SMS 598: Calibration and Validation for Ocean Color Remote Sensing

Lecture 4 – Phytoplankton

What are phytoplankton?
How are phytoplankton assessed?
Why are phytoplankton important?

Mary Jane Perry, UMaine
9 July 2013

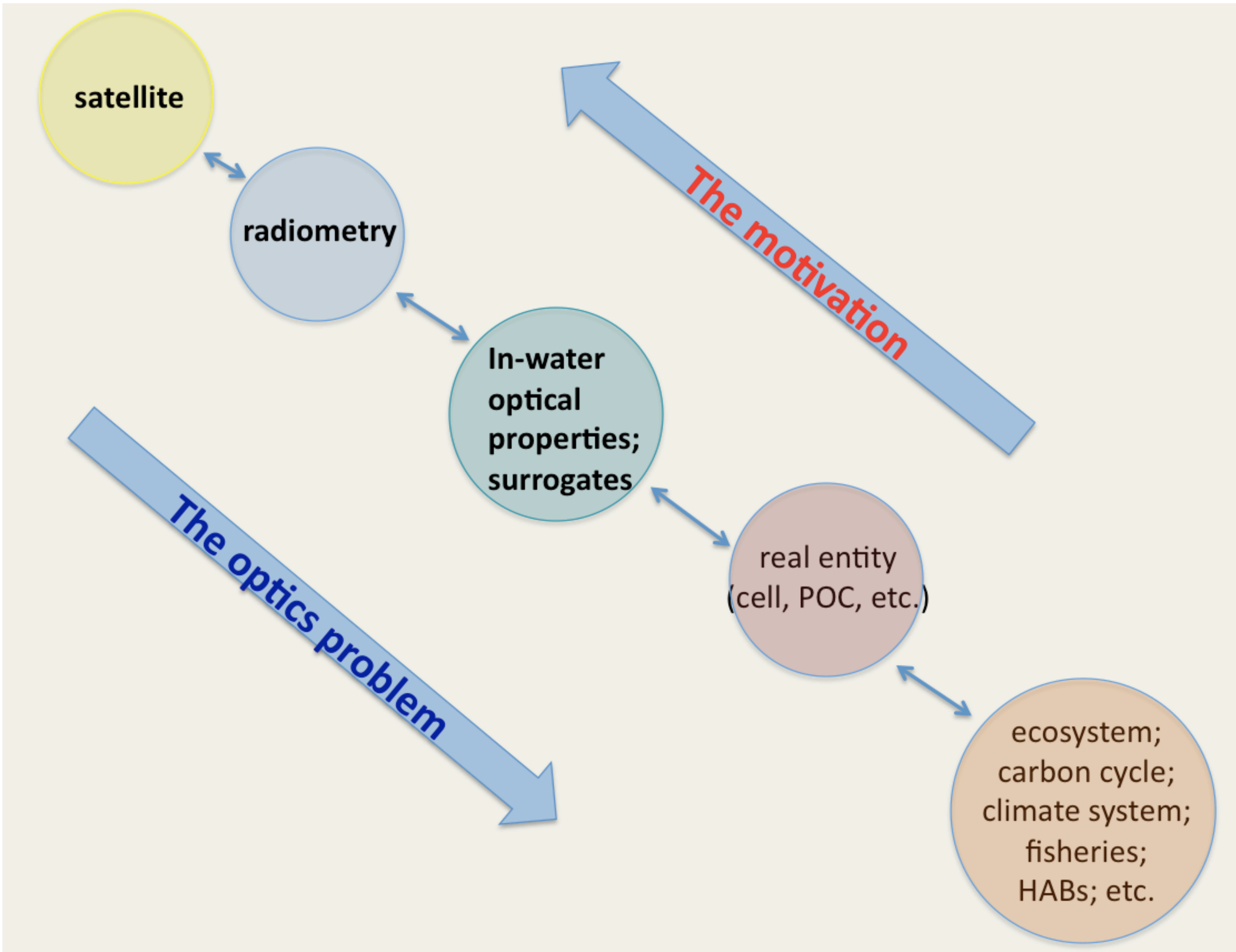
On the piece of paper provided,
answer these three questions:

What are phytoplankton? (one sentence max)

How are phytoplankton assessed? (top approach)

Why are phytoplankton important? (top reason)

Your interest or science question may determine your answers.
(Ken Carder would say, the question determines your 'look angle'.)



Optics to study biogeochemistry

Variable interacts with light

↘ Volts or Counts

↘ IOP or AOP

↘ Proxy

↘ Variable –> the real entity

(so what is a phytoplankton anyway?)

What are phytoplankton?

Aerobic (oxygenated environment)
Photosynthetic (pigmented)
Oxygenic (oxygen producing; use sunlight)
Small, single-celled **particle** (usually) but some form chains)
Not all round and uniform (limitation for Mie modeling)

Three Super Kingdoms (phytoplankton in two; most are NOT plants)

Eukaryotes → Internal organelles

Eubacteria, Archaea → Prokaryotes (no nucleus or chloroplast) single cells and consortia

Bottom line:
great genetic <u>diversity</u> of organisms that interact with light in the ocean

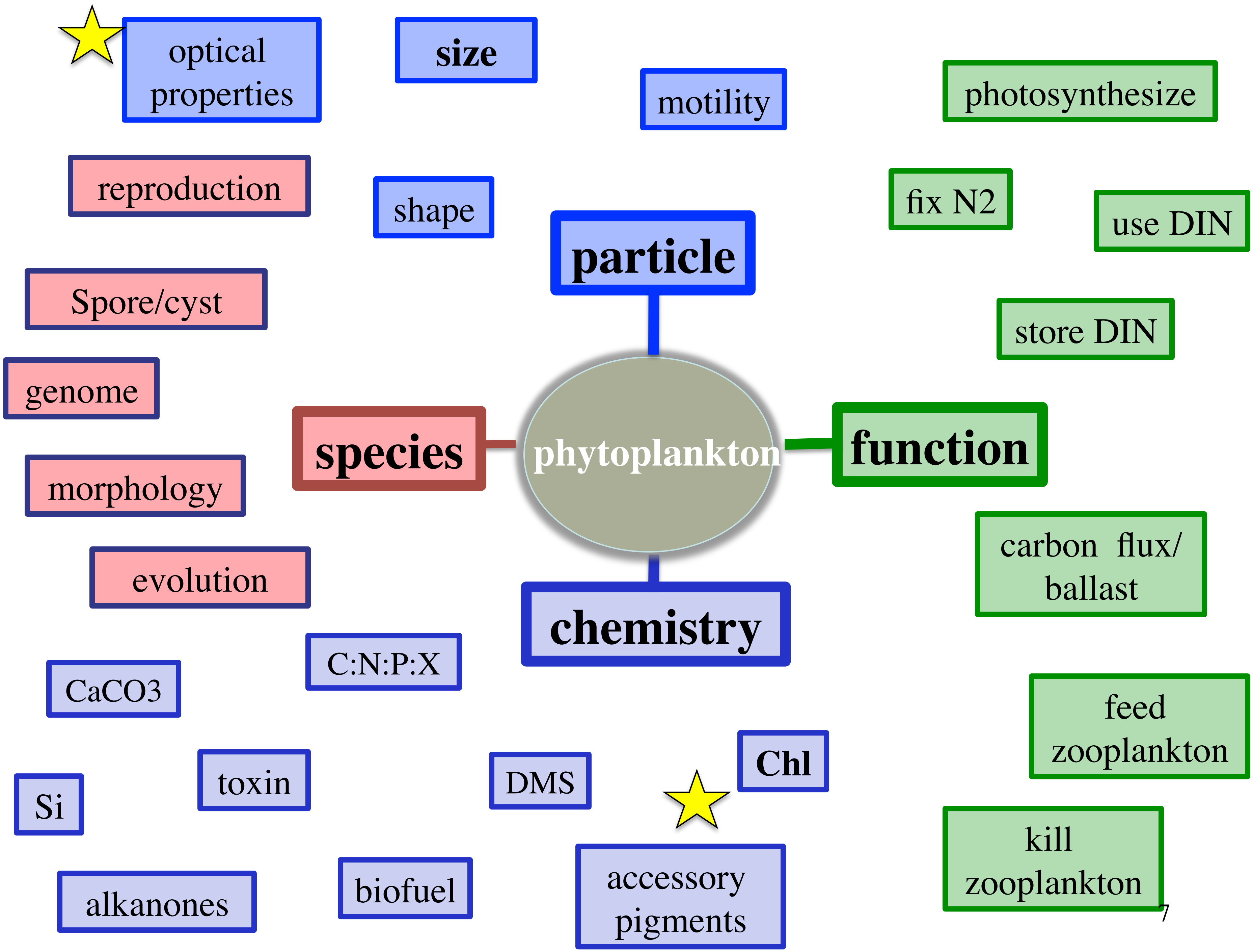
(See Keeling et al. 2004, Science 306: 2191, endosymbiotic evolution)

This lecture:

1. Introduce you first to phytoplankton,
   and a little bit about their role in the ocean

2. Phytoplankton interactions with light are basis for optical proxies
   – particles scatter light
   – pigments absorb light
   – chlorophyll *a* and phycoerythrin fluoresce light

3. Physiology can change the relationship between
   phytoplankton and some of their optical proxies
   (plasticity is intrinsic to their survival, potential annoyance to us)

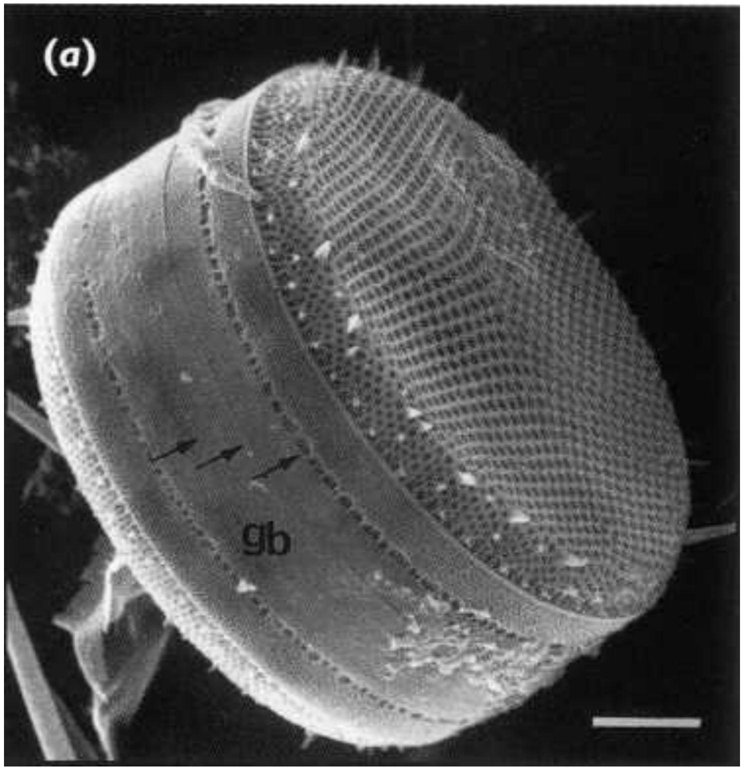

optical properties
size
motility
photosynthesize
reproduction
shape
fix N2
use DIN
particle
Spore/cyst
store DIN
genome
species
phytoplankton
function
morphology
carbon flux/ ballast
evolution
chemistry
C:N:P:X
CaCO3
feed zooplankton
Chl
toxin
DMS
Si
kill zooplankton
accessory pigments
alkanones
biofuel

Phytoplankton as species (or taxa) –when does knowing species matter?

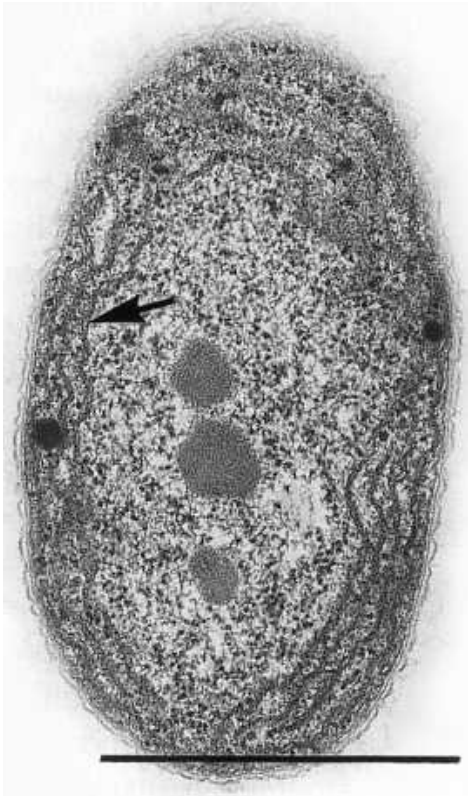
Eubacteria – **cyanobacteria** (aerobic, oxygenic, autotrophic eubacteria)

also aerobic, anoxygenic species and anaerobic, anoxygenic (sulfur bacteria)

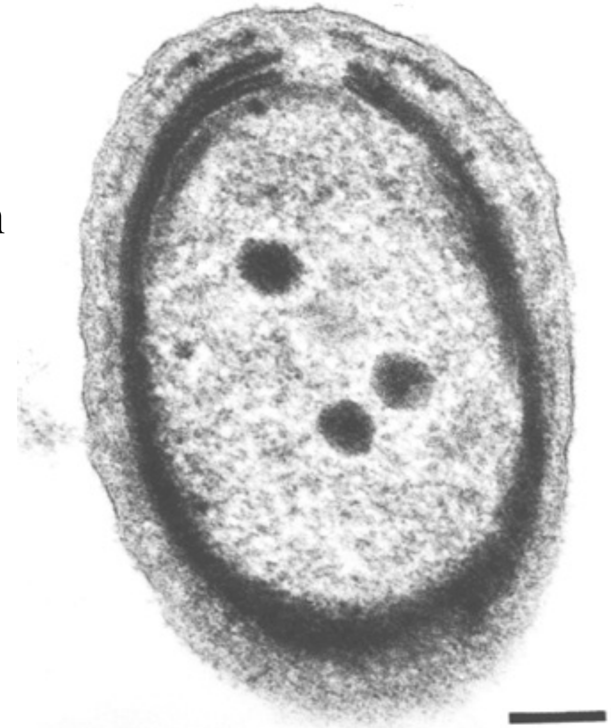
Eukaryotes – **protists** (very diverse) and **chlorophytes** (closer to land plants)



Morphology
to characterize species (here, diatom frustule structure)



electron micrographs of
prokaryotic phytoplankton

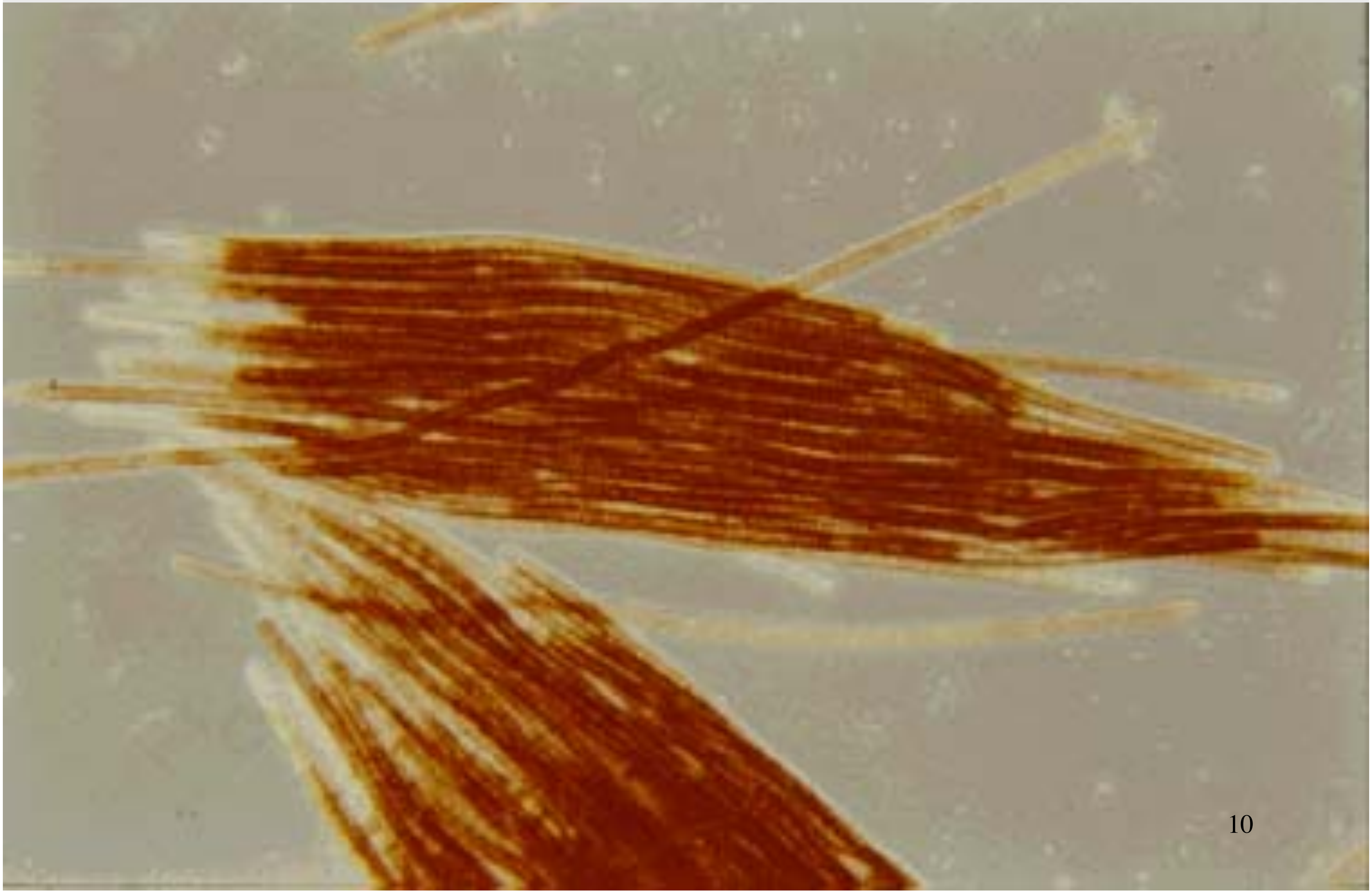


Electron micrograph of a longitudinal section of Prochlorococcus (isolate MIT 9313). Tightly appressed intracytoplasmic lamellae are present near the cell periphery, and carboxysomes are visible within the cell interior. Scale bar, 0.1mm. (C. Ting, J. King, S.W. Chisholm, 1999)

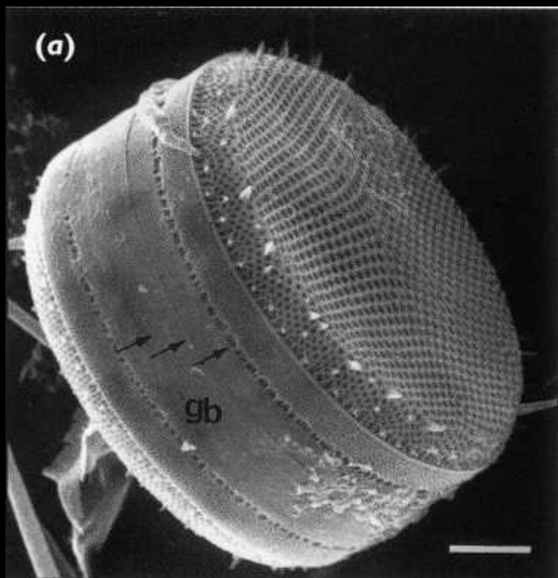
Synechococcus (~ 1 μm)
Arrow denotes thylakoid membrane which has both photosynthetic and respiratory functions.
Diagnostic: phycoerthyrin pigment fluoresces orange (in contrast to chlorophyll, which fluoresces red.

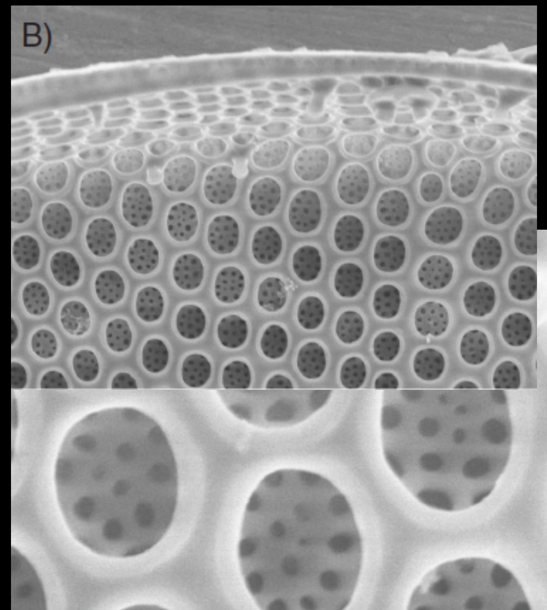
Prochlococcus (~ 0.7 μm)
Diagnostic: very small size, lack of orange fluorescence, divinyl chlorophyll a & b. Found only in tropics/subtropics.

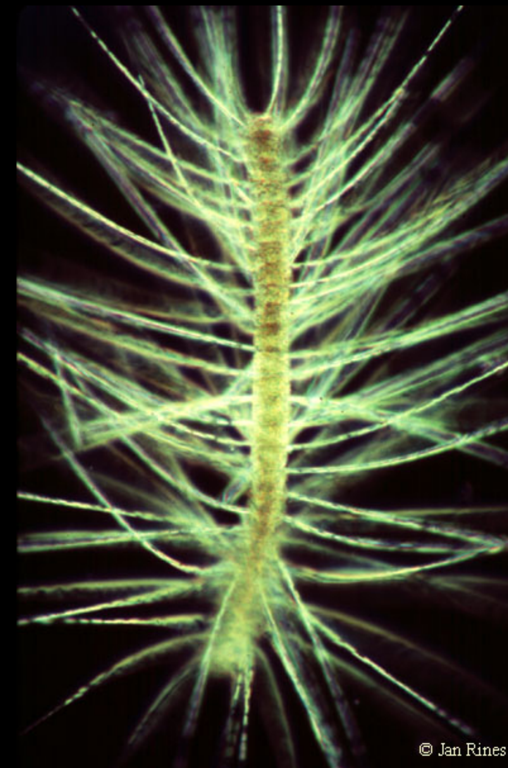
Trichodesmium (cyanobacterial nitrogen fixer; warm waters; patchy; Fe may regulate abundance)



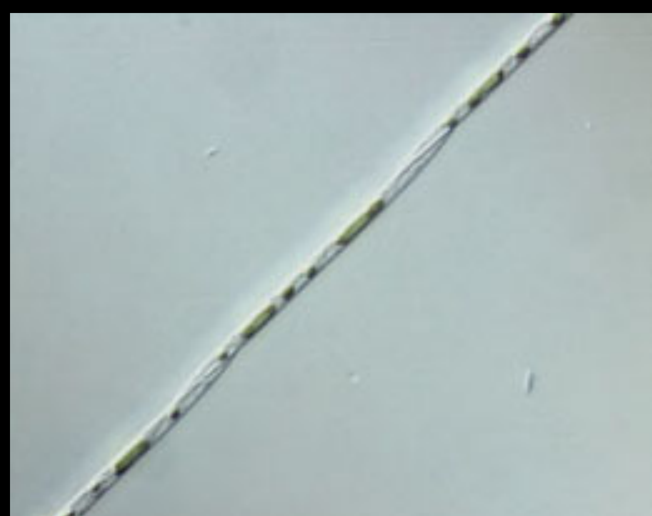
Centric Diatoms, single cell *Thalassiosira* and chained *Chaetoceros*







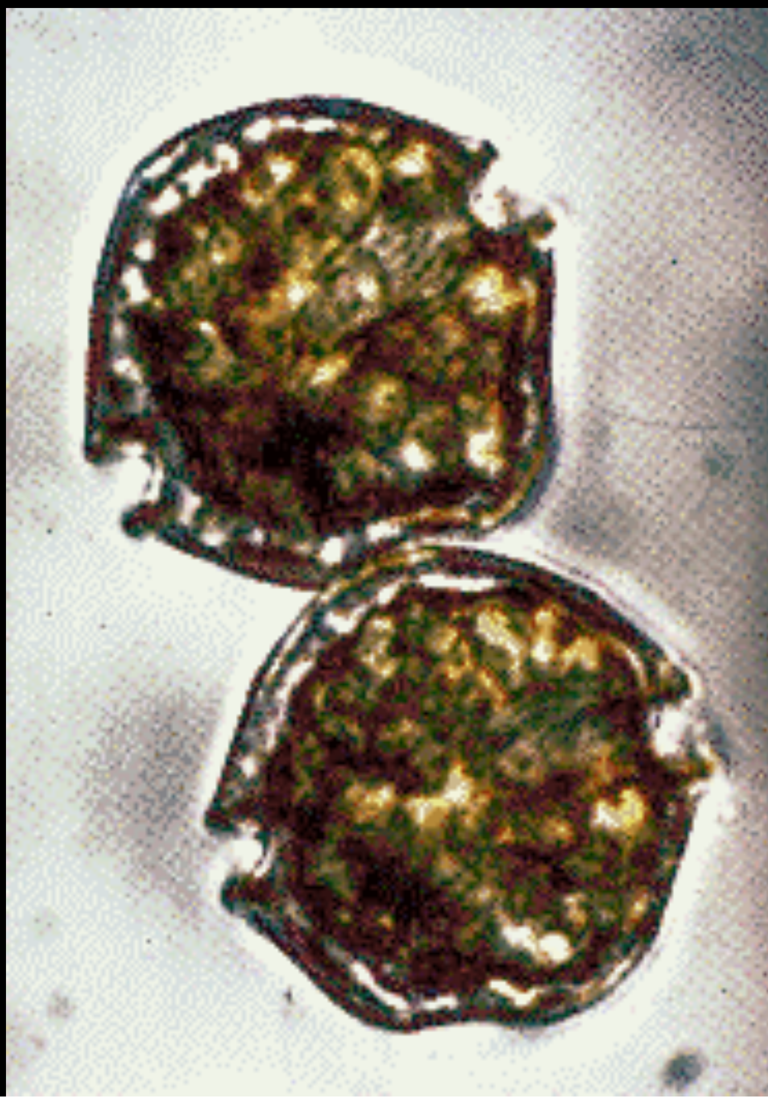
SEM – Coscinodiscus Townley et al. 2008. Adv. Funct. Mat. 18: 369.



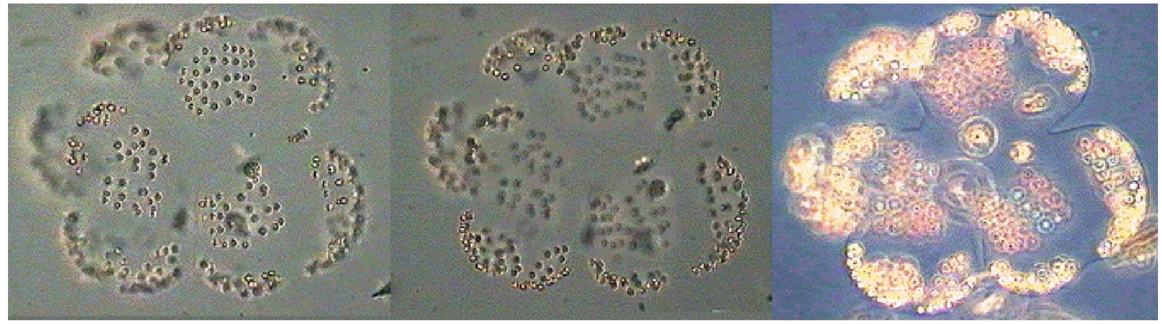
Pennate, *Pseudonitzschia* (some species have domoic acid) http://vimeo.com/33031310

Dinoflagellates

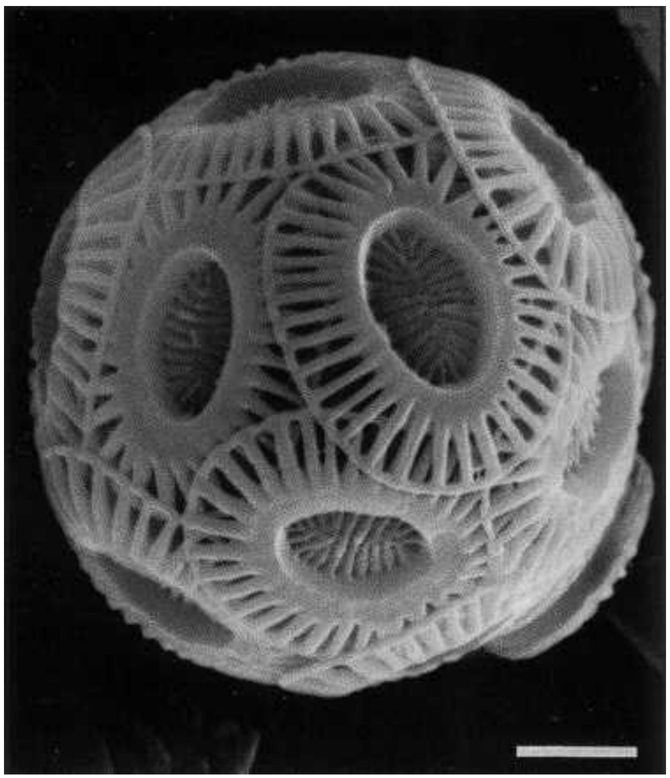
Alexandrium tamarense

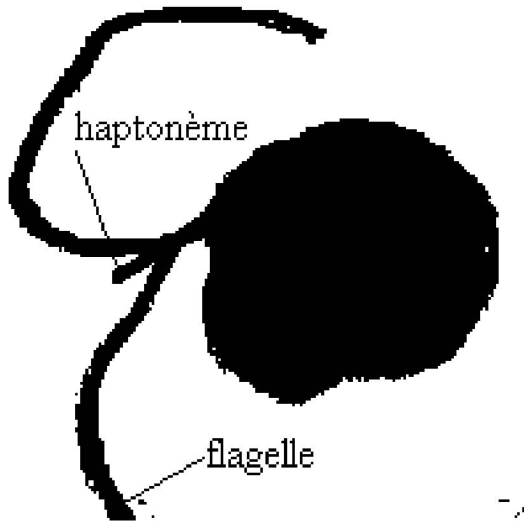


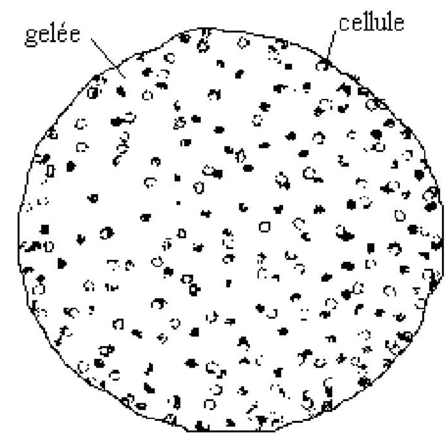
Ceratium



Coccolithophorid, with calcite plates (coccoliths); blooms visible from space





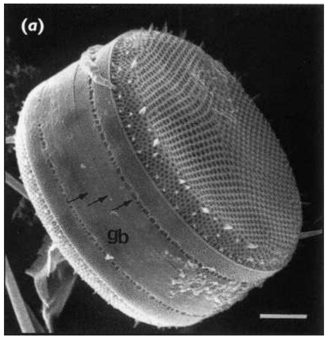


Phaeocystis
(colonical and single cell)
famous for producing foam on northern European beaches

Most small (pico phytoplankton) look like this

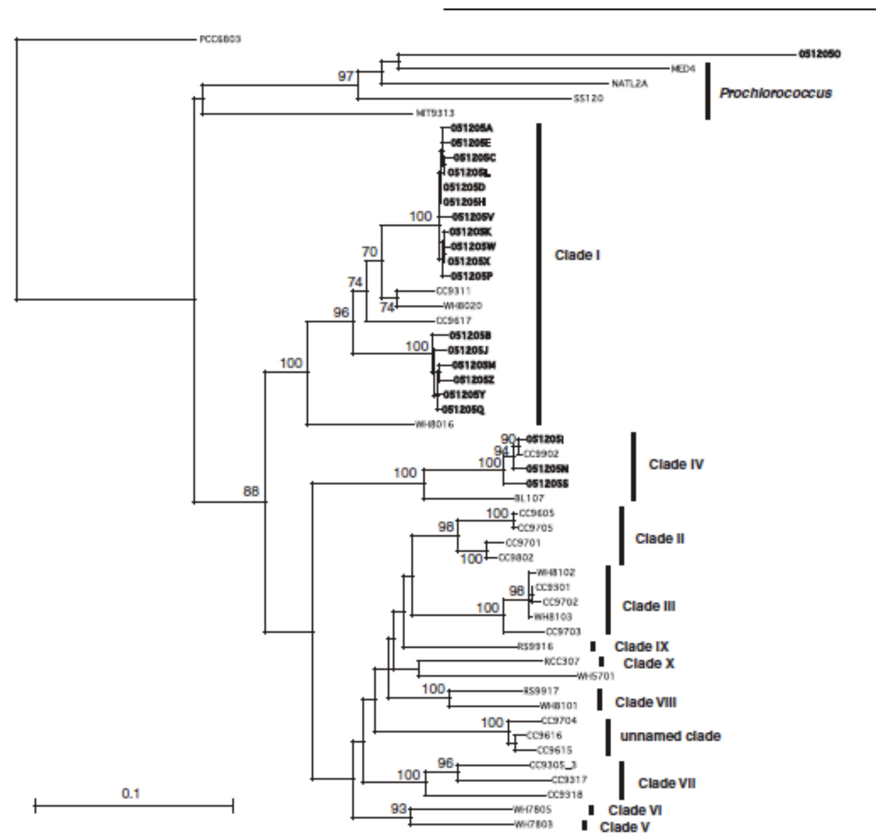
– small and non-descript under microscope

Phytoplankton species (or taxa)



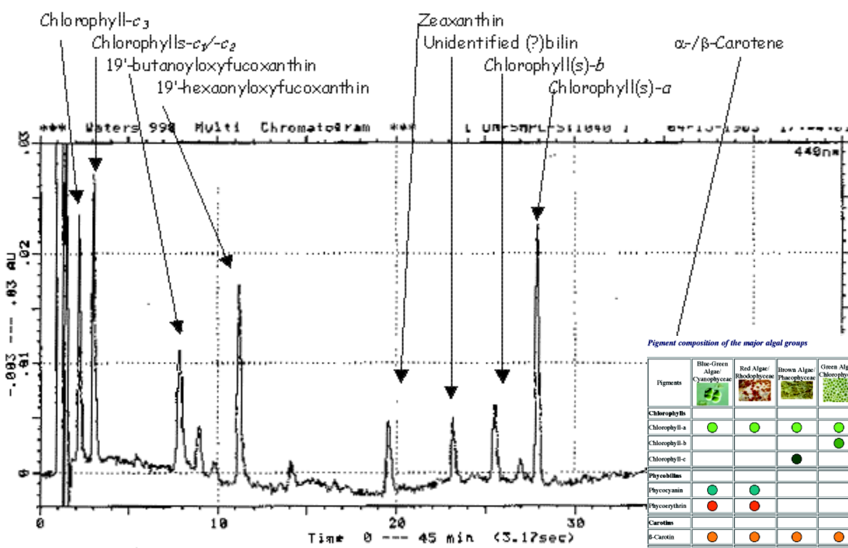
Morphology
to characterize species
(diatom frustule
structure)

Molecular characterization
(clades of *Synechococcus*)



Tai & Palenik, 2009, ISME 3: 908

HPLC pigment clustering & Chemtax
(some cautions here)



Pigment composition of the major algal groups

Pigments	Blue-Green Algae/ Cyanophyceae	Red Algae/ Rhodophyceae	Brown Algae/ Phaeophyceae	Green Algae/ Chlorophyceae	Dinoflagellates/ Dinophyceae	Diatoms/ Bacillariophyceae	Naked Flagellates
Chlorophylls							
Chlorophyll-a	●	●	●	●	●	●	●
Chlorophyll-b				●			
Chlorophyll-c			●		●	●	●
Phycobilins							
Phycocyanin	●	●					
Phycoerythrin	●	●					
Carotins							
ß-Carotin	●	●	●	●	●	●	●
Xanthophylls							
Diadinoxanthin			•		•	●	•
Fucoxanthin			●		•	●	●
Lutein		●		●			
Peridinin					●		
Alloxanthin							●
Zeaxanthin	●	●	•	●			

Phytoplankton as particles

– in the ocean, **size matters** and is related to function

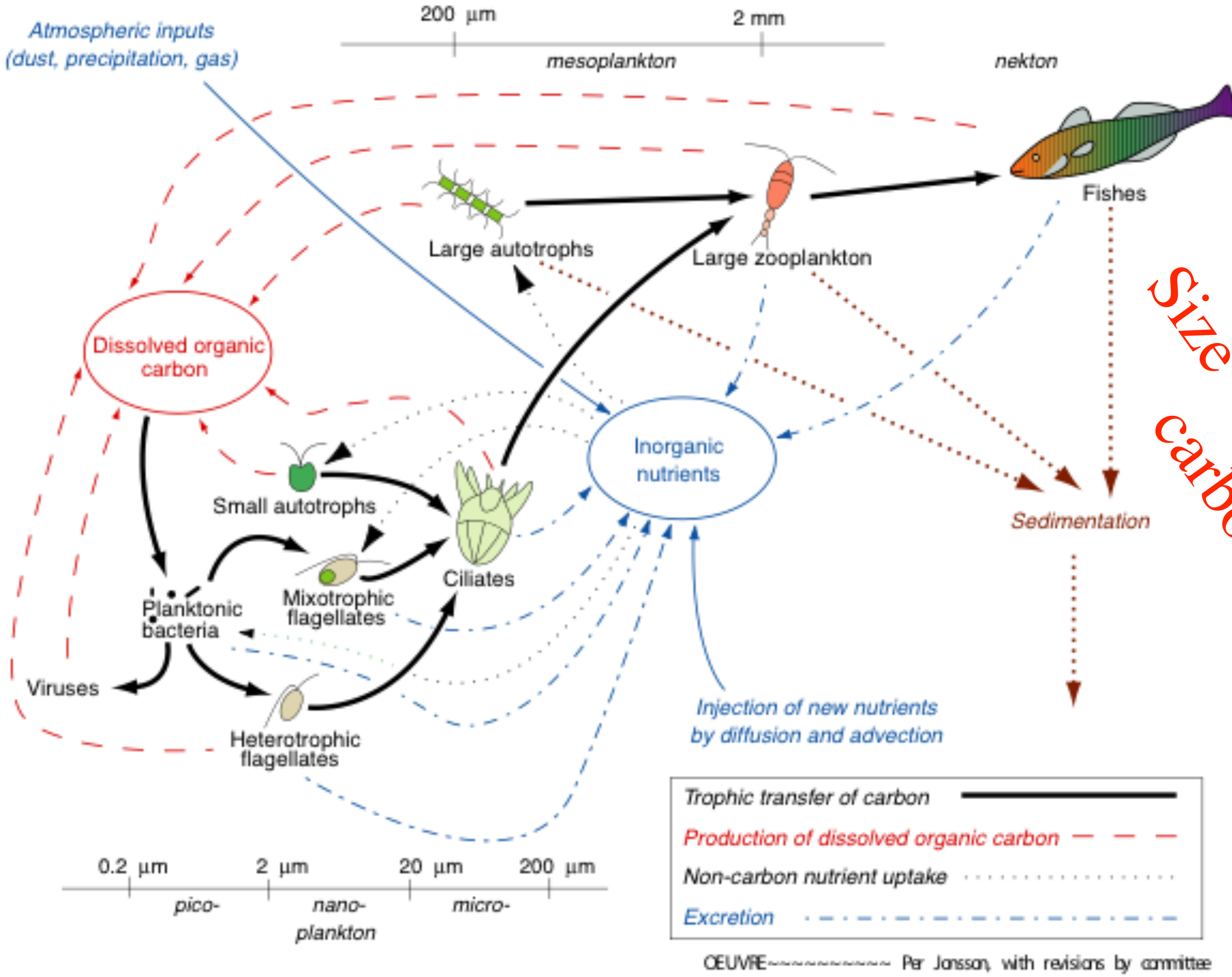
historical nomenclature:

net $> 20\mu m$ nano $< 20\mu m$

pico $< 5\mu m$ ultra $< 2\mu m$ (smallest mostly prokaryotes)

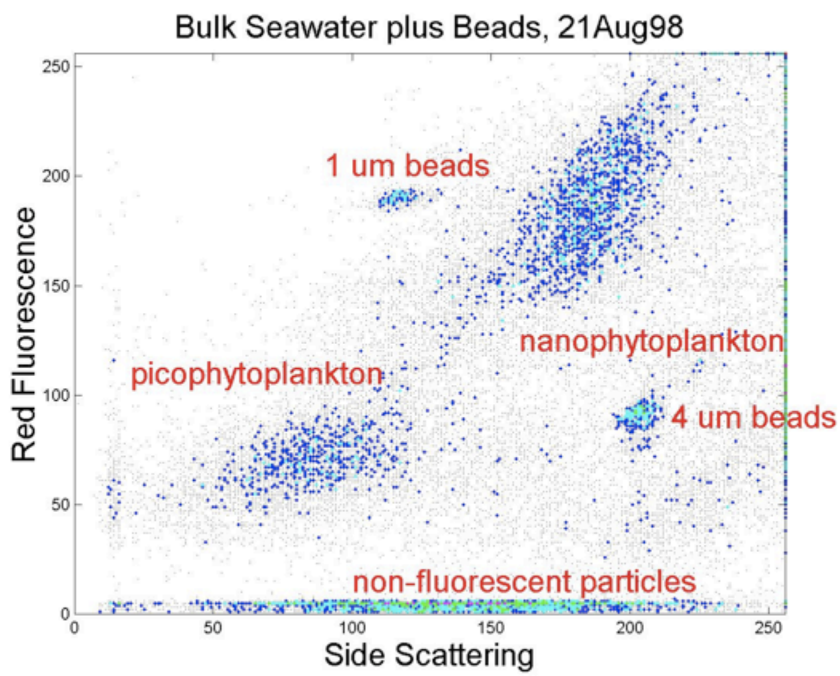
Size

* small cells are mostly spherical; larger cells often non-spherical
* efficiency of dissolved solute capture (diffusion – smaller cells better)
* efficiency of encounter – surface area for contact
* efficiency of aggregation increases with size
* settling – Stokes Law (carbon cycling – small cells don't sink)
* exposure to light (packaging; a*) and UV damage greater for small cells
* carbon content – cell carbon density higher for small cells
* metabolic rates scale to size (specific rate decreases with increasing size)
* **size determines carbon export efficiency** (number of trophic interactions)

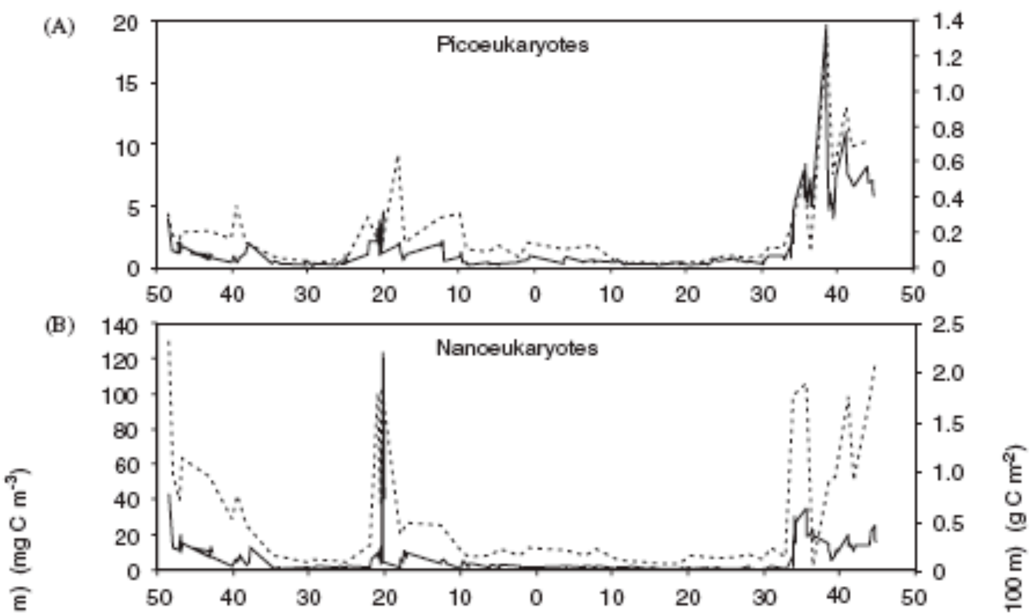


Size ➔ food webs; carbon cycling

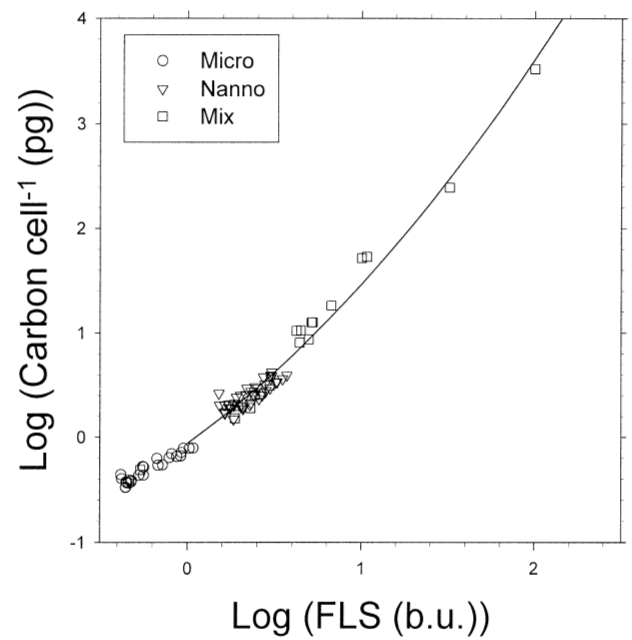
. community consensus view; details and color at <http://www.joss.ucar.edu/joss_psg/project/oce_workshop/oeuvre/report/>



Common way to measure picoeukaryotes, as well as determine their carbon, is by flow cytometry – measures single particle (chl and PE fluorescence and FS optical size)



Tarran et al. 2006 DSR II 53: 1516



DuRand 2002 J. Phycol. 39: 1132

Phytoplankton as functional types:

Functional type

- autotrophic, oxygenic, oxygen evolving
- size and shape (previous comments….)
- transformer of specific nutrient (N_2 fixer, $CaCO_3$ 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 ??

Chemical composition – relates to function, species, etc. For optics, pigments are key (& sometimes unique) chemicals. But it C we really want?

This lecture:

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   and a little bit about their role in the ocean

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3. Physiology can change the relationship between
   phytoplankton and some of their optical proxies

   (plasticity is intrinsic to their survival, potential annoyance to us)

How are we going to measure phytoplankon?

Count them – microscope, flow cytometer/FlowCAM/imaging

Gene sequence them – presence/absent or not yet quantitative

Optics – related to absorption (unique), scattering (no unique), fluorescence (unique).

Historically – various measures related to **chlorophyll** used as proxies for phytoplankton mass (but what do we really want ???)

Chlorophyll *a* – most common entity used to denote presence of phytoplankton and attempt to quantify concentration (mass).

Term 'chlorophyll' biomass often used – anathema to some.

Is chlorophyll a perfect proxy for phytoplankton? Yes / No

Chlorophyll a (or divinyl Chl a) is found in all phytoplankton and not in heterotrophs (exception: mixotrophs, digesting predators).

Relationship between carbon and Chl allows estimation of phytoplankton carbon. But beware Mobley's Law of Conservation of Misery; C/Chl ratio influenced by physiology.

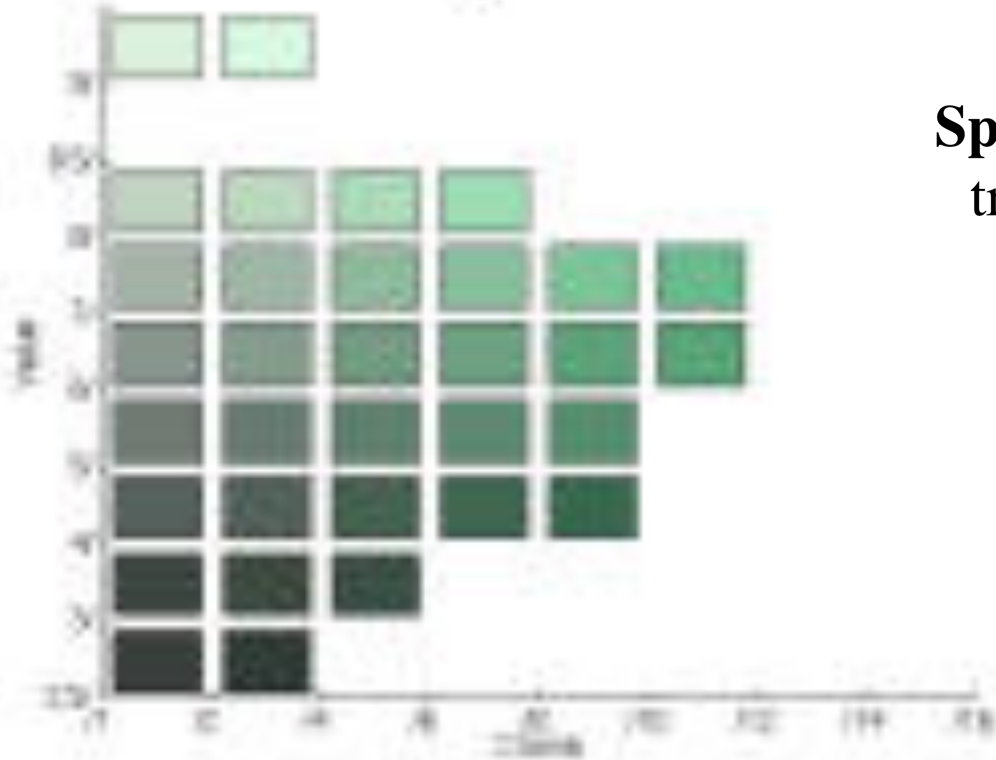
Some measure of assessing chlorophyll can be used at all scales – from drop of water, ship, mooring, autonomous platform, satellite.

Different measures of assessing 'chlorophyll' need to be aligned; not measuring exactly same thing.

Brief history of measurement of ‘bulk’ chlorophyll & related entities

Harvey Plant Pigment Unit (**HPPU**) - up to ~ 1950

– standardized color on filters (Munsell chart); eyeball reflectance measurements. Still used for soils and tobacco.

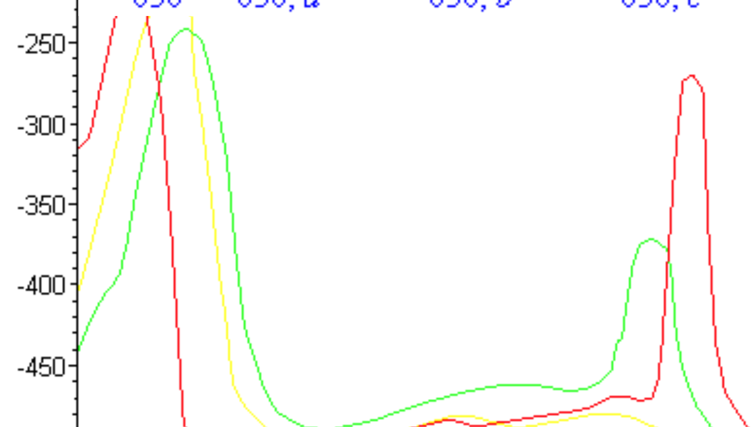


Spectrophotometry, extracts in solvent; trichromatic eq. to separate pigments. ~ 1950’s – 1960’s

$$OD_{664} = \varepsilon_{664,a}\, a\, L + \varepsilon_{664,b}\, b\, L + \varepsilon_{664,c}\, c\, L$$

$$OD_{647} = \varepsilon_{647,a}\, a\, L + \varepsilon_{647,b}\, b\, L + \varepsilon_{647,c}\, c\, L$$

$$OD_{630} = \varepsilon_{630,a}\, a\, L + \varepsilon_{630,b}\, b\, L + \varepsilon_{630,c}\, c\, L$$



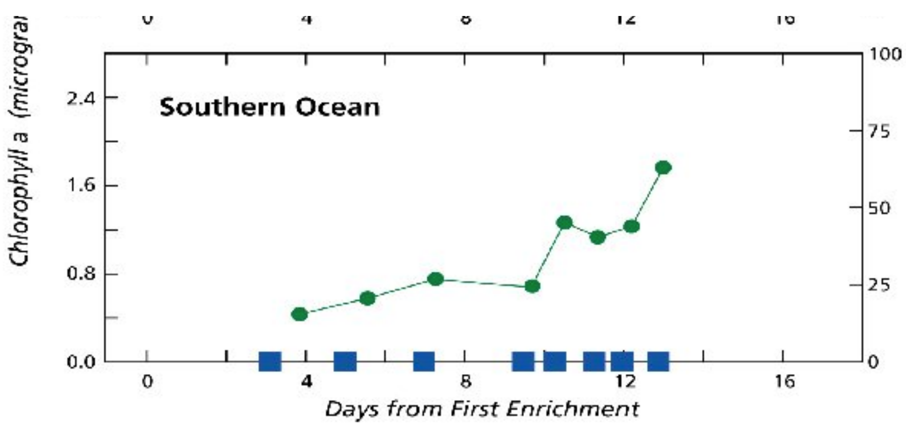
Brief history of measurement of ‘bulk’ chlorophyll & related entities

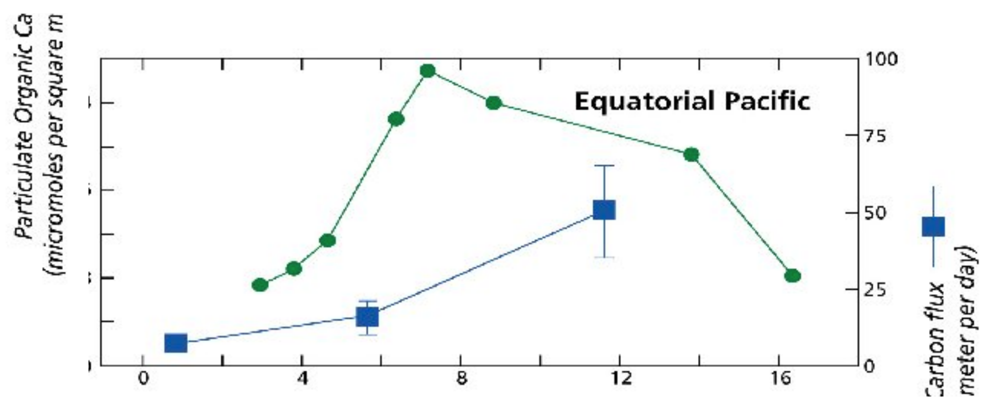
Early 1960’s, **solvent extracts** of filtered water samples, measured by **fluorescence**.

Attraction was it is reasonably fast.

Still benchmark method. (Mobley’s Conservation of Misery)

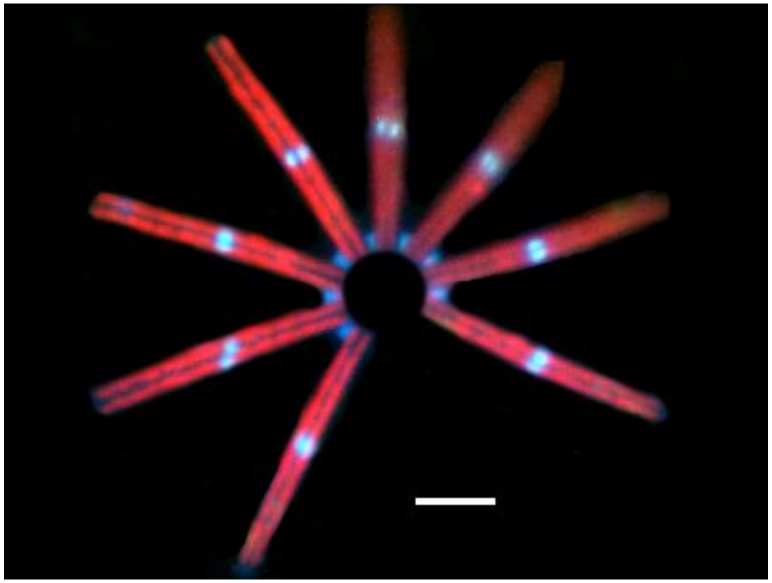
Lots of good information. For example: phytoplankton response to iron-fertilization; Chl *a* (μg L^{-1}) provided an index of bulk phytoplankton response: Southern Ocean vs. Equatorial Pacific.





http://cafethorium.whoi.edu/Fe/1999-Annualreport.html

Brief history of measurement of 'bulk' chlorophyll & related entities



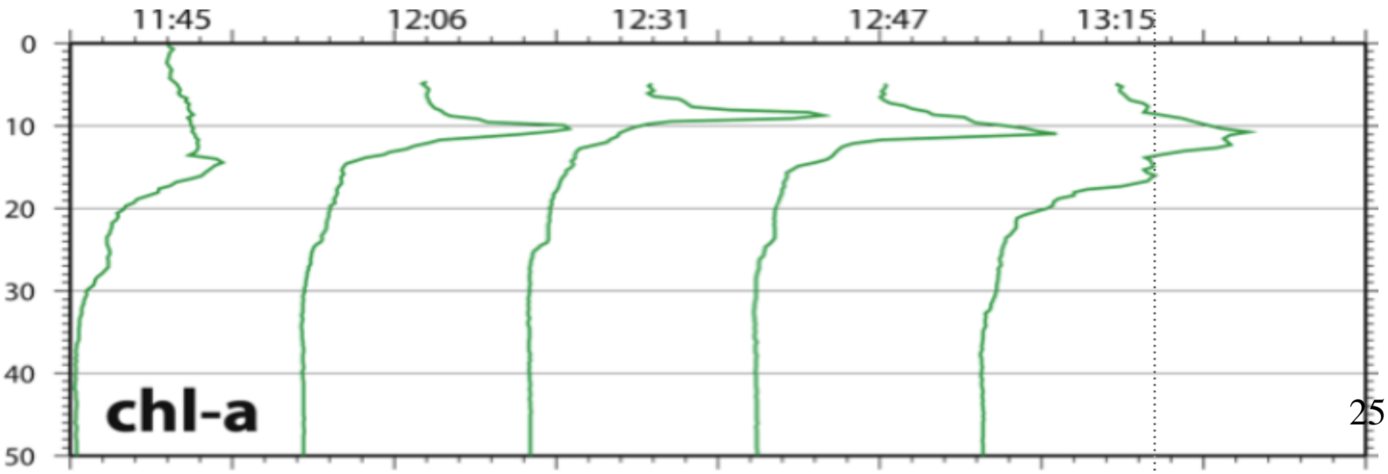
Late 1960's, **fluorescence profiles of fluorescence in living cells** – measure directly in the ocean.

Fast! and high vertical resolution.

(Mobley's Conservation of Misery)

Used on CTD, mooring, floats, gliders, etc.

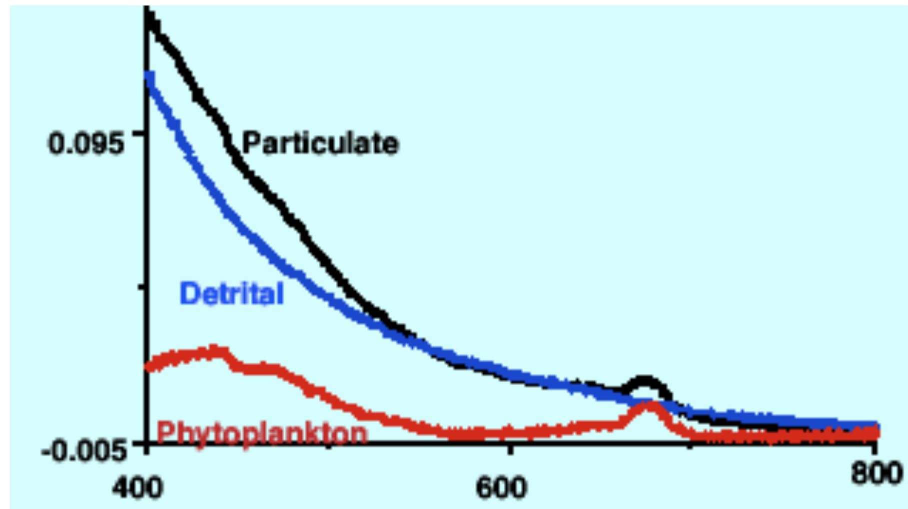
Example below of thin layers in Monterey Bay.

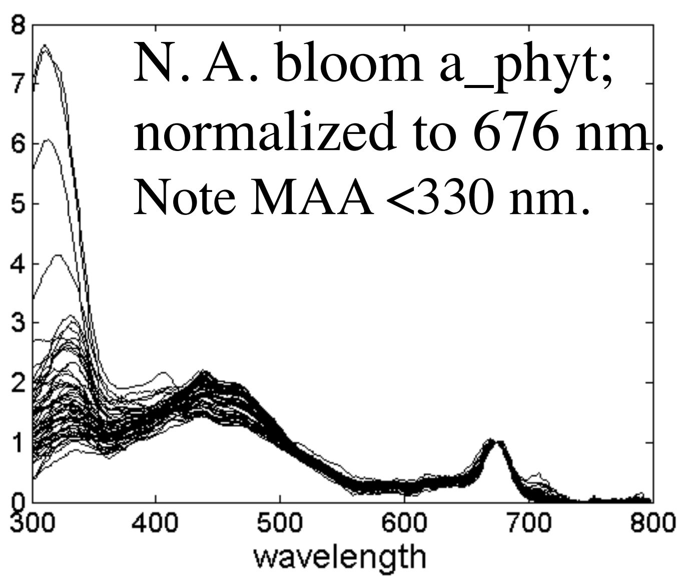


Brief history of measurement of ‘bulk’ chlorophyll & related entities

QFT – Quantitative Filter Technique (filter pad absorption) ~ 1980’s

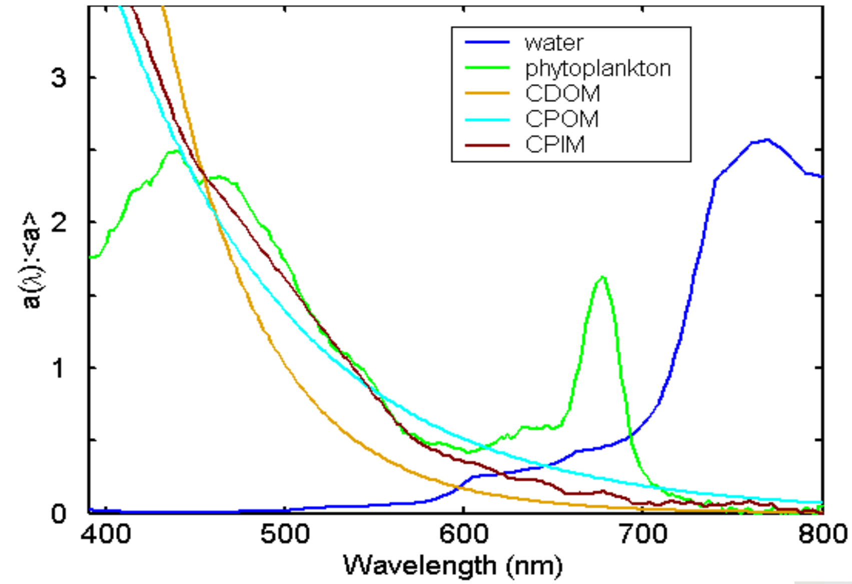
(Quantitative version of HPPU)





a_particulate
a_phytoplankton: a_PS and a_PP
a_NAP: not solvent extractable, e.g.,
a_mineral, a_dead stuff

Brief history of measurement of ‘bulk’ chlorophyll & related entities

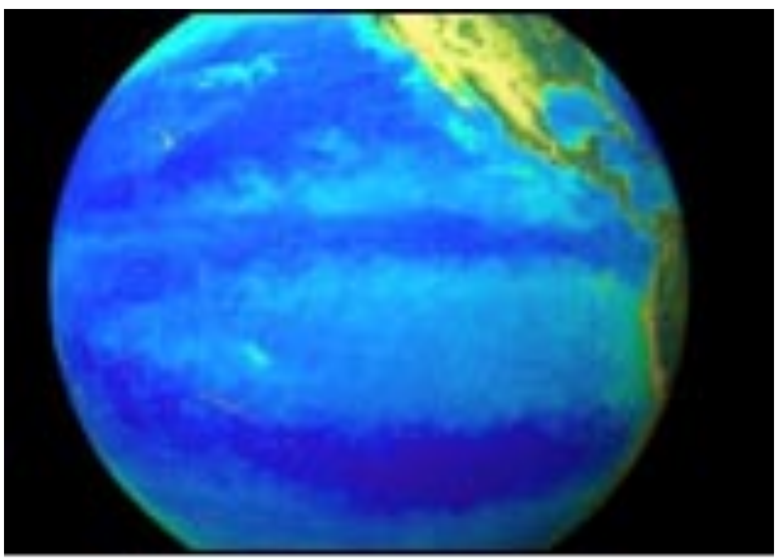


Remote sensing reflectance is based on selective absorption by phytoplankton pigments; empirical algorithms, need local tuning. ~ 1980’s

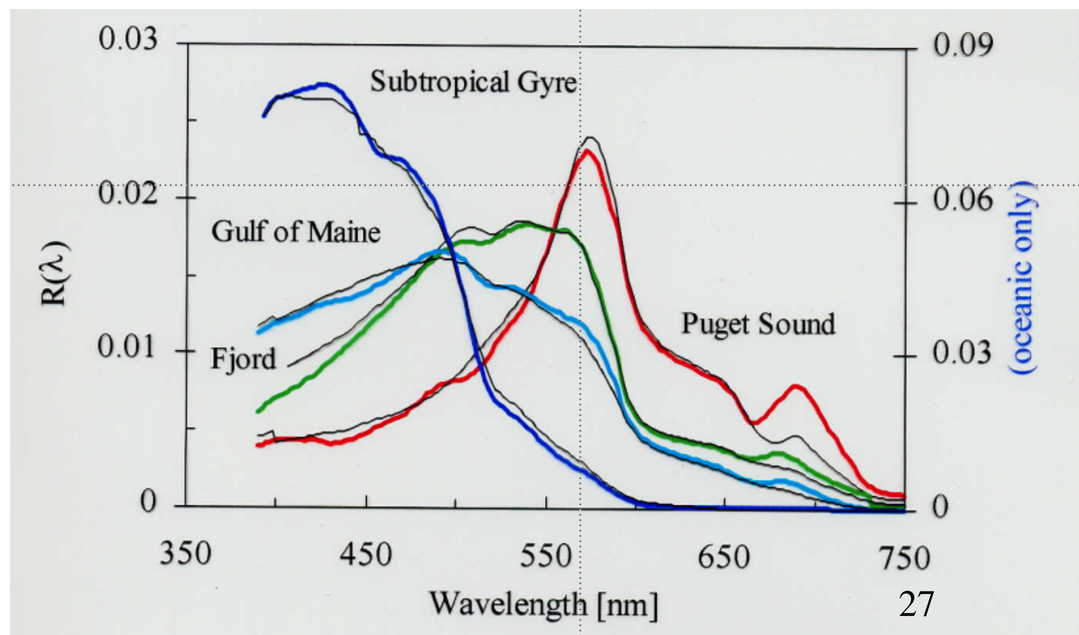
$$R_{rs} \sim [b_b / (a + b_b)]$$

a ~ phytoplankton (Chl? absorption?)

b_b ~ particles and carbon



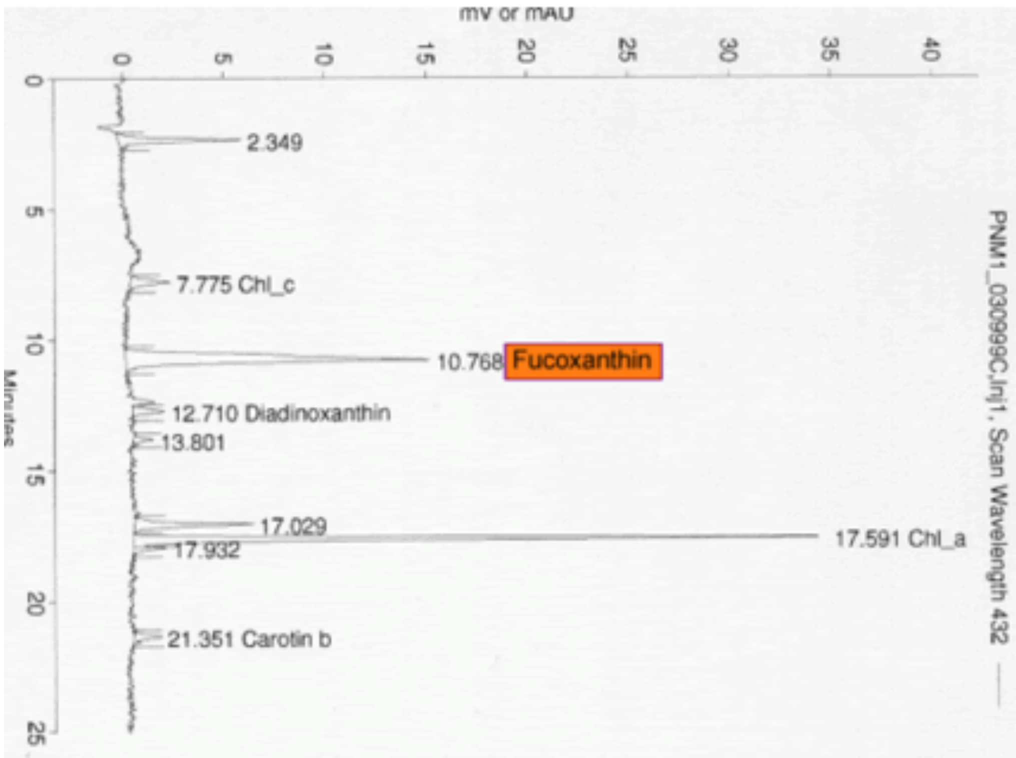
El Nino

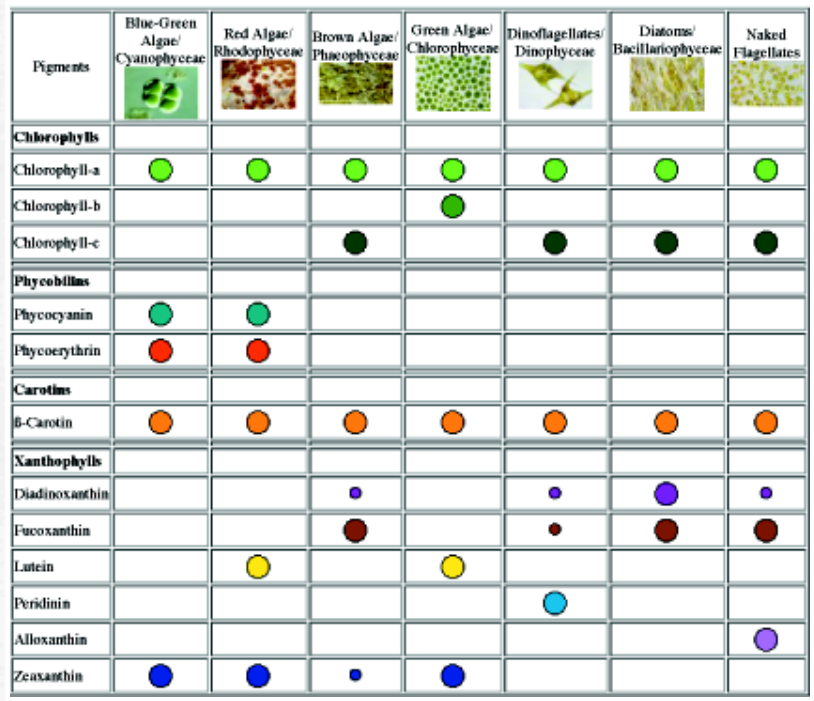


Roesler & Perry, 1995

Brief history of measurement of ‘bulk’ chlorophyll & related entities

HPLC pigments – resolve most of phytoplankton pigments. ~1990’s.
Chemtax – for taxonomic assessment (requires training).
Filter lots of water; sample ~ $80/





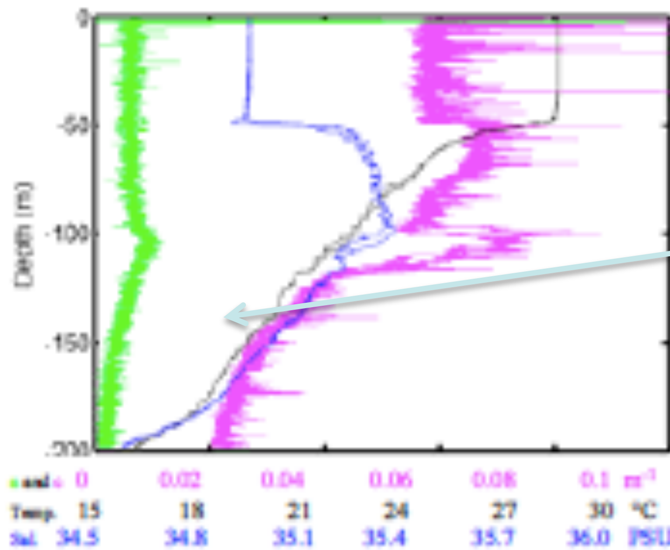
Pigment composition of the major algal groups

Pigments	Blue-Green Algae/ Cyanophyceae	Red Algae/ Rhodophyceae	Brown Algae/ Phaeophyceae	Green Algae/ Chlorophyceae	Dinoflagellates/ Dinophyceae	Diatoms/ Bacillariophyceae	Naked Flagellates
Chlorophylls							
Chlorophyll-a	●	●	●	●	●	●	●
Chlorophyll-b				●			
Chlorophyll-c			●		●	●	●
Phycobilins							
Phycocyanin	●	●					
Phycoerythrin	●	●					
Carotins							
ß-Carotin	●	●	●	●	●	●	●
Xanthophylls							
Diadinoxanthin			•		•	●	•
Fucoxanthin			●		•	●	●
Lutein		●		●			
Peridinin					●		
Alloxanthin							●
Zeaxanthin	●	●	•	●			

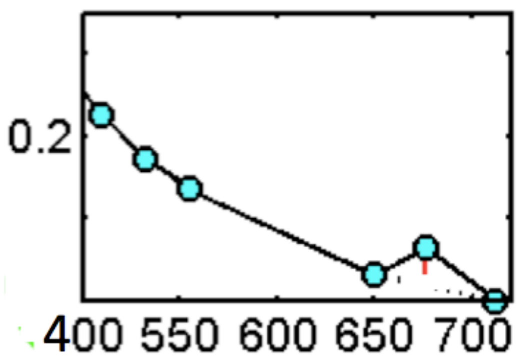
Quantitative version of trichromatic equations.
(Mobley’s Conservation of Misery – not all dinoflagellates have peridinin.Δ\
Used to ground truth satellite PFT algorithms.

Brief history of measurement of ‘bulk’ chlorophyll & related entities

ac-9 and ac-s - absorption and attenuation meters for profiles ~ 1990’s



a_phyt (676) is a good estimator of chlorophyll concentration in cell (Roesler leader in use & interpretation)



In-situ measurements demonstrate instrument stability and precision. Absorption (673nm, green line), Beam attenuation (650nm, magenta line), Temperature (black line) and Salinity (blue line) profiles taken at the Hawaii Ocean Time Series (HOTS) Aloha site near 22.75°N, 158°W (approximately 100 km north of Oahu, Hawaii) on August 11, 2004. The data were obtained during one down and up profile.

http://www.wetlabs.com/Research/presentations/ONR%20ac-s.pdf

Let's explore more pigments

Definition: absorbing compound

Role: light harvesting for photosynthesis (PS – photosynthetic)
light protection if too much light (PP – photoprotective)

Types:

chlorophylls

chlorophyll *a* - primary PS pigment in all oxygen producers
chlorophyll *b* or *c* - accessory PS pigments; expand λ range; transfer energy to chlorophyll *a*
(divinyl chl *a* and *b* in *Prochlorococcus*)

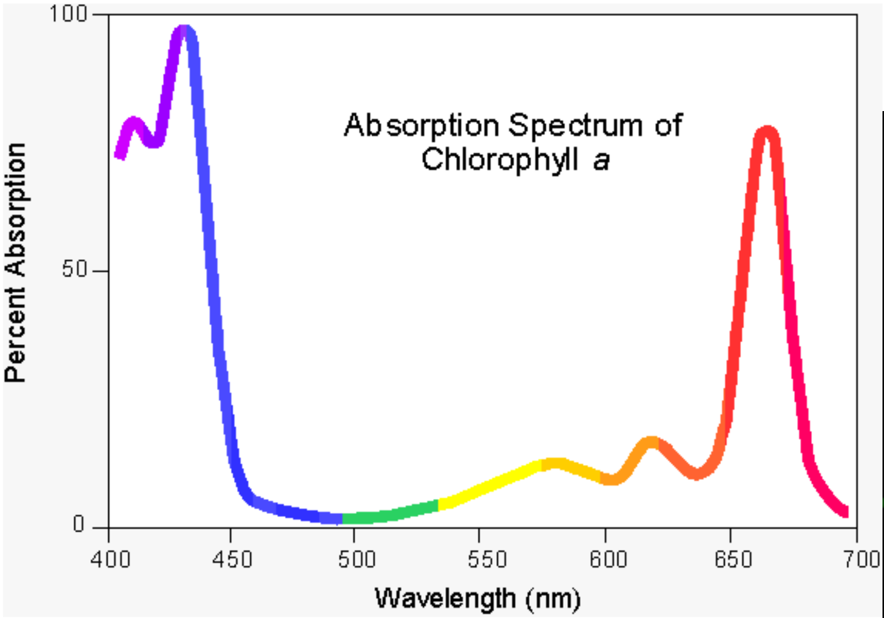
carotenoids

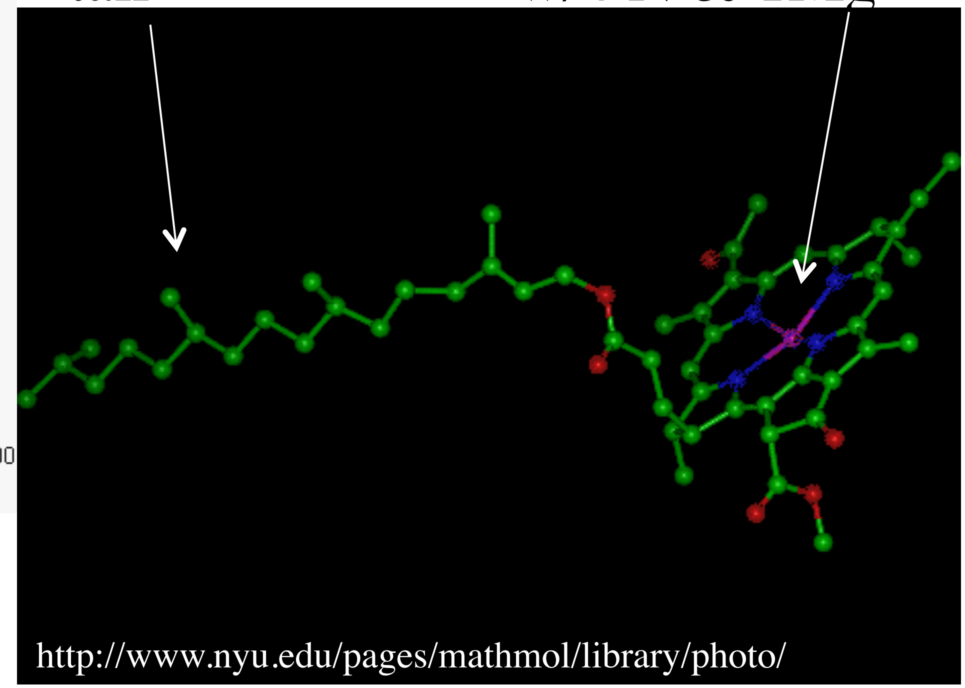
light harvesting for photosynthesis (PS)
light protection when too much light (PP)

phycobilins

water soluble pigments; phycoerythrin can fluorescence phytol tail

Porphyrin ring w/4 N & 1Mg^{++}





Chlorophyll *a*

(absorption peaks will vary, depending on environment – protein complex in membrane, polarity of solvent)

Degraded pigments:

Pheophytin
lost Mg^{++}; peak shifts to ~415

Pheophorbide
lost Mg^{++} and phytol tail

www.ch.ic.ac.uk/local/projects/ steer/cloroads.gif

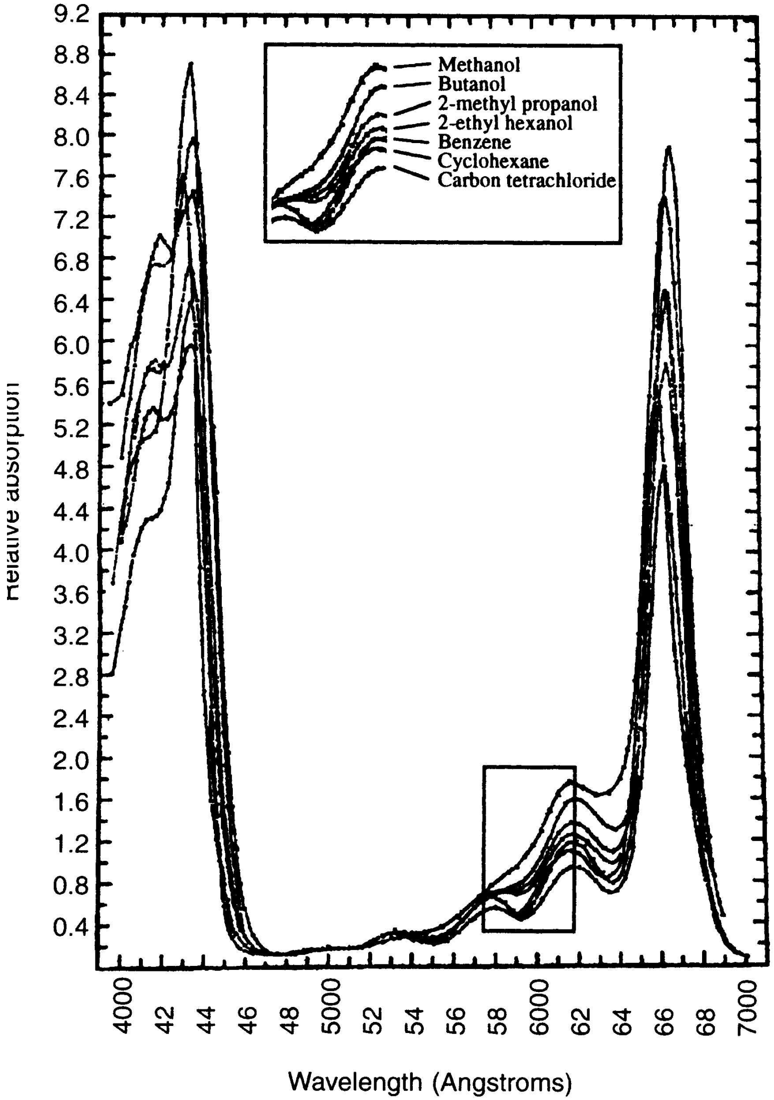
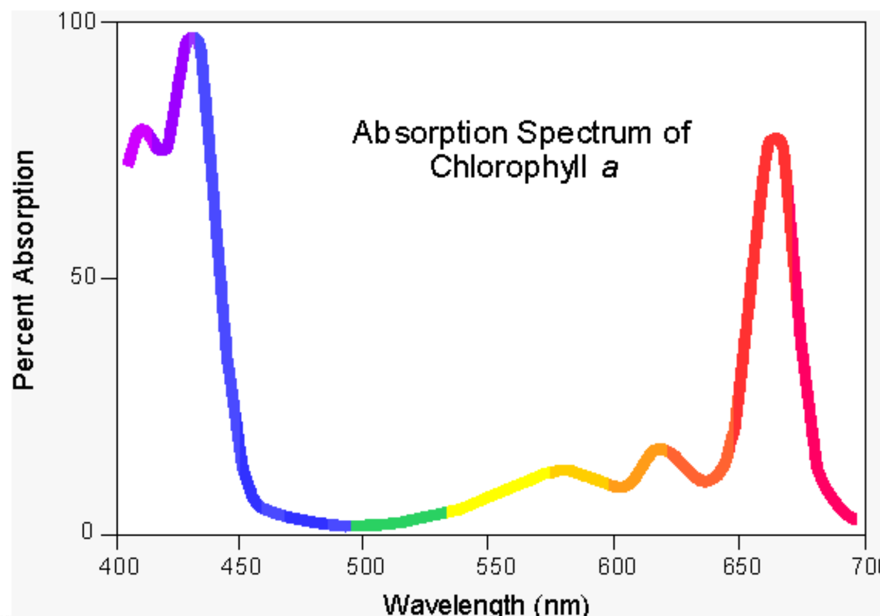


Figure 7.2
Absorption spectra of highly purified chlorophyll *a* in different solvents.
Original, after Harris and Zscheile (1943).

Note of caution: solvent effects the position of absorption peak



Accessory pigments:

Chl *b* and *c*

inside chl a max peaks

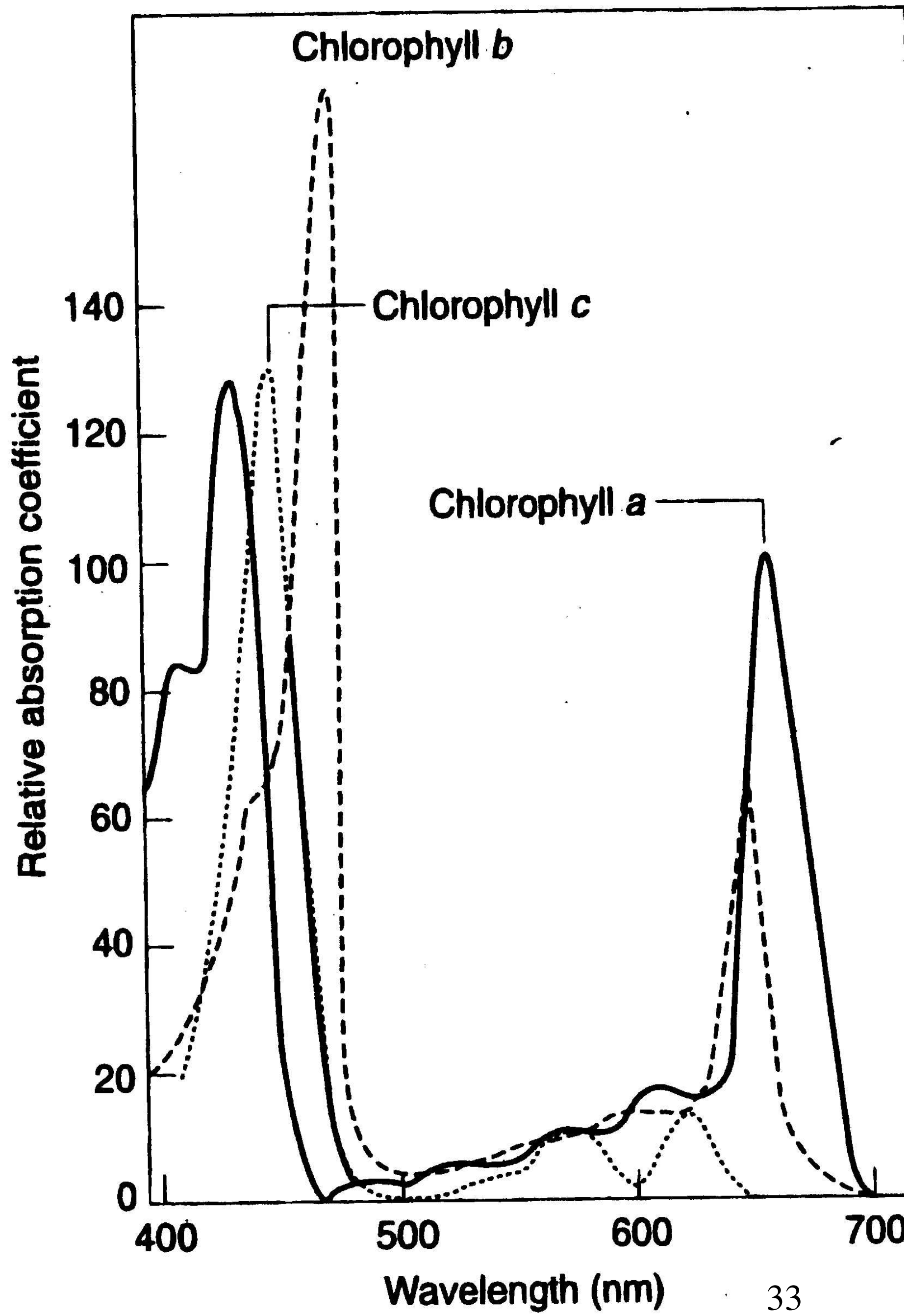
minor modification of ring

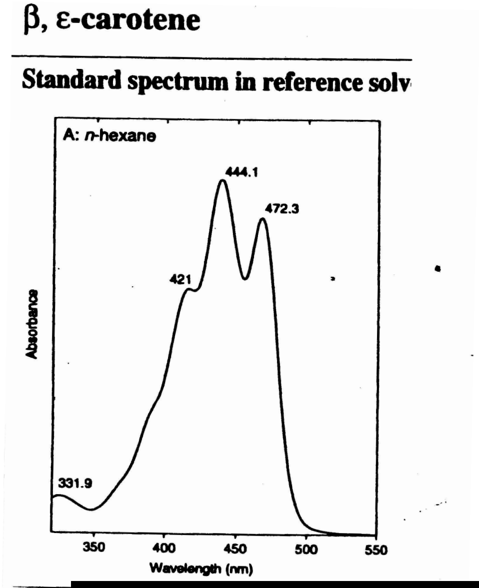
Chl *b*

in vitro fluorescence

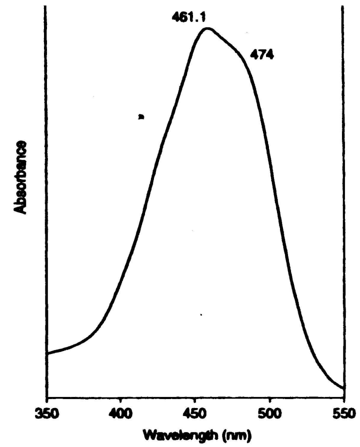
Chl *c*

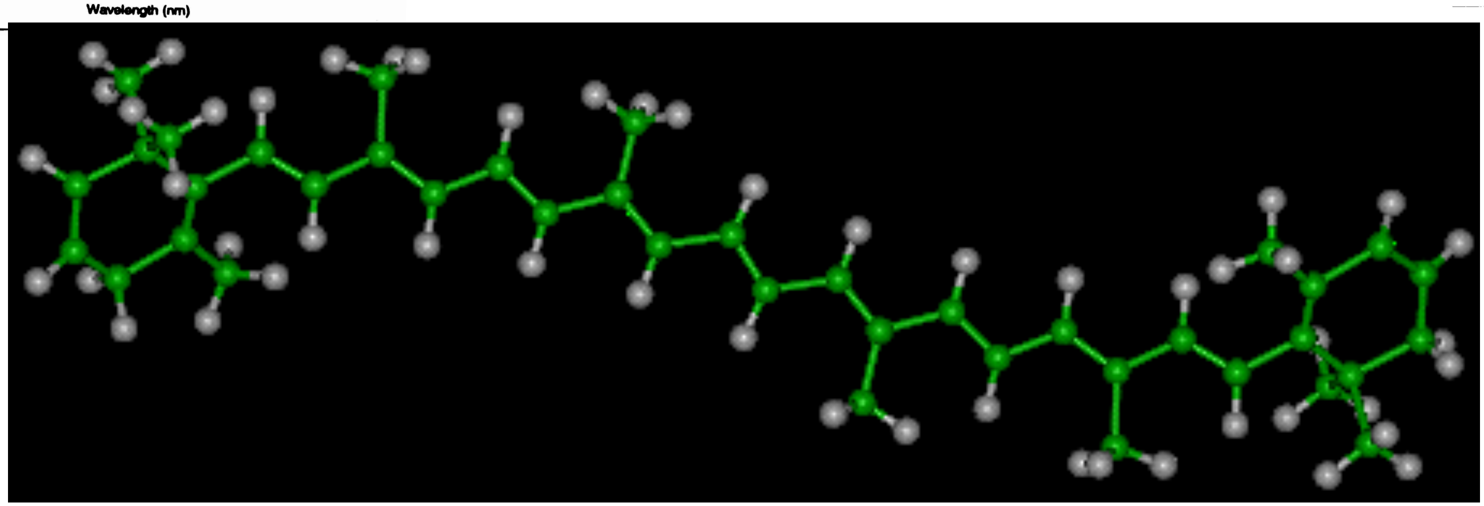
lacks phytol tail





Carotenoids





conjugated double bonds; some taxon specificity; role in <u>photosynthesis</u> (PS - absorb blue-green-yellow λs) and <u>photoprotection</u> (PP - absorb excess photons, quench free radicals & triplet oxygen)

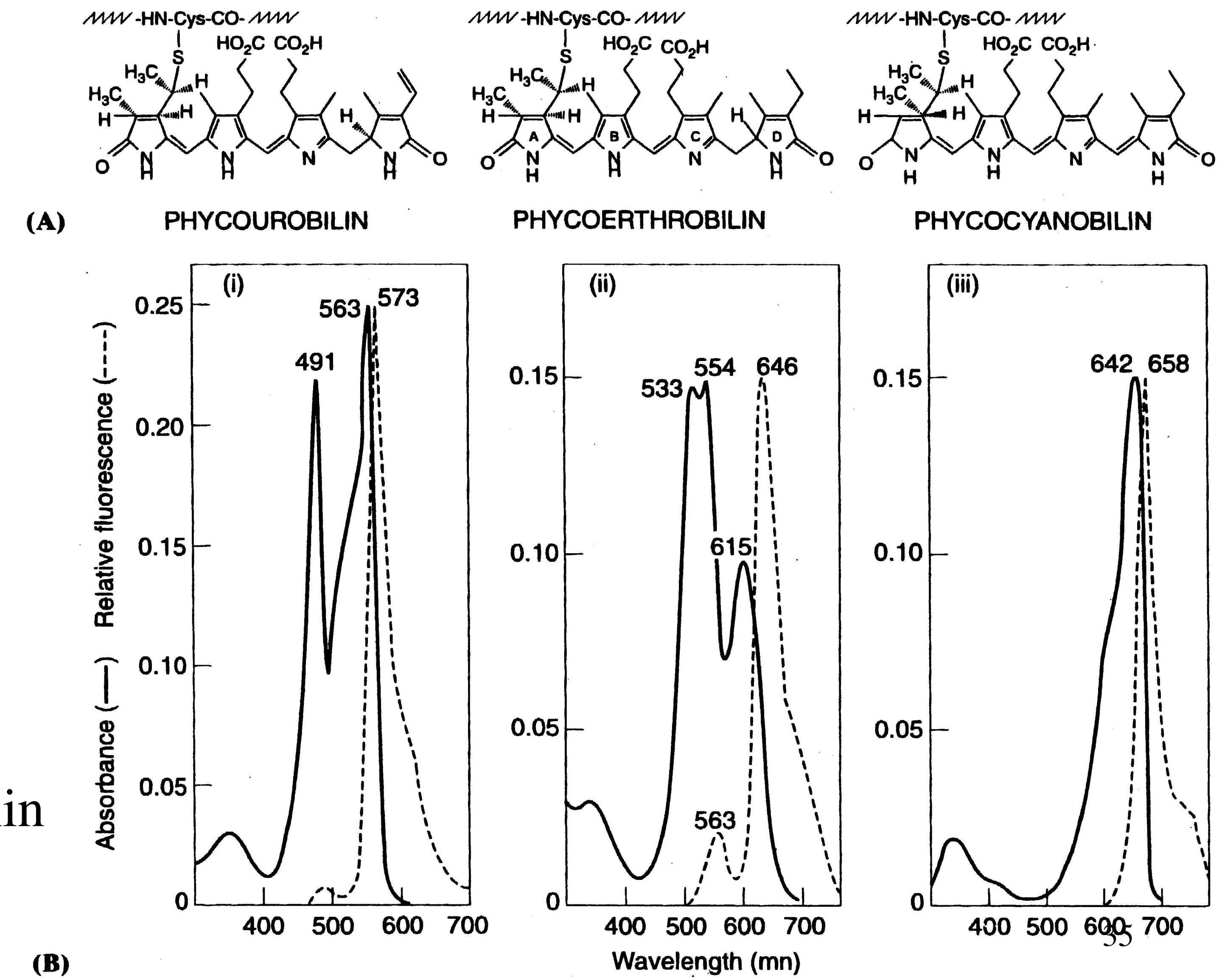
Phycobilins (phycobiliproteins) – water soluble
cyanobacteria and chryptomonads

PUB
phycourobilin

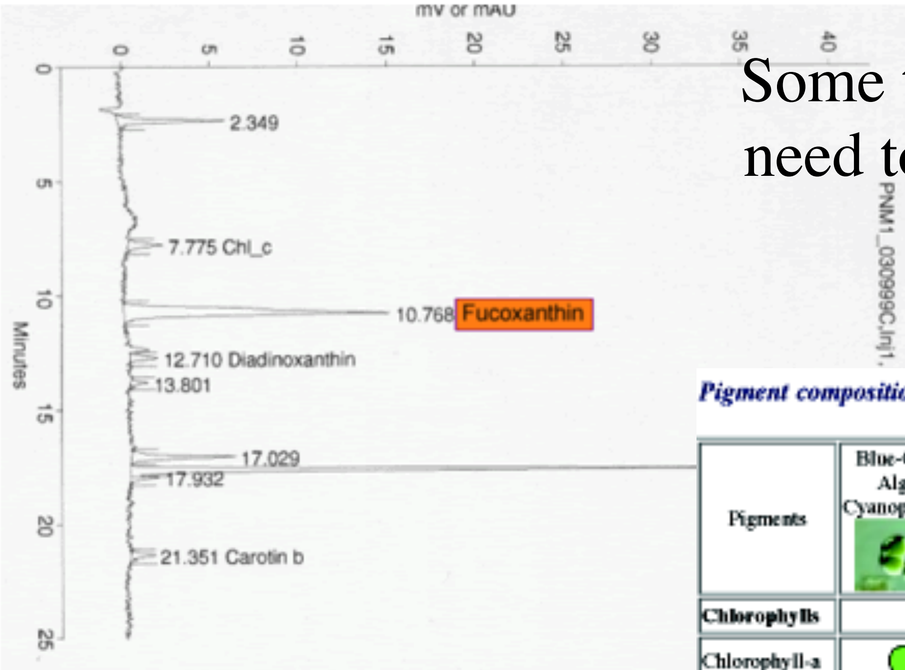
PE
phycoerthyrin
(fluoresces
orange)

PC
phycocyanin

APC
allophycocyanin



Some taxonomic information in pigments, need to assess against species information (local tuning needed)

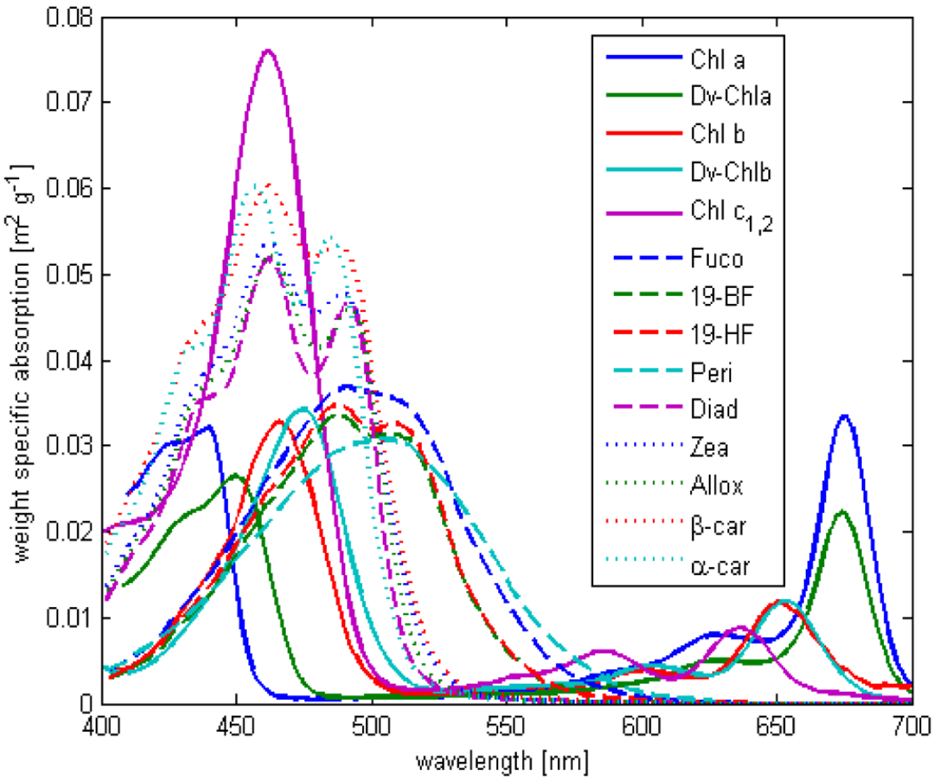


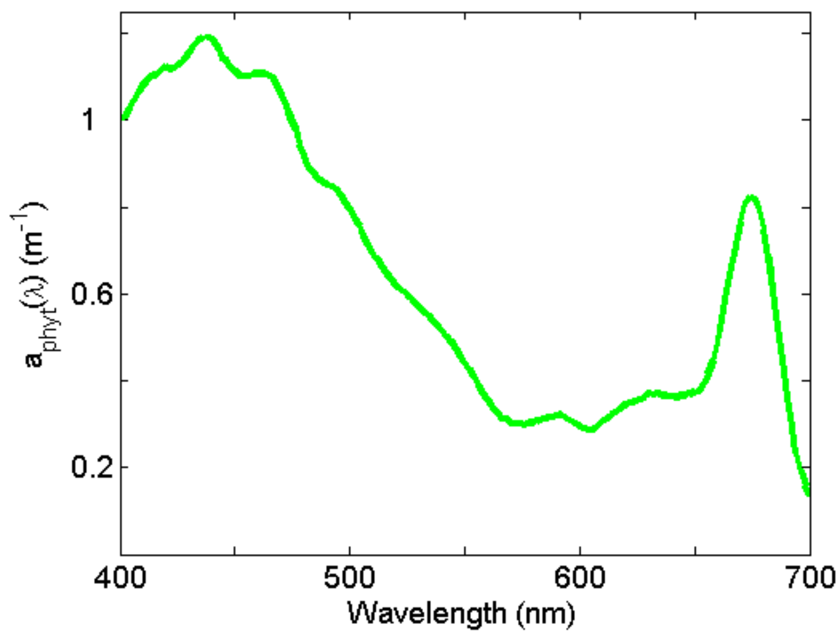
Pigment composition of the major algal groups

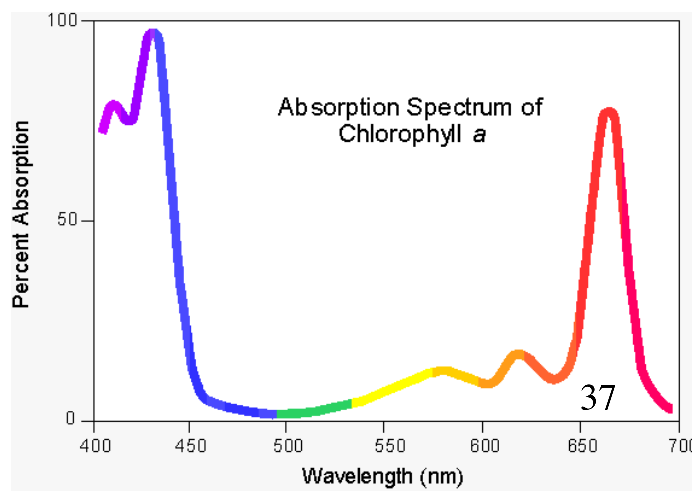
Pigments	Blue-Green Algae/ Cyanophyceae	Red Algae/ Rhodophyceae	Brown Algae/ Phaeophyceae	Green Algae/ Chlorophyceae	Dinoflagellates/ Dinophyceae	Diatoms/ Bacillariophyceae	Naked Flagellates
Chlorophylls							
Chlorophyll-a	●	●	●	●	●	●	●
Chlorophyll-b				●			
Chlorophyll-c			●		●	●	●
Phycobilins							
Phycocyanin	●	●					
Phycoerythrin	●	●					
Carotins							
β-Carotin	●	●	●	●	●	●	●
Xanthophylls							
Diadinoxanthin			•		•	●	•
Fucoxanthin			●		•	●	●
Lutein		●		●			
Peridinin					●		
Alloxanthin							●
Zeaxanthin	●	●	•	●			

Composite absorption – why have multiple pigments?

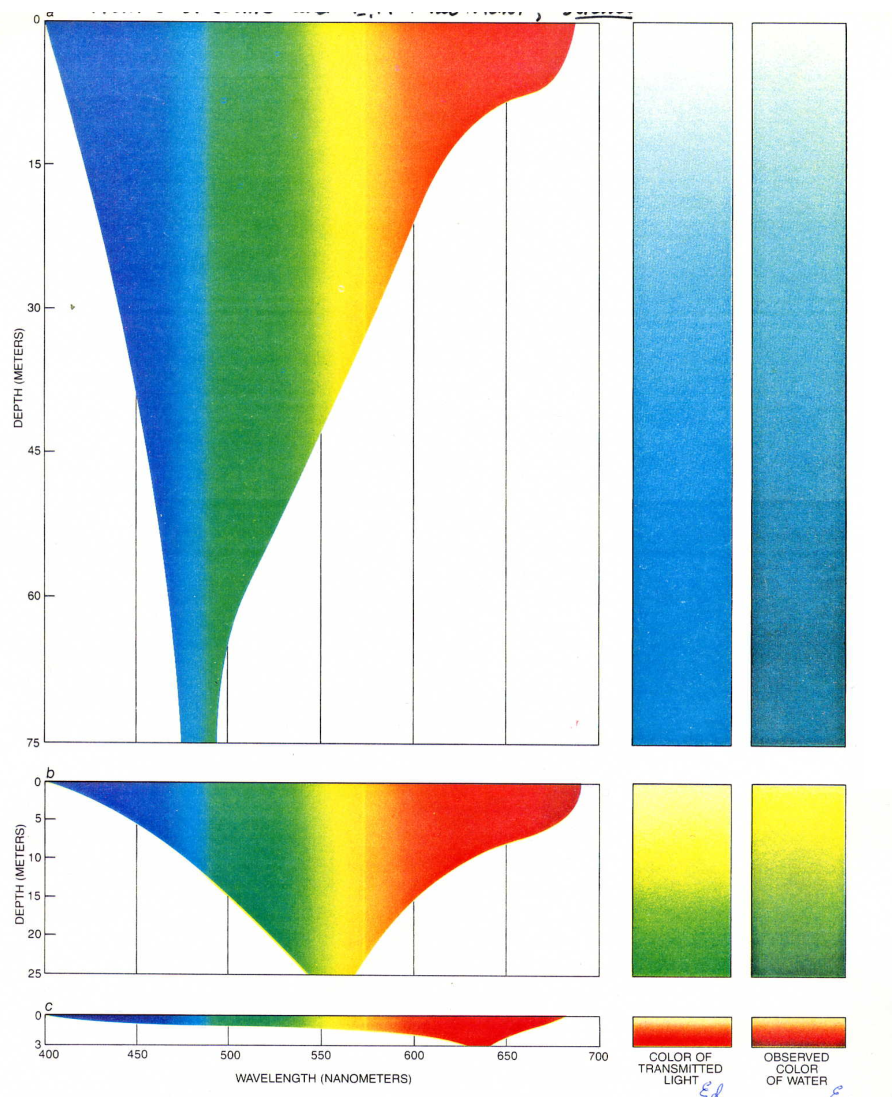
Chlorophyll *a* and *b* is good enough for spinach & other plants.



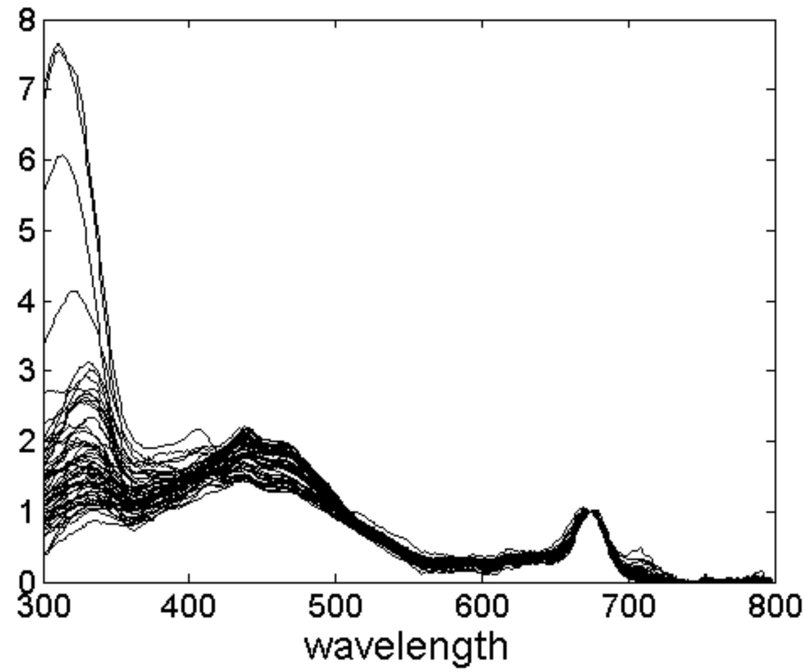




Composite absorption – multiple pigments expand livable environment



TRANSMISSION OF LIGHT by water is dependent on the color or wavelength of the light. In clear oceans and lakes (*a*) the light becomes increasingly monochromatic and blue as its path length increases. In fresh water that carries green organic matter (*b*) light at all wavelengths is absorbed more quickly than it is in clear water, but the light becomes greener with path length. In rivers, swamps and marshes that carry large amounts of the products of plant and animal decay (*c*) absorption is rapid and the spectral distribution of the light shifts to the red. Such waters are called black because the human eye is relatively insensitive to light at long wavelengths; a less anthropomorphic name would be infrared water. The depths given for the maximum penetration of light are typical, but they vary widely.



Chlorophyll *a* – most common entity used to denote presence of phytoplankton and attempt to quantify concentration (mass).

Is chlorophyll a good proxy for phytoplankton?

Chlorophyll a (or divinyl Chl a) is found in all phytoplankton and not in heterotrophs (exception: mixotrophs, digesting predators).

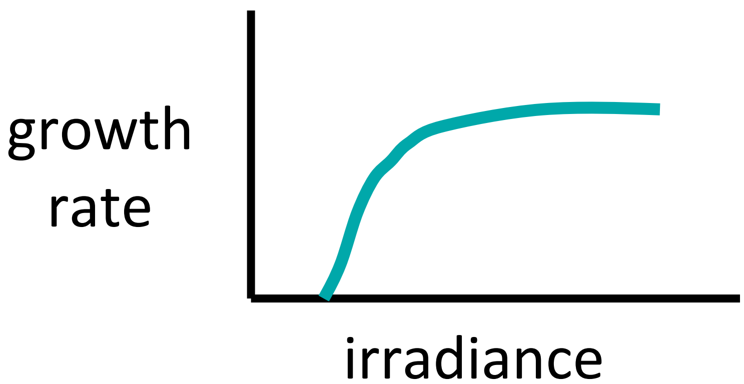
Relationship between carbon and Chl allows estimation of phytoplankton carbon. But beware Mobley's Law of Conservation of Misery: **C/Chl ratio influenced by physiology.**

Some measure of assessing chlorophyll can be used at all scales – from mooring, ship, autonomous platform, satellite.

Different measures of assessing 'chlorophyll' need to be aligned; not measuring exactly same thing. Remember need for closure.

Variability in Chl / cell

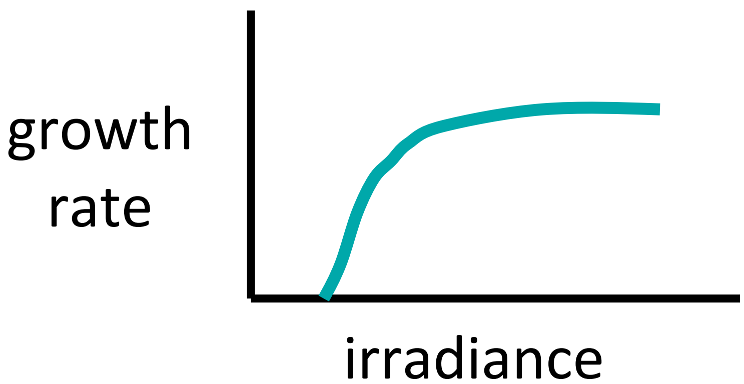
Physiological adaptation to low light is to increase amount of light collectors (chlorophyll molecules).

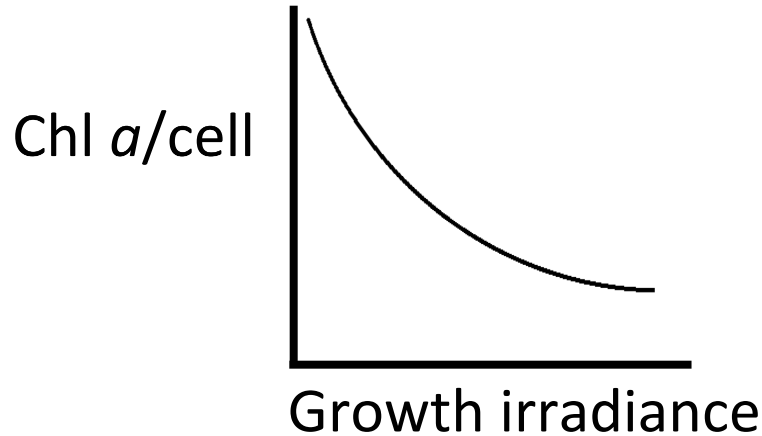


With constant chlorophyll/cell, growth rate would be very low at low light

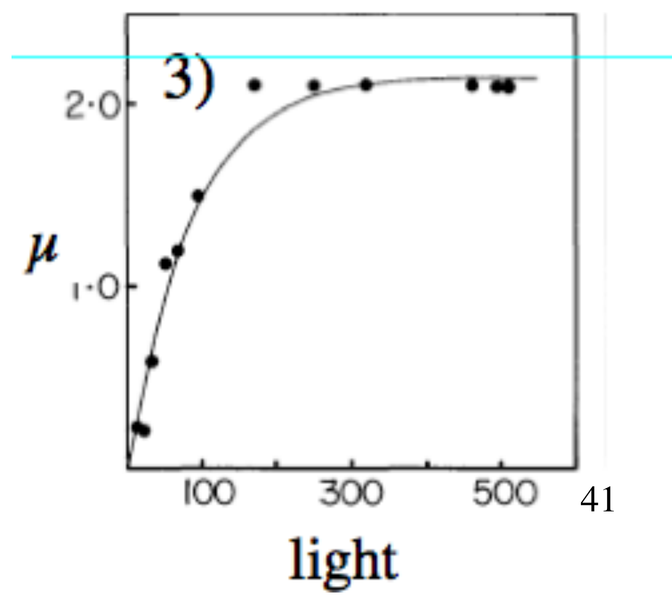
Variability in Chl / cell

Physiological adaptation to low light is to increase amount of light collectors (chlorophyll molecules).

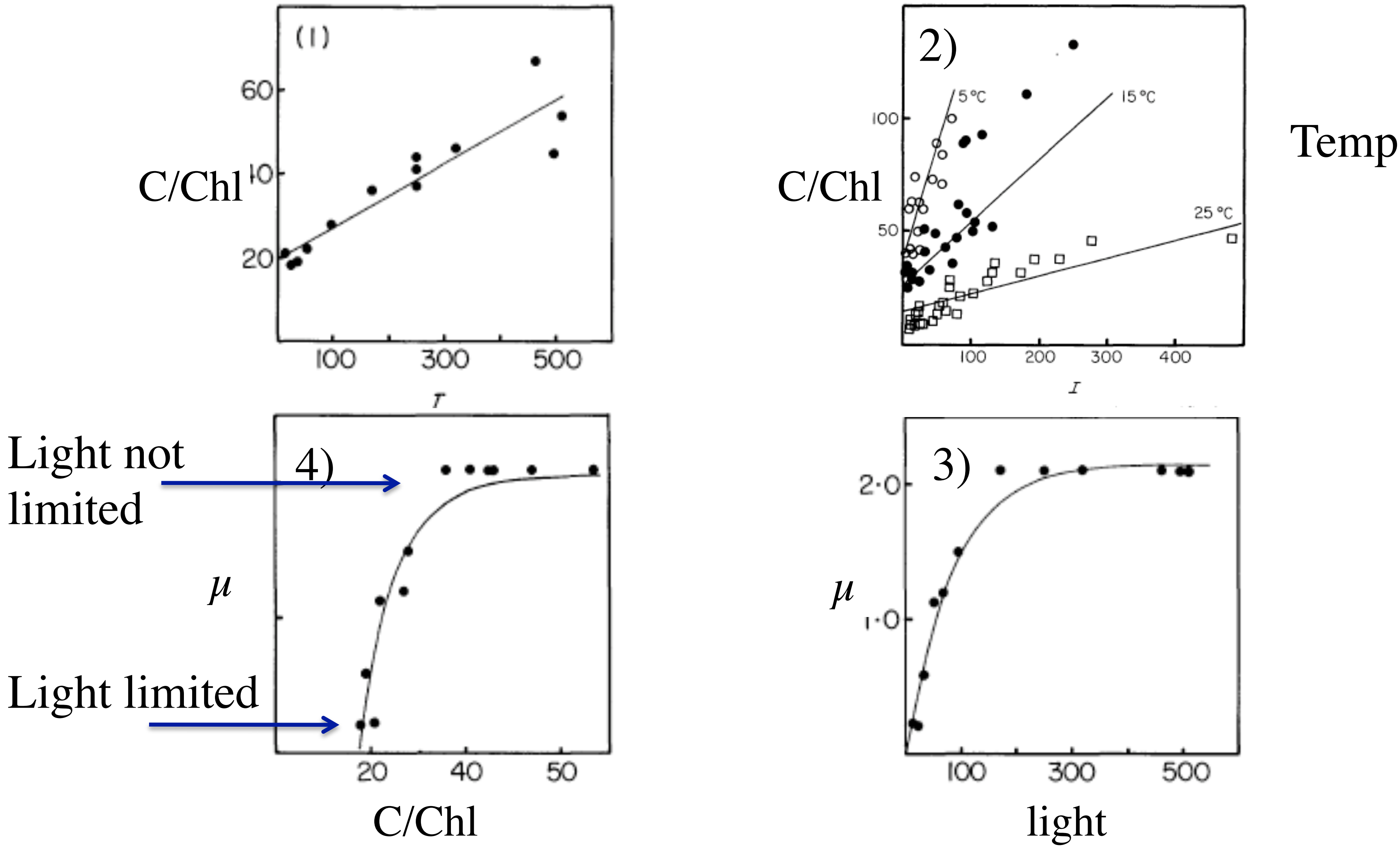




But with ability to increase Chl a/ cell at low light, growth rate is higher than would otherwise be.



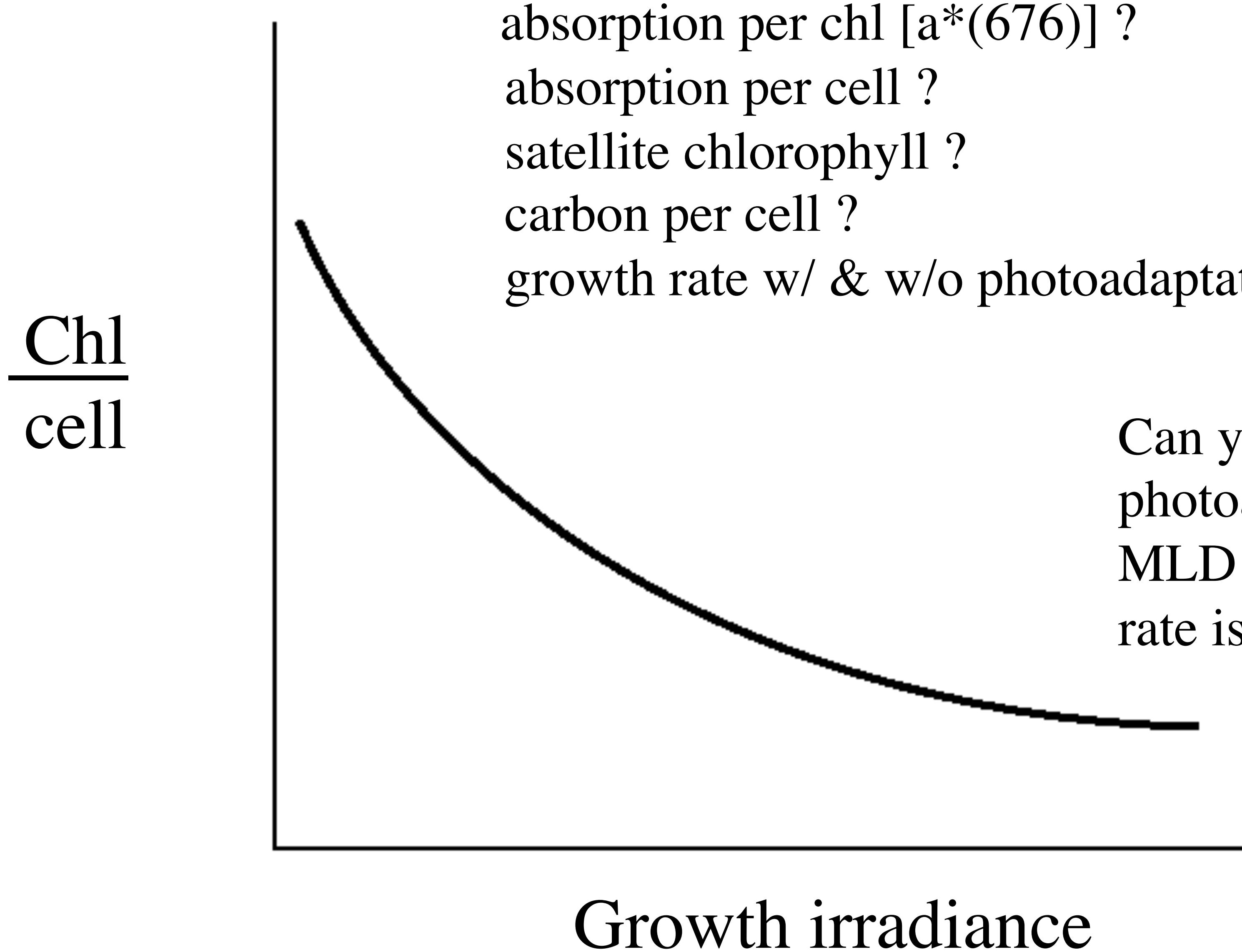
Real data - higher concentrations of chlorophyll and other pigments allow cells to grow better at lower irradiances



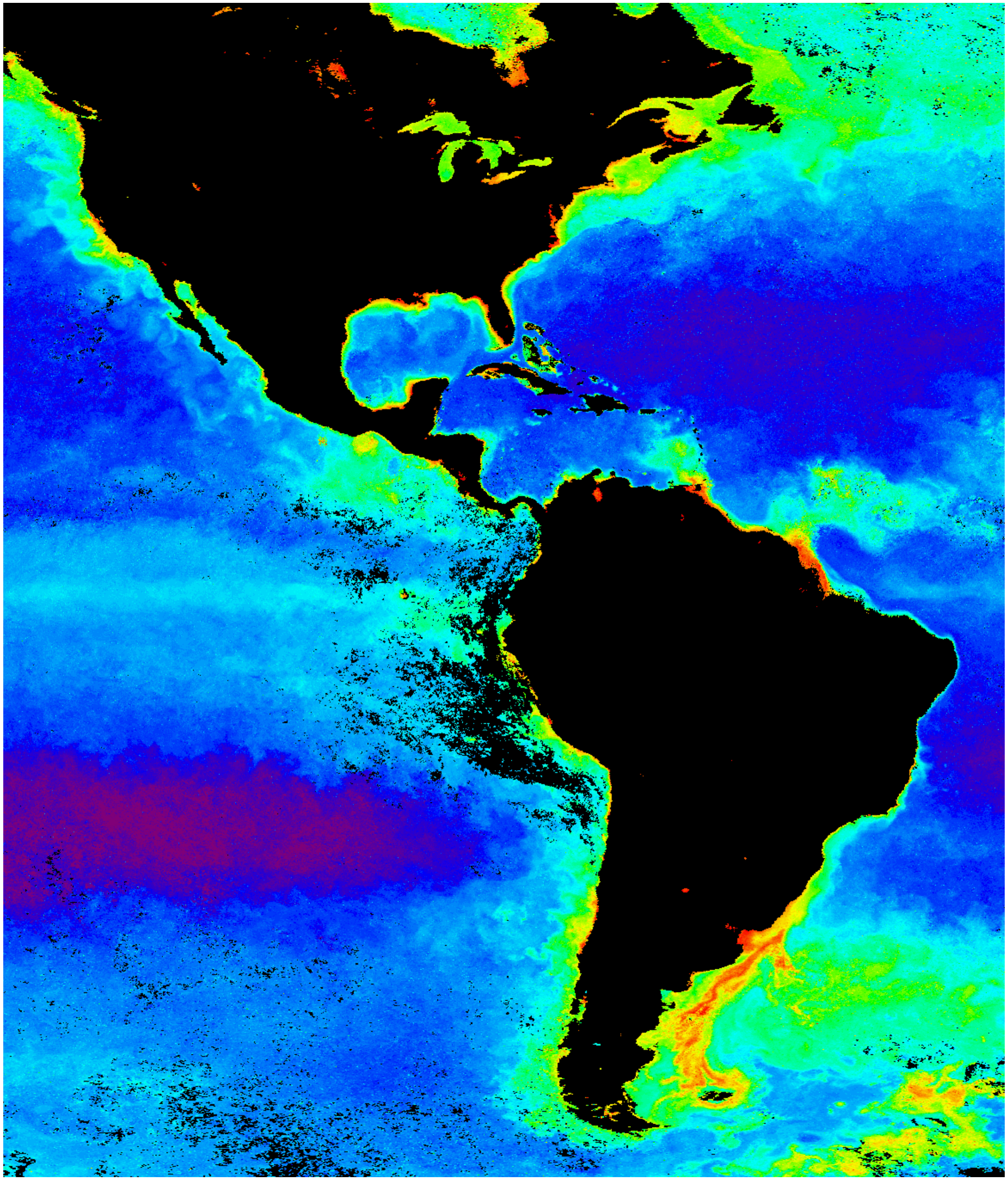
Geider. 1987. New Phytologist 106 :1

What is the consequence of photo adaptation on:

absorption per chl [a*(676)] ?
absorption per cell ?
satellite chlorophyll ?
carbon per cell ?
growth rate w/ & w/o photoadaptation ?



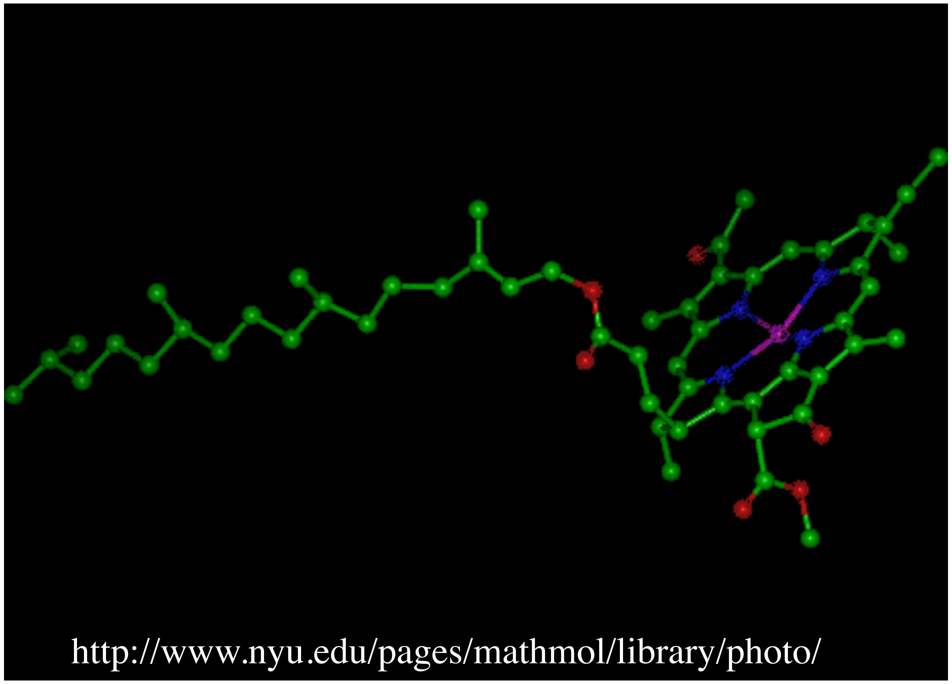
Can you assess photoadaptation if MLD or mixing rate is known?

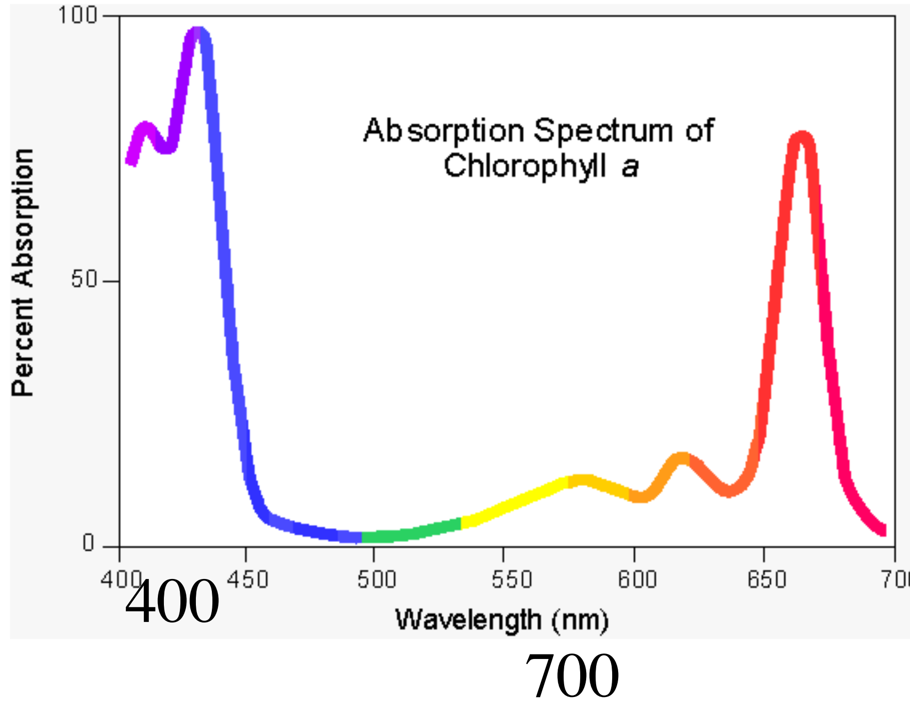


Chlorophyll
- the molecule that let's us measure phytoplankton from the scale of a water droplet to the global ocean.

Organization of chlorophyll in the cell – following slides.

Chlorophyll *a* – chemical structure & absorption spectrum





Extract chlorophyll *a*:

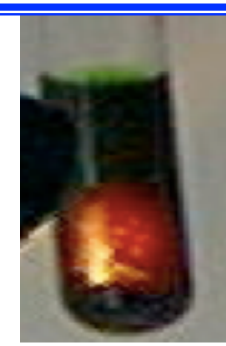
* filter cells GF/F filter

* extract w/ 90% acetone

* measure in fluorometer blue source, red emission

* concentration of molecule ~ red light emitted

What's the relationship of extracted Chl to its organization in cell?



Chlorophyll molecule is attached to binding protein.

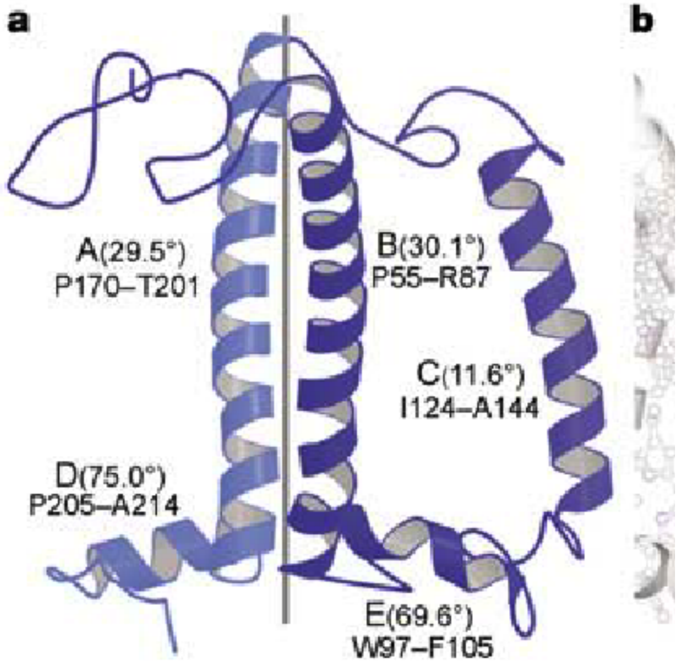
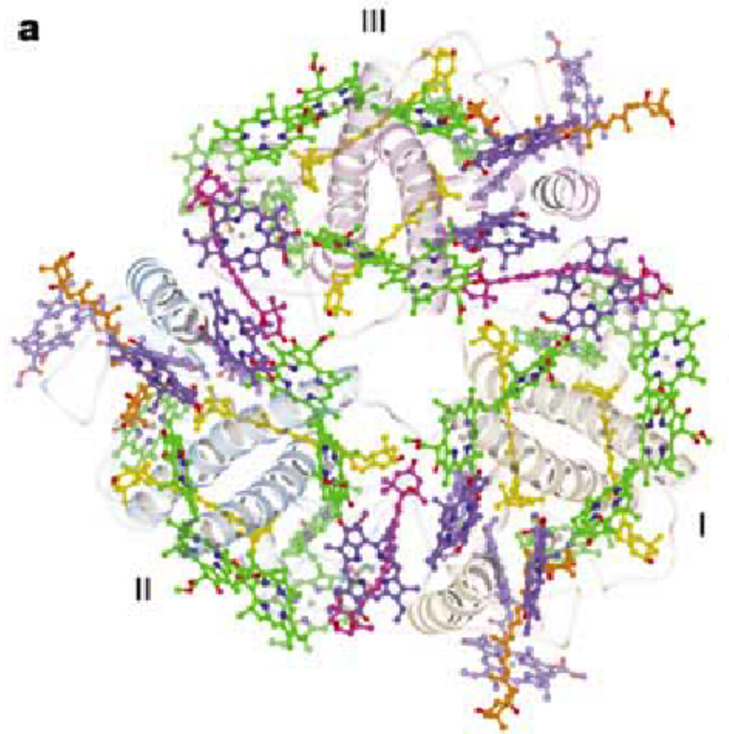


Figure 3 Secondary structure of monomeric LHC-II

protein backbone of monomeric LHC-II protein complex, from electron density mapping

Trimeric complexes of Chl and binding protein.



3 monomers = 1 trimer

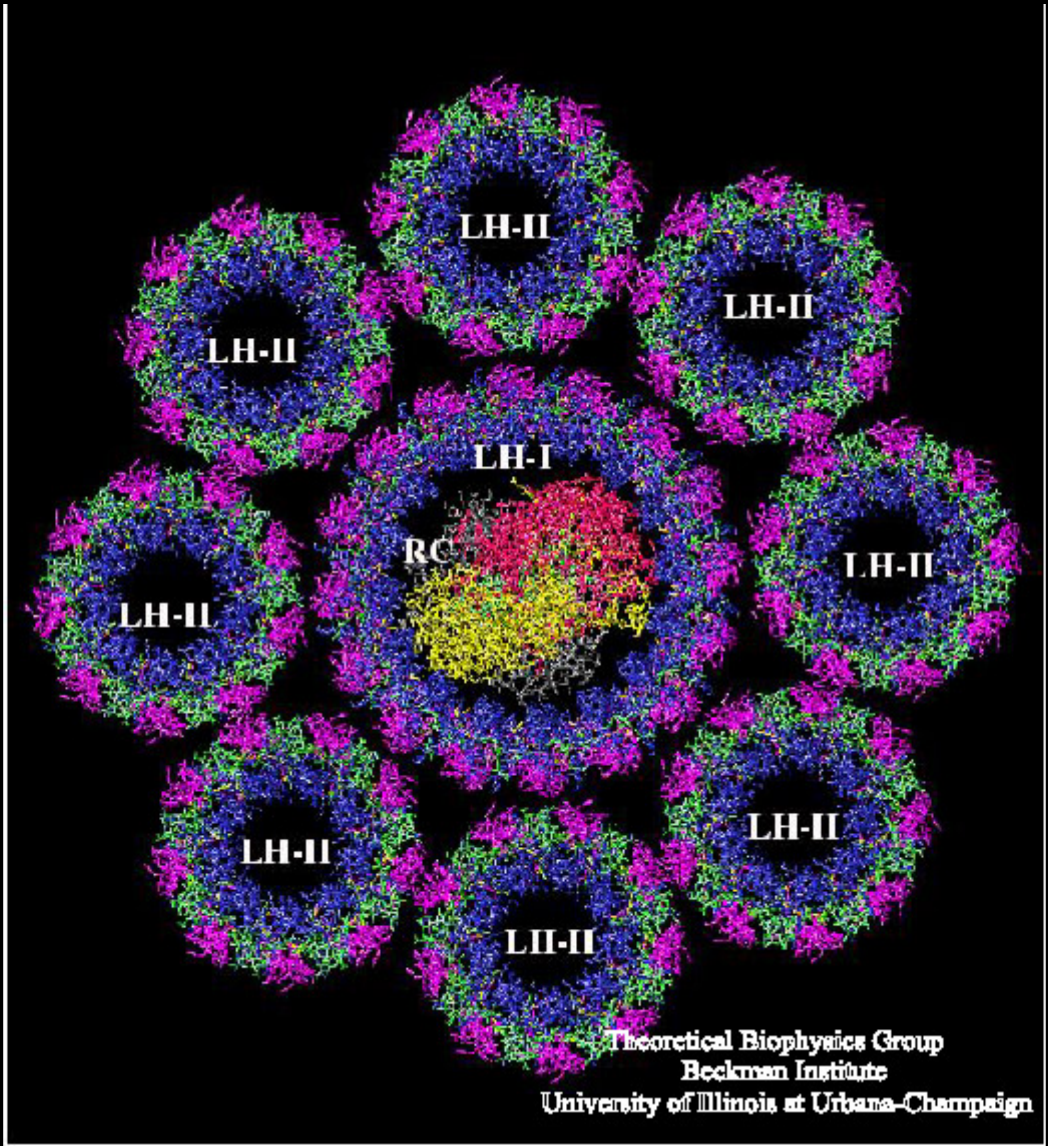
green: chl *a*; blue: chl *b*

yellow/orange: P carotenoids

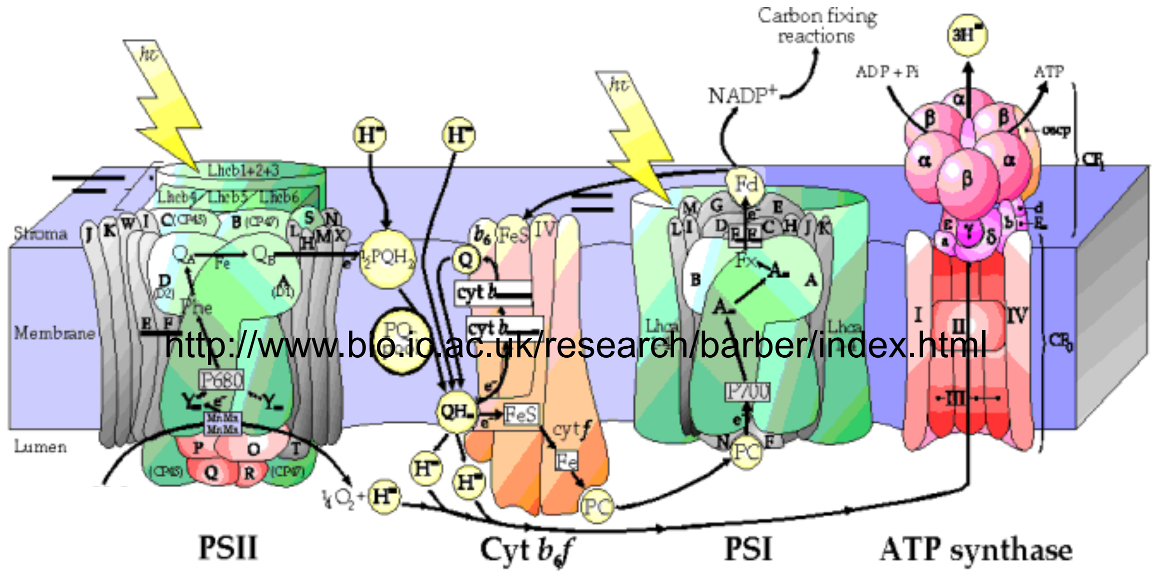
magenta: PP carotenoids

Lui et al., 2004, Nature 428: 287ff for spinach LHC-II)

Many light harvesting trimers around reaction center (PS II)
to form a light harvesting complex.



Light harvesting complexes and other functional complexes are located in thylakoid membrane.



http://www.bio.ic.ac.uk/research/barber/index.html

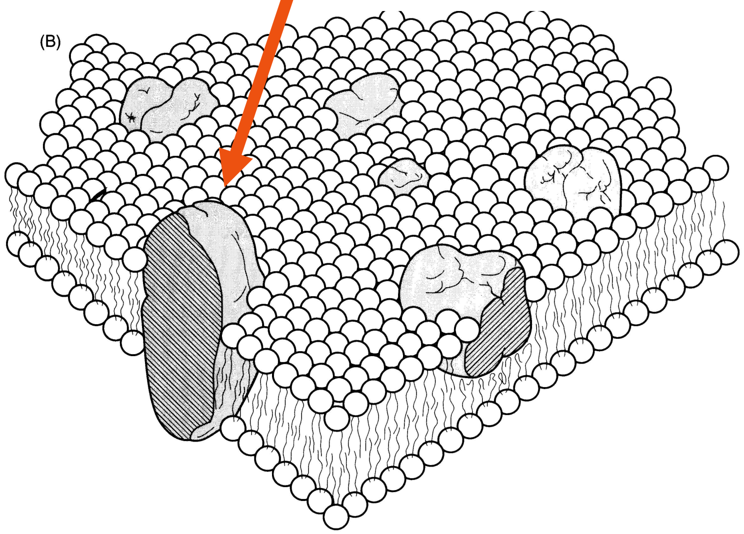
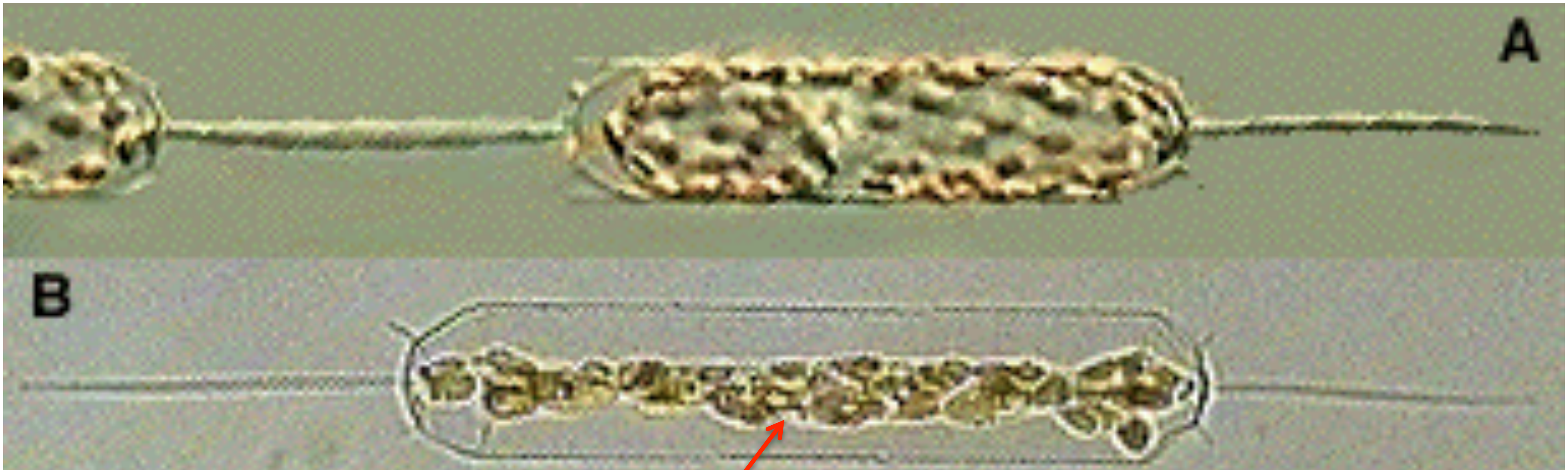


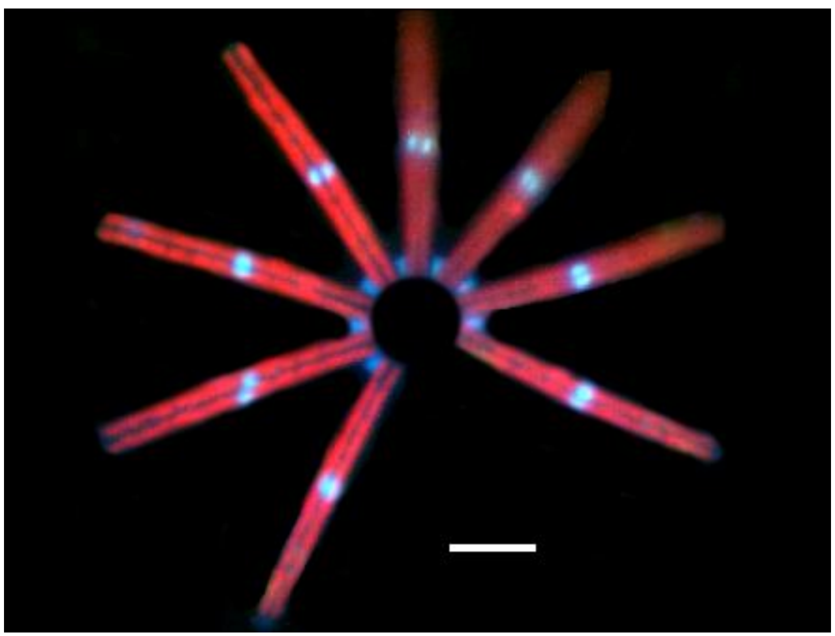
Figure 1.2 (A) Structure of two of the most important lipids that make up thylakoid membranes: monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG). In the formation of membranes, the polar sugar groups face the aqueous phases, while opposing nonpolar alkyl groups are oriented toward each other to form a lipid bilayer. The width of the bilayer is approximately 4 nm. (B) A schematic diagram of a thylakoid membrane (modified from Singer, Nicolson 1972). Thylakoid membranes are largely composed of MGDG and DGDG with other polyunsaturated fatty acids. Proteins are oriented within the membrane in a nonrandom fashion. Some proteins span the membrane, whereas others may only partially protrude. The proteins will have specific "sidedness," with some functional groups facing the lumen and others facing the stroma.

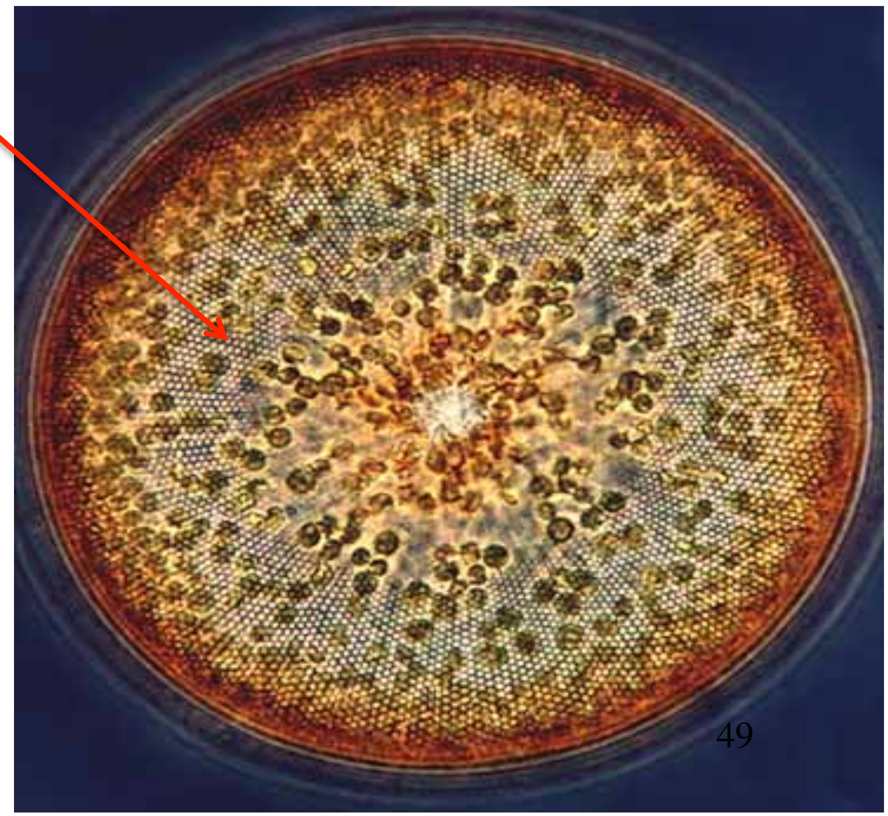
Thylakoid membranes in chloroplast



Diatom chloroplasts

In vivo chlorophyll fluorescence





What is a phytoplankton?
Cell, species, particle of some size, carbon or chlorophyll or ???

What are potential surrogates for phytoplankton:

* extracted chlorophyll or other pigments (HPLC)
* chlorophyll fluorescence
* absorption coefficients
    - a_{phyt}, all pigments
    - a_{ϕ} photosynthetically competent pigments
* beam c or backscatter
* particle size distribution
* particle size distribution
* what else ?