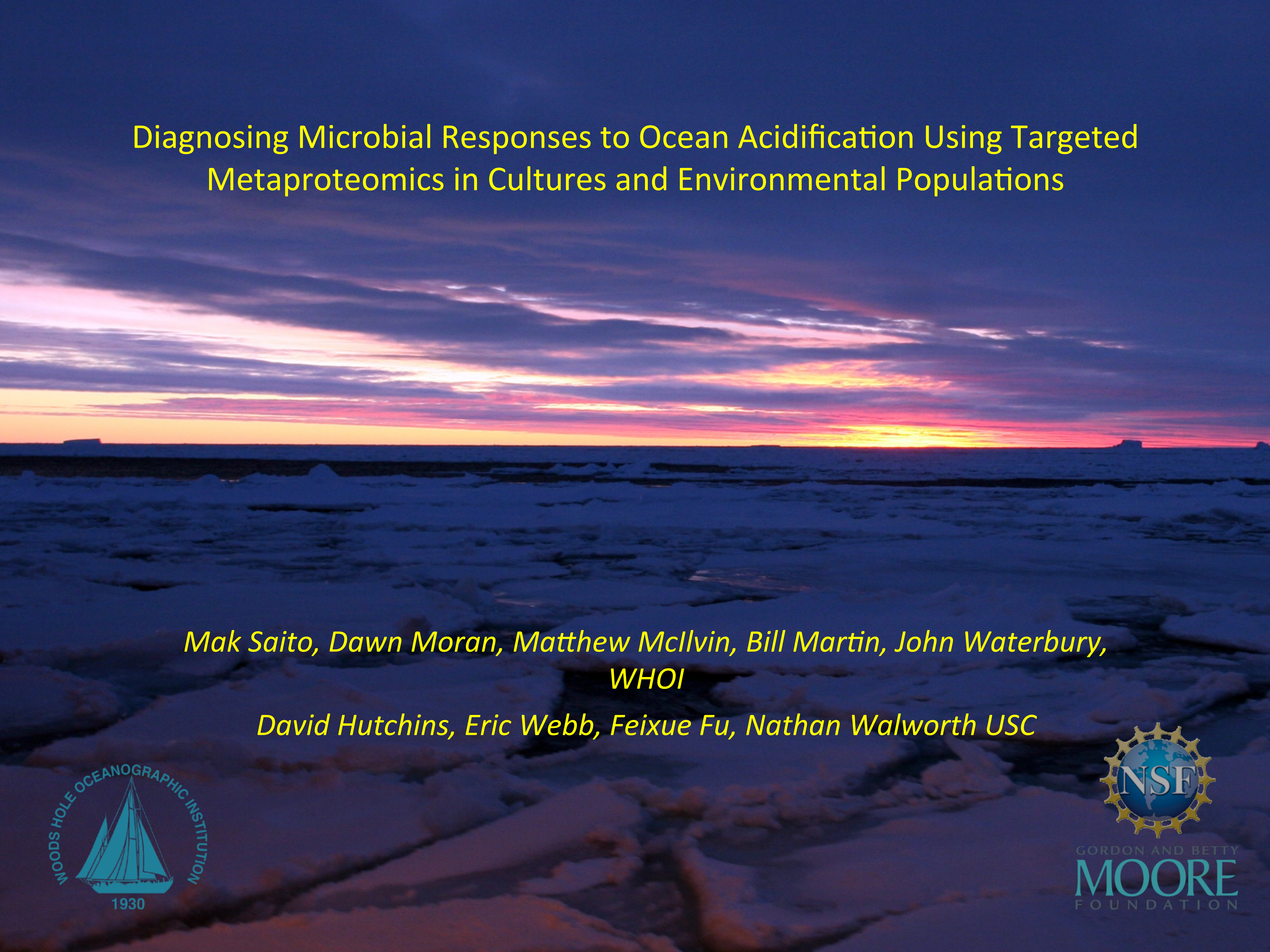


Diagnosing Microbial Responses to Ocean Acidification Using Targeted Metaproteomics in Cultures and Environmental Populations

Mak Saito, Dawn Moran, Matthew McIlvin, Bill Martin, John Waterbury, WHOI

David Hutchins, Eric Webb, Feixue Fu, Nathan Walworth USC

Challenges of studying ecosystem and biogeochemical changes

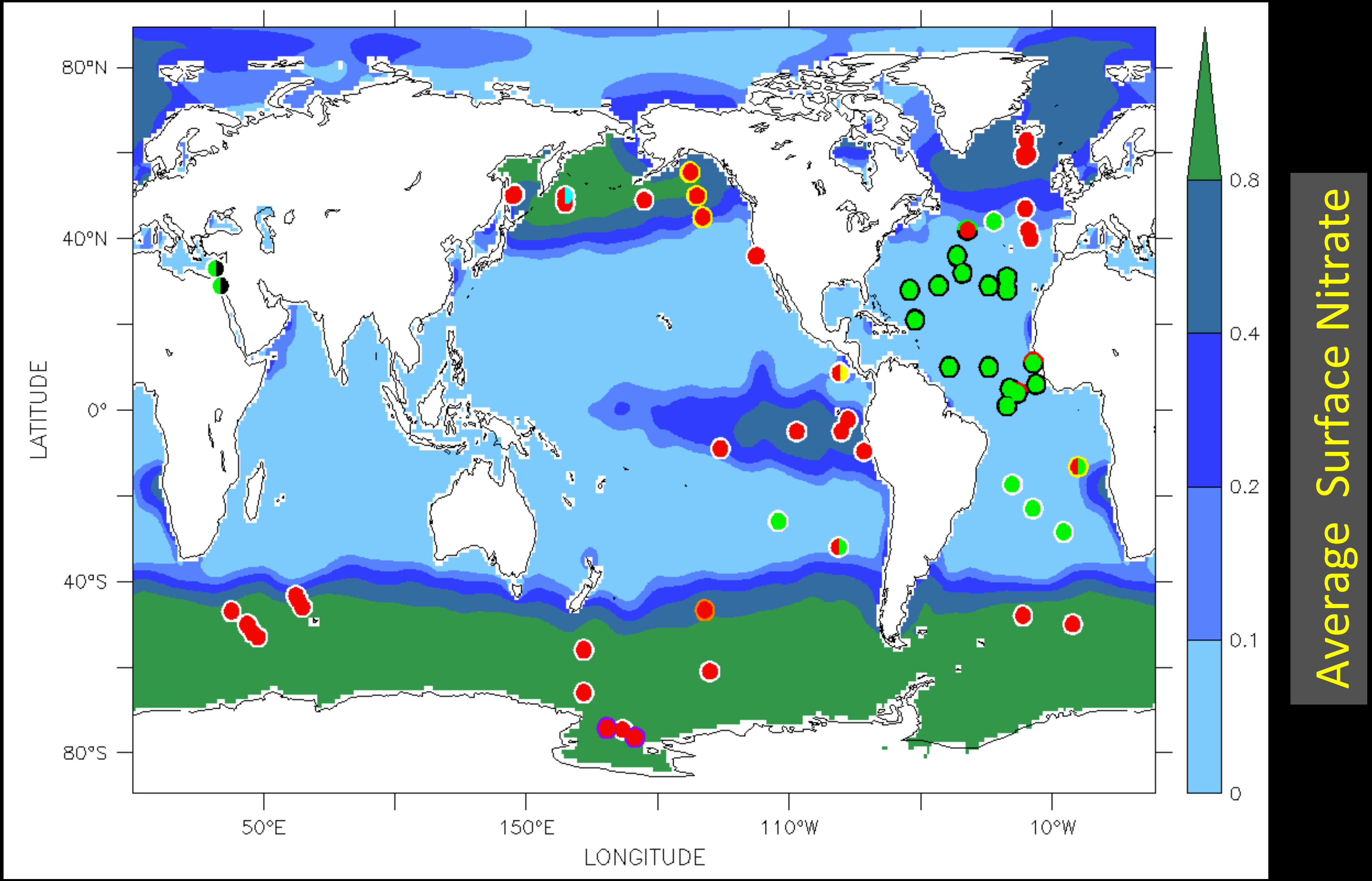
- The oceans are vast: Difficult to extrapolate from limited spatial and temporal sampling
- Potential for multiple stressors

- Developed a new method: *Targeted Metaproteomics*
  - *Can contribute significantly to these challenges*

Described in two recent/upcoming papers:

- Saito et al., Multiple nutrient stresses at intersecting Pacific Ocean Biomes detected by proteomic biomarkers. *Science 2014*
- Saito et al., Targeted Metaproteomics: Finding sub-species needles in the vast blue sea. *Proteomics in press.*

Observations of Multiple Nutrient Stressors on Marine Primary Productivity



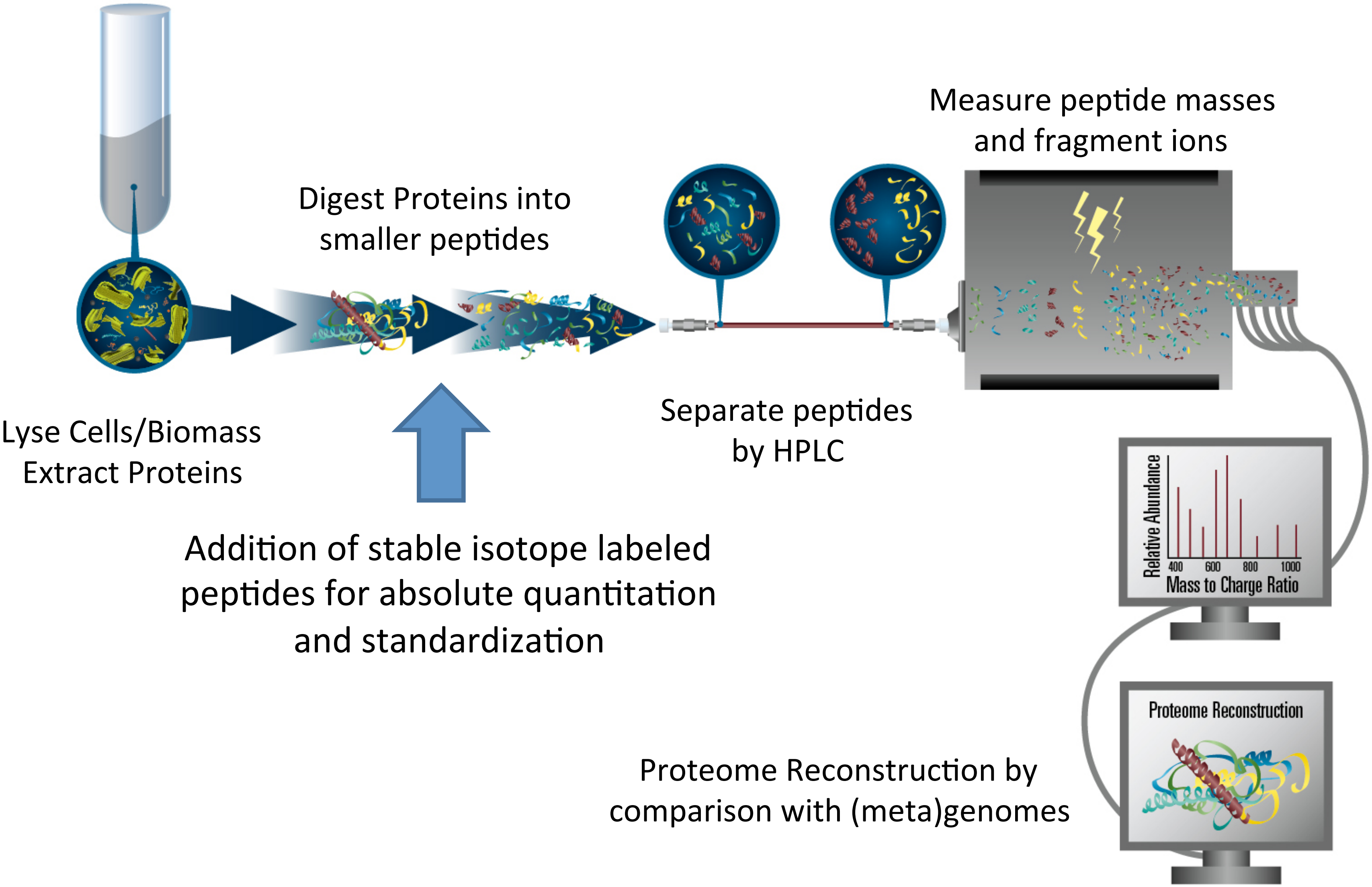
Circle *Interior*=primary limiting nutrient; Circle *Exterior*=secondary limiting nutrient

Red=Iron Green=Nitrogen Black=Phosphate; Purple=B12 Yellow=Cobalt Cyan=Zn

Moore et al., Nature Geosciences 2013

Can we measure biomarkers that can measure nutrient stresses?

Proteomics – the measurement of many proteins simultaneously (typically by mass spectrometry)



Lyse Cells/Biomass
Extract Proteins

Digest Proteins into
smaller peptides

Addition of stable isotope labeled
peptides for absolute quantitation
and standardization

Separate peptides
by HPLC

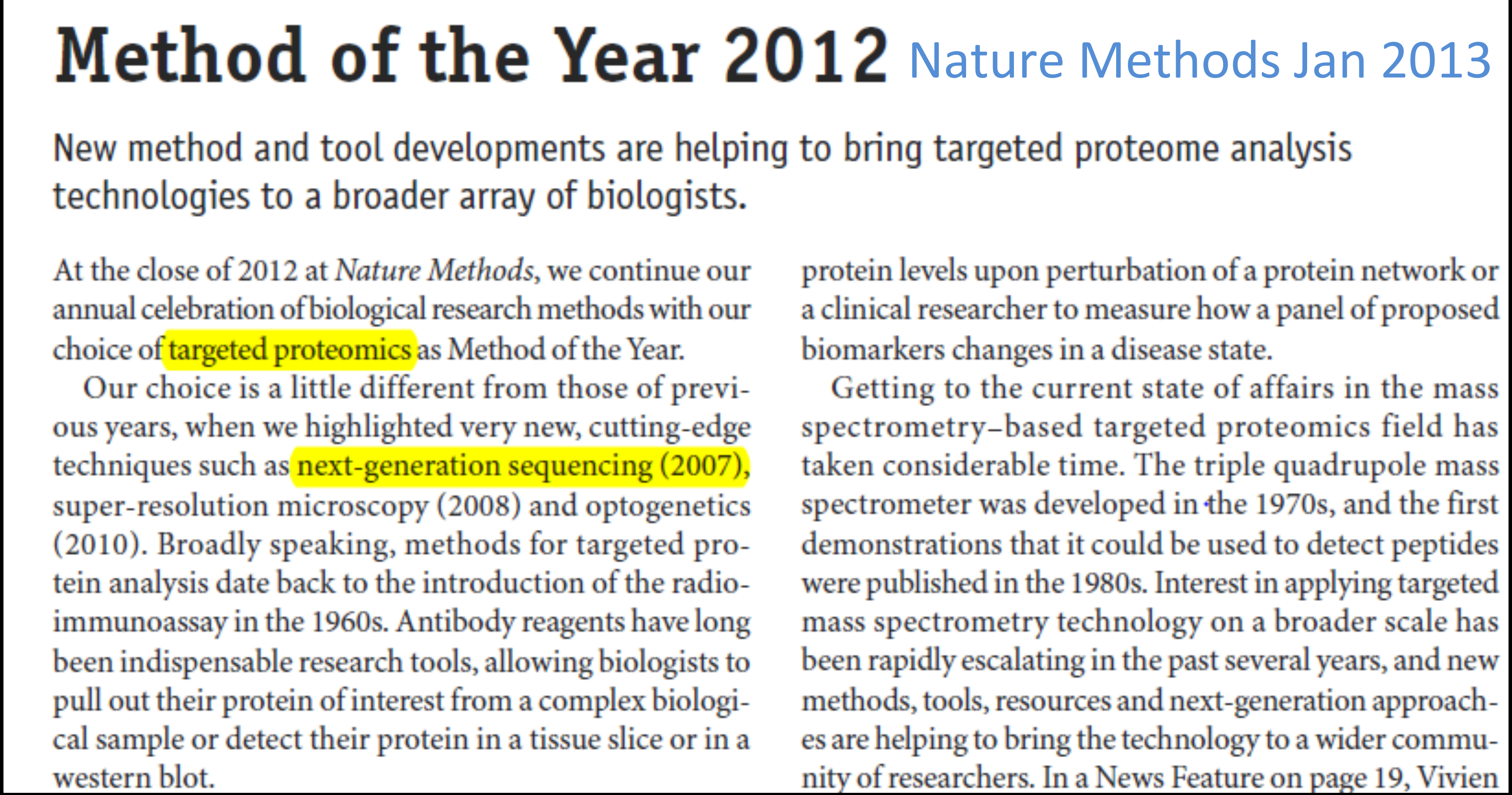
Measure peptide masses
and fragment ions

Proteome Reconstruction by
comparison with (meta)genomes

"Targeted Metaproteomics"

Meta'omics – the study of diverse environmental populations

Saito et al., Targeted Metaproteomics: Finding sub-species
needles in the vast blue sea. *Proteomics in press.*



Method of the Year 2012

Nature Methods Jan 2013

New method and tool developments are helping to bring targeted proteome analysis technologies to a broader array of biologists.

At the close of 2012 at *Nature Methods*, we continue our annual celebration of biological research methods with our choice of targeted proteomics as Method of the Year.

Our choice is a little different from those of previous years, when we highlighted very new, cutting-edge techniques such as next-generation sequencing (2007), super-resolution microscopy (2008) and optogenetics (2010). Broadly speaking, methods for targeted protein analysis date back to the introduction of the radioimmunoassay in the 1960s. Antibody reagents have long been indispensable research tools, allowing biologists to pull out their protein of interest from a complex biological sample or detect their protein in a tissue slice or in a western blot.

protein levels upon perturbation of a protein network or a clinical researcher to measure how a panel of proposed biomarkers changes in a disease state.

Getting to the current state of affairs in the mass spectrometry–based targeted proteomics field has taken considerable time. The triple quadrupole mass spectrometer was developed in the 1970s, and the first demonstrations that it could be used to detect peptides were published in the 1980s. Interest in applying targeted mass spectrometry technology on a broader scale has been rapidly escalating in the past several years, and new methods, tools, resources and next-generation approaches are helping to bring the technology to a wider community of researchers. In a News Feature on page 19, Vivien

Translating protein quantitation technology for the oceans
This startup uses the same technology applied to tumors…

PROTEOMICS AND GENOMICS

from the same FFPE tissue

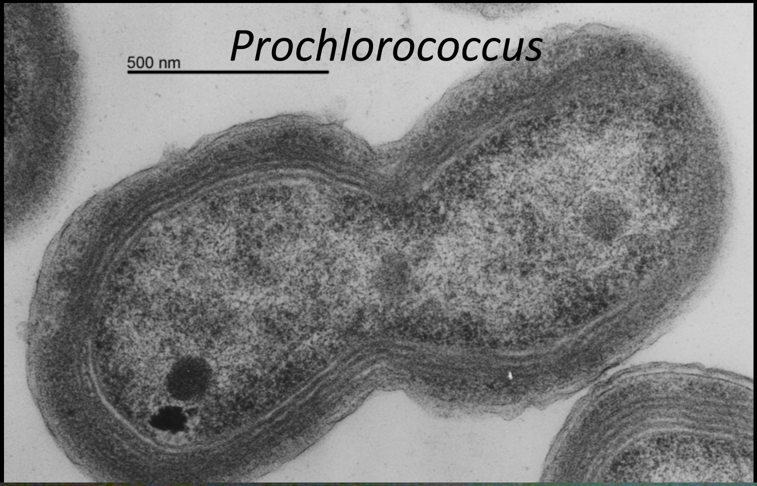
View OncoPlexDx news and upcoming events

Absolute quantification of protein markers and Genomic Mutation identification for

How will microbes respond to environmental changes?

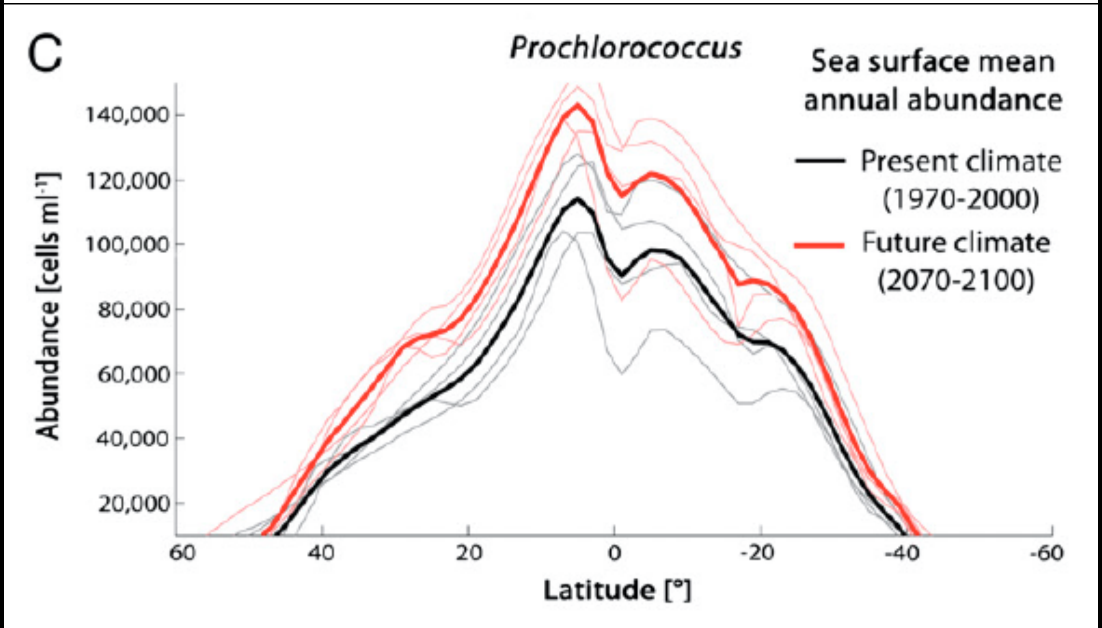
Prochlorococcus – Abundant primary producer in the oceans

Trichodesmium – Major nitrogen fixer in oligotrophic oceans

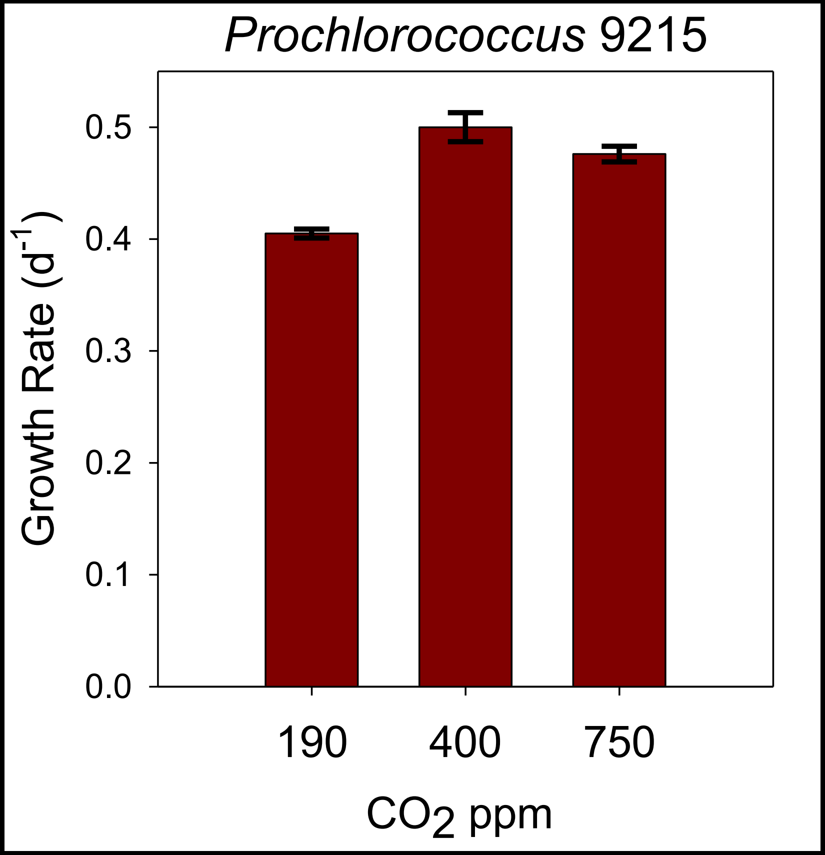


Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*

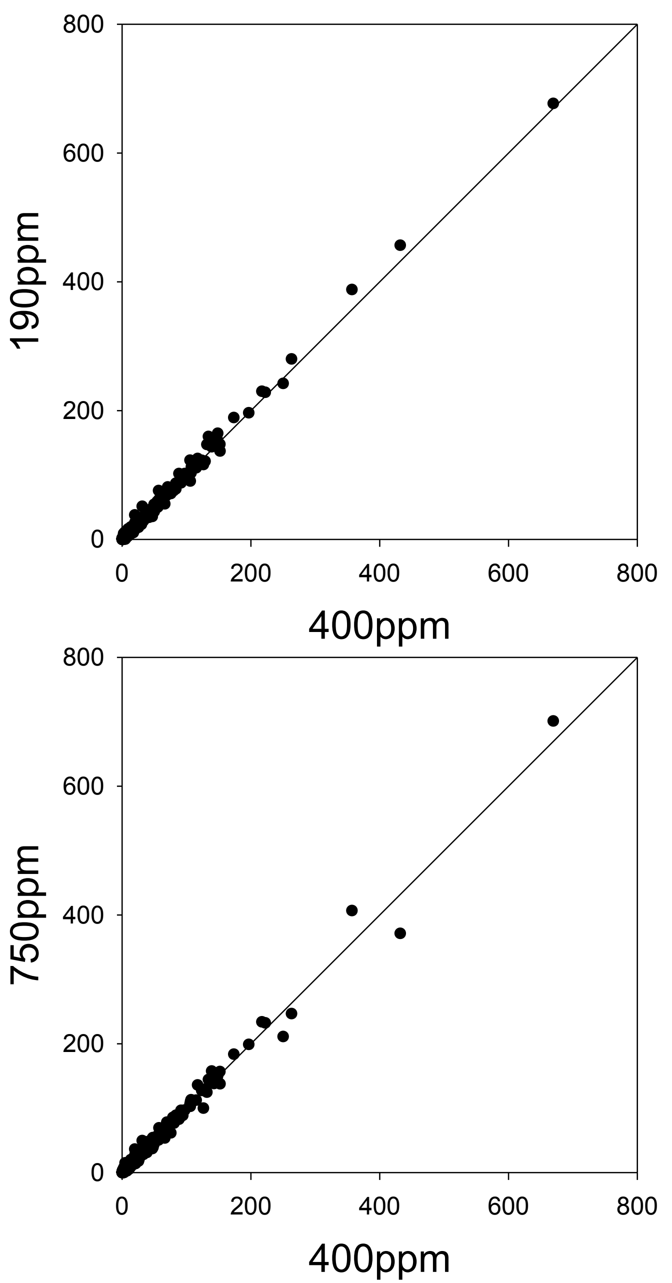
Pedro Flombaum[a,b], José L. Gallegos[a], Rodolfo A. Gordillo[a], José Rincón[a], Lina L. Zabala[b], Nianzhi Jiao[c], David M. Karl[d,1], William K. W. Li[e], Michael W. Lomas[f], Daniele Veneziano[g], Carolina S. Vera[b], Jasper A. Vrugt[a,h], and Adam C. Martiny[a,i,1]



Prochlorocccus steady state pCO_2 experiment



- Two acclimation transfers
- Biological triplicates
- Carbonate chemistry



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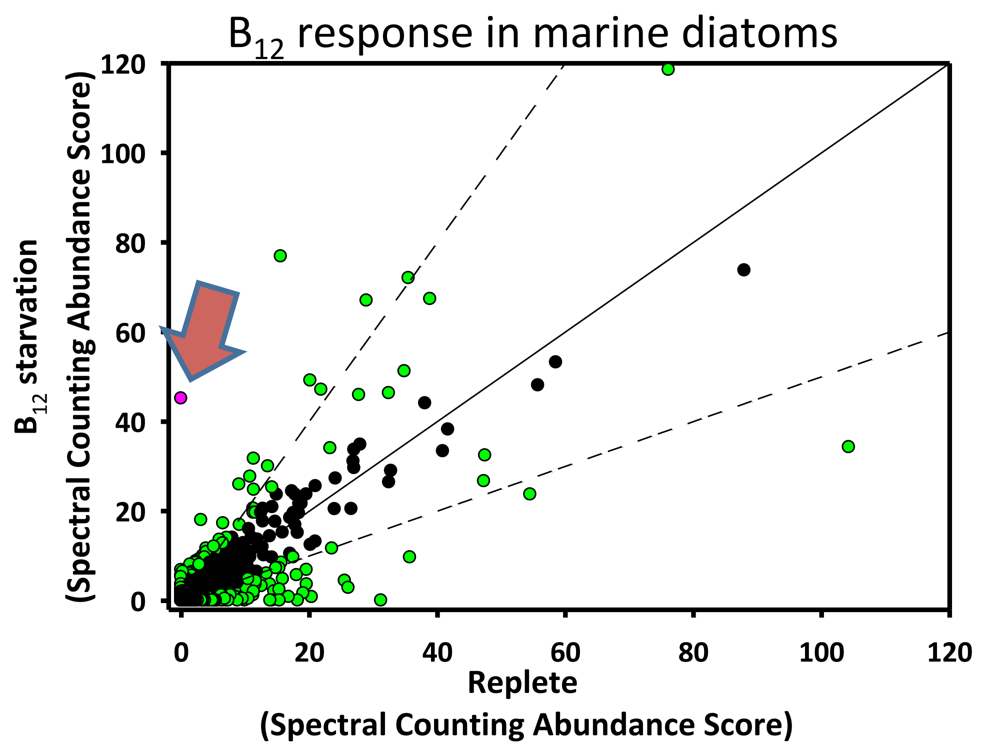
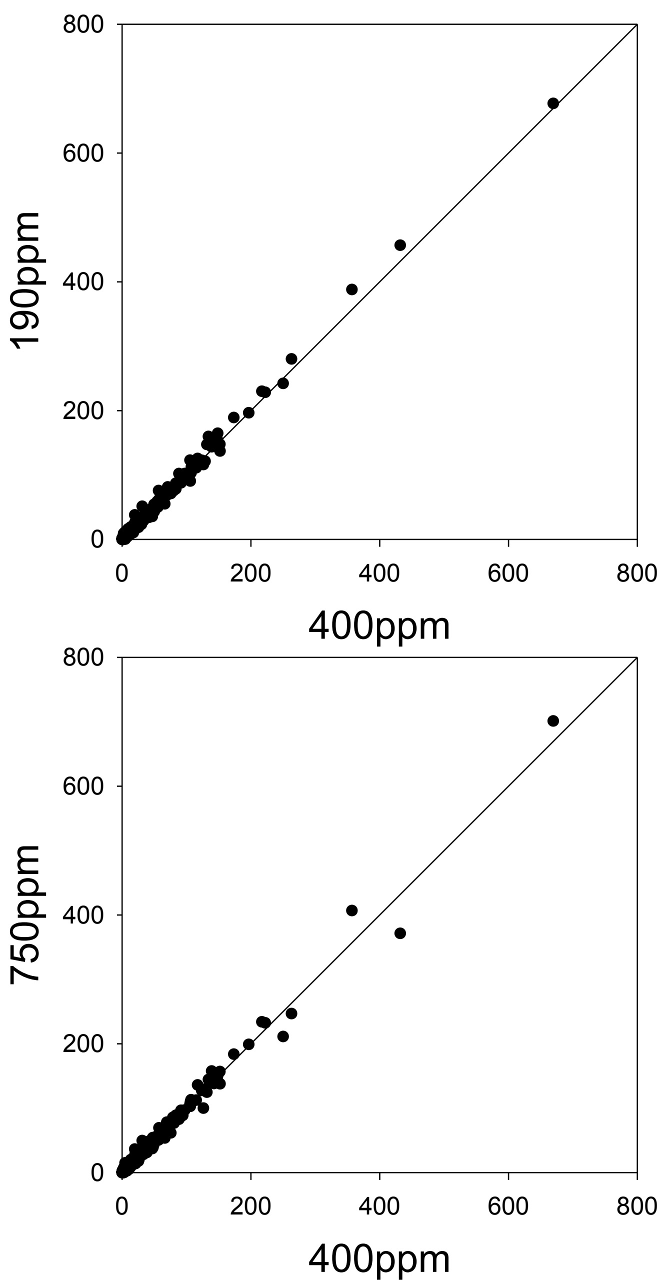
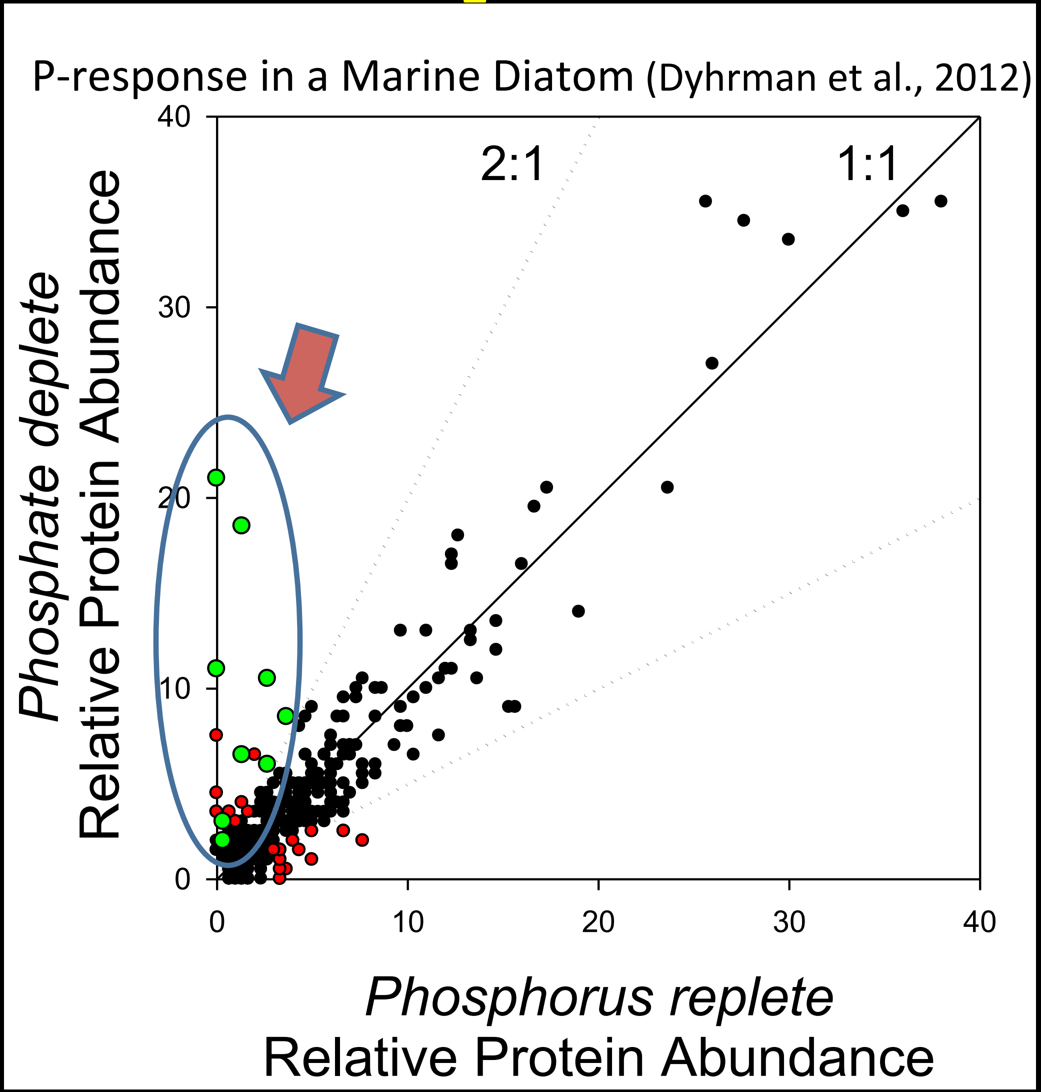
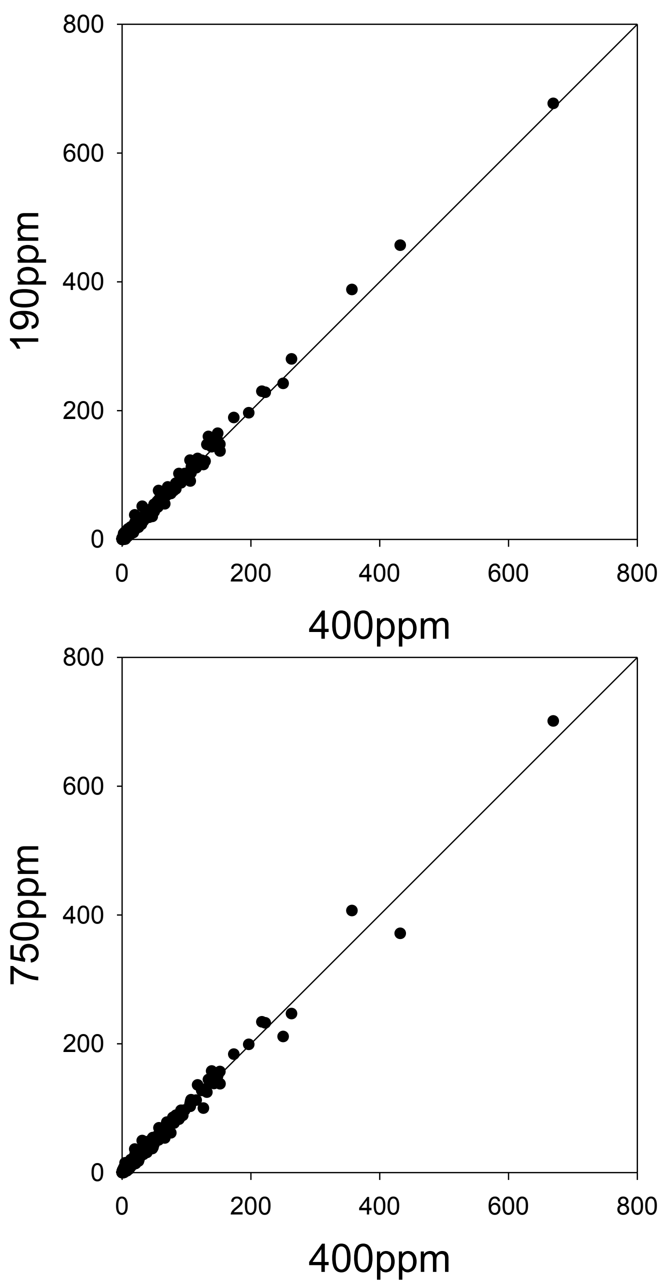




Discovery of a novel B_{12} transporter and biomarker - Bertrand et al., 2013 PNAS

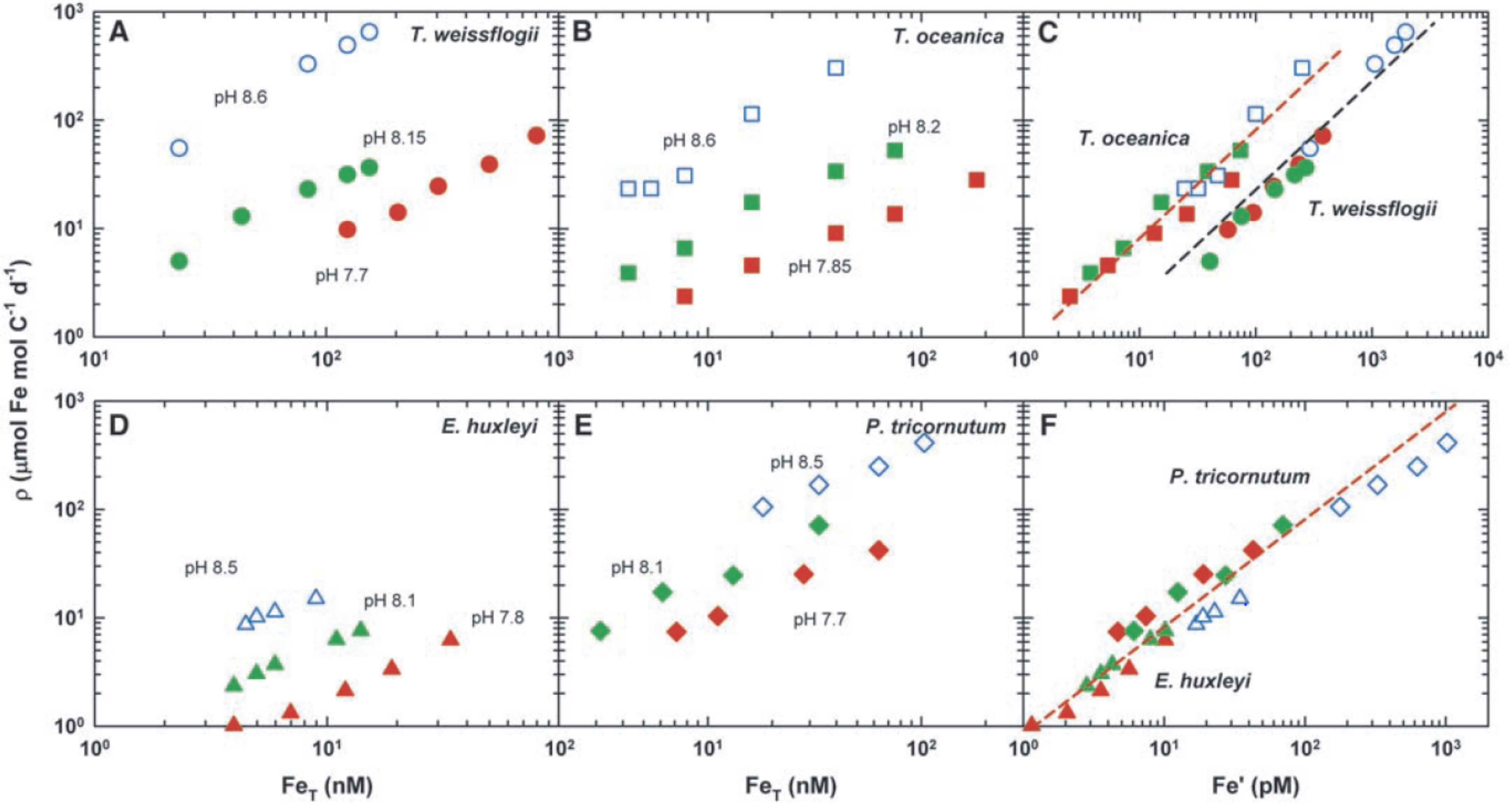
Prochlorocccus steady state pCO$_2$ experiment



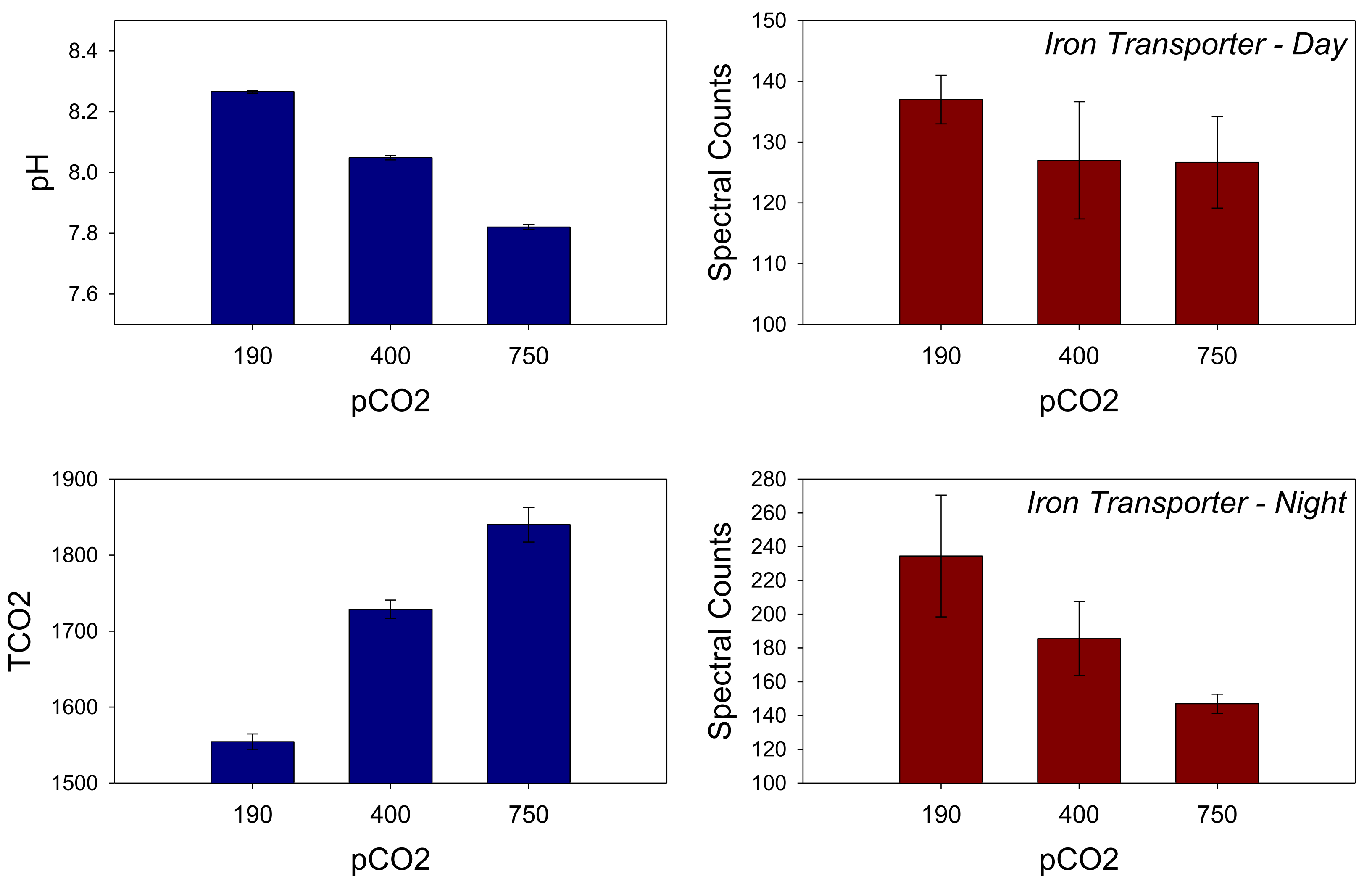


Effect of Ocean Acidification on Iron Availability to Marine Phytoplankton

Dalin Shi,* Yan Xu, Brian M. Hopkinson, François M. M. Morel

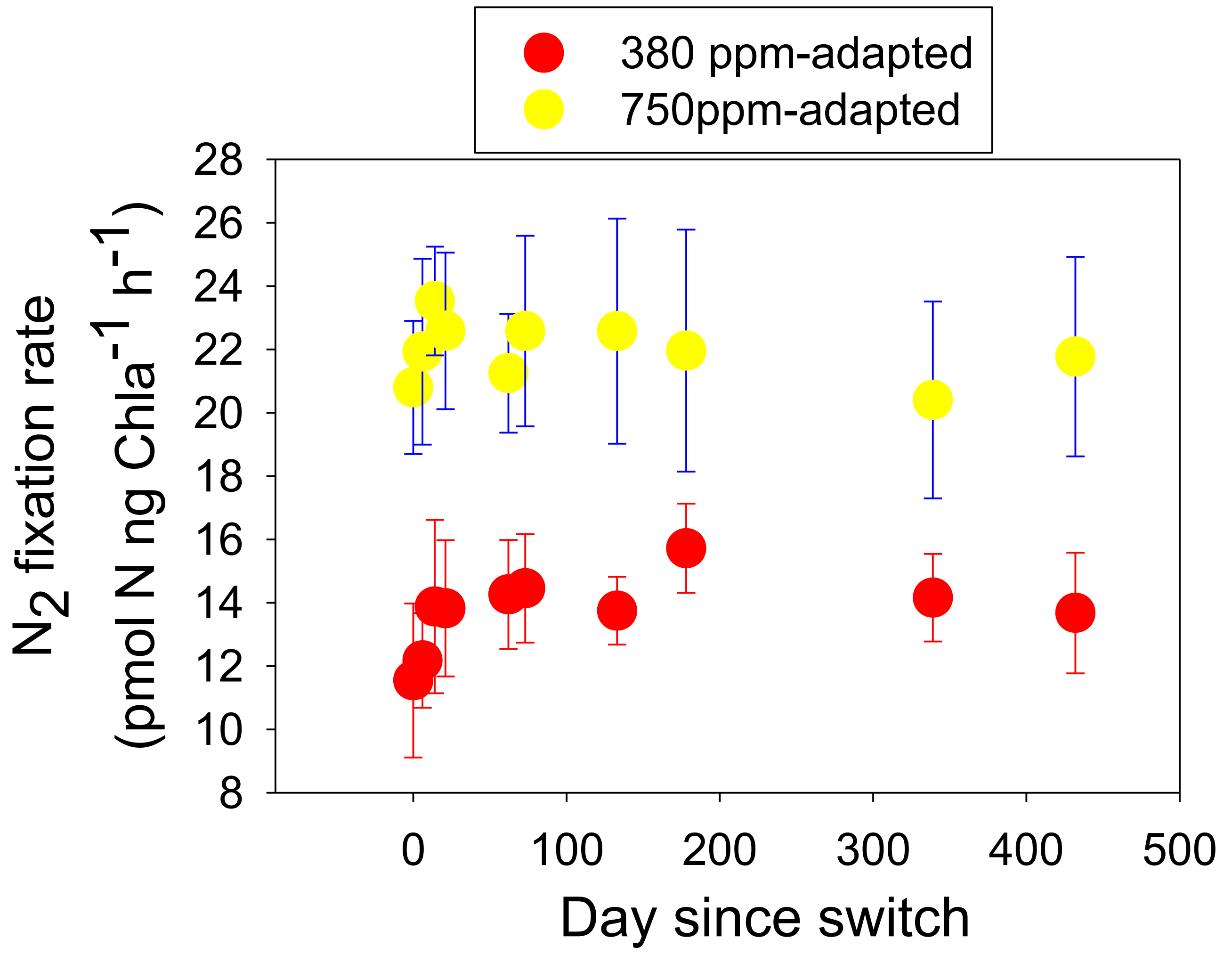


Ocean acidification decreases iron stress in *Prochlorococcus*



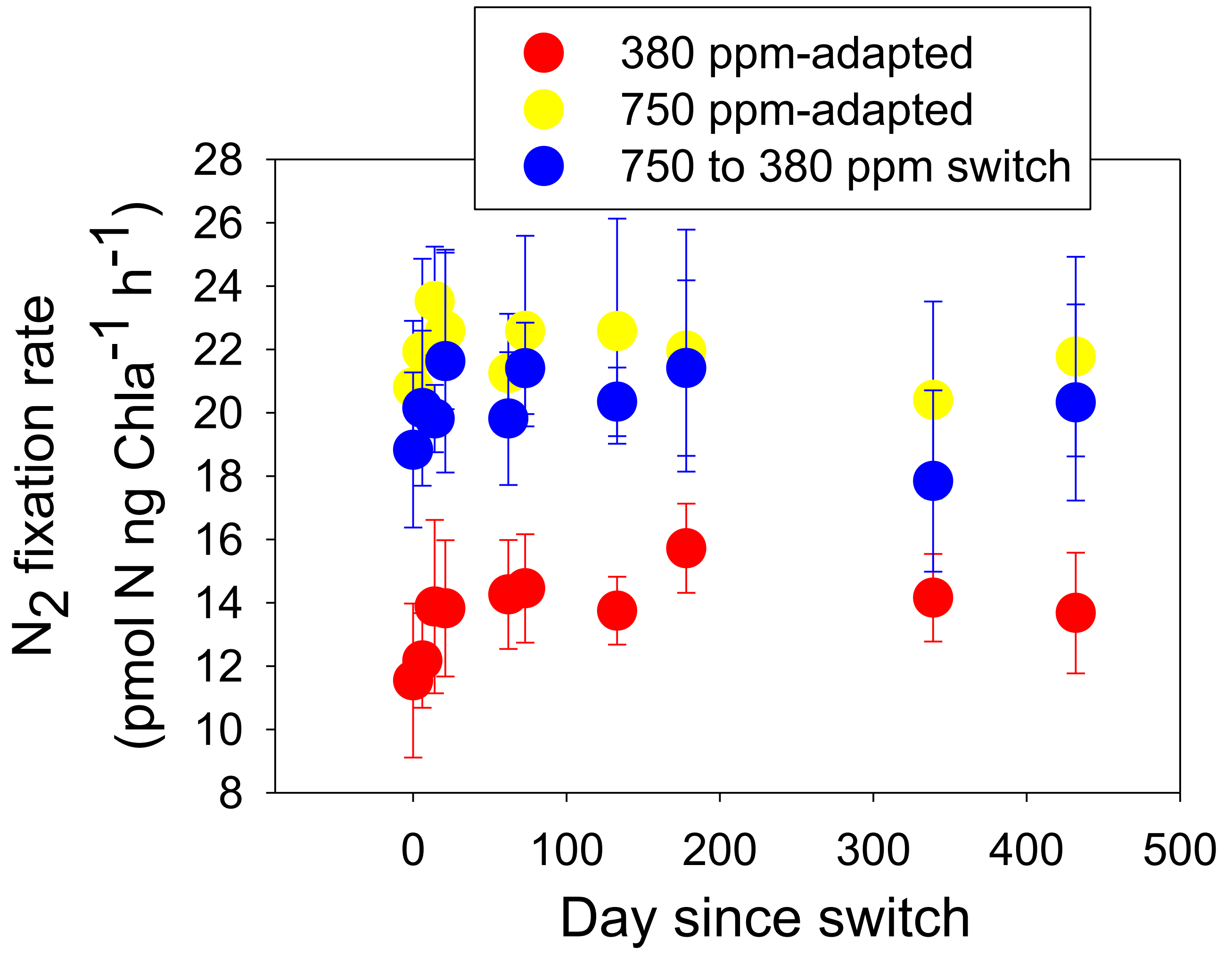
Carbonate Chemistry by Bill Martin, WHOI

N_2 fixation rates of *Trichodesmium* 380- and 750- adapted cell lines over the subsequent 15 months



Hutchins et al. in revision

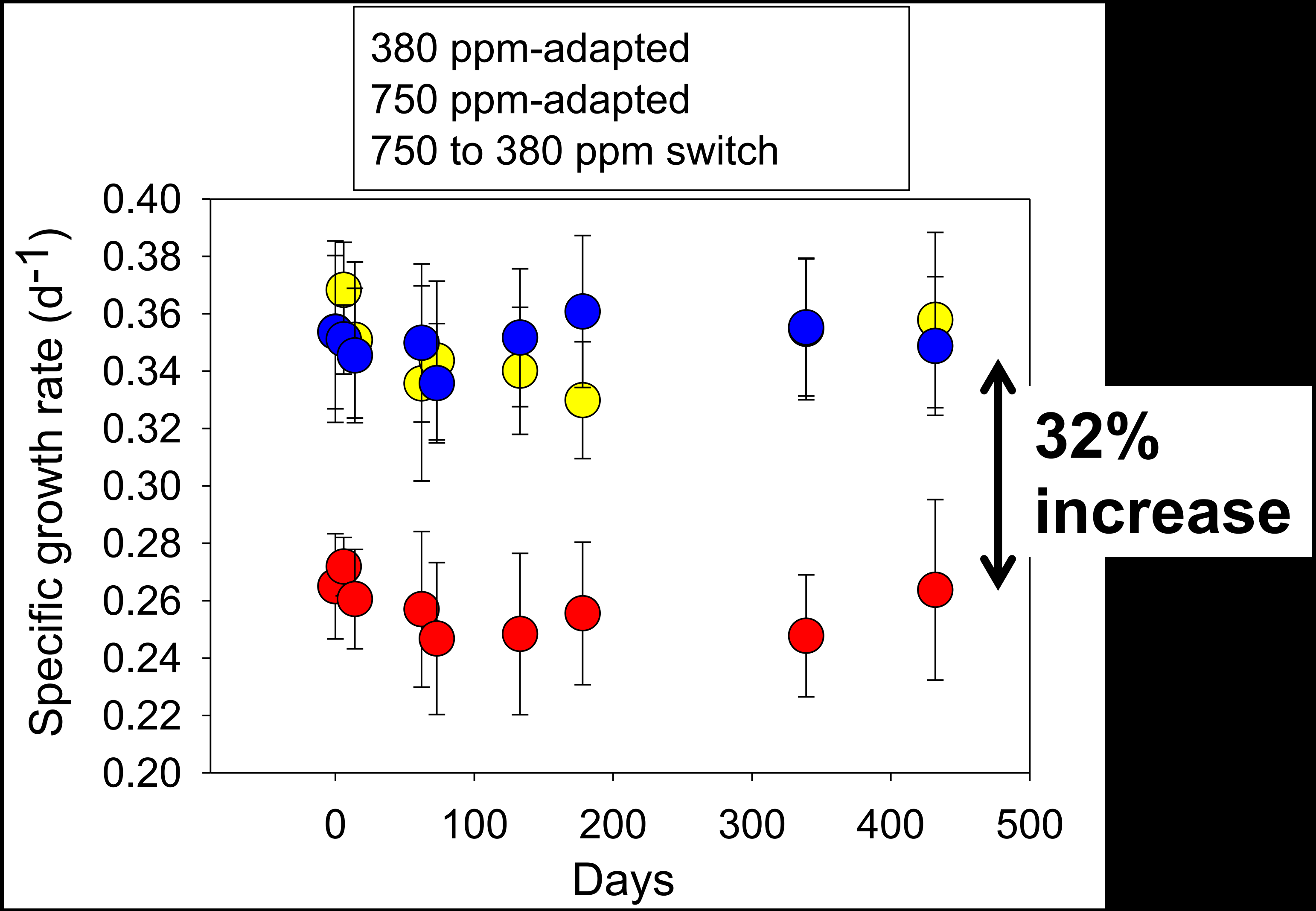
N_2 fixation rates of *Trichodesmium* 380- and 750-adapted and 750 to 380 switch cell lines over the subsequent 15 months



Hutchins et al. in revision

Constitutive up-regulation of N_2 fixation rates in all six 750 to 380 switch cell lines…

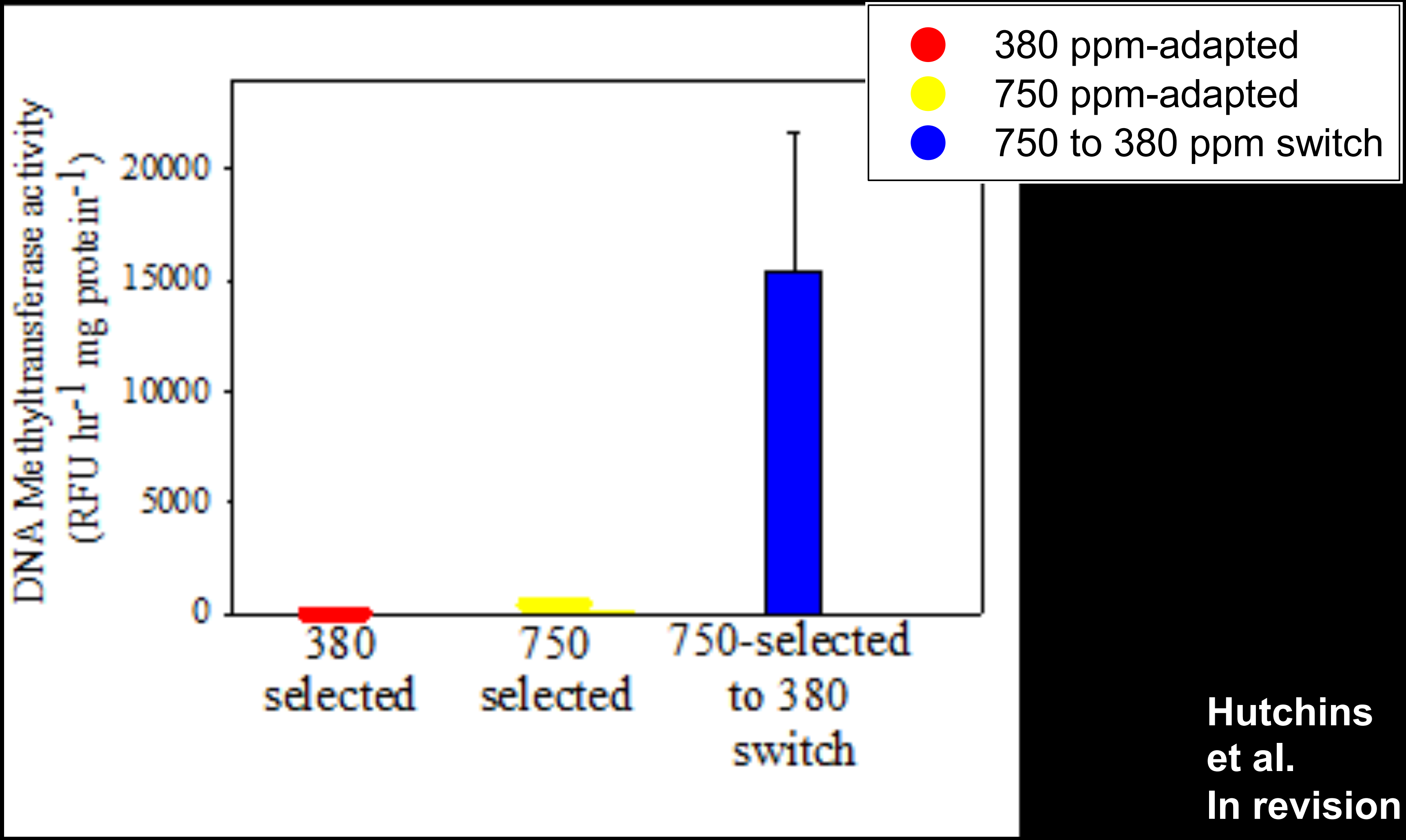
Growth rates over the subsequent 15 months



Hutchins
et al.
In revision

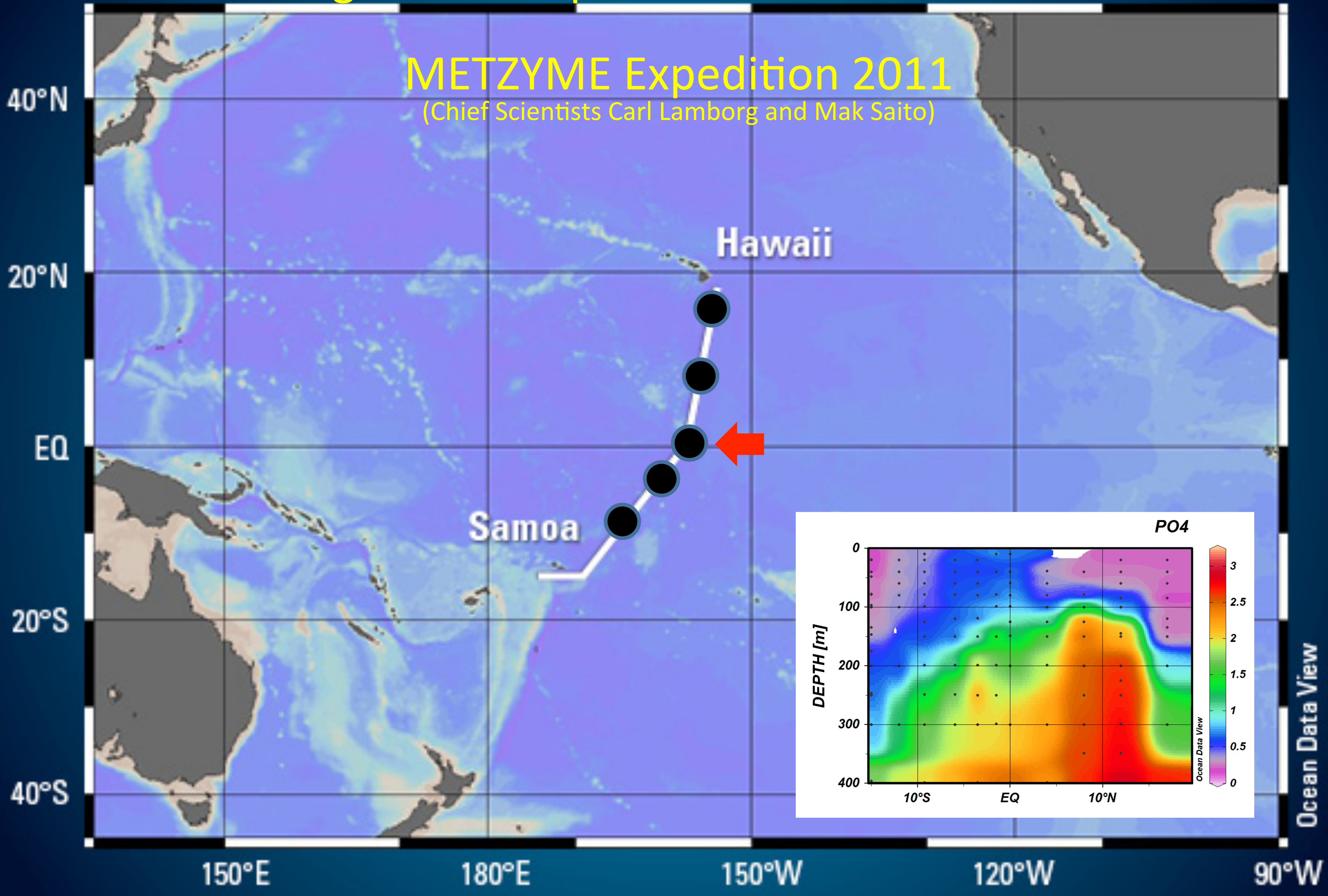
In evolutionary terms, a 32% increase in fitness-
But what is unprecedented is that they are more fit in the ancestral environment, not in the selection environment!

And an enzyme that methylates DNA and so plays a role in epigenetic gene expression is highly active in the 750 to 380 switch cell lines



Hutchins et al.
In revision

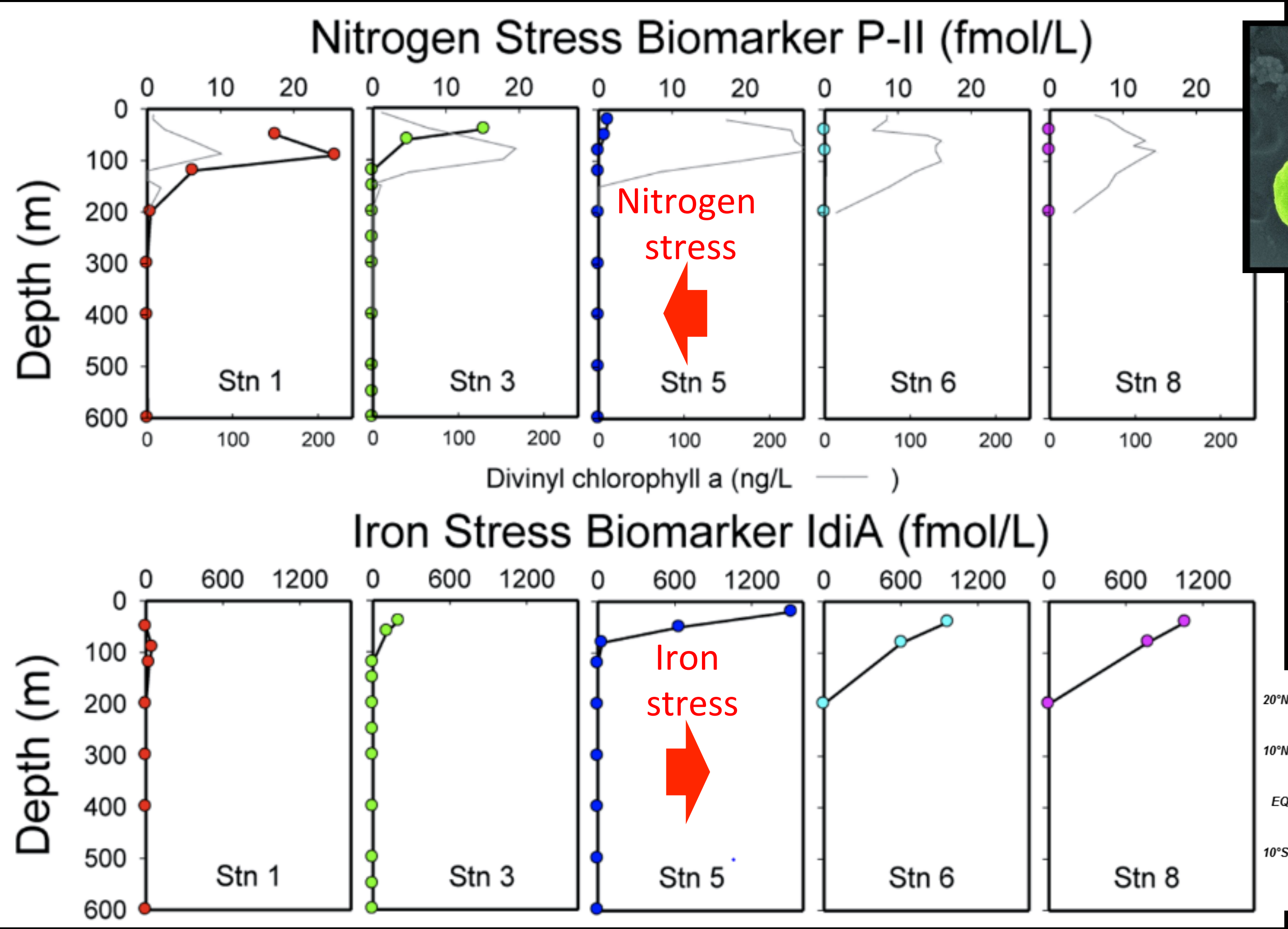
Targeted Metaproteomics in the Oceans

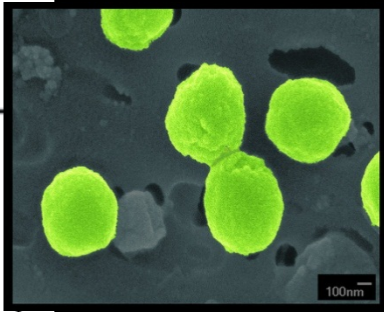


METZYME Expedition 2011

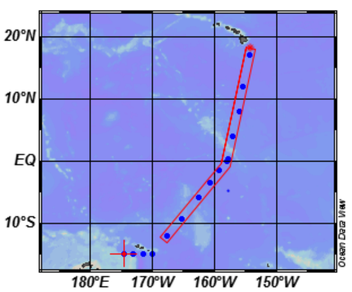
(Chief Scientists Carl Lamborg and Mak Saito)

Profiles of *Prochlorococcus* nutrient stress biomarkers



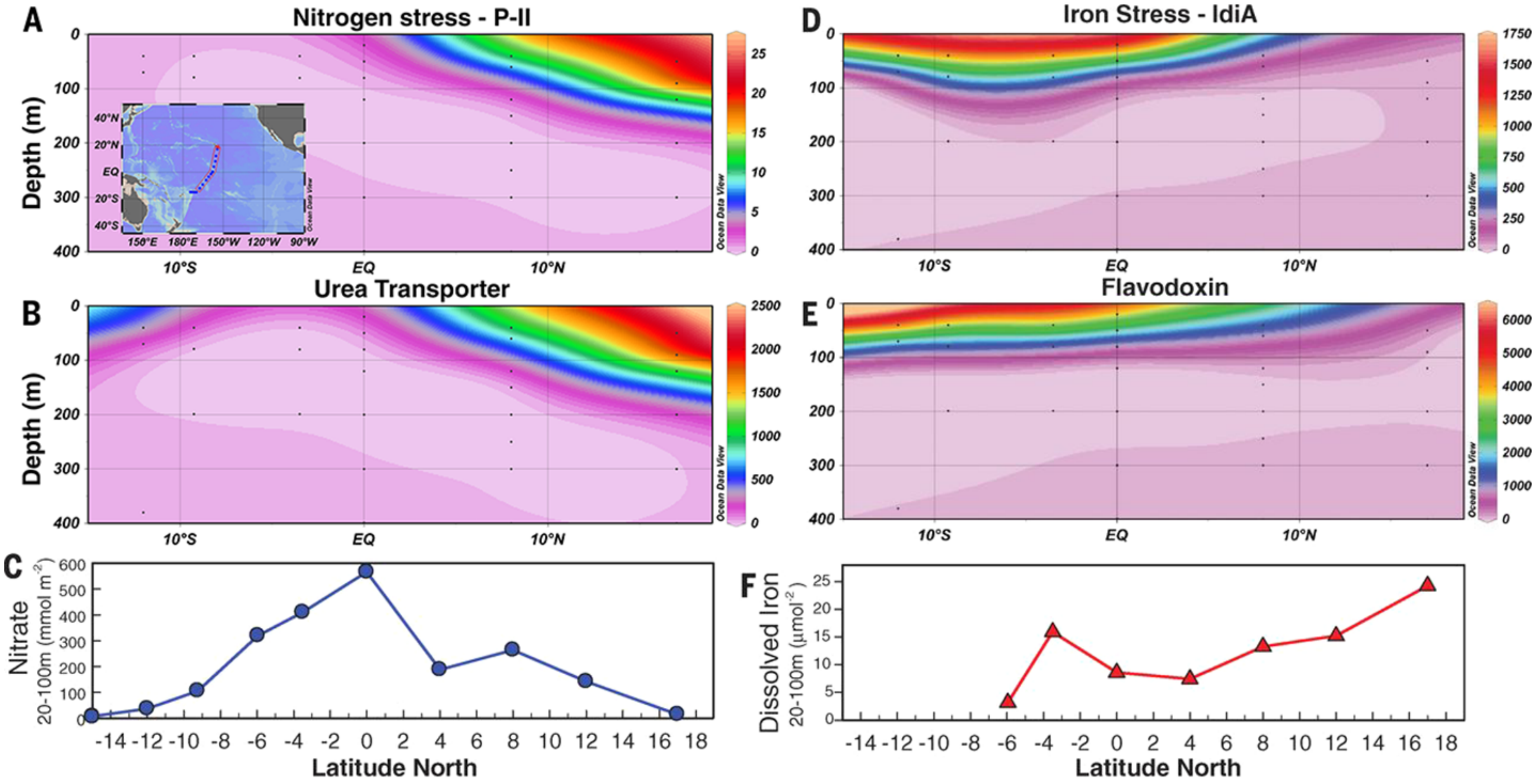


SEM by Anne Thompson



Saito, McIlvin, Moran, Goepfert, DiTullio, Post, Lamborg, Science 2014

Compile Suites of Protein Profiles into ~4500km Biochemical Sections



Saito, McIlvin, Moran, Goepfert, DiTullio, Post, Lamborg Science 2014

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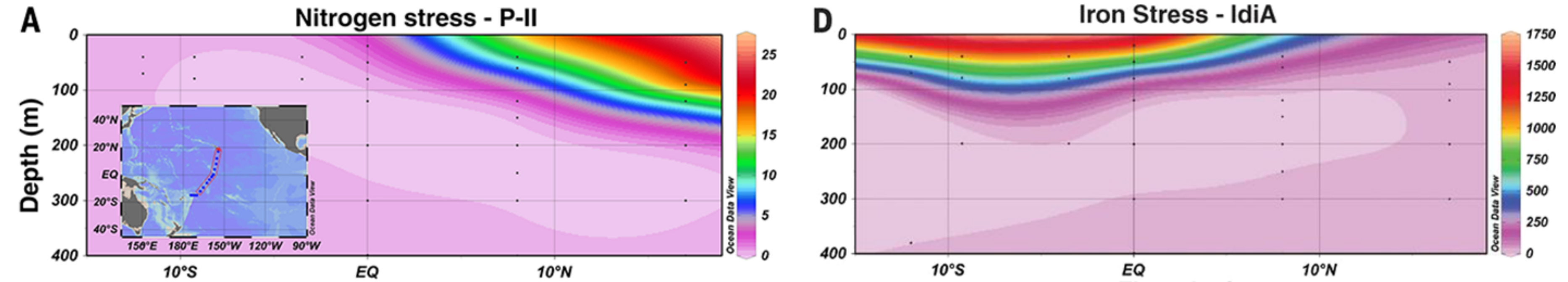
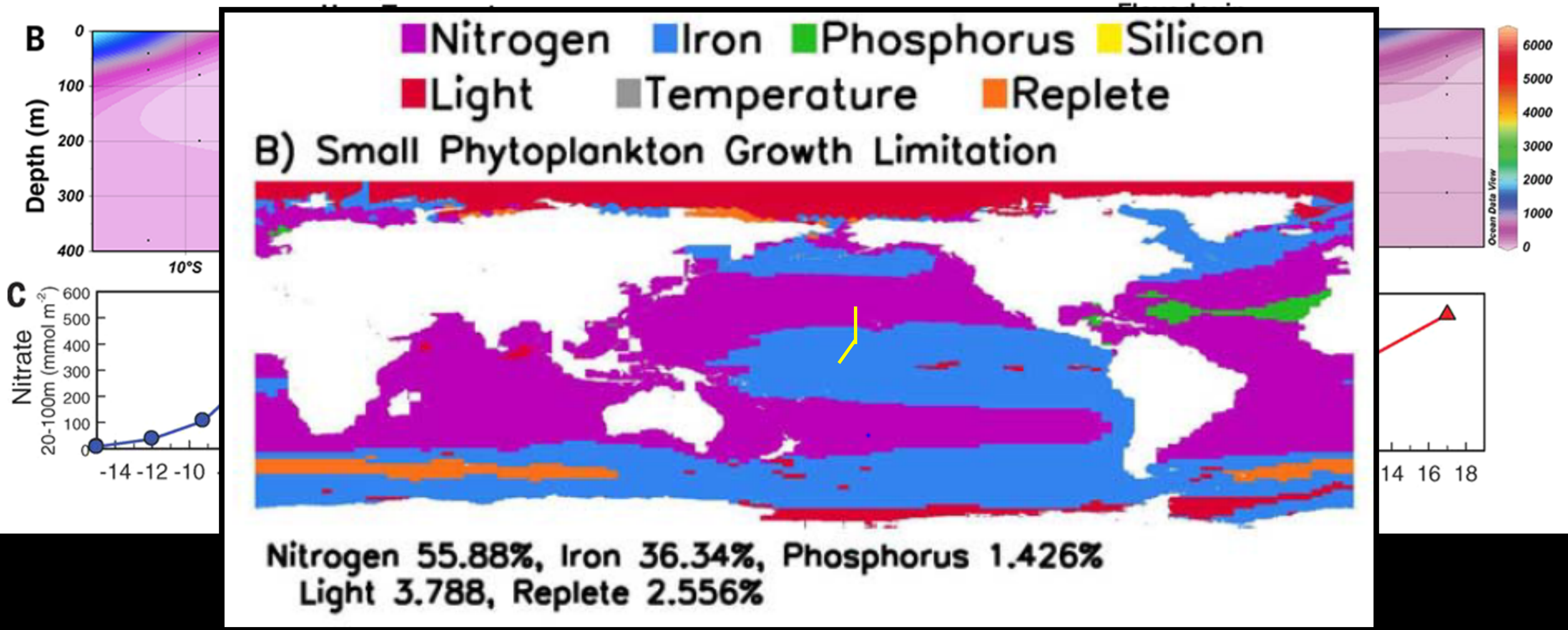




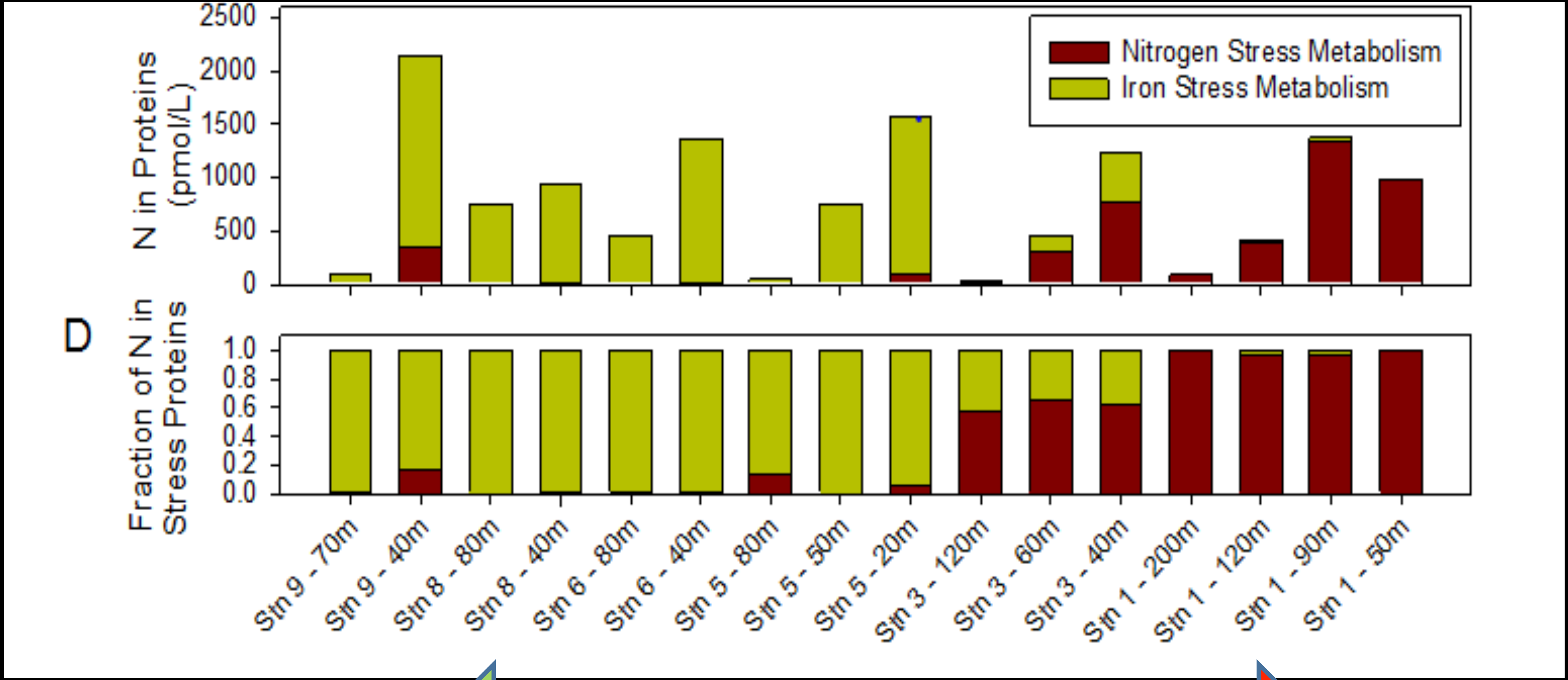
B) Small Phytoplankton Growth Limitation

Nitrogen 55.88%, Iron 36.34%, Phosphorus 1.426%
Light 3.788, Replete 2.556%

Moore, Doney, and Lindsey GBC 2004

Saito, McIlvin, Moran, Goepfert, DiTullio, Post, Lamborg Science 2014

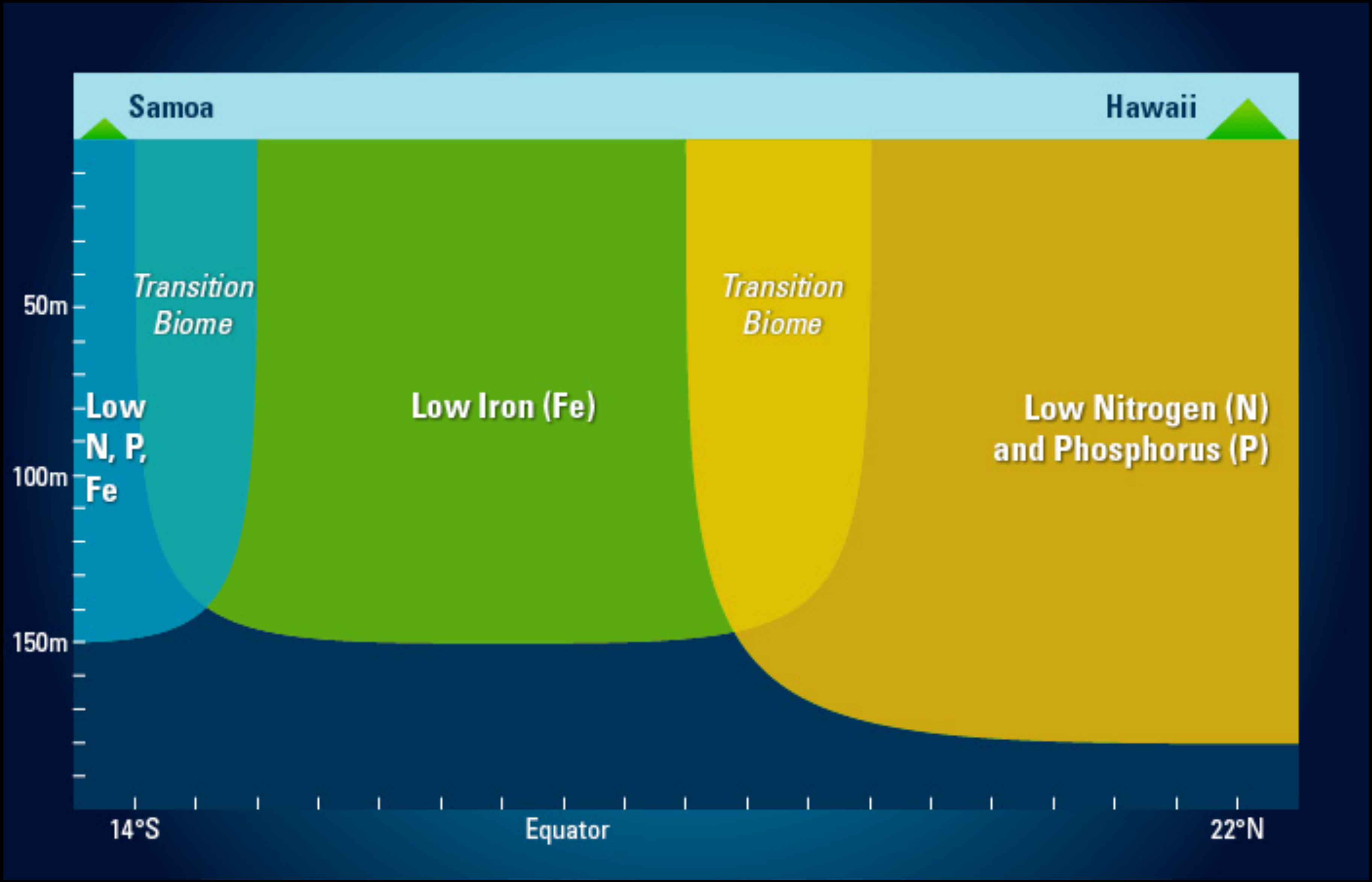
Stoichiometry: Nitrogen Use in 5 key proteins associated with iron and N metabolism (IdiA, flavodoxin, urea transporter, urease, glutamine synthetase)



Fe Stress

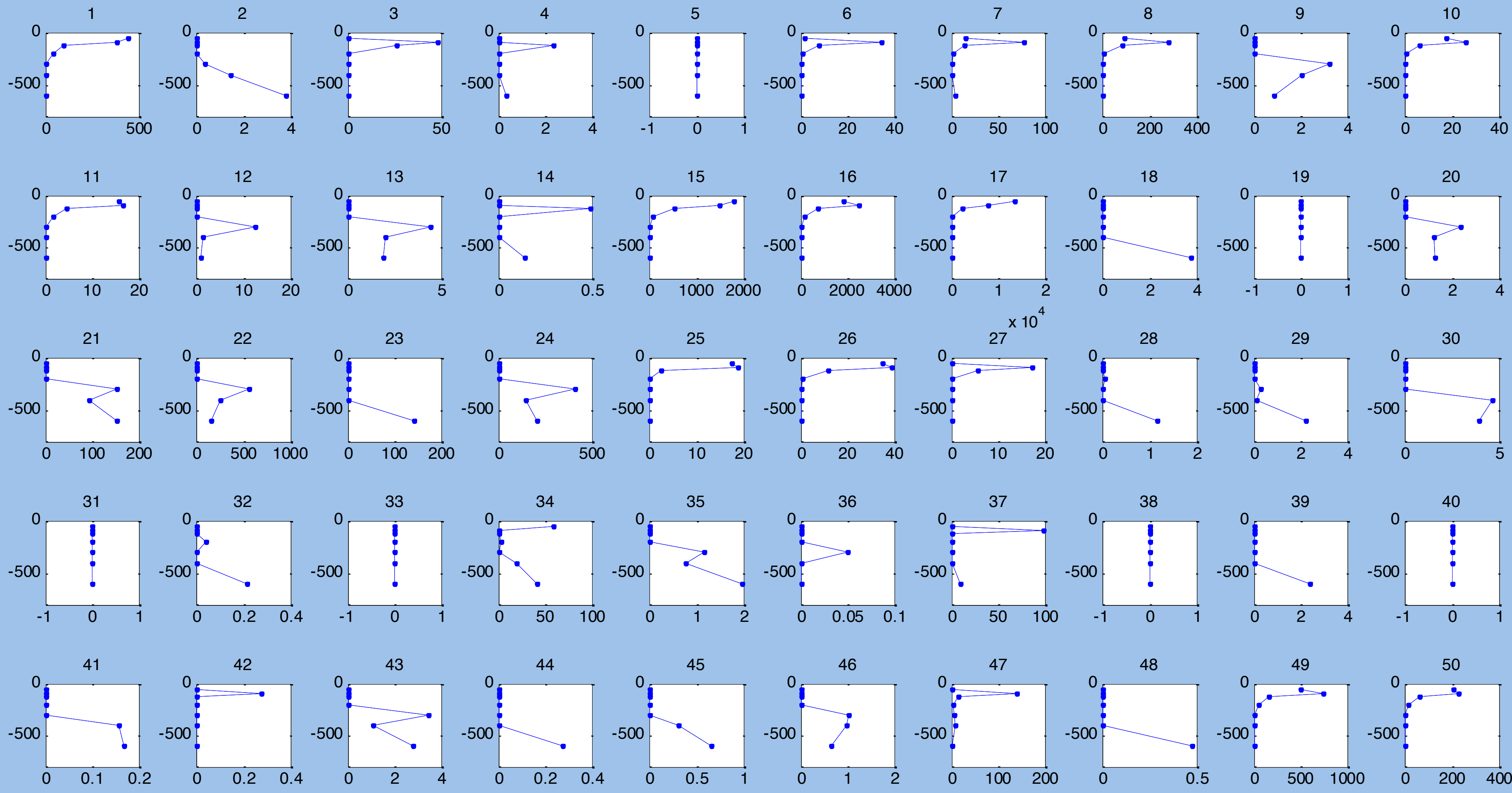
N stress

An Ocean Ecosystem Diagnosis Capability



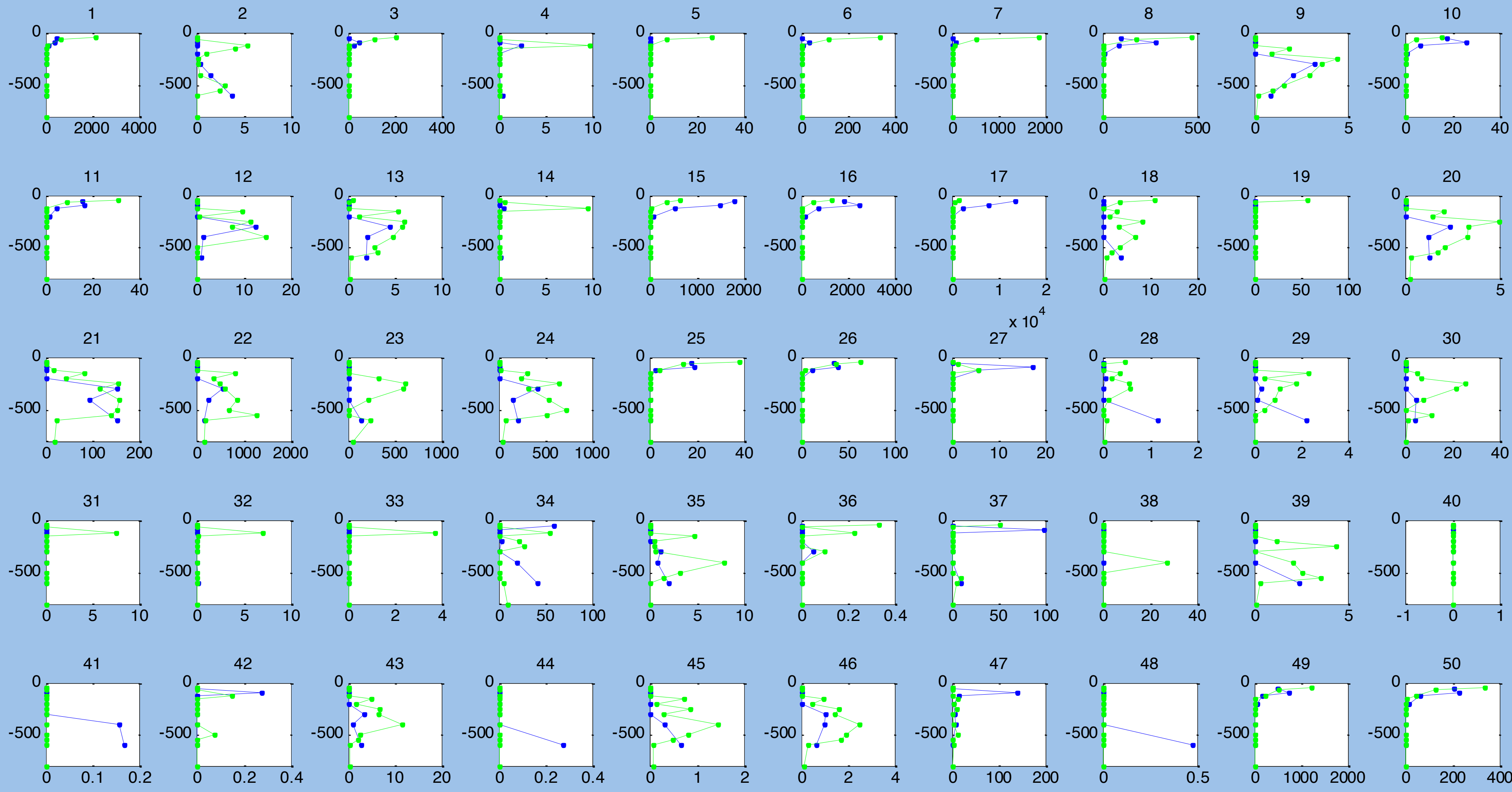
Fifty targets of proteins in the Pacific: Towards a biochemical assessment of the microbial community

Stn 1



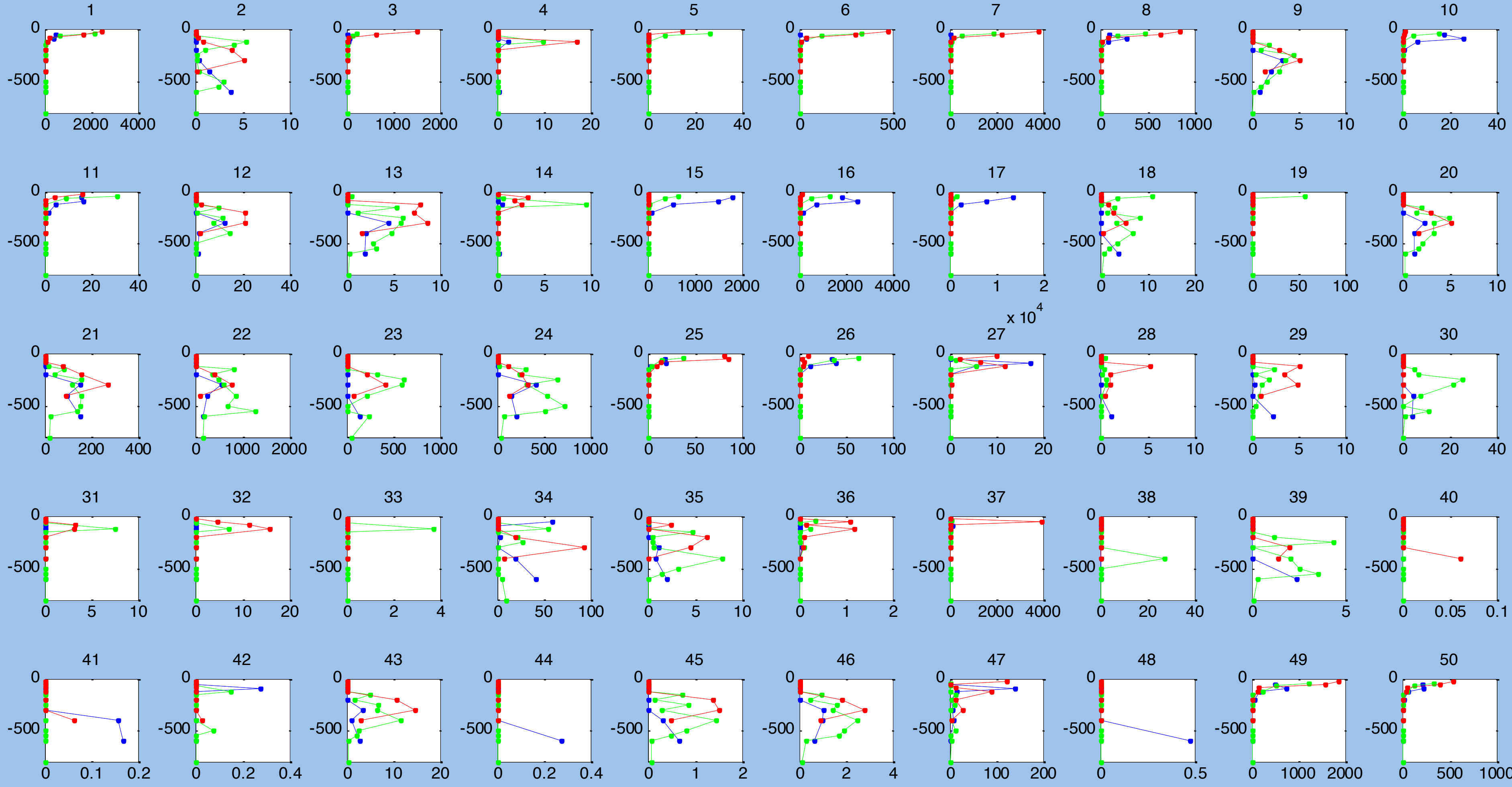
Fifty targets of proteins in the Pacific:
Towards a biochemical assessment of the microbial community

Stn 1 Stn 3

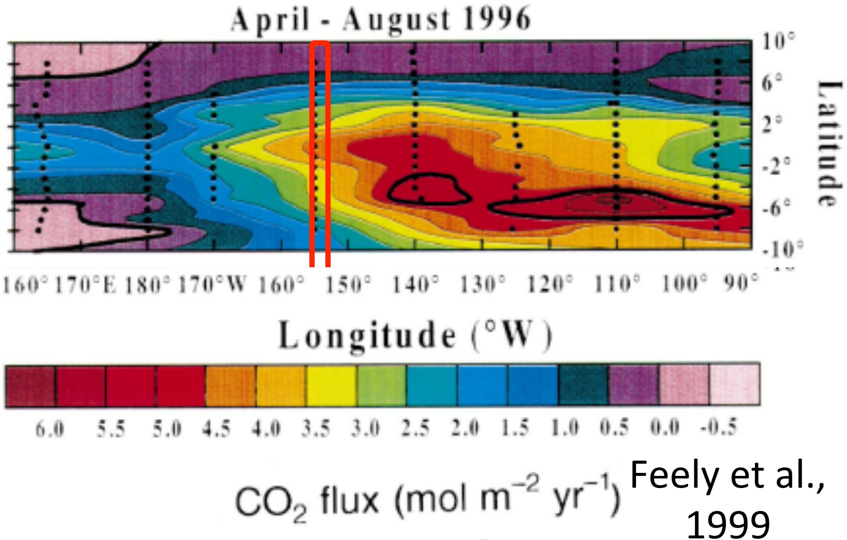


Fifty targets of proteins in the Pacific: Towards a biochemical assessment of the microbial community

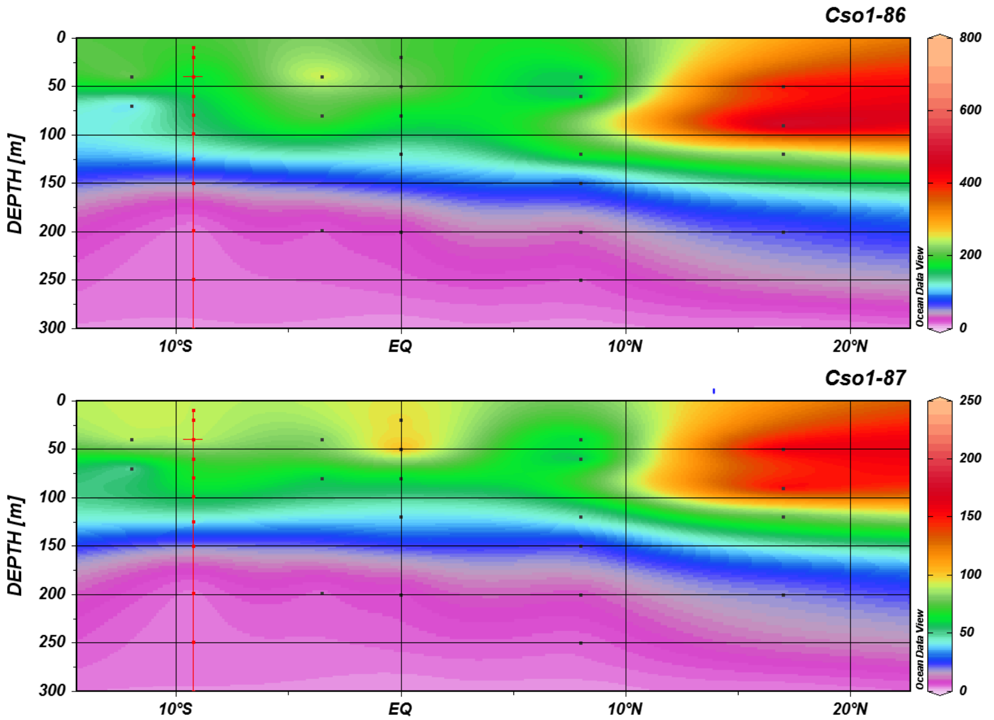
Stn 1 Stn 3 Stn 5

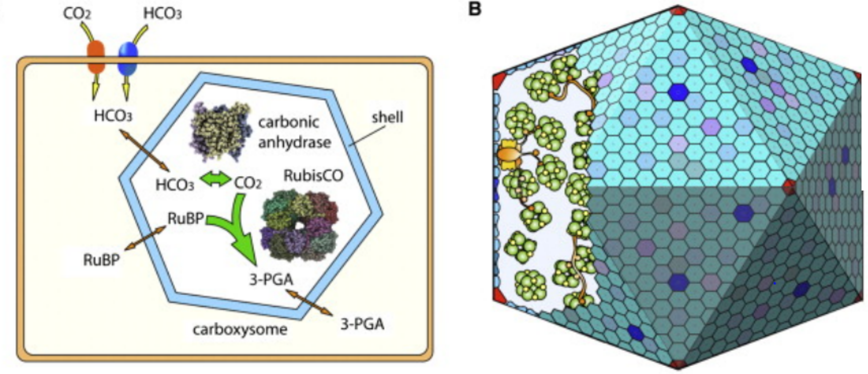


Carboxysome Abundance and pCO2 flux gradients in the Pacific Ocean



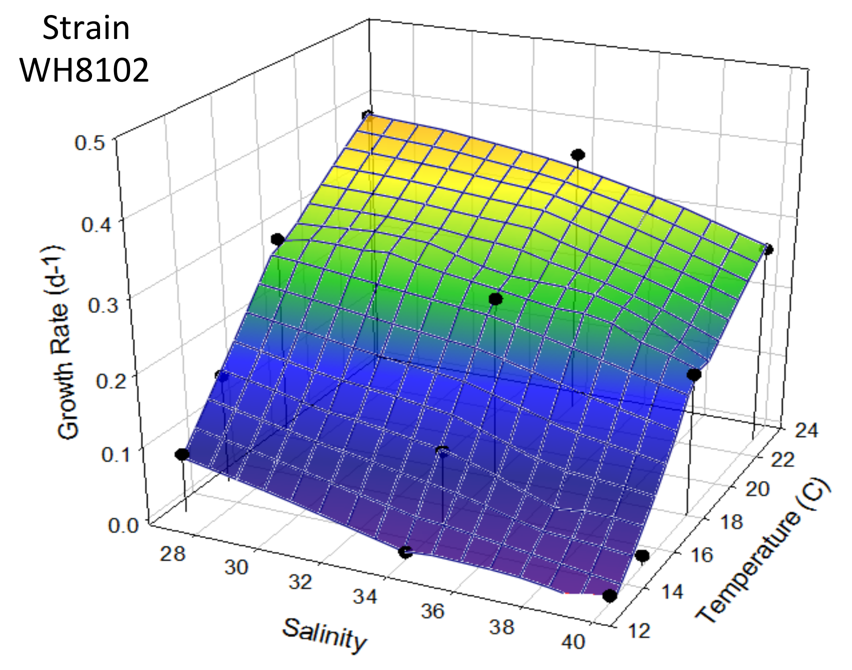
Feely et al., 1999



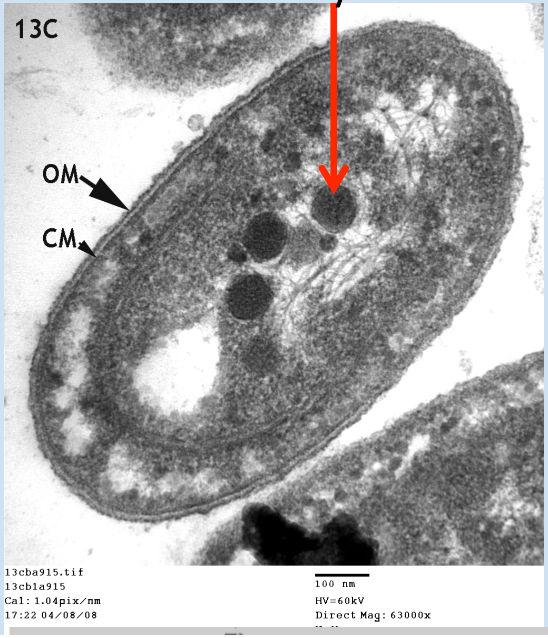


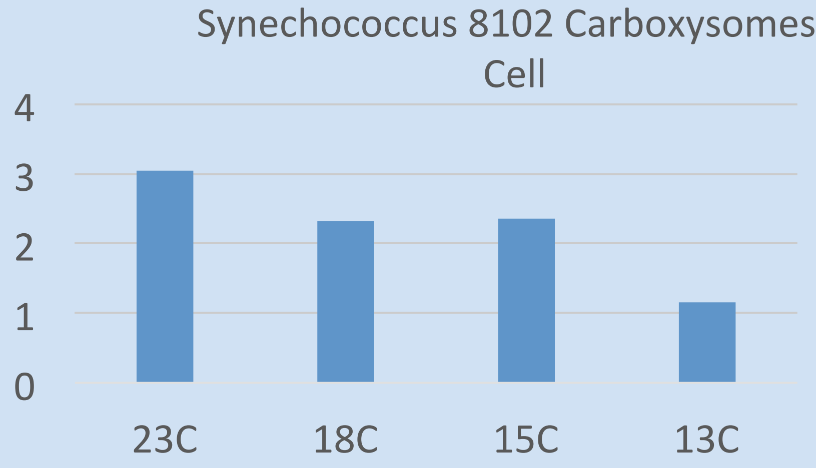
- Increased carboxysome shell protein Cso1 in the North Pacific Subtropical Gyre
- pCO$_2$ flux increases in Equatorial Upwelling
- What controls carboxysome distributions? How do we reconcile with culture studies?

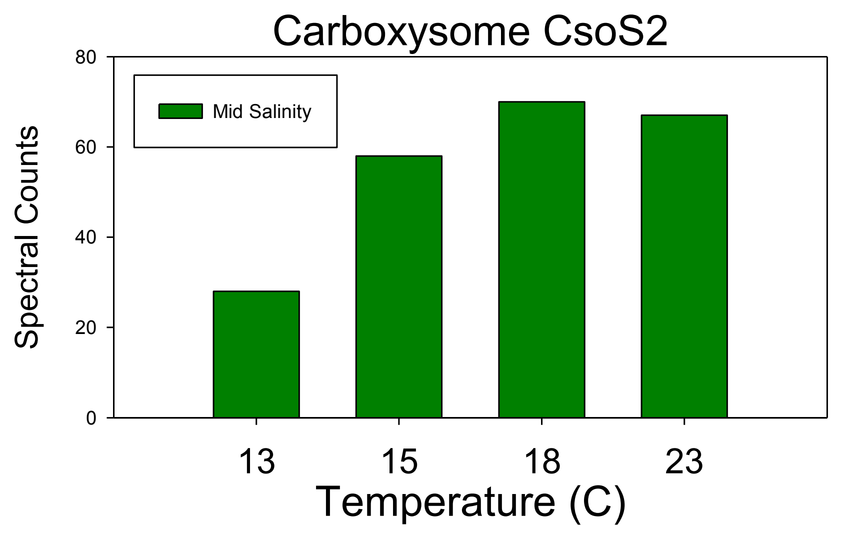
Growth rate is a controller of carbon acquisition machinery: A decrease in carboxysomes with temperature in *Synechococcus*



carboxysome

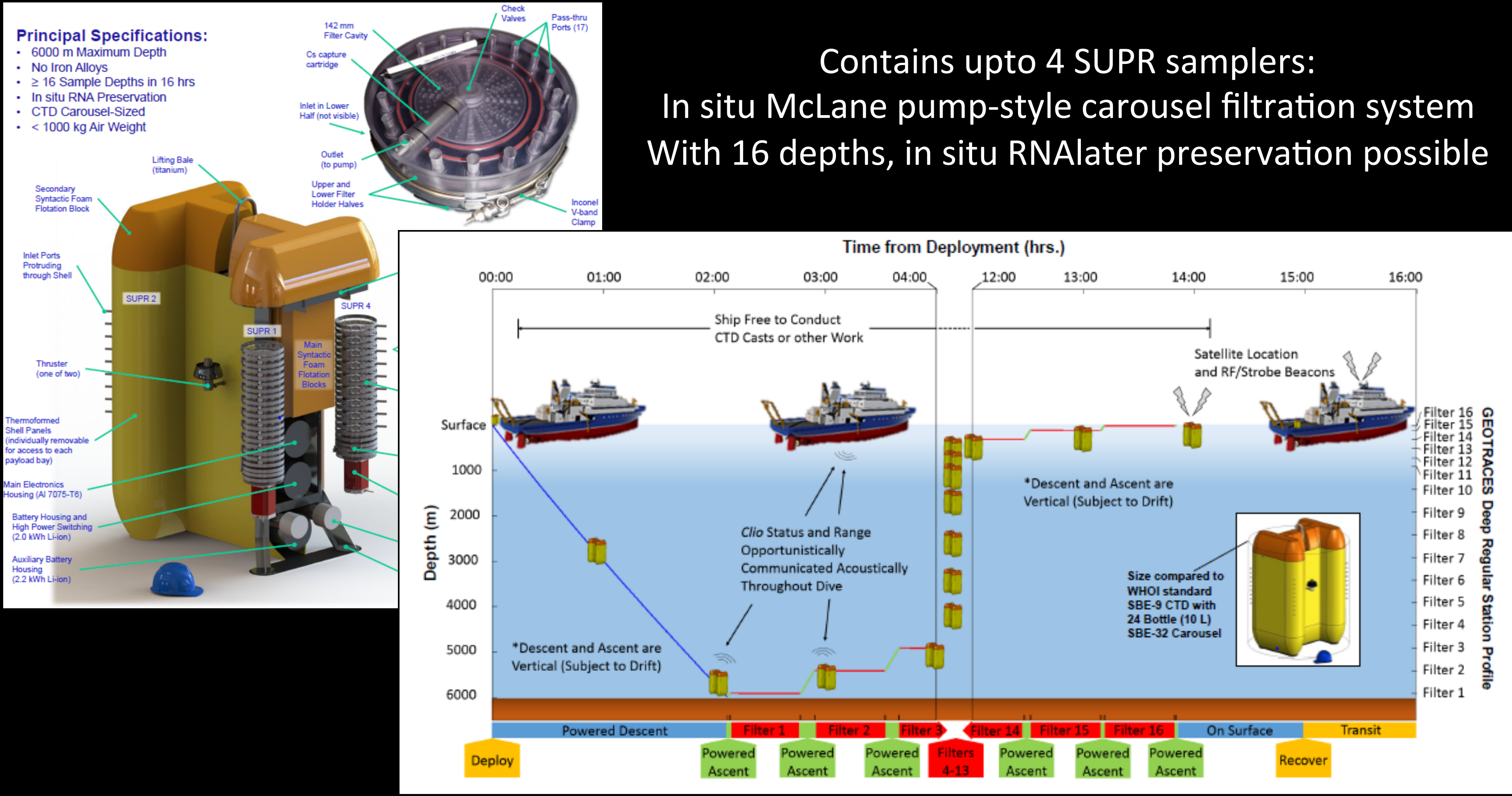






Clio: A New Biogeochemical Vertical AUV

Chip Breier, Mike Jakuba, Daniel Gomez-Ibanez, Mak Saito, Greg Dick



Principal Specifications:

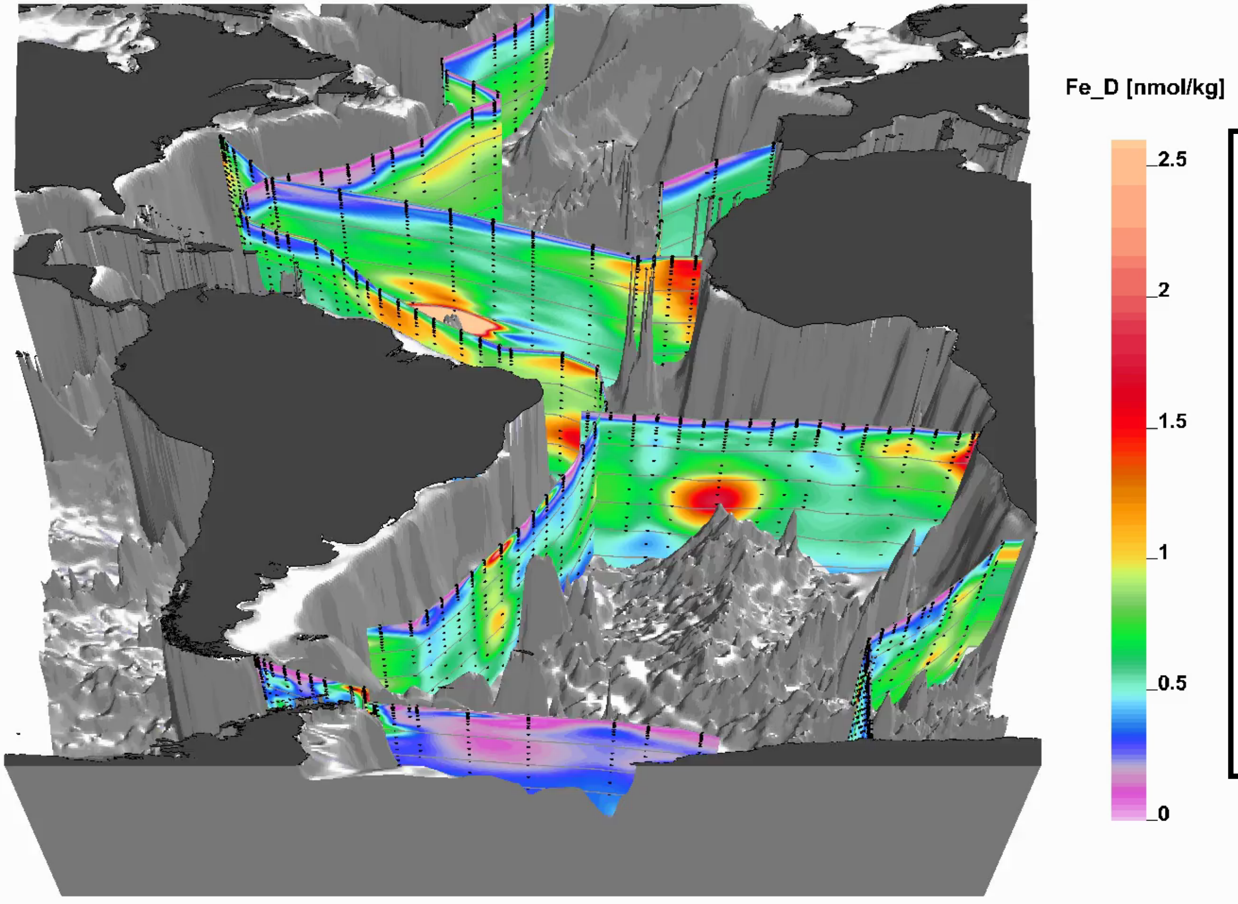
- 6000 m Maximum Depth
- No Iron Alloys
- ≥ 16 Sample Depths in 16 hrs
- In situ RNA Preservation
- CTD Carousel-Sized
- < 1000 kg Air Weight

Contains upto 4 SUPR samplers:
In situ McLane pump-style carousel filtration system
With 16 depths, in situ RNAlater preservation possible

Enable ship-based, full depth, high-throughput acquisition of filtered biochemical, genomics, transcriptomics, and chemical samples on ocean sections and time series

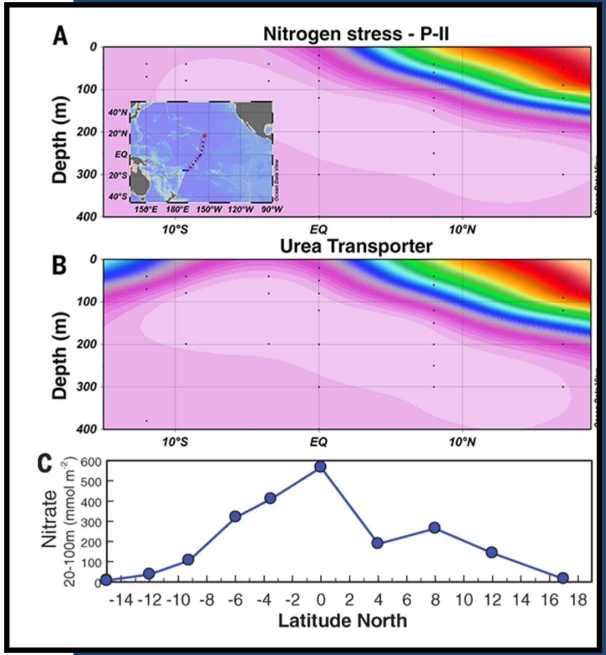
Present: Ocean Sections of Micronutrients (GEOTRACES)
In the Future: Ocean Sections of Biochemical Biomarkers

Dissolved Iron in the Atlantic (www.egeotraces.org)



Data: **Ken Bruland, Tim Conway, Hein de Baar, Fanny Chever, Seth John, Maarten Klunder, Patrick Laan, Francois Lacan, Rob Middag, Abigail Noble, Micha Rijkenberg, Mak Saito, Geraldine Sarthou, Jingfeng Wu**
Graphics: **Reiner Schlitzer**

Nitrogen Stress in *Prochlorococcus*



Potential to connect geochemistry with biochemistry on global scales

Conclusions

- OA relieves iron stress at night in *Prochlorococcus*
  - Evidence of ferrous iron use
- Increased N fixation in *Trichodesmium* does not revert
  - Likely caused by epigenetic or post-translational modifications
- Targeted metaproteomic analyses in the Pacific detects multiple stressors for *Prochlorococcus*
- Emerging proteomics methods applicable to any biological system with genomic data

Quantitative Protein Measurements - Pipeline

Sampling
High-Throughput

- In situ pumping by suite of McLane Pumps
- Development of AUV *Clio*

Protein Extraction and Digestion

- Total protein extraction (detergent-based)

Discovery Proteomics

- Identification & relative quantitation of 10,000’s of proteins
- Mapping to metagenomes and genome by HPC

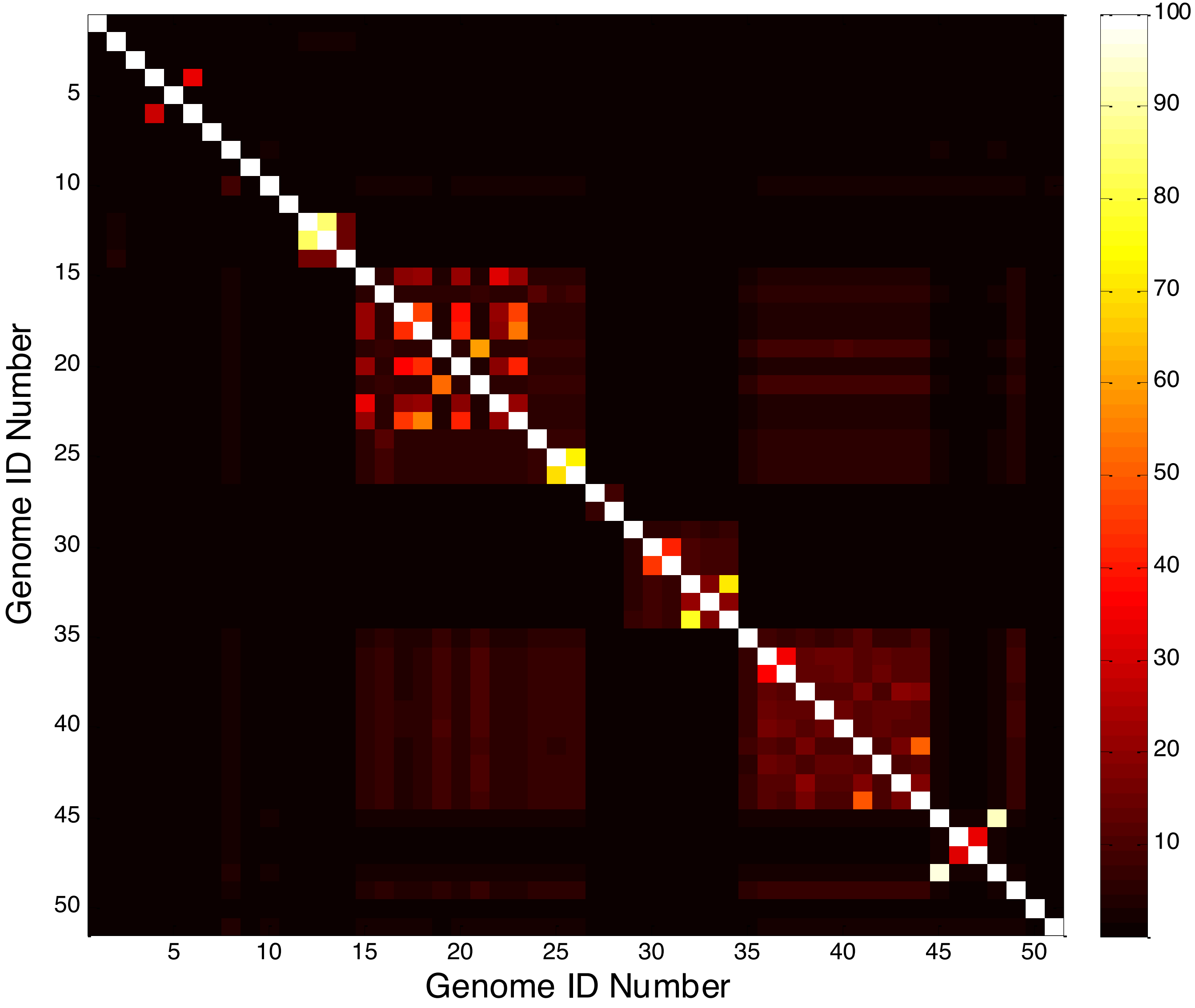
Targeted Proteomics

- Measurement by Selected Reaction Monitoring (QQQ)
- Calibration by isotope labeled peptide standards

Ocean Profiles and Sections of Proteins

- *Many possible applications…*
- Diagnosis using nutrient stress biomarkers; stoichiometry
- Identify biogeochemical reactions by enzyme abundances

Shared Peptides: Intra and Inter Species

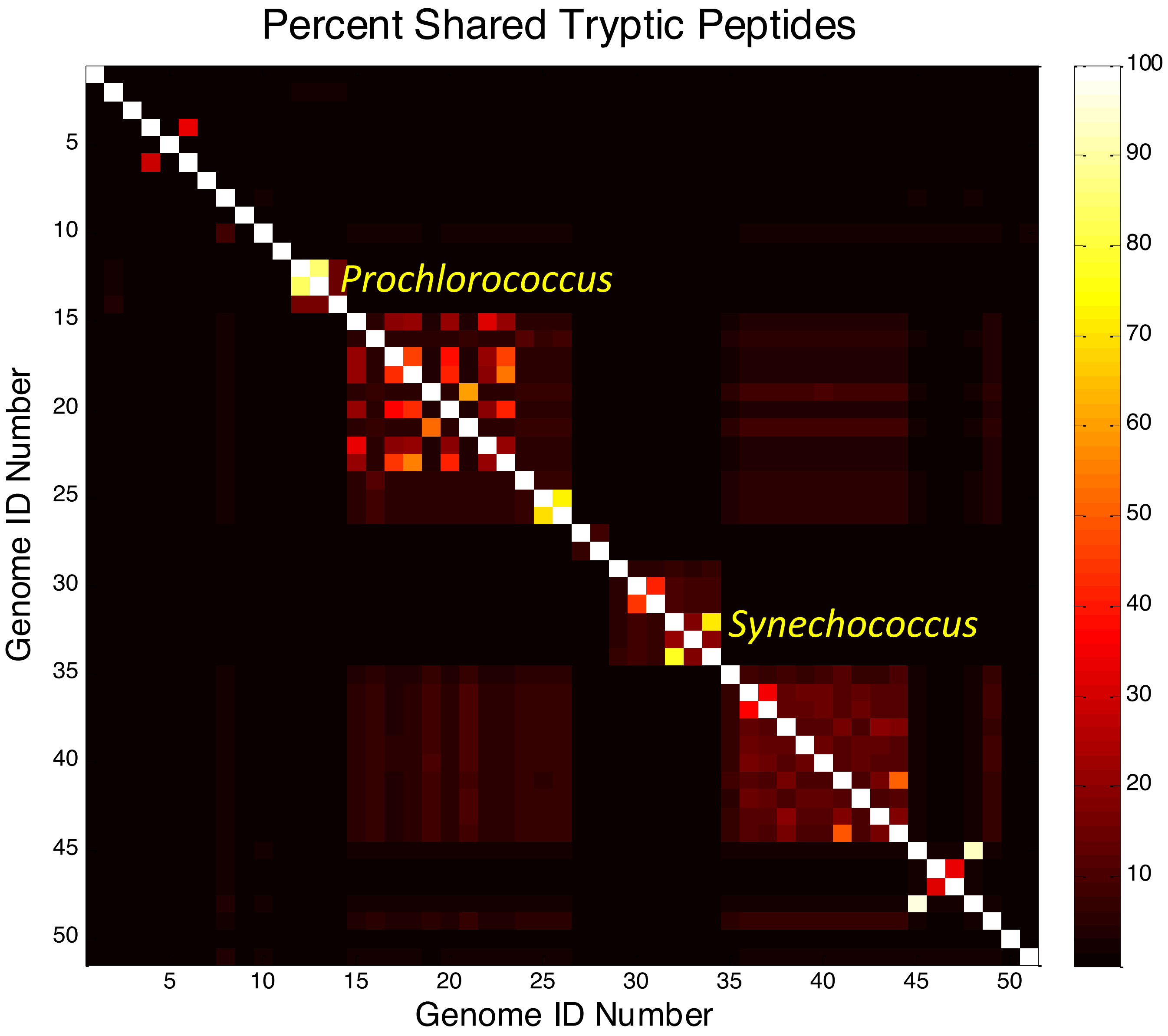


Percent Shared Tryptic Peptides

Saito et al., Proteomics, in press

1E coli GCA
2HIMB114
3HIMB59
4Nitrobacter 311
5Nitrobacter defluvii
6Nitrobacter winogradski
7Nitrobacter Nb-211
8UCYN-A
9cenarchaeum_symbiosumA
10Crocosphaera 8501
11Kuenenia
12Peligibacter 1002
13Peligibacter 1062
14Pelagibacter72111
15Prochlorococcus 1986
16Prochlorococcus 9211
17Prochlorococcus 9215
18Prochlorococcus 9301
19Prochlorococcus 9303
20Prochlorococcus 9312
21Prochlorococcus 9313
22Prochlorococcus 9515
23Prochlorococcus AS9601
24Prochlorococcus 1375
25Prochlorococcus NATL1A
26Prochlorococcus NATL2a
27Pseudomonas PA7
28Pseudomonas putida
29RoseoMED193
30roseodenitrificans114
31roseolit149
32sulfitobacter_ee36
33sulfitobacter_gai101
34sulfitobacter_nas14
35Synechococcus 5701
36Synechococcus 7803
37Synechococcus 7805
38Synechococcus 8102
39Synechococcus 9916
40Synechococcus 9917
41Synechococcus bl107
42Synechococcus cc9311
43Synechococcus cc9605
44Synechococcus 9902
45Synechocystis pcc6803
46Synechcoccus ja23ba
47Synechococcus ja33ab
48Synechococcus pcc7942
49Synechococcus rcc307
50thiomicrospira_crunogena
51Tricho IMS101

Shared Peptides: Intra and Inter Species



Saito et al., Proteomics, in press

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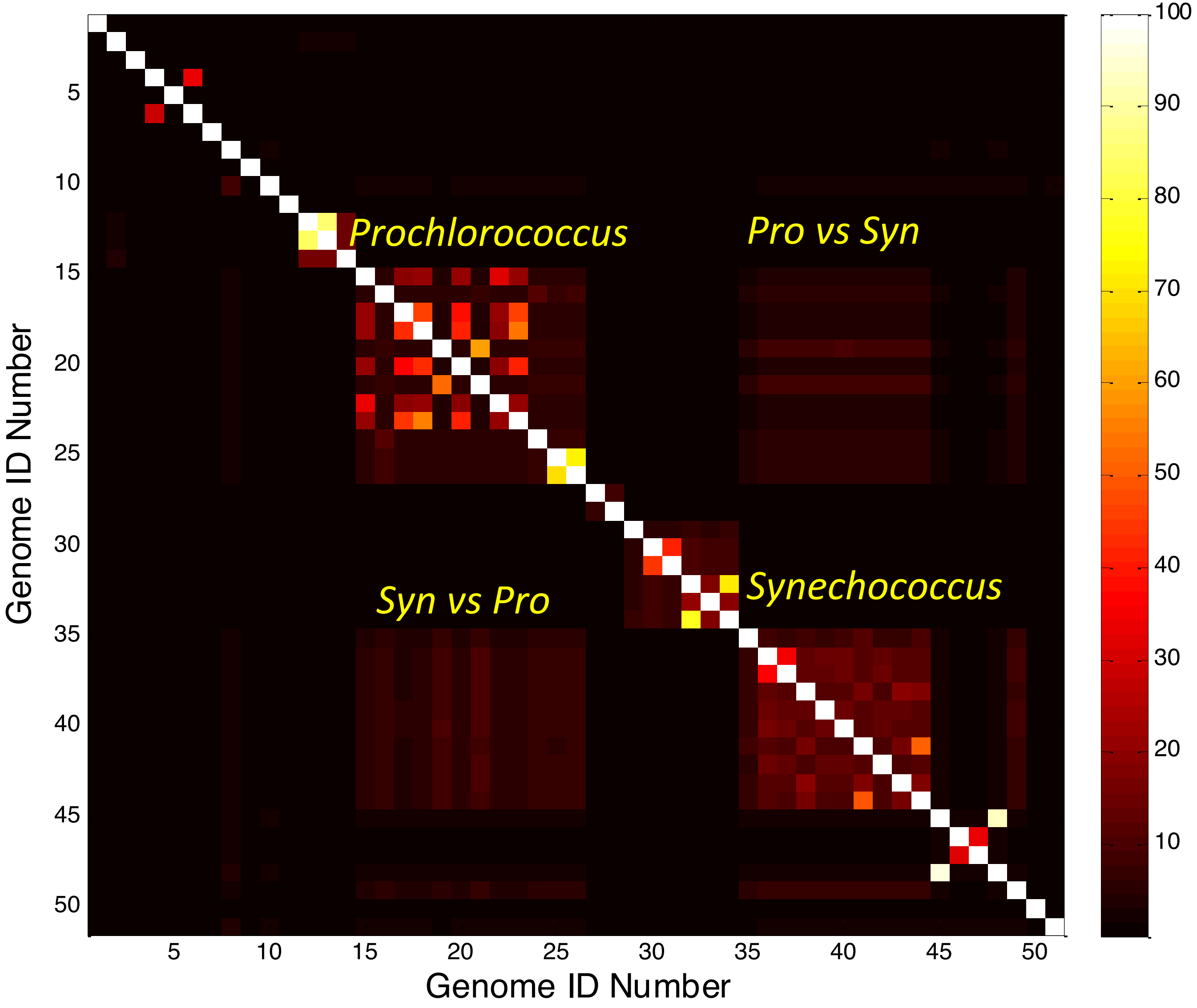
22Prochlorococcus 9515
23Prochlorococcus AS9601
24Prochlorococcus 1375
25Prochlorococcus NATL1A
26Prochlorococcus NATL2a
27Pseudomonas PA7
28Pseudomonas putida
29RoseoMED193
30roseodenitrificans114
31roseolit149
32sulfitobacter_ee36
33sulfitobacter_gai101
34sulfitobacter_nas14
35Synechococcus 5701
36Synechococcus 7803
37Synechococcus 7805
38Synechococcus 8102
39Synechococcus 9916
40Synechococcus 9917
41Synechococcus bl107
42Synechococcus cc9311
43Synechococcus cc9605
44Synechococcus 9902
45Synechocystis pcc6803
46Synechcoccus ja23ba
47Synechococcus ja33ab
48Synechococcus pcc7942
49Synechococcus rcc307
50thiomicrospira_crunogena
51Tricho IMS101

Shared Peptides: Intra and Inter Species

Percent Shared Tryptic Peptides



Saito et al., Proteomics, in press

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19Prochlorococcus 9303
20Prochlorococcus 9312
21Prochlorococcus 9313
22Prochlorococcus 9515
23Prochlorococcus AS9601
24Prochlorococcus 1375
25Prochlorococcus NATL1A
26Prochlorococcus NATL2a
27Pseudomonas PA7
28Pseudomonas putida
29RoseoMED193
30roseodenitrificans114
31roseolit149
32sulfitobacter_ee36
33sulfitobacter_gai101
34sulfitobacter_nas14
35Synechococcus 5701
36Synechococcus 7803
37Synechococcus 7805
38Synechococcus 8102
39Synechococcus 9916
40Synechococcus 9917
41Synechococcus bl107
42Synechococcus cc9311
43Synechococcus cc9605
44Synechococcus 9902
45Synechocystis pcc6803
46Synechcoccus ja23ba
47Synechococcus ja33ab
48Synechococcus pcc7942
49Synechococcus rcc307
50thiomicrospira_crunogena
51Tricho IMS101