

A pilot program to characterize the geography and impact of microbial biomes

A progress report from “The Molecular Biology of Biogeochemistry”, an OCB sponsored workshop

Held in Los Angeles, November 2010

Chairs: Jim Moffett (USC), Eric Webb (USC),

Steering Committee: Bob Anderson (Columbia), Ginger Armbrust (UW), Kevin Arrigo (Stanford), Bess Ward (Princeton), Keith Moore (UCI), Benjamin Van Mooy (WHOI), Jed Fuhrman (USC)

Workshop Objective

The goal of this workshop was to explore whether a sectional-survey approach would be useful for marine microbiology and if so, whether the activities of the existing programs, especially GEOTRACES, could complement a new program in some way.

Sectional Survey Programs in Chemical Oceanography

- GEOSECS was a major achievement in Chemical Oceanography that transformed our understanding of major nutrient cycles in the oceans.
- GEOTRACES is building on that foundation by examining a suite of trace elements and isotopes that are of geochemical and biological relevance. Its value was justified to the community by documenting a list of critical problems in the field that cannot be addressed at present because we are data-limited.

How useful are sectional surveys in Biological Oceanography?

Traditional emphasis on rates and processes not very compatible with sampling-intensive survey approach.

Less interest in euphotic-zone interactions with deep ocean regimes, so the surface to seafloor sampling in these surveys of less obvious value.

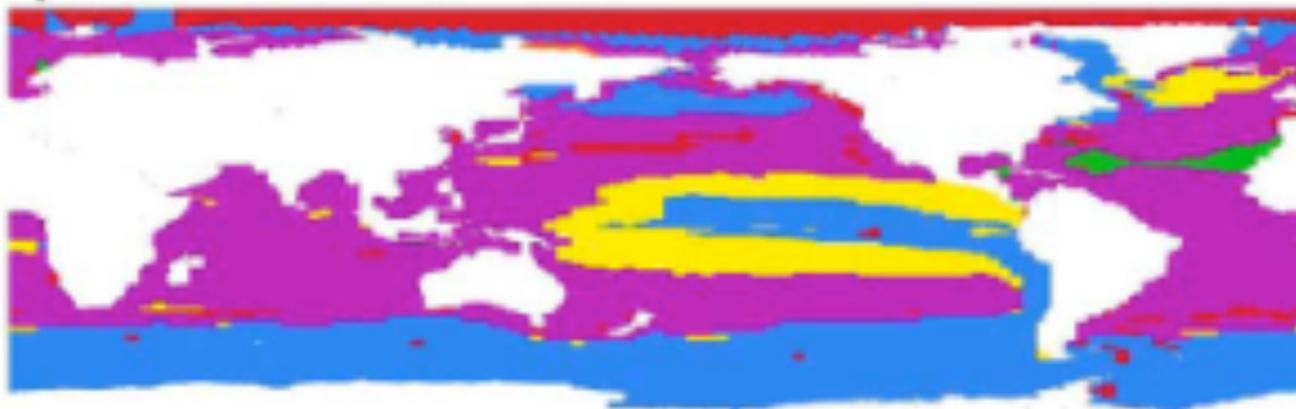
The New Tools of Molecular Biology

- Enable the characterization of microbial community composition, activities of key biogeochemical processes, and probe the relationship between these processes and limiting factors including light, nutrients and micronutrients. *Discrete sampling.*
- Many of these tools can be applied to entire communities (e.g. metagenomics and meta transcriptomics) and can be applied to deep sea communities as well as surface ones, to foster linkage between studies throughout the water column.

Biogeochemical Cycles and Sectional Surveys

- Biogeochemical cycling always important in GEOTRACES. Example Moore and Doney (2004) cited heavily in the GSP.

A) Diatom Growth Limitation



Nitrogen 55.73%, Iron 27.67%, Silica 12.54%, Phosphorus 1.405%
Light 2.645%, Replete 0.000%

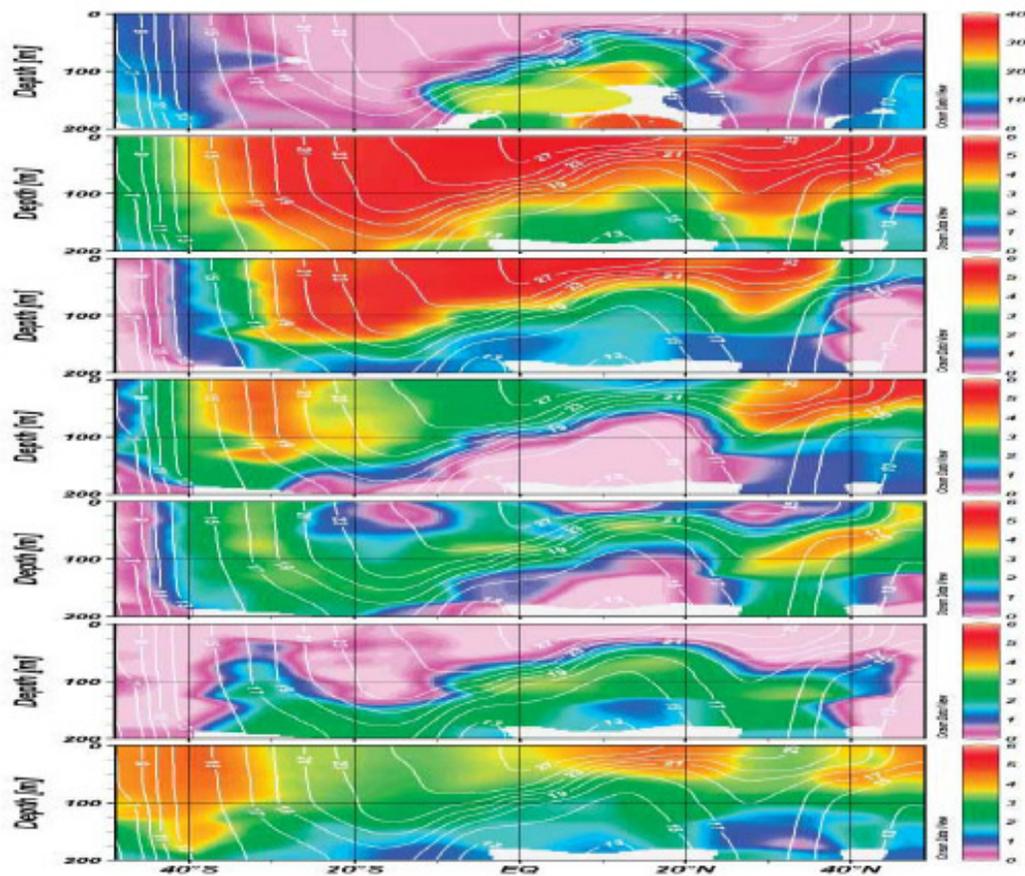
■ Nitrogen ■ Iron ■ Phosphorus ■ Silicon
■ Light ■ Temperature ■ Replete

Original Intent of the Workshop

- Carefully selected suite of molecular biological parameters, for example diagnostics of Fe limitation in diatoms and diazotrophs, and cyanobacterial diversity to compliment key parameters on GEOTRACES
- Inspired by the “Biogeotraces” program developed by Phil Boyd (GEOTRACES SSC)

BIOGEOTRACES

- Samples collected on GEOTRACES cruises of multiple nations for the following parameters
- Diagnostics of N fixation (Julie LaRoche)
- Archaea (Gerhard Herndl)
- *Prochlorococcus* diversity (Chisholm)
- Biogeotraces ([http://www.obs-
vlfr.fr/GEOTRACES/index.php/science/biogeotraces](http://www.obs-vlfr.fr/GEOTRACES/index.php/science/biogeotraces))



Nitrate

Prochl.

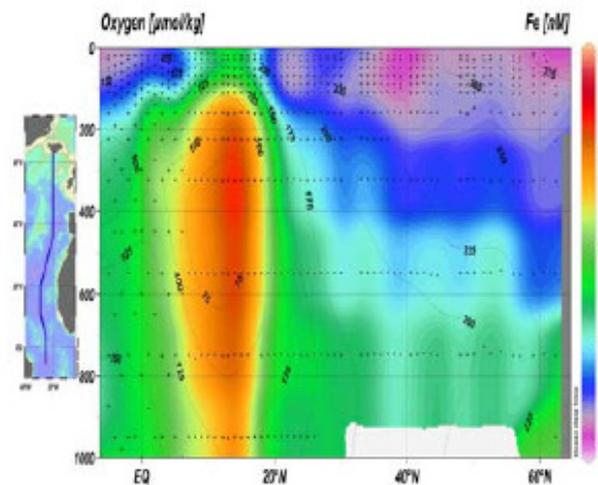
eMIT9312

eMED4

eNATL2A

eMIT9313

Syn.



Workshop Approach

- Identify critical questions in marine microbiology that are difficult to address because we are data-limited.
- Identify ways in which a synthesis of geochemical and molecular biological data could help answer these questions.
- Identify over-arching questions that would make the case for a survey program.

Critical Questions Identified in the Workshop

- 1. *What is the relationship between the photoautotrophic community and heterotrophic community in the euphotic zone?*** How is this relationship influenced by chemical and physical parameters? How does it ultimately influence community structure, nutrient utilization and the f-ratio?
- 2. *What is the relationship between heterotrophy and heterotroph community structure in the deep ocean (including the “twilight zone”) and the overlying biogeochemical regime?*** How does this affect vertical scale of organic carbon regeneration (e.g., the Martin curve) and, ultimately, the oceanic control of atmospheric CO₂ (e.g., Kwon et al., 2009)? What is the relative importance of silicate versus PIC-dominated phytoplankton systems in contributing to carbon export in the deep ocean?
- 3. *How is climate change likely to influence upper ocean ecosystems?*** Will it be primarily through changing the boundaries of existing biogeochemical provinces or by establishing new, unprecedented ones? An example would be high-pCO₂ oligotrophic surface waters. In the contemporary surface ocean, pCO₂ is only high in nutrient-rich upwelling regions.

4. ***What really controls the growth rates of diatoms in different biogeochemical provinces of the oceans?*** What defines the boundaries? What species-specific differences exist within this diverse group of organisms that confounds quantification of bottom-up controls?

5. ***What controls the inventory of fixed N in the ocean?*** How important is denitrification vs. N₂ fixation and how will climate change affect the relative importance?

6. ***What is the relative importance of anammox versus denitrification, especially in oxygen minimum zones (OMZs)?*** How does their relative importance affect how the overall loss of fixed nitrogen is determined by physical and chemical parameters? What factors control the balance between these processes today, and how might this balance be altered by climate change, with concomitant feedbacks that may include N₂O?

Microbial biogeography

Can the concept of microbial biogeography, becoming a valuable tool in many habitats (Martiny et al., 2006), be applied to open ocean marine microbial communities in a rigorous way?

Can we define microbial provinces or biomes where the distributions of phylogenetically unrelated taxa exhibit common boundaries? What is their relationship to biomes defined previously, as in Longhurst (2007), Moore et al., (2004) and Sarmiento et al., (2004)?

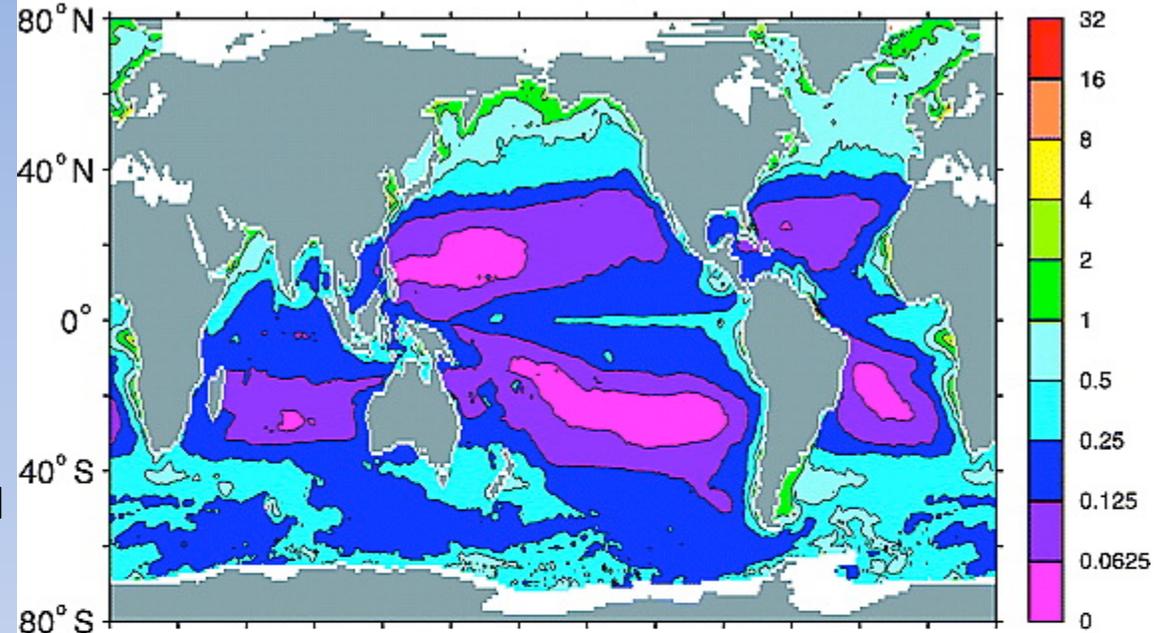
What is the relationship between biome boundaries in the upper ocean and deep ocean?

What parameters *determine* these boundaries? What parameters are most useful in *defining* these boundaries?

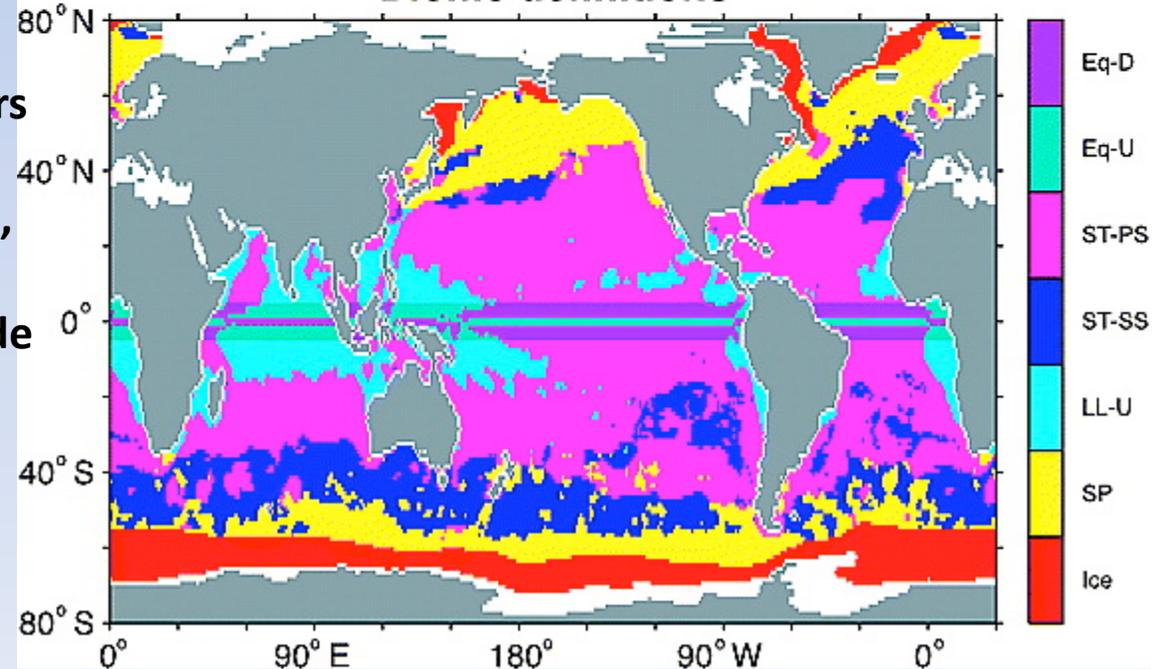
SARMIENTO, J. L. and others. 2004.
Response of ocean ecosystems to
climate warming. *GBC 18*: GB3003.

- (a) Annual mean SeaWiFS
chlorophyll from Yoder and
Kennelly (2005)
- (b) Biome classification scheme
calculated using mixed layer
depths obtained from observed
density and from upwelling
calculated from the wind stress
divergence using observed winds.
Eq-U The equatorially influenced
biome areas where upwelling occurs
Eq-D where downwelling occurs
“Ice” is the marginal sea ice biome,
“SP” is the subpolar biome, the
“LL-U” (light blue) is the low-latitude
upwelling biome,
“ST-SS” is the seasonally mixed
subtropical gyre biome,
“ST-PS” is the permanently
stratified subtropical gyre biome.

SeaWiFS Chlorophyll (mg m^{-3})



Biome definitions



The Case for a Stand-Alone Program

Logistically impossible to accommodate the full sampling and personnel needs for extensive molecular biology on GEOTRACES cruises, which are already strained for space and ship time.

The GEOTRACES sampling strategy, with a focus on high-resolution sampling throughout the water column, was seen by the workshop participants to have too much focus on the deep ocean and too little in the upper water column.

Rationale for complete, boundary to boundary sections less compelling.

Some process work (e.g., rate measurement) is desirable, and this required fewer stations to allow more time for these experiments.

Measurements to be made on this program

Sampling scheme

Parameter to be measured midday and midnight

Core stations

- Metals, Nutrients (dissolved inorganic and dissolved organic), PAR, size fractionated Chla, POC, PON, Flow cytometry Bacterial/picoeuk counts,
- Microscopy counts of larger Euks, samples for microarrays, metagenomics and metatranscriptomics, Size spectrum from smallest viruses to largest protists.

'Rate' stations

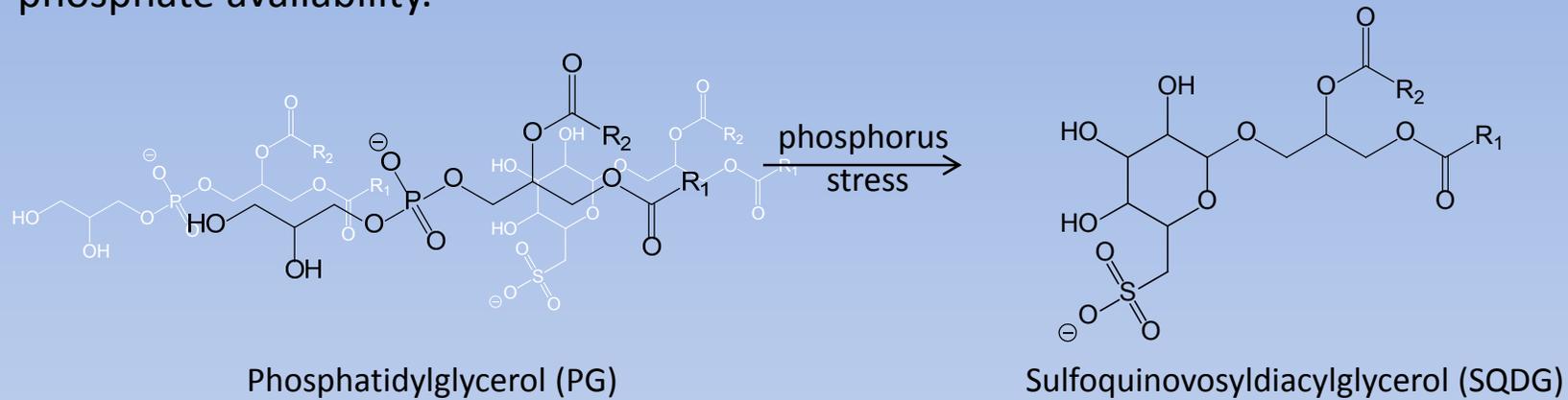
- Metabolomics, Proteomics, Nitrogen fixation, Denitrification, ¹⁵N isotopic labeling for N cycling, Primary and Bacterial production, vitamin utilization, Stable isotope probing of nitrogen source utilization,
- Anammox, Manipulation experiments to test limitation

Metabolomics: Definition and relevance

Metabolomics, defined in the broadest and most ambitious sense to include all biochemistry left uncharacterized by the aforementioned RNA, DNA, and protein analyses.

The distribution of compounds measured within the metabolome probably don't determine biome boundaries, but they may have more influence than nutrients and micronutrients in determining the relationships amongst taxa and they may be exceedingly useful in defining biome boundaries

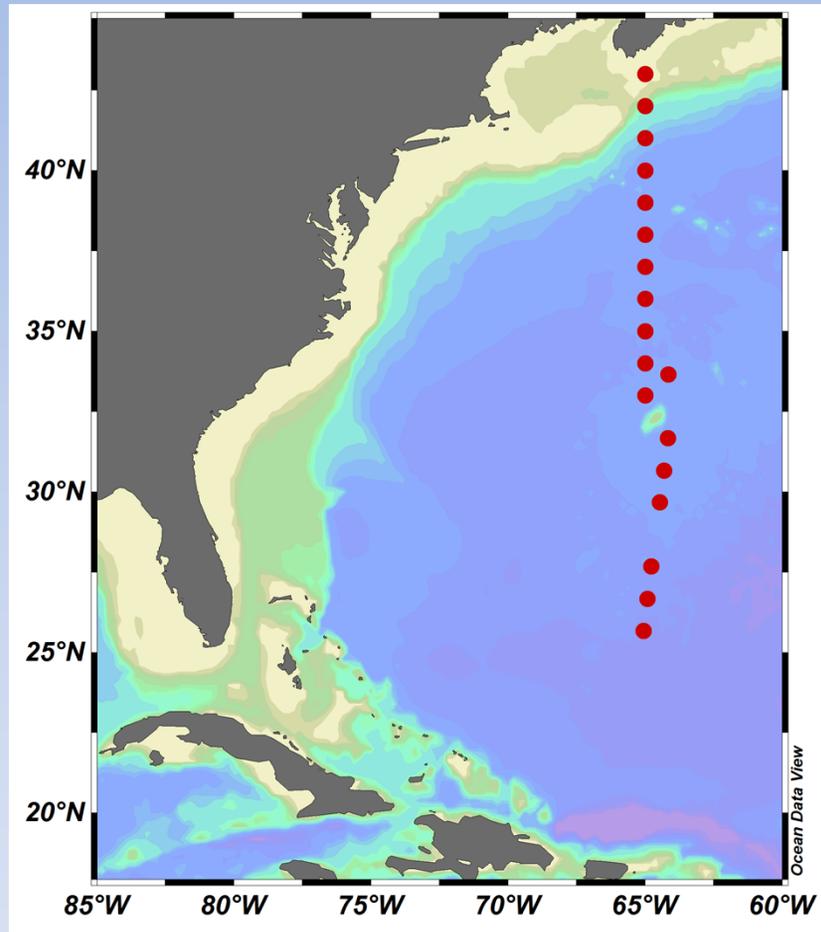
Using meta-lipidomics to assess ecosystem-scale physiological responses to changes in phosphate availability.



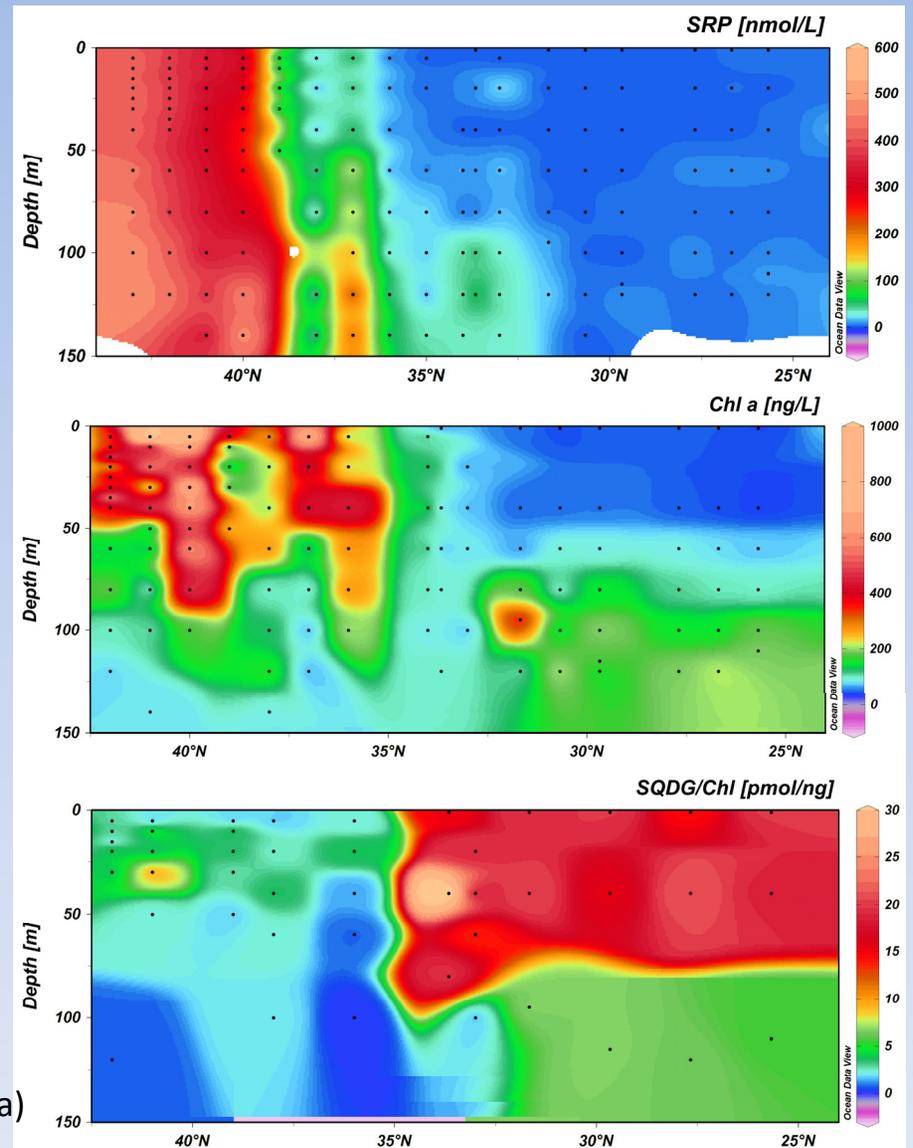
- Substituting SQDG for PG is a physiological mechanism for phytoplankton to decrease P demand (leading to increased C:P).
- Offers quantitative physiological insights on the coupling between SRP and C:P.

Van Mooy and Lomas (unpublished data)

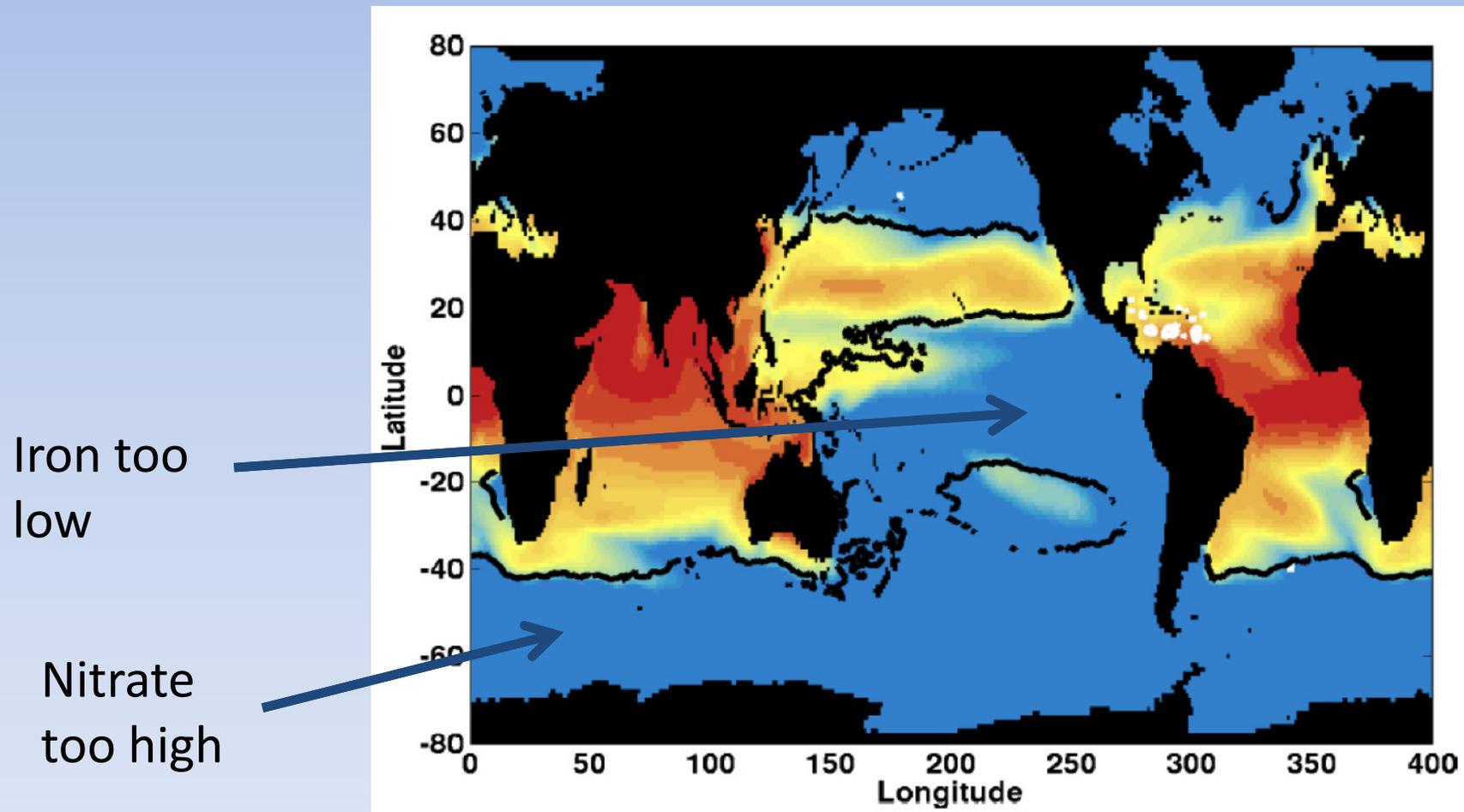
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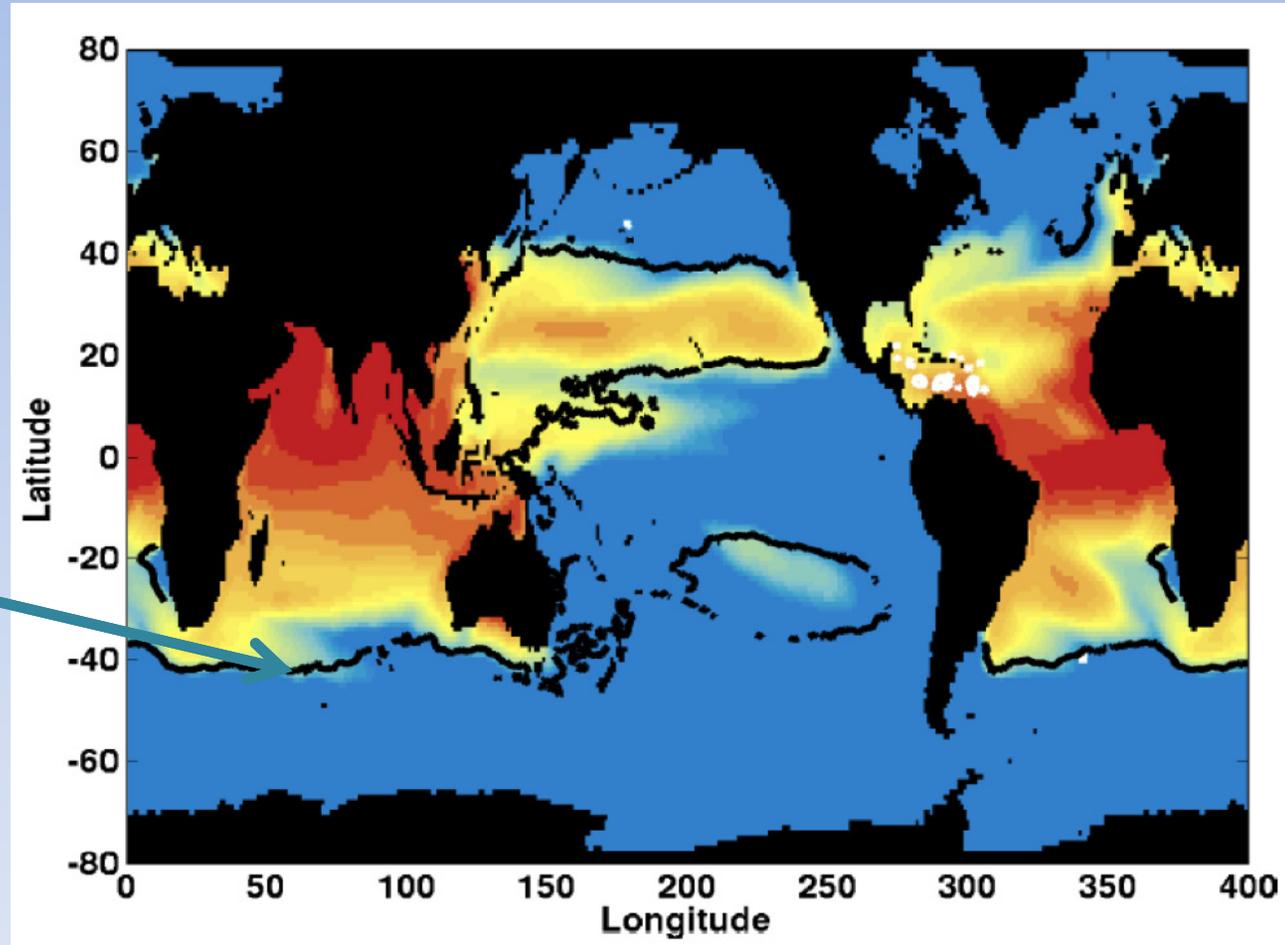
Molecular markers for Fe limitation of N fixation



(Model simulation from Mick Follows, 2012)

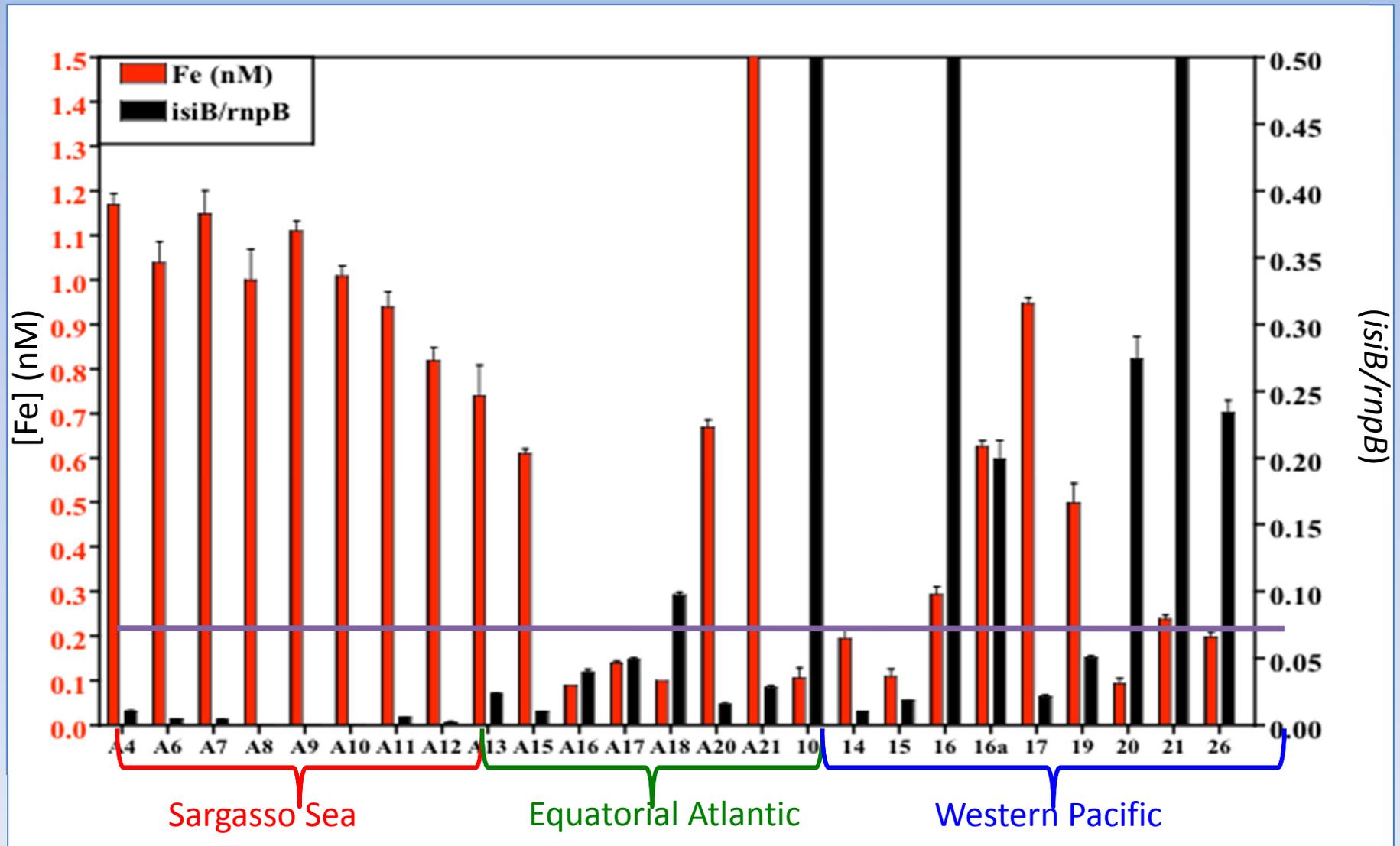
Formalize relationship between iron and diazotroph range

$$\overline{Fe} = \frac{K_{Fe} m}{\mu_{\max} - m}$$



Trichodesmium Experiences Fe limitation in the Field

Chappell, P.D., Moffett, J.W., Hynes, A.M. and Webb, E.A. (2012) MOLECULAR EVIDENCE OF IRON LIMITATION AND AVAILABILITY IN THE GLOBAL DIAZOTROPH TRICHODESMIUM. (In Press) The ISME Journal.

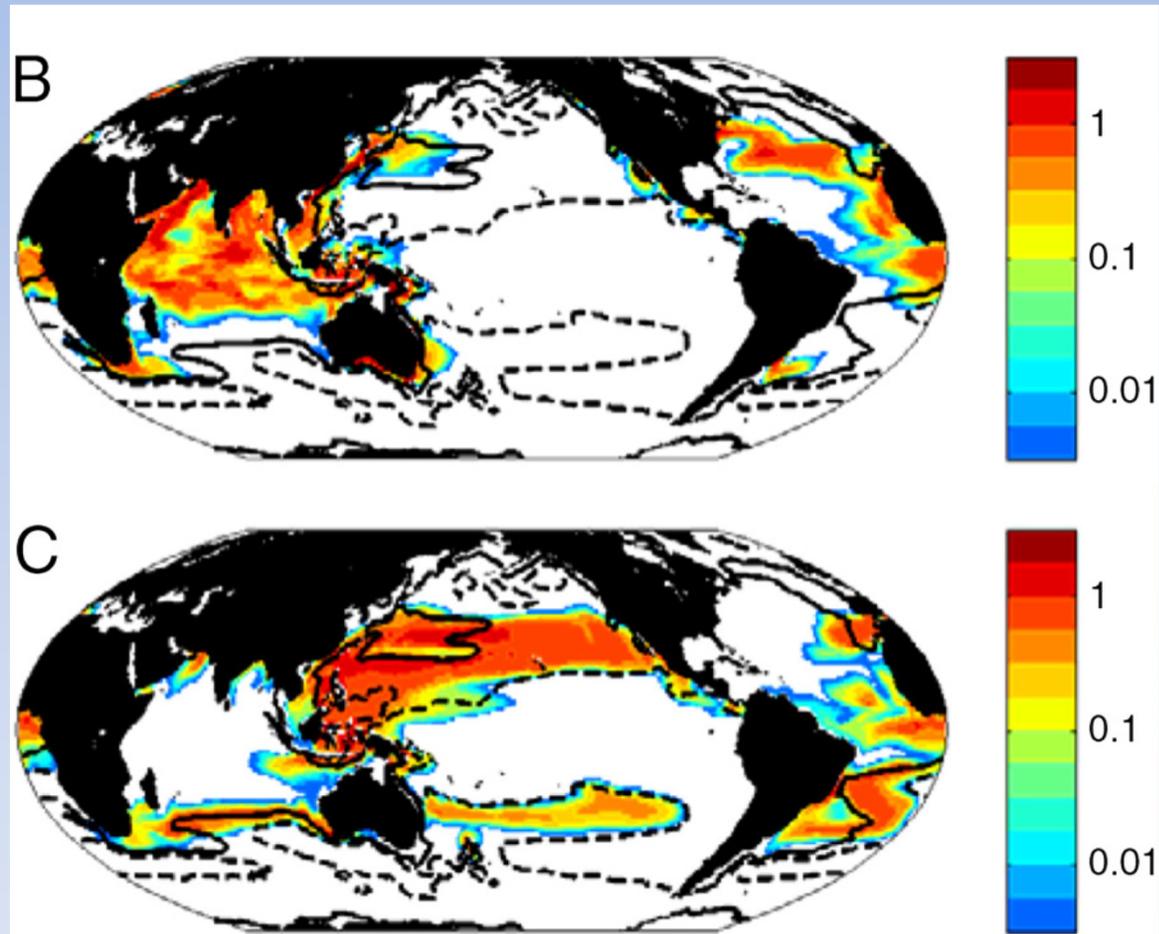


Another biomarker for a different diazotroph: *Crocosphaera*.

High iron quota
High growth rate

(mg C m^{-3})

Low iron quota
Low growth rate
“Hotbunkers”



Saito et al (2011)

Stephanie Dutkiewicz

Metagenomics

- DNA sequencing of entire communities to characterize complete assemblages
- New insights about processes e.g. Novel light harvesting protein proteorhodopsin and related proteins.
- No hydrographic or biogeochemical context in earliest studies.

Going Deeper: Metagenome of a Hadopelagic Microbial Community

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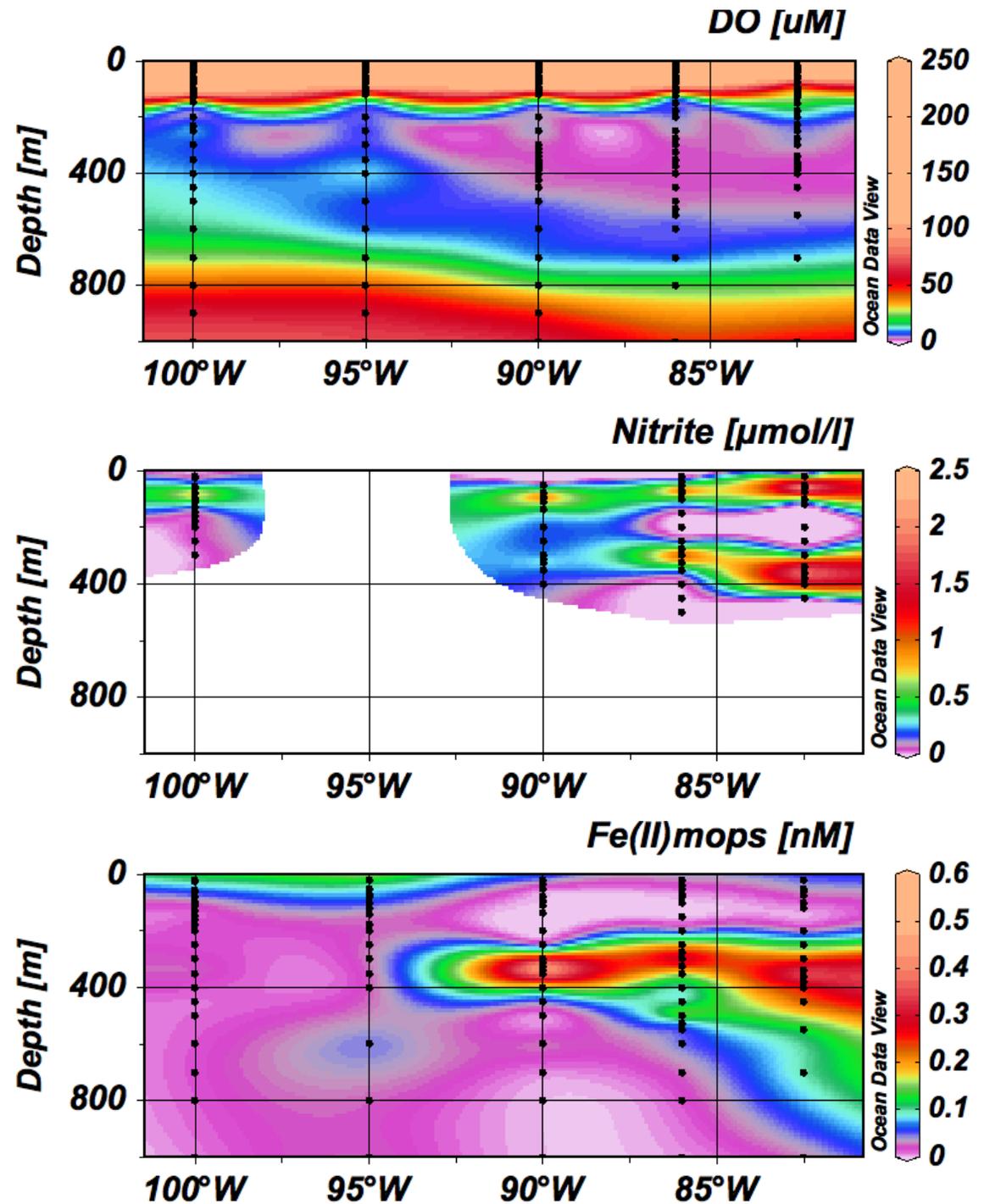
1 Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, United States of America, **2** Microbial and Environmental Genomics, J. Craig Venter Institute, La Jolla, California, United States of America, **3** J. Craig Venter Institute, Rockville, Maryland, United States of America, **4** Division of Biological Sciences, University of California San Diego, La Jolla, California, United States of America

Abstract

The paucity of sequence data from pelagic deep-ocean microbial assemblages has severely restricted molecular exploration of the largest biome on Earth. In this study, an analysis is presented of a large-scale 454-pyrosequencing metagenomic dataset from a hadopelagic environment from 6,000 m depth within the Puerto Rico Trench (PRT). A total of 145 Mbp of assembled sequence data was generated and compared to two pelagic deep ocean metagenomes and two representative surface seawater datasets from the Sargasso Sea. In a number of instances, all three deep metagenomes displayed similar trends, but were most magnified in the PRT, including enrichment in functions for two-component signal transduction mechanisms and transcriptional regulation. Overrepresented transporters in the PRT metagenome included outer membrane porins, diverse cation transporters, and di- and tri-carboxylate transporters that matched well with the prevailing catabolic processes such as butanoate, glyoxylate and dicarboxylate metabolism. A surprisingly high abundance of sulfatases for the degradation of sulfated polysaccharides were also present in the PRT. The most dramatic adaptational feature of the PRT microbes appears to be heavy metal resistance, as reflected in the large numbers of transporters present for their removal. As a complement to the metagenome approach, single-cell genomic techniques were utilized to generate partial whole-genome sequence data from four uncultivated cells from members of the dominant phyla within the PRT, Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes and Planctomycetes. The single-cell sequence data provided genomic context for many of the highly abundant functional attributes identified from the PRT metagenome, as well as recruiting heavily the PRT metagenomic sequence data compared to 172 available reference marine genomes. Through these multifaceted sequence approaches, new insights have been provided into the unique functional attributes present in microbes residing in a deeper layer of the ocean far removed from the more productive sun-drenched zones above.

Dissolved oxygen, nitrite and Fe(II) on a zonal transect off Peru at 10° S

Data show a secondary nitrite maximum at 350m that coincides with Fe(II).



Data Synthesis

- Cluster core phylogenetic markers (e.g. 16sRNA)
- Compare these clustered or binned sequences with geochemical parameters statistically or visually
- A useful approach for community level parameters and individual taxa (e.g. Martiny et al., 2009)
- Community level interactions can also be studied as networks

Timeline

- Nov 2010 OCB Workshop

2011

- Ginger Armbrust agrees to lead an effort to establish a stand-alone program
- Town meeting at ASLO meeting, led by Ben Van Mooy and Bethany Jenkins
- Workshop Report Released
- Ginger travels to NSF to develop a strategy for launching a pilot program

Timeline (continued)

2012

- EAGER award to Armbrust, Moffett, Cutter, Ingalls, Twining, Morris to stage a Pilot Cruise GEOMICS (Genome-Enabled Ocean Microbiology Integrated with Chemical Surveys)
- May, Cruise staged along Line P involving 17+ research groups spanning a range of disciplines
- Objective: Characterize gradients across a coastal/ oceanic boundary

GEOMICS Cruise

- Seven Stations along Line P spanning shelf and blue-water regimes. Samples collected using the GEOTRACES Rosette and ship's rosette as well as large volume pumping systems.

Rationale for this section:

- Well studied line with major gradients in phytoplankton community structure.
- Tractable plan given short time frame and need for non-NSF ship-time.

Participants on GEOMICS Cruise

PI	Measurement		
Ginger Armbrust	Chief Scientist Underway flow cytometry Nitrate sensor Temperature, salinity Eukaryotic metatranscriptomics DIC, O ₂ measurements nutrients, chlorophyll a, fixed samples for flow cytometry, microscopy, Bsi	<i>Jim Moffett</i>	Dissolved Fe, Cu, Mn and Zn
		<i>Adrian Marchetti</i>	Fast repetition fluorescence, iron status of Pseudo-nitzschia, diatom isolates
		<i>Carl Carranos</i>	molecular detection of photoreactive siderophores
		<i>Bethany Jenkins</i>	molecular detection of Thalassiosira iron status
<i>Mary Ann Moran</i>	Prokaryotic metatranscriptomics	<i>Sergio Sanudo-Wilhelmy</i>	B vitamins (particulate) B vitamins (dissolved)
<i>Al Devol</i>	nitrification rates		
<i>Ben van Mooy</i>	intact polar membrane lipids for nutrient physiology	<i>Gabrielle Rocap</i>	cyano distributions
Bob Morris	metaproteomics, total bacterial counts, fixed samples for FISH DNA for bacterial, archaeal community composition	<i>Anitra Ingalls</i>	stable isotope probing, nitrogen uptake, vitamin, targeted metabolites qPCR for nitrification,
<i>Liz Kujawinski</i>	metabolites high resolution DOM characterization POC, bulk DOC, TOC	<i>Ben Twining</i>	particulate metals; cell quotas
		<i>Ann Pearson</i>	Archeal proteomics and metabolomics
		<i>Kathy Barbeau</i>	Iron speciation
		<i>Mak Saito</i>	Proteomics, Cobalt

Next Steps

- December 2012 Analyses completed for parameters measured on cruise. Results shared amongst all participating groups.
- Spring 2013 A Data Synthesis workshop that may be combined with a planning meeting to develop a new program.
- May 2013 Gene sequences submitted to NCBI.

Acknowledgments

- Eric Webb and the speakers and participants of the OCB Workshop
- Heather Benway and Mary Zawoysky
- Ginger Armbrust
- NSF: Dave Garrison, Don Rice, Mike Sieracki and Roberta Marinelli

2013 Chemical Oceanography Gordon Conference

Jim Moffett, Chair, Kathleen Ruttenberg,
Vice-Chair

Theme: Chemical Geography of the Sea