Phytoplankton size and the discovery of *Prochlorococcus*

Chisholm

C-MORE Summer Course

June 1, 2012

Overview of 4 lectures

June 1: Size and intro to *Prochlorococcus*

June 6: *Prochlorococcus* and its phage

June 9: *Prochloroccus* as a model system

July 2: Synthetic biology to geoengineering

Lecture 1 – Guide to Readings

Phytoplankton Size:

Chisholm 1992:	An old review
Finkel 2010:	Excellent overview, including stoichiometry
Kempes 2011:	Analytical treatment

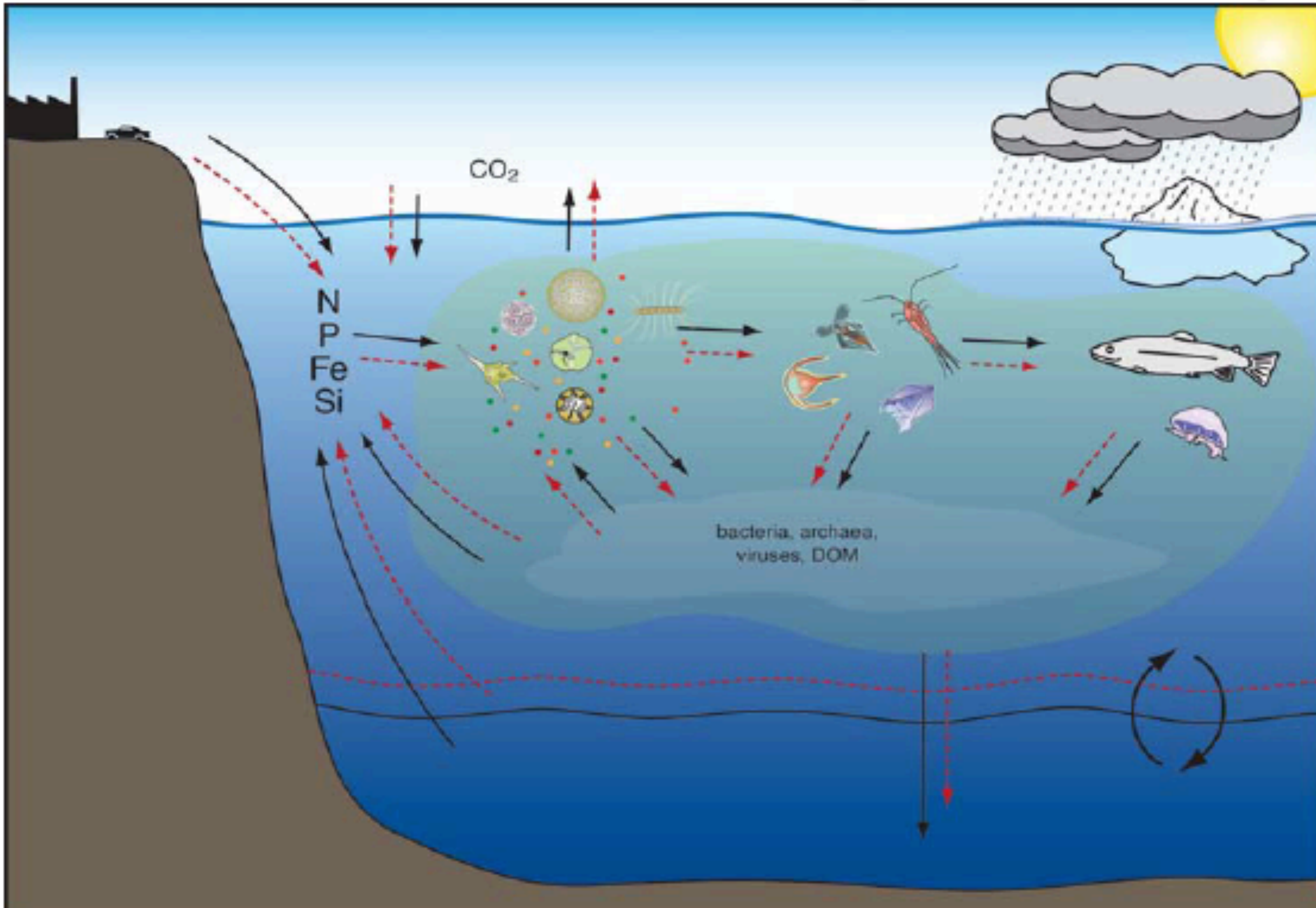
Prochlorococcus – ancient history

Chisholm et al 1988:	the "Discovery"
Chisholm et al 1992:	the "Naming"
Chisholm 2011:	An "easy reading" overview of the whole story

Prochlorococcus – Ecotypes

Johnson-Zinser et al 2006	Ecotype distributions along gradients

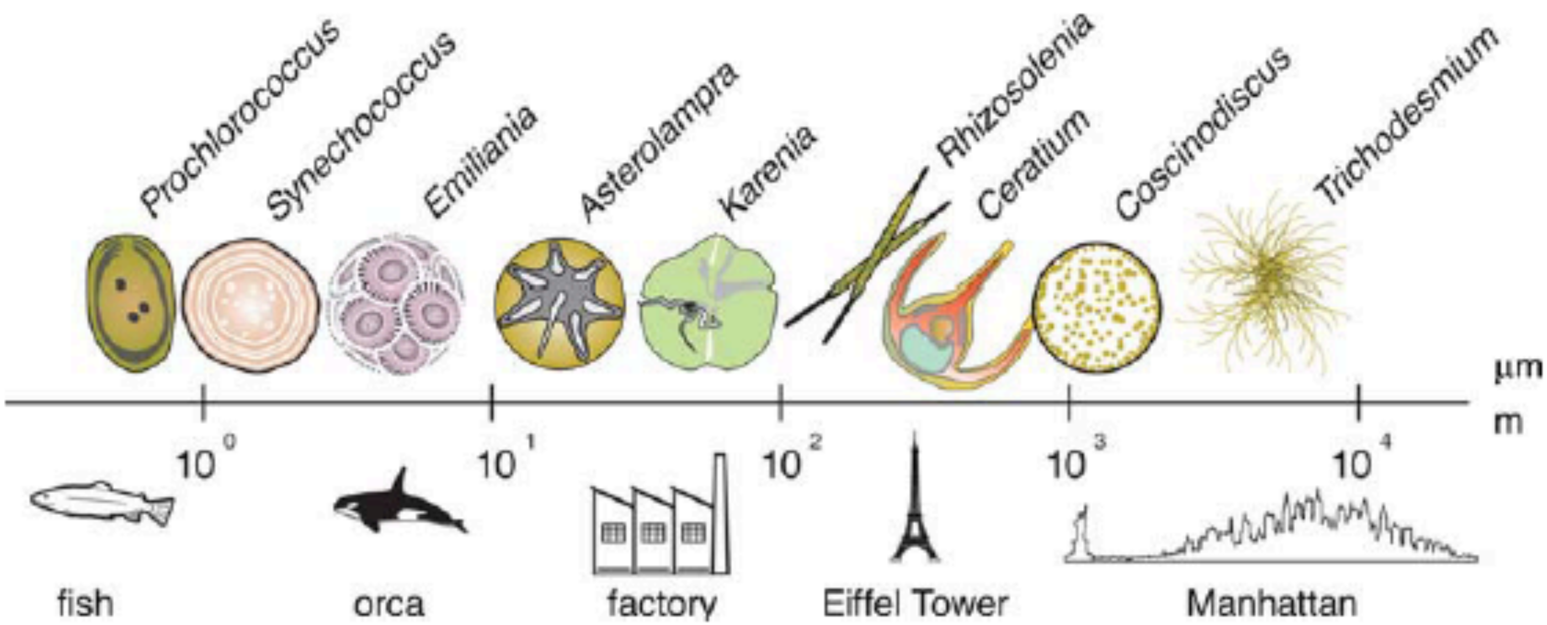
Ocean food webs and biogeochemistry



Finkel et al J. Plankton Res 2010

We have a hard time imagining log scales

(Size influences abundance, and distances between cells)



Finkel et al J. Plankton Res 2010

Allometry: The classic size-density relationship

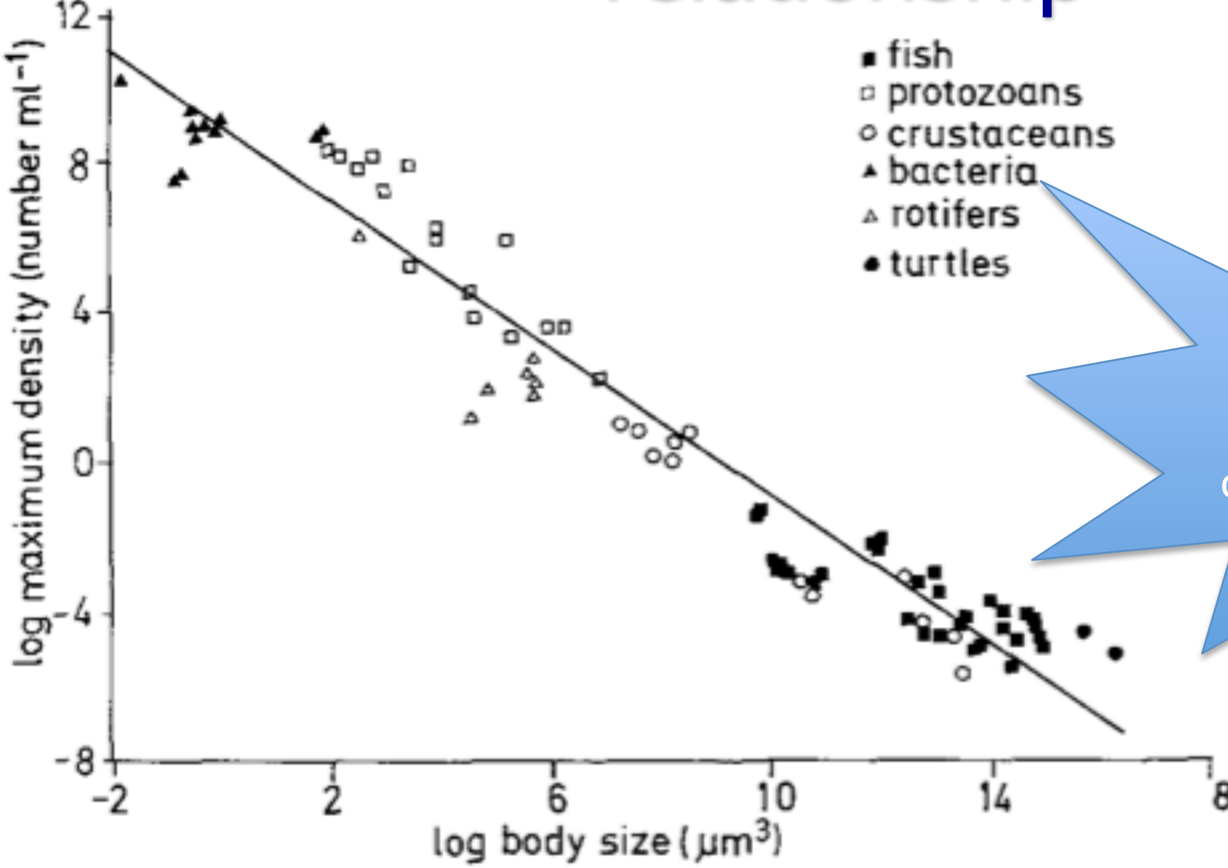


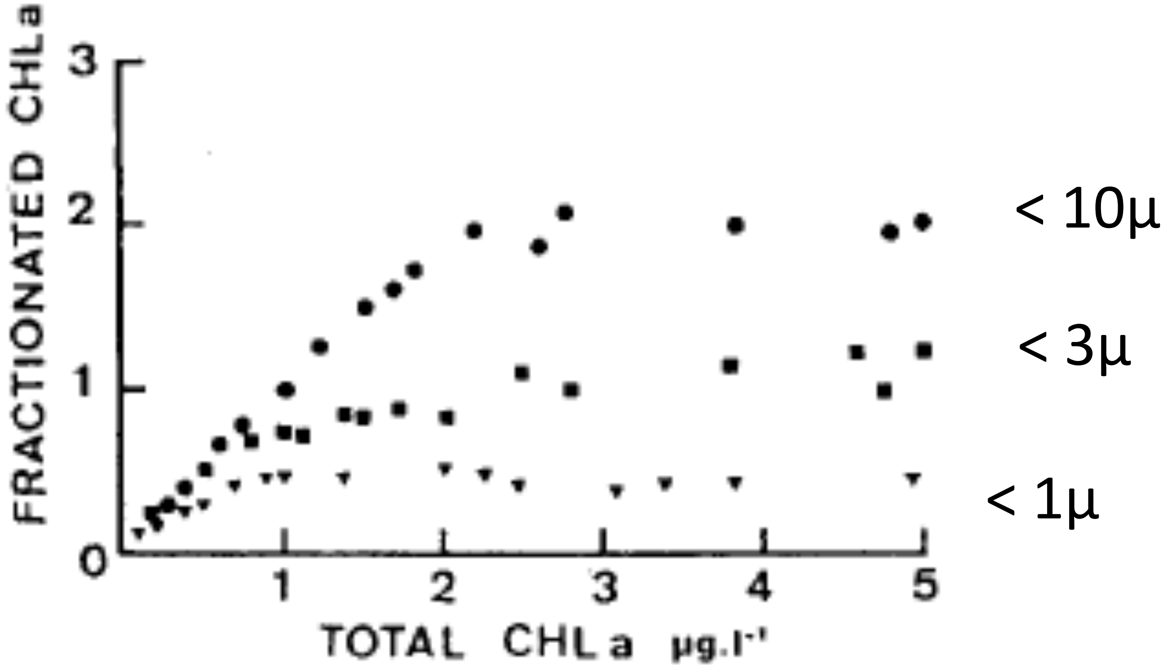
Fig. 1. The relationship between body size and the maximum density of aquatic organisms. The heavy line represents Equation 1

Challenge: What does it look like if you add all organisms, including humans?

$$\log_{10}(D_{max}) = 8.53 - 0.95 \log_{10}(V)$$

Duarte et al 1987

Size and Chlorophyll

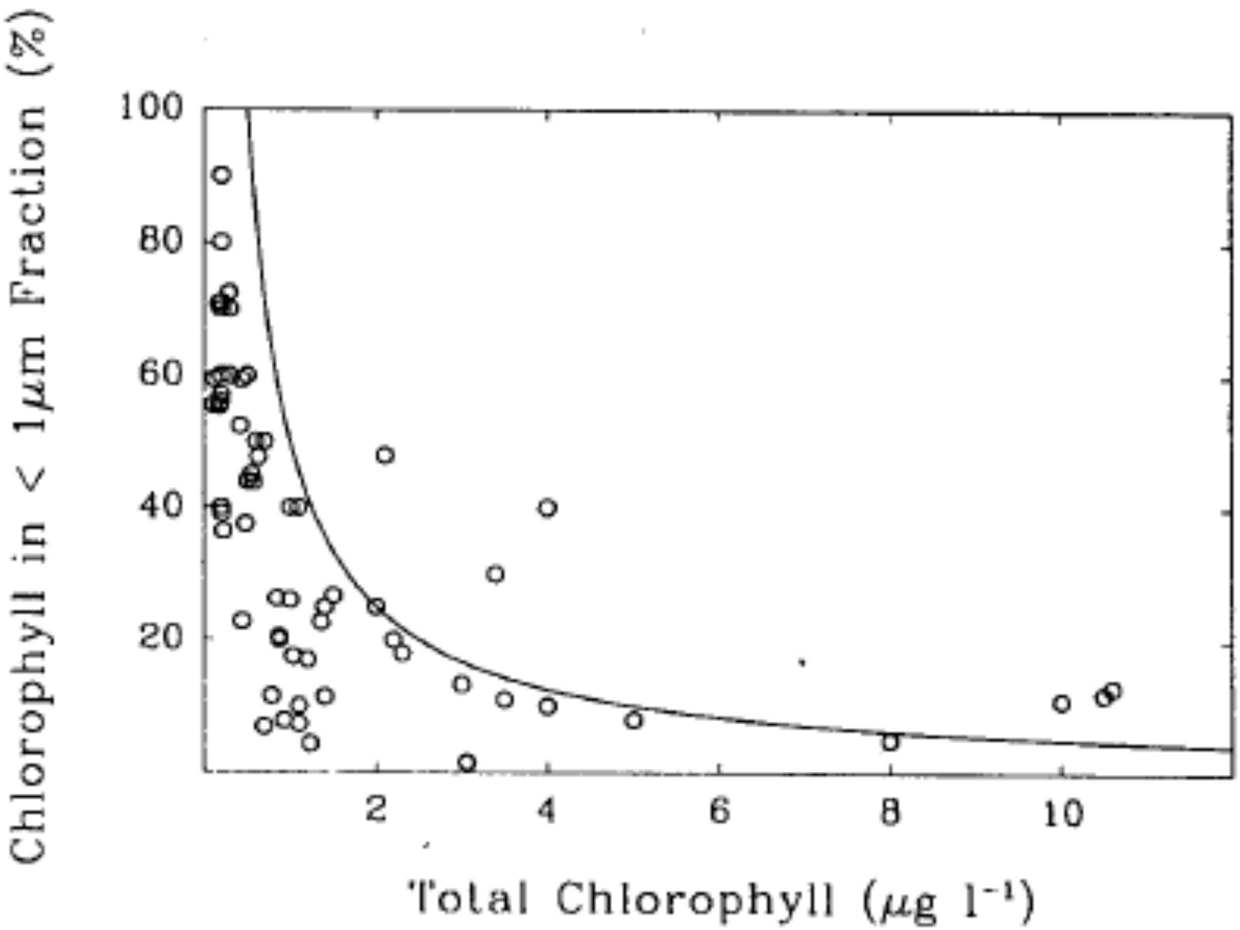


Relationships between total chlorophyll a and the maximum amount of chlorophyll *a* in the <1 µm (triangles), <3 µm (squares) and <10 µm (circles) fraction in the Mediterranean Sea (from Raimbault et al., 1988).

Chisholm 1992

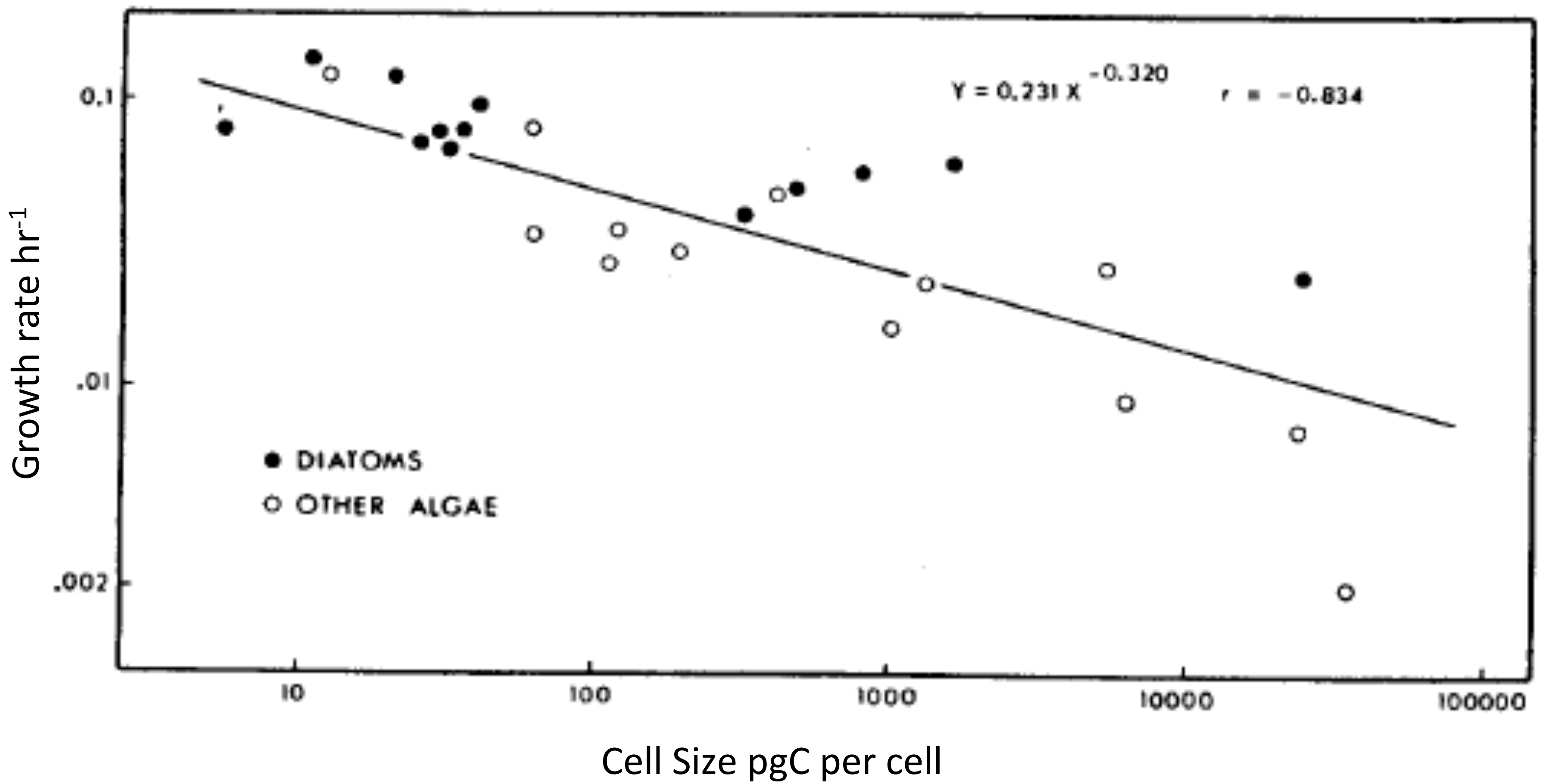
Ecosystems add chlorophyll by adding bigger species

(Or low chlorophyll oceans are dominated by small cells)



Chisholm 1992

Size and Growth Rate



ig. 1. Relationship between phytoplankton cell size and the maximum specific growth rate of species grown under optimal conditions of light and temperature. Growth rates were normalized to 20°C when necessary using a Q_{10} of 1.88. From Schlesinger et al., (1981). Note that the slope for diatoms is significantly less negative than that of the data set as a whole.

Chisholm 1992

Growth/size relationship falls apart for small cells

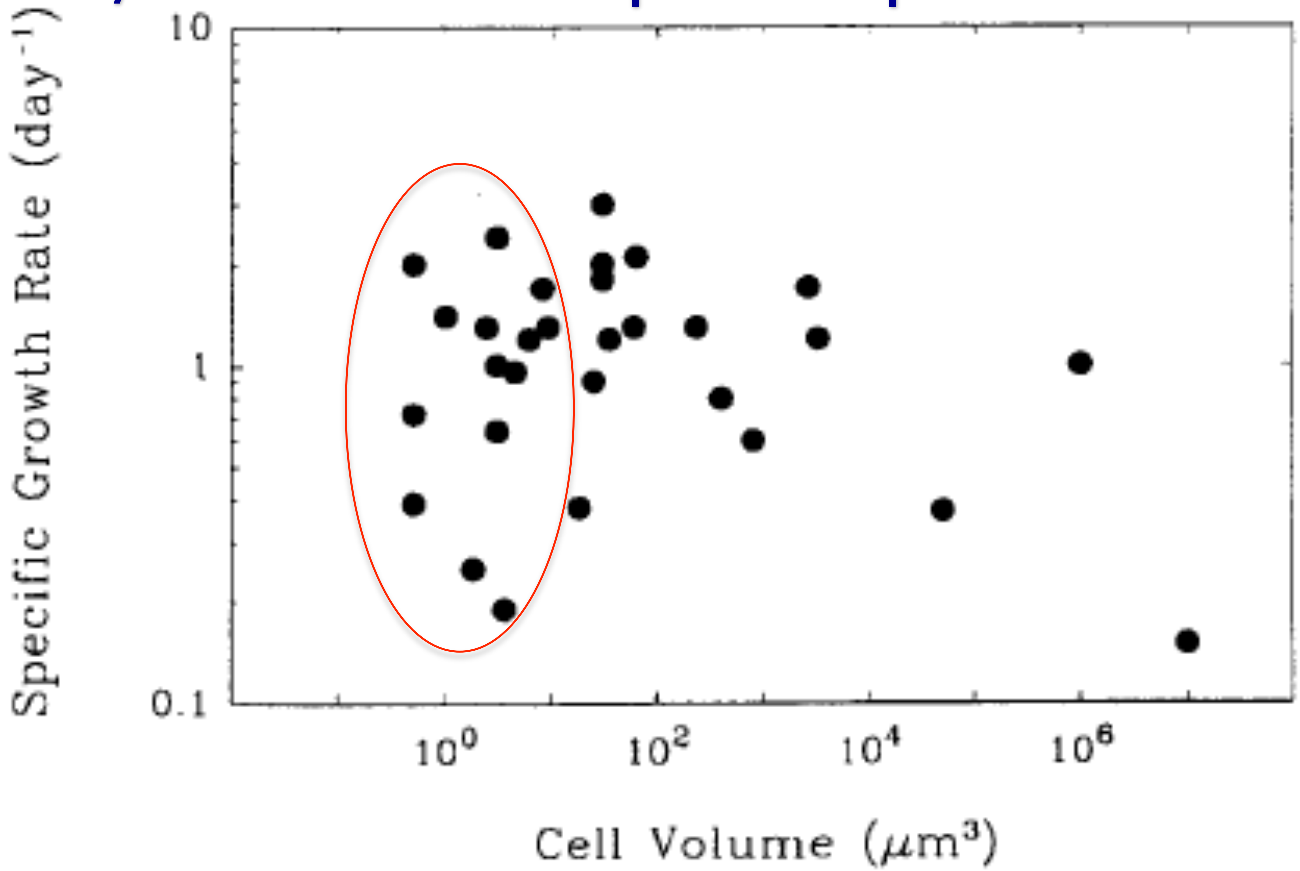
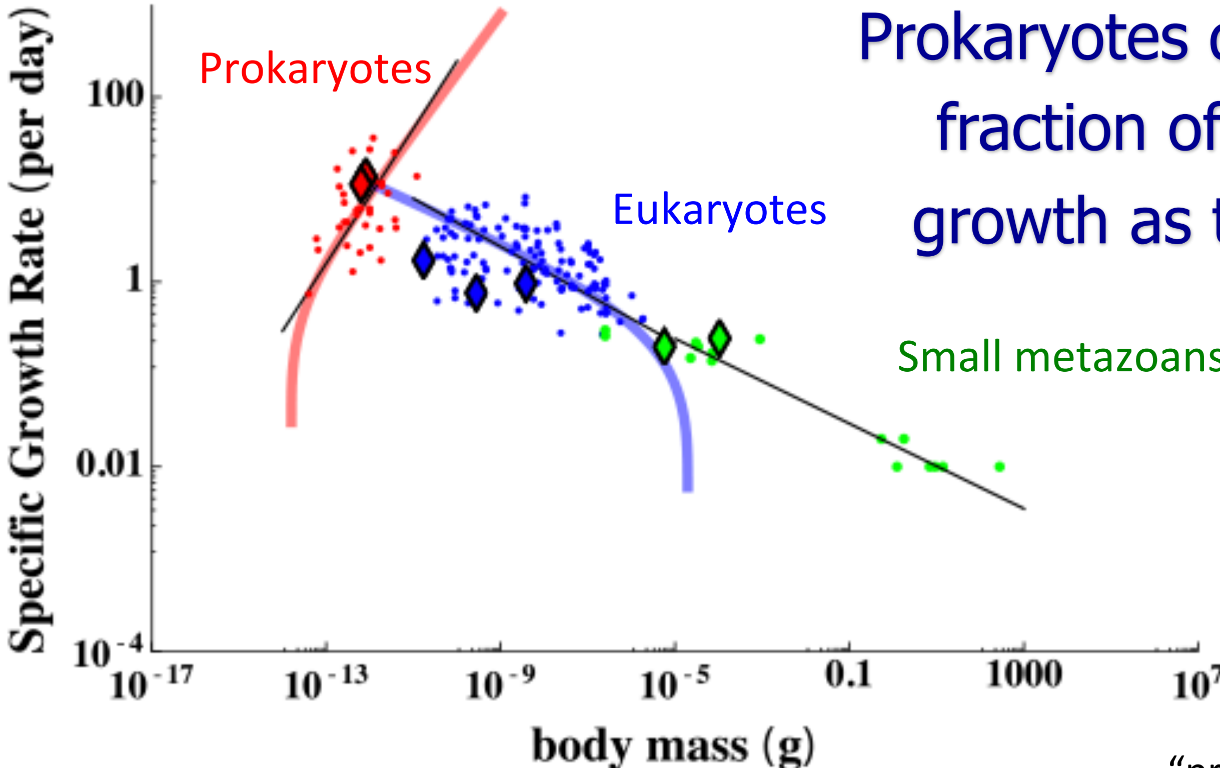
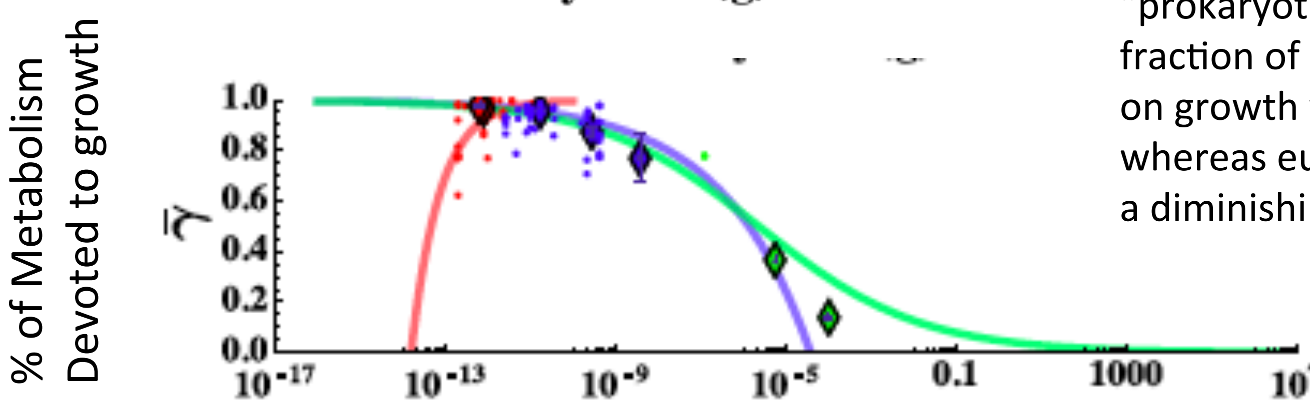


Fig. 2. Relationship between phytoplankton cell size and the maximum specific growth rate of species grown under optimal conditions of light and temperature. Data includes all of the autotrophic organisms in Table 4 from Raven (1986). Growth rates were normalized to 20°C using a Q_{10} of 2.0. Note, for comparison with (a), that a cell 75 μm^3 in volume (5 μm in diameter) has roughly 10 pg C. The organisms smaller than this deviate significantly from the general relationship.

Chisholm 1992

Prokaryotes devote decreasing fraction of metabolism to growth as they get smaller

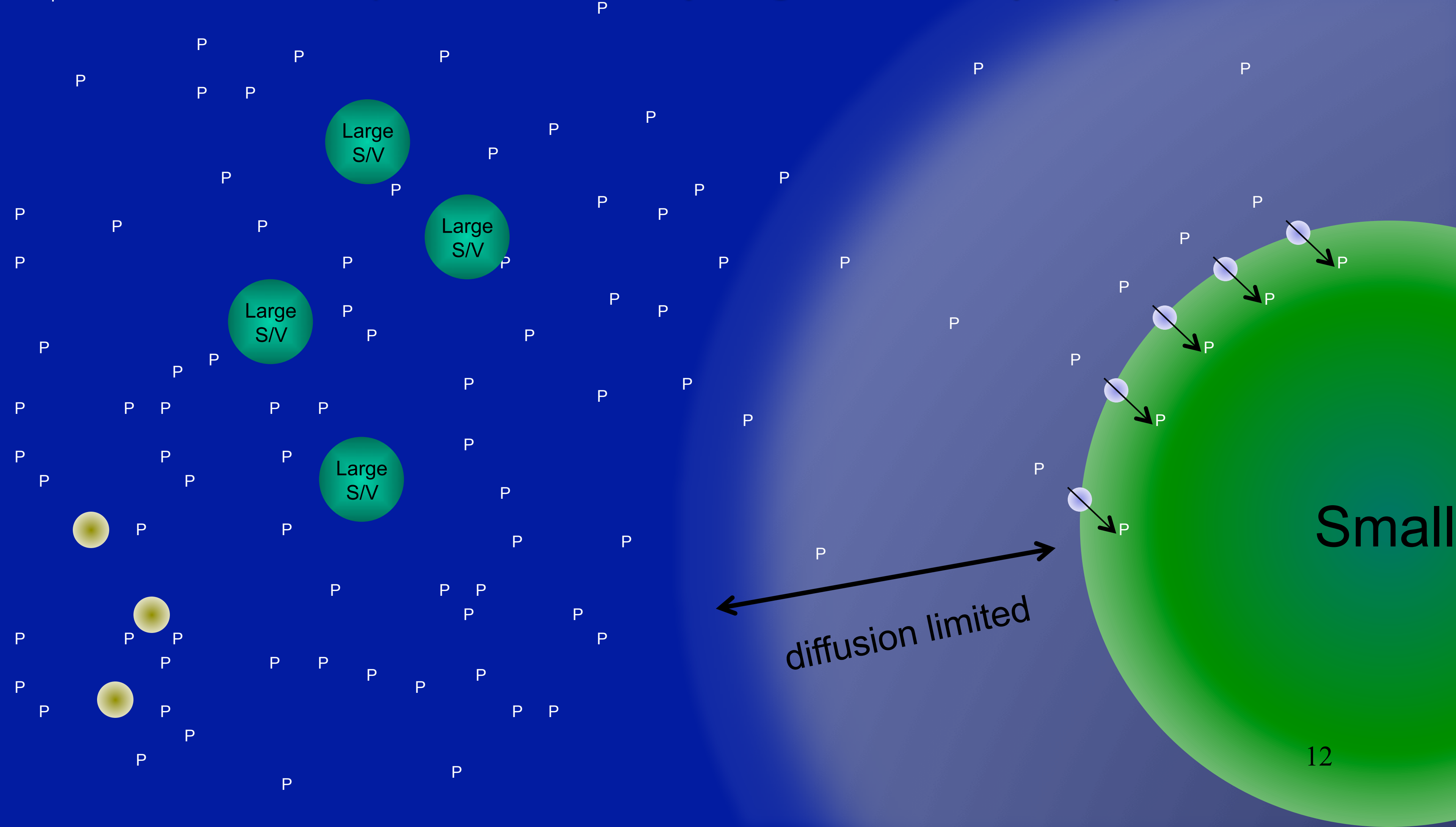




"prokaryotes spend an increasing fraction of their entire metabolism on growth with increasing cell size, whereas eukaryotes devote a diminishing fraction"

Size and nutrient acquisition: It's all about surface/volume ratios

(but how do very large cells compete?)



Size and Nutrient Acquisition:

At what N concentration would a cell of a particular size become diffusion limited if it were growing at 1 day^{-1}?

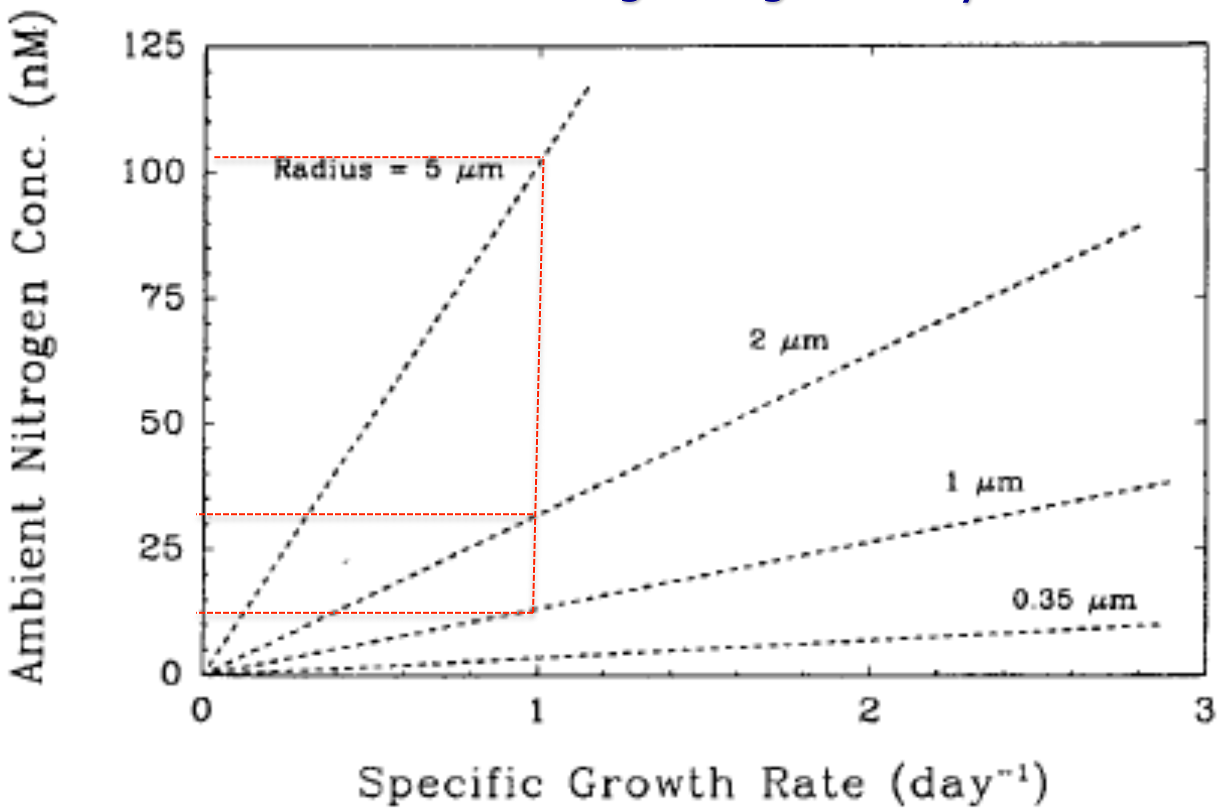


Fig. 10. Ambient concentration of nitrogen at which cells of different sizes would be diffusion limited as a function of growth rate. A cell of radius 5 μm growing at 1 day^{-1}, for example, would be diffusion limited at nitrogen concentrations less than 100 nM, whereas a cell of radius 0.35 μm growing the same rate would not be diffusion limited until concentrations dropped below 5 nM.

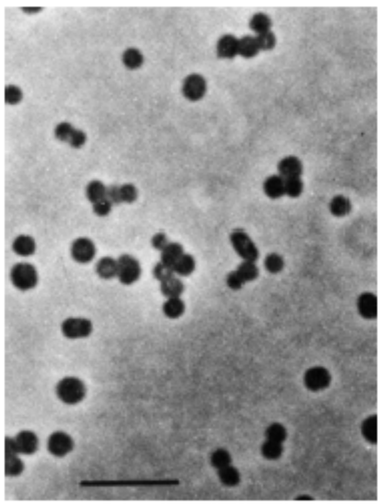
Chisholm 1992

Intro to *Prochlorococcus*

- The early evidence
- The 'rediscovery'
- What we were able to learn pre-genomics
- Intro to "ecotypes"

…The BIG discovery - began the paradigm shift

Waterbury *et. al.* 1979



[Fig]. 1 Phase contrast photomicrograph of *Synechococcus* sp. [(st]rain Syn-48) illustrating general cell morphology (scale bar, 5.0 μm).

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Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium

IN marked contrast to their freshwater counterparts, marine planktonic cyanobacteria are restricted to a few nostocalean genera, of which only *Trichodesmium* is capable of forming extensive water blooms[1–3]. We report here the widespread occurrence of a small, marine, chroococcalean cyanobacterium belonging to the genus *Synechococcus*.

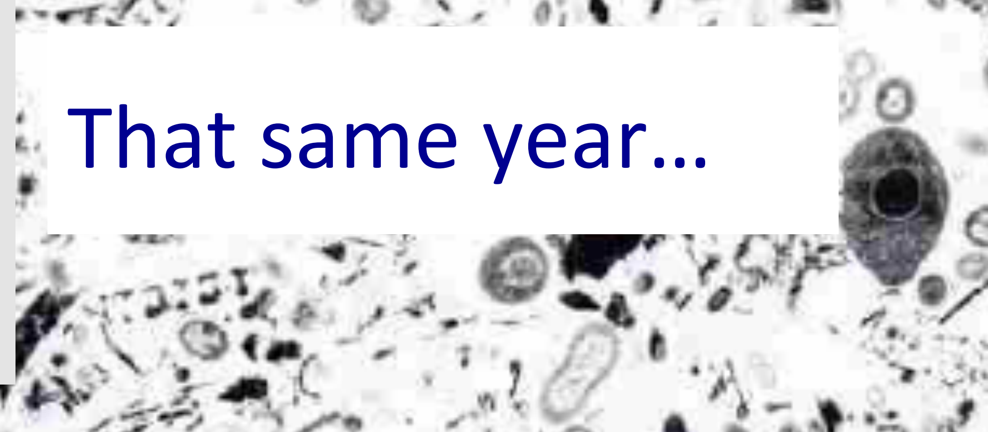
Natural water samples were filtered through 0.2 μm Nuclepore filters, counterstained with Irgalan black[4]. The filters were examined with a Zeiss Standard microscope equipped with Neofluar objectives and an epifluorescent illumination system containing a 100-W halogen lamp, a BP 450–500 excitation filter, a LP 528 barrier filter and a FT 510 chromatic beam splitter. Using this system, phycoerythrin-containing cyanobacteria fluoresce orange and can be distinguished from phytoplankters that fluoresce red.

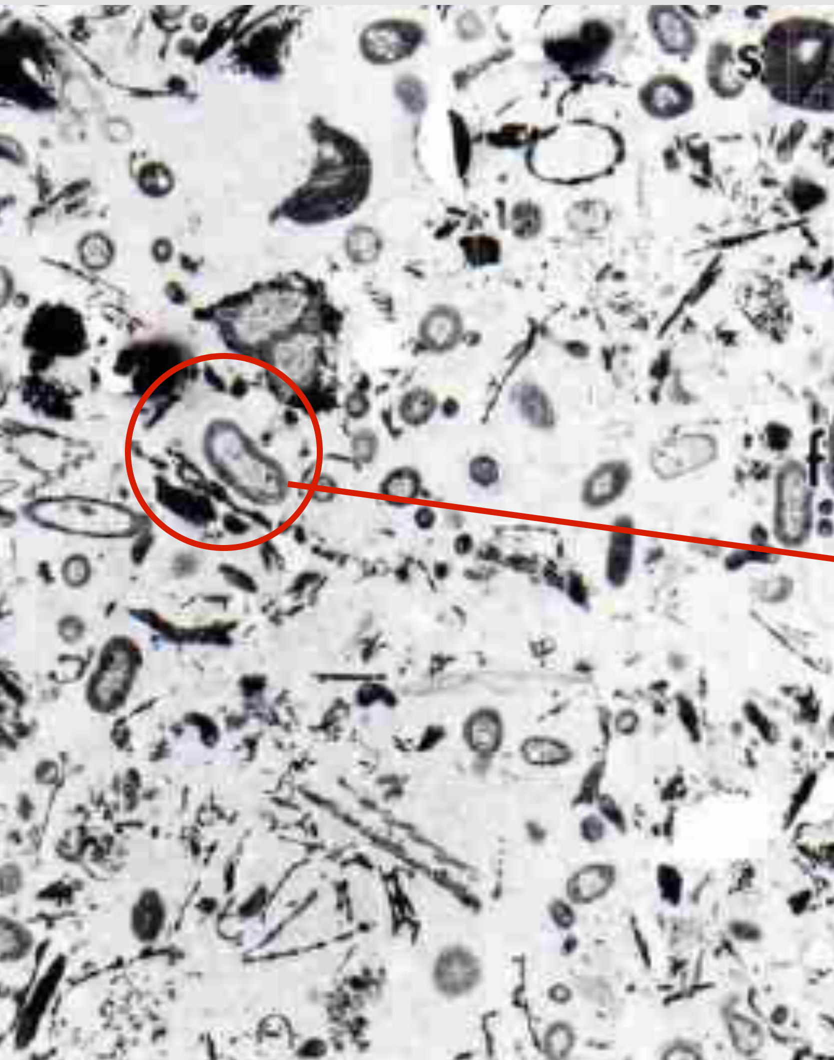
Phycoerythrin-rich unicellular cyanobacteria were observed at seven stations in the Arabian Sea in January 1977, at three stations off the coast of Peru in March 1978, in Slope Water north of the Gulf Stream in April 1978, and periodically in Woods Hole Harbor. In the relatively rich waters of the Arabian Sea and off the coast of Peru, the population varied from 10^4 to 10^5 cells ml^{-1} within the euphotic zone (Table 1). The greatest number of cells was found within the top 20 m of the water column, with occasional cells being observed as deep as 400 m. In contrast, the surface sample collected from Slope Water north

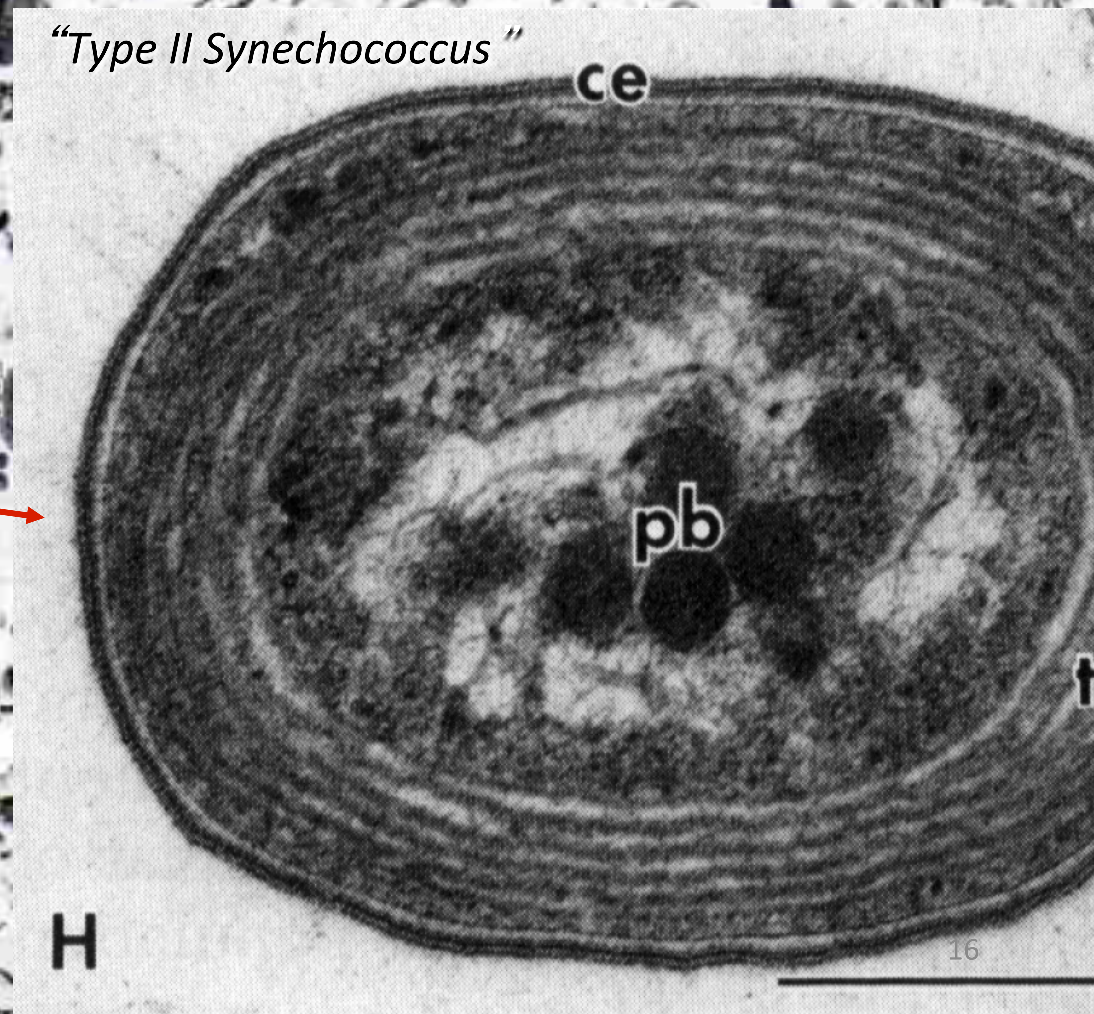
Johnson and Seiburth 1979

Chroococcoid cyanobacteria in the sea:
A ubiquitous and diverse phototrophic biomass.
Limnology and Oceanography 24(5):928-935.

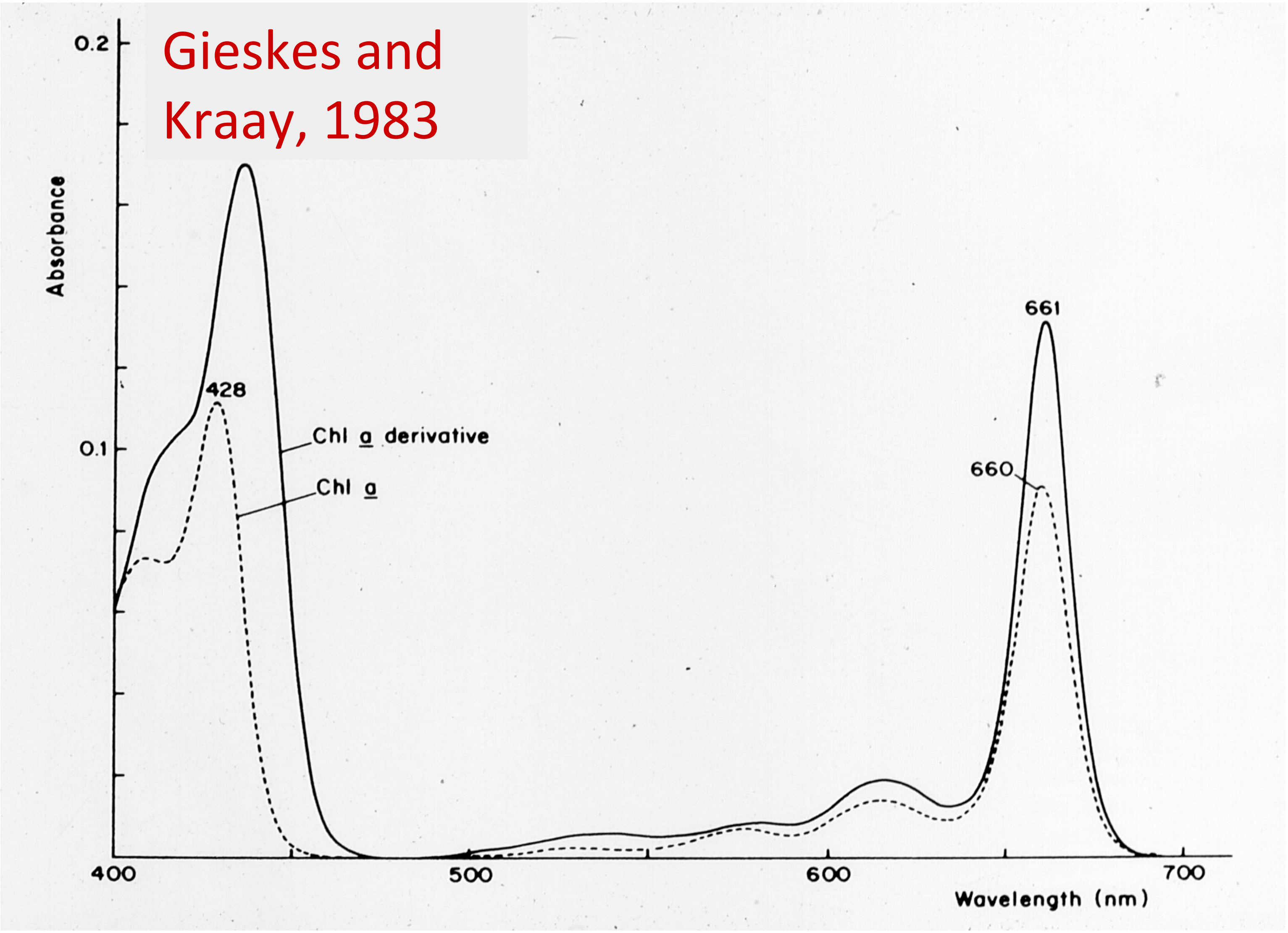
That same year…



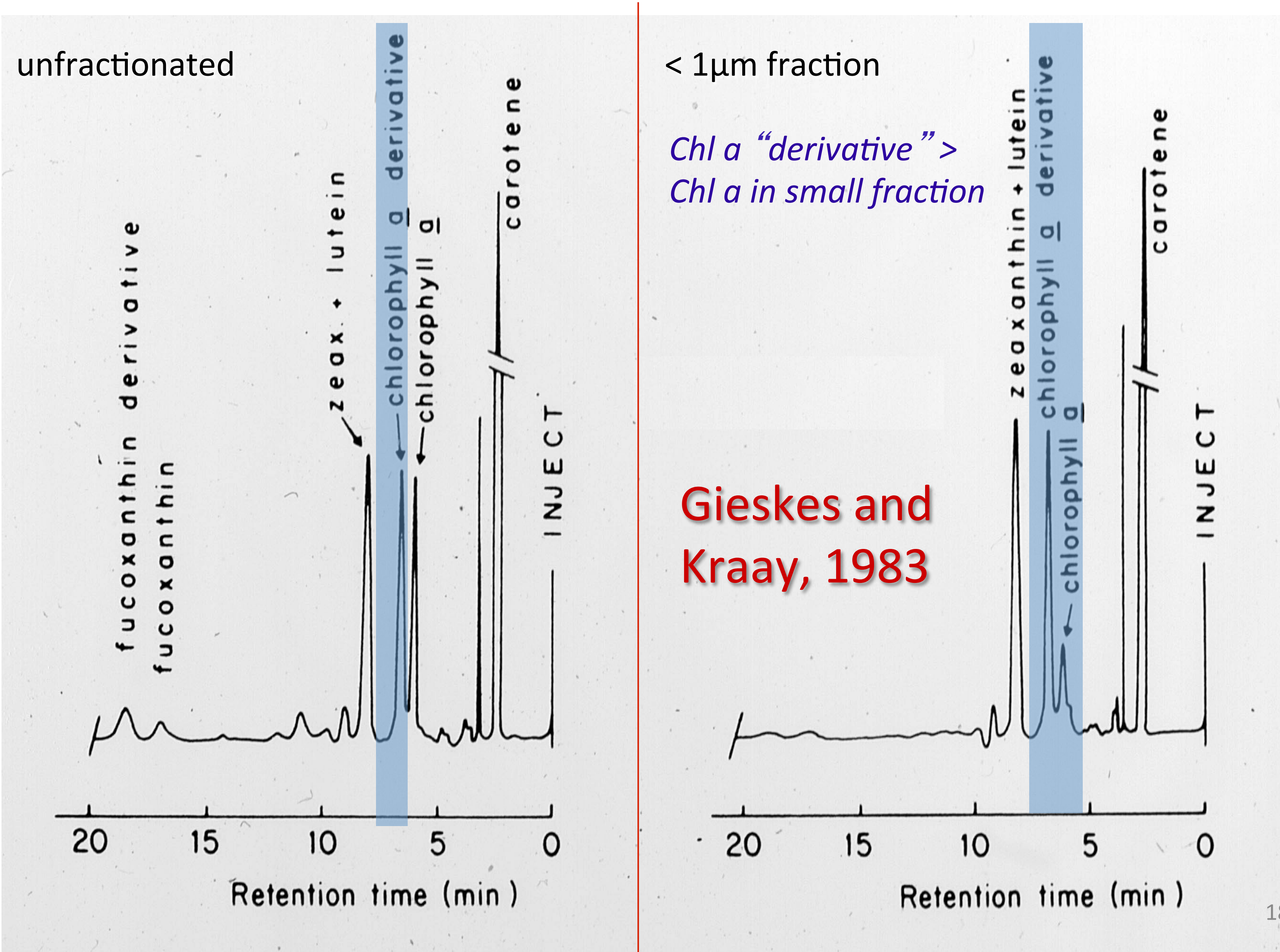




Meanwhile, a few years later, a new pigment was discovered…

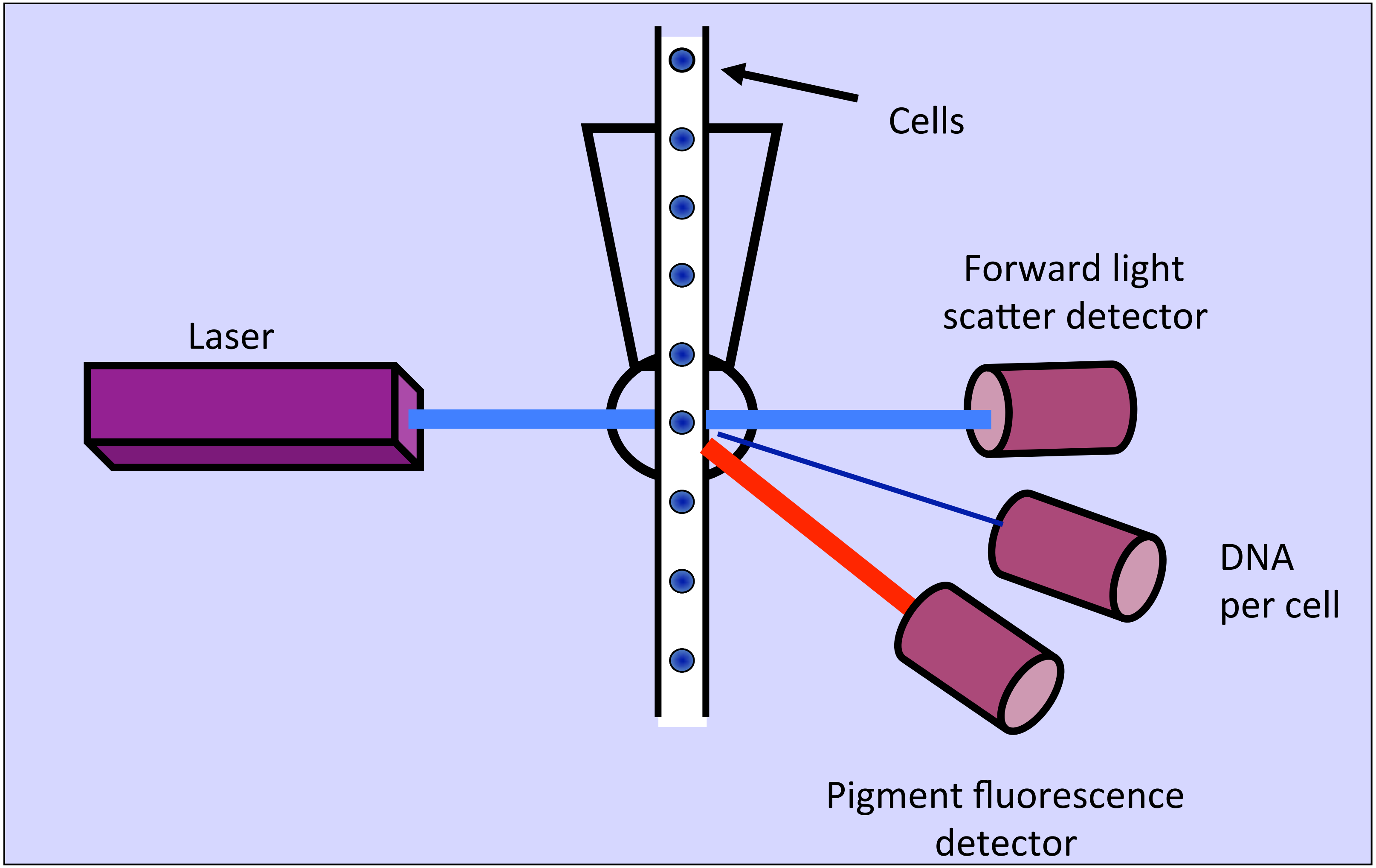


And that pigment is enriched in the < 1µm fraction

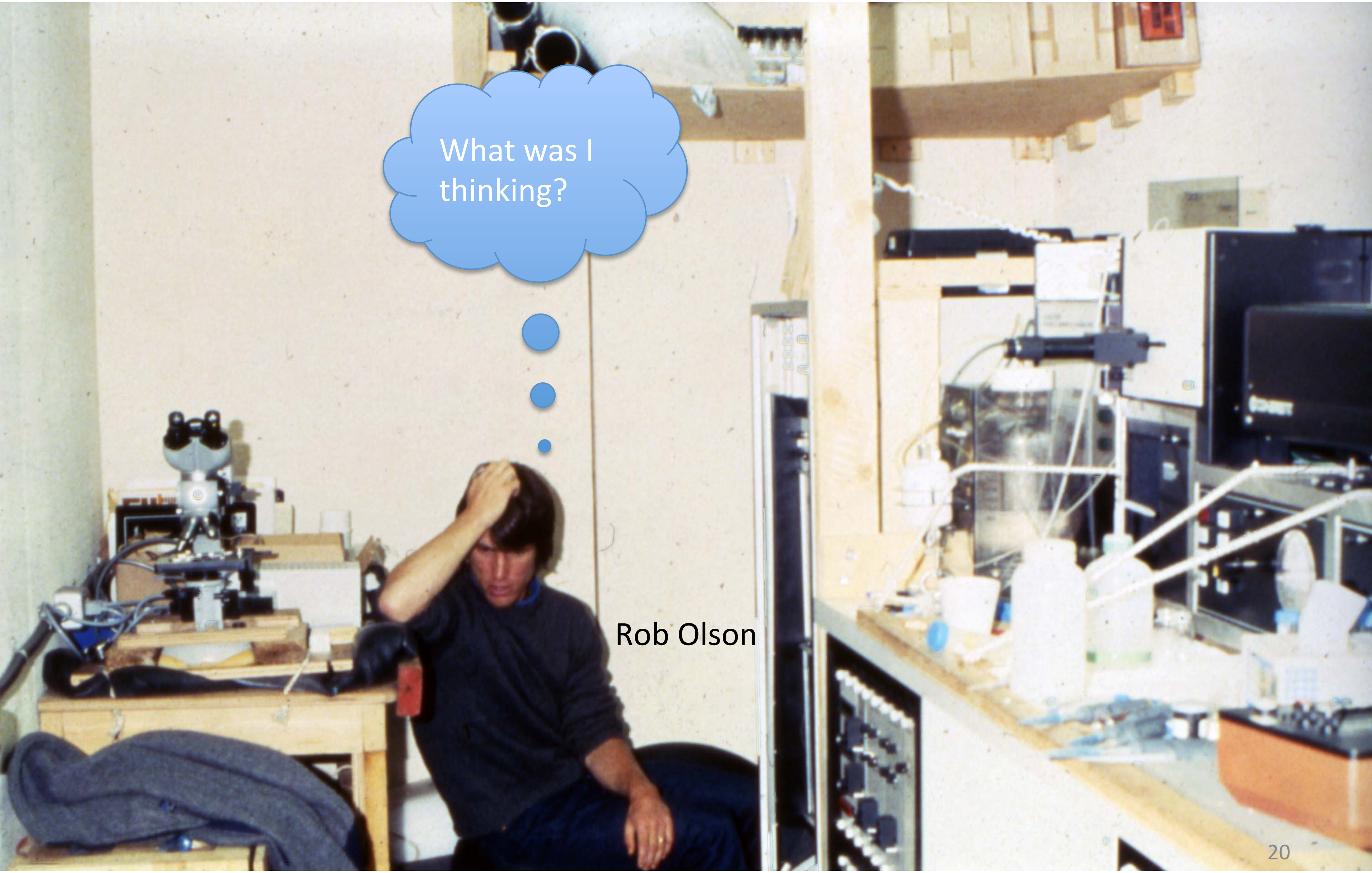


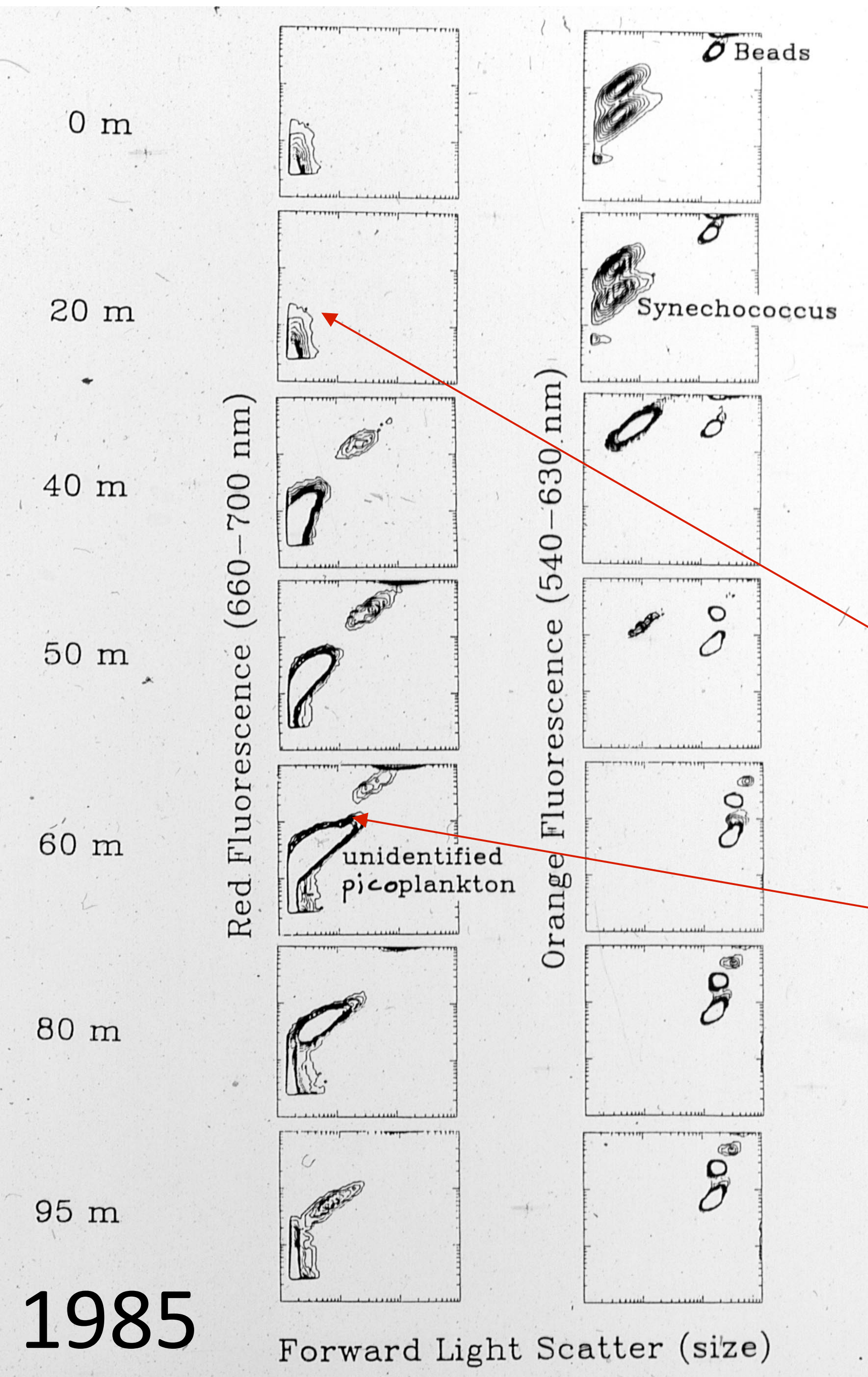
Enter Flow Cytometry…..

…for the study of single cells



And someone with the courage to take it to sea…





We set out to study *Synechococcus*, and mess about with flow cytometry

And noticed something else…

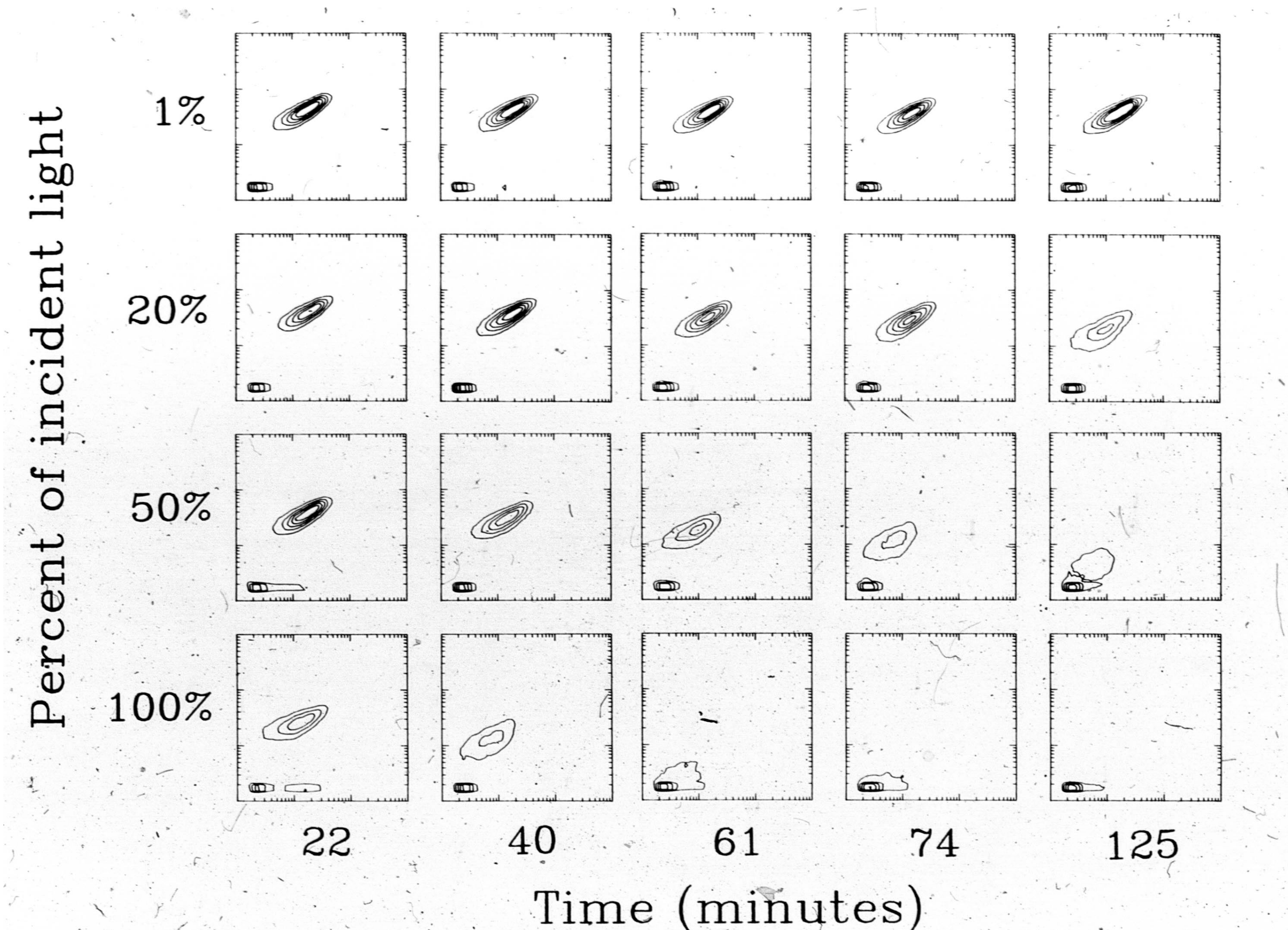
The noise

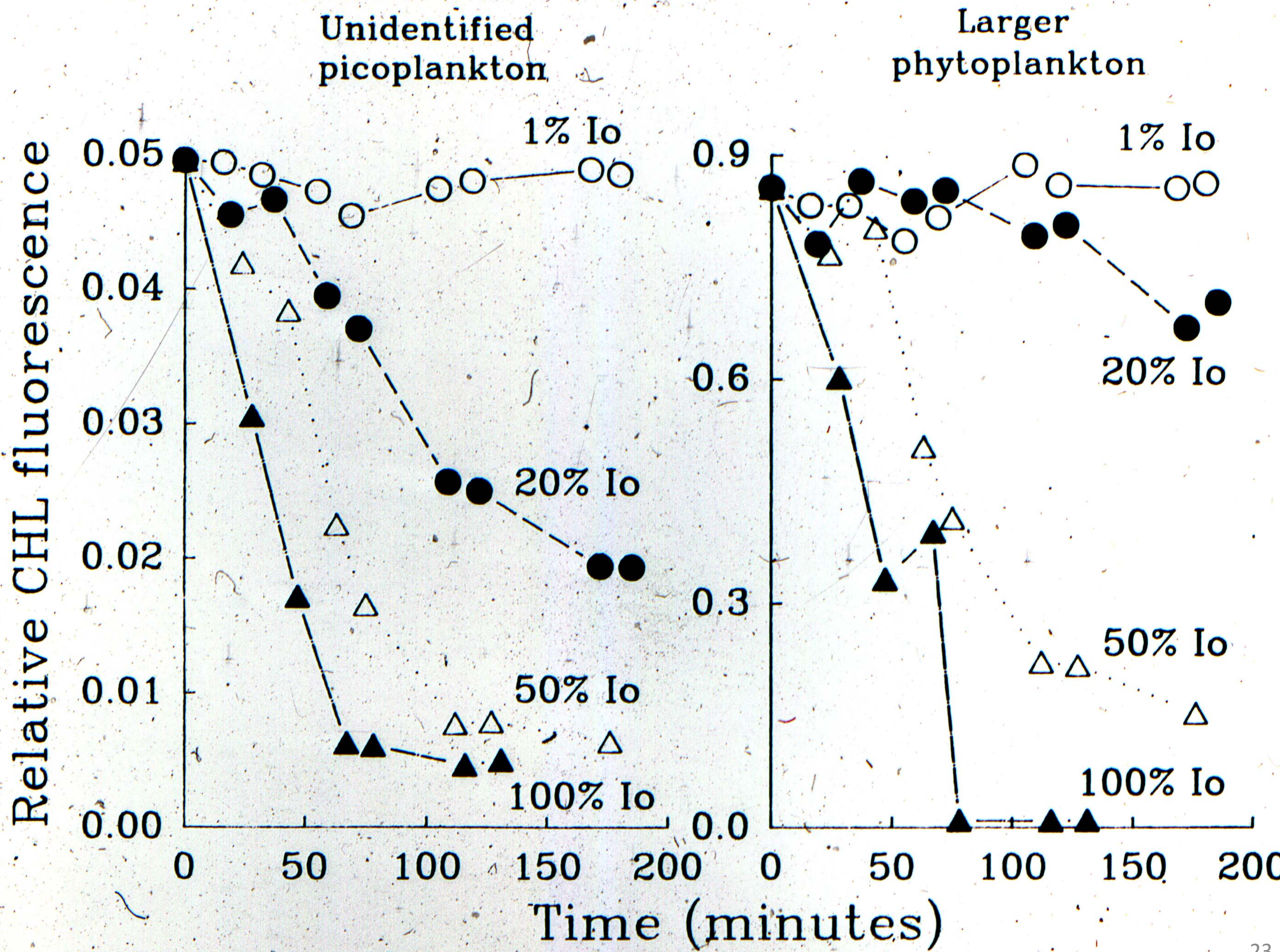
Emerging from the noise!

Based on their fluorescence excitation/emission spectrum we suspected they had chl b, typical of green algae.
Called them "**Little Greens**"

Electronic noise or living cells? (some ancient unpublished stuff)

– Collect cells from 1% light level and change their light intensity.

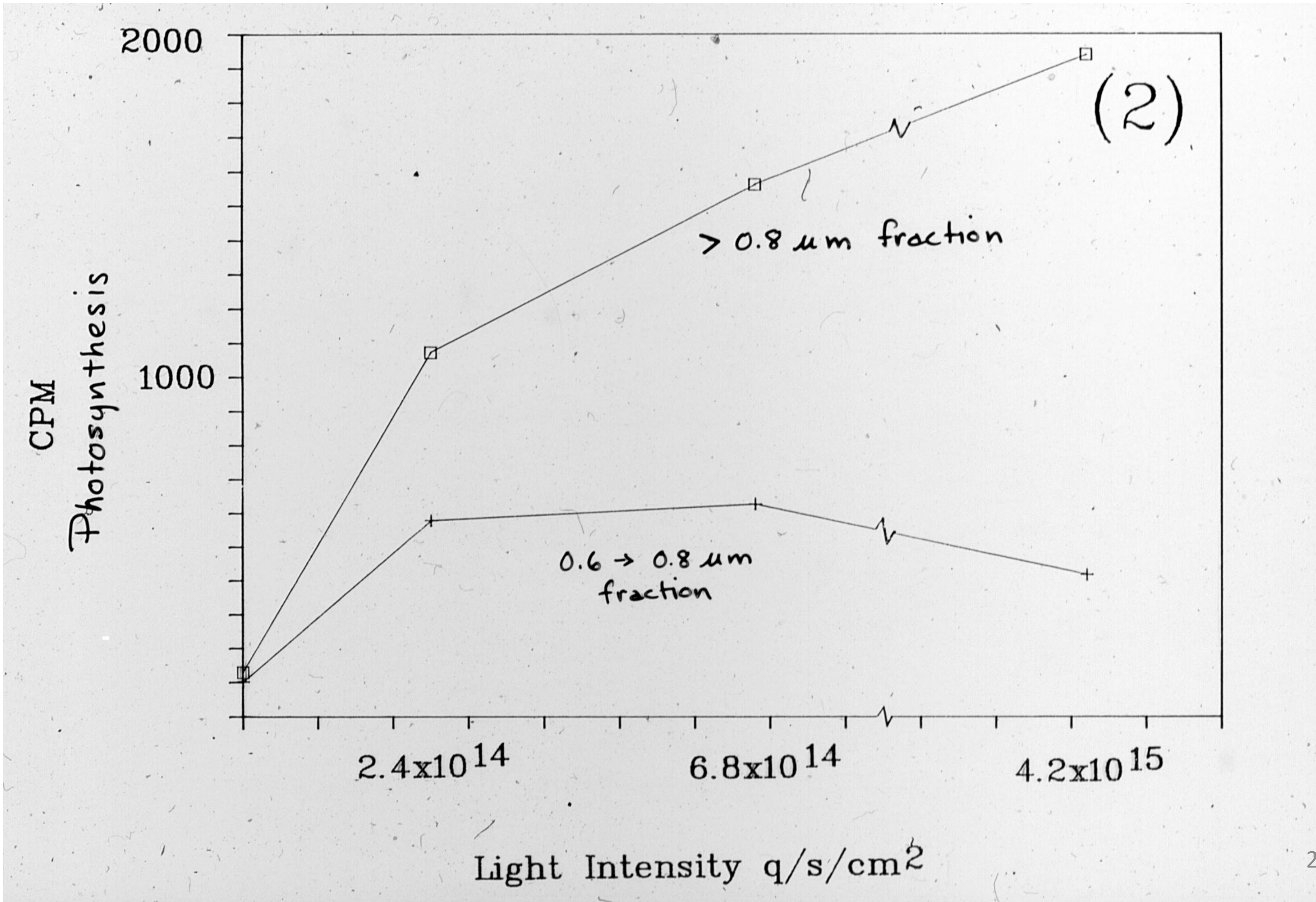




Unidentified picoplankton
Larger phytoplankton
1% Io
20% Io
50% Io
100% Io
Relative CHL fluorescence
0.05
0.04
0.03
0.02
0.01
0.00
0.9
0.6
0.3
0.0
0
50
100
150
200
Time (minutes)

But do they photosynthesize?

YES!



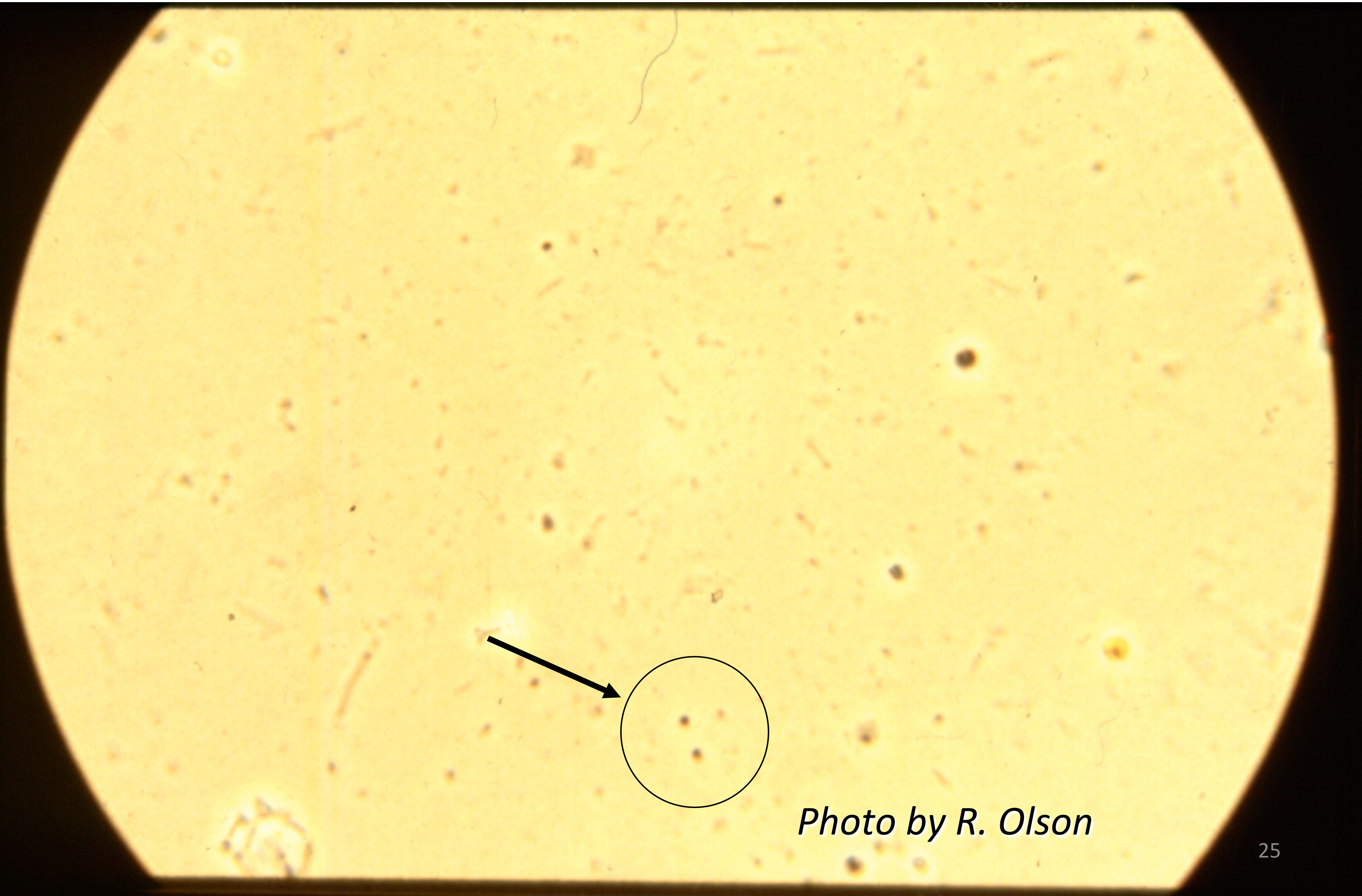


Photo by R. Olson

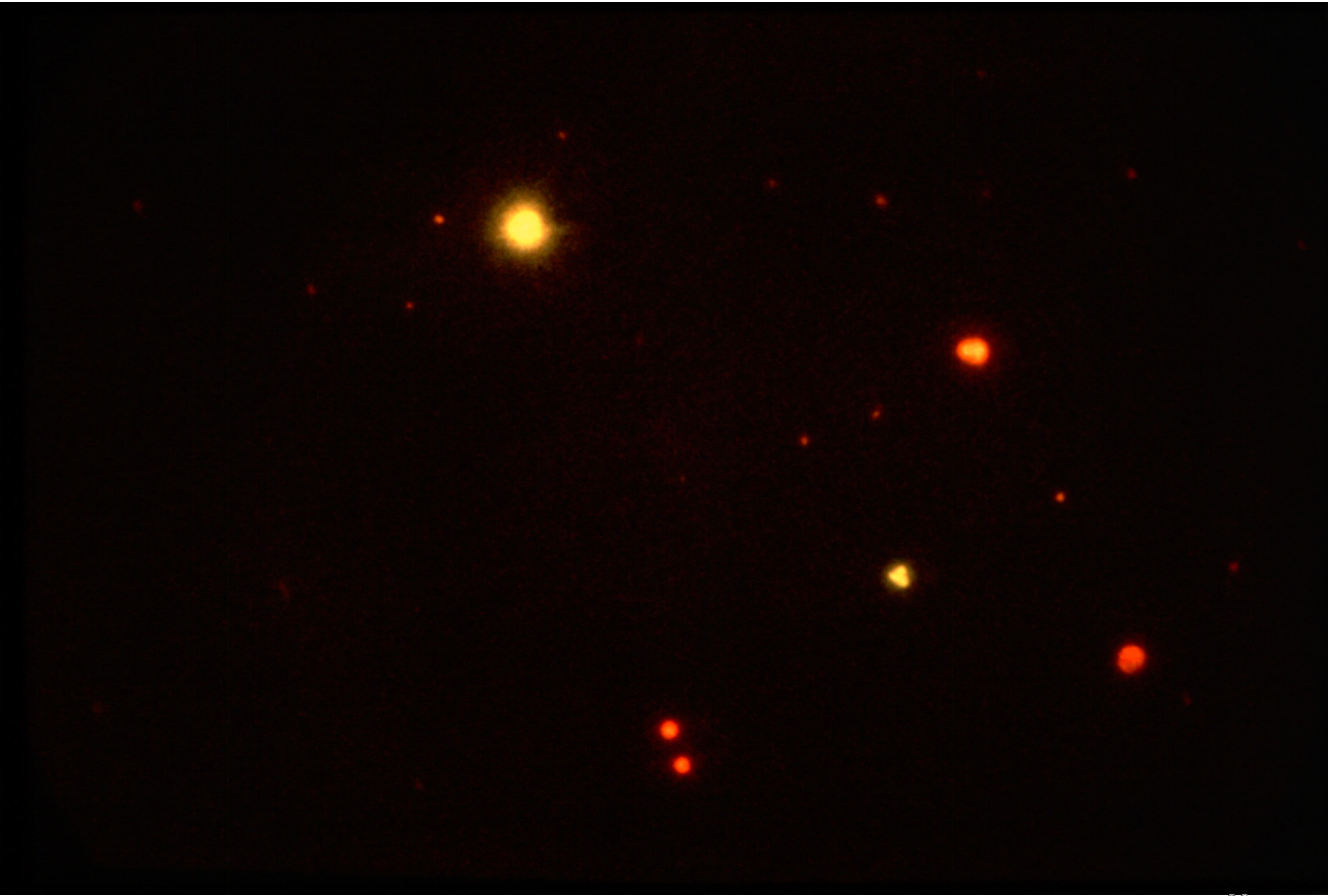
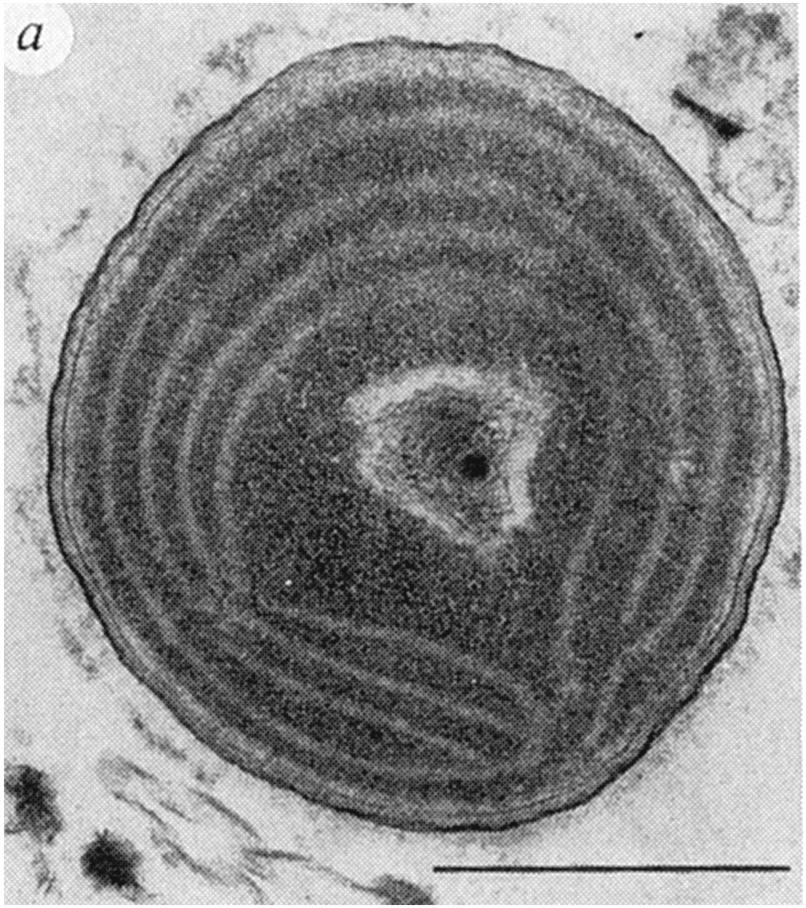


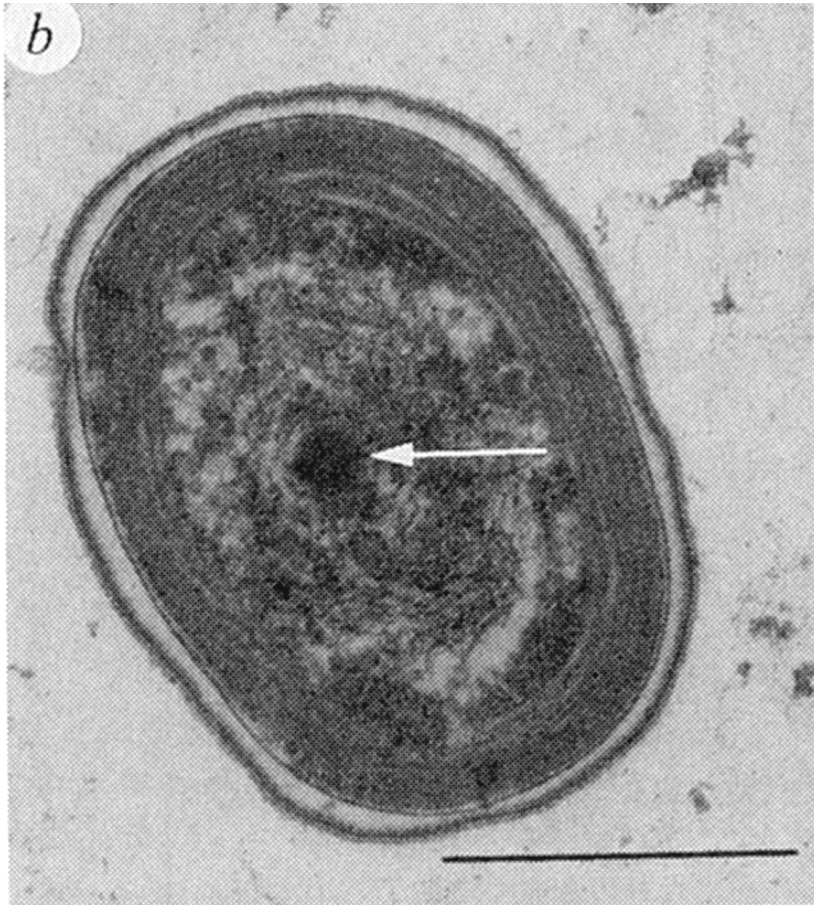
Photo by R. Olson

Finally, and electron micrograph – It’s a prokaryote!

Synechococcus



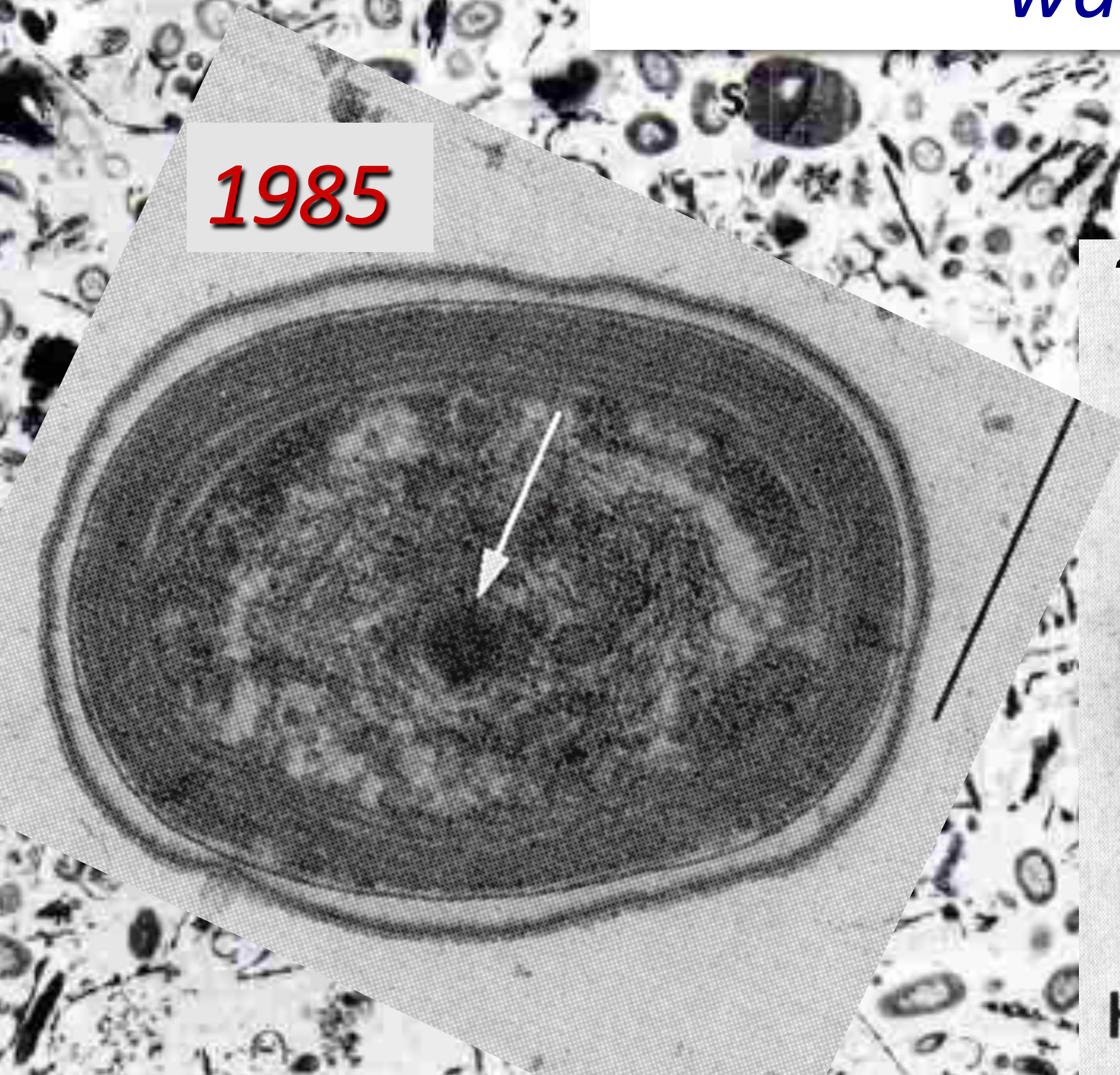
“Little Greens”

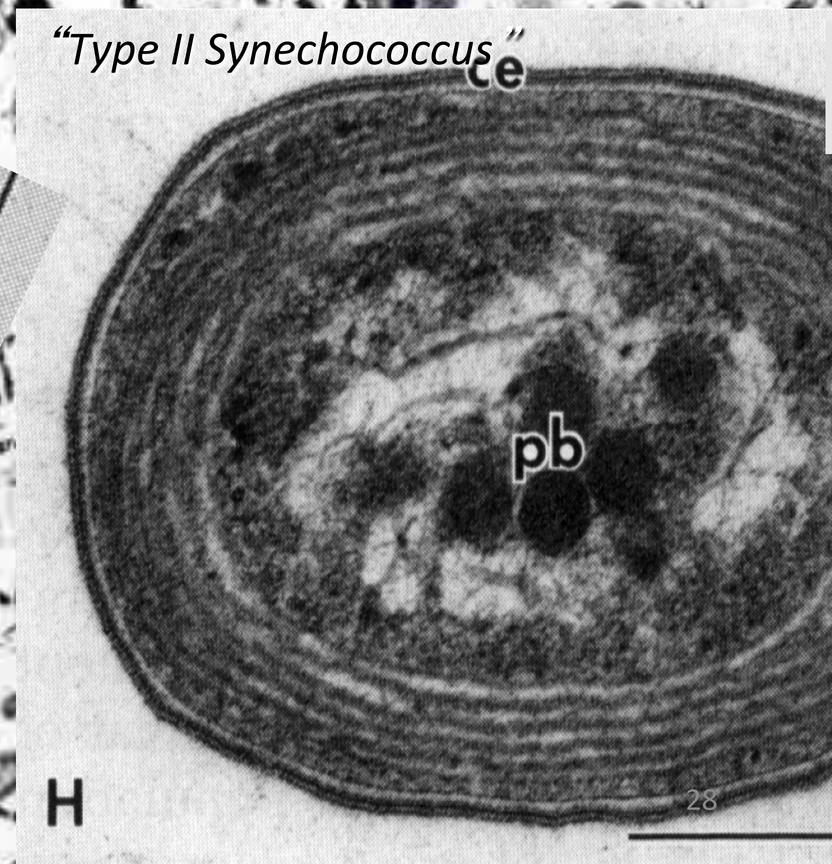


John Waterbury

CLOSURE I: Johnson and Sieburth

“Type II Synechococcus
was the same as our ce





What is their pigment composition?

Divinyl Chl

Ralph Goericke

HPLC pigment analysis of unidentified picoplankton

		0.8 μm filtrate (⟩95% pure)	FCM sort
= *divinyl chl a*	chlorophyll a_1	2.0 fg $cell^{-1}$	present
= *divinyl chl b*	chlorophyll b_1	2.7 fg $cell^{-1}$	present
	zeaxanthin	0.6 fg $cell^{-1}$	
	α carotene	0.3 fg $cell^{-1}$	
	lutein	not detected	
	chlorophyll a_2	not detected	
	β carotene	not detected	

These pigment properties are restricted to the < 0.8 micron fraction

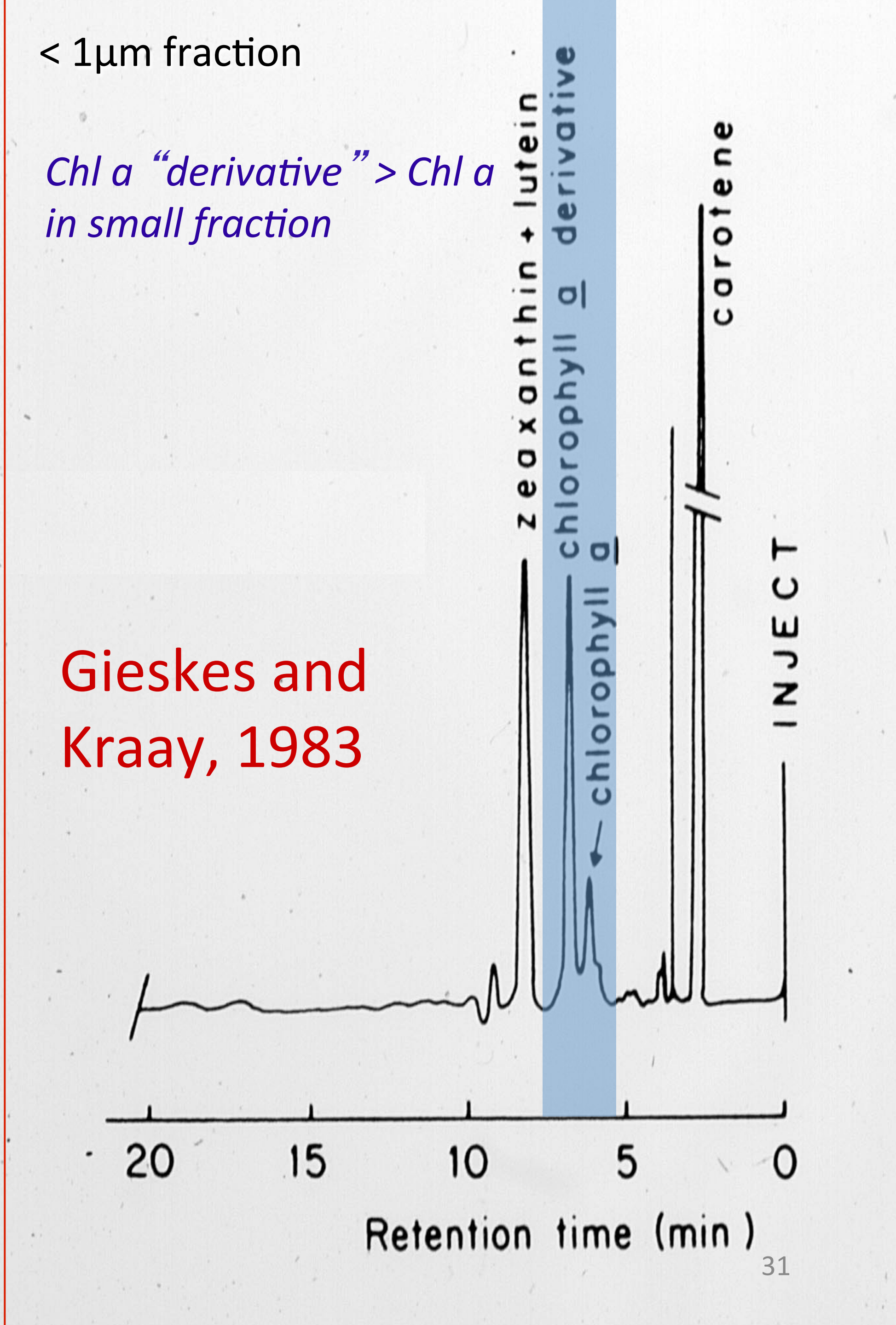
Pigments	Whole Sea Water	<0.8 μm Fraction
Div-Chl *a* per cell	1.4	1.1
Div-Chl *b* per cell	1.8	1.4
α-Carotene per cell	0.2	0.2
Div-Chl *a*/Div-Chl *b*	0.8	0.8
Div-Chl *a*/α-Carotene	7.1	7.9
Relative FLS per cell	5.6	3.9
Relative Fluorescence per cell	1.4	1.1

Sample from Sargasso Sea 80-100m

Goreicke thesis 1990

CLOSURE II:

Gieskes and Kraay's "chlorophyll *a* derivative" was the divinyl chlorophyll *a* of the "Little Greens"

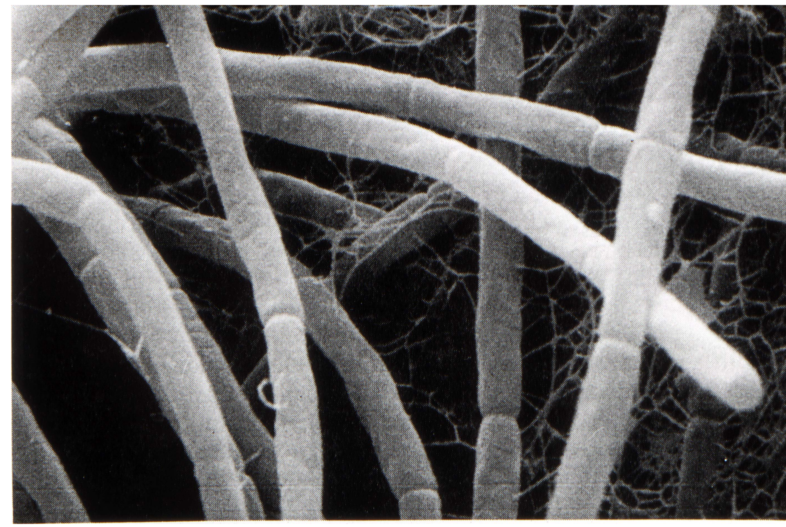


But what do we call them?

What else is prokaryotic and has Chlorophyll b?

Plate 2 . *Didemnum molle*, brown and white colonies with one cut open to show *Prochloron* cells embedded in mucus. Palau. Scale, approx. × 1.4. (Photo: L. Muscatine.)

Prochloron



Prochlorothrix

So we called them "Prochlorophytes"

1988: The Birth Announcement

340 LETTERS TO NATURE NATURE VOL. 334 28 JULY 1988

A novel free-living prochlorophyte abundant in the oceanic euphotic zone

Sallie W. Chisholm, Robert J. Olson*, Erik R. Zettler*, Ralf Goericke†, John B. Waterbury* & Nicholas A. Welschmeyer†

48-425 Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
* Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
† Harvard University, Cambridge, Massachusetts 02138, USA

Few noticed except…

PHYSICAL SCIENCE

Mouth Lightning

Go into a dark closet with a few friends and some wintergreen candies. Start chewing. Then stand back. You might be hit by lightning—the tiny bolts you'll see inside each others' mouths.

Nearly 400 years ago the philosopher Francis Bacon noted that some solid materials give off light when they are broken or crushed. Such materials were later labeled *triboluminescent* (TRY-boh-lou-meh-NES-int), from the Greek *tribein*, to rub.

Why do wintergreen candies triboluminesce? Crush a bunch, measure the colors of light they give off, and you may find out. That's what happened to chemistry professor Dr. Linda M. Sweeting and her colleagues at Towson State University in Baltimore. Here's what they say:

It has to do with crystals of *sucrose* (sugar) and menthol, two of many materials known to triboluminesce. Both are found in wintergreen candies. As you chew, you create tiny cracks in the crystals. Patches of positive and negative charges form on opposite sides of these cracks. When enough charge builds, electrons (negatively charged particles) dart across the gap and neutralize the charged patches. Some of the electrons bombard nitrogen molecules in the nearby air, causing the nitrogen to give off tiny bolts of blue-white light.

"It's exactly the same as real lightning, only much weaker," says Dr. Sweeting.

Wintergreen candies, because they also contain wintergreen flavor molecules, can be even more . . . illuminating. When these flavor molecules absorb some of the tiny lightning given off by nearby nitrogen molecules, they respond by giving

off their own blue-gr… process is called *phot*…

What you see in … mouths then is a com… boluminescence, fro… and menthol, and … cence, from the wint… molecules.

LIFE SCIENCE

Smallest Sea Life

They are probably the most plentiful form of life on Earth. But, surprisingly, nobody ever noticed them until now.

"They" are tiny one-celled organisms that live in virtually all the world's oceans in such huge numbers that there are about half a million in every teaspoonful of seawater.

In an ordinary 10-gallon aquarium of ocean water, there would be as many of these microorganisms as there are human beings on the entire planet—about five billion. Taking all the oceans together, there are probably more of them than there are of any other species known.

The microorganisms, which have not yet been named, were discovered by Sallie W. Chisholm, a marine biologist at the Massachusetts Institute of Technology. "Nobody ever saw them before because they're just so small," Chisholm says. "You wouldn't see them unless you went looking for something that size."

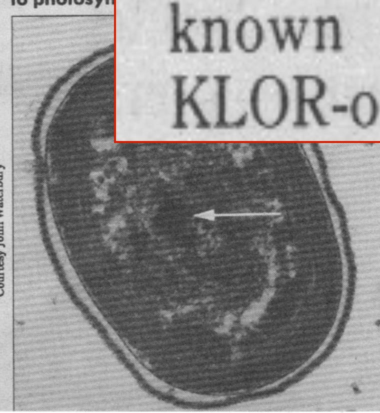
The organisms are about the size of a bacterium—so small that 50 equal the width of a human hair.

Chisholm says they are a primitive kind of plant, belonging to a group known as *prochlorophytes* (pro-KLOR-o-fights). Other members of the group are already known, but they are not common. All contain chlorophyll, the same substance that big plants use to trap sunlight and convert it into the chemical energy of sugar. This process is known as photosynthesis.

Because they are so plentiful, Chisholm suspects the microscopic plants may be the most important link in the ocean food ch…

All plants are *prim*… cause they are the start of the food chain, the first link. Plants convert solar energy… animals eat. … prochlorophy… sharks or wh…

Microscopic … points to a structure important to this cell's ability to photosyn…



Courtesy John Waterbury

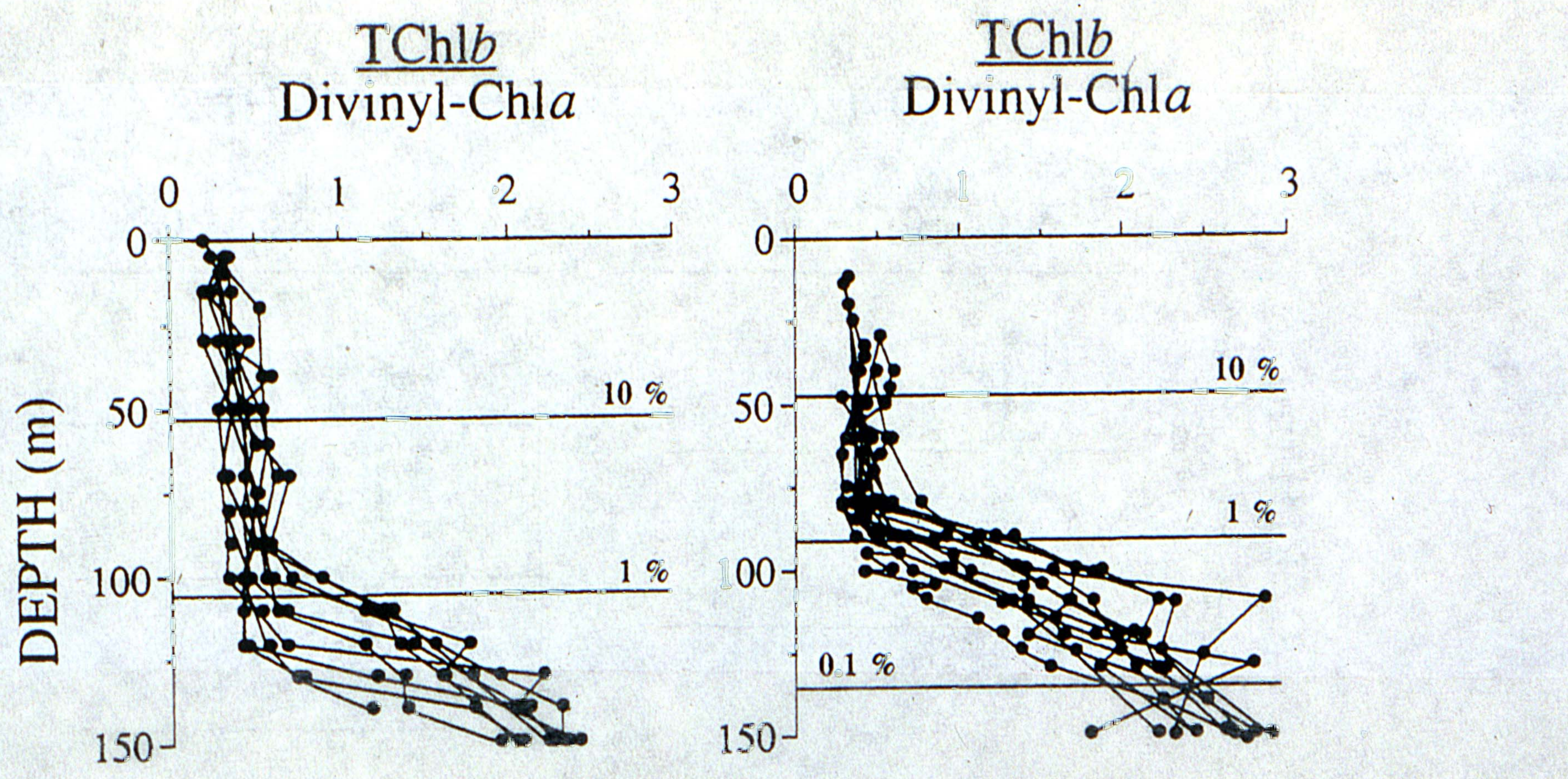
In an ordinary 10-gallon aquarium of ocean water, there would be as many of these microorganisms as there are human beings on the entire planet—about five billion.

—Boyce Rensberger

known as *prochlorophytes* (pro-KLOR-o-fights).

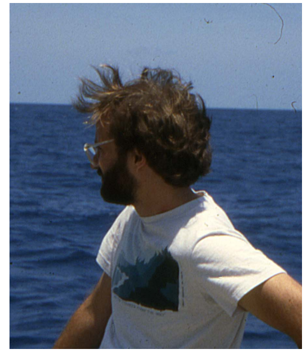
Chl b/Divinyl Chl a ratio shows photoacclimation in *Prochlorophytes (in hindsight, the two ecotypes)*

Tropical Atlantic Ocean



Claustre and Marty 1995

1992 Cultures

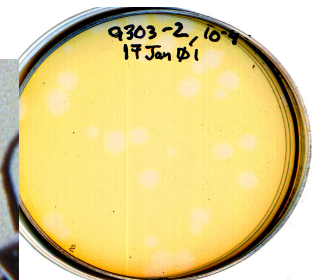


Brian Palenik

John Waterbury

Freddie Valois

Sometime later…..



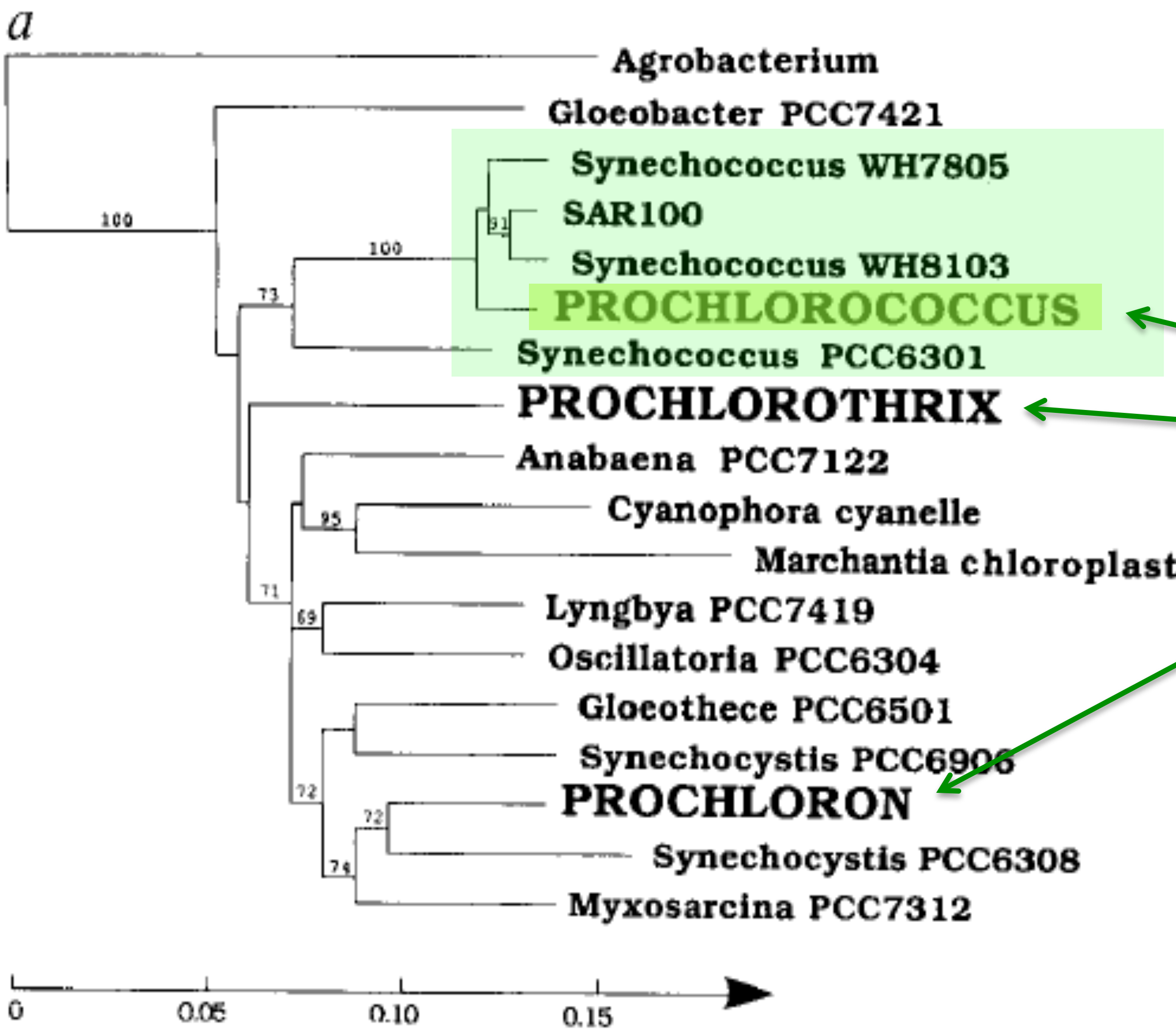
Mak Saito

Lead to the name: *Prochlorococcus*

("coccoid prokaryote with chl b")

Enter Molecular Phylogeny: We were wrong!

It *IS* closely related to *Synechococcus,* and not to *Prochloron* or *Prochlorothrix*



Chl b trait evolved independently in these three groups

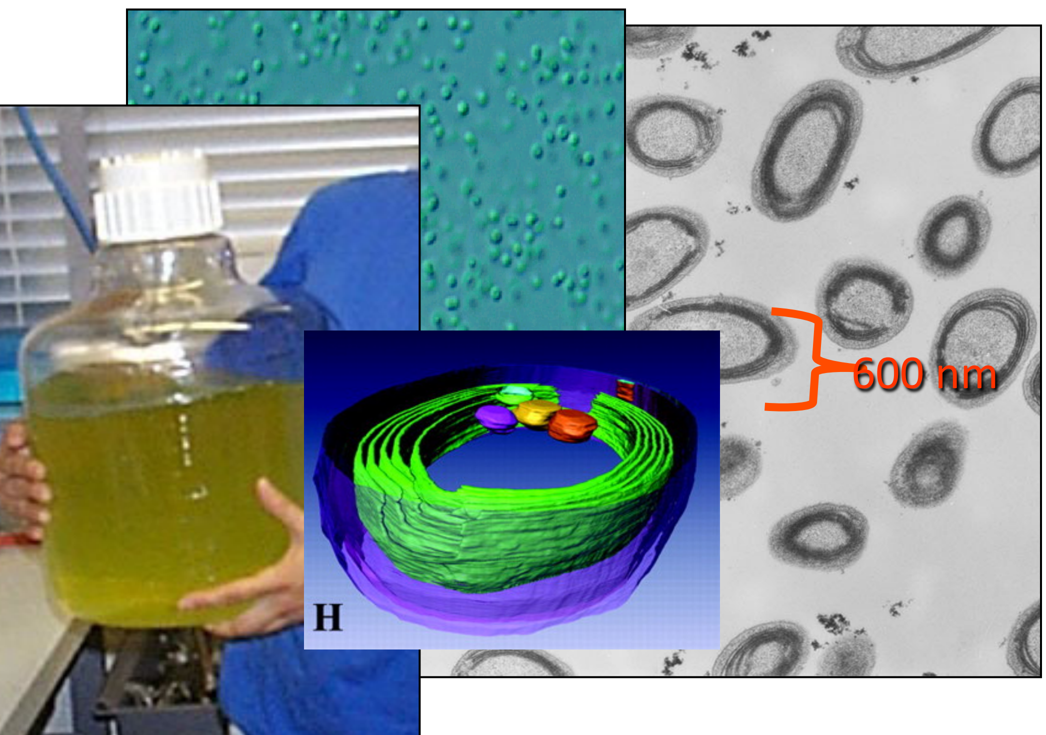
Ena Urbach

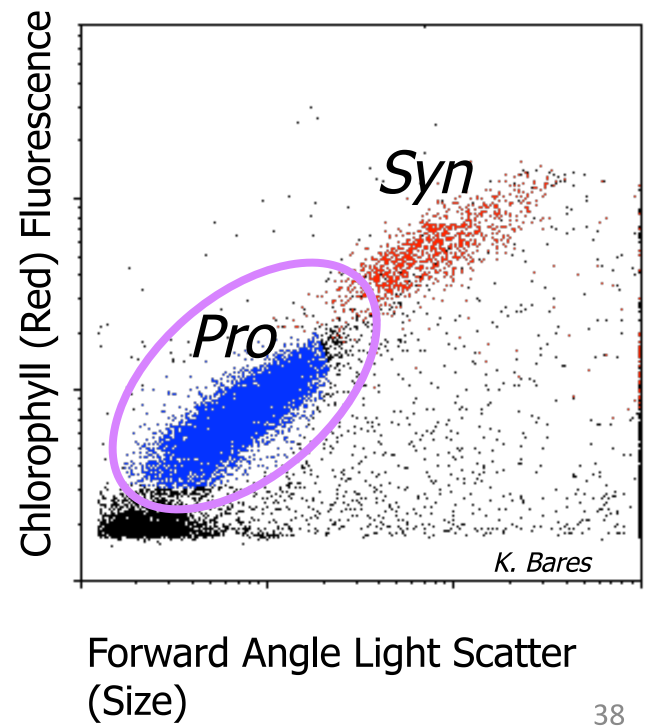
Mitch Sogin

Brian Palenik

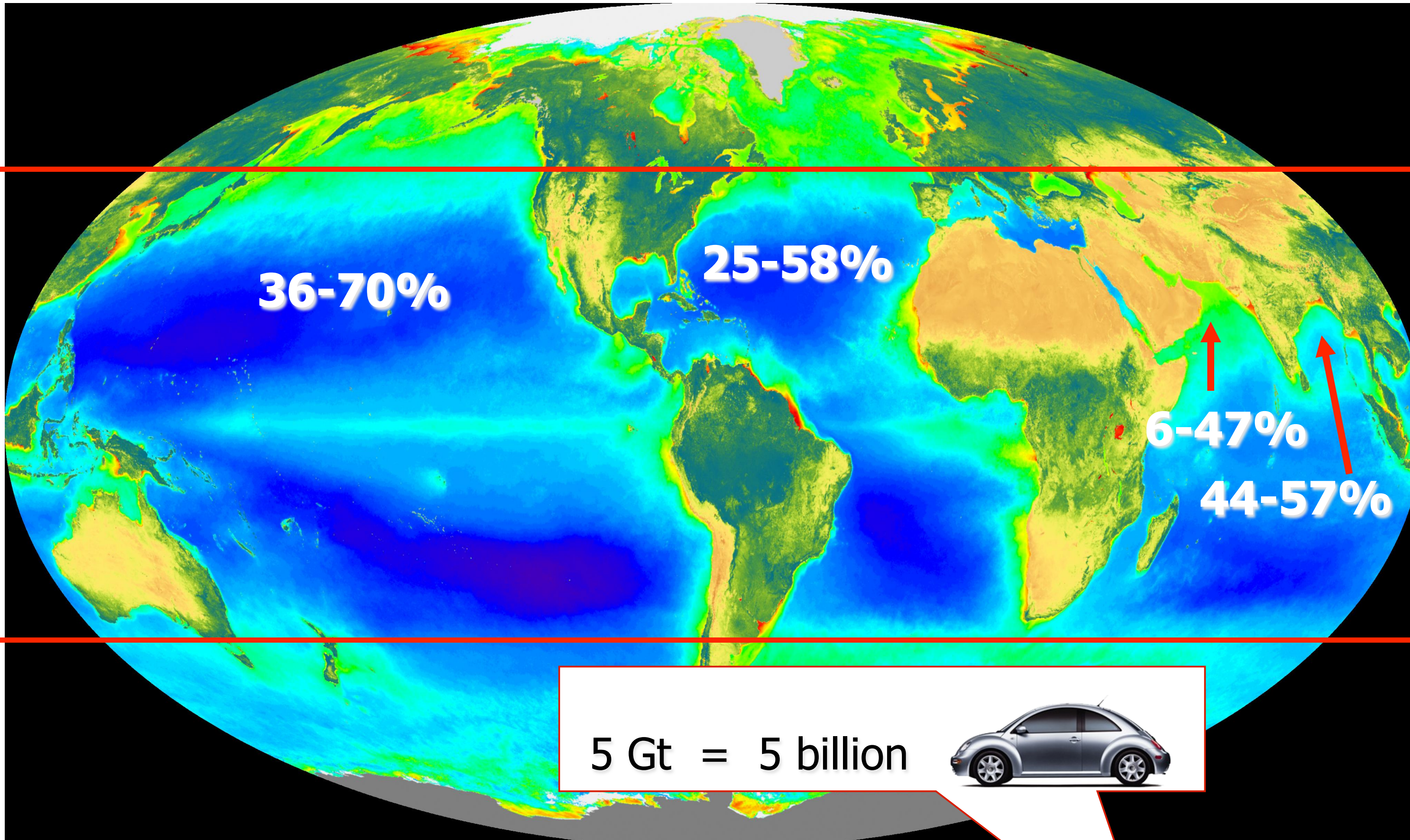
What is *Prochlorococcus*?

- Smallest cell in the oceans that fluoresces red
- Contains Divinyl Chl a and Chl b
- Oceanic cyanobacterium, 0.6 - 0.8 μm diameter
- Smallest (size and genome), and most abundant photosynthetic cell on Earth





A significant fraction of global chlorophyll

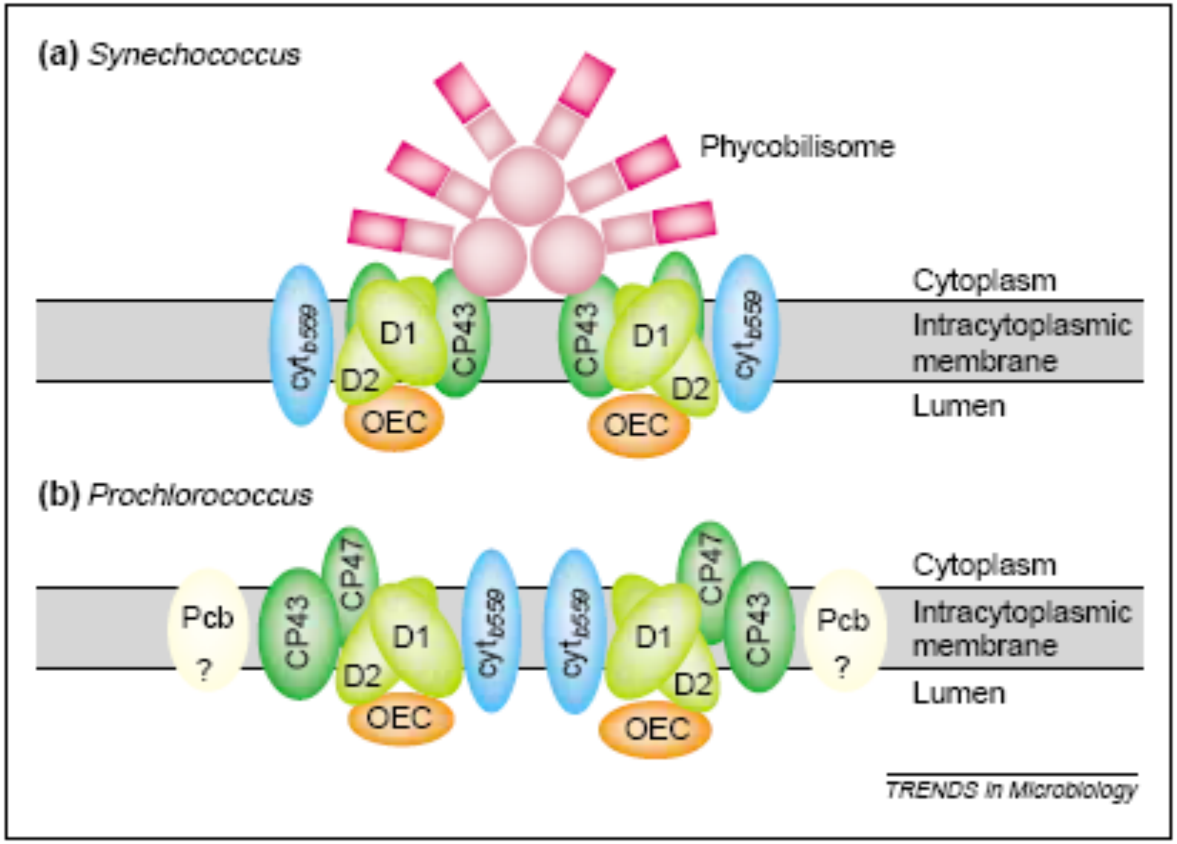


Record concentration:

700,000 cells ml^{-1}

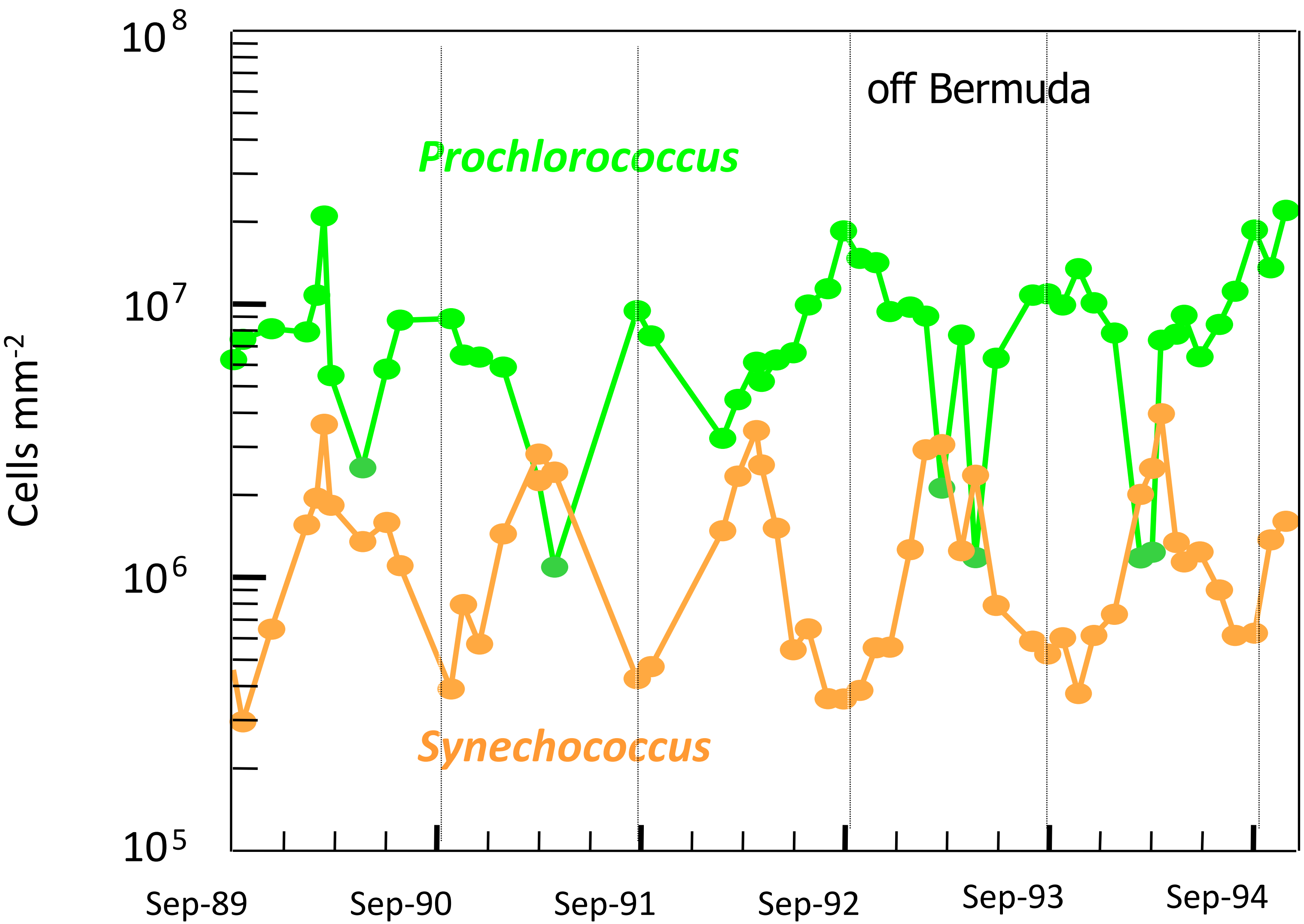
Global photosynthesis ≈ 5 Gt C yr^{-1}

It's basically a slightly smaller *Synechococcus* with a different light harvesting system



Ting et al

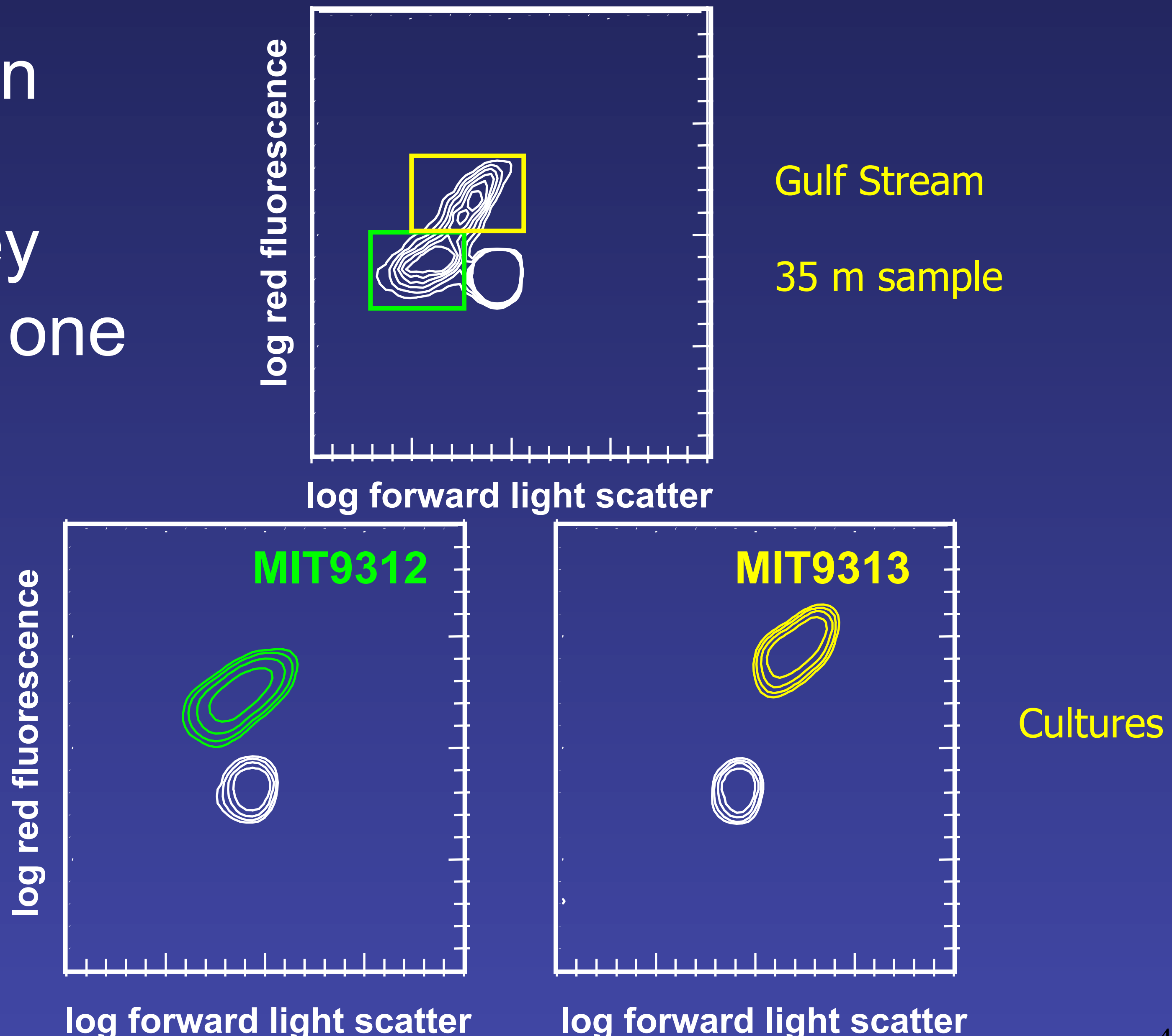
Prochlorococcus and *Synechococcus* partition the "small size" niche



1995

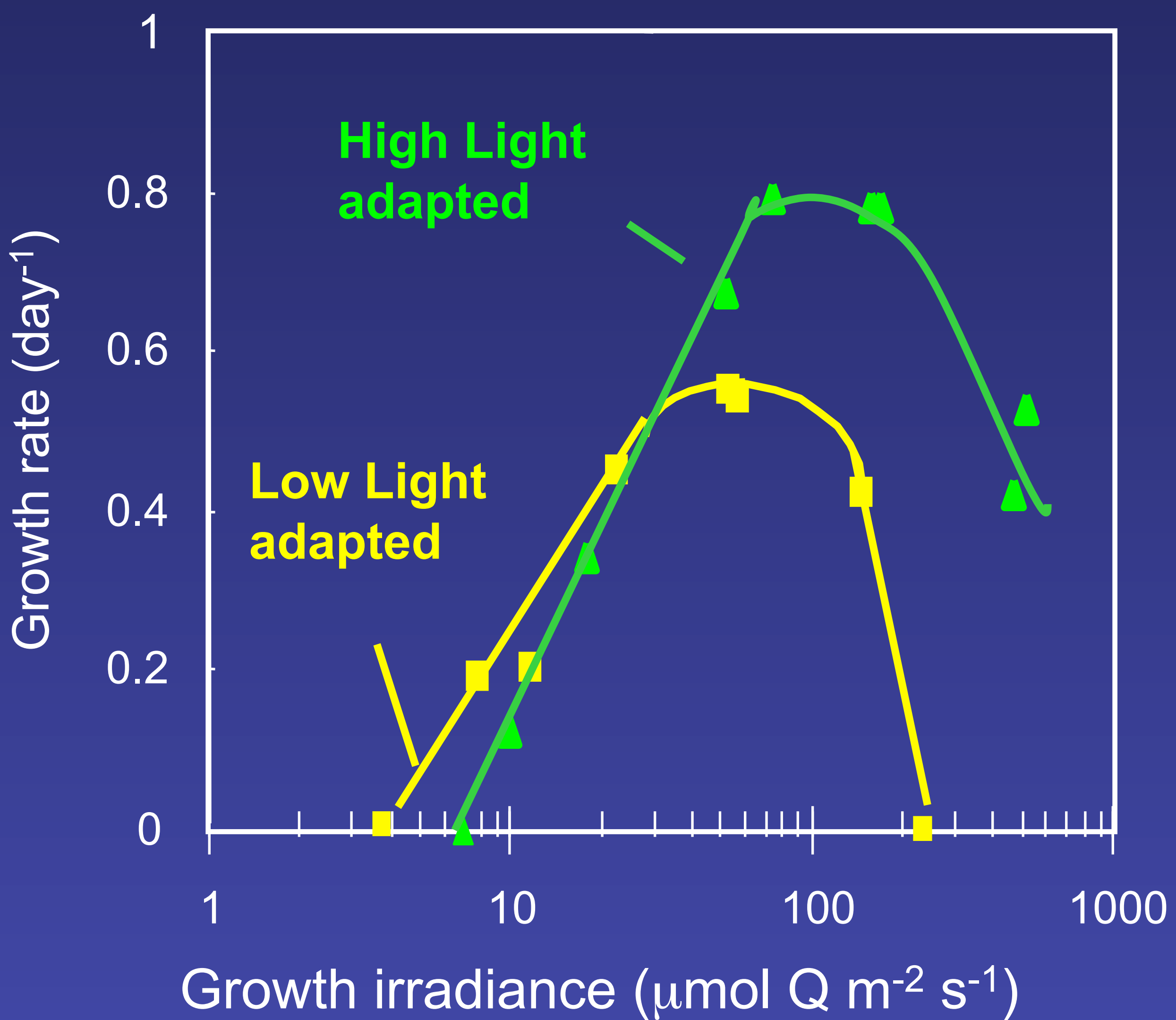
DuRand et al 2001

We soon learned that they are not one thing…



Moore *et al.* 1998

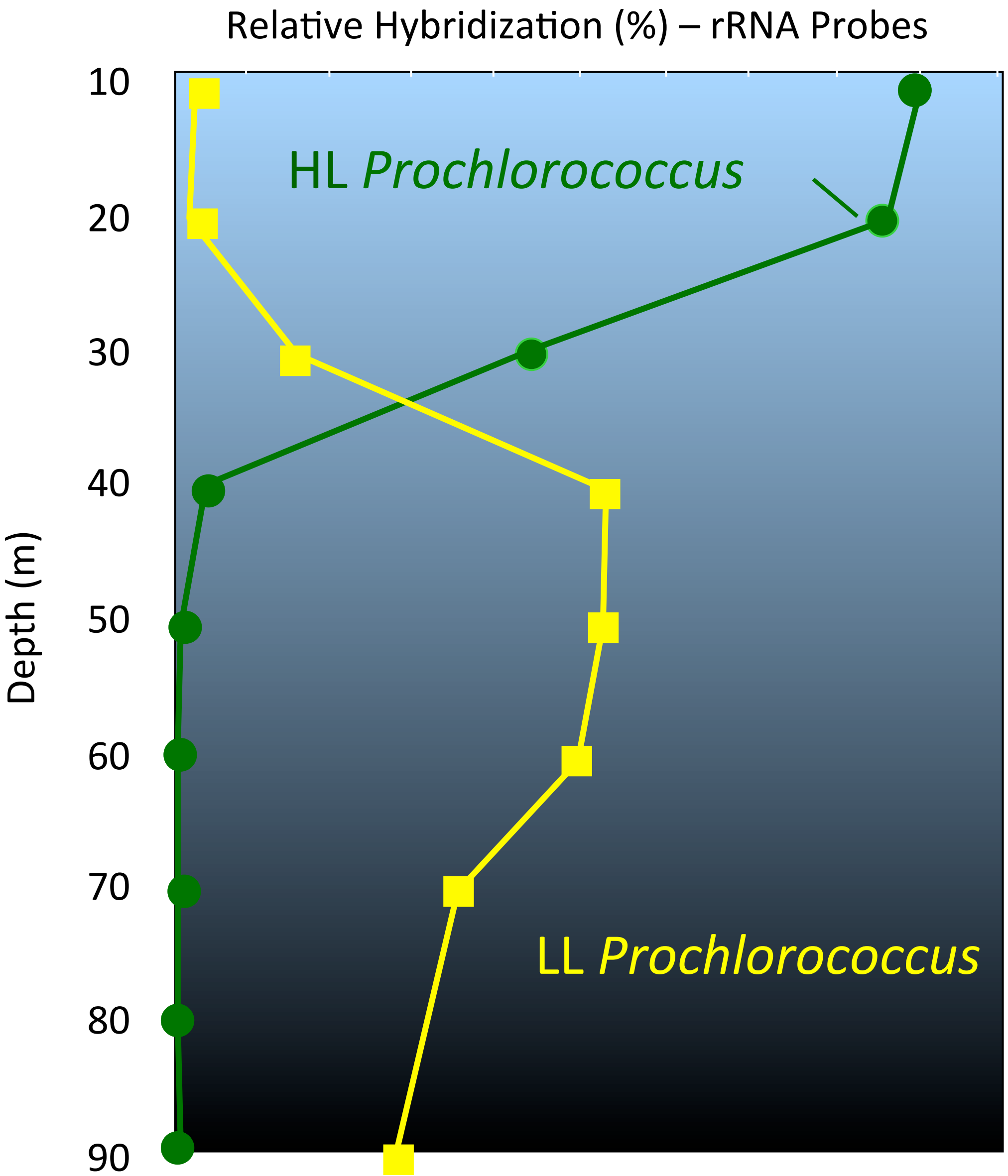
Closely related, coexisting, isolates differ in their physiology



Moore, Rocap et al….

1999

Niche Partitioning in the euphotic zone



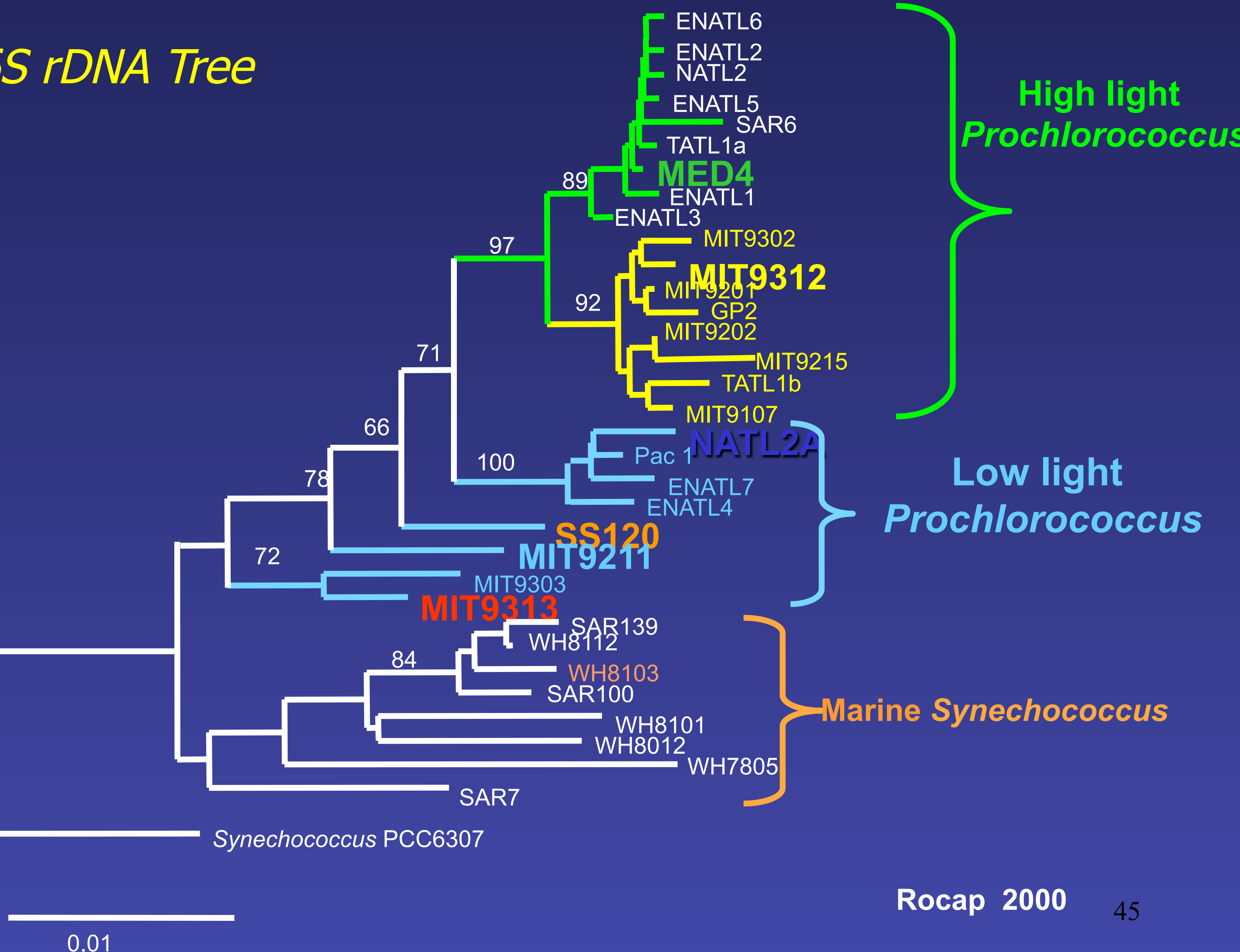
Dave Scanlan

Still…*Prochlorococcus* is a single species by traditional microbial standards

16S rDNA Tree

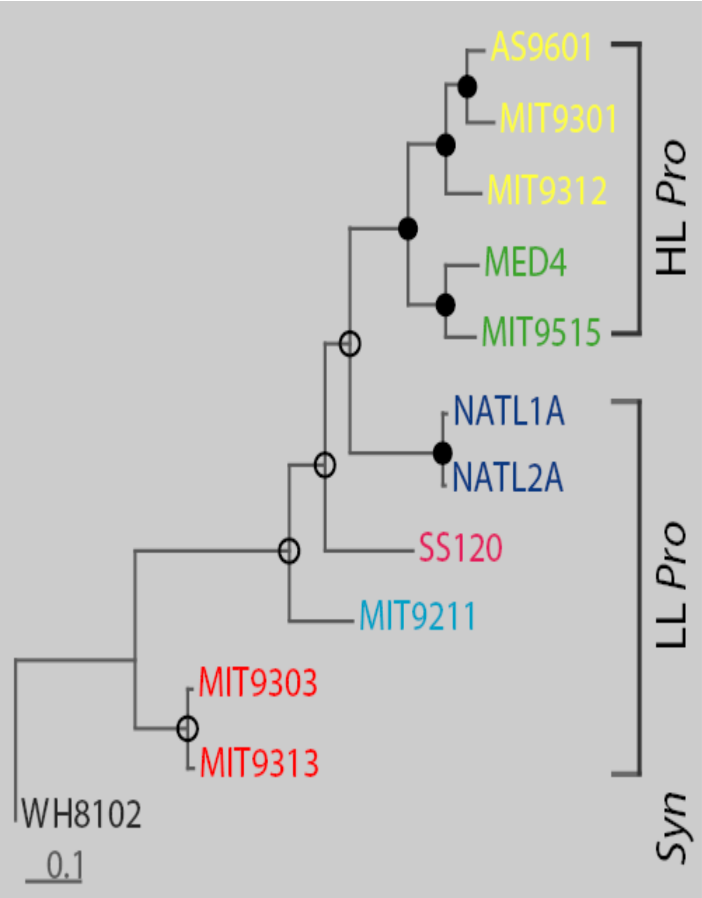
Lisa Moore

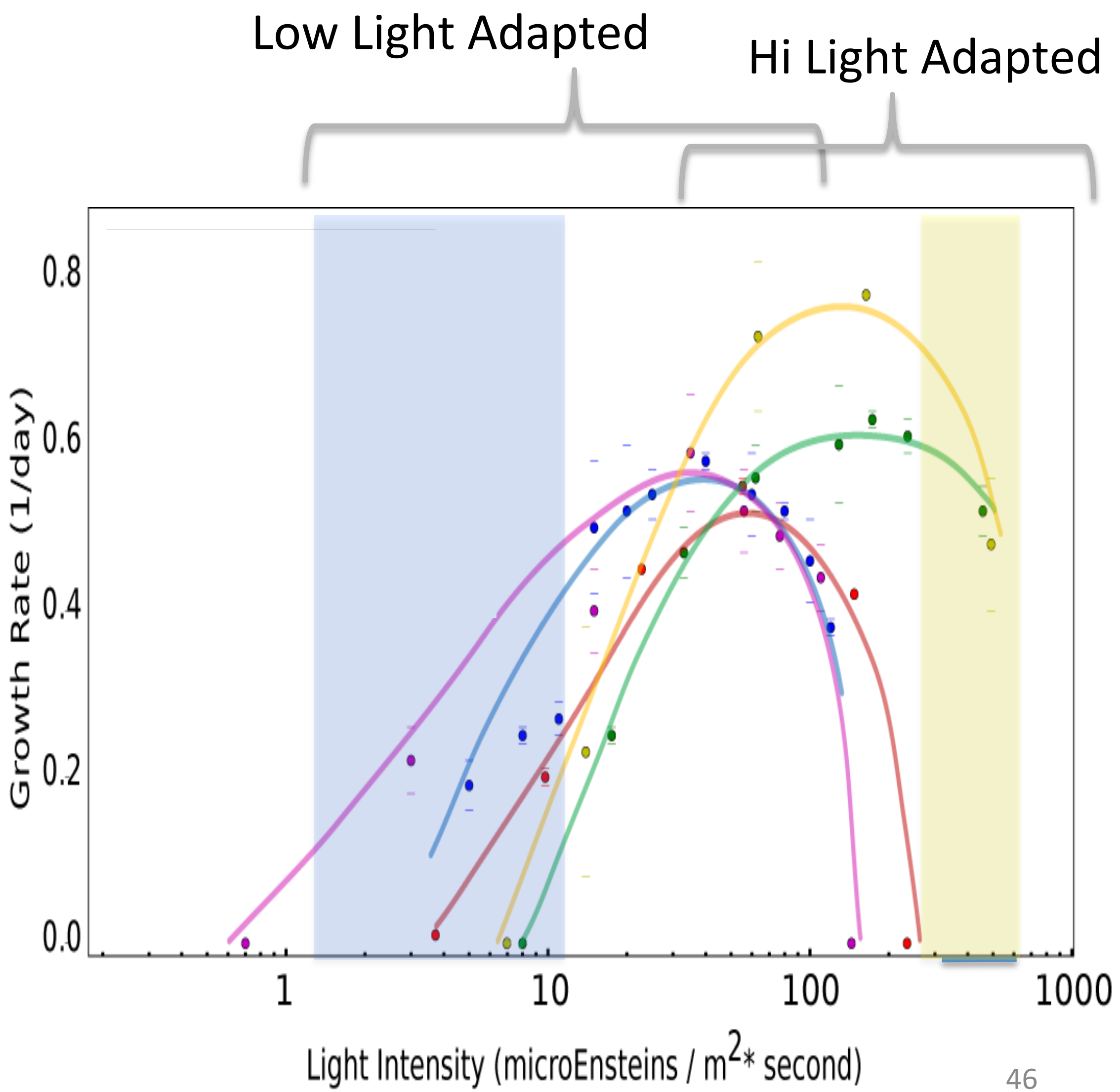
Gabrielle Rocap



Light adaptation defines ecotypes…

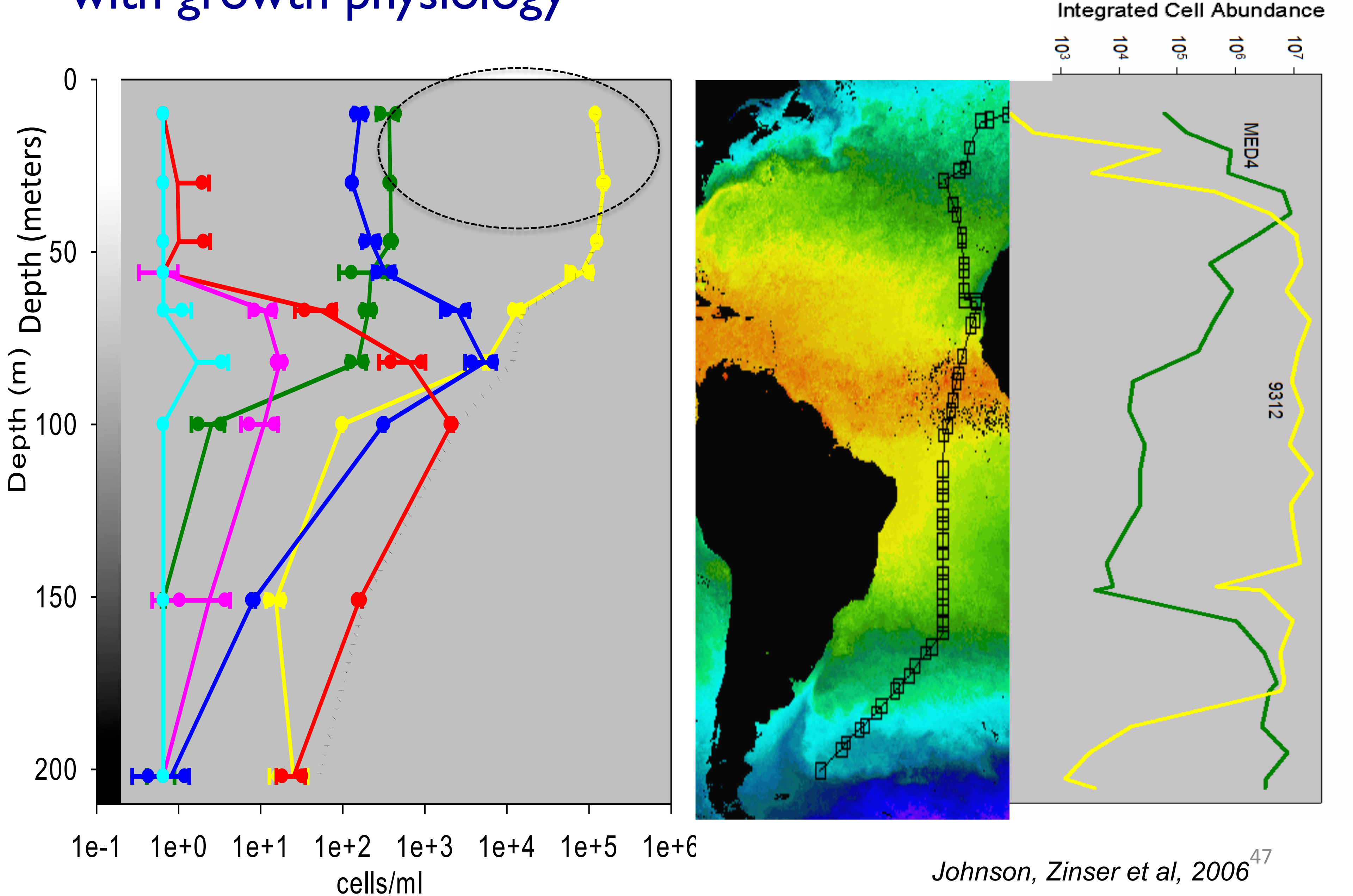
Whole Genome Phylogeny





Moore, Rocap, Kettler and more, 1990 - 2006

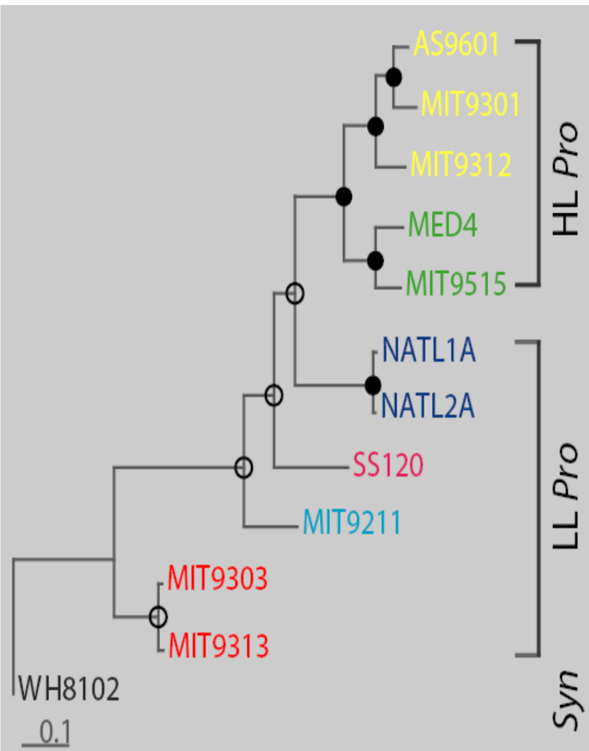
Ecotype distributions in the wild are consistent with growth physiology

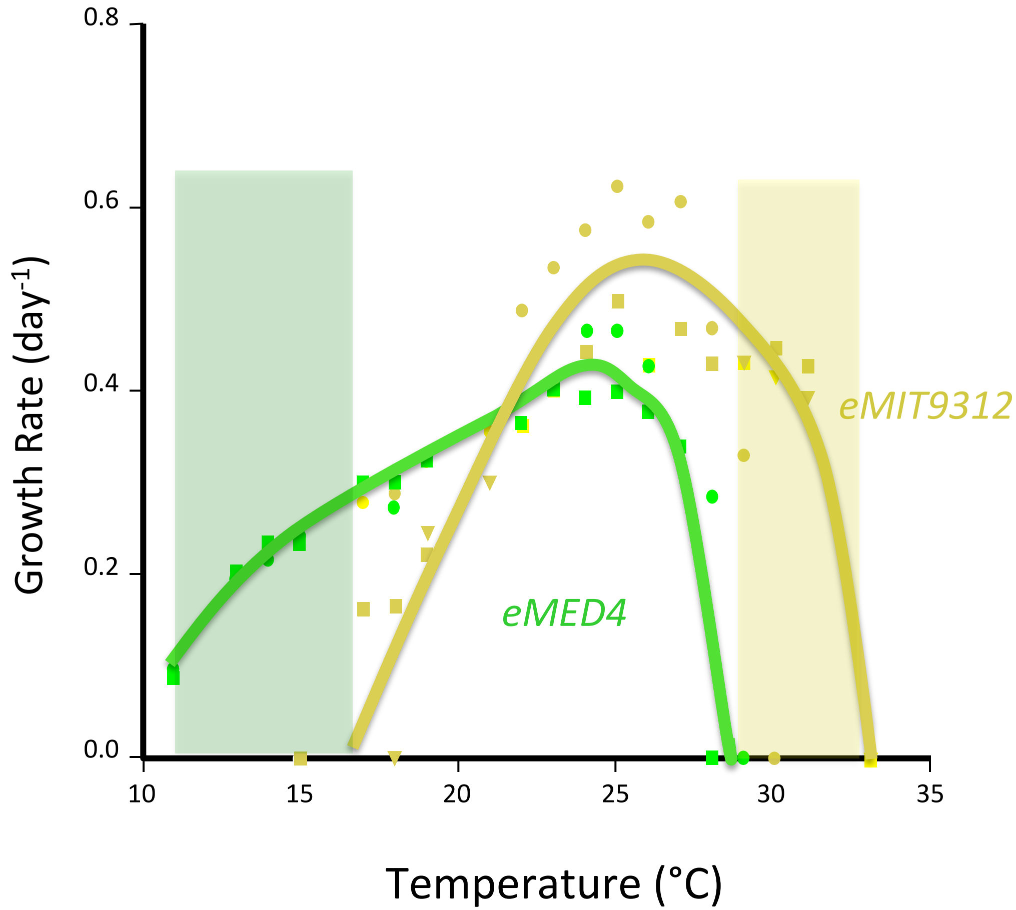


Johnson, Zinser et al, 2006

Temperature tolerance differentiates the two HL ecotypes

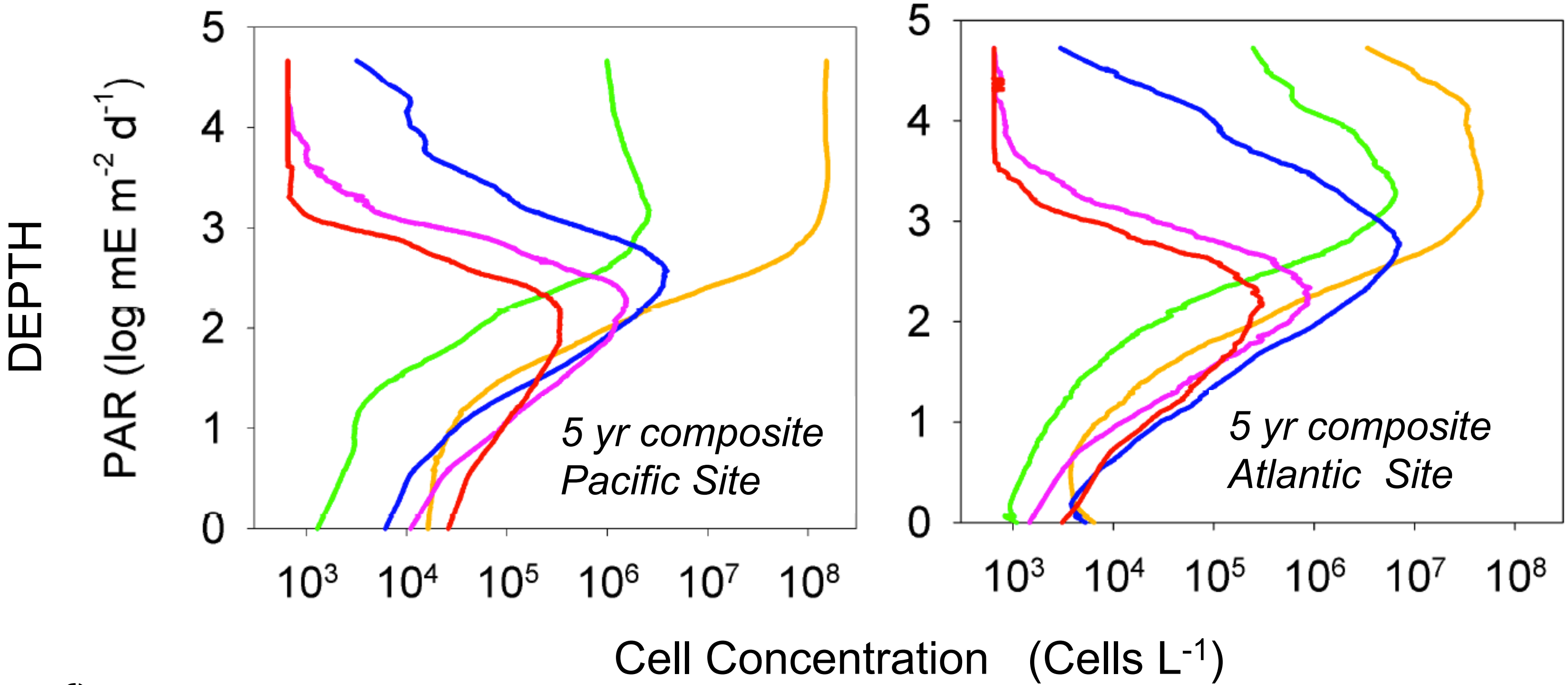
Sequenced genome tree

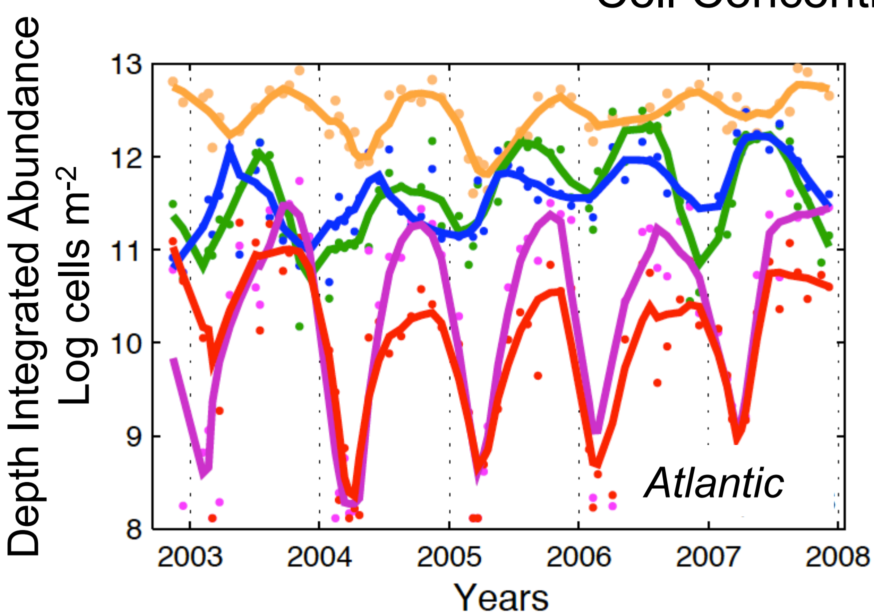


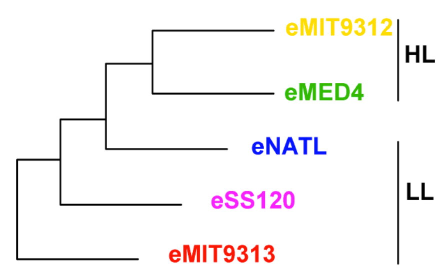


Johnson, Zinser et al 2006

…leading to niche differentiation







Malmstrom et al 2010

Take Home Messages

- Size matters
- Small cells dominate oligotrophic oceans
- *"Discovery"* happens in chapters. Some are lucky enough to be there when the critical chapter is written and the story unfolds
- A "species" can have many phenotypes
- And many many many genotypes (Coming next week!)