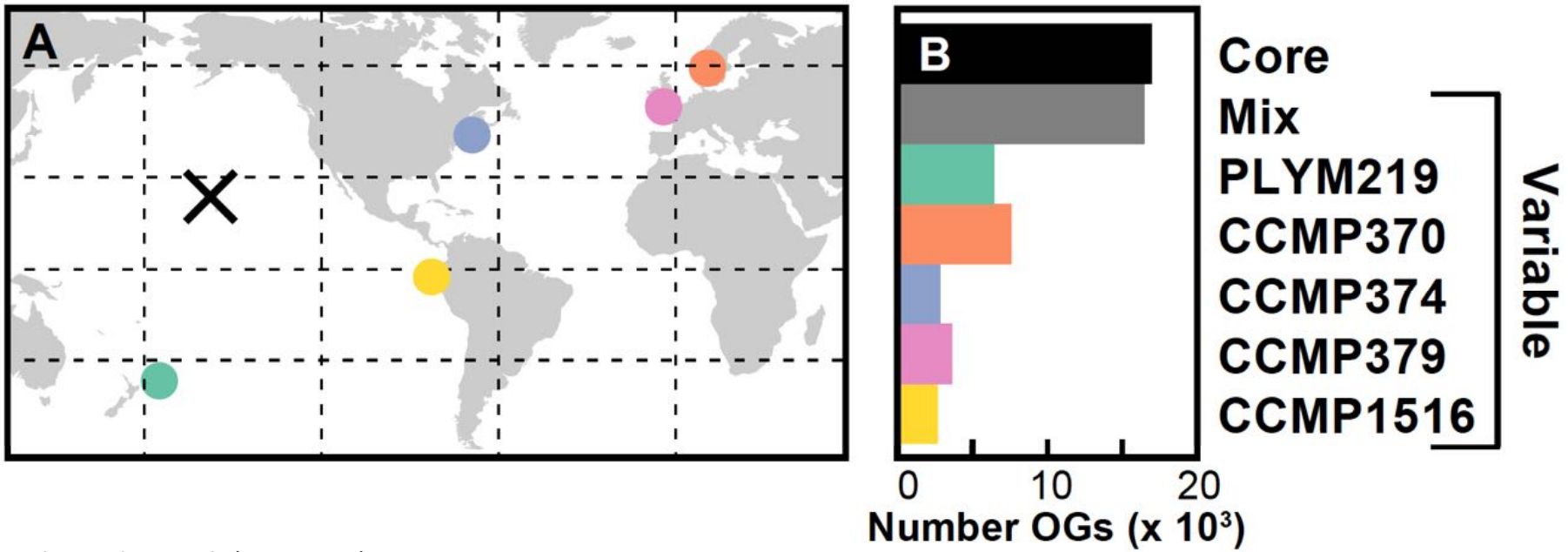
In both the 2012 and 2015 data, rank order changes during blooms (+DSW), although *E. huxleyi* was consistently the dominant haptophyte.

Alexander et al. 2015b *PNAS*

Sampling and pipeline



Orthologous group analysis



Alexander et al. (in review)

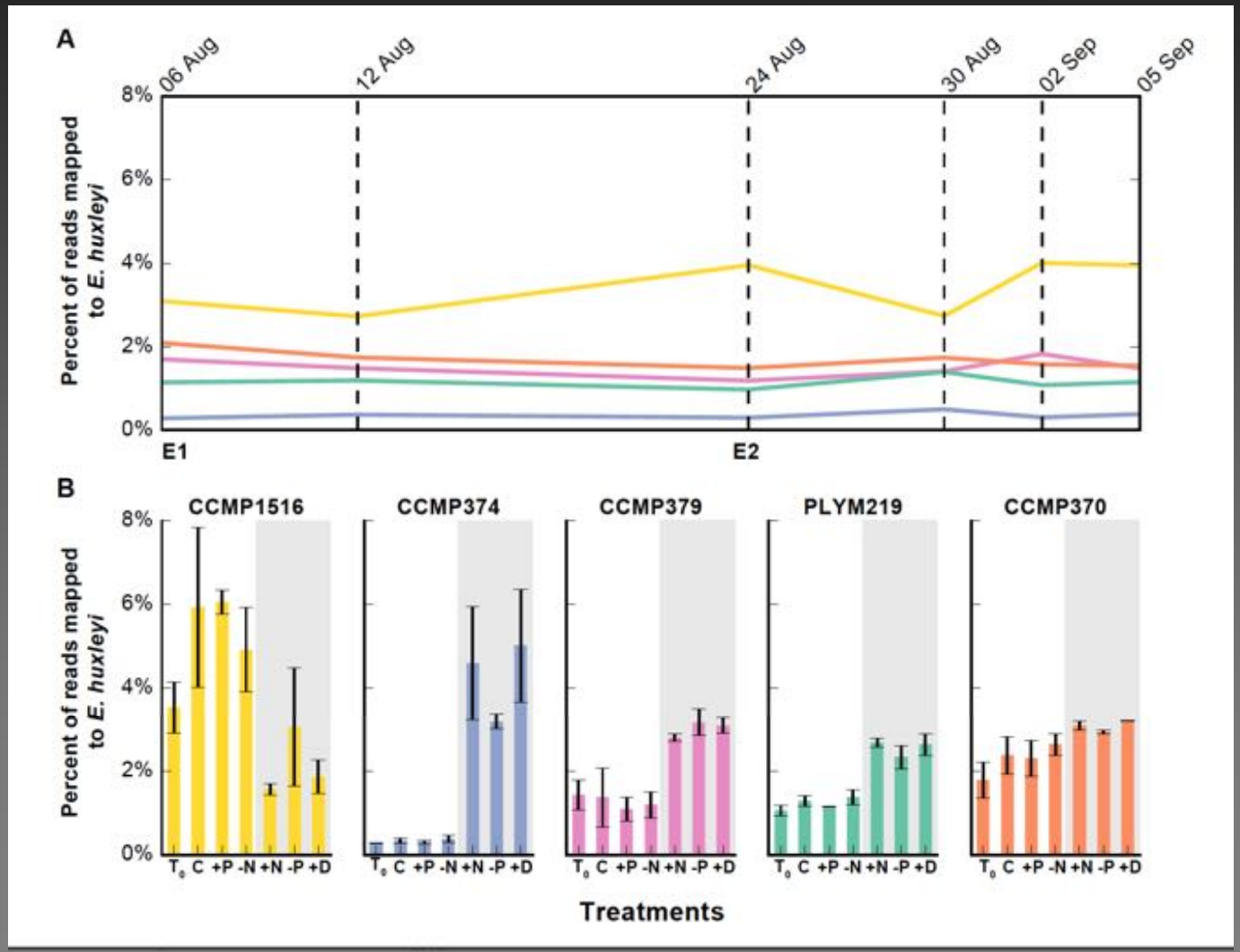
Reference strains isolated from disparate conditions.

Use diagnostic OGs to track strain distribution

Baas Becking hypothesis

"Everything is every where, but the environment selects"

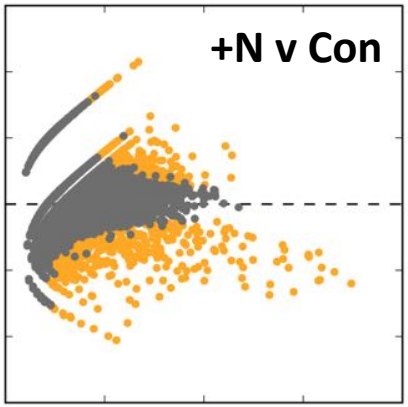
Strain distribution *in situ* and in the incubations

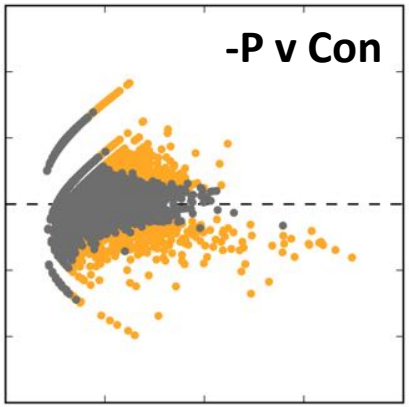


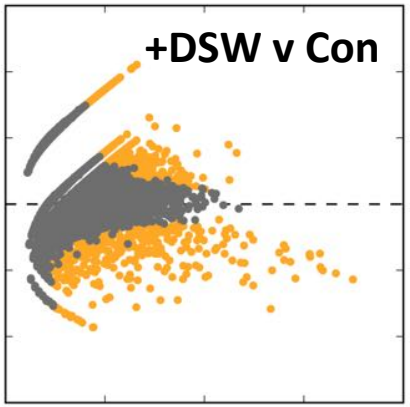
Alexander et al. (in review)

E. huxleyi physiological response suggests N control

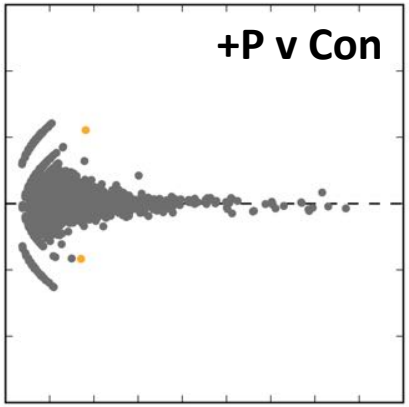
N added (Increased N:P)

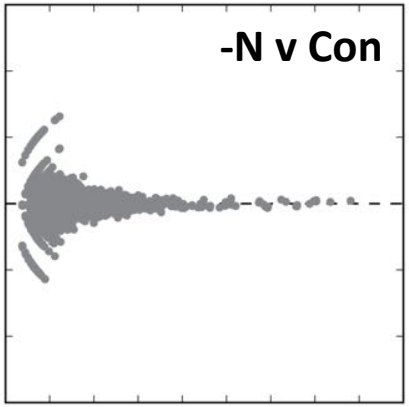






No N added (Decreased N:P)

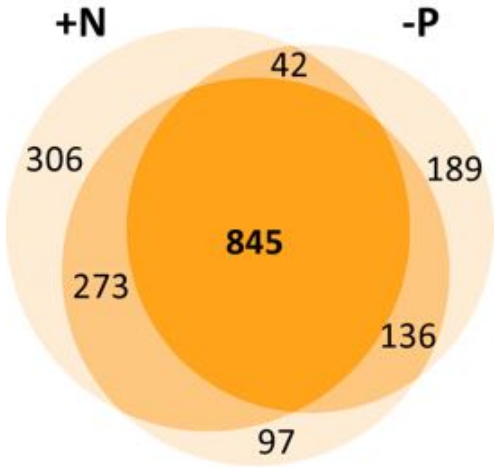




Log_2 Fold Change

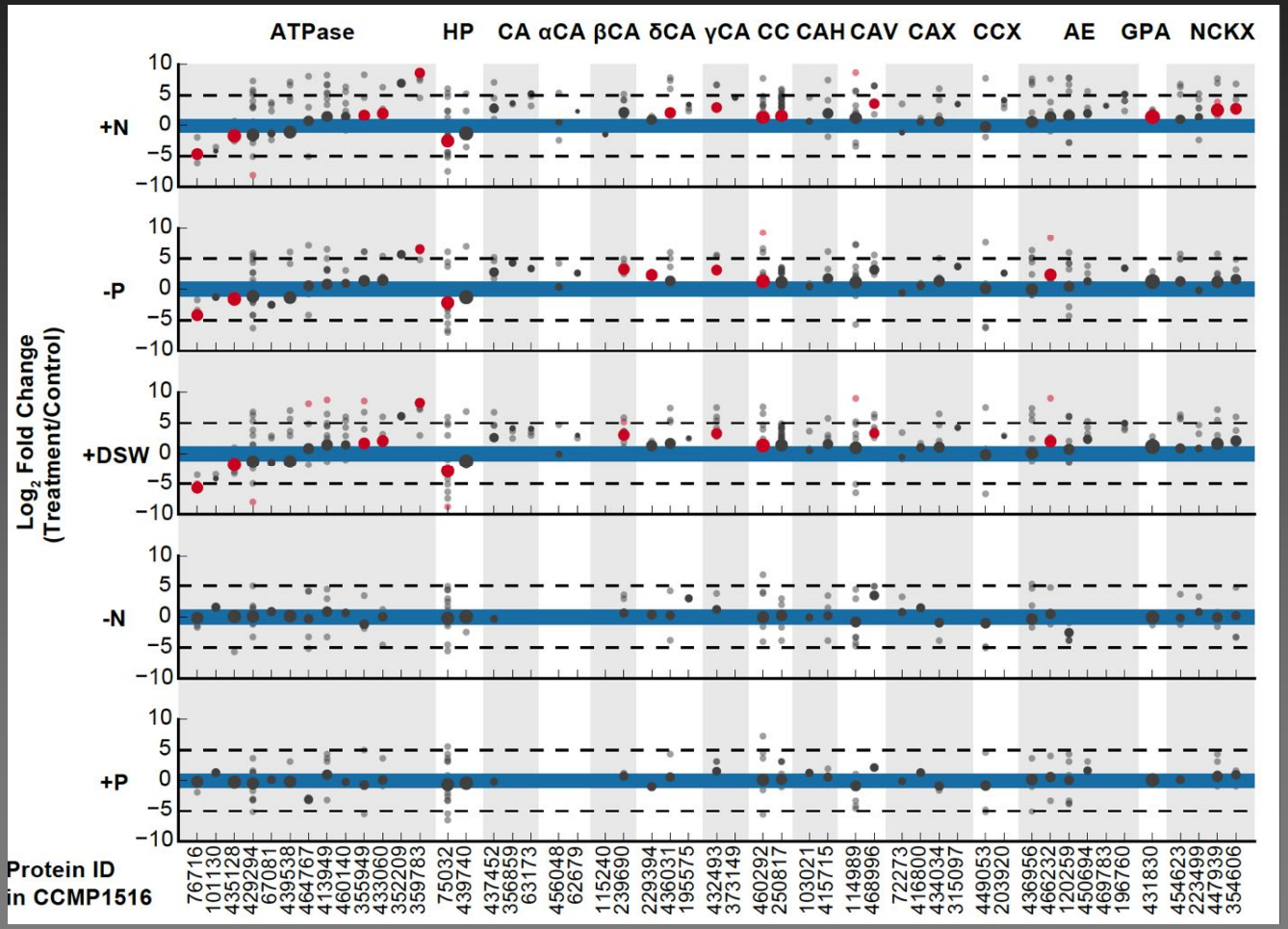
Log_2 Average Abundance

Significantly Differentially Abundant Genes



Alexander et al. (in review)

E. huxleyi physiological response - calcification



Markers indicate an increase in calcification with N addition

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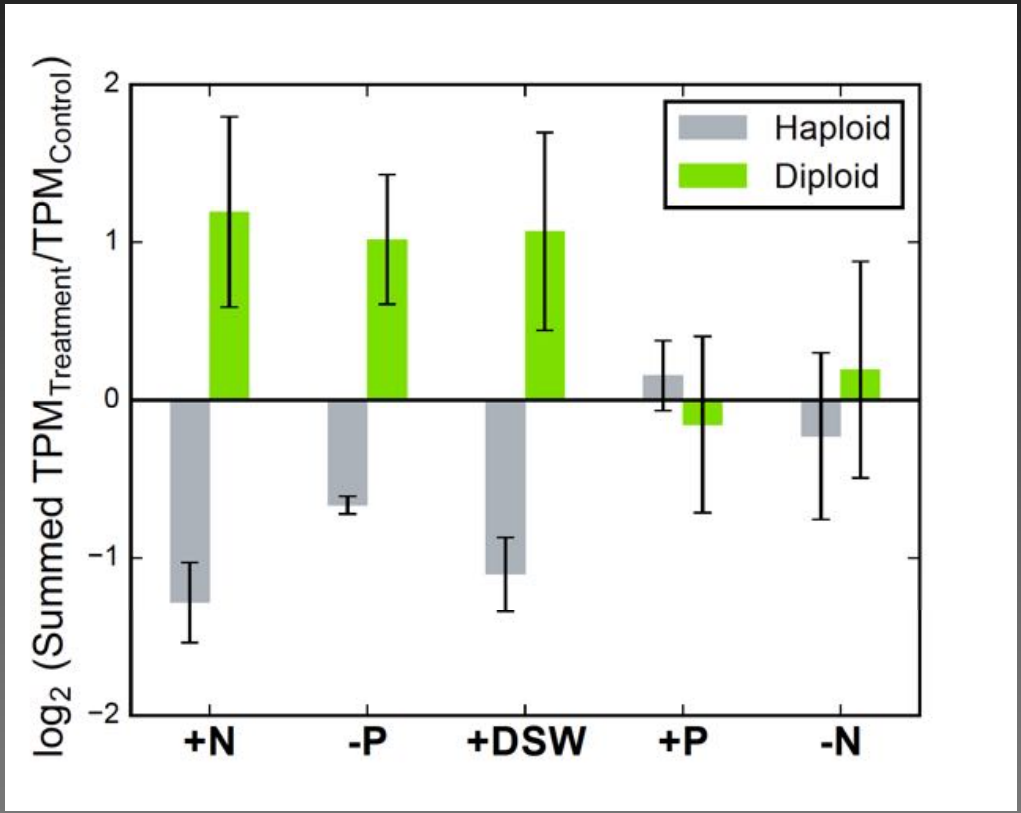
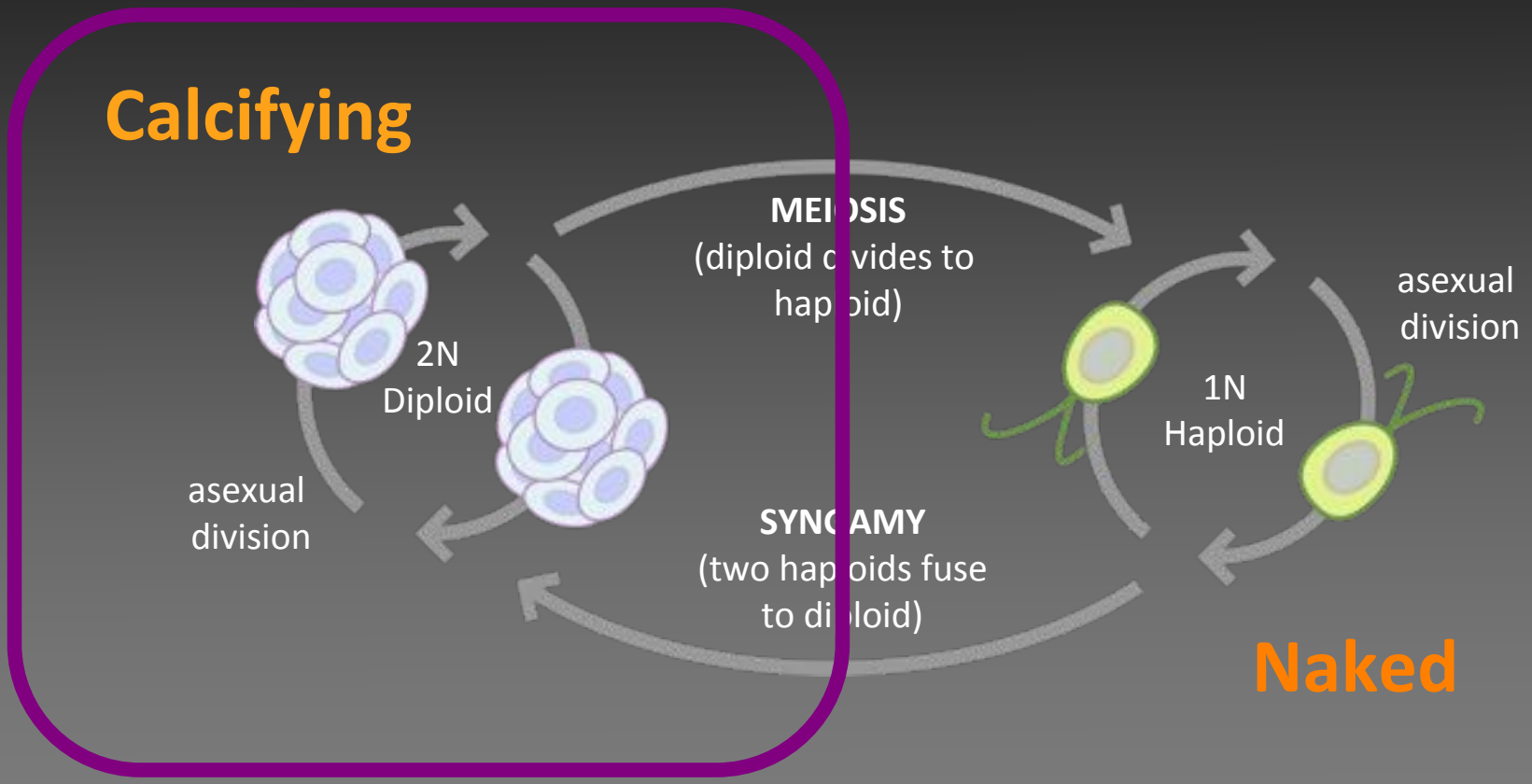
E. huxleyi response - shifts between haploid and diploid



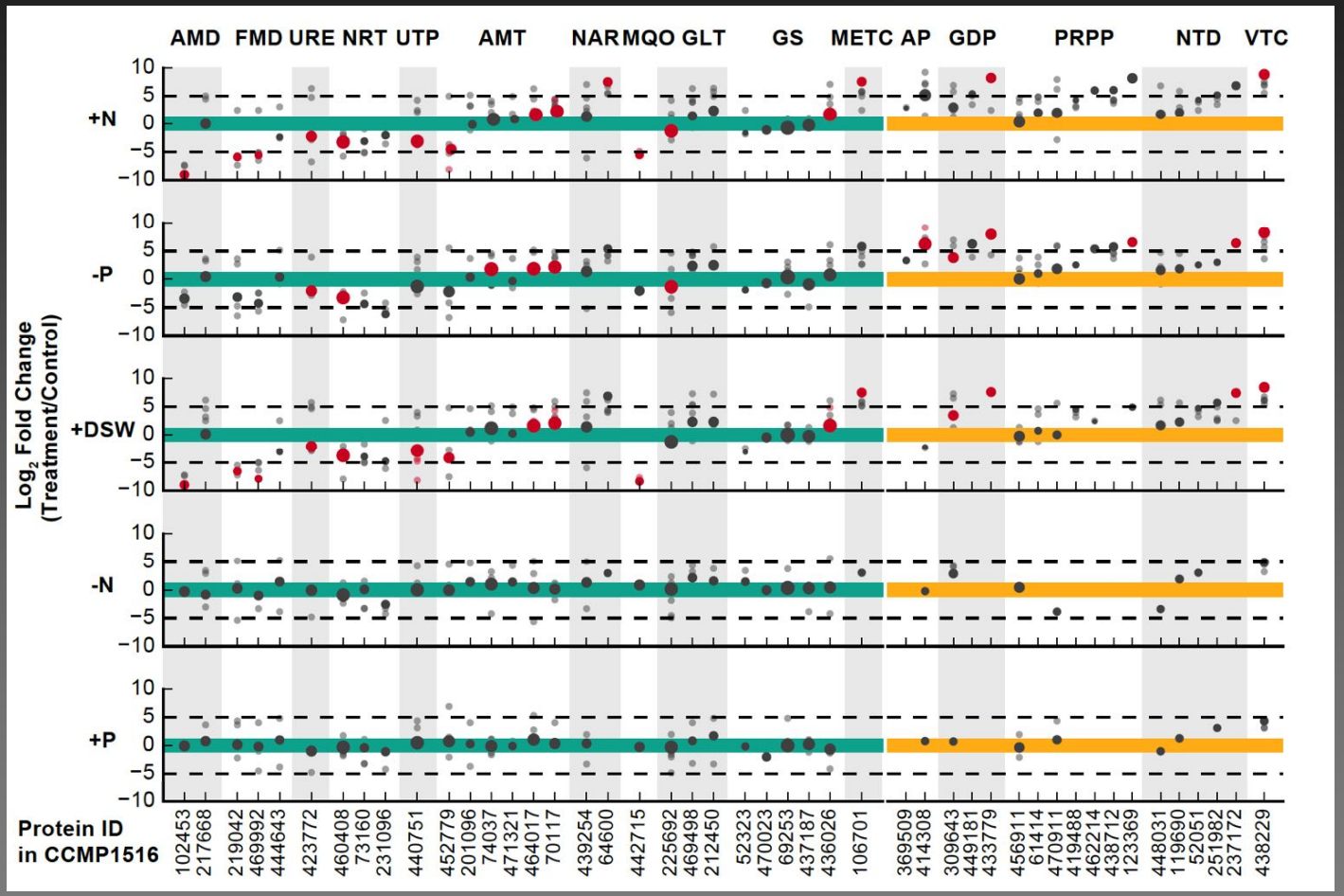
Haploid transcript signal declines, diploid increases with N addition - possible shift towards diploid phase with N

Alexander et al. (in review)

E. huxleyi life cycle



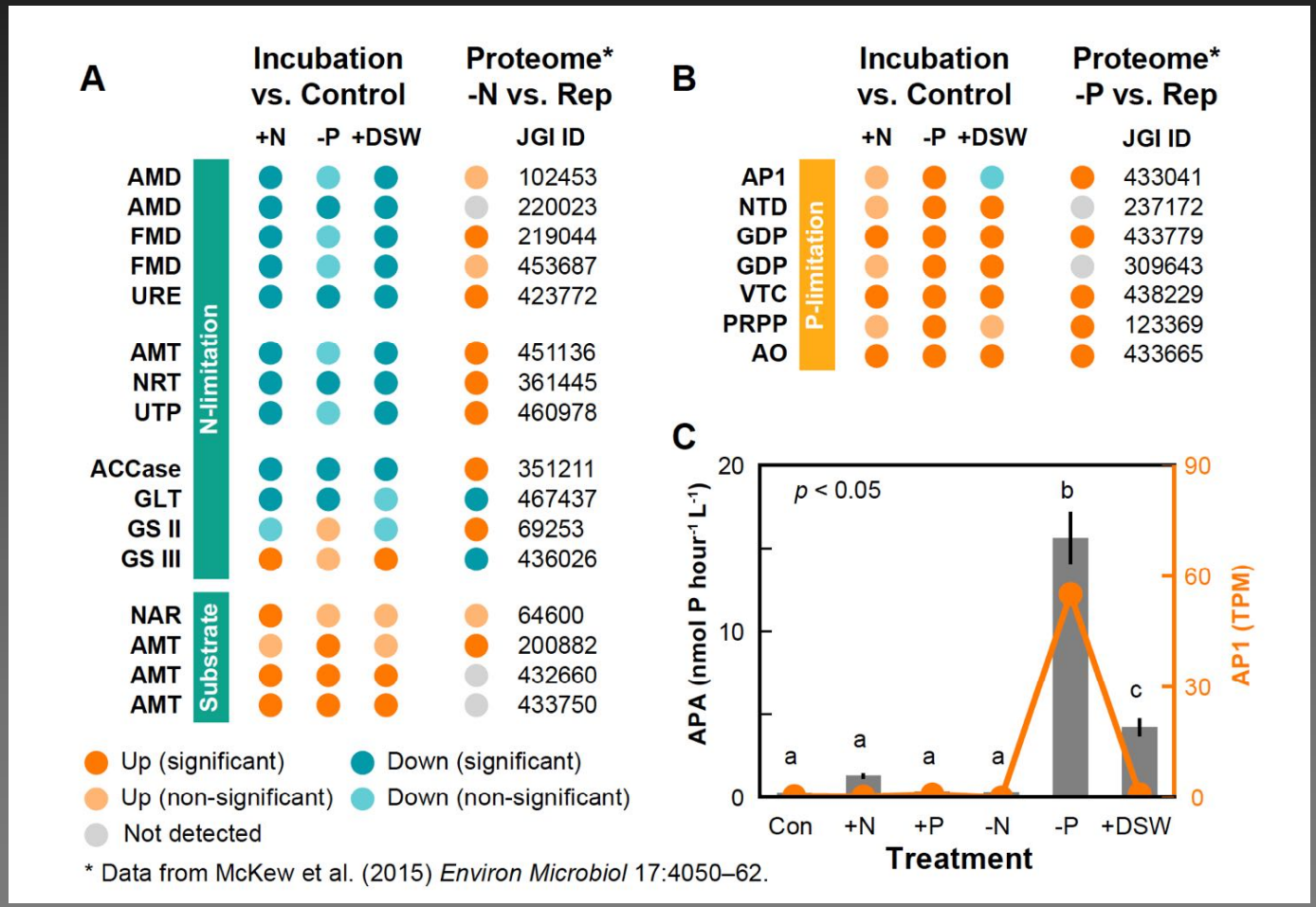
E. huxleyi physiological response - Nitrogen and Phosphorus



Markers of N and P limitation are modulated by treatments in expected manner

Alexander et al. (in review)

E. huxleyi physiological response



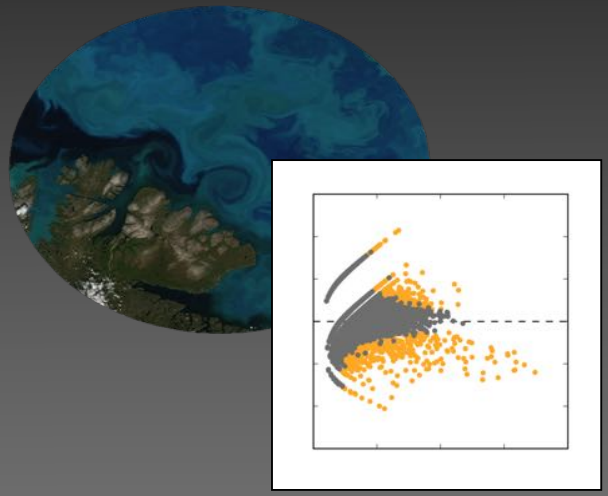
Excellent choreography between N and P related metatranscriptome shifts and changes in proteins and activities.

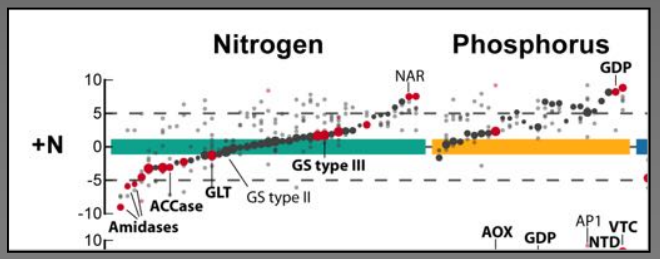
Alexander et al. (in review)

Summary - Metabolic plasticity and diversity

How do diversity and metabolism influence the physiological ecology of a keystone group?

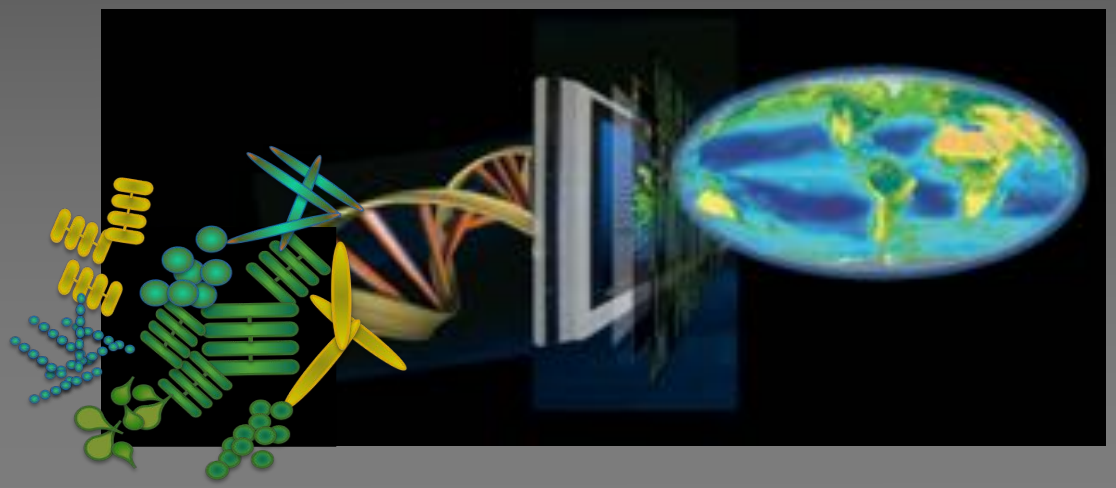
- Metatranscriptome data can be parsed at the strain level with appropriate reference data.
- Apparent abundance of Ehux strains and their physiology is driven by nitrogen addition
- Everything is perhaps everywhere and the environment selects for what is dominant





Conclusions

Transcriptome-enabled approaches are providing new tools to identify drivers of bloom formation in keystone eukaryotic phytoplankton. Increasing genome/transcriptome databases will further resolution moving forward.



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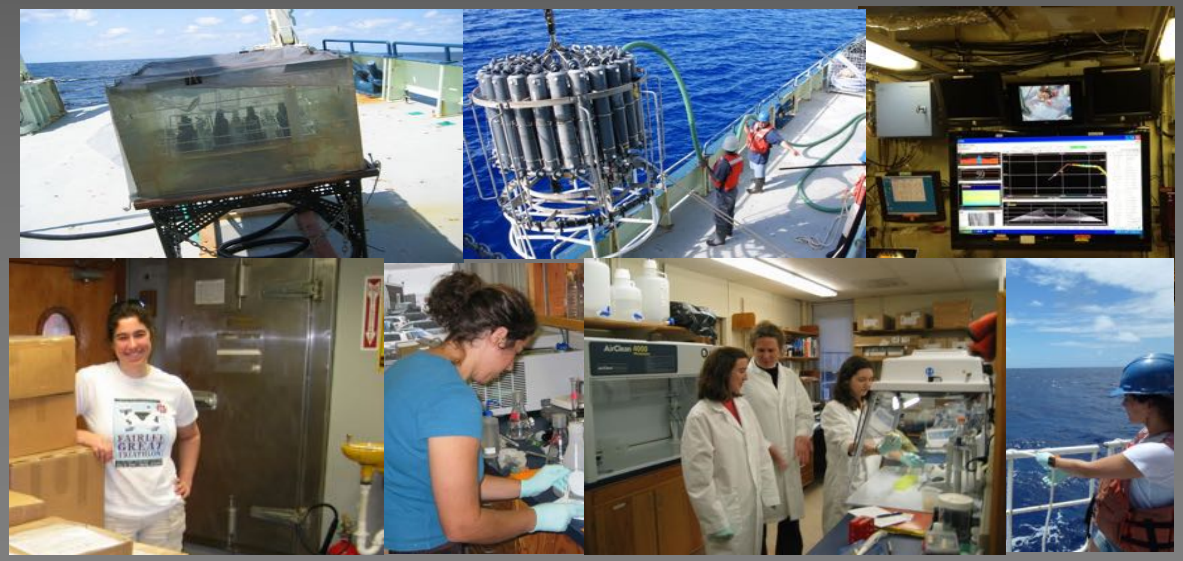
- Angell Foundation

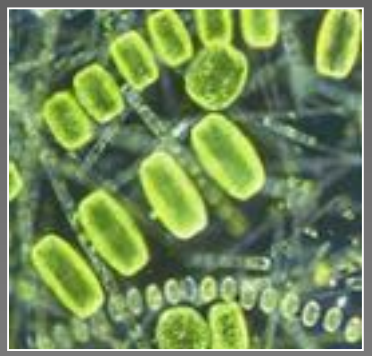


Vignettes

- From genome to biome: Tracking the metabolism and microbiome of a keystone N_2 fixer

Genome - enabled

- Co-existing in a sea of competition: Leveraging transcriptome data to identify the physiological ecology of phytoplankton from key groups



Transcriptome - enabled