

The Oceanography of Plankton

Guest Lecture:

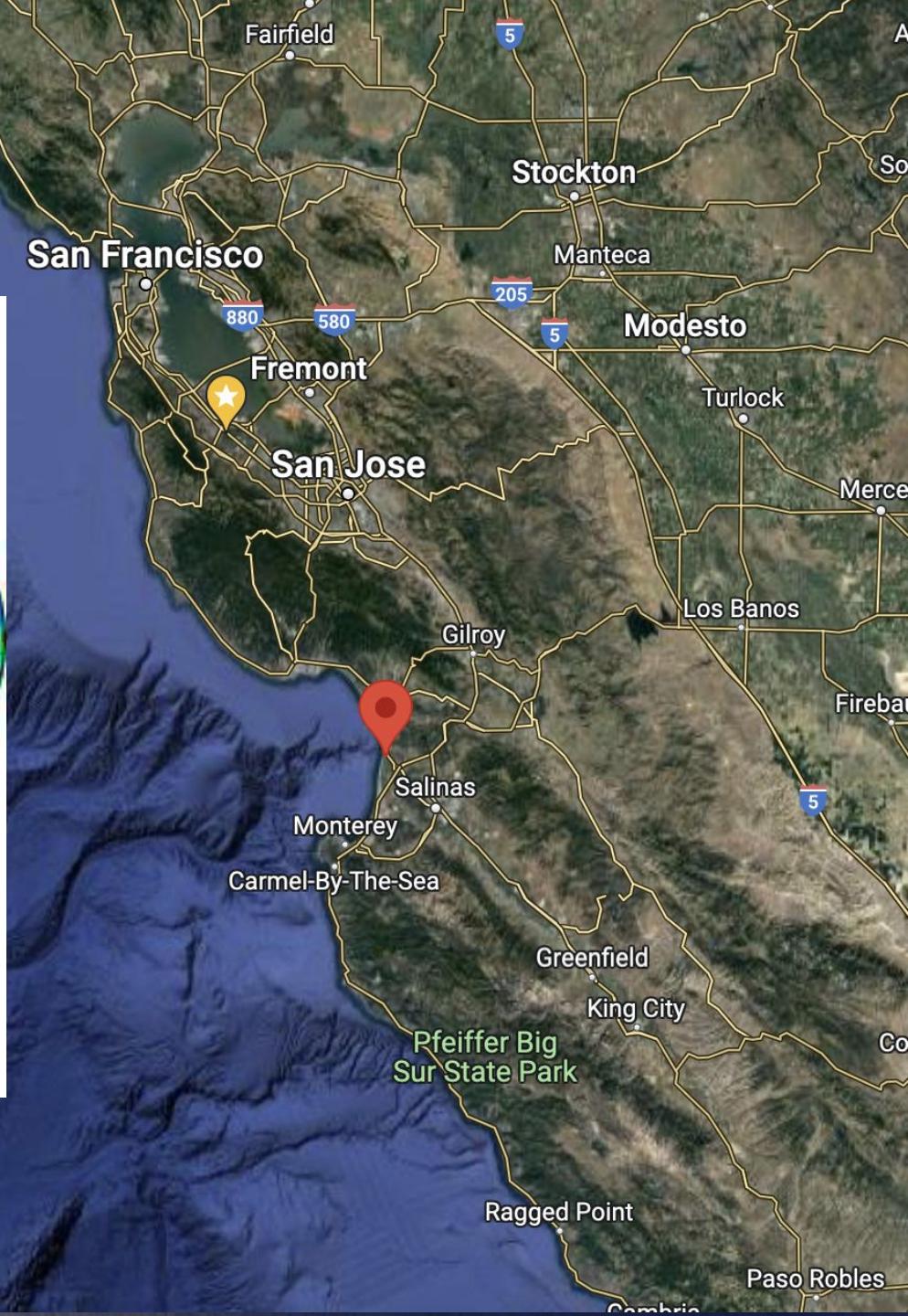
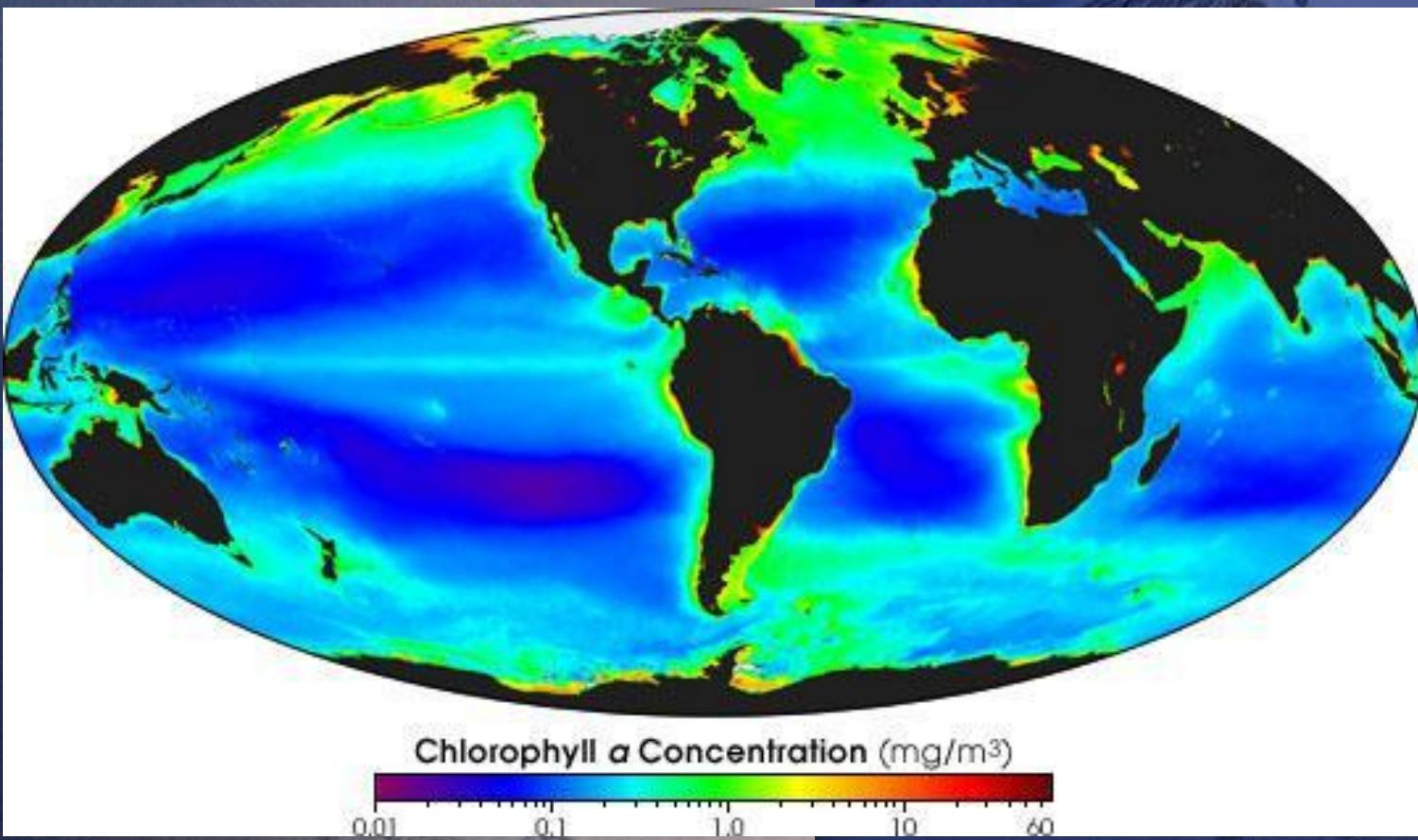
Biology 145 / Computer Science 185C: Marine Bioinformatics

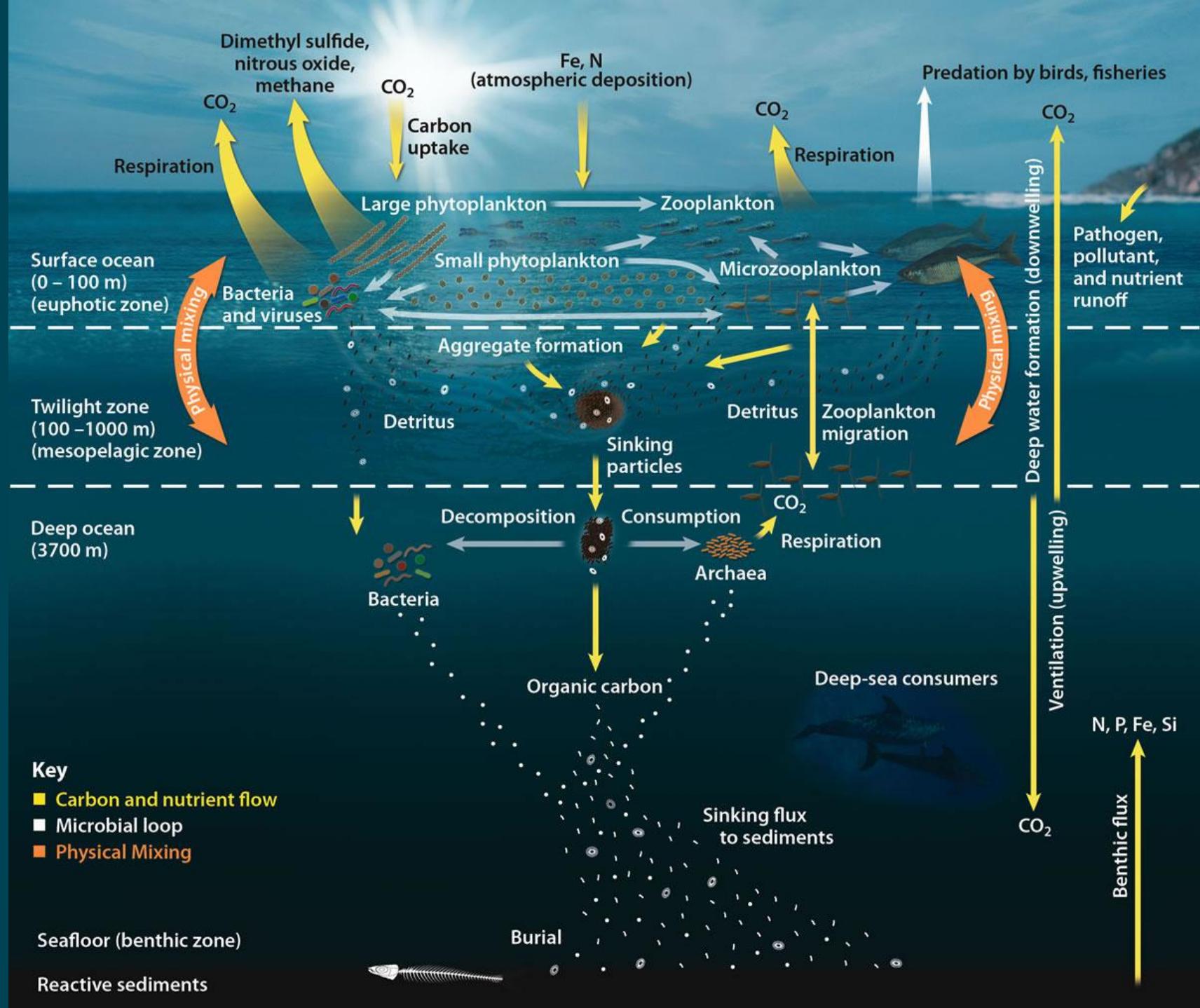
Sarah R. Smith

Assistant Professor, Moss Landing Marine Laboratories

Learning Outcomes

- Define two major functional categories of plankton
- Contextualize plankton diversity relative to diversity on earth
- Explain the difference between Biological Oceanography and Marine Biology as it relates to the scale of processes
- Recognize why molecular tools are important to study plankton diversity
- Recall major field campaigns that pioneered the use of molecular biology to study plankton
- Understand why diatoms have emerged as a model system for the study of marine phytoplankton





Phytoplankton and Zooplankton

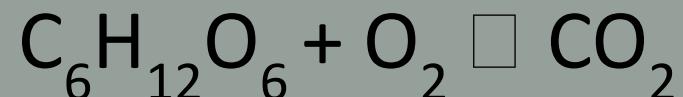
Photosynthesis



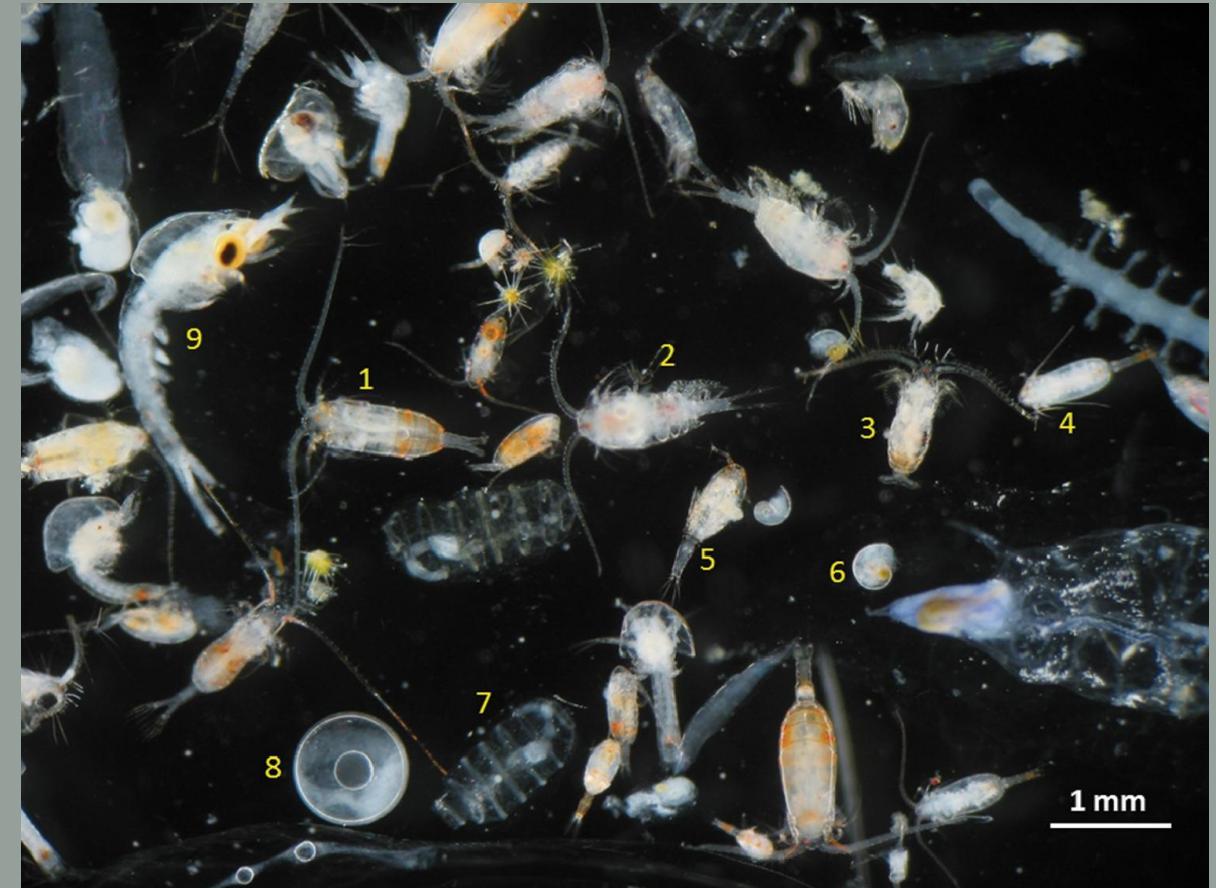
Inorganic Nutrients



Zooplankton



“Nutrients” from ingesting food

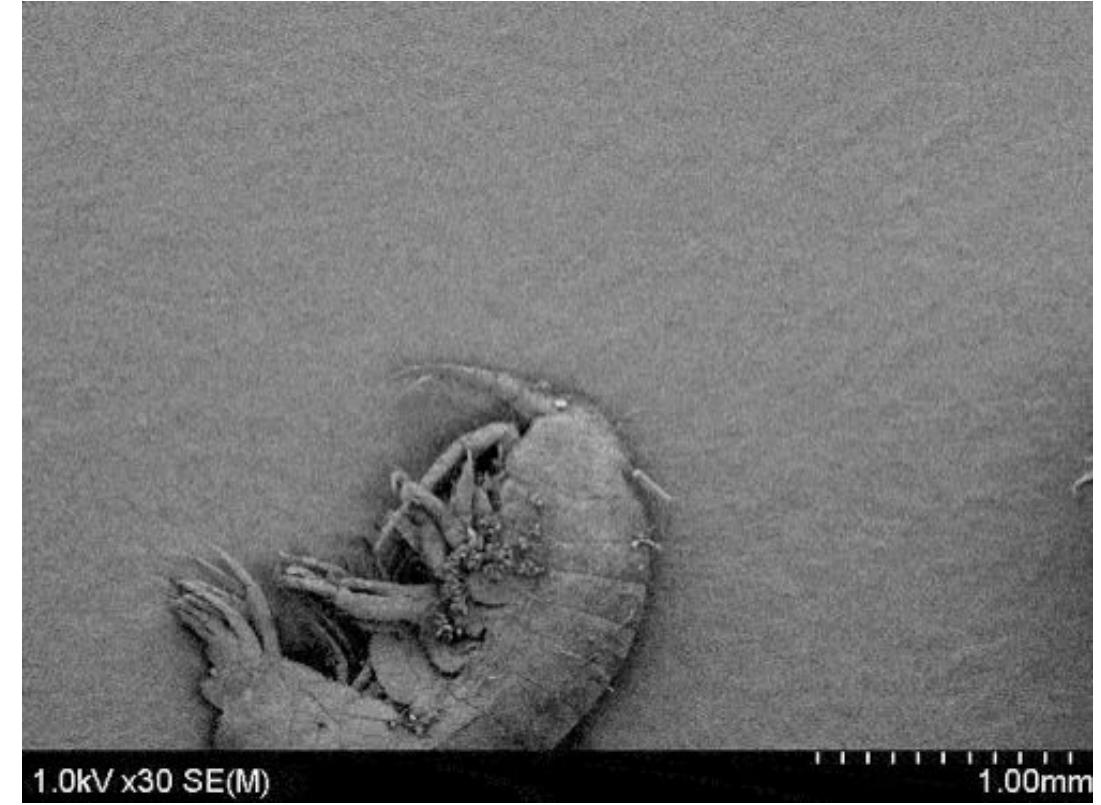
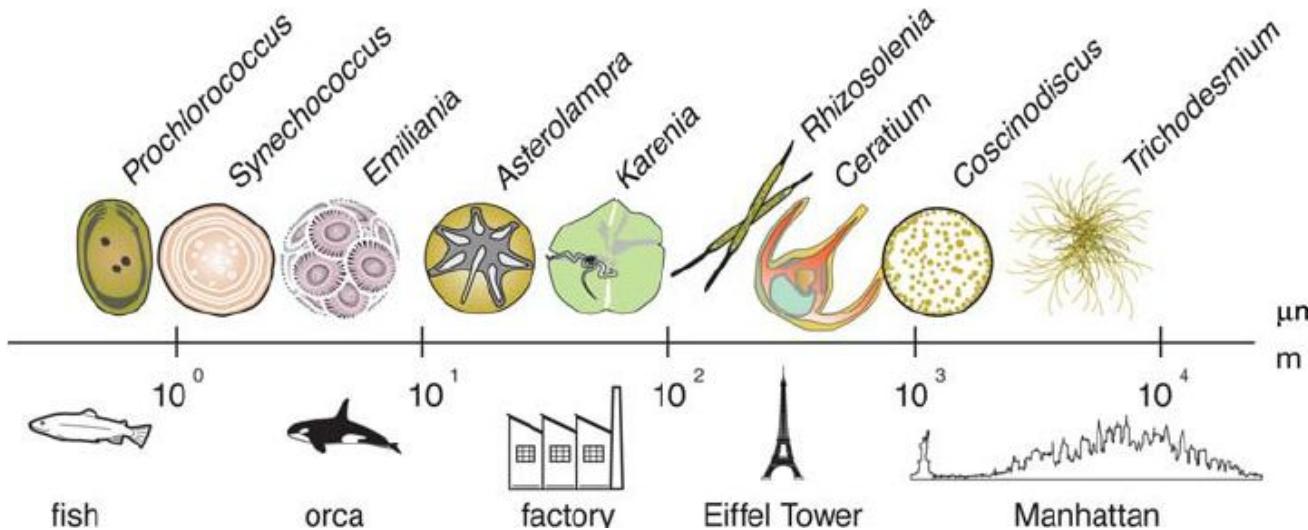
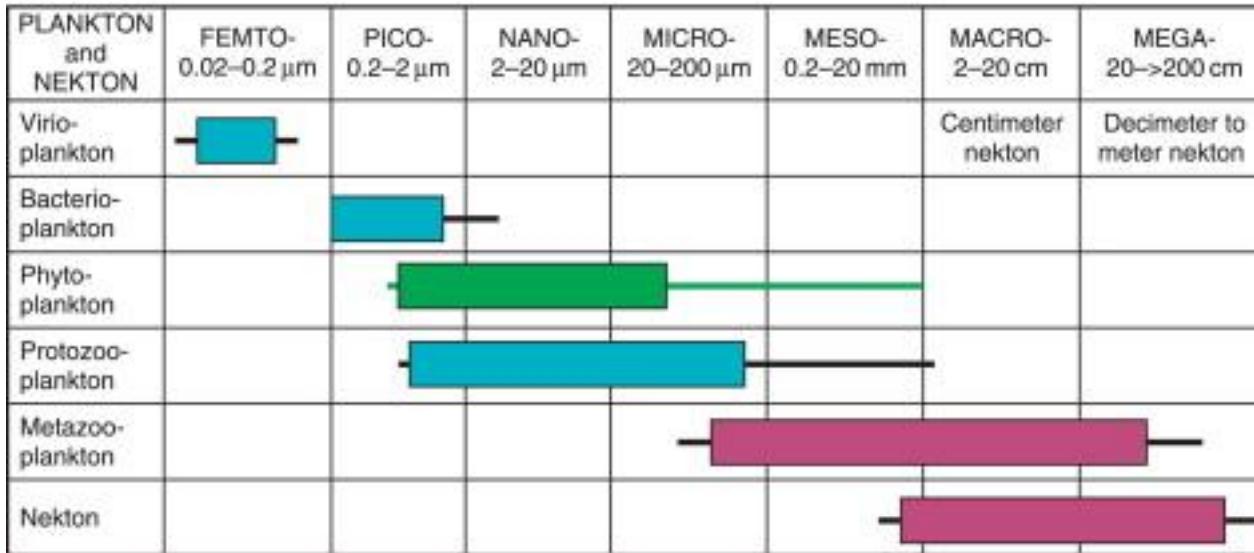


How small is small?

Primary
producers

Microbial
consumers

Multicellular
consumers



Kingdoms of Life

BIOLOGY



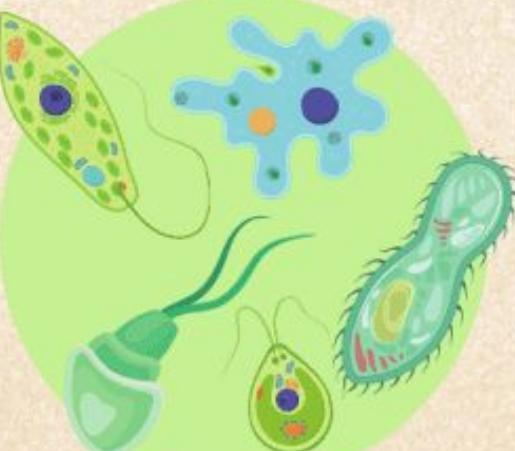
Animalia



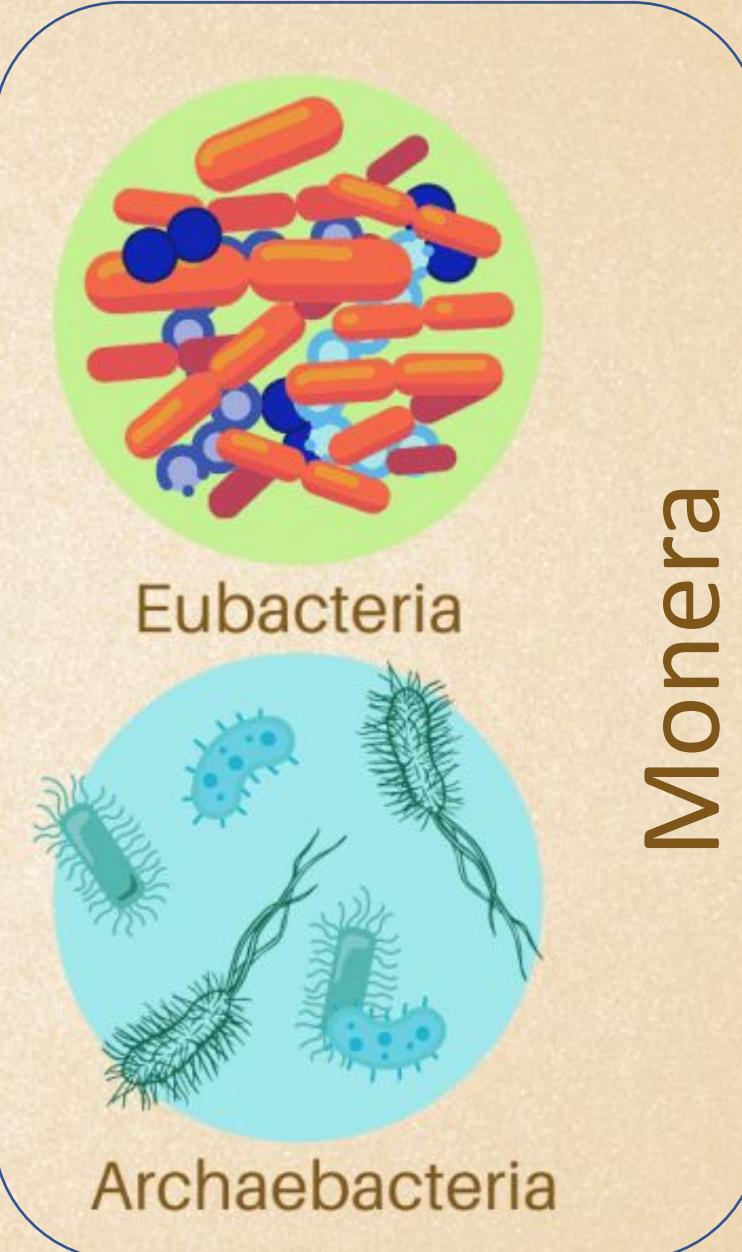
Fungi



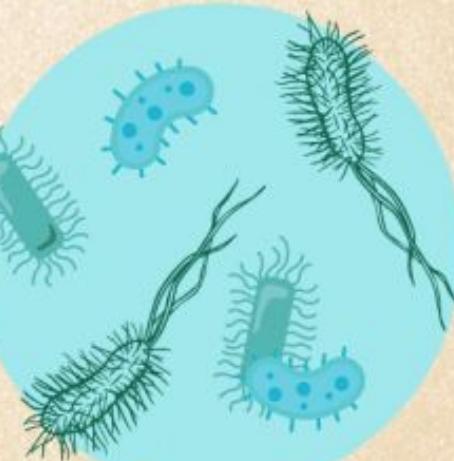
Plantae



Protista



Eubacteria



Archaeabacteria

Monera

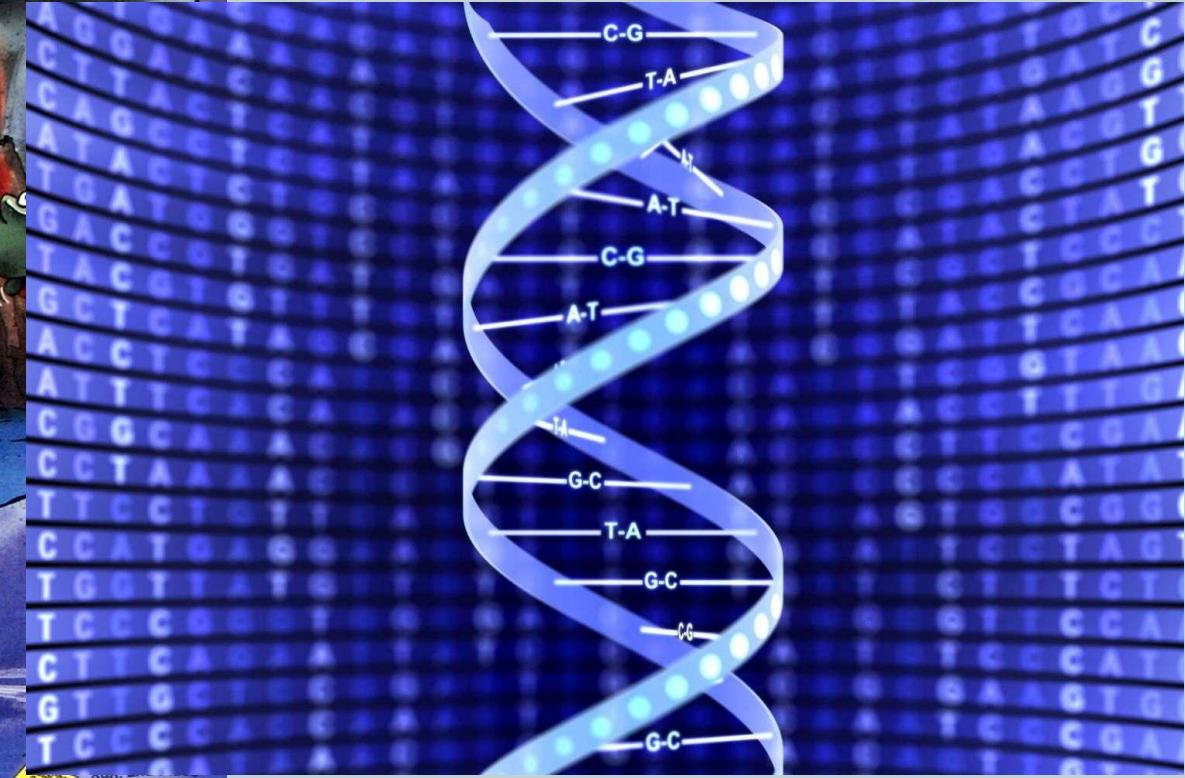
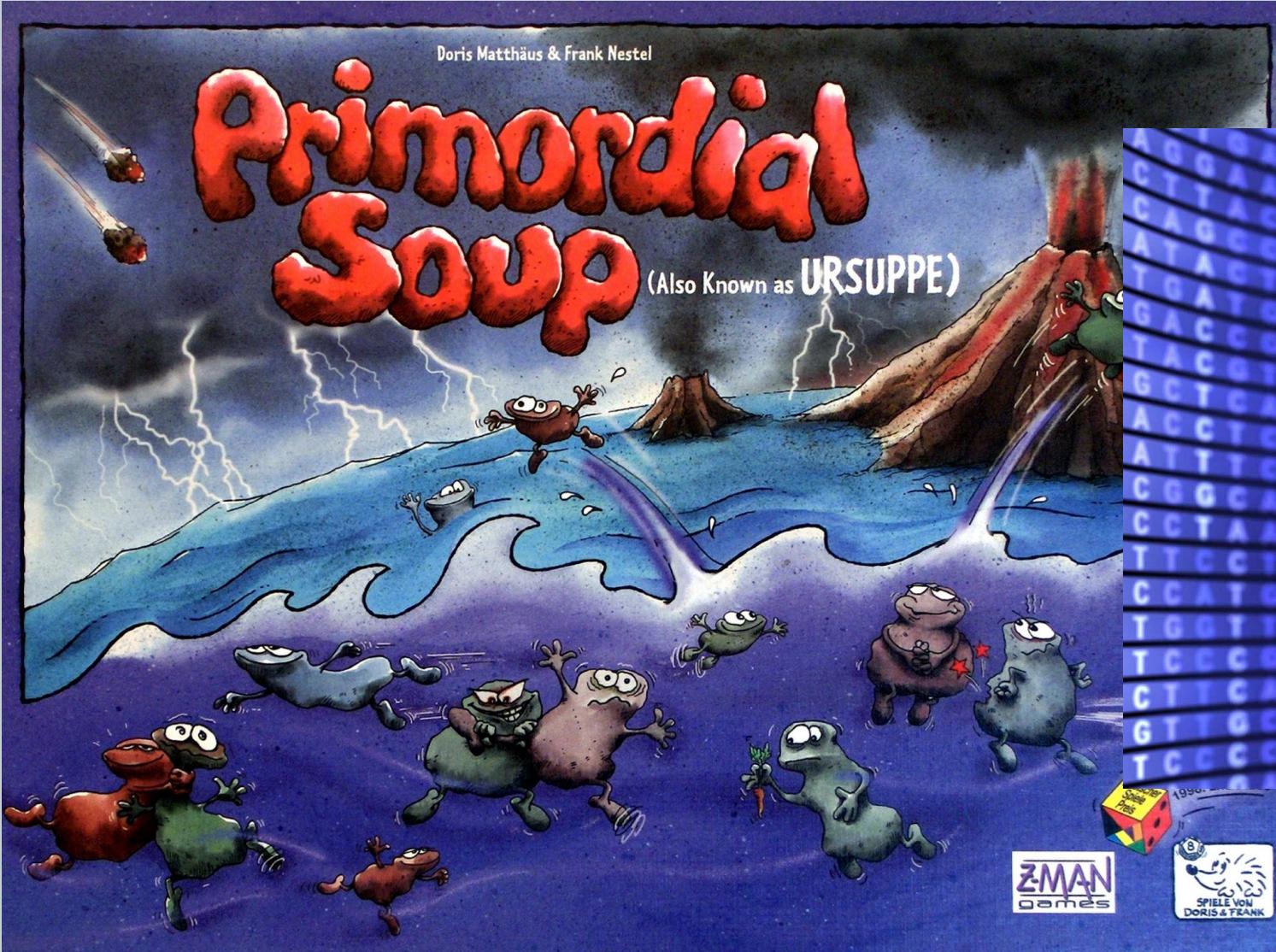
Beyond traditional kingdoms [edit]

While the concept of kingdoms continues to be used by some taxonomists, there has been a movement away from traditional kingdoms, as they are no longer seen as providing a [cladistic](#) classification, where there is emphasis in arranging organisms into natural groups.^[41]

Cladistics is an approach to [biological classification](#) in which [organisms](#) are categorized in groups ("[clades](#)") based on hypotheses of most recent [common ancestry](#).

Linnaeus 1735 ^[23]	Haeckel 1866 ^[24]	Chatton 1925 ^{[25][26]}	Copeland 1938 ^{[27][28]}	Whittaker 1969 ^[29]	Woese et al. 1977 ^{[30][31]}	Woese et al. 1990 ^[32]	Cavalier- Smith 1993 ^{[33][34][35]}	Cavalier- Smith 1998 ^{[36][37][38]}	Ruggiero et al. 2015 ^[39]
—	—	2 empires	2 empires	2 empires	2 empires	3 domains	3 superkingdoms	2 empires	2 superkingdoms
2 kingdoms	3 kingdoms	—	4 kingdoms	5 kingdoms	6 kingdoms	—	8 kingdoms	6 kingdoms	7 kingdoms
—	Protista	Prokaryota	Monera	Monera	Eubacteria Archaeabacteria	Bacteria	Eubacteria Archaeabacteria	Bacteria	Bacteria Archaea
Vegetabilia	Plantae	Eukaryota	Plantae	Protista	Protista	Archaea	Archezoa Protozoa	Protozoa	Protozoa
Animalia	Animalia		Animalia	Animalia	Plantae Fungi	Eucarya	Chromista Plantae Fungi	Chromista Plantae Fungi	Chromista Plantae Fungi

If the goal is to organize all life on earth, based on the last common ancestor, you need something organisms have retained since the origin of life



A JOURNEY THROUGH THE HISTORY OF DNA SEQUENCING

A Journey Through The History Of DNA Sequencing



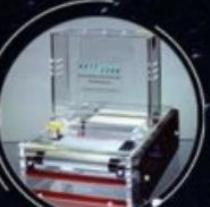
1869

Friedrich Miescher isolates DNA, in the form of chromatin



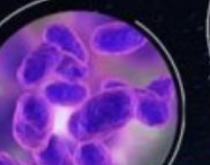
1975

Frederick Sanger introduces "PLUS AND MINUS" sequencing platform, using DNA sequencing gels to separate newly synthesized DNA by size



1984

Fritz P. Non-radioactive sequencing platform



1984



1999

"sequencing-by-synthesis" method that uses fluorescent dyes



2002

Complete human genome published



2007

SOLID system, which uses sequencing by synthesis



2011

Pacific Biosciences launches first commercial product - PacBio RS - based on SINGLE MOLECULE REAL TIME technology



1953

Crick, Watson and Franklin discovered the structure of DNA as a DOUBLE HELIX



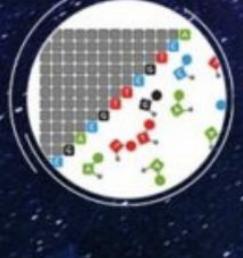
1972

Walter Fiers sequences first whole gene: coding for a MS2 virus protein



1977

Frederick Sanger establishes the CHAIN TERMINATION METHOD and uses it to sequence first complete genome: bacteriophage ϕ X174



1987

Leroy Hood and ABI develop first semi-automated DNA sequencing machines



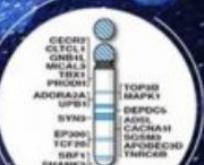
1996

Ronald H. Uhlen and Nyren introduce PYROSEQUENCING, a "sequencing-by-synthesis" method

S. CEREVIAE genome is sequenced

1999

First human chromosome 22 is sequenced



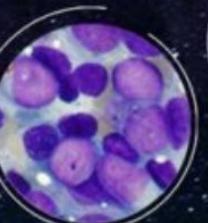
2005

The 454 SYSTEM, based on pyrosequencing, becomes the first next-generation sequencer to come on the market



2008

Whole DNA sequence of a CANCER is decoded for the first time

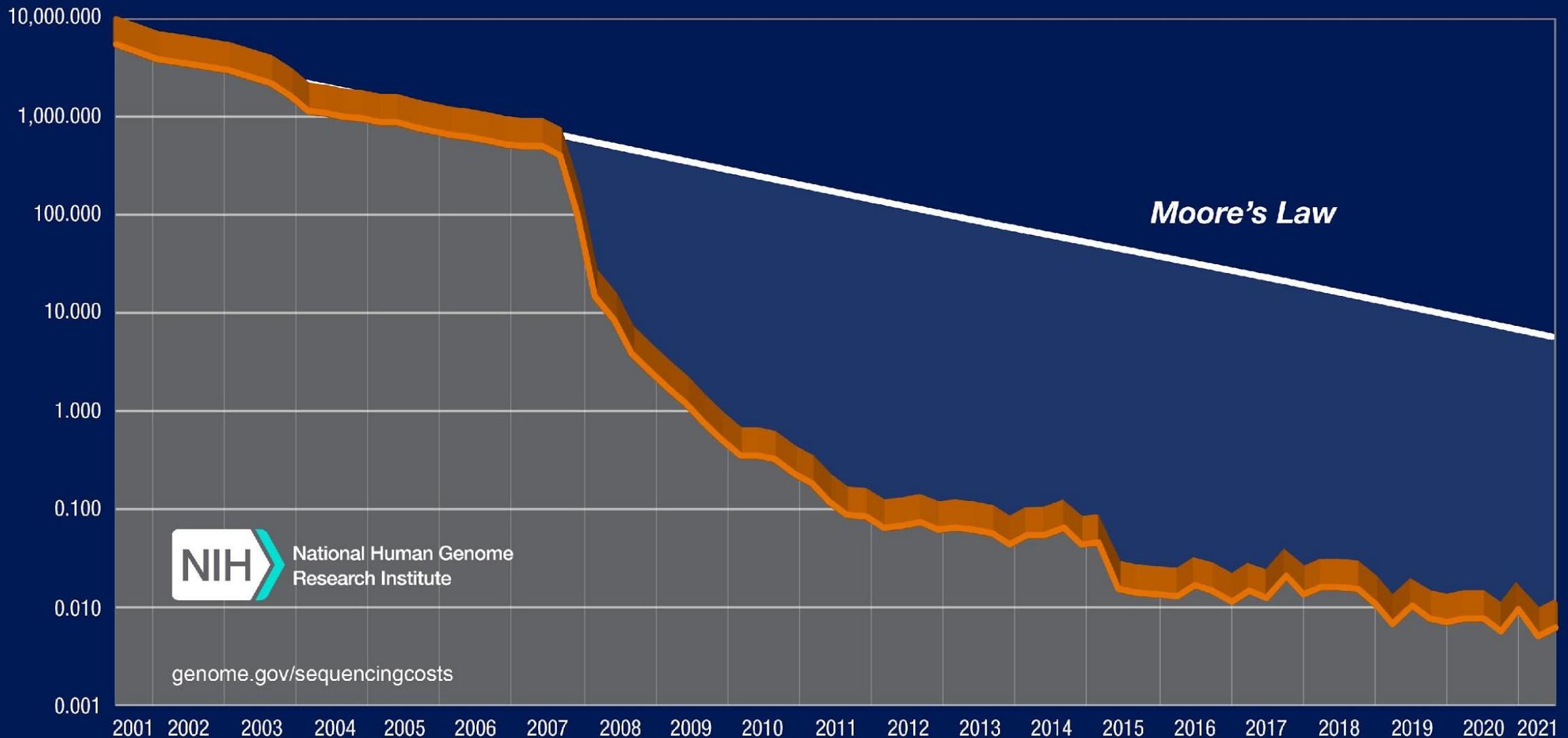


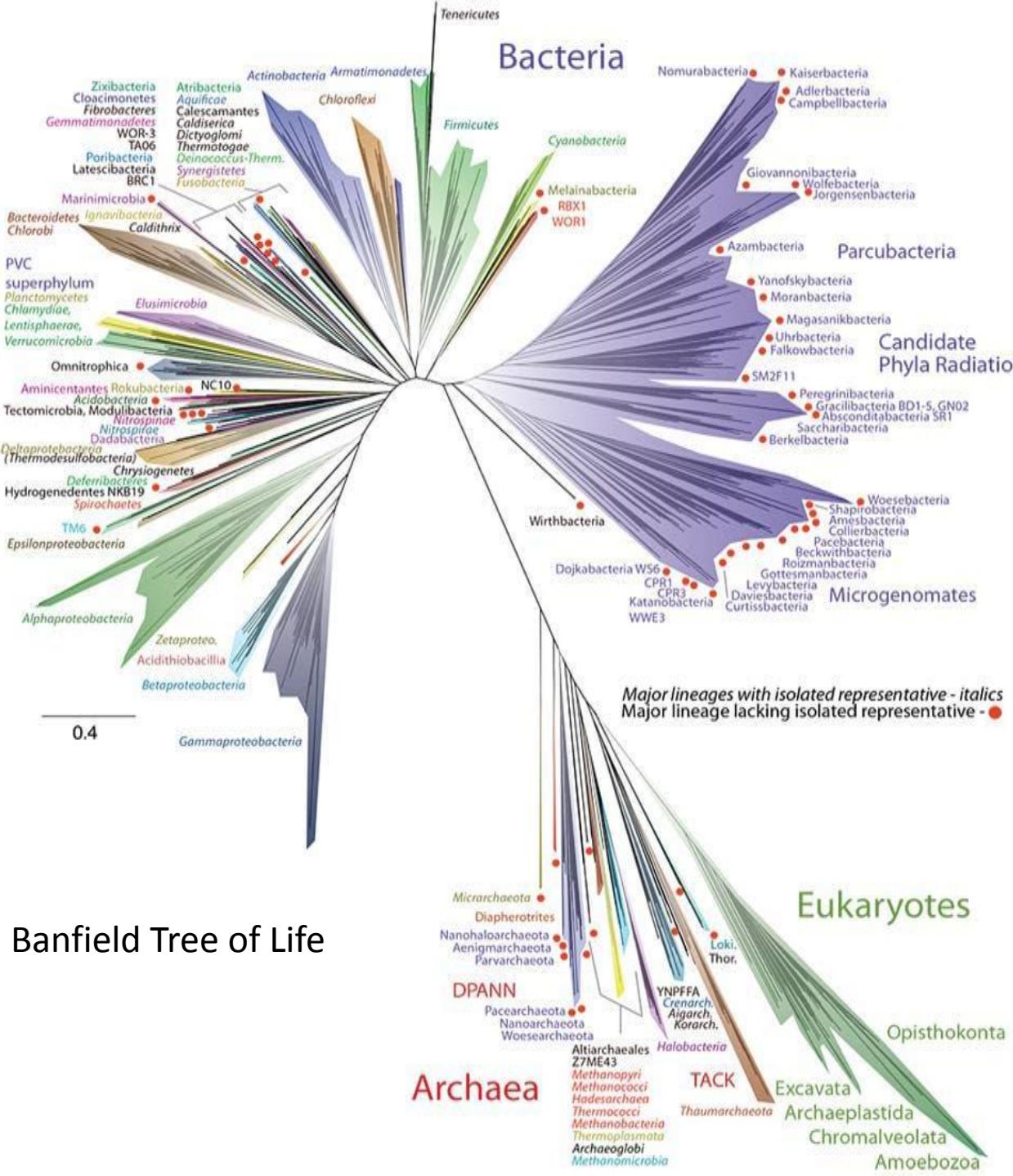
2012

Oxford Nanopore Technologies commercializes NANOPORE-SEQUENCING



Cost per Raw Megabase of DNA Sequence





Banfield Tree of Life

Prokaryotes: Bacteria & Archaea



*Unicellular, Metabolically Diverse
Autotrophic and heterotrophic*

Eukaryotes:

*Unicellular and multicellular
Autotrophic and heterotrophic*

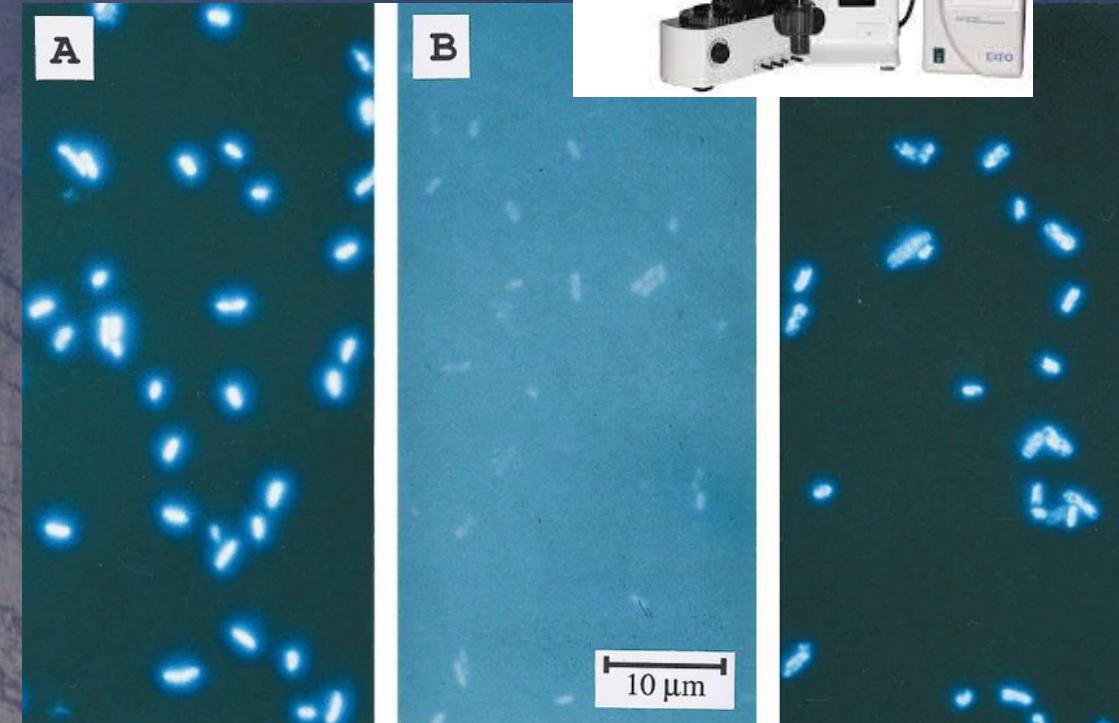


Great Plate Count Anomaly

0.1-1% of total marine bacteria could be cultivated



Epifluorescence microscopy and DNA stains like Acridine Orange, allowed scientists to count bacteria directly



Genetic diversity in Sargasso Sea bacterioplankton

Stephen J. Giovannoni, Theresa B. Britschgi,
Craig L. Moyer & Katharine G. Field

Department of Microbiology, Oregon State University, Corvallis,
Oregon 97331, USA

BACTERIOPLANKTON are recognized as important agents of biogeochemical change in marine ecosystems, yet relatively little is known about the species that make up these communities. Uncertainties about the genetic structure and diversity of natural bacterioplankton populations stem from the traditional difficulties associated with microbial cultivation techniques. Discrepancies between direct counts and plate counts are typically several orders of magnitude, raising doubts as to whether cultivated marine bacteria are actually representative of dominant planktonic species^{1–3}. We have phylogenetically analysed clone libraries of eubacterial 16S ribosomal RNA genes amplified from natural populations of Sargasso Sea picoplankton by the polymerase chain reaction⁴. The analysis indicates the presence of a novel microbial group, the SAR11 cluster, which appears to be a significant component of this oligotrophic bacterioplankton community. A second cluster of lineages related to the oxygenic phototrophs—cyanobacteria, prochlorophytes and chloroplasts—was also observed. However, none of the genes matched the small subunit rRNA sequences of cultivated marine cyanobacteria from similar habitats. The diversity of 16S rRNA genes observed within the clusters suggests that these bacterioplankton may be consortia of independent lineages sharing surprisingly distant common ancestors.

Our purpose was to identify genetic markers that could be used to examine the distributions and diversity of bacterioplankton with nucleic acid hybridization probes in routine studies.

NATURE · VOL 345 · 3 MAY 1990

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Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5685–5689, June 1992
Ecology

Archaea in coastal marine environments

(archaeabacteria/phylogeny/bacterioplankton/molecular ecology)

EDWARD F. DELONG*

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Communicated by George N. Somero, March 17, 1992 (received for review February 4,

ABSTRACT Archaea (archaeabacteria) are a phenotypically diverse group of microorganisms that share a common evolutionary history. There are four general phenotypic groups of archaea: the methanogens, the extreme halophiles, the sulfate-reducing archaea, and the extreme thermophiles. In the marine environment, archaeal habitats are generally limited to shallow or deep-sea anaerobic sediments (free-living and endosymbiotic methanogens), hot springs or deep-sea hydrothermal vents (methanogens, sulfate reducers, and extreme thermophiles), and highly saline land-locked seas (halophiles). This report provides evidence for the widespread occurrence of unusual archaea in oxygenated coastal surface waters of North America. Quantitative estimates indicated that up to 2% of the total ribosomal RNA extracted from coastal bacterioplankton assemblages was archaeal. Archaeal small-subunit ribosomal RNA-encoding DNAs (rDNAs) were cloned from mixed bacterioplankton populations collected at geographically distant sampling sites. Phylogenetic and nucleotide signature analyses of these cloned rDNAs revealed the presence of two lineages of archaea, each sharing the diagnostic signatures and structural features previously established for the domain Archaea. Both of these lineages were found in bacterioplankton populations collected off the east and west coasts of North America. The abundance and distribution of these archaea in oxic coastal surface waters suggests that these microorganisms represent undescribed physiological types of archaea, which reside and compete with aerobic, mesophilic eubacteria in marine coastal environments.

macroaggregates. PCR primers based on eukaryotic genes surprisingly, archaeal lineages and characterized

Bacterioplankton were collected and filtered. Bacterioplankton were filtered water (Amicon) fitted with a 0.22-μm cutoff. Two volumes of 10% sucrose centrifuged at 10,000 × g for 1 h. Cell pellets were washed with seawater and resuspended in 1 ml of fixative (2% paraformaldehyde) and fixed for 1 h. Cells were then rinsed with 1 ml of PBS and filtered through a 0.22-μm filter.

Extraction of DNA from the cell pellets was performed using a modified protocol described (5) for the preparation of bacterial genomic DNA. Briefly, the

SAR11 Bacteria: The Most Abundant Plankton in the Oceans

Annual Review of Marine Science

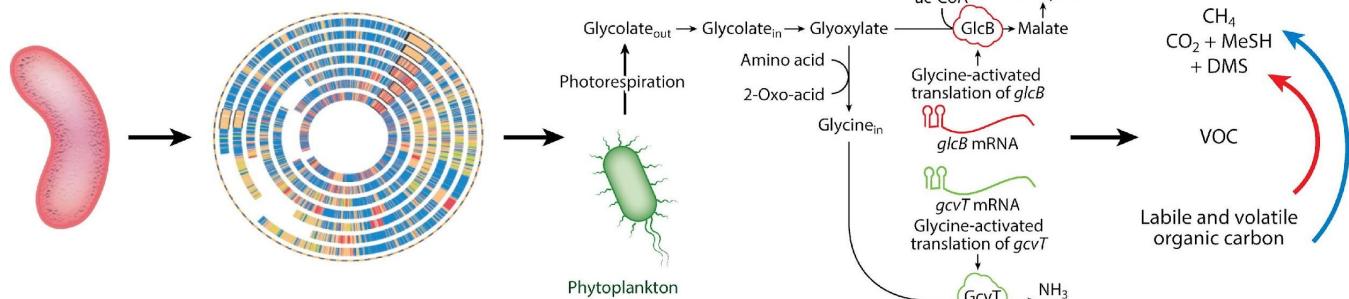
Vol. 9:231–255 (Volume publication date January 2017)

First published online as a Review in Advance on September 28, 2016

<https://doi.org/10.1146/annurev-marine-010814-015934>

Stephen J. Giovannoni

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331; email: steve.giovannoni@oregonstate.edu



Giovannoni SJ. 2017.

Annu. Rev. Mar. Sci. 9:231–55

Abstract

SAR11 is a group of small, carbon-oxidizing bacteria that reach a global estimated population size of 2.4×10^{28} cells—approximately 25% of all plankton. They are found throughout the oceans but reach their largest numbers in stratified, oligotrophic gyres, which are an expanding habitat in the warming oceans. SAR11 likely had a Precambrian origin and, over geological time, evolved into the niche of harvesting labile, low-molecular-weight dissolved organic matter (DOM). SAR11 cells are minimal in size and complexity, a phenomenon known as streamlining that is thought to benefit them by lowering the material costs of replication and maximizing transport functions that are essential to competition at ultralow nutrient concentrations. One of the surprises in SAR11 metabolism is their ability to both oxidize and produce a variety of volatile organic compounds that can diffuse into the atmosphere. SAR11 cells divide slowly and lack many forms of regulation commonly used by bacterial cells to adjust to changing environmental conditions. As a result of genome reduction, they require an unusual range of nutrients, which leads to complex biochemical interactions with other plankton. The study of SAR11 is providing insight into the biogeochemistry of labile DOM and is affecting microbiology beyond marine science by providing a model for understanding the evolution and function of streamlined cells.

Global Ocean Sampling Expedition (GOS) 2004 - 2006

Overview

In 2004, after a successful pilot project of shotgun metagenomics sequencing at the Bermuda Atlantic Time Series site, J. Craig Venter, PhD, and a Venter Institute team launched the Sorcerer II Global Ocean Sampling (GOS) Expedition. Inspired by 19th Century sea voyages like Darwin's on the H.M.S. Beagle and Captain George Nares on the H.M.S. Challenger, The Sorcerer II circumnavigated the globe for more than two years, covering a staggering 32,000 nautical miles, visiting 23 different countries and island groups on four continents.



JCVI J. CRAIG VENTER
INSTITUTE®

The Origin of Marine Metagenomics

(untargeted environmental sequencing)

Data has been deposited to GenBank



Nucleotide

Nucleotide

venter global ocean survey

Create alert Advanced

Species

Bacteria (2)

Customize ...

Molecule types

genomic DNA/RNA (2,633,897)

Customize ...

Source databases

INSDC (GenBank) (2,633,897)

Customize ...

Sequence Type

Nucleotide (3)

GSS (2,633,894)

Sequence length

Custom range...

Release date

Custom range...

Revision date

Custom range...

[Clear all](#)

[Show additional filters](#)

Summary ▾ 20 per page ▾ Sort by Default order ▾

Send to: ▾

Items: 1 to 20 of 2633897

<< First < Prev Page of 131695 Next > Last >>

[1095516108092 Global-Ocean-Sampling_GS-31-01-01-1P3-1P8KB marine metagenome genomic](#)

1. [clone 1061005964225 3'. genomic survey sequence](#)

748 bp linear DNA

Accession: EK42933.1 GI: 128192458

[BioProject](#) [BioSample](#) [PubMed](#) [Taxonomy](#)

[GenBank](#) [FASTA](#) [Graphics](#)

[1095516108091 Global-Ocean-Sampling_GS-31-01-01-1P3-1P8KB marine metagenome genomic](#)

2. [clone 1061005964223 3'. genomic survey sequence](#)

894 bp linear DNA

Accession: EK429332.1 GI: 128192457

[BioProject](#) [BioSample](#) [PubMed](#) [Taxonomy](#)

[GenBank](#) [FASTA](#) [Graphics](#)

[1095516108090 Global-Ocean-Sampling_GS-31-01-01-1P3-1P8KB marine metagenome genomic](#)

3. [clone 1061005964221 3'. genomic survey sequence](#)

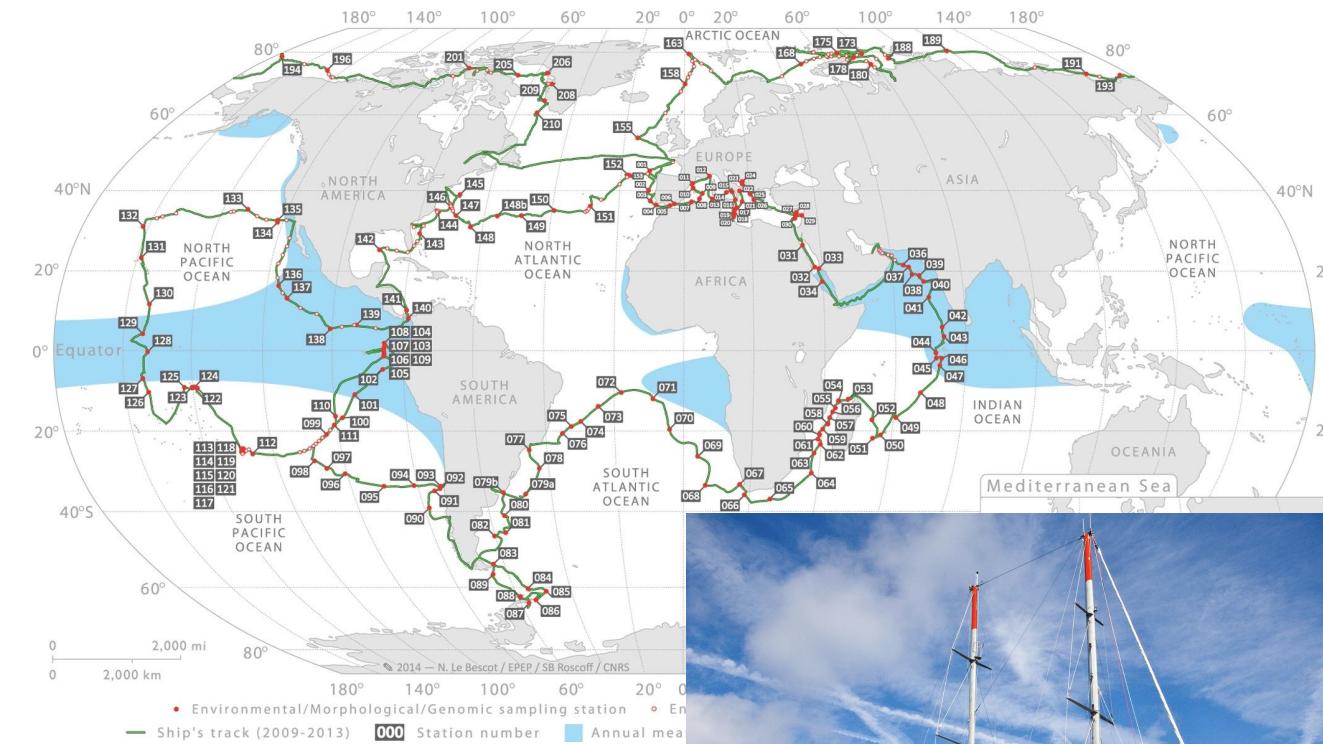
757 bp linear DNA

Accession: EK429331.1 GI: 128192456

[BioProject](#) [BioSample](#) [PubMed](#) [Taxonomy](#)

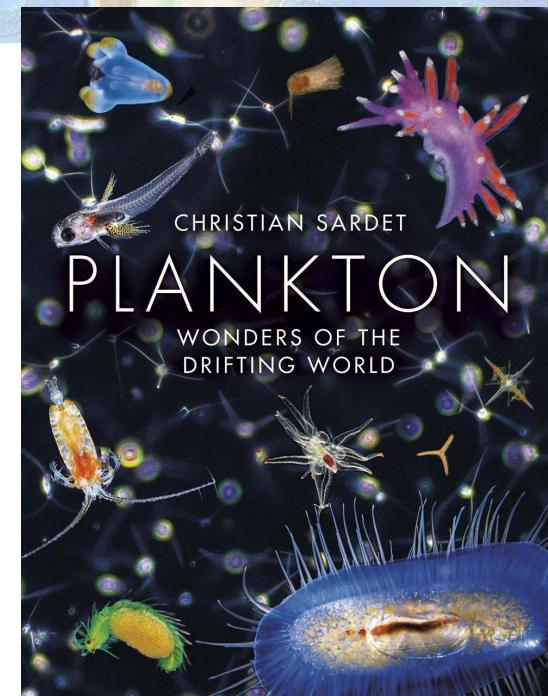
[GenBank](#) [FASTA](#) [Graphics](#)

TARA Oceans Expedition (2009 – 2013)



Ocean Atlas Database

Coffee Table
Book □
Only \$45!



DNA Sequencing impacts on biological oceanography

- Redefined organization of the diversity of life on earth
- Provided tools to evaluate diversity of microbial life in the ocean, and the functional roles of microbes that are difficult to study otherwise (i.e. SAR11 bacteria)
- What else?

PLANKTON CHRONICLES

EPISODES

NEWS

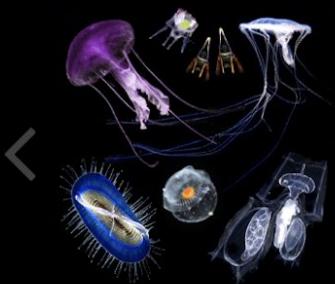
THE PROJECT

THE BOOK

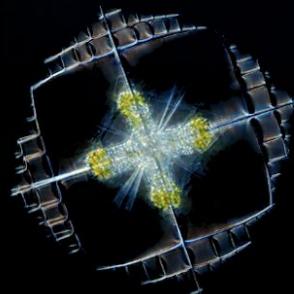
CONTACT US



THE WORLD OF PLANKTON



PLANKTON OF THE
RIVIERA



ANIMAL+VEGETAL



CERATIUM



PROTISTS 2



SIPHONOPHORES

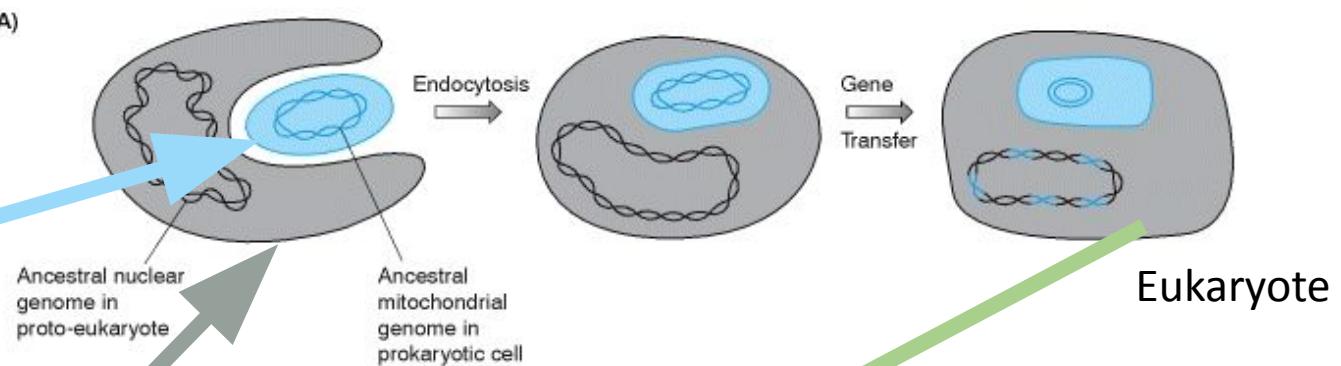
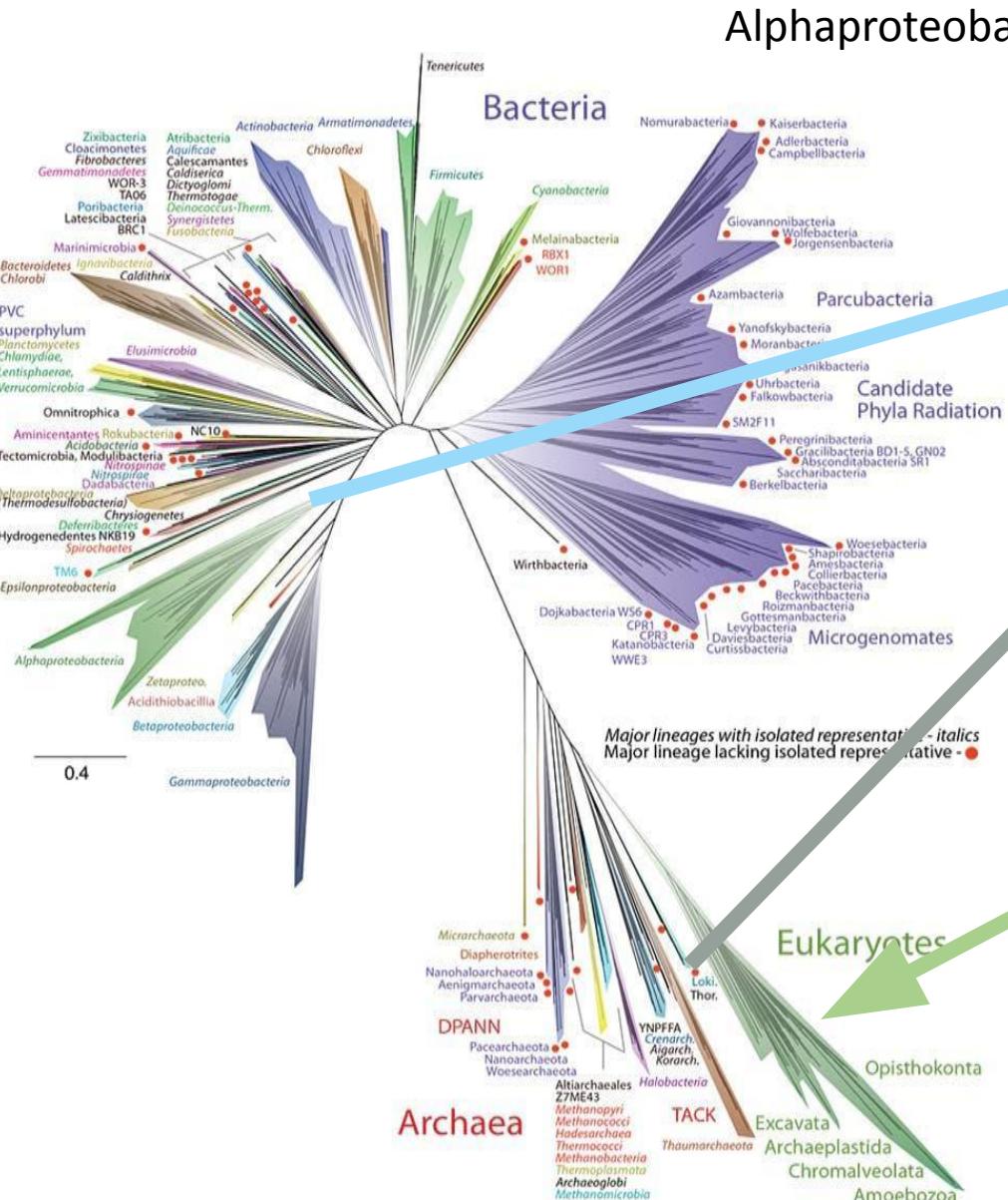


DIATOMS

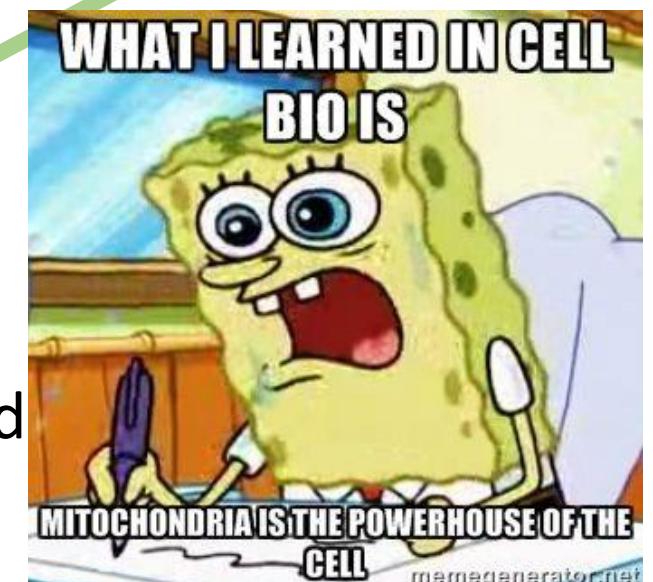
ALL PLANKTON VIDEOS

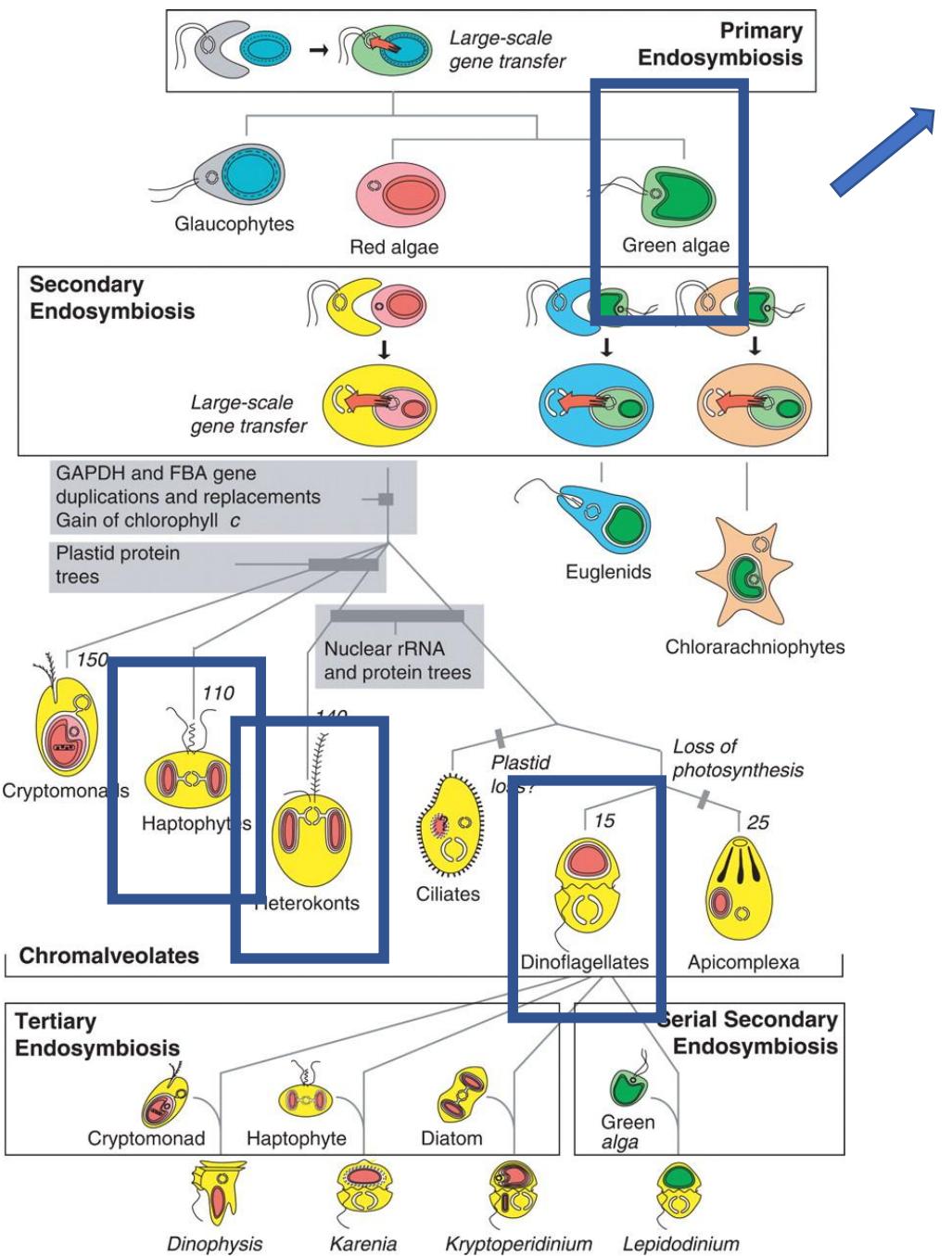
The origin of eukaryotes

Lateral gene transfer



Endosymbiosis led
the origin of
eukaryotes (&
mitochondria)





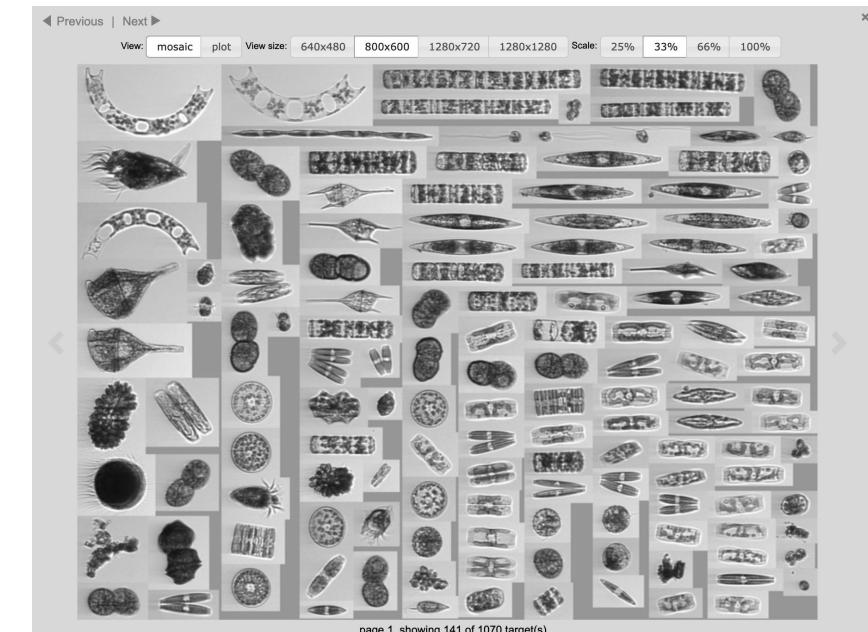
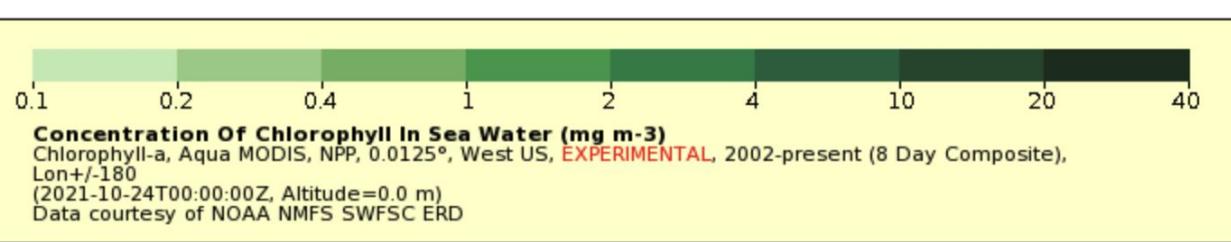
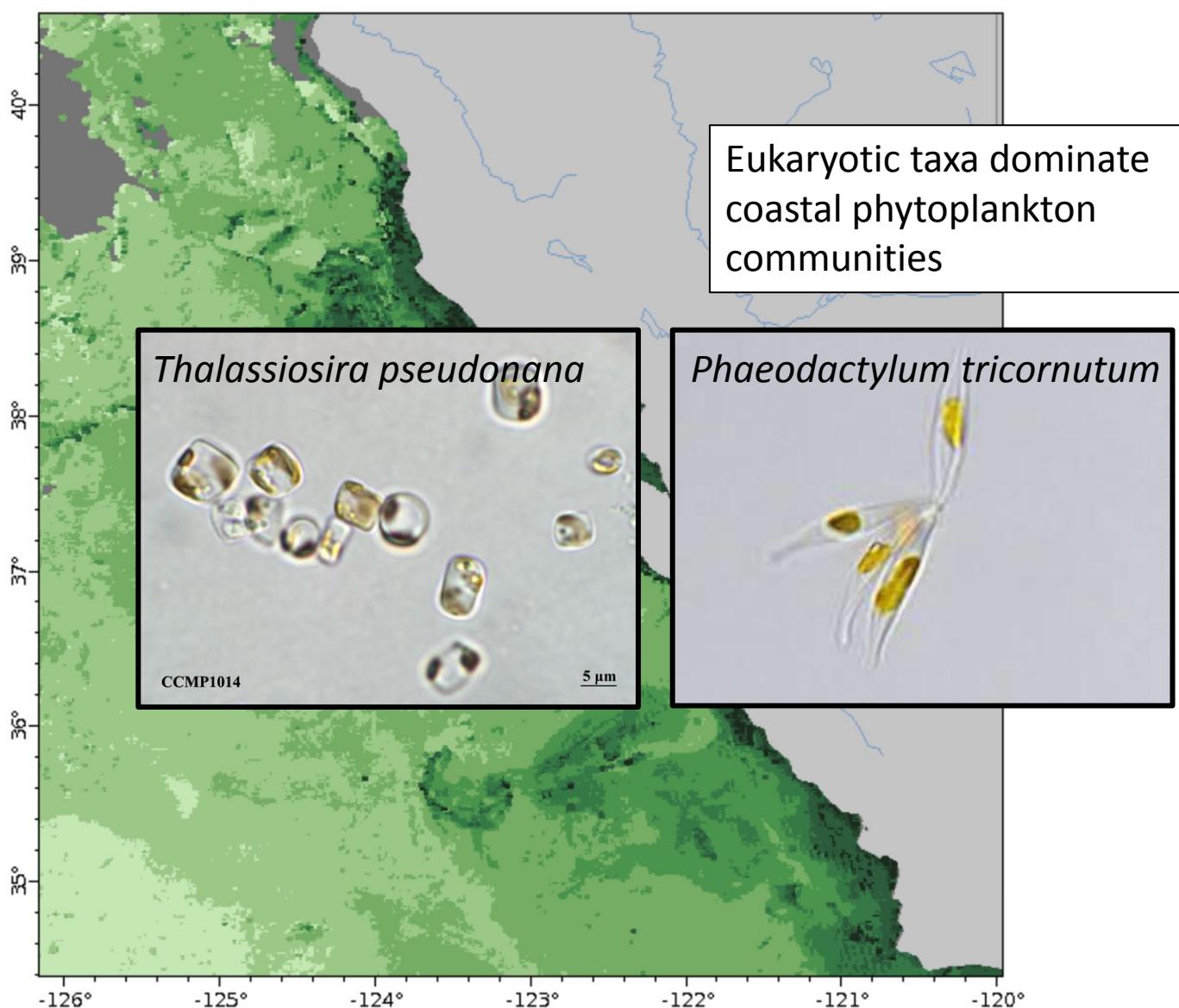
All land plants



Most dominant bloom-forming phytoplankton taxa are from the red-plastid derived secondary endosymbionts

Do red plastid-derived secondary endosymbionts possess traits that make them more successful than green lineages?

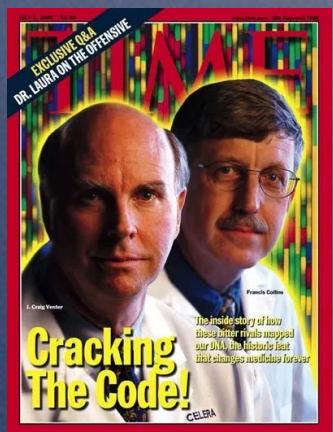
Do groups within the red-plastid lineage possess traits that distinguish them from one another?



July 17, 2021



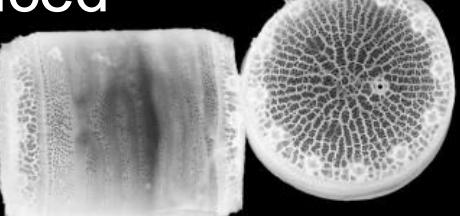
October 20, 2021



Human Genome
Sequenced
2000/2001

Global
Ocean
Survey
2004

2004
First diatom genome
sequenced



Armbrust et al. 2004, Science



First pennate
diatom genome
sequenced
2008

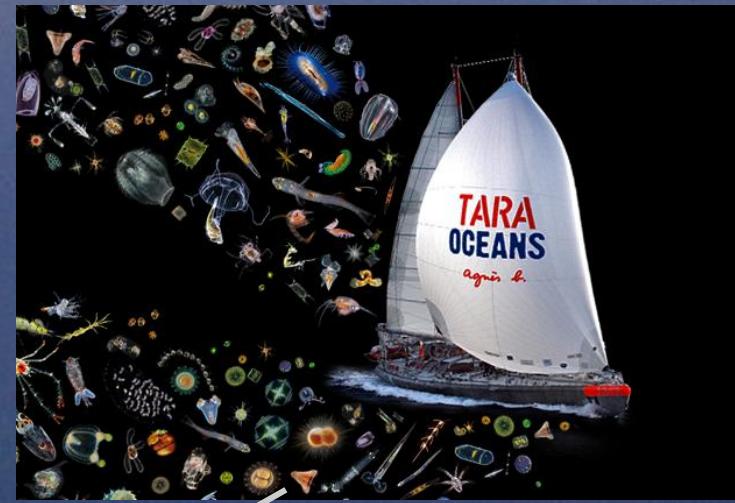
Bowler et al. 2008, Nature

High throughput sequencing
becomes mainstream
2007

Genome
editing tools
available
TALEN and
CRISPR/Cas
2012/2013

2011
Marine Microbial
Eukaryote
Transcriptome
Sequencing Project
(MMETSP) Launched
650+ marine eukaryotes

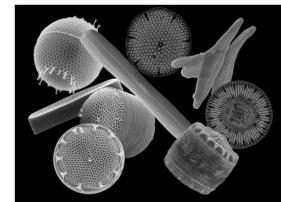
Protists



Oxford Nanopore &
PacBIO

100 Diatom Genomes

Diatoms are the most species-rich algal class with estimated \approx 100,000 species. They contribute to 20% of global carbon fixation and oxygen production. However, with genomic information from only about 10 diatoms, our ability to harness their unique biology is very limited. Consequently, this project will sequence 100 diatom species for providing unique insights into their roles as key players for capturing carbon dioxide and as the foundation of diverse aquatic food webs. These insights will also be critical for advancing diatom-based biotechnology and synthetic biology platforms.

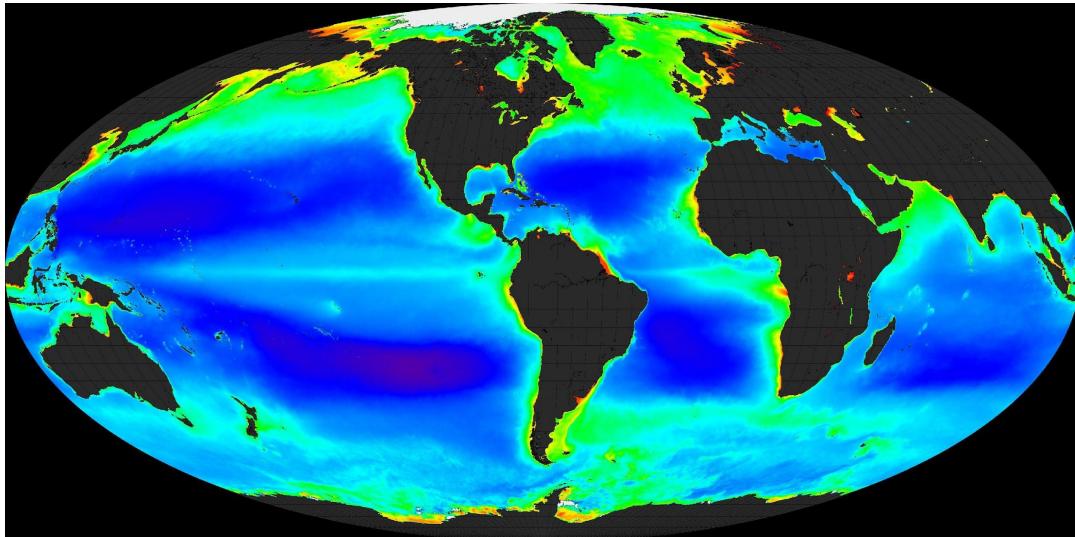


Proposer: Thomas Mock, University of East Anglia (UK)
Proposal: 100 Diatom Genomes Project

Scanning electron micrographs of diverse diatoms.
(Credits: Diana Sarno, Marina Montresor, Nicole Poulsen, Gerhard Dieckmann)

John Martin and The Iron Hypothesis

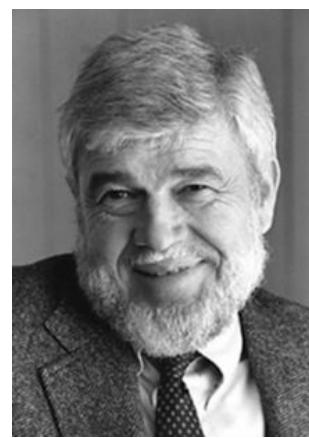
“Give me a half a tanker of iron and I'll give you the next ice age” - John Martin



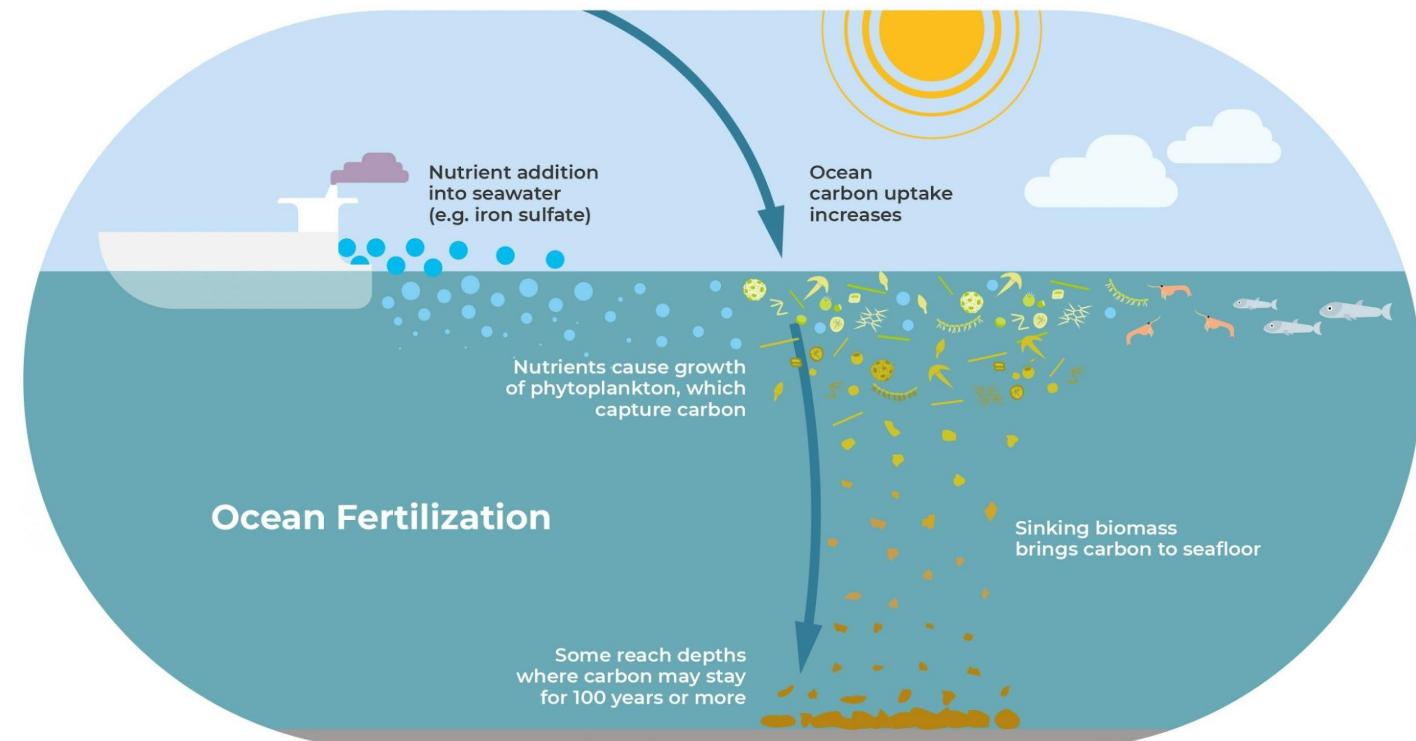
High-nutrient, low chlorophyll (HNLC)

Despite adequate sunlight, and nutrients, there are fewer phytoplankton than you'd expect

Martin showed that Fe limits productivity in these regions

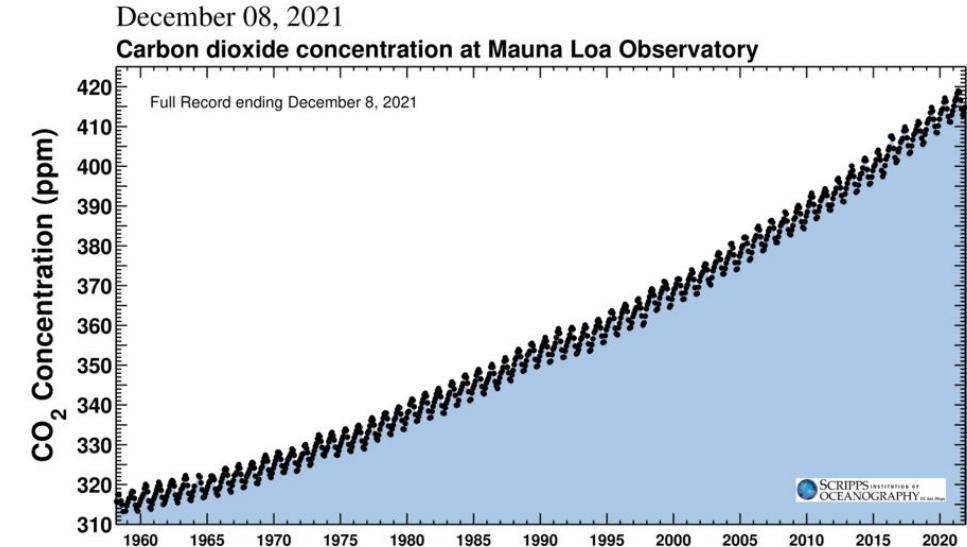
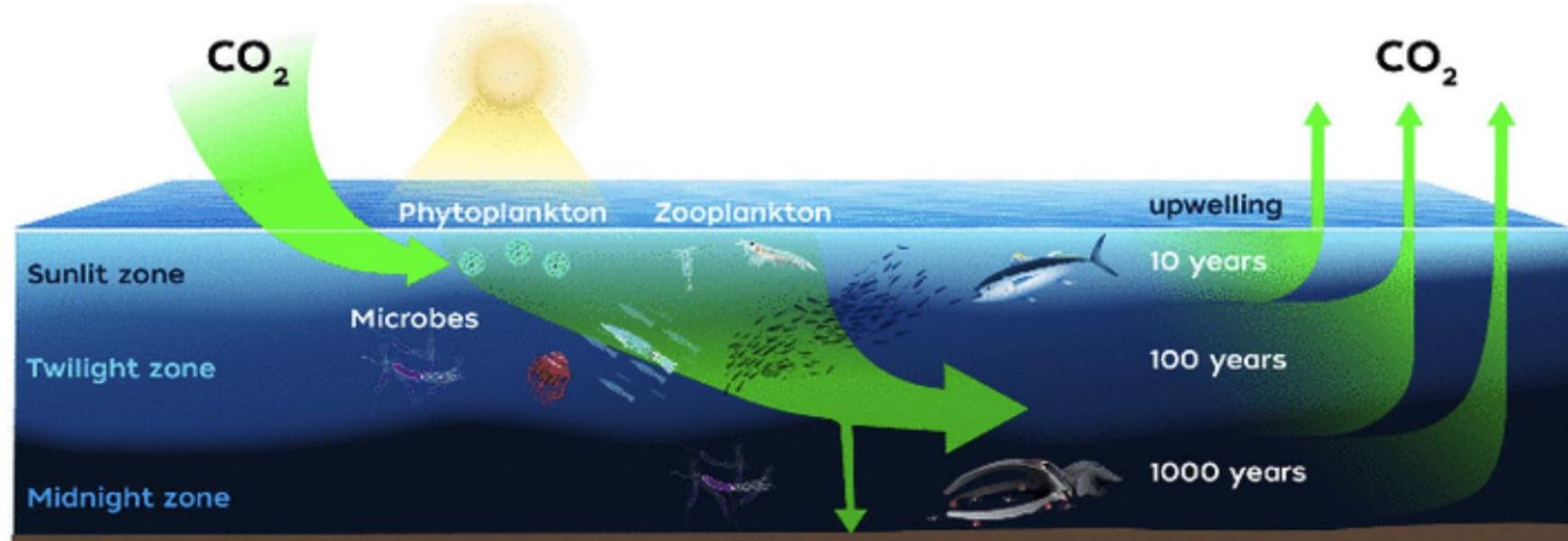
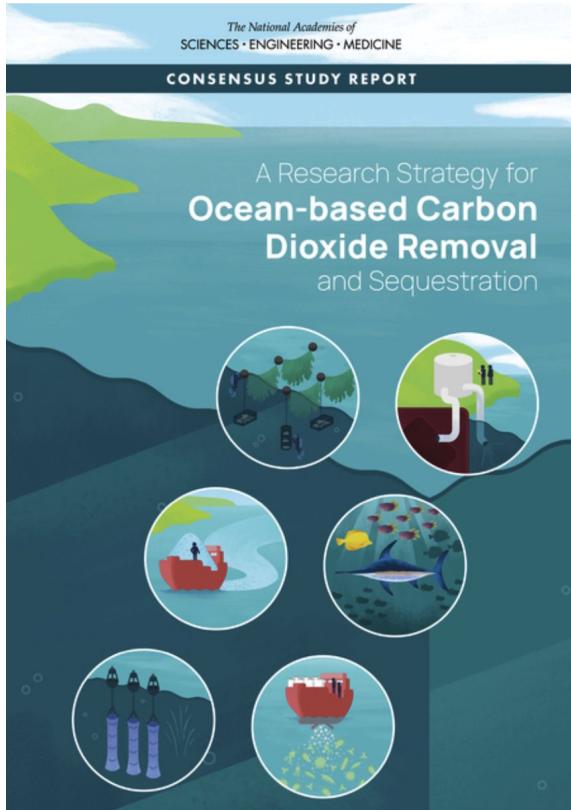


Former MLML Director



Iron fertilization as a geoengineering strategy for CDR (or CCS)

- NASEM (National Academies of Science Engineering Medicine) report published Dec 8 2021





EXPLORING OCEAN
IRON SOLUTIONS

THE PROBLEM POTENTIAL SOLUTIONS OUR PLAN WHO WE ARE

NEWS & EVENTS

Exploring responsible uses of the ocean to address our climate crisis

Every year, humans pump about 40 billion tons of carbon dioxide into the atmosphere. The heat from this greenhouse gas is disrupting planetary systems and reshaping life on Earth. The ocean may be able to help us reverse this trend, but we need to proceed with caution.

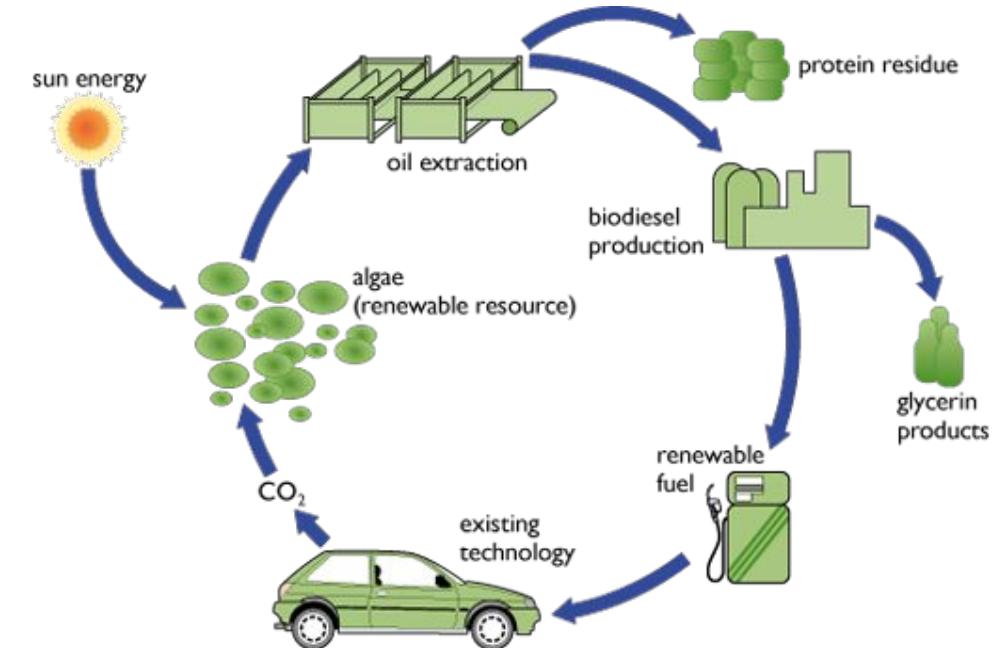
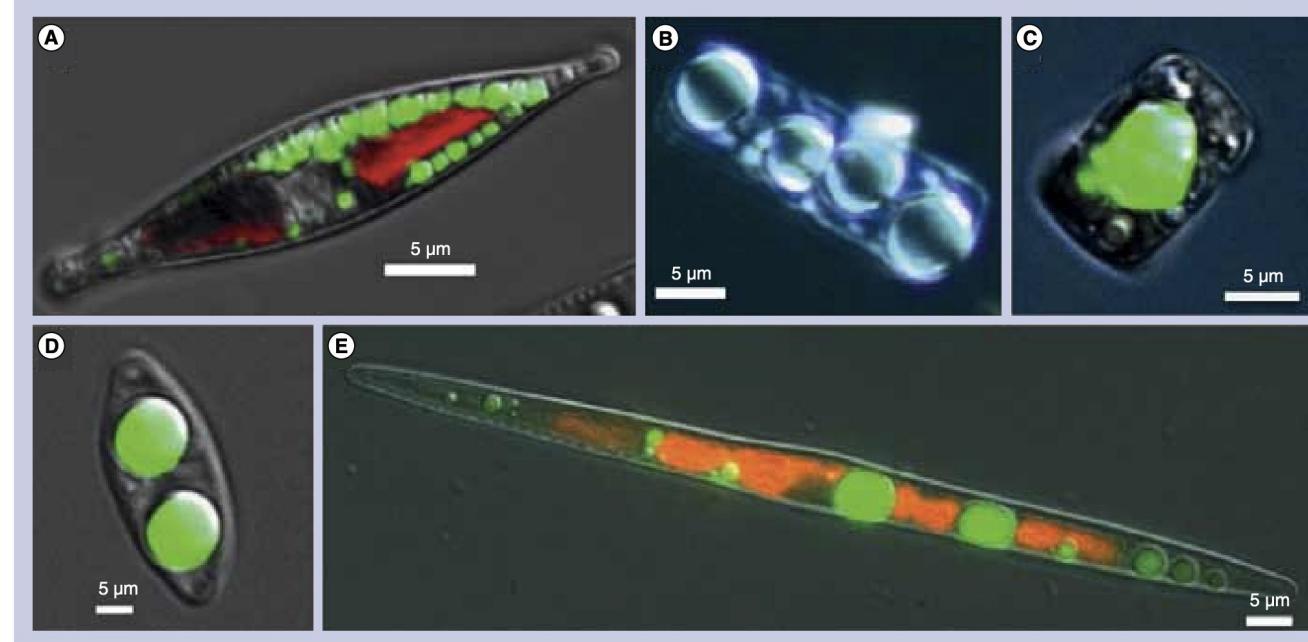
[READ MORE ABOUT THE PROBLEM](#)

<https://oceaniron.org/>

Find out more



Sustainable products from algae?



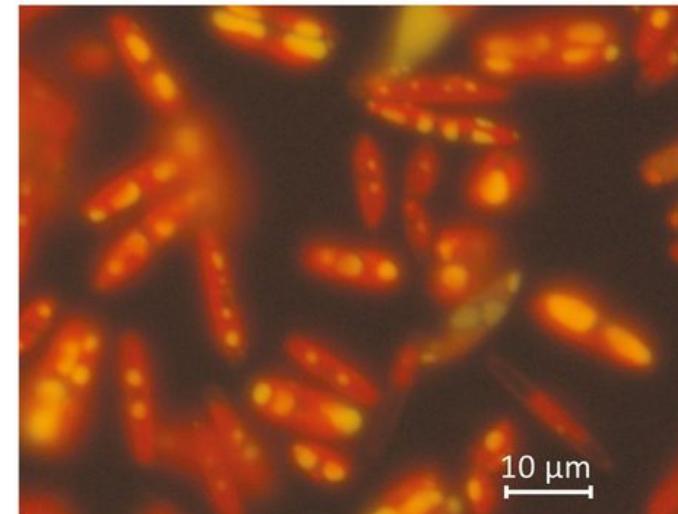
Article | Open Access | Published: 02 August 2021

Diploid genomic architecture of *Nitzschia inconspicua*, an elite biomass production diatom

Aaron Oliver, Sheila Podell, Agnieszka Pinowska, Jesse C. Traller, Sarah R. Smith, Ryan McClure, Alex Beliaev, Pavlo Bohutskyi, Eric A. Hill, Ariel Rabines, Hong Zheng, Lisa Zeigler Allen, Alan Kuo, Igor V. Grigoriev, Andrew E. Allen, David Hazlebeck & Eric E. Allen

Scientific Reports 11, Article number: 15592 (2021) | [Cite this article](#)

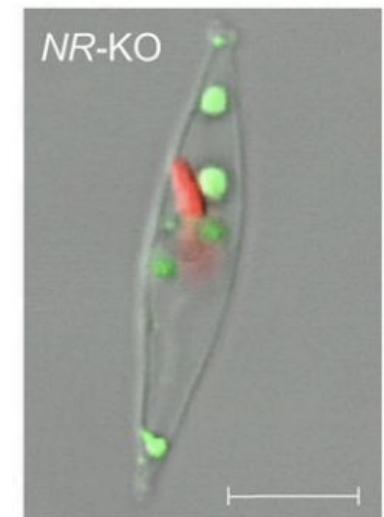
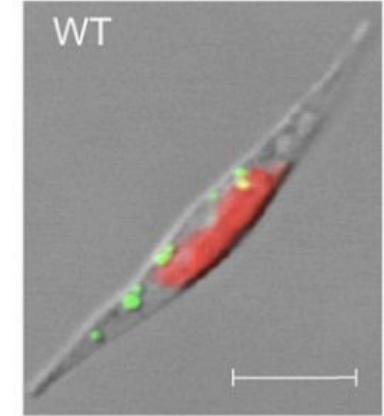
890 Accesses | 1 Altmetric | [Metrics](#)



Diatoms outcompete other phytoplankton taxa for nitrate

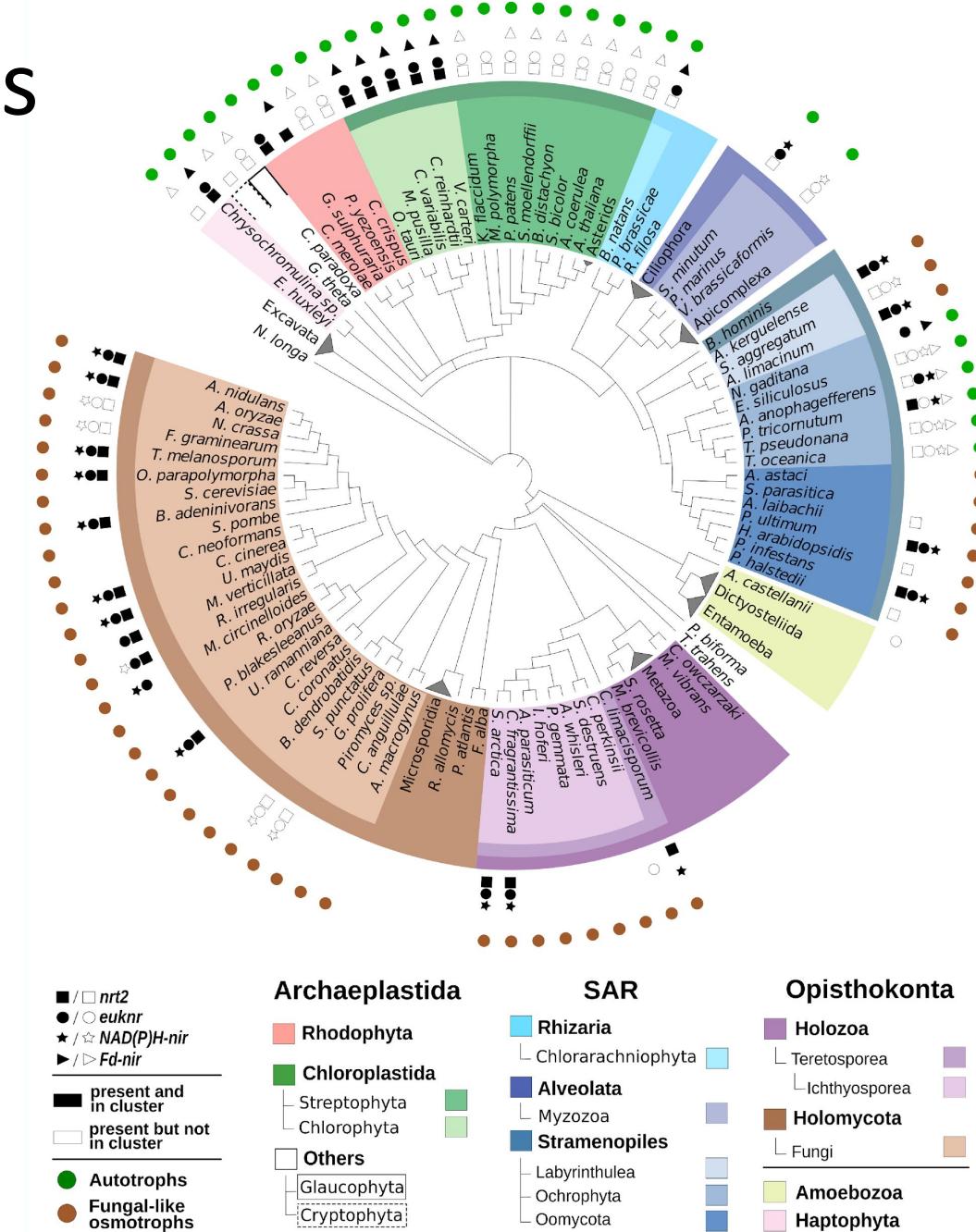
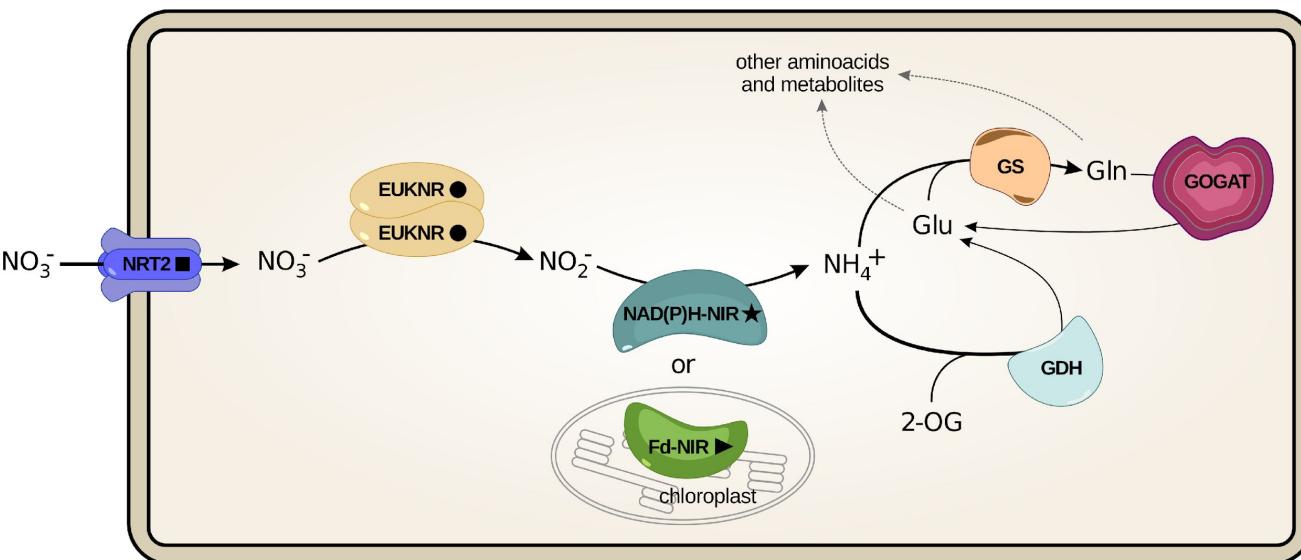
- First to bloom in HNLC regions
- Luxury uptake and vacuolar storage
- Other unique metabolic features that contribute to this success?
 - Urea cycle (Armbrust et al. 2004, Allen et al. 2011)
- **Little is known about the genetic components involved in sensing, regulation, and metabolic assimilation of different forms of nitrogen and how diatoms arrived at the current configuration throughout evolution**

36 hours after
 NO_3^- addition



Nitrate assimilation in eukaryotes

- Nitrate assimilation is restricted to autotrophs and osmotrophs



Ocaña-Pallarès et al. 2019 Reticulate evolution in eukaryotes: Origin and evolution of the nitrate assimilation pathway. PLOS Genet. 15:e1007986

ARTICLE

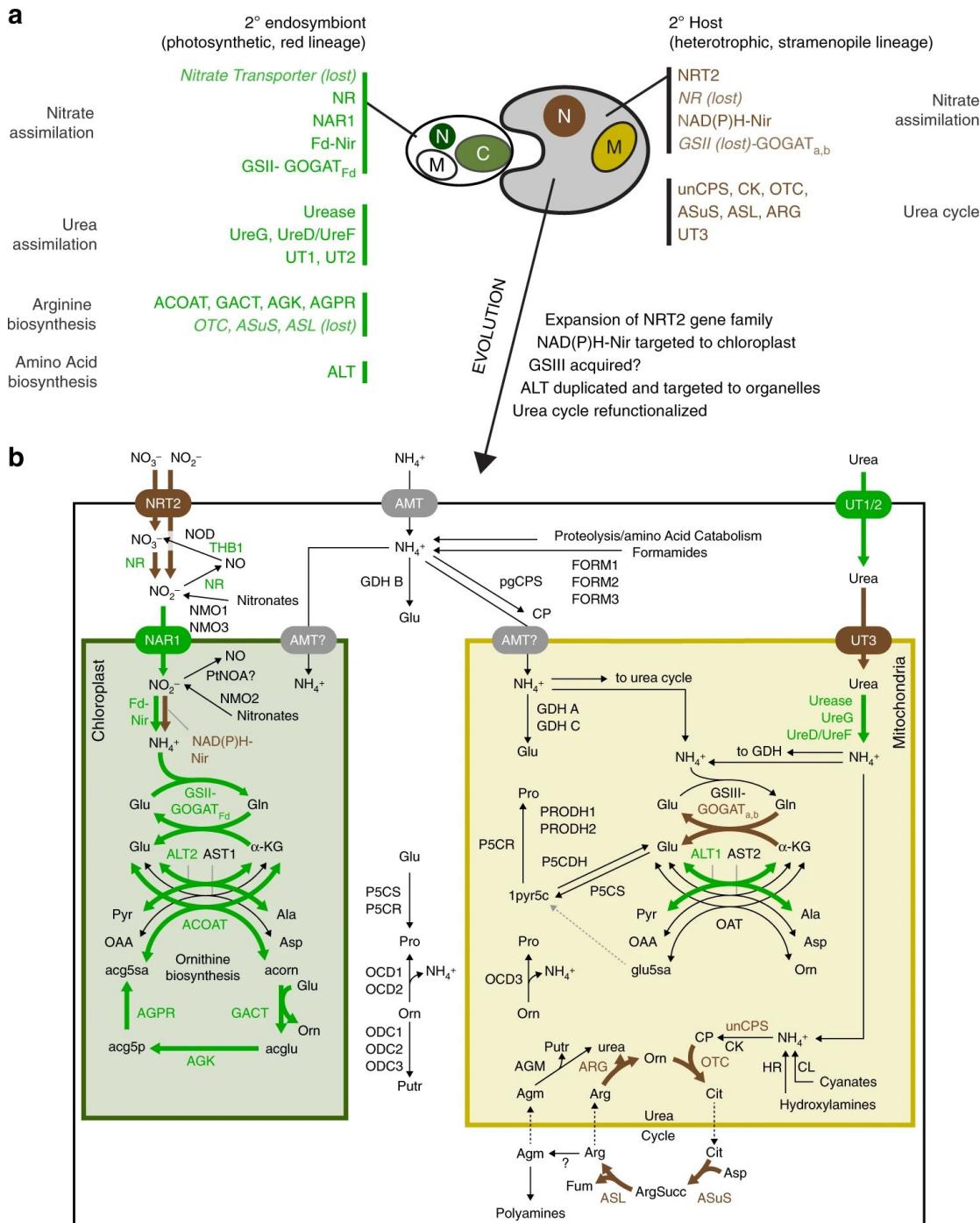
<https://doi.org/10.1038/s41467-019-12407-y>

OPEN

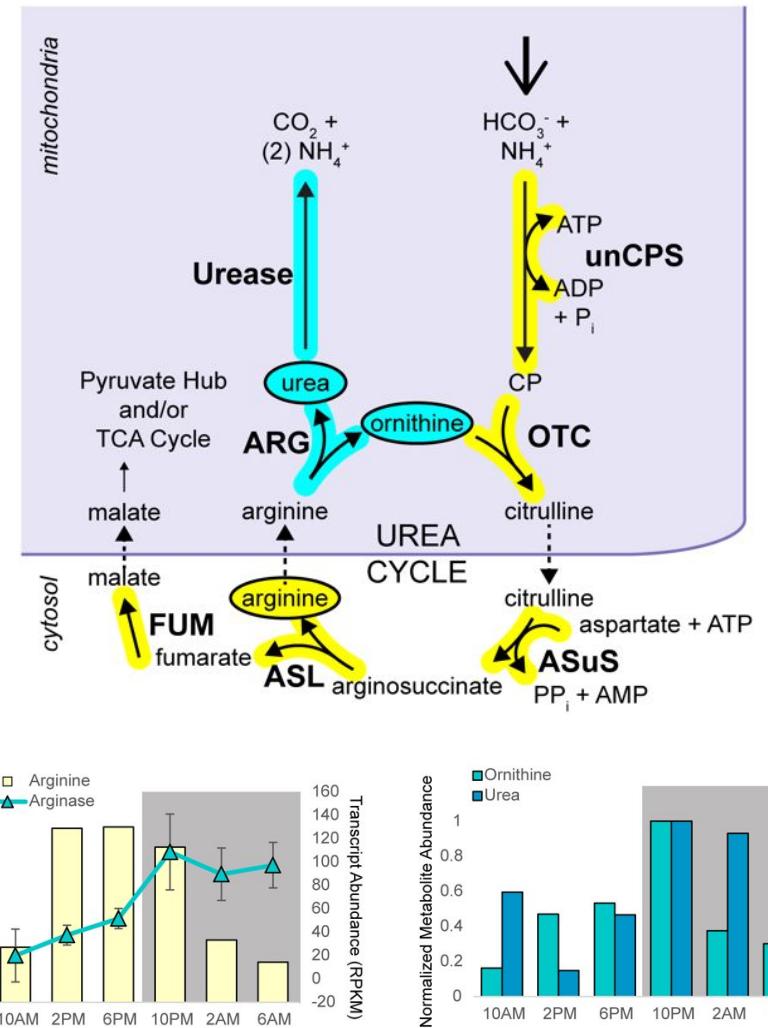
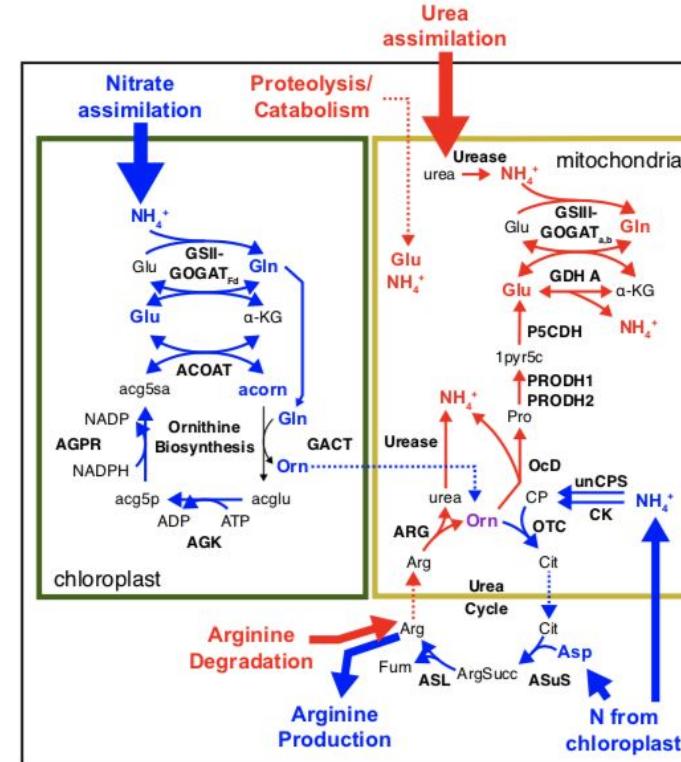
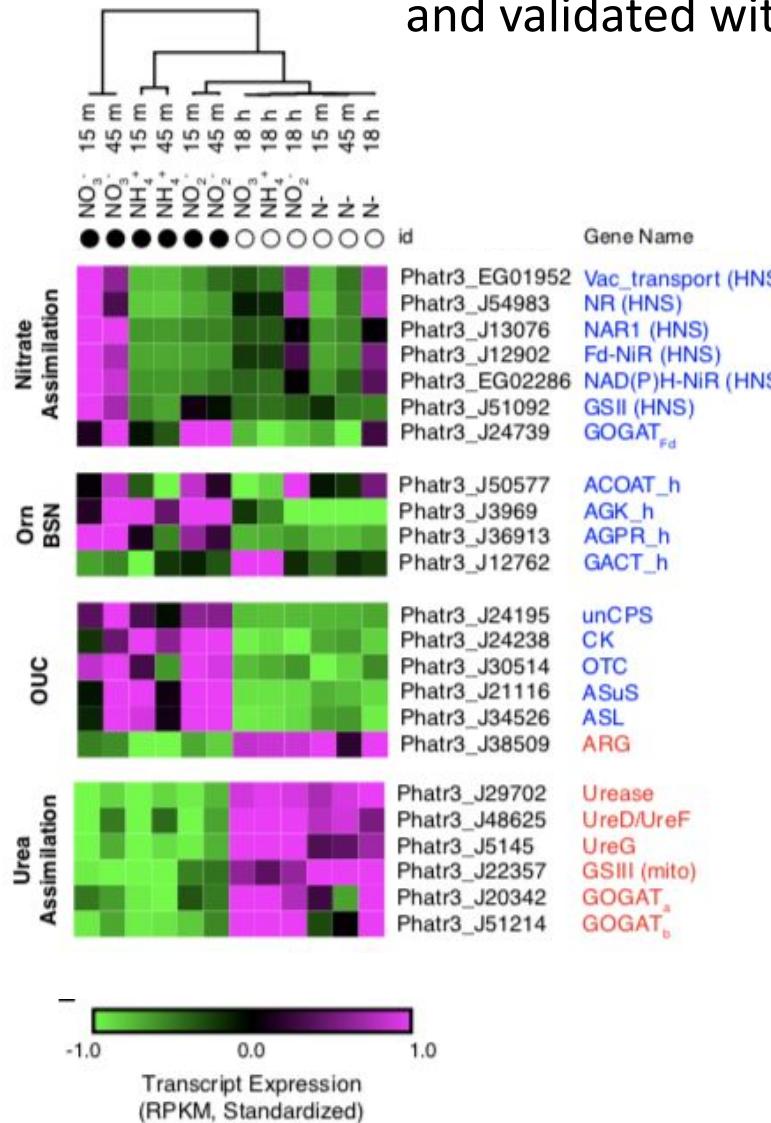
Evolution and regulation of nitrogen flux through compartmentalized metabolic networks in a marine diatom

Sarah R. Smith¹, Chris L. Dupont¹, James K. McCarthy^{ID 1}, Jared T. Brodrick^{2,3}, Miroslav Oborník^{4,5}, Aleš Horák^{4,5}, Zoltán Füssy^{ID 4,5}, Jaromír Cihlář^{4,5}, Sabrina Kleessen⁶, Hong Zheng¹, John P. McCrow¹, Kim K. Hixson^{ID 7}, Wagner L. Araújo^{ID 8,9}, Adriano Nunes-Nesi⁸, Alisdair Fernie¹⁰, Zoran Nikoloski¹¹, Bernhard O. Palsson^{ID 3} & Andrew E. Allen^{ID 1,12*}

- Developed a compartmentalized model of nitrogen metabolism
 - 42 phylogenies of 74 nitrogen-metabolism genes curated from annotations, literature, and knowledge from other organisms
 - Validated sub-cellular localizations
- Functional genomics for insights into regulation and pathway usage
 - Transcriptomics, proteomics
 - Cis-regulatory element promoter analysis of key nitrogen genes
- Flux Balance Analysis using genome-scale metabolic modeling
 - Metabolomics and isotope labeling experiments: ¹⁵N-nitrate and ¹⁵N-urea



Flux inferred (short-term transcriptomics experiments and validated with fluxomics and flux balance analysis



Smith et al. *PLoS Genetics* (2016)

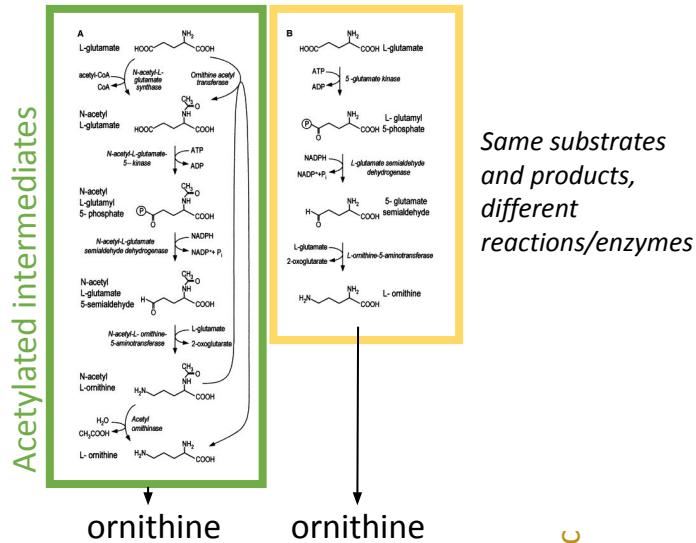
Arginine biosynthesis and degradation pathway?

Arginine biosynthesis in plants and animals

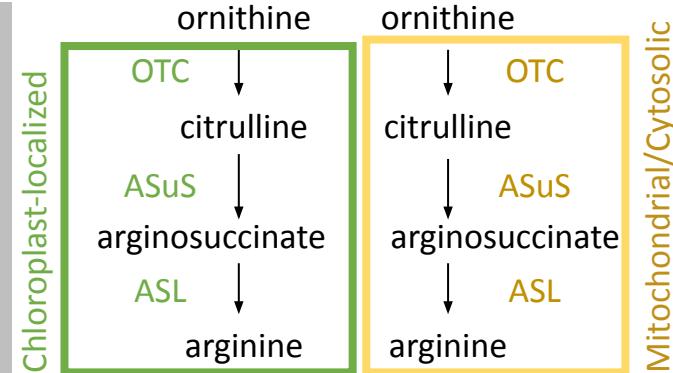
First Half

Arginine Biosynthesis Pathway

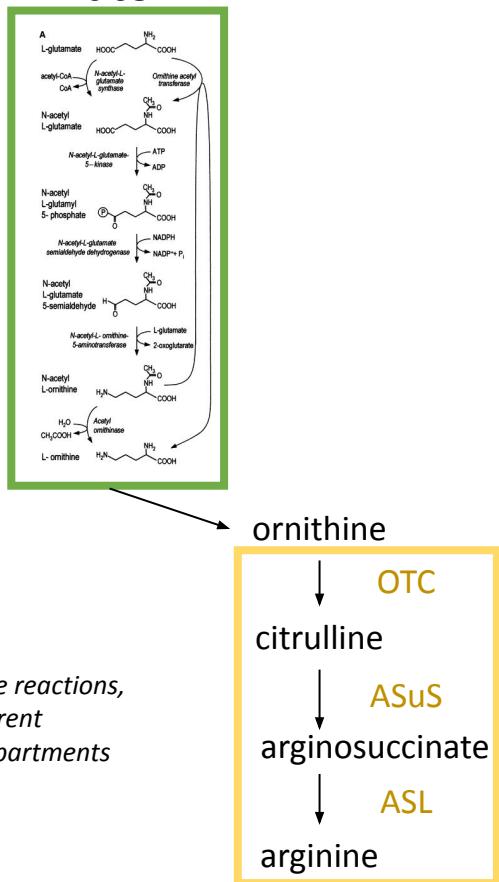
Plant **Animal**



Second Half

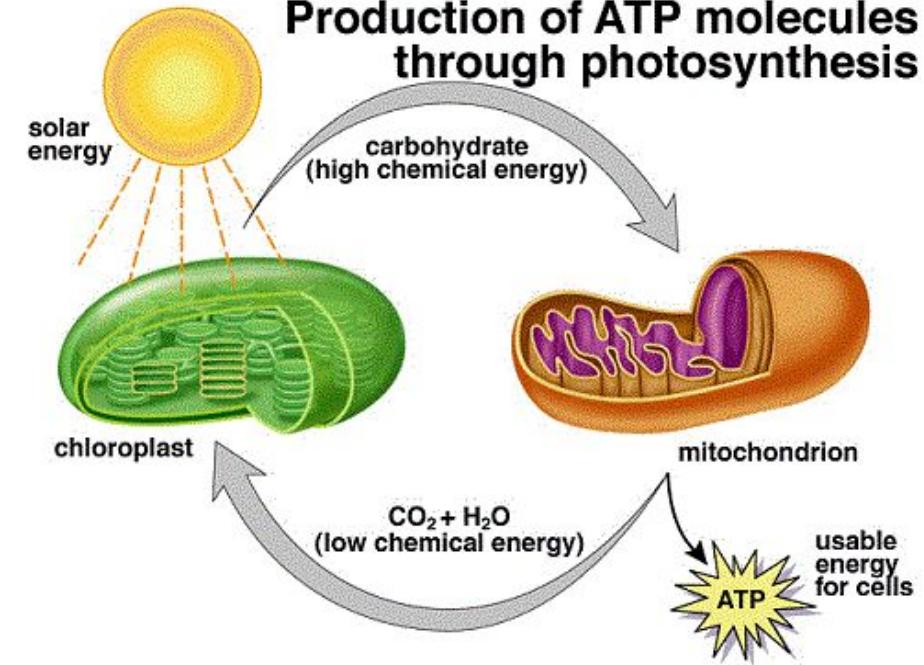


Diatom



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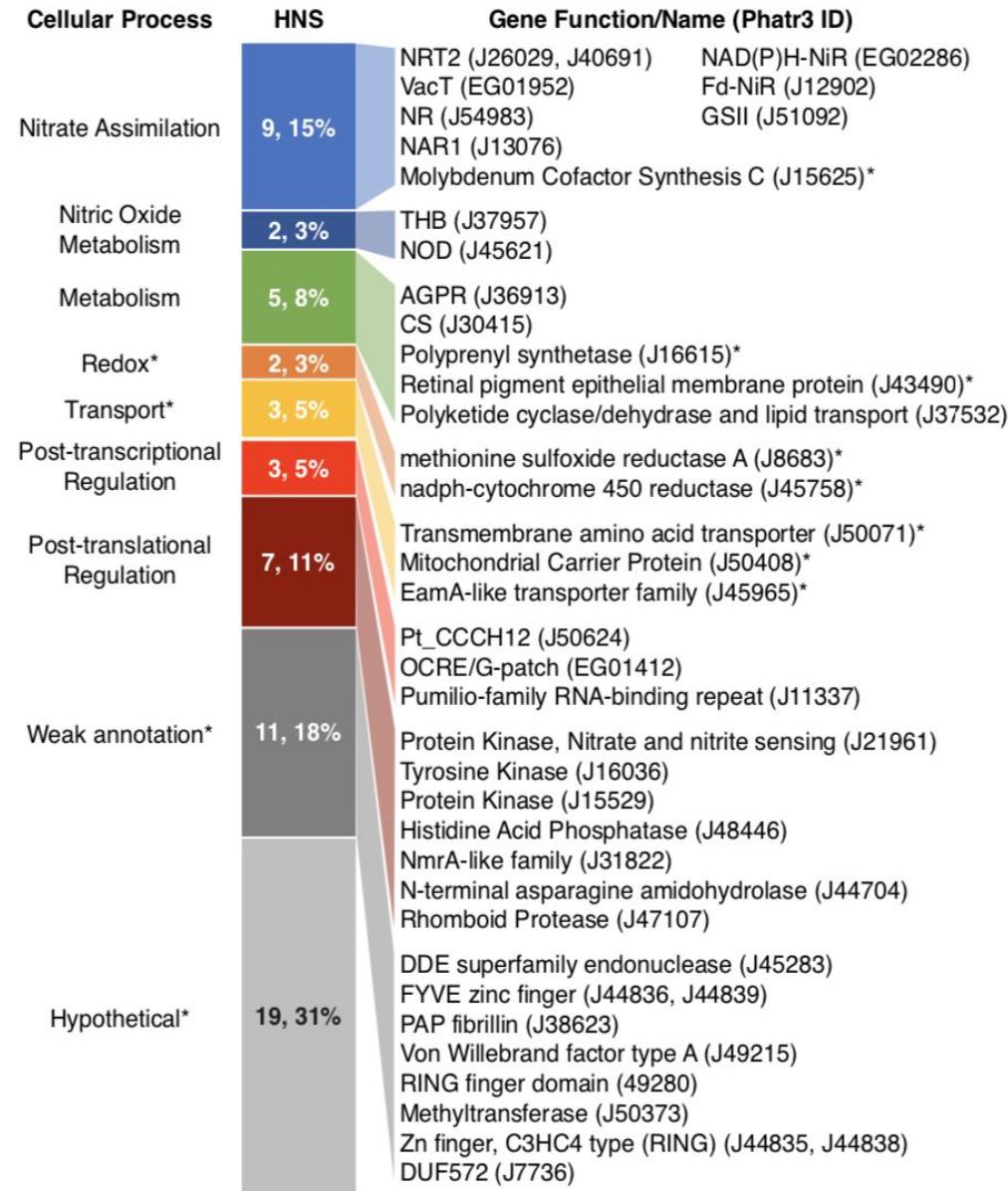
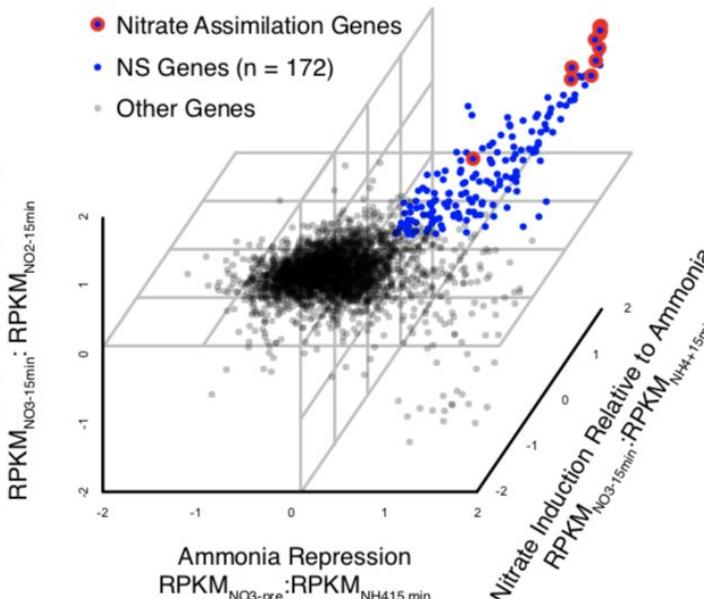
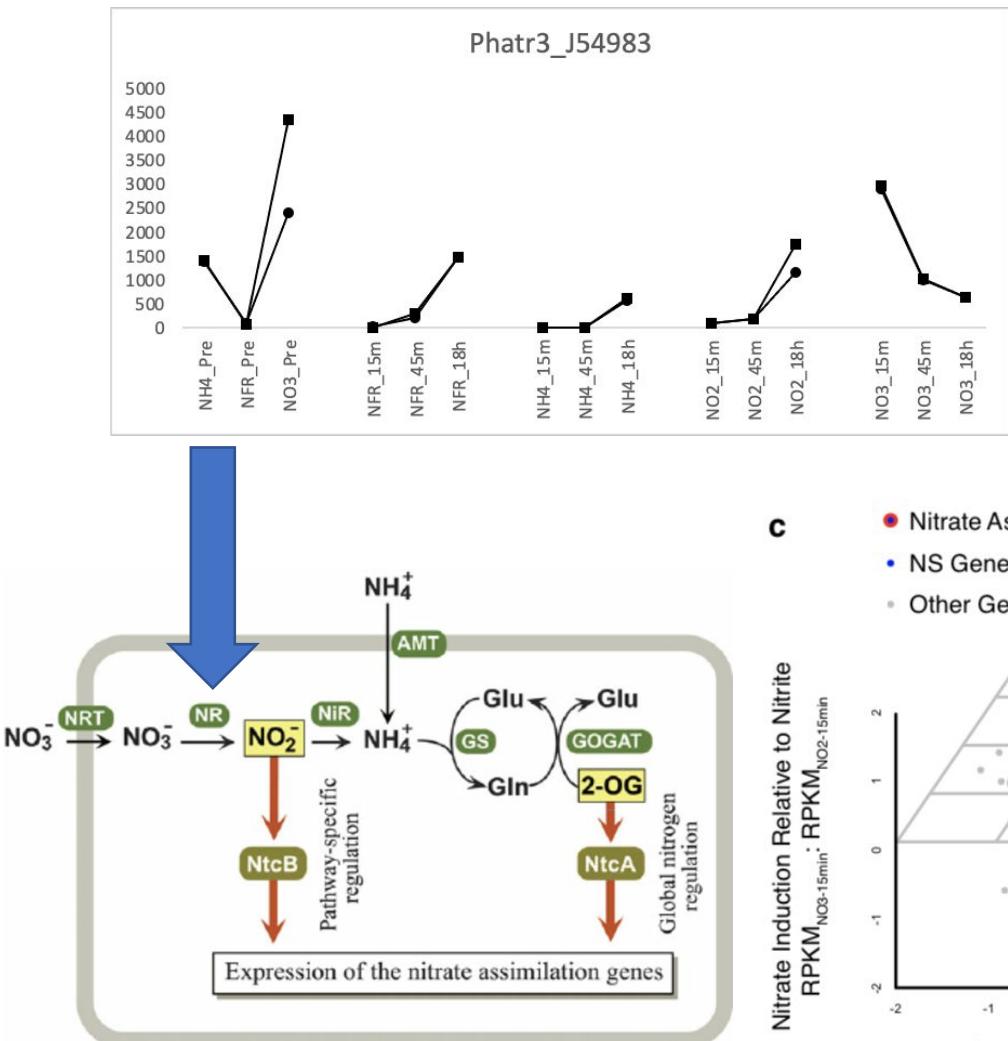
Production of ATP molecules through photosynthesis



Splitting arginine biosynthesis across organelles ties N status communication to overall energy status

Giving diatoms an advantage over other phytoplankton taxa in highly dynamic nutrient environments

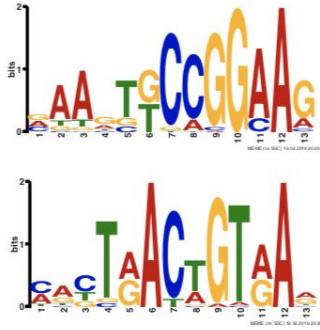
The Nitrate Assimilation Gene Regulon = “HNS”



Transcription factor binding site(s?) identification in highly nitrate sensitive genes and NR bidirectional promoter

Promoter Training Set
HNS (n = 61)

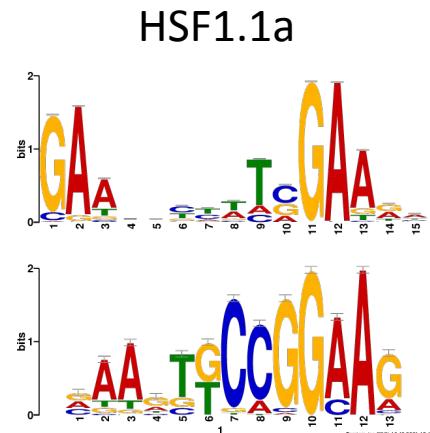
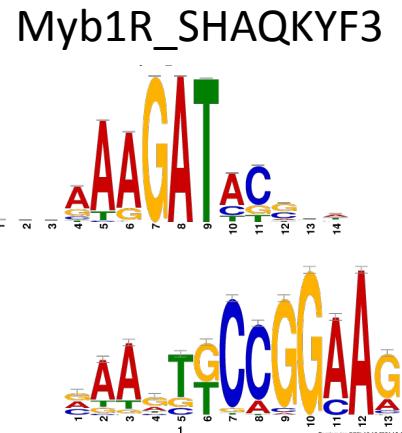
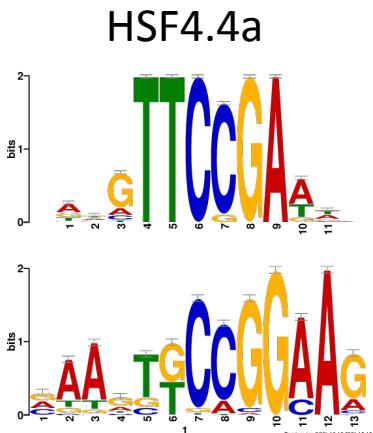
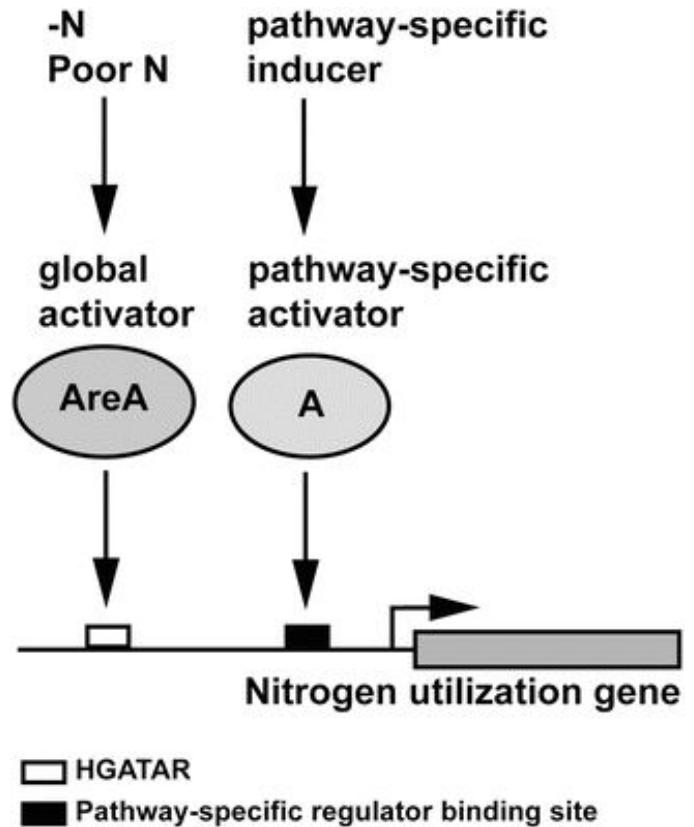
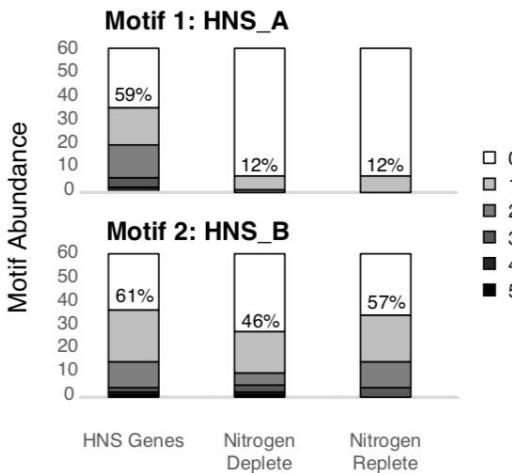
Motif Discovery
MEME Suite 5.0.4
(Bailey & Elkan 1994)



Motif 1: HNS_A
9.1e-018
48 sites, 36/61 promoters

Motif 2: HNS_B
2.5e-013
54 sites, 37/61 promoters

b

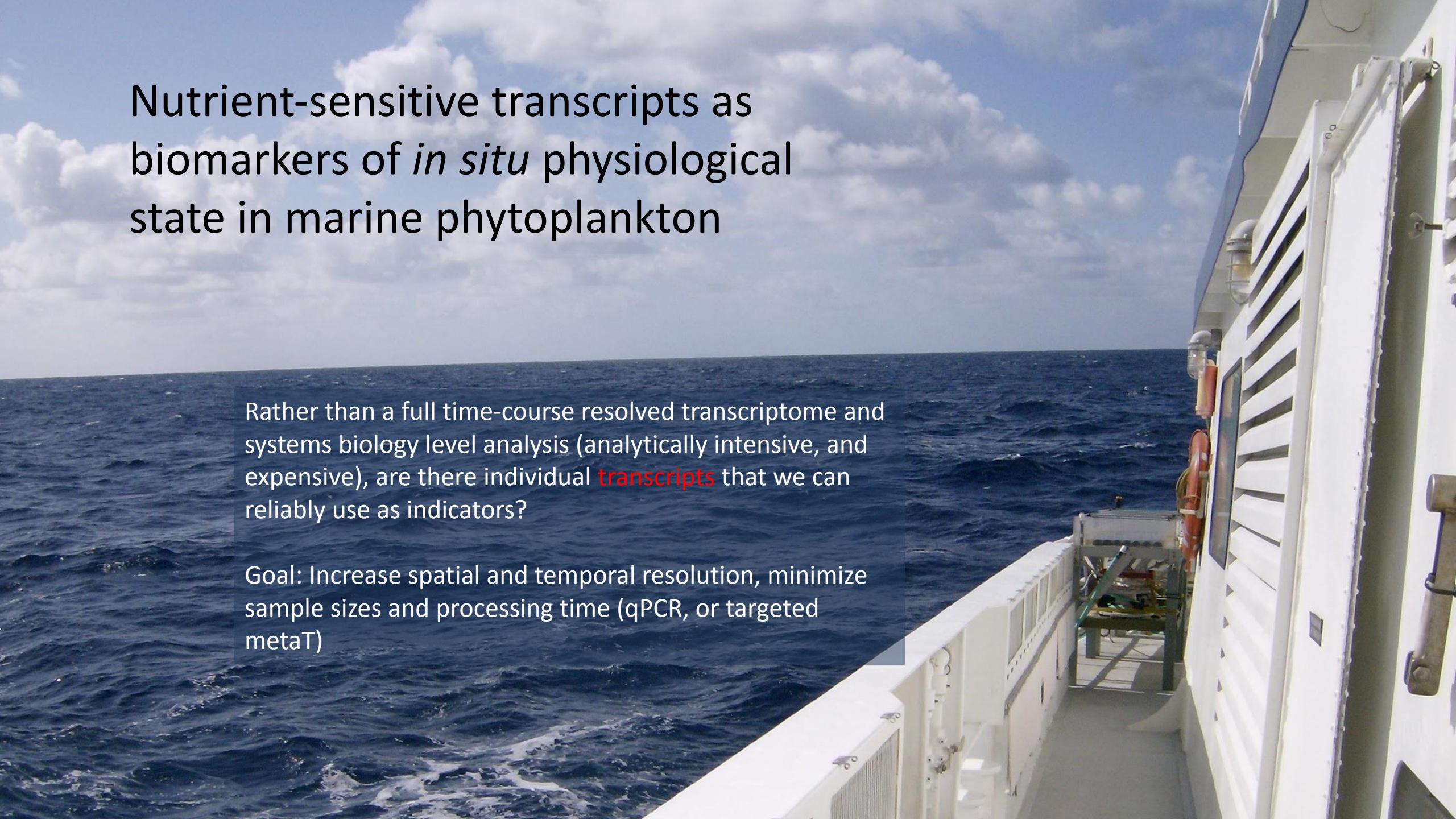


Todd 2016 Regulation of fungal nitrogen metabolism in The Mycota III

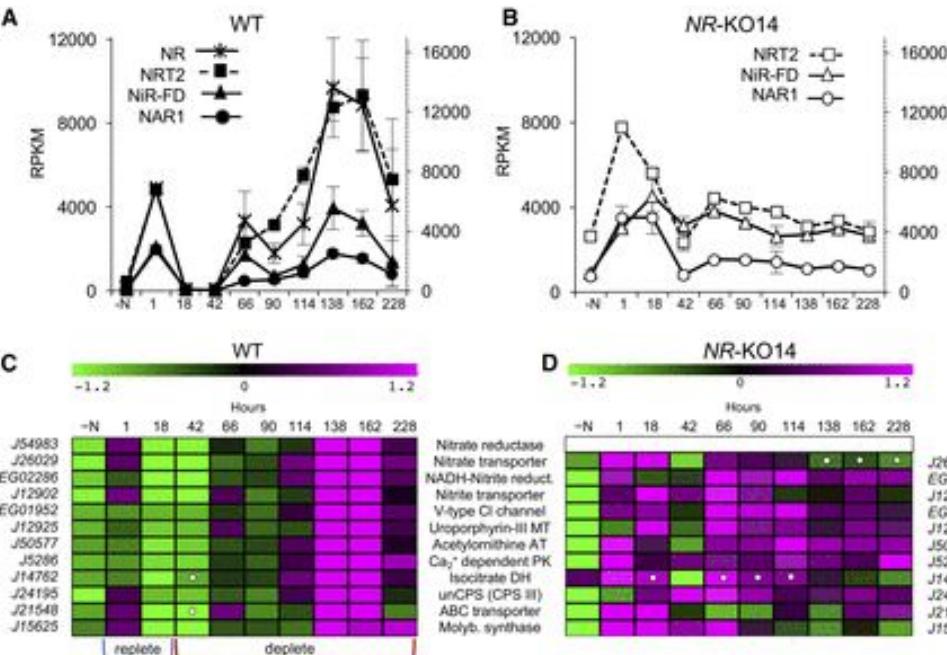
Nutrient-sensitive transcripts as biomarkers of *in situ* physiological state in marine phytoplankton

Rather than a full time-course resolved transcriptome and systems biology level analysis (analytically intensive, and expensive), are there individual **transcripts** that we can reliably use as indicators?

Goal: Increase spatial and temporal resolution, minimize sample sizes and processing time (qPCR, or targeted metaT)

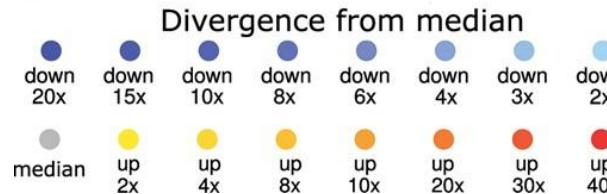
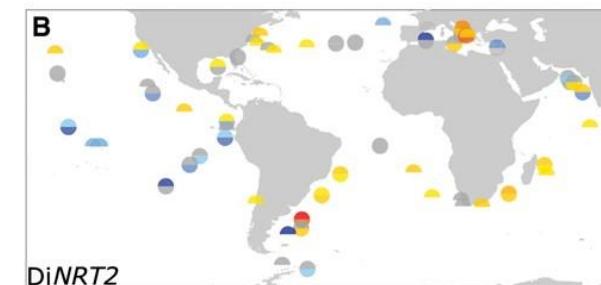


Nitrate transporter as an ecogenomic sensor



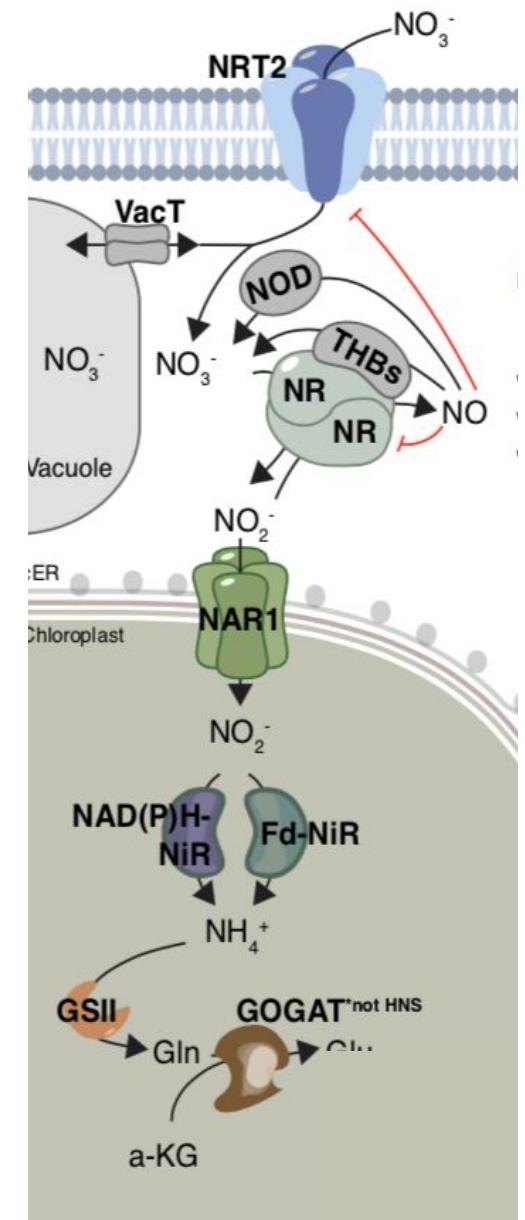
McCarthy et al. 2017 The Plant Cell

- Logical choice (function of gene is known)
- Highly expressed (detectable in nature)
- *Not a reliable indicator*

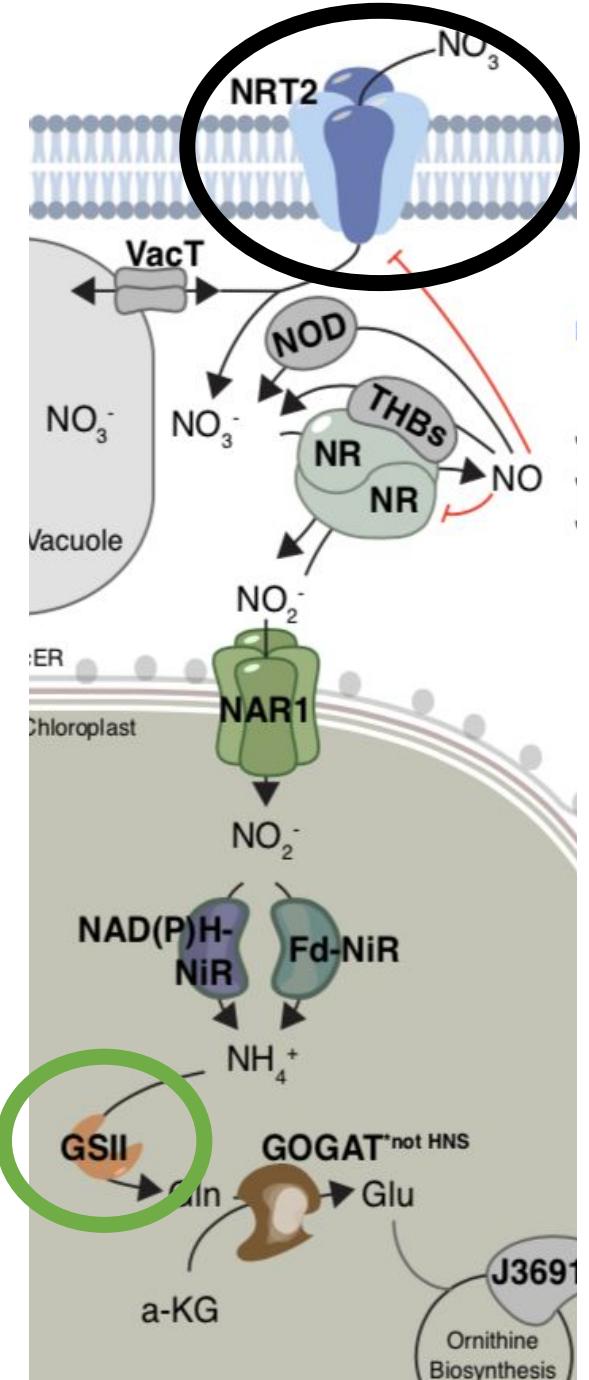
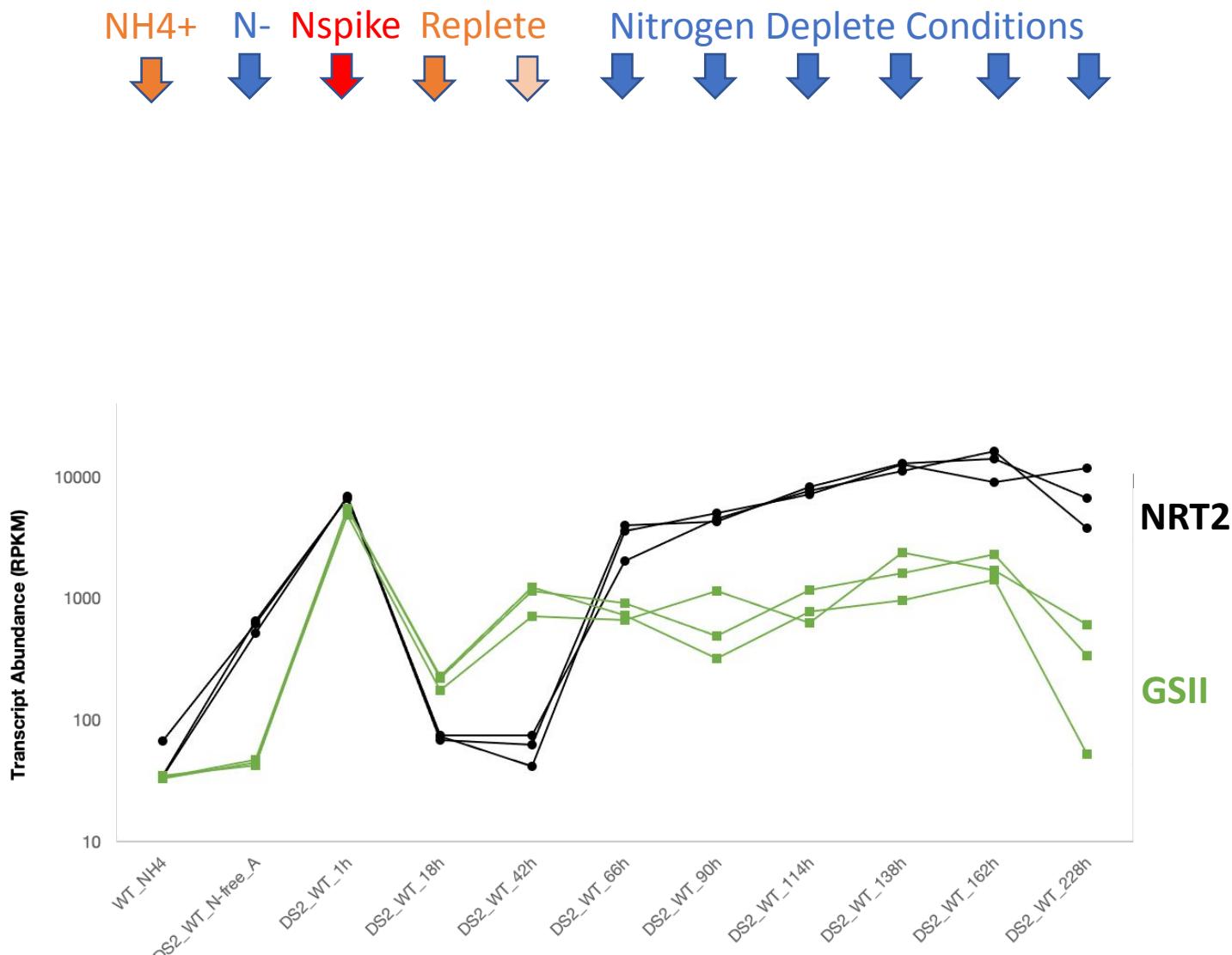


“DiNRT2 [is] abundant in areas of low N availability”

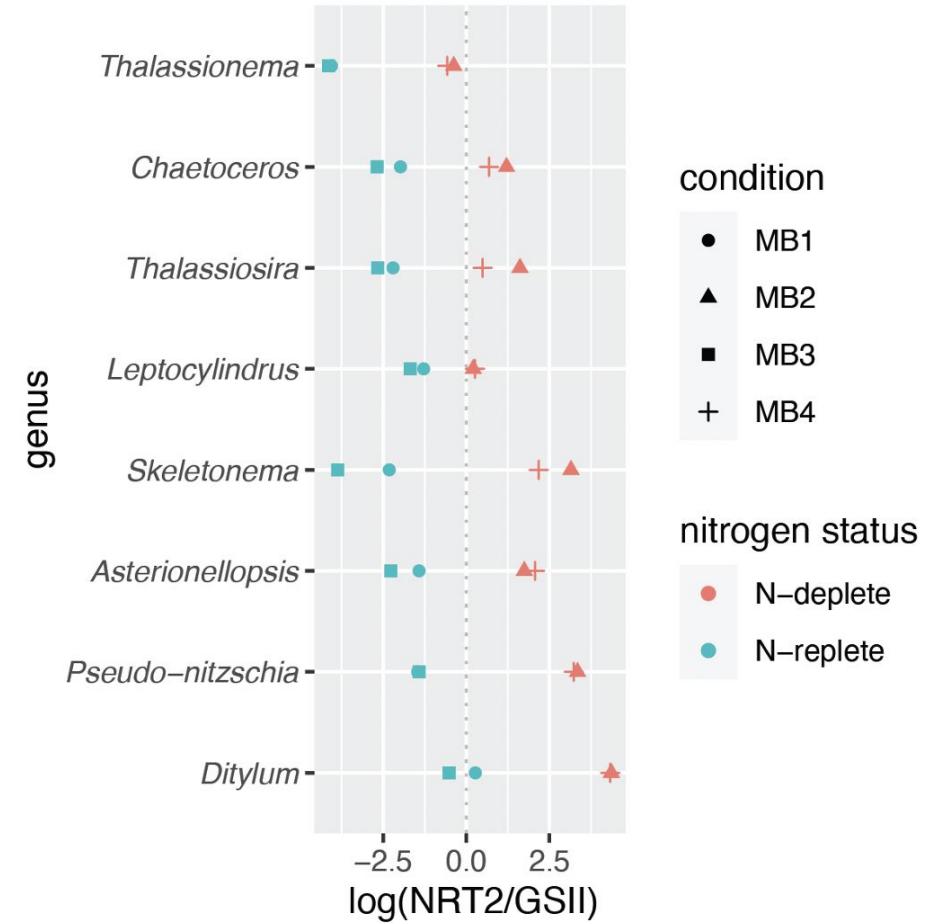
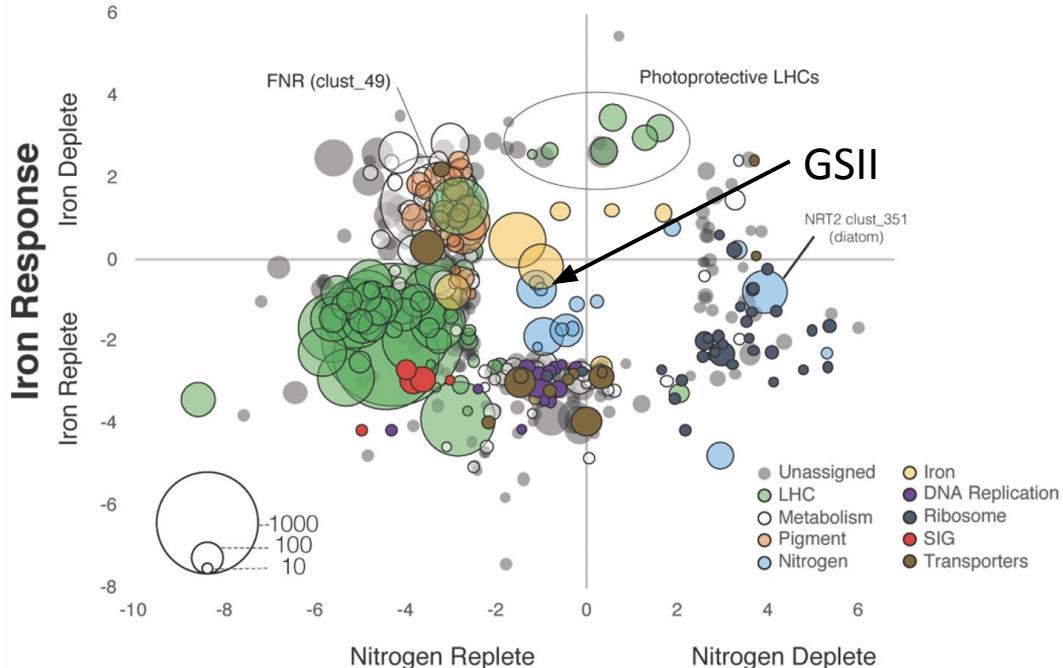
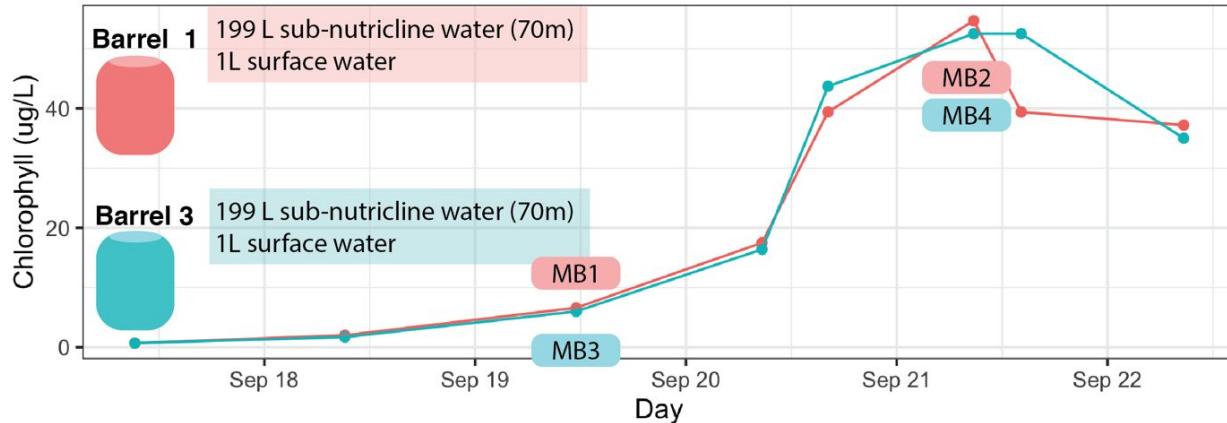
Busseni et al. 2019 MBE 36:2522-2535



NRT2/GSII is an intracellular N status sensor

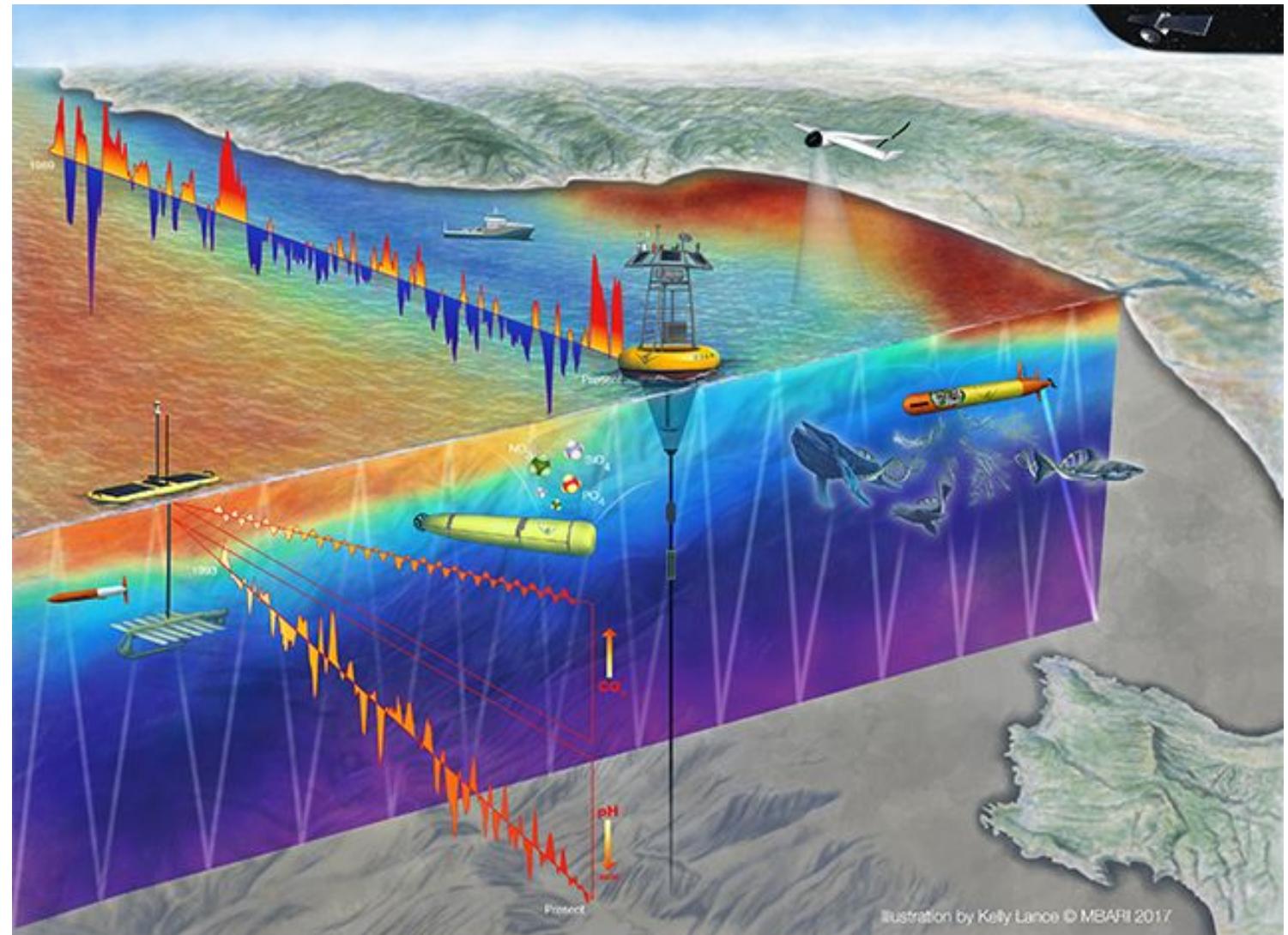


Community-level gene expression in response to experimental manipulation



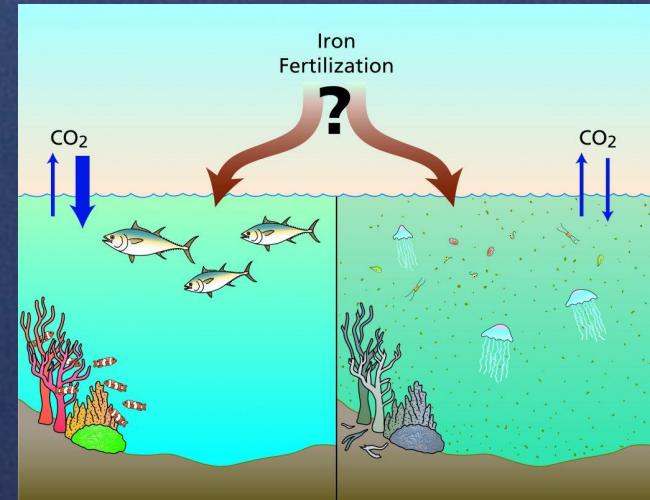
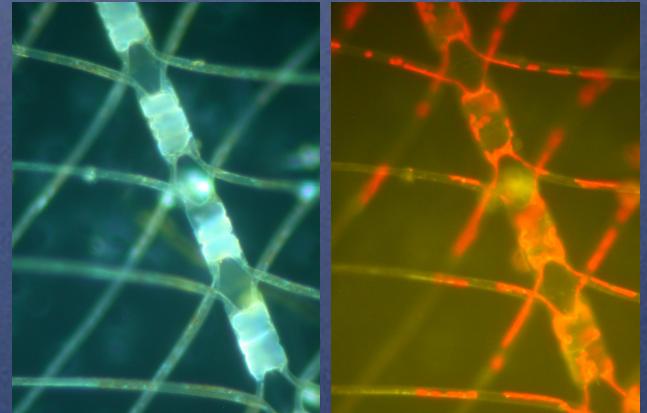
November 20, 2018

Careers in oceanography— a changing world



Science Talk: Take Home

- Insights into the evolution and ecology of eukaryotic phytoplankton
- Enabling development of new tools
 - model coastal ecosystems
 - evaluate impacts of geoengineering schemes like Fe fertilization
- Optimization of systems for sustainable production of foods or fuels



Nexus of technological innovation, DNA sequencing revolution, data analysis/computing and environmental need



Some parting words – and unsolicited advice

- **If you're interested in marine science/grad studies – be a scientist first**
 - Cultivate basic science skills (math, physics, chemistry, organic chemistry, computer science, statistics, biostatistics, analytical methods)
 - Everyone loves the ocean, passion isn't enough to distinguish yourself!
 - Grades don't have to be perfect
- **Identify your goals**
 - If you don't want to be a marine researcher/scientist, but you want to do something related to marine science – recognize how to best develop that skillset! (Conservation/policy? Education? Economics?)
- **Don't be afraid to fail or look “stupid”!**
 - Science is incredibly interdisciplinary, and becoming more so!
 - We need specialists, which means you can't know everything.
 - If you're not failing, you're not trying
- **Refine your communication skills**
 - Learning how to break down complicated concepts that require specialized training for other very smart people (don't be patronizing)
 - Remember to view the world from outside your bubble of knowledge

All of human knowledge

