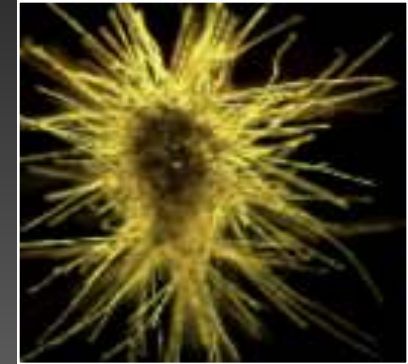


Vignettes

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

Genome - enabled

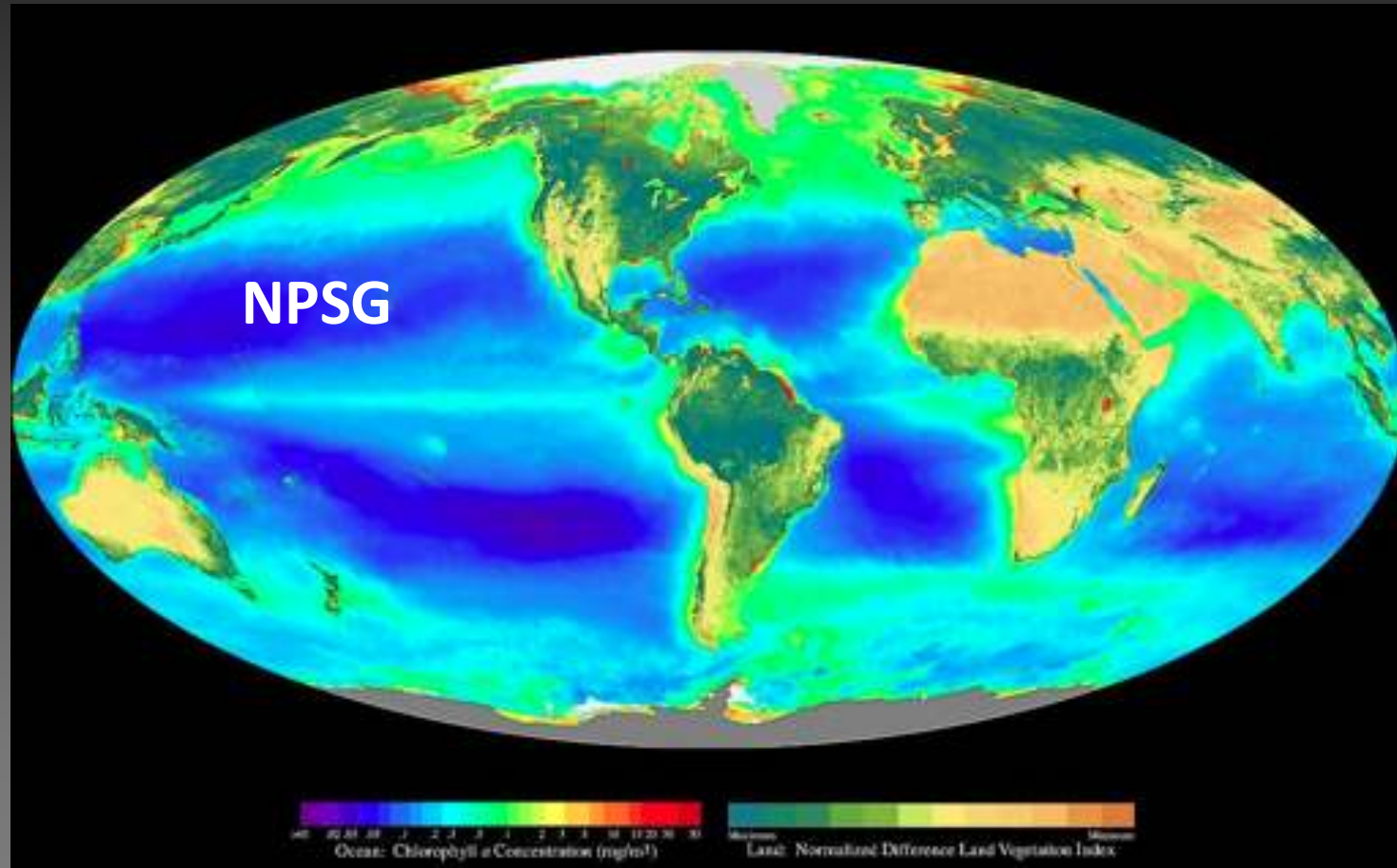


- Co-existing in a sea of competition: Leveraging transcriptome data to track the physiological ecology of phytoplankton from key groups

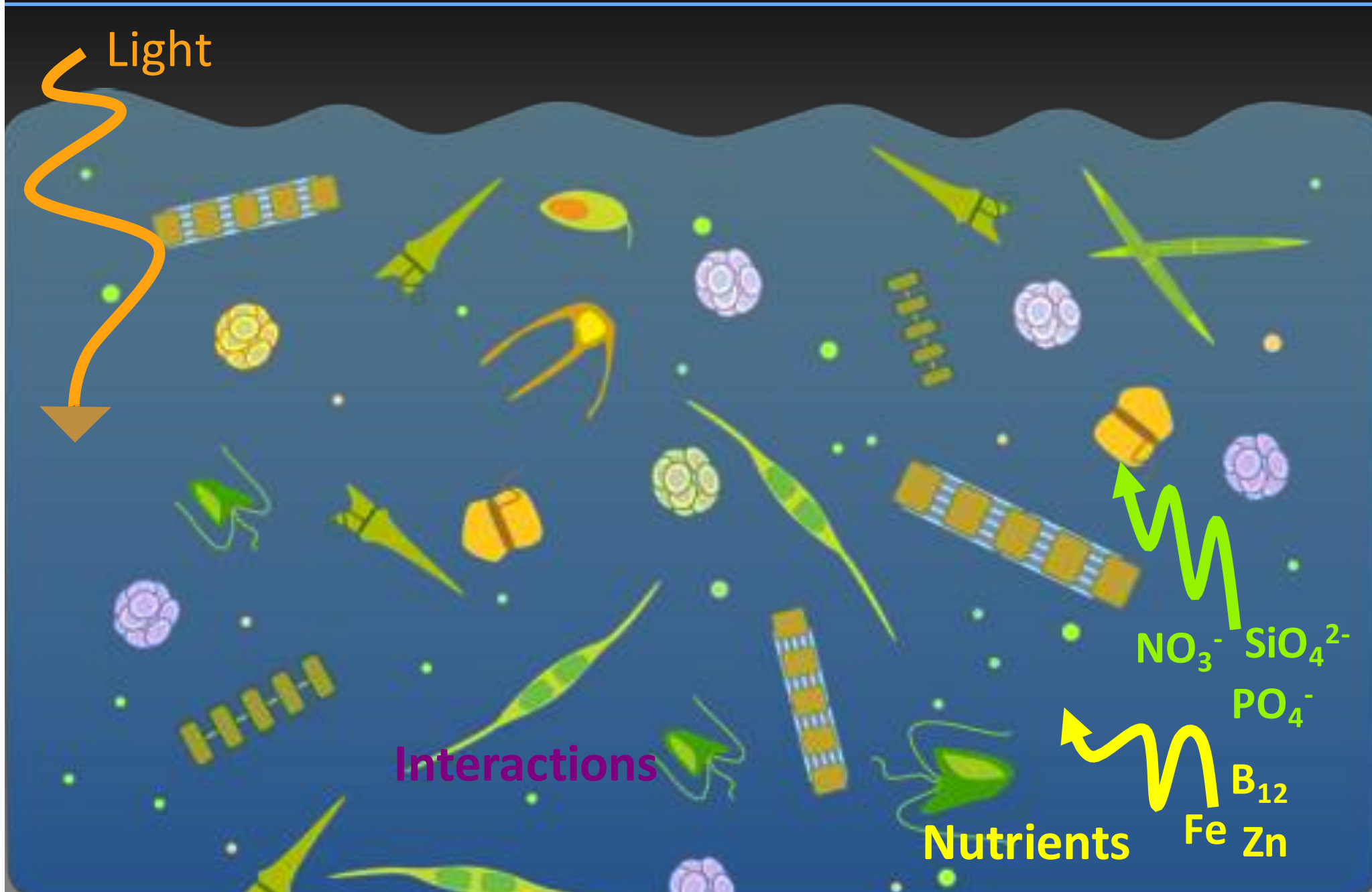
Transcriptome - enabled



North Pacific is one of the largest biomes on the planet



Complex community dynamics driven in part by resources



Tracking phytoplankton physiological ecology

Ecosystem function and biogeochemistry

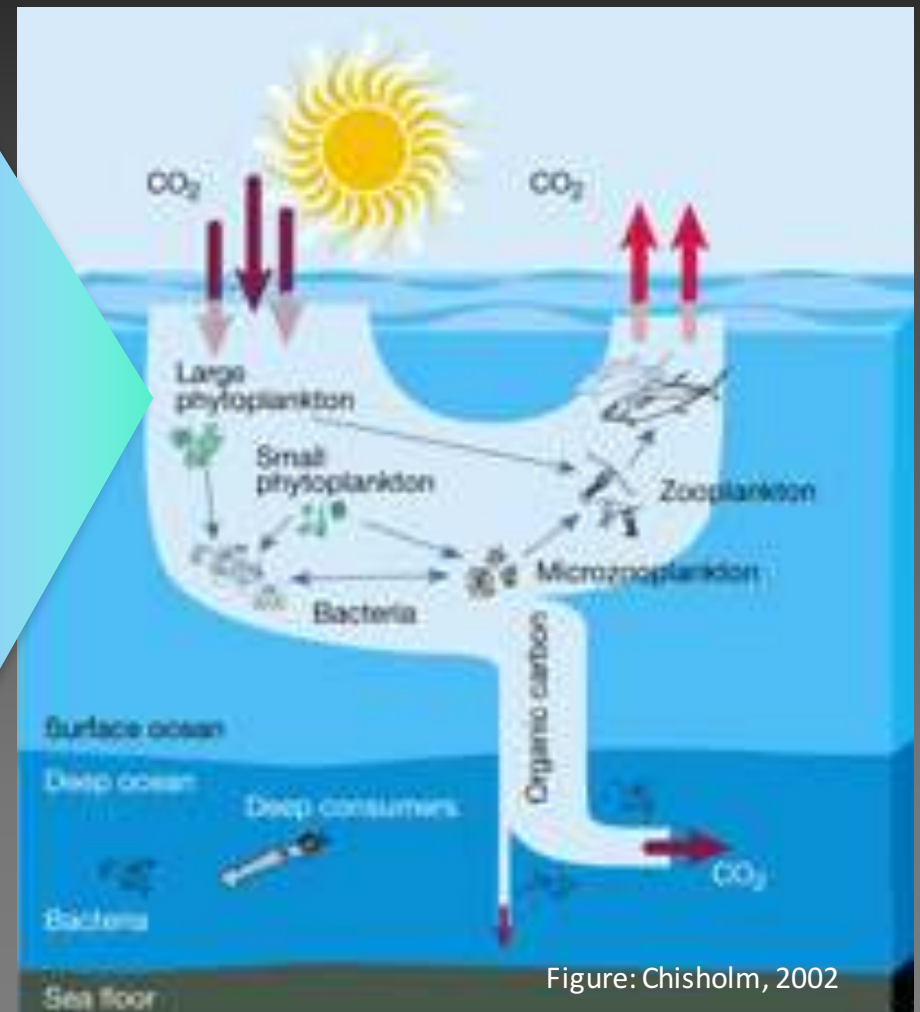
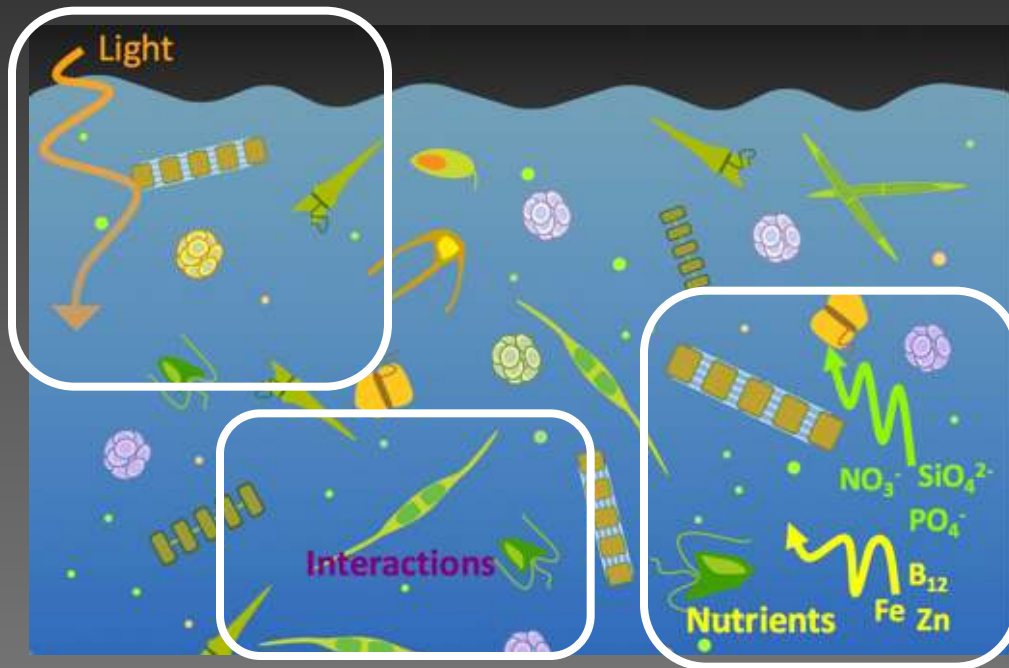


Figure: Chisholm, 2002

How do these factors drive phytoplankton distributions and activities?

Core questions

Nutrient responses

- Proof of concept: How do different groups respond to nutrient pulses?

Light responses

- How does light drive metabolic cascades across phyla?

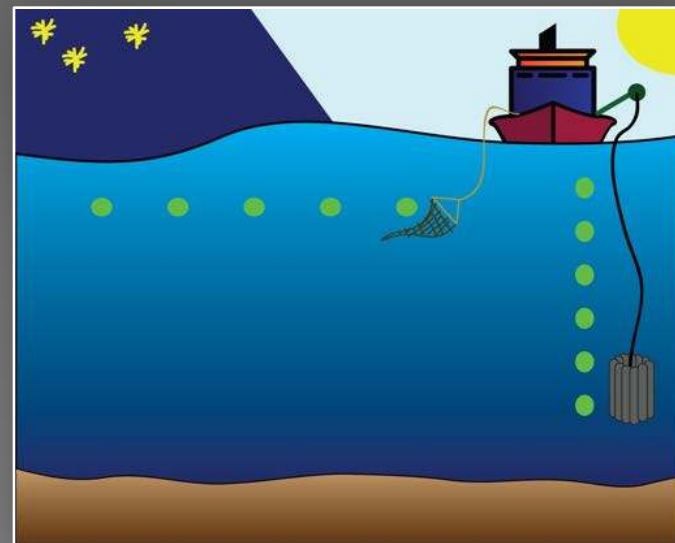
Interactions

- Are there patterns of coordinated expression that underpin host-symbiont physiology?
- How does light drive interactions between the microbiome and host?

HOE:DYLAN



MAHALO



Challenges and opportunities in microbial oceanography

- Long standing challenges:
 - Populations are dilute
 - Few species-specific assays
 - Few genome or transcriptome sequences
 - Meta 'omics approaches for marine eukaryotes are still in their infancy
 - Diverse communities
- Can we get enough RNA?
- How deep do we sequence?
- Can we ID reads with sufficient resolution?

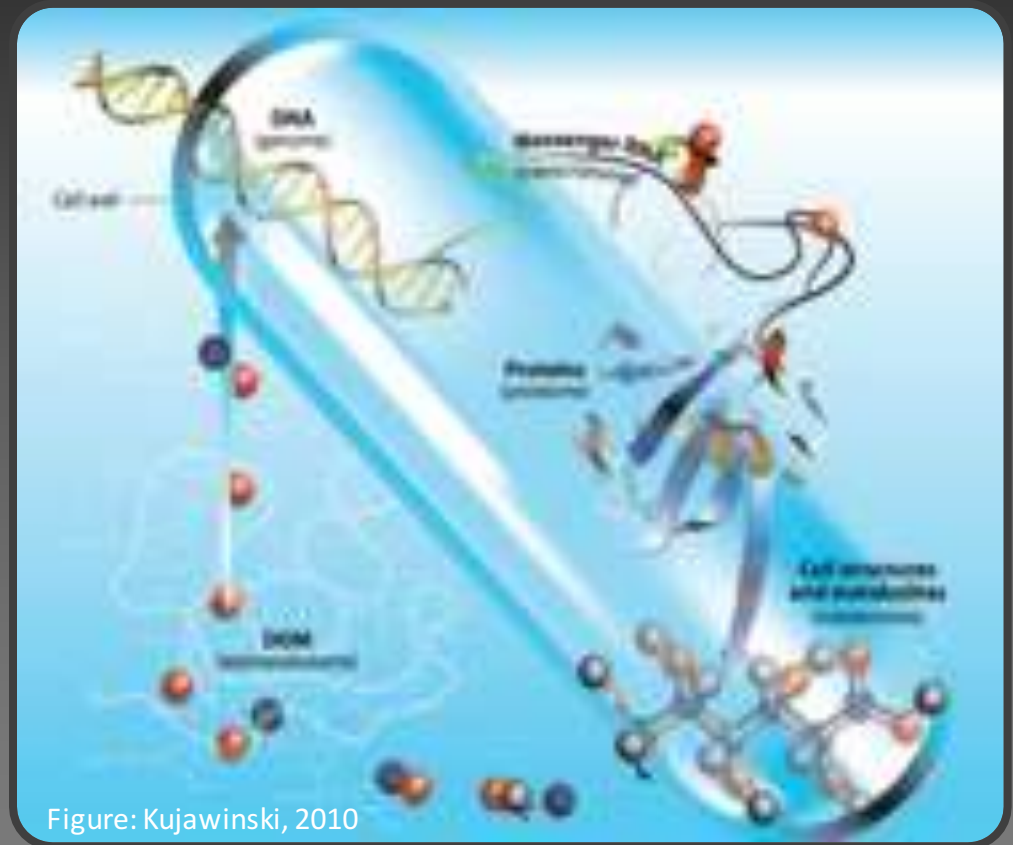


Figure: Kujawinski, 2010

Core questions

Nutrient responses

- Proof of concept: How do different groups respond to nutrient pulses?

Light responses

- How does light drive metabolic cascades across phyla?

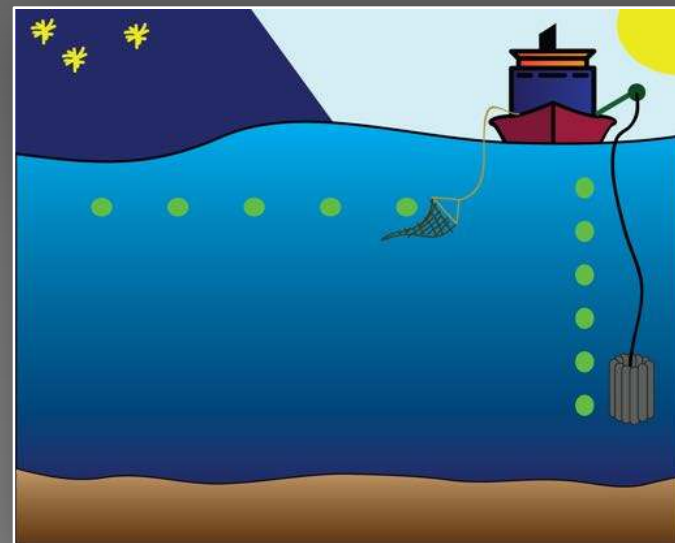
Interactions

- Are there patterns of coordinated expression that underpin host-symbiont physiology?
- How does light drive interactions between the microbiome and host?

HOE:DYLAN

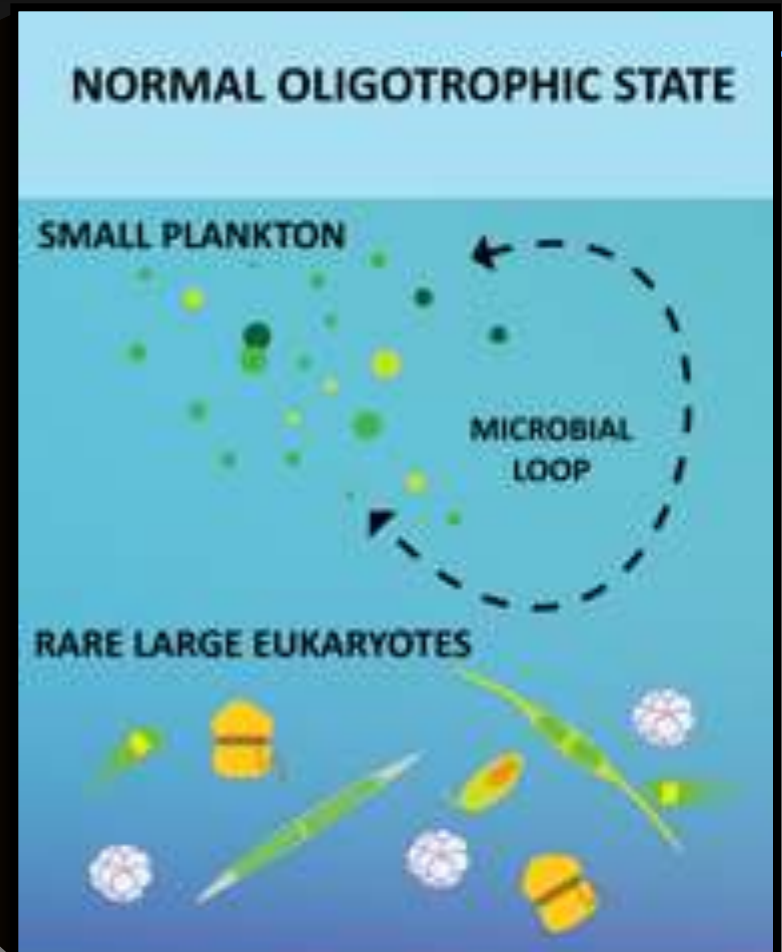
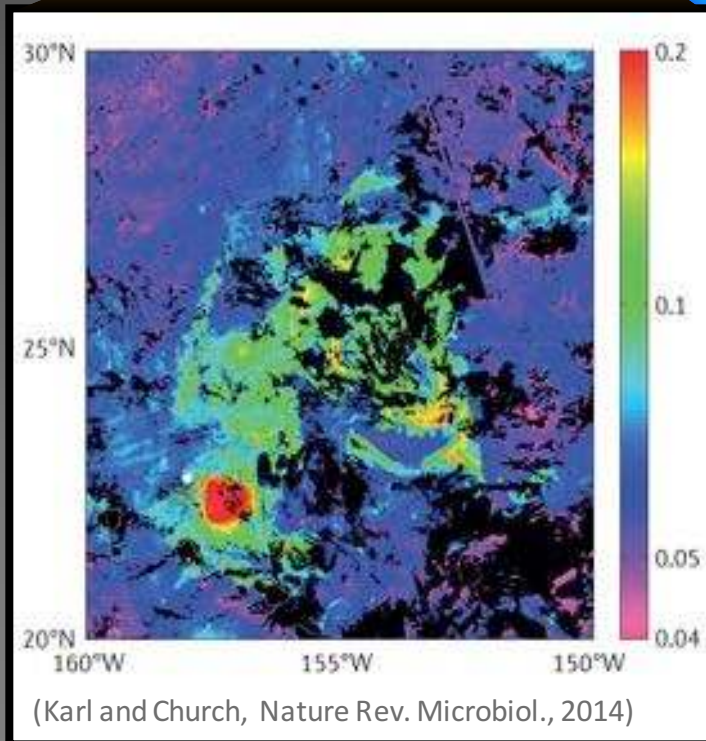


MAHALO

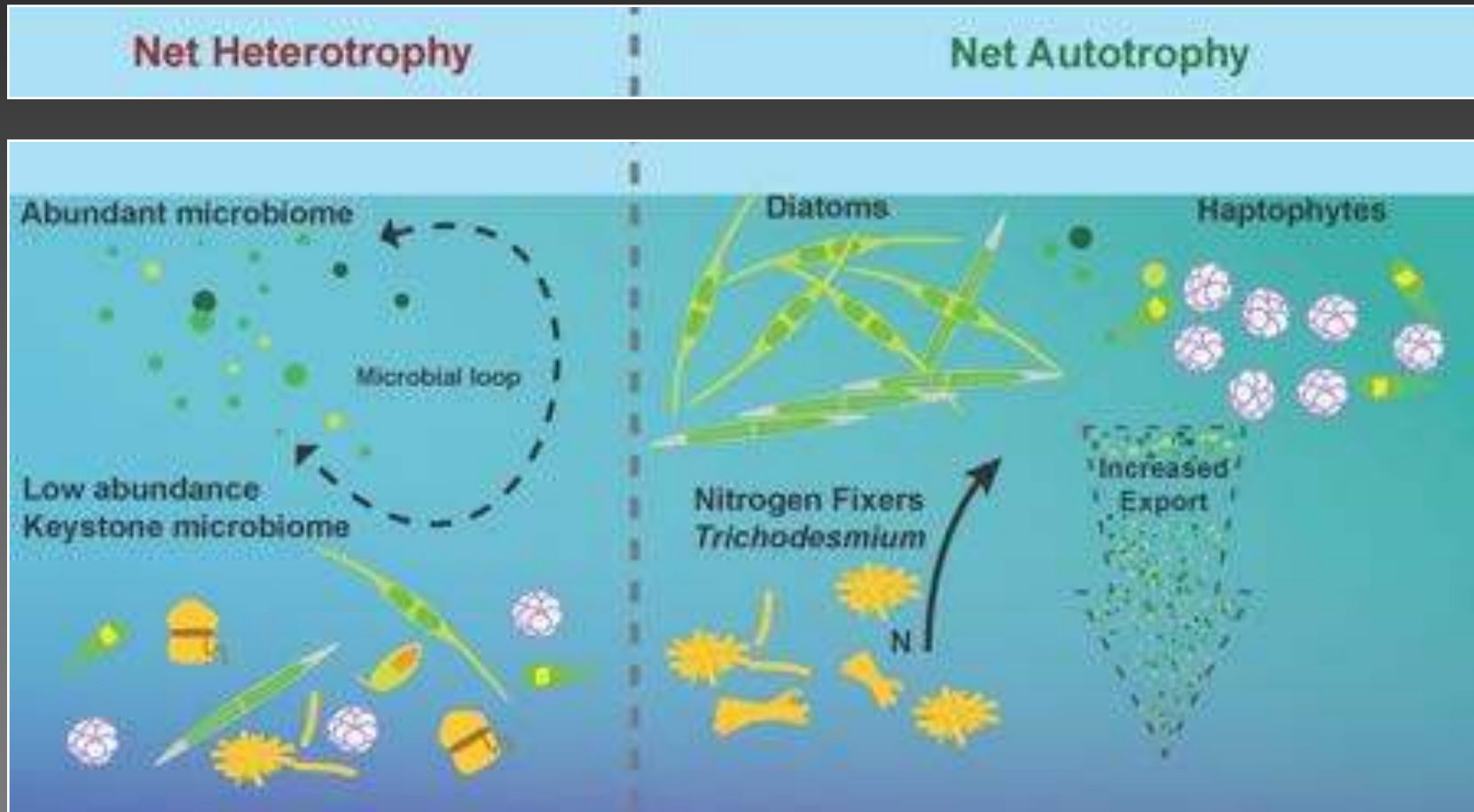


The significance of the NPSG

North Pacific
Subtropical
Gyre



Blooms of keystone species can shift ecosystem state



Nutrient input, or other forcings

Hawaii Ocean Experiment: Dynamics of Light and Nutrients



Global Biogeochemical Cycles

AN AGU JOURNAL

[Explore this journal >](#)

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

Wilson et al. 2015 *GBC*

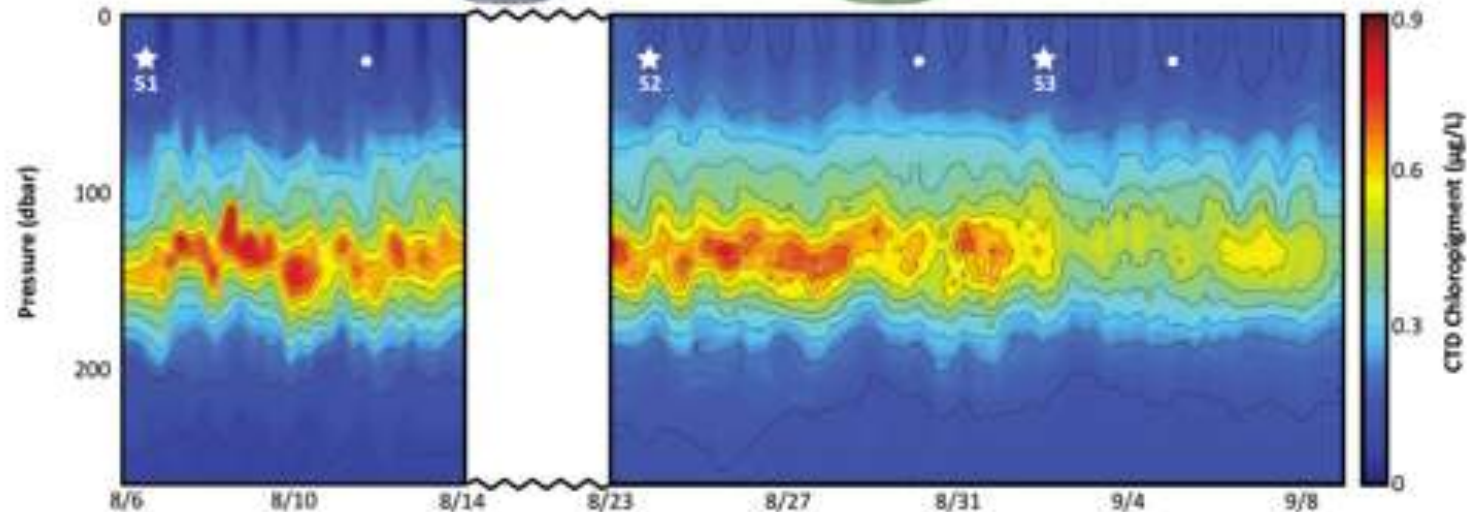
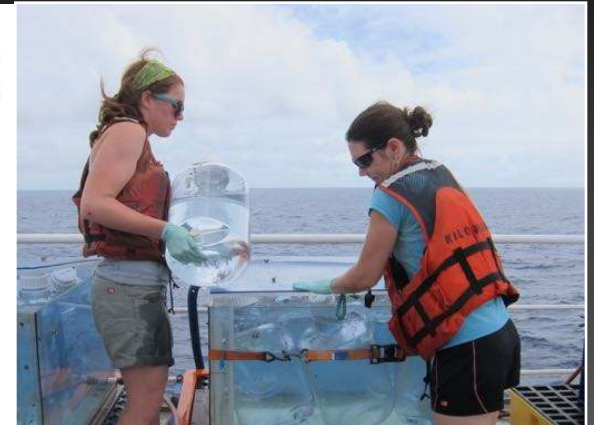


center for microbial oceanography: research and education
CMORE *linking genomes to biomes*

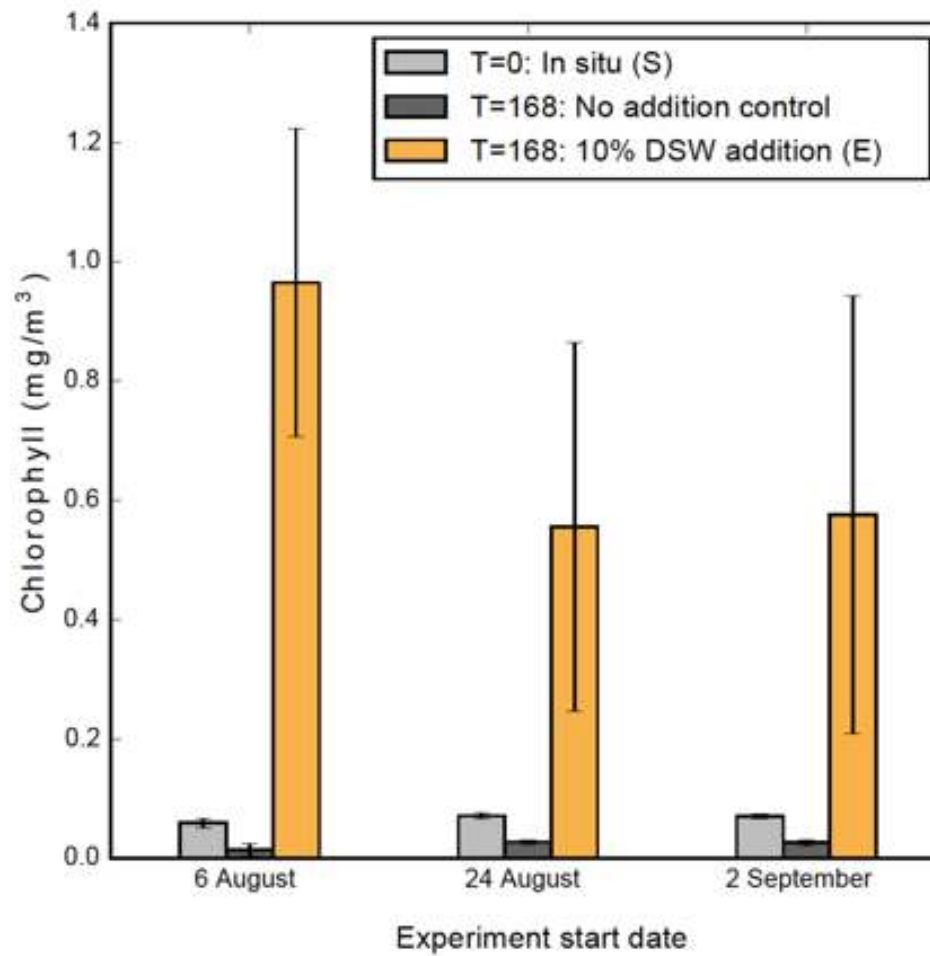
(HOE-DYLAN)

Sampling a bloom

Sampling at Station ALOHA coupled with bloom simulations

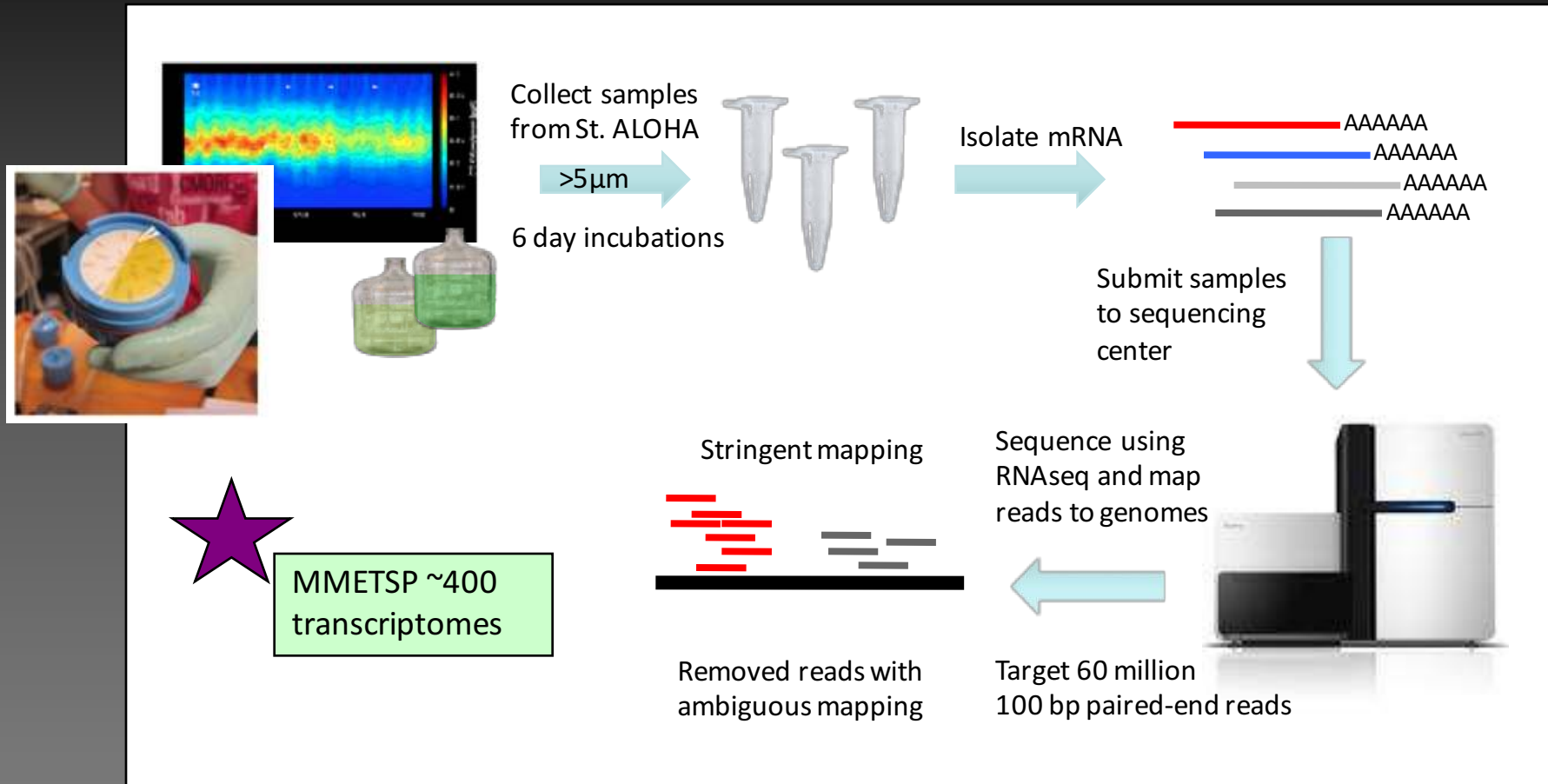


Deep water addition led to simulated “bloom”



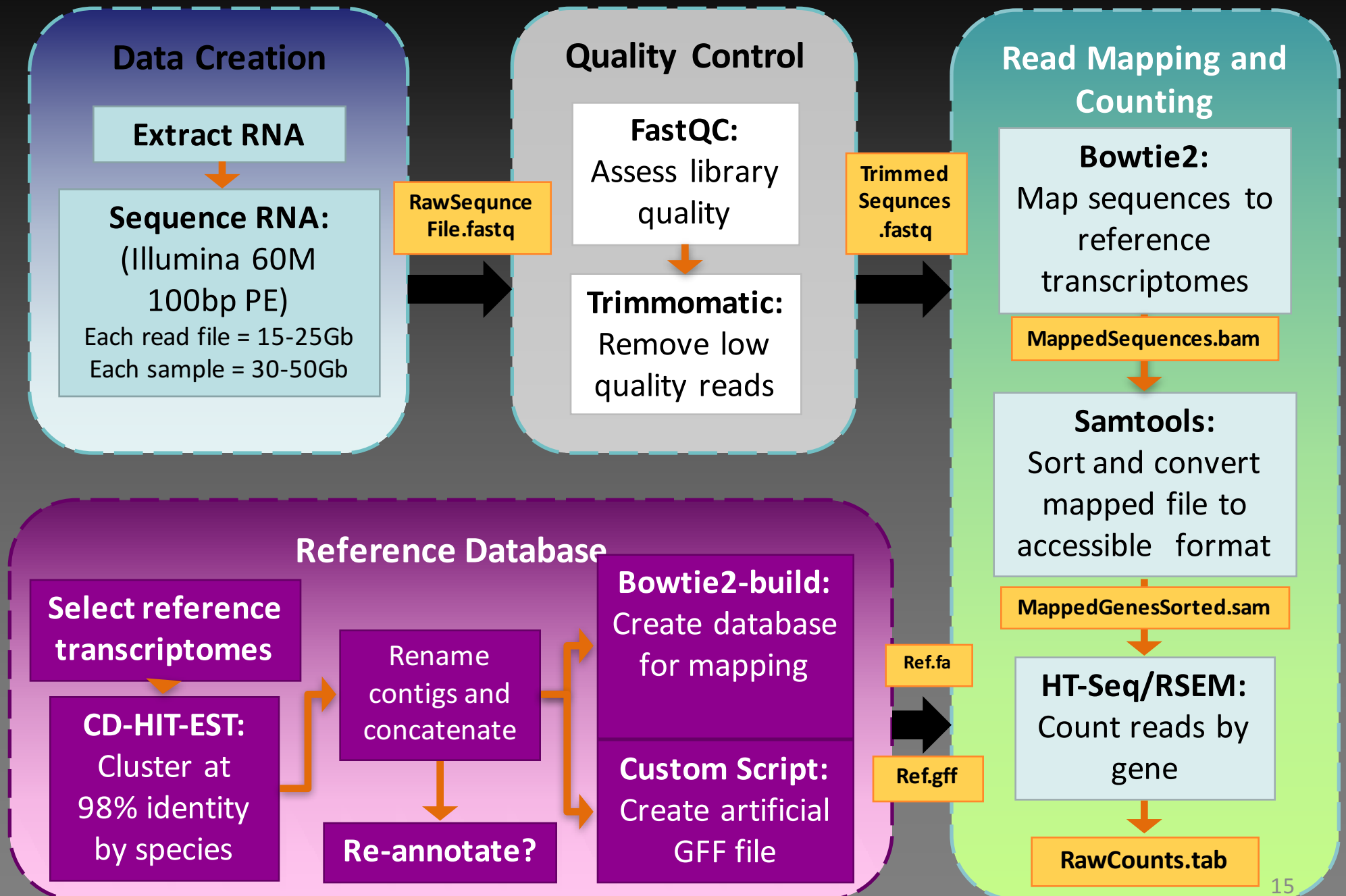


Sampling and pipeline

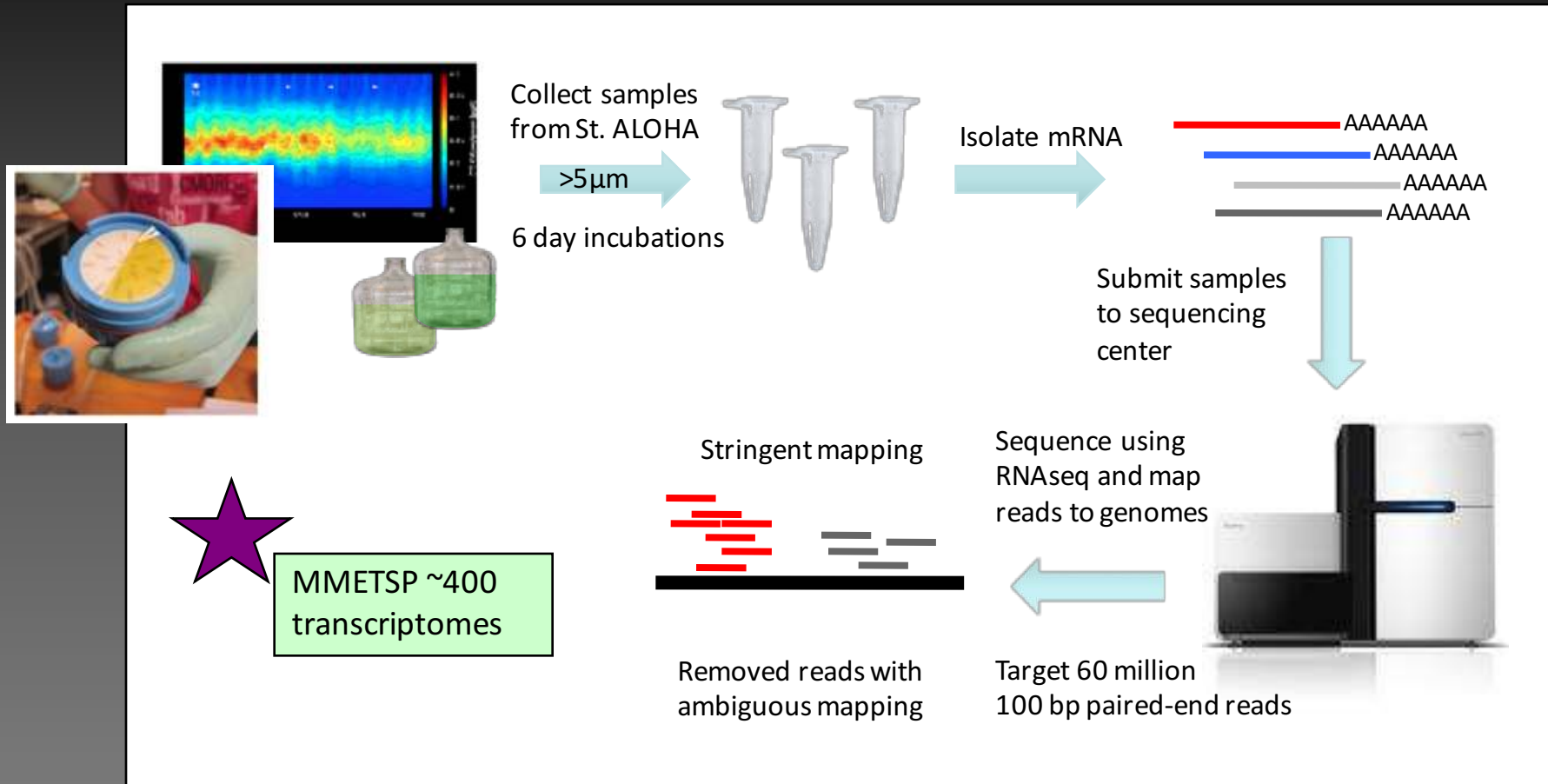


MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Metatranscriptome Data Pipeline - the gory details ...



Sampling and pipeline



MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Marine Microbial Eukaryote Transcriptome Sequencing Project

OPEN ACCESS Freely available online

PLOS BIOLOGY

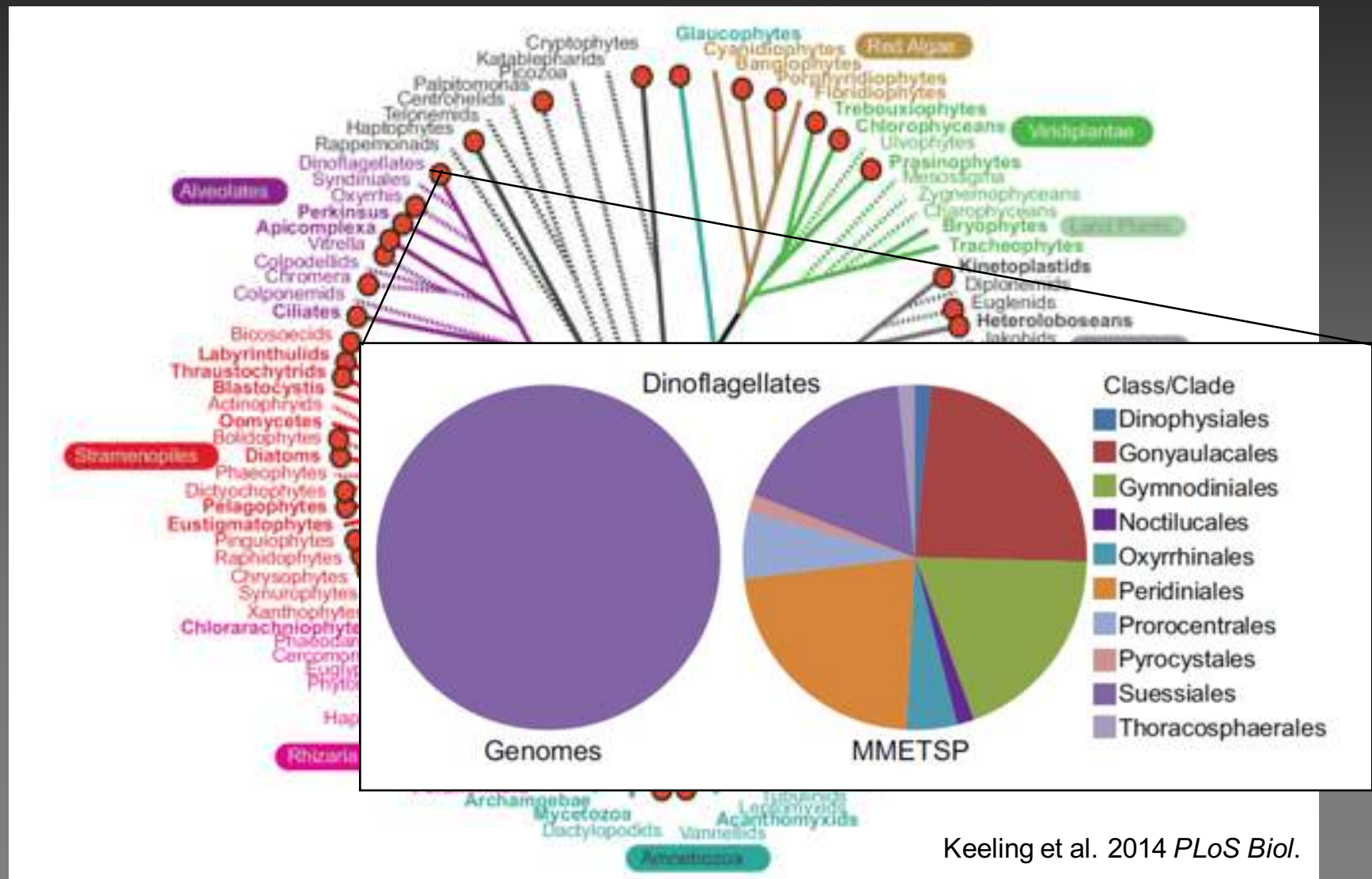
Community Page



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

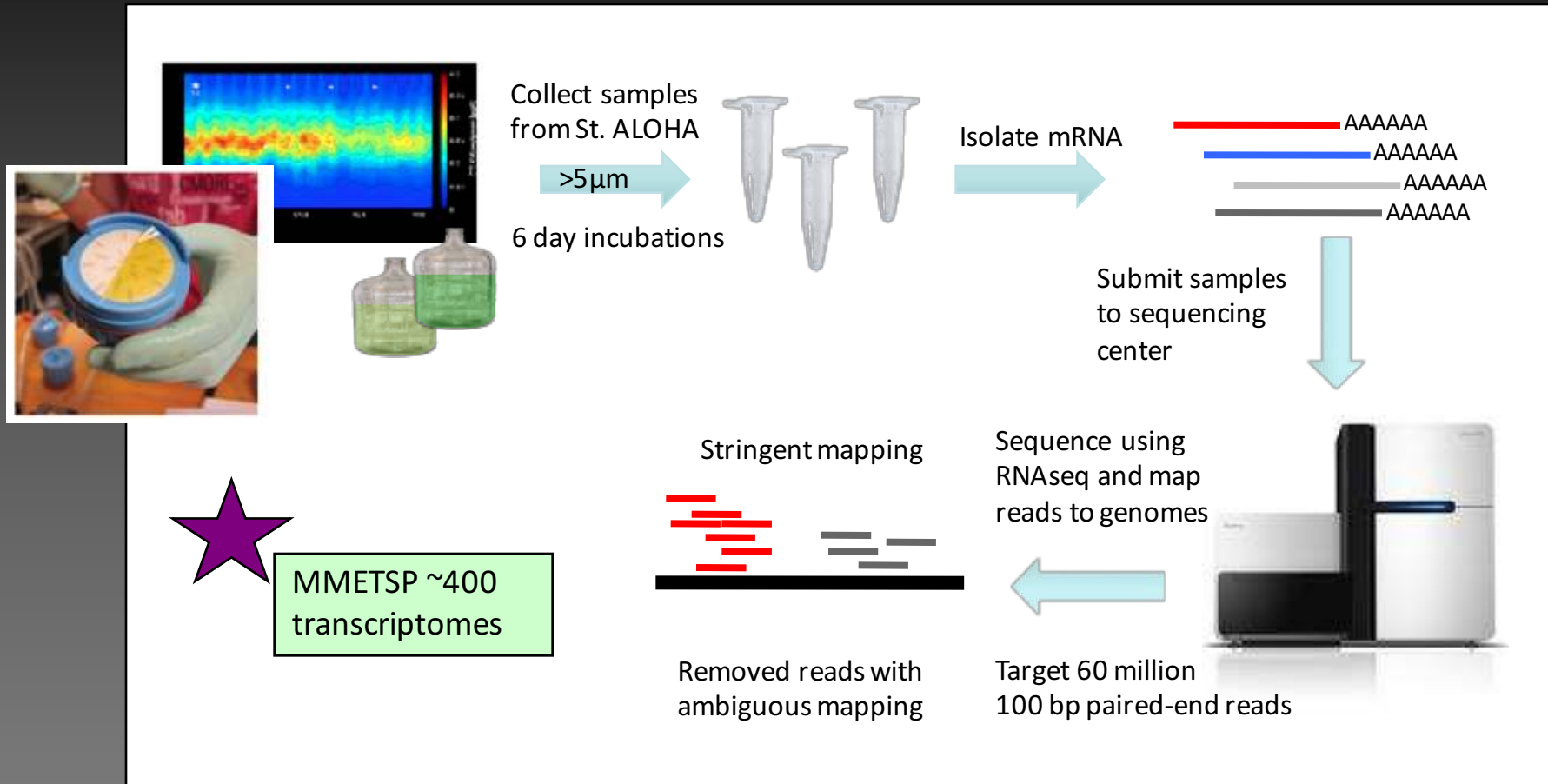
Patrick J. Keeling^{1,2*}, Fabien Burki¹, Heather M. Wilcox³, Bassem Allam⁴, Eric E. Allen⁵, Linda A. Amaral-Zettler^{6,7}, E. Virginia Armbrust⁸, John M. Archibald^{2,9}, Arvind K. Bharti¹⁰, Callum J. Bell¹⁰, Bank Beszteri¹¹, Kay D. Bidle¹², Connor T. Cameron¹⁰, Lisa Campbell¹³, David A. Caron¹⁴, Rose 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. Slamovits^{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.*

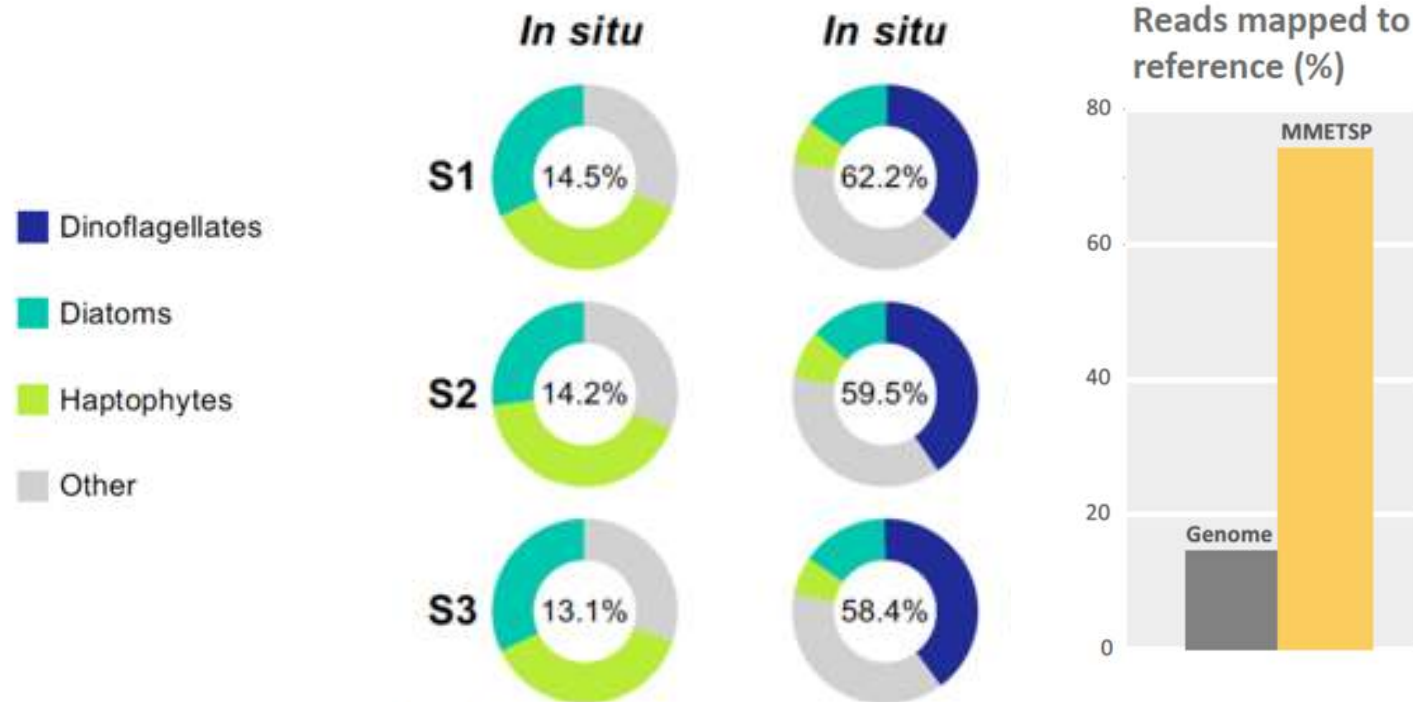
Sampling and pipeline



MMETSP = Marine Microbial Eukaryotic Transcriptome Project

MMETSP improves read identification

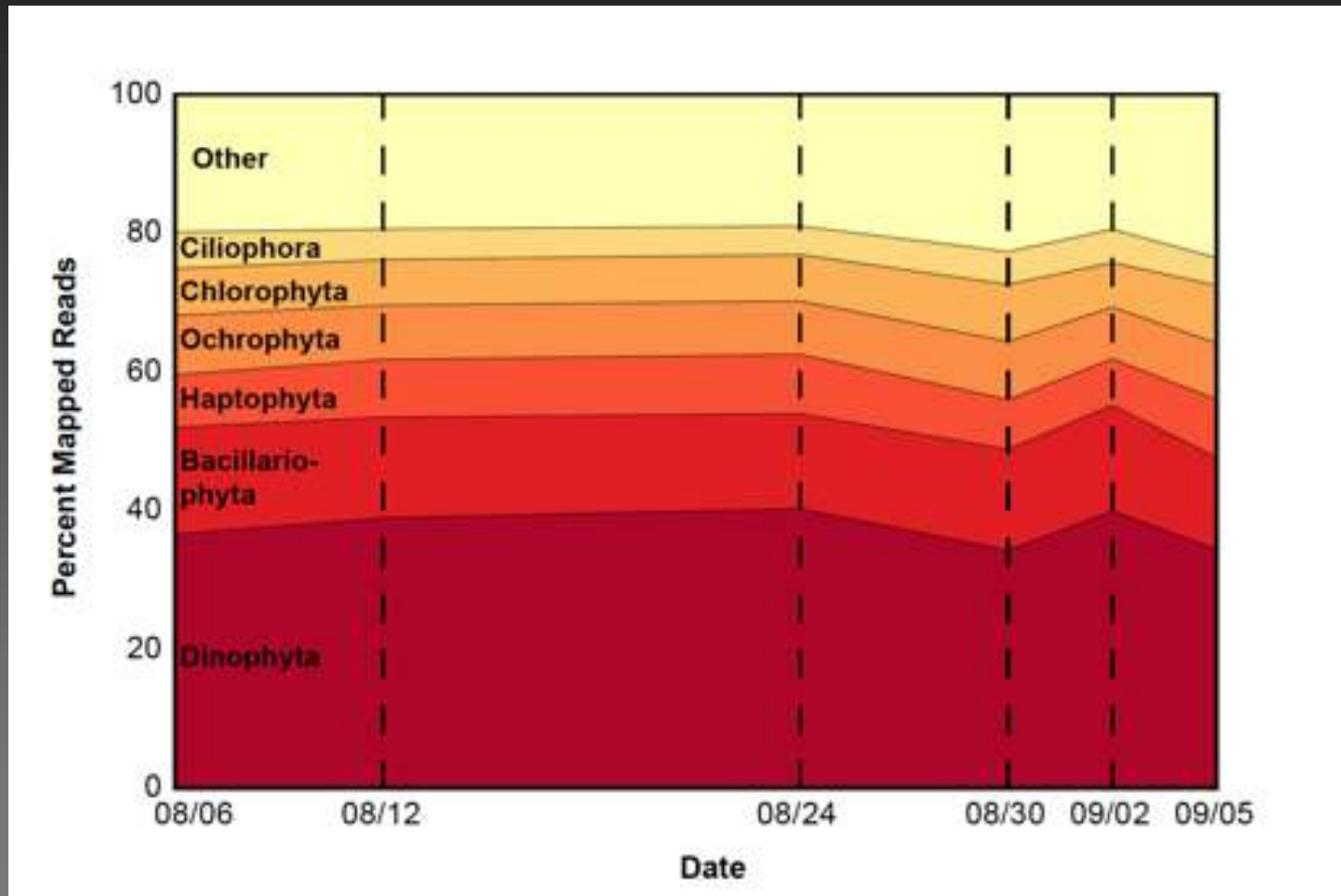
Read mapping against genomes v. MMETSP at St. ALHOA



Alexander et al. 2015b *PNAS*

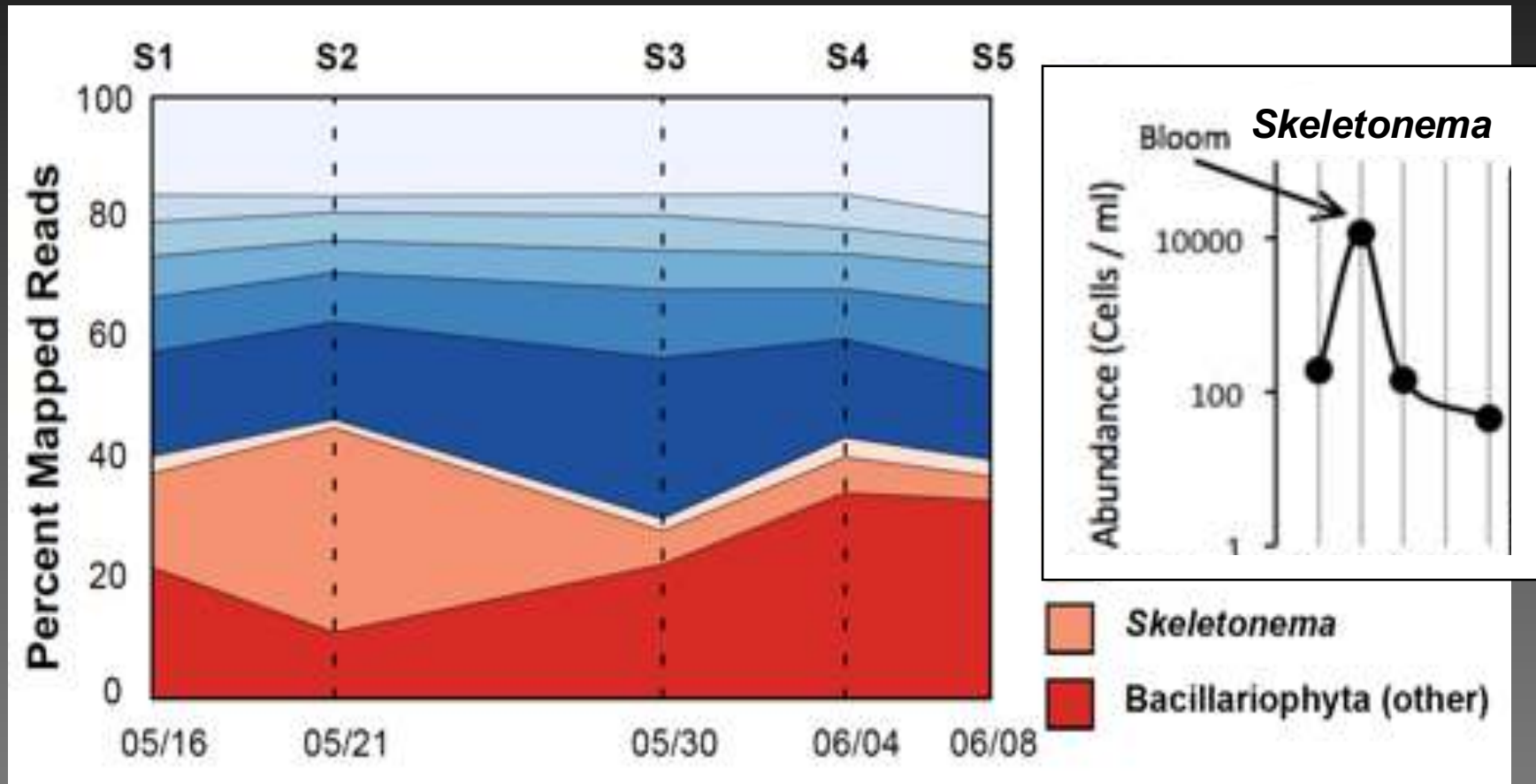
Sequence read identification is substantially improved over using phytoplankton genomes which do not capture the same diversity.

Taxonomic distribution of reads



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

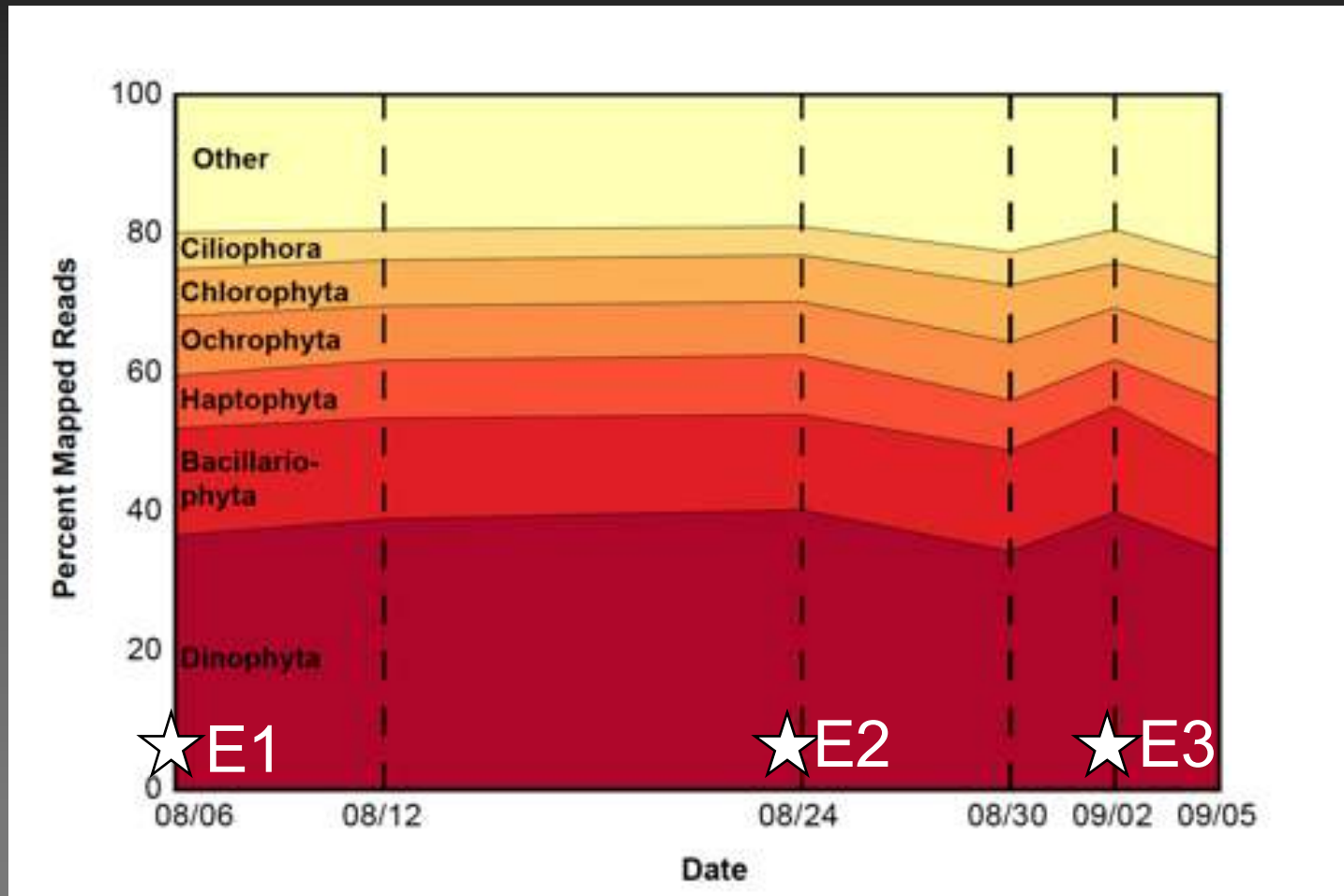
Taxonomic distribution of reads - coastal bloom



Alexander et al. 2015 *PNAS*

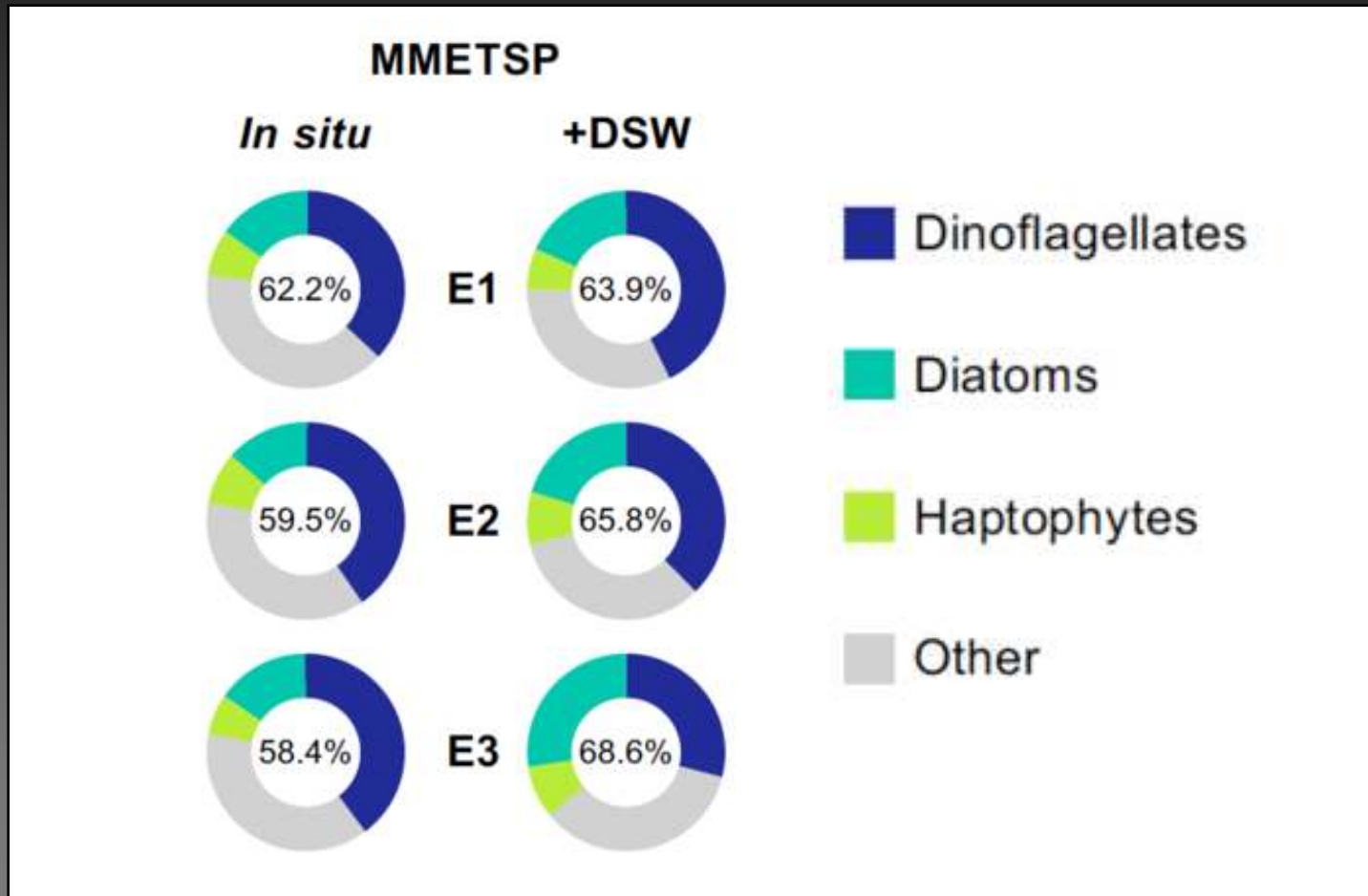
Taxonomic distribution of reads is more variable in a coastal system

Taxonomic distribution of reads



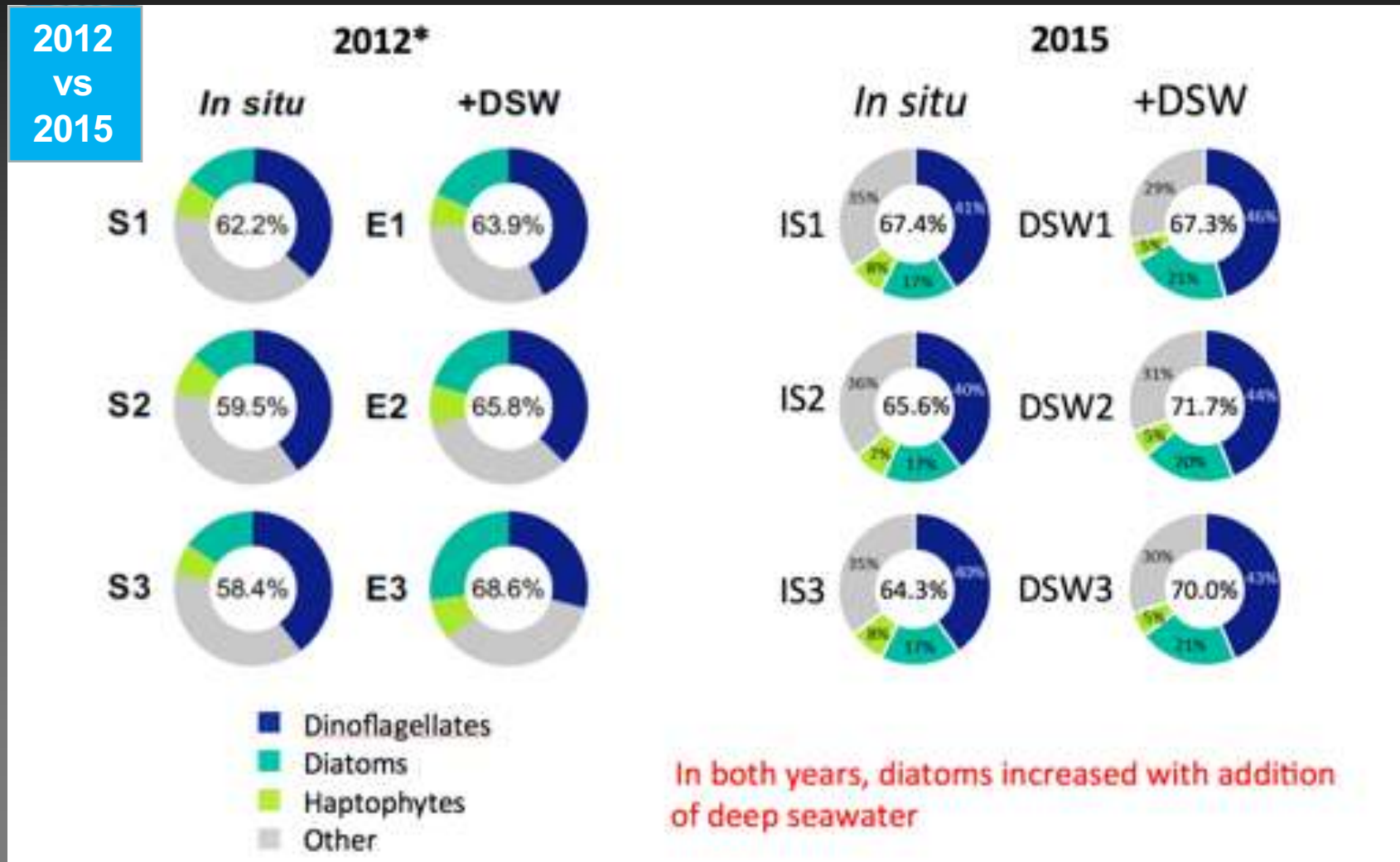
Alexander et al. 2015b *PNAS*

Increase in diatoms during simulated blooms



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

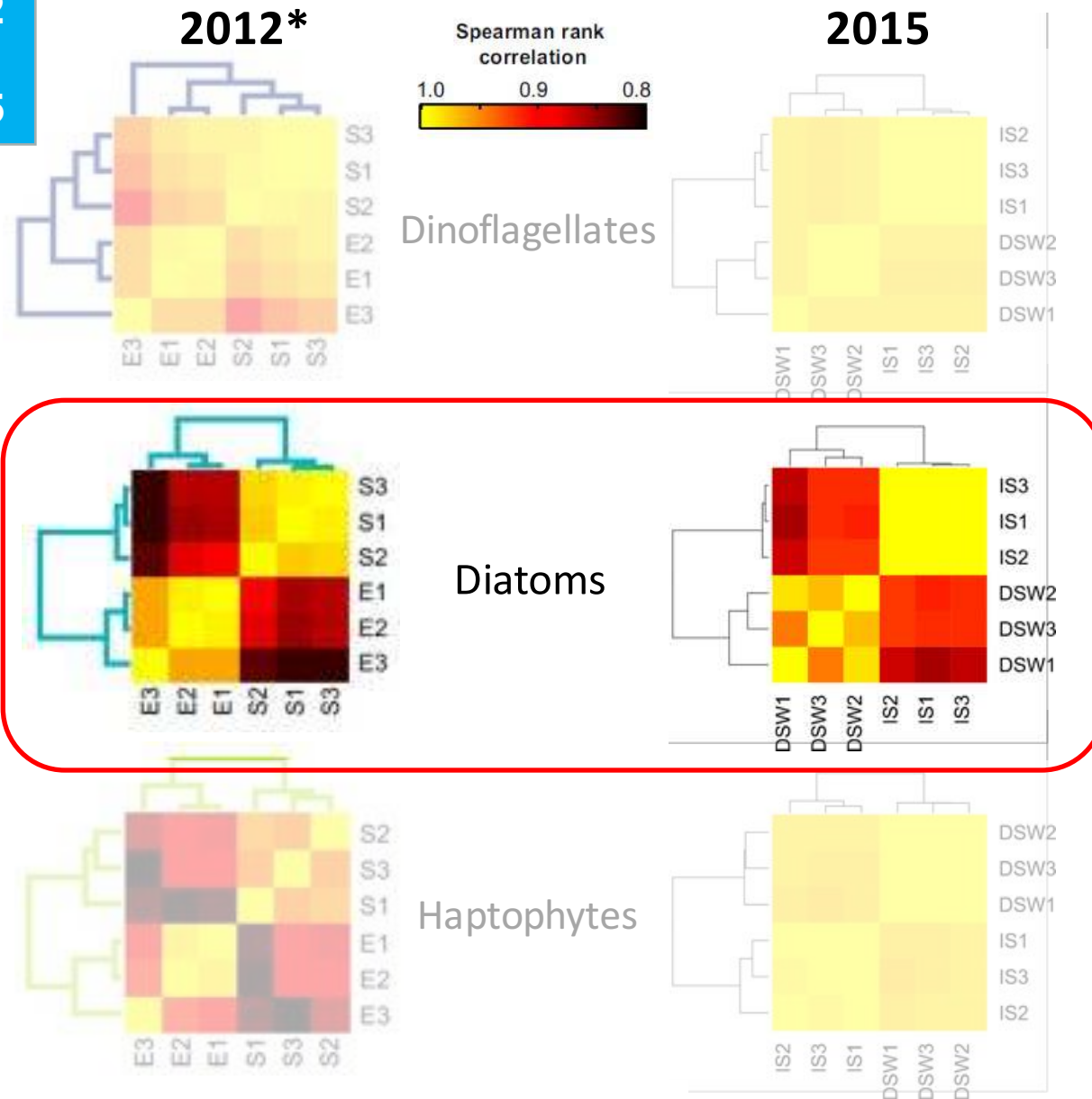
Simulated bloom response was reproducible 3 years later



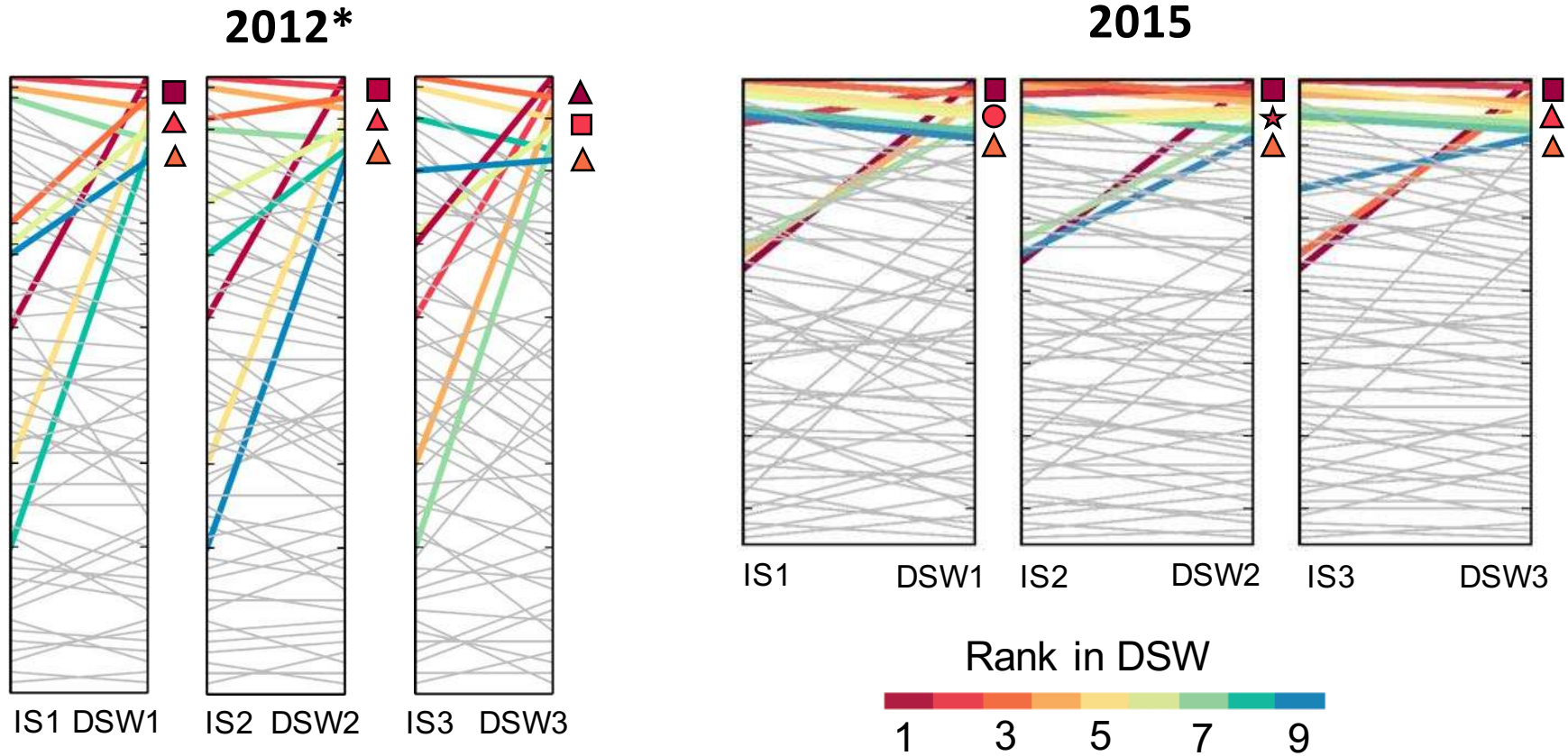
Read identification increases with larger database, remarkable similarity in response between years

Community composition shifts are reproducible between years

2012
vs
2015



Its even the same species changing...



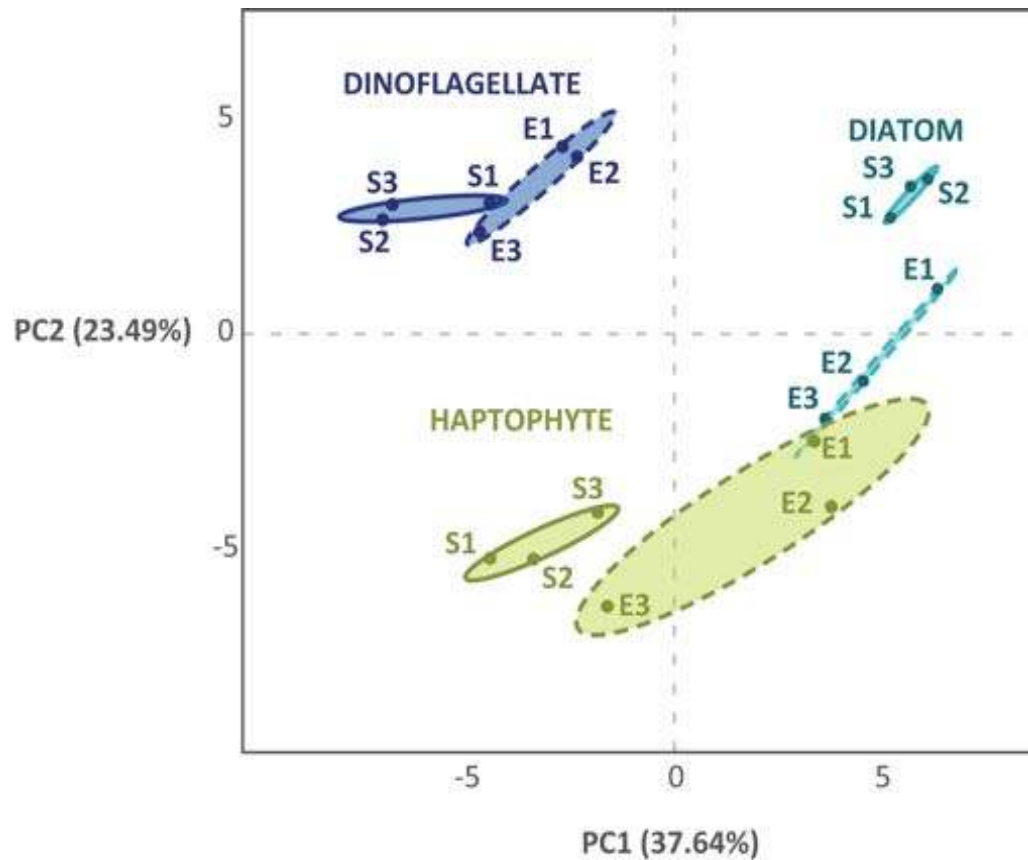
- *Cylindrotheca* sp.
- △ *Pseudo-nitzschia* sp.
- *Chaetoceros* sp.
- ☆ *Ditylum* sp.

2012
vs
2015

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

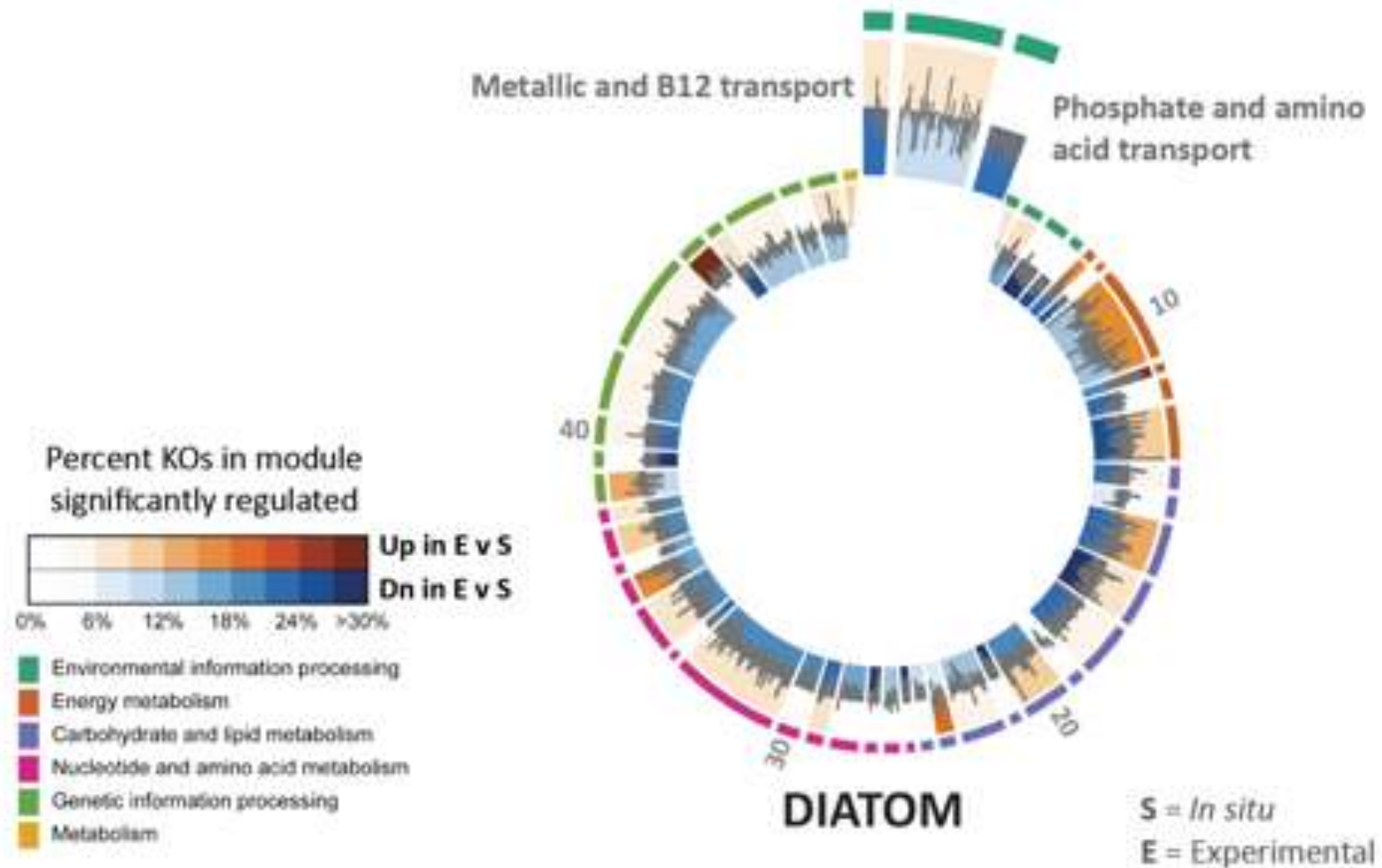
Patterns of metabolism

Alexander et al. 2015b PNAS

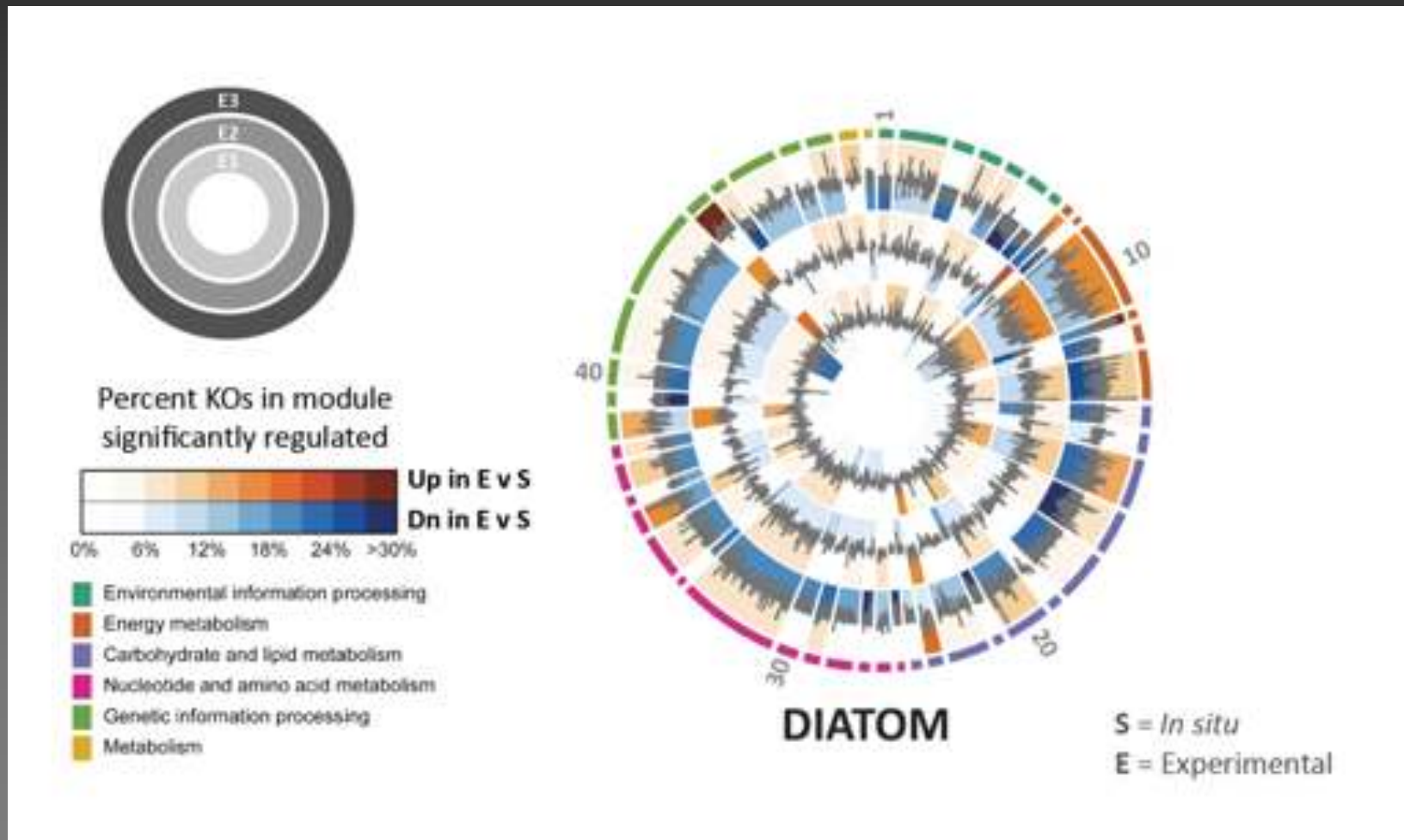


Species are distinct from each other with 95% confidence, and all but dinoflagellates significantly shift in the simulated blooms

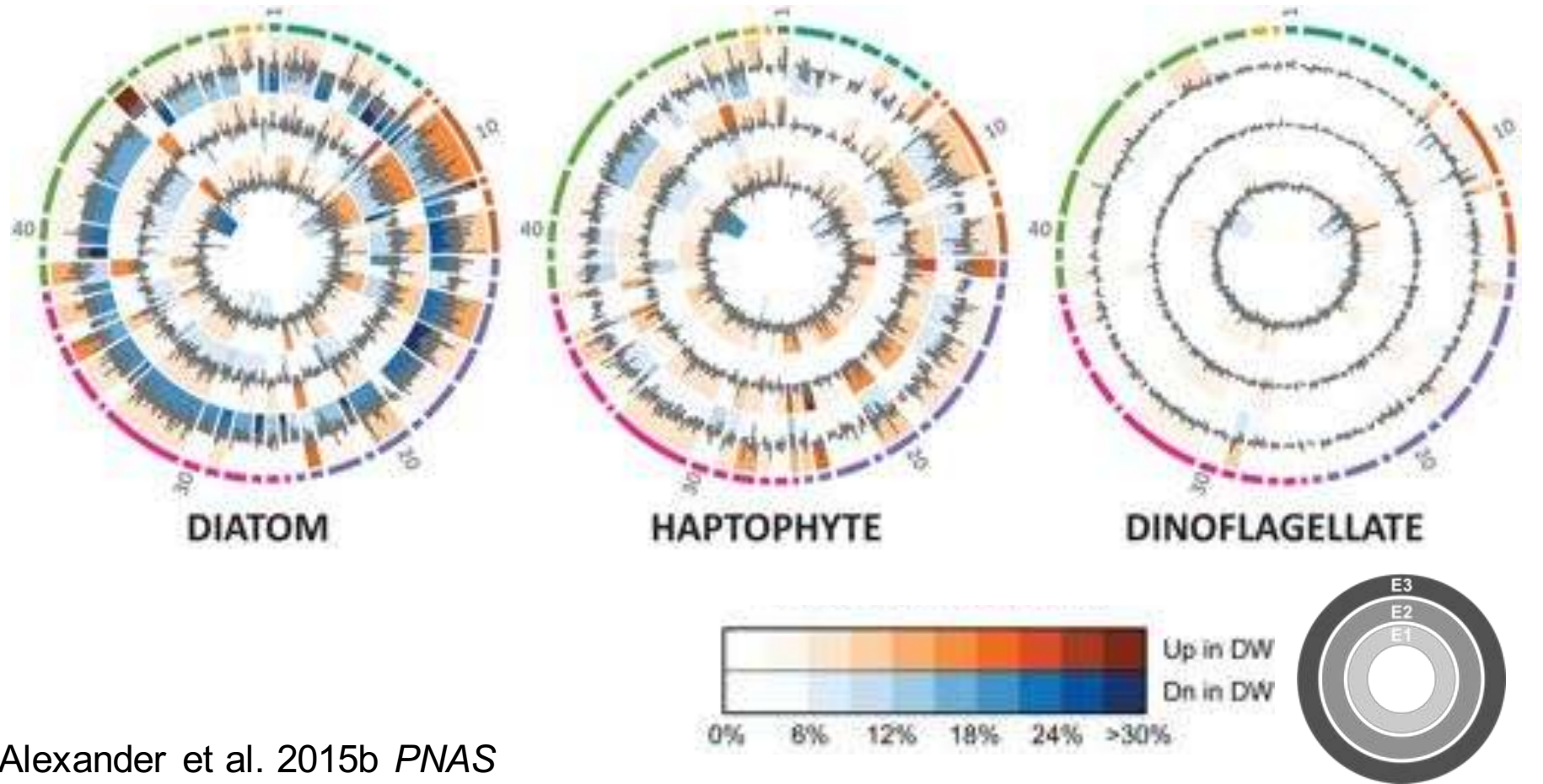
Transcriptional shifts in diatoms



Transcriptional shifts in diatoms



Transcriptional response during simulated blooms

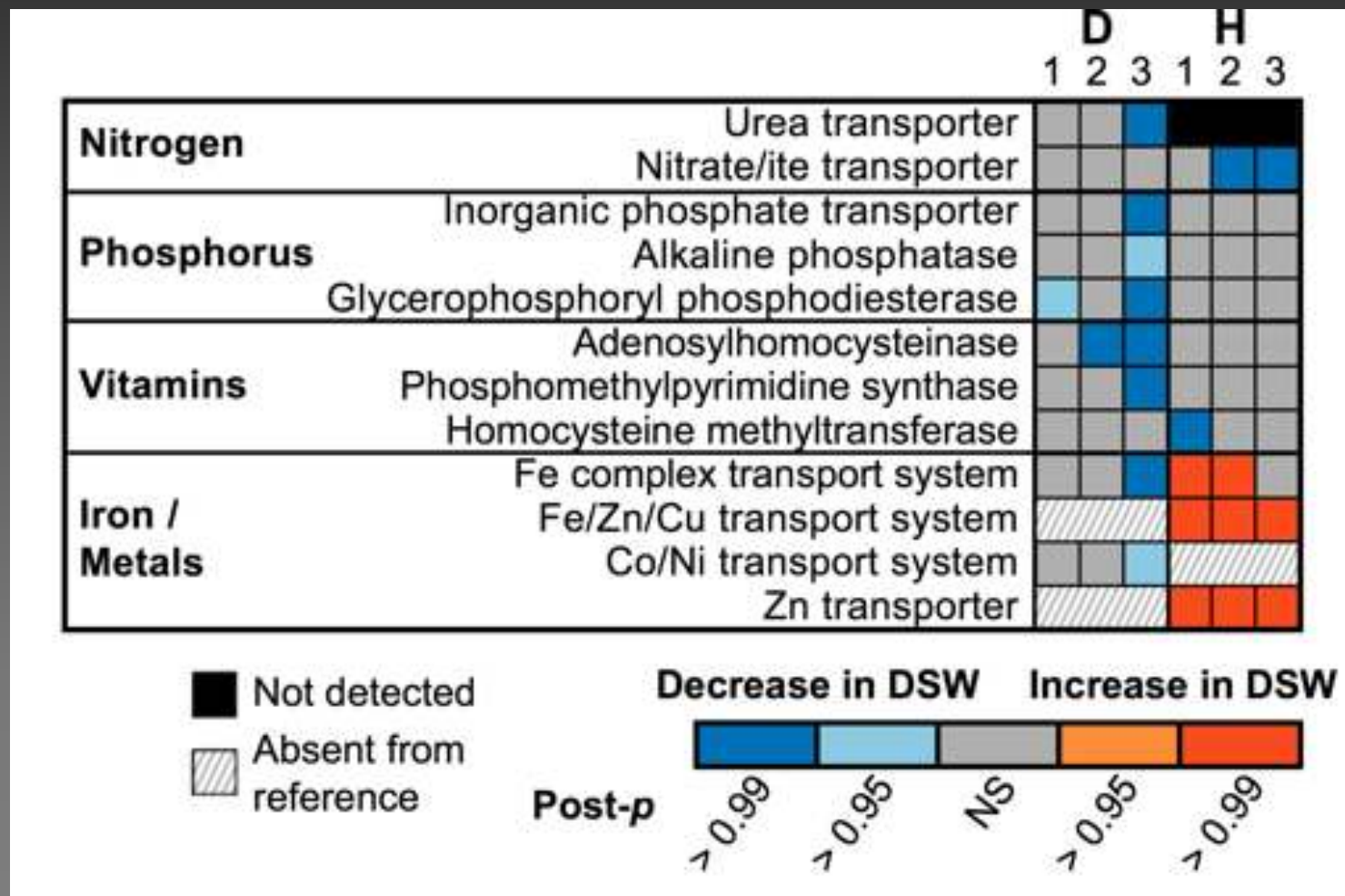


Alexander et al. 2015b *PNAS*

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

Diatoms appear to be released from nutrient limitation

Fold change of genes related to resource acquisition



Transcriptional responses underscore functional group traits

Dinoflagellates



FEW significant shifts



Alternative trophic modes?

Diatoms



BROAD transcript decreases reflecting release from limitation

TARGETED transcript increase related to growth



“the scavengers”
r-selected

Haptophytes



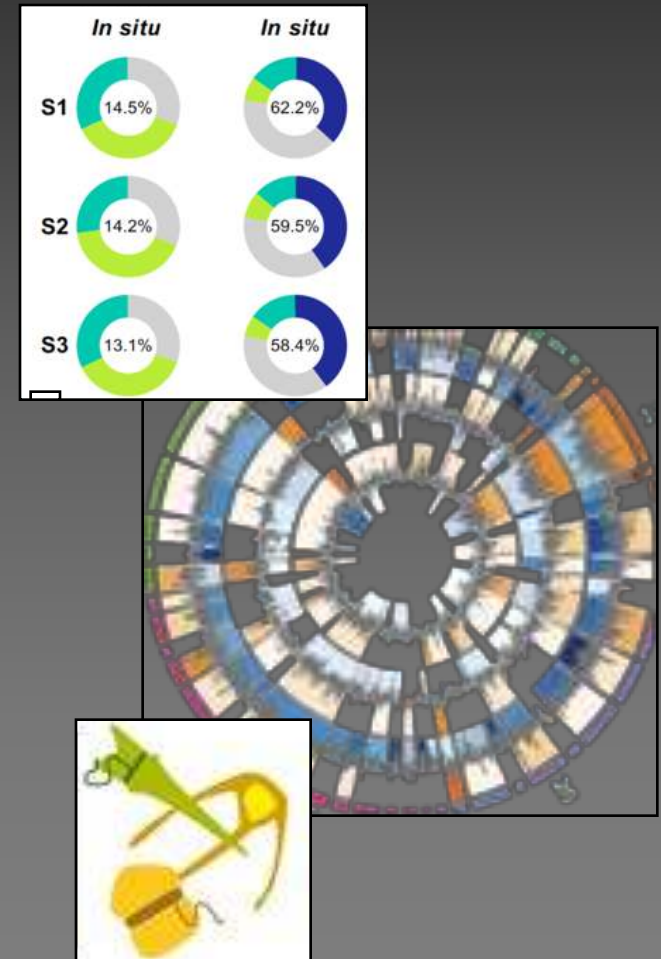
MUTED increase and decreases



“the survivalists”
K-selected

Summary – Nutrient responses

- **Transcriptomes:** The better the reference database (MMETSP) the better resolution
- **Metatranscriptomes:** Phytoplankton groups appear to be limited by resource availability in the NPSG with remarkable year to year consistency
- **Metatranscriptomes:** Dinoflagellates have little transcriptional response to nutrient input - will need other methods
- Diatoms are particularly responsive nutrient input which likely underpins their dominance in blooms



Core questions

Nutrient responses

- Proof of concept: How do different groups respond to nutrient pulses?

Light responses

- How does light drive metabolic cascades across phyla?

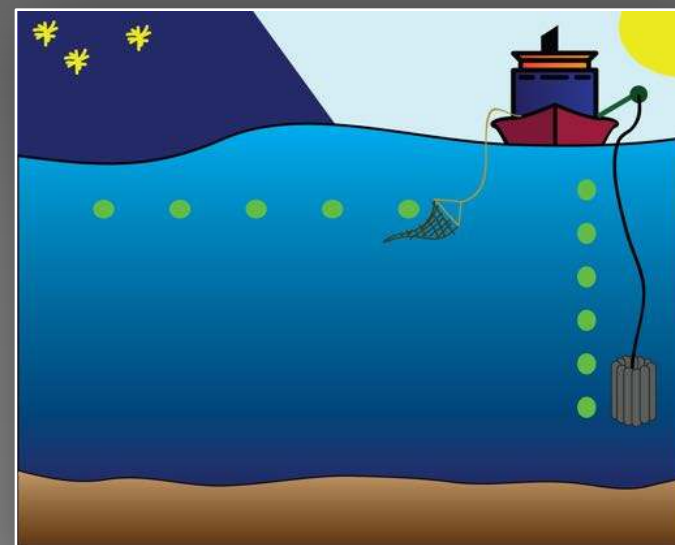
Interactions

- Are there patterns of coordinated expression that underpin host-symbiont physiology?
- How does light drive interactions between the microbiome and host?

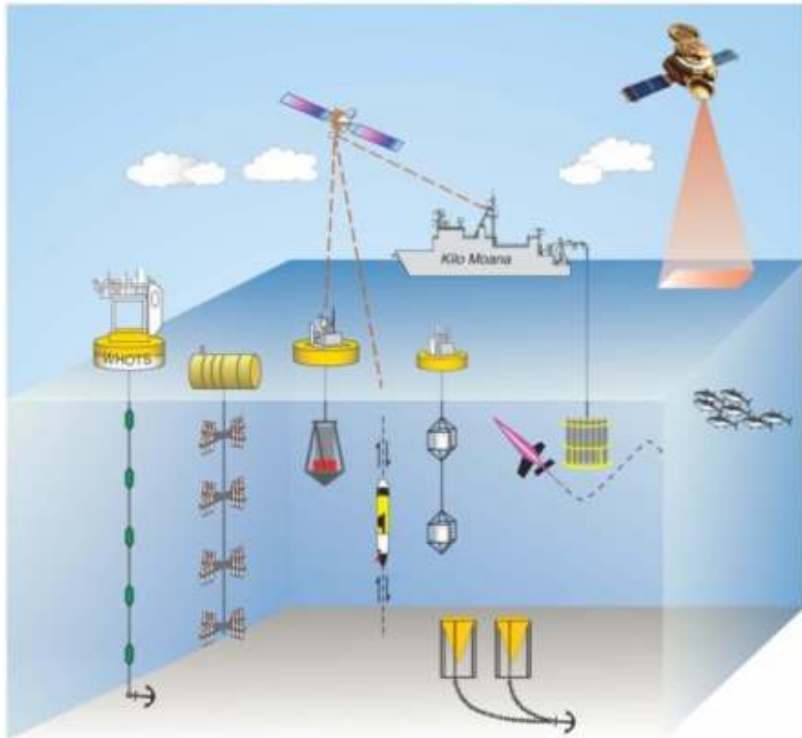
HOE:DYLAN



MAHALO



Hawaii Ocean Experiment: Dynamics of Light (MAHALO)



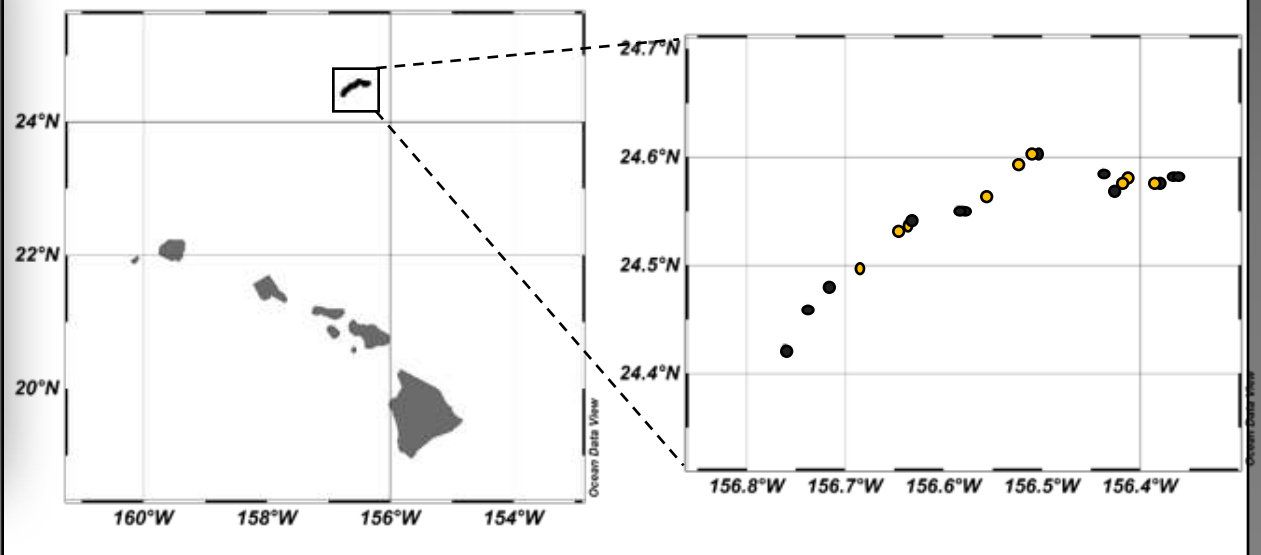
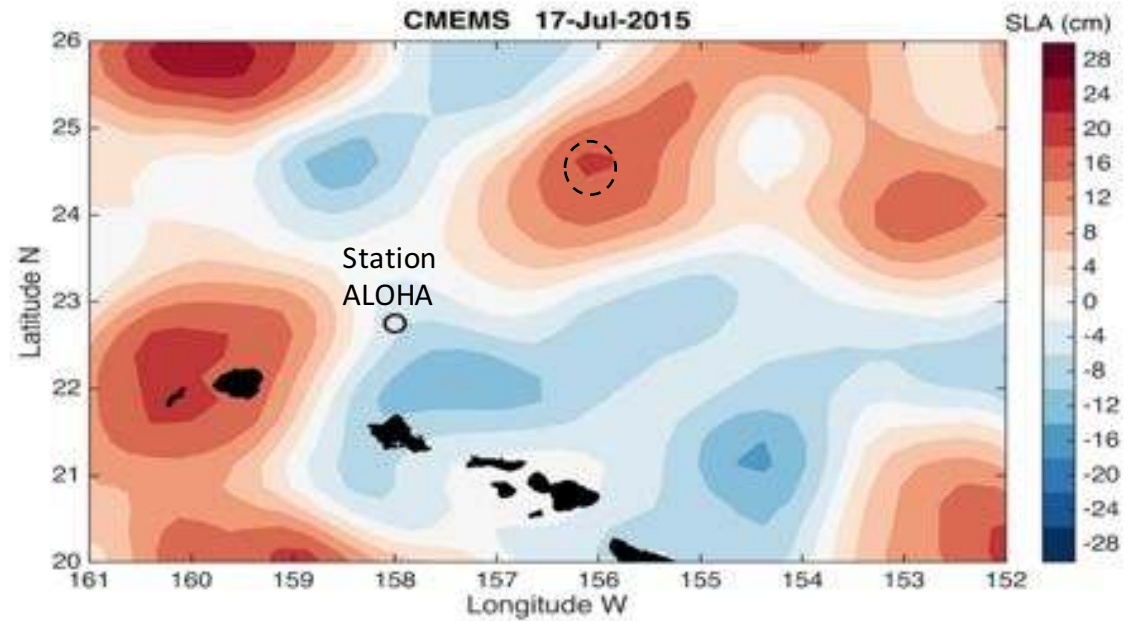
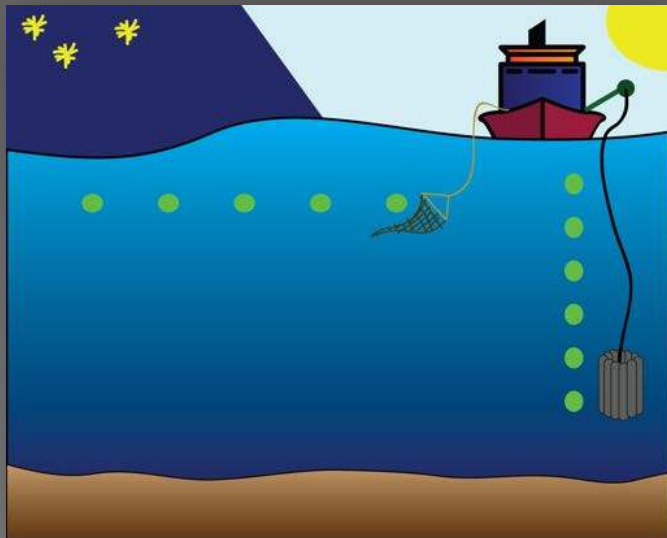
MAHALO: Special thanks to Daniel Muratore and Joshua Weitz for synthesis graphics



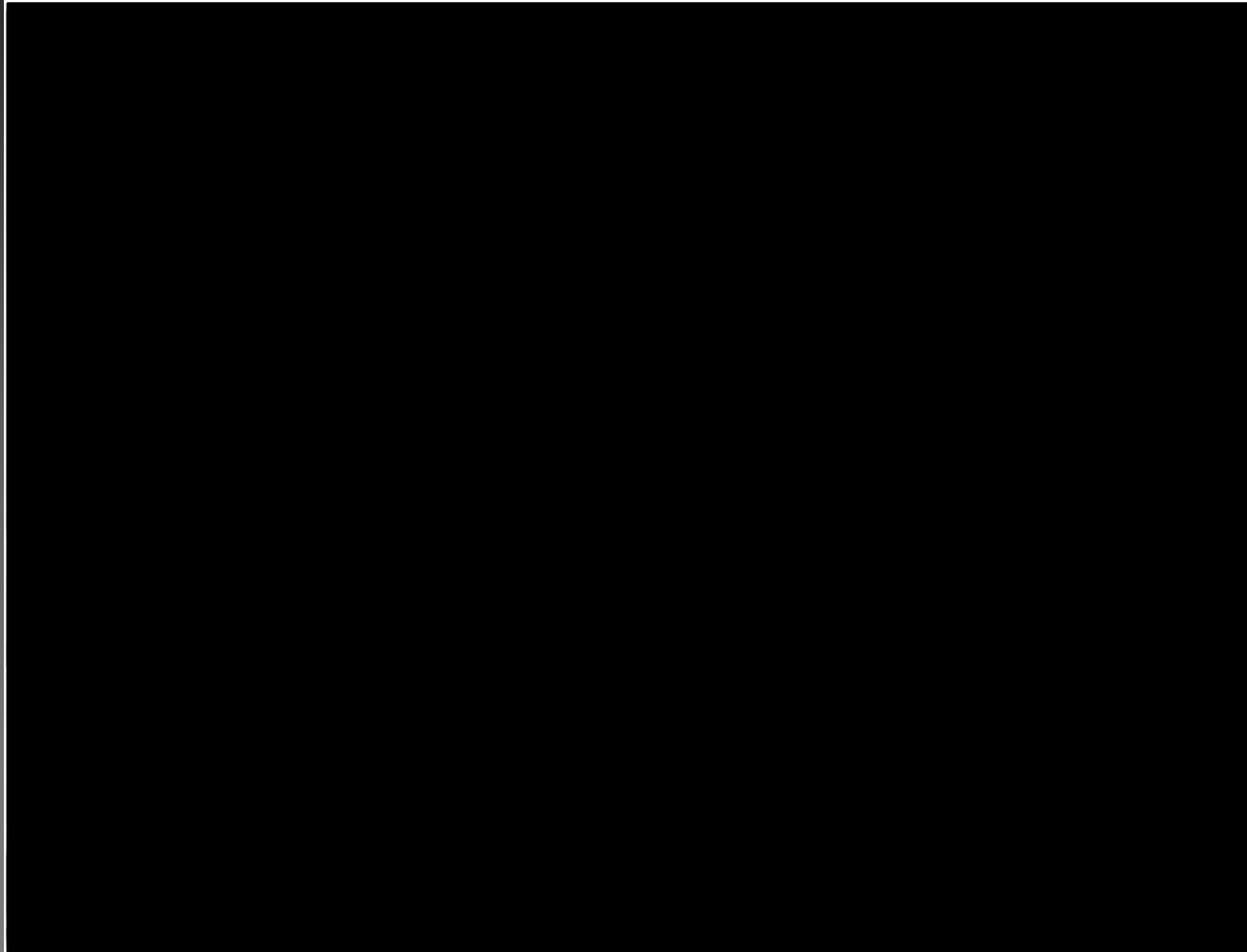
Sampling light driven responses in the NPSG

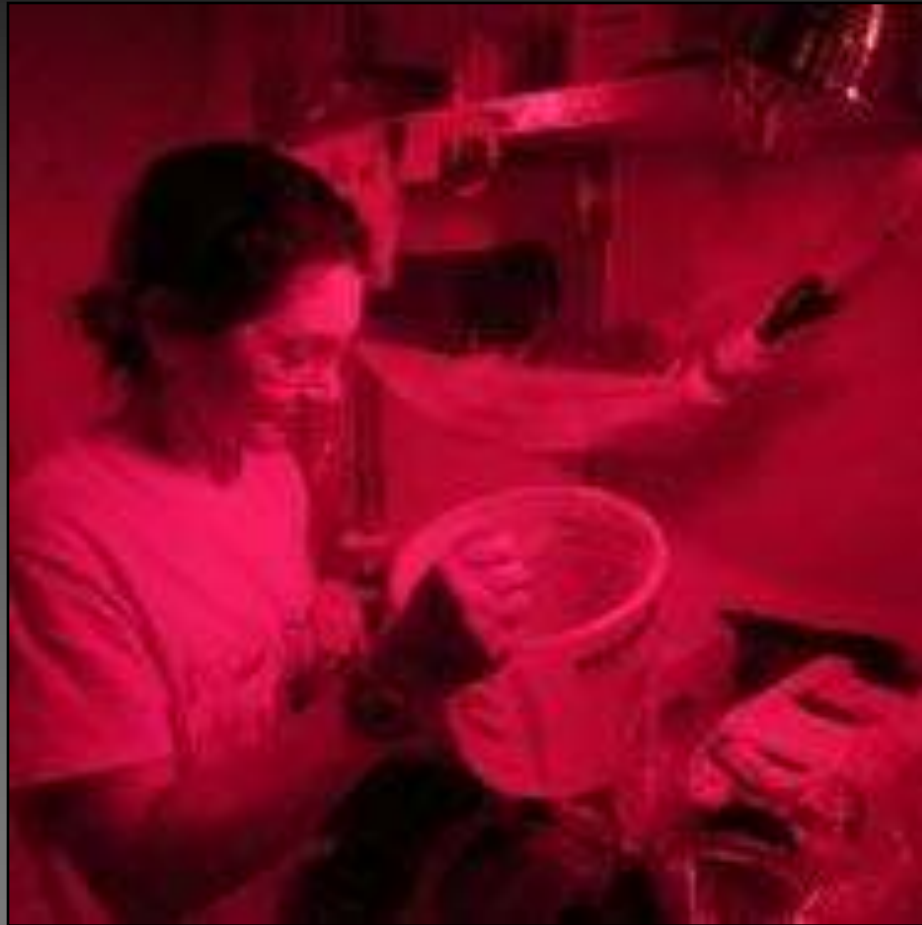


R/V Kilo Moana



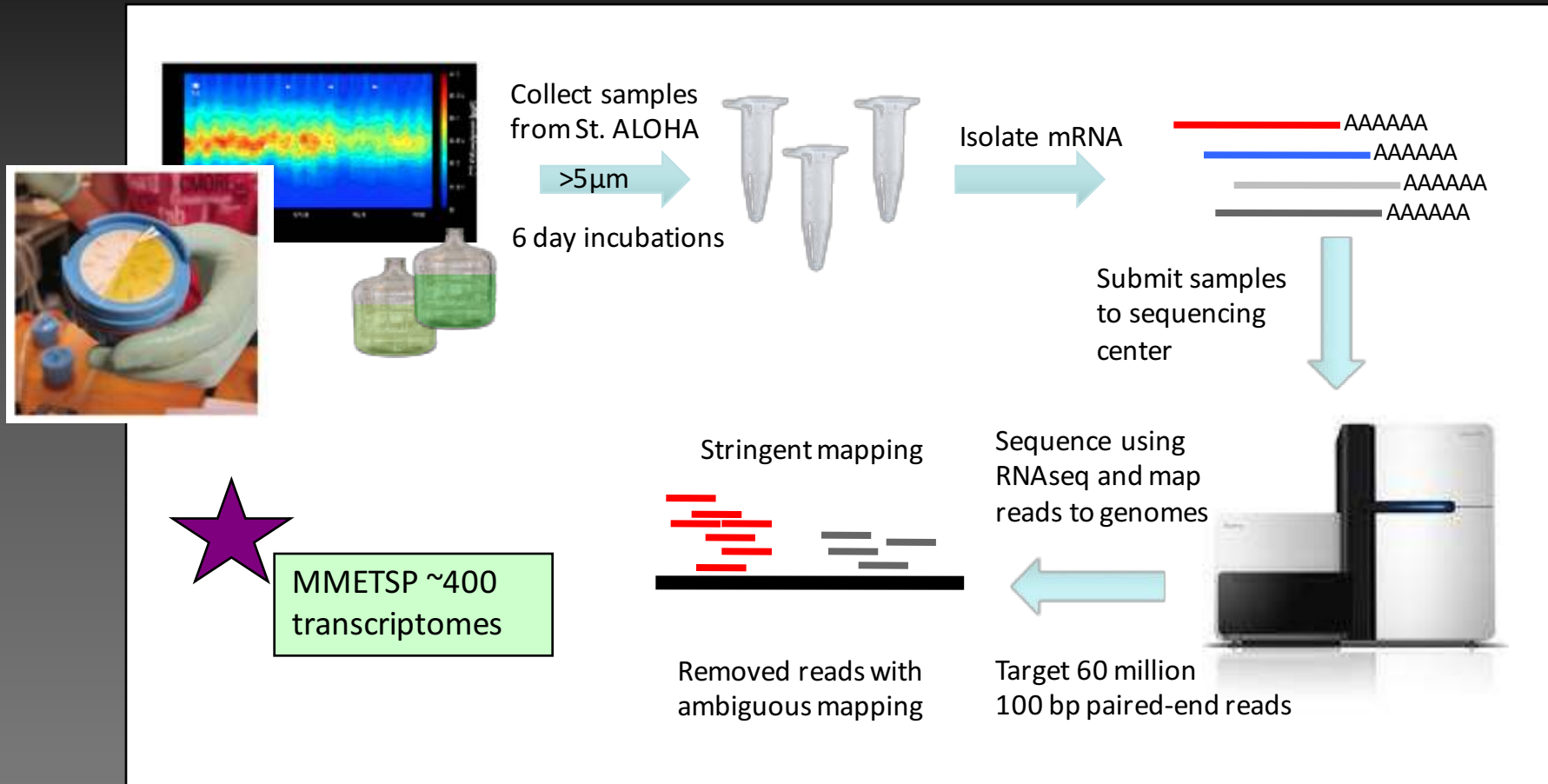
Sampling every 4hrs for 3.5 days





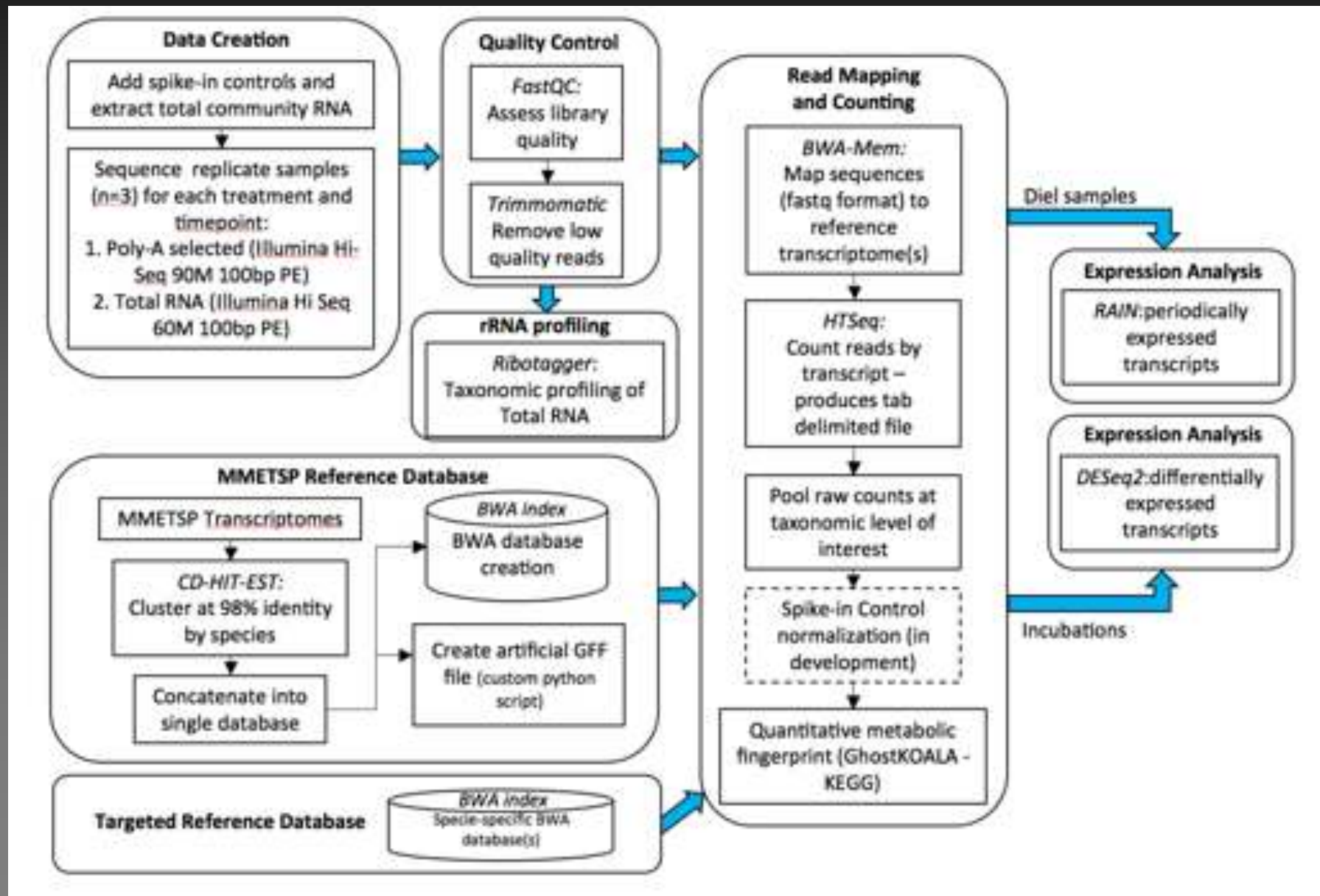


Sampling and pipeline

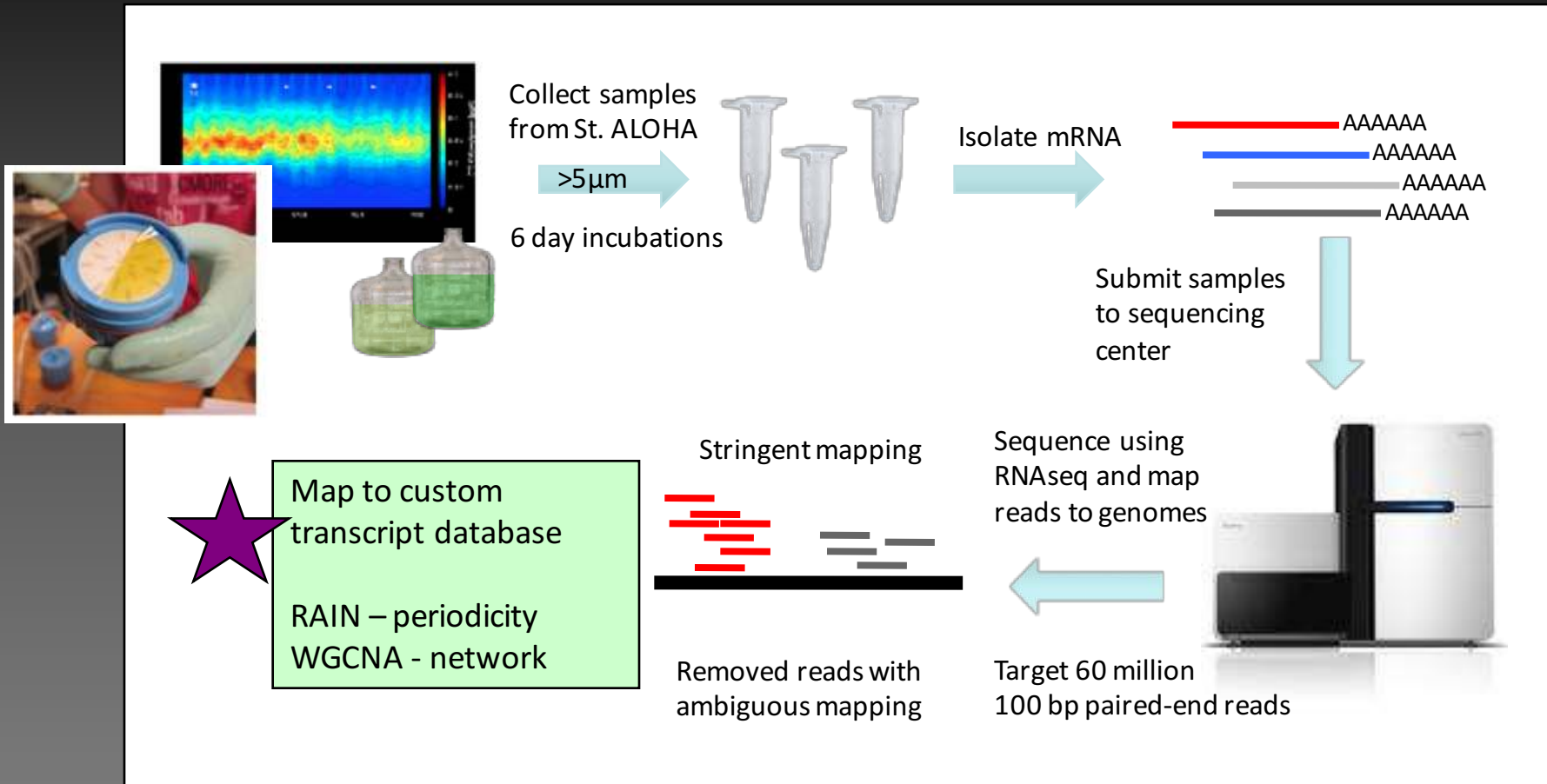


MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Sequencing and analysis strategy



Sampling and pipeline



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

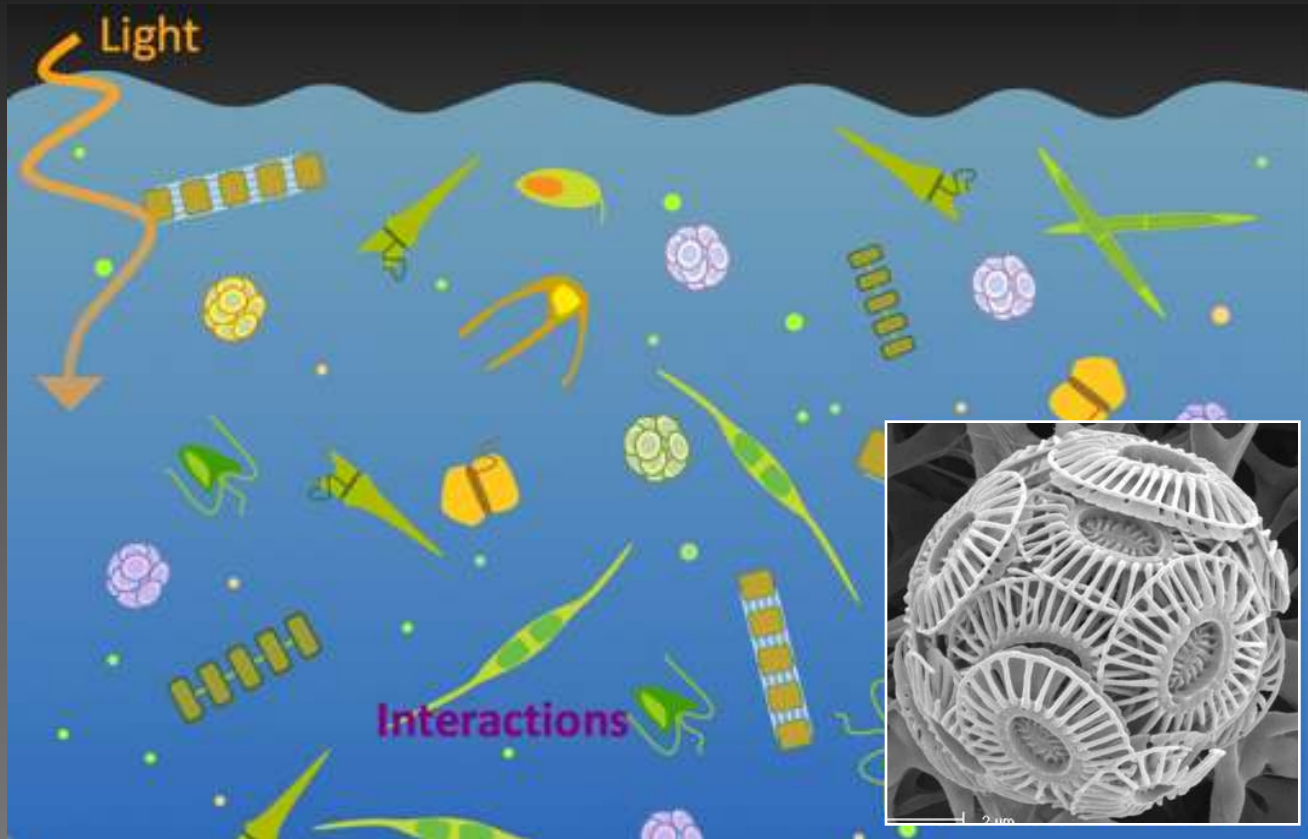
<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-9-559>

NMDS plot shows phased peaks in transcription

Different photosynthetic phyla have different patterns

Some overlap of functions between phyla

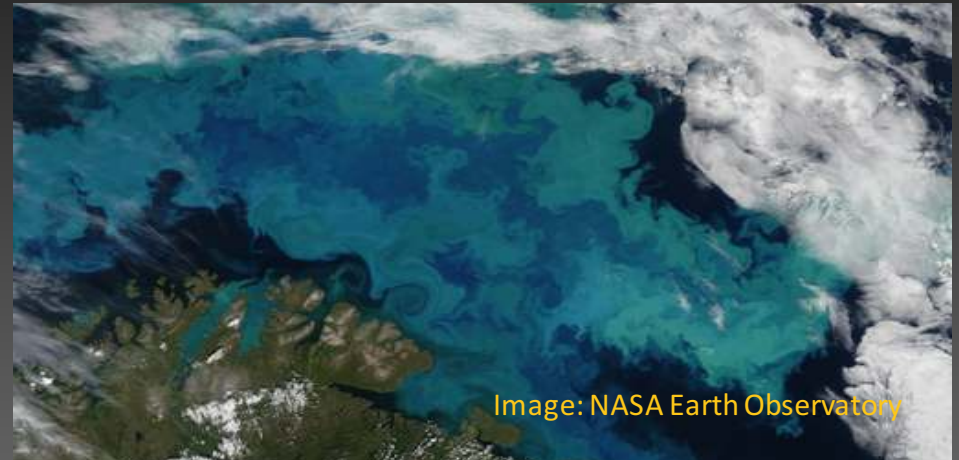
Diving beyond phyla to the species level ...



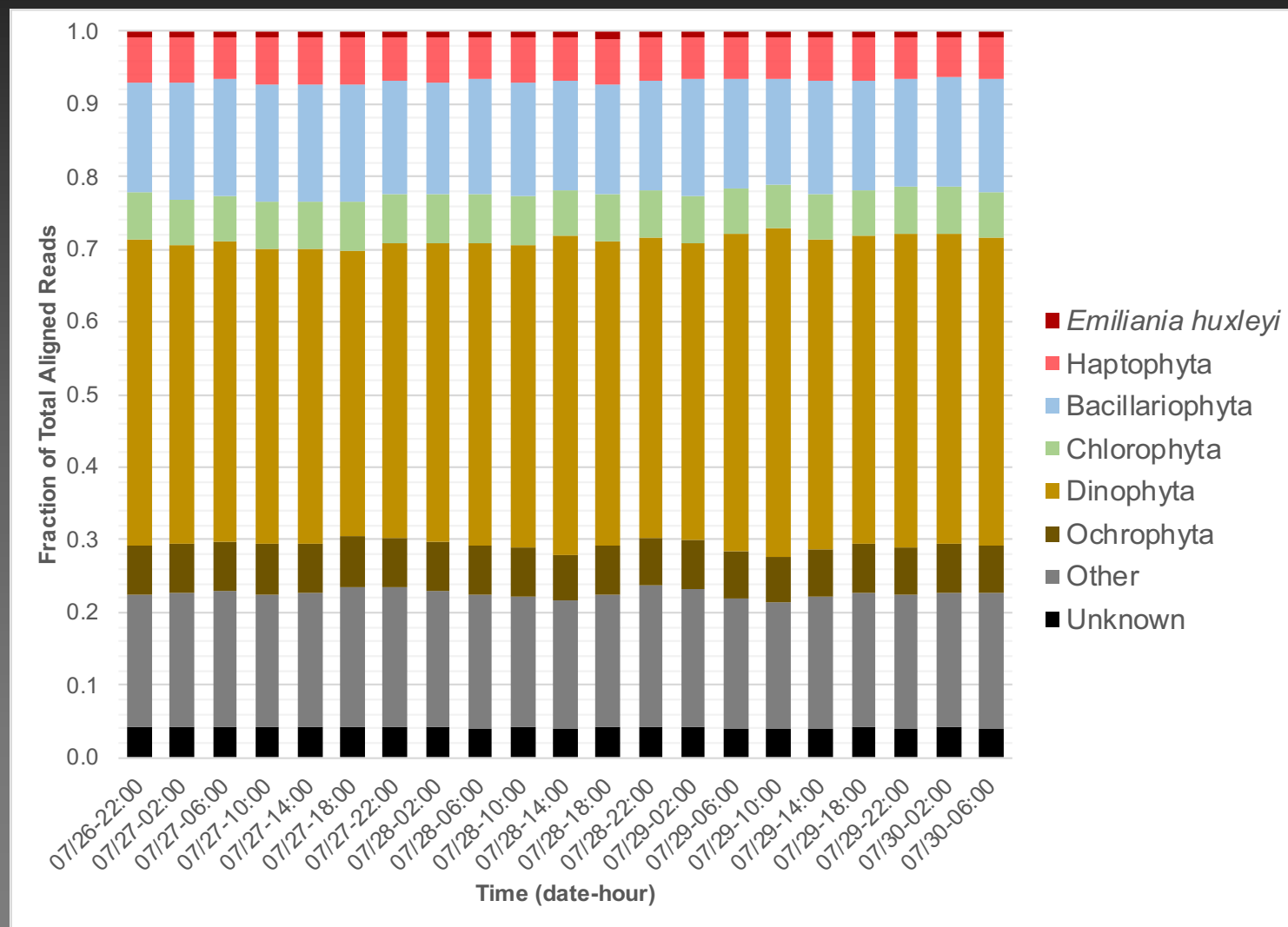
Tracking strain specific responses in the calcifying haptophyte:
Emiliana huxleyi (Ehux)

Emiliana huxleyi: a cosmopolitan, globally significant species

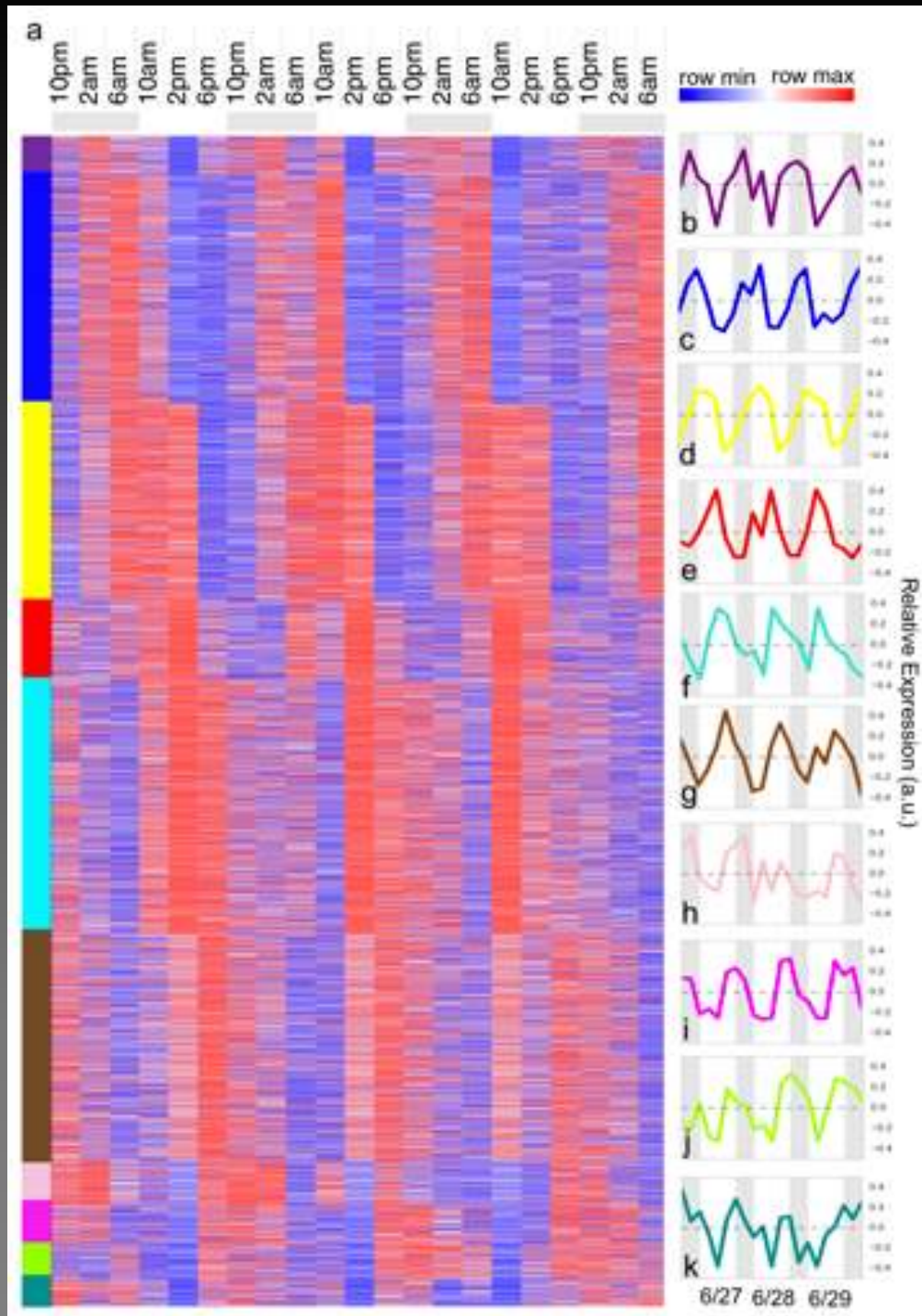
- Calcification - critical role in global carbon cycle and strongly linked to climate driven ocean acidification
- Source of paleoproxies for climate reconstructions
- Form dense blooms, drivers largely unknown
- First marine phytoplankton to have multiple strains sequenced, identifying pan genome



Data richness by phyla – *E. huxleyi* dominates haptophyte signal

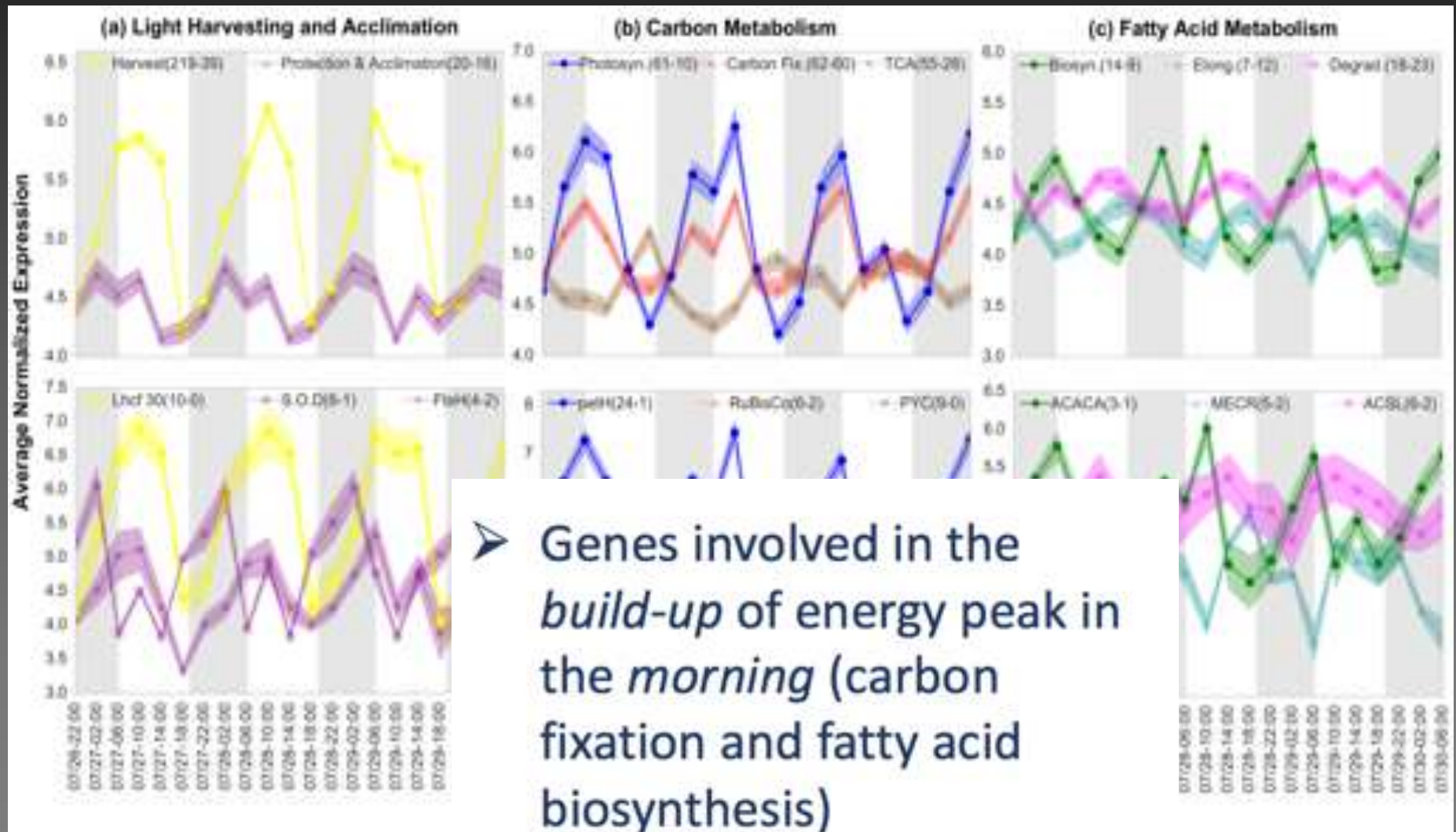


Hernandez et al. (in prep)

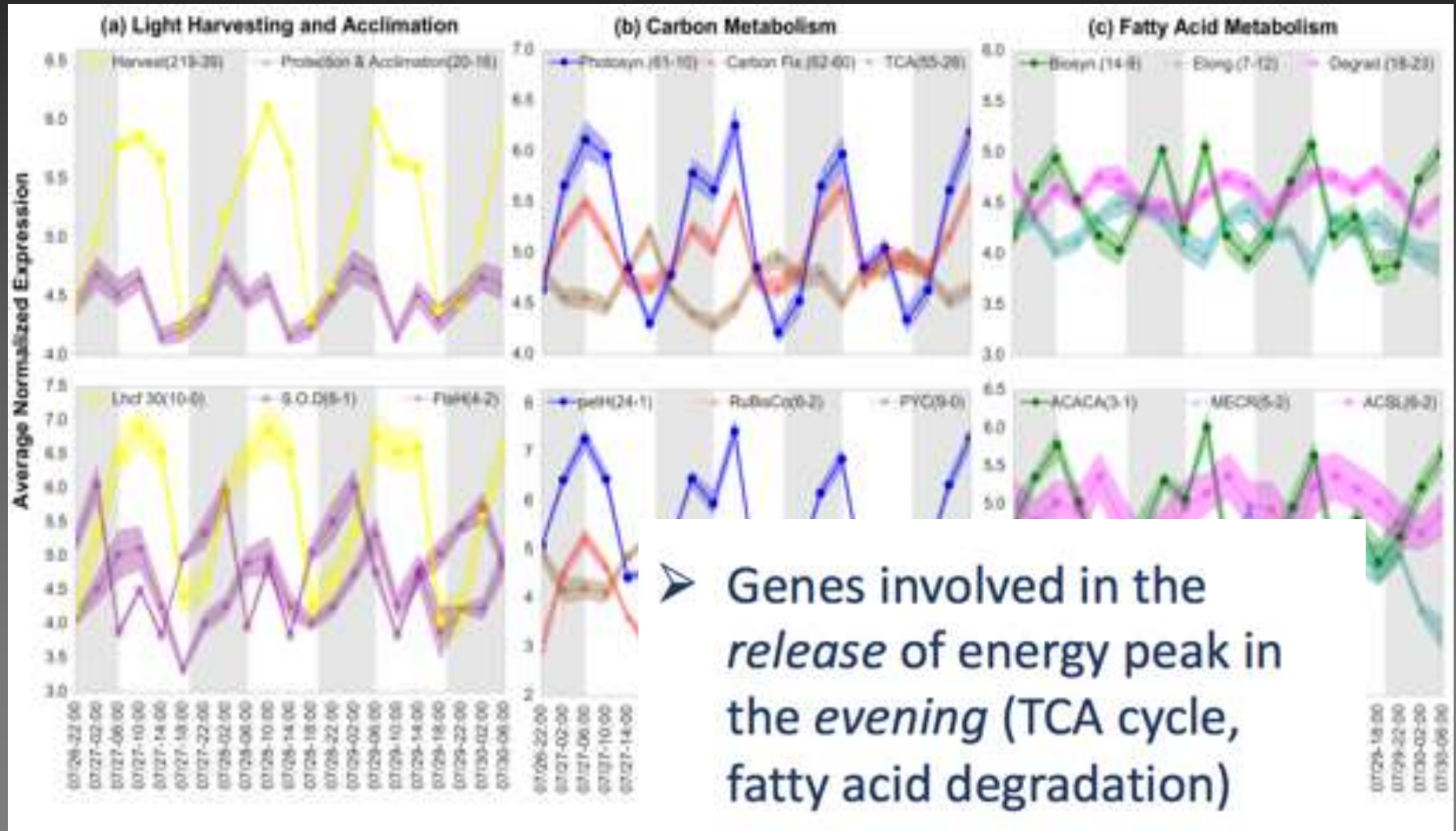


- 25% of total genes exhibit diel pattern
- Most peak in morning and evening

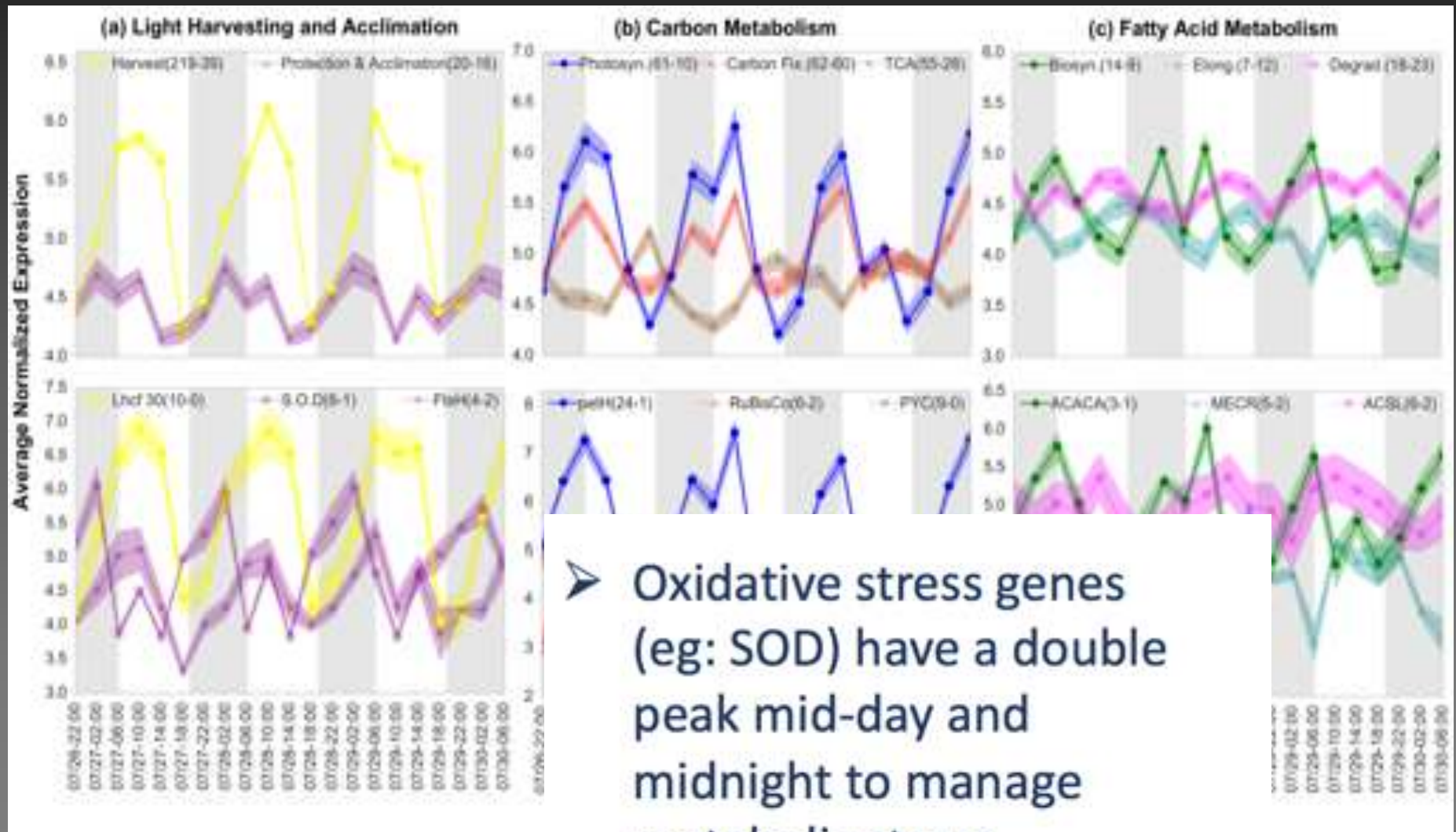
Diel partitioning of metabolism



Diel partitioning of metabolism

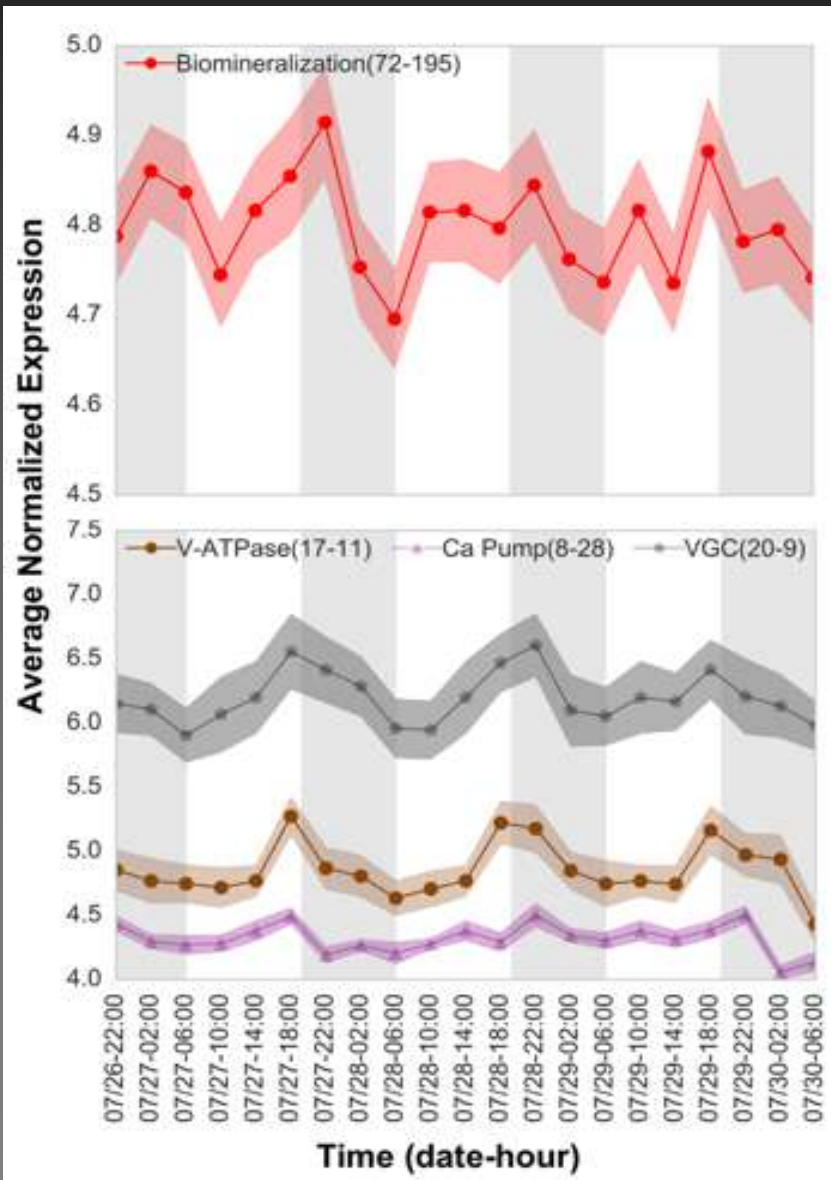


Diel partitioning of metabolism



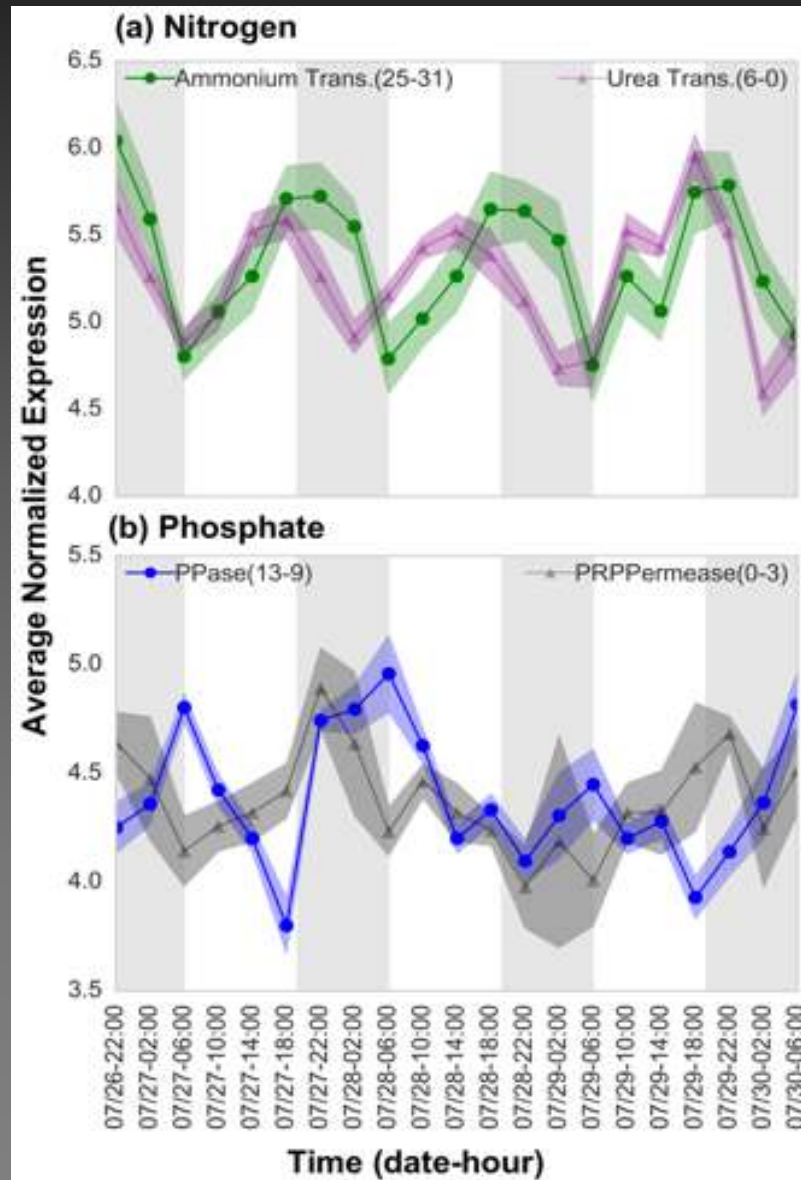
Hernandez et al. (in prep)

Patterns of putative biomineralization pathways



- Little evidence of phased biomineralization (calcification).
- Diel patterns in some putative biomineralization genes, but these pathways are poorly understood.
- Caution! Sampling time matters in comparing between samples/stations.

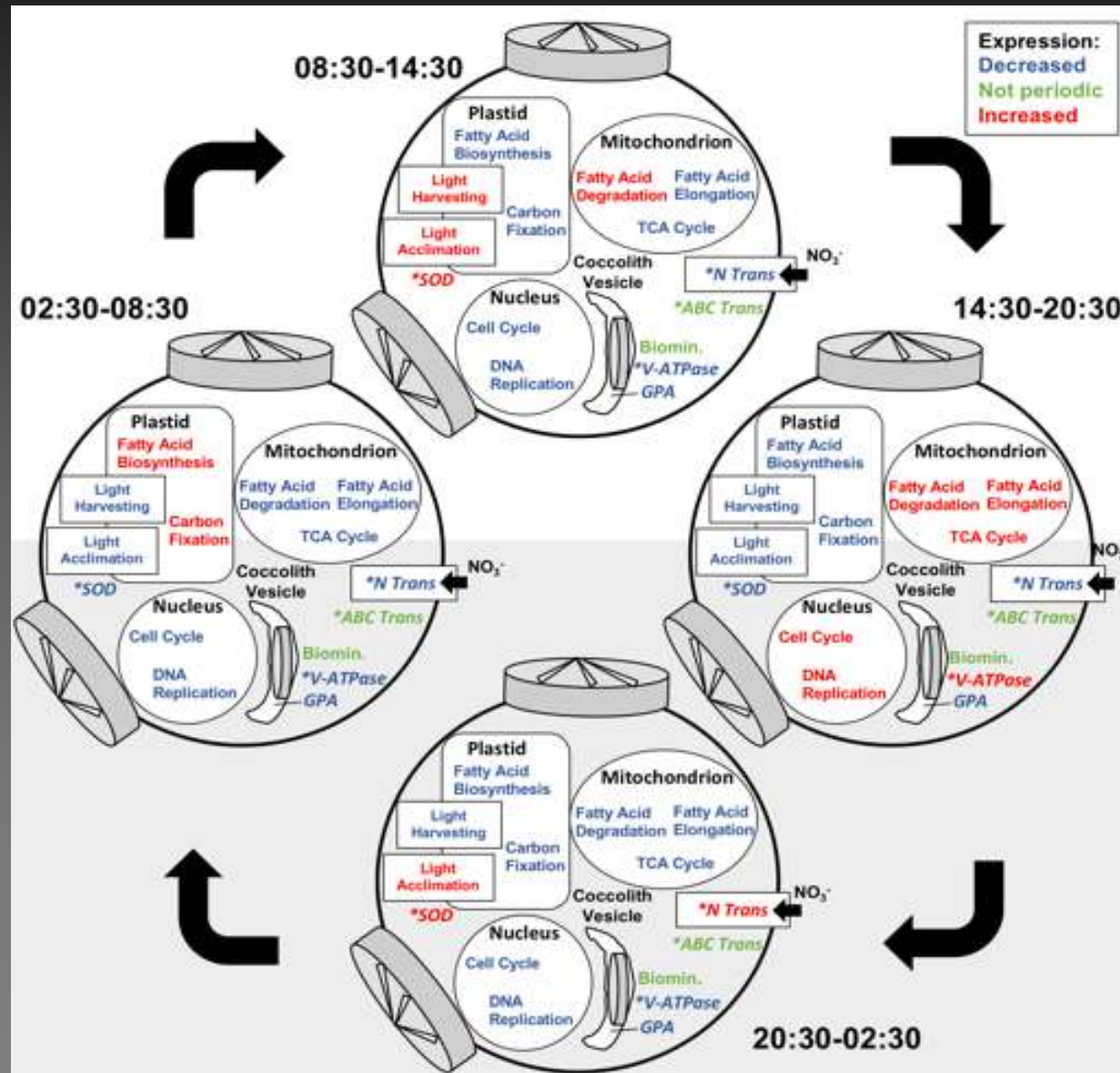
Patterns of resource acquisition



- Strong diel patterns in N-related transporters, fewer diel signals in P-related transport and metabolism
- Caution! Sampling time matters in comparing between samples/stations.

Hernandez et al. (in prep)

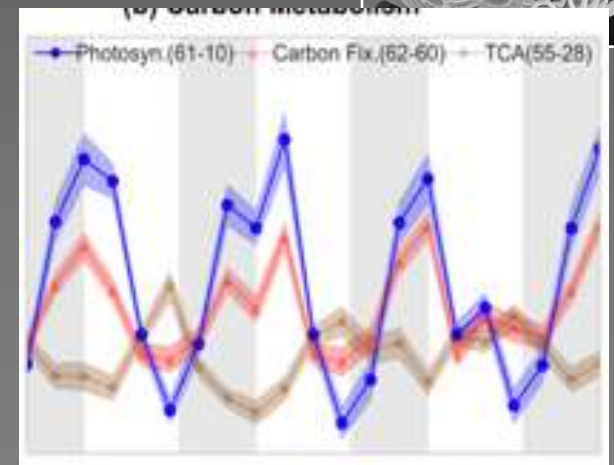
Light drives a reproducible cascade of metabolism



Summary – Light responses

How does light drive metabolic cascades across phyla?

- **Metatranscriptomes:** Light drives phyla specific metabolic cascades which underpin carbon cycling in the NPSG
- **Metatranscriptomes:** Functional responses related to photosynthesis are shared between phyla
- **Metatranscriptomes:** Species-specific resolution of *E. huxleyi* tracks a day in the life of key primary producers in the NPSG



Core questions

Nutrient responses

- Proof of concept: How do different groups respond to nutrient pulses?

Light responses

- How does light drive metabolic cascades across phyla?

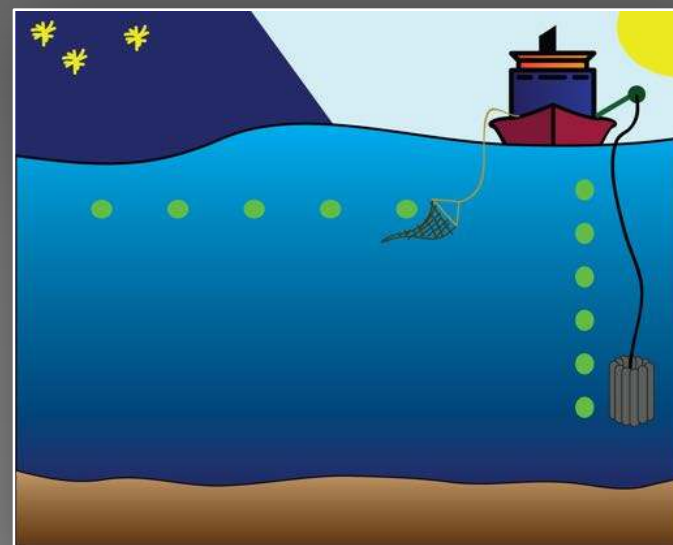
Interactions

- Are there patterns of coordinated expression that underpin host-symbiont physiology?
- How does light drive interactions between the microbiome and host?

HOE:DYLAN

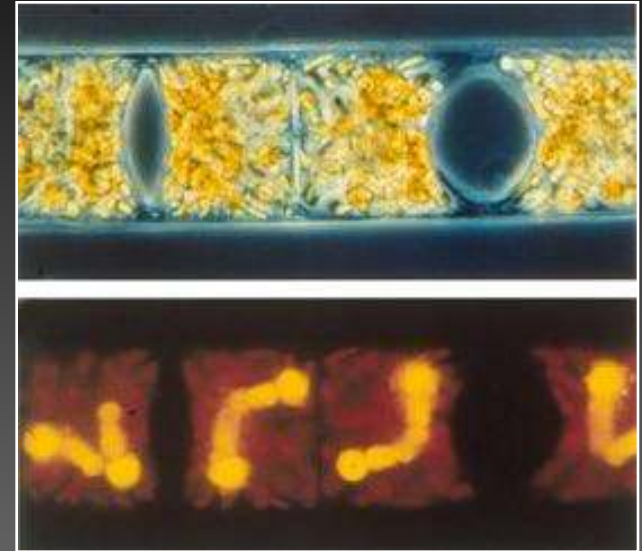


MAHALO



Using light to study interactions

- Some of the most important microbial eukaryotes live in symbioses or associations.
- Diatom diazotroph associations (DDAs) drive summer export of carbon to the deep sea, sequestering CO₂.
- Diazotroph supplies fixed N₂ to host.
- There are no long term reference cultures, resulting in limited knowledge about the activities of these symbioses in the field.



Are there patterns of coordinated expression that underpin host - symbiont physiology?



3x20L from 15m
(21 time points, every 4 h, 3.5 days)

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



Extract RNA

Selected



**Map to reference
database of MMETSP
diatoms**
(Including *Rhizosolenia*)

Unselected

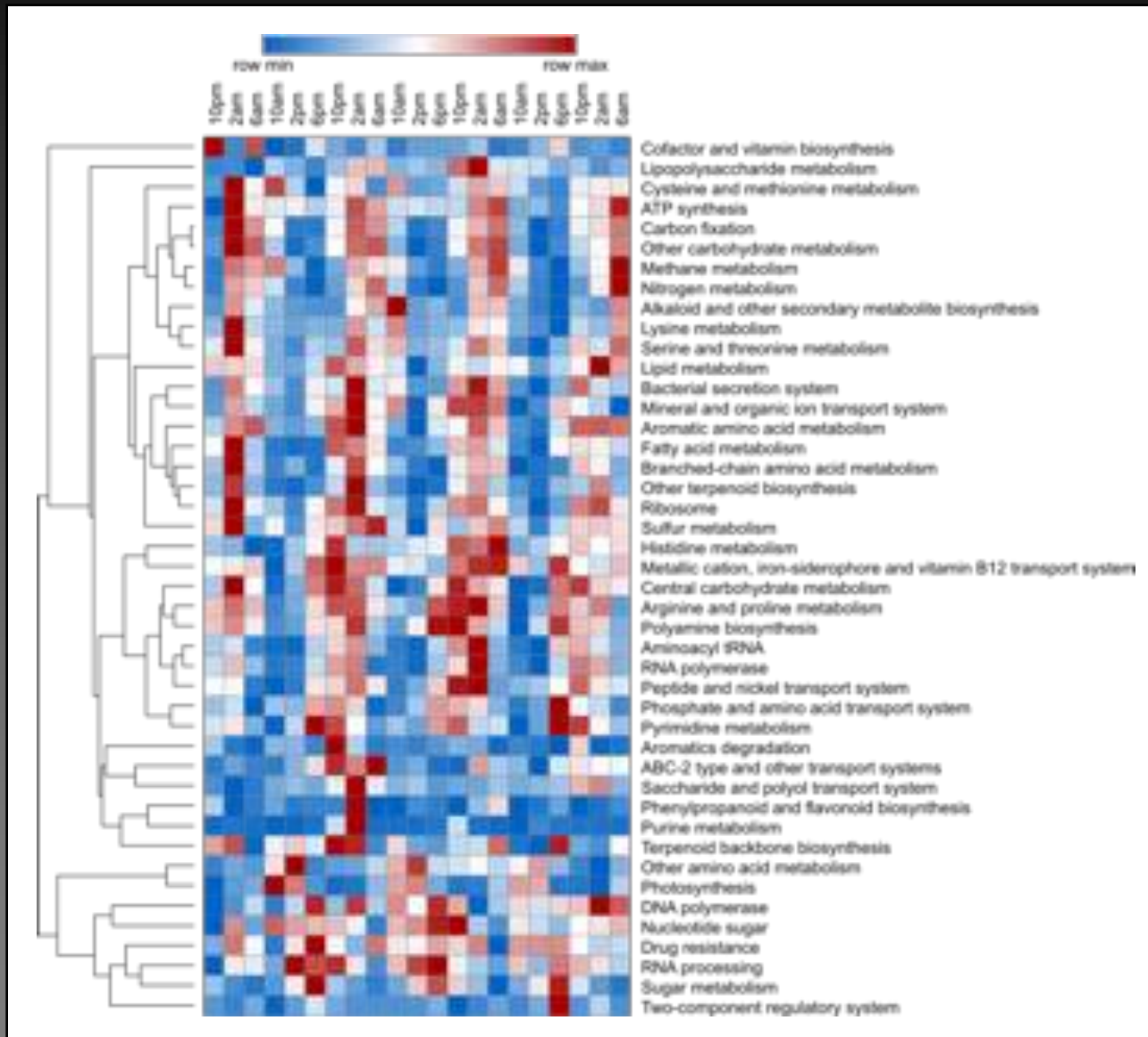


**Map to reference
database of
diazotrophs**
(Including *Richelia*)

- Each sample generates between 4-6 Gb of sequencing data
- Current data set :
 - ~ 19 billion reads
 - ~ 2 terabytes of data

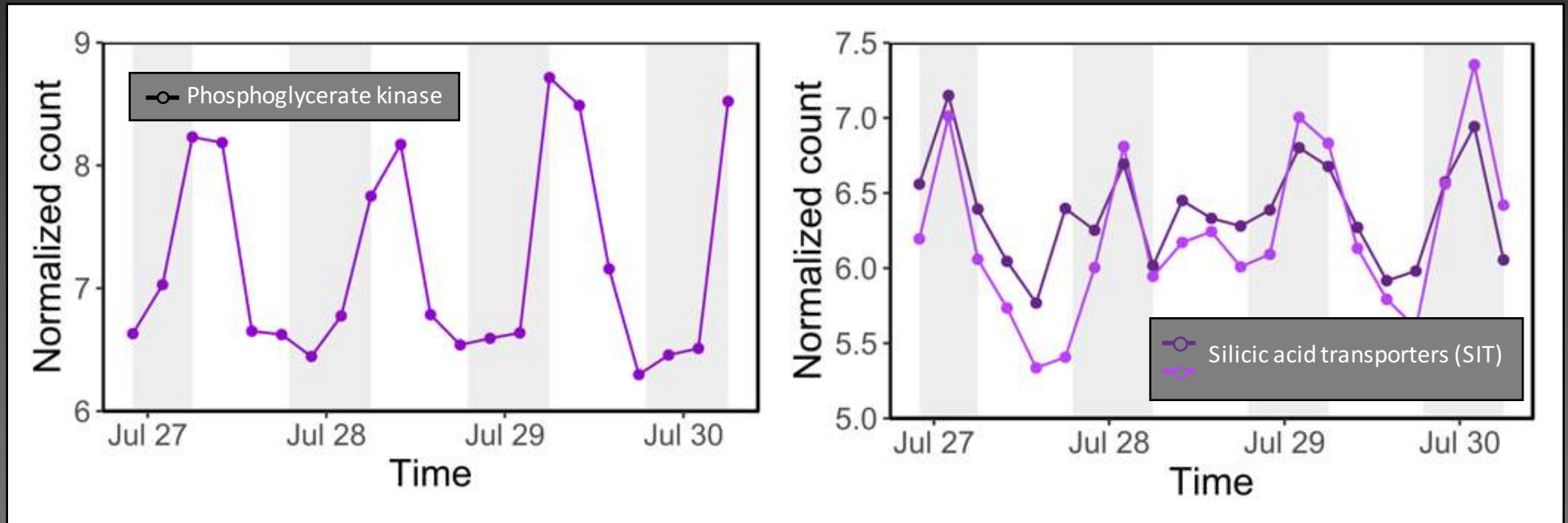
RAIN and WGCNA

Diel patterns in *Rhizosolenia host*



Harke et al. (2018) *ISMEJ*

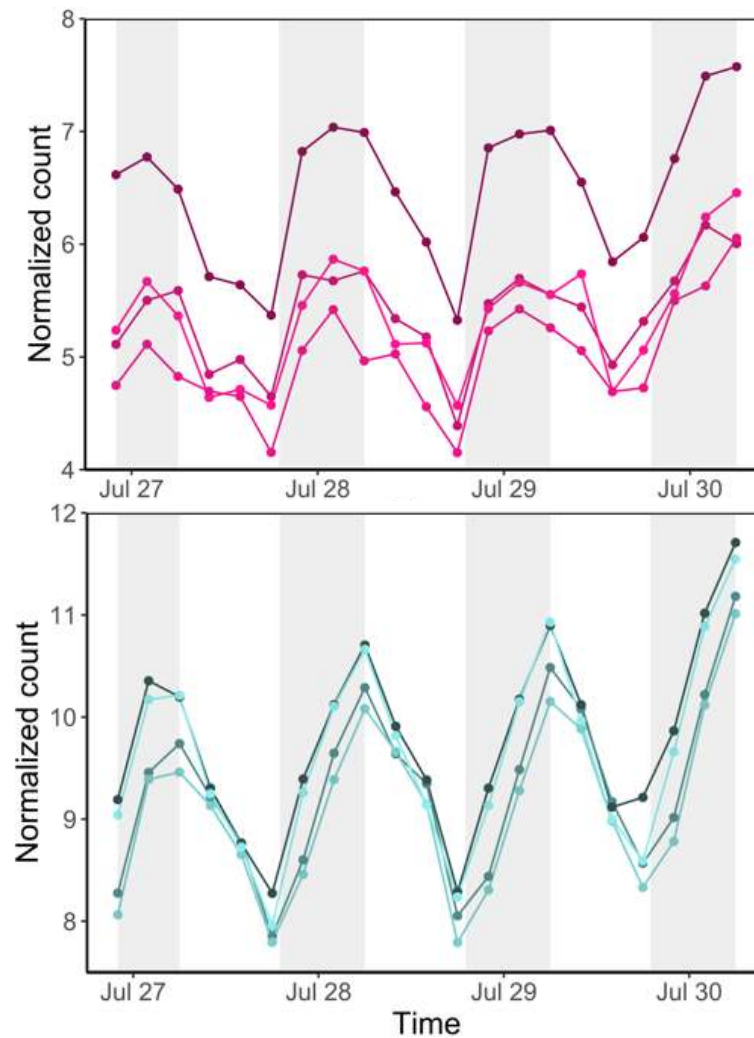
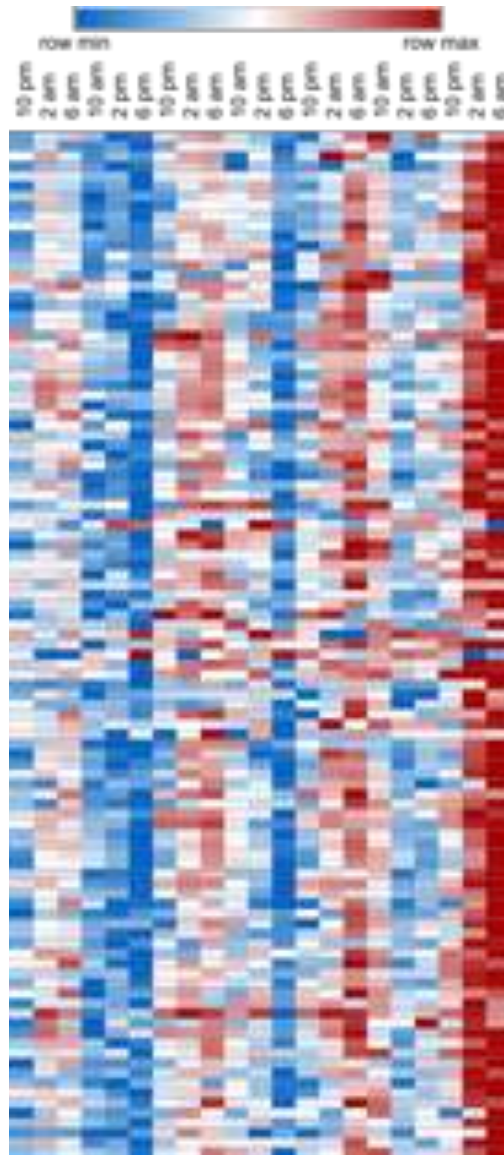
Significantly diel genes in *Rhizosolenia* host



Harke et al. (2018) *ISMEJ*

Carbon fixation and growth (Si uptake - cell wall formation) are tightly linked to the light-dark cycle

Richelia symbiont has strong diel patterns

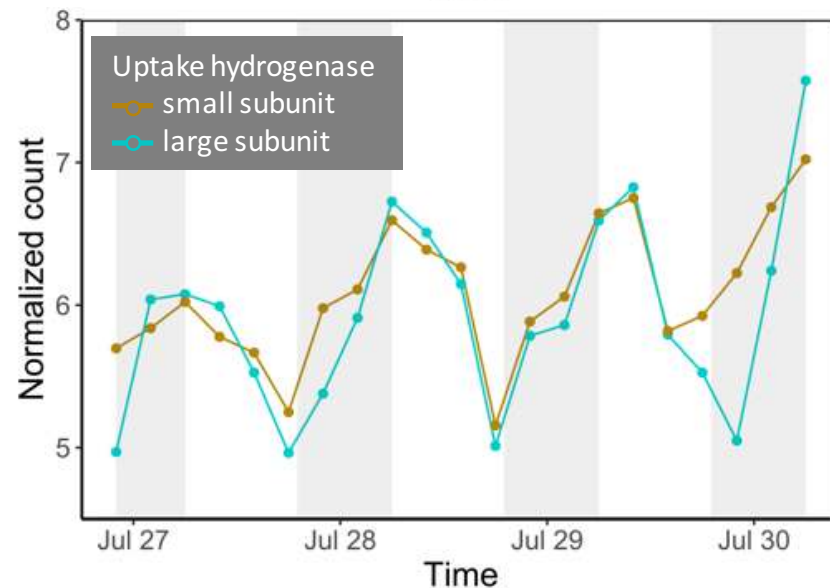
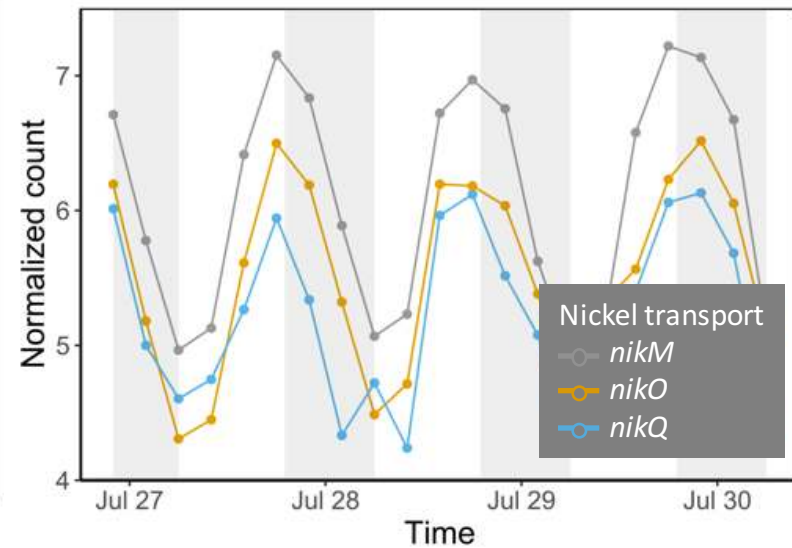
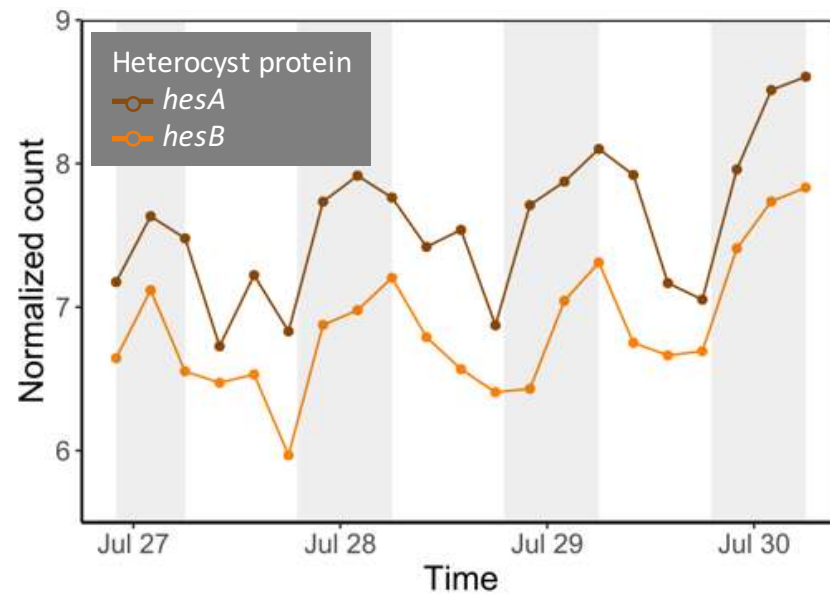


Nitrogenase genes

-  *nifE*
-  *nifT*
-  *nifW*
-  *nifX*
-  *nifD*
-  *nifH*
-  *nifH*
-  *nifK*

Harke et al. (2018) *ISMEJ*

Coordinated expression of genes related to N₂ fixation in *Richelia*



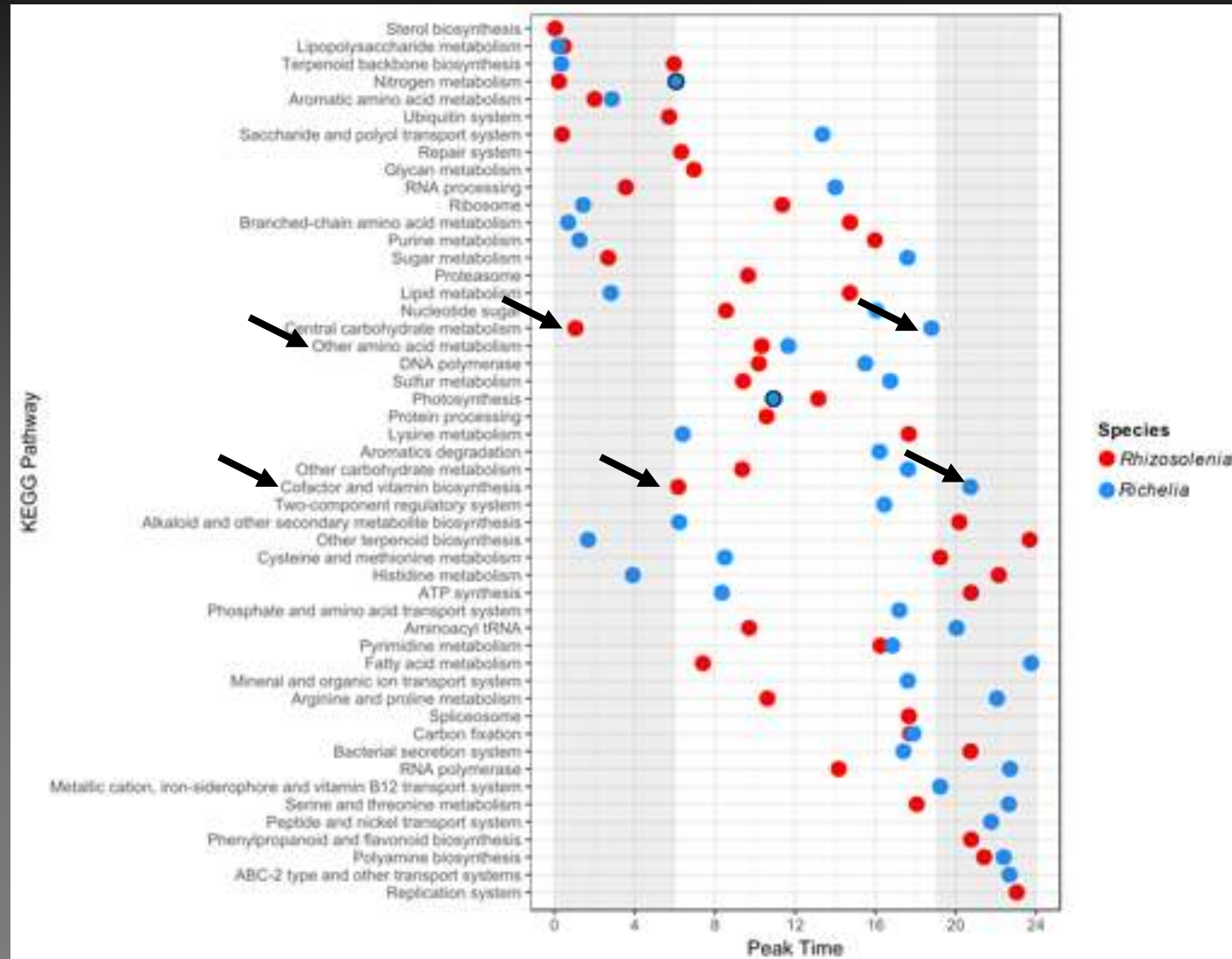
Harke et al. (2018) *ISMEJ*

Timing of expression suggests coordination of processes supporting N₂ fixation

Coexpression between host and symbiont

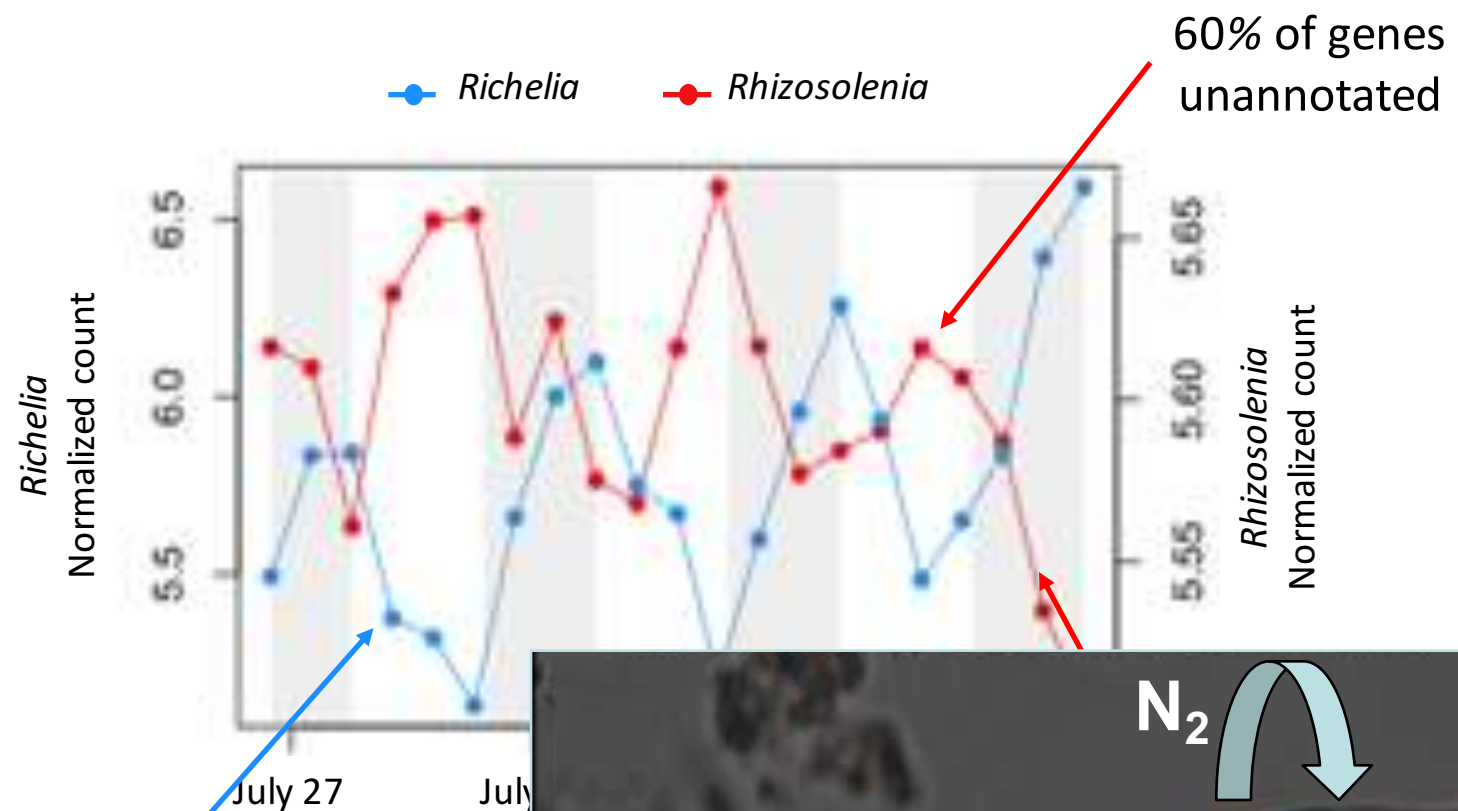


Cascade in metabolic timing between host and symbiont

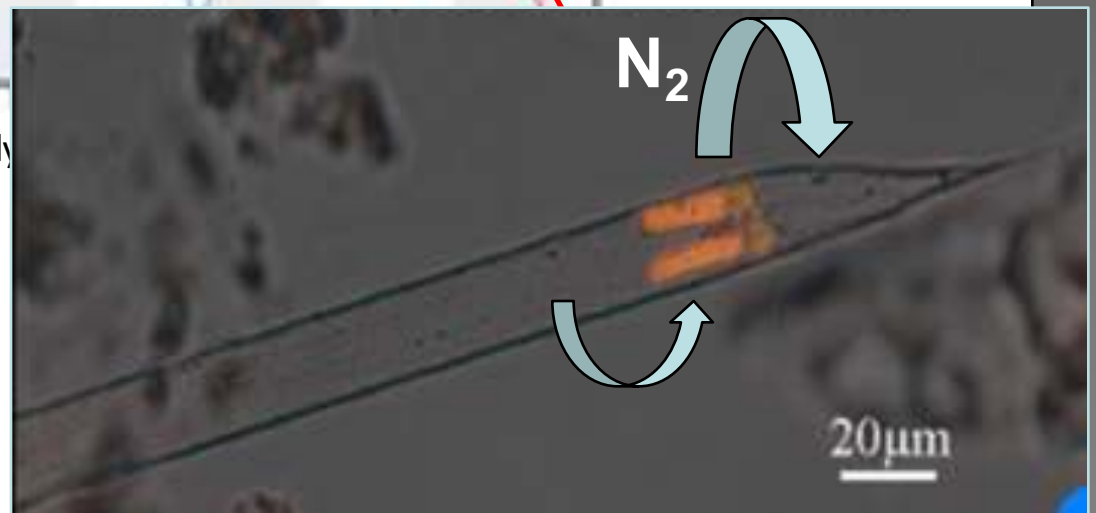


Metabolic offset for many KEGG pathways - although both are photosynthetic, core metabolism is offset

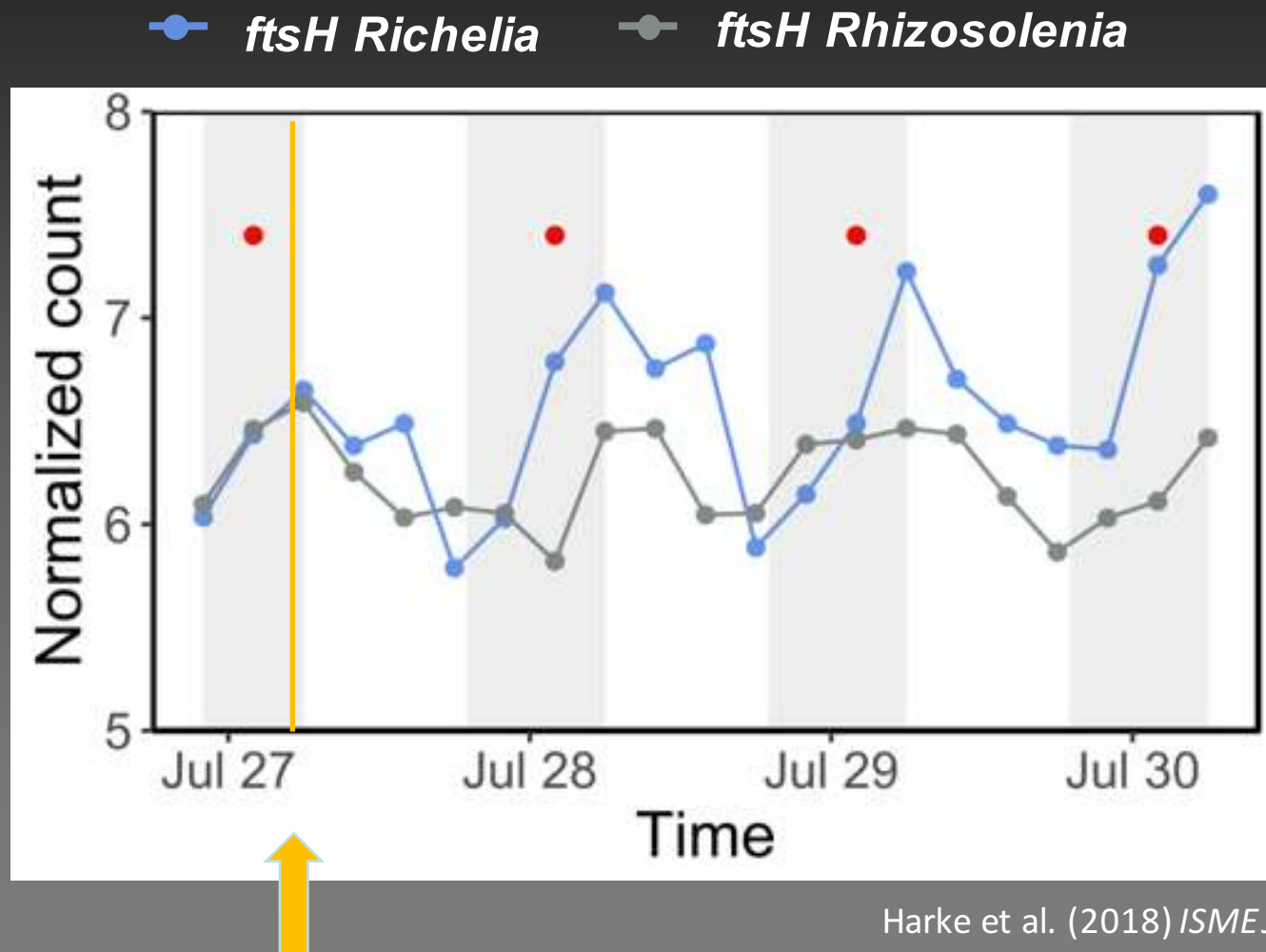
Coordinated expression between host and symbiont in N_2 fixation module using WGCNA



Majority of N_2 fixation genes

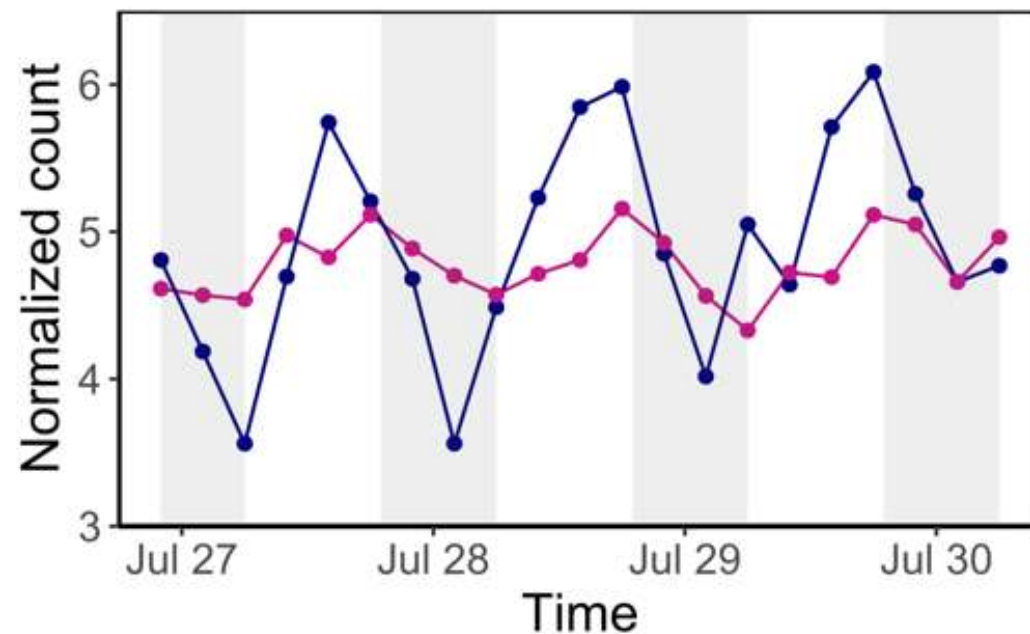


Is cell division coordinated to maintain association?



Division timing
Villareal et al. 1989

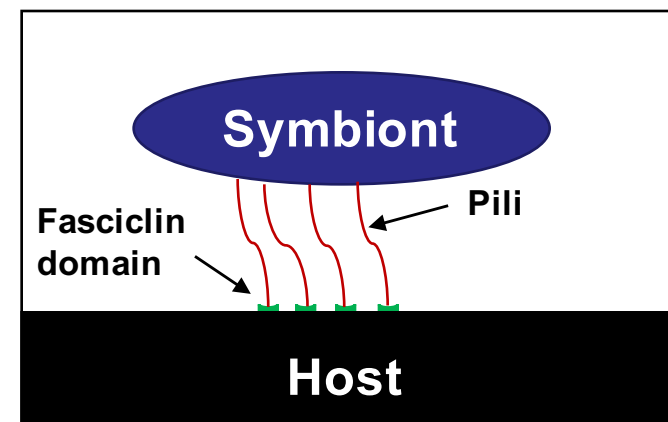
How is the association maintained?



—●— *Richelia* type IV pilus
—●— *Rhizosolenia* fasciclin domain

- Genes involved in cell adhesion have similar peak expression timing

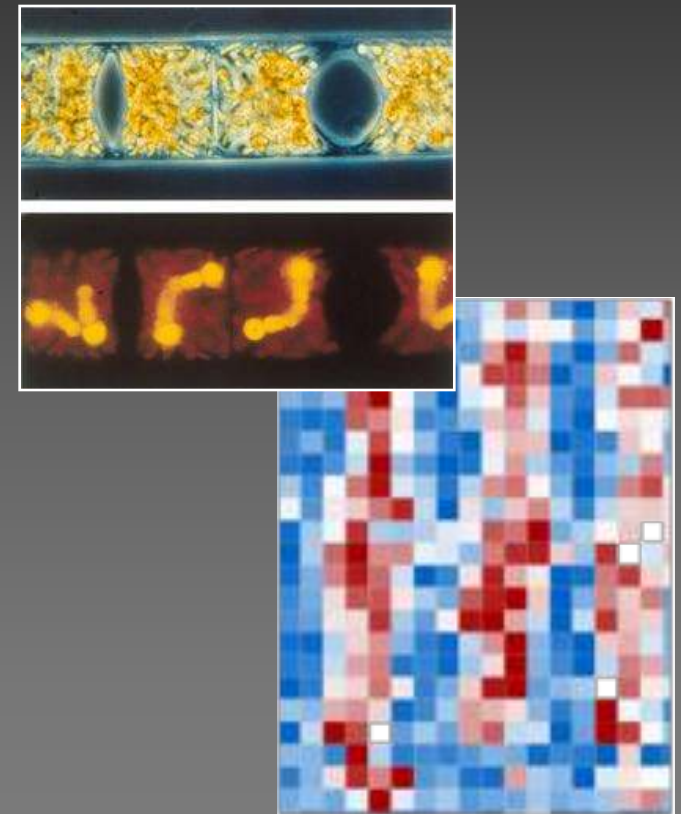
Harke et al. (2018) *ISMEJ*



Summary – Interactions coordinated responses in DDA

Are there patterns of coordinated expression that underpin host - symbiont physiology?

- **Selected Metatranscriptomes:** Host physiology is driven independently by light
- **Unselected Metatranscriptomes:** Symbiont physiology is driven independently by light.
- **Dual Metatranscriptomes:** Light drives coordinated patterns that underpin N exchange, cell division, and maintenance of symbiosis



Core questions

Nutrient responses

- Proof of concept: How do different groups respond to nutrient pulses?

Light responses

- How does light drive metabolic cascades across phyla?

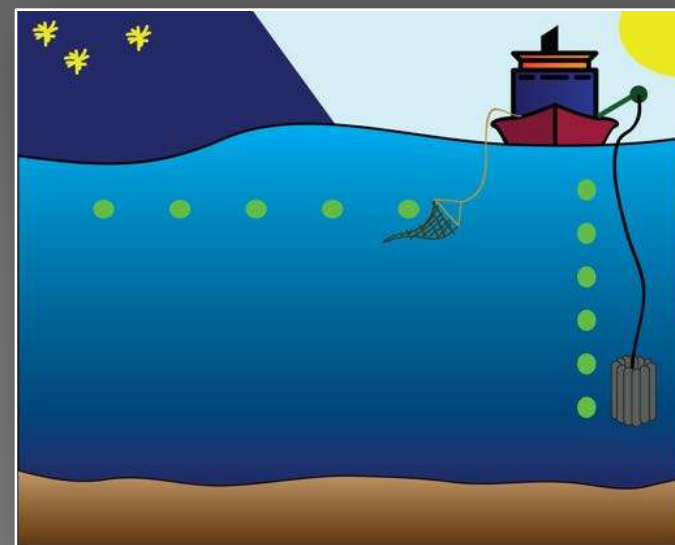
Interactions

- Are there patterns of coordinated expression that underpin host-symbiont physiology?
- How does light drive interactions between the microbiome and host?

HOE:DYLAN

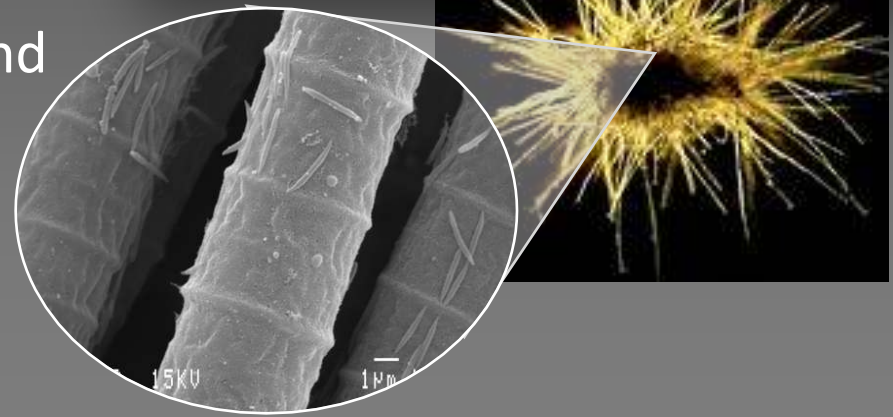
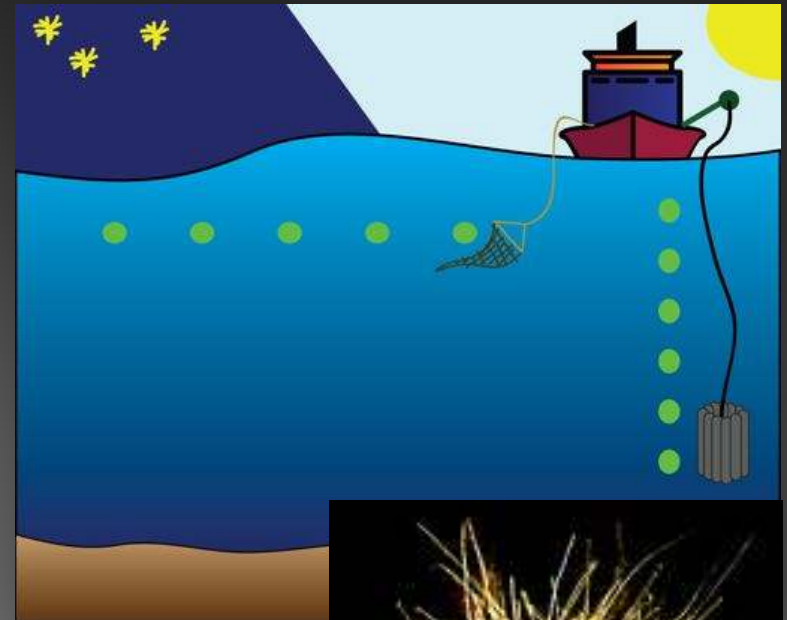


MAHALO



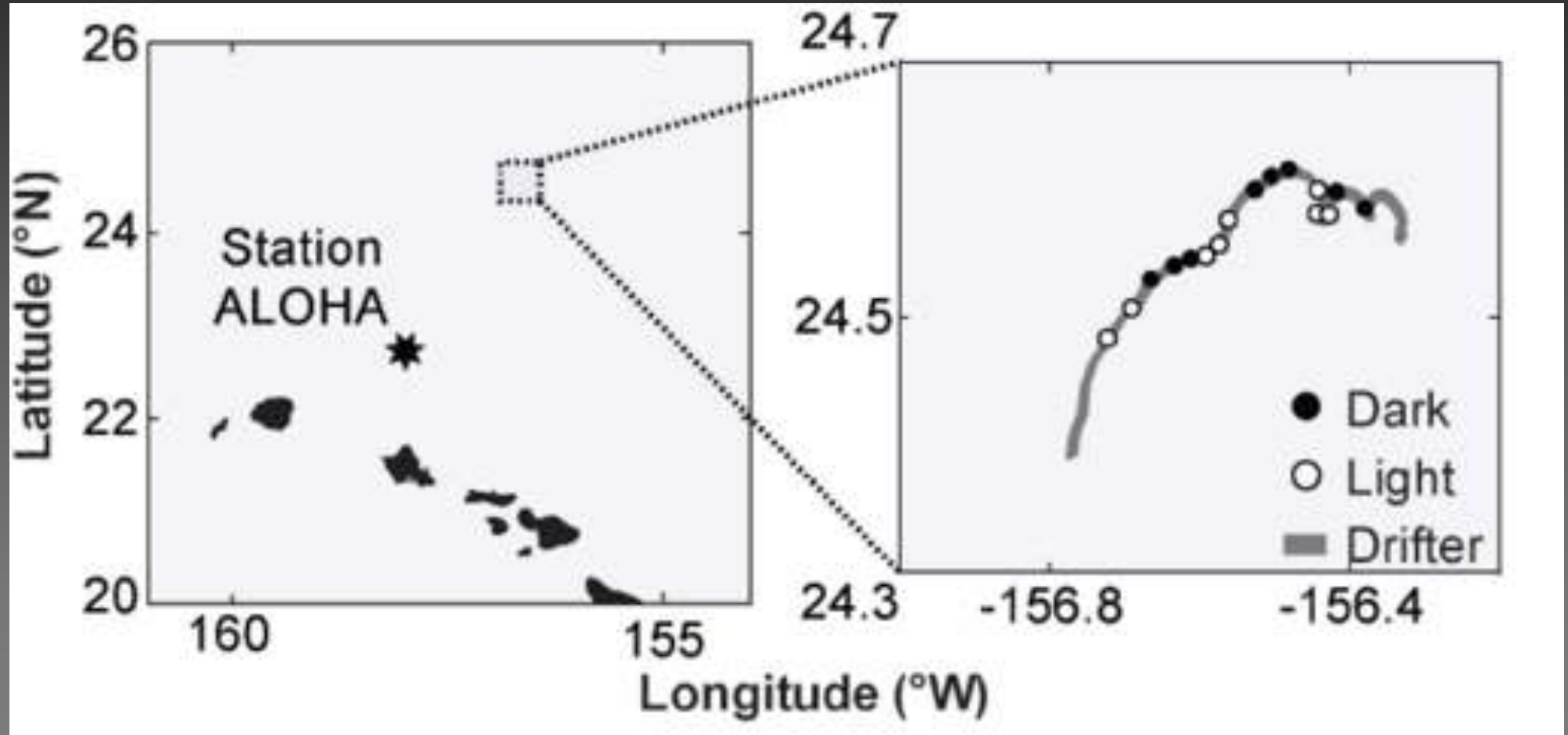
Using light to study interactions

- *Trichodesmium* plays a critical role in the ecology of the NPSG by fixing N_2 that is otherwise limiting.
- Little is known about the interactions between this colonial cyanobacteria and its heterotrophic epibiotic microbiome
- Do epibionts have diel responses, and what might they tell us about interactions between the host and microbiome?



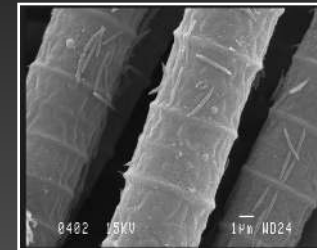
How does light drive interactions between the microbiome and host?

Studying the holobiont ... using a time series of daily rhythms



Colony samples were taken every 4 hours for 72 hours, twice!

Light-driven dynamics in host and microbiome



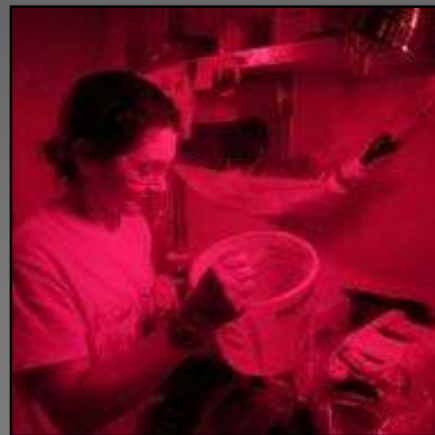
Metatranscriptomes



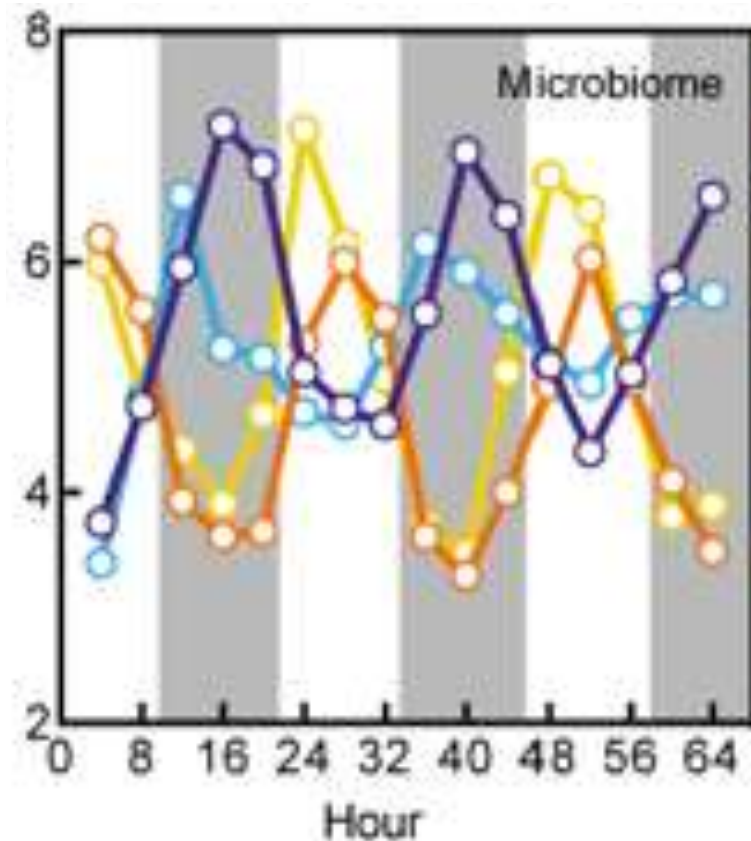
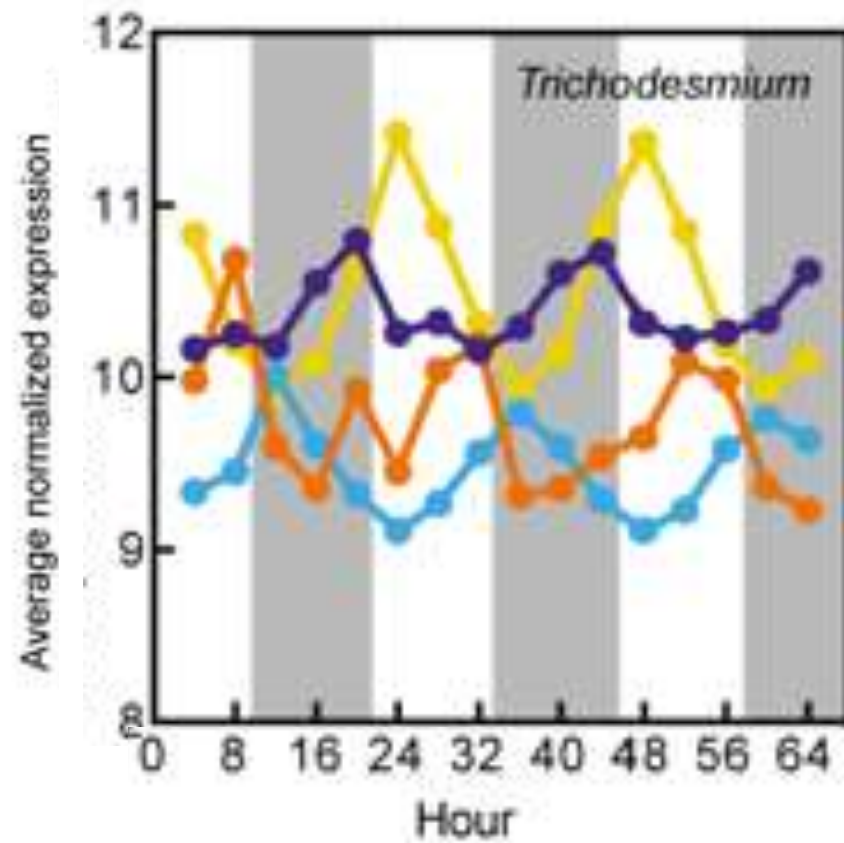
Orthologous group
analysis



Periodicity: RAIN
Co-expression: WGCNA

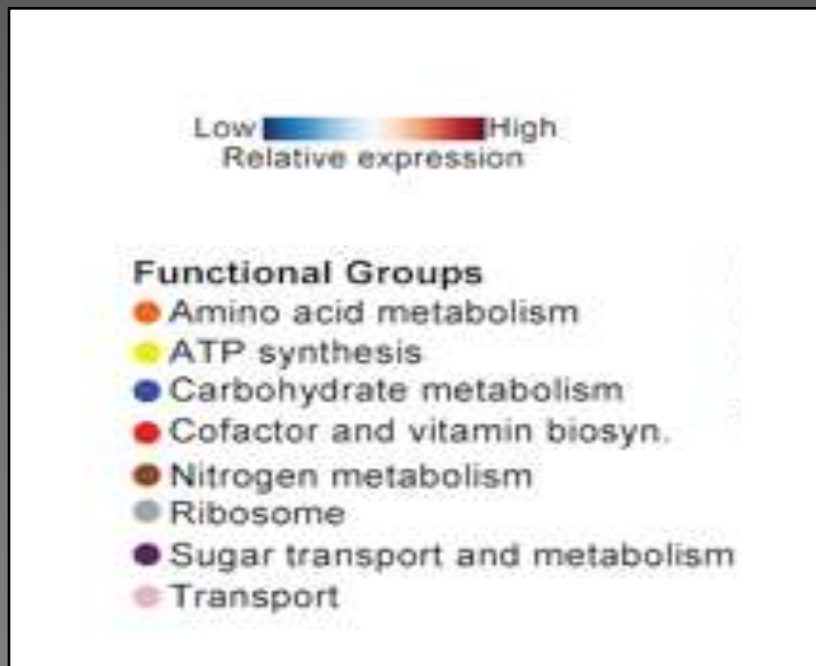


Diel modulation of transcripts in *Trichodesmium*

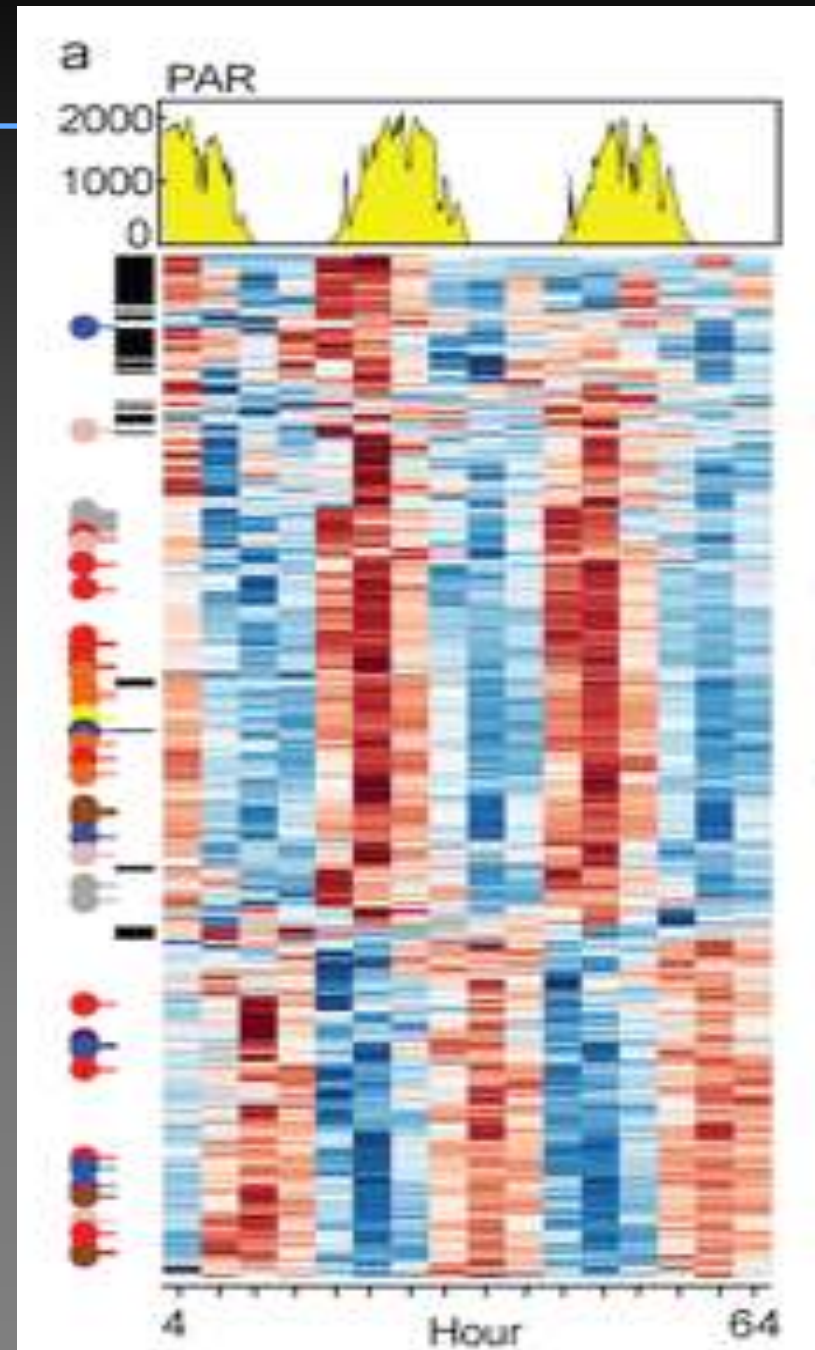


WGCNA co-expression network

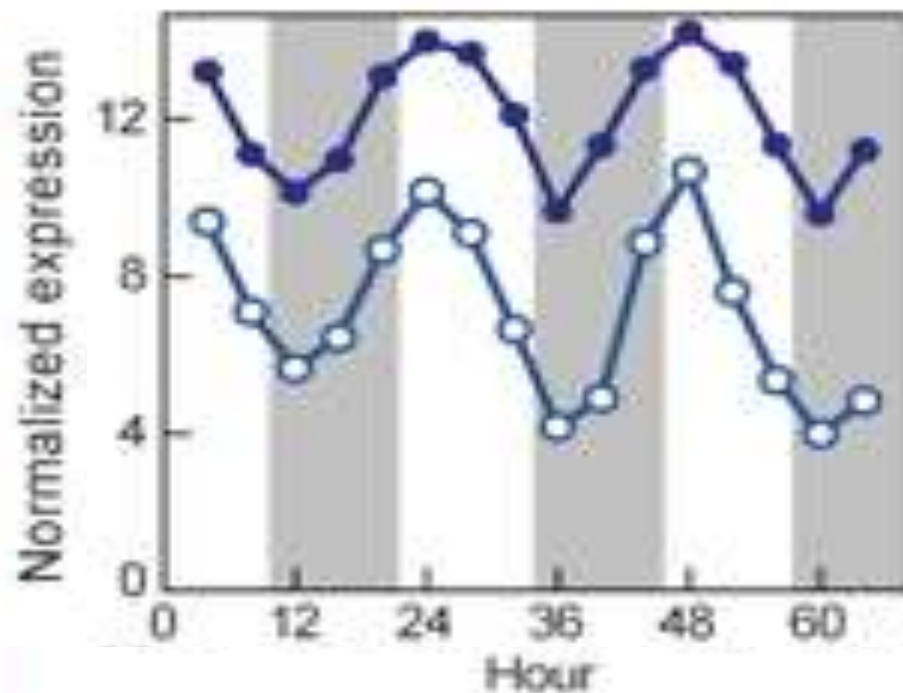
Module example: Significantly coordinated expression patterns between *Trichodesmium* and microbiome.



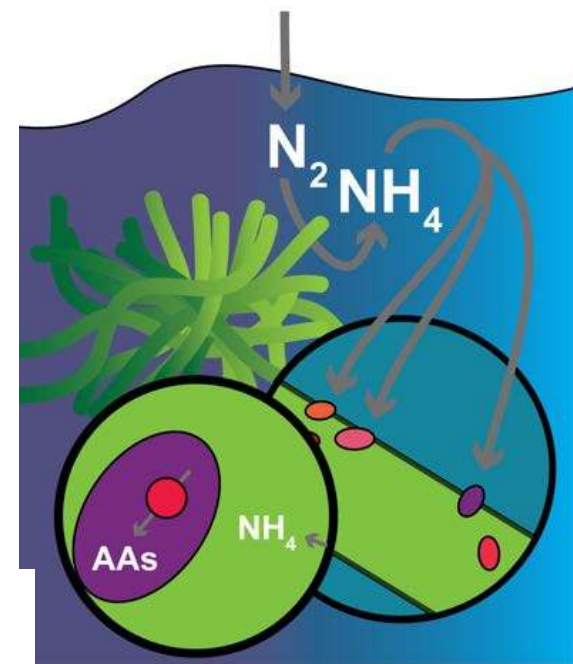
Frischkorn et al. (2018) *ISMEJ*



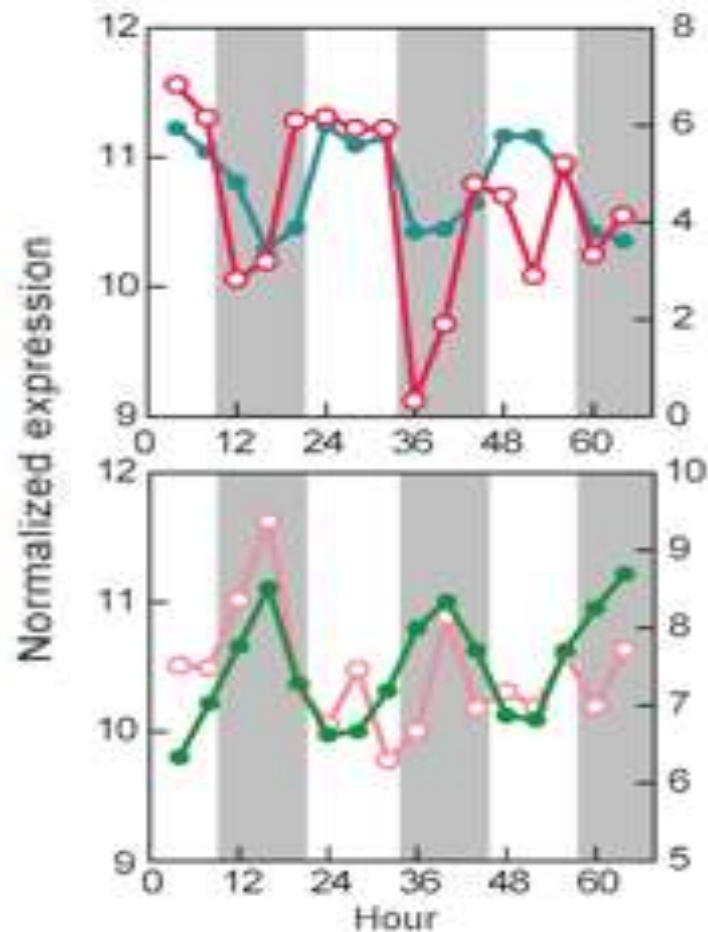
Diel modulation of transcripts suggest coordination: Nitrogen



● *Tricho.* nitrogenase avg.
○ Microbiome N metab. avg.



Diel modulation of transcripts suggest coordination: Carbon



$\text{CO}_2 \rightarrow \text{DOC} \rightarrow \text{CO}_2$
Trichodesmium Microbiome

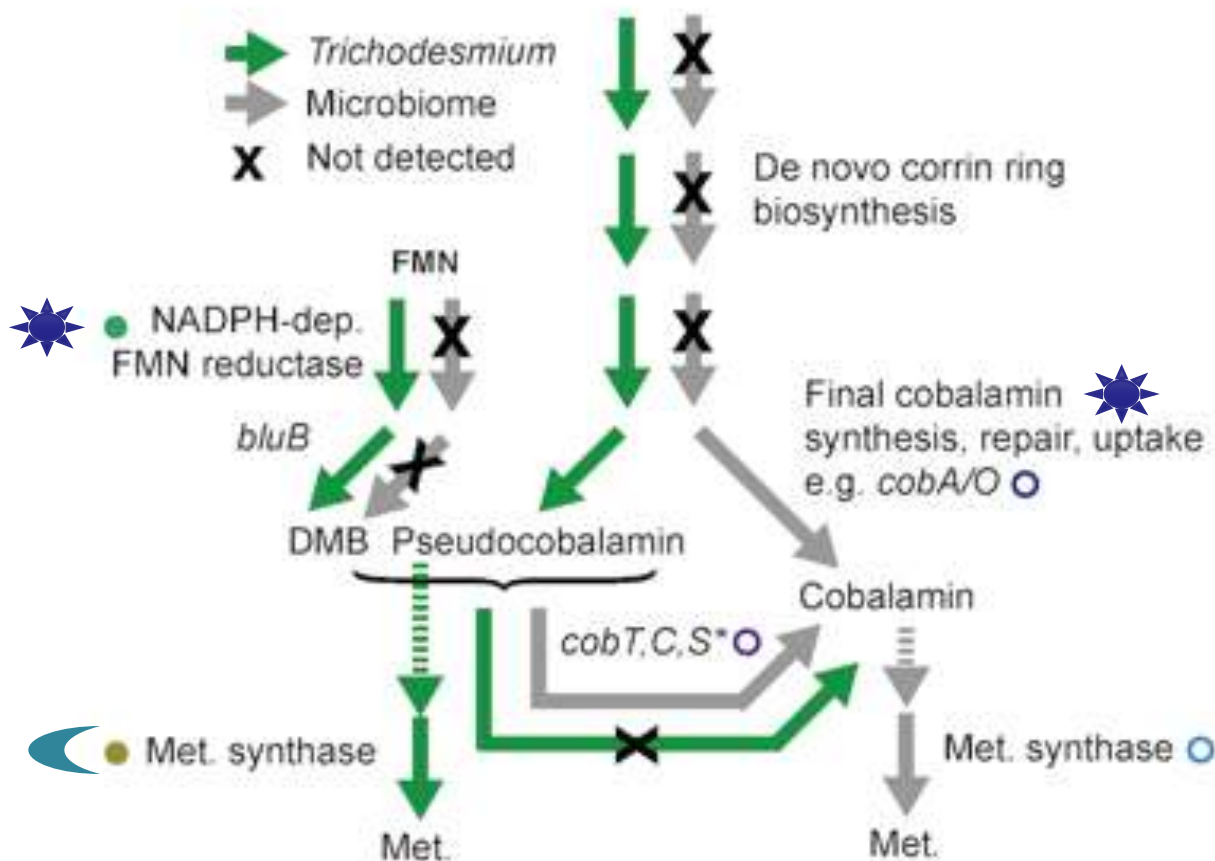
Light Peak

- *Tricho.* photosystem & C fixation avg.
- Microbiome respiration avg.

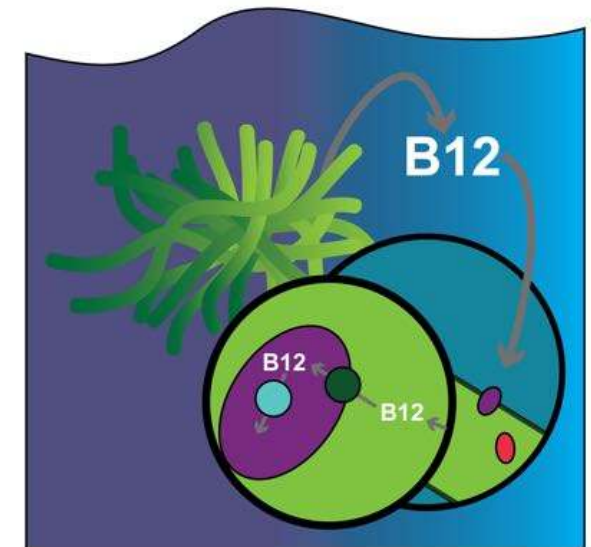
Dark Peak

- *Tricho.* photosystem & C fixation avg.
- Microbiome respiration avg.

Diel modulation of transcripts suggest coordination: Vitamins



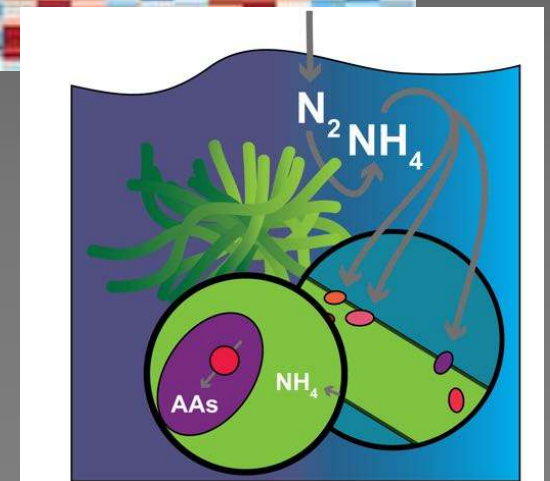
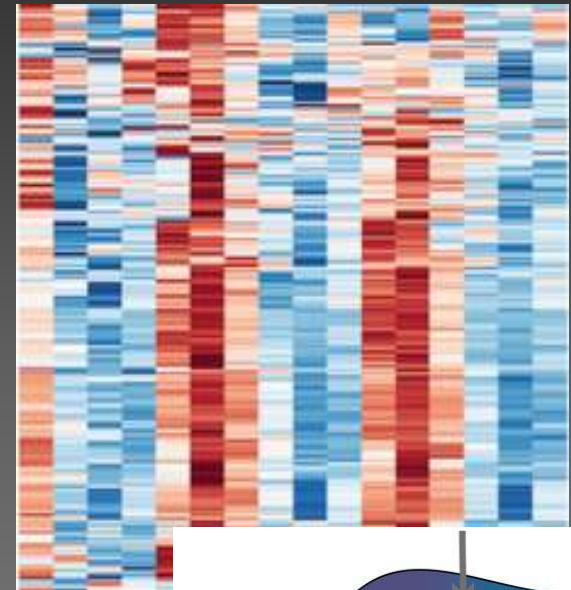
☾ Night peak ★ Day peak



Summary – Interactions coordinated responses between host and heterotrophic microbiome

How does light drive interactions between the microbiome and host?

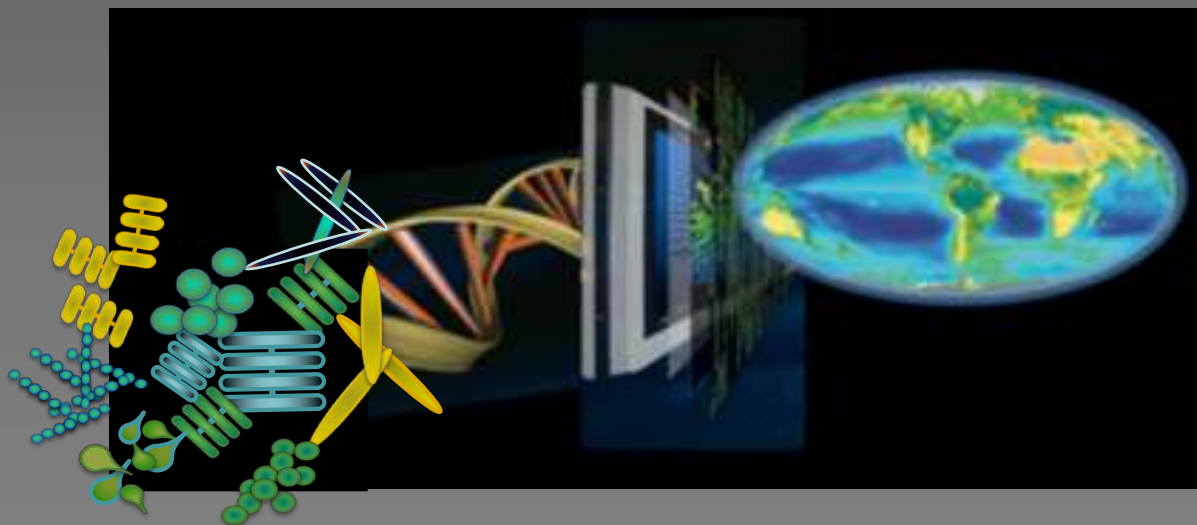
- **Metatranscriptomes:** Heterotrophic epibionts have strong diel patterns, which are coordinated with the patterns present in the host
- **Metatranscriptomes:** Dynamics suggest the exchange of nitrogen, carbon, and vitamins



Conclusions

Transcriptome-enabled approaches are providing new tools to identify the physiological ecology of single species and their role in ecosystem structure and function.

Lessons learned: We need more reference sequences and to think creatively about how we approach and mine sequence data. Need to sample at the same time of day to avoid diel variability.



Acknowledgements

- Dyhrman Lab: Dr. Rachel Wisniewski Jakuba, Dr. Elizabeth Orchard, Dr. Louie Wurch, Abigail Heithoff, Dr. Harriet Alexander, Dr. Kyle Frischkorn, Dr. Alena Sevucu, Dr. Julia Diaz, Dr. Monica Rouco Molina, Dr. Matt Harke, Dr. Gwenn Hennon, Hannah Joy-Warren, Maria Hernandez, Sheean Haley and many undergraduate interns.
- Collaborators: Ben Van Mooy, C-MORE partnership, Mike Lomas and the BATS Program, Jim Ammerman and the ATP³ Program, Eric Webb, Claudia Benitez-Nelson, Ellery Ingall, Dan Repeta, Dennis McGillicuddy, John Waterbury, Cabell Davis, Mak Saito, Bethany Jenkins, Tatiana Ryneerson, SCOPE

- C-MORE
- Simons Foundation
- NSF
- EPA
- NOAA
- DOE, JGI
- WSL
- Angell Foundation



Other things to talk to me about

- Experimental evolution
- Future ocean dynamics
- Harmful algal blooms
- Proteomics and metabolomics
- Working in Antarctica
- Learning in virtual environments

Whyville



