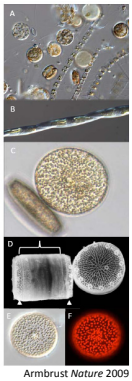
Molecular insights from diatoms living in iron-deprived oceans: individual genes to community transcriptomics

Adrian Marchetti
University of North Carolina at Chapel Hill

OCB Workshop July 2013

Diatoms are young, important and beautiful!



- Highly diverse (>100,000 species)
- Present in almost all environments
- Range in size from µm to mm
- Base of most aquatic food chains
- Some produce marine toxins
- Important to global biogeochemistry

Armbrust *Nature* 2009

Take home messages

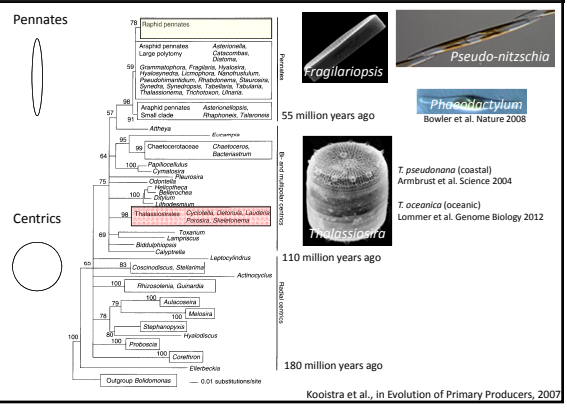
1. There is a high amount of genetic diversity in diatoms which translates into physiological and biogeochemical diversity (i.e. not all diatoms are created equal!).

1. Metatranscriptomics provides a new way of elucidating plankton-environment interactions (but there are still limitations).

Paul J. Harrison

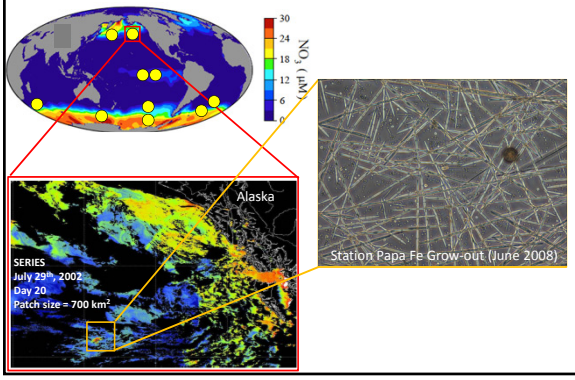
Why do pennate diatoms bloom when iron is added to HNLC regions?

Diatom phylogeny

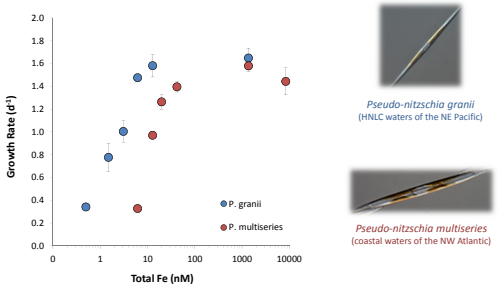


Kooistra et al., in Evolution of Primary Producers, 2007

Iron limitation and iron fertilization



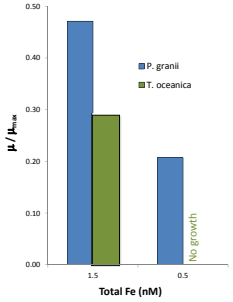
Oceanic versus coastal diatoms



Pseudo-nitzschia granii
(HNLC waters of the NE Pacific)

Pseudo-nitzschia multiseries
(coastal waters of the NW Atlantic)

Pennate versus centric oceanic diatoms



Pseudo-nitzschia granii
(HNLC waters of the NE Pacific)

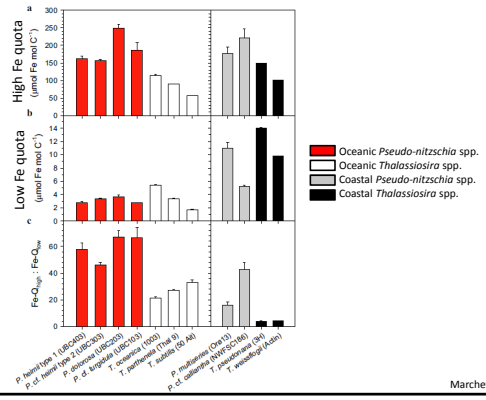
Thalassiosira oceanica
(oligotrophic waters of the N Atlantic)

Diatom acclimation to variable iron conditions

When dealing with low iron, diatoms will:

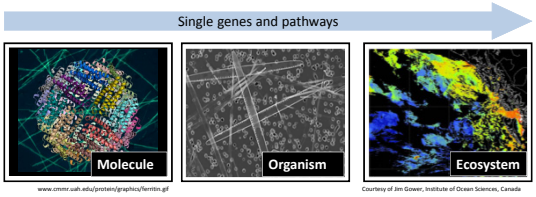
- change their cell morphology
- reduce their iron requirements
- increase their iron uptake affinity
- have an extensive iron storage capacity

Iron requirements in coastal versus oceanic diatoms



Marchetti et al. L&O 2006

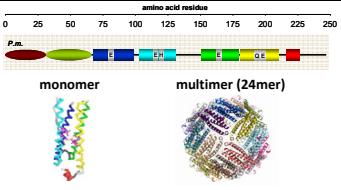
Using molecular approaches to study eukaryotic plankton



www.cmmr.uah.edu/protein/graphics/ferritin.gif

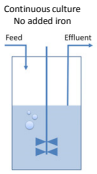
Courtesy of Jim Gower, Institute of Ocean Sciences, Canada

Ferritin is used for iron storage in pennate diatoms

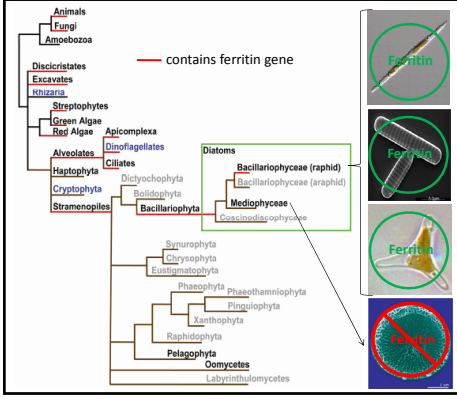


Marchetti, Parker et al. *Nature* 2009

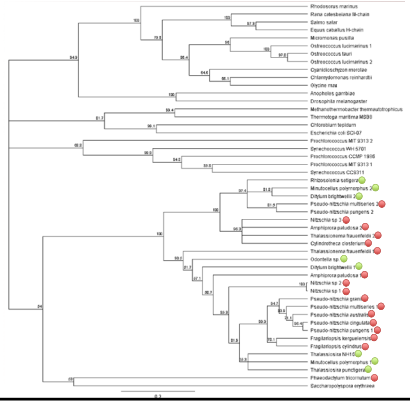
P. granii and *T. oceanica* continuous cultures



Ferritin diversity in eukaryotes



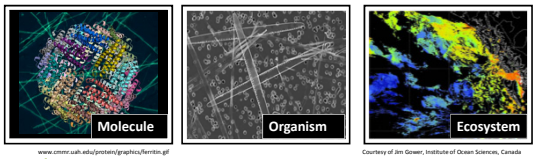
An updated FTN phylogeny



1. All examined pennates have FTN
2. Some, but not all, examined centrics have FTN

centric
pennate

Using molecular approaches to study eukaryotic plankton

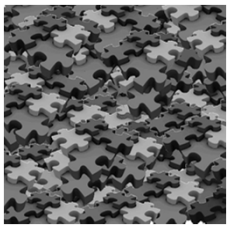


Community genomics and transcriptomics ("meta-omics")

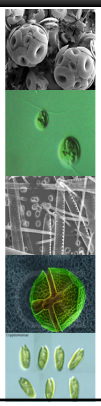
Metatranscriptomics in the marine environment

- gene expression in microorganisms not easily cultured and in their natural environment
- previously there were few metatranscriptomic studies in marine eukayotes due to:
  - a) poorly established methods
  - b) few reference genomes

However, we are making progress...



Eukaryotic phytoplankton reference organisms



Haptophytes (includes coccolithophores)
Emiliana huxleyi
Isochrysis galbana
Phaeocystis antarctica and globosa

whole genomes
Expressed Sequence Tags (ESTS)

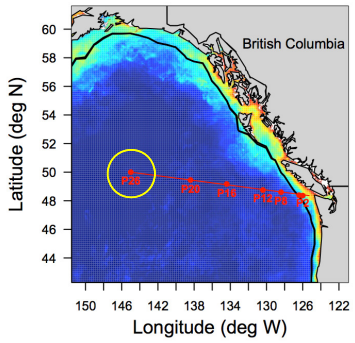
Chlorophytes (includes green algae)
Ostreococcus tauri
Ostreococcus lucimarinus
Chlamydomonas reinhardii
Volvox carteri
Chlorella sp.
Micromonas pusilla

Heterokontophyta (includes diatoms)
Thalassiosira pseudonana
Pseudo-nitzschia multiseries
Fragilariopsis cylindrus
Chaetoceros sp.
Phaeodactylum tricornutum
Pseudo-nitzschia granii
Aureococcus anophagefferens
Pseudo-nitzschia australis

Dinoflagellata (includes dinoflagellates)
multiple species

Cryptophyta
Guillardia theta
multiple species

Ocean Station Papa (P26: 145° W, 50° N) in June 2008



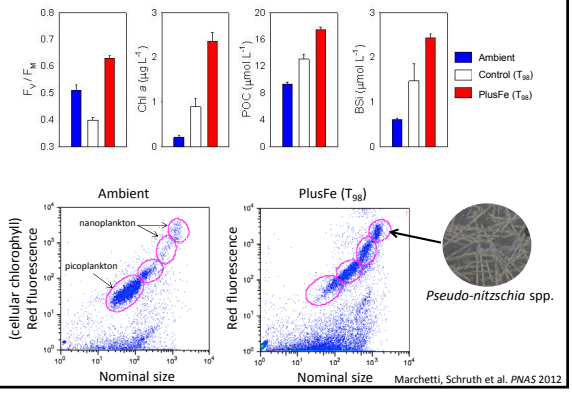
- well-characterized iron-limited, HNLC region
- site of SERIES iron-enrichment experiment in July 2002

Comparative metatranscriptome at Station Papa (Papa-GO)

Ambient: ~1000L of seawater were filtered from ambient OSP (T_0)

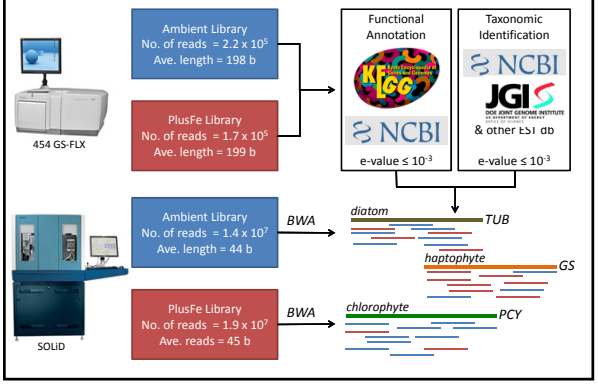
PlusFe and Ctl Treatments: 24 x 10L were filled with seawater and spiked with 4 nM $FeCl_3$. 3 x 10L were left unamended → After 98 h, seawater was filtered (T_{98})

Diatoms exhibited the largest response to iron enrichment

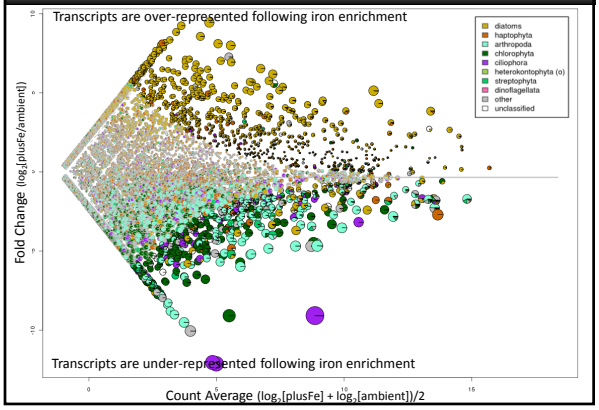


Marchetti, Schruth et al. *PNAS* 2012

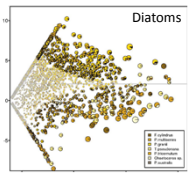
Metatranscriptomic annotation pipeline

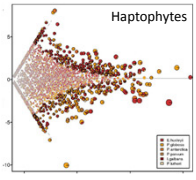


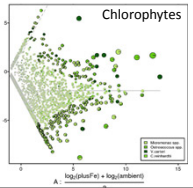
MANTA SOLiD: 3003 genes are significantly differentially expressed



MANTA Plots were created for taxonomic subsets





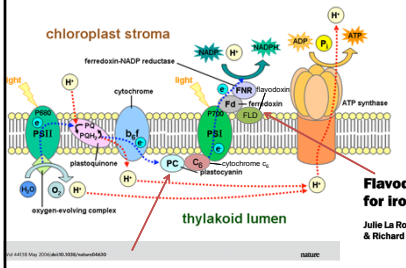


Proportion of reads with taxonomic hits assigned to major phytoplankton groups (from 454 reads)

	Ambient	plusFe
Diatoms	11%	35%
Haptophytes	30%	33%
Chlorophytes	26%	11%

Marchetti, Schruth et al. *PNAS* 2012

Photosynthetic architecture in diatoms differs depending on iron status



Flavodoxin as an *in situ* marker for iron stress in phytoplankton

Julie La Roche*, Philip W. Boyd†, R. Michael L. McKay*‡ & Richard J. Geider§

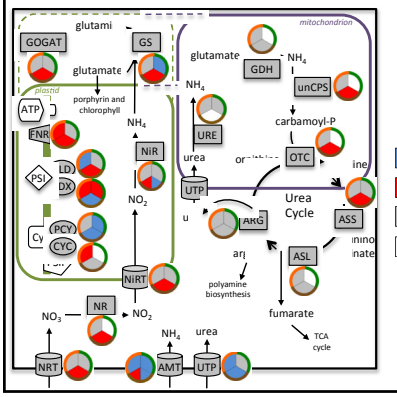
Nature 1996

LETTERS

Copper-containing plastocyanin used for electron transport by an oceanic diatom

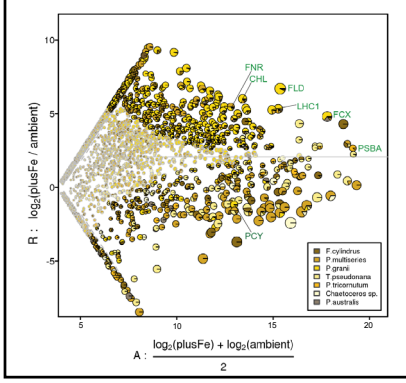
Graham Peers† & Neil M. Price†

Phytoplankton gene expression response to iron enrichment



Marchetti, Schruth et al. *PNAS* 2012

Diatom gene expression response to iron enrichment



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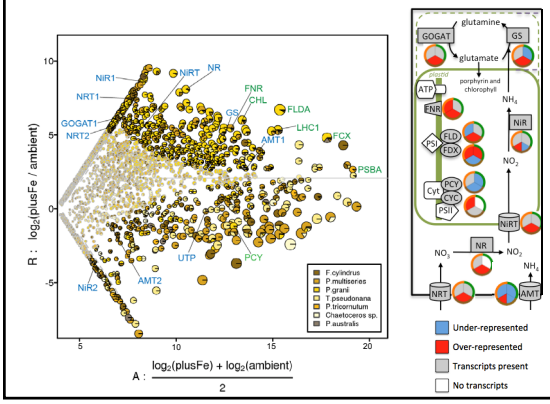
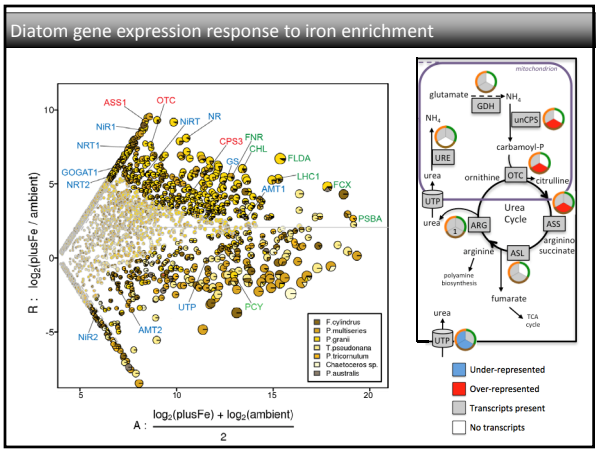
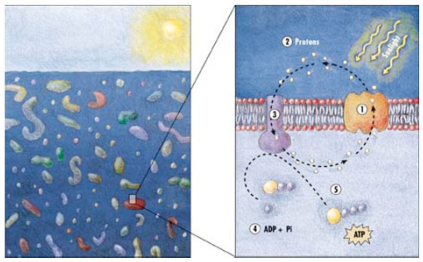


Diatom gene expression response to iron enrichment



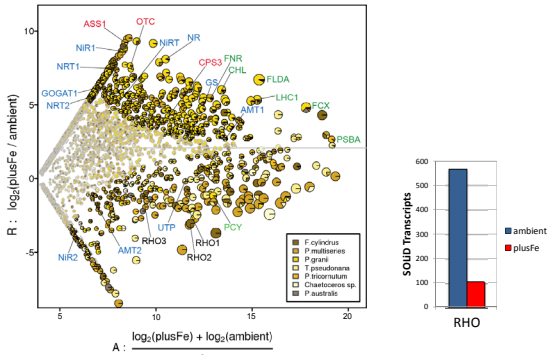
Proteorhodopsins in marine microbes



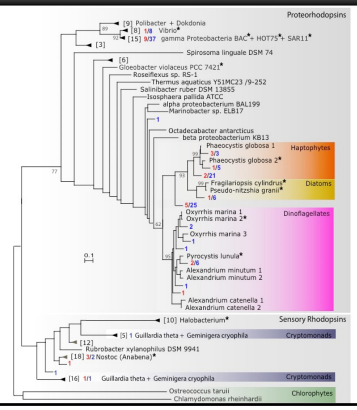
Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea

Beja et al. Science 2000

Diatom gene expression response to iron enrichment



HGT of rhodopsins from bacteria to eukaryotic phytoplankton?

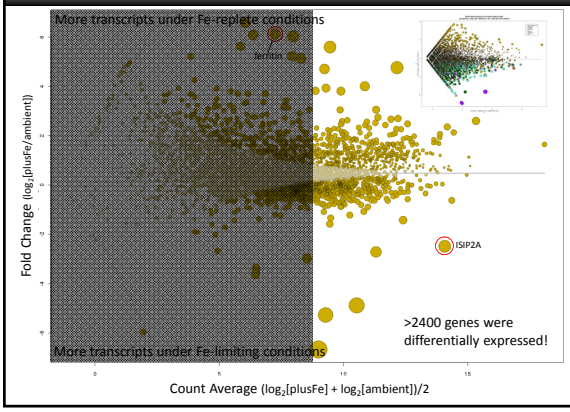


- RHO is not present in all diatoms
- Eukaryotic phytoplankton fall within a xanthorhodopsin clade of eubacteria

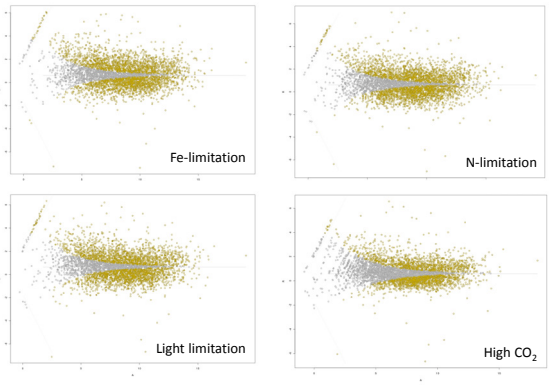
Conclusions

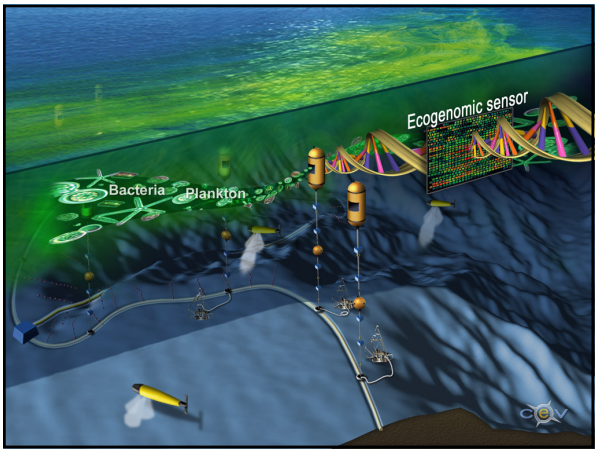
- Immediately following iron enrichment, oceanic diatoms continue to express iron-free protein encoding genes (e.g flavodoxin, plastocyanin, non-Fe SODs, etc.)
- Most highly expressed genes are characteristic of diatoms pathways (urea cycle, nitrate assimilation, Si transporters and polyamine synthesis, PUFA and chrysolaminarin biosynthesis)
- Oceanic diatoms contain ferritin and rhodopsin-type genes that may provide a competitive advantage in iron-limited environments

Pseudo-nitzschia granii transcriptome in response to iron



Future work: Transcriptome profiling ecologically important diatoms





Acknowledgements

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