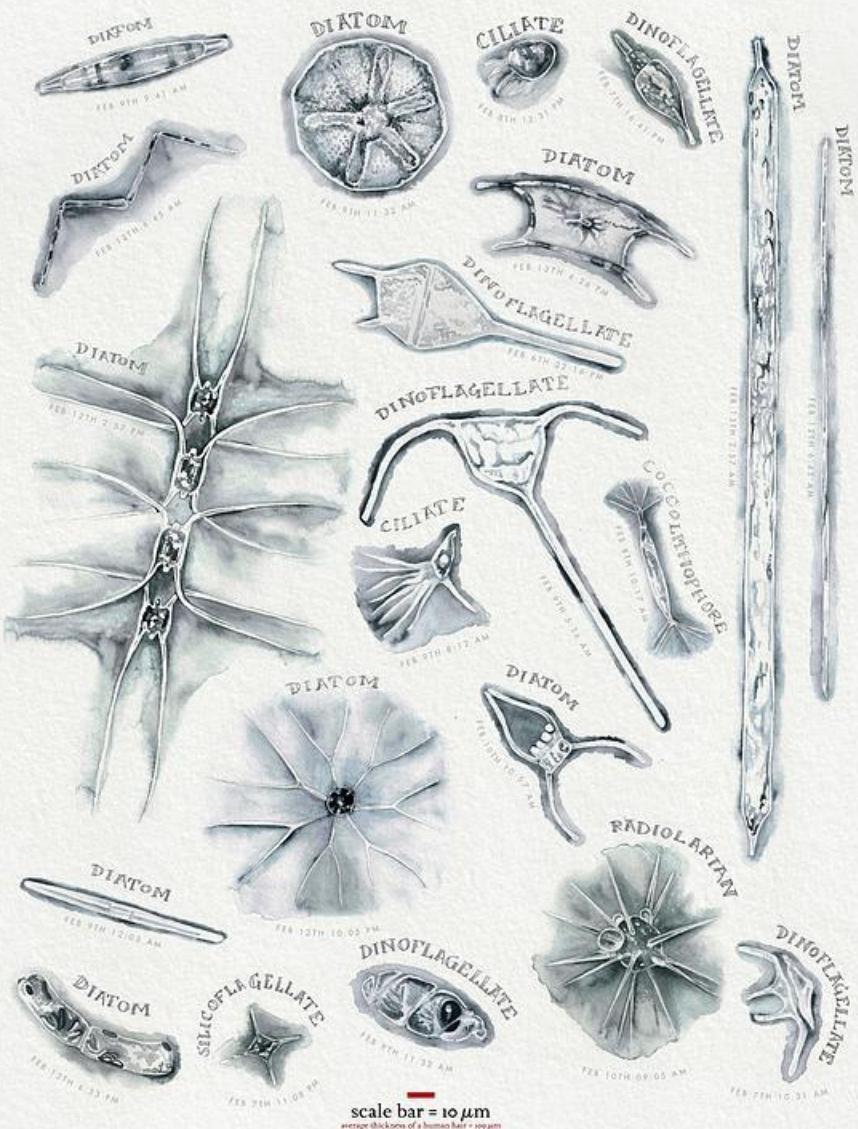




PLANKTON

SEA TO SPACE PARTICLE INVESTIGATION • JANUARY 24 TO FEBRUARY 20, 2017



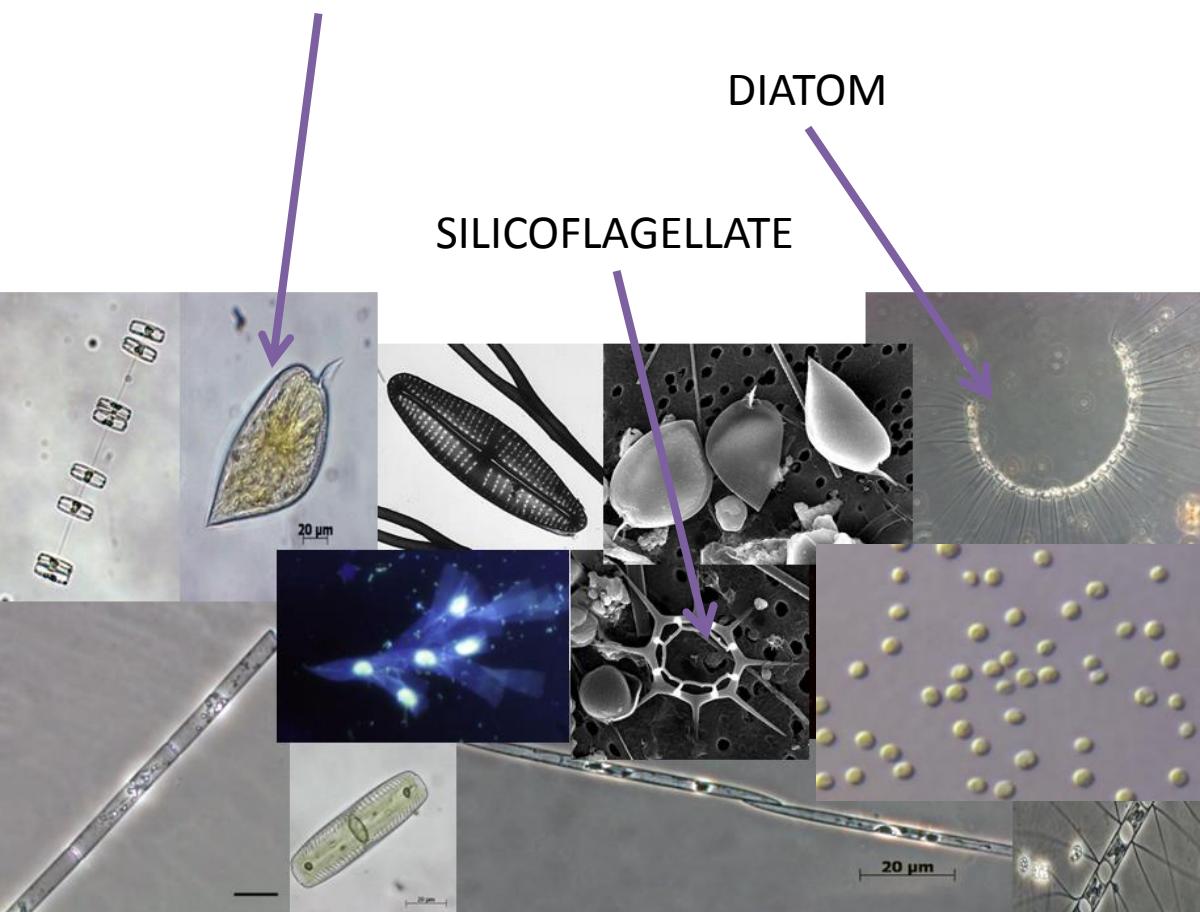
WHAT ARE PHYTOPLANKTON?

IVONA CETINIĆ
NASA GSFC / USRA
@teuta

PHYTOPLANKTON

“drifting plants”, 1887

DINOFLAGELLATE



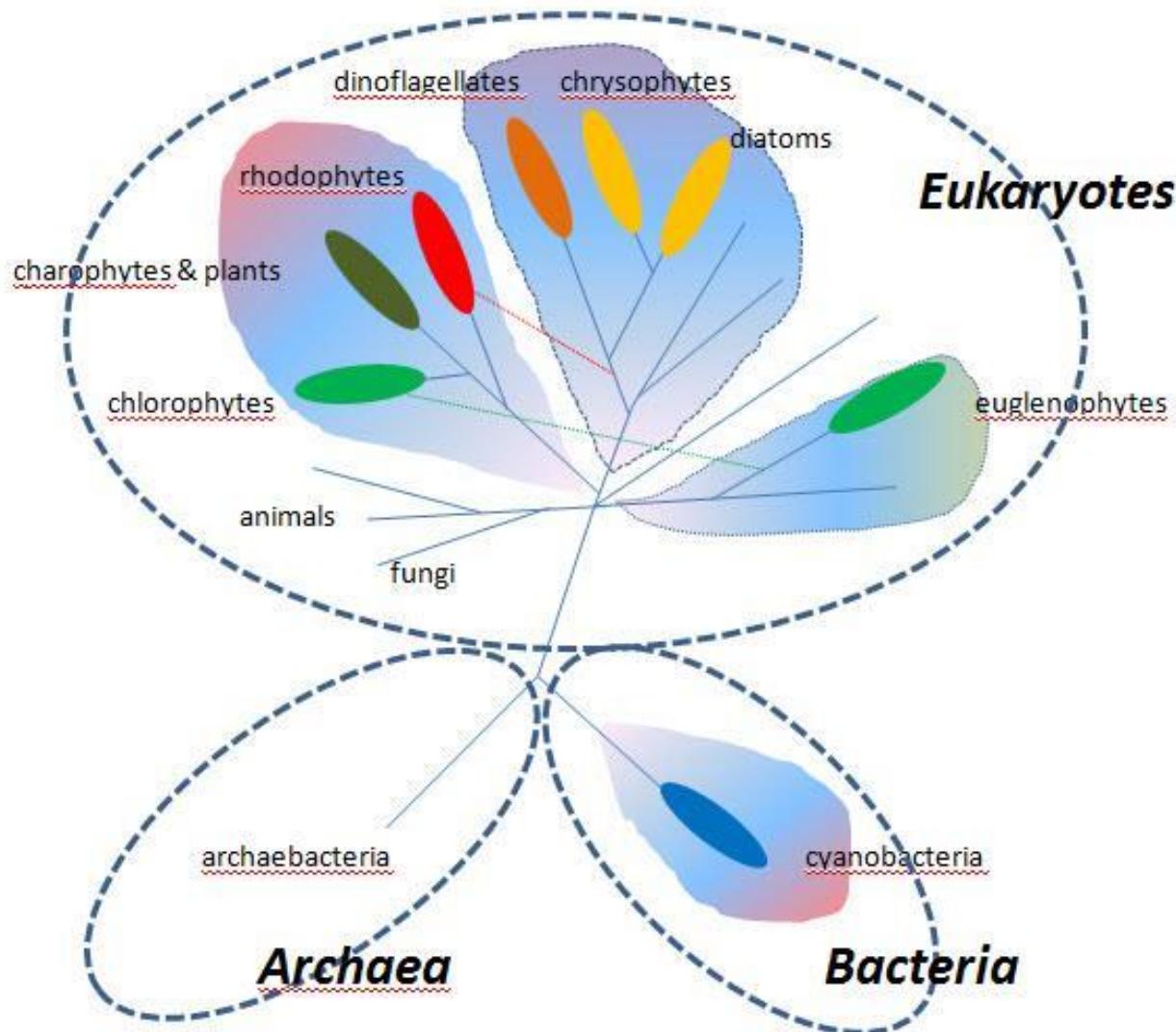
DIATOM

COCCOLITHOPHORE

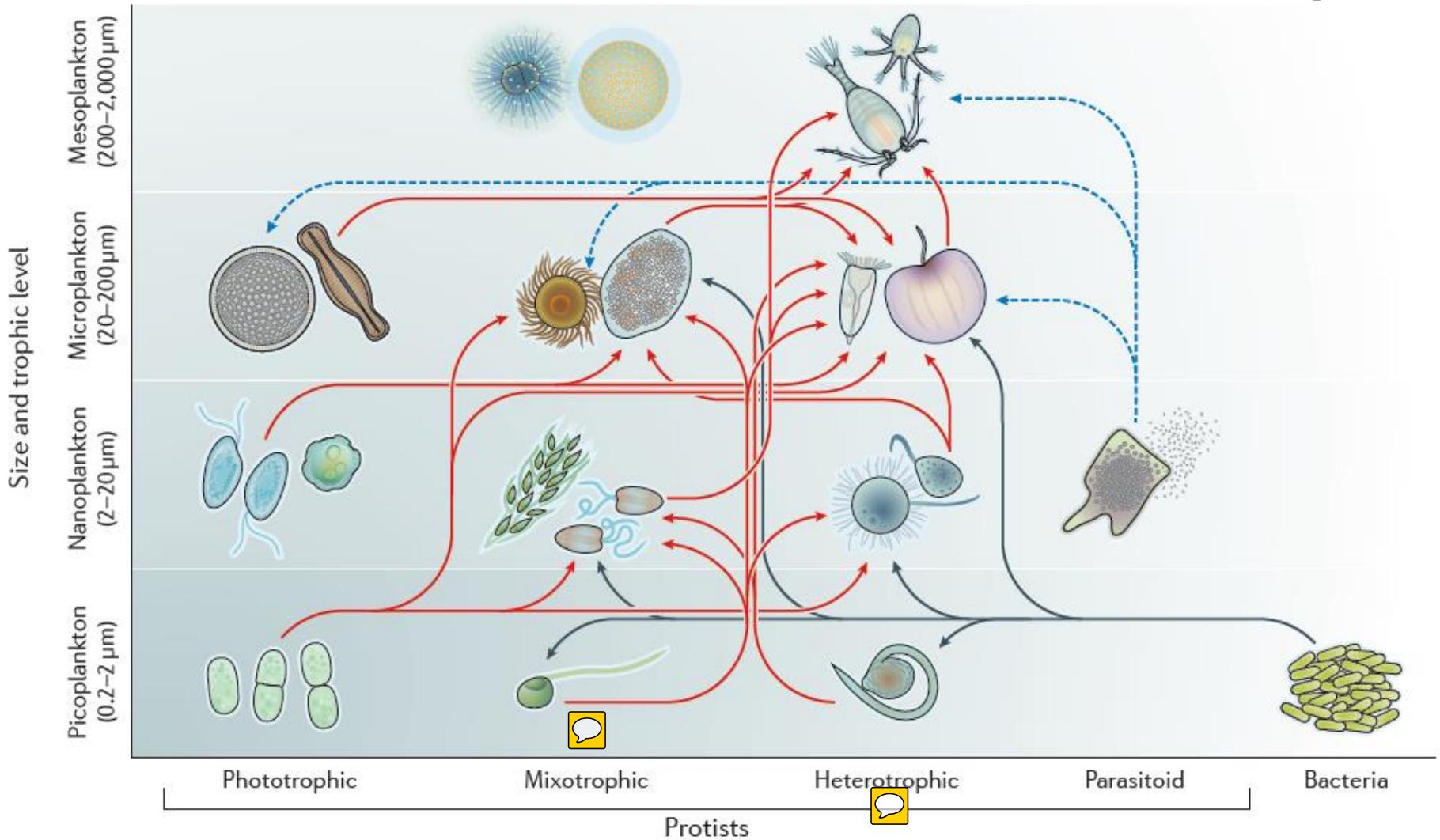


Ljubesic & Bosak

GREAT GENETIC DIVERSITY OF ORGANISMS THAT INTERACT WITH LIGHT IN THE OCEAN



PLANKTON



Caron et al, 2017

PHYTO

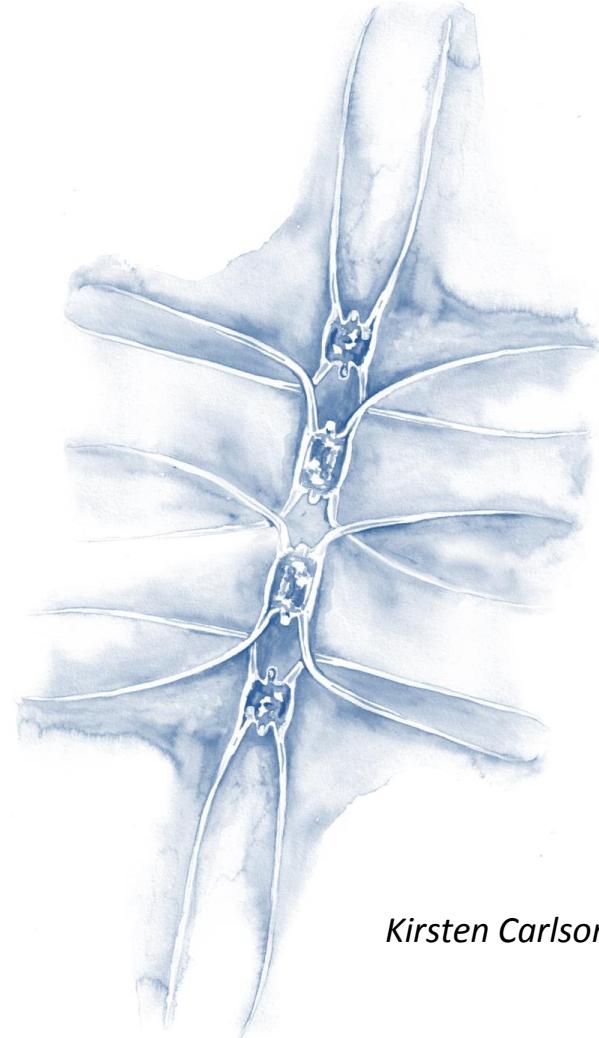
This is why they are so important and why we study them

process of photosynthesis:

DRAW DOWN CARBON DIOXIDE

**MAKE CARBOHYDRATES
(BASE OF MARINE FOOD WEB)**

PRODUCE OXYGEN

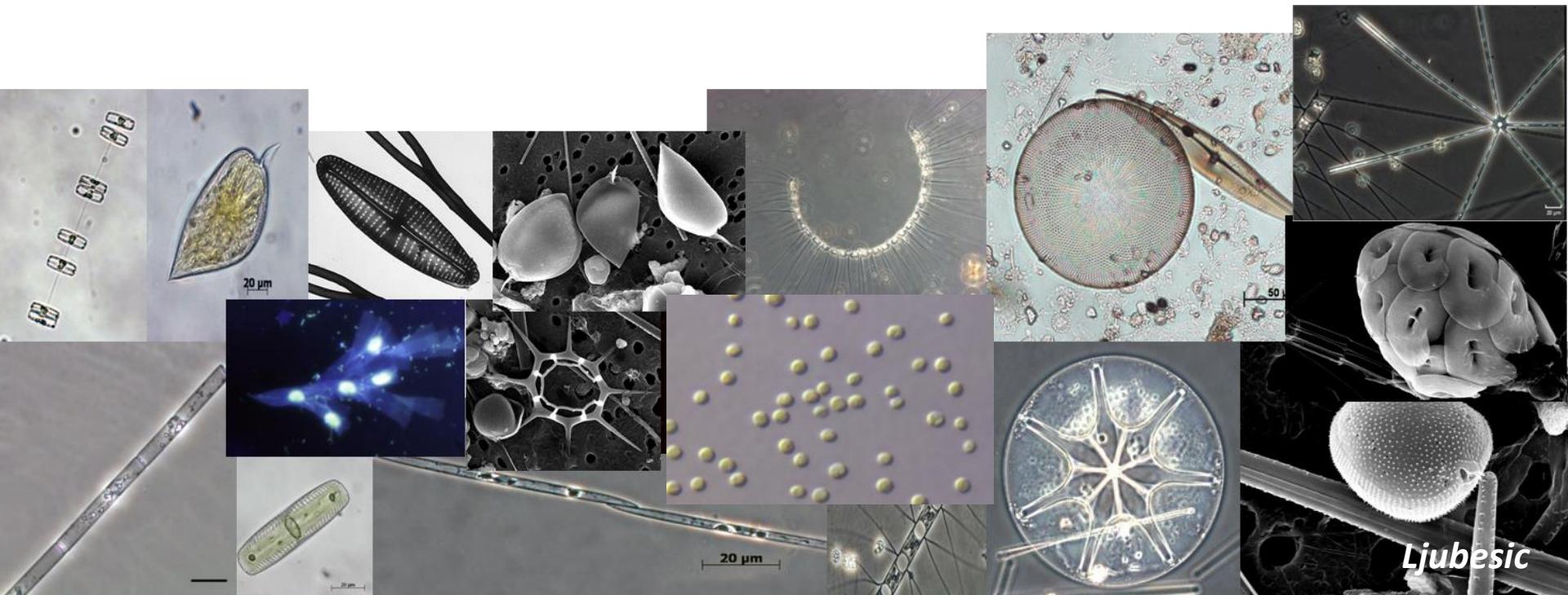


Kirsten Carlson SOI



PHYTOPLANKTON

*Many shapes, colors and sizes..
...that define their role in marine ecosystem and oceanic carbon cycle*

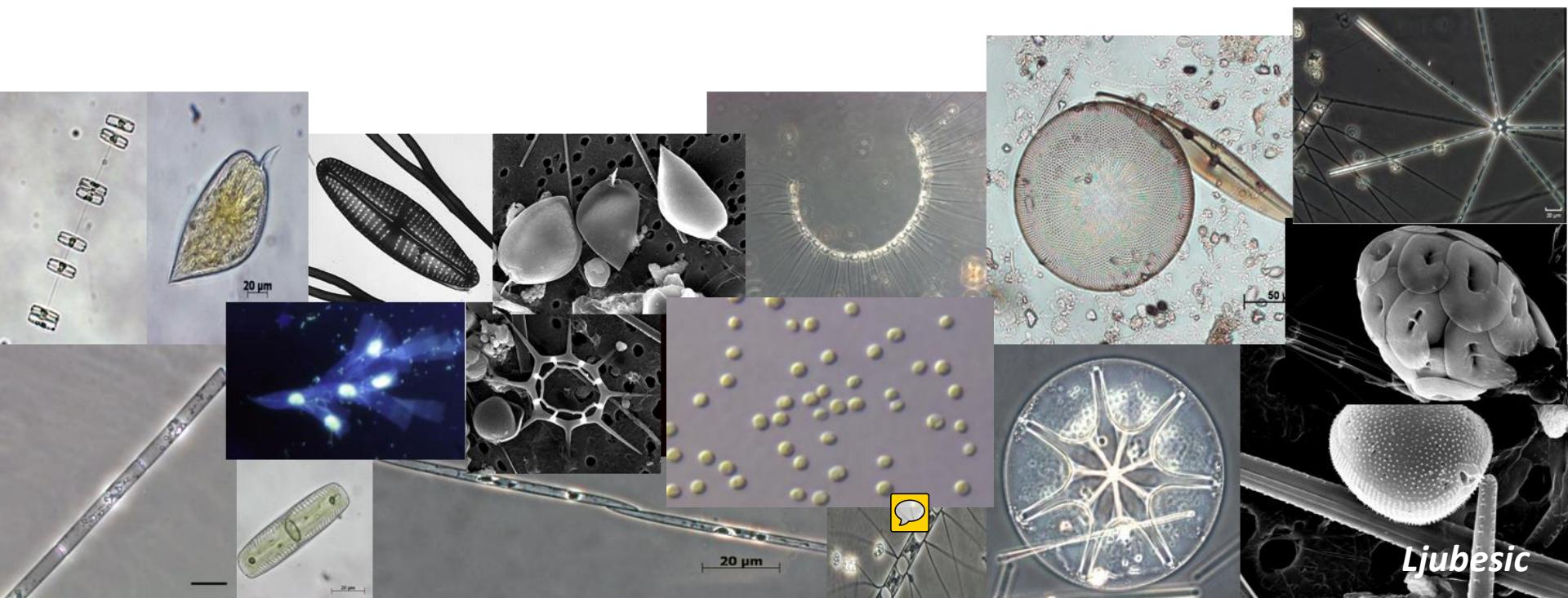


Connect optics to
carbon cycle and
ecosystem

shapes, colors and
size drive both
characteristics

PHYTOPLANKTON

*Many shapes, colors and sizes..
...that define their optical signal*



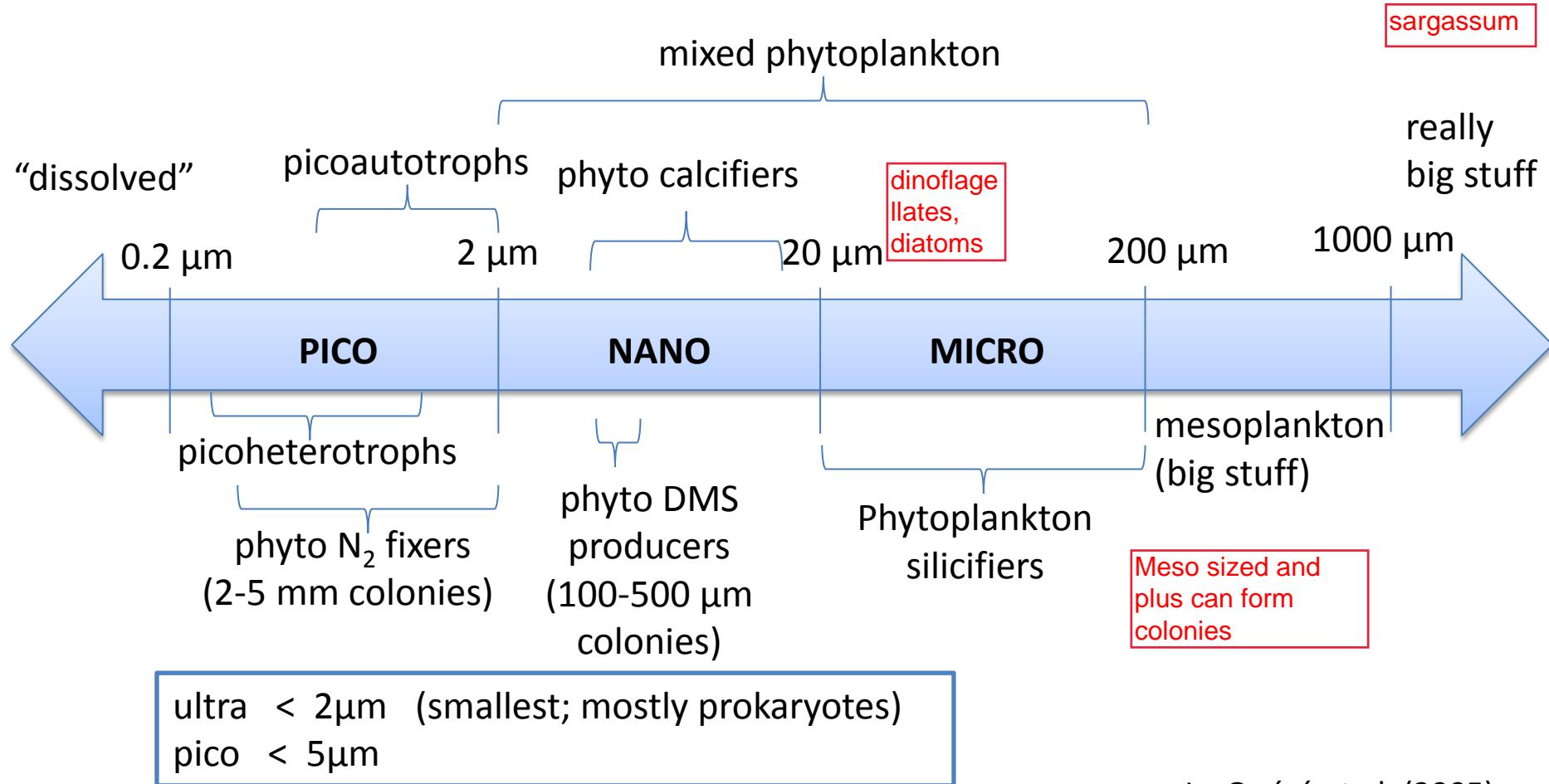
PHYTOPLANKTON

- Particle (size, shape, morphology)
- Taxa (Taxonomic approach)
- Chemistry (pigments, minerals, metabolites..)
- Function (role)

silicates

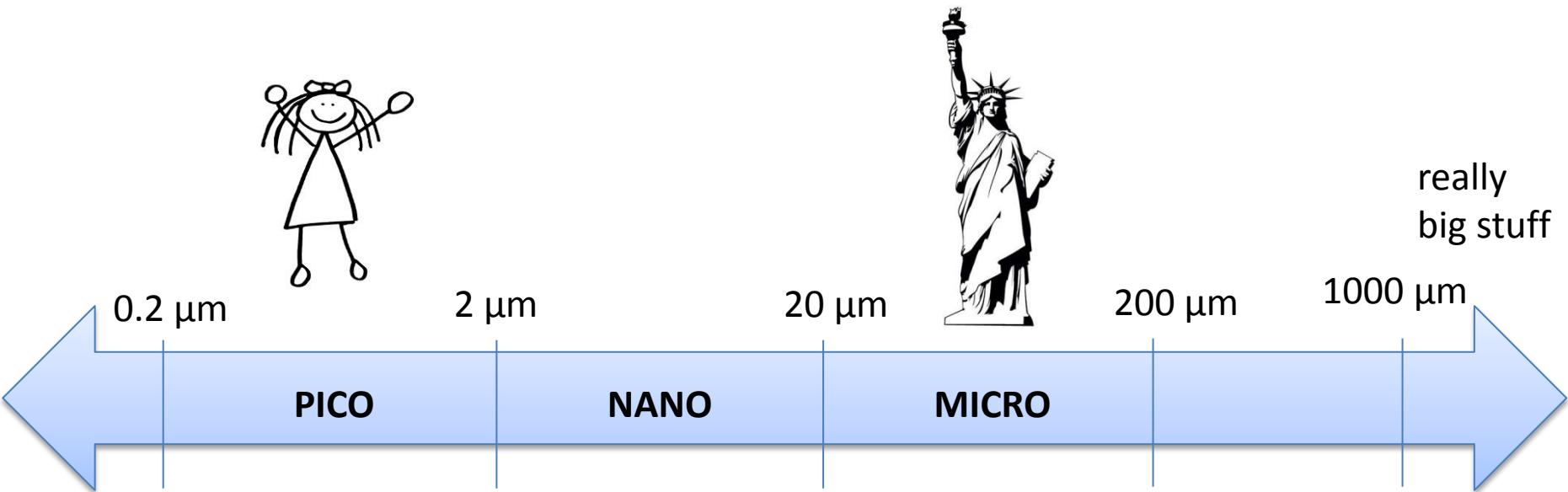
toxins are
metabolites,
influence cloud
formation

PHYTOPLANKTON AS PARTICLES



Le Quéré et al. (2005)

PHYTOPLANKTON AS A PARTICLE...



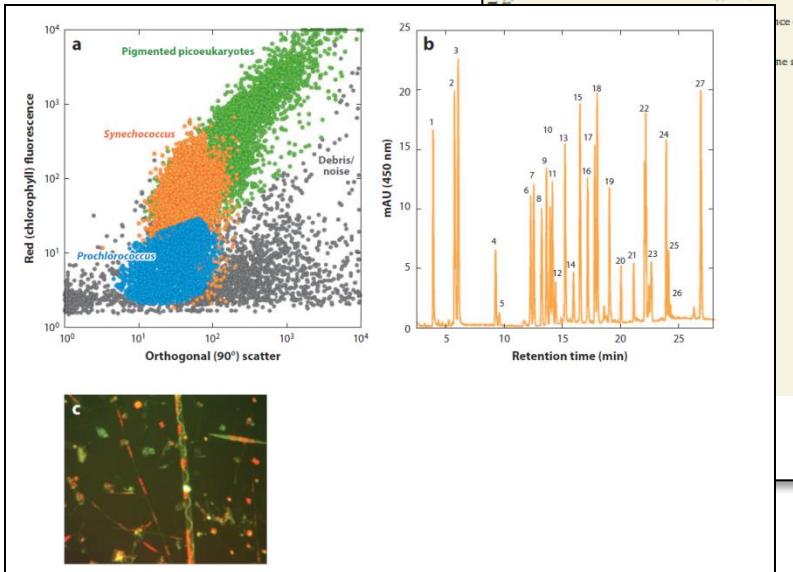
More about particle properties on Friday

PHYTOPLANKTON

- Particle (size, shape, morphology)
- Taxa (Taxonomic approach)
- Chemistry (pigments, minerals, metabolites..)
- Function (role)

PHYTOPLANKTON AS TAXA

- Evolution of concept of taxa
- Driven by the development of the methods



Techniques for Quantifying Phytoplankton Biodiversity

Zackary I. Johnson¹ and Adam C. Martiny²

¹Marine Laboratory (Nicholas School of the Environment) and Department of Biology, Duke University, Beaufort, North Carolina 28516; email: zj@duke.edu

²Department of Earth System Science and Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697; email: amartiny@uci.edu

Keywords

phytoplankton diversity, next-generation sequencing, molecular ecology

Abstract

The biodiversity of phytoplankton is a core measurement of the state and activity of marine ecosystems. In the context of historical approaches, we review recent major advances in the technologies that have enabled deeper characterization of the biodiversity of phytoplankton. In particular, high-throughput sequencing of single loci/genes, genomes, and communities (metagenomics) has revealed exceptional phylogenetic and genomic diversity whose breadth is not fully constrained. Other molecular tools—such as fingerprinting, quantitative polymerase chain reaction, and fluorescence *in situ* hybridization—have provided additional insight into the dynamics of this diversity in the context of environmental variability. Techniques for characterizing the functional diversity of community structure through targeted or untargeted approaches based on RNA or protein have also greatly advanced. A wide range of techniques is now available for characterizing phytoplankton communities, and these tools will continue to advance through ongoing improvements in both technology and data interpretation.

299

TAXONOMY - ALL ROLLS BACK TO THE EVOLUTION OF THE TAXA CONCEPT

Modern system of algal classification (20-ish yrs old)

- DNA, RNA, protein based (genetics, genomics, transcriptomics... proteomics) + ultrastructure electron microscopy

Cool & easy read - Caron (2013), *Journal of Eukaryotic Microbiology*

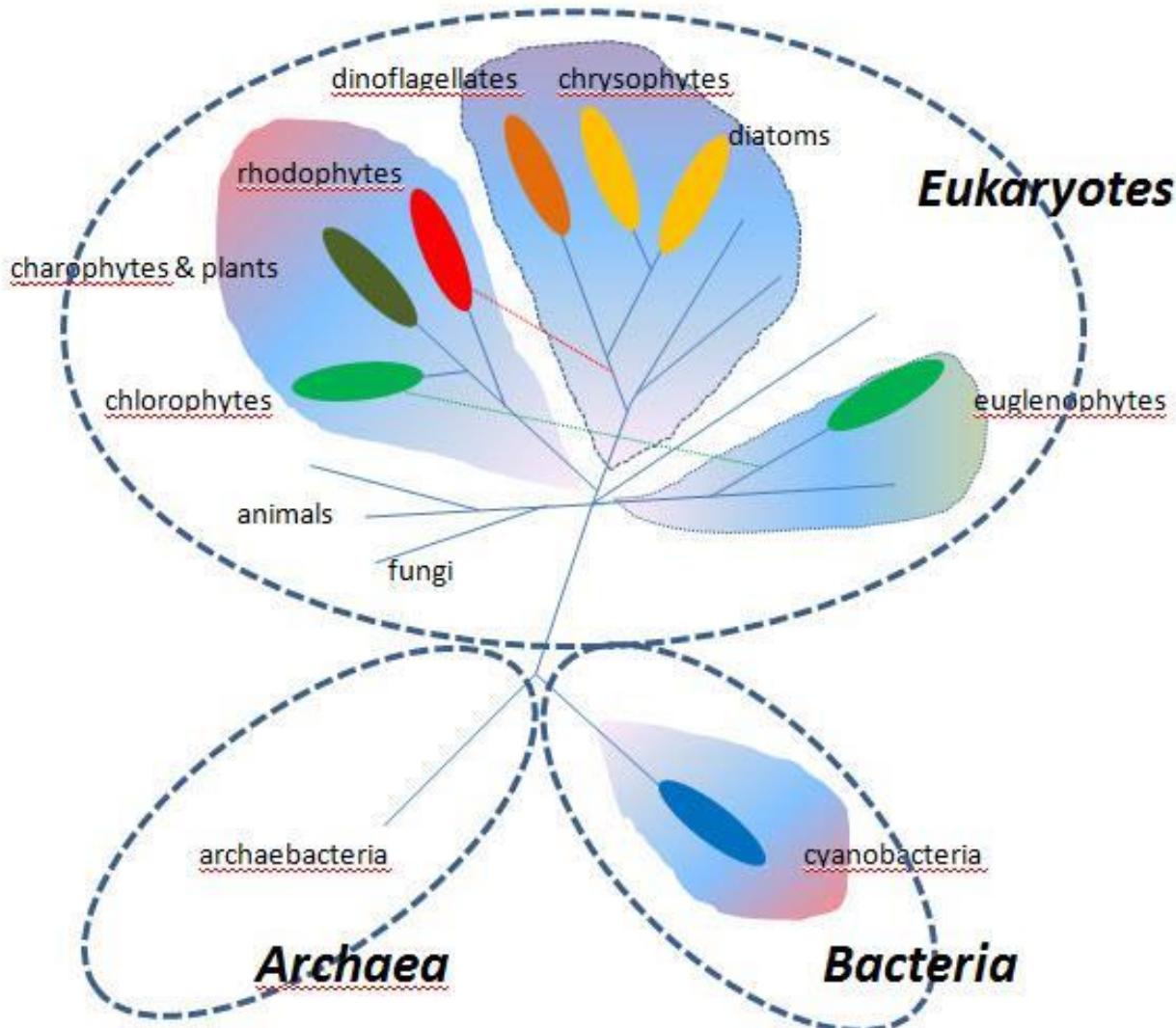
Classical system (~ 350 yrs old)

- Classification based on morphological characteristics based purely on light microscopy
- Electron microscopy (ultrastructure)
- Pigmentation

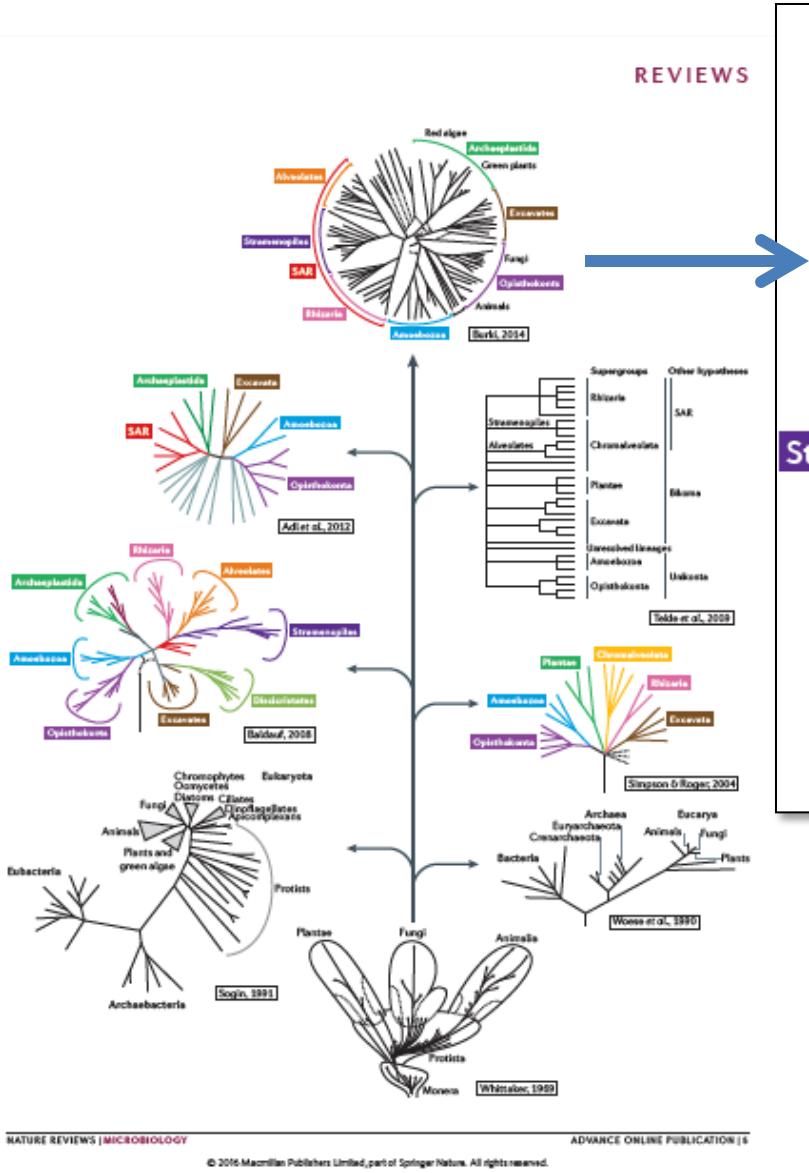
Dinoflagellates,
diatoms, and
flagellates are
terms from this
classical system

VERY SIMPLE TREE OF LIFE (EMPHASIS ON PHYTOPLANKTON)

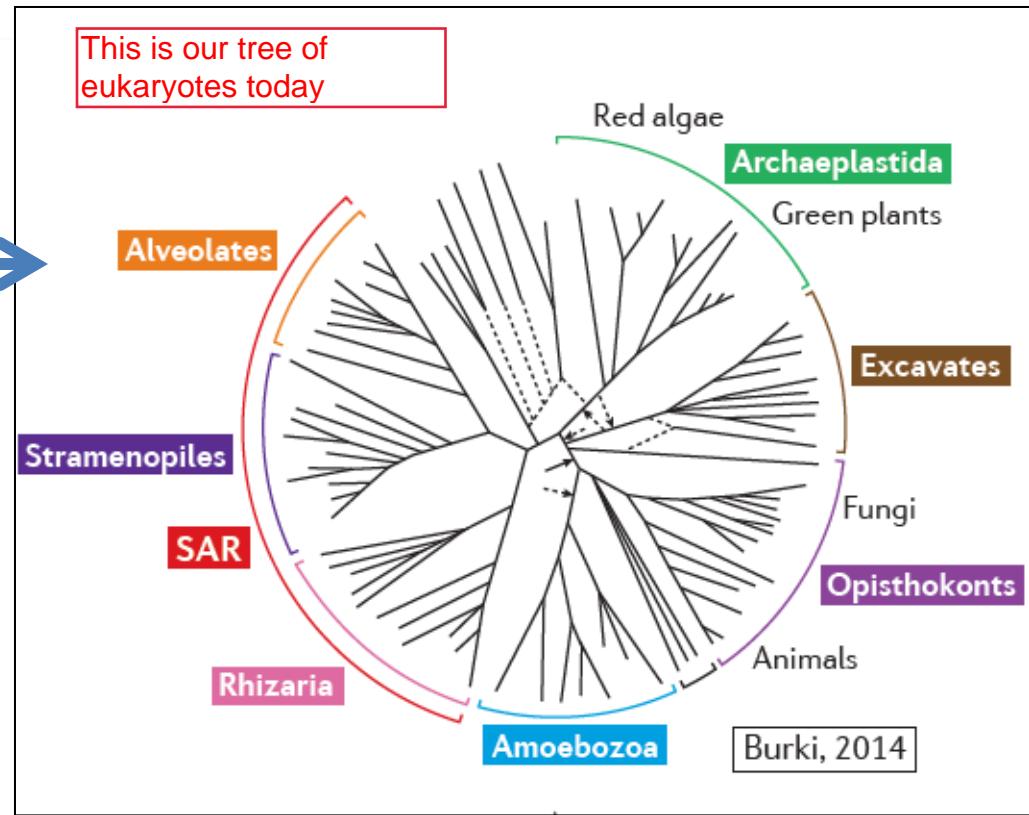
Ph



EUKARYOTE PHYLOGENETIC TREE



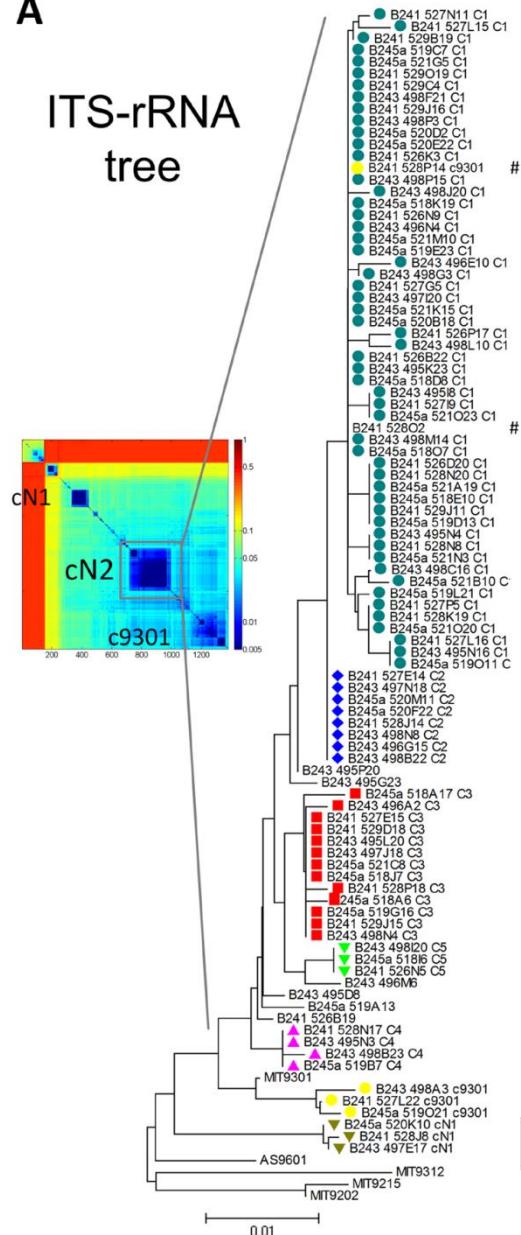
This is our tree of eukaryotes today



PROCHLOROCOCCUS PHYLOGENETIC TREES

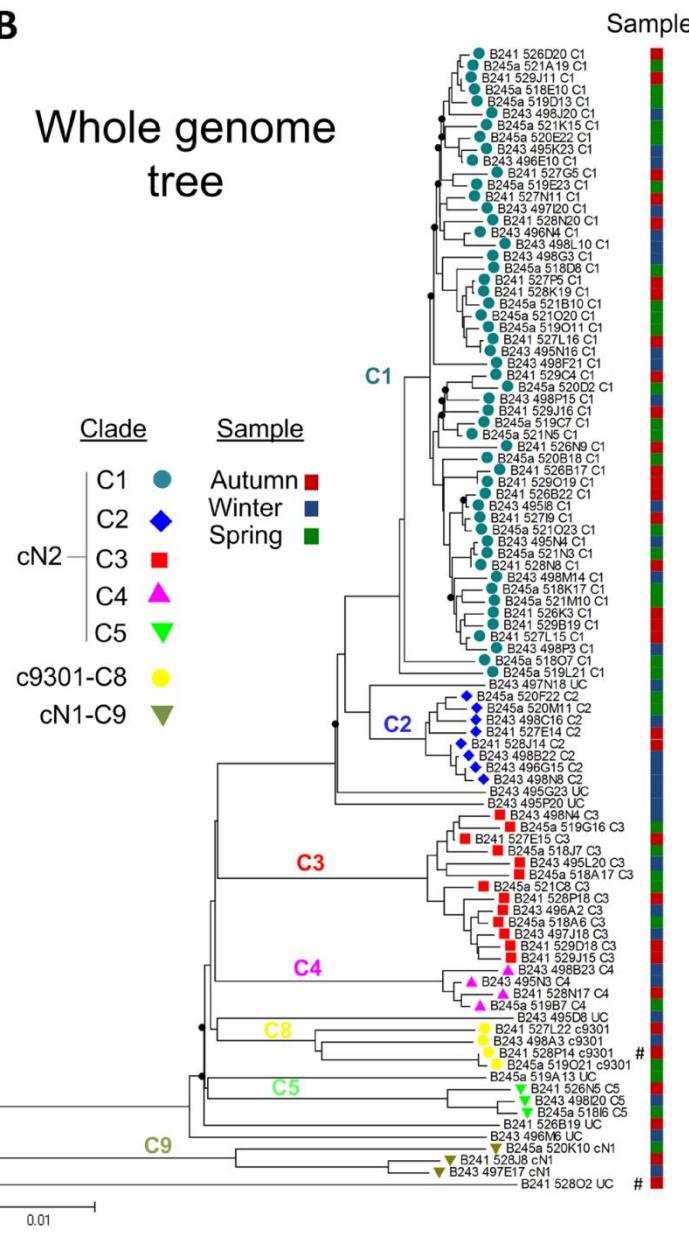
A

ITS-rRNA
tree



B

Whole genome
tree



prokaryotic
bacterial
autotrophs found
everyone in the
ocean



Kashtan,
Nadav, et al. (2014)

TAXONOMY

1) Old, classical system (~ 350 yrs old)

- Classification based on morphological characteristics based purely on light microscopy
- Electron microscopy (ultrastructure)
- Pigmentation

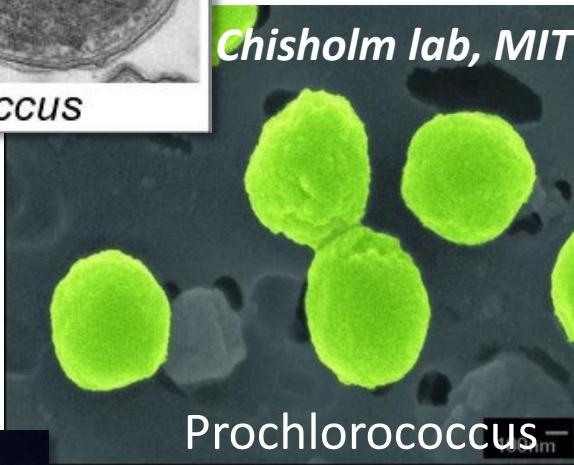
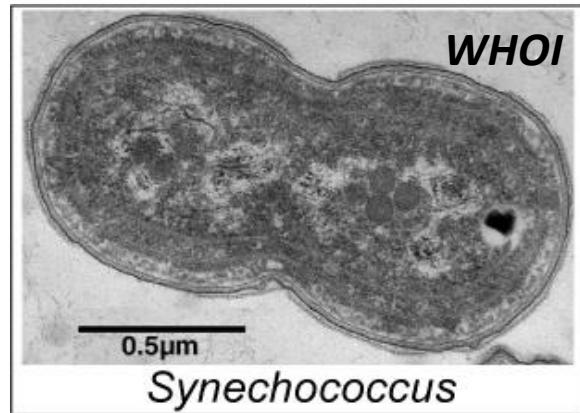
Dinoflagellate &
diatom



Caron lab, USC

CYANOBACTERIA

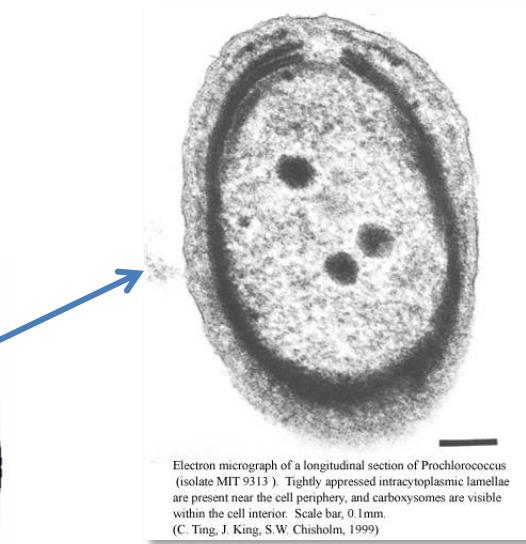
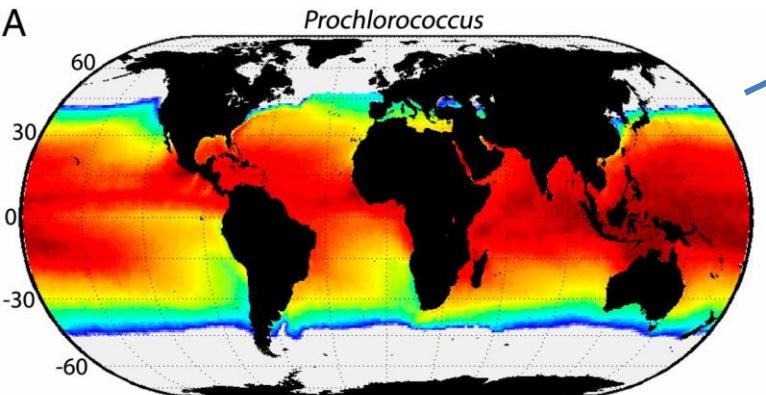
Prokaryote, teeny tiny, DNA piled up inside, Penny Chisholm found them by accident like 20 years ago



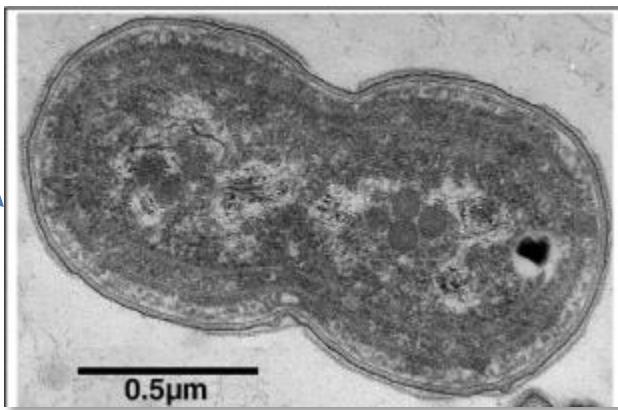
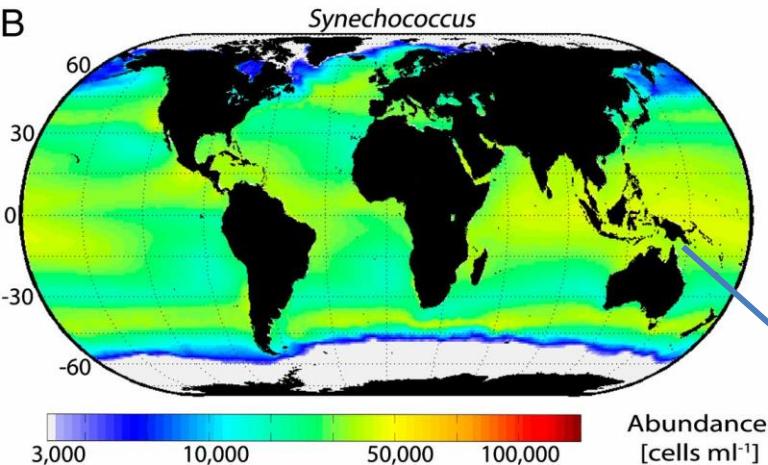
Trichodesmium (puff)

- Includes many of the picoplankton
- Many do Nitrogen Fixation 
- Smallest and most abundant phytoplankton in the ocean
- Tropical to cosmopolitan

Prochlorococcus
Smallest and most abundant.
($\sim 0.7 \mu\text{m}$)
Diagnostic: very small size, lack of orange fluorescence, divinyl chlorophyll a & b.



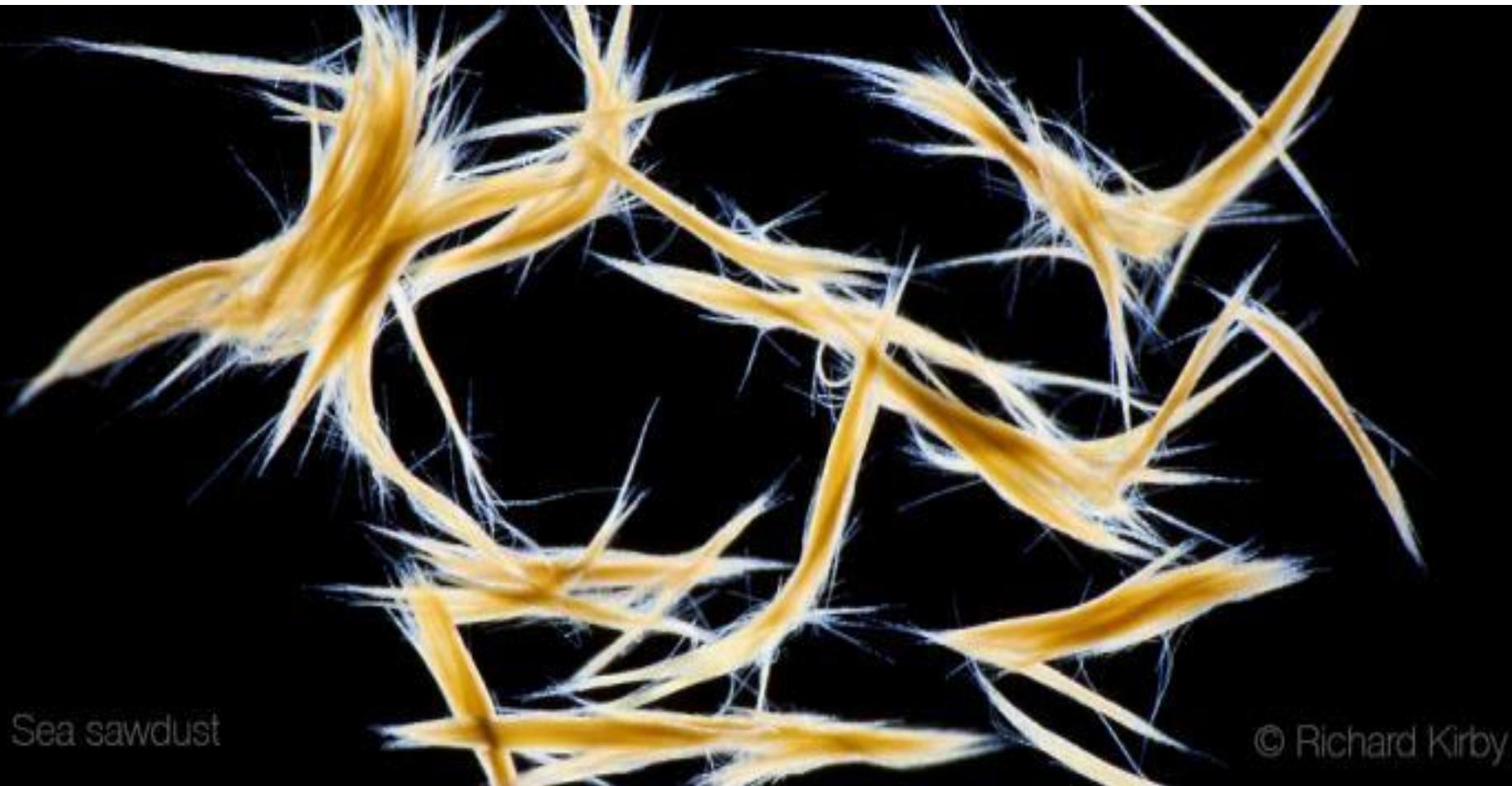
Electron micrograph of a longitudinal section of *Prochlorococcus* (isolate MIT 9313). Tightly appressed intracytoplasmic lamellae are present near the cell periphery, and carboxysomes are visible within the cell interior. Scale bar, 0.1 mm.
(C. Ting, J. King, S.W. Chisholm, 1999)



Synechococcus
($\sim 1 \mu\text{m}$)
Diagnostic: phycoerthrin pigment fluoresces orange (in contrast to chlorophyll, which fluoresces red).

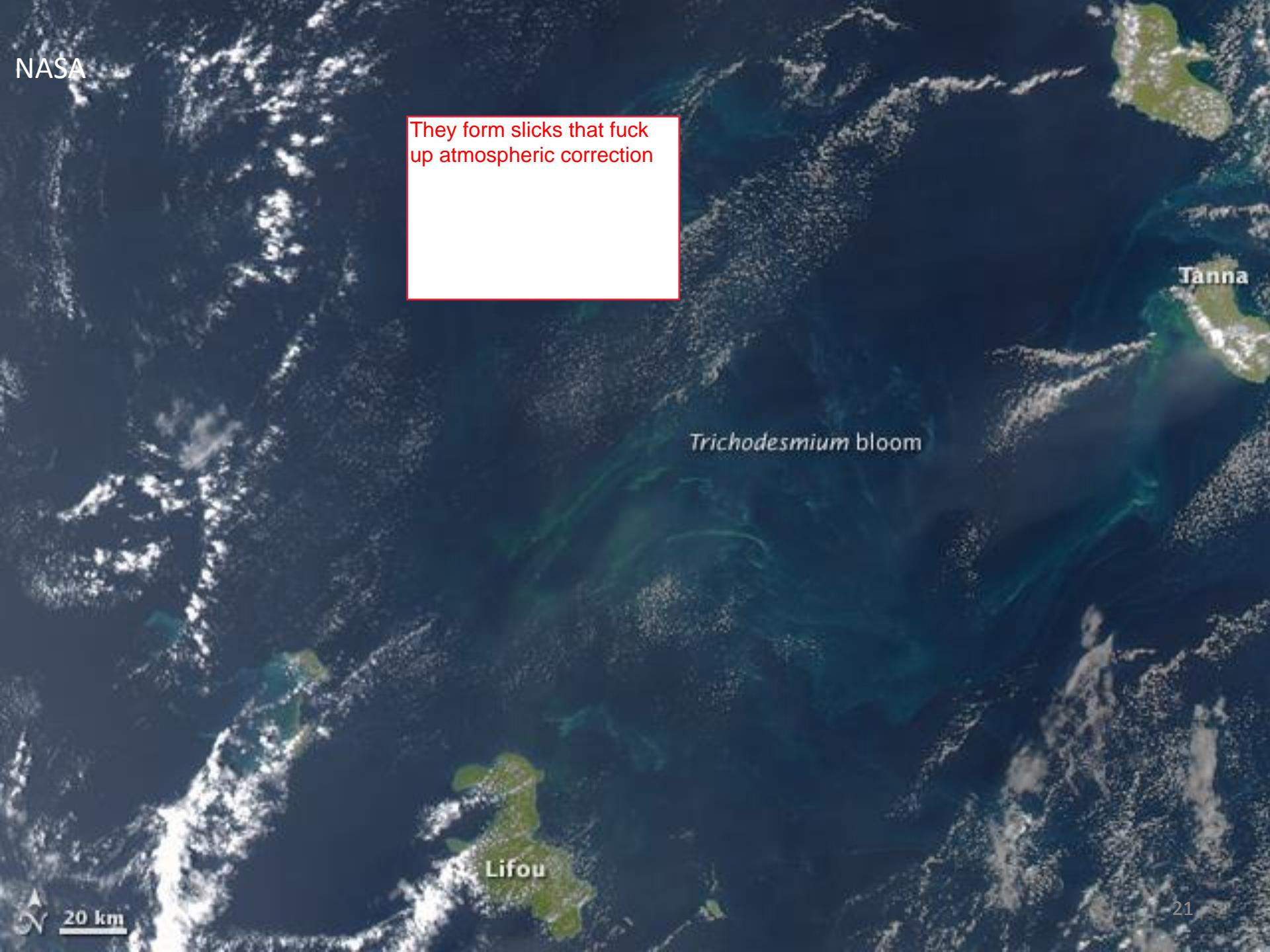
Trichodesmium

cyanobacteria, nitrogen fixer, warm waters, puffs and tuffs, phycoerthrin, Fe may regulate abundance



Sea sawdust

© Richard Kirby



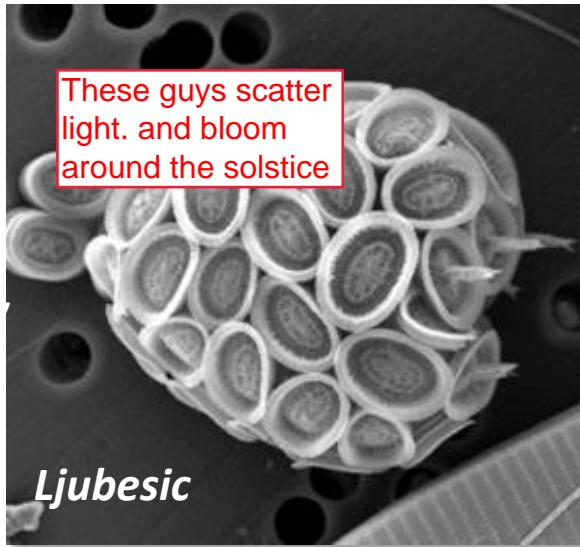
They form slicks that fuck
up atmospheric correction

Trichodesmium bloom

Lifou

Tanna

PRYMNESIOPHYTES



7-10 nm



Phaeocystis, makes floating with hundreds of cells embedded in a polysaccharide gel matrix

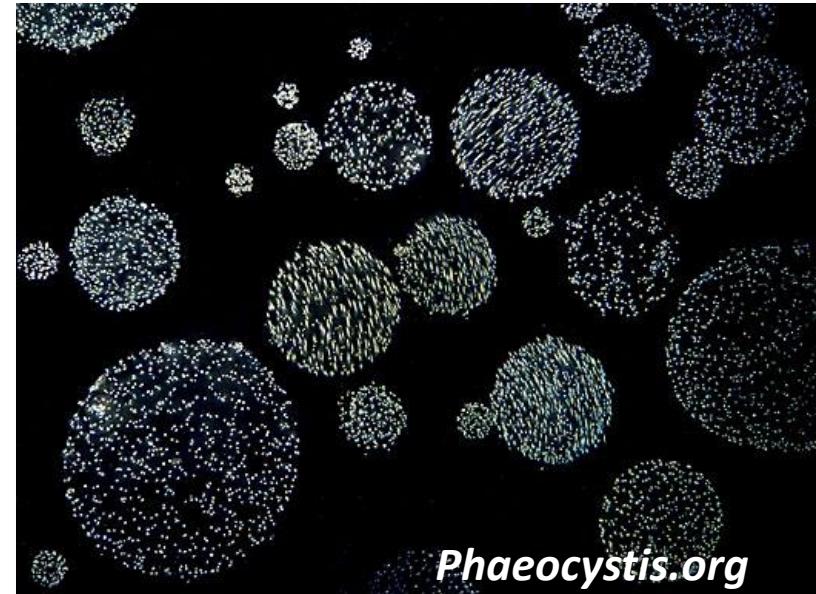
DMSP producers

Form ugly foam on beaches in UK (smell of the seaside?)

Coccolithophores, calcareous phytoplankton – calcium carbonate “shells”, nano-micro

Sensitive to sea surface temperature
- important tool in paleontology
Sensitivity to pH

Important for Carbon Export, climate studies

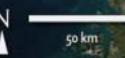


NASA

We can see
coccolithophores
from space; makes
them milky

Barents Sea

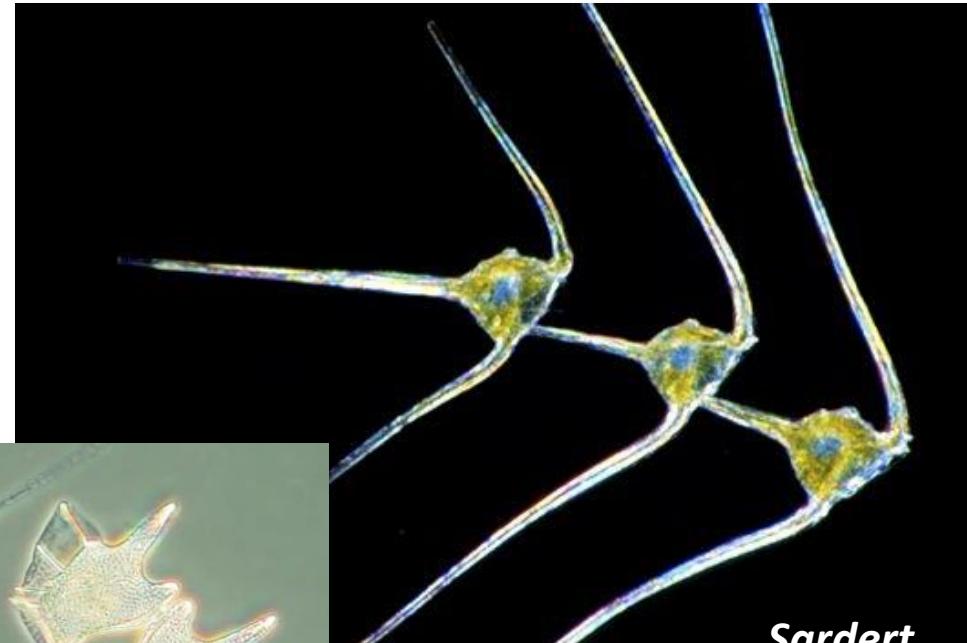
Norway



DINOFLAGELLATES



These are the
blooms of the
spring



- Flagellate containing algae
- Plant-like, but sometimes animal like (heterotroph), even predators
- Mostly coastal, warm waters
- Are also often symbionts of benthic and pelagic “heterotrophs”
- Red tide organisms, some toxic



Alexandrium

Not toxic, but
causes hypoxia

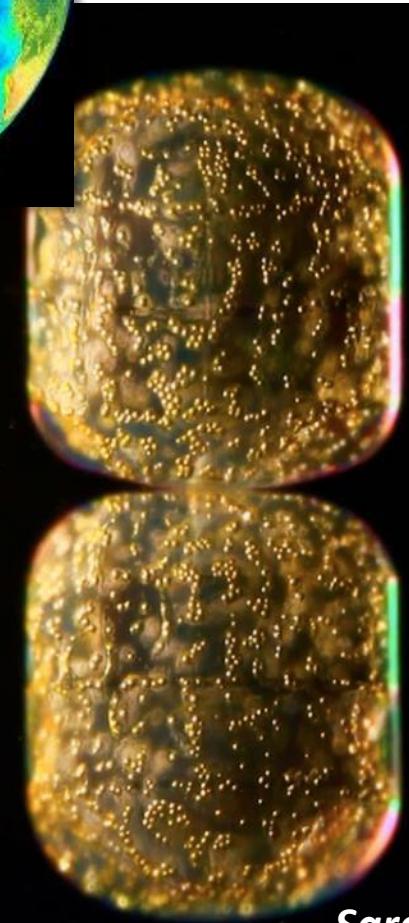
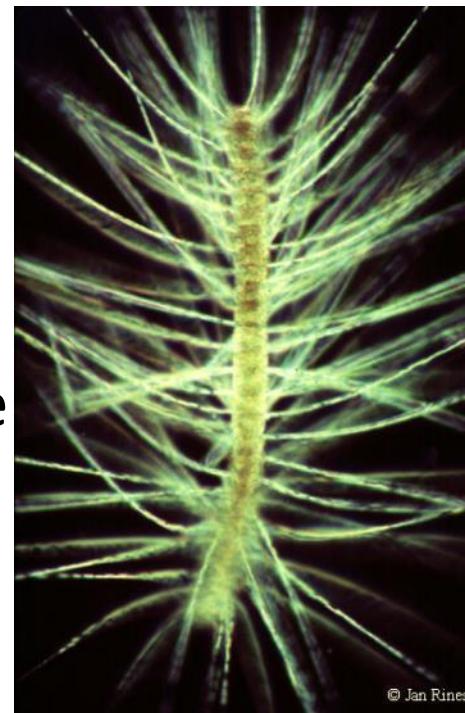
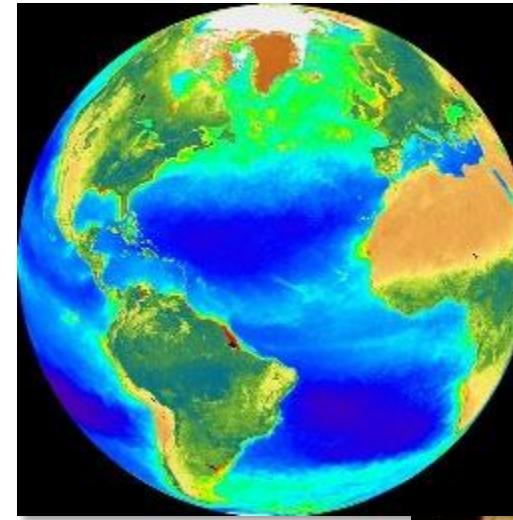




Usually in nano
and microsize

DIATOMS

- Most common type of phytoplankton
- Have silica shells
- Single cells occasionally form chains
- Two forms: pennate, centric
- Some toxic (domoic acid)
- Spring bloomers, effective carbon exporters



Sardet

PHYTOPLANKTON

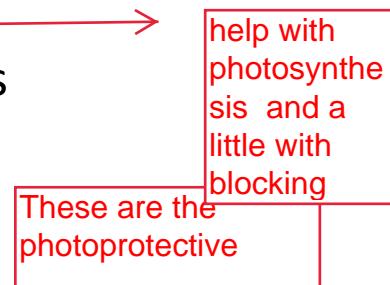
- Particle (size, shape, morphology)
- Taxa (Taxonomic approach)
- Chemistry (pigments, minerals, metabolites..)
- Function (role)

PHOTOSYNTHETIC PIGMENTS

(Ps-Light harvesting, PP – photoprotective)

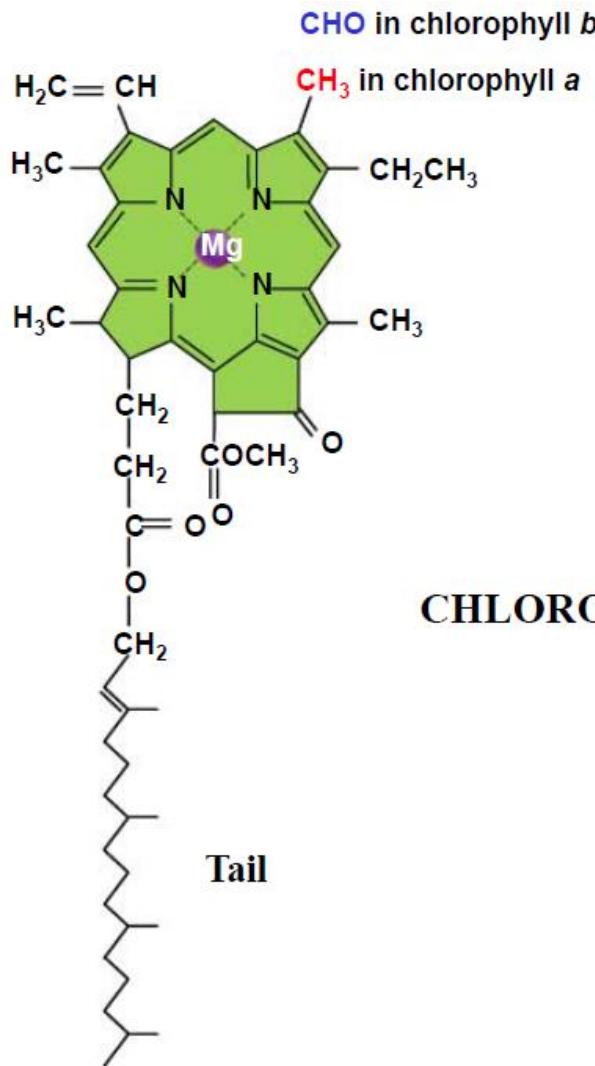
- **Chlorophylls (green)**
 - Chlorophylls a, b, c, d
 - Divinyl chlorophyll
- Carotenoids(brownish/orangish)
 - Carotenes
 - Beta-carotenes
 - Xanthophylls
 - Peridinin
 - Alloxanthin
 - Zeaxanthin.....
- Phycobilins (blue-green/redish)
 - Phycocyanin
 - Phycoerithrin

Intense light damages the system of the cell, can drive your ability to do photochemistry to zero
That is where photoprotective pigments come in

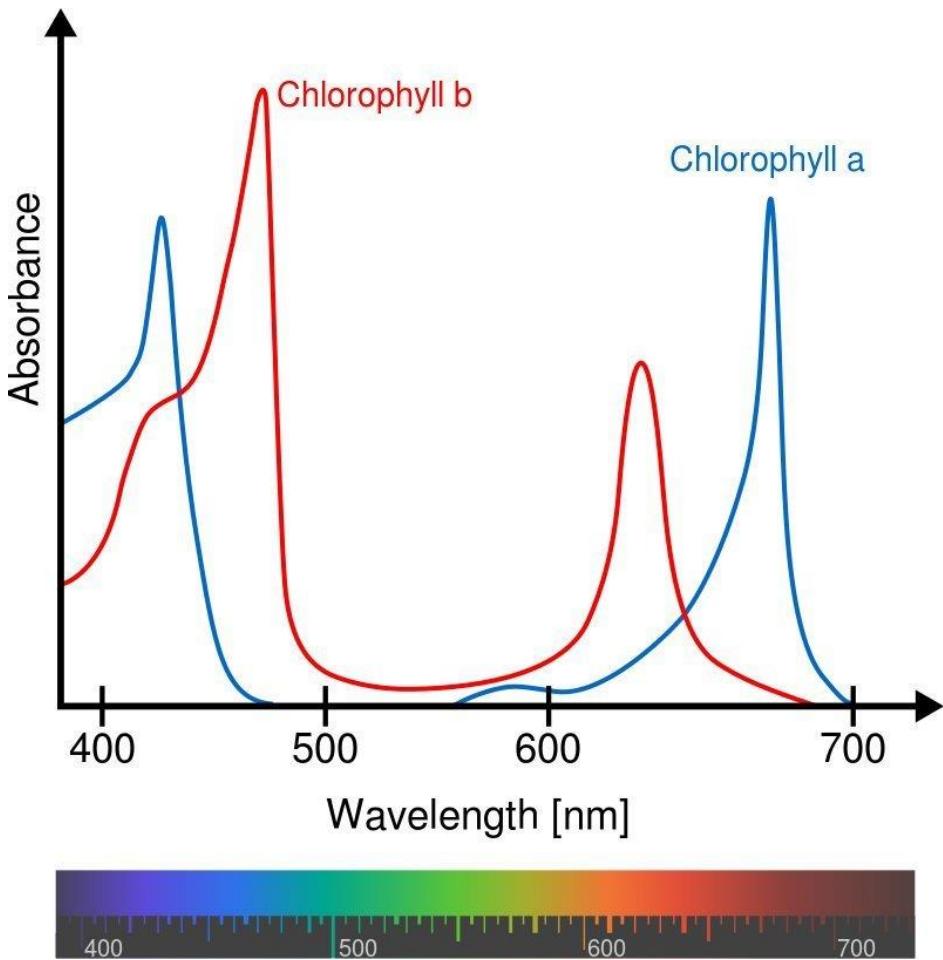




HRH CHLOROPHYLL



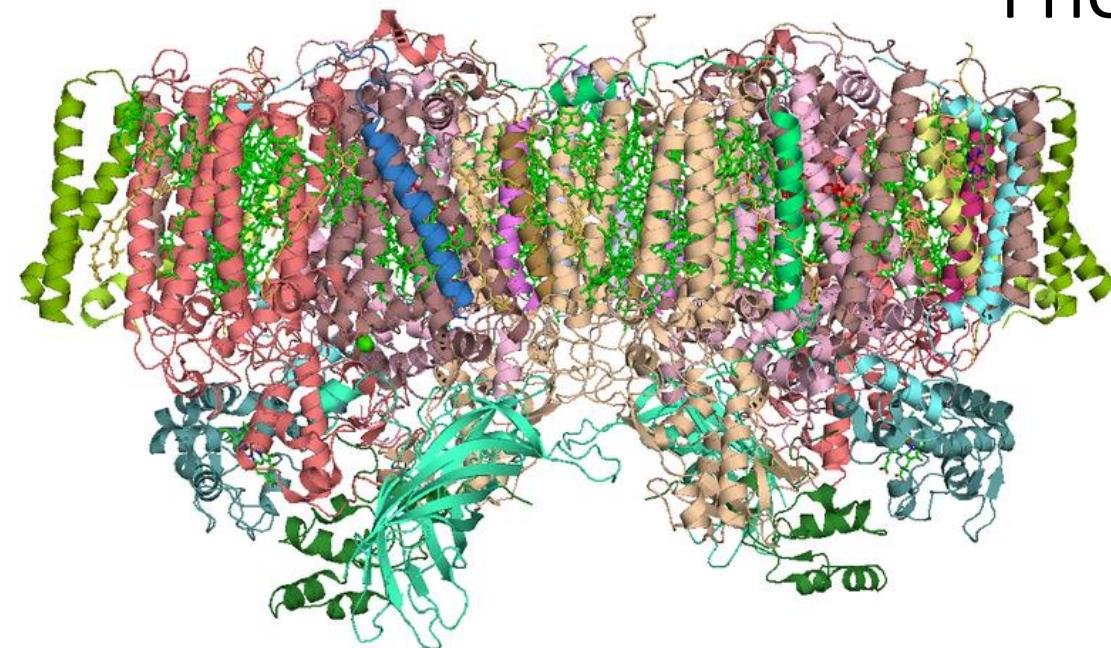
Chlorophyll absorption spectra (in vitro)



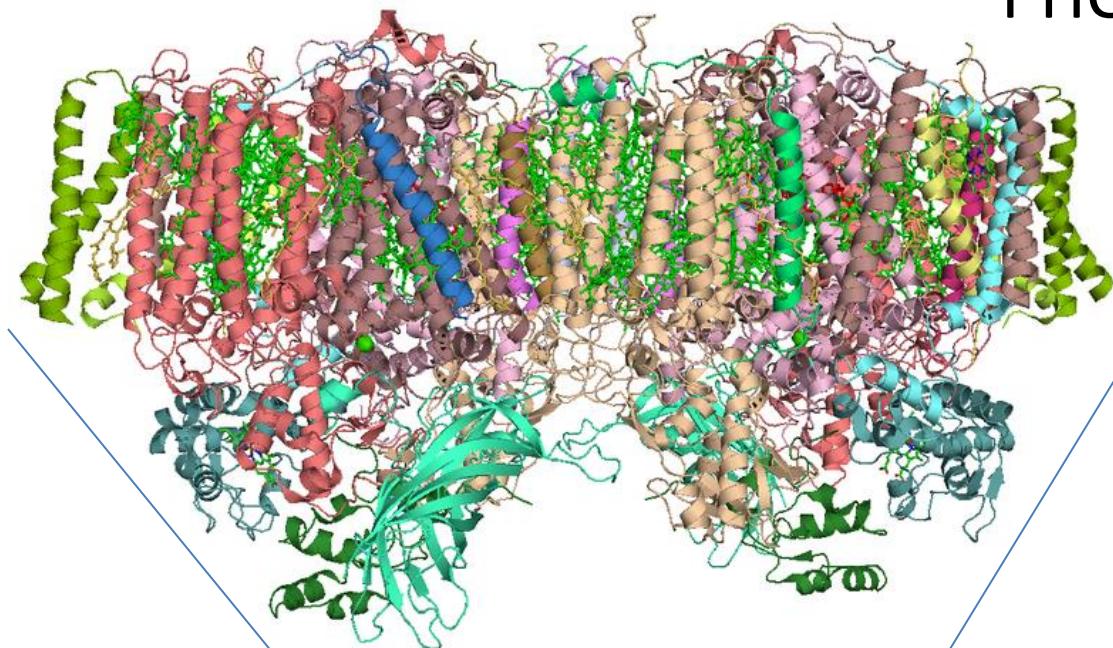
Pigment	Solvent	λ_{max} (nm)
Chl a	Acetone	430, 662
	Diethyl ether	428, 660
	95% Ethanol	432, 664
	Methanol	432, 665
Chl b	Acetone	457, 646
	Diethyl ether	453, 642
	95% Ethanol	464, 649
	Methanol	469, 652

Adapted from Roy et al, 2012

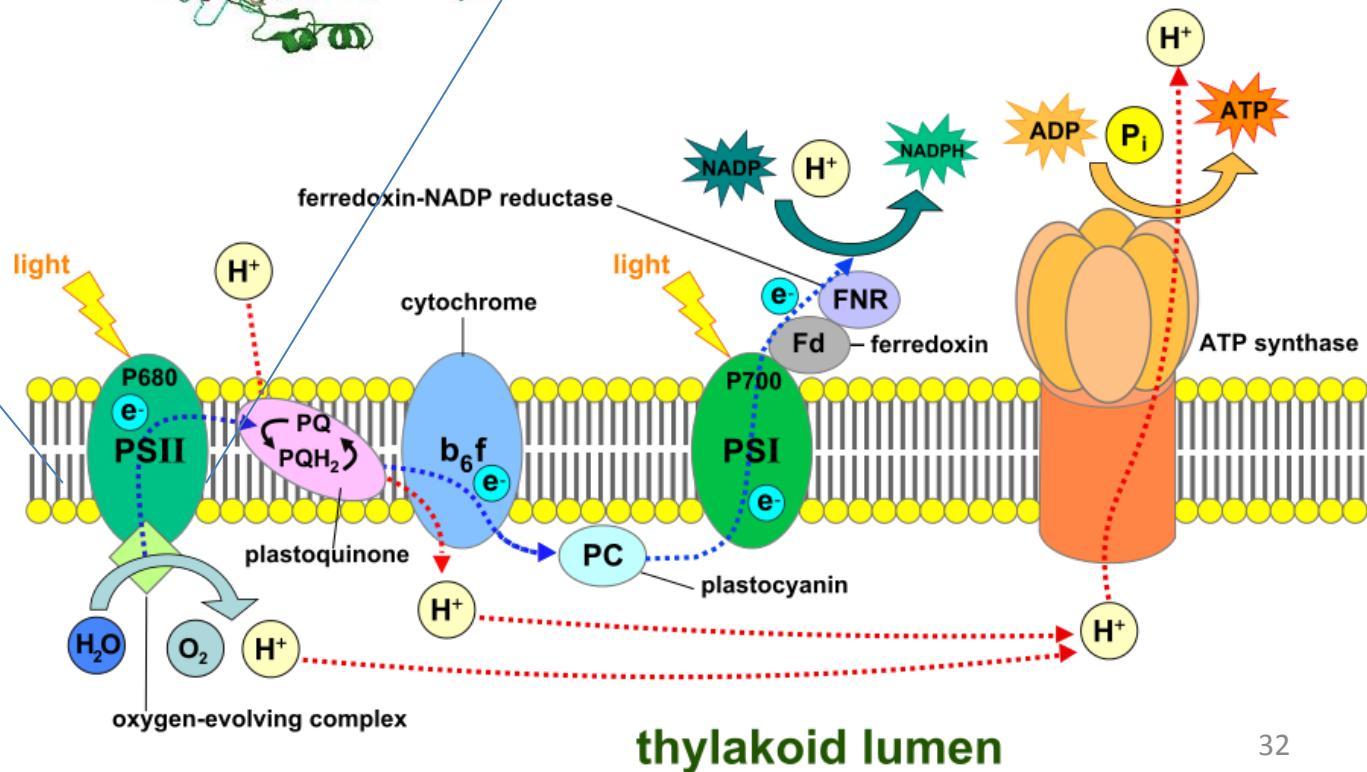
PHOTOSYSTEM II



PHOTOSYSTEM II



chlorophyll is not floating in the cell. It is embedded in a protein complex that is in a membrane that is inside a photosystem is inside a plastid in eukaryotes.
In prokaryotes, the photosystems are in thylakoids not in an organelle



The majority of energy absorbed goes to heat - not every cell but on a global level. If the cell is stressed those ratios change

Fluorescence is a tiny part

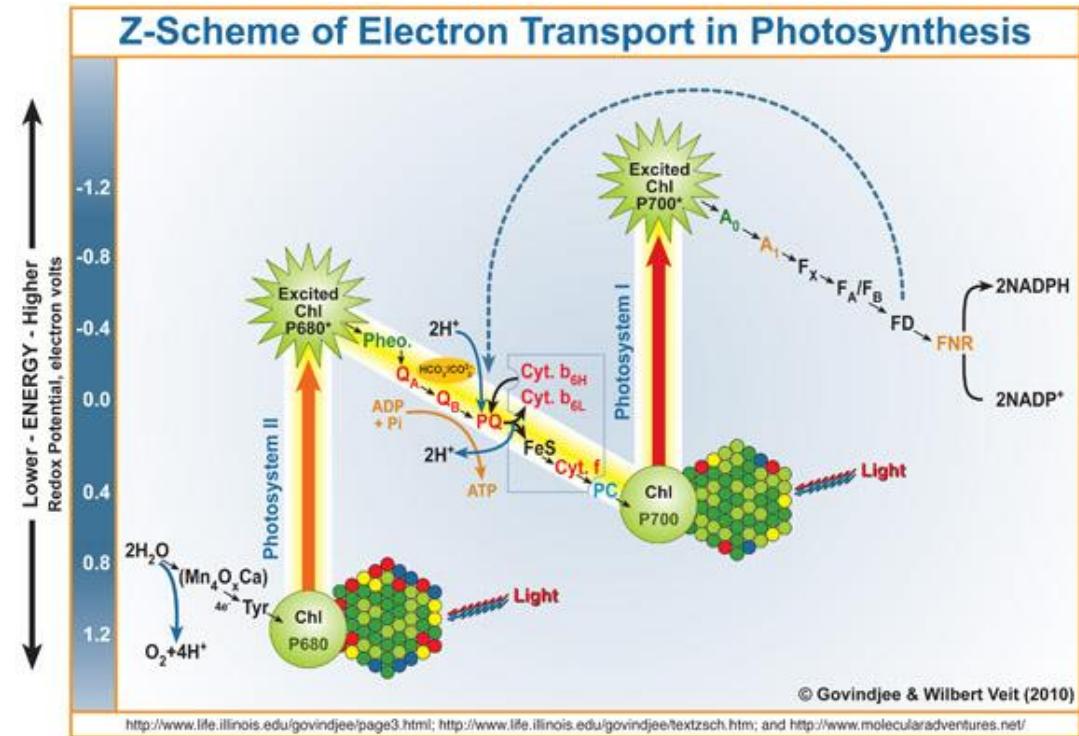
Different colors of dots are different pigments

MAGIC OF CHLOROPHYLL

NOT ALL ENERGY ABSORBED GOES TO PHOTOSYNTHESIS !!!

- 1) Heat (~60%)
- 2) Fluorescence (~7%)
- 3) Photosynthesis (~33 %)

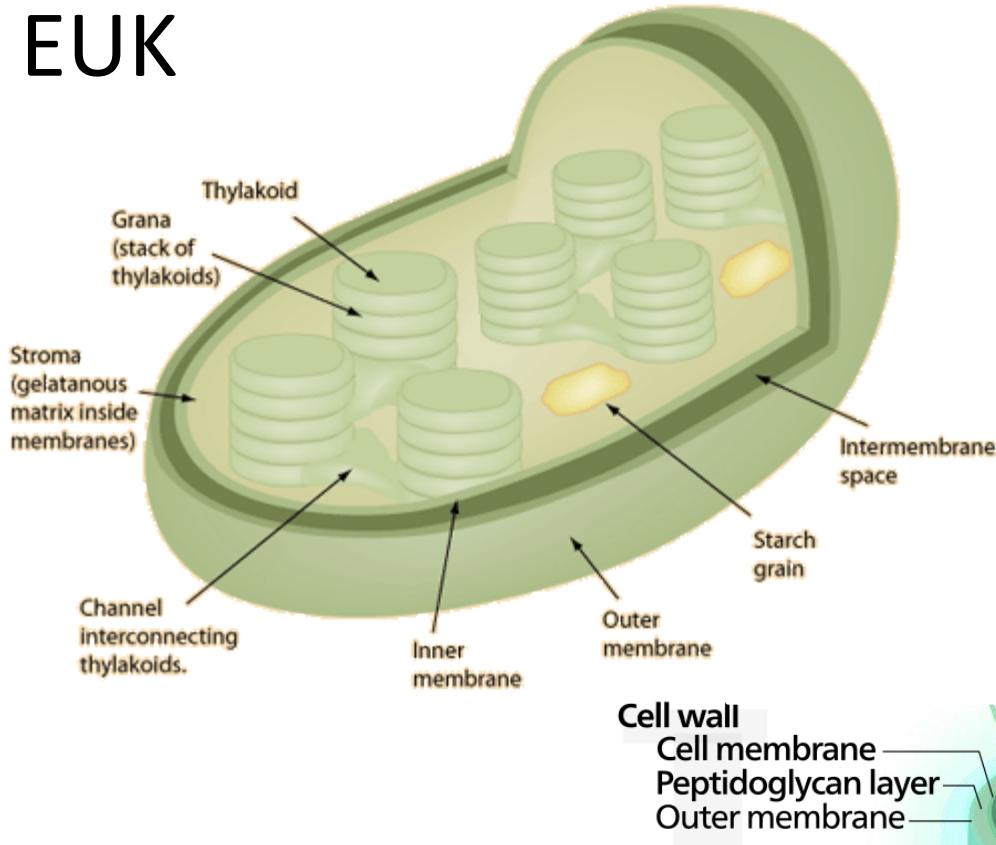
(Lin et al, 2016)



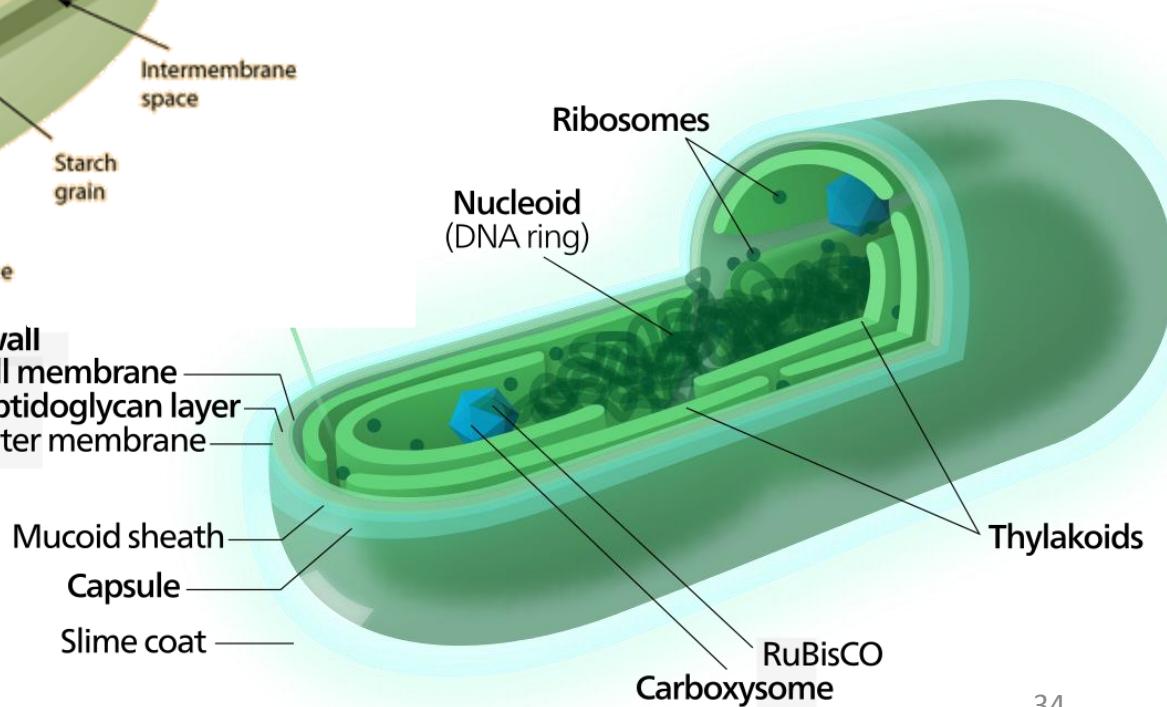
(Collin's lecture!!!)

CHLOROPHYLL (IN VIVO)

EUK

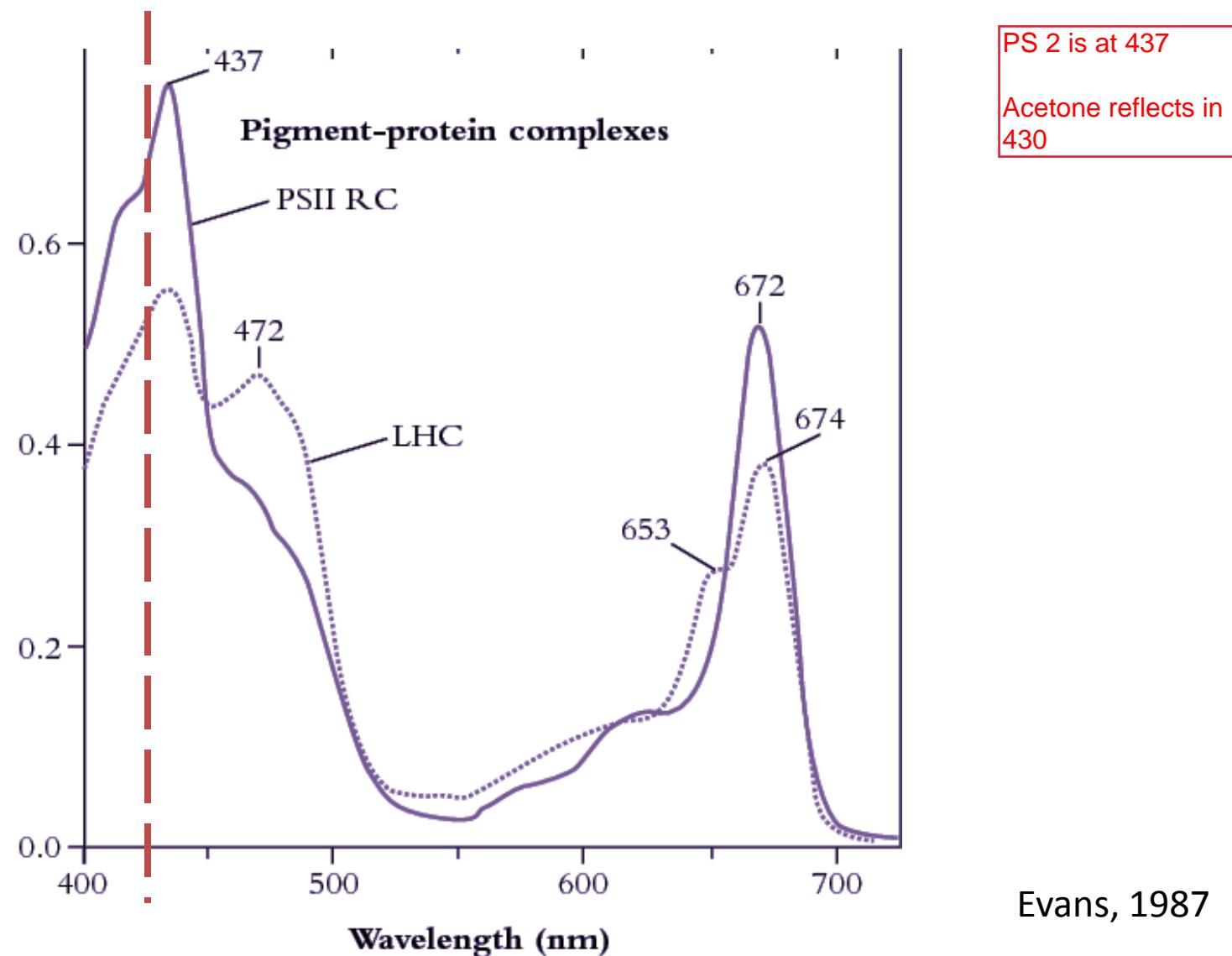


PRO



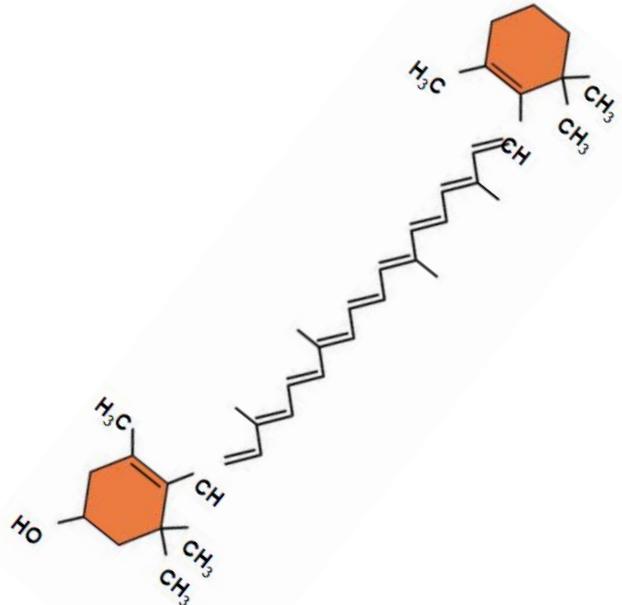
Chlorophyll absorption spectra (in vivo)

Acetone (430 nm)



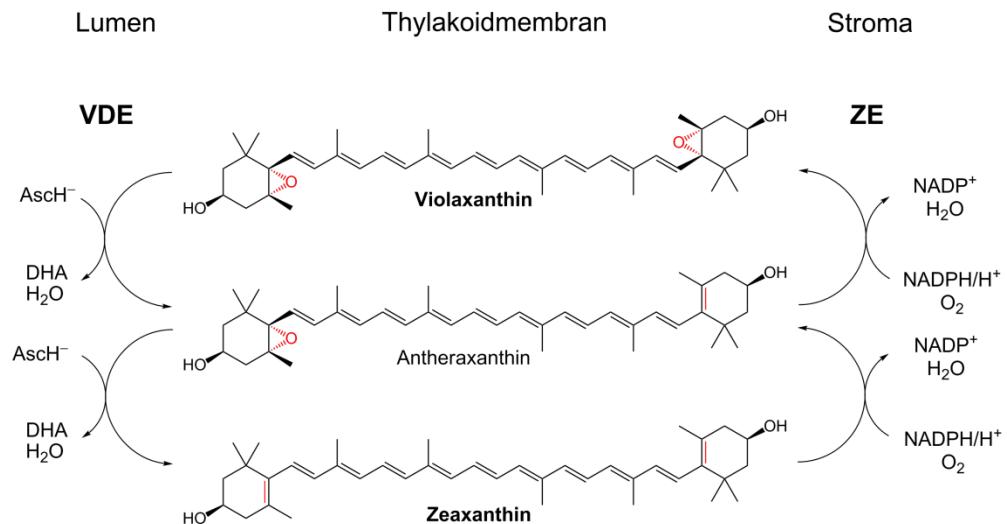
Evans, 1987

CAROTENOIDS



β – carotene (**Carotenes**)

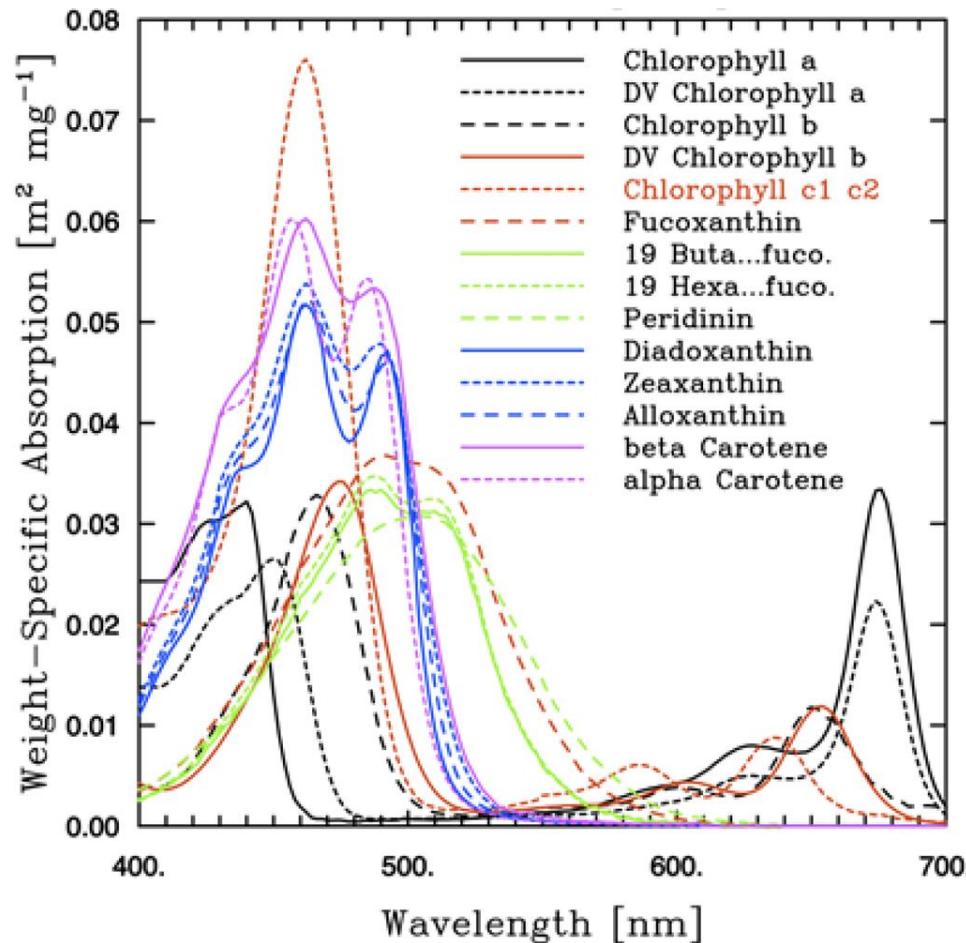
- Photosynthetic/photoprotective pigment
- Both in Photosystem I and II



Xanthophylls

- Photoprotective pigments
- Xanthophyll cycle – protective mechanism against photoinhibition...

Many other pigments



Devred et al, 2013

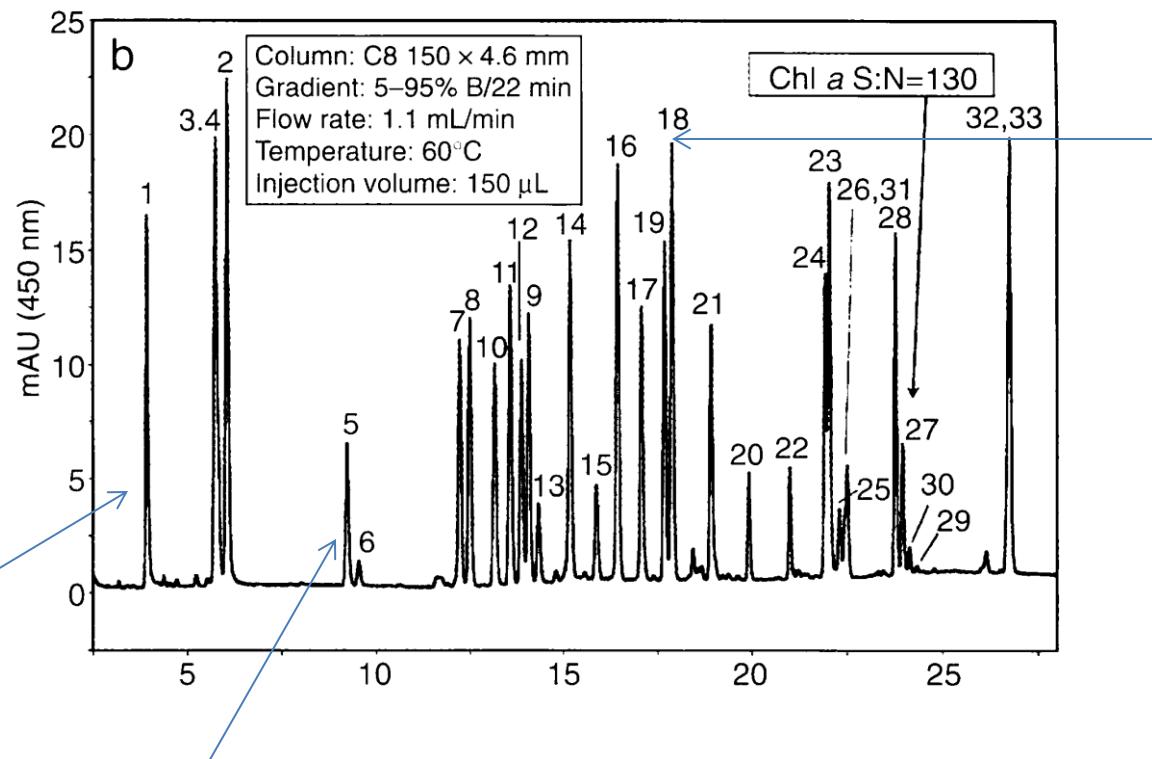
Pigments...

How we take the
pigments out of the
cell and analyze
them.

High Performance Liquid Chromatography (HPLC)

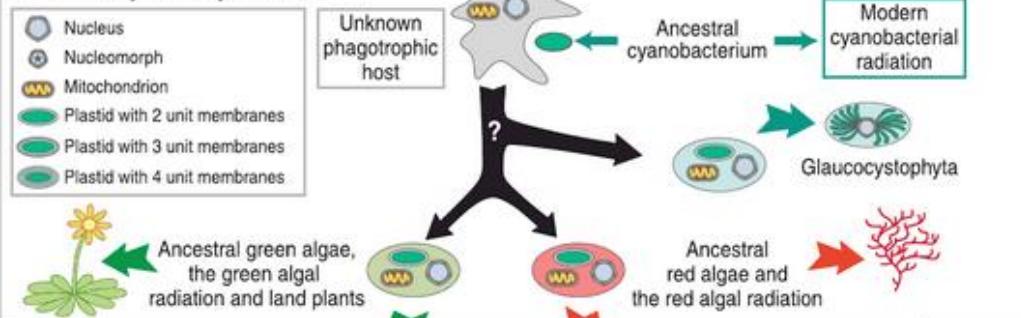
These are
absorption
heights

Each peak
is a
different
pigment



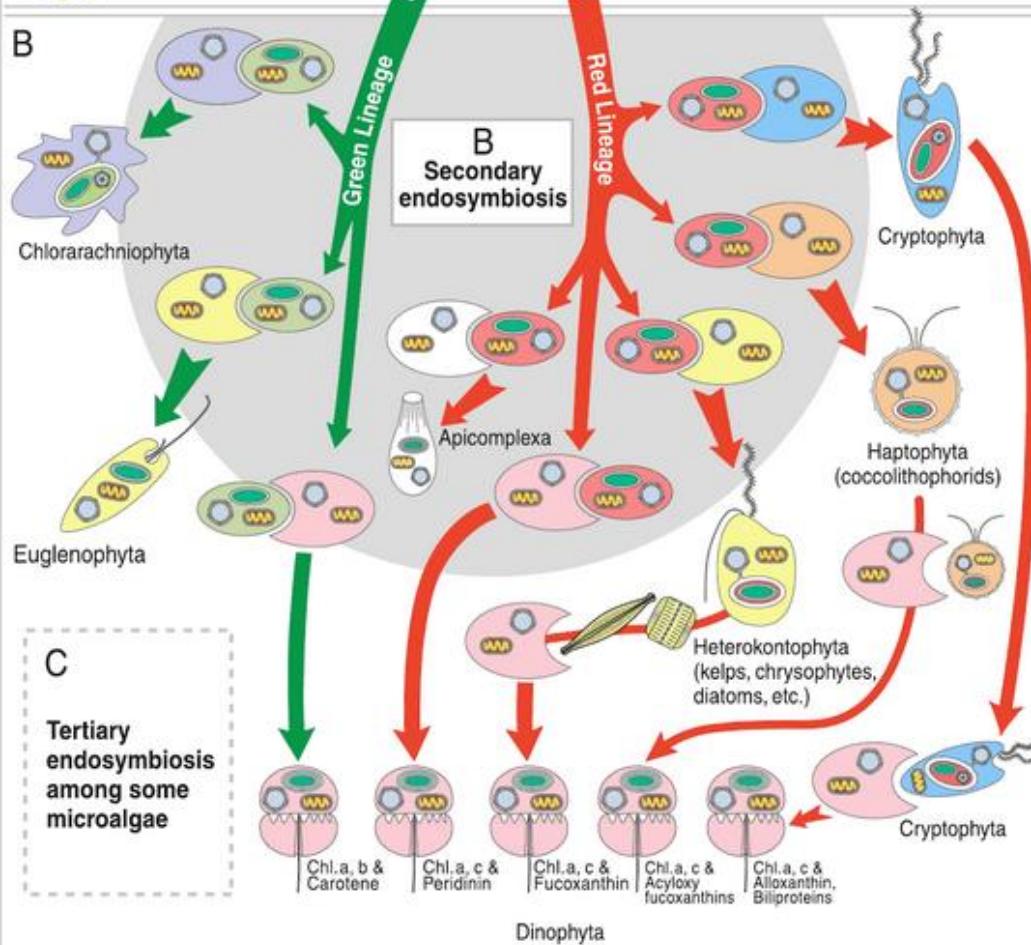
Bidigare, R. R., Van Heukelem, L., & Trees, C. C. (2005)

A Primary endosymbiosis



Different cells have different pigments, so we can use HPLC to identify different types

B



Origin of the species and the plastids in the cell will define their pigment structure..

PIGMENTS AS A TAXONOMICAL TOOL

- CHEMTAX – powerful tool if smart person uses it— careful of environmental condition and local flavors
- Other clustering methods

Vol. 144: 265–283, 1996 MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser Published December 5

CHEMTAX—a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton

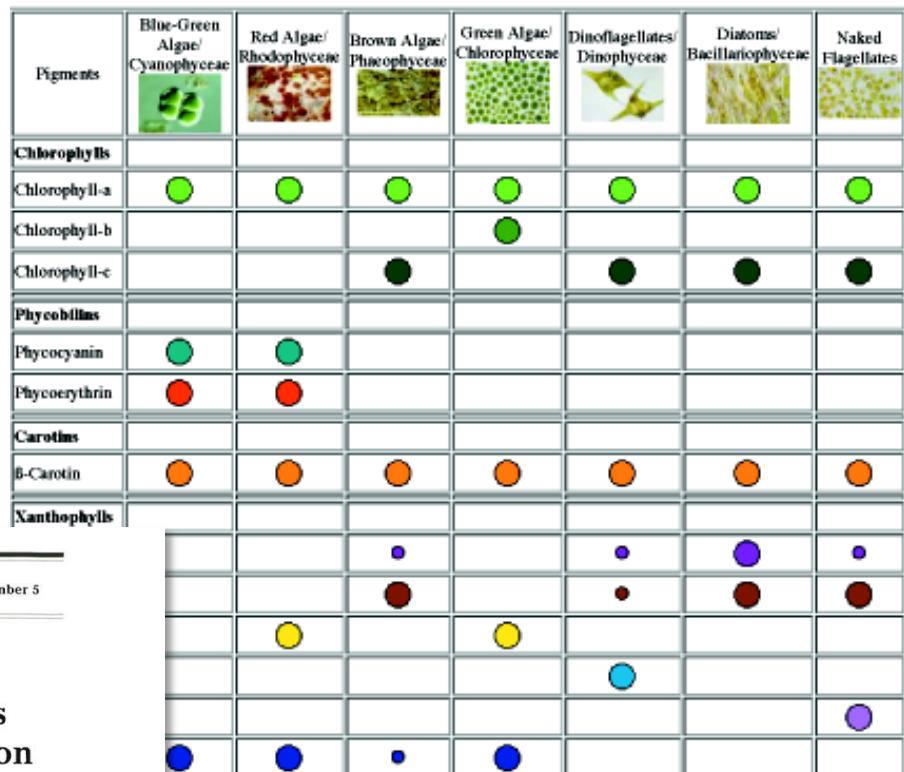
M. D. Mackey^{1,2}, D. J. Mackey^{2,*}, H. W. Higgins², S. W. Wright³

¹University Chemical Laboratory, Lensfield Rd, Cambridge CB2 1EW, United Kingdom

²CSIRO Division of Oceanography, PO Box 1538, Hobart, Tasmania 7001, Australia

³Australian Antarctic Division, Channel Highway, Kingston, Tasmania 7050, Australia

Pigment composition of the major algal groups



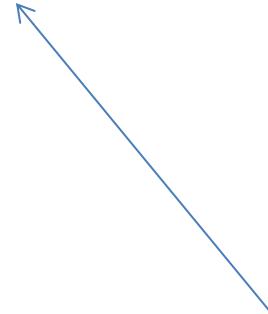
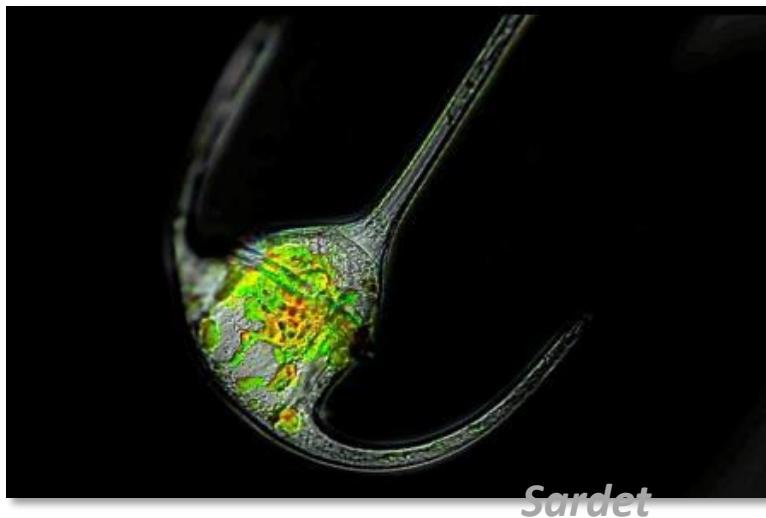
PHYTOPLANKTON

- Particle (size, shape, morphology)
- Taxa (Taxonomic approach)
- Chemistry (pigments, minerals, metabolites..)
- Function (role)

PHYTOPLANKTON FUNCTIONAL TYPES - physiological and ecological criteria

“...group of organisms (irrespective of taxonomic affiliation) that carry out a particular function, e.g. a chemical process such as calcification, silicification, nitrogen fixation, or dimethyl sulfide production; functional groups are also sometimes referred to as ‘biogeochemical guilds’.”

IOCCG Report 15, (2014)



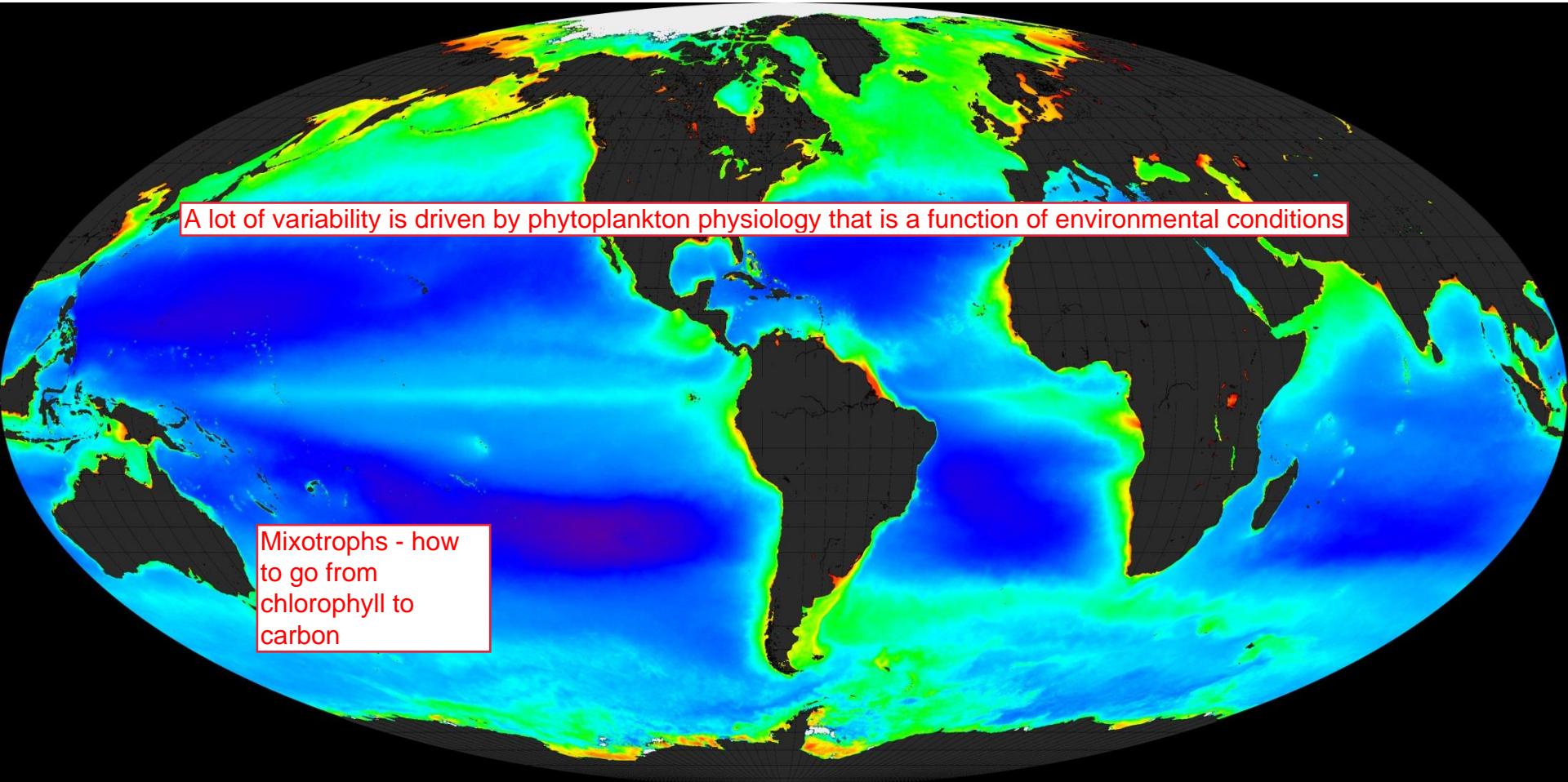
Another great read

PHYTOPLANKTON AS FUNCTIONAL TYPES

Functional type

- autotrophic, oxygenic, oxygen evolving
- size and shape
- transformer of specific nutrient (N₂ fixer, CaCO₃ precipitator, silica polymerizer, etc.); ballasting to enhance C flux; specialized nutrient-up take pathways, sequestering mechanisms; unique C:N:P:trace metal ratio
- nutritional value to higher trophic organisms, such as essential fatty acids, toxins or development disrupters, paleo markers
- ability to live in turbulent vs. stratified environment
- motility for enhancing nutrient acquisition, encounter gametes, avoiding predation
- what else ??

Is it straightforward? Lets look at chlorophyll... (20yrs!)



Variability... 2-3 orders of a magnitude

Postulates:

- From chlorophyll we can get cell abundances
- From chlorophyll we can get cell carbon
- From change in chlorophyll we can get growth rates

Plant in your kitchen problem

Postulates:

- From chlorophyll we can get cell abundances



- From chlorophyll we can get cell carbon
- From change in chlorophyll we can get growth rates

Packaging - basil will get darker when you move it from the light.

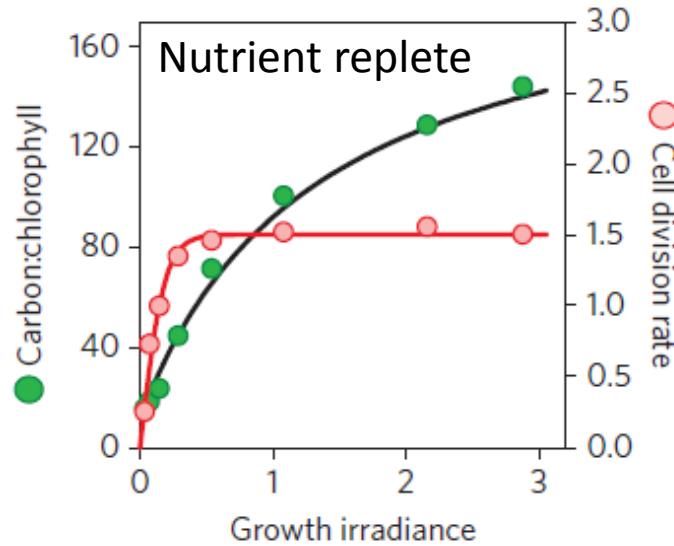


Areas with less light, you can have one algae with a chlorophyll to cell ratio and a totally different ratio in an area with more light

Plant in your kitchen problem

Postulates:

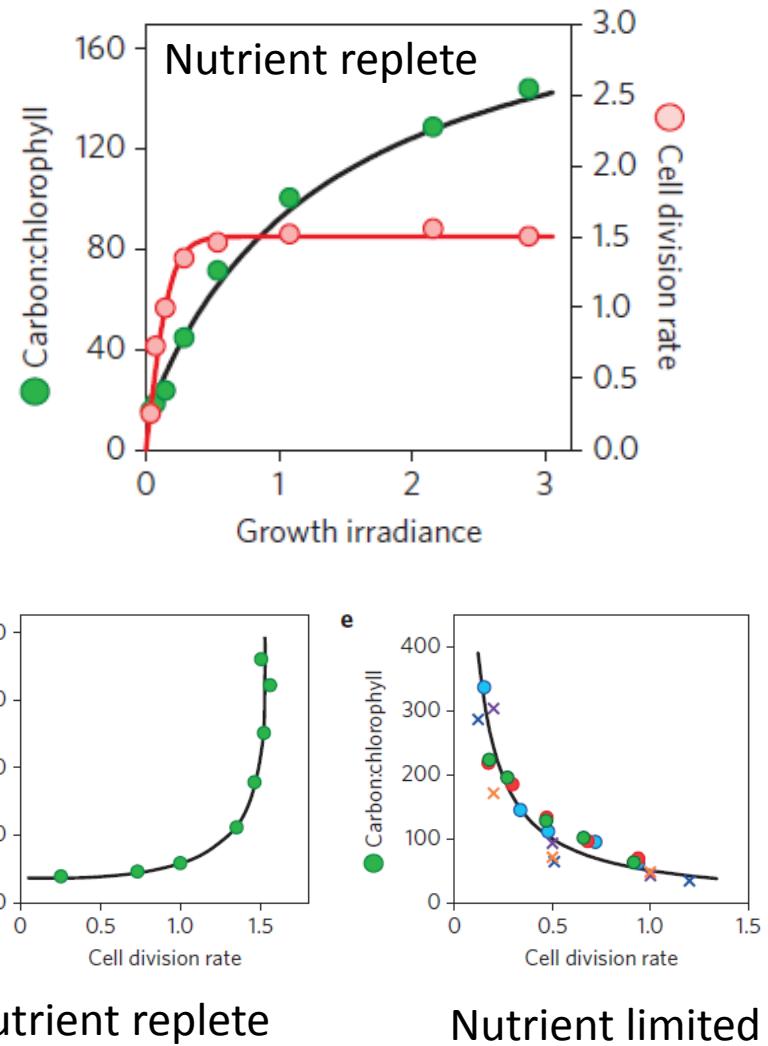
- From chlorophyll we can get cell abundances
- From chlorophyll we can get cell carbon
- From change in chlorophyll we can get growth rates



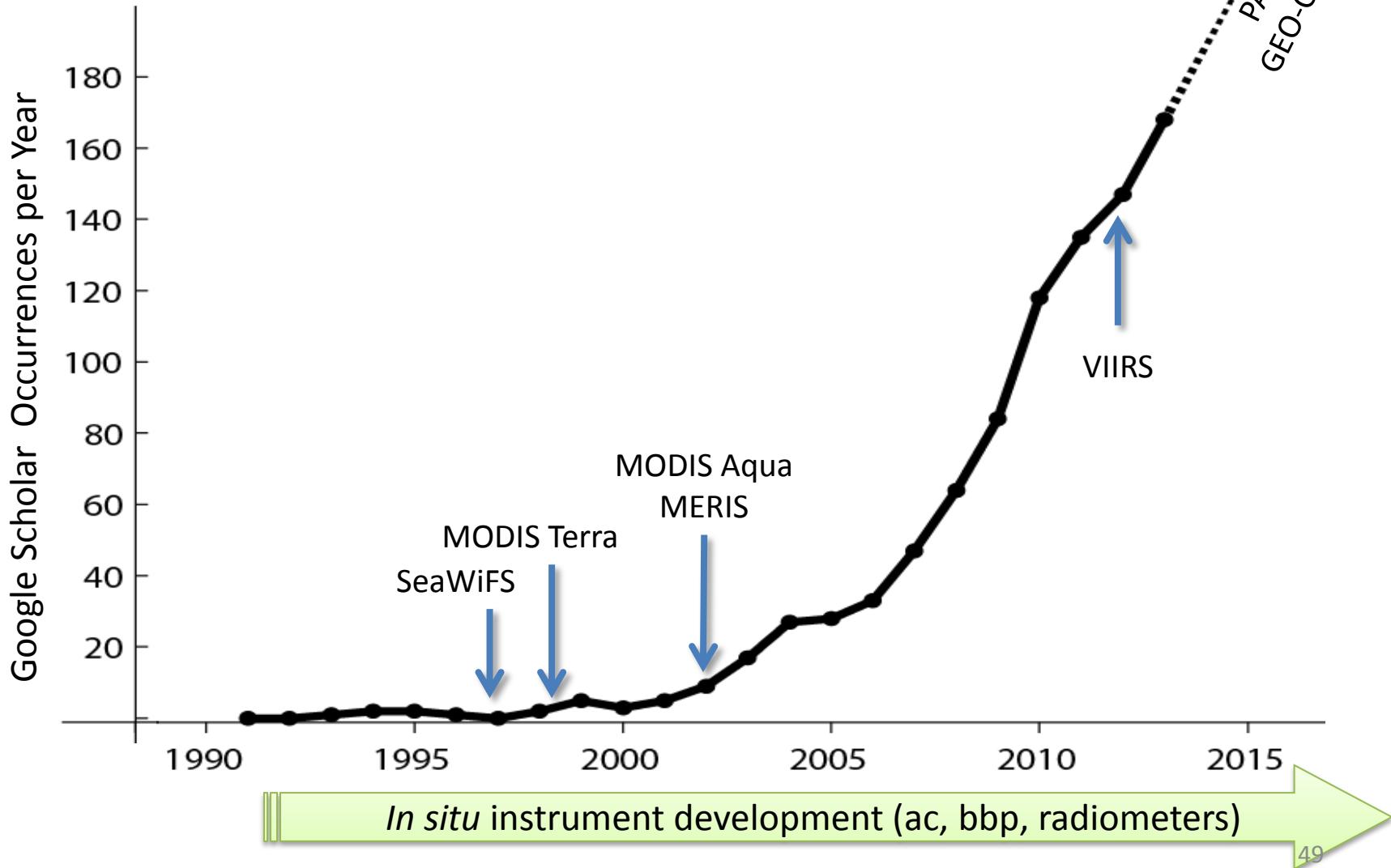
Plant in your kitchen problem

Postulates:

- From chlorophyll we can get cell abundances
- From chlorophyll we can get cell carbon
- From change in chlorophyll we can get growth rates



PHYTOPLANKTON FUNCTIONAL TYPES



HOW TO STUDY PHYTOPLANKTON FUNCTIONAL TYPES with OPTICS?

Focus on specific morphological and structural features that impact light

- Specific pigment structure leads to specific optical signal
- Specific size will lead to specific optical signal (then we talk about Particle Size Classes)
- Mind the ecology (and environment)

Focus on taxa specific ecological traits and trophic states

- E.g. Certain chlorophyll/ IOP concentration infers specific community composition

HOW (Feasible is) TO STUDY PHYTOPLANKTON FUNCTIONAL TYPES with OPTICS?

You will be able to answer that question in the end of this class

Remember!

- Know your friends and your enemies
- Be realistic
- Validate