What are phytoplankton?! An introduction to taxonomy and in situ measurements

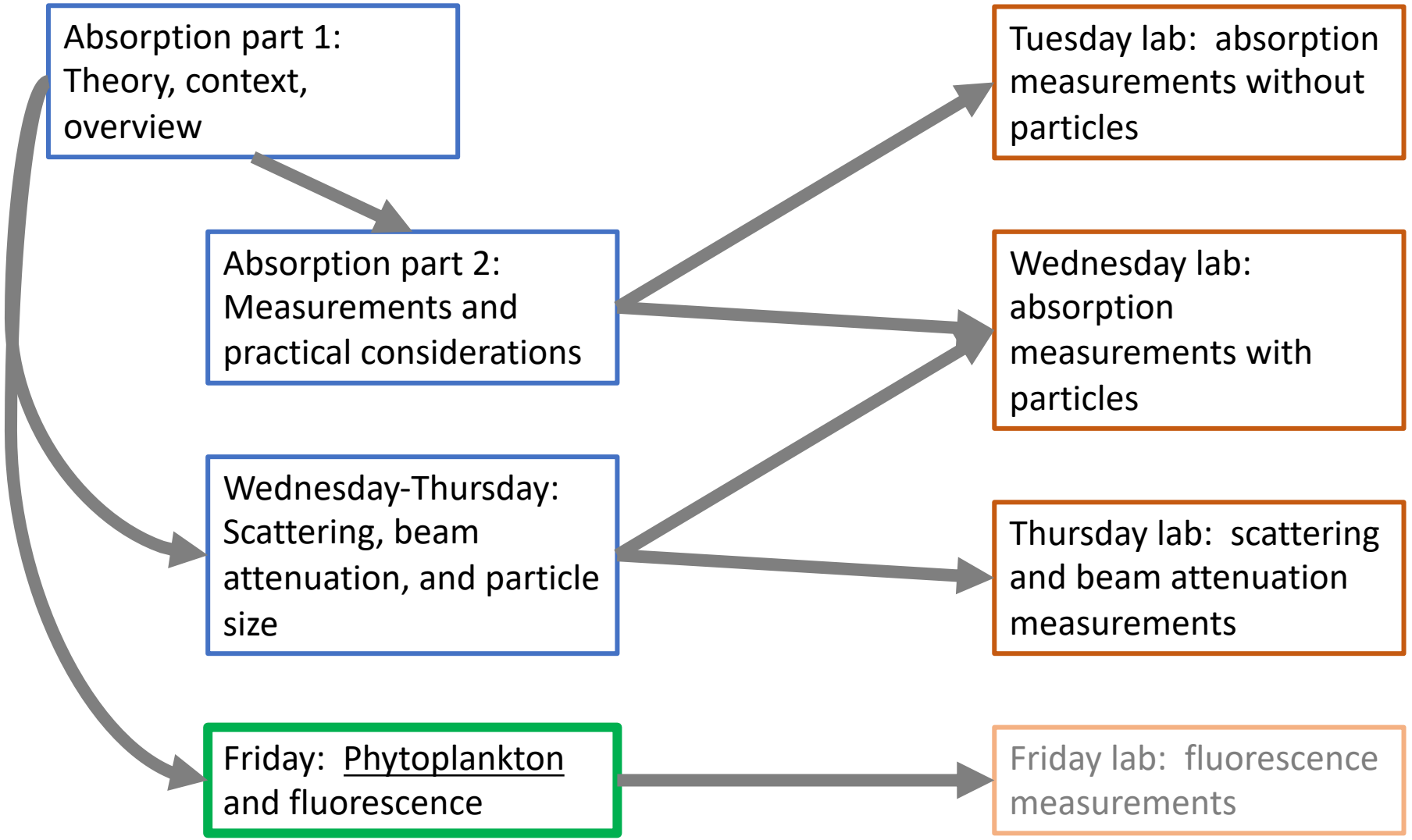
Sasha J. Kramer (MBARI) · skramer@mbari.org

(with inspiration from Ivona Cetinić, Collin Roesler, Ali Chase, Jeremy Werdell, Dylan Catlett, and Colleen Durkin)

NASA Ocean Optics course

Summer 2023

Class context: Week 1 roadmap



Can you define “phytoplankton”?

At the most basic:

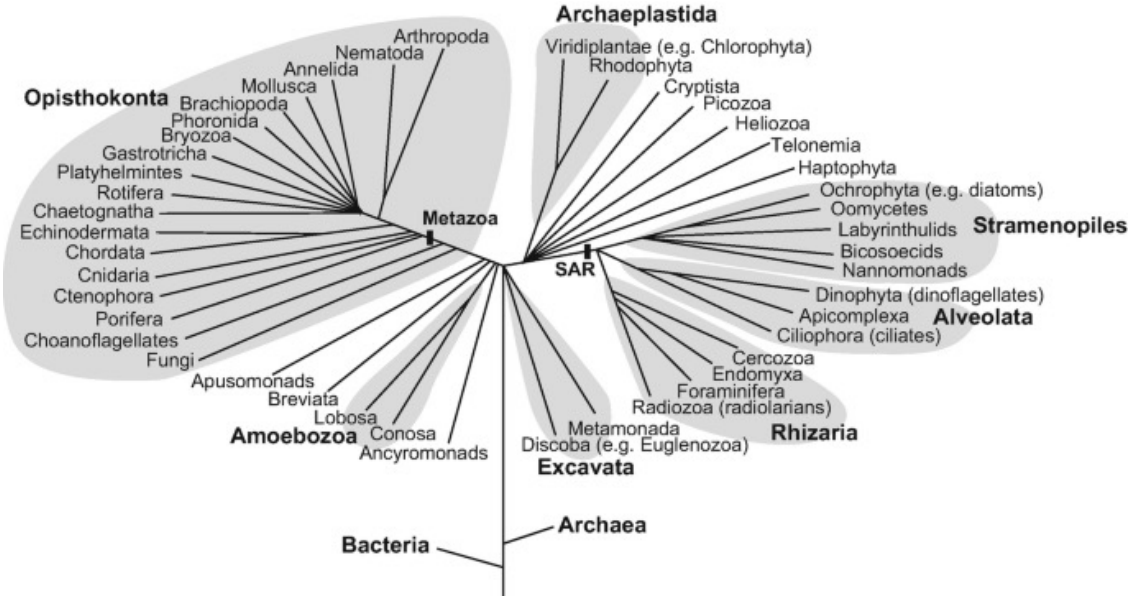
From the Greek “*phyto*” (light) and “*planktos*” (to wander or drift).

An intro oceanography course might tell you that phytoplankton are microscopic photosynthesizers that are found in the sunlit global ocean…

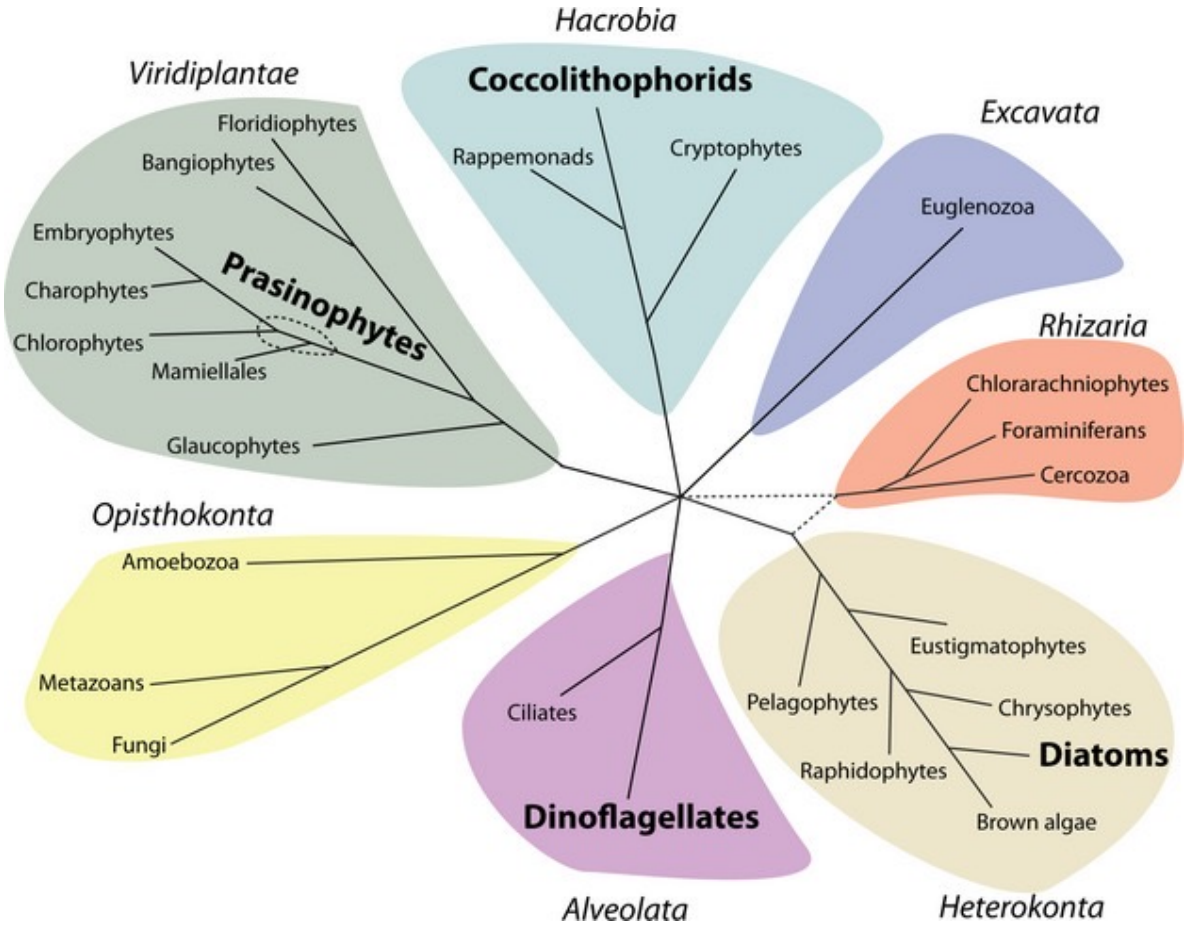
BUT the more you know, the trickier it becomes to give one simple definition for “phytoplankton”!

Phytoplankton have high phylogenetic diversity

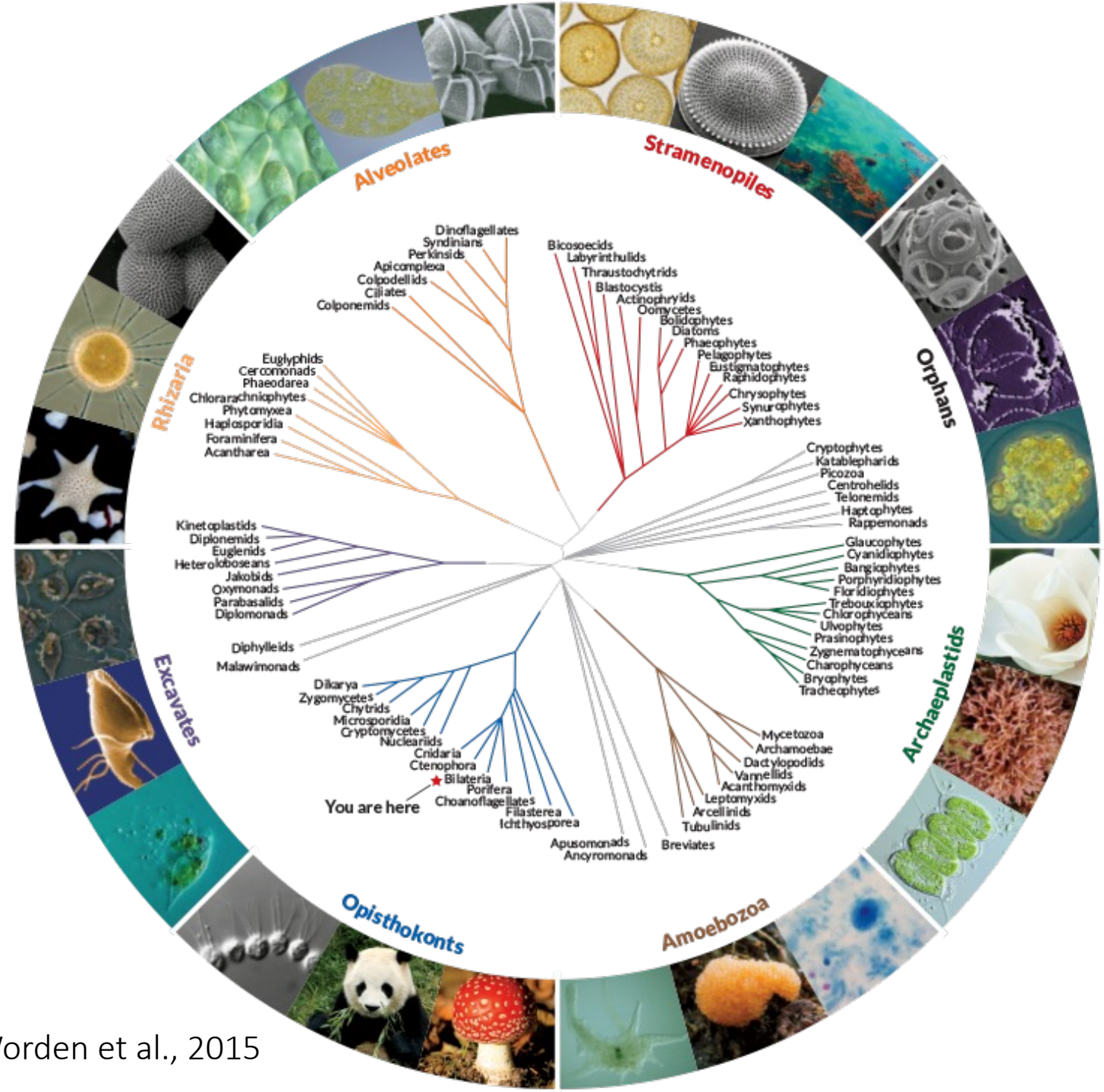
Phytoplankton comprise multiple branches of the tree of life (mainly bacteria and eukarya).



Inaba et al., 2020



Collins et al., 2014

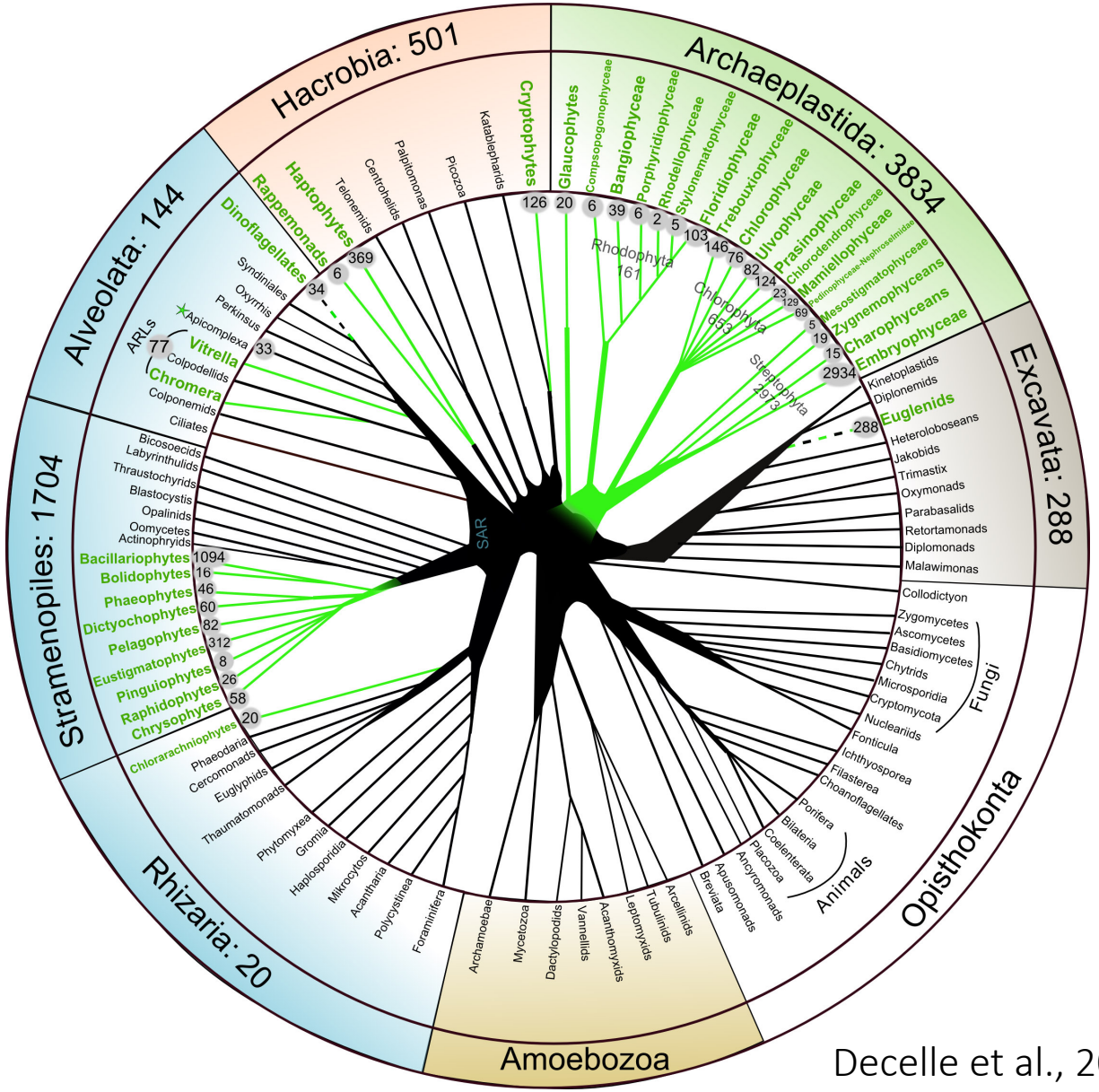


Worden et al., 2015

Phytoplankton have very high taxonomic diversity

The organisms we describe as “phytoplankton” include thousands of species, and even distinct strains within the same species!

>25,000 species have been identified - but this number is only going up with advances in sequencing technology.



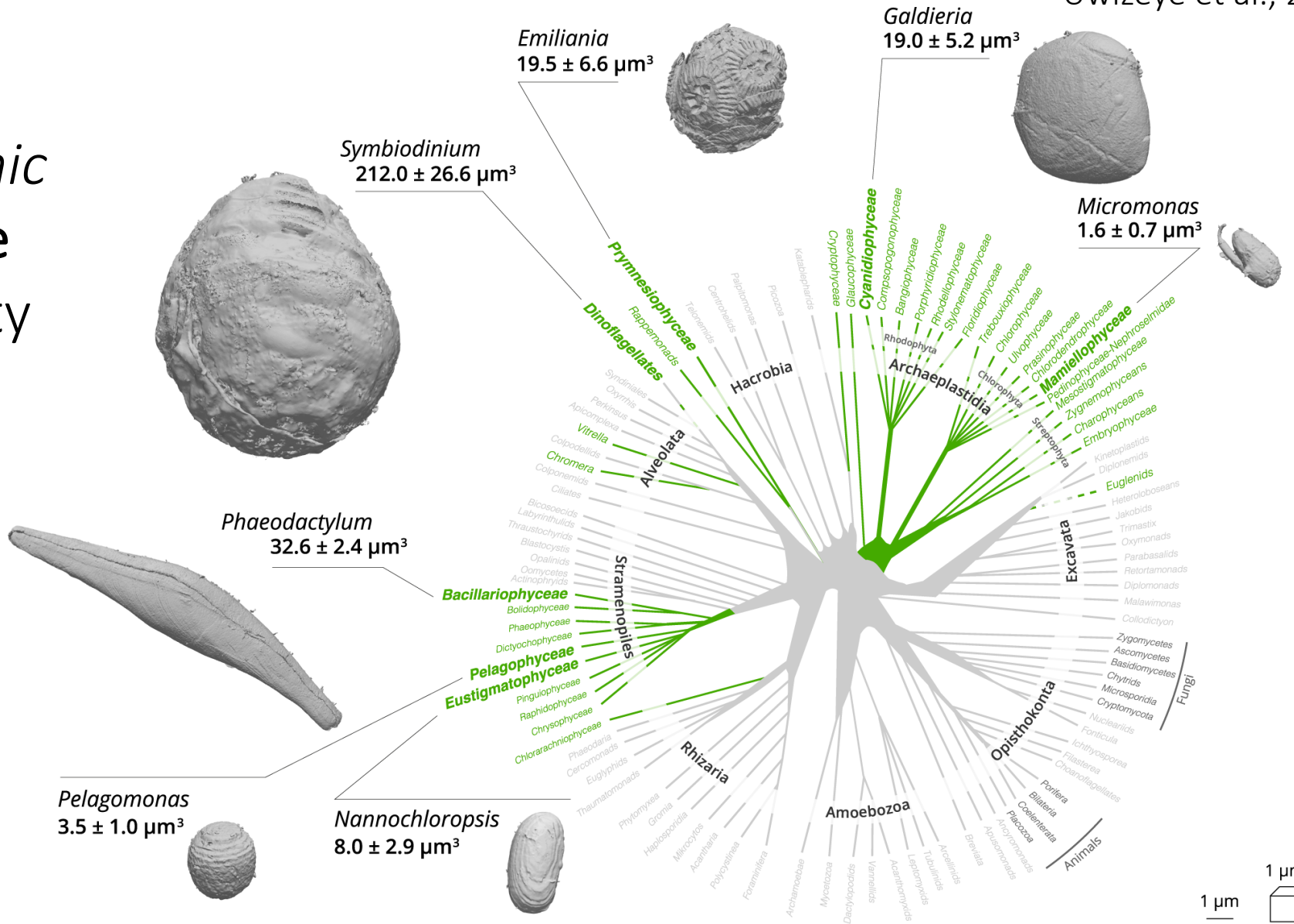
Decelle et al., 2015

Phytoplankton come in many shapes & sizes

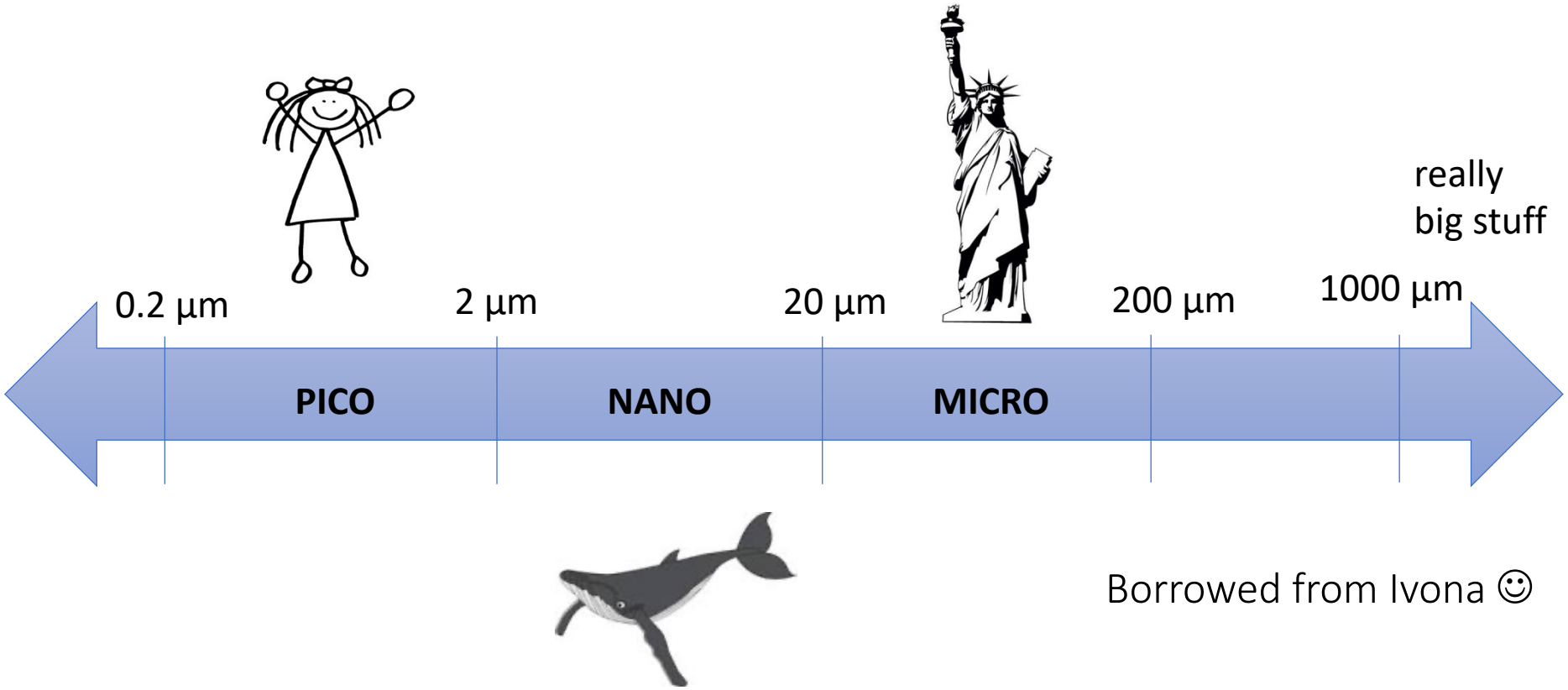
Uwizeye et al., 2021

Within that *phylogenetic/taxonomic* diversity, there is **huge** *morphological* diversity

Large variability in the shapes and **sizes** of phytoplankton cells



Phytoplankton come in many shapes & sizes

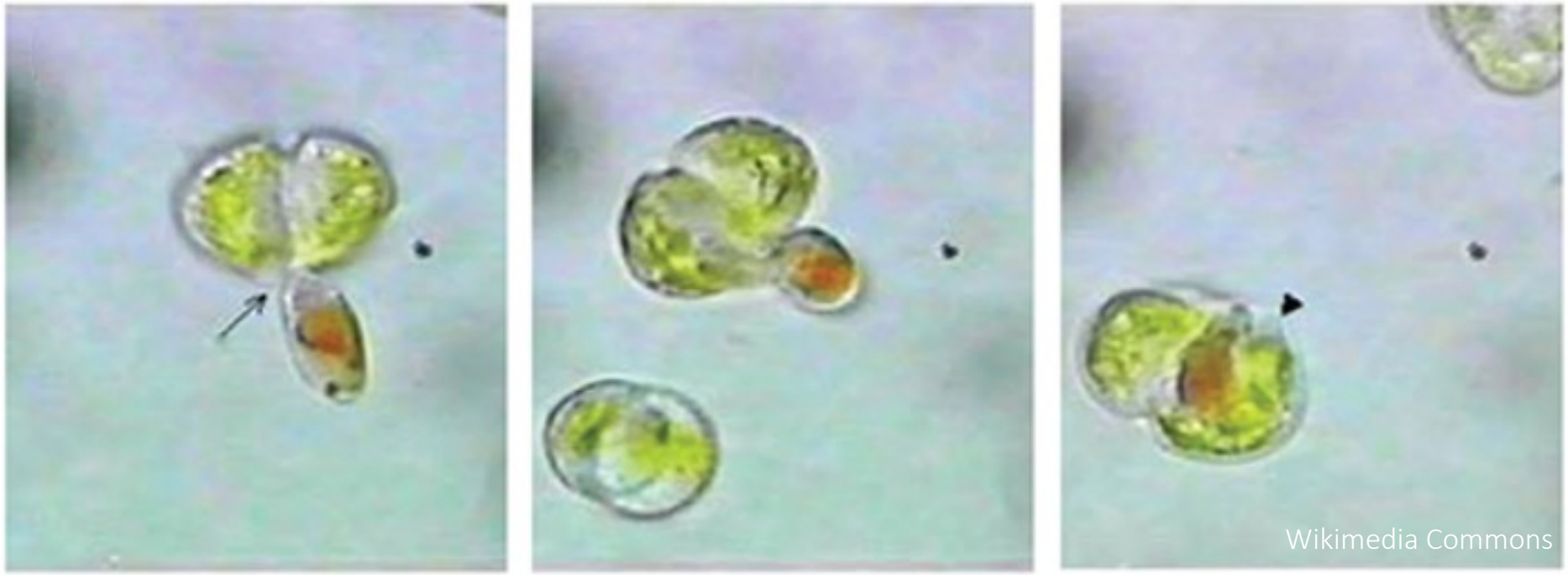


Borrowed from Ivona ☺

Phytoplankton nutritional strategies vary widely

Even the descriptor of phytoplankton as “photosynthetic” can be oversimplified – organisms that we call “phytoplankton” use varied feeding strategies (phototrophy, heterotrophy, mixotrophy, parasitism).

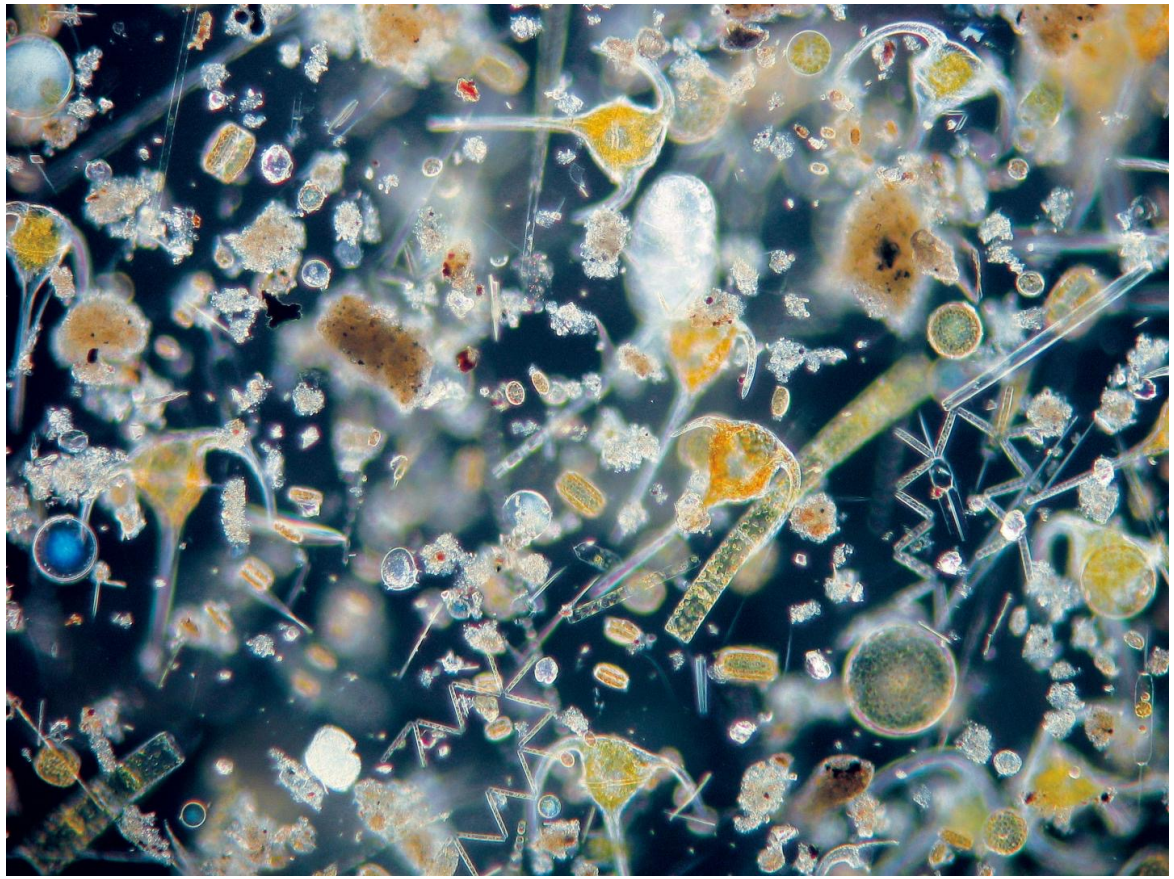
Example of a mixotrophic *Karlodinium* sp. (a dinoflagellate) that contains chloroplasts consuming another cell



What are some of the ways that we define phytoplankton community composition?

Taxonomy
Particle size (and shape)
Chemistry (pigments or minerals)
Function (role in the environment)

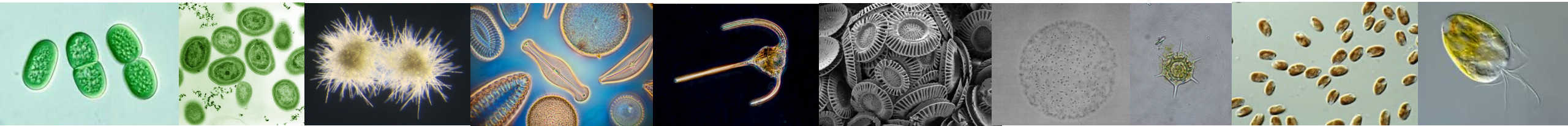
Some of these traits are also targeted with remote sensing or important for inclusion in Earth system models



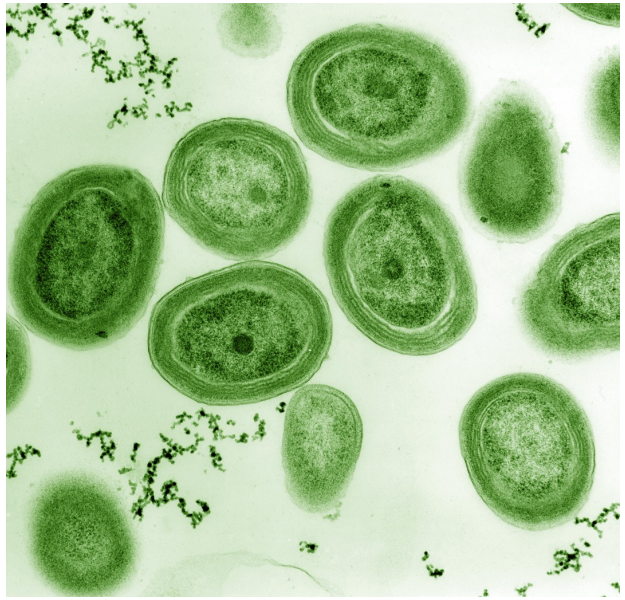
A brief overview of some major important phytoplankton groups:

Synechococcus spp.

- Cyanobacteria
- Very small (0.8-1.5 µm diameter)
- Super abundant in the global ocean
- Contain phycoerythrin (pigment that fluoresceces orange)

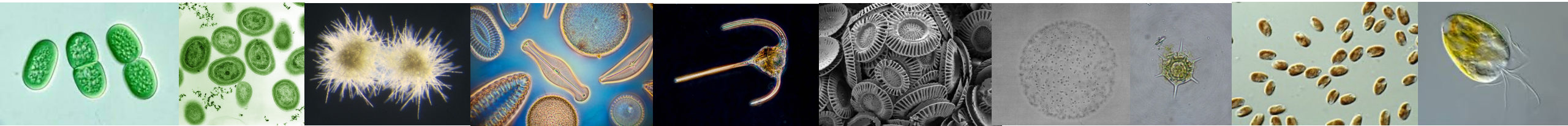


A brief overview of some major important phytoplankton groups:

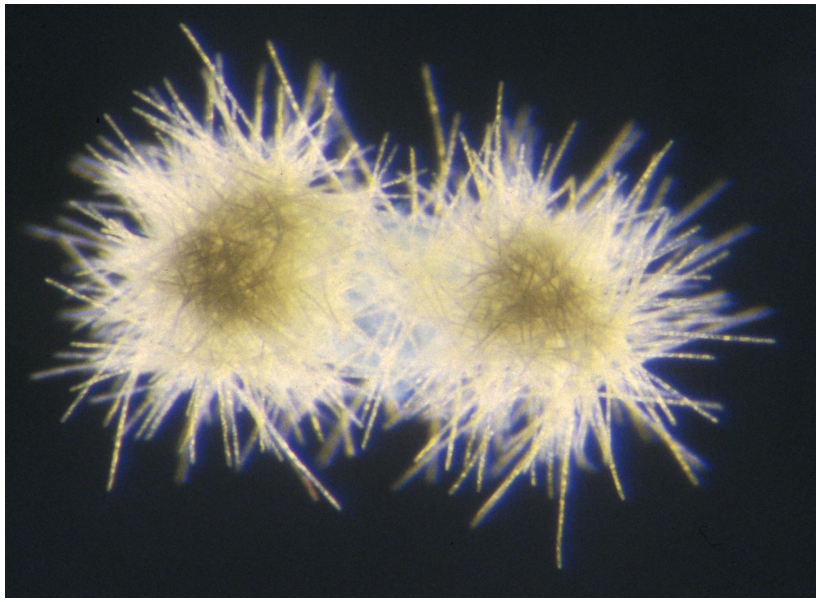


Prochlorococcus spp.

- Cyanobacteria
- Smallest known phytoplankton (~0.7 µm diameter)
- Super abundant in the global ocean
- Uniquely contain divinyl chl-a and divinyl chl b

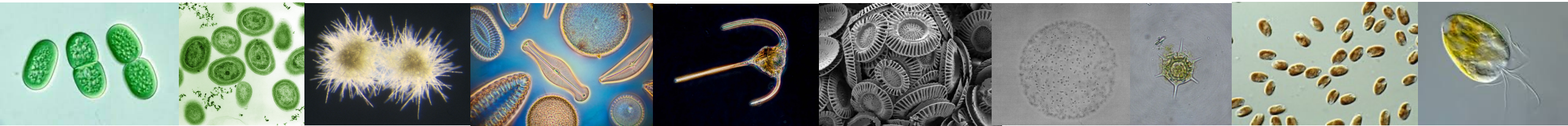


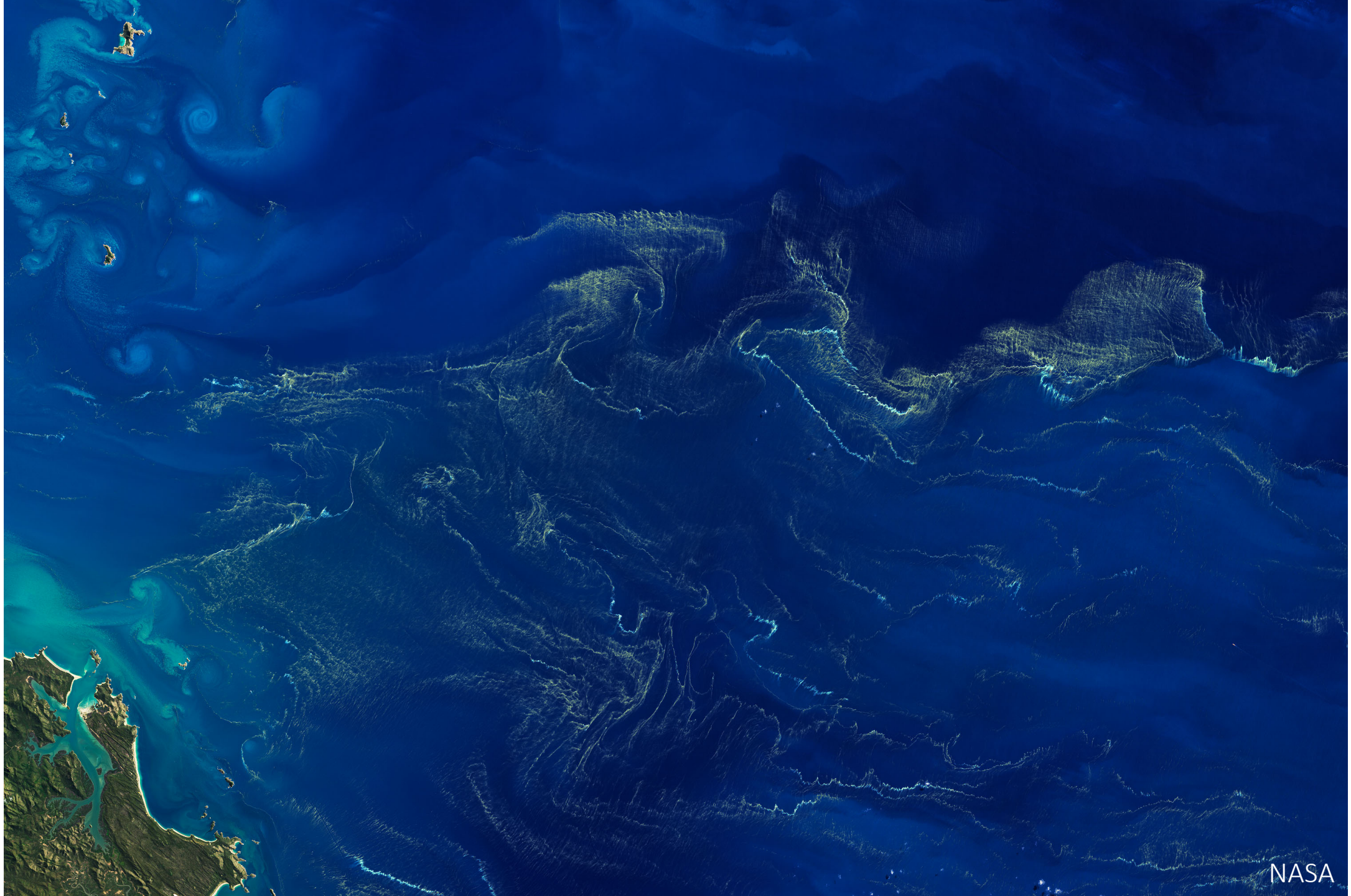
A brief overview of some major important phytoplankton groups:



Trichodesmium spp.

- Cyanobacteria
- Small phytoplankton that form puffs/tufts (can be visible from space!)
- Nitrogen fixers
- Like *Synechococcus*, contain phycoerythrin

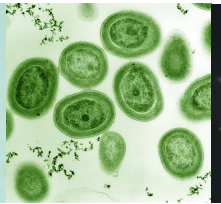
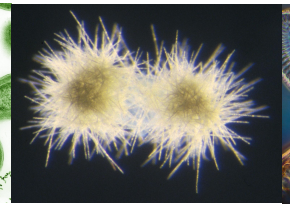



NASA

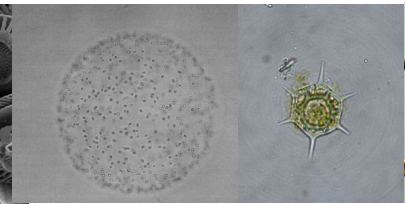
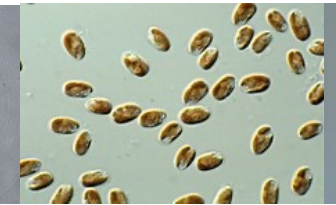
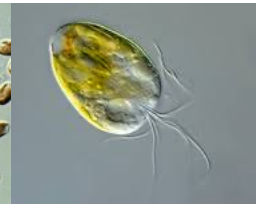
A brief overview of some major important phytoplankton groups:

Diatoms

- Eukaryotes (red algae)
- Huge range of sizes for individual cells (~3->200 µm diameter) + can form chains
- Cell walls are made of silicon
- Efficient carbon exporters

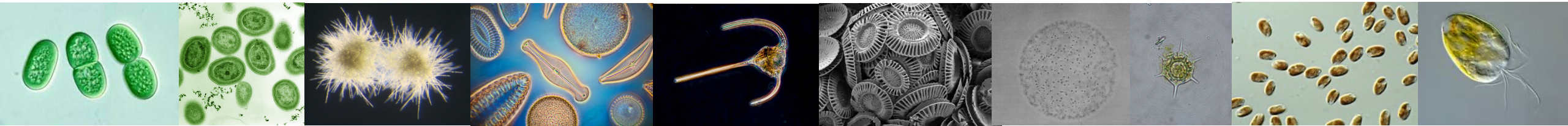


A brief overview of some major important phytoplankton groups:

Dinoflagellates

- Eukaryotes (red algae)
- Huge range of sizes (~5->200 µm diameter)
- Commonly mixotrophic or heterotrophic
- Can form harmful blooms (most “red tide” species are dinoflagellates)

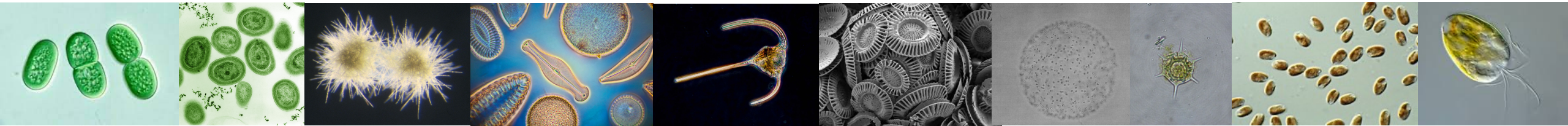


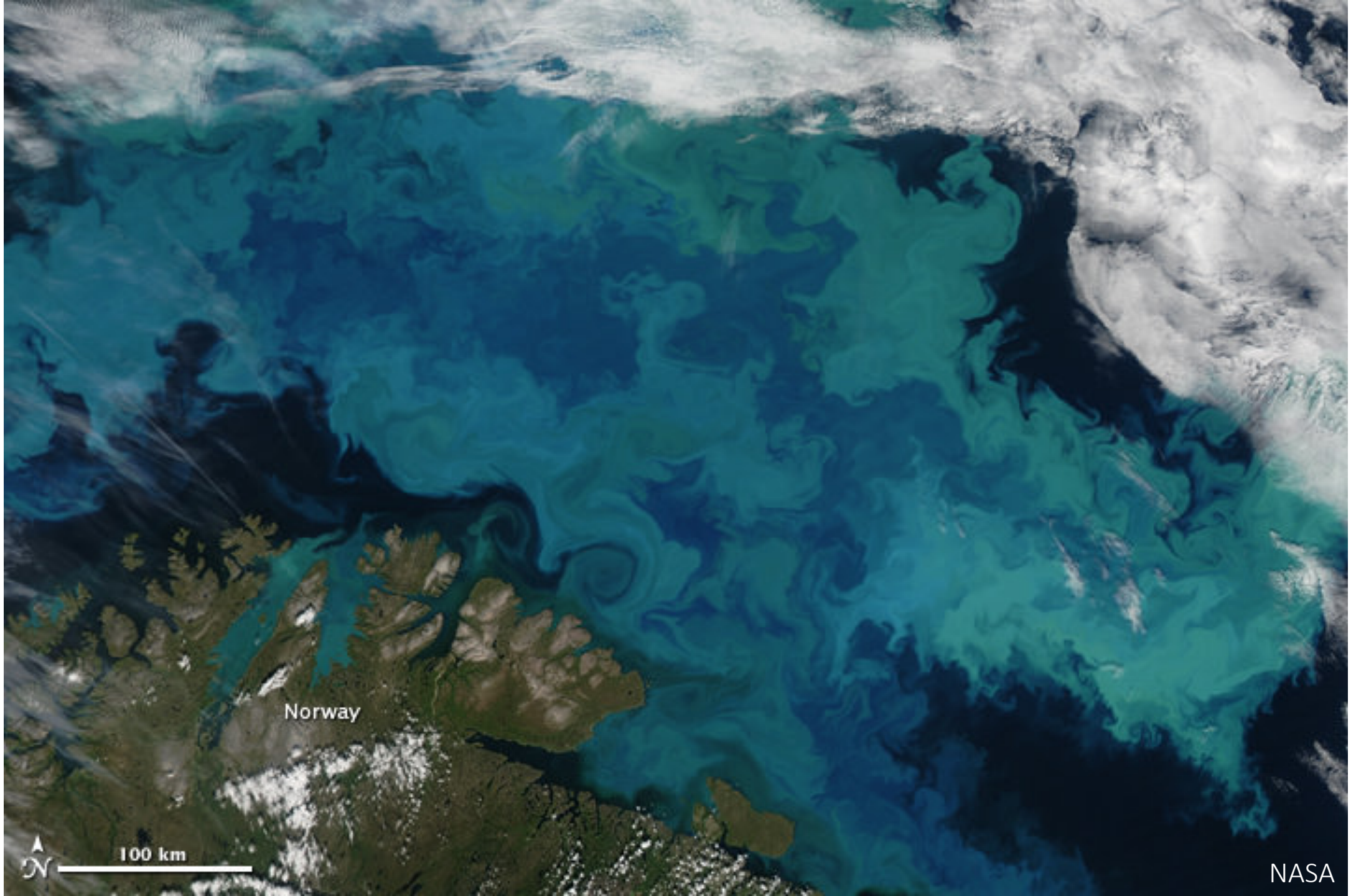
National Geographic

A brief overview of some major important phytoplankton groups:

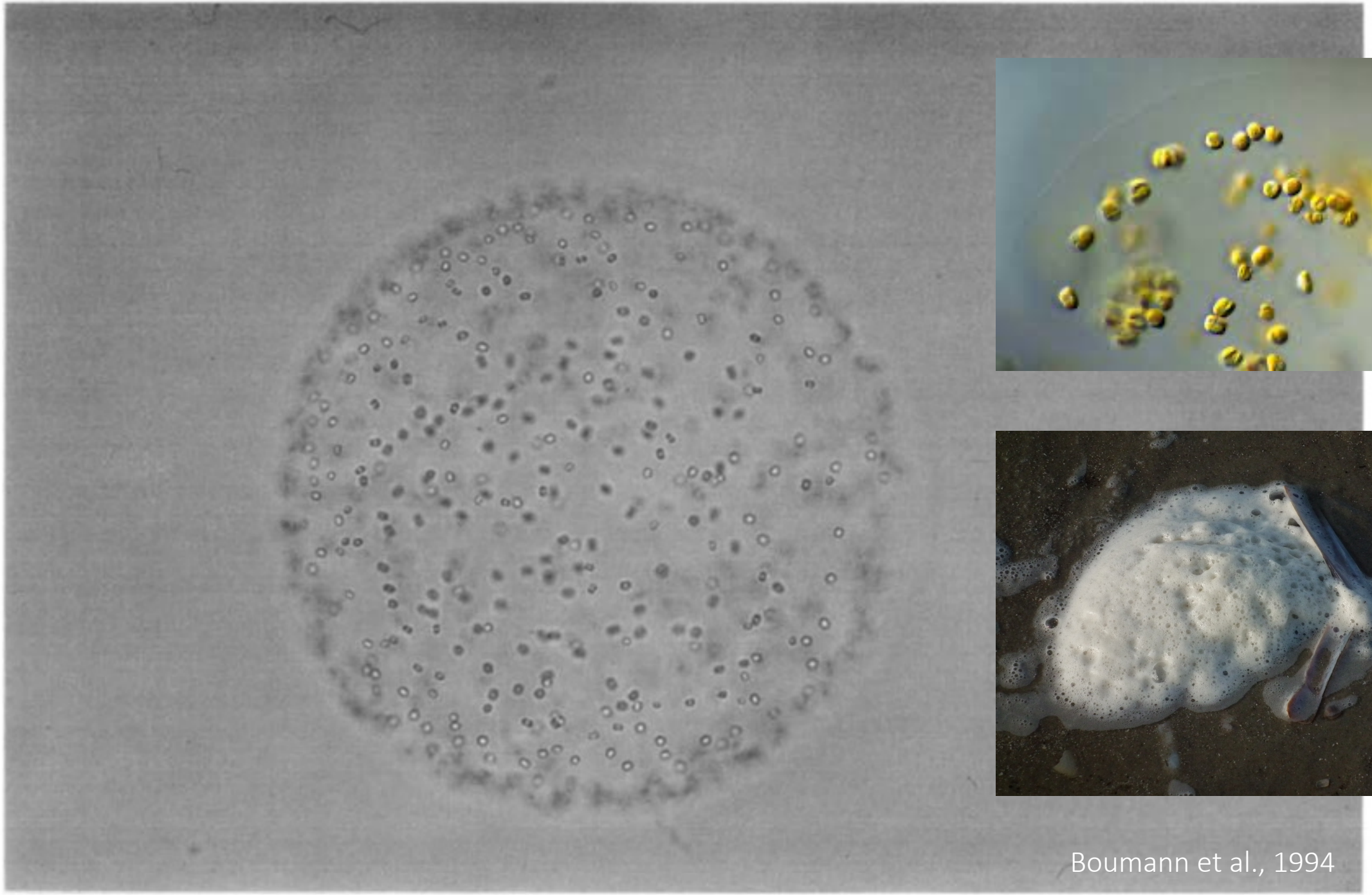
Prymnesiophytes

- Eukaryotes (red algae)
- Large size range (~2-50 µm diameter)
- Includes coccolithophores (calcified, high scattering) and *Phaeocystis* sp. (DMSP producers, can form clumps or foam)



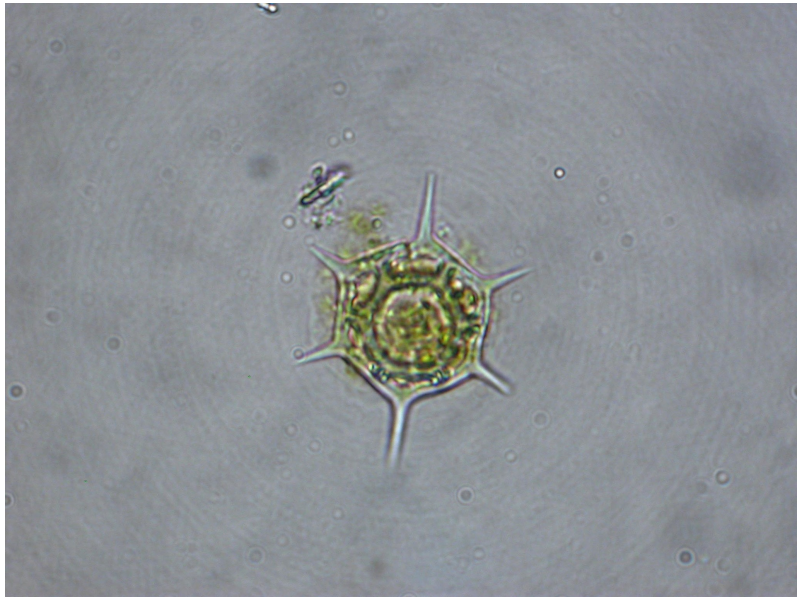


NASA



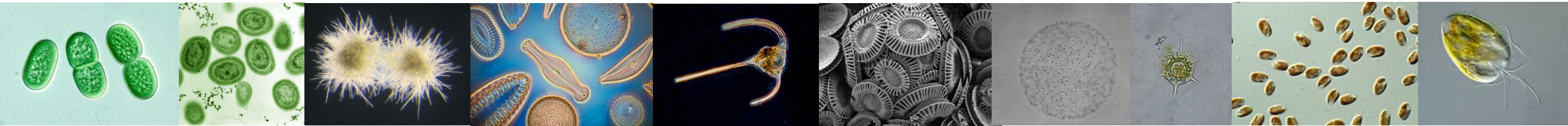
Boumann et al., 1994

A brief overview of some major important phytoplankton groups:

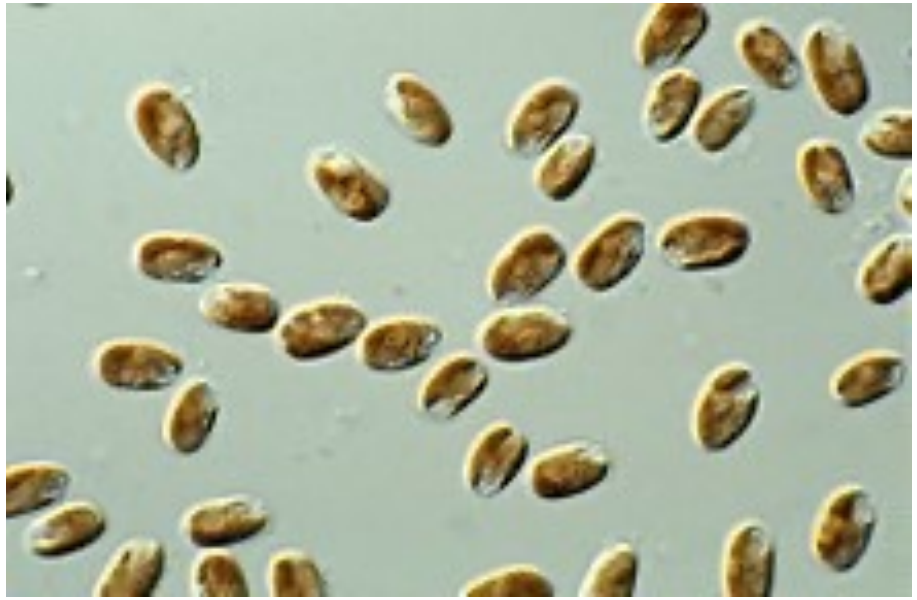


Dictyochophytes

- Eukaryotes (red algae)
- Mostly observed in the 10-20+ µm range
- Silicifying phytoplankton

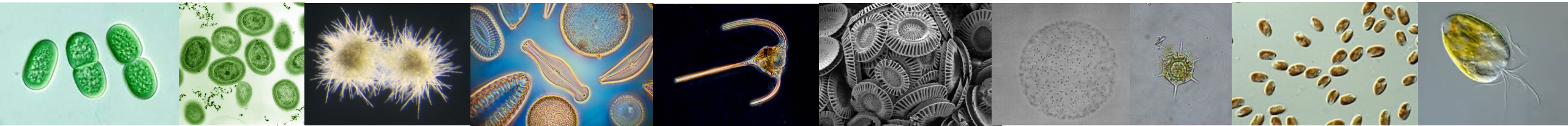


A brief overview of some major important phytoplankton groups:



Cryptophytes

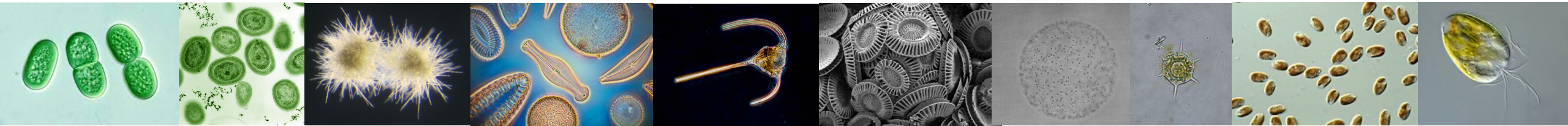
- Eukaryotes (red algae)
- In a broad taxonomic class with prymnesiophytes (Hacrobia)
- Mostly observed in the 3-20 µm range
- Uniquely contain the pigment alloxanthin

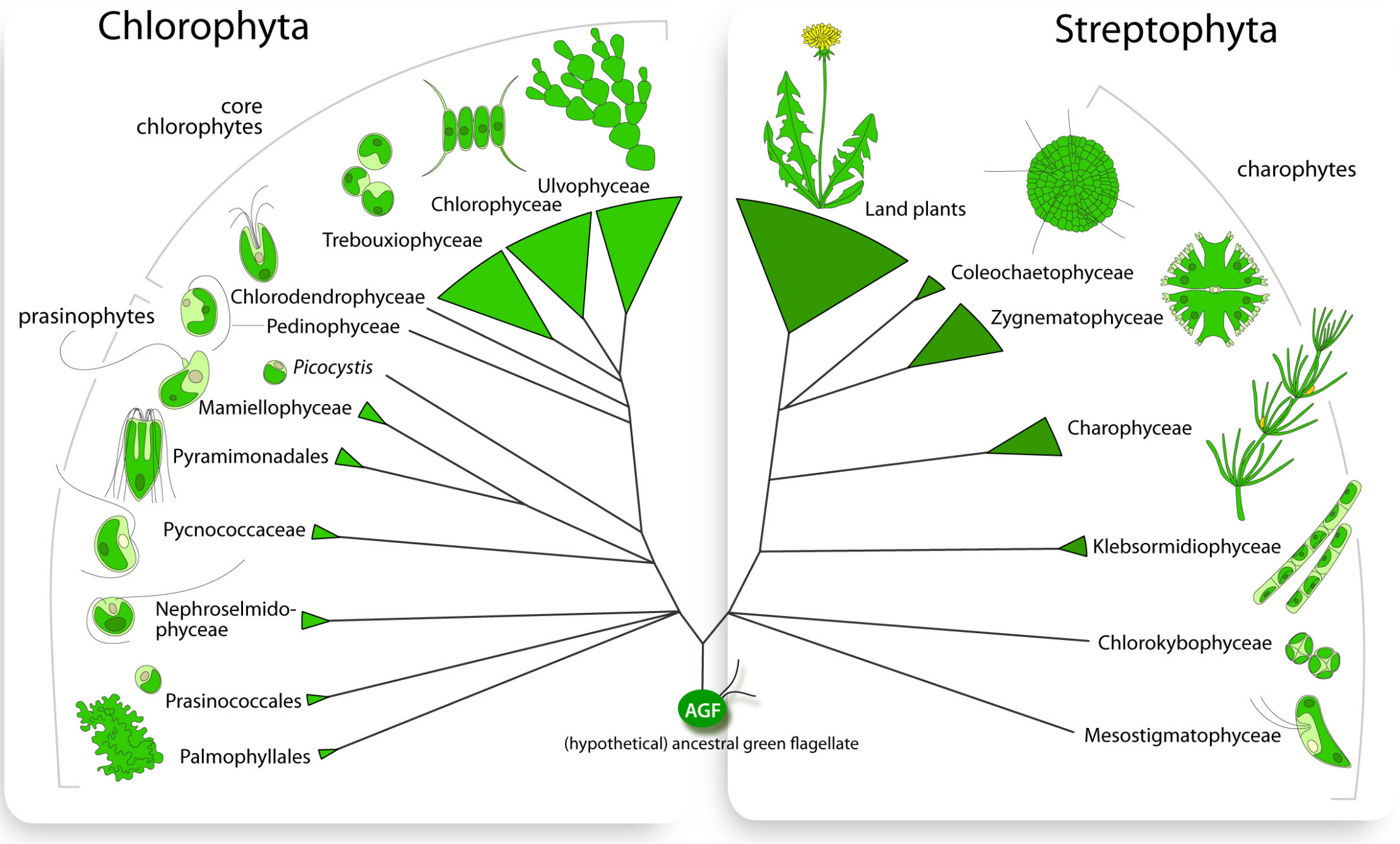


A brief overview of some major important phytoplankton groups:

Chlorophytes

- Eukaryotes (green algae)
- HUGE taxonomic and morphologic diversity
- Mostly observed in the 2-20 µm range
- Contain chlorophyll b

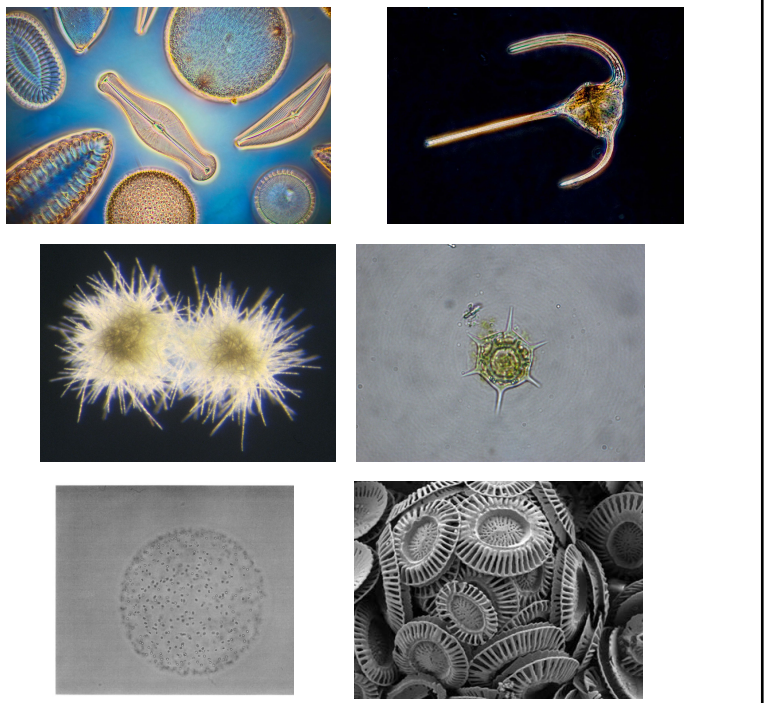




Leliaert et al., 2012

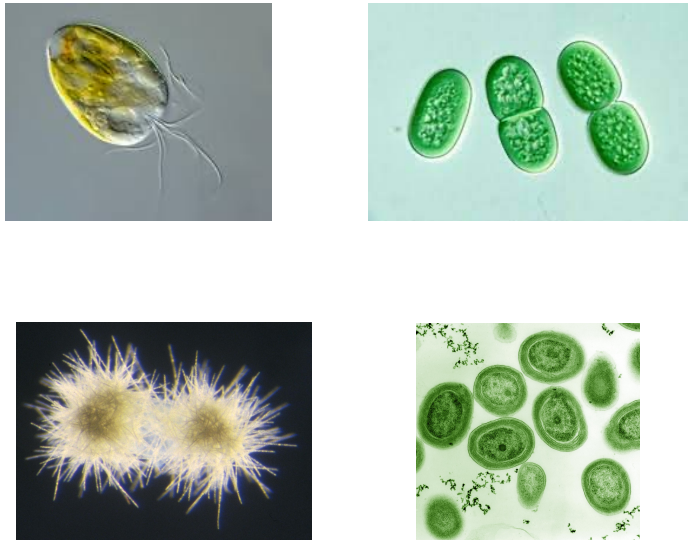
Let’s try this with our pre-defined phytoplankton groups…by size

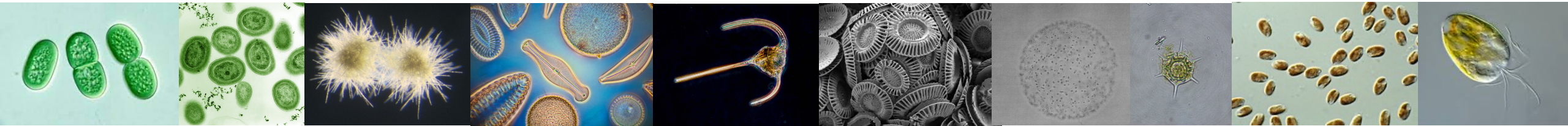
Microplankton (>20 µm)



Nanoplankton (2-20 µm)

Picoplankton (<2 µm)

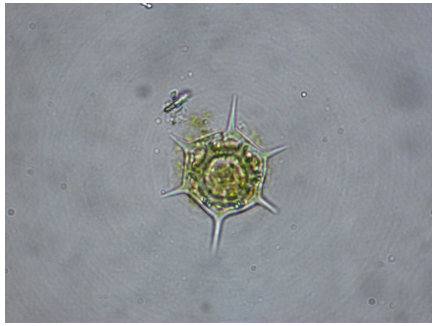




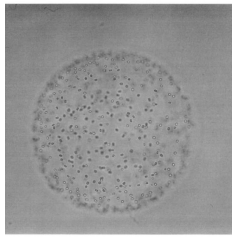
Let’s try this with our pre-defined phytoplankton groups…by function

Silicifying phytoplankton

Also Chrysophytes (relatively rare in the ocean)

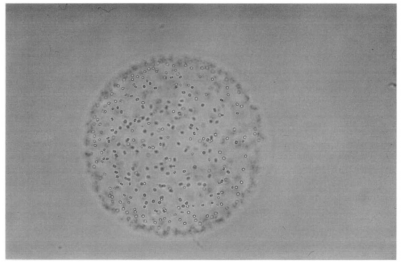


Can form harmful blooms



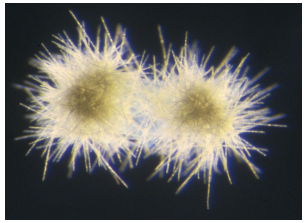
+ other Cyanobacteria

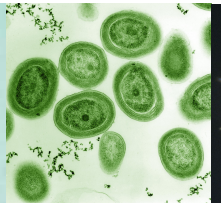
DMSP producers

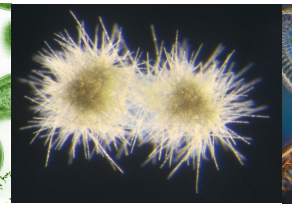


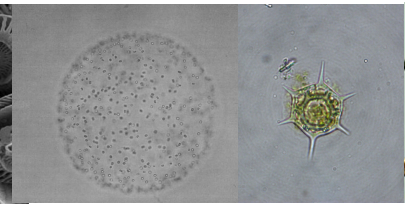
Calcifiers

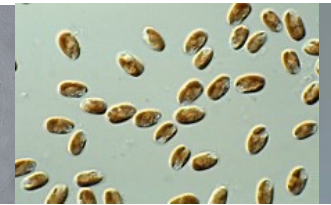
Nitrogen fixers

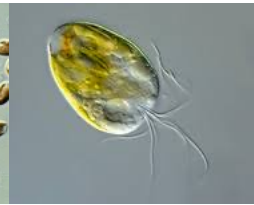






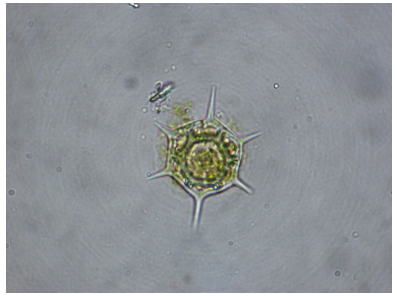


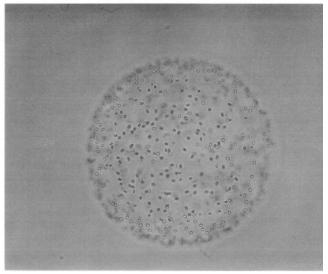




Let’s try this with our pre-defined phytoplankton groups…by pigment composition

Contain fucoxanthin (diatom “biomarker”)

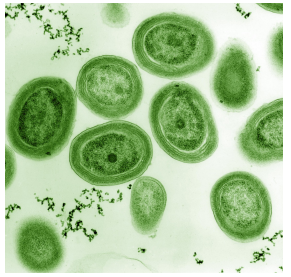
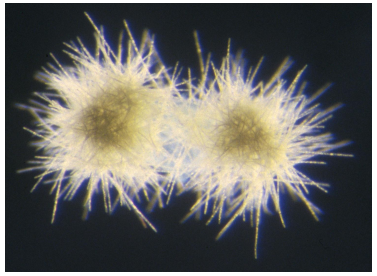



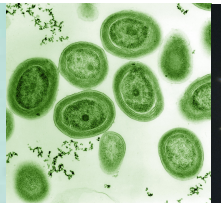
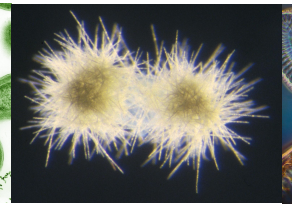
Also Chrysophytes, Pelagophytes, Bolidophytes

Do not contain fucoxanthin

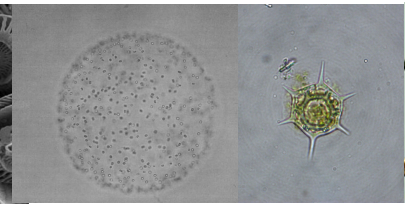
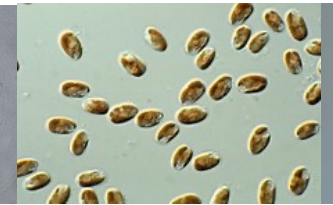
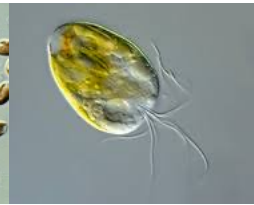


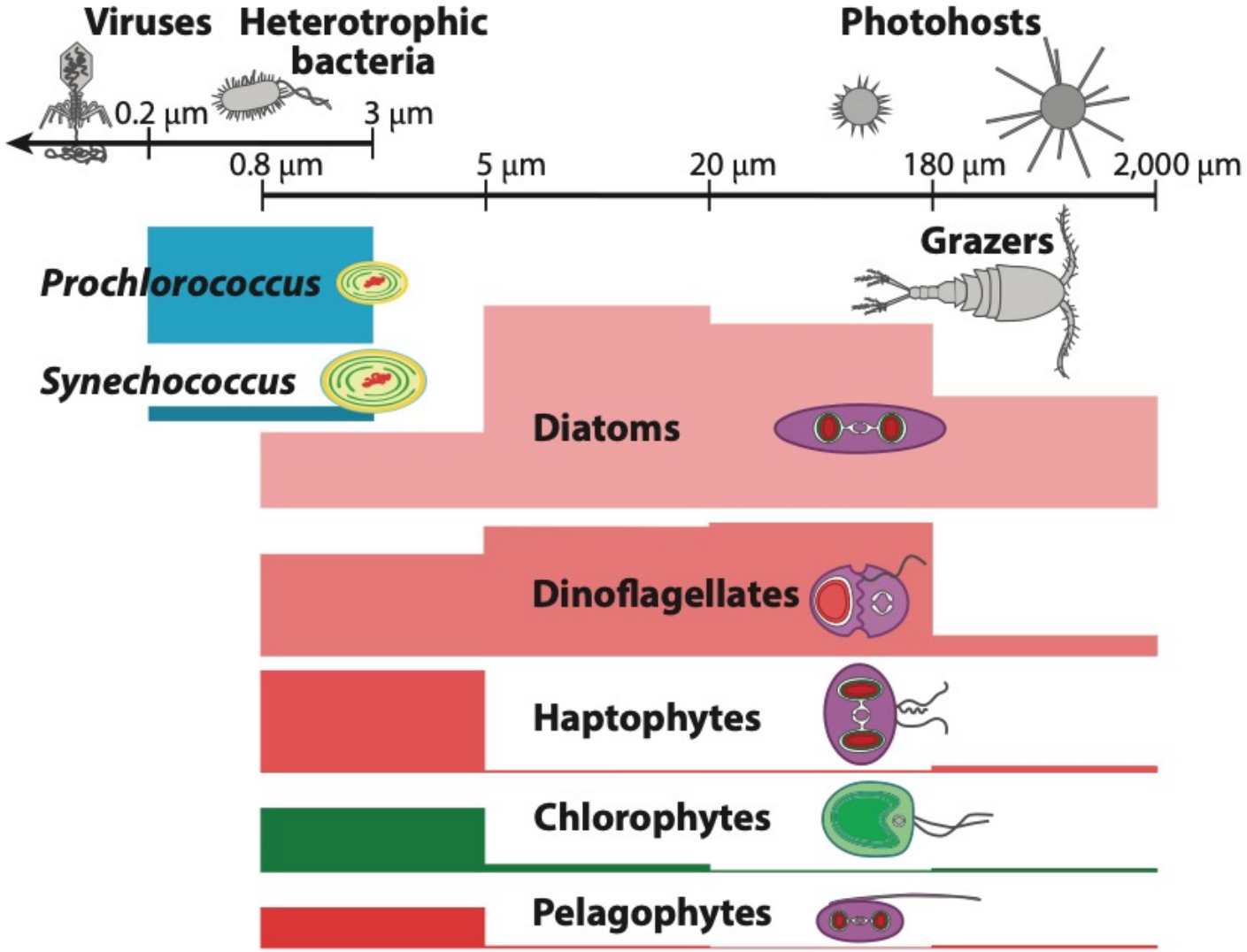




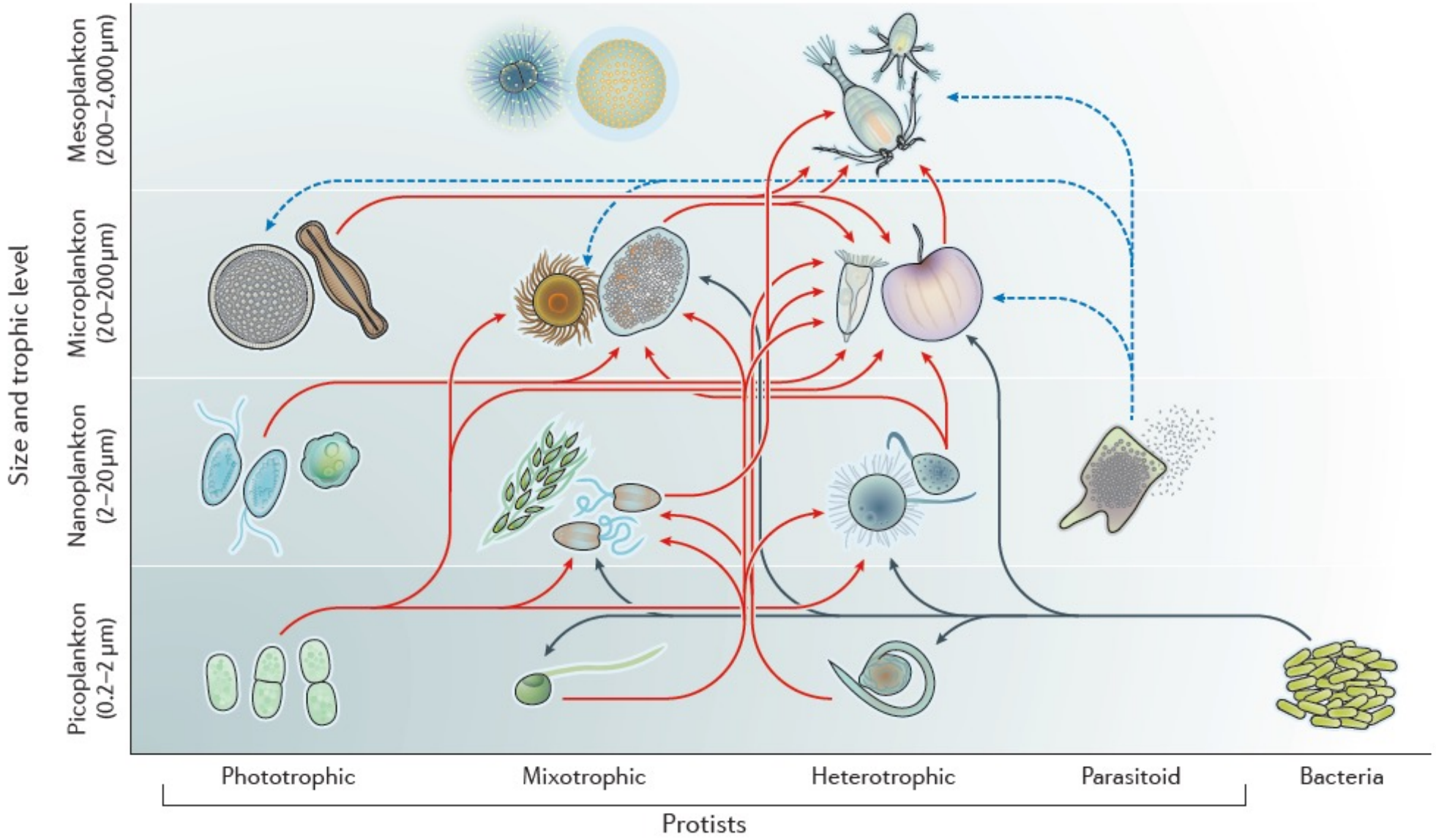




Phytoplankton size + taxonomy



Pierella Karlusich et al., 2020a

Feeding strategy + size + taxonomy…



Caron et al., 2017

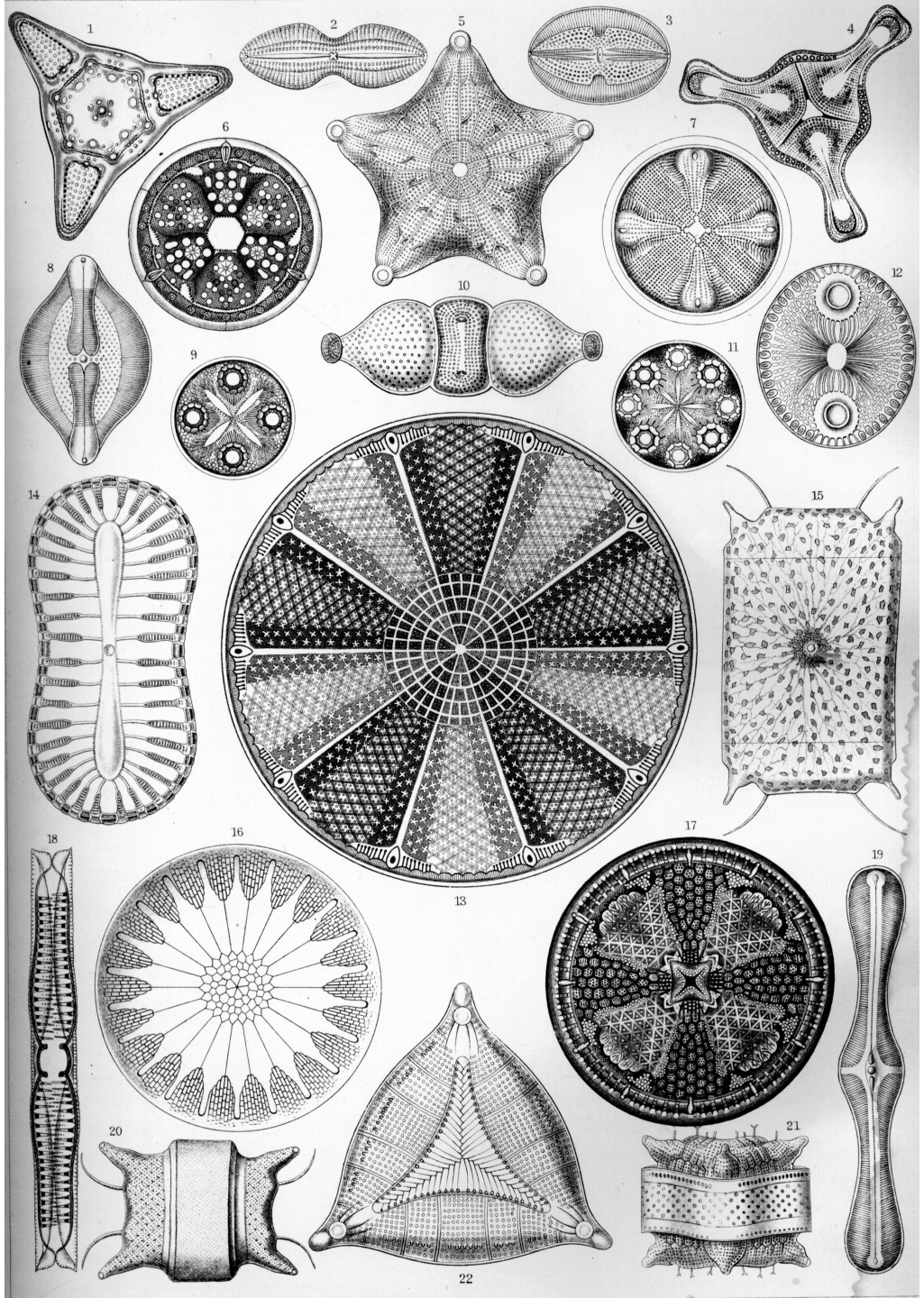
How do we measure phytoplankton community composition?!

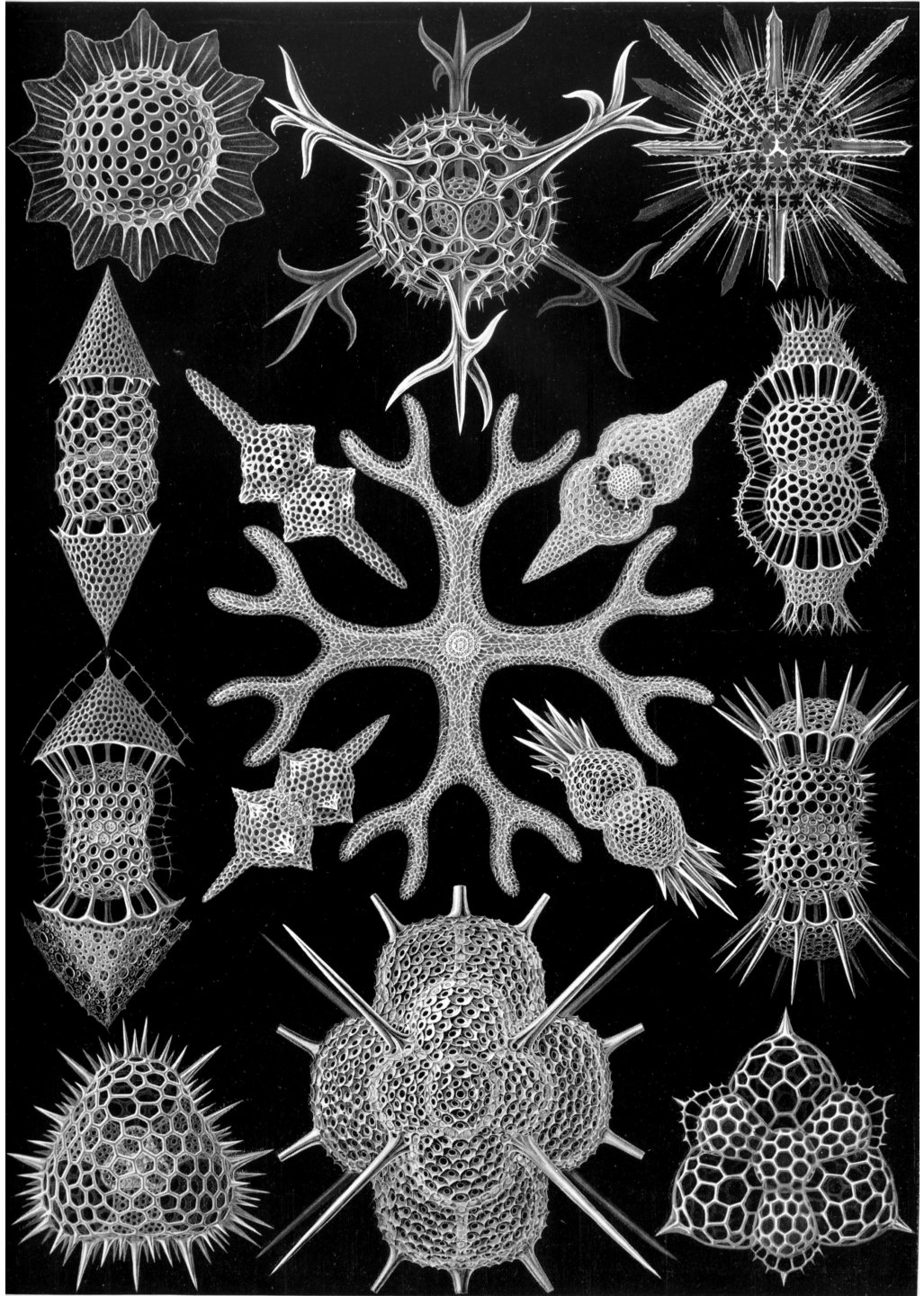
Given these challenges in describing the vast diversity of phytoplankton and the importance of capturing this variability, how do we select a method for measuring phytoplankton in situ?

NASA

Early efforts focused on larger planktonic organisms

Map from Pierella Karlusich et al., 2020b

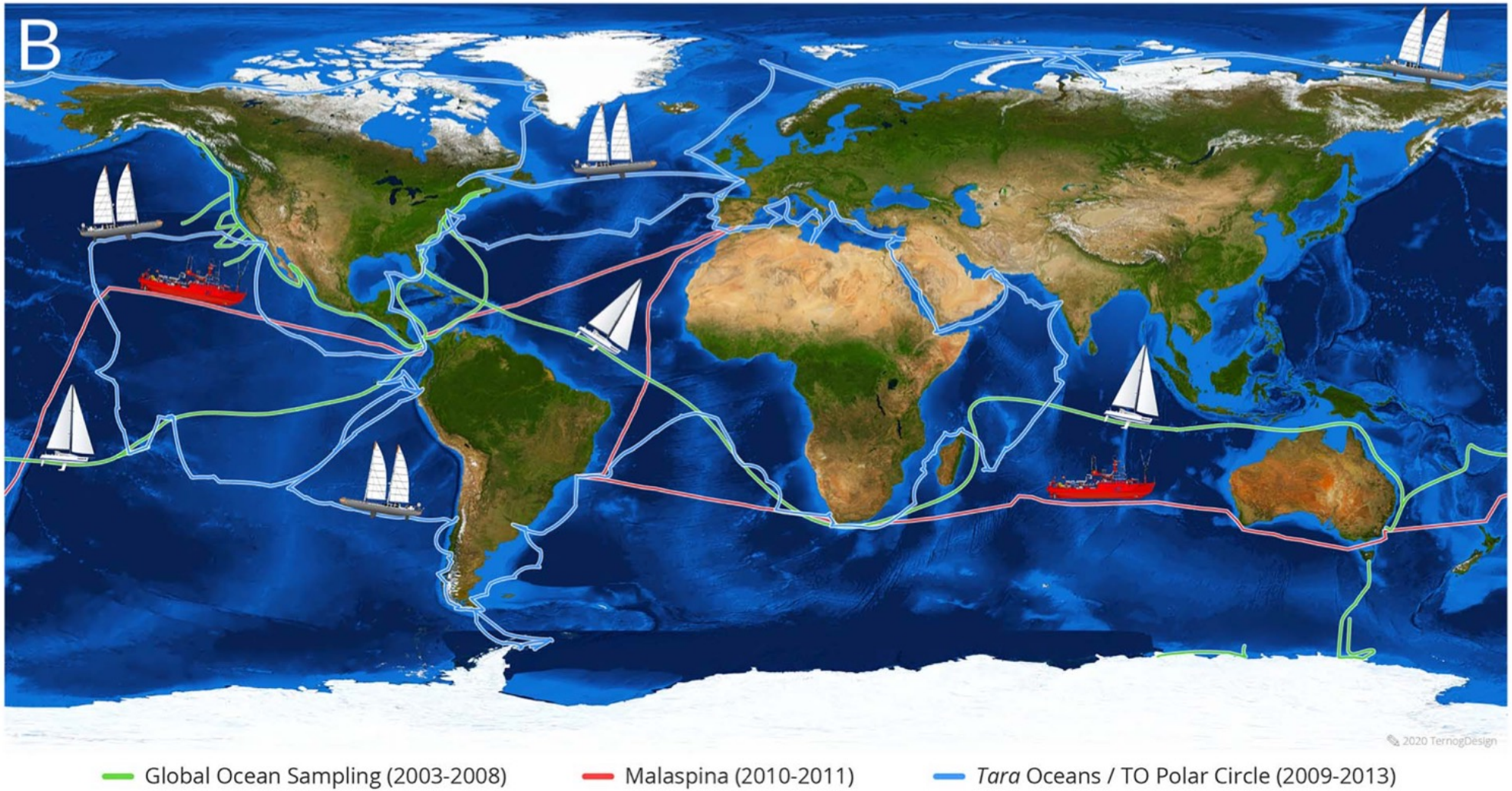

1
2
5
3
4
6
7
8
10
12
9
11
14
15
13
16
17
18
19
20
21
22



Understanding of plankton biogeography was limited by sampling and tools (as it is today!)

- Methods were limited to microscopy (on lower quality microscopes)
- Sampling was concentrated on the coasts (much easier to access!)
- *Synechococcus* only discovered in 1977! *Prochlorococcus* was only discovered in 1986!

Modern efforts have replicated global scale sampling…

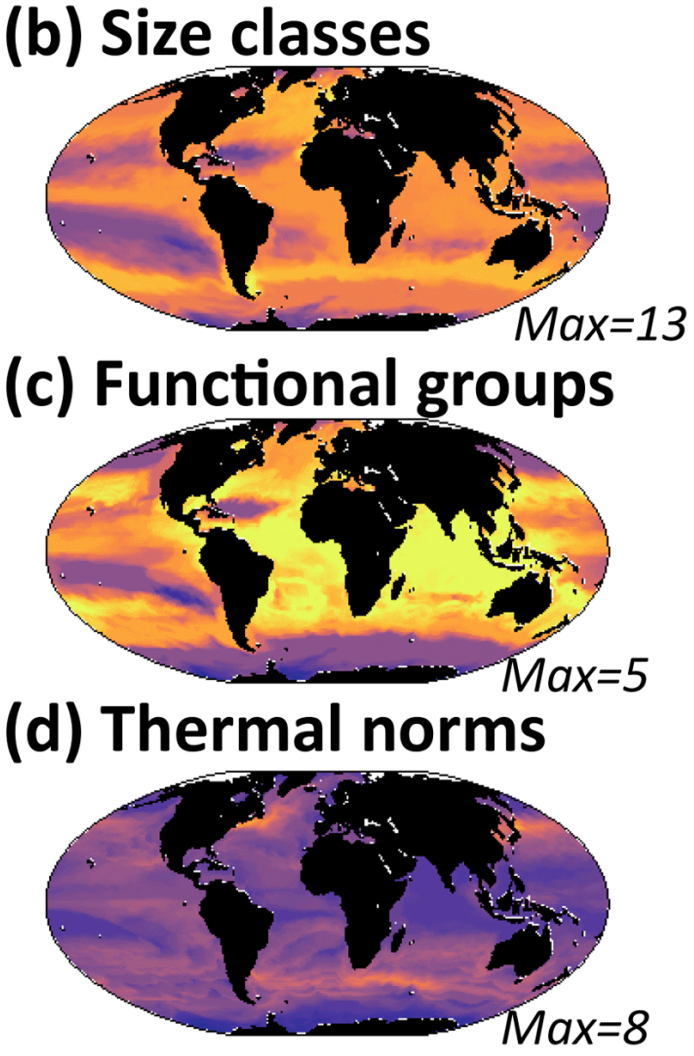


Map from Pierella Karlusich et al., 2020b

But with higher resolution methods!

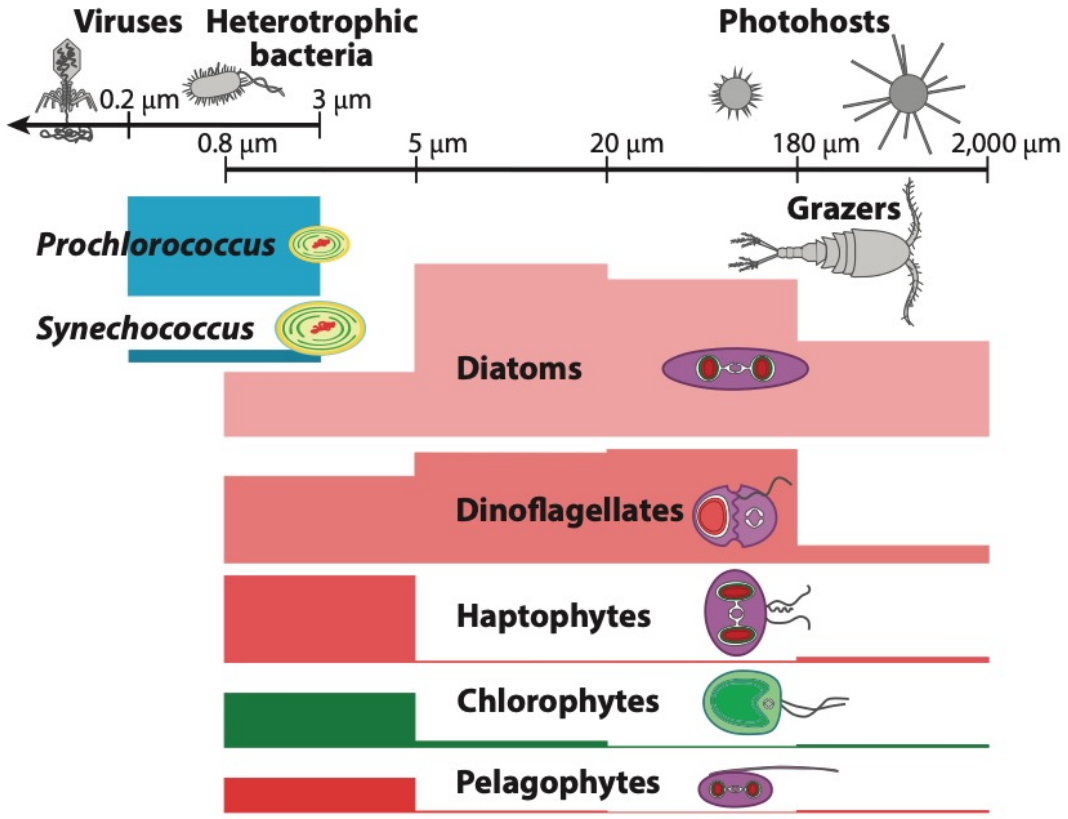
How do we measure phytoplankton community composition?!

- Microscopy
- Flow cytometry
- Quantitative cell imaging (IFCB)
- Molecular methods
- Pigments
- Optics proxies (absorption by phytoplankton and pigments)

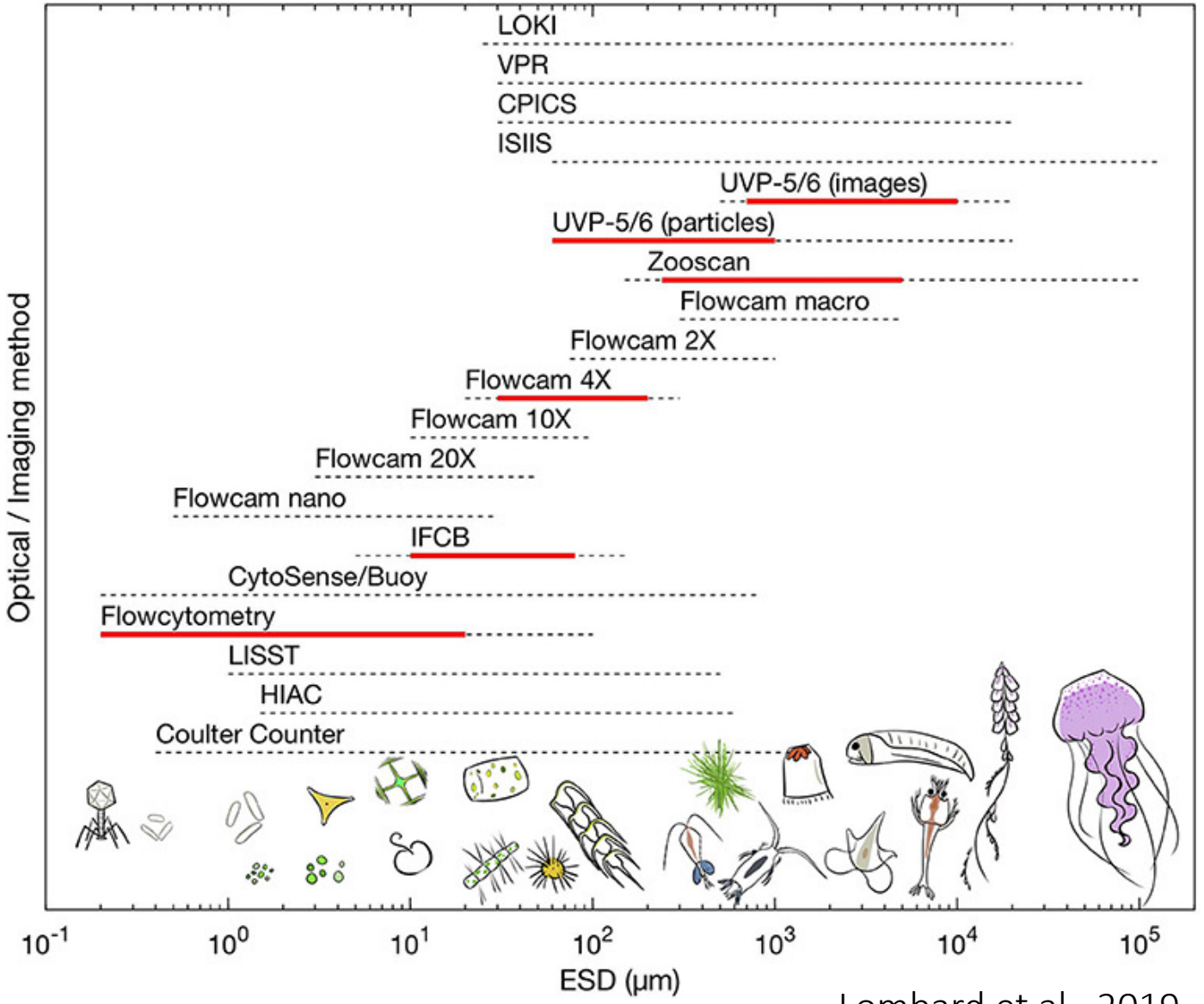


Dutkiewicz et al., 2020

One challenge: choosing the right tool for the job



Pierella Karlusich et al., 2020a



Lombard et al., 2019

Light microscopy

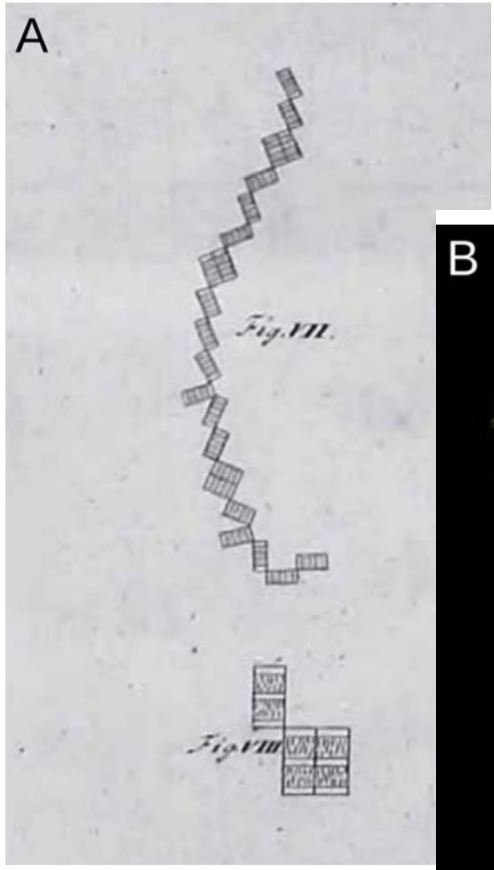
Quick method summary: Small sample (<1mL) collected, cells enumerated and identified under magnification. Can be stained / dyed for further info

Strengths: Useful for larger cells (many HABs), compare well to historical record

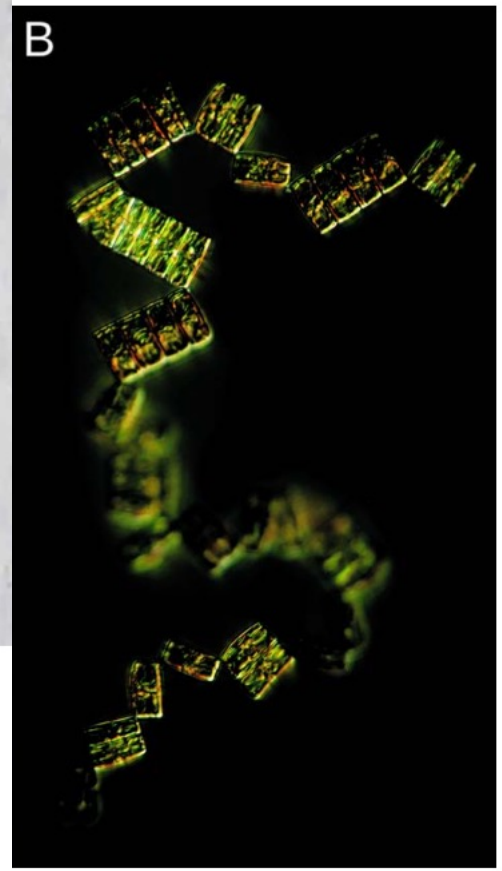
Weaknesses: Biased toward larger cells, very small sample volume, human error in ID, can't revisit IDs

Output: Cell counts per volume

1703 diatom



2019 diatom



Pierella Karlusich et al., 2020b

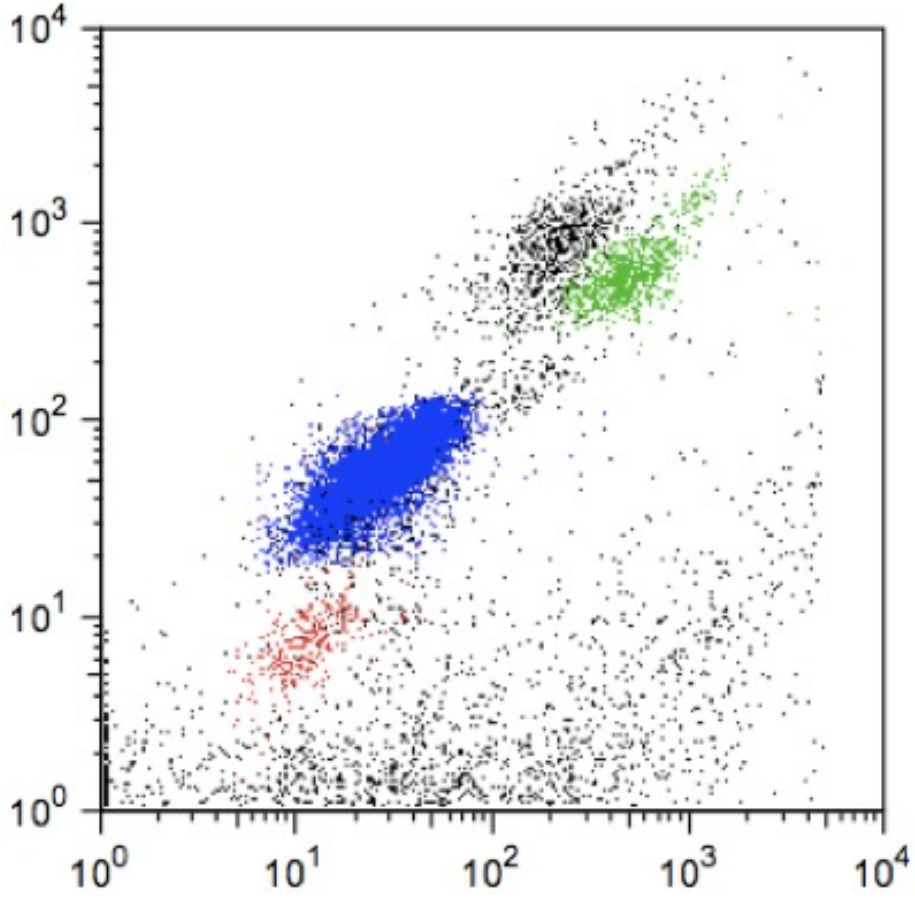
Flow cytometry

Quick method summary: Small sample (~1mL) collected, small cells enumerated and identified using fluorescence + scattering properties. Can be stained or dyed

Strengths: Useful for smallest cells (separates *Syn*, *Pro*, small eukaryotes)

Weaknesses: Only samples small cells, very small sample volume, requires calibration

Output: Cells/volume, biomass/volume (estimated)



Bigelow Labs

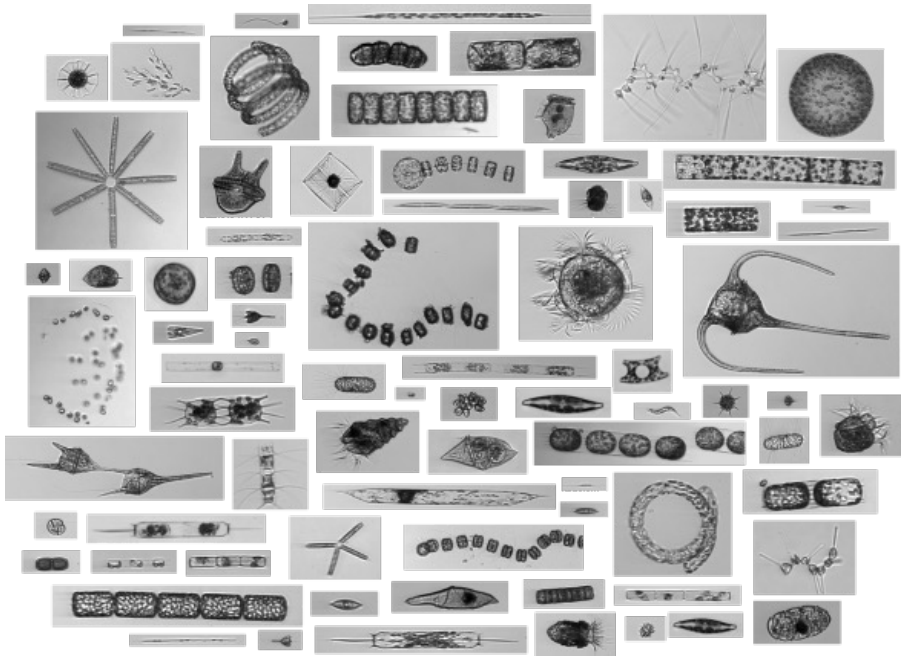
Quantitative cell imaging/imaging-in-flow cytometry

Quick method summary: Small sample (~2-5mL) collected, larger cells enumerated and imaged (also fluorescence + scattering info)

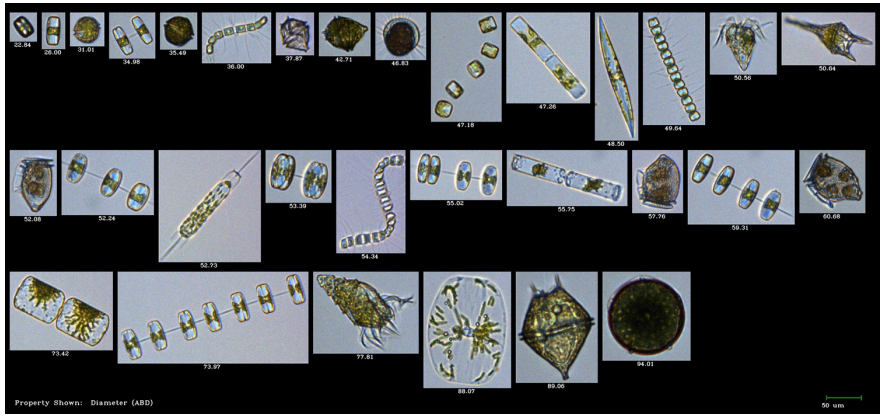
Strengths: Can operate autonomously, high taxonomic resolution, image collection allows for iterative ID (manual or automated)

Weaknesses: Only samples slightly larger cells, small sample volume, requires calibration + classification of images

Output: Cells/volume, biomass/vol (estimated)



IFCB (Sosik/McLane)



FlowCam (Fluid Imaging Tech)

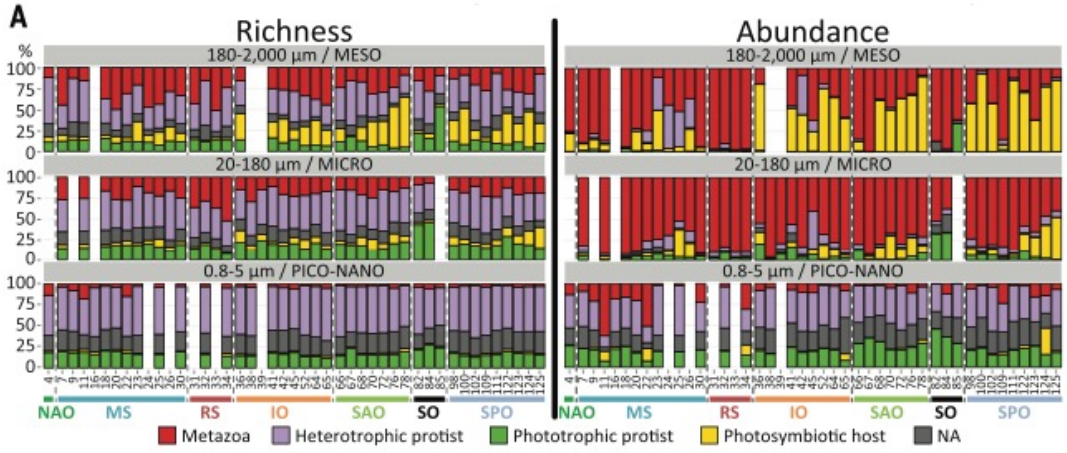
Molecular methods

Quick method summary: Large sample (2-10L) collected, filtered, DNA is extracted, and “barcode” genes are targeted for amplification and sequencing

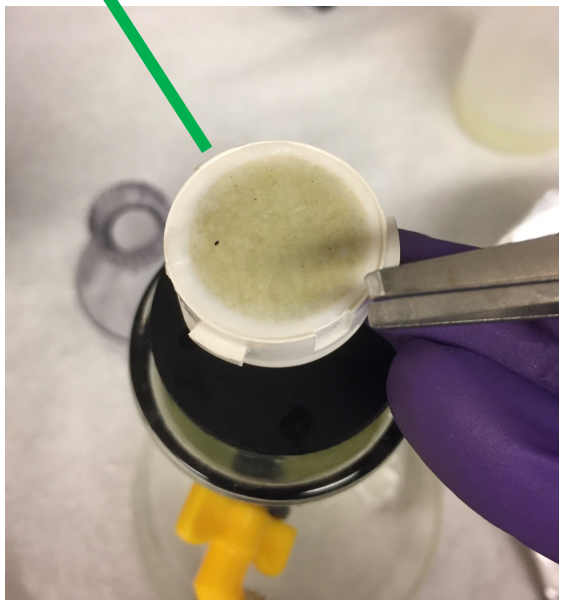
Strengths: High taxonomic resolution (often to genus or species level), can compare well to other methods (microscopy, pigments)

Weaknesses: Primers may be limited for some groups, gene copies =\= abundance

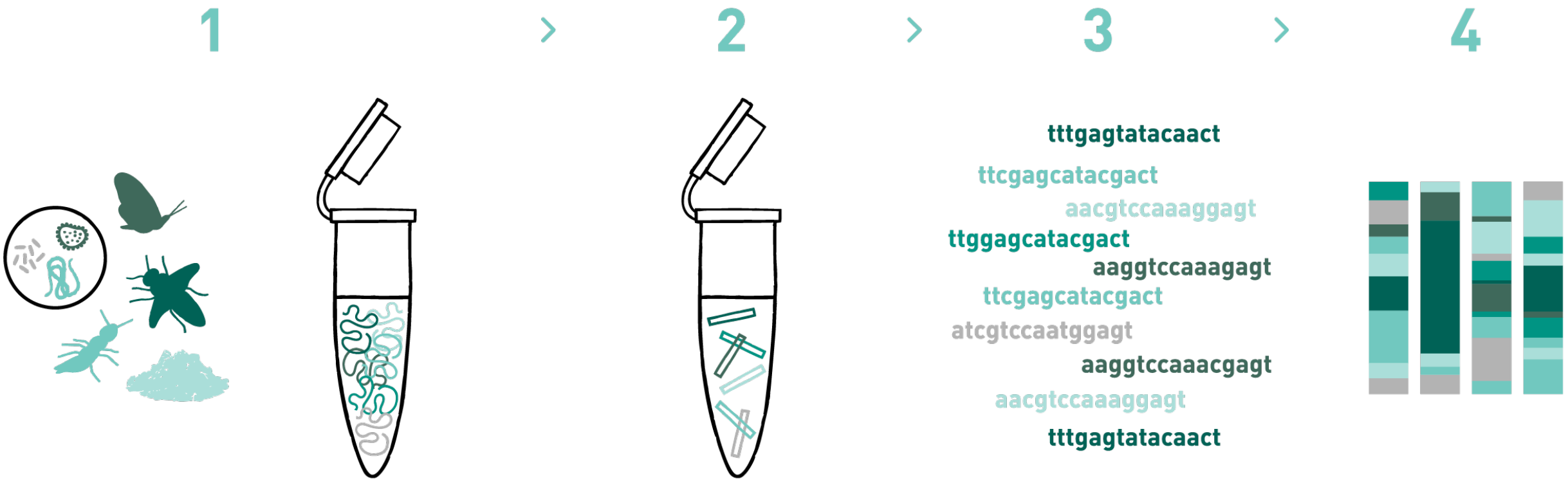
Output: *Relative* sequence abundances



de Vargas et al., 2015



Molecular methods

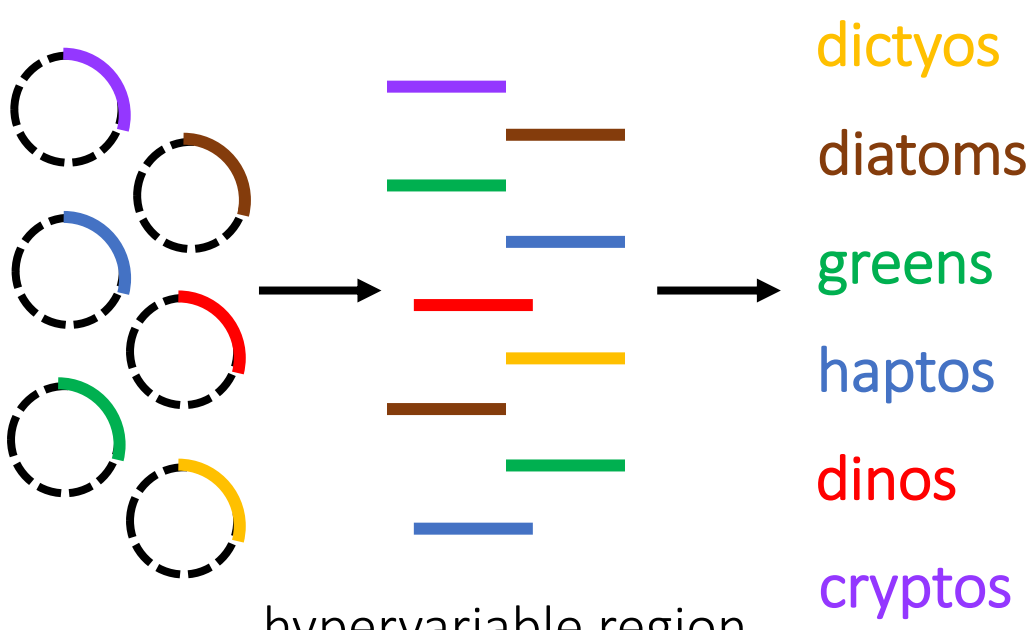


1) Collect sample and extract DNA, 2) Amplify gene of interest,
3) Sequence gene of interest, 4) Analyze relative abundance of sequences

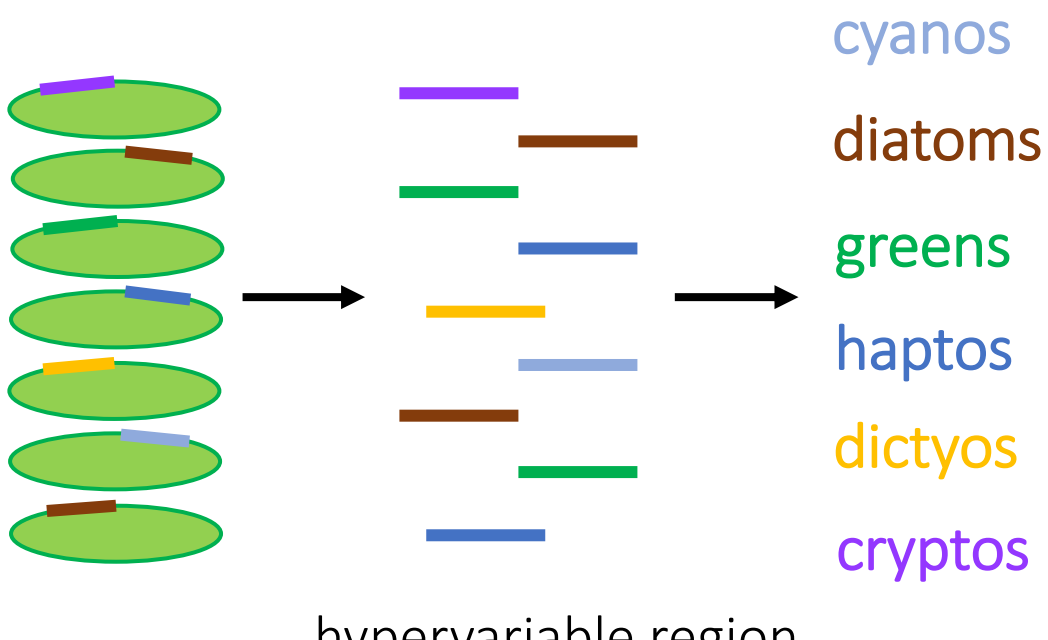
Molecular methods

2 main approaches for phytoplankton: 18S gene and 16S gene (other approaches are being developed to be more “universal”)

18S gene: only in eukaryotes

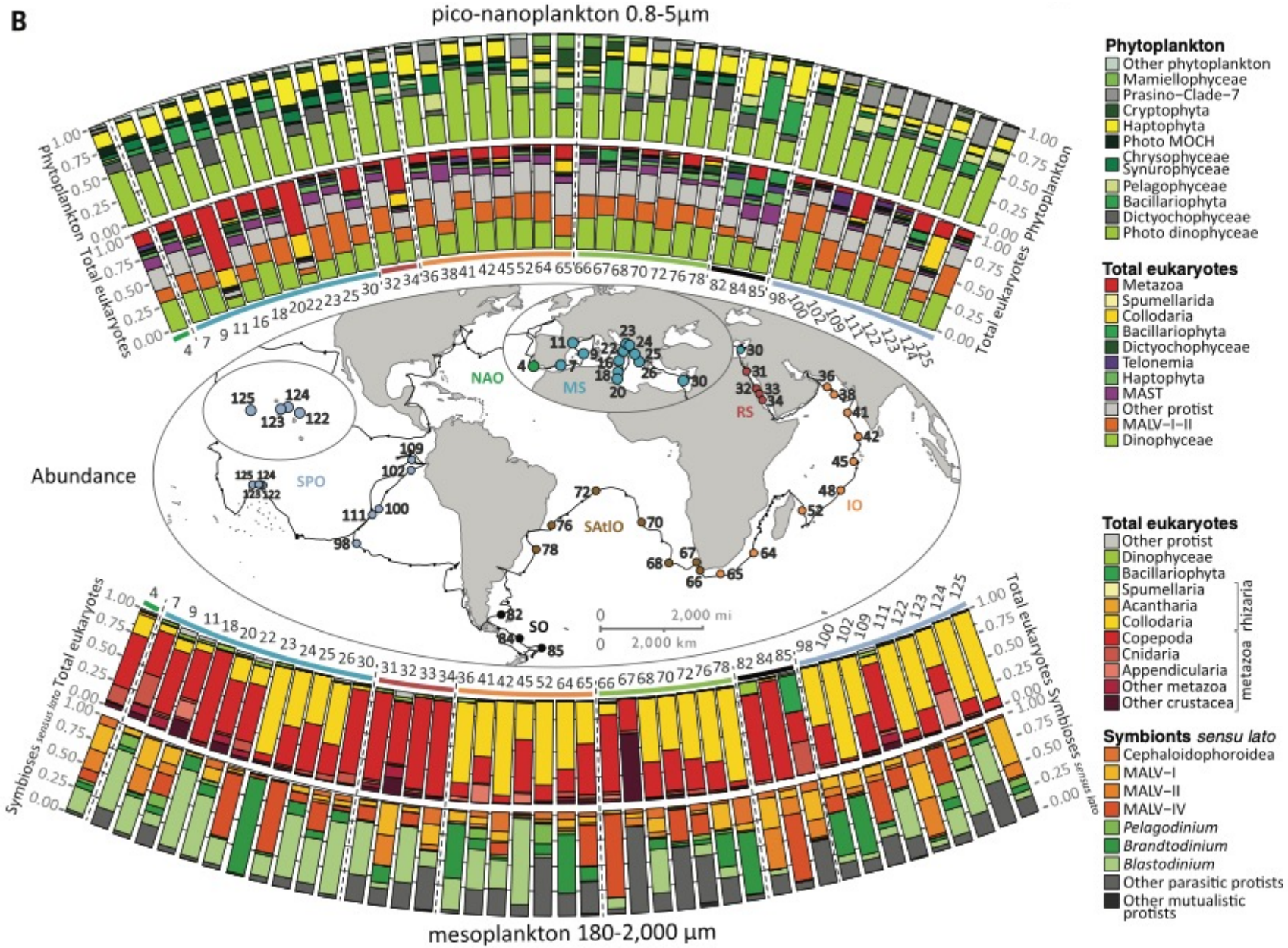


16S gene: in the chloroplast for euks



Molecular methods

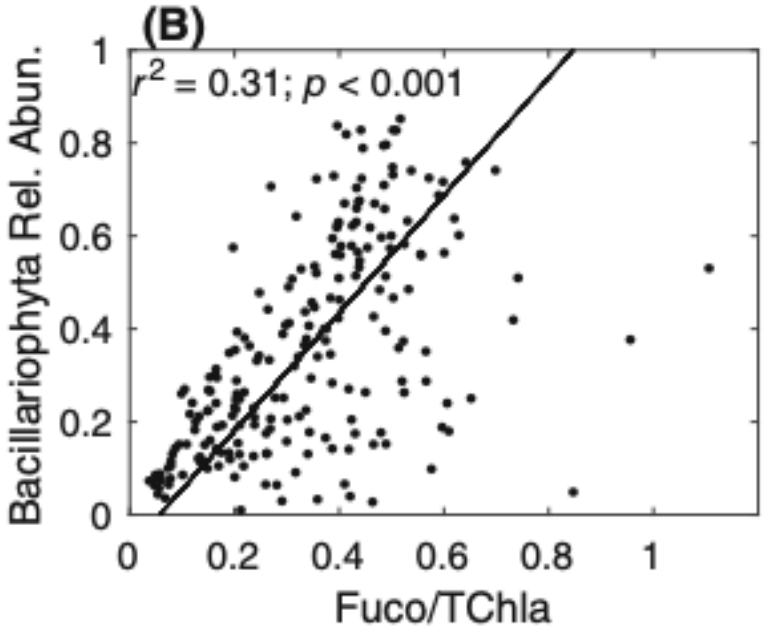
de Vargas et al., 2015



Molecular methods

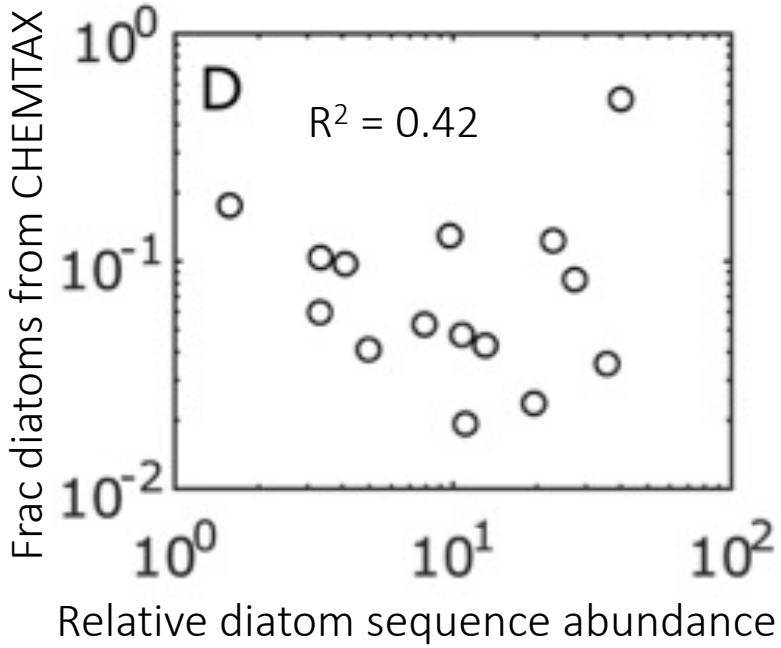
Comparing DNA metabarcoding to other methods: results can really depend on ecosystem dynamics

Santa Barbara Channel



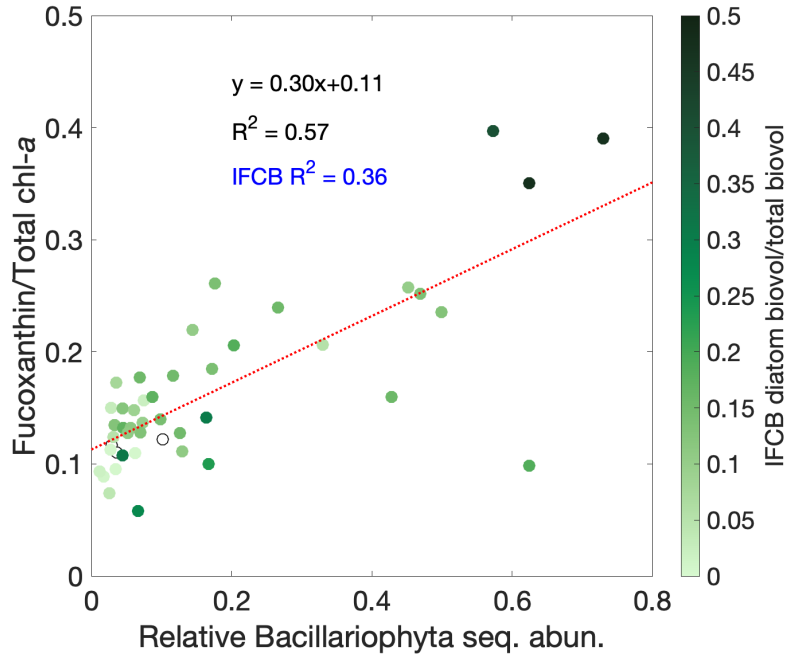
Catlett et al., 2022

West Antarctic Peninsula



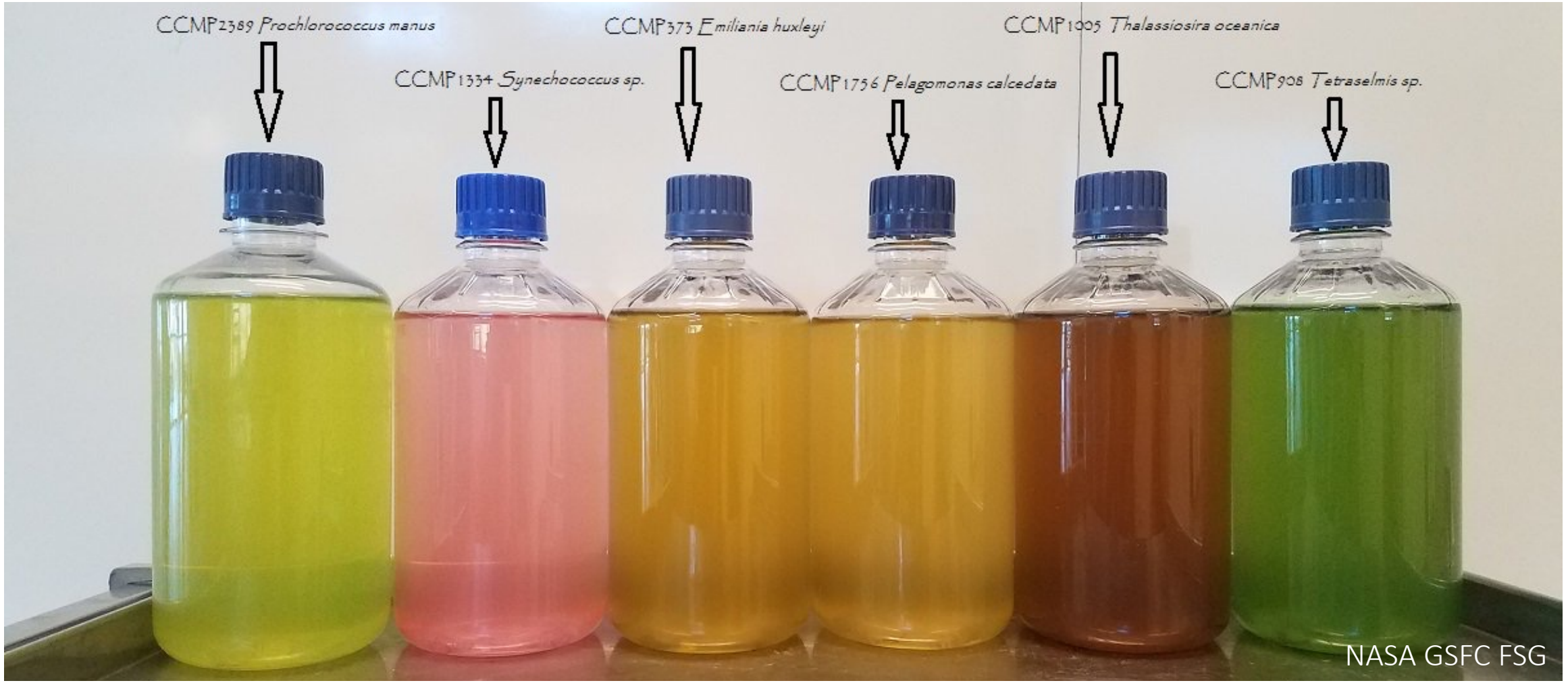
Lin et al., 2019

Open ocean (N. Atl + N. Pac)



Kramer et al., *in prep*

Phytoplankton pigments



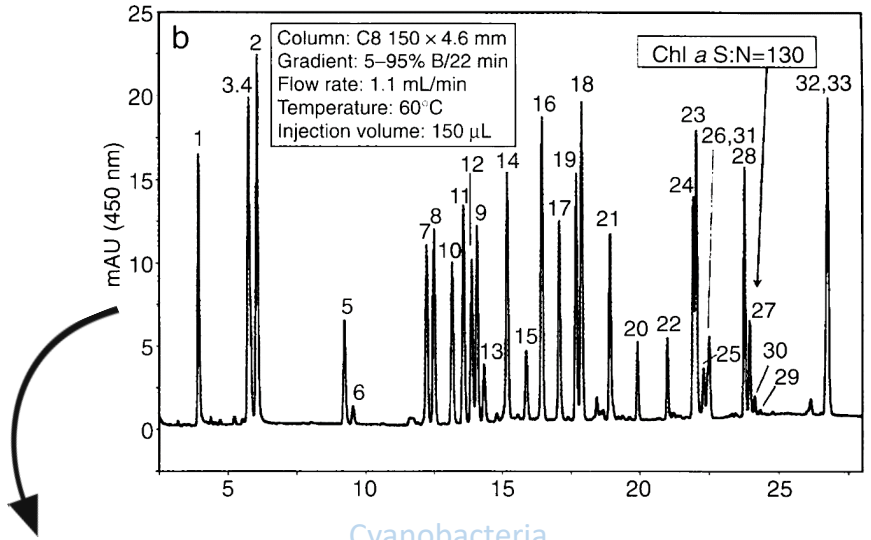
Phytoplankton pigments

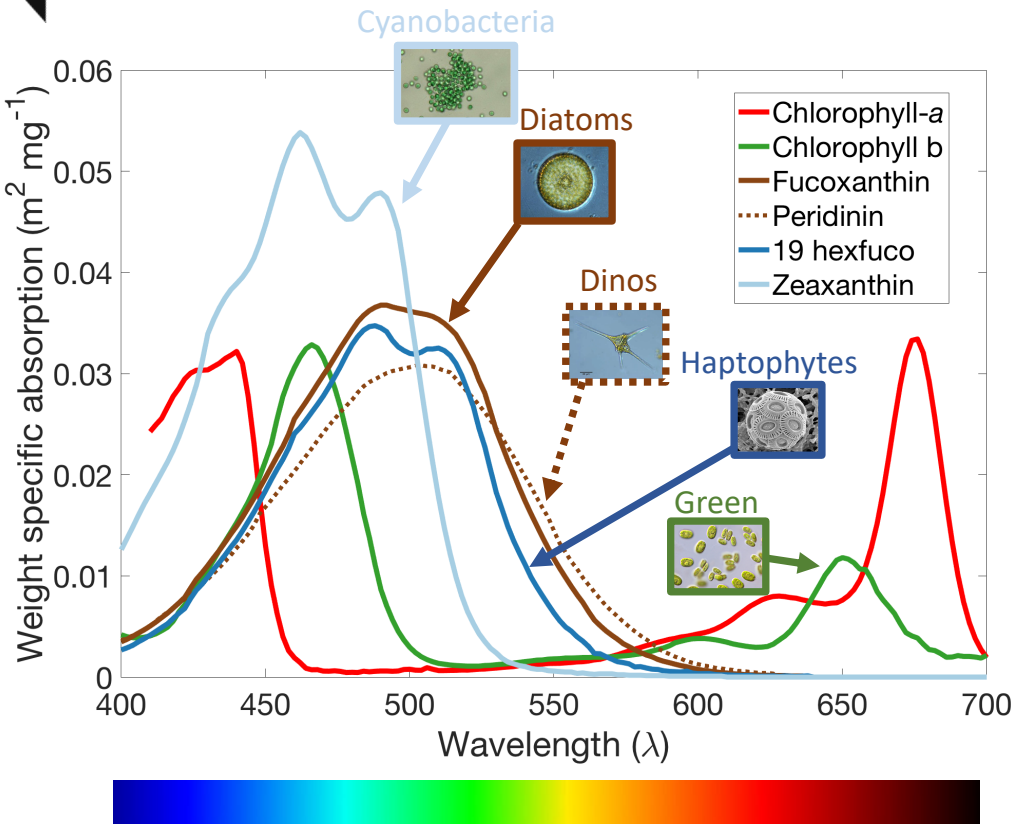
Quick method summary: Large sample (1-2L) collected, filtered, pigments are measured using high performance liquid chromatography (HPLC)

Strengths: Captures cells across size classes, direct link to absorption and ocean color

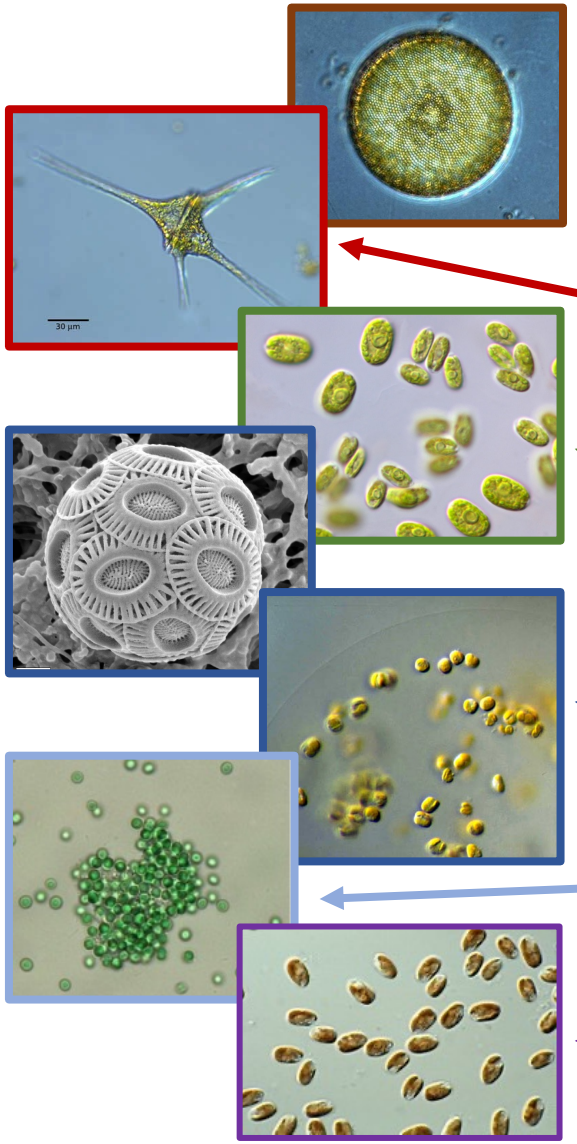
Weaknesses: Many major pigments are shared between phytoplankton groups!

Output: Pigment concentrations



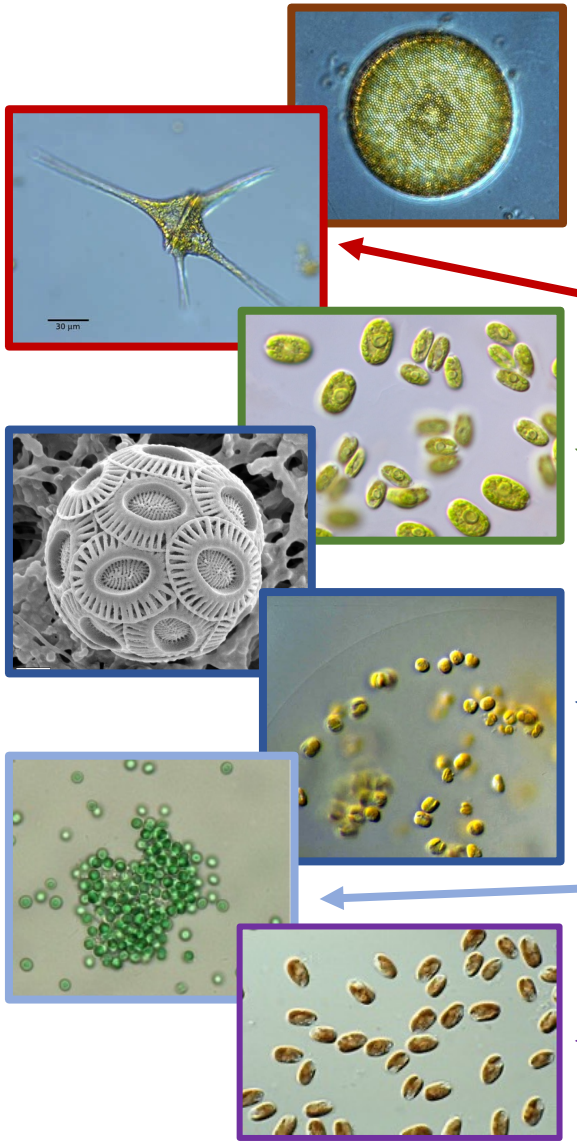


Phytoplankton pigments



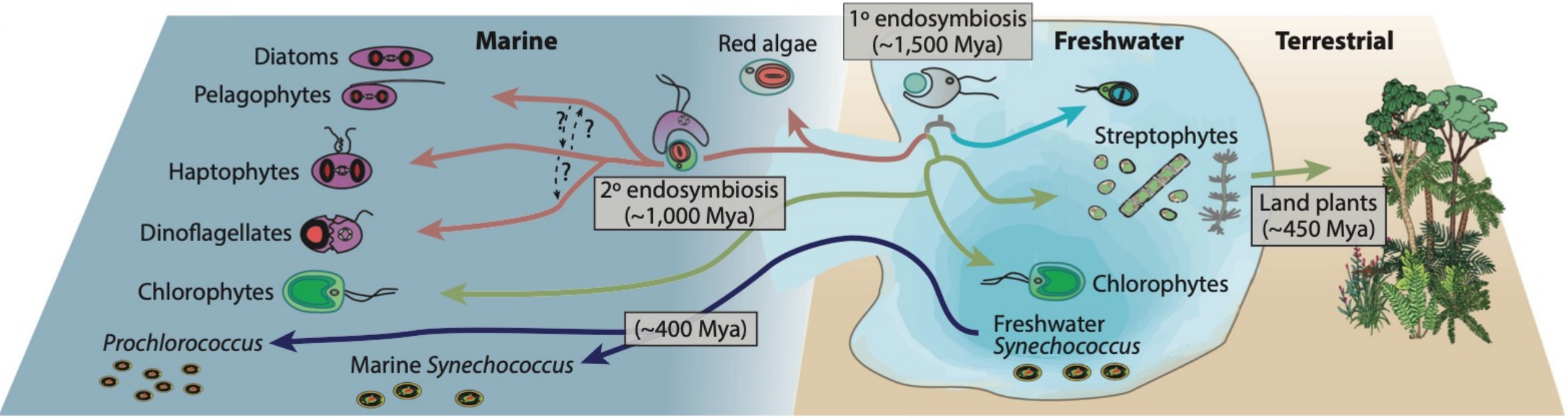
Phytoplankton group	Associated marker pigment(s)
Diatoms	Fucoxanthin
Dinoflagellates	Peridinin
Green algae	MV chlorophyll b
Haptophytes	19’-hexanoyloxyfucoxanthin
Cyanobacteria	Zeaxanthin, DV chlorophyll-*a*
Crytophytes	Alloxanthin

Except……



Phytoplankton group	Associated pigment(s)
Diatoms	Fucoxanthin
Dinoflagellates	Peridinin (but not always!), Fucoxanthin
Green algae	MV chlorophyll b
Haptophytes	19’-hexfuco, Fucoxanthin, 19’butfuco
Cyanobacteria	Zeaxanthin, DV chlorophyll-*a*
Crytophytes	Alloxanthin

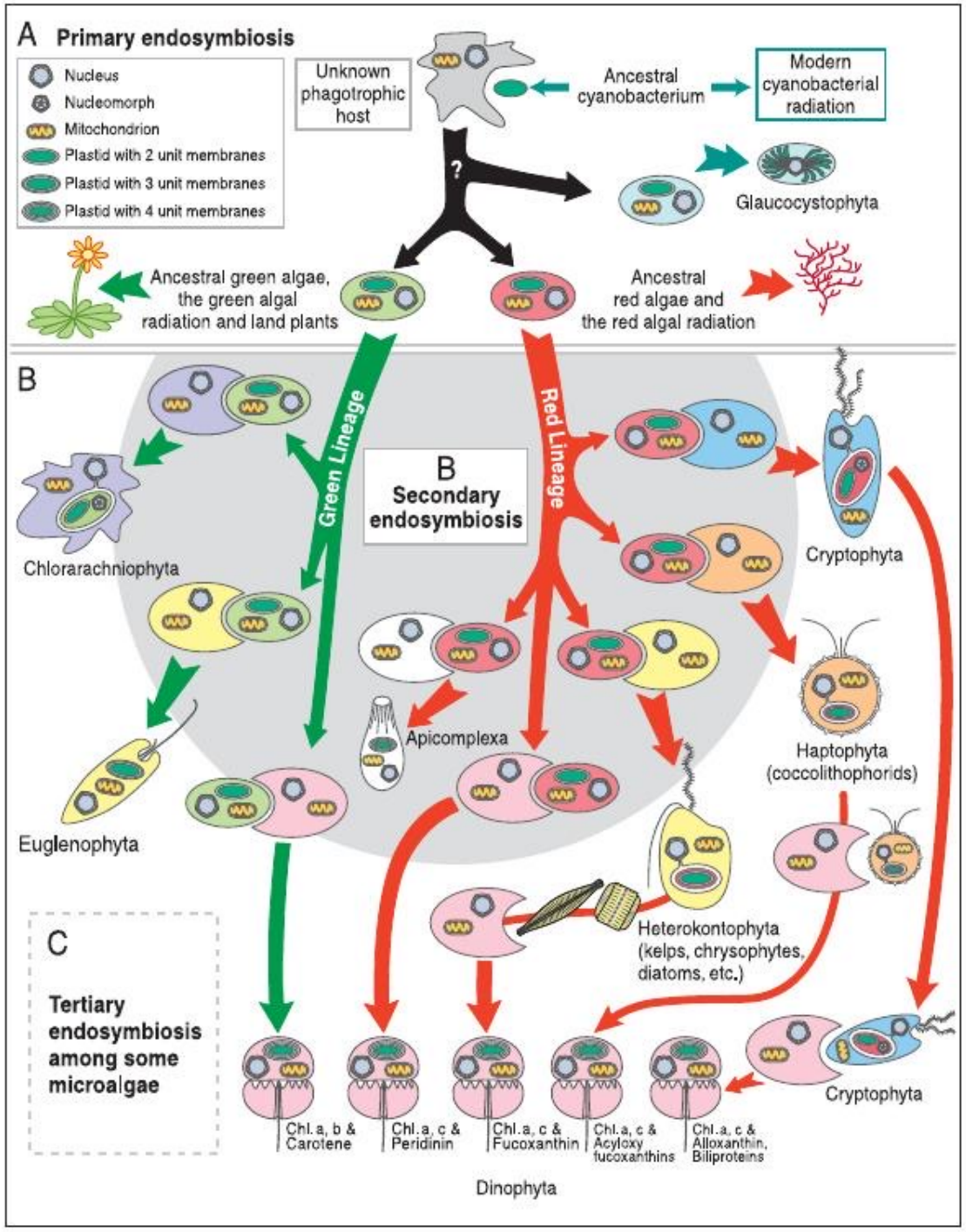
Phytoplankton evolution gives important information about limitations of in situ methods



Pierella Karlusich et al., 2020a

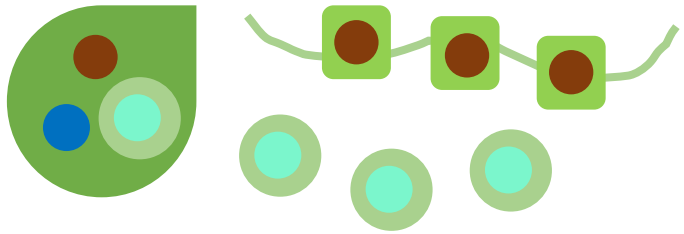
Phytoplankton share some major accessory pigments due to multiple endosymbiosis events throughout their evolutionary history

Dinoflagellates also share chloroplast DNA with these groups! Challenges for 16S sequencing

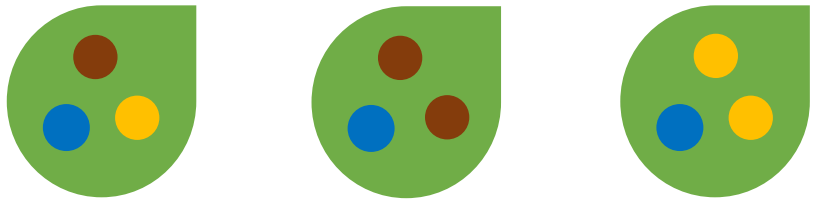


Three major challenges for pigment-based PCC

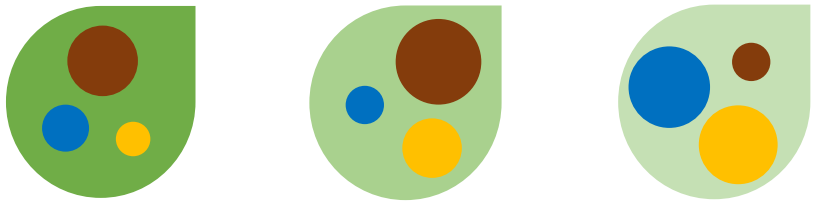
1. Many phytoplankton groups share pigments, either due to evolution or mixotrophy.



2. Within the same broad phytoplankton group, there can be variations in pigment composition.



3. Pigment concentration and composition can be affected by the environment (light, nutrients).



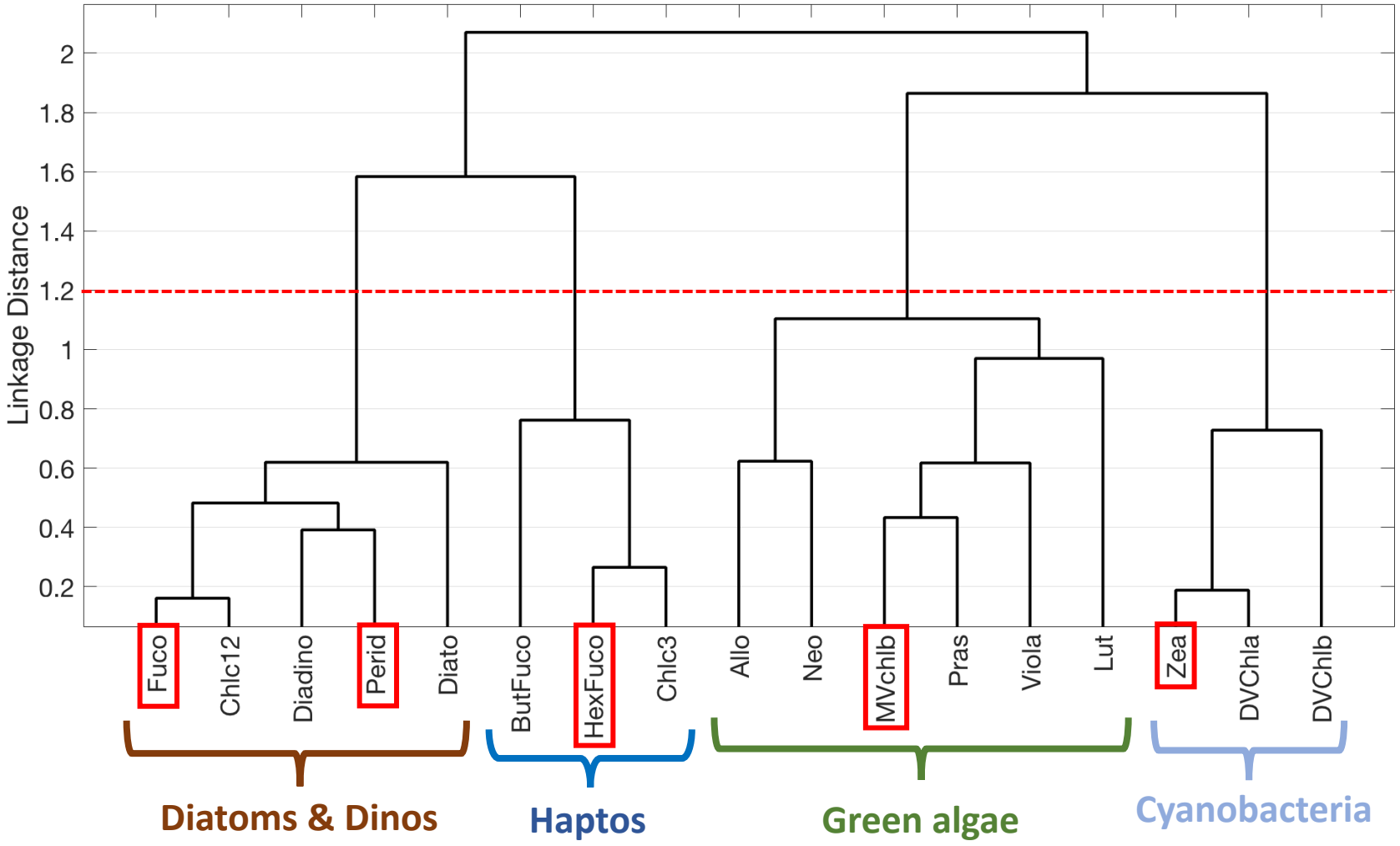
How do we use this information to maximize the utility of pigments without taking our conclusions about PCC out of context?

What can we as optical oceanographers do to make sure that our pigment measurements are used RESPONSIBLY??

The pigment data will tell you its limitations!!

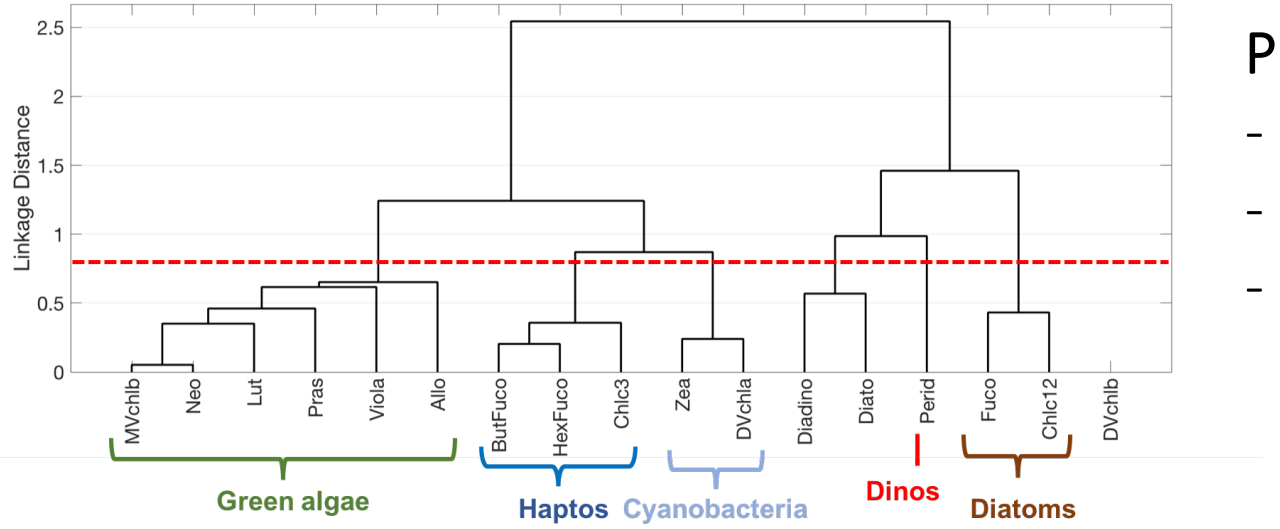
Using hierarchical cluster analysis to separate pigments based on the strength of correlations.

Four major phytoplankton groups separate.



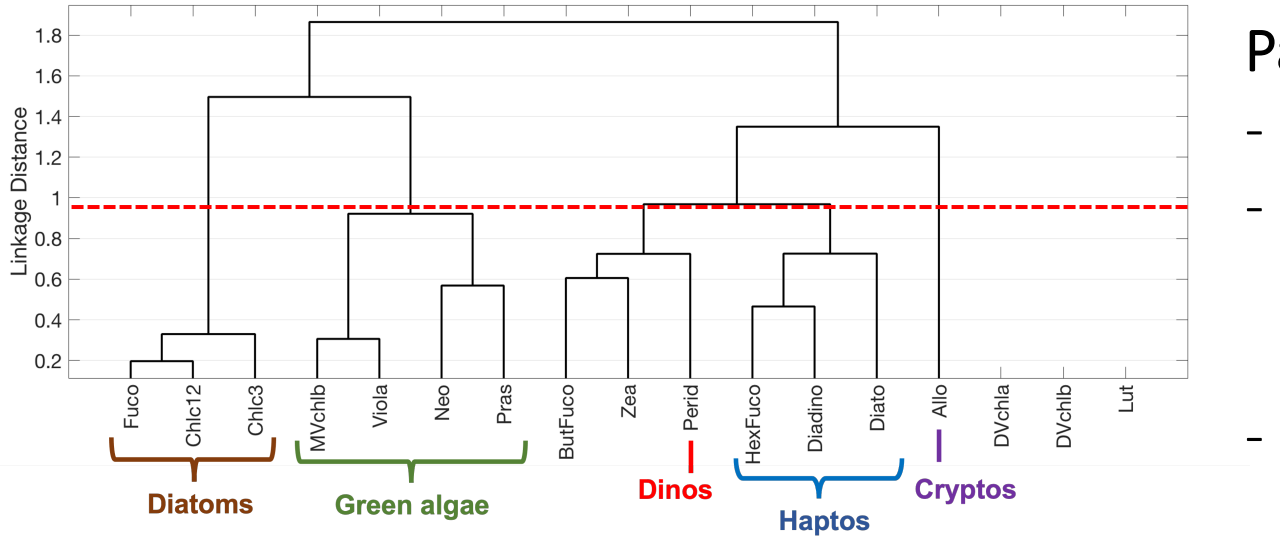
Kramer and Siegel, 2019

Those results may vary across ecosystems…



Plumes and Blooms (SB Channel):

- Five groups separate using pigments
- Dinoflagellates separate from diatoms
- More and different groups from global



Palmer Station (West Antarctic Peninsula):

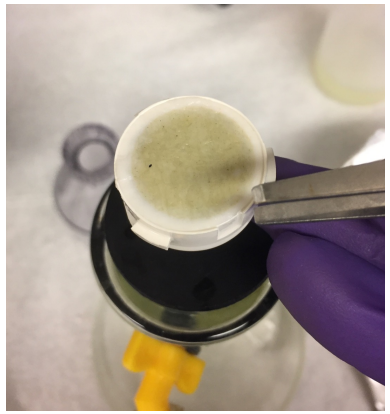
- Five groups separate using pigments
- Dinoflagellates separate from diatoms, cryptophytes separate from all others, no cyanobacteria group
- More and different groups from global

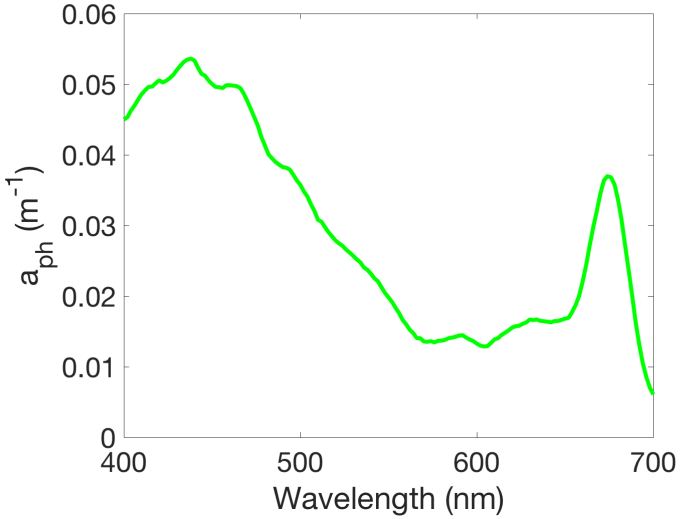
Kramer and Siegel, 2019

NASA

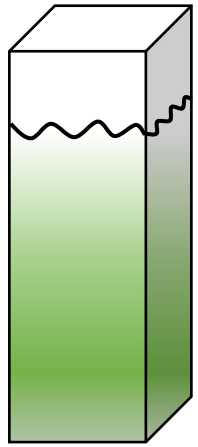
Optical proxies: absorption by phytoplankton and their pigments

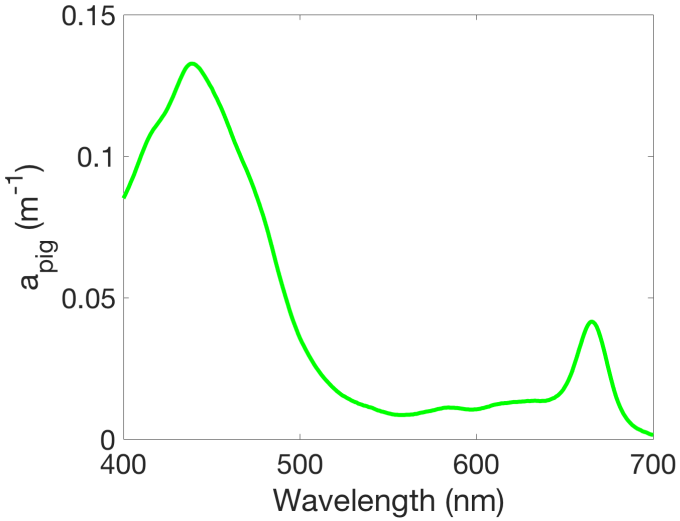
You learned about particulate absorption and a_{ph} measurements earlier this week (and got to practice these techniques in Lab 3).





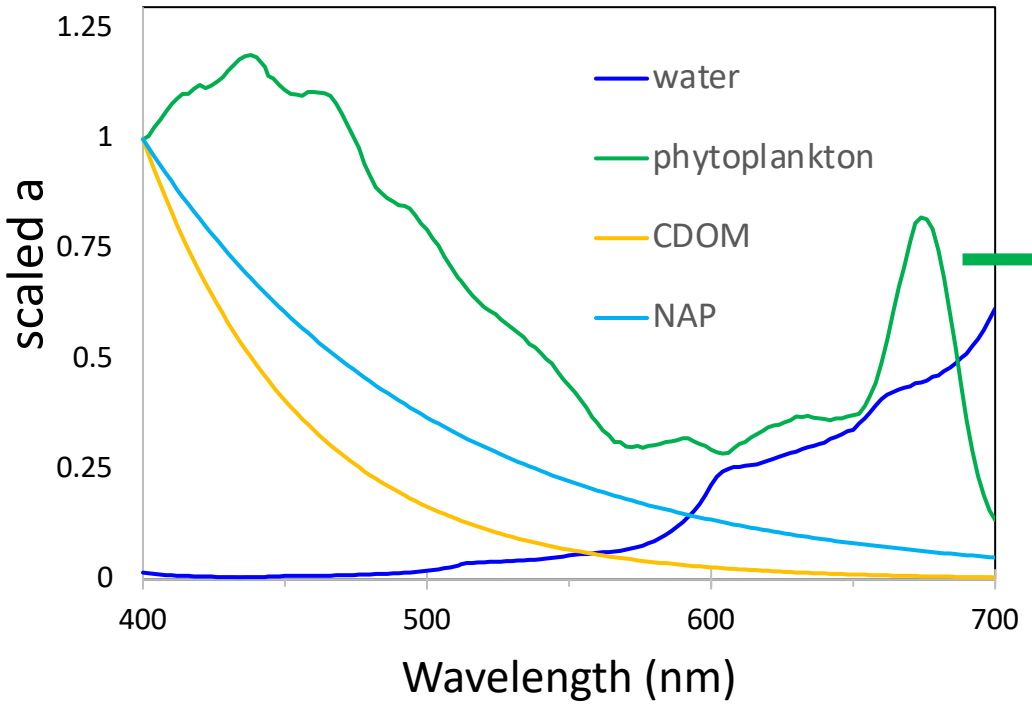
We can decompose a_{ph} or a_p measurements into pigment absorption. Phytoplankton pigments can also be extracted from the cell and we can measure pigment absorption in solvent.

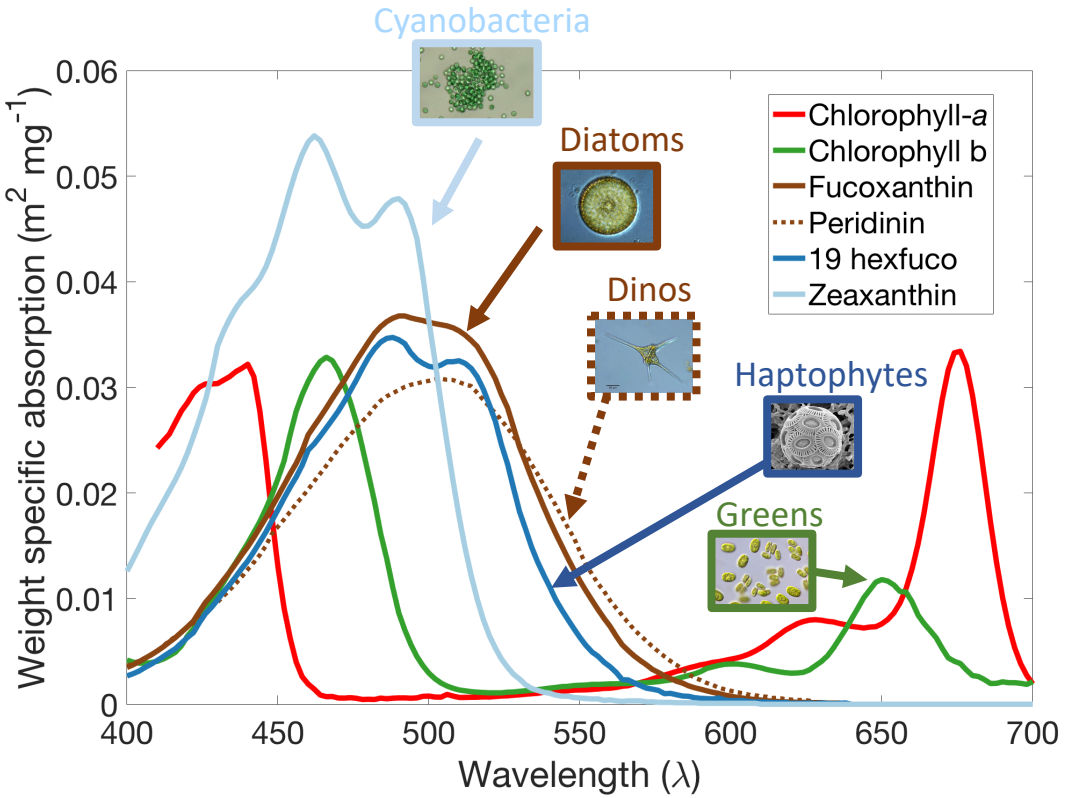




Optical proxies: absorption by phytoplankton and their pigments

$$R_{rs}(\lambda) \approx \frac{b_b(\lambda)}{a(\lambda) + b_b(\lambda)} \qquad a = a_{water} + a_{NAP} + a_{CDOM} + a_{ph}$$

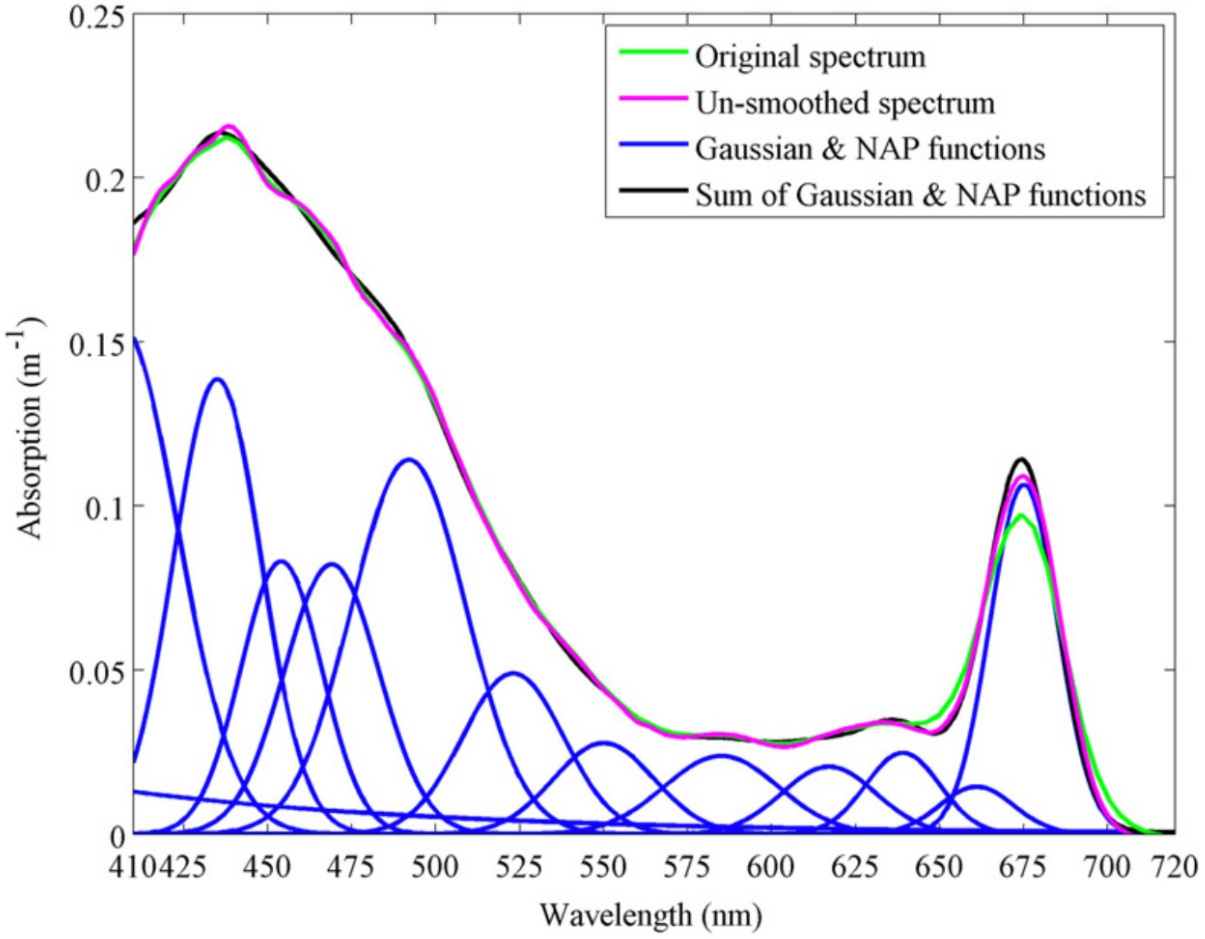




IOP spectra from Roesler and Perry 1995

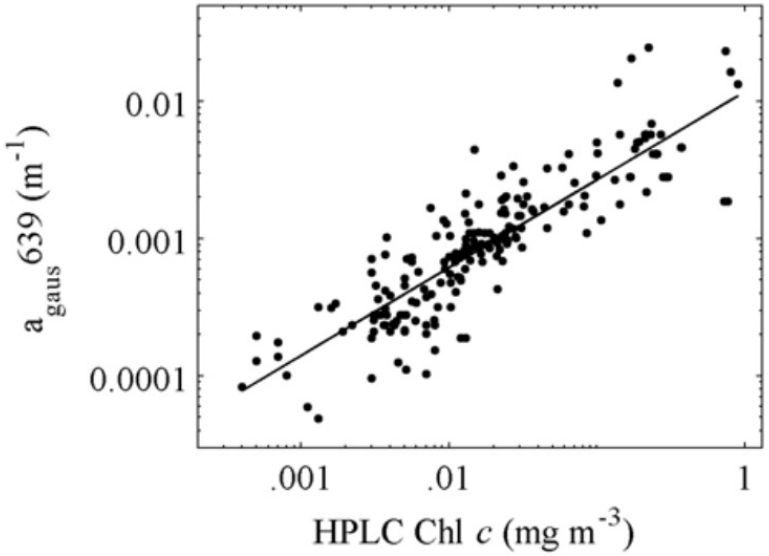
Optical proxies: absorption by phytoplankton and their pigments

Spectral decomposition from particulate absorption – Chase et al., 2013



Decomposing a_p spectra into contributions from PPCs and PSCs:

$$X^2 = \sum_{\lambda=410}^{720} \frac{\left(a_p(\lambda) - \left(\sum_{i=1}^{12} a_{gaus}(\lambda_i) \exp\left\{-0.5\left[\frac{\lambda-\lambda_i}{\sigma_i}\right]^2\right\} + a_{NAP}(400)\exp\{-0.01\,(\lambda-400)\}\right)\right)^2}{\sigma_{SD}^2(\lambda)}$$



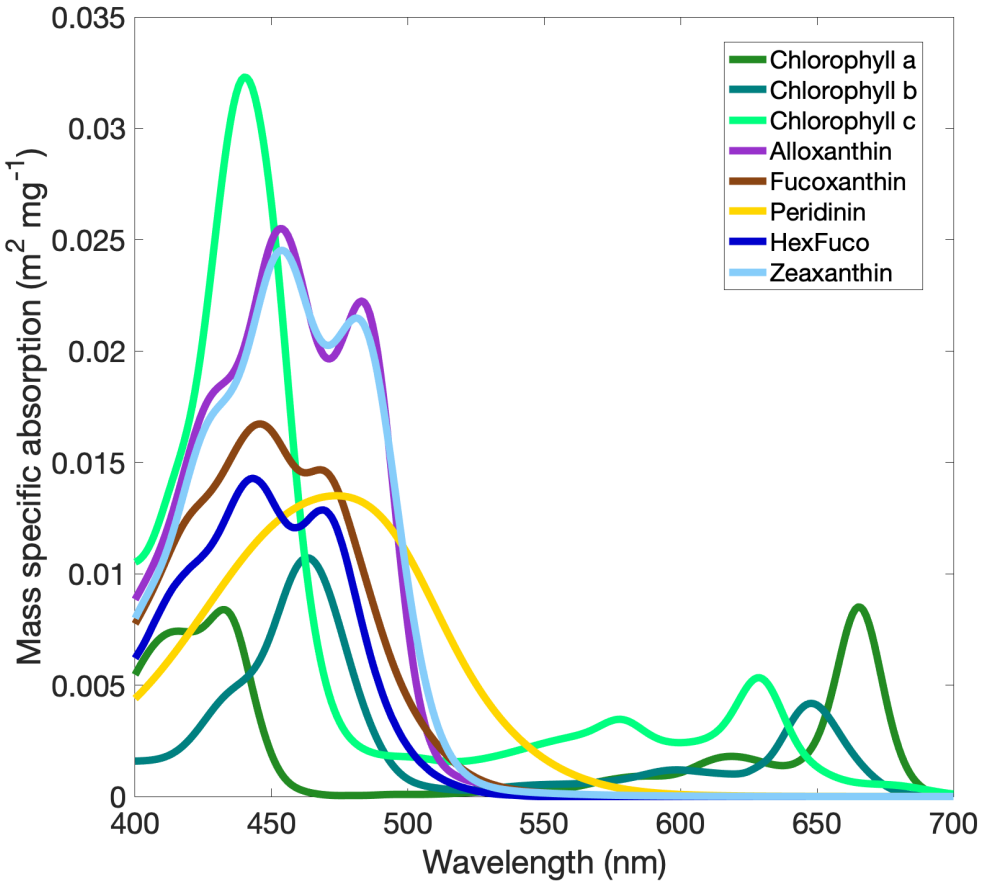
Optical proxies: absorption by phytoplankton and their pigments

Pure pigment absorption data for each pig from Thrane et al. (2015) and digitized from Jeffrey & Wright (1987), Jeffrey (1997), and Clementson & Wojtasiewicz (2019).

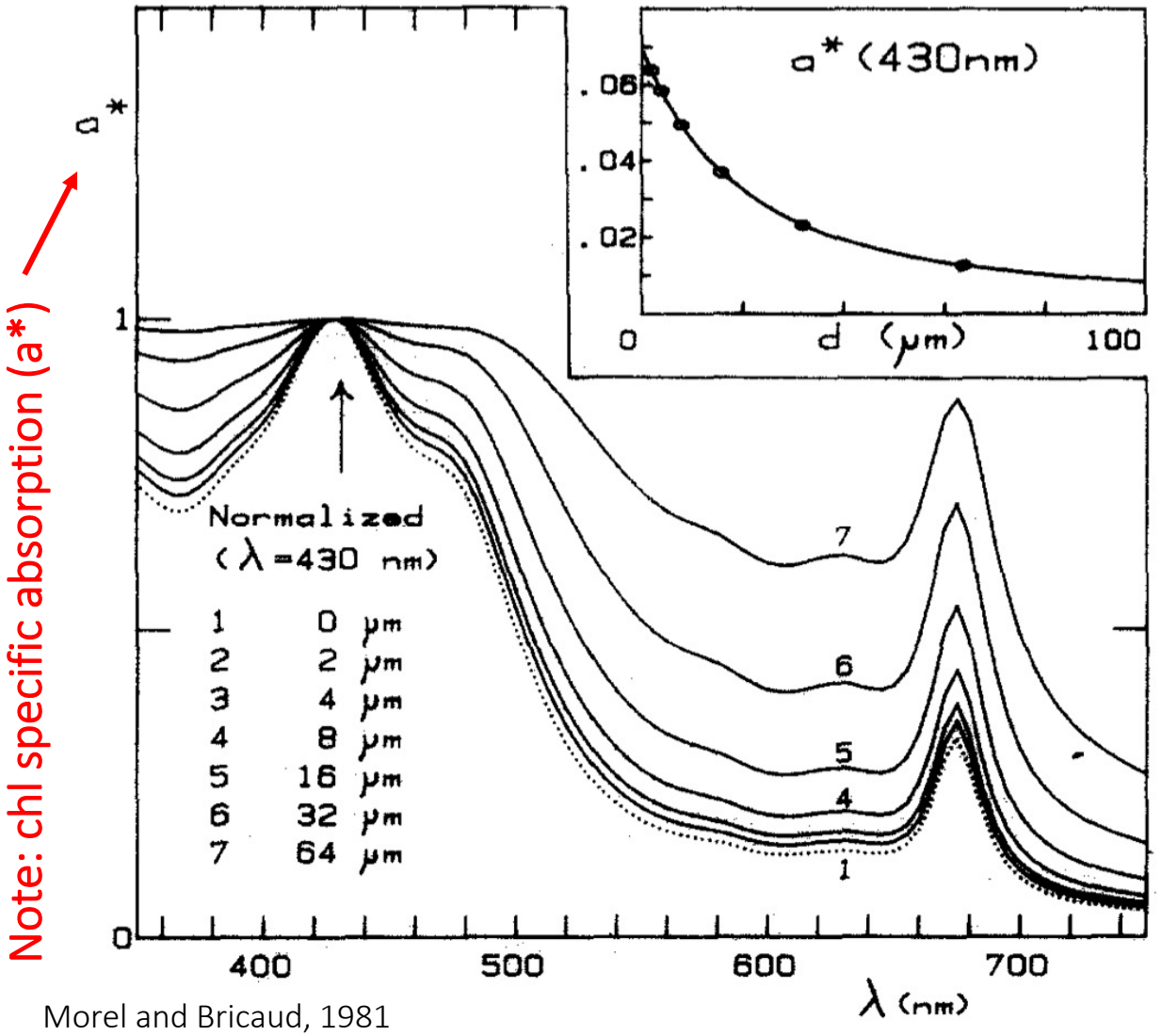
Can forward-model absorption from pigment concentration data ($[pig]$) and pure pigment absorption spectra (a^*_{pig}):

$$a_{pig,i} = [pig] * a^*_{pig,i}$$

$$a_{pig,tot} = \sum_{i=1}^{\#\ pigs} a_{pig,1} + a_{pig,2} + \cdots + a_{pig,i}$$

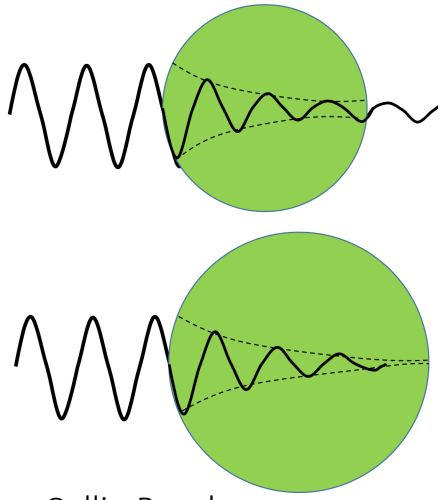


Optical proxies: absorption by phytoplankton and their pigments



Morel and Bricaud, 1981

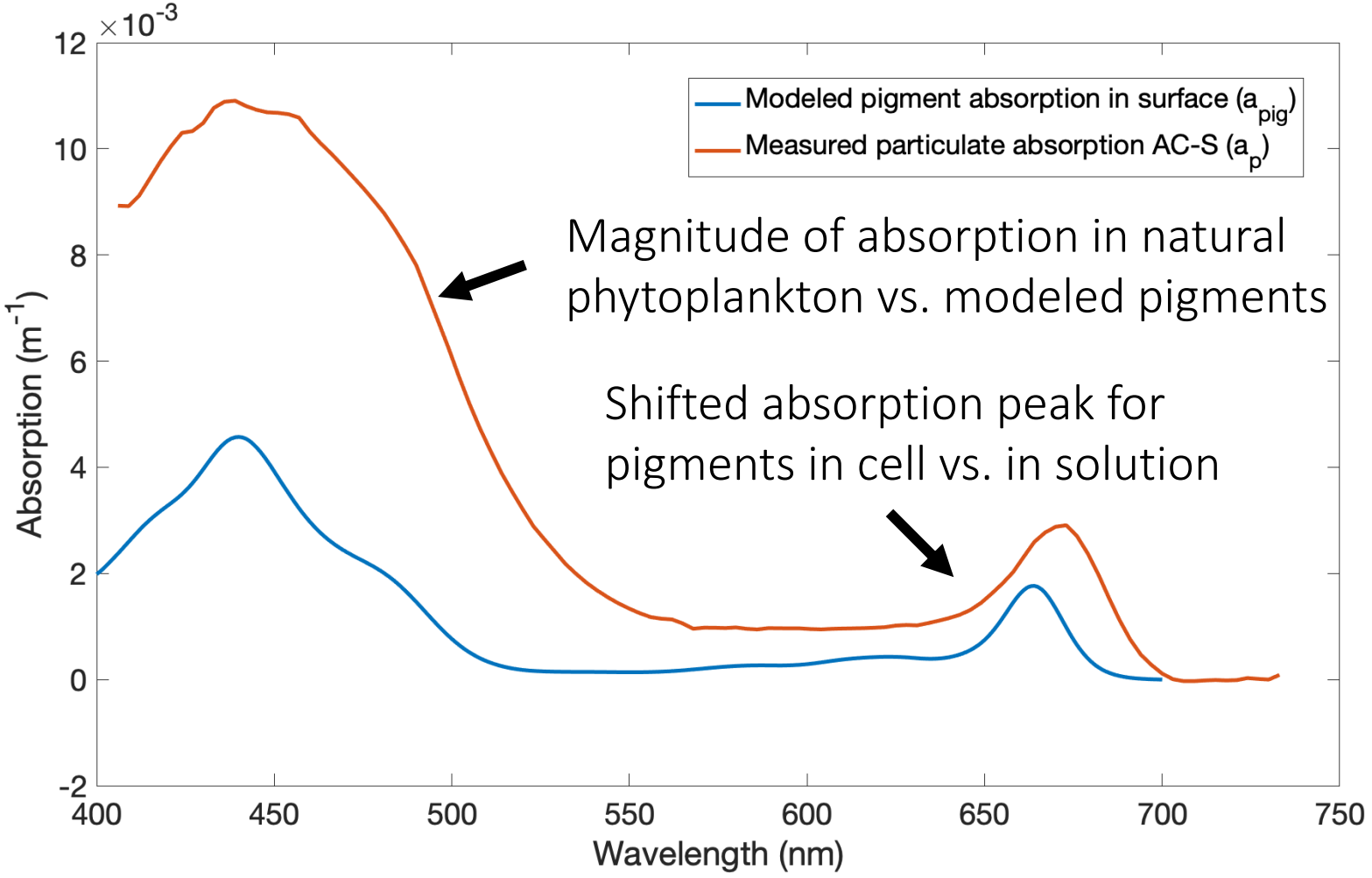
Package effect = pigments in a chloroplast in a cell absorb light differently than extracted pigments in solvent…



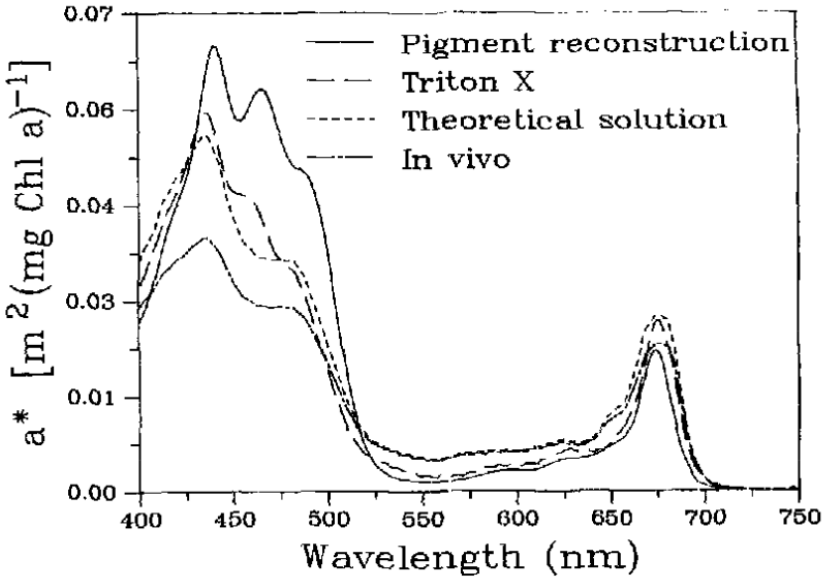
Collin Roesler

Also impacted by cell size. When pathlength through the cell increases, absorption properties change.

Comparing AC-S a_p to modeled a_{pig} highlights challenges



Example measured AC-S and modeled a_{pig} spectra from Tara Oceans (surface sample from oligotrophic Pacific)



Sosik and Mitchell, 1991

(Further methods for "stretching"/ correcting pure pigment absorption spectra in Sosik and Mitchell, 1995)

Don’t forget to keep track of units!!! By Dr. Ali Chase

Absolute

- Concentrations (cells/L)
- Biovolume concentration (mg/m^3)
- Biomass or carbon per volume (mg/m^3)
- Pigment concentration ($\mu g/L$, mg/m^3)

Relative (compositional)

- Fraction (%) of total chlorophyll-*a*
- Fraction (%) of total cell biovolume concentration
- Relative sequence abundance
- Fraction of some subset of the total community (e.g., % of all microplankton) - “dominant” group (in what units?)

Probability of occurrence (at some threshold?)

So, now that you have more familiarity with common methods for describing phytoplankton community composition in situ, let’s practice choosing the right tool for the job…

Case Study 1

Coastal observatory with easy access by boat

Seasonal HABs formed by dinoflagellates are an issue

Active fishery (needs timely warnings/early alert)

Bowdoin

Case Study 2

OSU

Open ocean site with monthly time series sampling

Interested in characterizing seasonal succession of *all* phytoplankton

Budget is no issue!

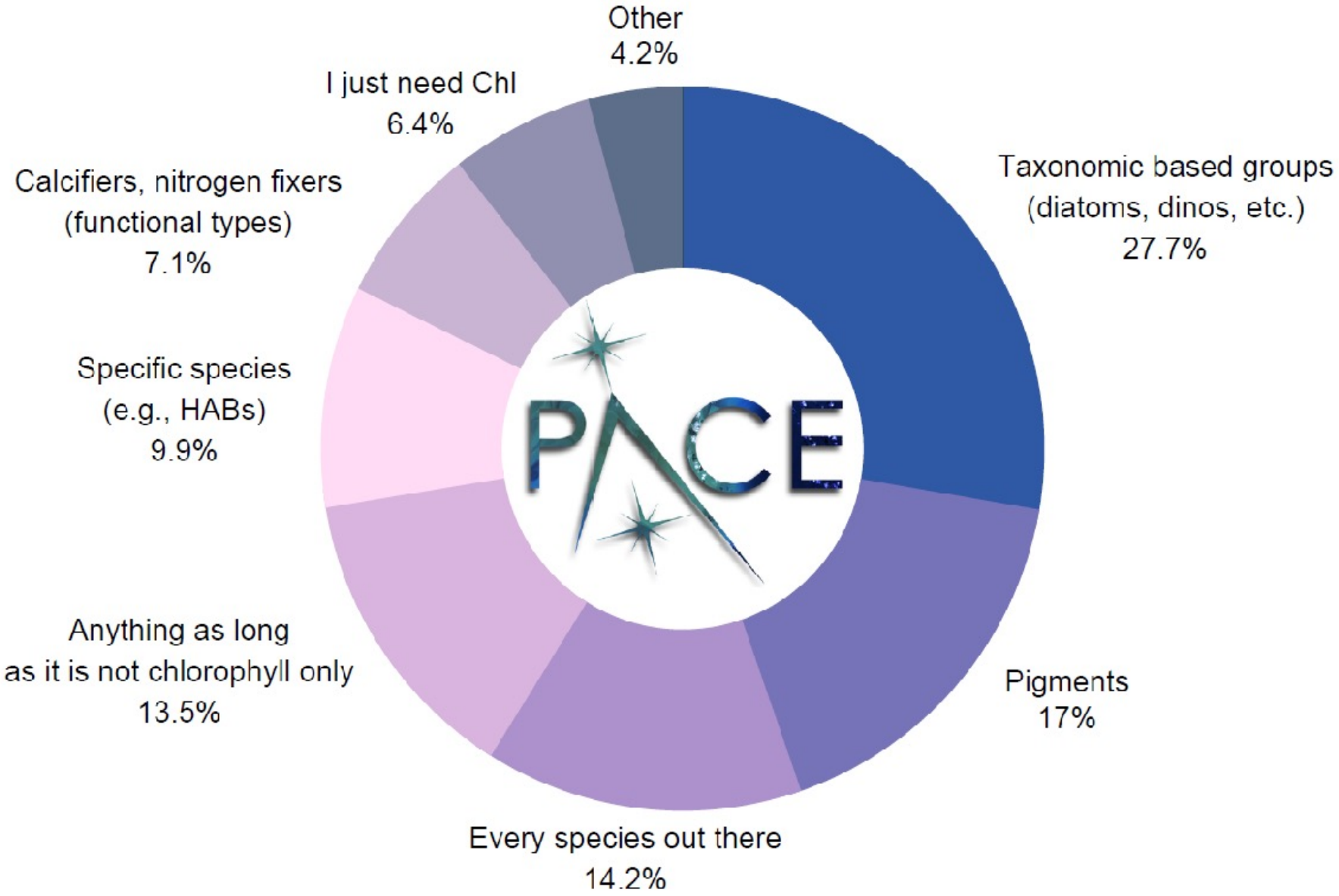
Case Study 3

Month-long research cruise to dynamic high-latitude site

Want to understand links between phytoplankton taxonomy and carbon export

Need to develop remote sensing data products, too!

Results of the PACE user survey (Cetinić et al.)



Results of the PACE user survey (Cetinić et al.)

The strongest PCC models for the global ocean will combine high-resolution taxonomic information with remotely-sensible variables.

Combining in water methods shows us the strengths and limitations of individual approaches – any models we develop for ocean color remote sensing will need similar validation, calibration, and confirmation!!