

 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



Culture and field based approaches to physiological ecology

Culture-based experiments

Species-specific responses to wellcontrolled 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



Genome and transcriptome-enabled advances allowing to query cells in their environment in a species specific way

Key themes

Dinofagellates Diatoms Haptophytes

- Our focus is on groups that arguably serve a "keystone" role in the marine ecosystem
- Biogeochemical drivers of physiology:
 - What are the mechanistic underpinnings of resource utilization and how does resource utilization vary over time?
 - Eco-evolutionary traits:
 - Are there functional group traits that drive the structure and function of the NPSG?
- Metabolic plasticity and diversity:
 - How do diversity and metabolism influence the physiogical ecology of a keystone group?



Physiological ecology - a tale of two diatoms in Narragansett Bay



- Do closely related diatoms have the same expressed metabolic capacity?
- Is this metabolic profile consistent over time?
- Are the resource responses the same between species, or is there evidence of resource partitioning of niche space?



Sampling and pipeline



MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Metatranscriptome Data Pipeline - the gory details



Sampling and pipeline



Local isolates provide the most robust read mapping (15X increase for local isolate relative to species in database) - little mapping (<<1%) to diatoms not present in this system

Taxonomic distribution of reads





Expressed metabolic capacity

Quantitative Metabolic Fingerprint (QMF)

Plastic responses over time

Unique responses between diatom genera



Alexander et al. 2015 PNAS

QMF highlights the metabolic traits and tradeoffs



Variability in expressed metabolic capacity for N and P



Transcripts are choreographed with proteins and activities in the model diatom *Thalassiosira pseudonana*



Transcripts are choreographed with proteins and activities in the model diatom *Thalassiosira pseudonana*



Dyhrman et al. 2012 PLoS ONE

Variability in expressed metabolic capacity for N and P



Identifying the resource responsive gene set

• Incubations performed with shifted N:P ratio to provide context for in situ signals



- Resource responsive? Statistically identify resource responsive set (-N v. +N; -P v. +P)
- Stable? Statistically identify stable reference genes (no change over incubations) Alexander et al. 2012 *Front. Micro.*
- Normalize to reference genes (SGNC)



Resource responsive genes identified *in situ*



- Resource responsive sets are distinct from each other and enriched in pathways of N and P metabolism (e.g. urease, phosphatase, phosphate transport etc.)
- Can assign regulation patterns to novel gene families
- But how do we compare signals between species?



Alexander et al. 2015 PNAS

Proportionalize the resource responsive gene set



Visualizing resource responsive signals in the field



Alexander et al. 2015 PNAS



Species variability in response to the same environment



- Finely tuned species-specific variation each response (quadrant) is significantly different from the others
- Responses are orthogonal between species in the same resource environment

Summary - Biogeochemical drivers of physiology

What are the mechanistic underpinnings of resource utilization and how does resource utilization vary over time?

- New approaches for the normalization and comparison of metatranscriptome data.
- Diatom metabolism is highly variable in this estuarine environment
- Uniquely expressed metabolic capacity underpins *Skeletonema* bloom ... and trade offs in N and P metabolism that contribute to the dominance of one diatom over another
- Patterns in resource partitioning may reflect segregation of the realized niche space and explain why so many diatom species can cooccur



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The significance of the NPSG



The keystone microbiome

Net Heterotrophy

Net Autotrophy



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 (• MOGCE linking genomes to biomes (HOE-DYLAN)

Sampling a bloom



Harriet Alexander

Deep water addition led to simulated "bloom"



Sampling and pipeline



MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Marine Microbial Eukaryote Transcriptome Sequencing Project

OPEN a 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

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Greatly expanded reference sequences in the tree of life



Within lineage diversity also greatly expanded



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.

Increase in diatoms during simulated blooms



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



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



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

Variable transcript allocation underpins traits

Variable Transcript Allocation Ratio (VTAR)

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



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

Transcriptional responses underscore functional group traits

Dinoflagellates

Haptophytes

 FEW significant shifts
 Alternative trophic modes?

 Diatoms
 BROAD transcript decreases

 TARGETED transcript increase
 -> "the scavengers"

 r-selected

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.
- Dinoflagellates have little transcriptional response to nutrient input.
- Diatom are particularly responsive (VTAR>1) to nutrient input which likely underpins their dominance in blooms



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Diving beyond the functional group...



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



E. huxleyi life cycle



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





Emiliania huxleyi has many diverse isolates

LETTER

OPEN doi:10.1038/nature12221

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

Betsy A. Read¹, Jessica Kegel², Mary J. Klute³, Alan Kuo⁴, Stephane C. Lefebvre⁵, Florian Maumus⁶, Christoph Mayer^{7.8}, John Miller⁹, Adam Monier¹⁰, Asaf Salamov⁴, Jeremy Young¹¹, Maria Aguilar³, Jean-Michel Claverie¹², Stephan Frickenhaus^{2,13}, Karina Gonzalez¹⁴, Emily K. Herman³, Yao-Cheng Lin¹⁵, Johnathan Napier¹⁶, Hiroyuki Ogata¹², Analissa F. Sarno¹, Jeremy Shmutz^{4,17}, Declan Schroeder¹⁸, Colomban de Vargas¹⁹, Frederic Verret²⁰, Peter von Dassow²¹, Klaus Valentin², Vves Van de Peer¹⁵, 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. Grigoriev⁴



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

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



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center for microbial oceanography: research and education (• MOF Clinking genomes to biomes (HOE-DYLAN)

Responses to shifts in resource ratios





Control











Treatments with added N

Sampling and pipeline



MMETSP = Marine Microbial Eukaryotic Transcriptome Project

Summary - Metabolic plasticity and diversity

How do diversity and metabolism influence the physiogical 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



Summary

• Biogeochemical drivers of physiology:

 Plasticity in diatom physiology may allow for cooccurrence of diverse species.

Eco-evolutionary traits:

 Diatoms appear to be more efficient in turning over their transcriptome than haptophytes which likely underpins their success in response to nutrient pulses in the NPSG.

• Metabolic plasticity and diversity:

- *E. huxleyi* strains vary in the field in response to N, but harbor conserved responses to nutrient pulses in the NPSG.
- New resources and ways of parsing metatranscriptome data are leading to new insights!

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