

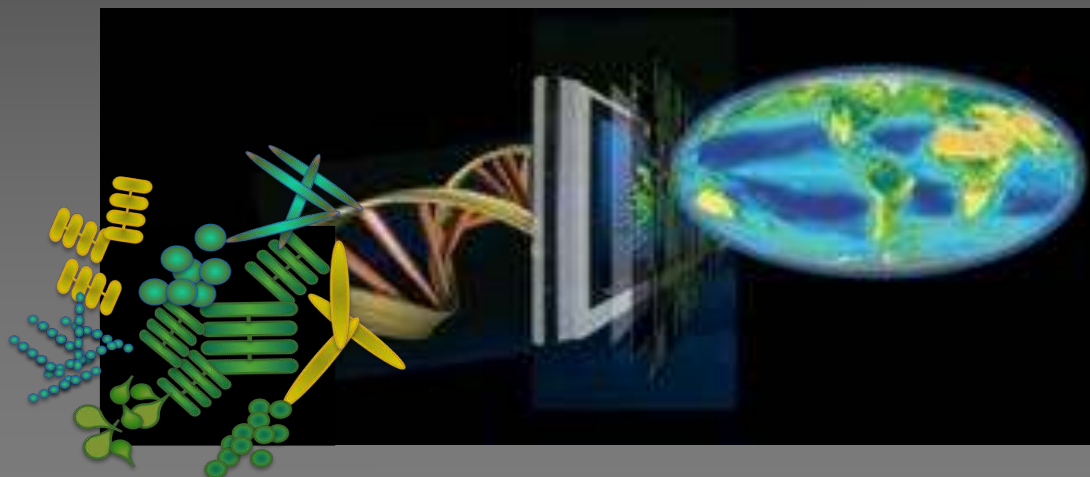
From flask to field: tracking the drivers of phytoplankton physiological ecology across marine ecosystems

Sonya Dyhrman

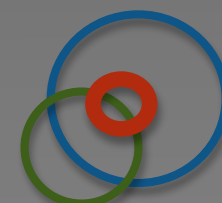
Department of Earth and Environmental Sciences

Lamont Doherty Earth Observatory

Columbia University



COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

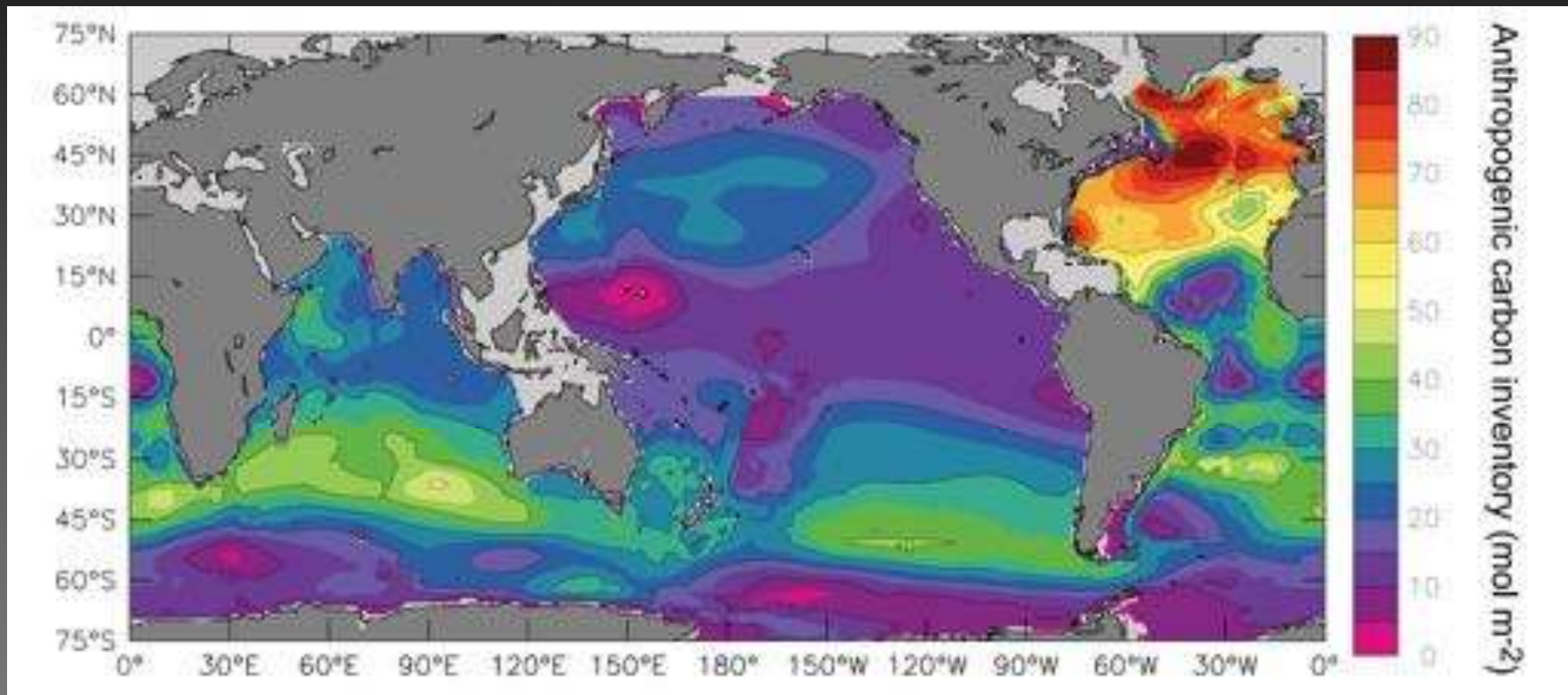


LAMONT-DOHERTY
EARTH OBSERVATORY

The ocean makes our planet livable



The ocean acts as a buffer for CO₂ in the atmosphere



Sabine et al. (2004) *Science*

Between 1800 - 1994, ocean has absorbed ~120 petagrams of CO₂
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*

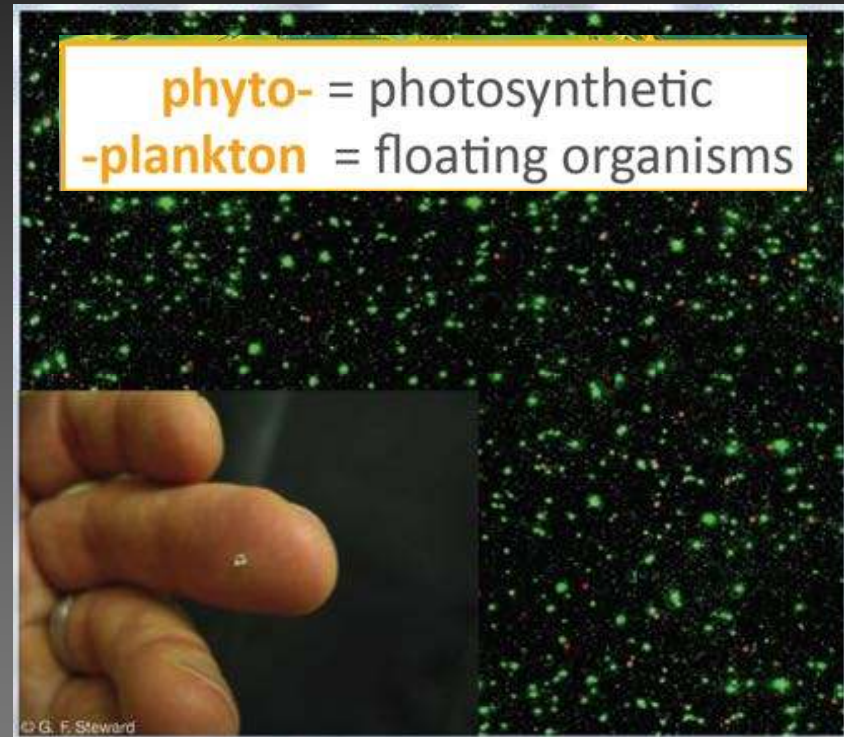
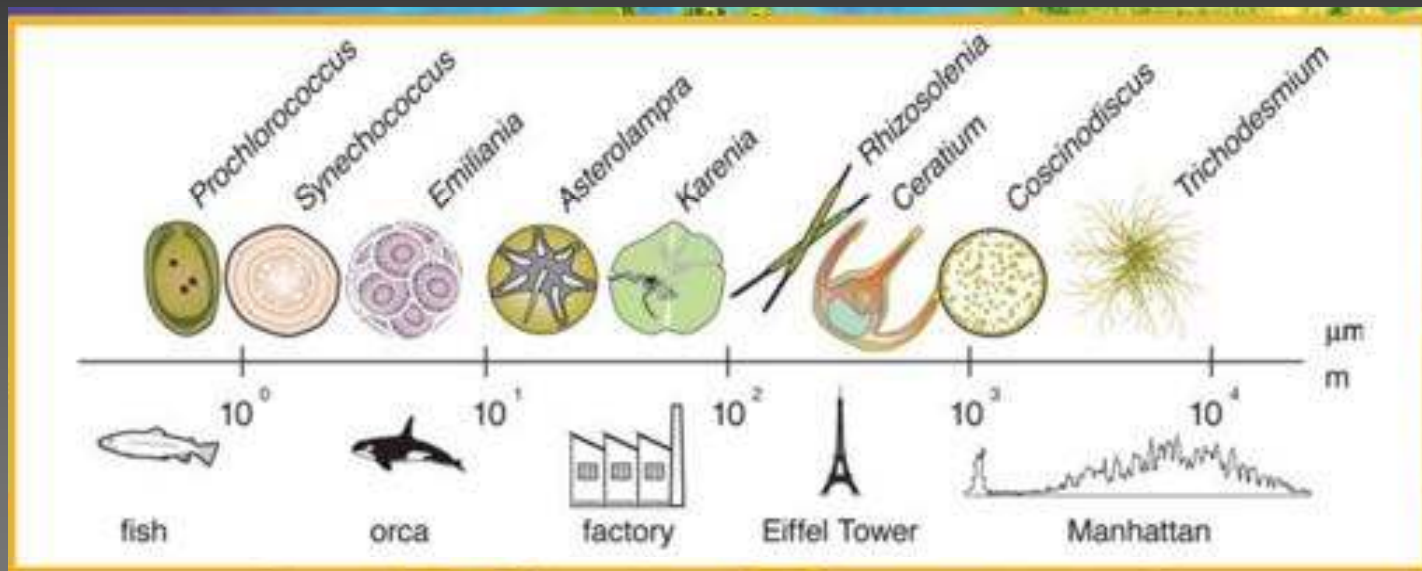
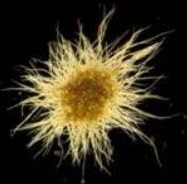
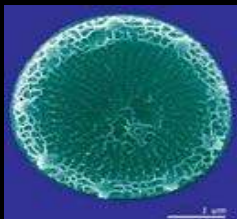
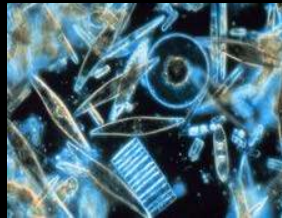
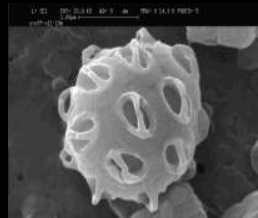
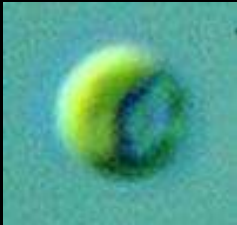
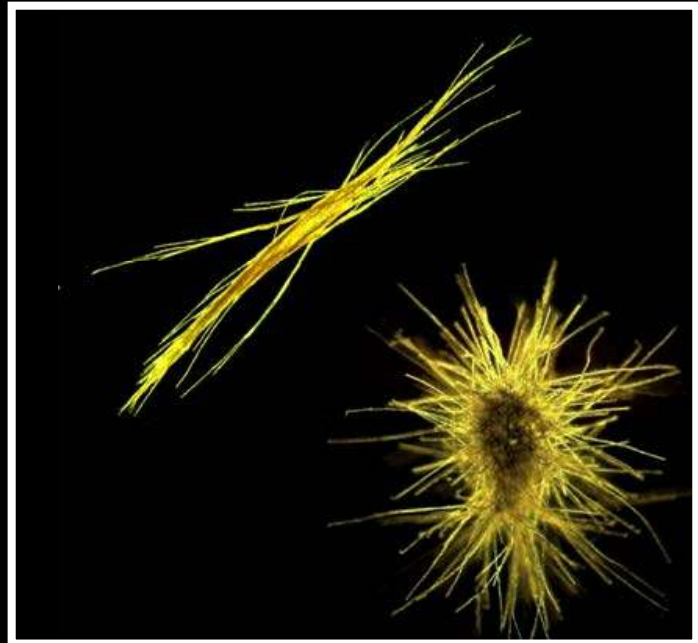
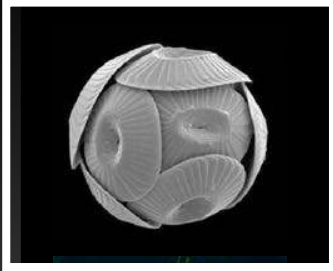


Image courtesy C-MORE

Marine phytoplankton are highly diverse





Focus on keystone groups

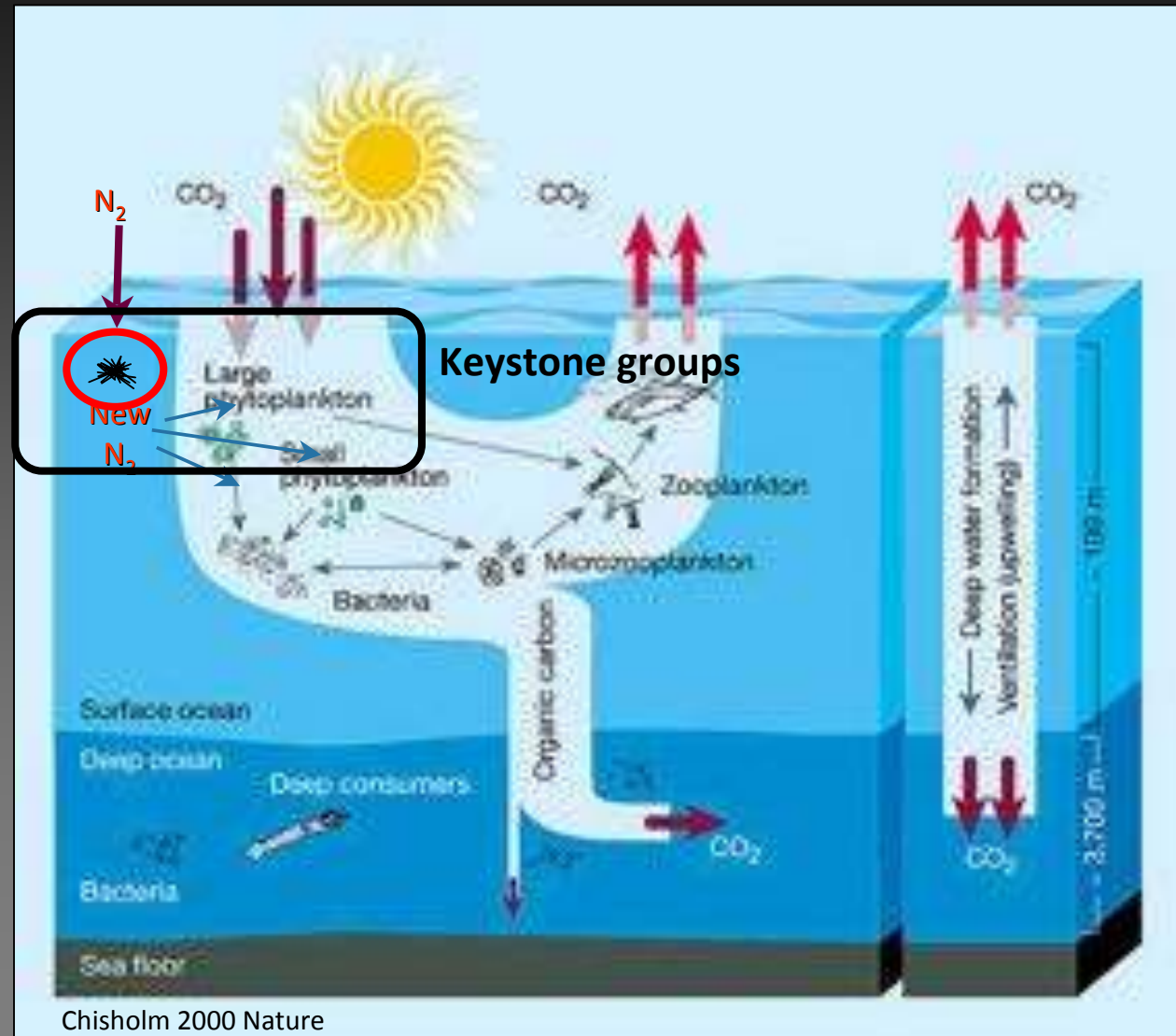
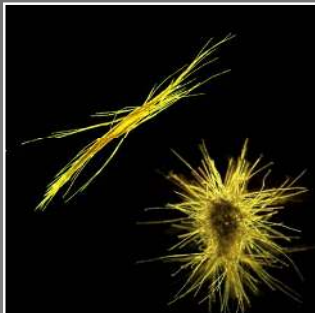
Haptophytes



Diatoms

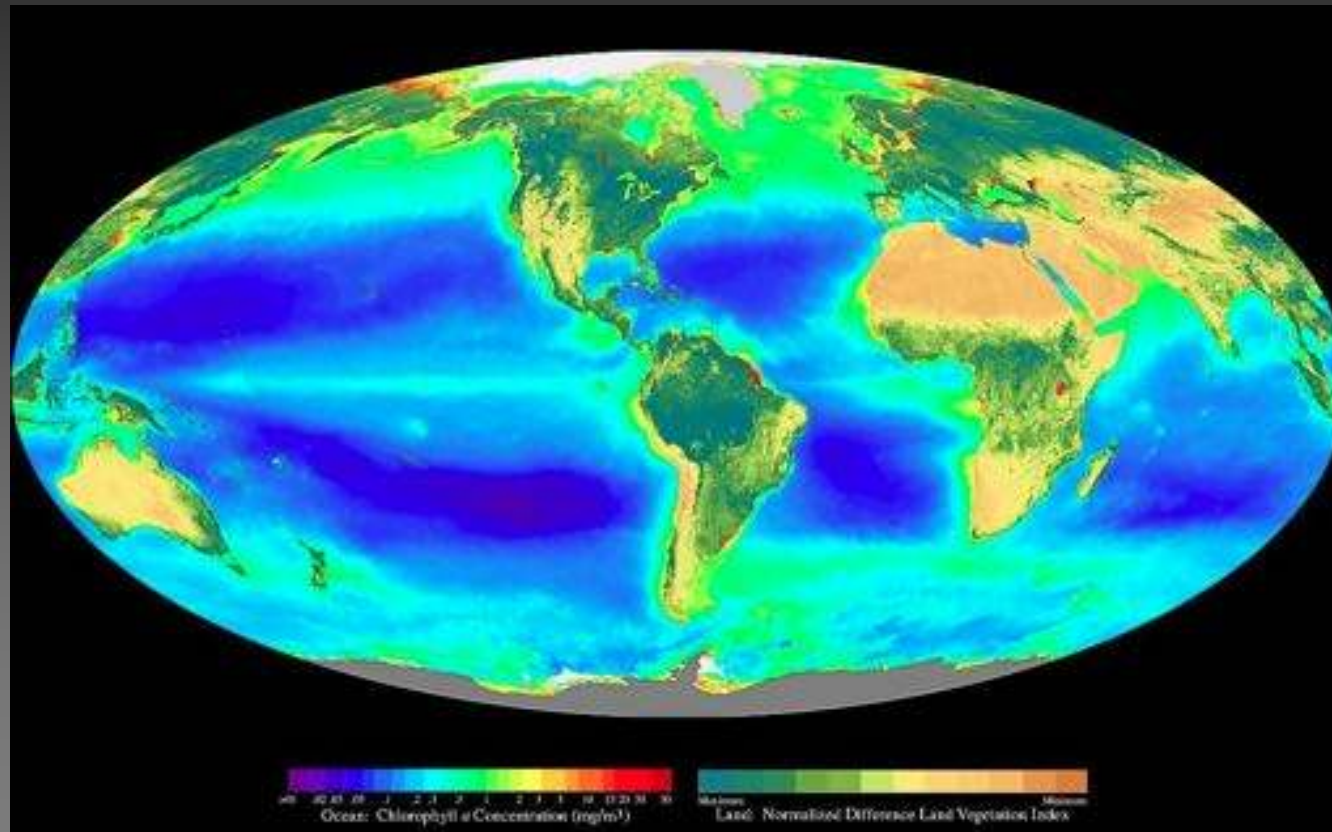


N₂ Fixers

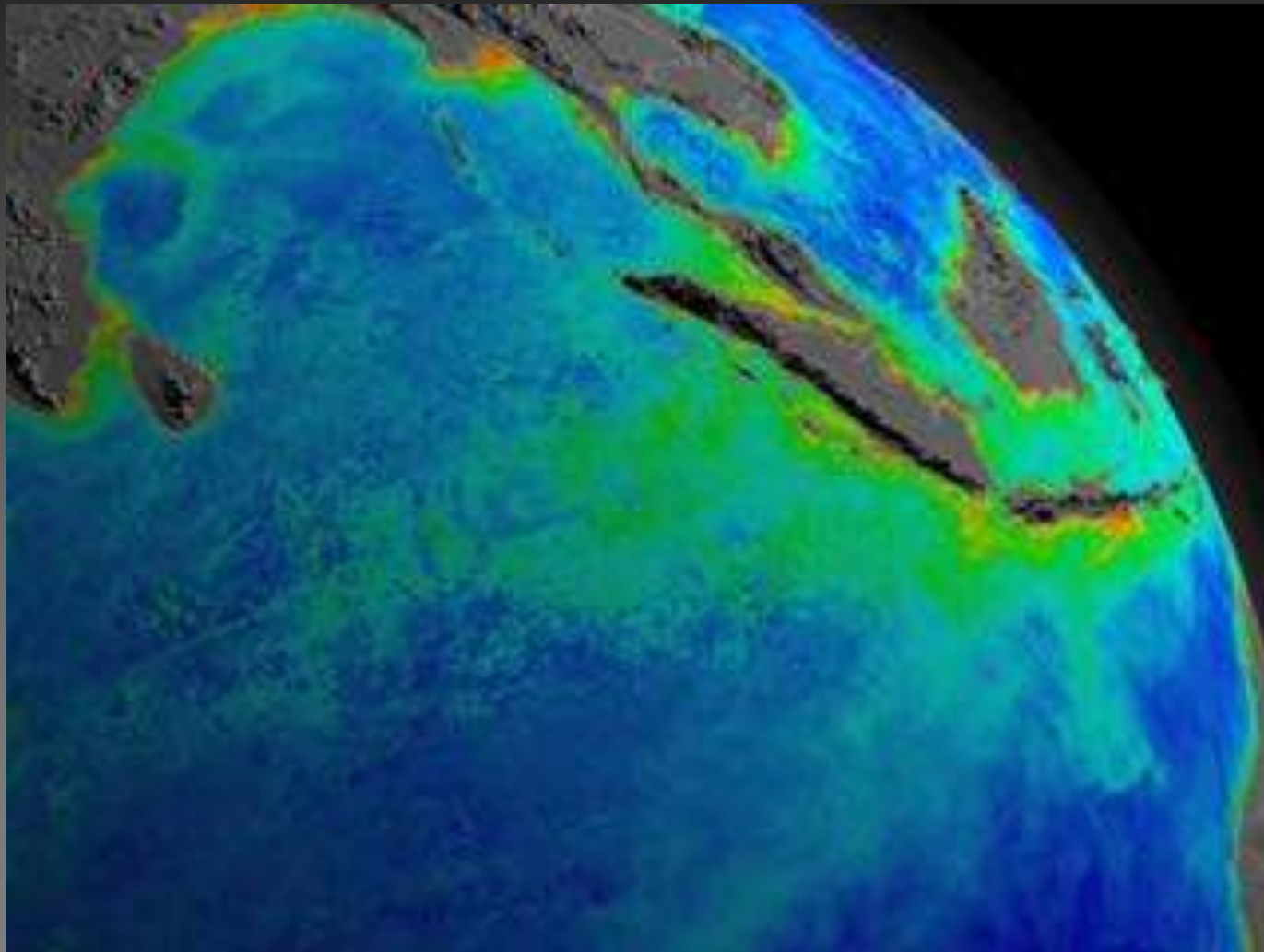


Phytoplankton play a profound role in the earth system

Half of global primary production



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



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

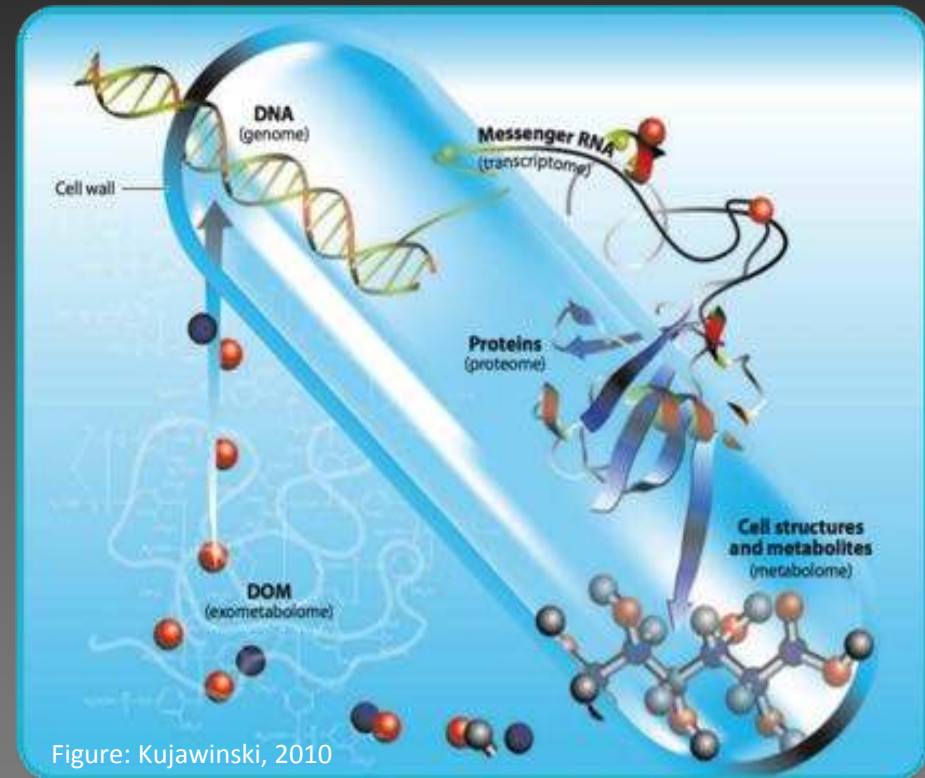
Micro/Mesocosm



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

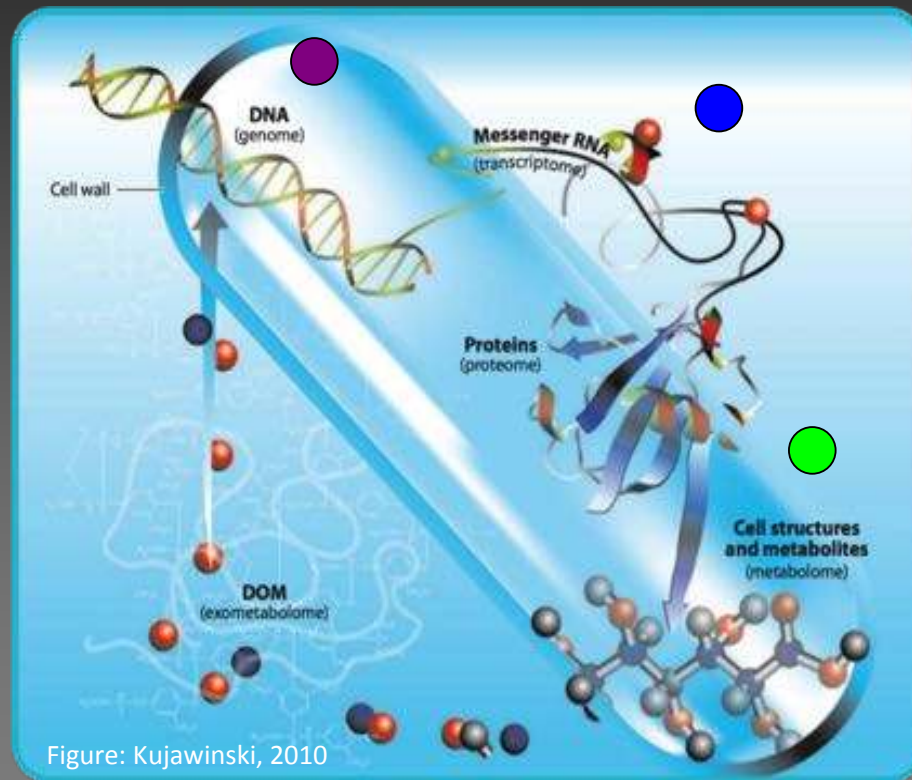
Challenges and opportunities in microbial oceanography

- 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



Increasingly able to use 'omic and 'metaomic approaches!

Leveraging 'omic data to study marine microbes



- **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



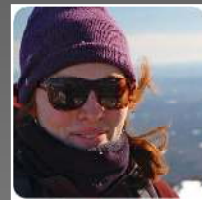
Kyle Frischkorn



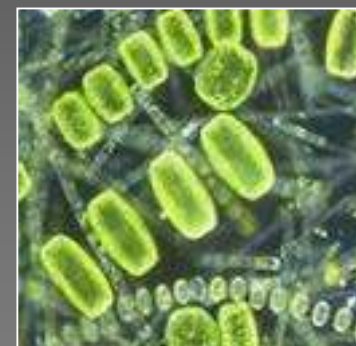
Matt Harke



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

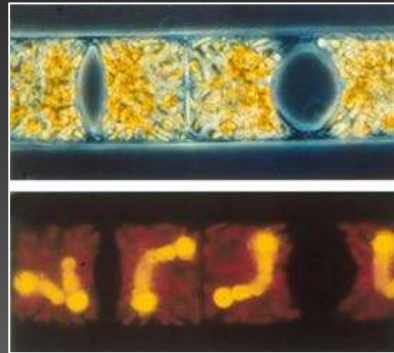
- Who is there? Microbiome diversity
- What are they doing? Microbiome functional diversity and interactions



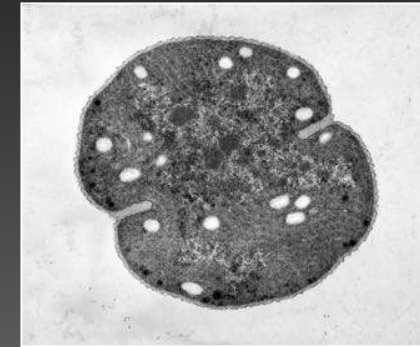
Nitrogen-fixing marine cyanobacteria

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

Richelia



Crococosphaera



Trichodesmium



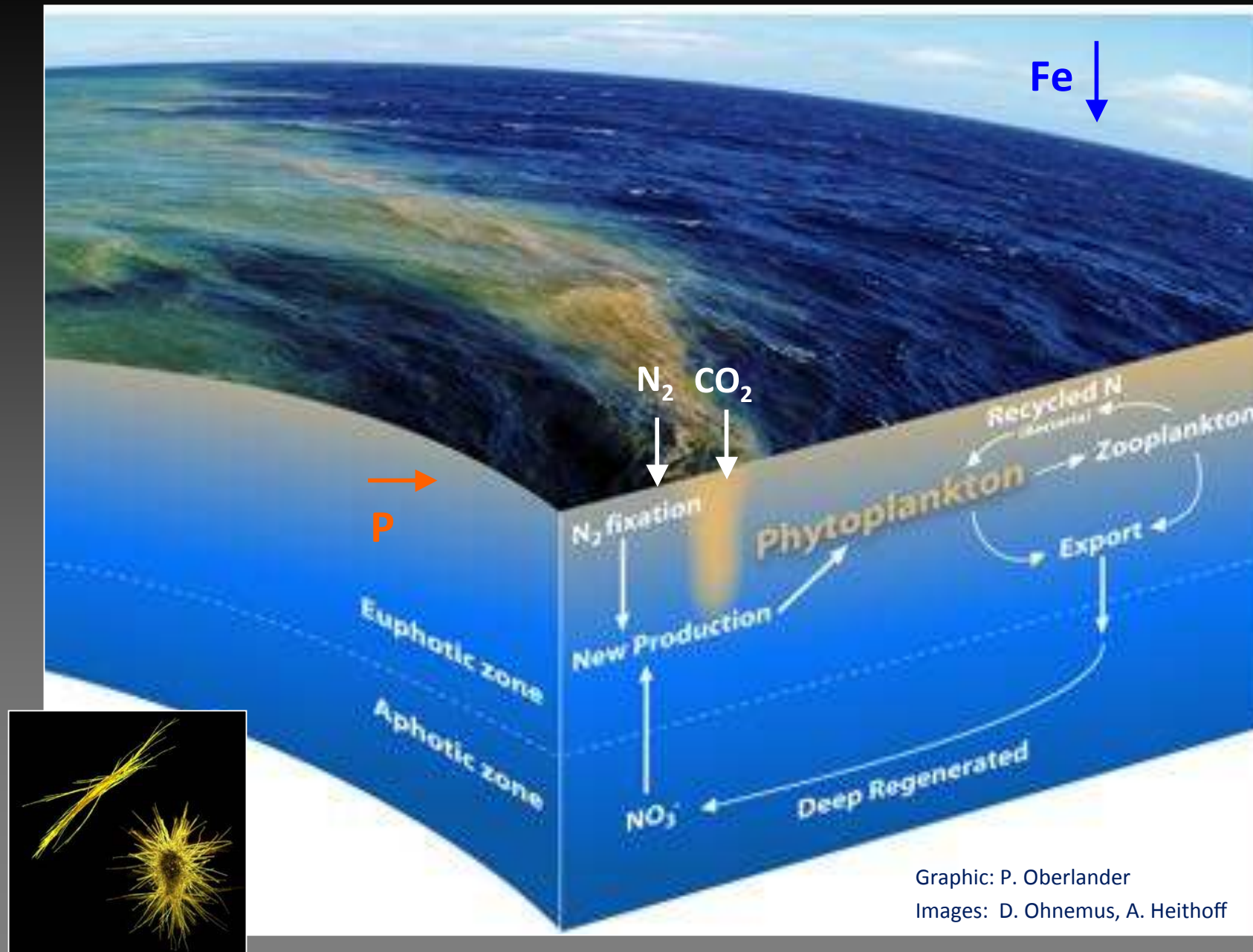
-*Trichodesmium contortum*
-*Trichodesmium erythraeum*

} *T. erythraeum* -
like Clade

-*Trichodesmium tenue*
-*Trichodesmium thiebautii*

} *T. thiebautii* -
like Clade

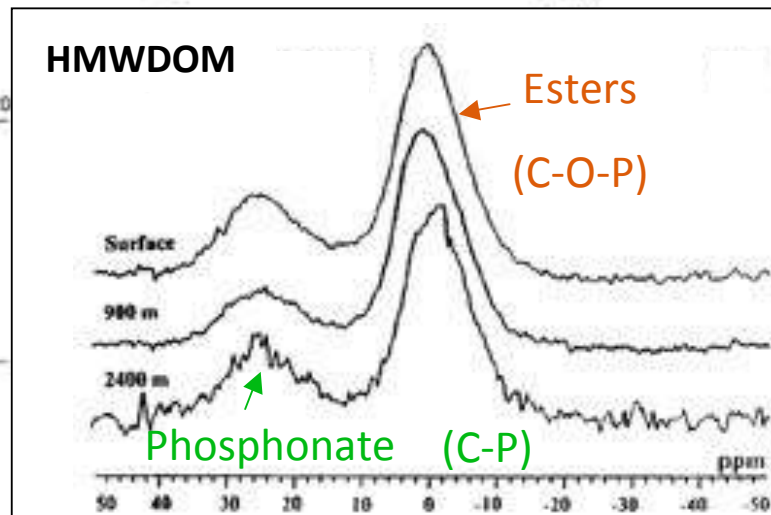
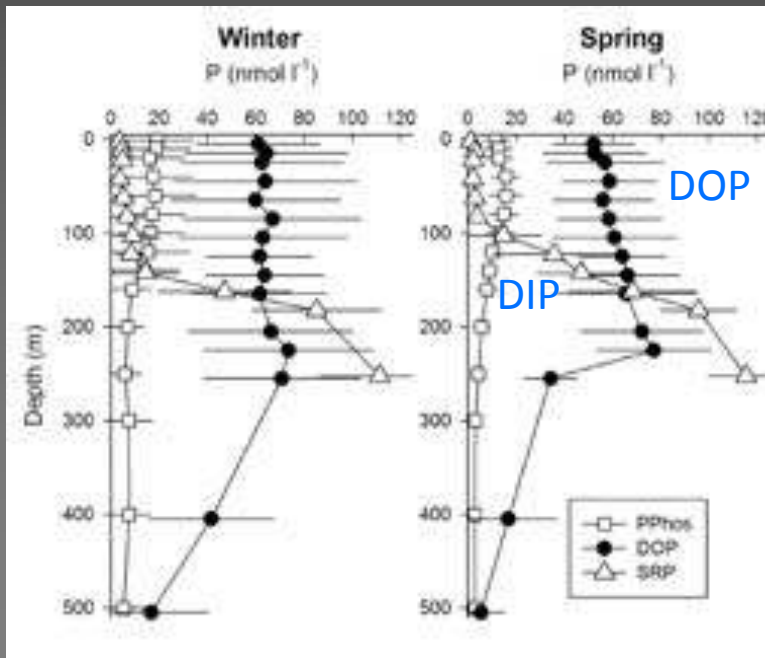
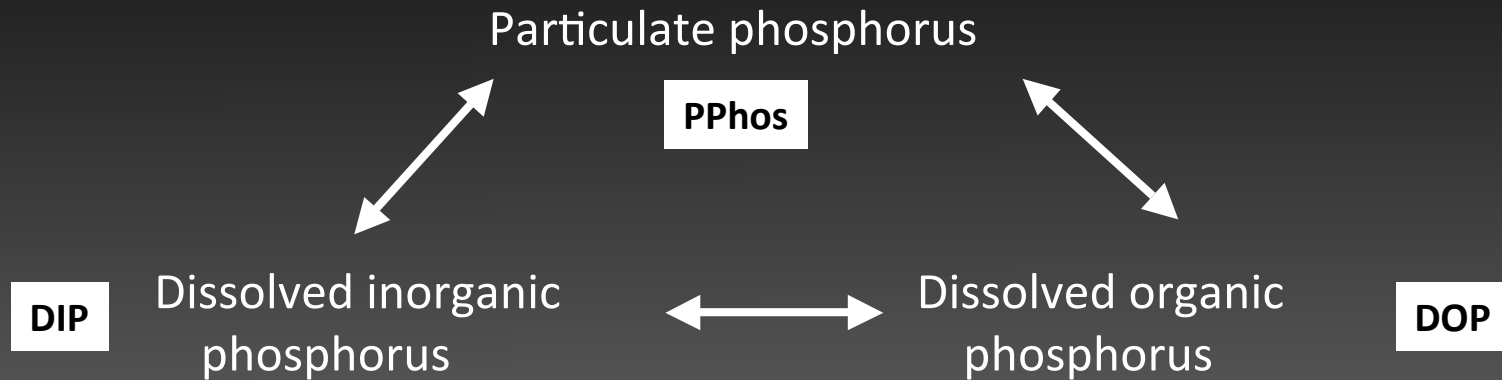
-*Trichodesmium spiralis*
-*Trichodesmium hildebrandtii*



Graphic: P. Oberlander

Images: D. Ohnemus, A. Heithoff

Phosphorus pools in the western North Atlantic



Kolowith et al. 2001 Limnol. Oceanogr.

Trichodesmium erythraeum IMS101 genome page



The screenshot shows a web browser window with the URL http://genome.jgi-psf.org/draft_microbes/trer/trer.html. The page features the JGI Microbes logo and a navigation bar with links: BLAST, Download, Draft Annotation, Info, and Home. A large image of *Trichodesmium erythraeum* cells is displayed on the left. To the right of the image is a text block describing the organism. At the bottom left, there is a small text block with contact information and a copyright notice.

JGI Microbes *Trichodesmium erythraeum* DRAFT GENOME

| BLAST | Download | Draft Annotation | Info | **Home**



Many filamentous cyanobacteria of the genus *Trichodesmium* play a major role in the tropical and subtropical oceans both as primary producers and suppliers of "new" nitrogen through their ability to fix atmospheric dinitrogen (N₂) (Capone et al., 1997). They are single unilaminated filamentous forms that divide in a single plane. They may occur as single filaments but more commonly as macroscopic colonial aggregates (0.3-2mm) containing many filaments. The colonies vary in color from yellowish-brown to deep red because they contain phycoerythrin as their primary light harvesting pigment. *Trichodesmium* spp. are planktonic and owe their buoyancy to the possession of gas vacuoles. The genus currently contains five species characterized from natural populations (Carpenter et al., 1993; Jensen et al., 1995). The species are distinguished by cell and colony morphology, pigmentation and buoyancy.

Questions about this project, contact: Paul Richardson
DOE Joint Genome Institute © 2002

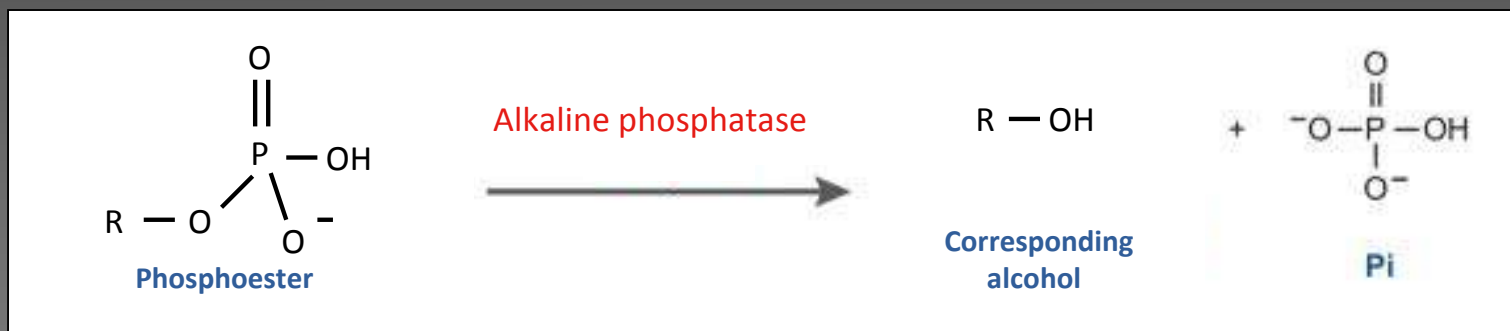
Phosphorus metabolic traits and trade-offs

- Phosphonate
 - C-P Lyase (Fe co-factor)
- Ester
 - *phoX* type alkaline phosphatase (Ca Fe co-factor)
 - *phoA* type alkaline phosphatase (Zn co-factor)

C-P

COP

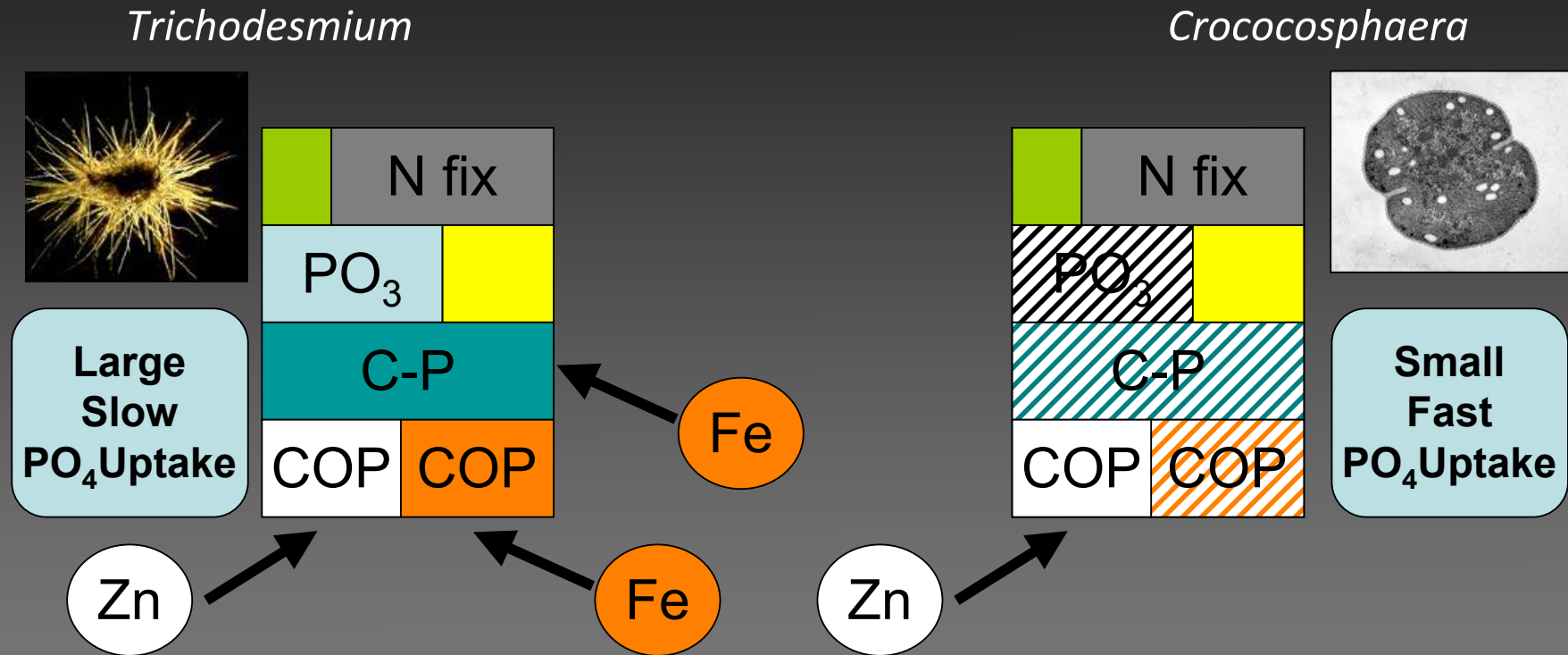
COP



- Phosphite
 - *ptxD* gene cluster

PO₃

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

Assaying P supply with isotope tracers

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

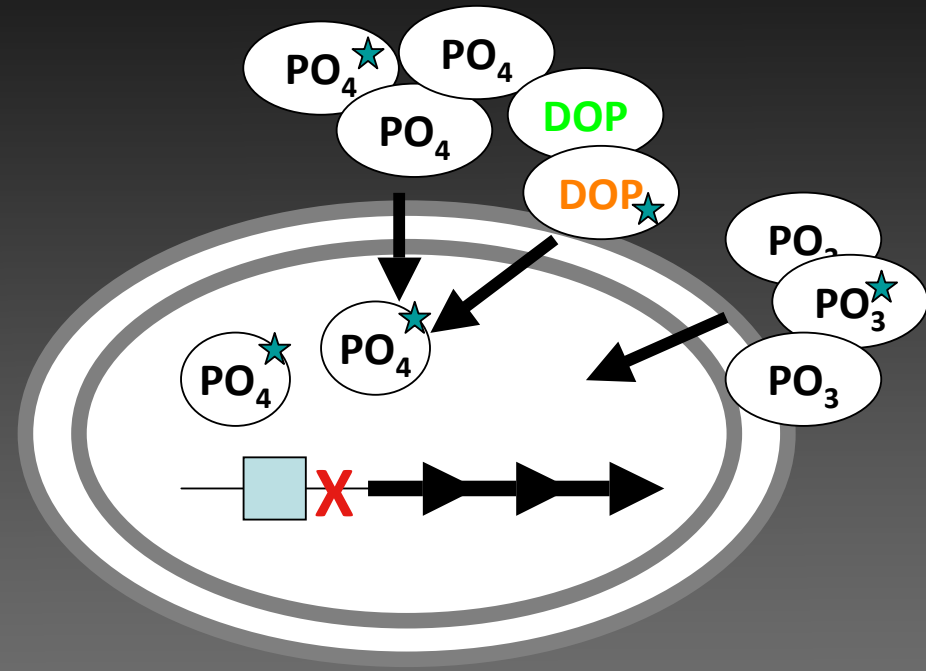
Trichodesmium:

~ 0.5 nmol $\text{PO}_4\text{h}^{-1}\mu\text{gChla}^{-1}$

~ 29 - 80 % ester DOP

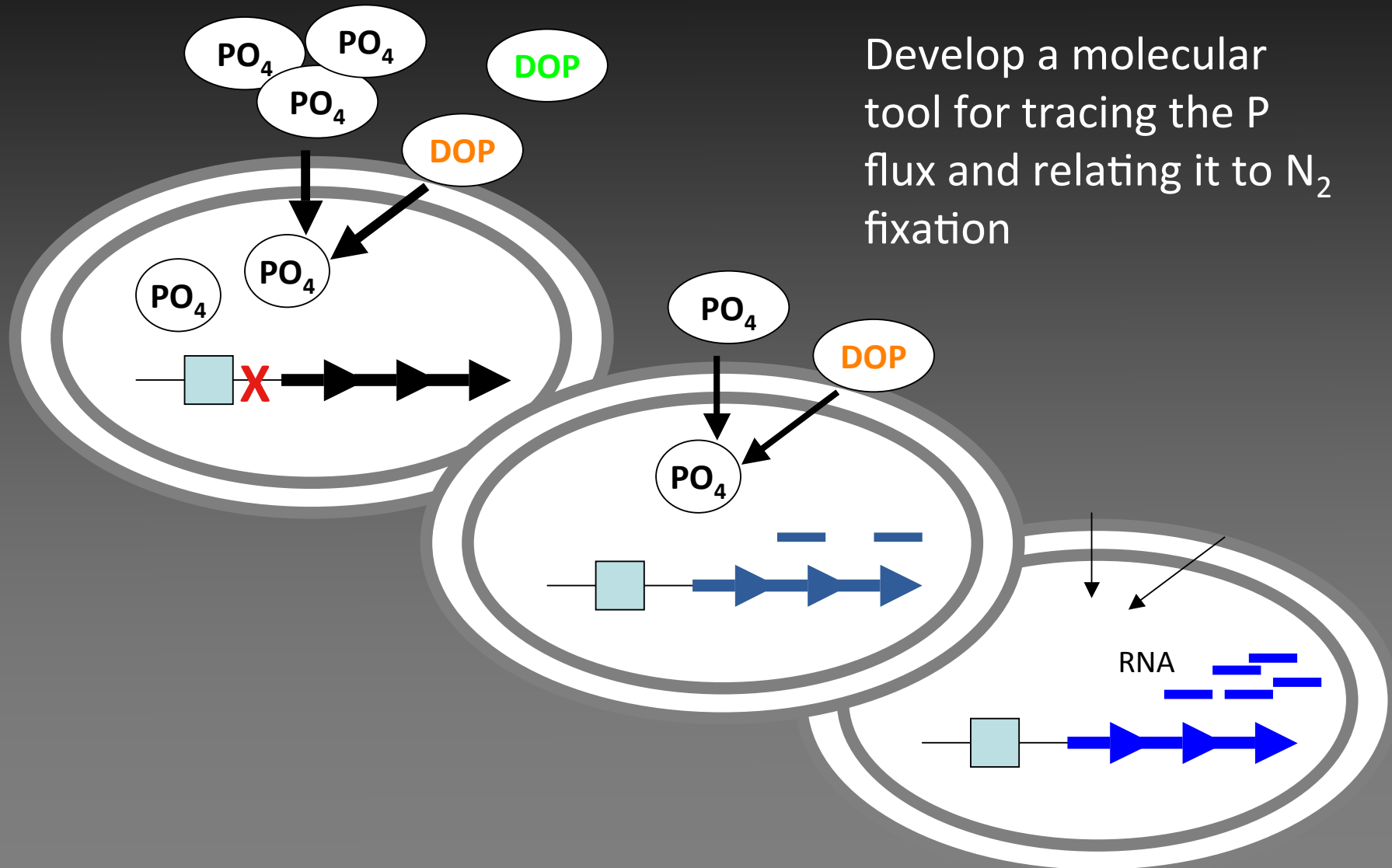
~ ?% phosphonate DOP

~ ?% phosphite (PO_3)



Orchard et al. 2010 *Limnol and Oceanogr*
Van Mooy et al. 2015 *Science*

Assaying P supply with a genome-enabled tool



Tracking genomic potential with expression studies



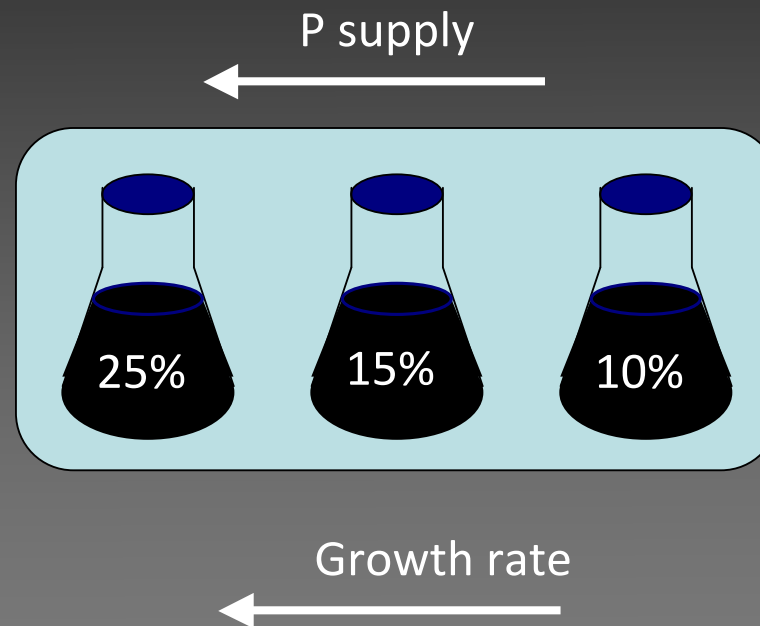
Culture cells



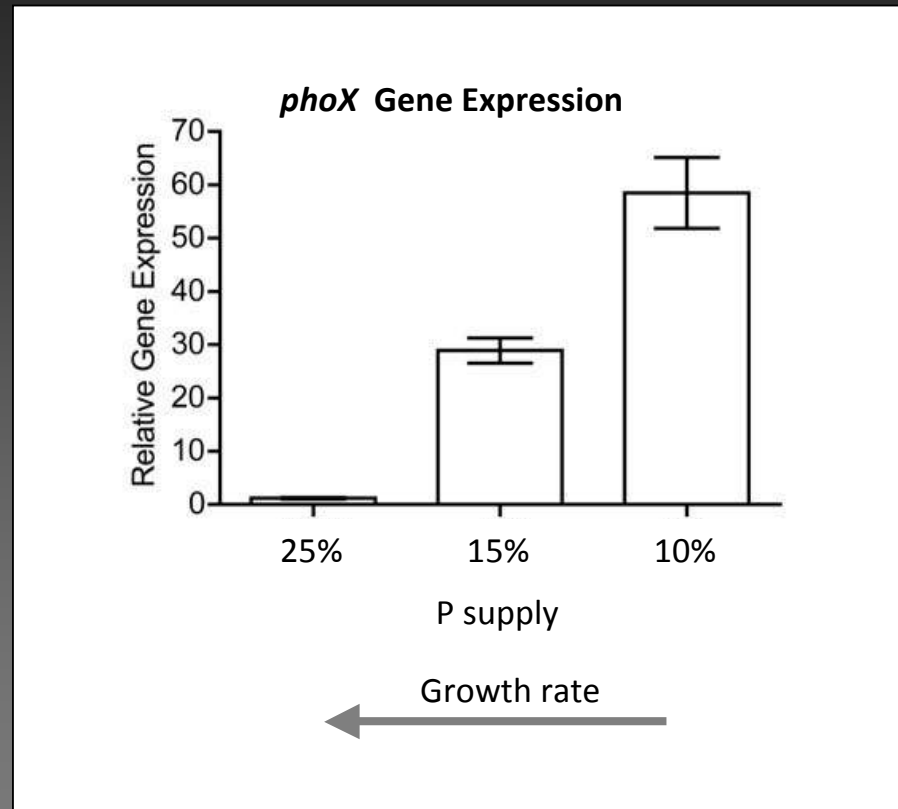
Harvest and preserve
samples



qRT-PCR of *phoX*
Activity



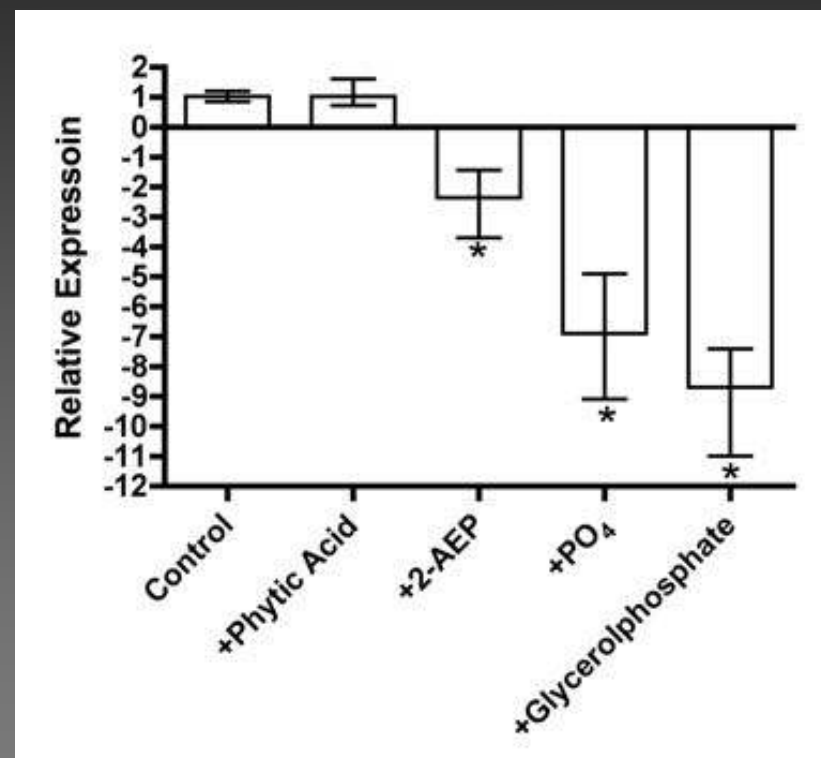
Calibrating gene expression to growth and N₂ fixation



Orchard and Dyhrman unpublished

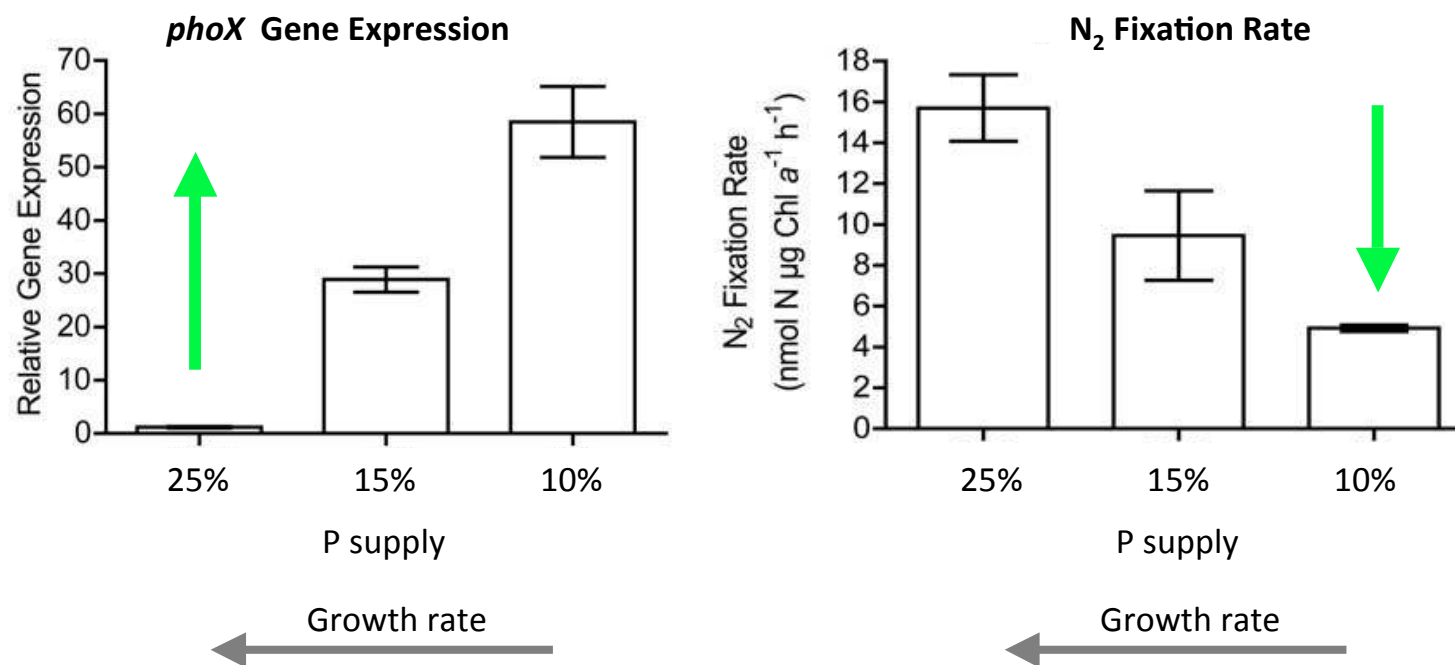
Calibrating gene expression to P supply

- *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



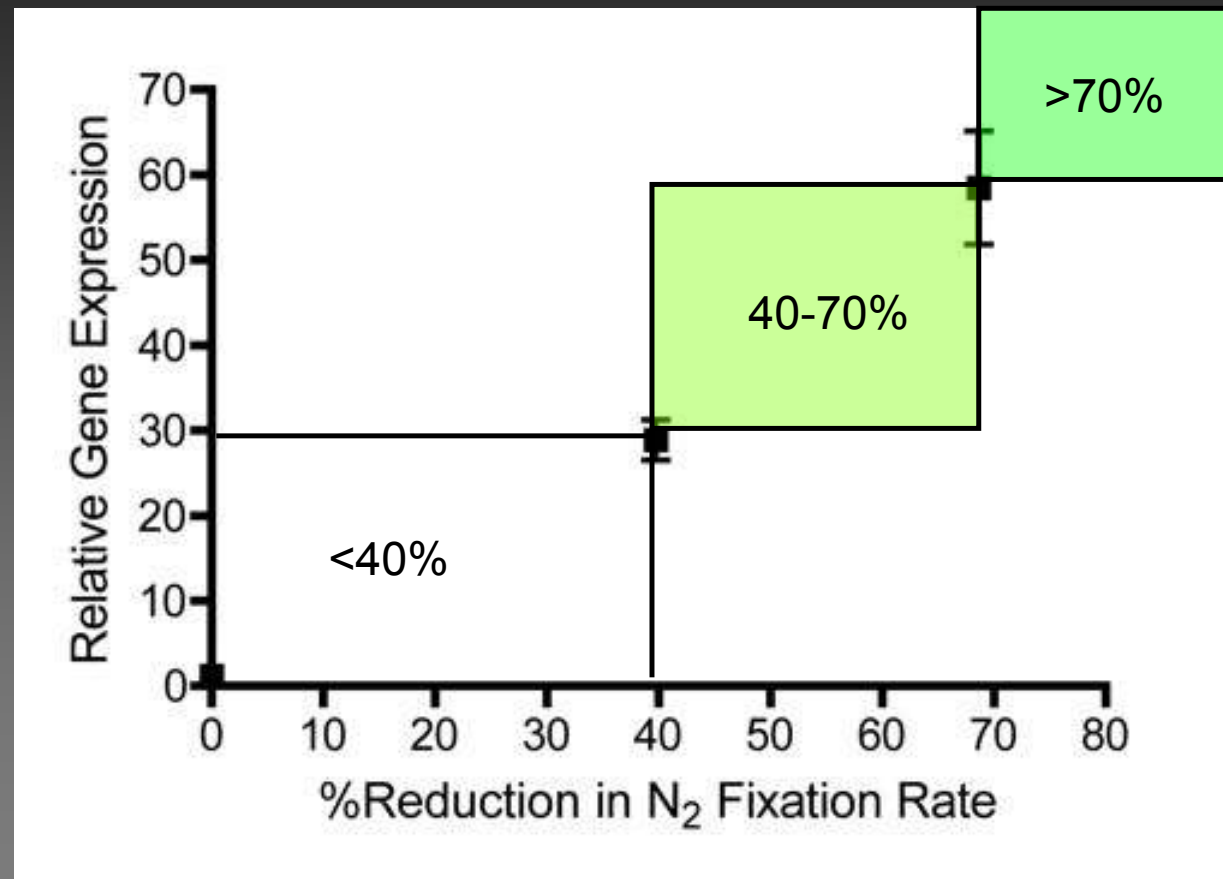
Orchard and Dyhrman unpublished

Calibrating gene expression to growth and N₂ fixation



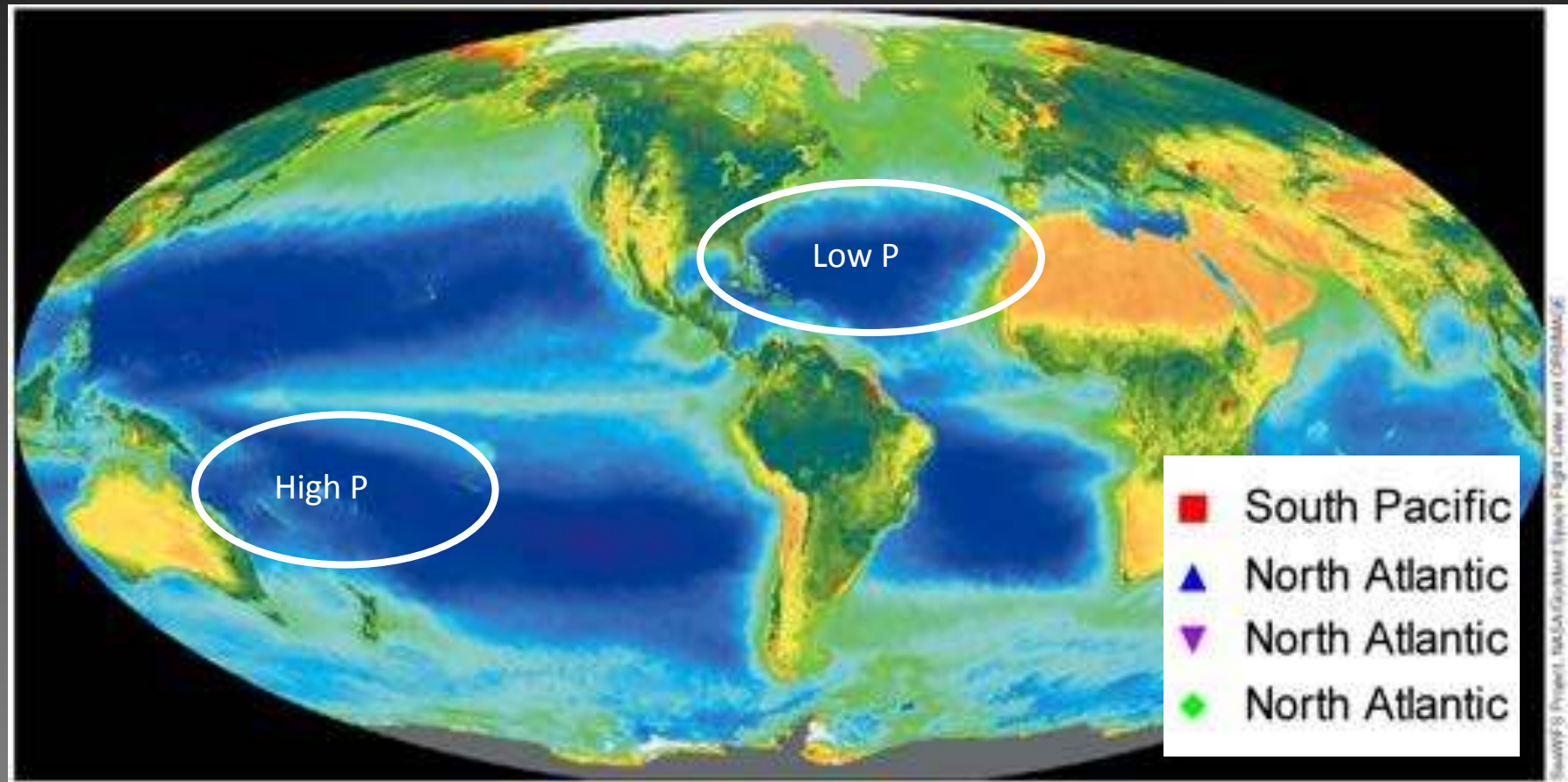
Orchard and Dyhrman unpublished

Calibrating gene expression to N₂ fixation



Orchard et al. in prep

Sampling different P regimes





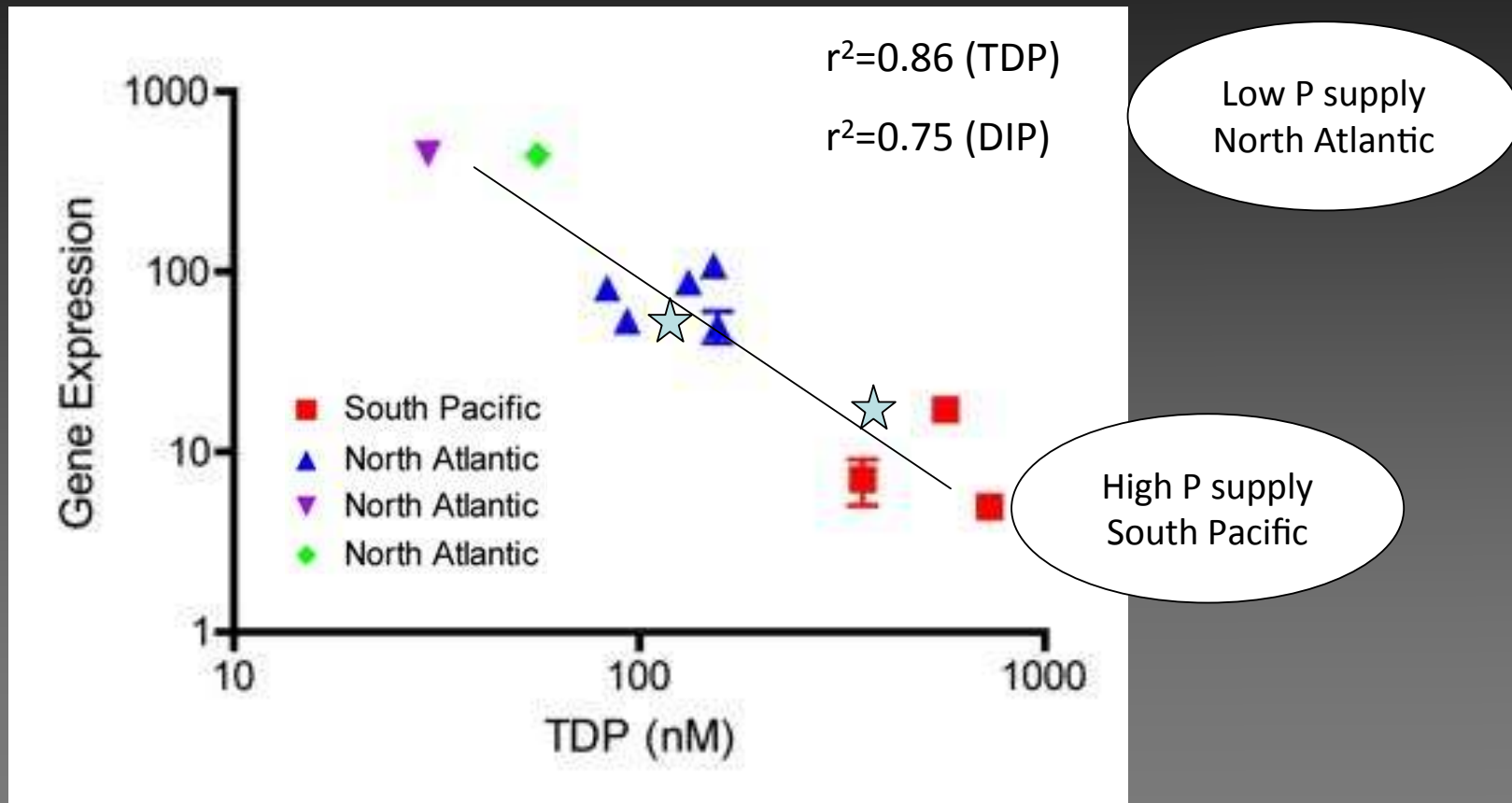


DIP, TDP
Measurements



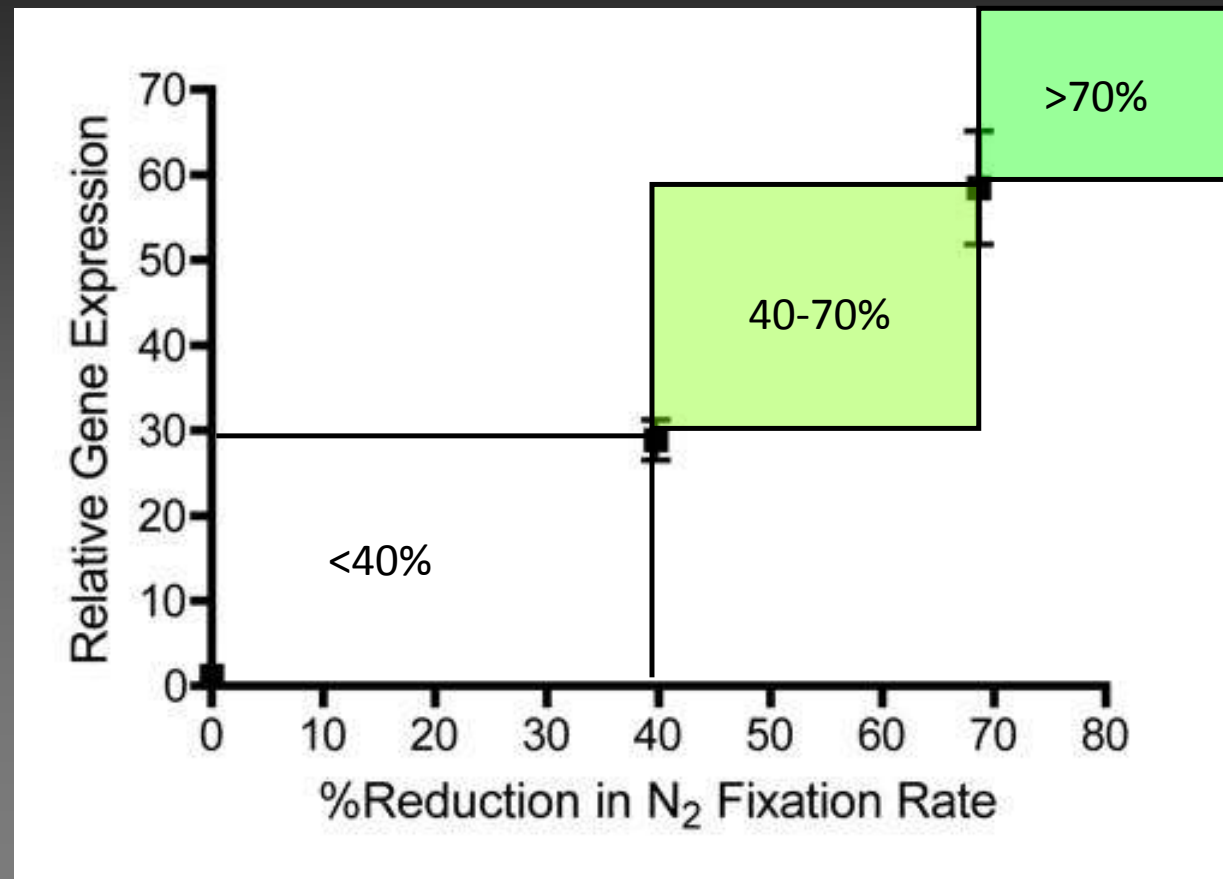
Measurements of
quantitative gene
expression for
Trichodesmium sp.

Gene expression increases at low phosphorus



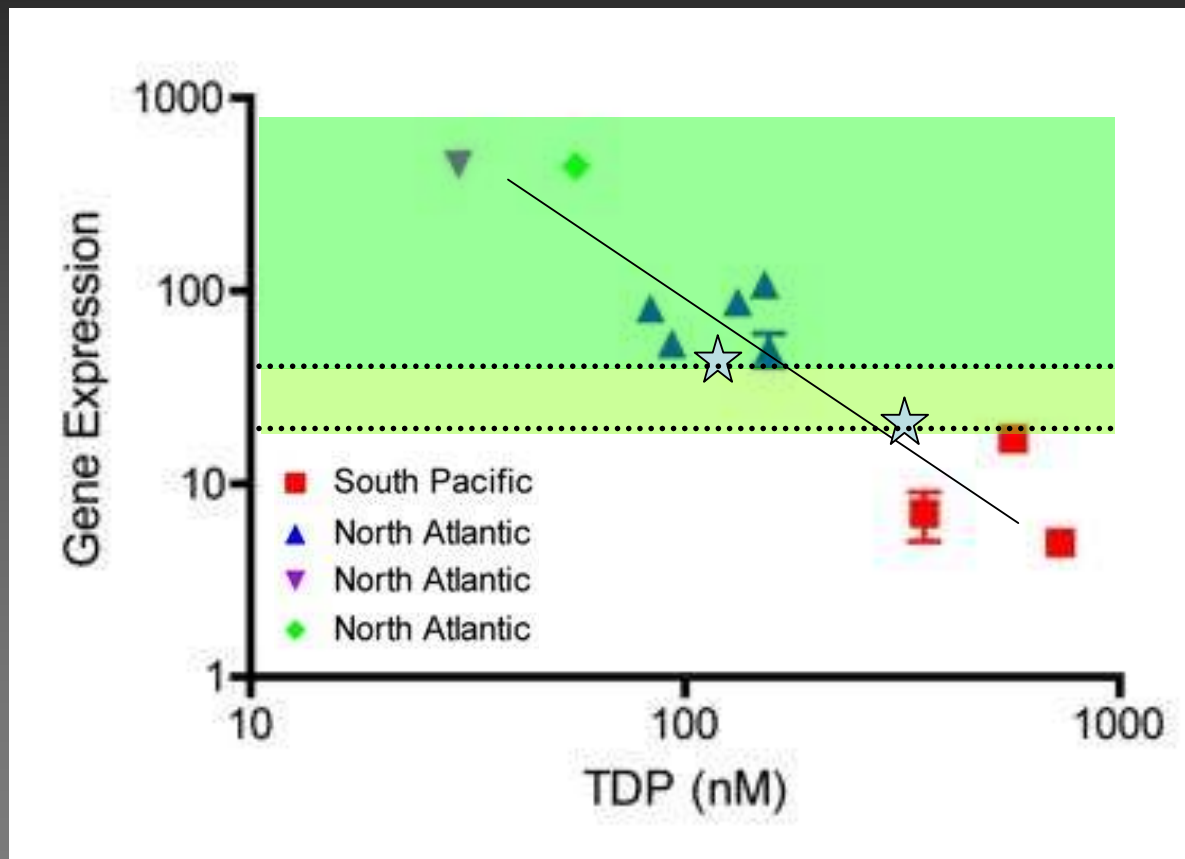
Orchard and Dyhrman unpublished

Calibrating gene expression to N₂ fixation



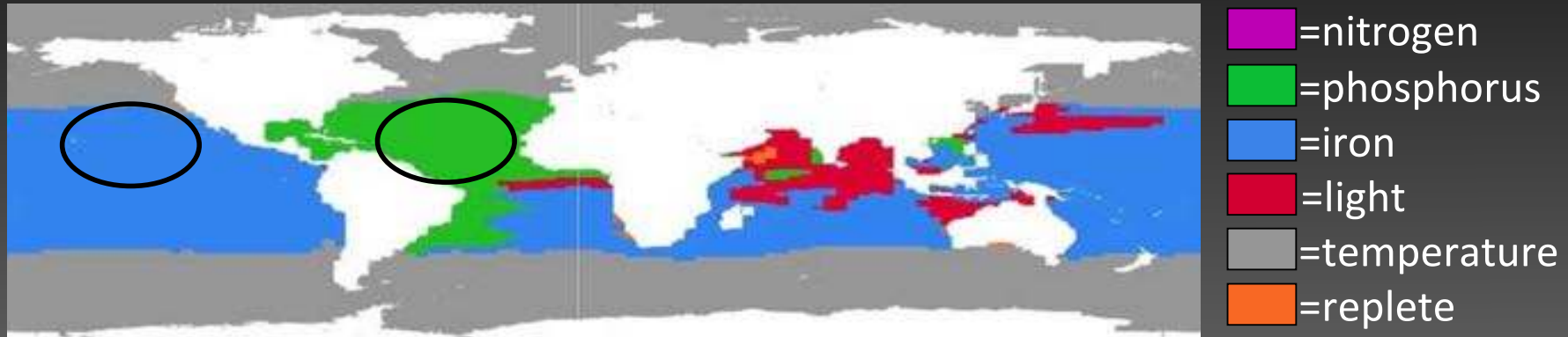
Orchard and Dyhrman unpublished

Data predicts that P supply limits N₂ fixation in the western N. Atlantic



Orchard and Dyhrman unpublished

Constraints on *Trichodesmium* N₂ fixation



(Moore et al. 2004)

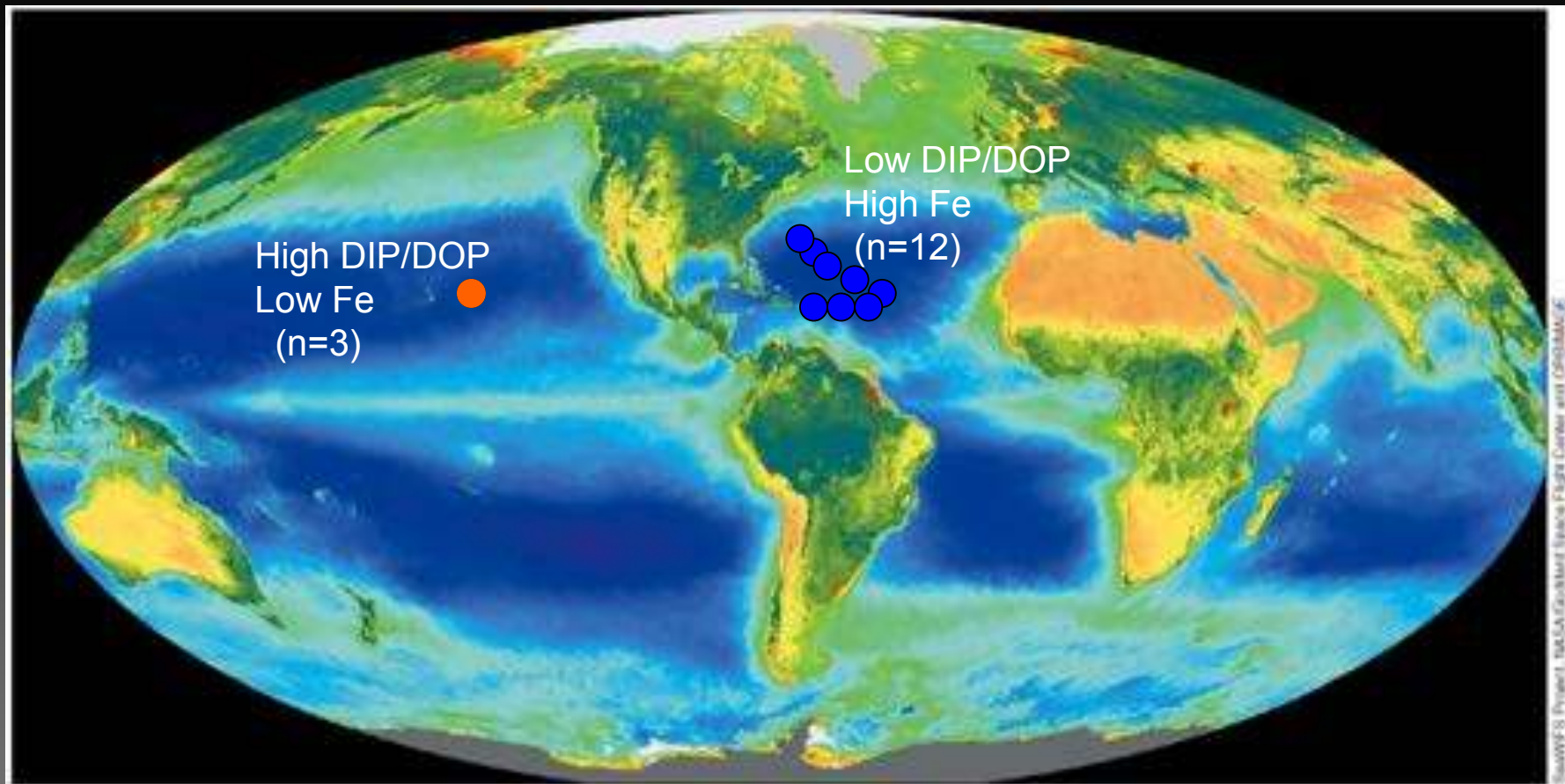
Molecular patterns corroborate predictive models in the western north Atlantic

phoX - P regulated ester metabolism (Orchard et al. 2009 *Environ. Micro.*)

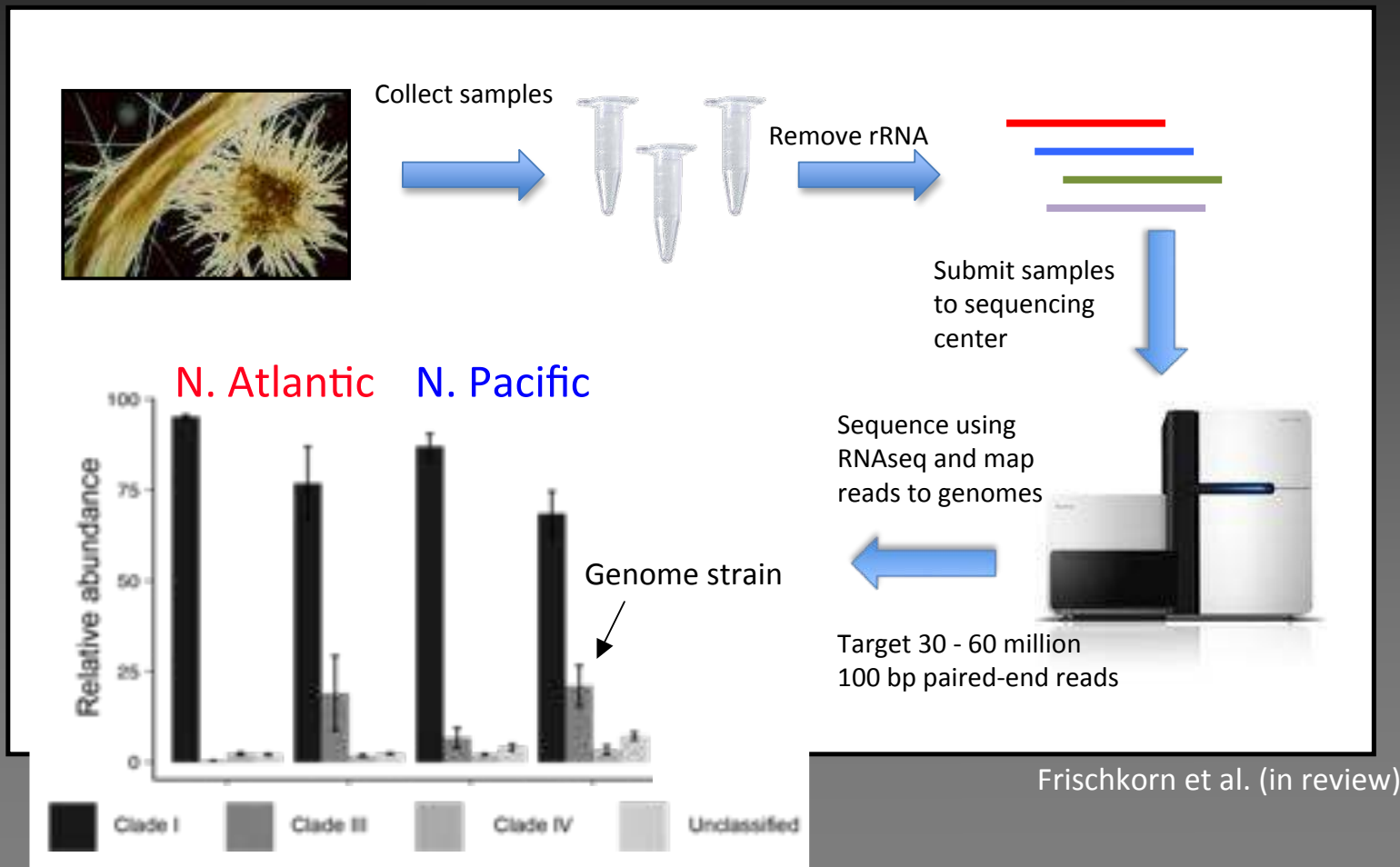
idiA - Fe regulated iron metabolism (Chappell et al. 2013 *ISME J.*)

rnpB - reference gene

nifH - N₂ - fixation



Analysis pipeline derived from metagenome analyses



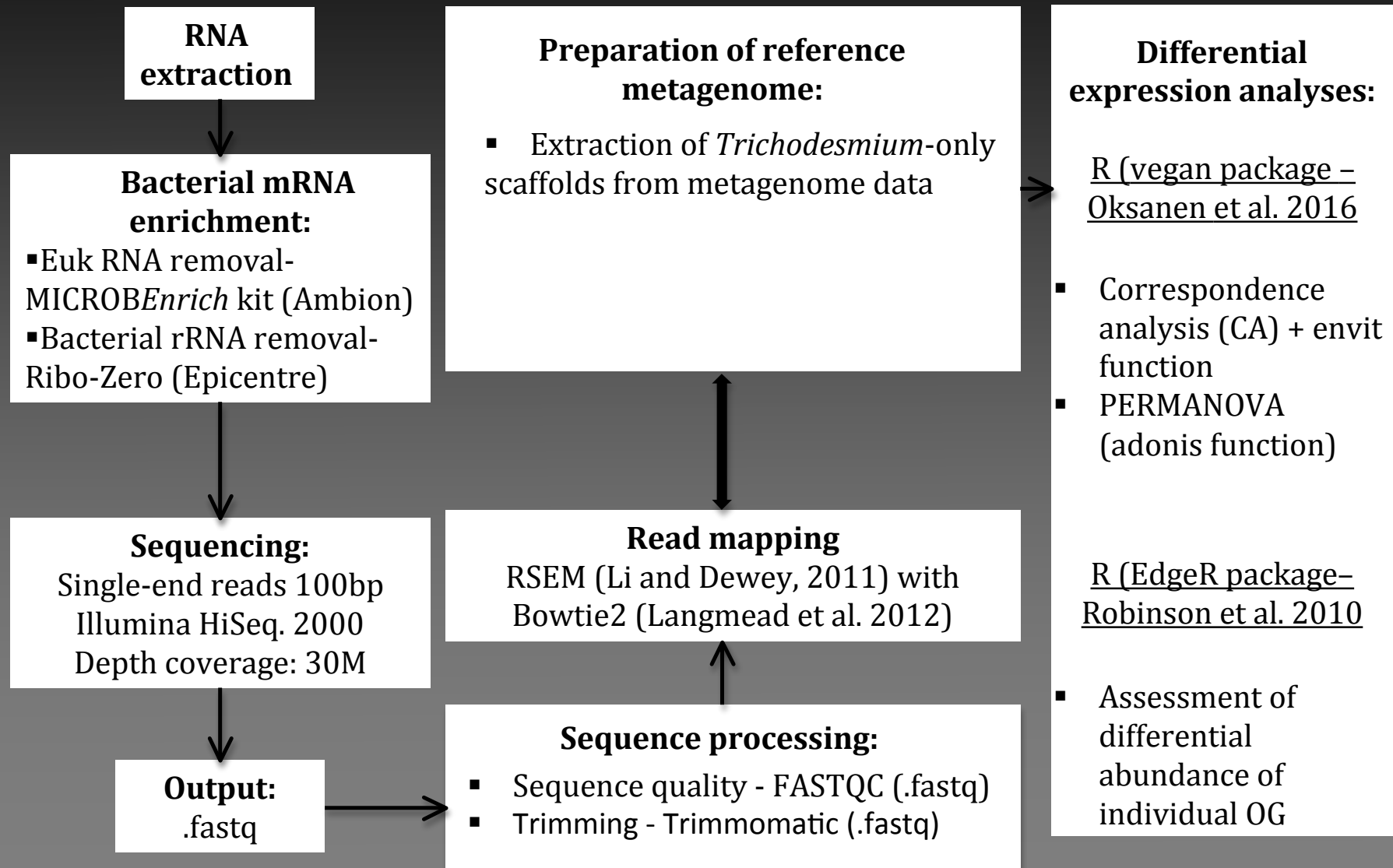
Frischkorn et al. (in review)

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

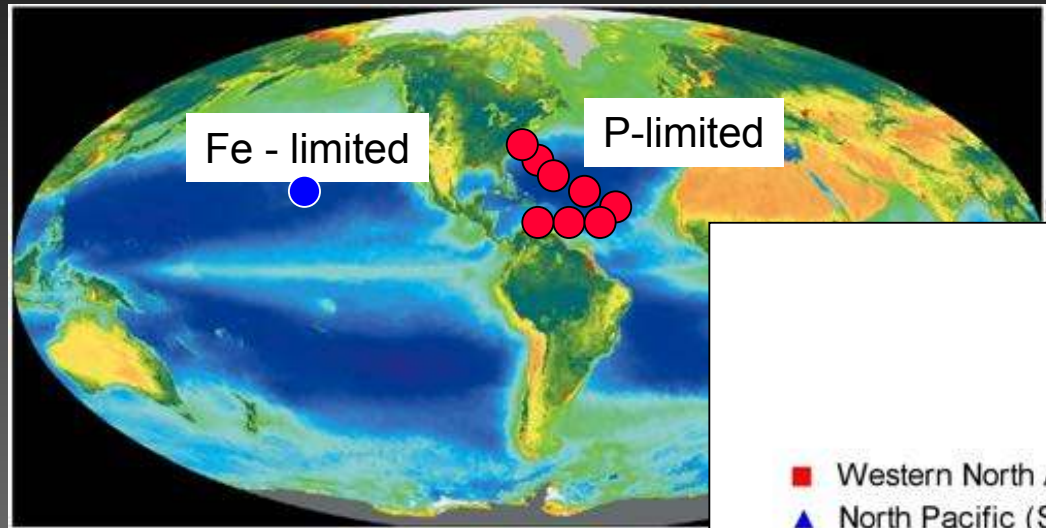
The reality.....

```
CAACAACATCATACCCCTTCCAAGGCACGGGCTTCAACGCCCGCCAAATCTCC
TGCGGCAGCAGGGATGGGACCCTGGGACTGTGTGGCGCTGTGGAGGCCG
+DHT4KXP1:1:1101:5930:2353#0/1
\W\^^aac^[ '[cfhh_Z'^eg[RJ`W^^000^aeae0_eehhULVMV\H\
\`ZSNW^aaQTRGX^BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@DHT4KXP1:1:1101:5904:2385#0/1
CTACCATTTTCATTTTCCATGTCTCTCCCATCTTGACACATTATTTTCT
ACCTCCATCACATCATGATCACGTTATACGATCTCTACAGTAGCCCCCA
+DHT4KXP1:1:1101:5904:2385#0/1
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iih11ih11h11h11iih11idgggggeeeecdddddccbcccccc_
@DHT4KXP1:1:1101:5781:2386#0/1
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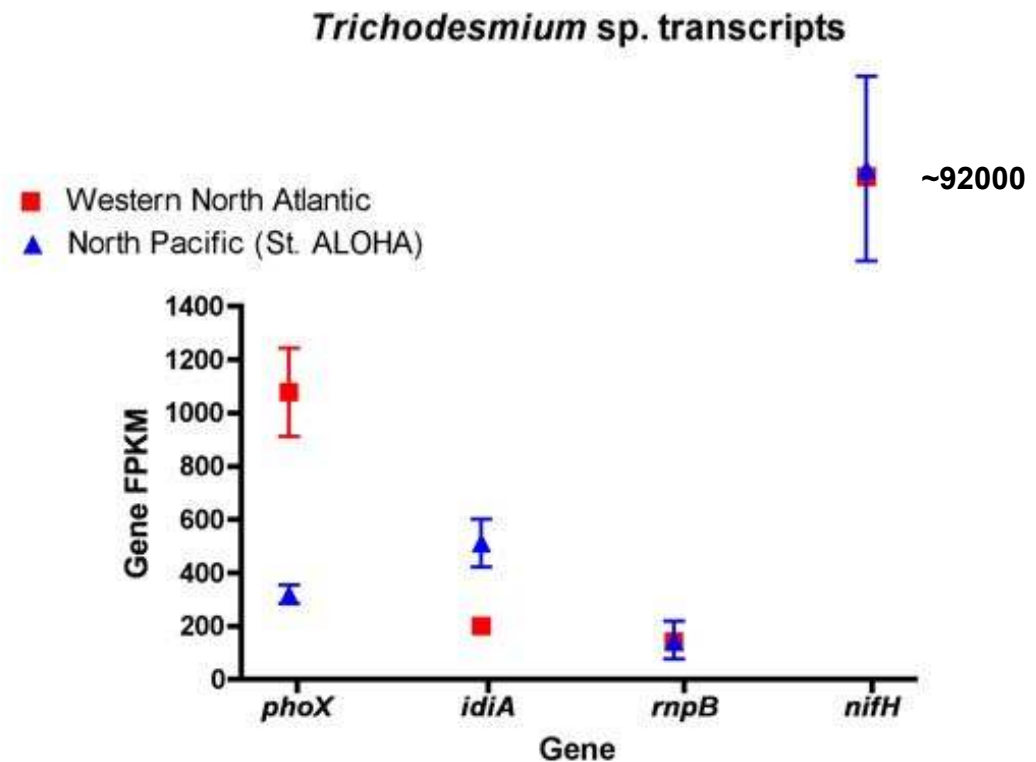
Metatranscriptome analysis



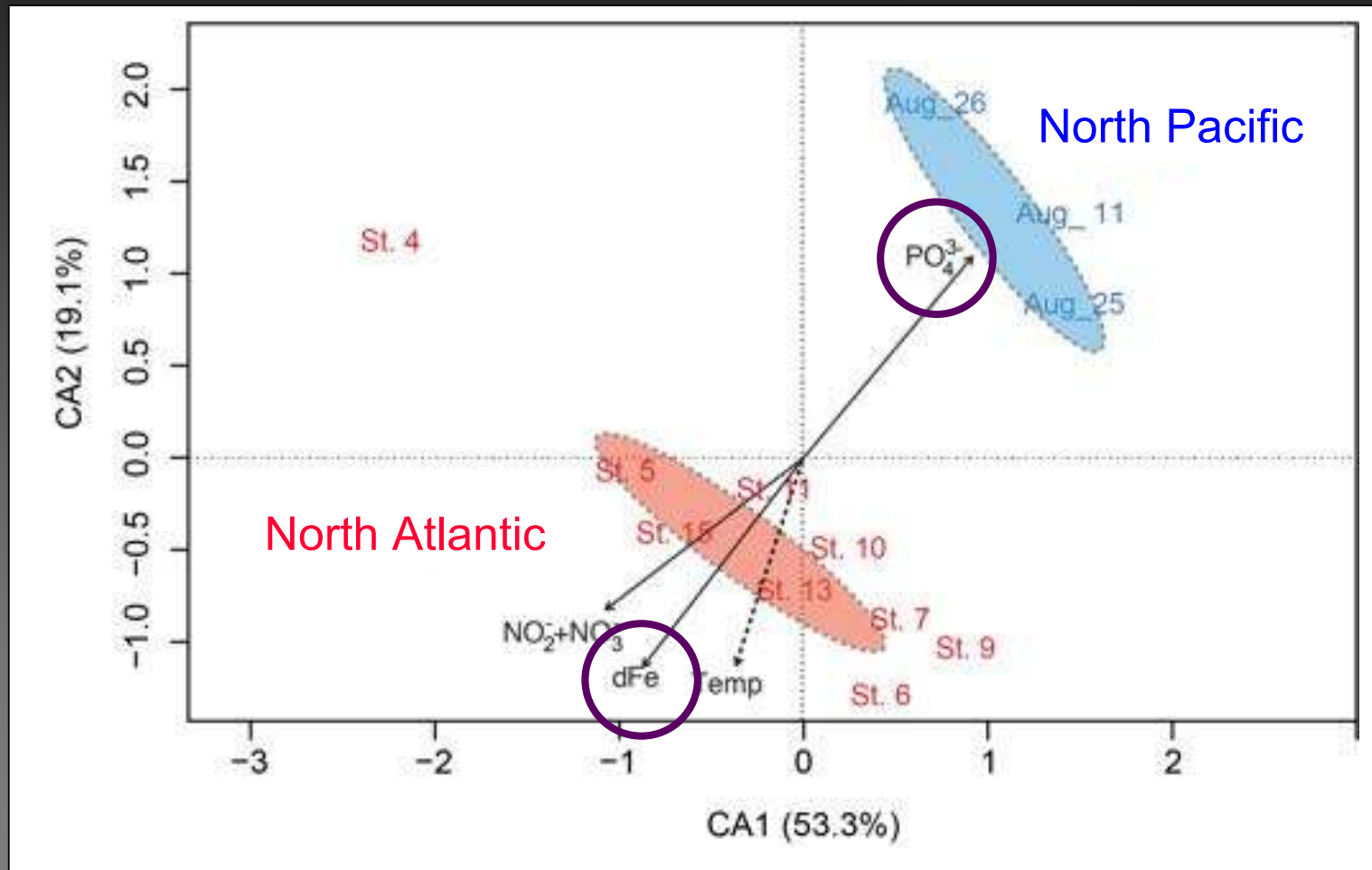
Biomarkers consistent with model prediction



Biomarkers confirm
model predictions.

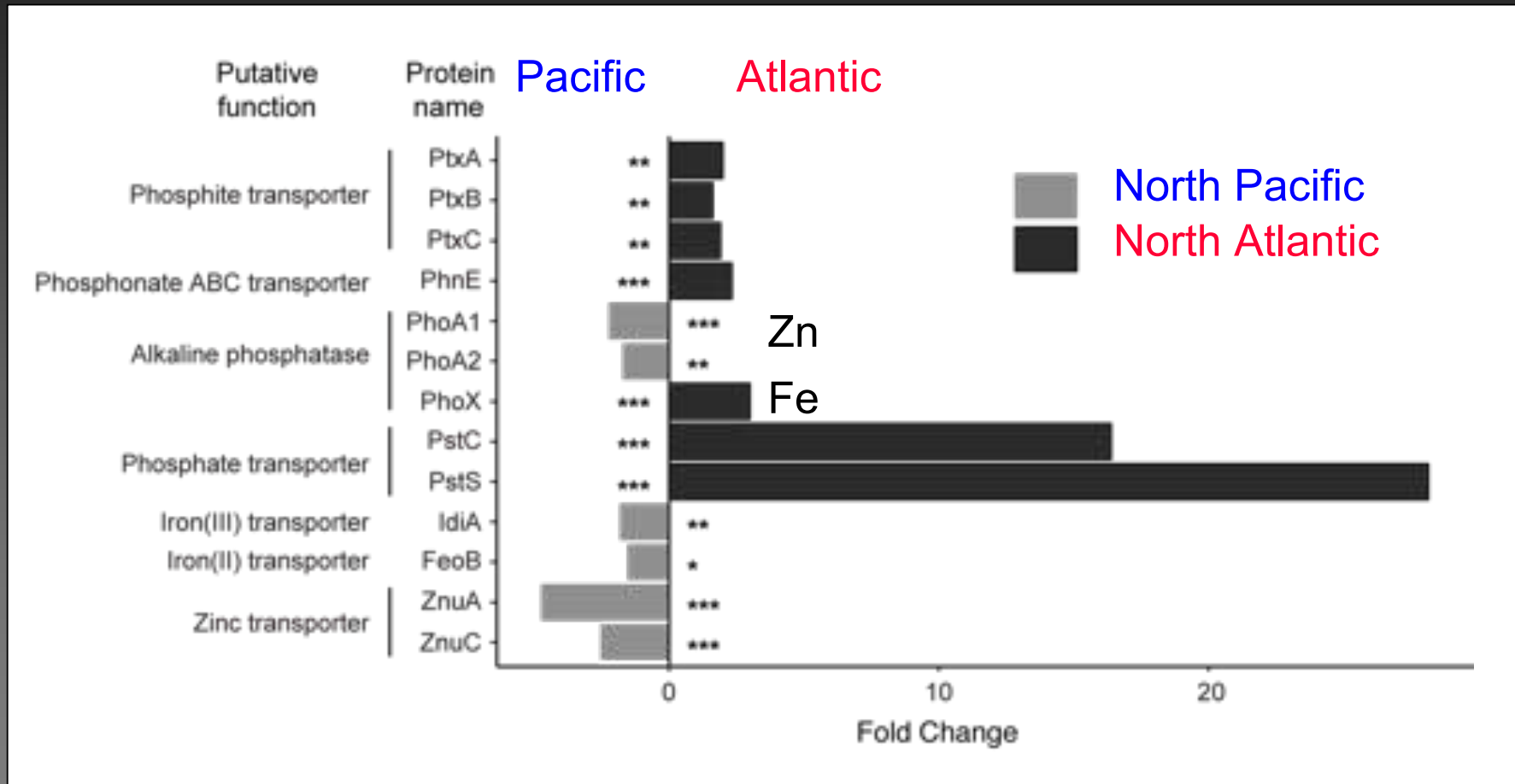


Significant differences in global transcription between the North Pacific and the North Atlantic



Ruoco et al. (in prep)

Transcription patterns indicate metalloenzyme trade-offs and geochemical controls

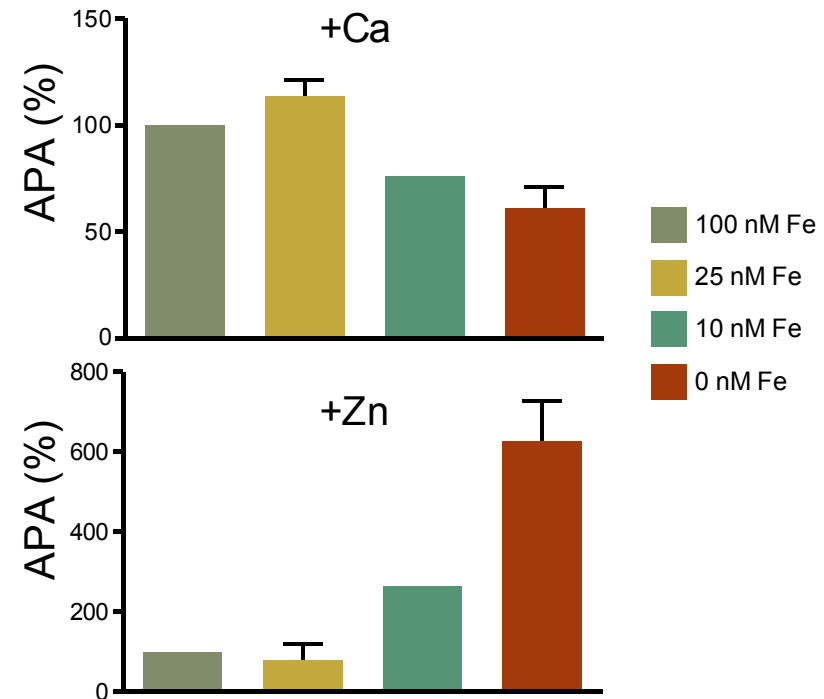
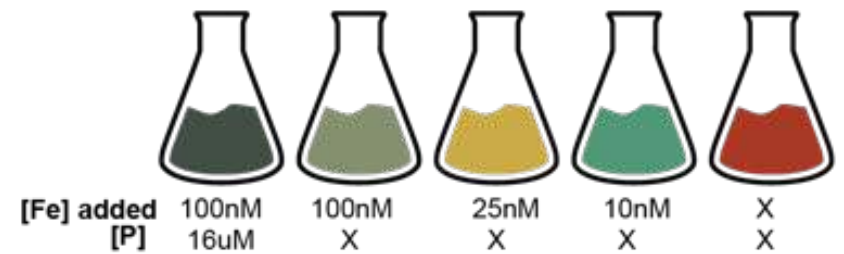


Contextualizing metatranscriptome data with culture experiments

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 N₂ fixation?

- *Trichodesmium* genome suggests bioavailability 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 N₂ fixation
- Predicted biogeochemical drivers of N₂ 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?



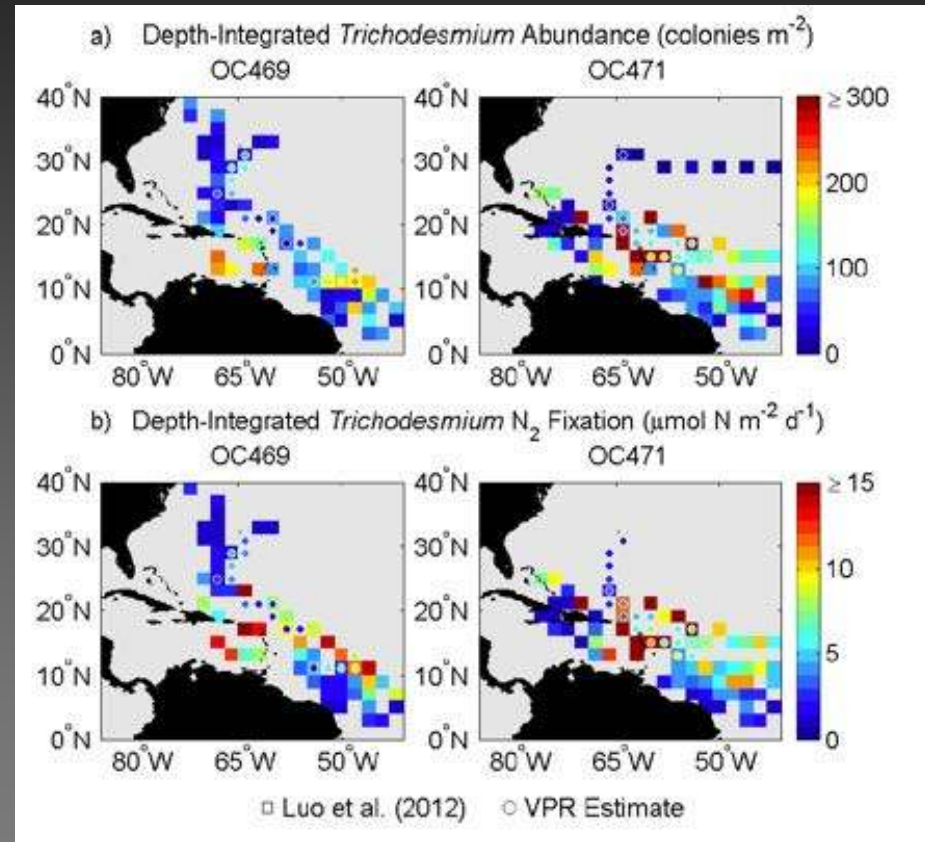
Host microbiome interactions

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



Modeling N₂ 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 N₂ 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*)

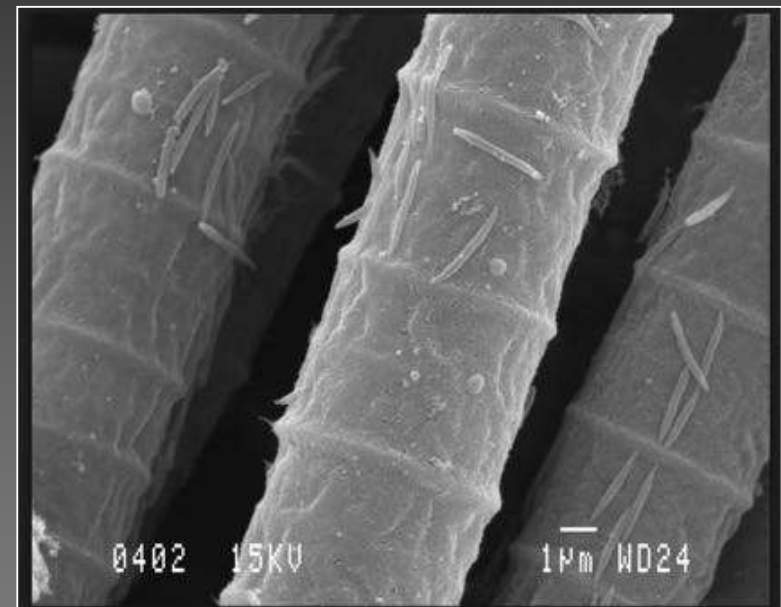
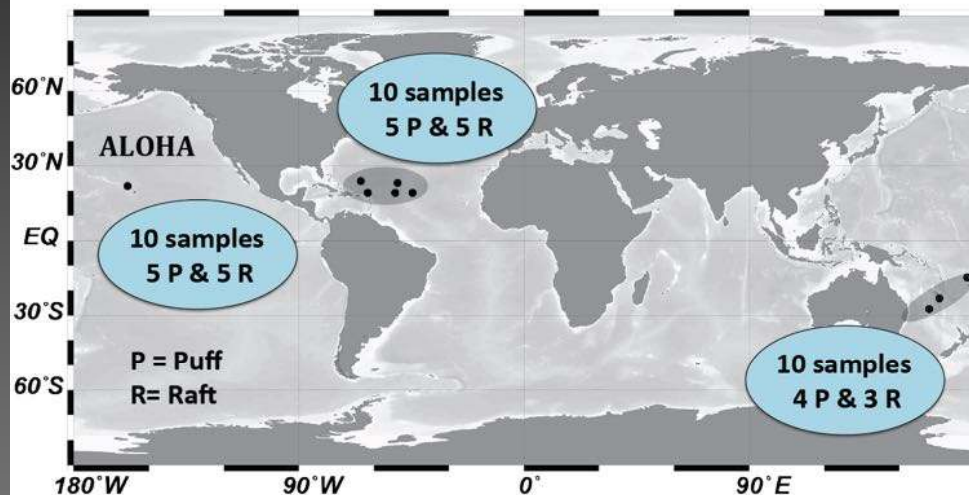


Image courtesy Tracy Mincer

Epibiont diversity

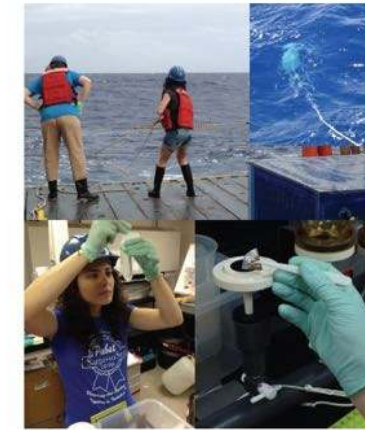
EXPERIMENTAL APPROACH



Collection of colonies from 3 ocean basins (~top 25m)

DNA extraction

Sequencing:
V4 region of 16S
rRNA gene
Miseq
(2x150 bp)



Mothur v. 1. 34. 0 (936,749 reads)

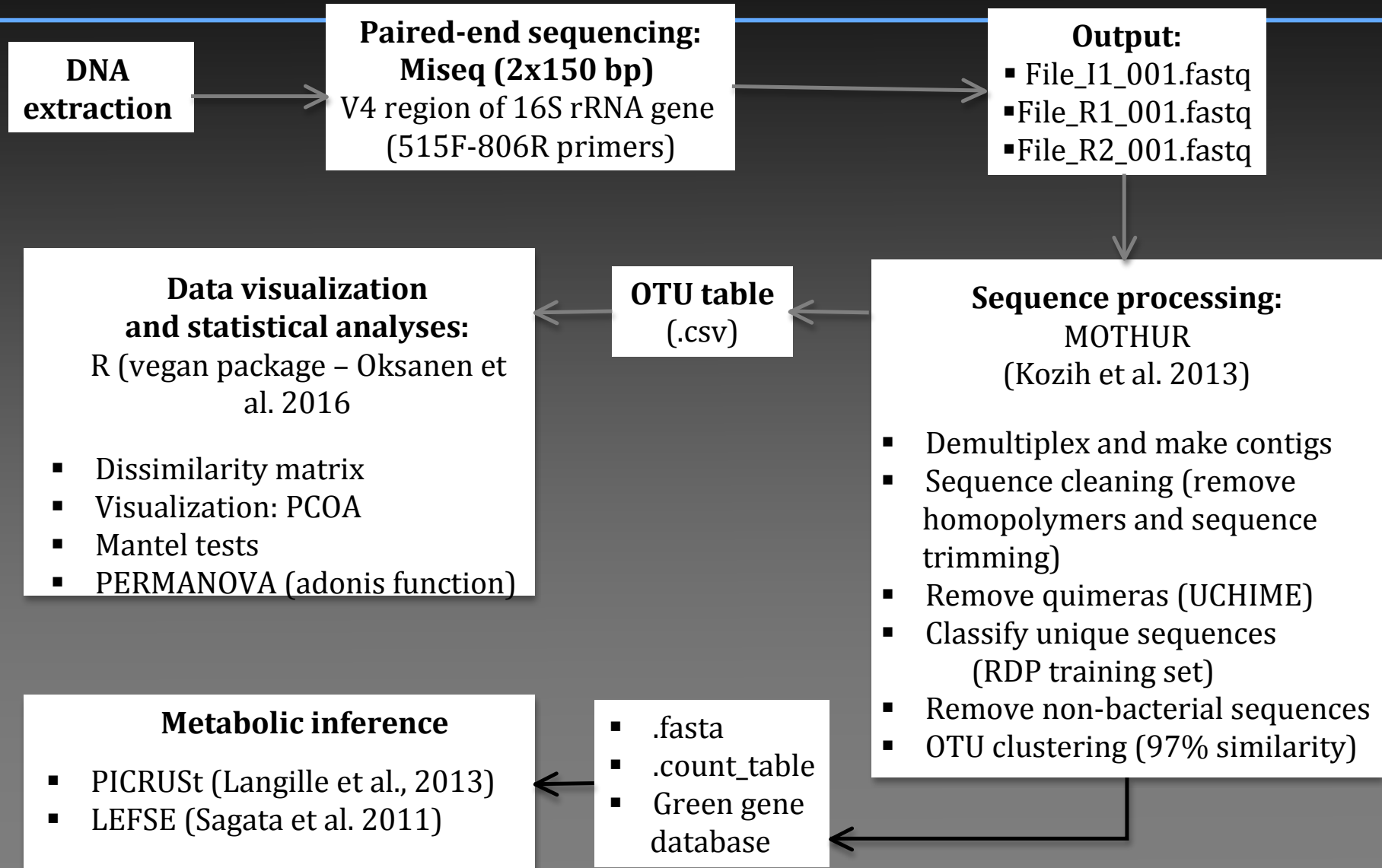
- *Trichodesmium*: ~67% of reads
- Epibionts: ~32% of reads

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

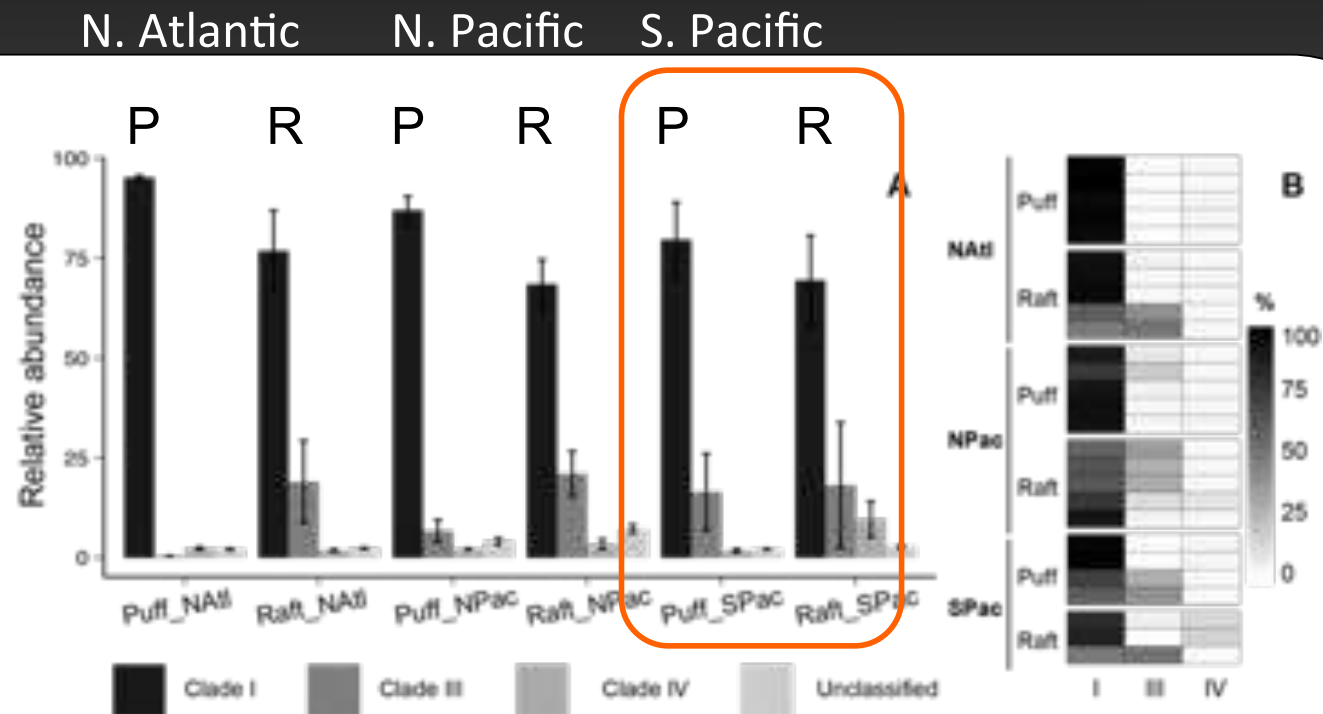


Mónica Rouco

16S rDNA analyses

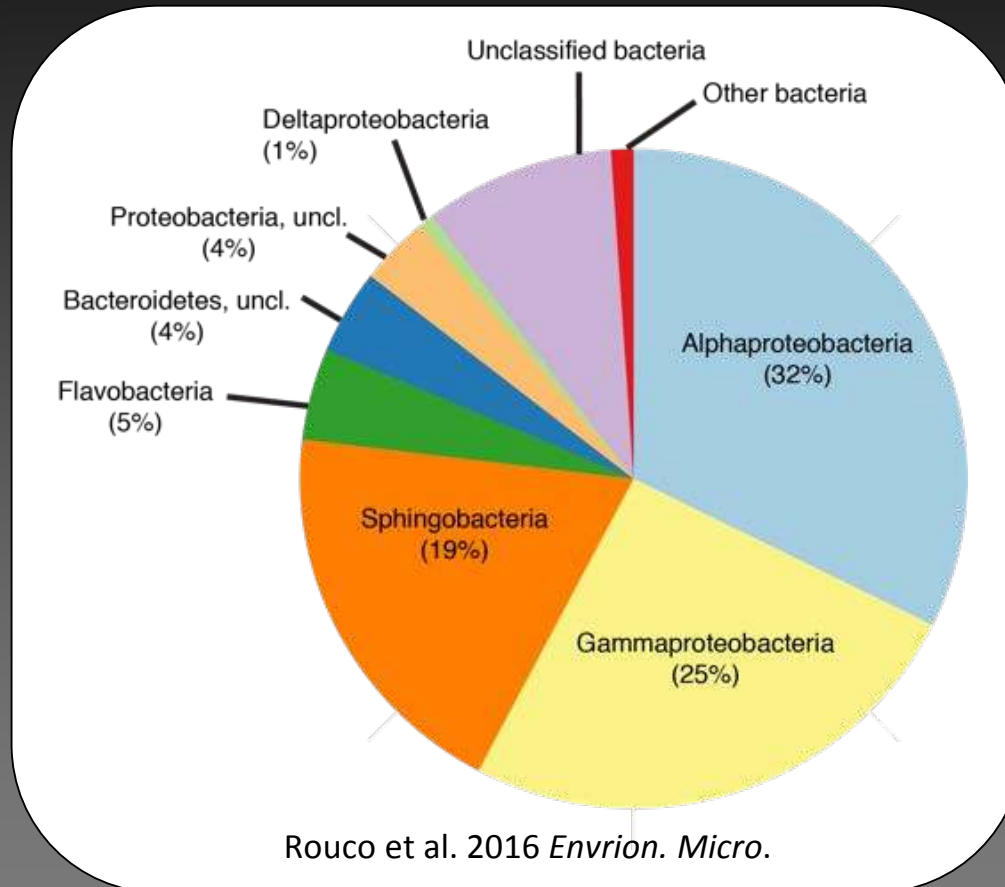


Colony composition by region



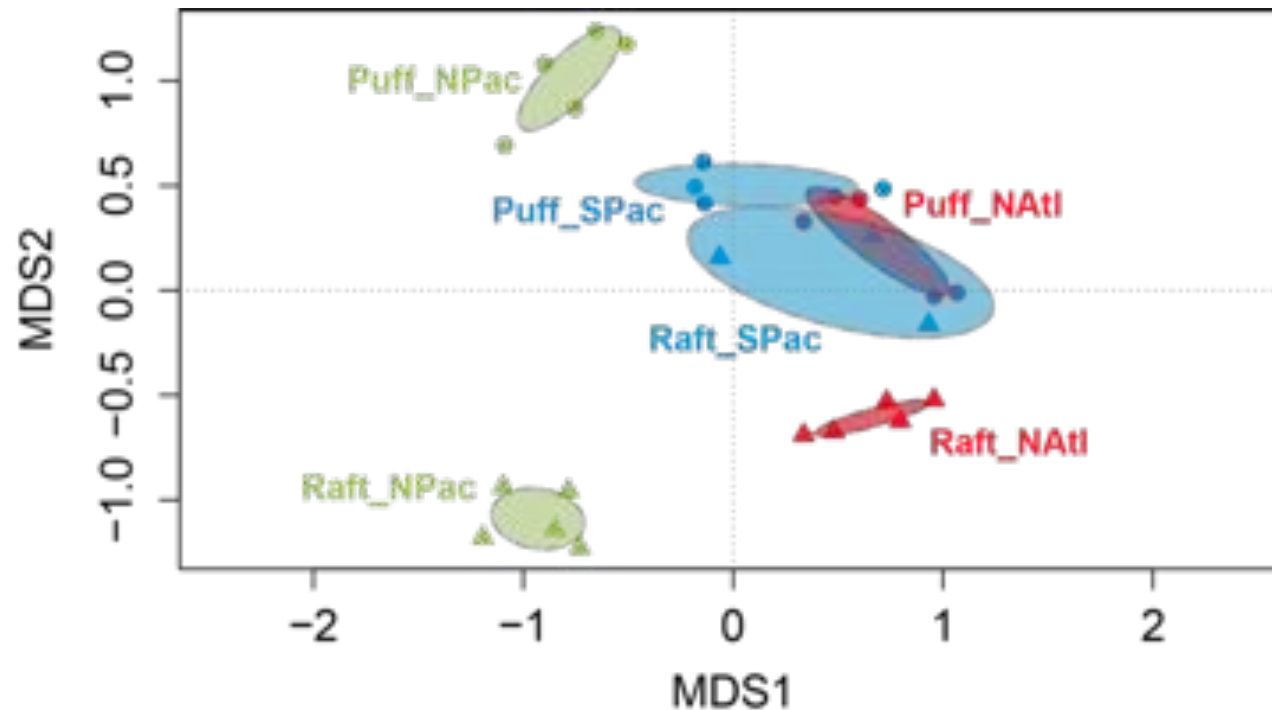
Colonies are not likely species specific, the raft morphology is more diverse except in the S. Pacific

Average epibiont community



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



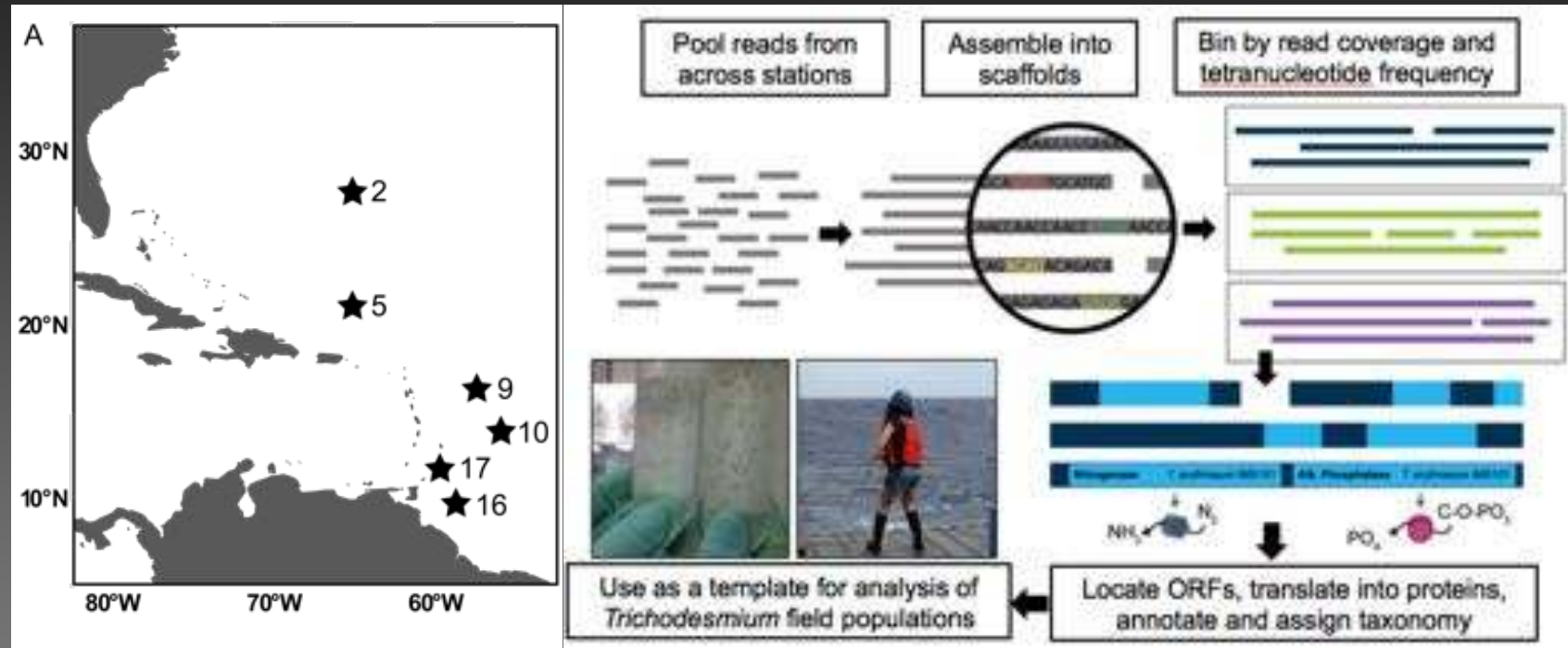
Rouco et al. 2016 *Environ. Micro.*

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?

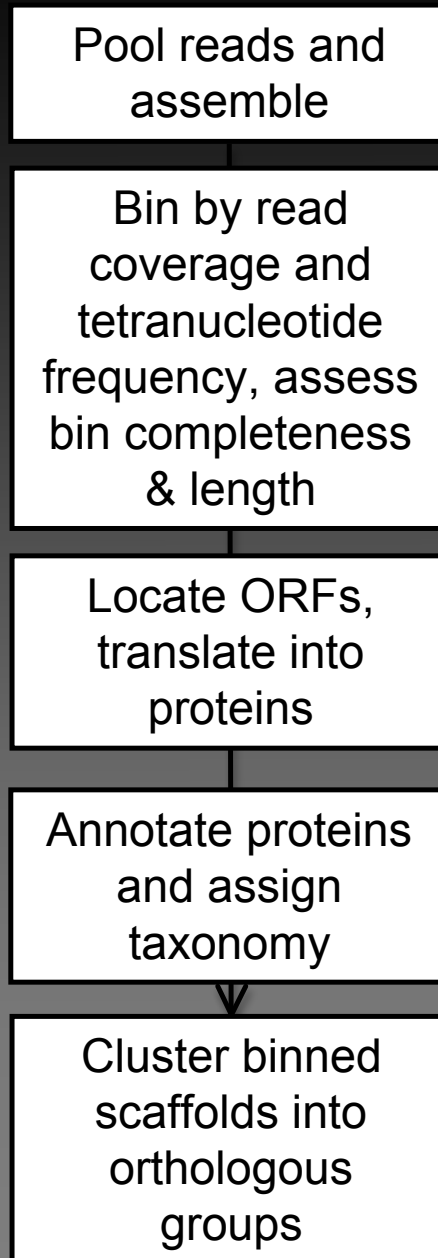
- *Niche?* What type of *Trichodesmium*, physiological ecology in the colony, environment..
- *Lottery?* Random selection of potential copiotrophs, role of taxonomic v. functional group uncertain...
- Working to examine the *Trichodesmium* holobiont with metagenomics/metatranscriptomics.

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



IDBA-UD

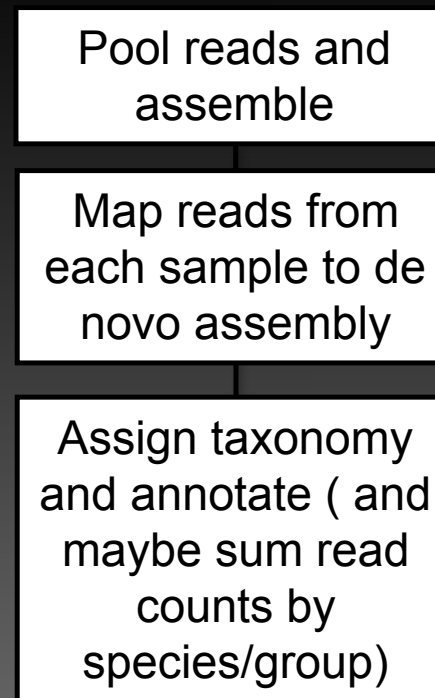
MaxBin

Prodigal

DIAMOND-BLAST
MEGAN?
KEGG

MCL

Metatranscriptome Pipeline



Eel Pond mRNAseq Protocol (Titus Brown)

RSEM

DIAMOND-BLAST (vs NR)
MEGAN
KEGG

Find genes/OGs with differential expression between samples

ASC (no reps)
EdgeR (reps)

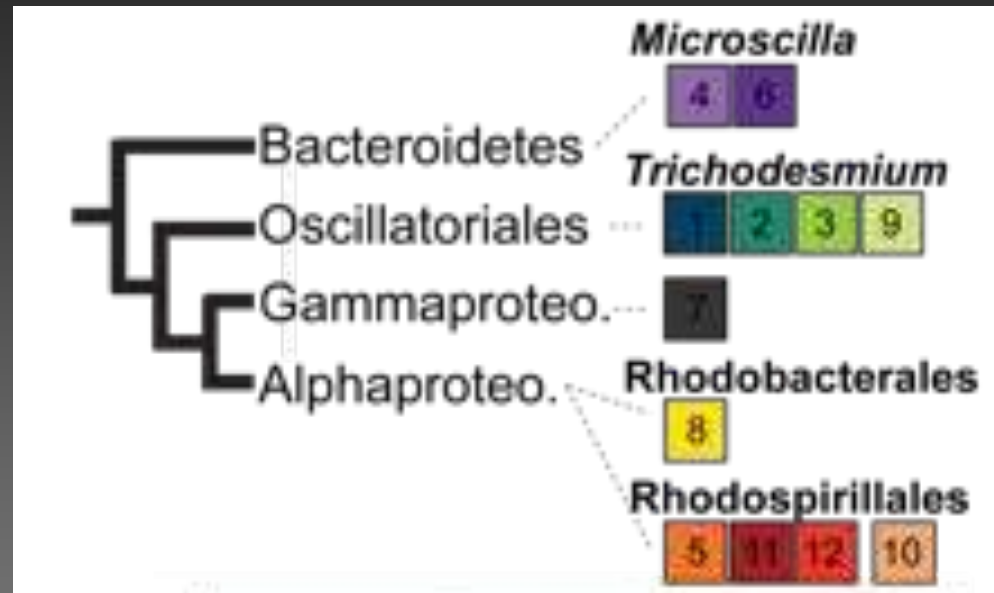
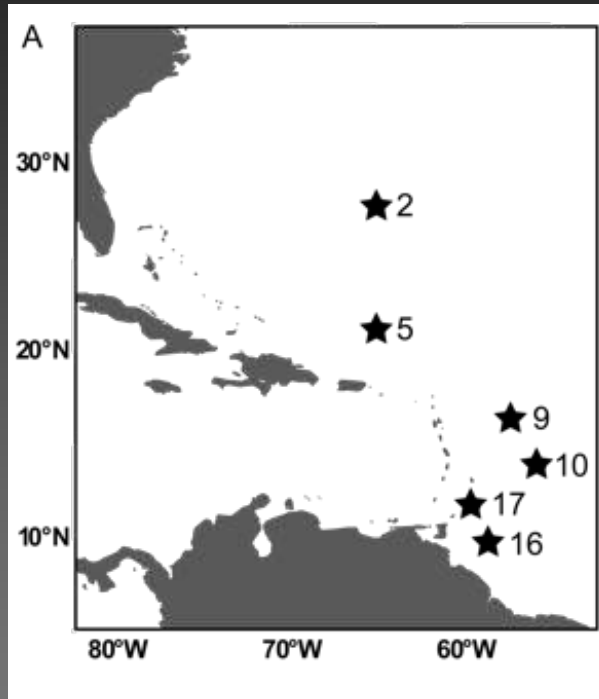
Normalize expression between different samples

EdgeR (variance stabilize)
TMM (NOISeq in R)
TPM

Find genes/OGs with key expr. patterns

RAIN (R)
Thaben and Westermarck, 2014

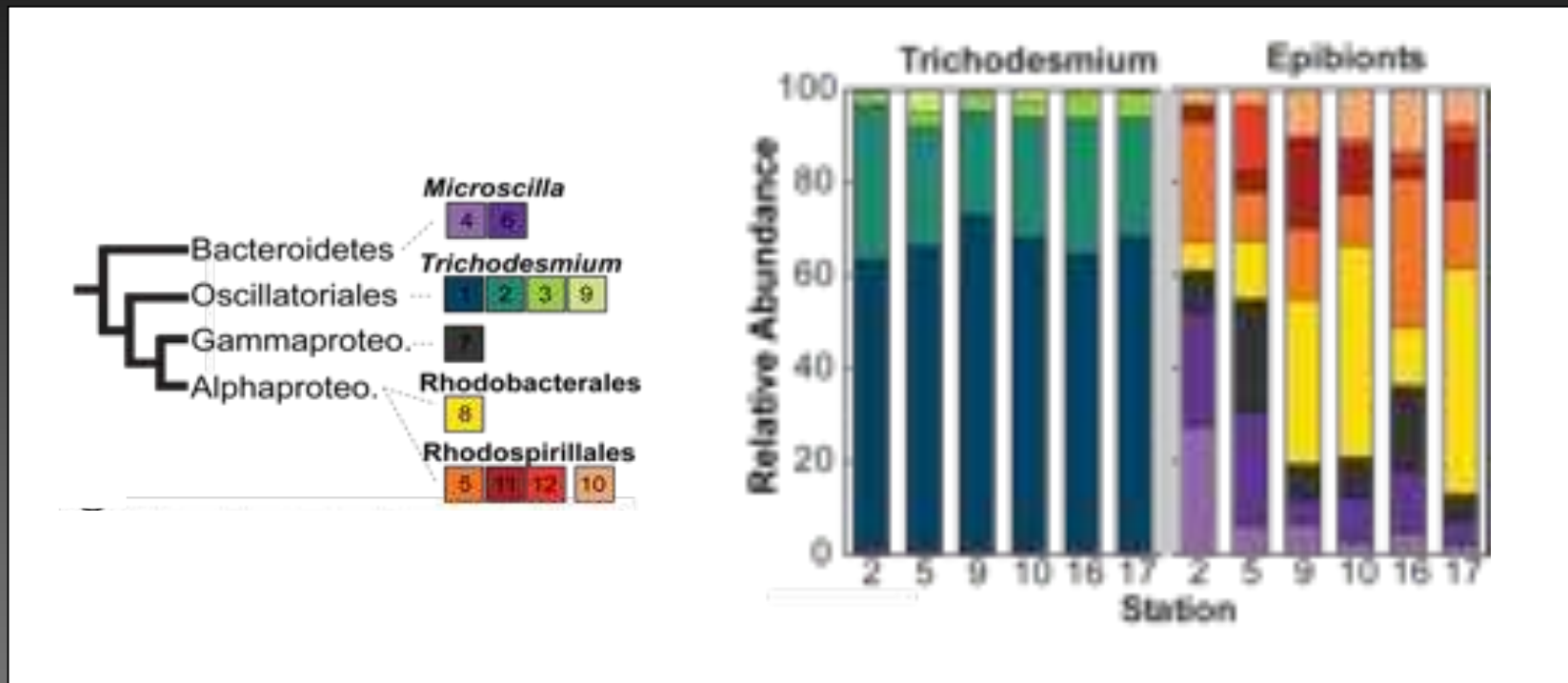
Composition of the holobiont



Frischkorn et al. (in review)

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

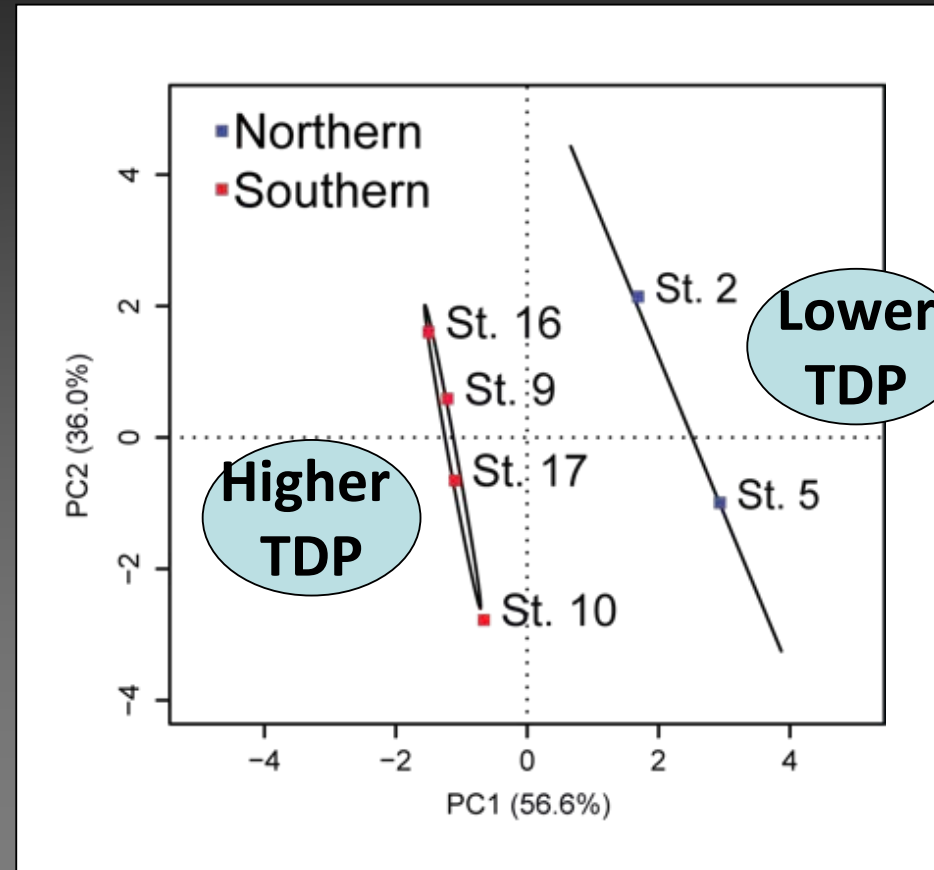
Distribution of holobiont



Frischkorn et al. (in review)

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

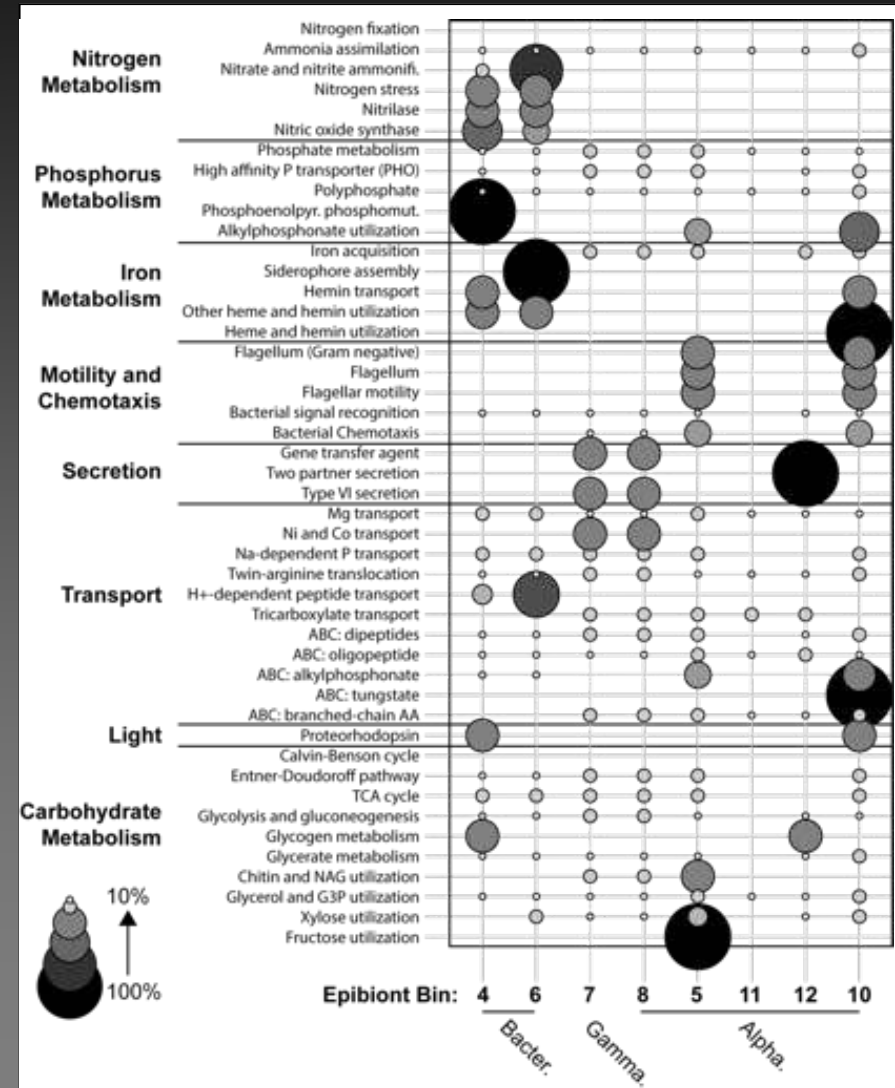
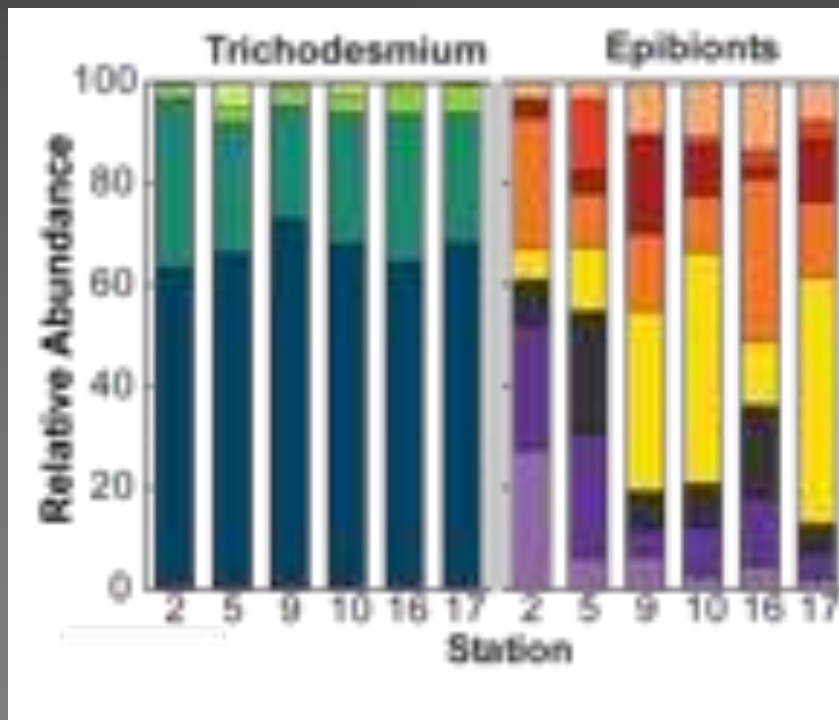
Microbiome diversity differs significantly between regions



Frischkorn et al. (in review)

Variable distribution of functional pathways among epibionts

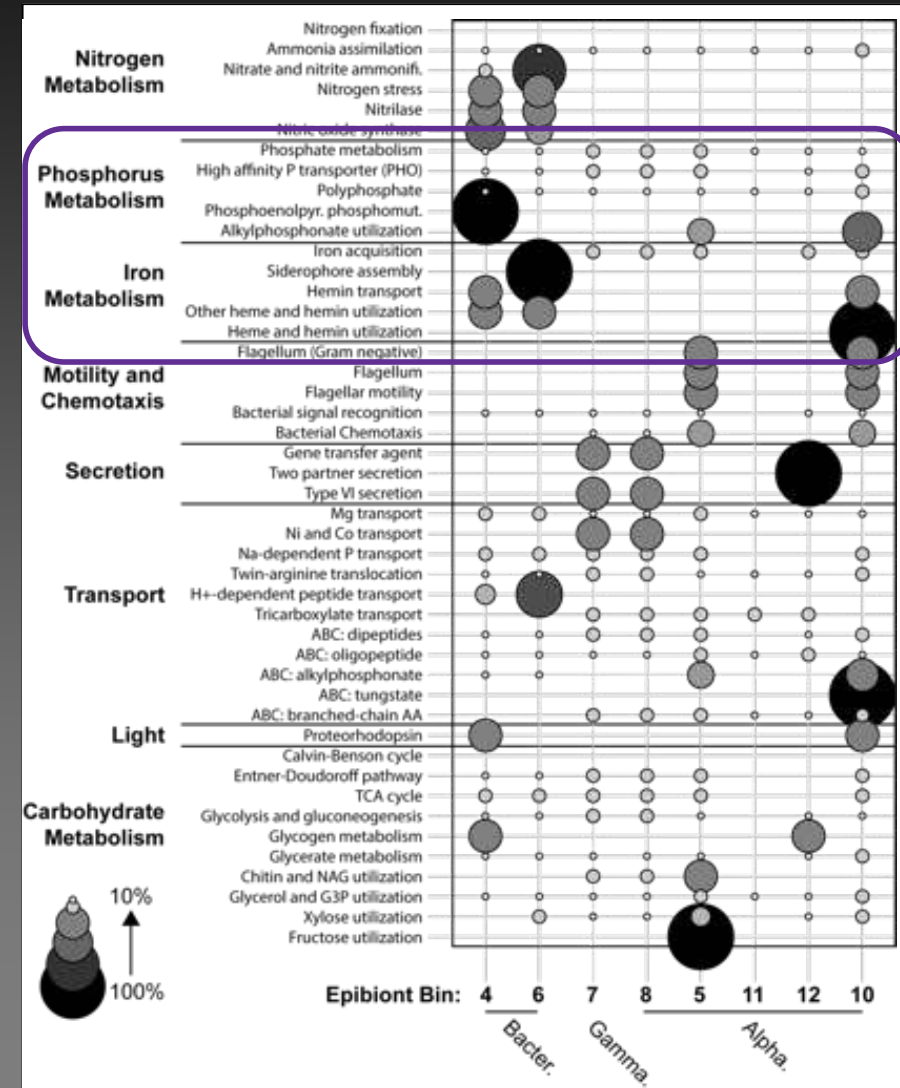
- Differential pathway enrichment consistent with a microbiome that is modulated as a function of environment



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*

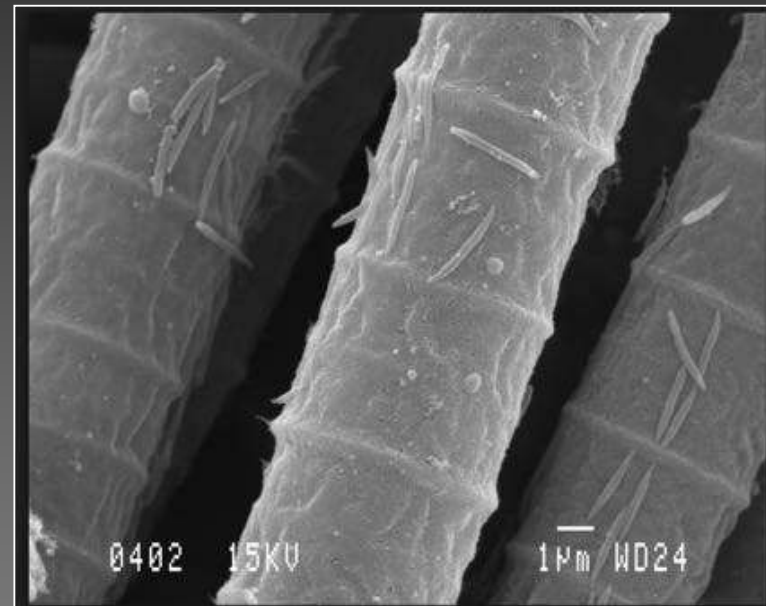
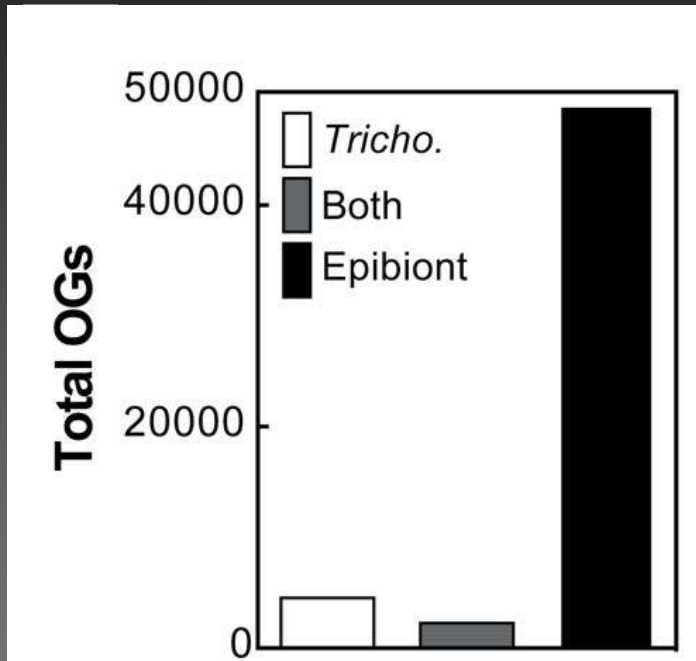


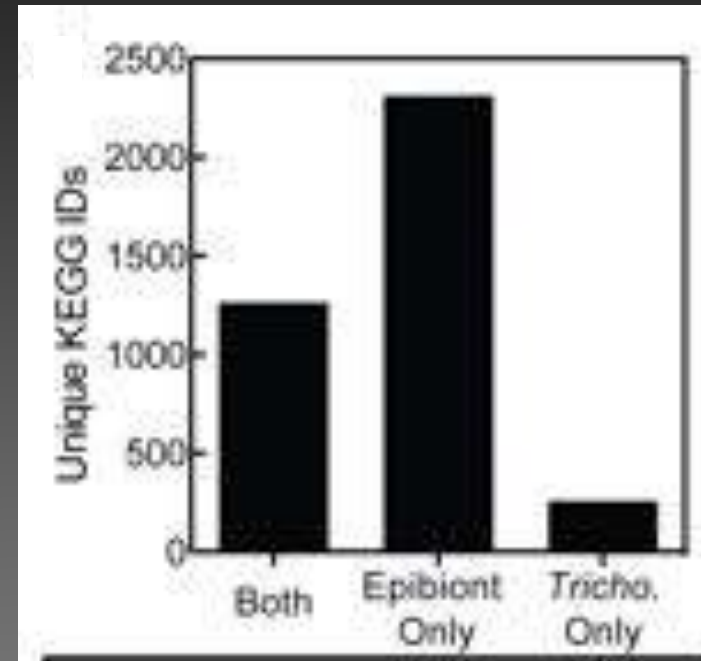
Image courtesy Tracy Mincer

Epibionts confer the majority of the metabolic potential

>90% OGs



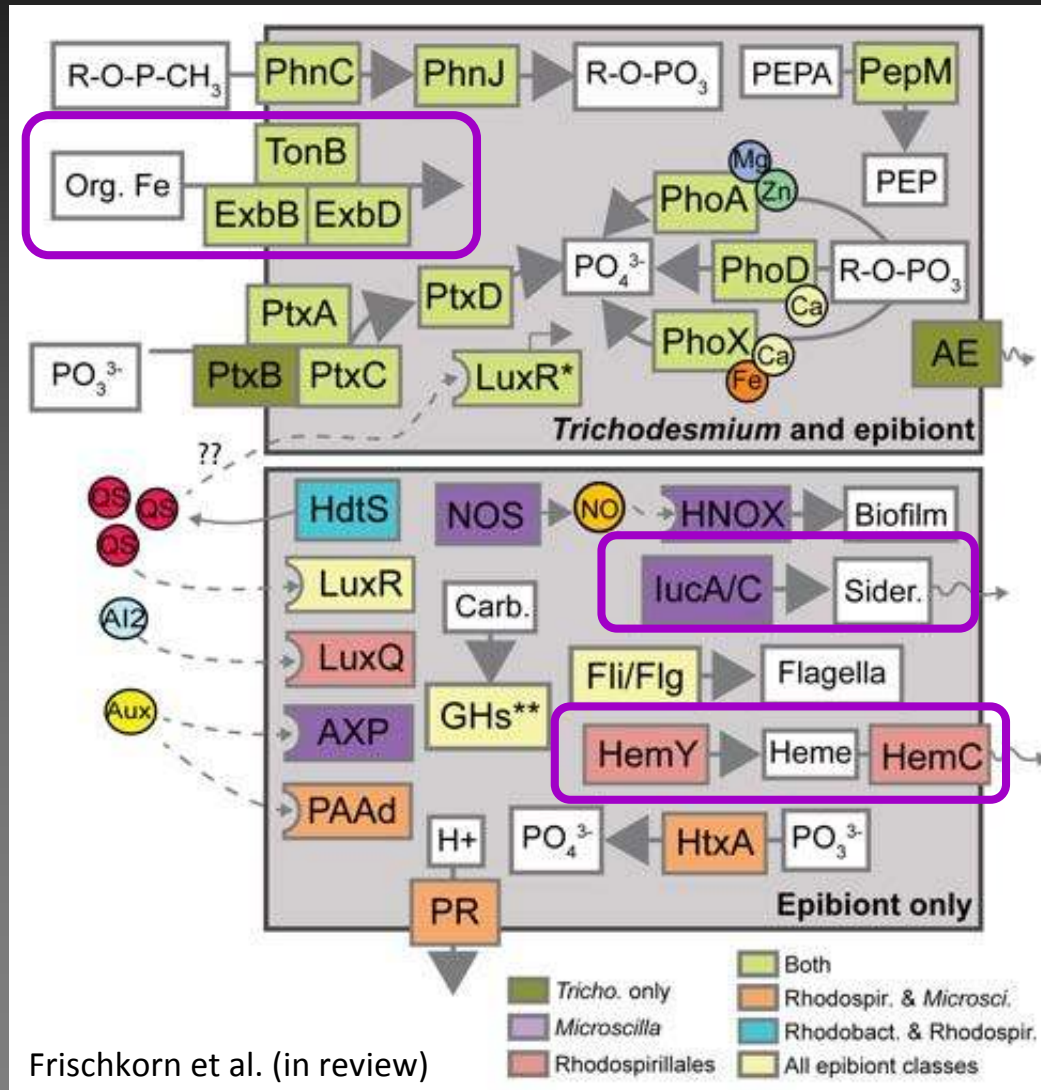
>10x Functions



Frischkorn et al. (in review)

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

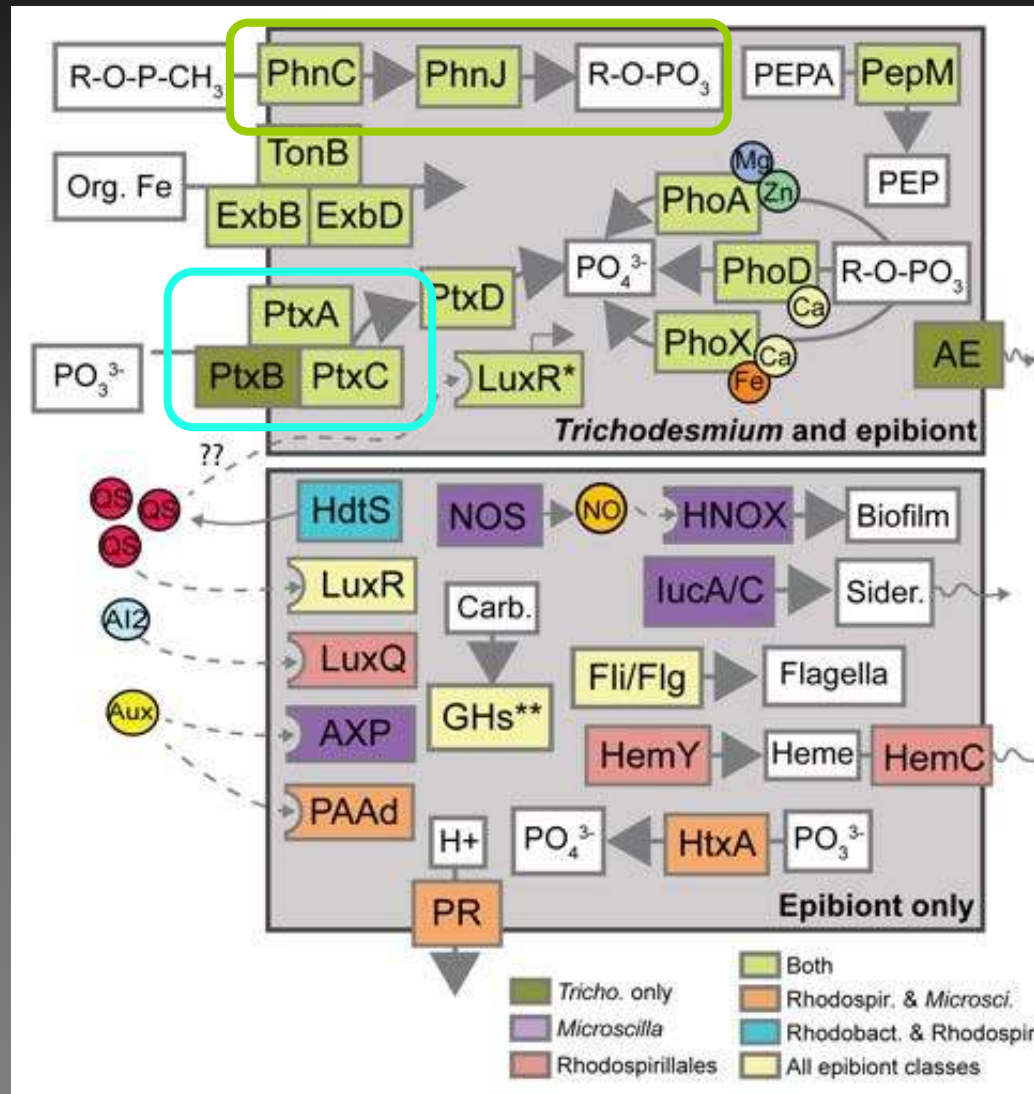
Metabolic partitioning within the *Trichodesmium* holobiont



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

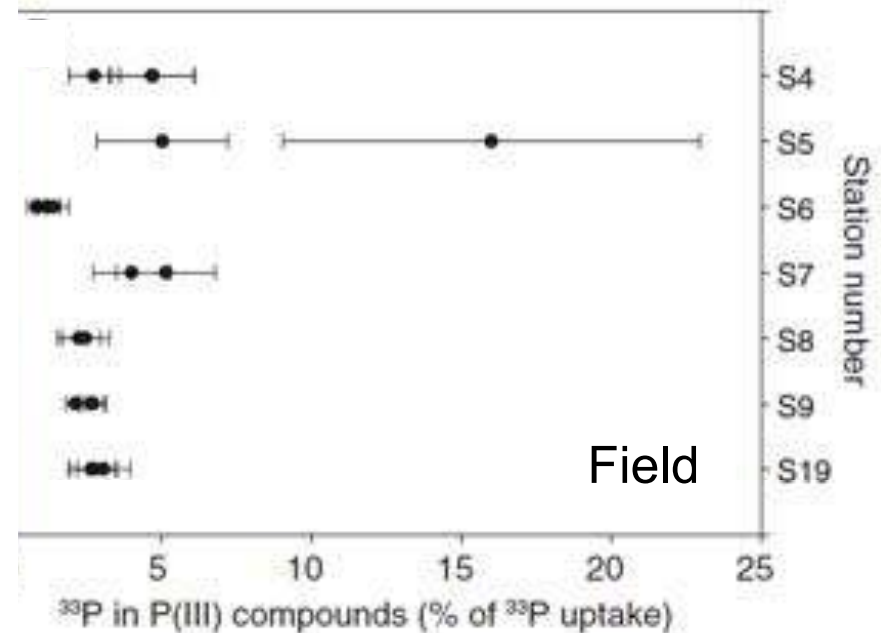
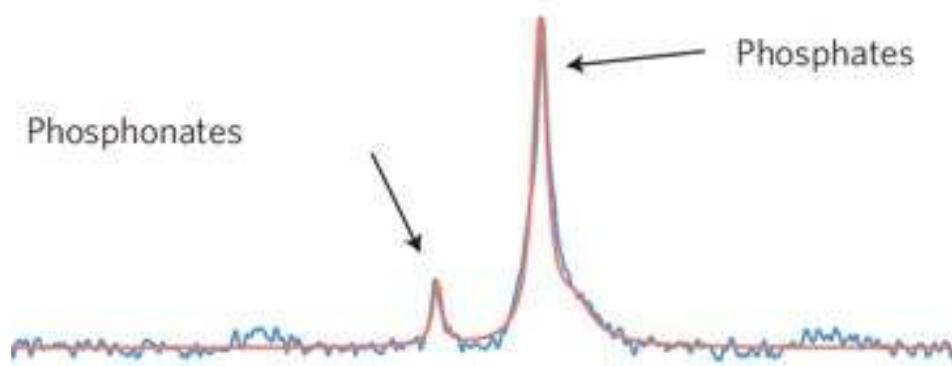
Phosphite
uptake

Phosphorus
metabolisms are
present in both
Trichodesmium and
the microbiome.

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

Phosphonate (C-P) biosynthesis

Culture

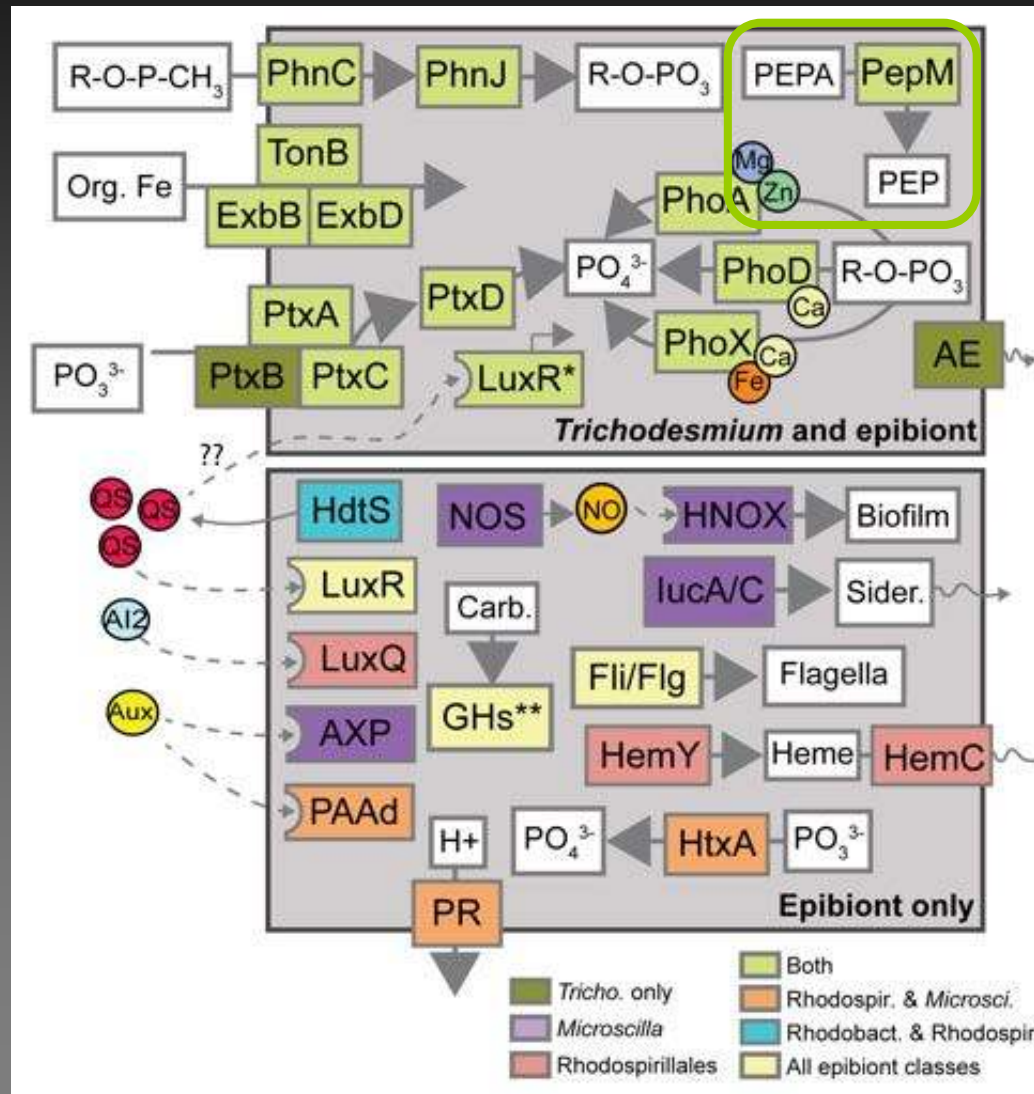


Dyhrman et al. (2009) *Nature Geo.*

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?

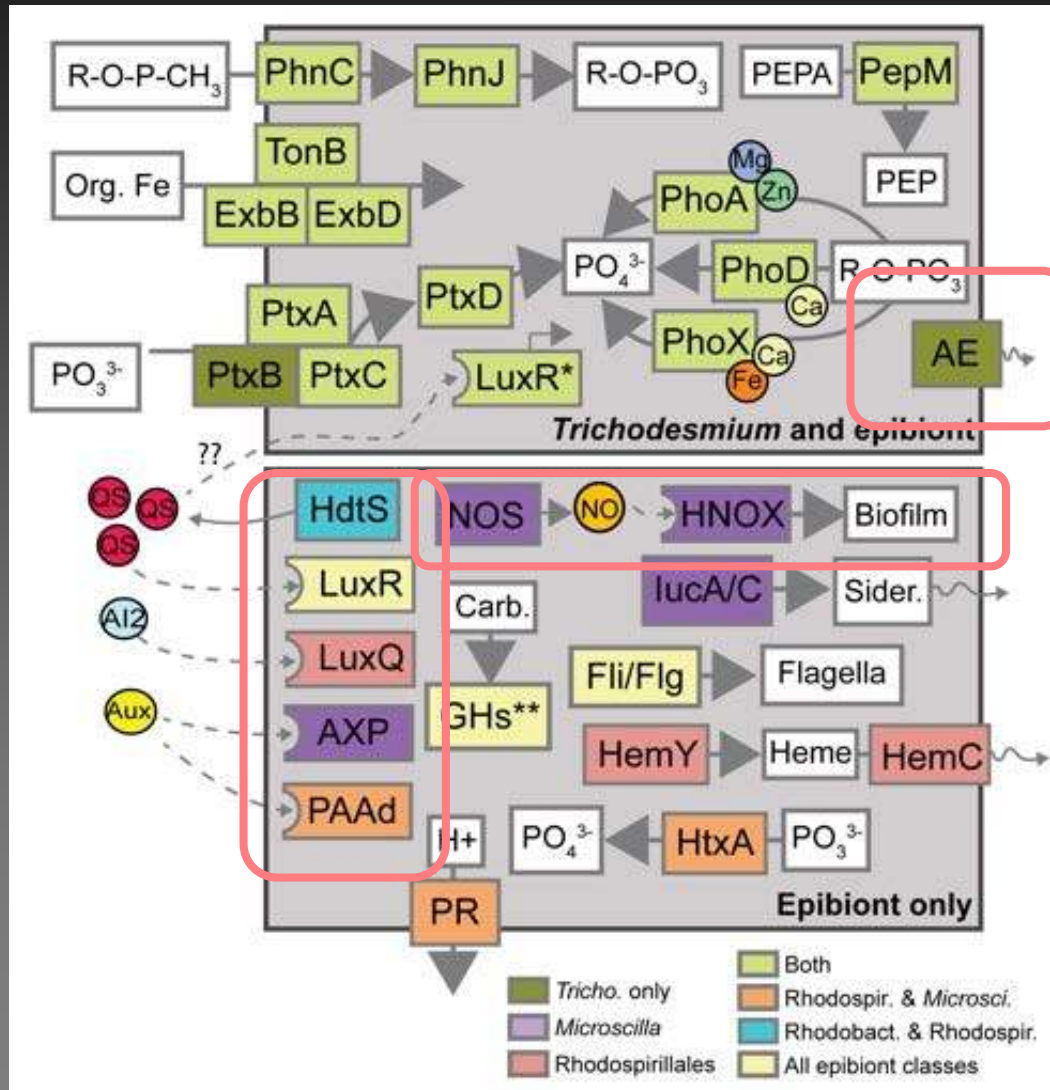
Phosphonate production is a shared metabolism



Phosphonate biosynthesis

Phosphonate produced by both *Trichodesmium* and the epibionts

Microbial cross talk within the *Trichodesmium* holobiont



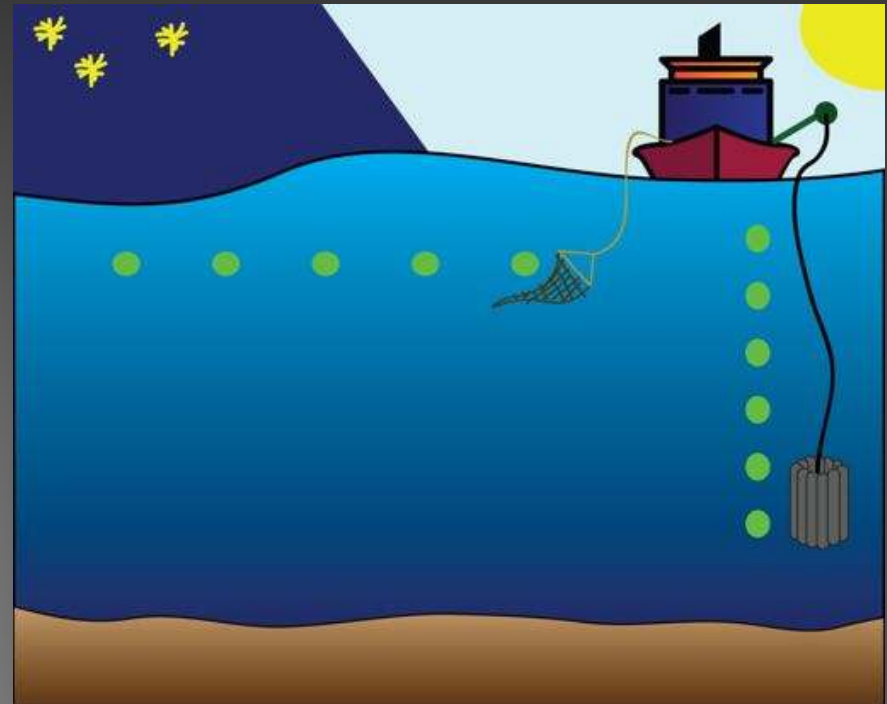
Tracking the interactome... with metatranscriptome profiling after addition of NO and QS molecules

QS and cell signaling

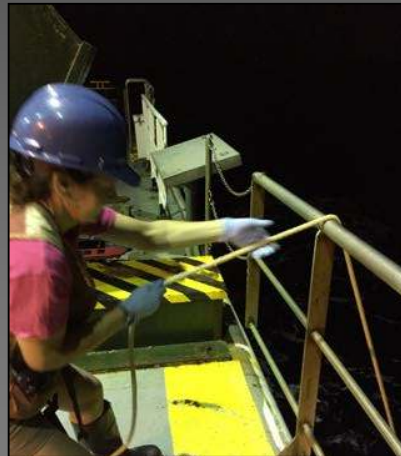
Trichodesmium holobiont interactome...

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

Are there diel signatures to epibiont genes?

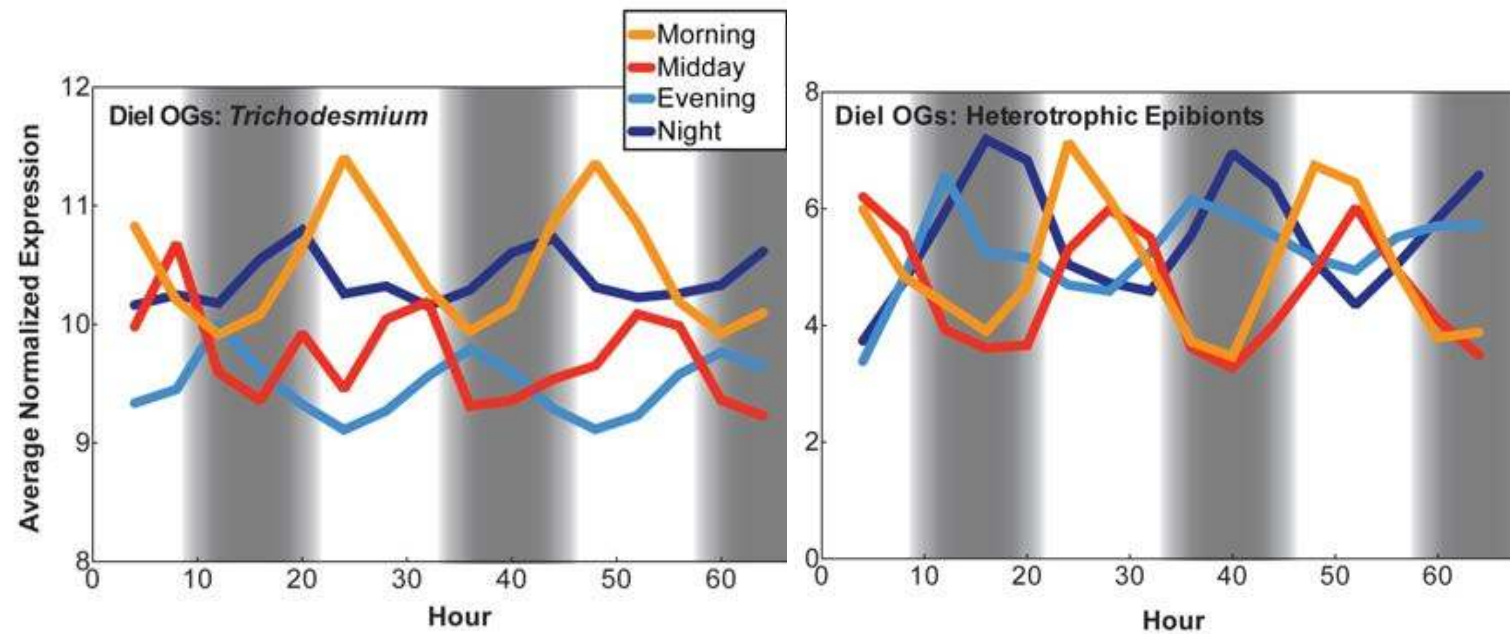


Sampling the interactome...

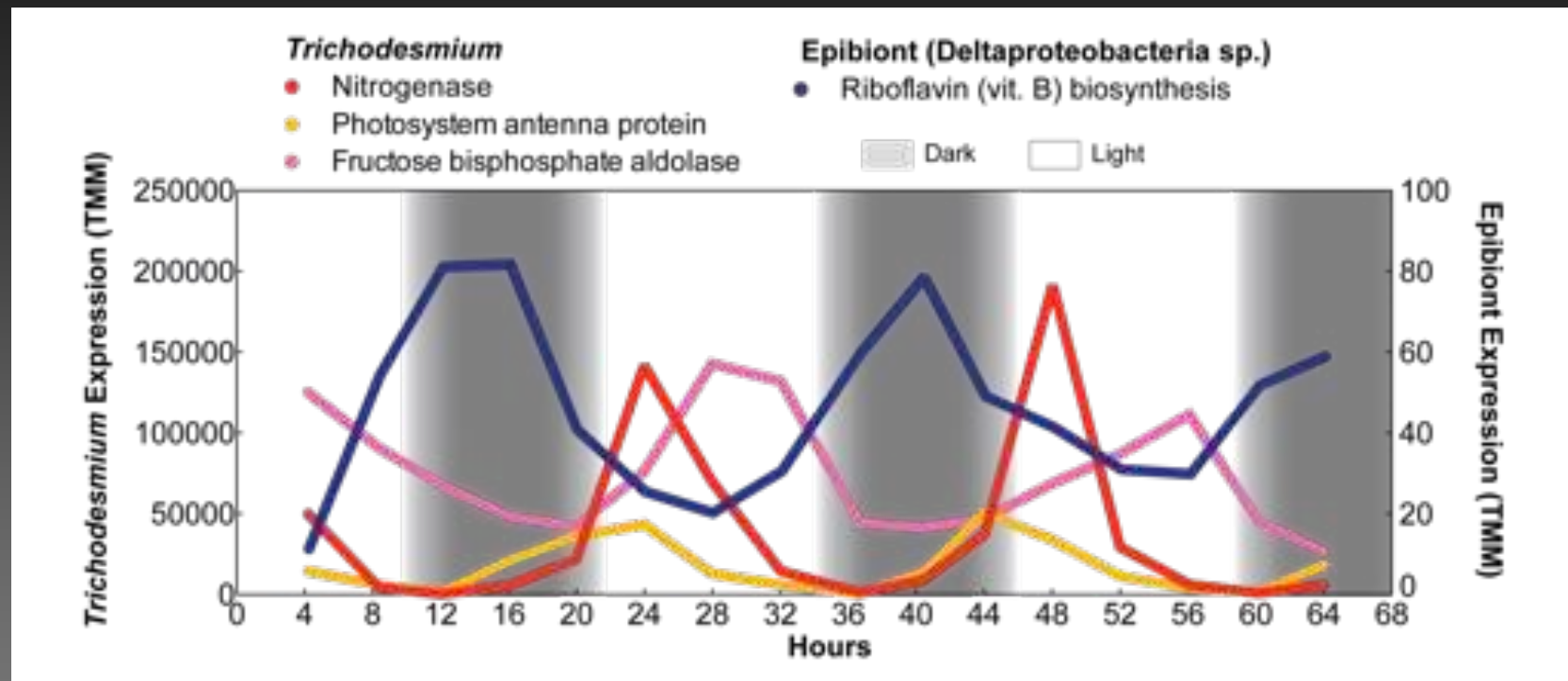


Colony samples were taken every 4 hours for 68 hours, twice!

Diel modulation of transcripts in *Trichodesmium*



Diel modulation of transcripts within the microbiome

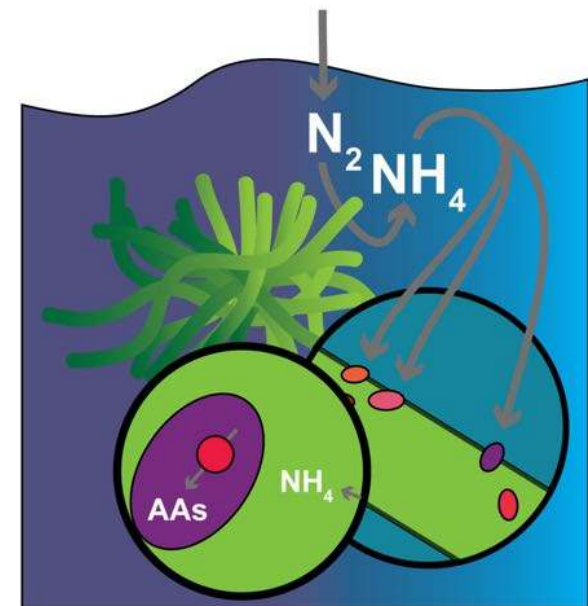
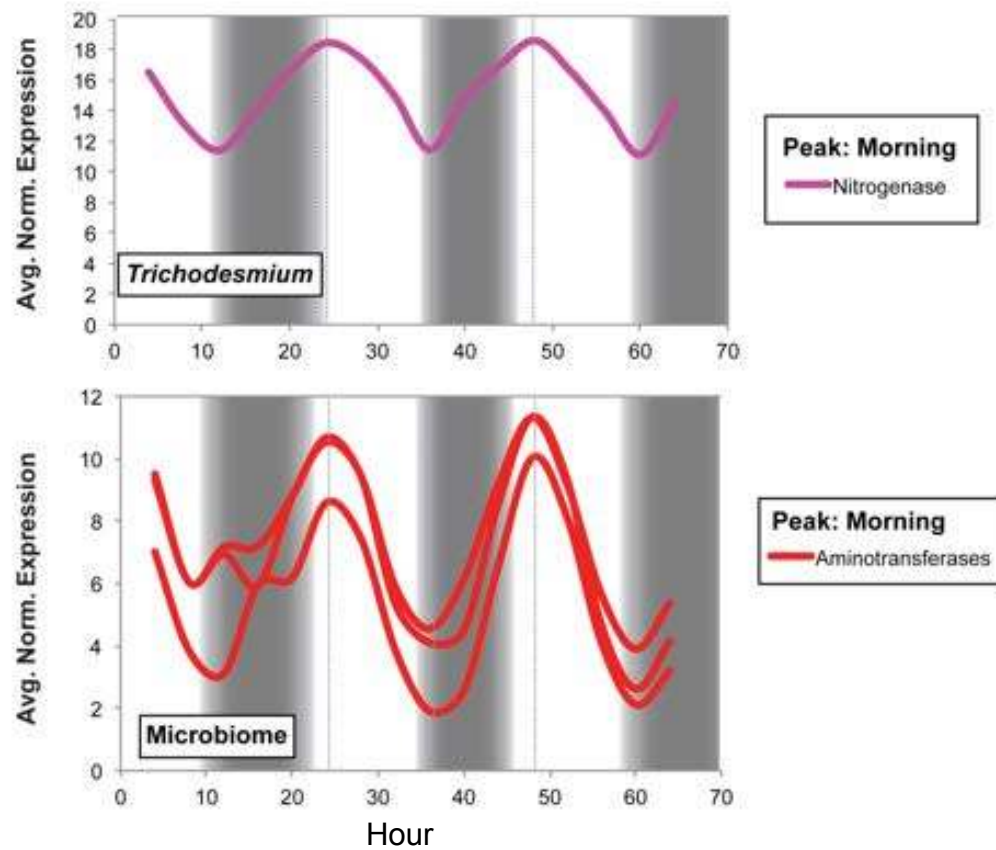


Epibiont riboflavin biosynthesis has a diel pattern oscillating with *Trichodesmium* photosynthesis, carbon fixation and nitrogen fixation.

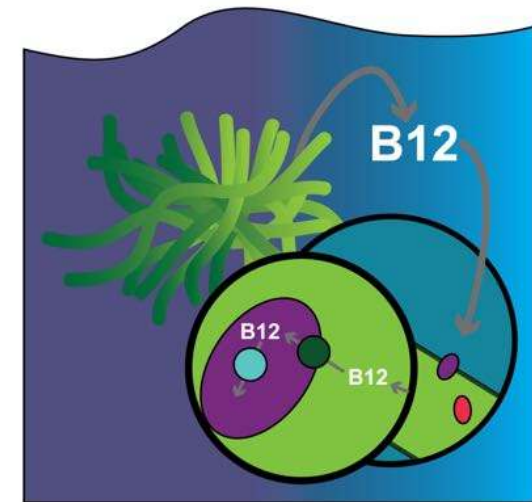
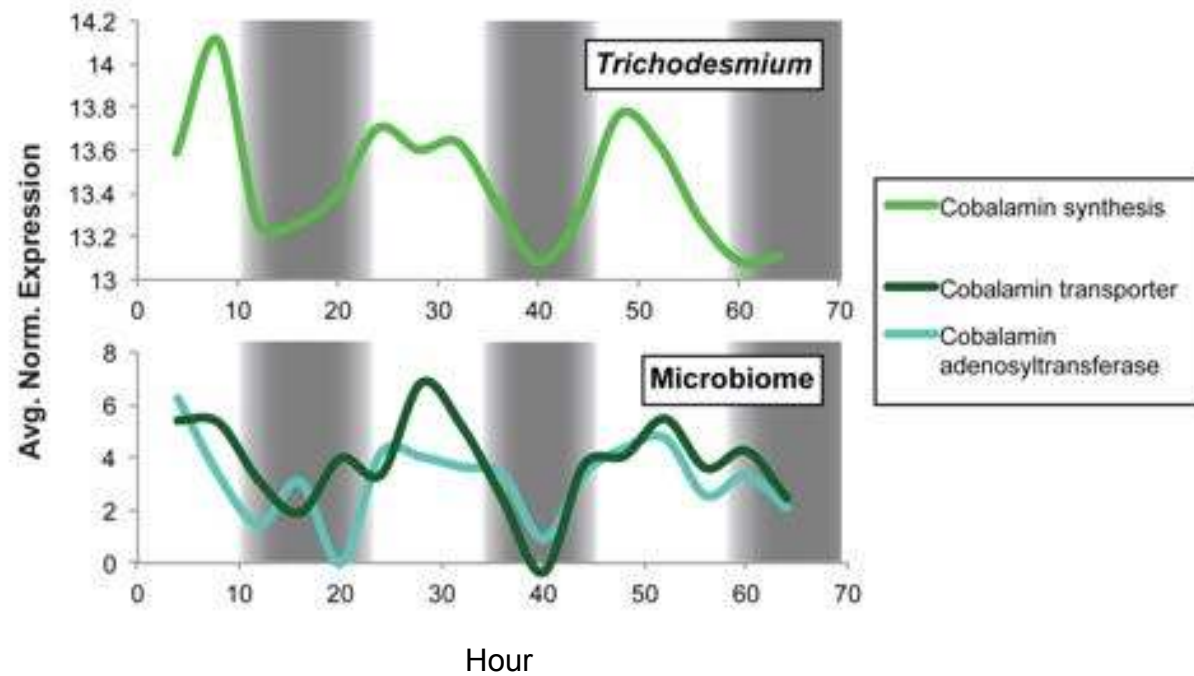
This is suggestive of the dynamic interplay between members of the holobiont.

Analyze key functions for coordination within the consortium

Diel modulation of transcripts suggest coordination: Nitrogen



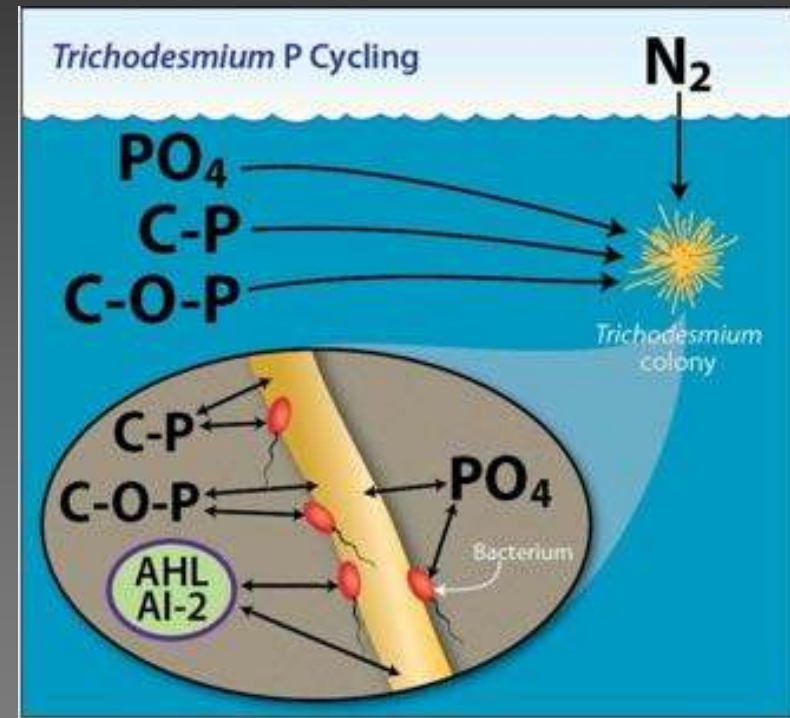
Diel modulation of transcripts suggest coordination: Vitamins



Summary - Host microbiome interactions

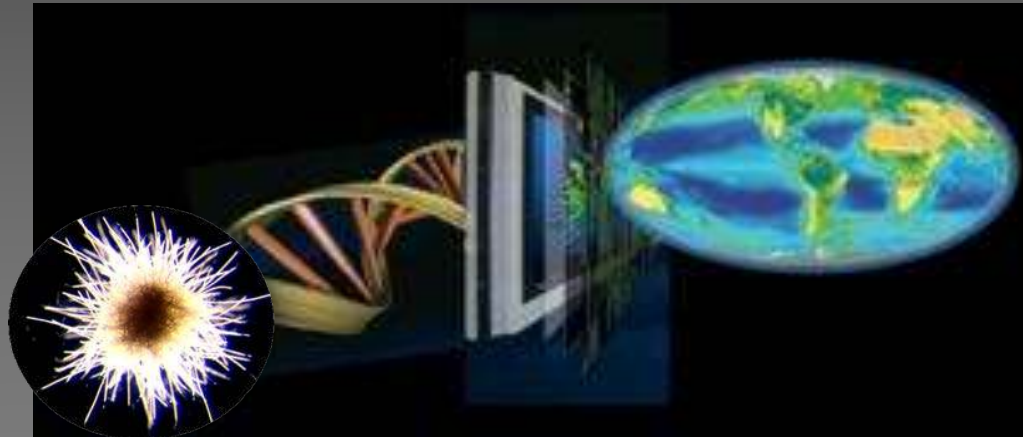
What is the role of the microbiome on *Trichodesmium* physiological ecology?

- 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 N₂ 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

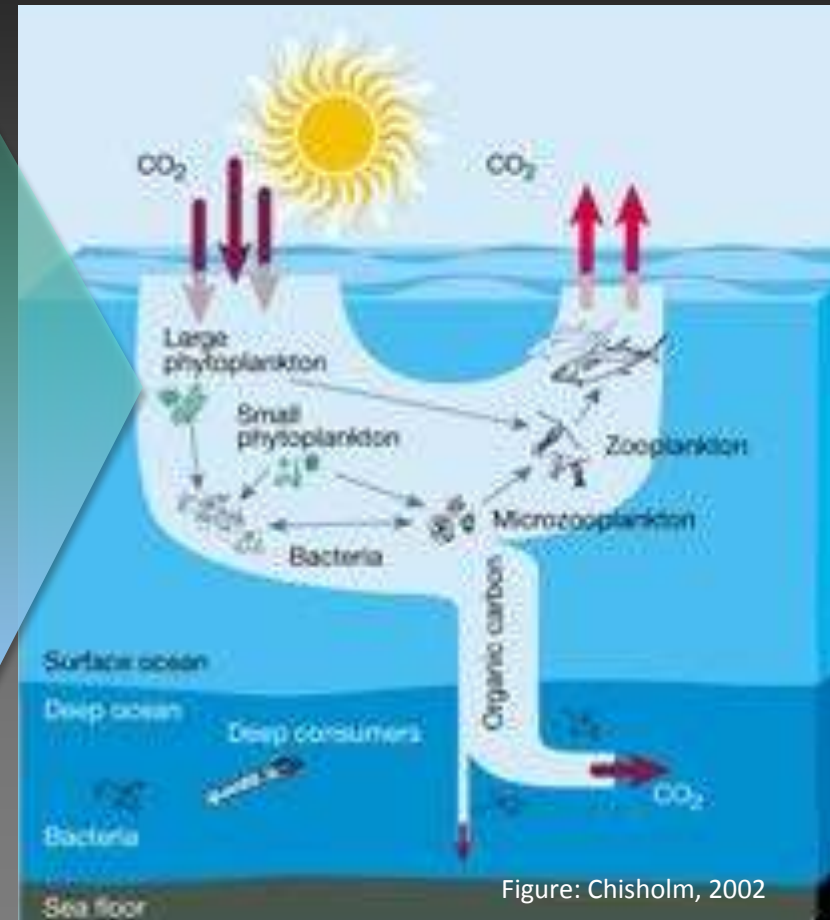
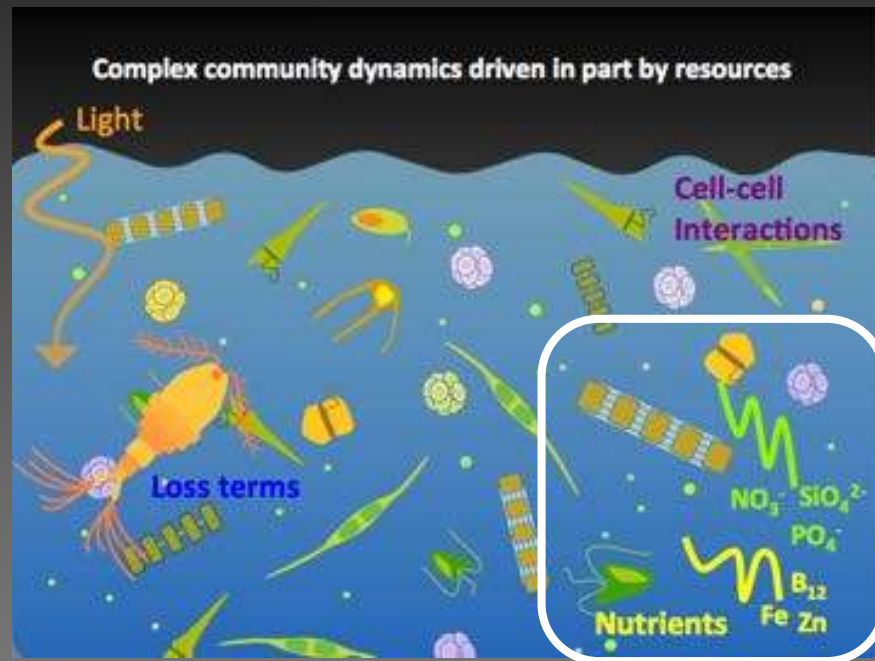


Complex community dynamics driven in part by resources



Tracking phytoplankton physiological ecology

Ecosystem function and biogeochemistry



How do resources drive phytoplankton distributions and activities?

Focus on keystone groups

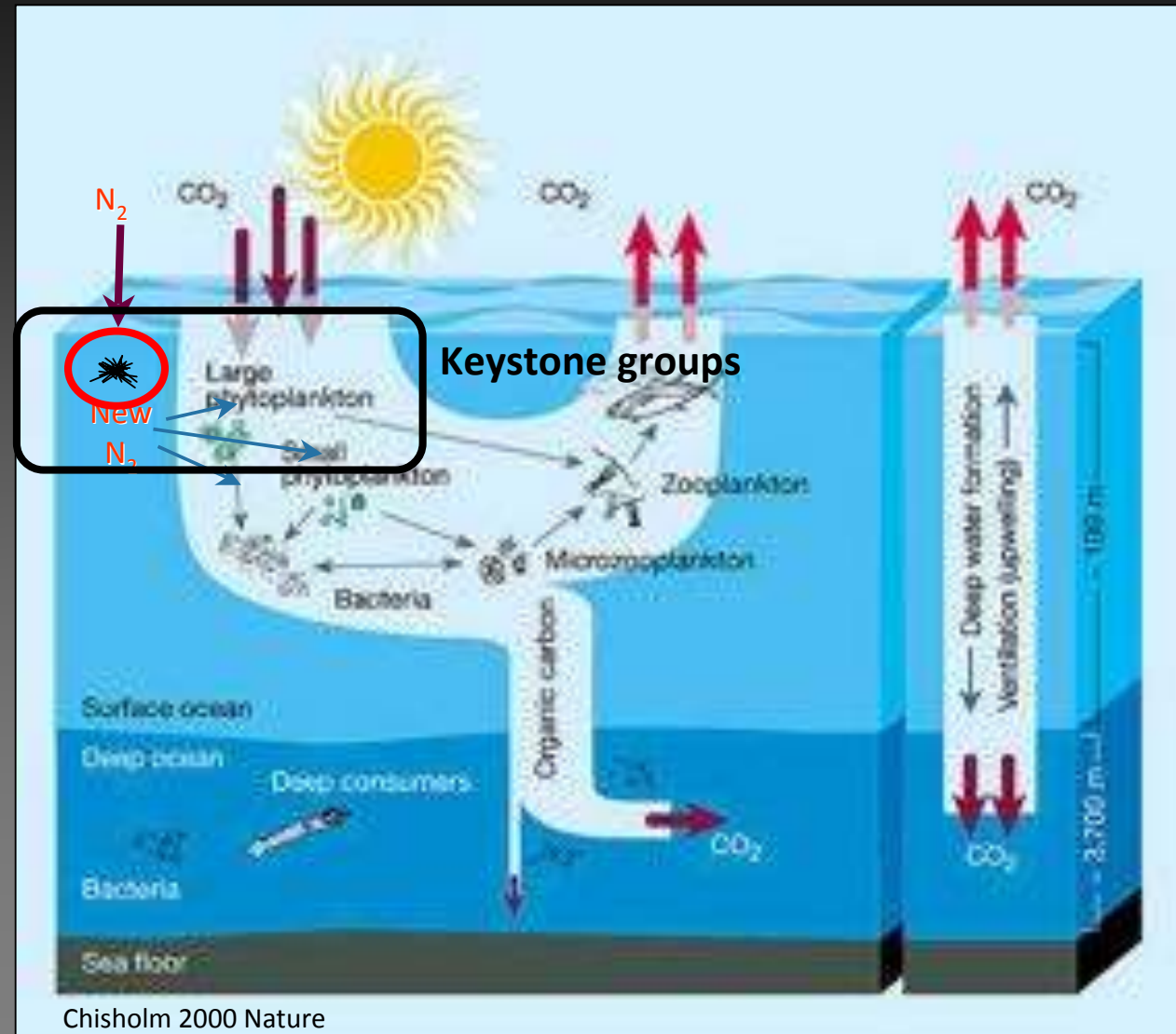
Haptophytes



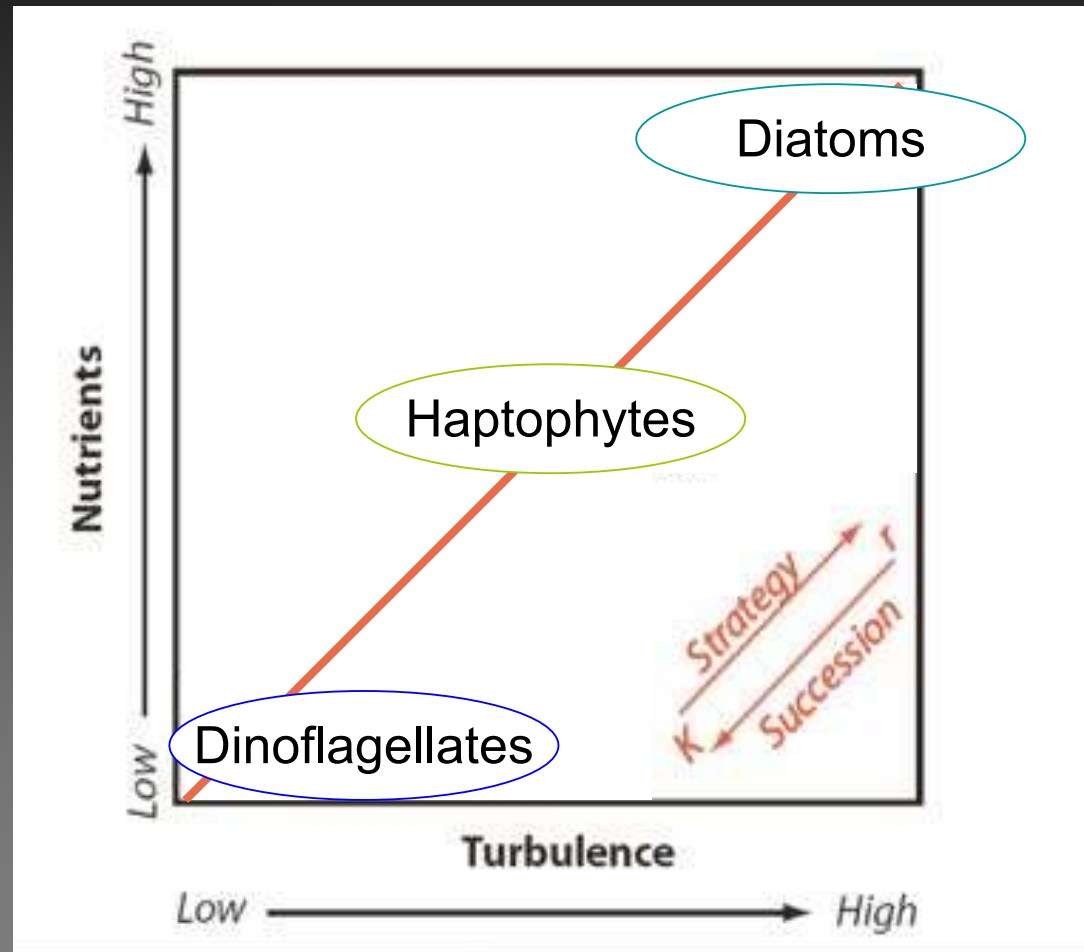
Diatoms



Dinoflagellates



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?



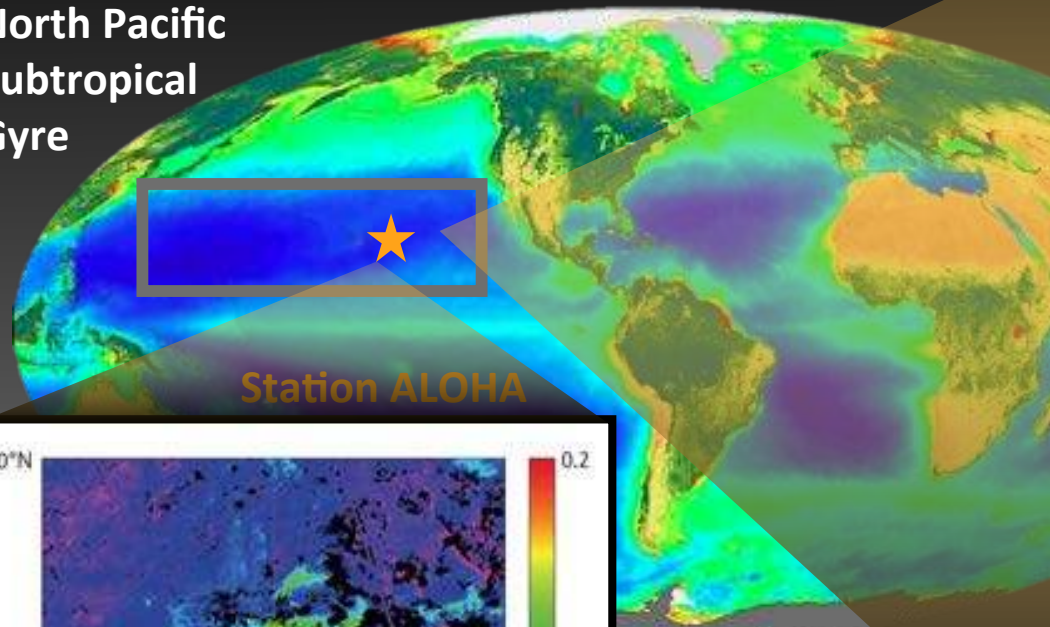
Metabolic plasticity and diversity

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

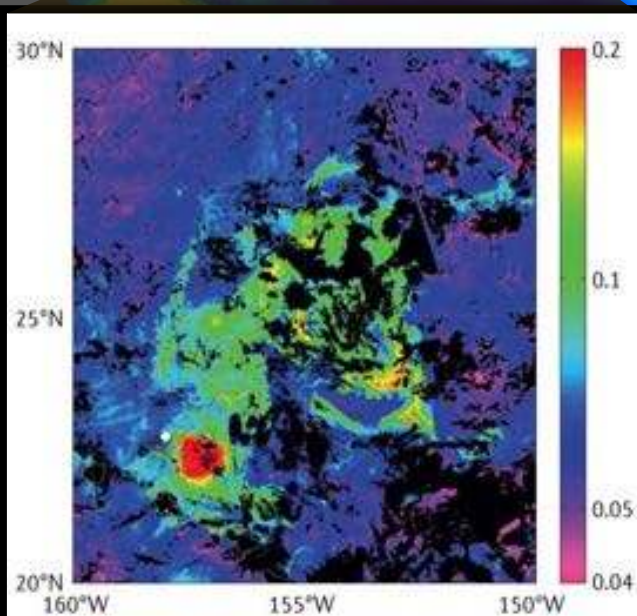


The significance of the NPSG

North Pacific
Subtropical
Gyre



Station ALOHA



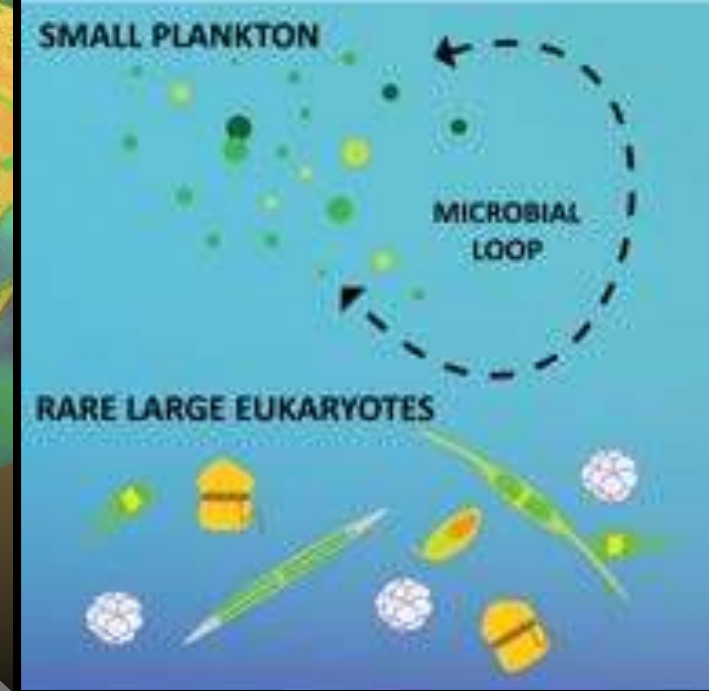
(Karl and Church, Nature Rev. Microbiol., 2014)

NORMAL OLIGOTROPHIC STATE

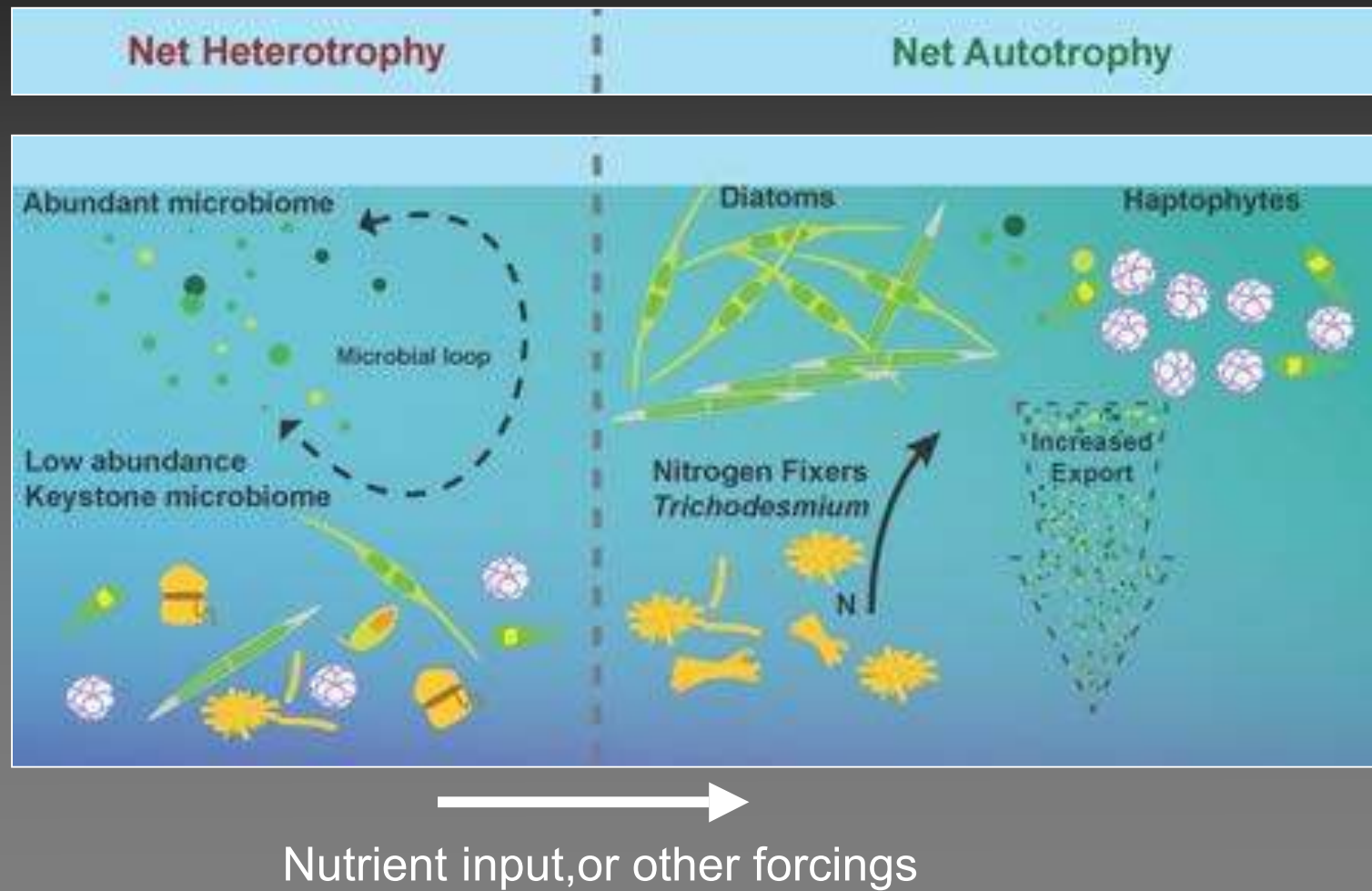
SMALL PLANKTON

MICROBIAL
LOOP

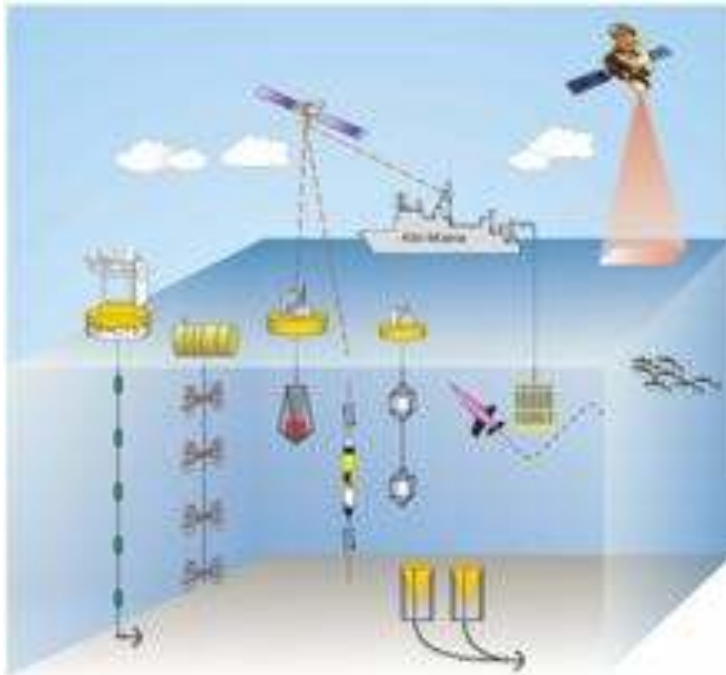
RARE LARGE EUKARYOTES



Blooms of keystone species can shift ecosystem state



Hawaii Ocean Experiment: Dynamics of Light and Nutrients



Wilson et al. 2015 *GBC*



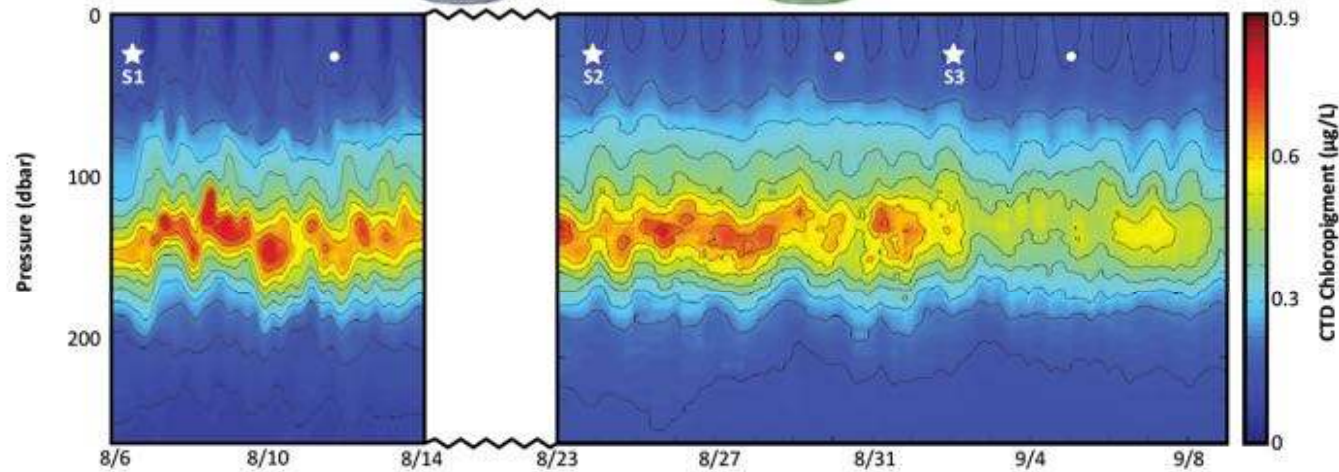
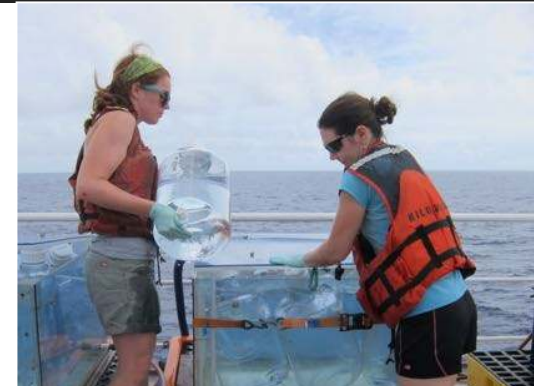
center for microbial oceanography: research and education

c.m.o.r.e. *linking genomes to biomes*

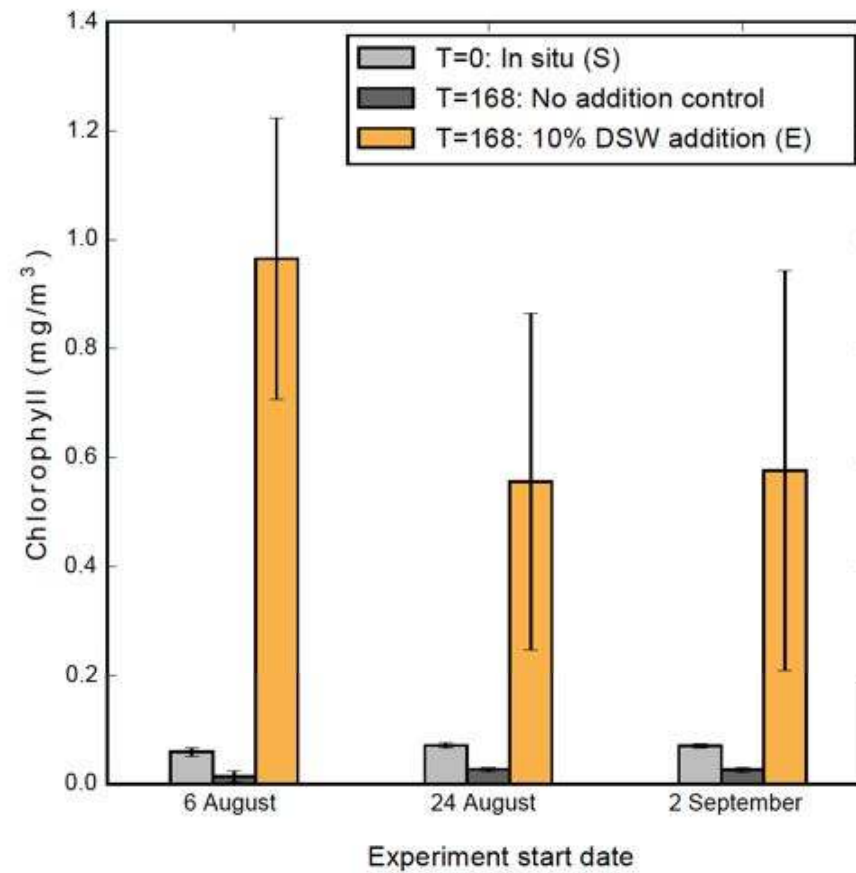
(HOE-DYLAN)

Sampling a bloom

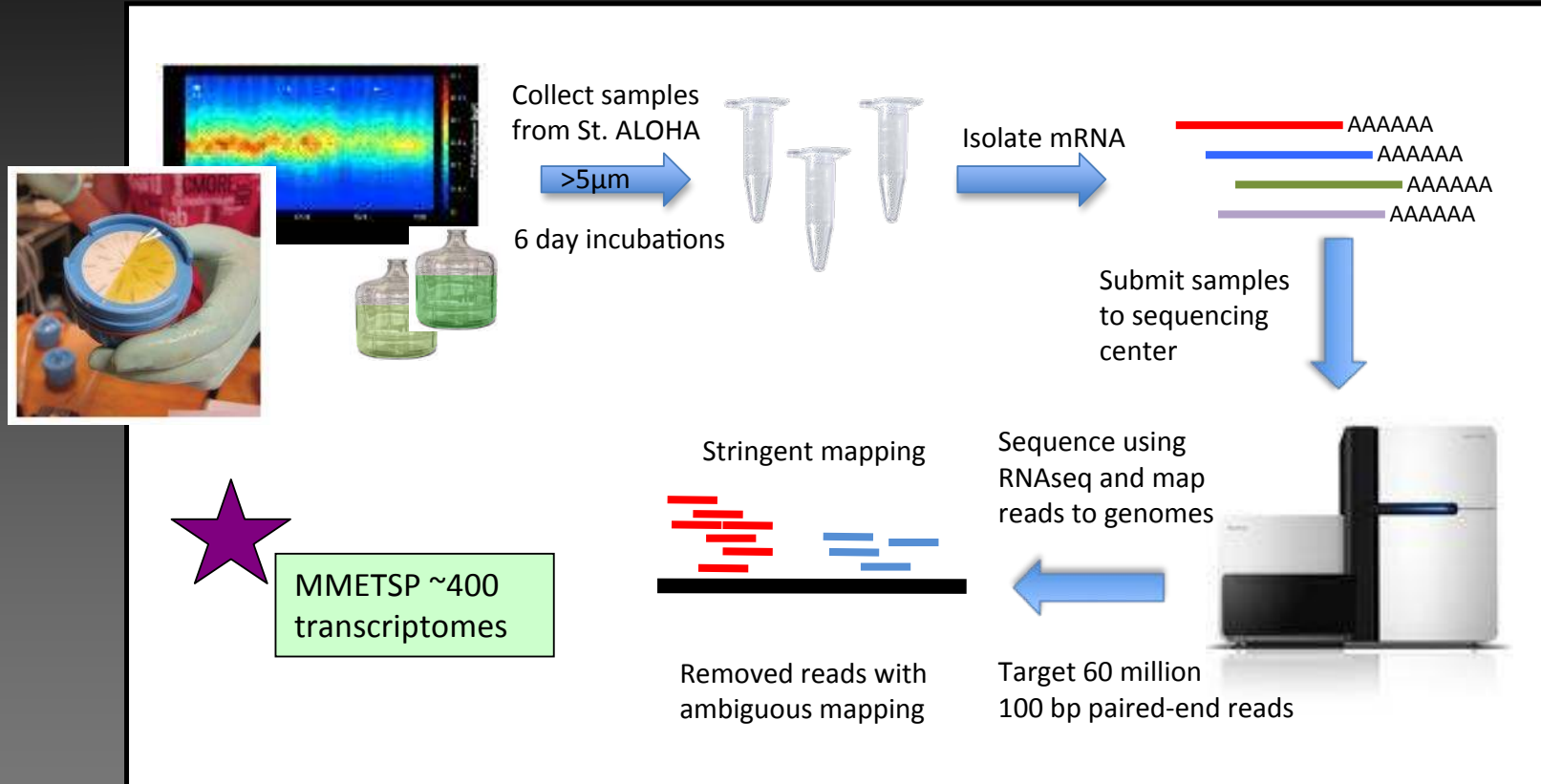
Sampling at Station ALOHA coupled with bloom simulations



Deep water addition led to simulated “bloom”

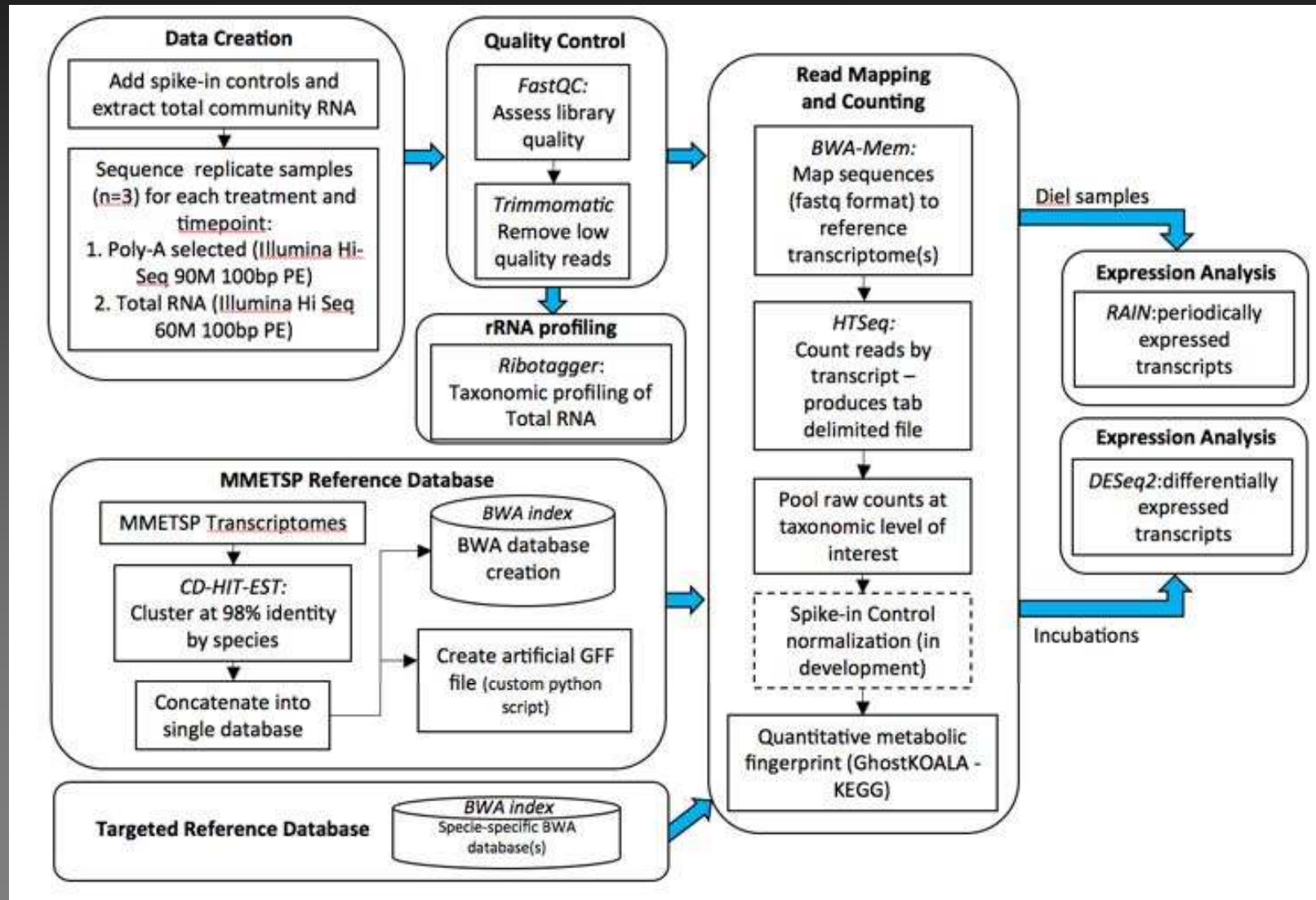


Sampling and pipeline

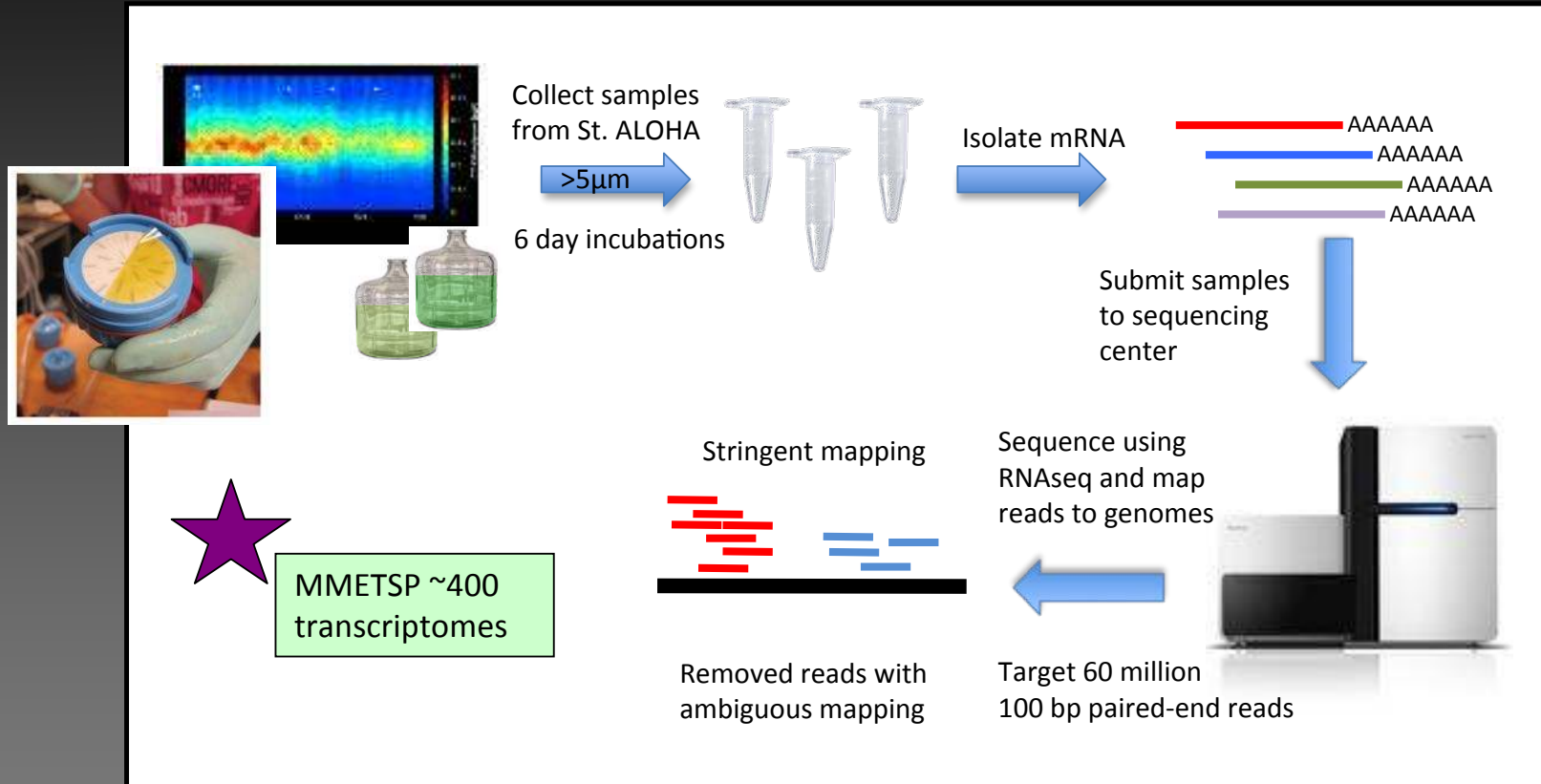


MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Sequencing and analysis strategy



Sampling and pipeline



MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Marine Microbial Eukaryote Transcriptome Sequencing Project

OPEN ACCESS Freely available online



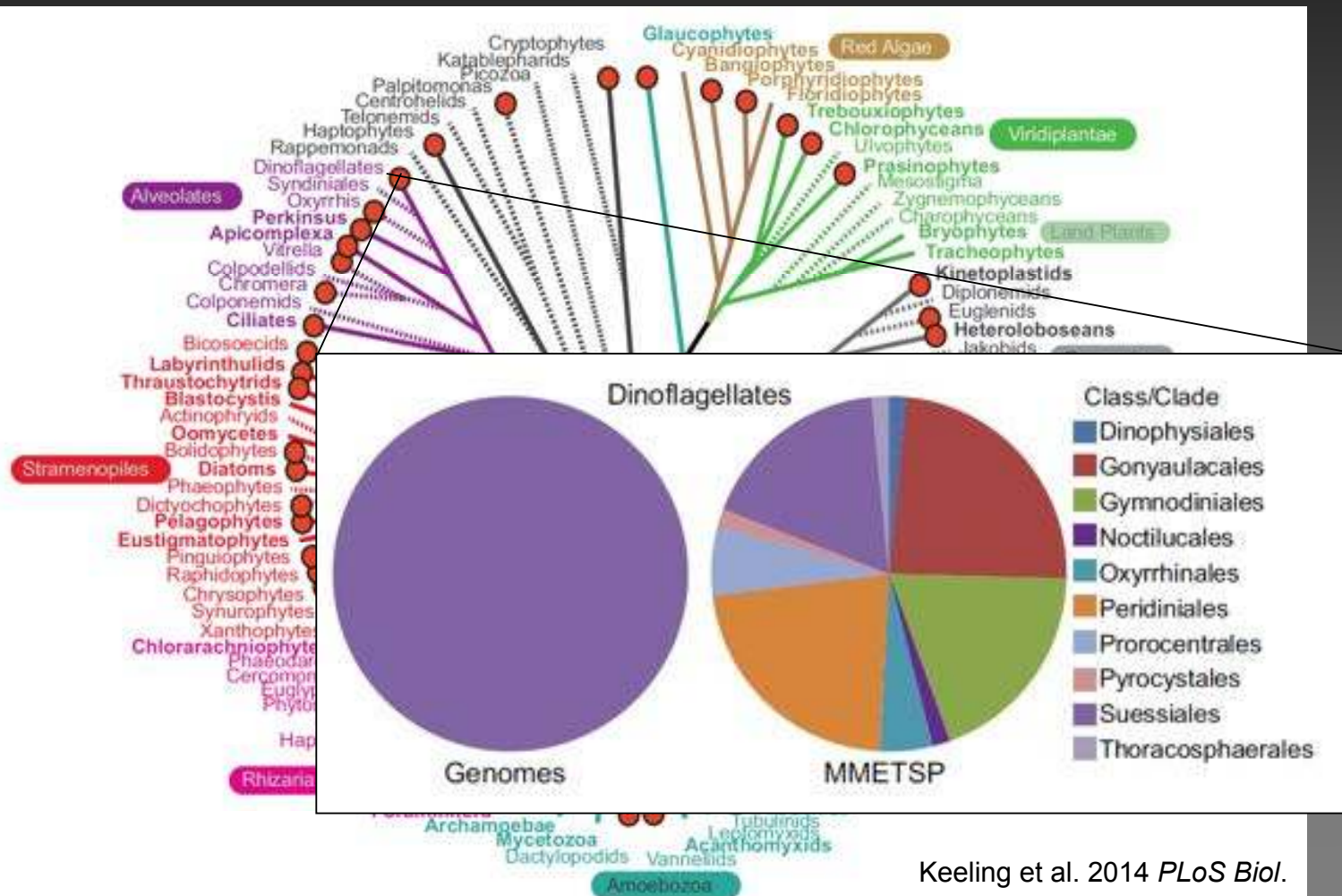
Community Page



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 Burki¹, Heather M. Wilcox³, Bassem Allam⁴, Eric E. Allen⁵, Linda A. Amaral-Zettler^{6,7}, E. Virginia Armbrust⁸, John M. Archibald^{2,9}, Arvind K. Bharti¹⁰, Callum J. Bell¹⁰, Bank Beszteri¹¹, Kay D. Bidle¹², Connor T. Cameron¹⁰, Lisa Campbell¹³, David A. Caron¹⁴, Rose Ann Cattolico¹⁵, Jackie L. Collier⁴, Kathryn Coyne¹⁶, Simon K. Davy¹⁷, Phillipe Deschamps¹⁸, Sonya T. Dyhrman¹⁹, Bente Edvardsen²⁰, Ruth D. Gates²¹, Christopher J. Gobler⁴, Spencer J. Greenwood²², Stephanie M. Guida¹⁰, Jennifer L. Jacobi¹⁰, Kjetill S. Jakobsen²⁰, Erick R. James¹, Bethany Jenkins^{23,24}, Uwe John¹¹, Matthew D. Johnson²⁵, Andrew R. Juhl¹⁹, Anja Kamp^{26,27}, Laura A. Katz²⁸, Ronald Kiene²⁹, Alexander Kudryavtsev^{30,31}, Brian S. Leander¹, Senjie Lin³², Connie Lovejoy³³, Denis Lynn^{34,35}, Adrian Marchetti³⁶, George McManus³², Aurora M. Nedelcu³⁷, Susanne Menden-Deuer²⁴, Cristina Miceli³⁸, Thomas Mock³⁹, Marina Montresor⁴⁰, Mary Ann Moran⁴¹, Shauna Murray⁴², Govind Nadathur⁴³, Satoshi Nagai⁴⁴, Peter B. Ngam¹⁰, Brian Palenik⁵, Jan Pawlowski³¹, Giulio Petroni⁴⁵, Gwenael Piganeau^{46,47}, Matthew C. Posewitz⁴⁸, Karin Rengefors⁴⁹, Giovanna Romano⁴⁰, Mary E. Rumpho⁵⁰, Tatiana Rynearson²⁴, Kelly B. Schilling¹⁰, Declan C. Schroeder⁵¹, Alastair G. B. Simpson^{2,52}, Claudio H. Slomovits^{2,9}, David R. Smith⁵³, G. Jason Smith⁵⁴, Sarah R. Smith⁵, Heidi M. Sosik²⁵, Peter Stief²⁶, Edward Theriot⁵⁵, Scott N. Twary⁵⁶, Pooja E. Umale¹⁰, Daniel Vaultot⁵⁷, Boris Wawrik⁵⁸, Glen L. Wheeler^{51,59}, William H. Wilson⁶⁰, Yan Xu⁶¹, Adriana Zingone⁴⁰, Alexandra Z. Worden^{2,3*}

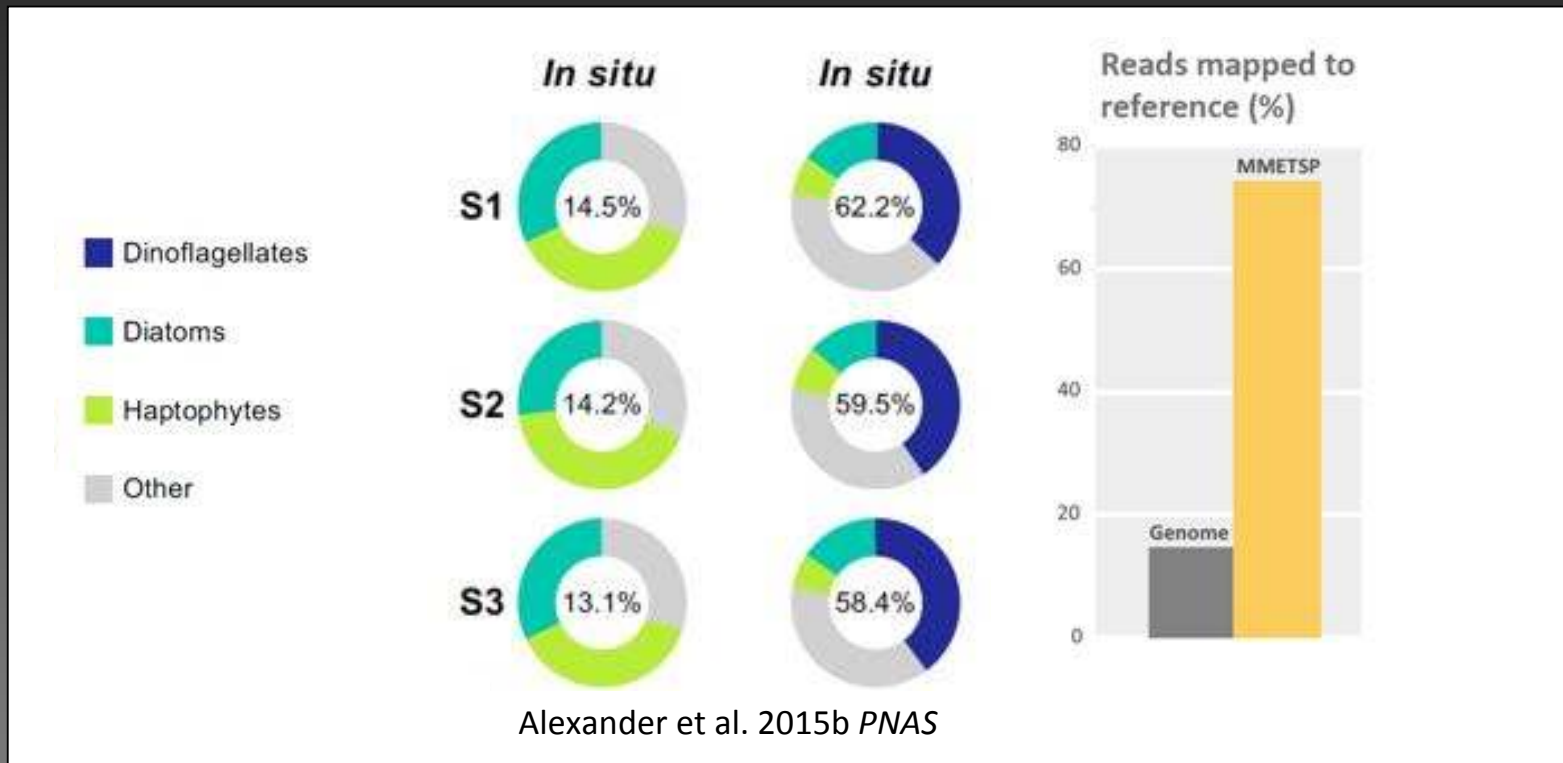
Greatly expanded reference sequences in the tree of life



Keeling et al. 2014 *PLoS Biol.*

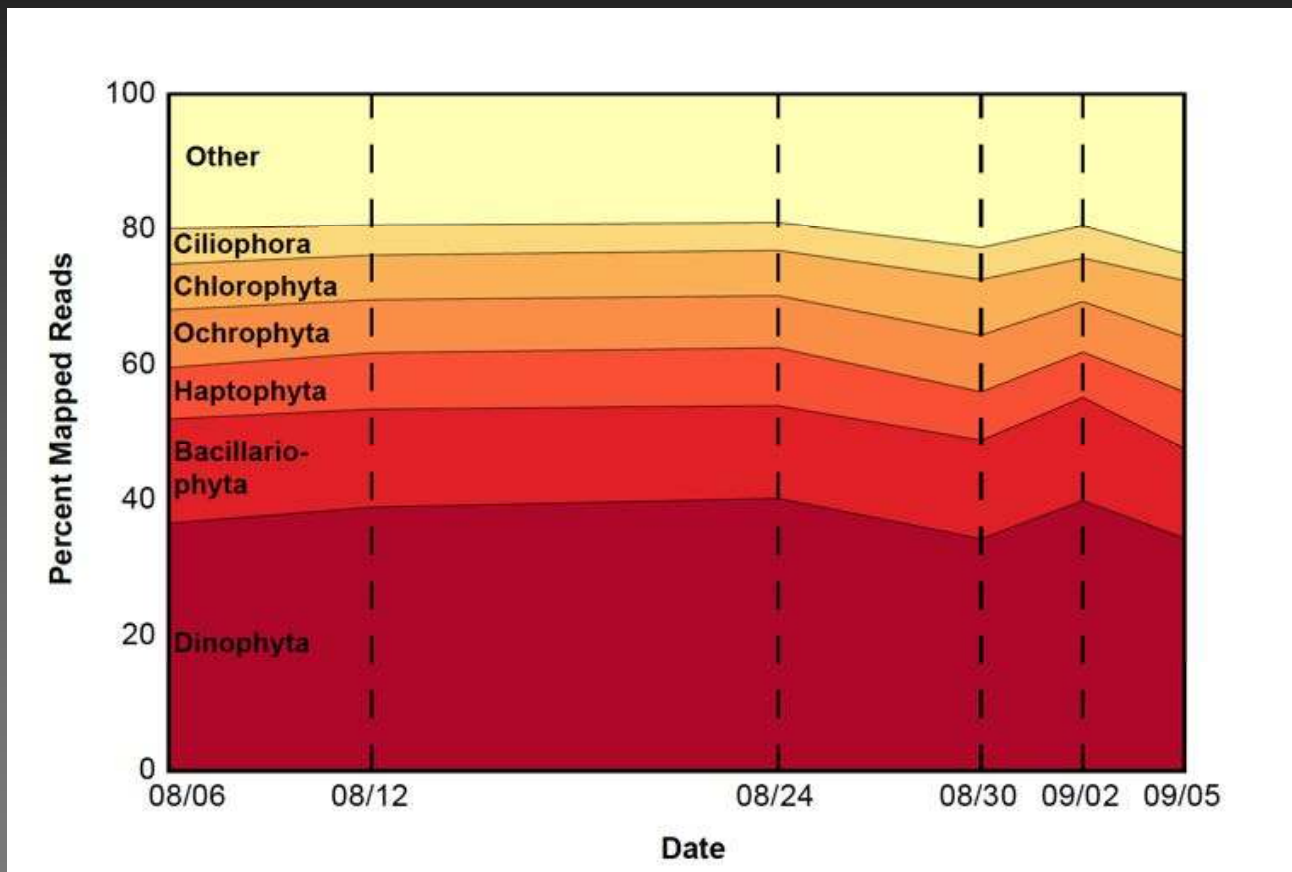
MMETSP improves read identification

Read mapping against genomes v. MMETSP at St. ALHOA



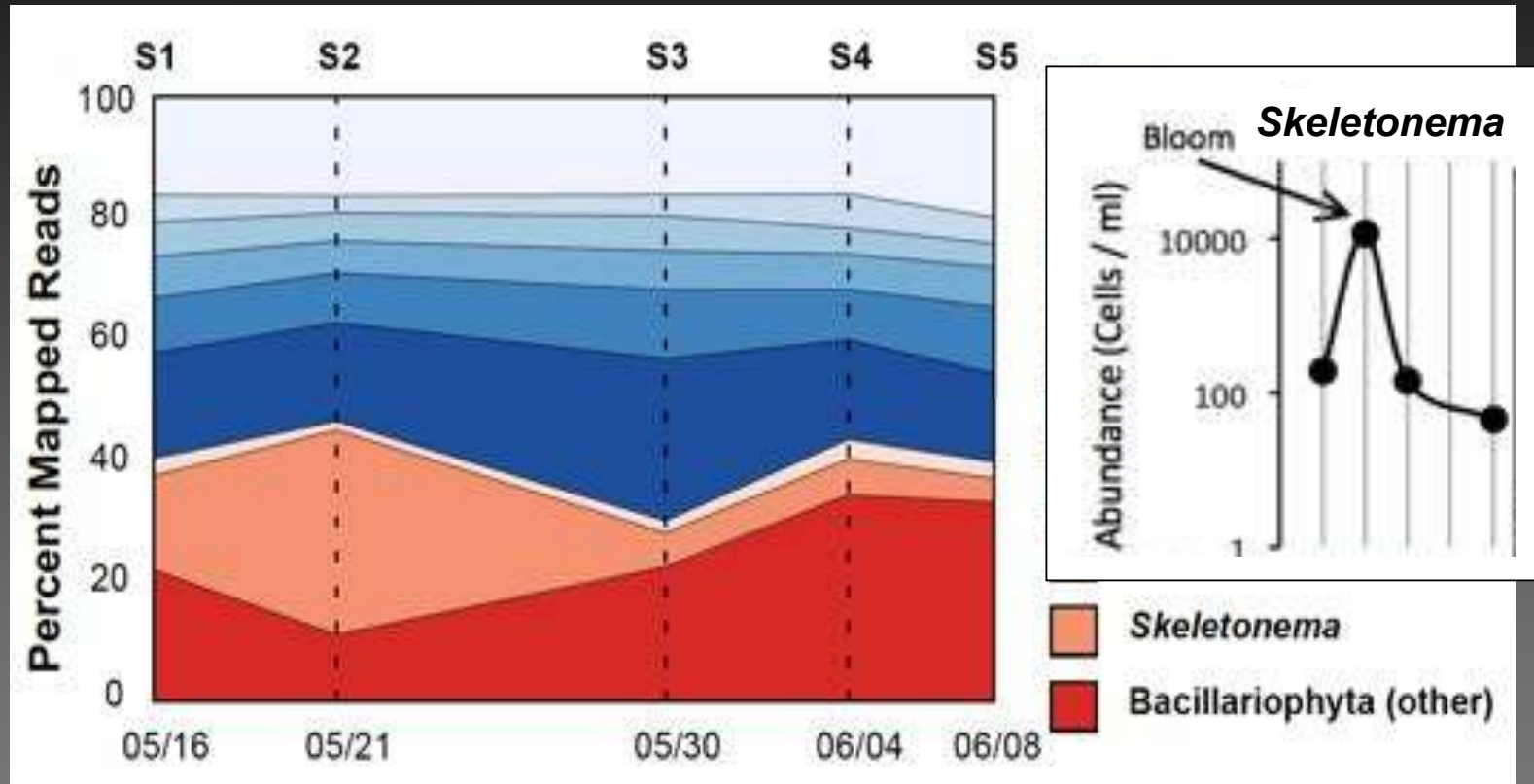
Sequence read identification is substantially improved over using phytoplankton genomes which do not capture the same diversity.

Taxonomic distribution of reads



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

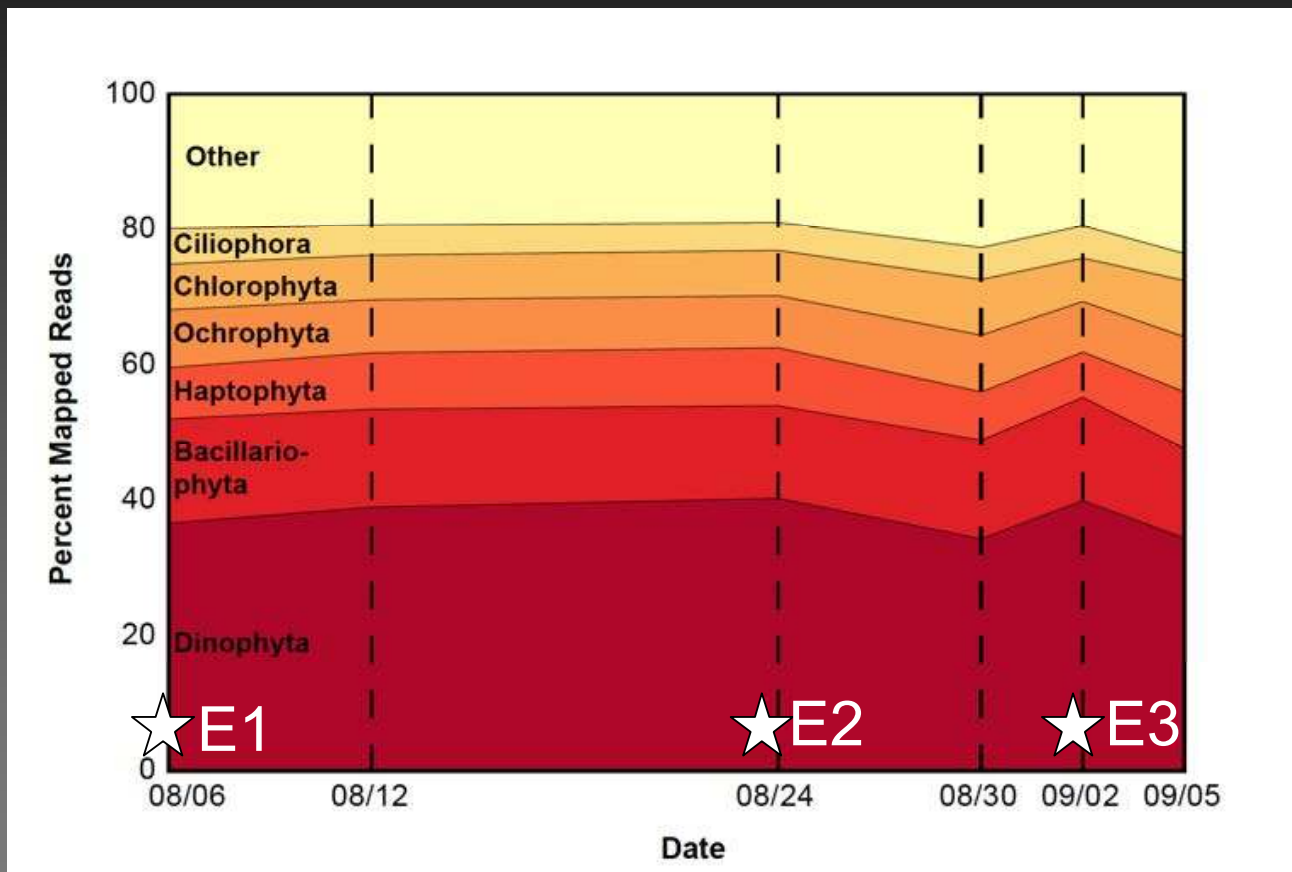
Taxonomic distribution of reads - coastal bloom



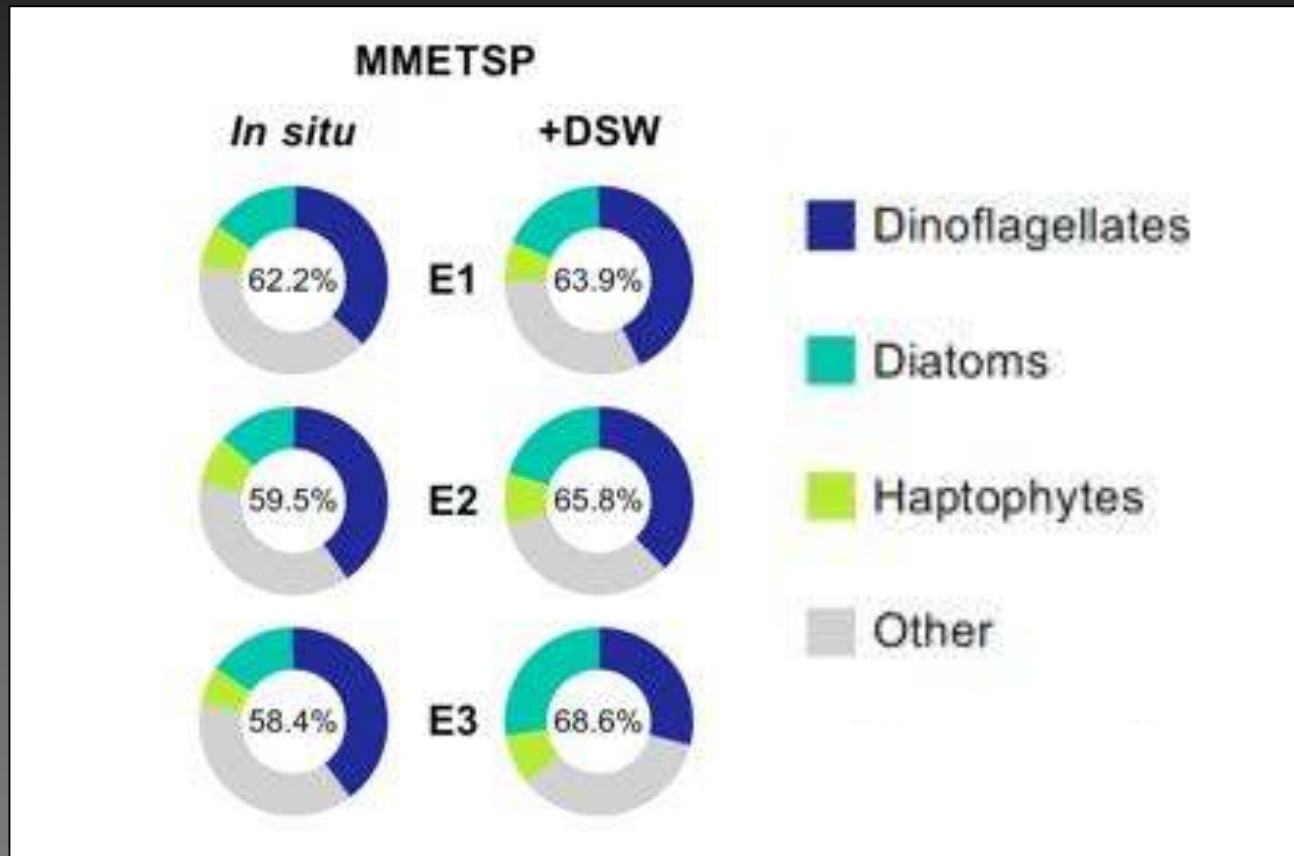
Alexander et al. 2015 *PNAS*

Taxonomic distribution of reads is more variable in a coastal system

Taxonomic distribution of reads

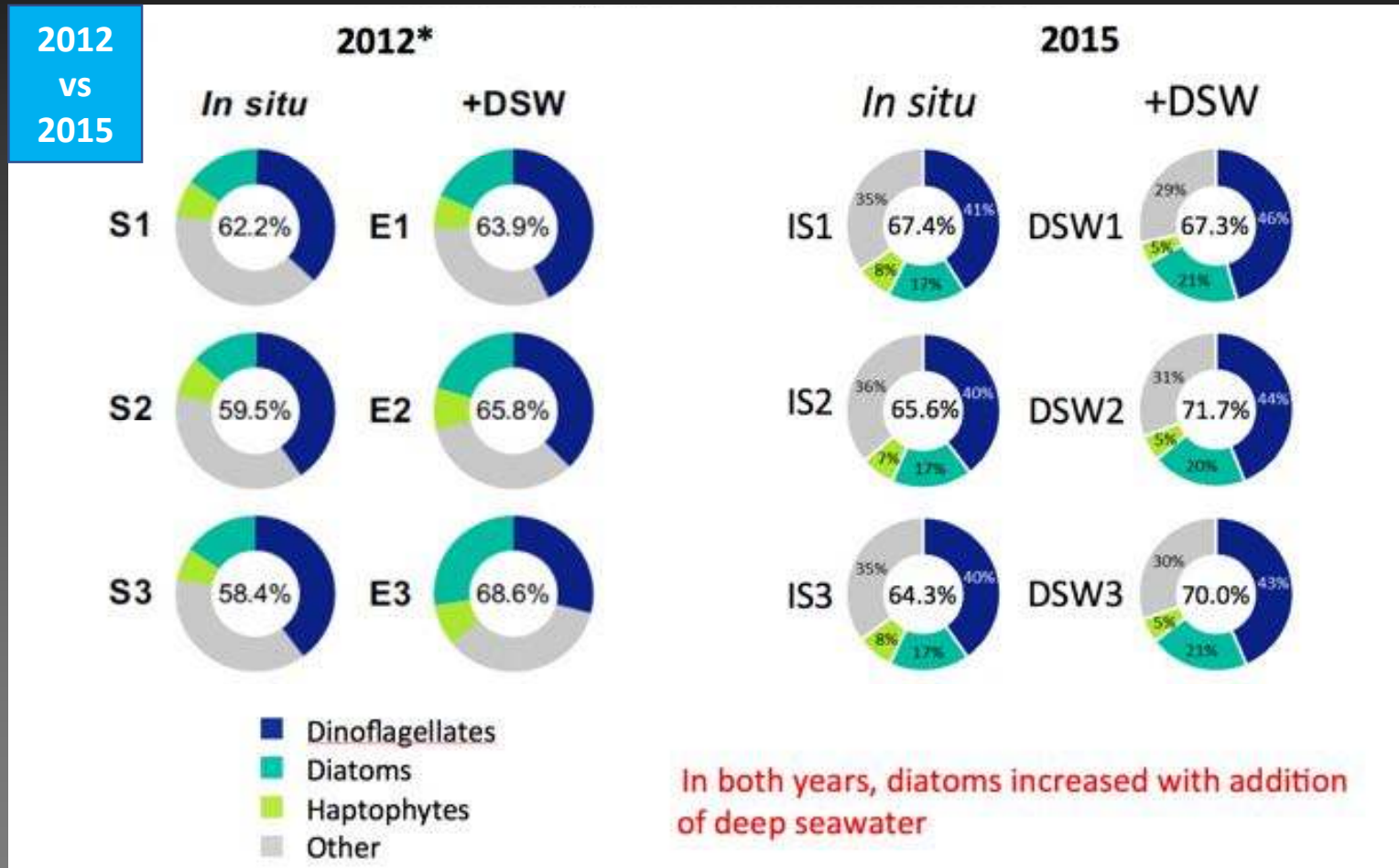


Increase in diatoms during simulated blooms



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

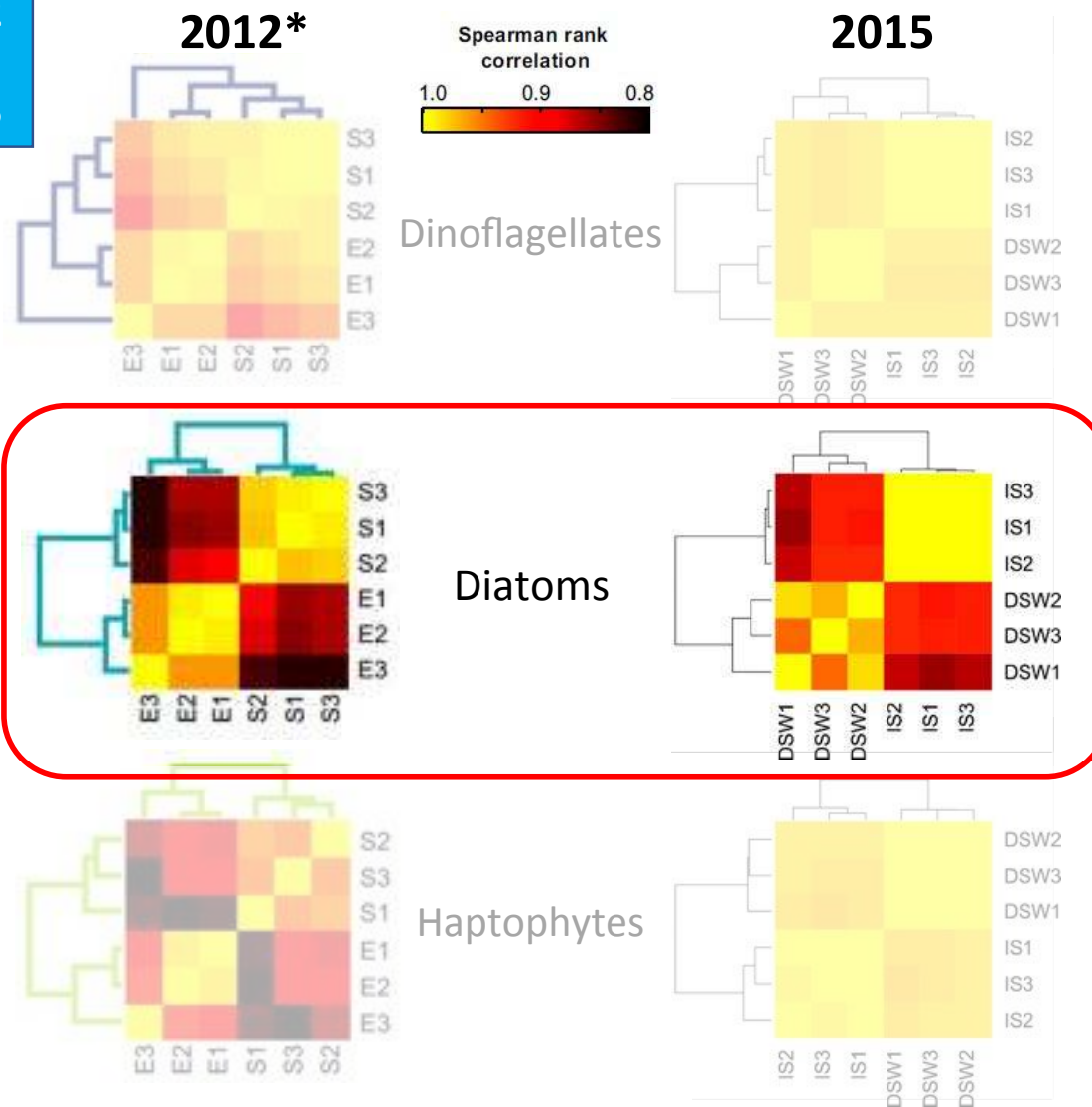
Simulated bloom response was reproducible 3 years later



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

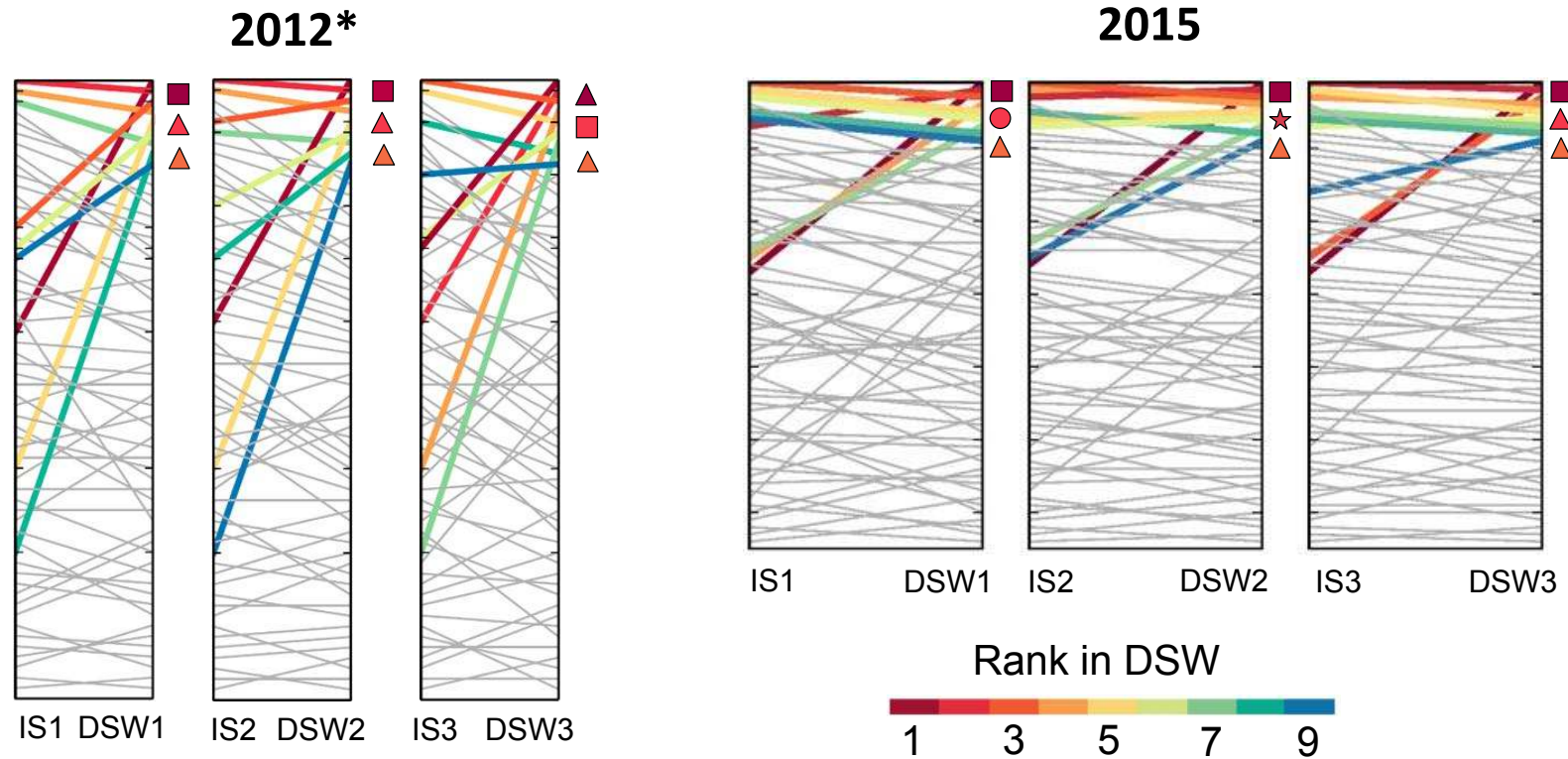
Community composition shifts between years

2012
vs
2015



E = +DSW S = *in situ*

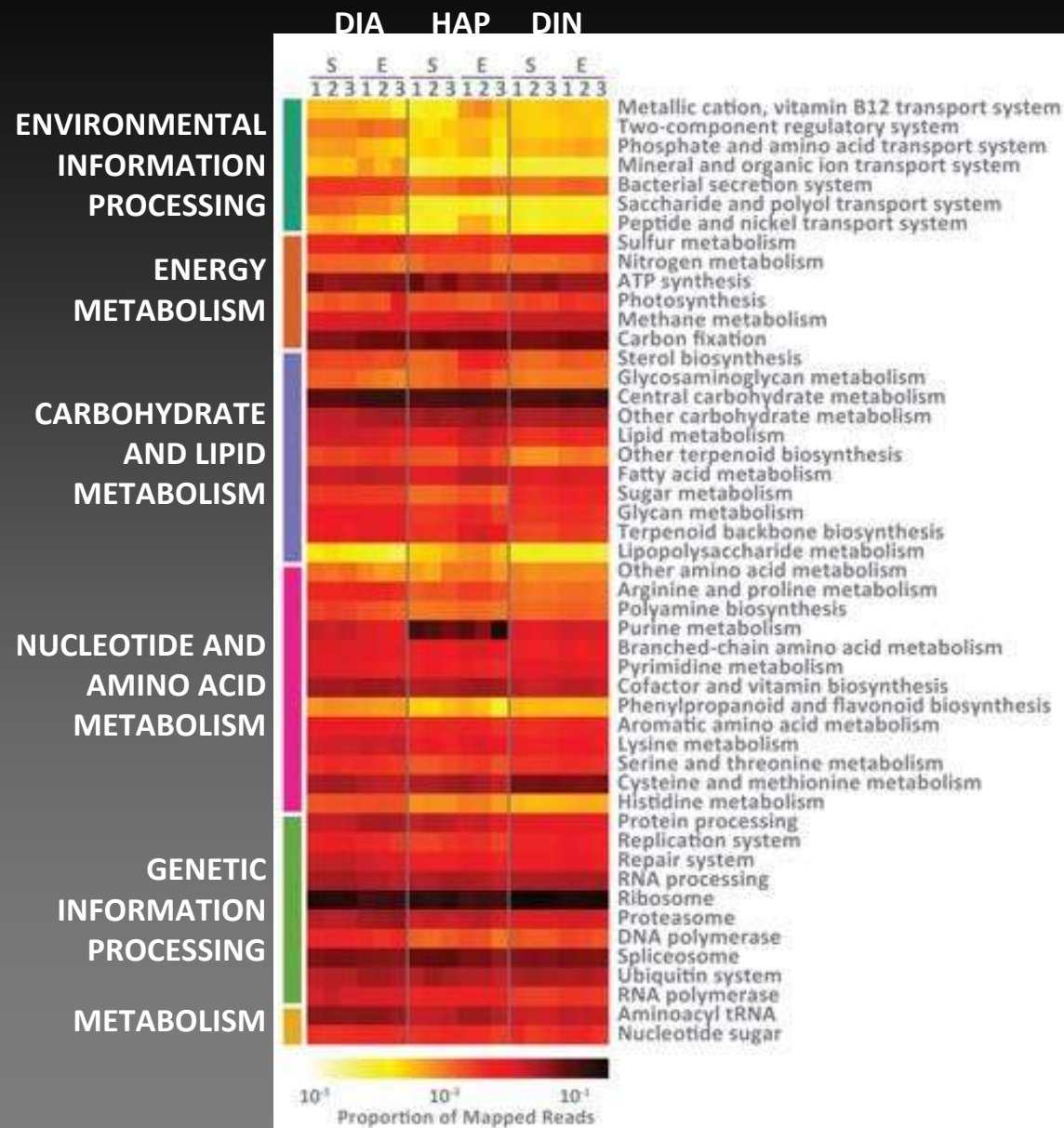
Its even the same species changing...



2012
vs
2015

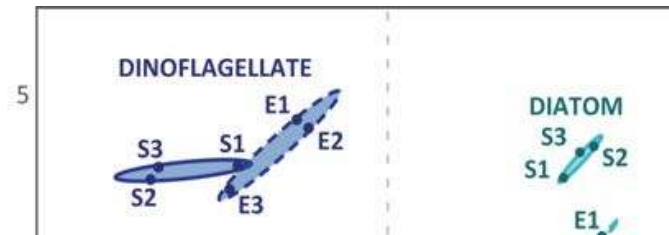
- *Cylindrotheca* sp.
- △ *Pseudo-nitzschia* sp.
- *Chaetoceros* sp.
- ☆ *Ditylum* sp.

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



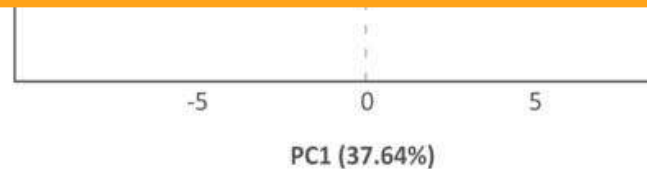
Alexander et al. 2015b *PNAS*

Quantitative metabolic fingerprint



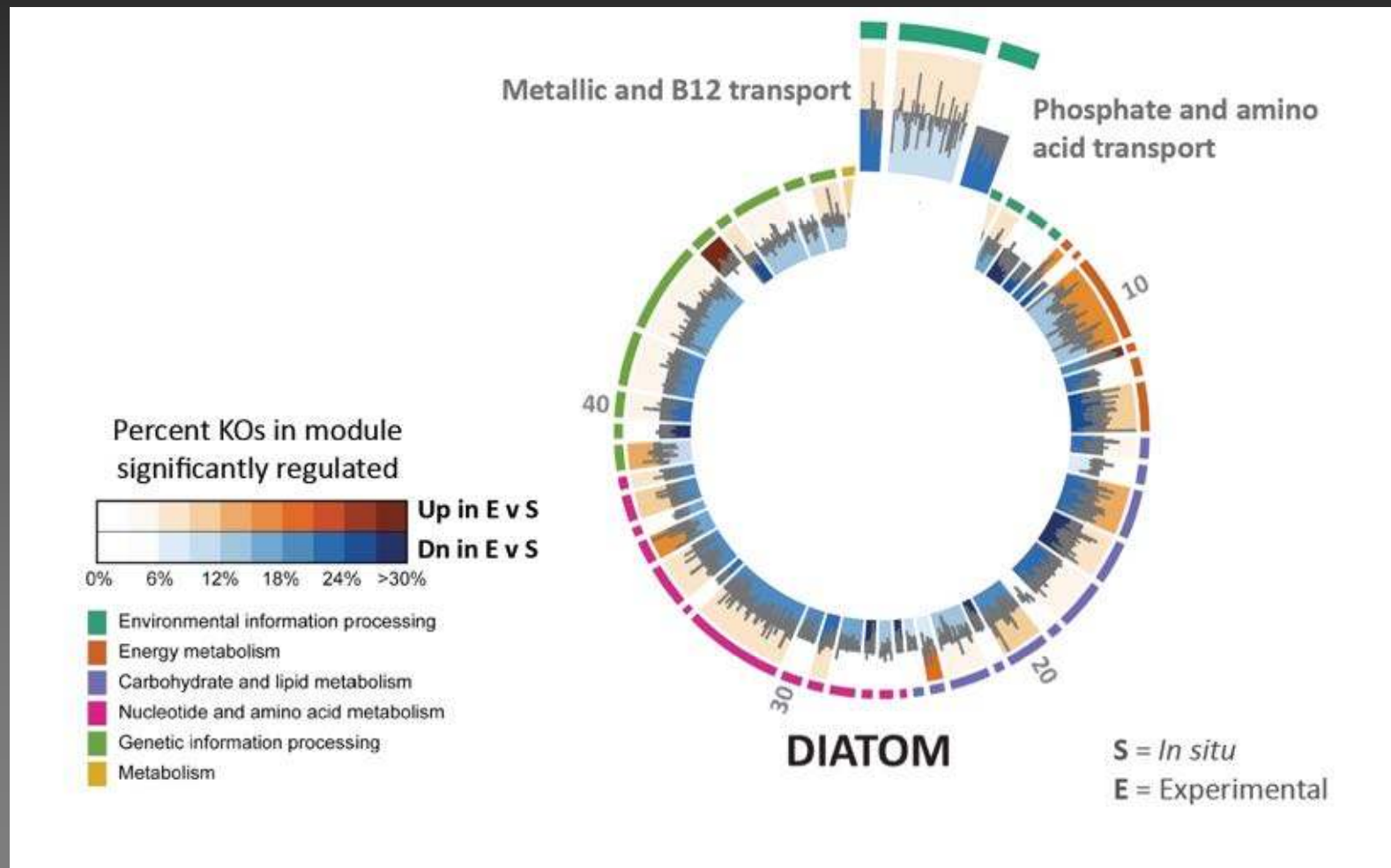
For each gene determine

Significance Post- $p > 0.95$ for 2-fold change

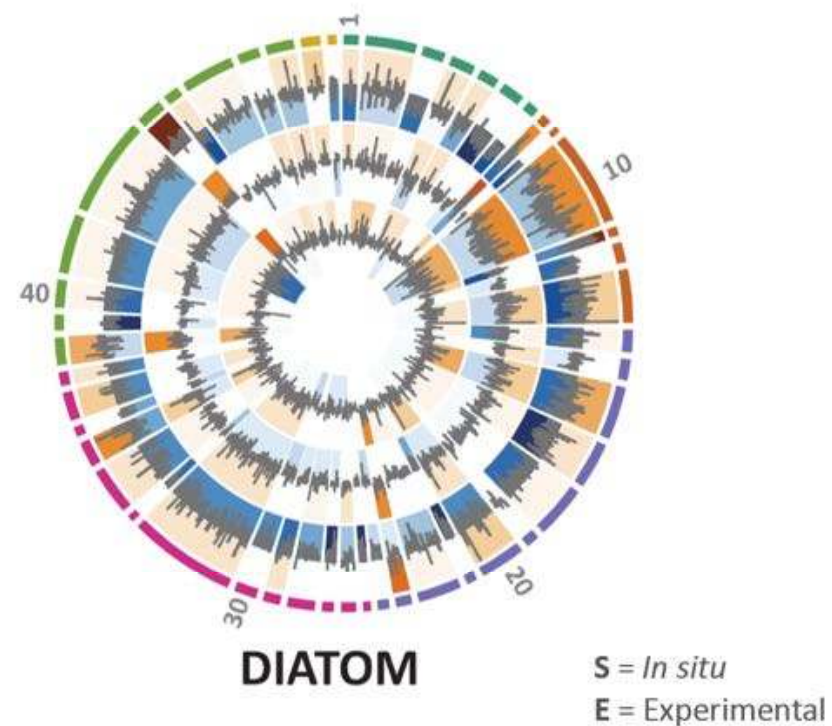
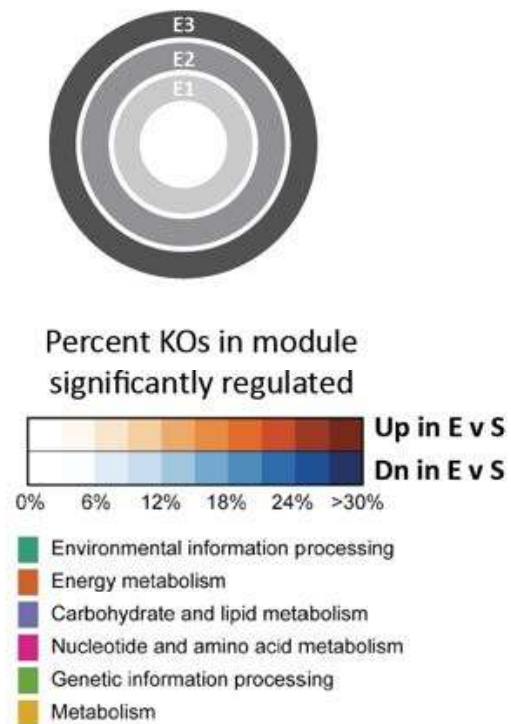


Species are distinct from each other with 95% confidence, and all but dinoflagellates significantly shift in the simulated blooms

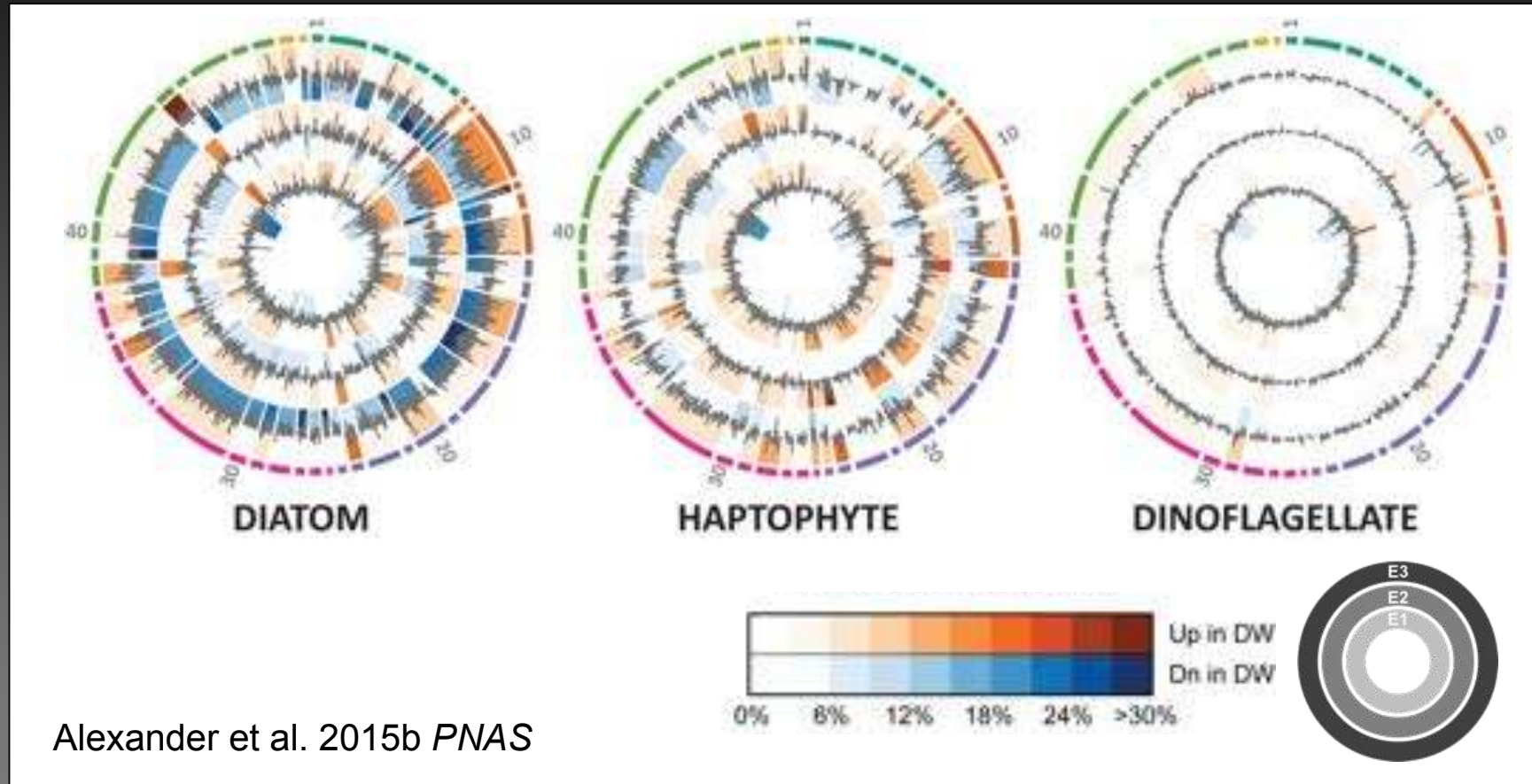
Gene shifts in diatoms



Gene shifts in diatoms



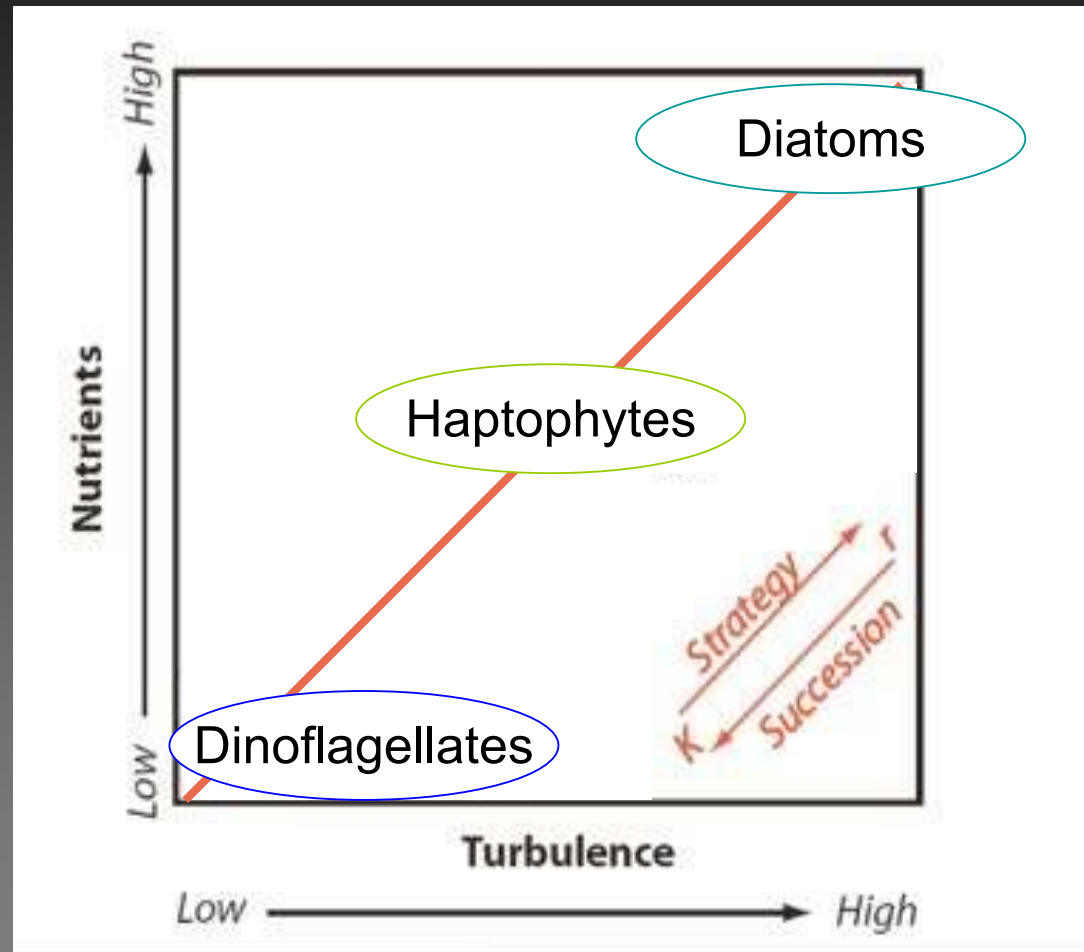
Transcriptional response during simulated blooms



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

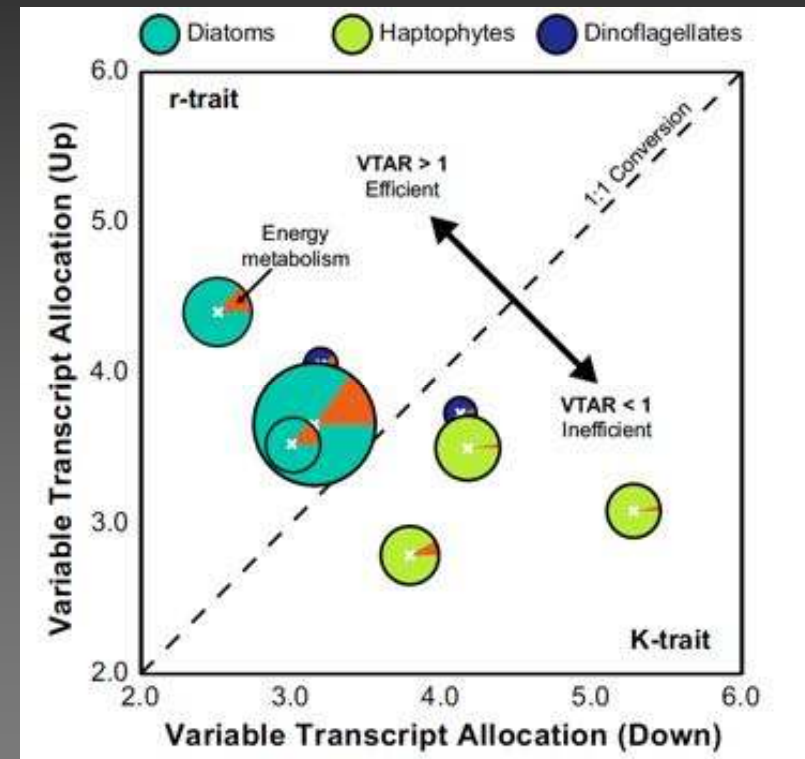


Adapted from Margalef 1978

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”; $VTAR > 1$

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

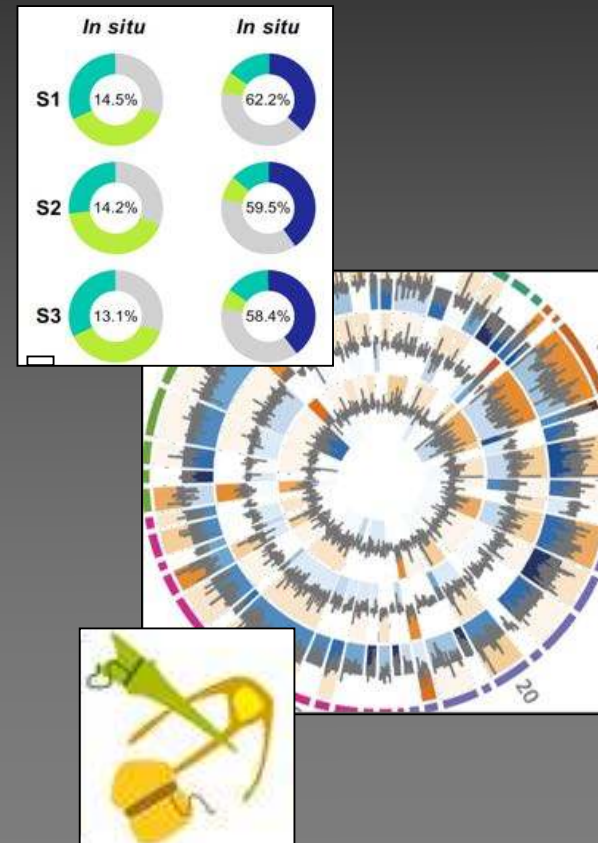


“the survivalists”
K-selected

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?

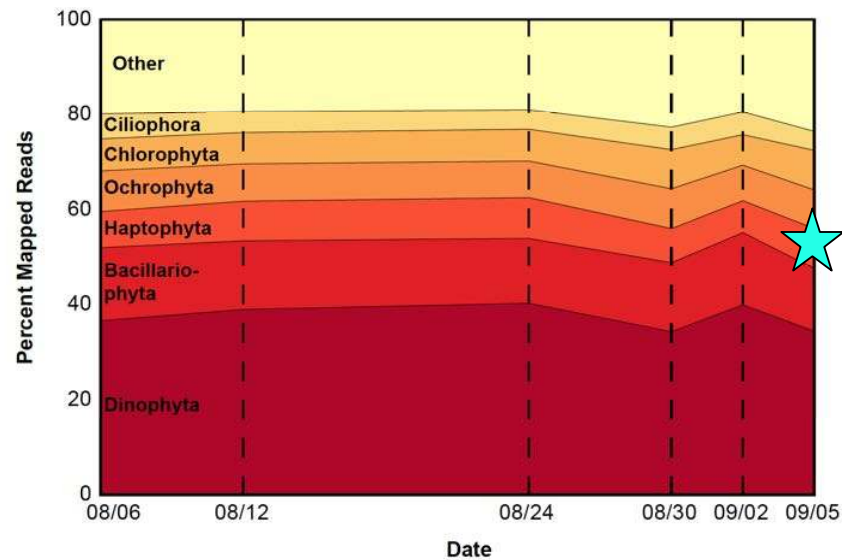


Metabolic plasticity and diversity

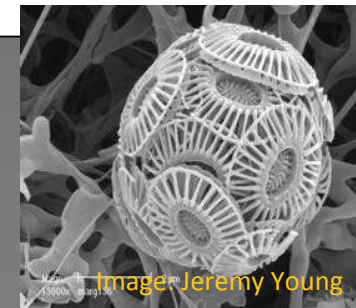
- How do diversity and metabolism influence the physiological ecology of a keystone group?



Diving beyond the functional group...

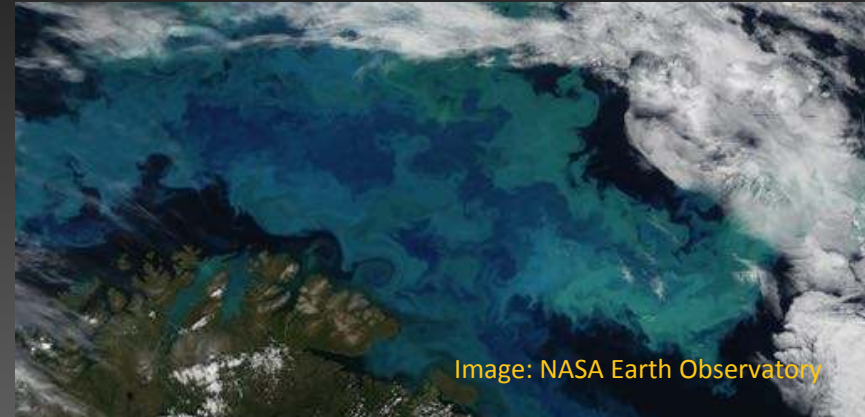


Tracking strain specific responses in the calcifying haptophyte, *Emiliana huxleyi* (Ehux)



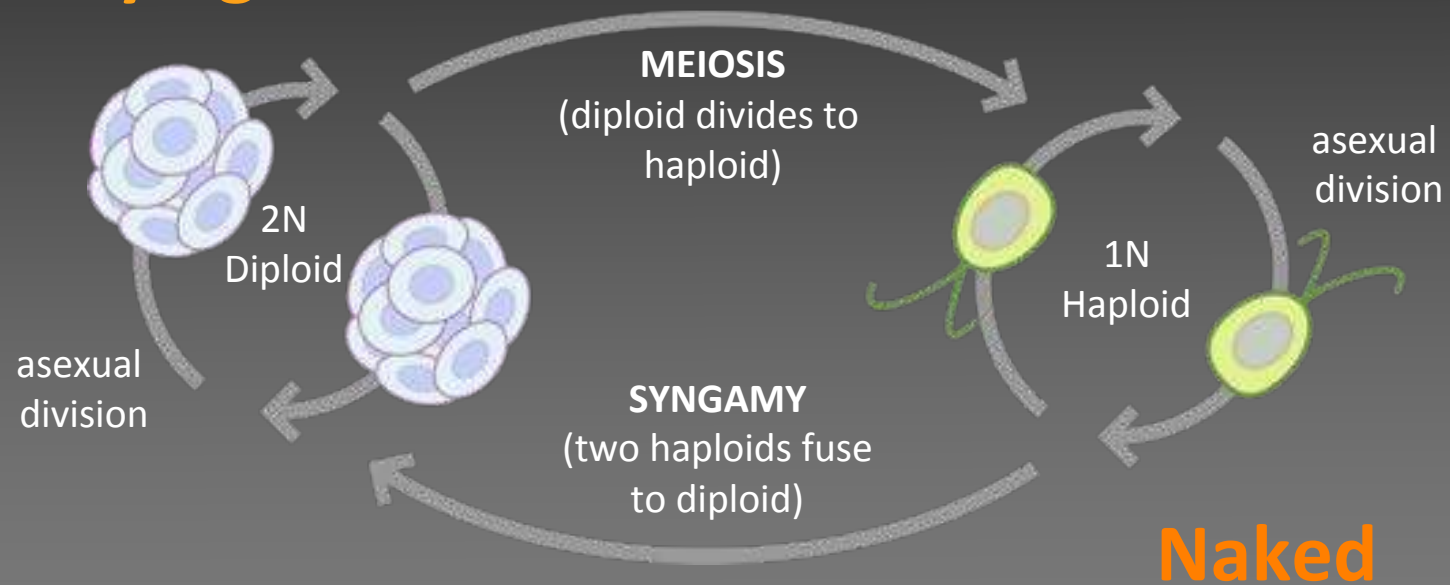
Emiliana huxleyi: a cosmopolitan, globally significant species

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

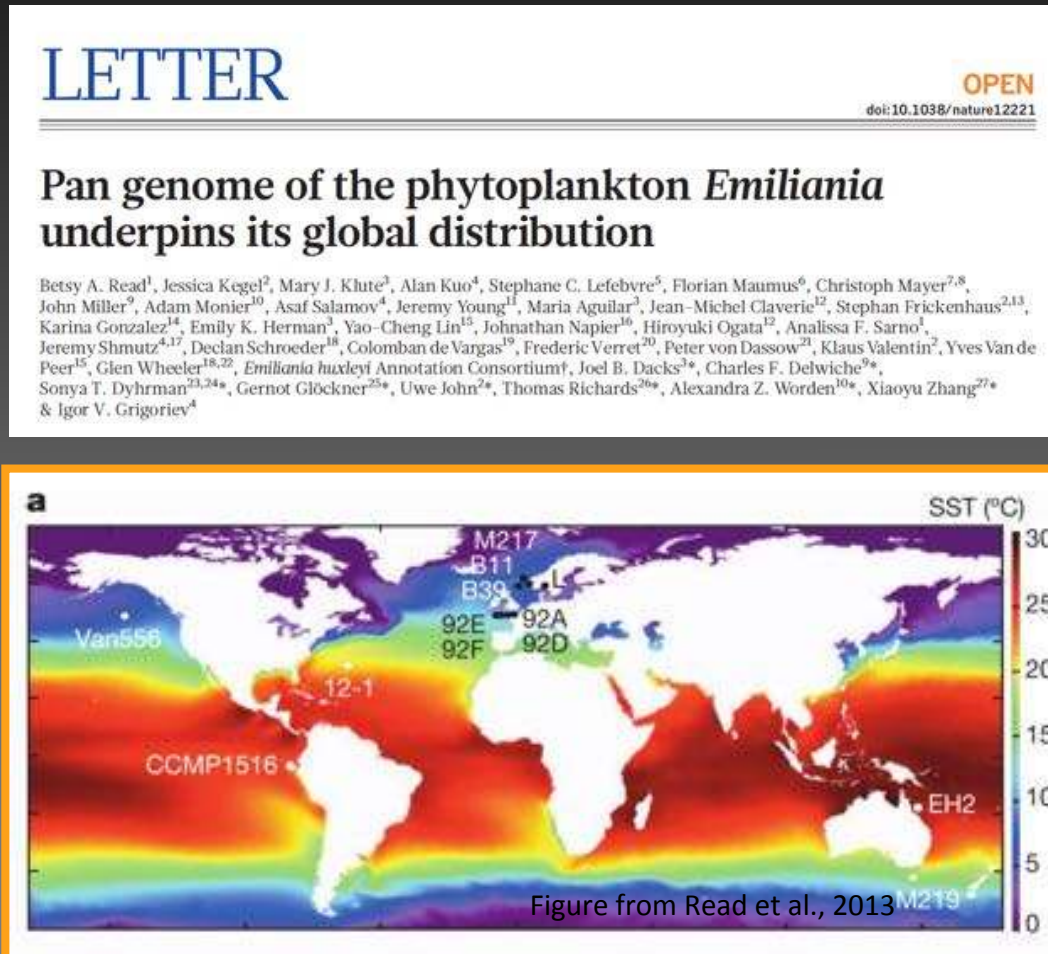


E. huxleyi life cycle

Calcifying

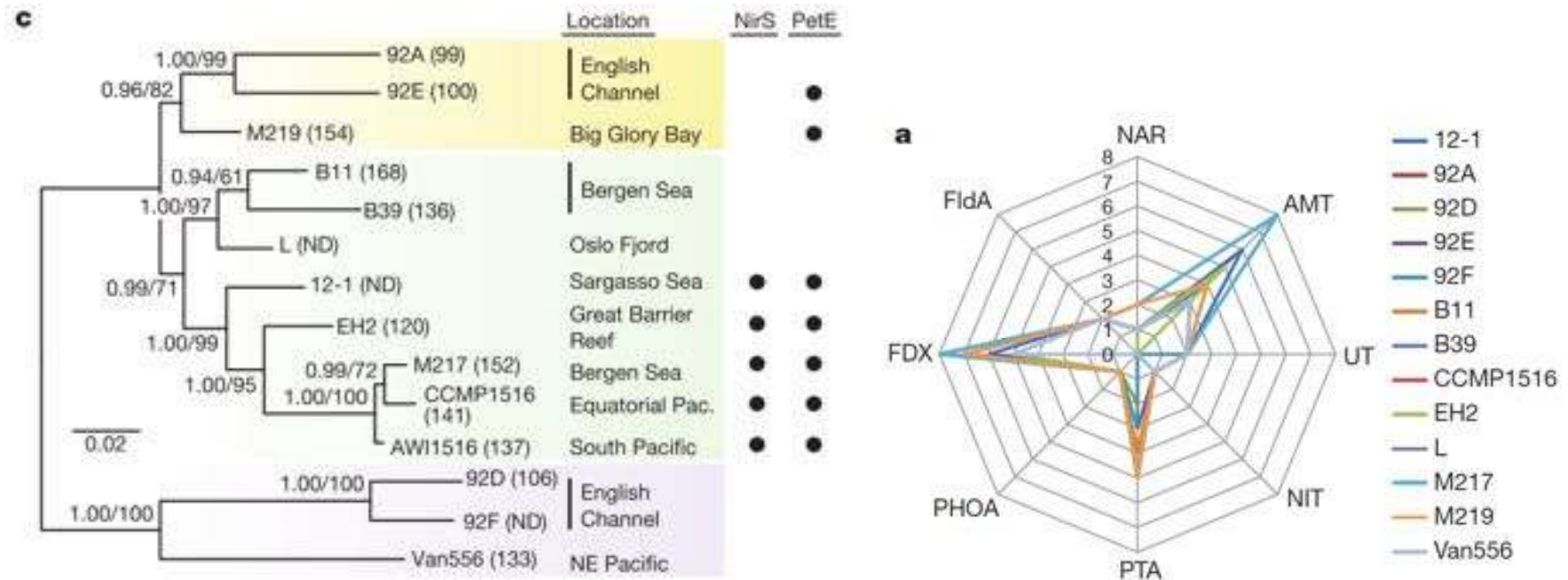


Emiliana huxleyi has many diverse isolates



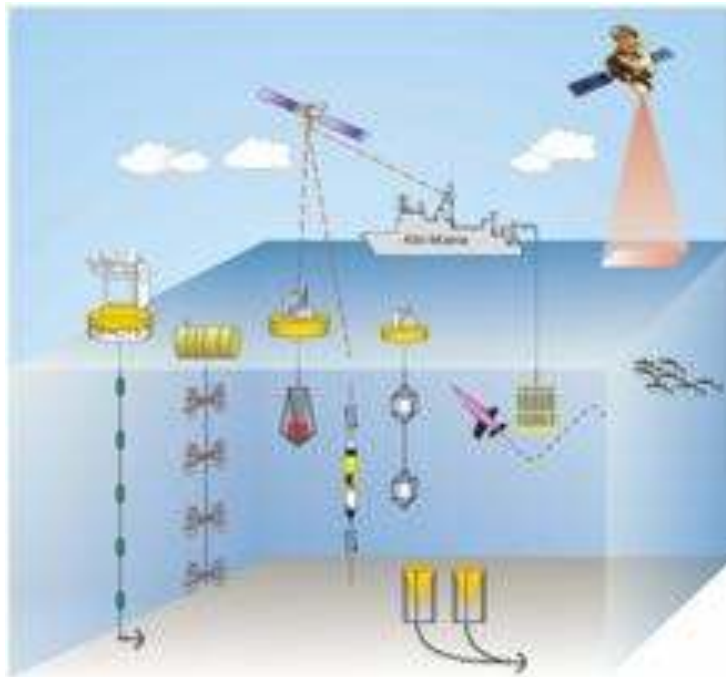
Cultured strains are highly diverse - isolated from a broad temperature range and displaying considerable physiological diversity

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*

Global Biogeochemical Cycles

AN AGU JOURNAL

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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 TDC](#)
Volume 29, Issue 8
August 2015
Pages 1145-1164

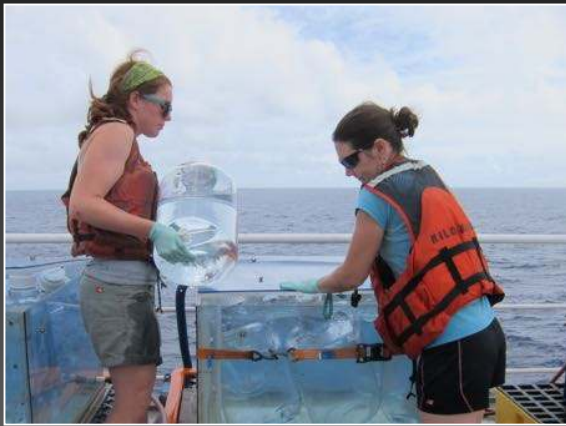


center for microbial oceanography: research and education

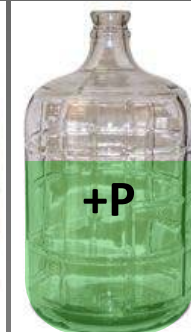
c·more *linking genomes to biomes*

(HOE-DYLAN)

Responses to shifts in resource ratios



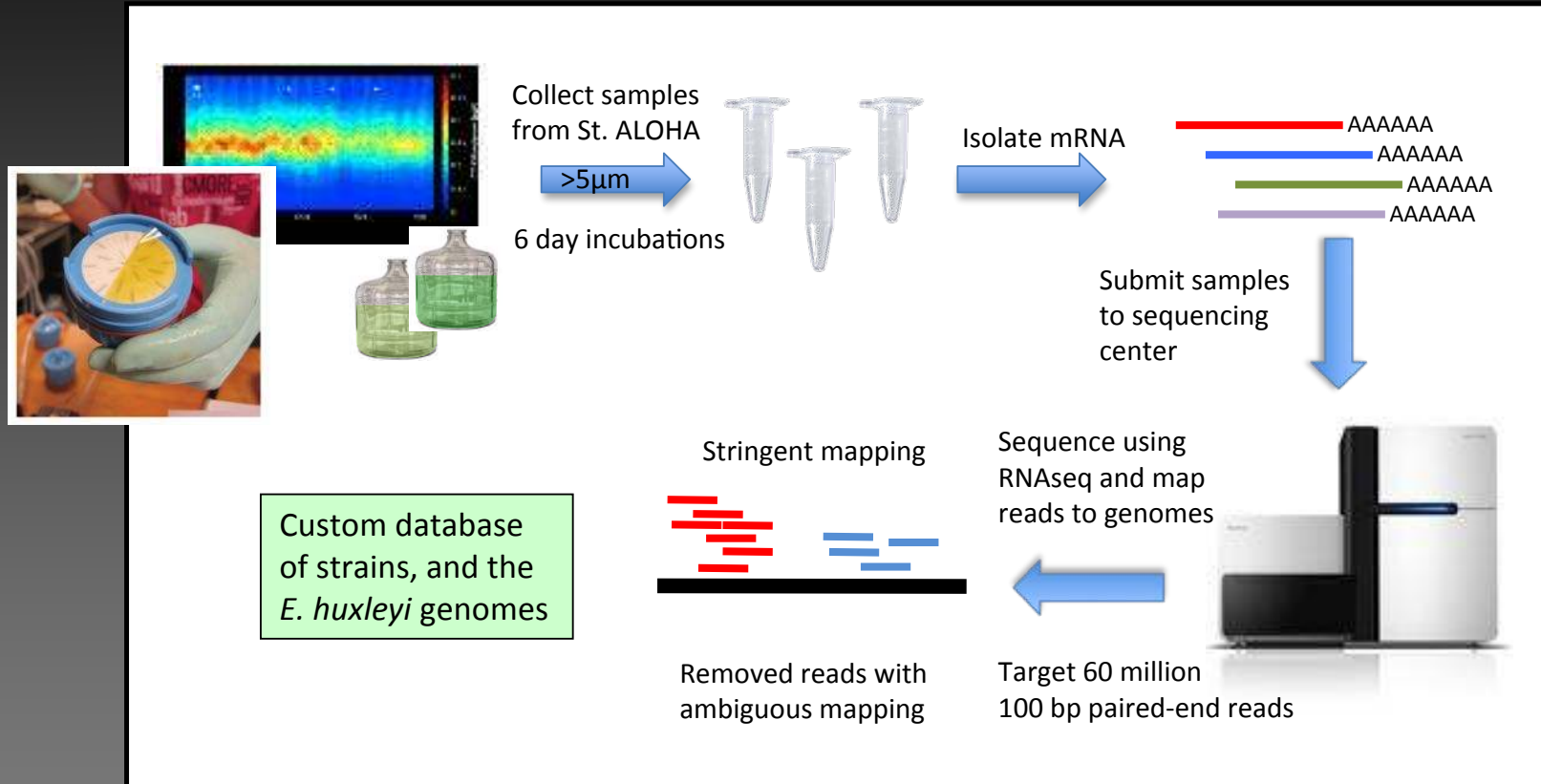
Control Deep seawater amendment



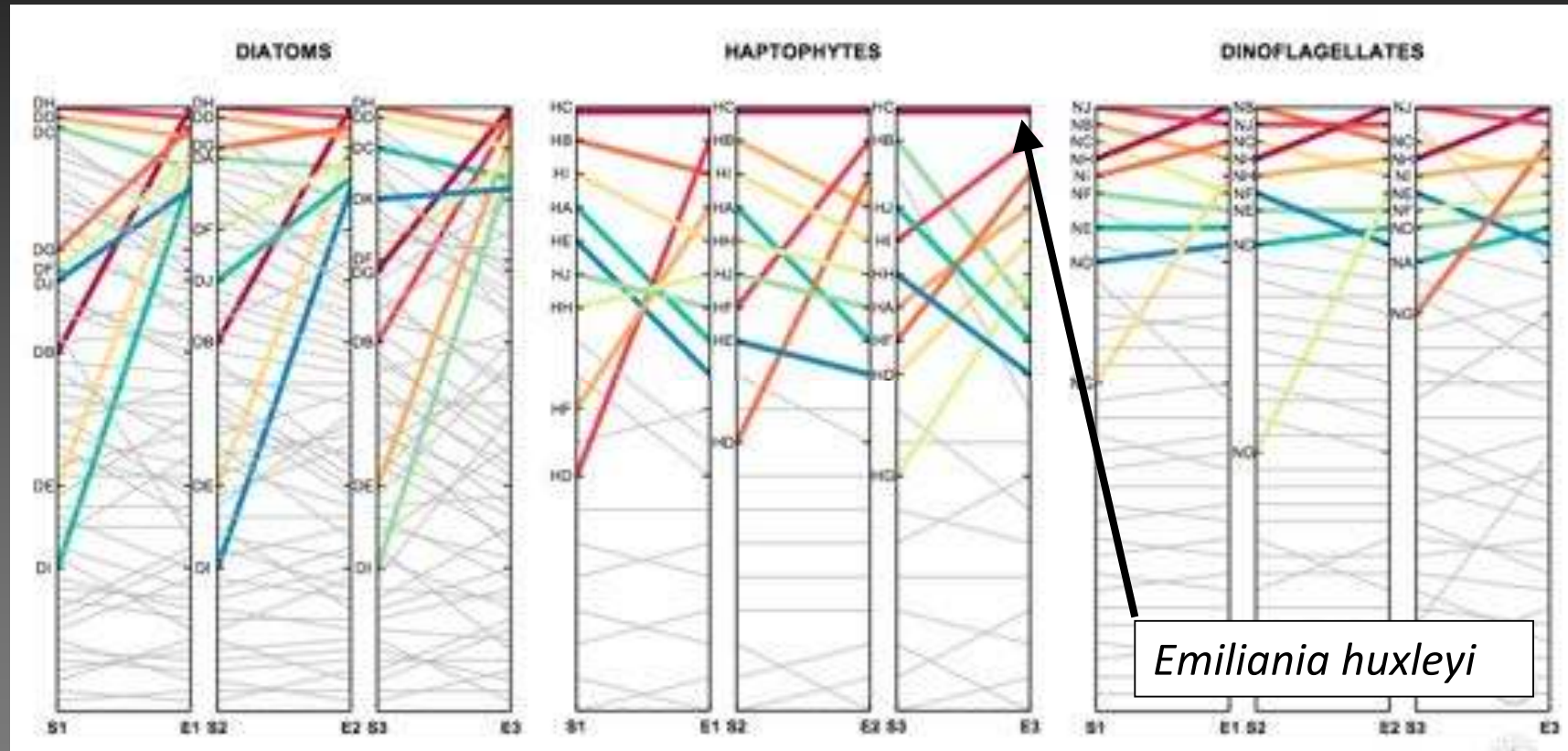
Treatments with added N



Sampling and pipeline

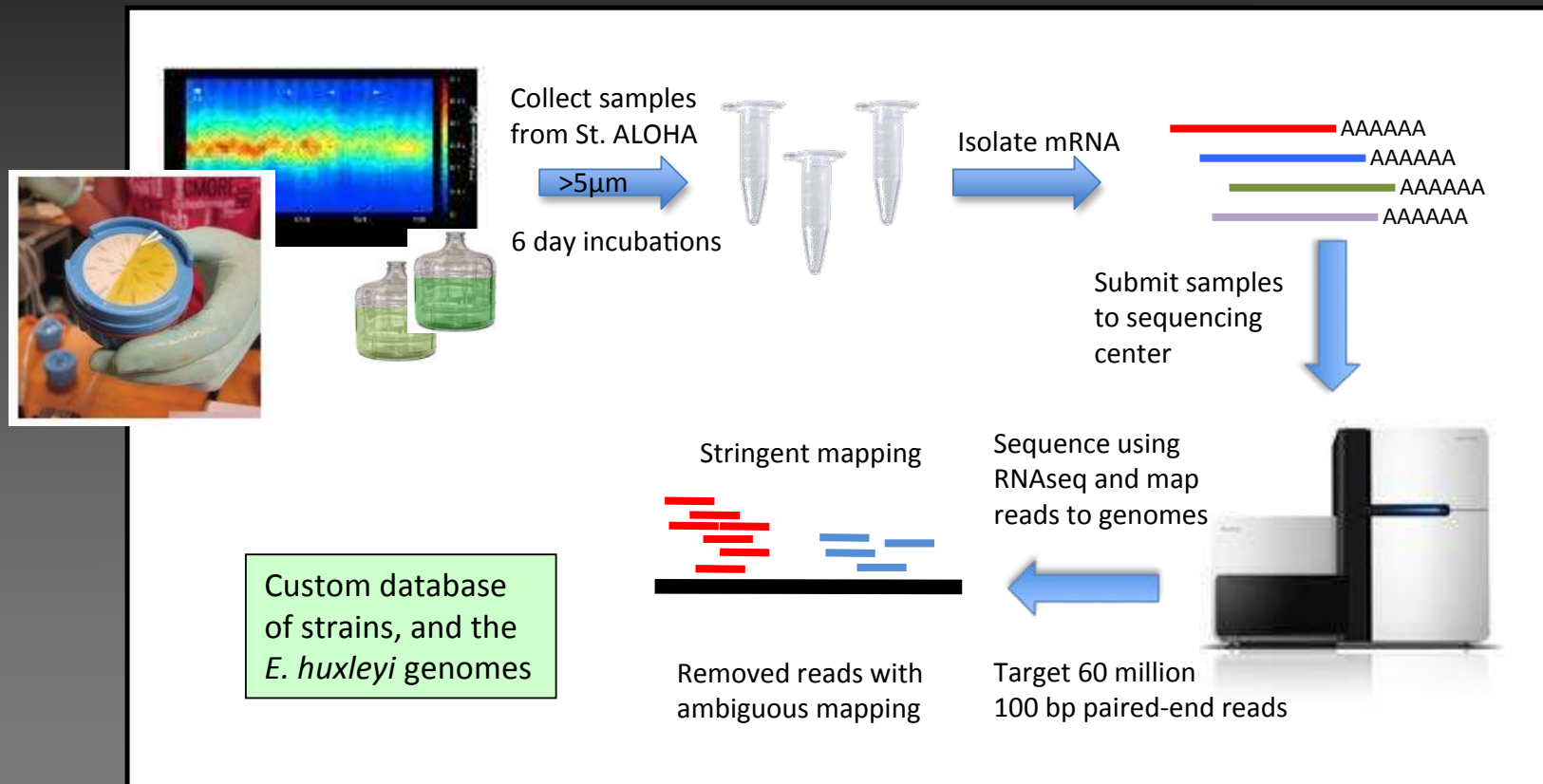


E. huxleyi consistently the dominant haptophyte

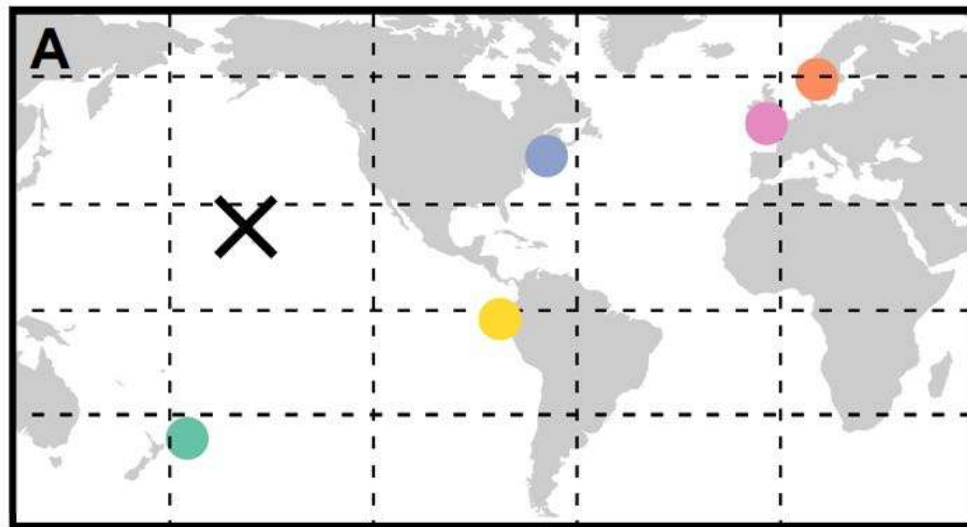


In both the 2012 and 2015 data, rank order changes during blooms (+DSW), although *E. huxleyi* was consistently the dominant haptophyte.

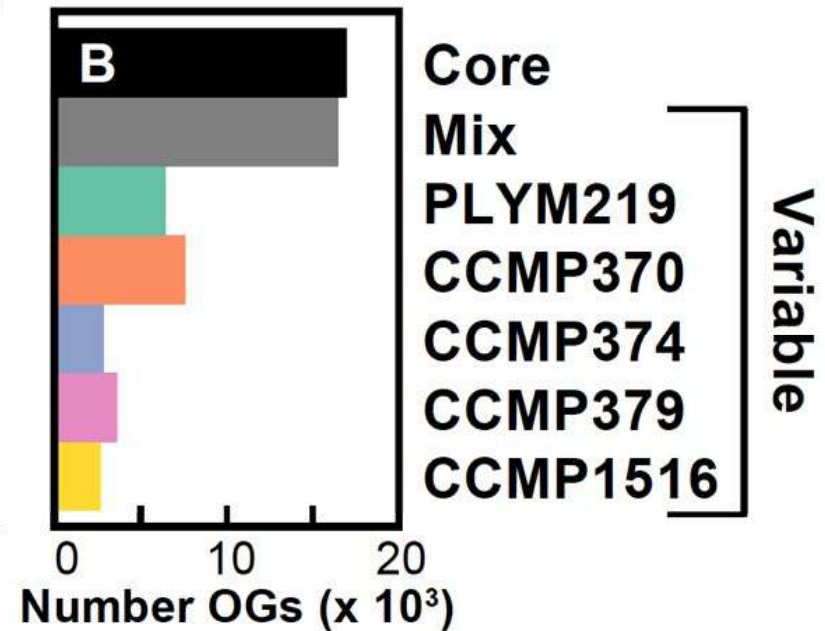
Sampling and pipeline



Orthologous group analysis



Alexander et al. (in review)



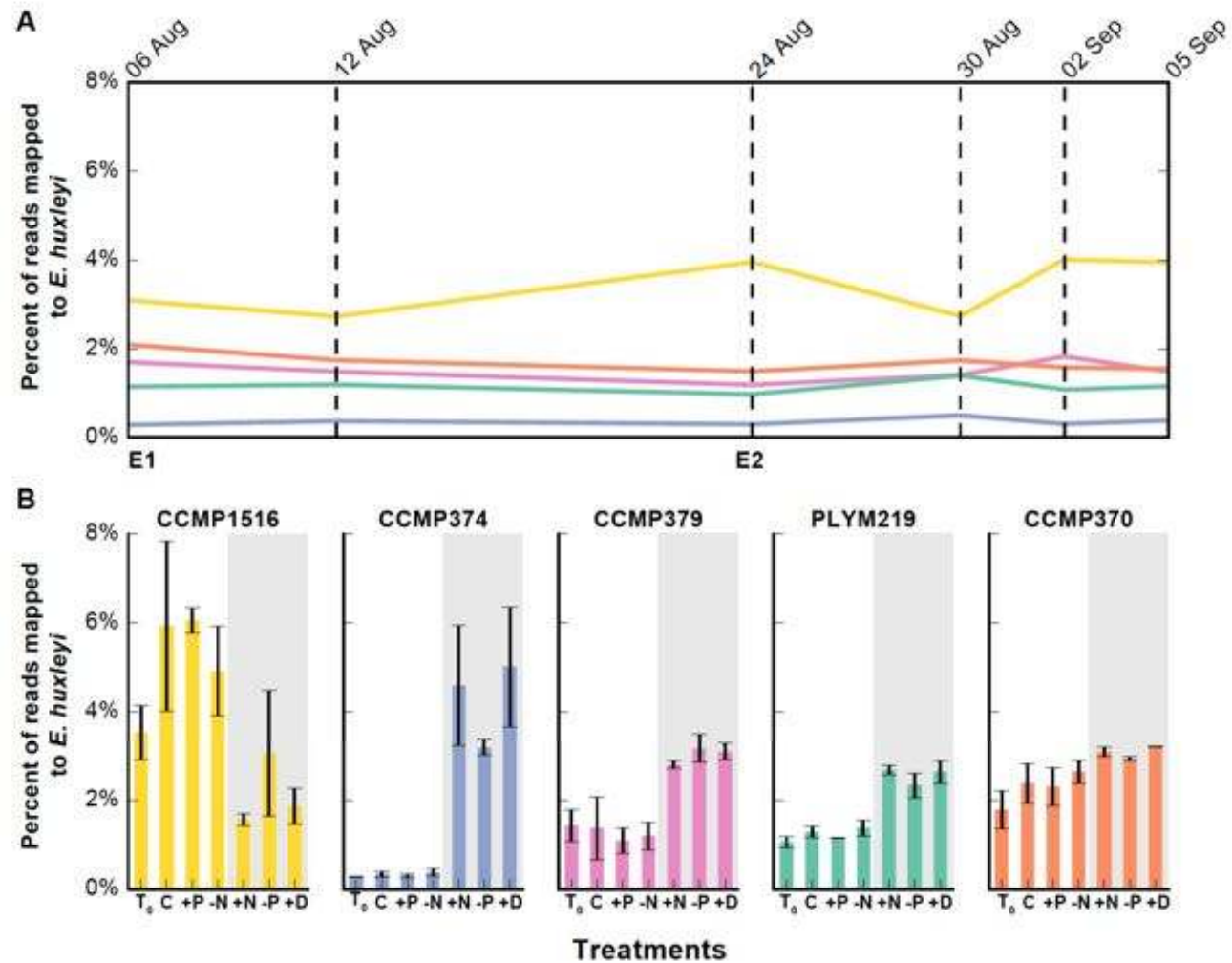
Reference strains isolated from disparate conditions.

Use diagnostic OGs to track strain distribution

Baas Becking hypothesis

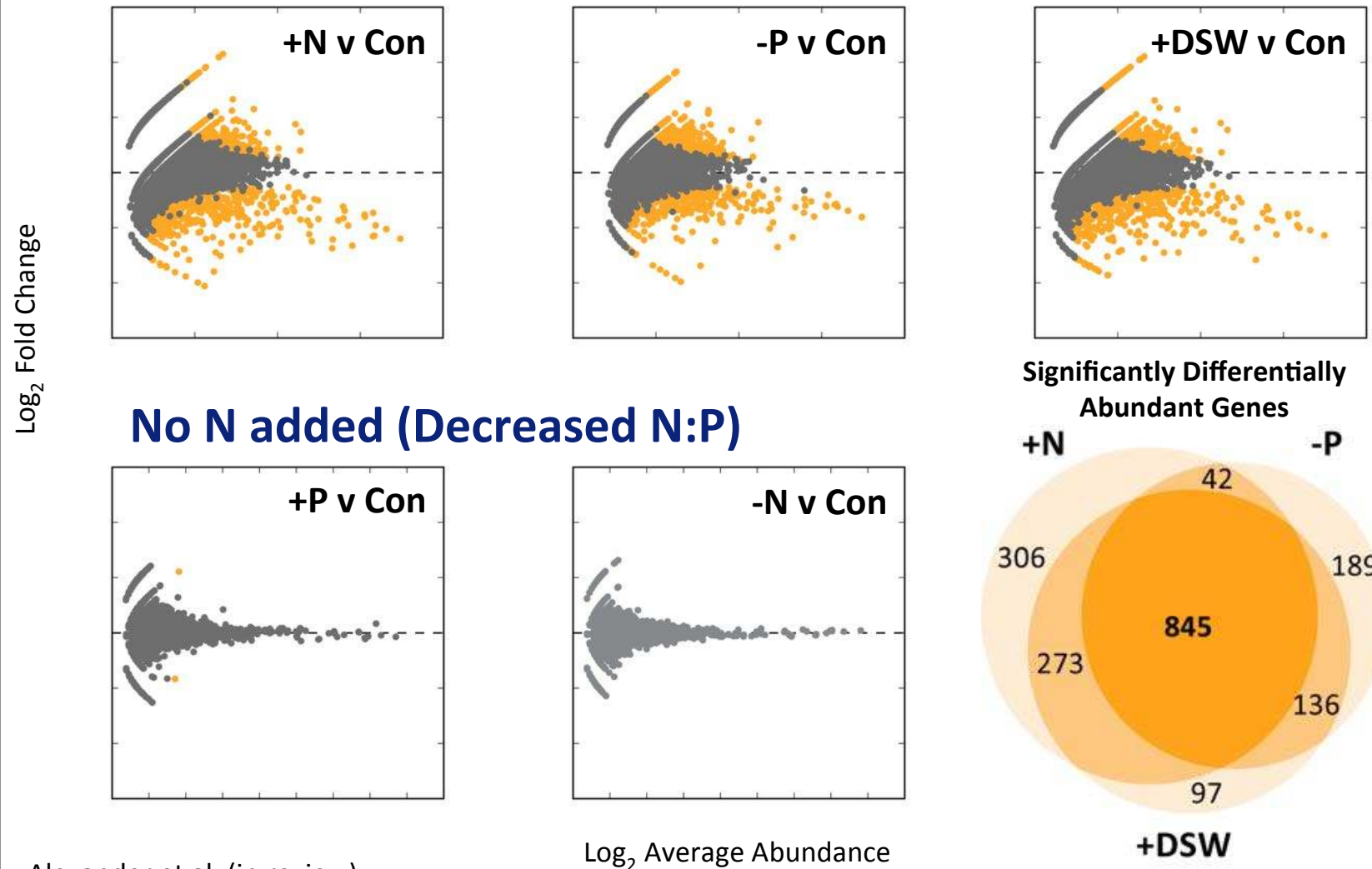
“Everything is every where, but
the environment selects”

Strain distribution *in situ* and in the incubations



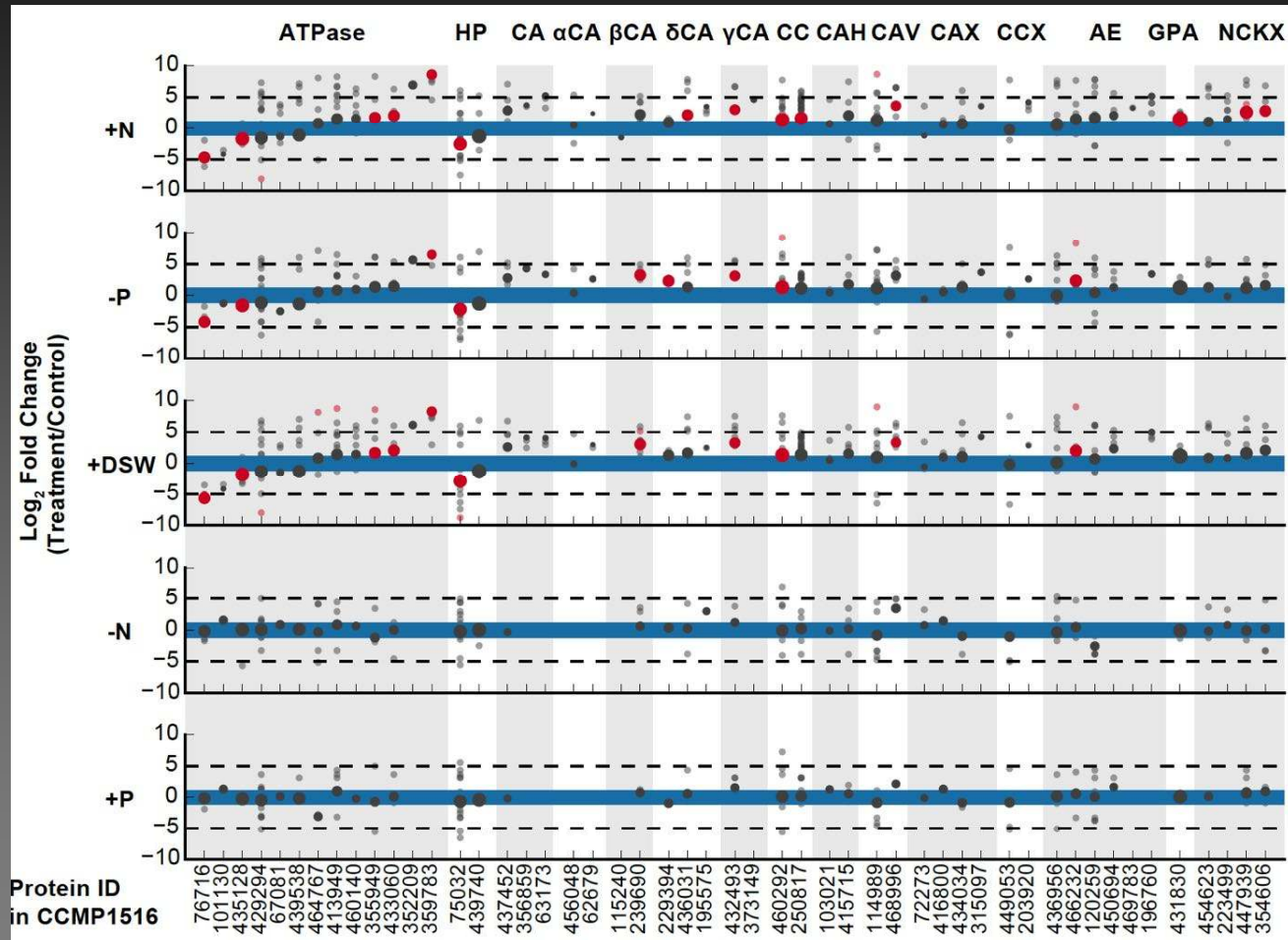
E. huxleyi physiological response suggests N control

N added (Increased N:P)



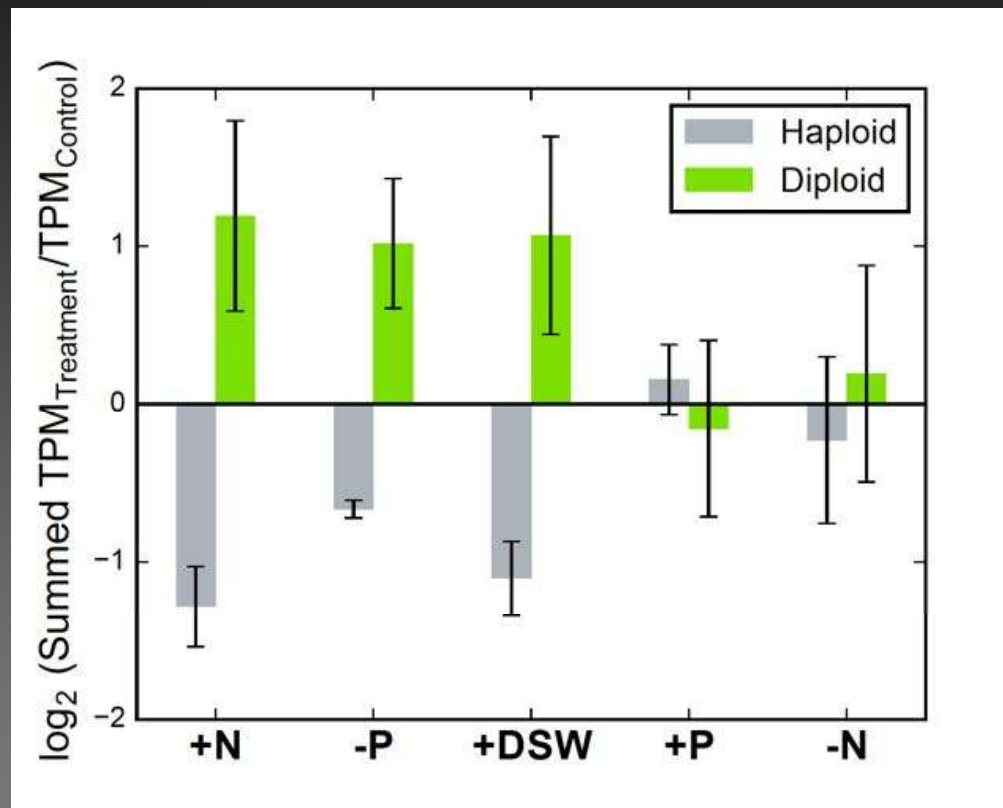
Alexander et al. (in review)

E. huxleyi physiological response - calcification



Markers indicate an increase in calcification with N addition

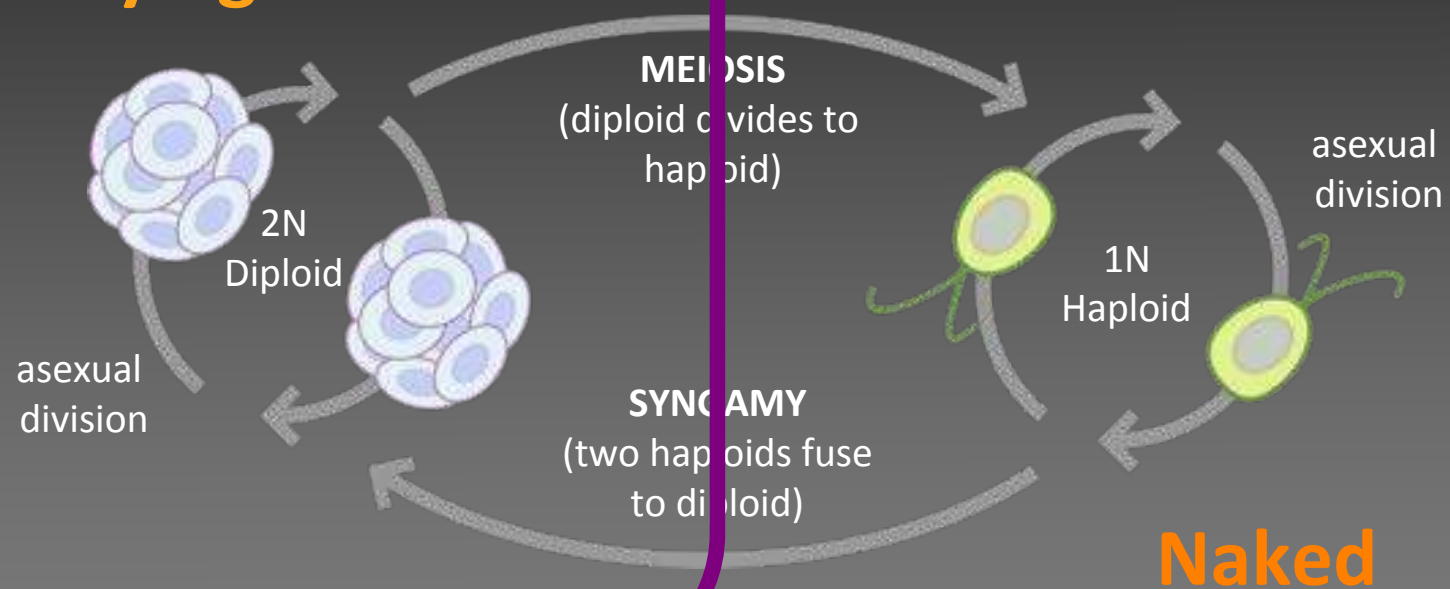
E. huxleyi response - shifts between haploid and diploid



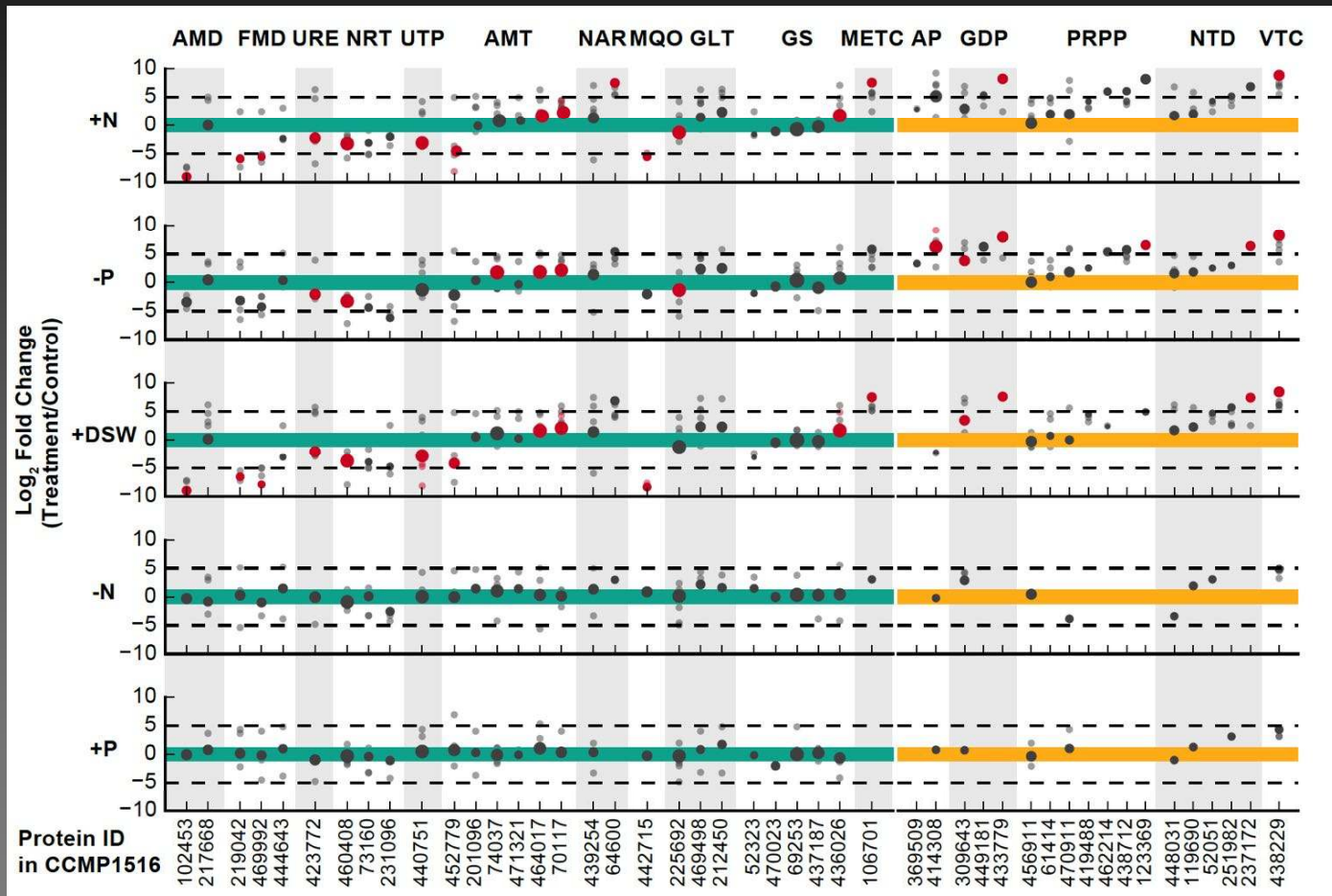
Haploid transcript signal declines, diploid increases with N addition - possible shift towards diploid phase with N

E. huxleyi life cycle

Calcifying



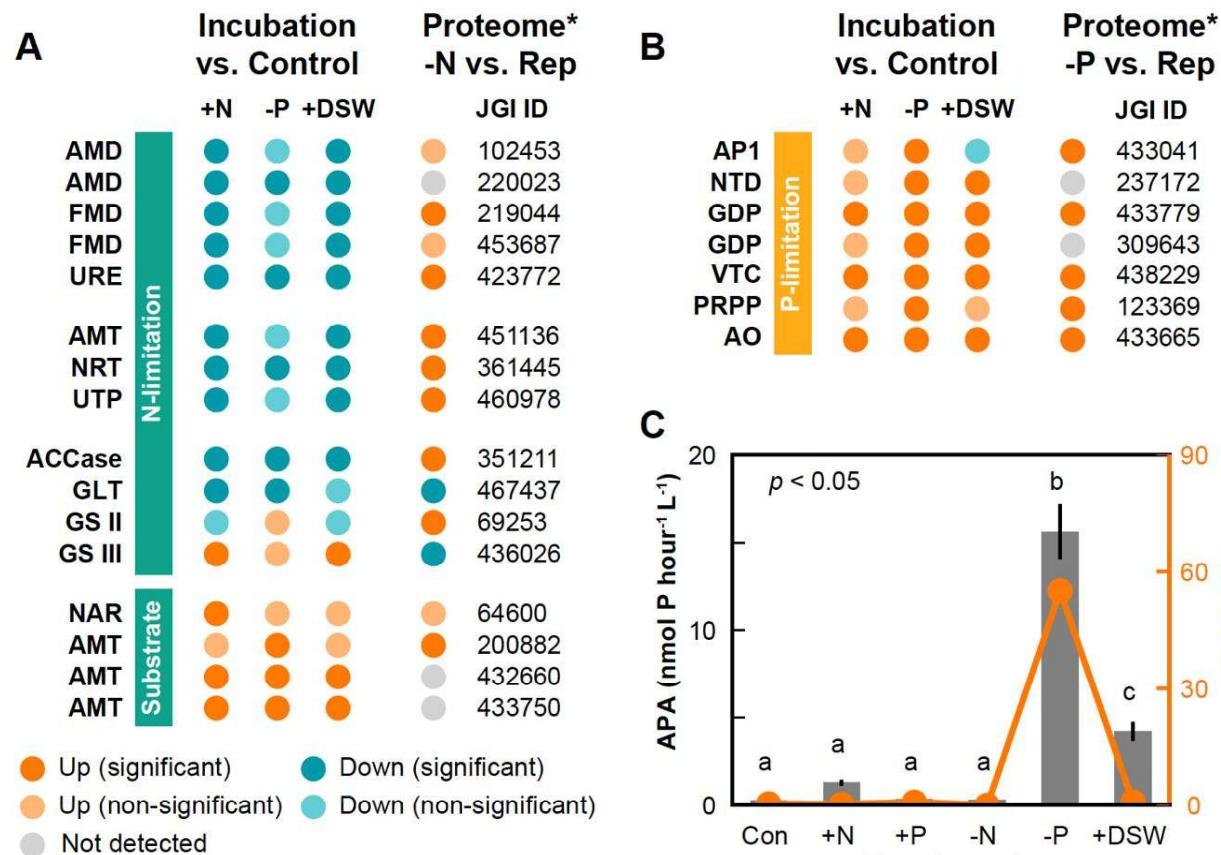
E. huxleyi physiological response - Nitrogen and Phosphorus



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

Alexander et al. (in review)

E. huxleyi physiological response



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

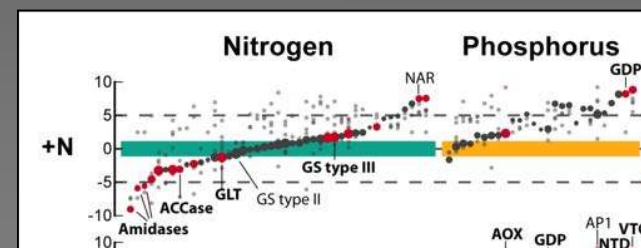
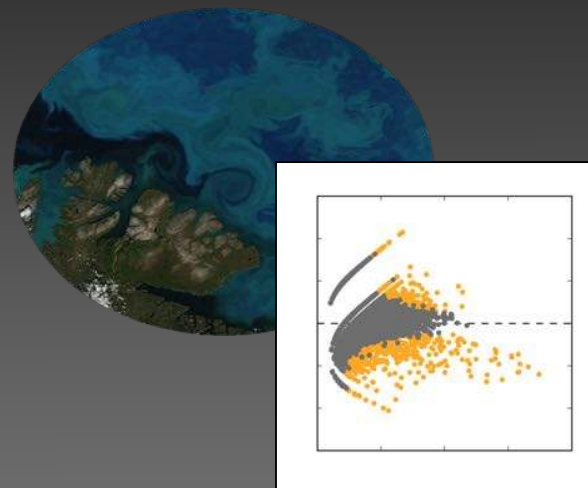
Excellent choreography between N and P related metatranscriptome shifts and changes in proteins and activities.

Alexander et al. (in review)

Summary - Metabolic plasticity and diversity

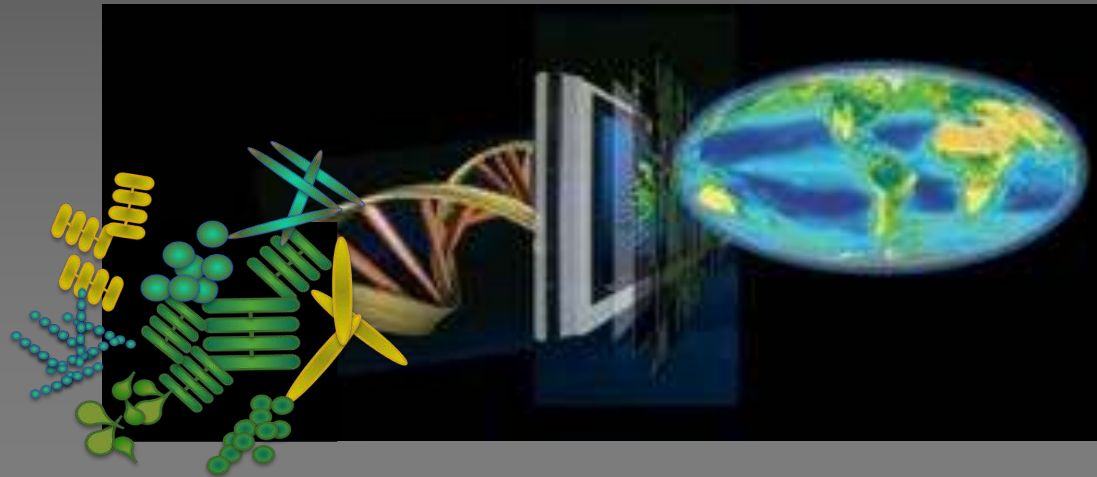
How do diversity and metabolism influence the physiological ecology of a keystone group?

- 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.



Acknowledgements

- Dyhrman Lab: Dr. Rachel Wisniewski Jakuba, Dr. Elizabeth Orchard, Dr. Louie Wurch, Abigail Heithoff, Harriet Alexander, Kyle Frischkorn, Dr. Alena Sevucu, Dr. Julia Diaz, Dr. Monica Rouco Molina, Hannah Joy-Warren, Sheean Haley and many undergraduate interns.
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- NOAA
- DOE, JGI
- WSL
- 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



