1865 THE UNIVERSITY OF MAINE
Celebrating 150 years

50 YEARS
Darling Marine Center
1965-2015

NASA

SMS 598: Calibration and Validation for Ocean Color Remote Sensing

Lecture 4b – More on phytoplankton

What are phytoplankton? (Ivona)

1 – photosynthetic ➔ocean life depends on phytoplankton

2 – taxonomically & functionally diverse

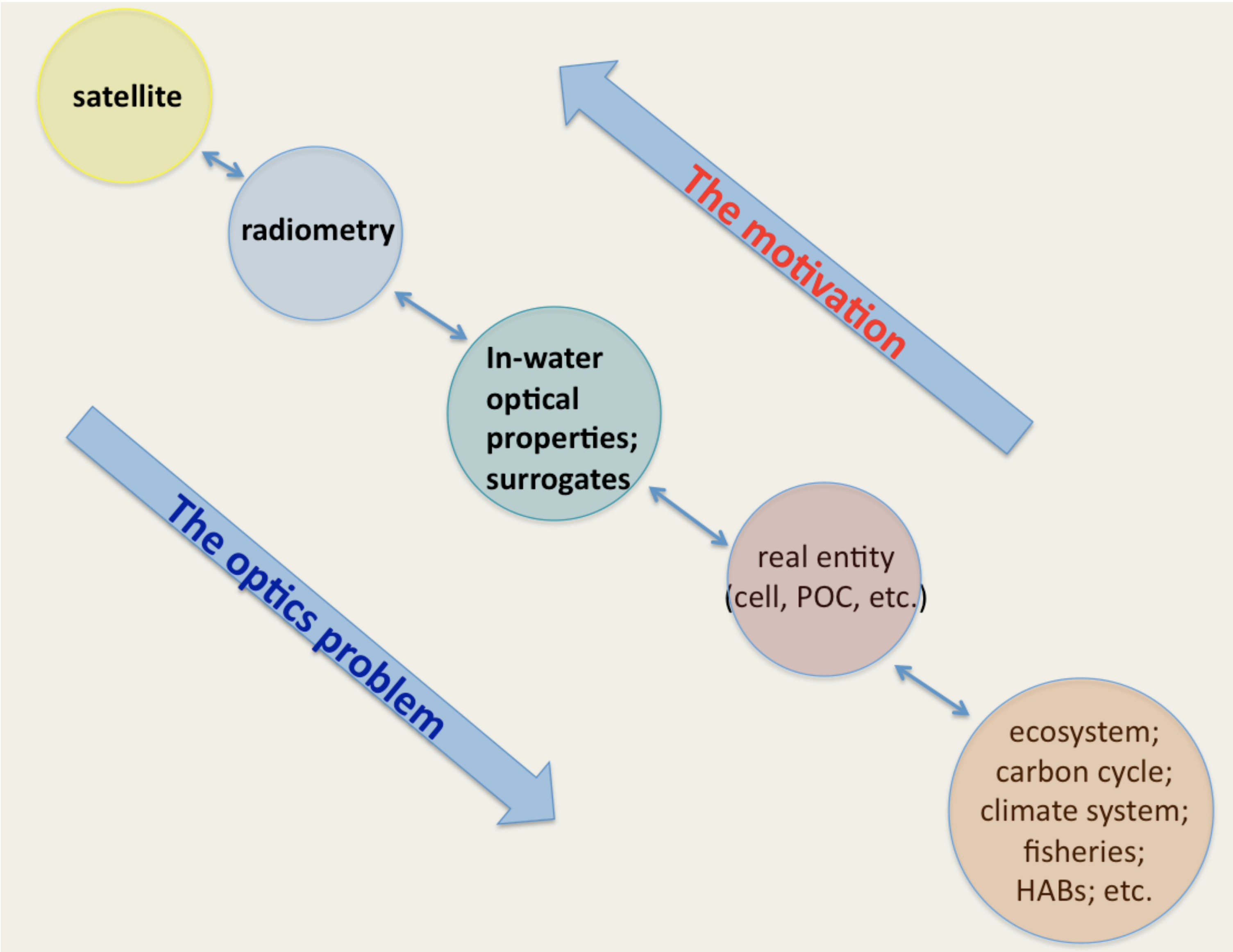
How are phytoplankton assessed?

3 – only ocean life form that can be measured on droplet & global scales ➔ due to chlorophyll a & other pigments

Mary Jane Perry, UMaine 7 July 2015

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?)

This lecture:

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 (somewhat unique), scattering (not unique), fluorescence (unique).

Phytoplankton interactions with light are basis for optical proxies

– particles scatter light

– pigments absorb light

– chlorophyll *a* and phycoerythrin fluoresce light

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

Physiology can change the relationship between

phytoplankton and some of their optical proxies

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

Phytoplankton pigments (chlorophyll and others)

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*)

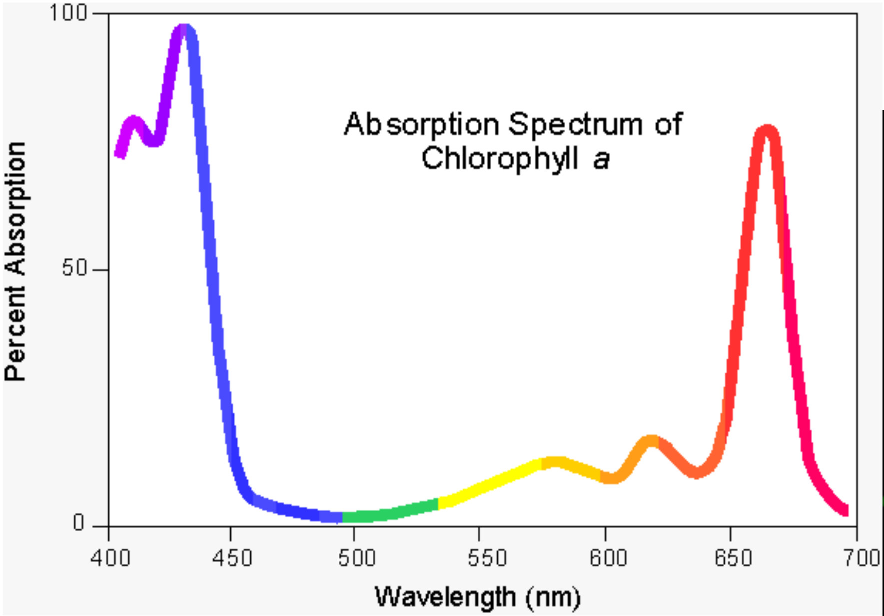
carotenoids

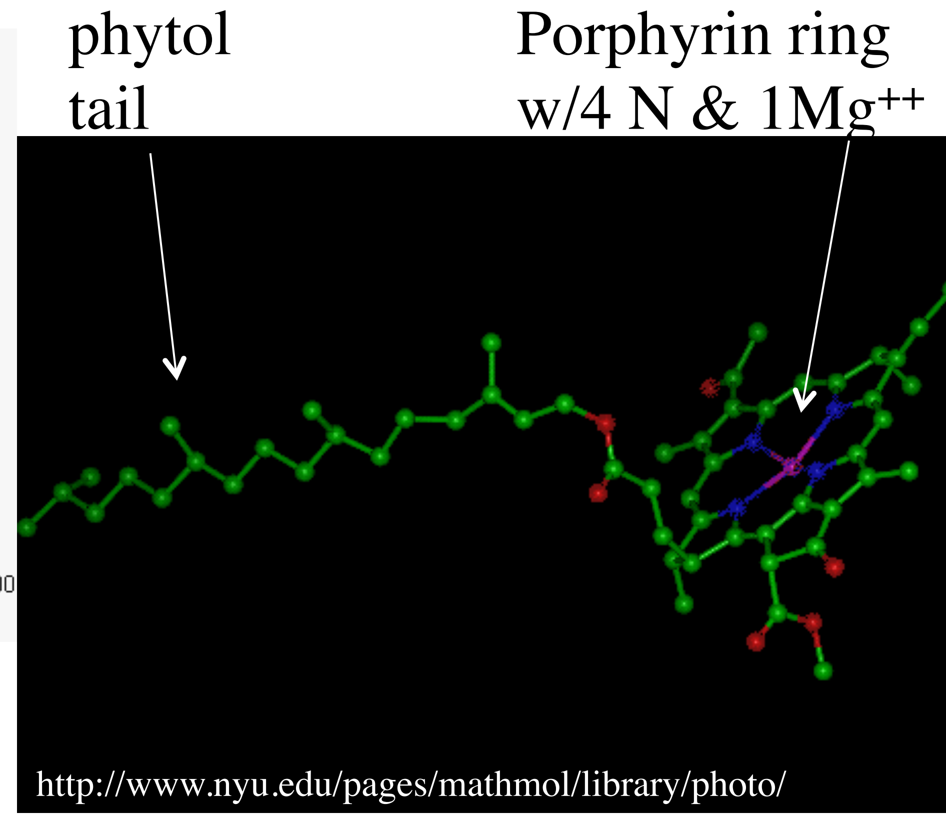
light harvesting for photosynthesis (PS)

light protection when too much light (PP)

phycobilins

water soluble pigments; phycoerythrin can fluorescence





Chlorophyll *a*

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

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

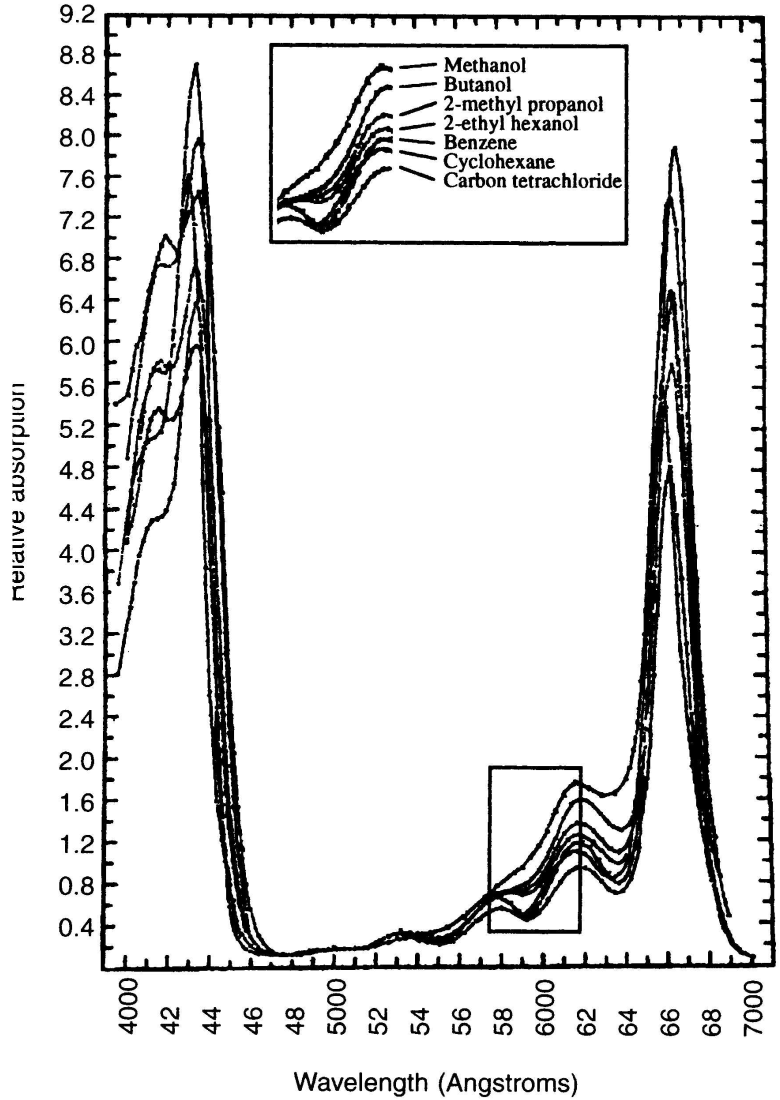
Degraded pigments:

Pheophytin

lost Mg^{++}; peak shifts to ~415; 676 nm decreased 54%

Pheophorbide

lost Mg^{++} and phytol tail



Note of caution: solvent effects the position of absorption peak

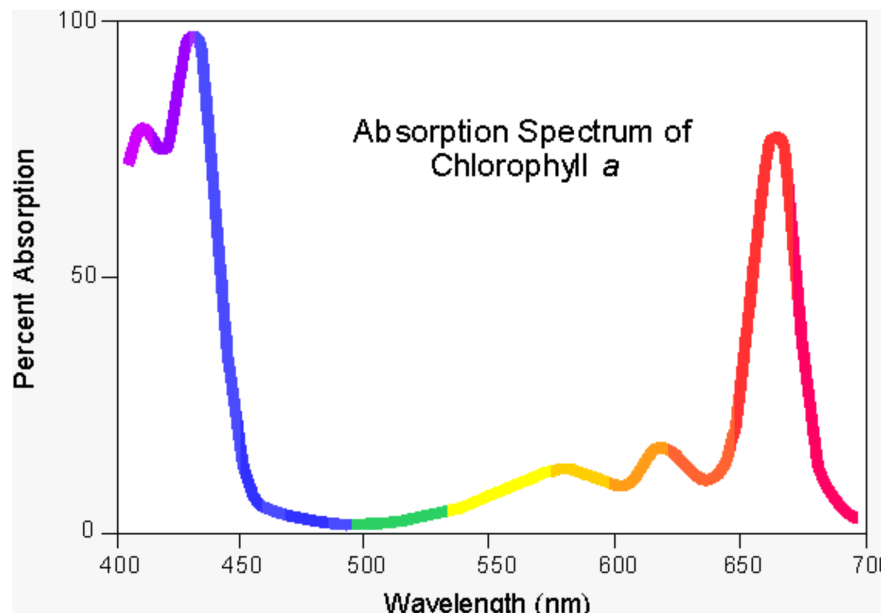


Figure 7.2
Absorption spectra of highly purified chlorophyll *a* in different solvents.
Original, after Harris and Zscheile (1943).

Accessory pigments:

Chl *b* and *c*

inside chl a max peaks

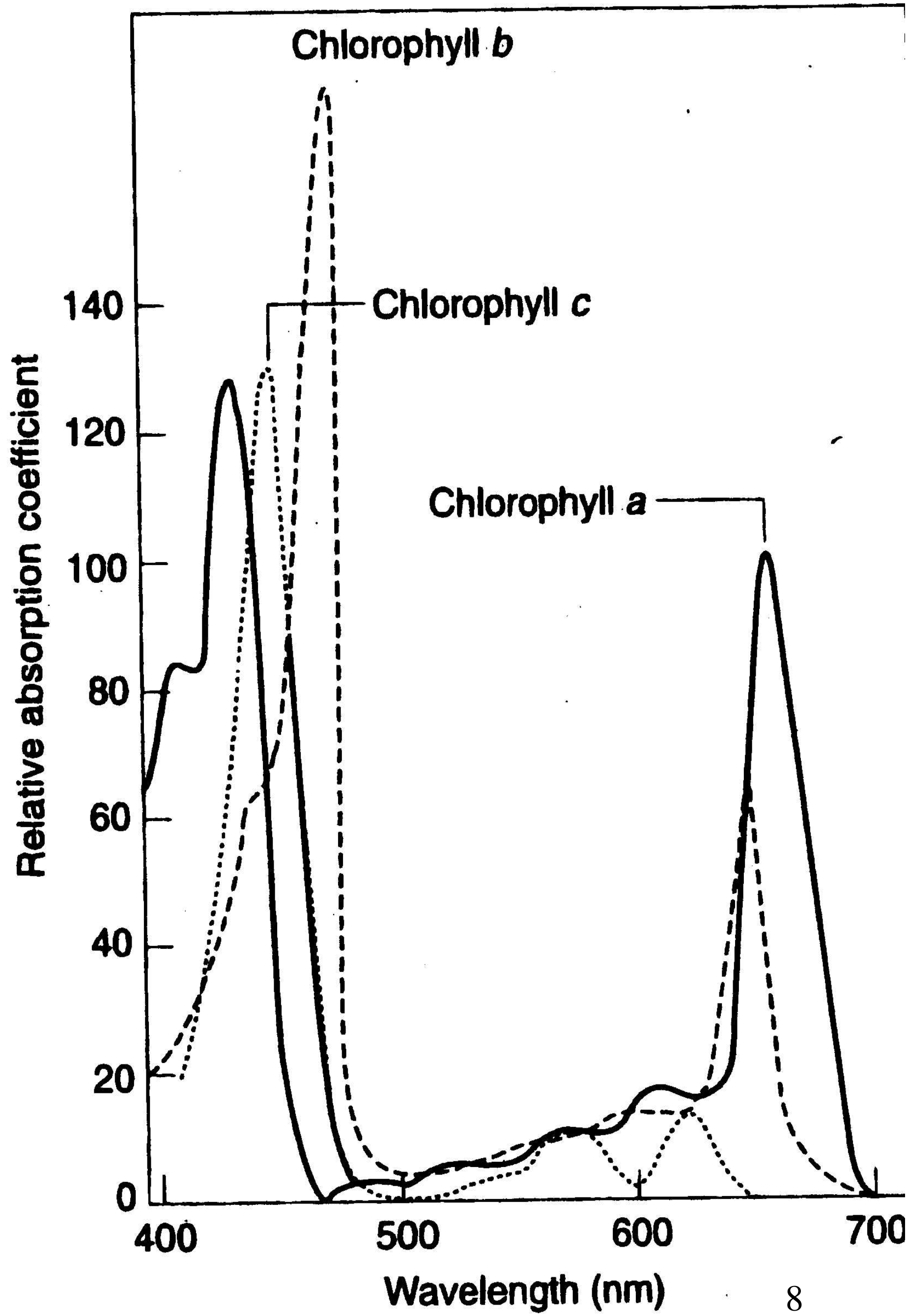
minor modification of ring

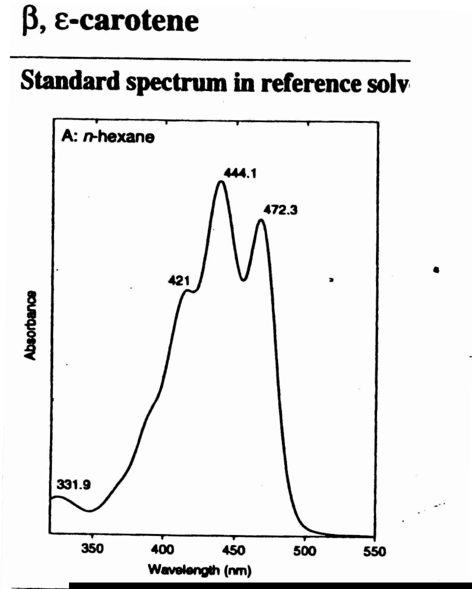
Chl *b*

in vitro fluorescence

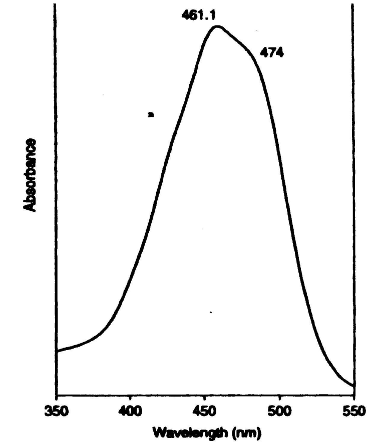
Chl *c*

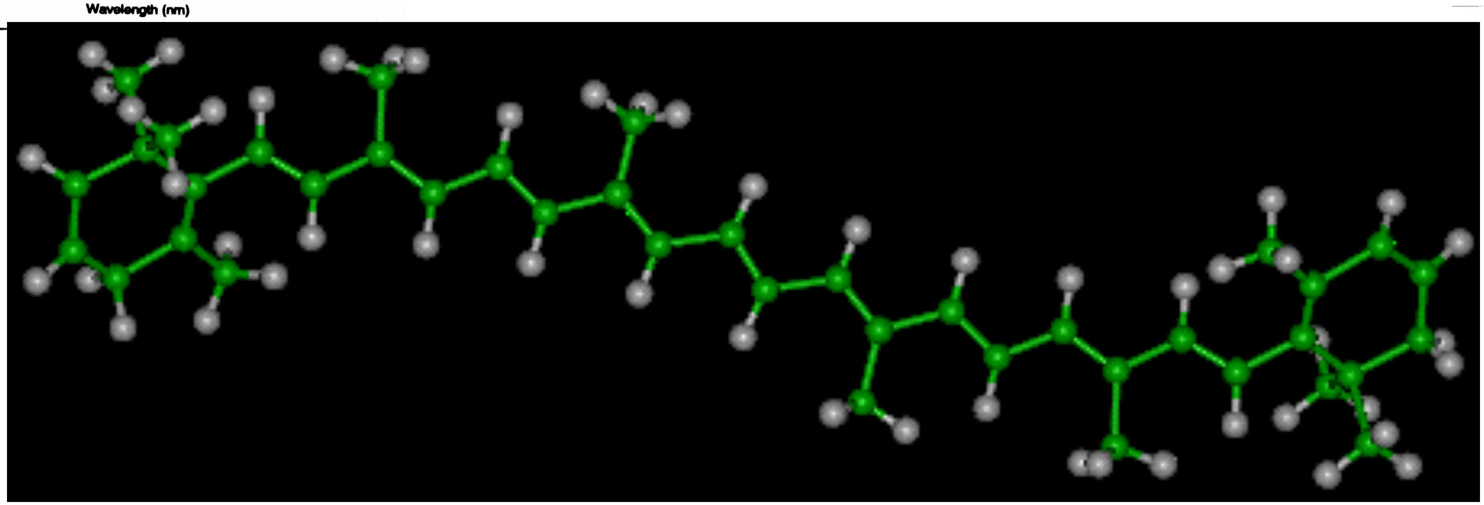
lacks phytol tail





Carotenoids





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

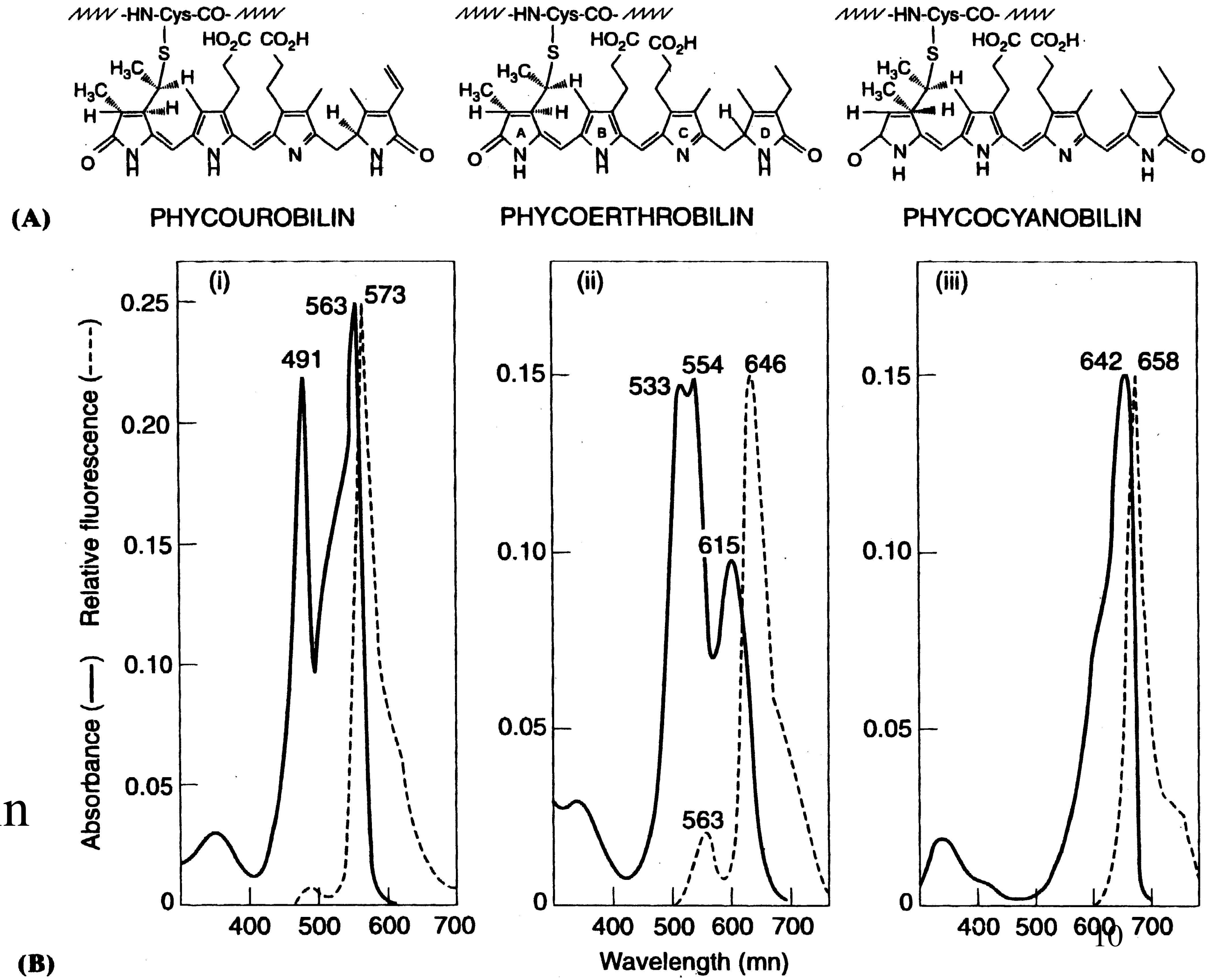
Phycobilins (phycobiliproteins) – water soluble cyanobacteria and chryptomonads

PUB
phycourobilin

PE
phycoerthyrin
(fluoresces
orange)

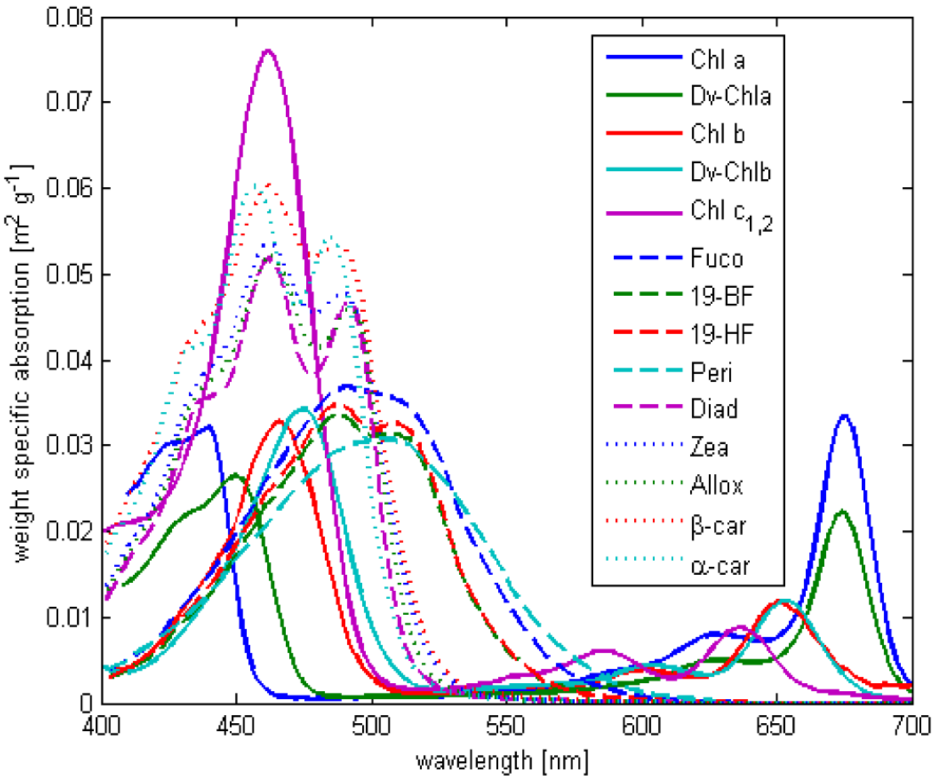
PC
phycocyanin

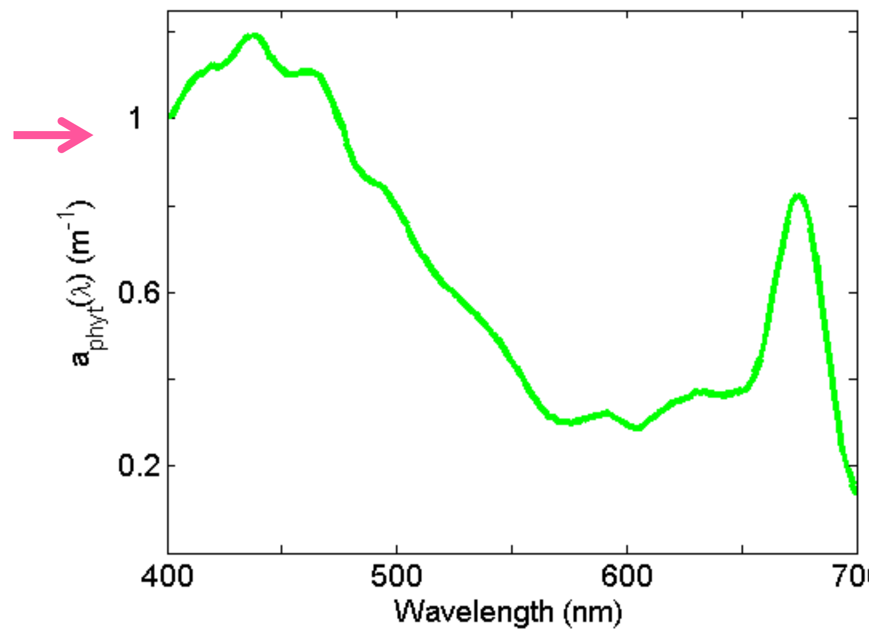
APC
allophycocyanin



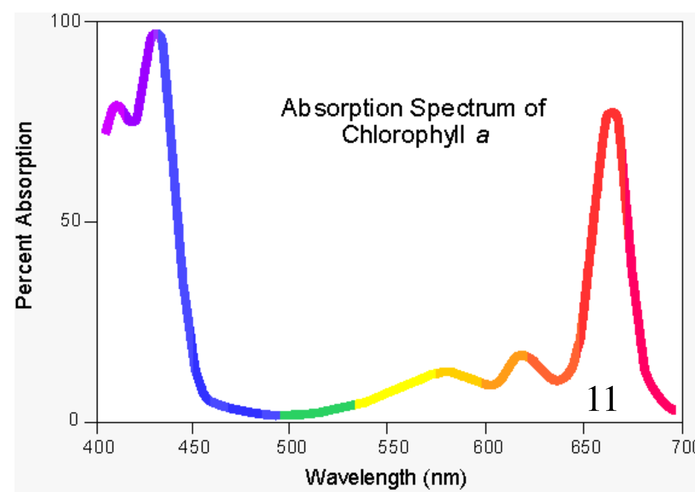
Composite absorption – why have multiple pigments?

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

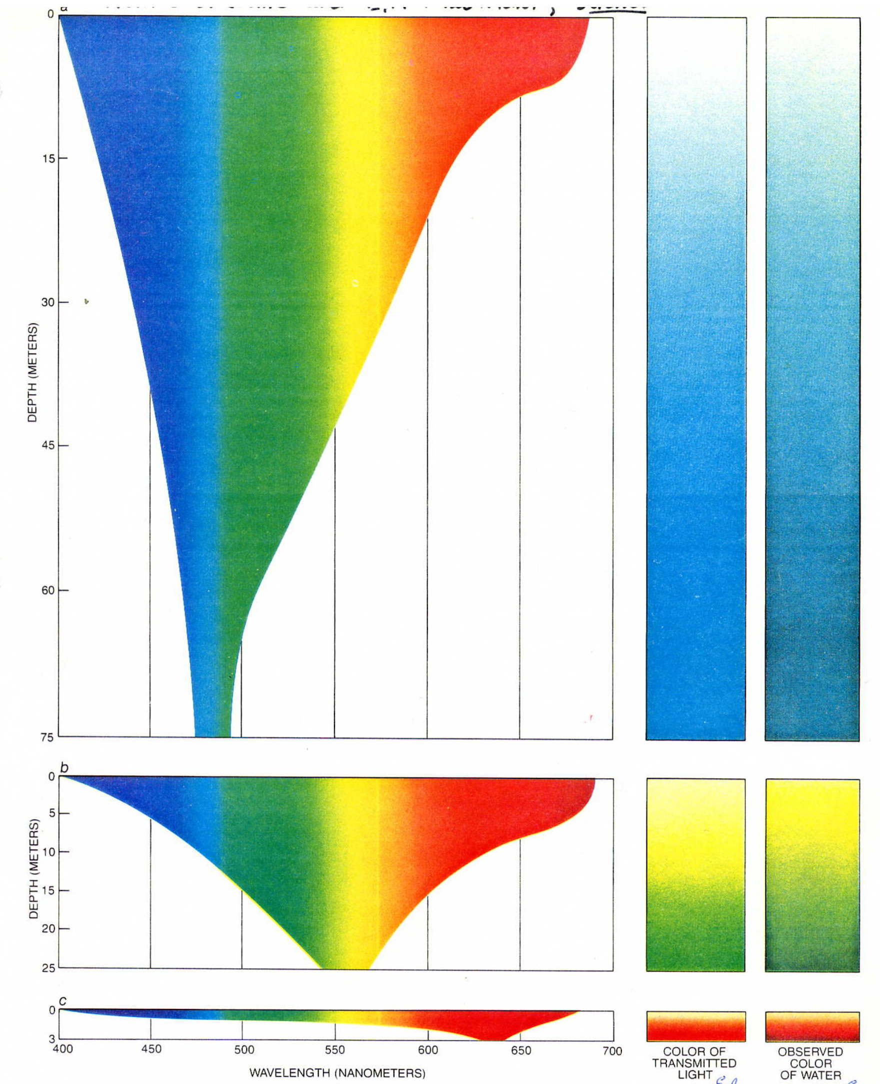




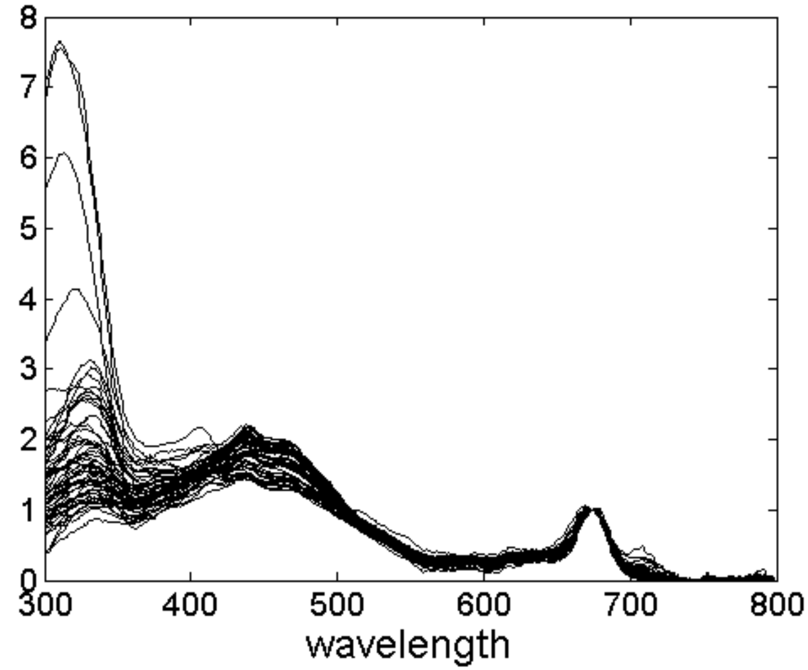
vs.

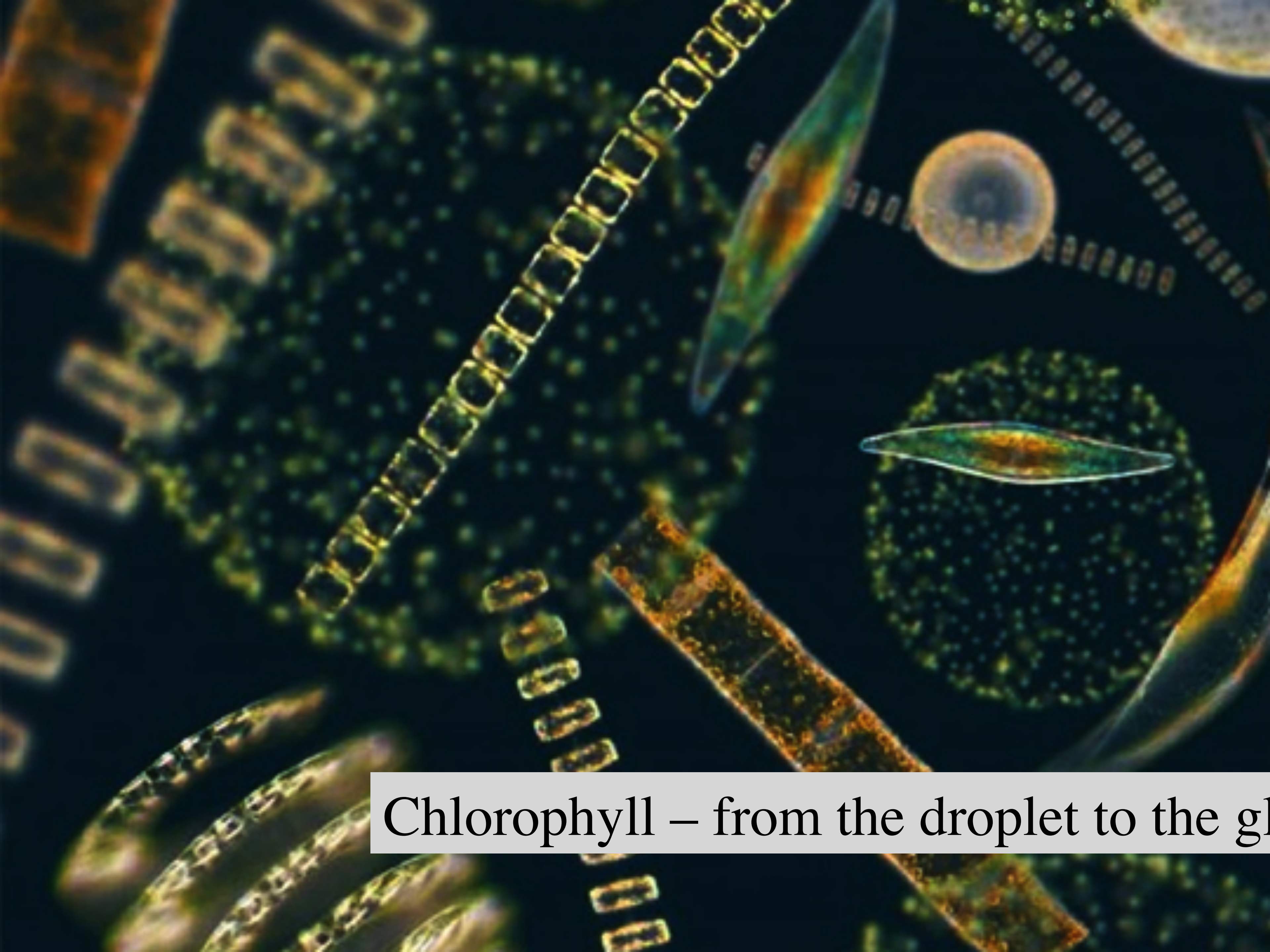


Composite absorption – multiple pigments expand livable environment

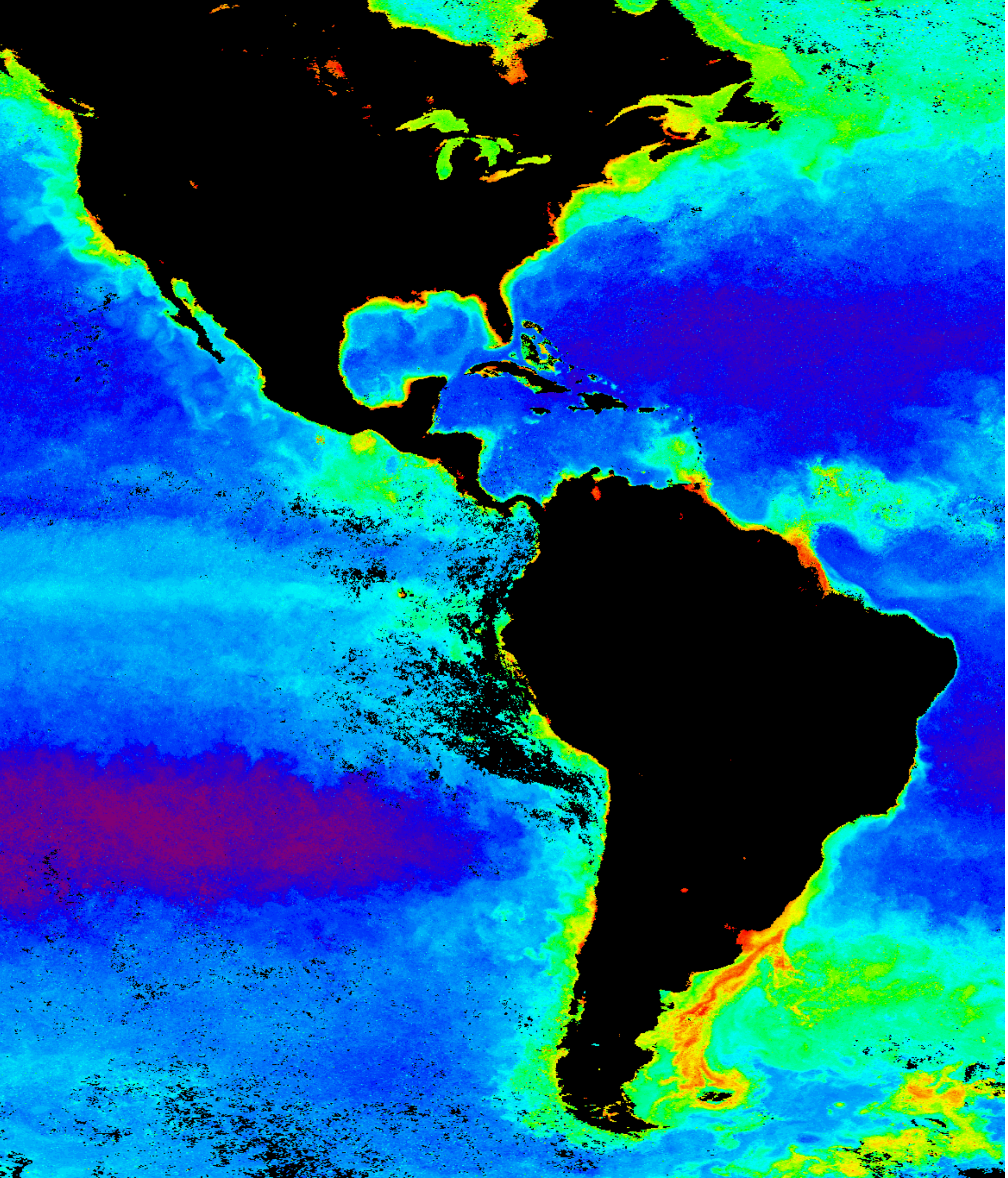


TRANSMISSION OF LIGHT by water is dependent on the color or wavelength of the light. In clear oceans and lakes (*a*) the light becomes increasingly monochromatic and blue as its path length increases. In fresh water that carries green organic matter (*b*) light at all wavelengths is absorbed more quickly 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.





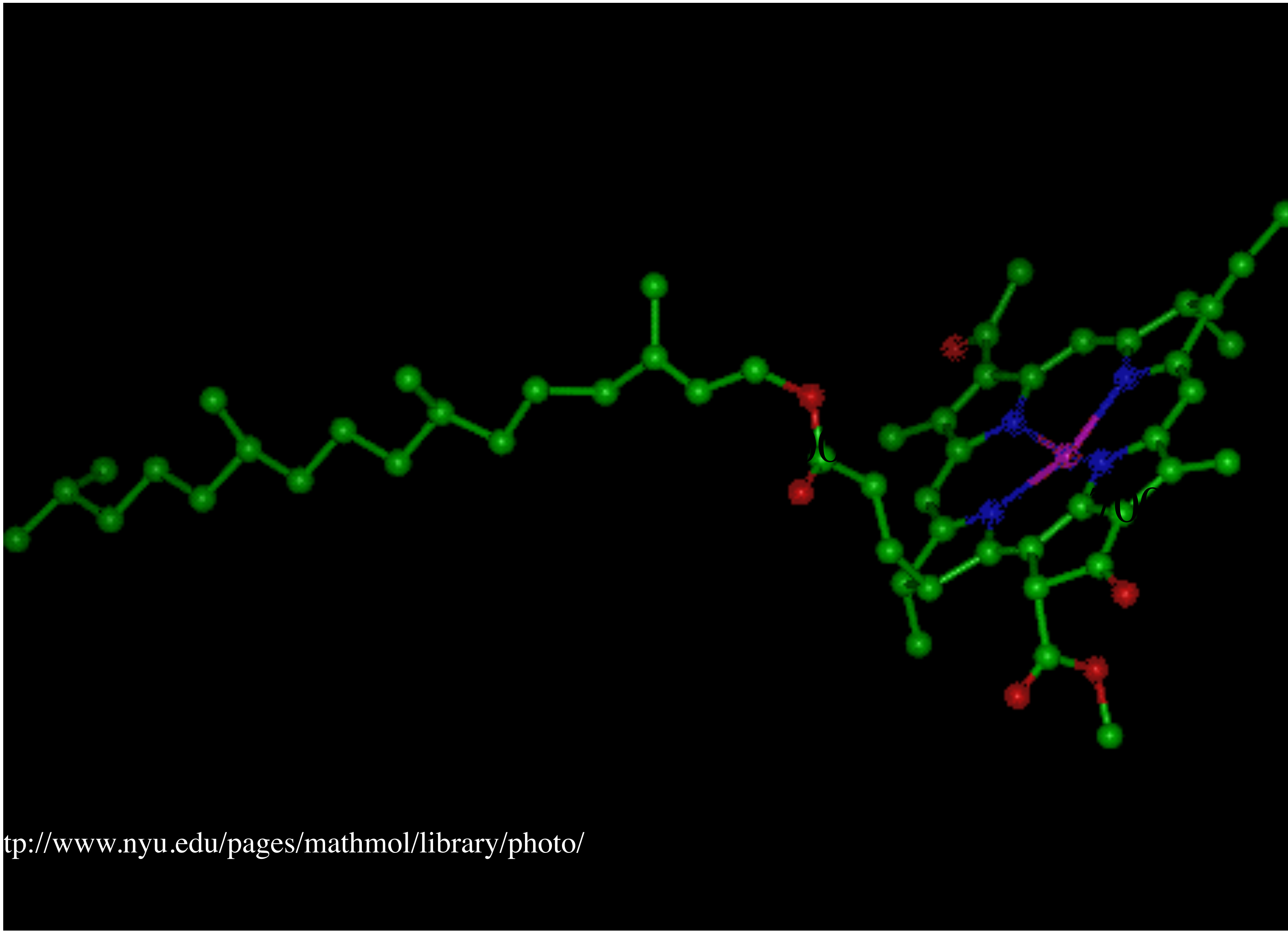
Chlorophyll – from the droplet to the gl



Chlorophyll a – 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 in following slides.

Chlorophyll *a*
– chemical structure & absorption spectrum



Chlorophyll molecule is attached to binding protein.

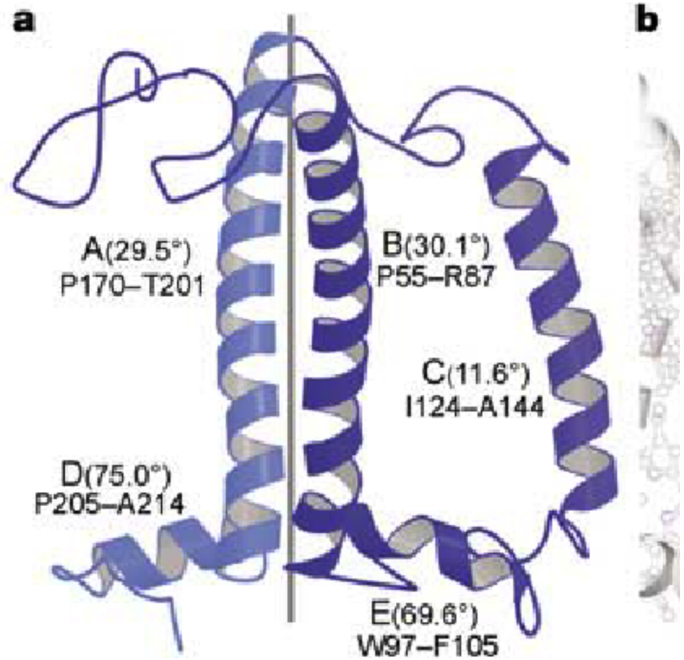
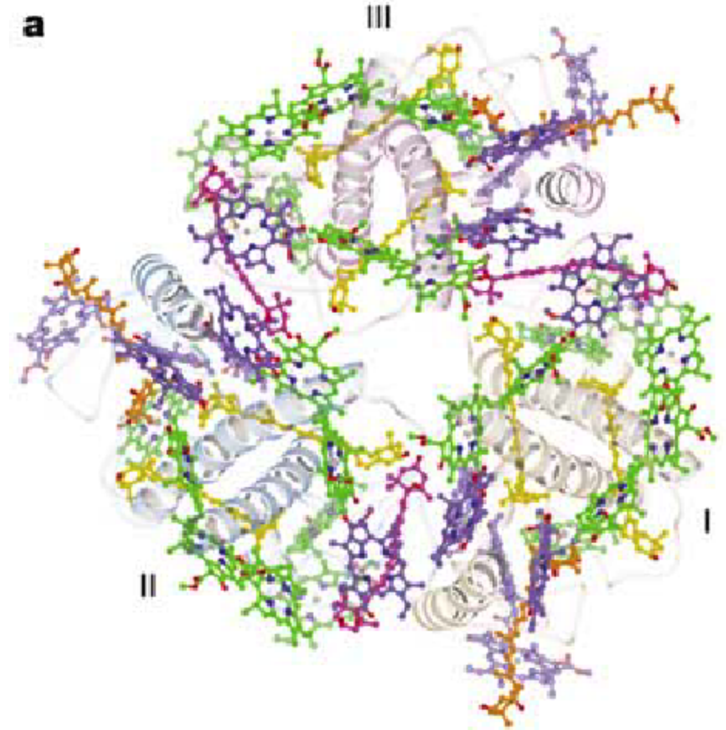


Figure 3 Secondary structure of monomeric LHC-II

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

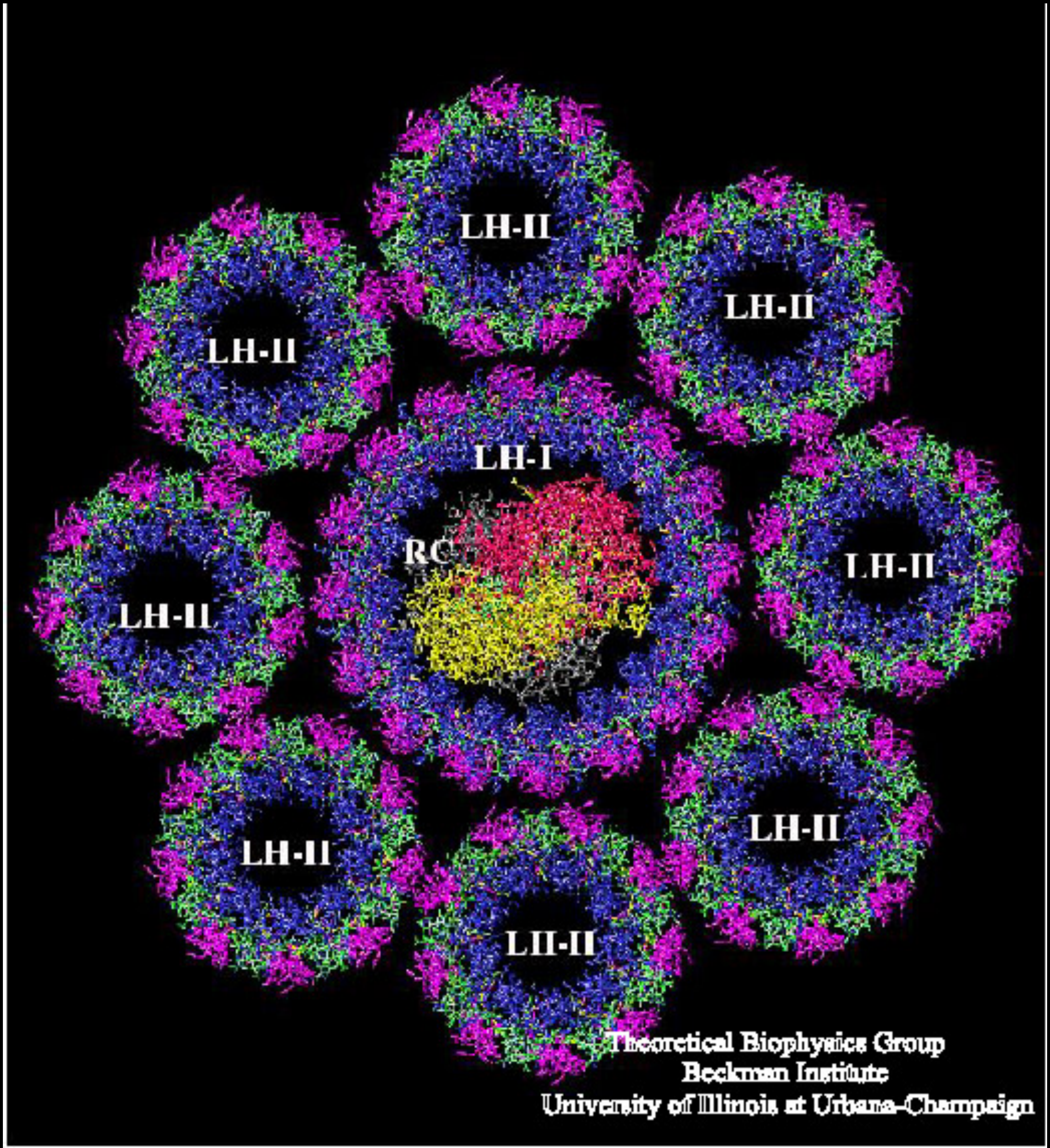
Trimeric complexes of Chl and binding protein.



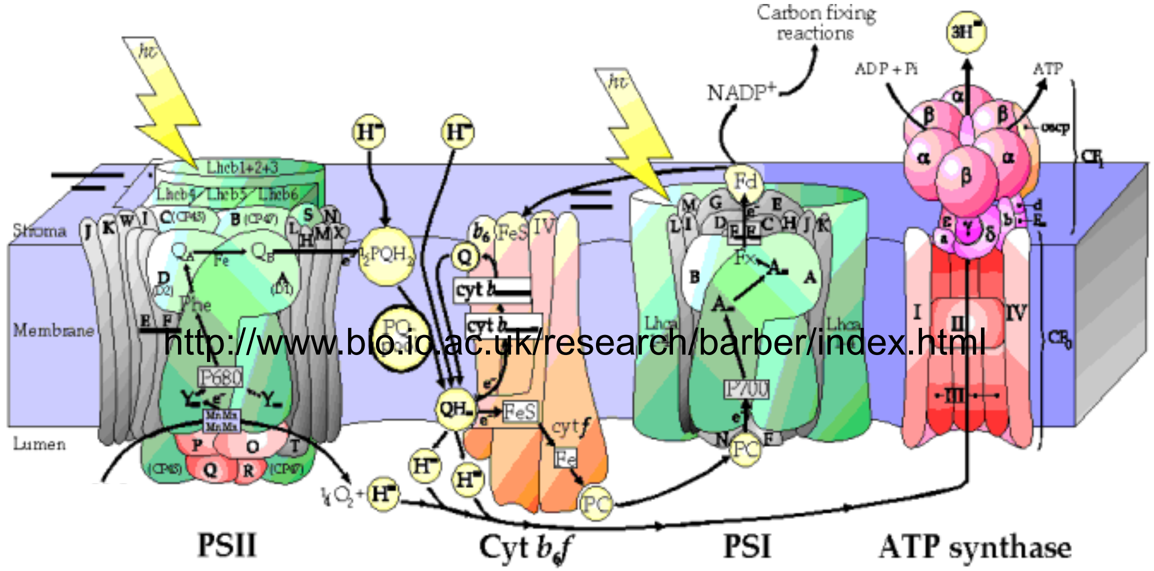
3 monomers = 1 trimer
- green: chl *a*; blue: chl *b*
- yellow/orange: P carotenoids
- magenta: PP carotenoids

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

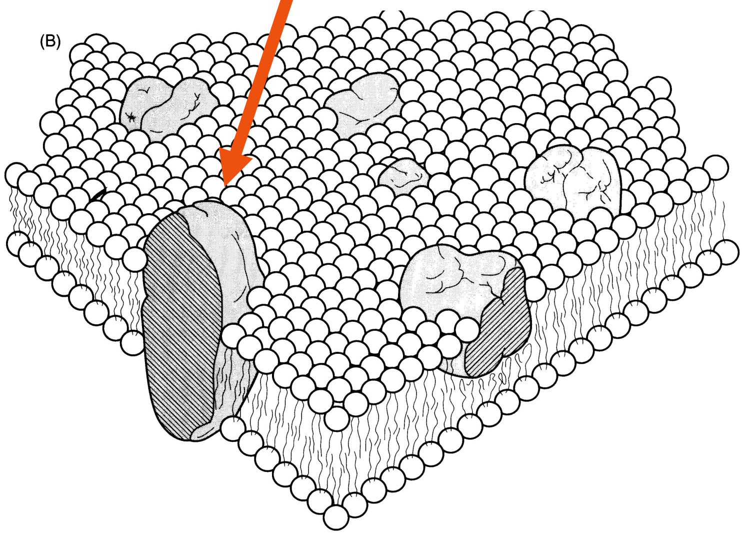
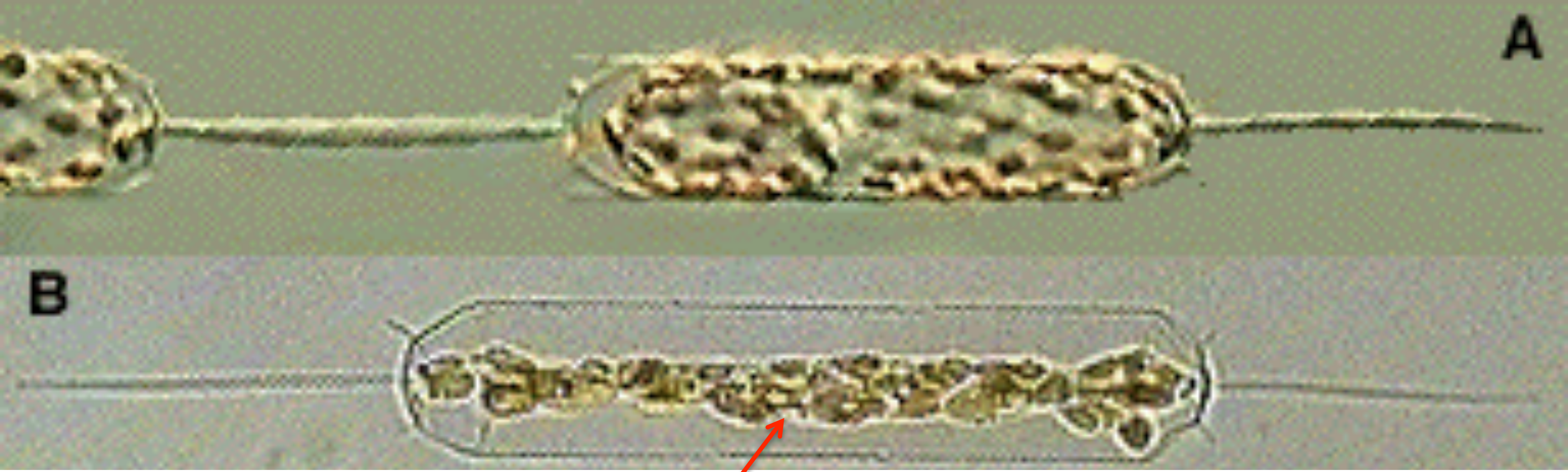


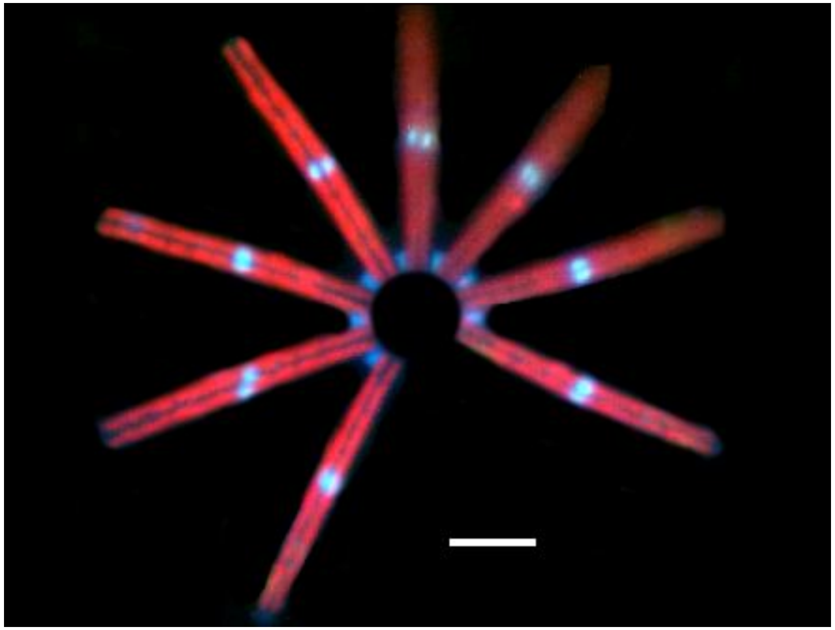
Figure 1.2 (A) Structure of two of the most important lipids that make up thylakoid membranes: monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG). In the formation of membranes, the polar sugar groups face the aqueous phases, while opposing nonpolar alkyl groups are oriented 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.

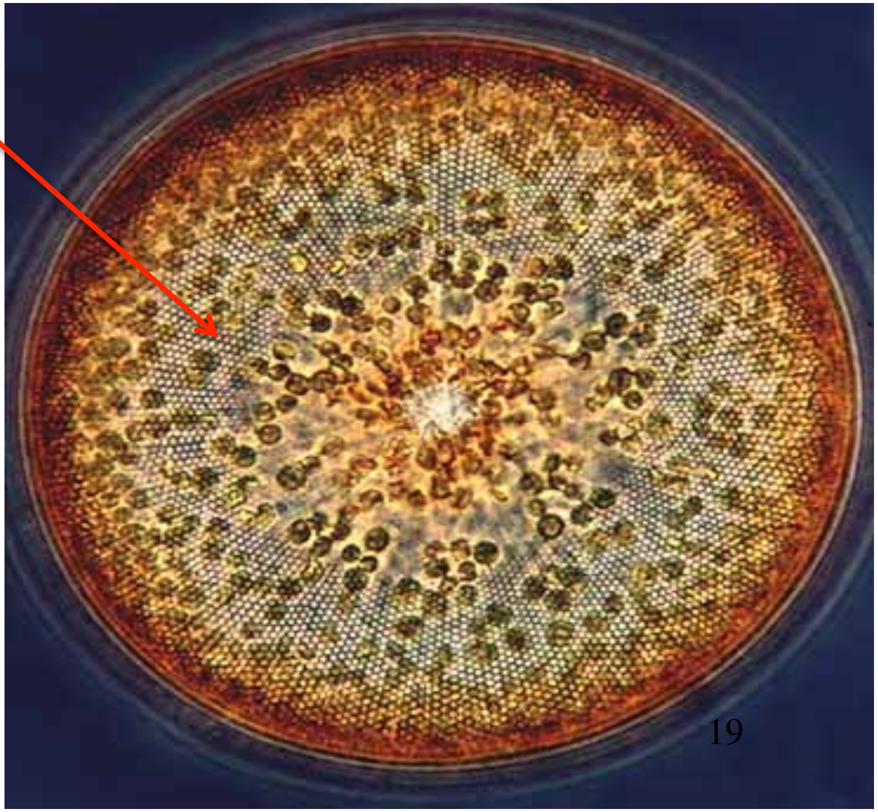
Thylakoid membranes in chloroplast



Diatom chloroplasts

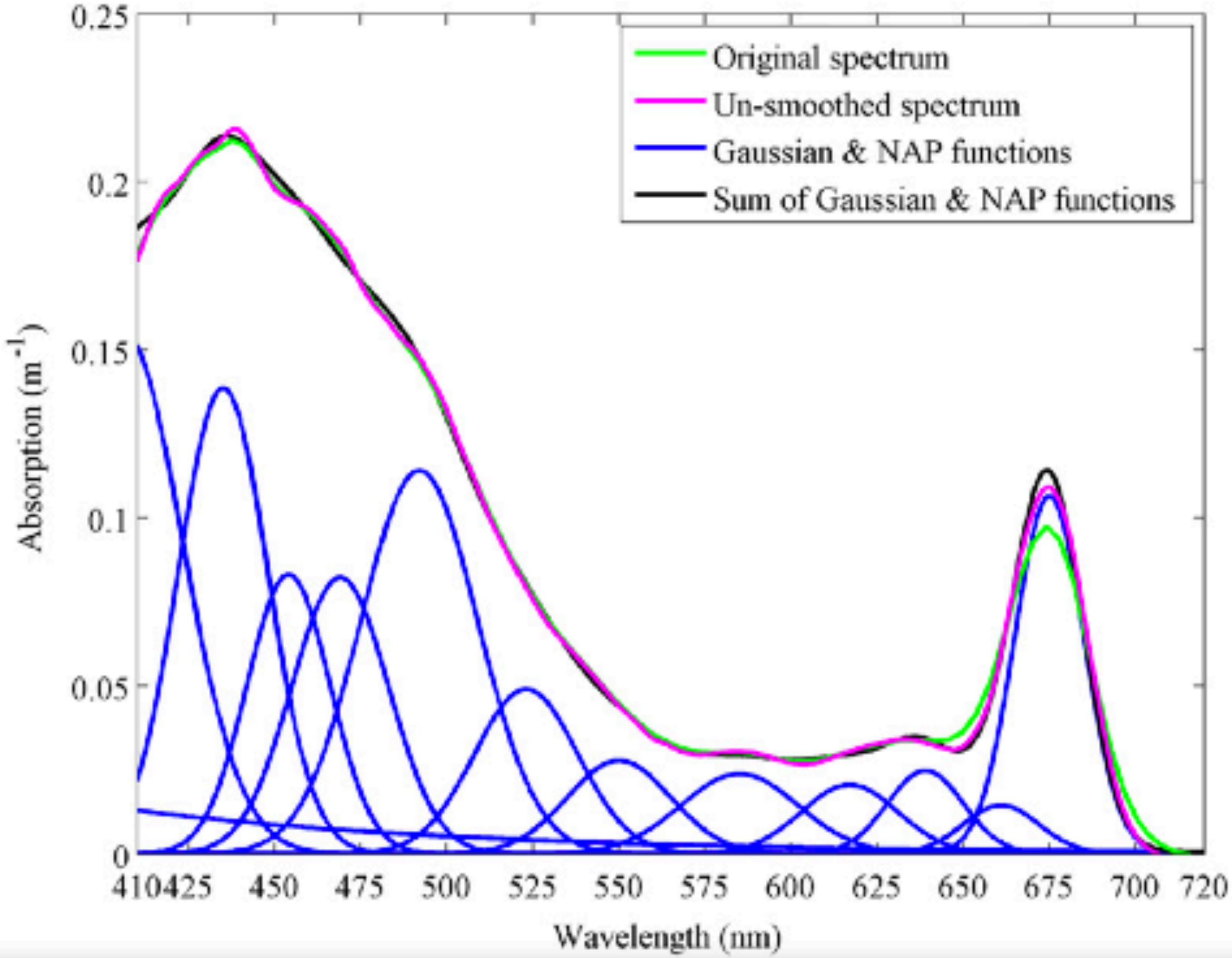
In vivo chlorophyll fluorescence

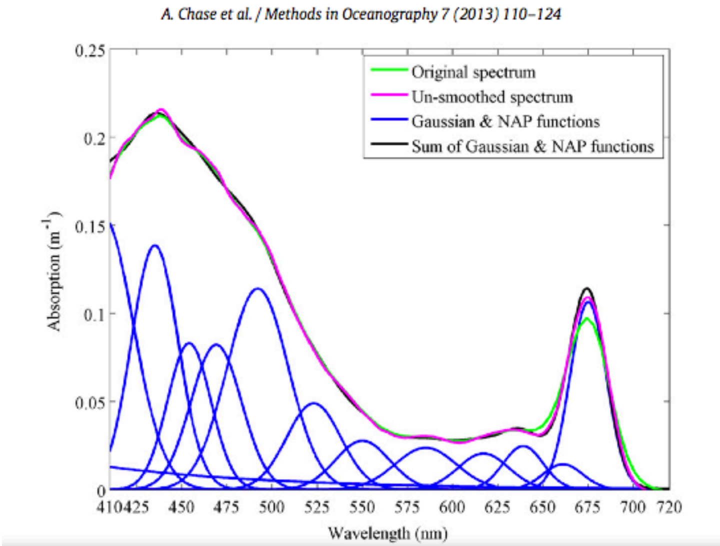


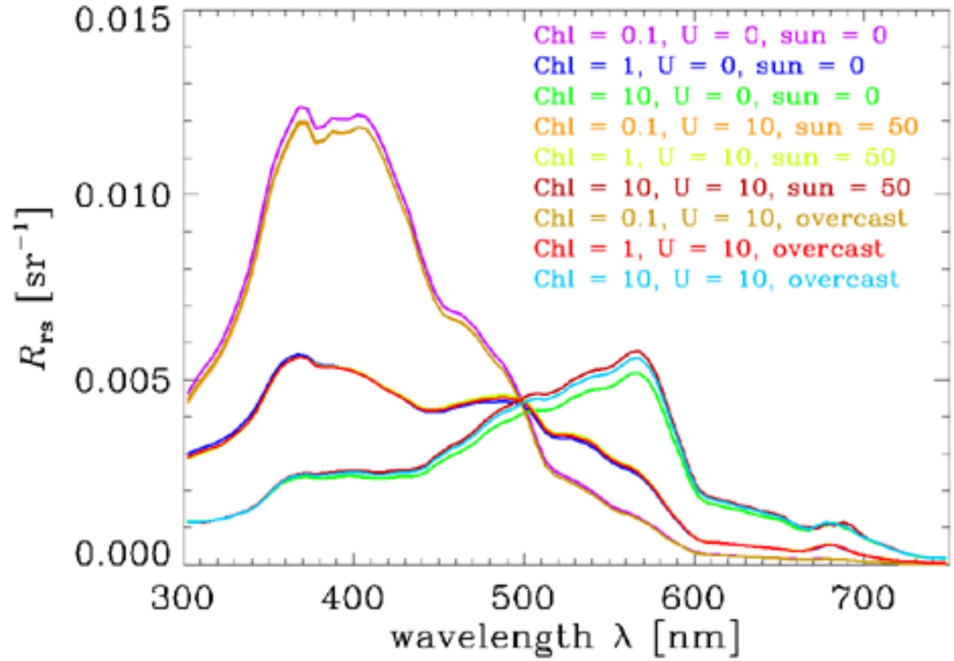


ac-s absorption

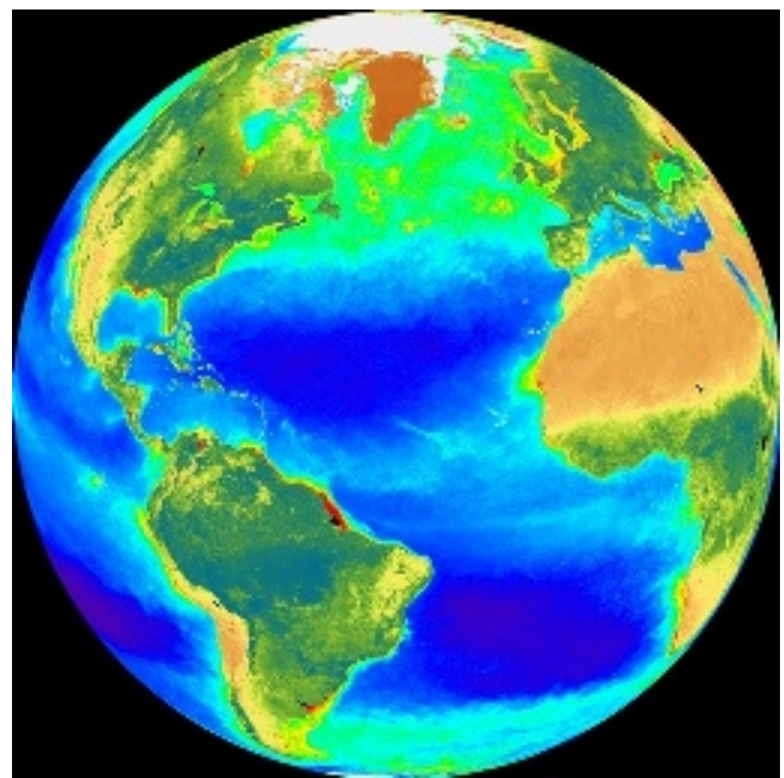
A. Chase et al. / Methods in Oceanography 7 (2013) 110–124



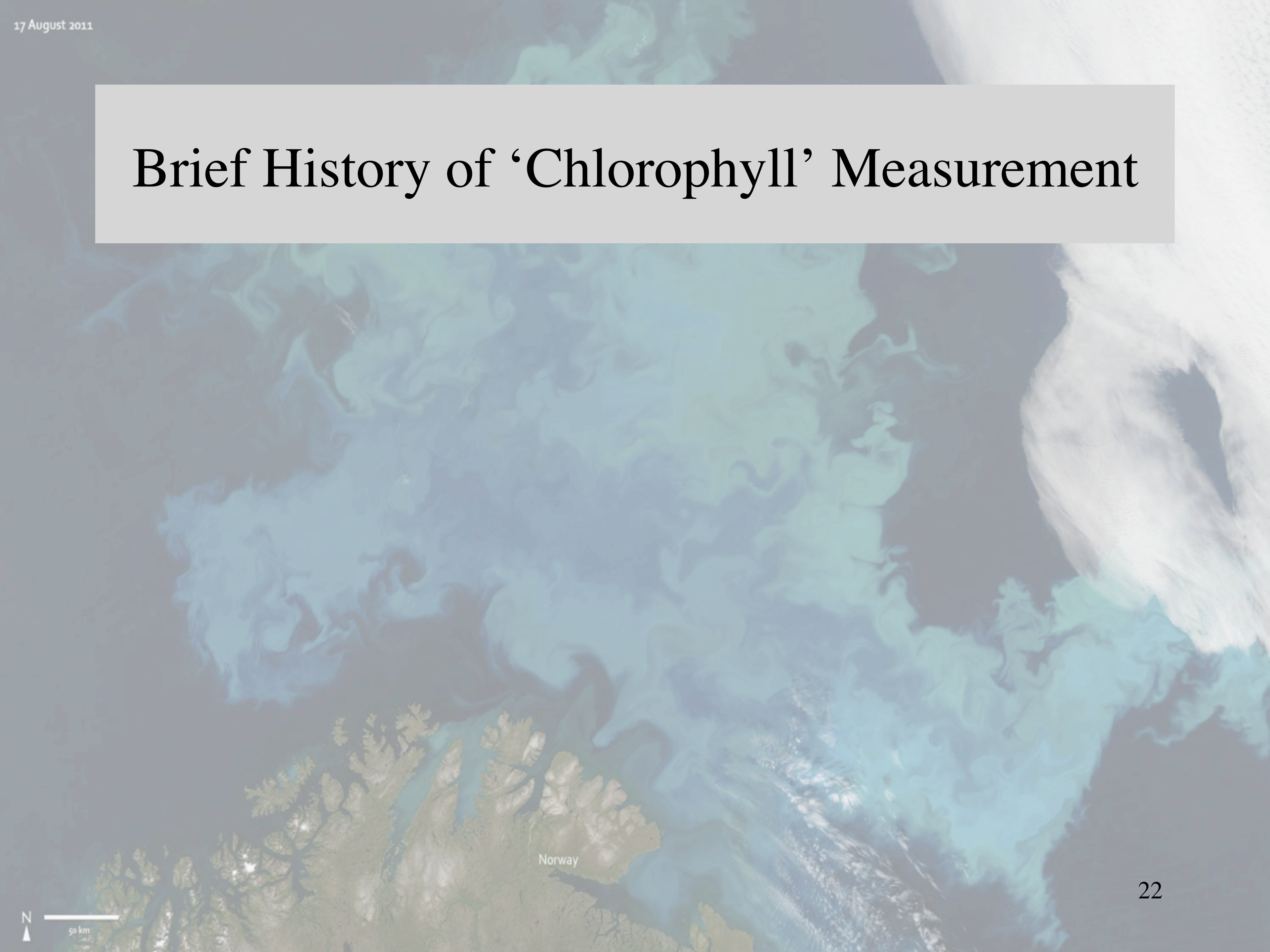




Chlorophyll
- from the molecule to the biology of the global ocean

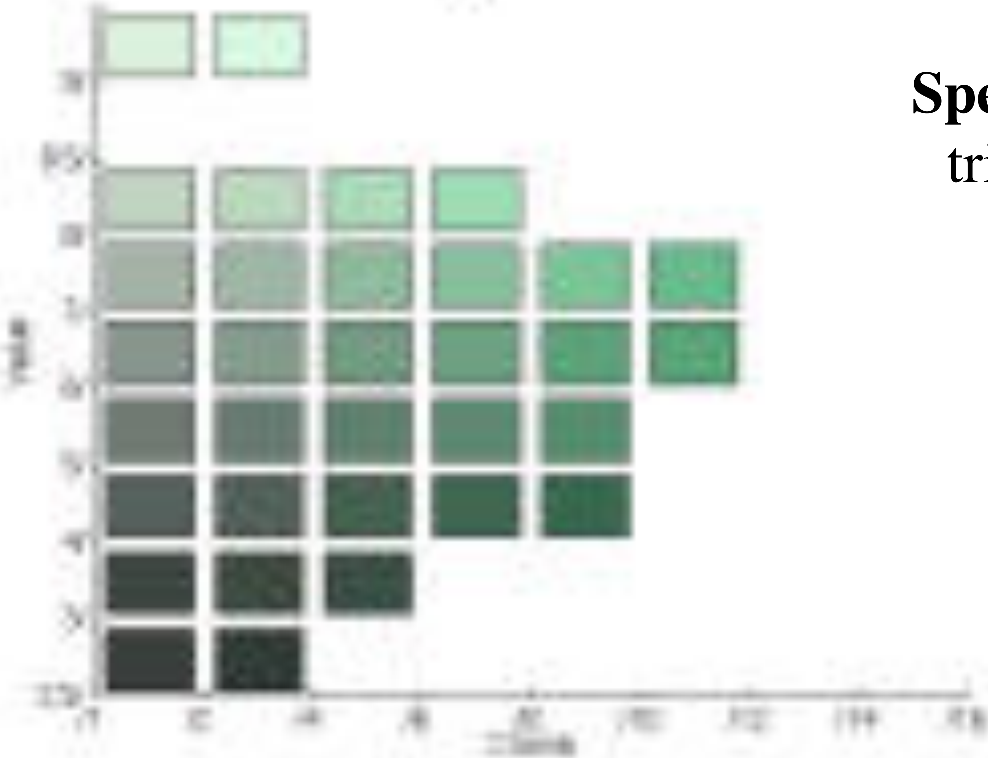


Brief History of ‘Chlorophyll’ Measurement



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

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

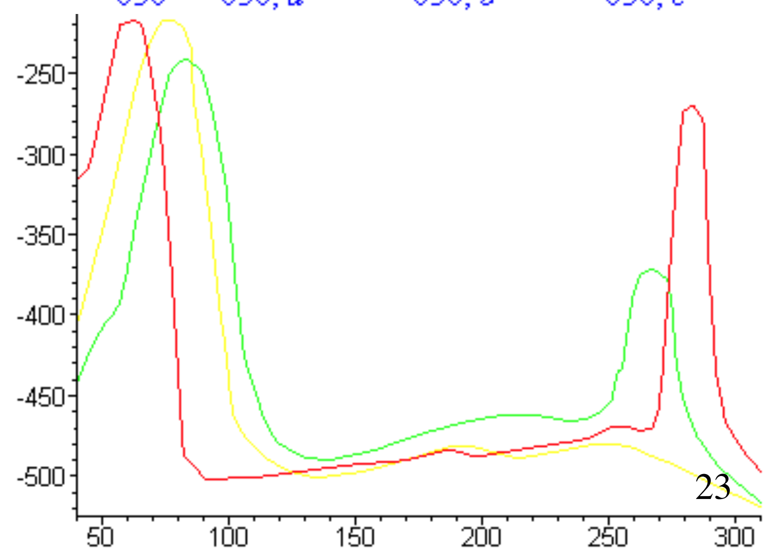


Spectrophotometry, extracts in solvent; trichromatic eq. to separate pigments.
~ 1950’s – 1960’s

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

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

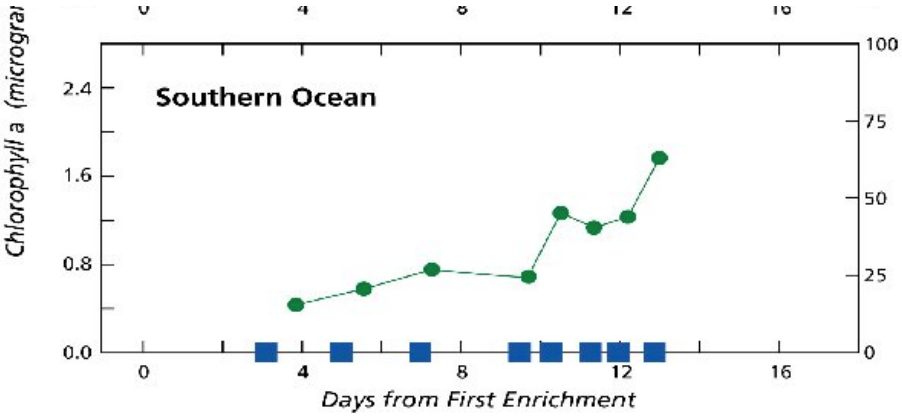
$$OD_{630} = \varepsilon_{630,a}\, a\, L + \varepsilon_{630,b}\, b\, L + \varepsilon_{630,c}\, c\, L$$

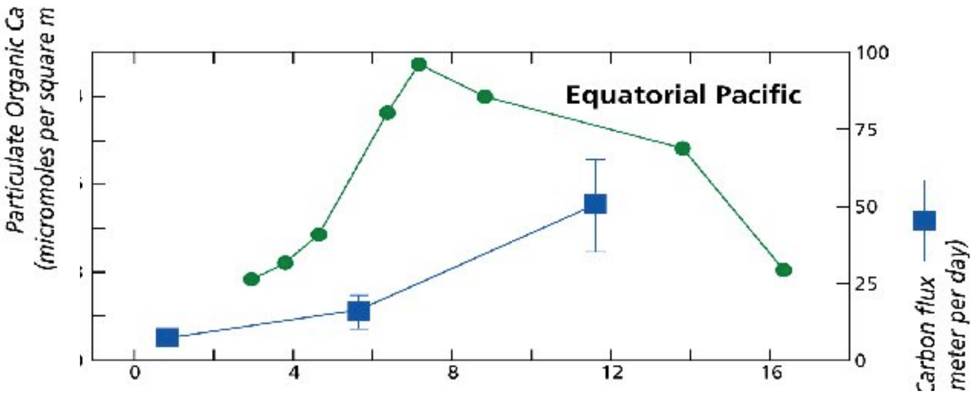


Brief history of measurement of ‘bulk’ chlorophyll & related entities

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)

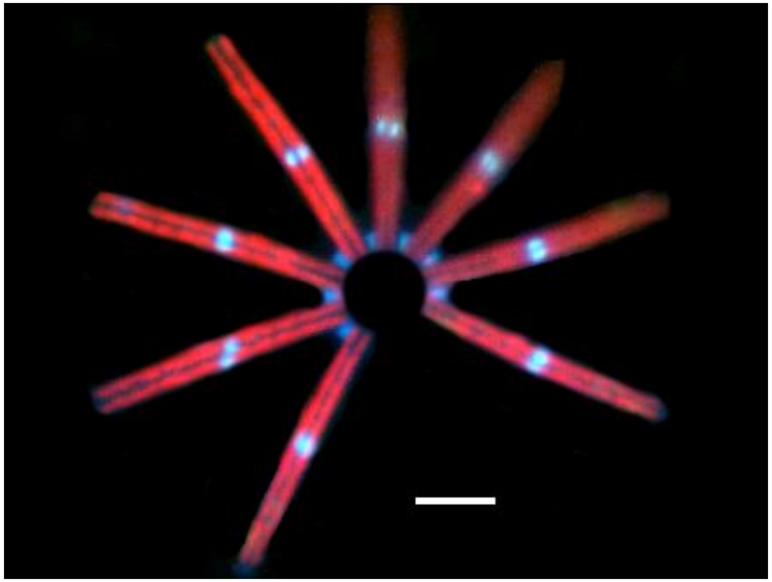
Lots of good information. For example: phytoplankton response to iron-fertilization; Chl *a* (μg L^{-1}) provided an index of bulk phytoplankton response:
Southern Ocean vs. Equatorial Pacific.





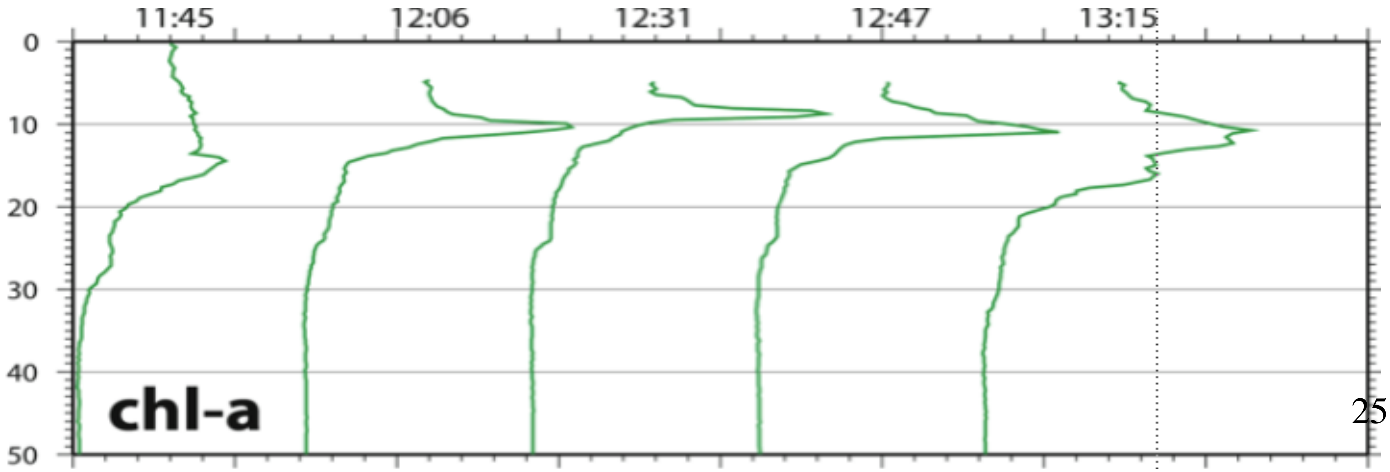
http://cafethorium.whoi.edu/Fe/1999-Annualreport.html

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



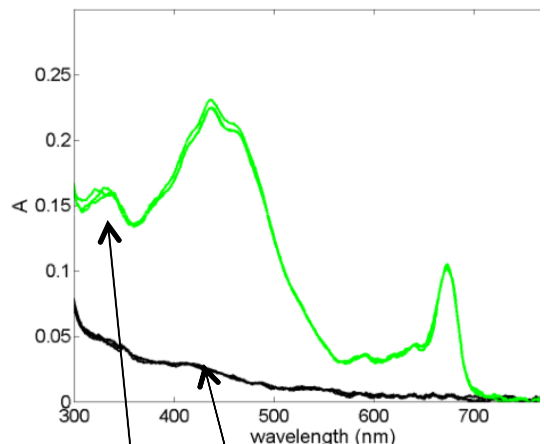
Late 1960’s, **fluorescence profiles of fluorescence in living cells** –
measure directly in the ocean.
Fast! and high vertical resolution.
(Mobley’s Conservation of Misery)

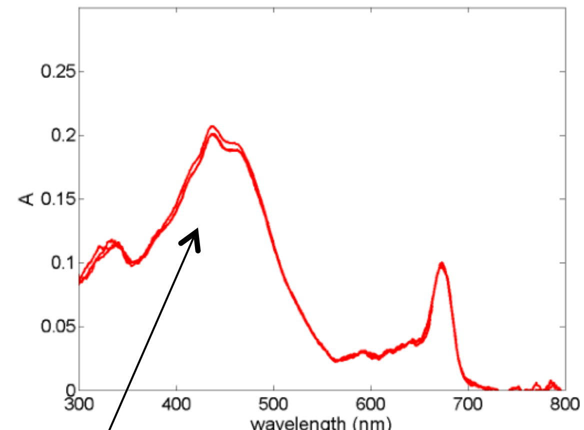
Used on CTD, mooring, floats, gliders, etc.
Example below of thin layers in Monterey Bay.

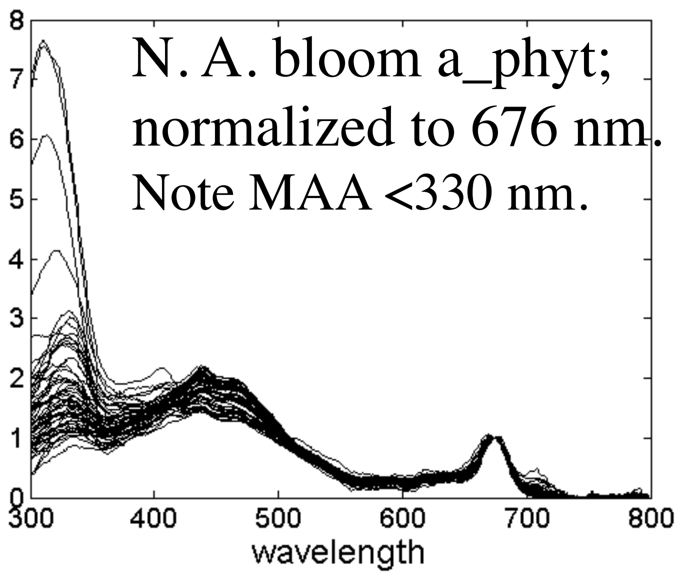


QFT – Quantitative Filter Technique (filter pad absorption) ~ 1980’s

(Quantitative version of HPPU)







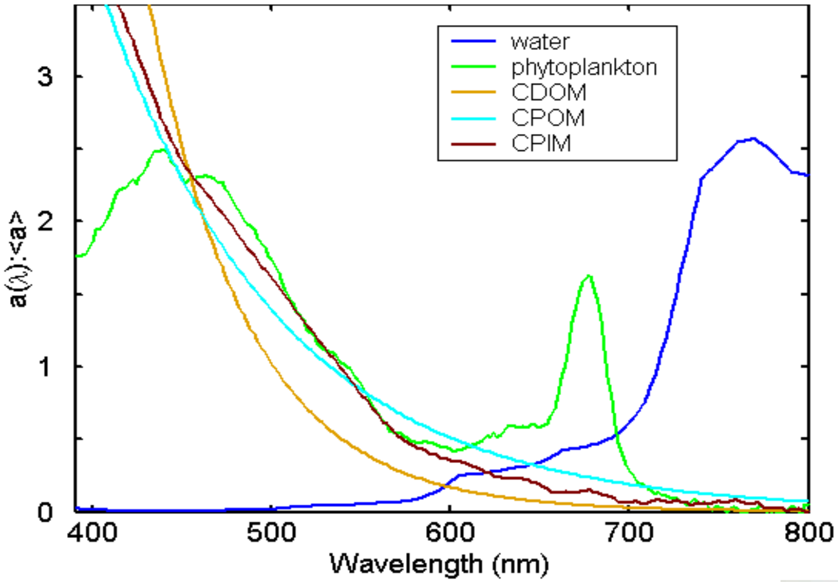
a_particulate

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

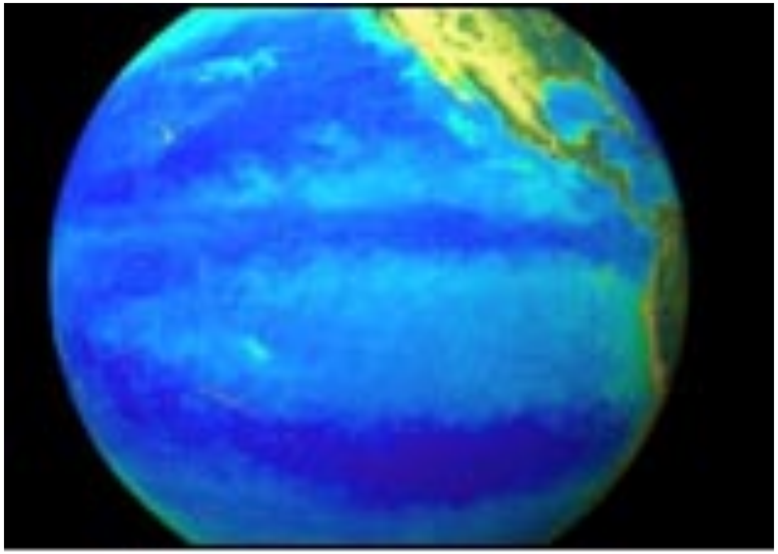


Remote sensing reflectance is based on selective absorption by phytoplankton pigments; empirical algorithms, need local tuning. ~ 1980's

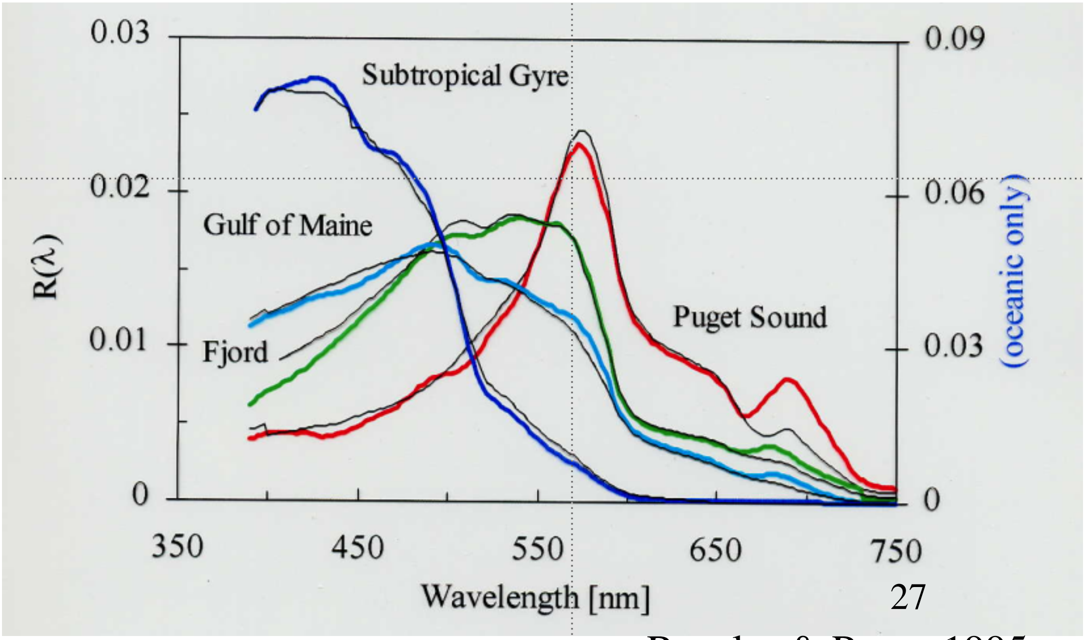
$$R_{rs} \sim [b_b / (a + b_b)]$$

a ~ phytoplankton (Chl? absorption?)

b_b ~ particles and carbon



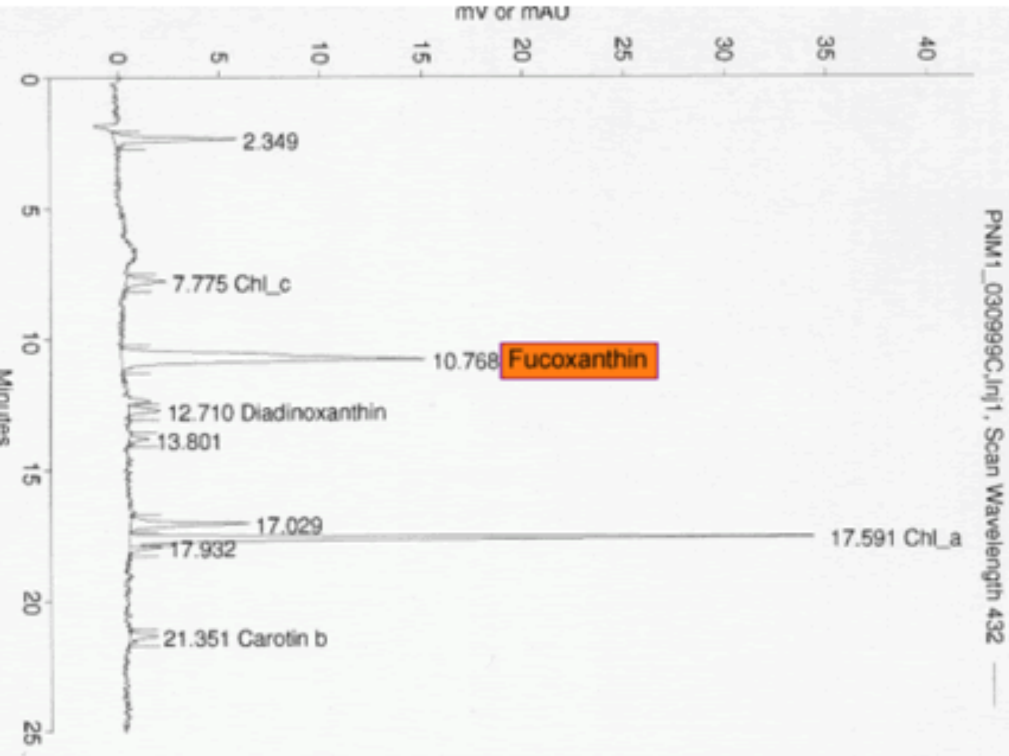
El Nino



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 ~ $100

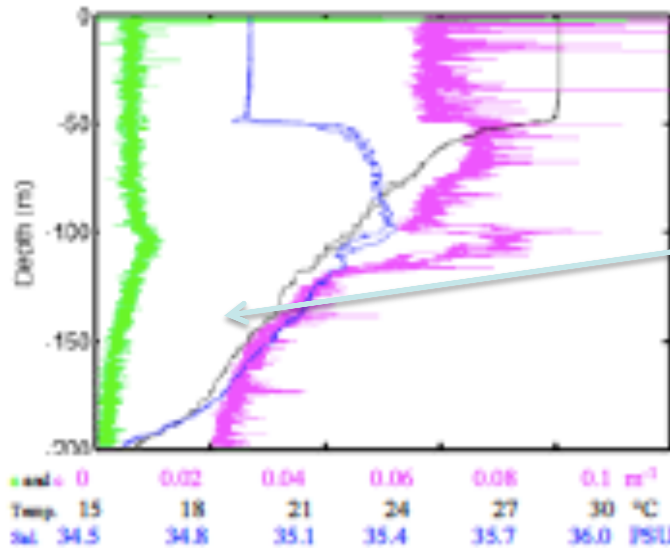


Pigment composition of the major algal groups

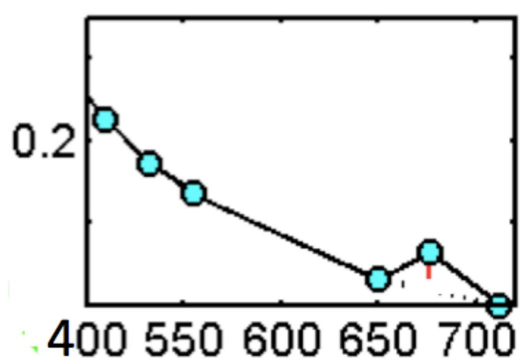
Pigments	Blue-Green Algae/ Cyanophyceae	Red Algae/ Rhodophyceae	Brown Algae/ Phaeophyceae	Green Algae/ Chlorophyceae	Dinoflagellates/ Dinophyceae	Diatoms/ Bacillariophyceae	Naked Flagellates
Chlorophylls							
Chlorophyll-a	●	●	●	●	●	●	●
Chlorophyll-b				●			
Chlorophyll-c			●		●	●	●
Phycobilins			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

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

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

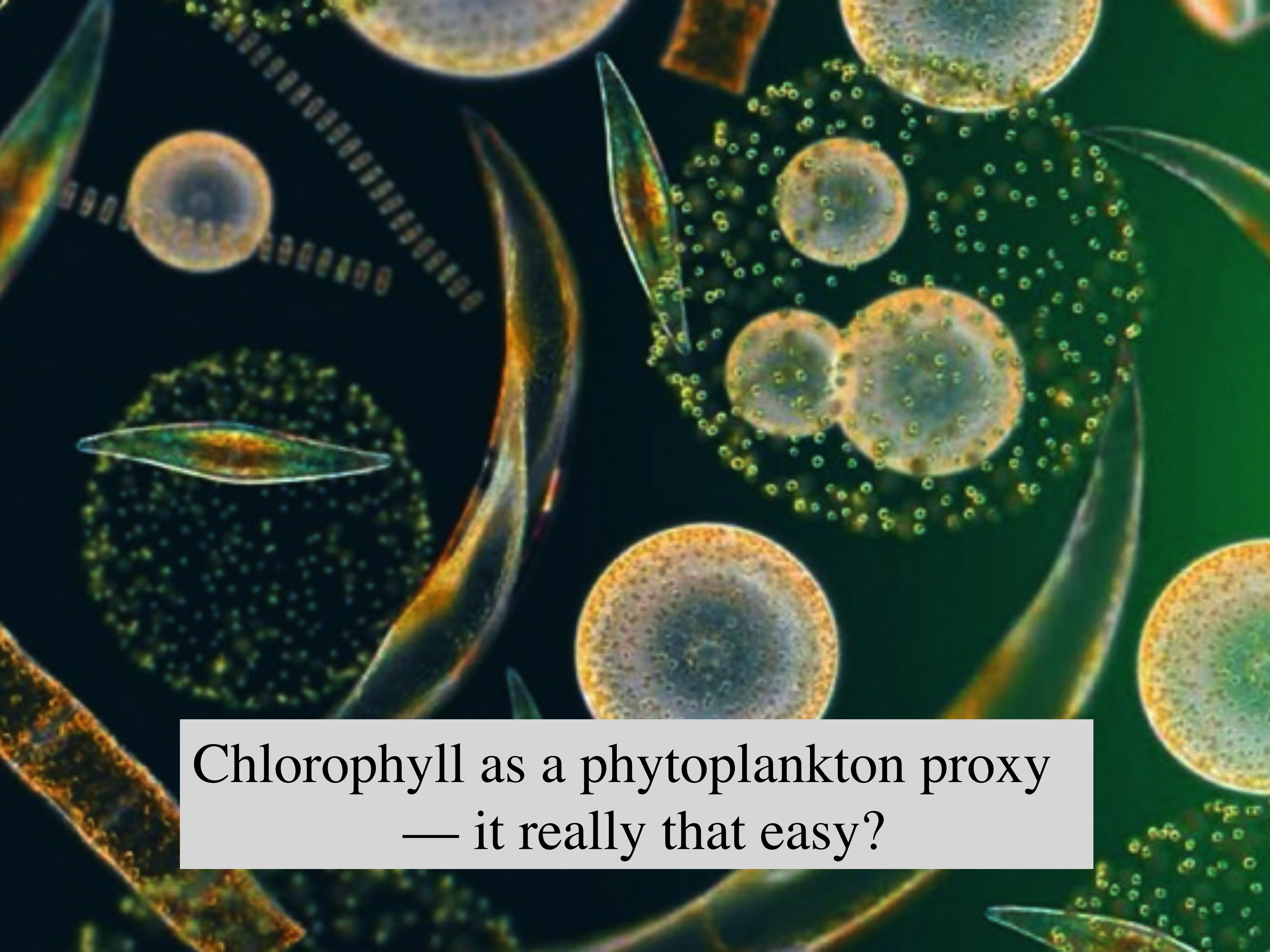


a_phyt (676) is a good estimator of chlorophyll concentration in cell (Roesler leader in use & interpretation)



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



Chlorophyll as a phytoplankton proxy — it really that easy?

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).

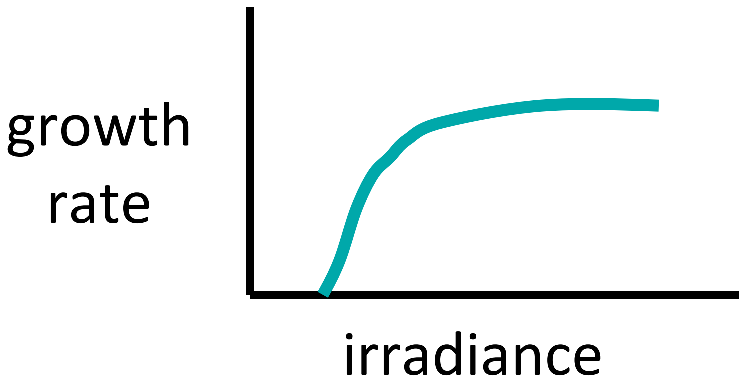
Relationship between carbon and Chl allows estimation of phytoplankton carbon. But beware Mobley's Law of Conservation of Misery; C/Chl ratio influenced by physiology.

Some measurement that assesses 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. Remember need for closure.

Variability in Chl / cell

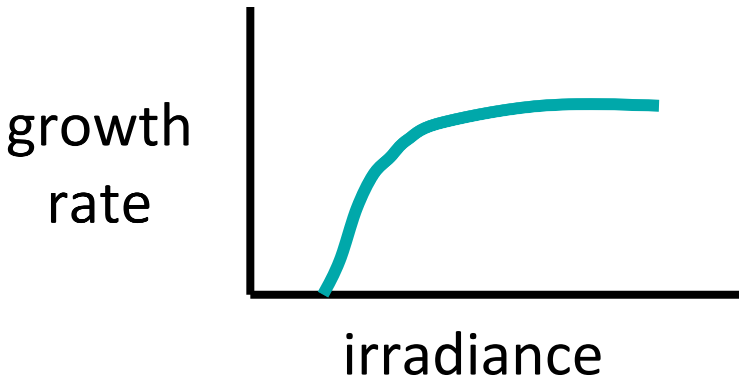
Physiological adaptation to low light is to increase amount of light collectors (chlorophyll molecules).

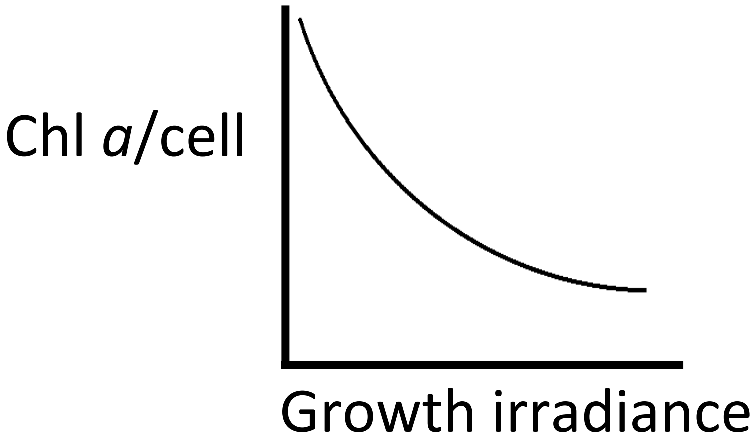


With constant chlorophyll/cell, growth rate would be very low at low light

Variability in Chl / cell

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





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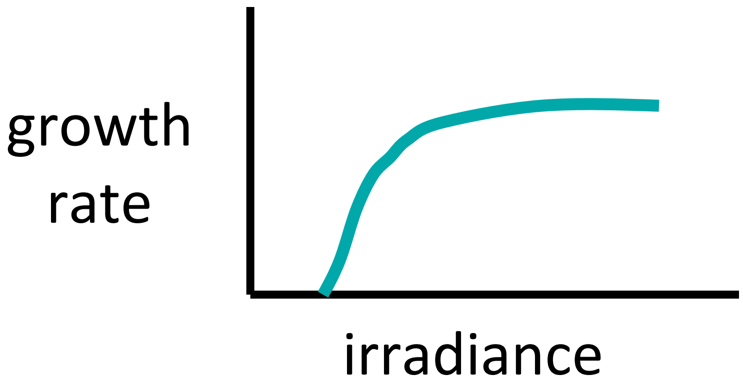
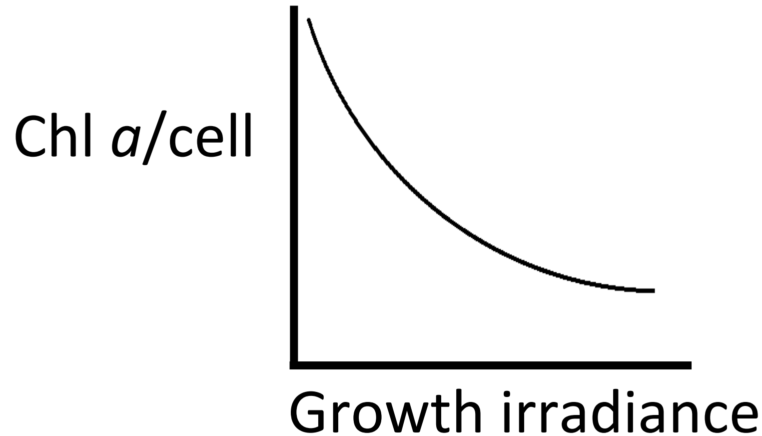
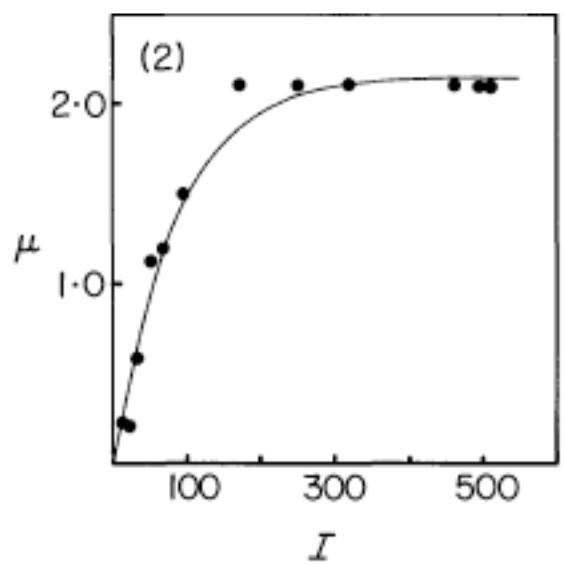


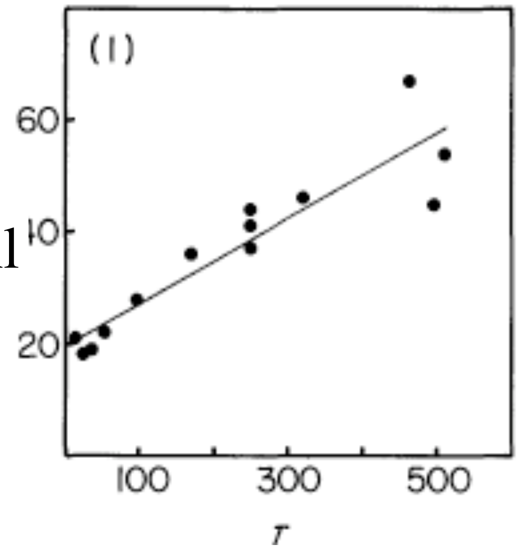


Ability to increase Chl a/ cell at low light enables growth rate to be higher than it otherwise would be.

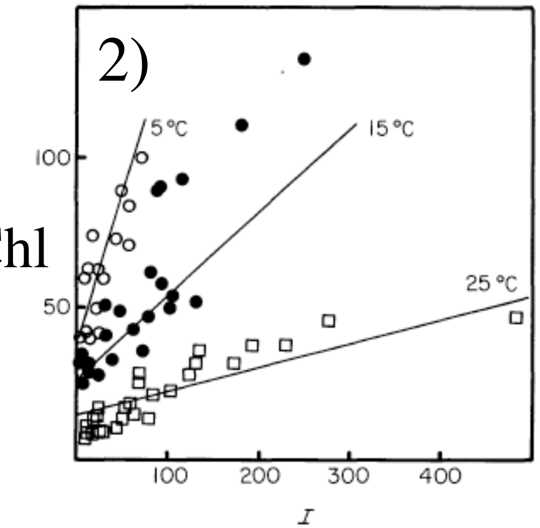


Real data - higher concentrations of chlorophyll and other pigments allow cells to grow better at lower irradiances

C/Chl



C/Chl

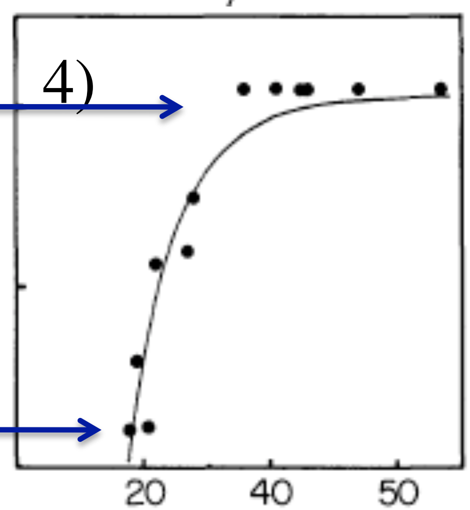


Temp

Light not limited

Light limited

μ

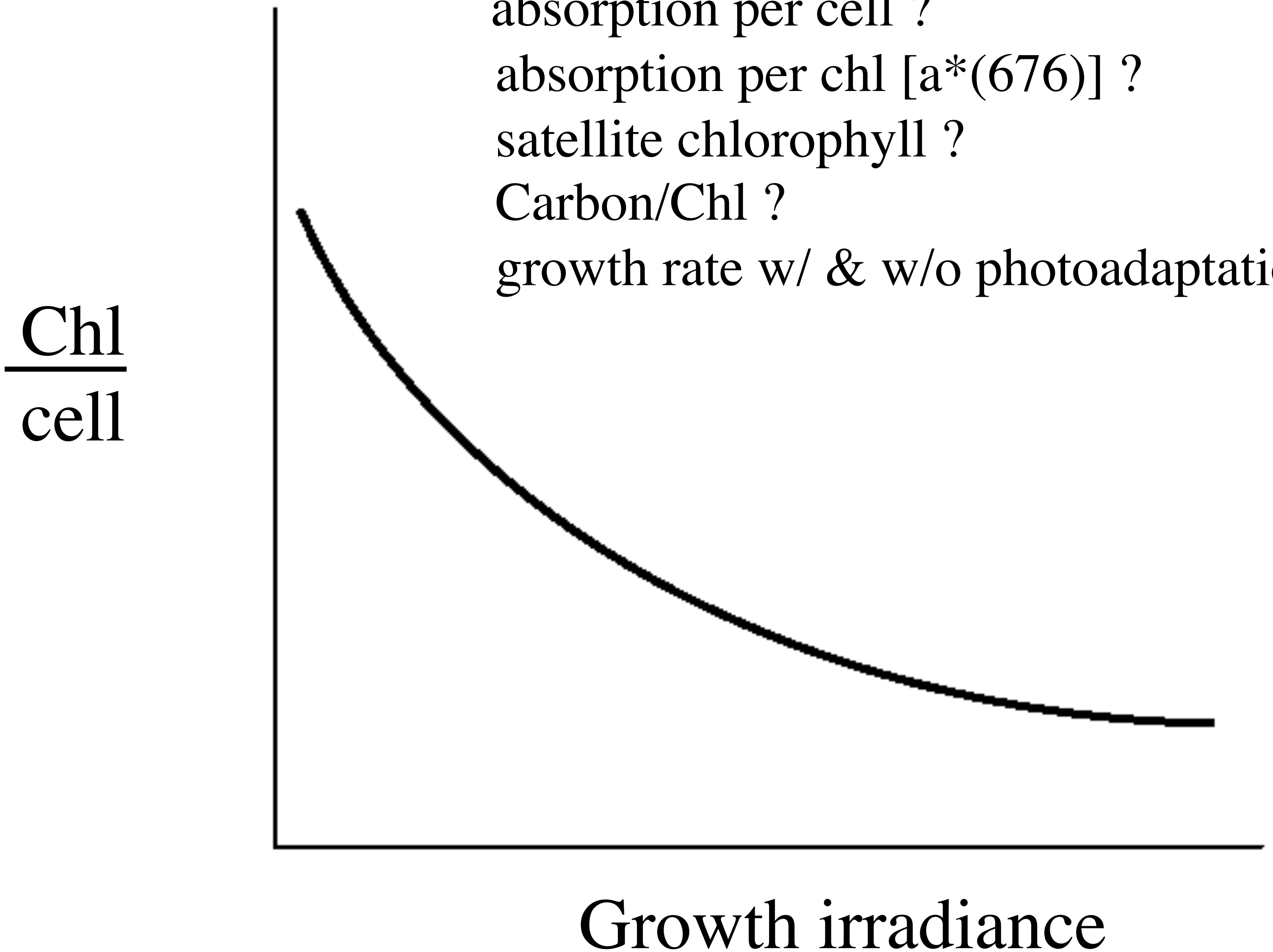


C/Chl

Geider. 1987. New Phytologist 106: 1

What is the consequence of photo adaptation on:

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



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 ?