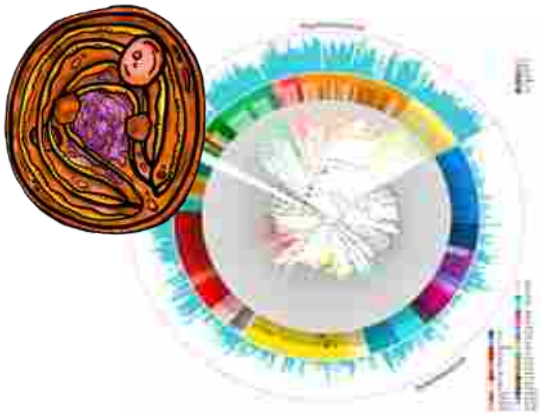
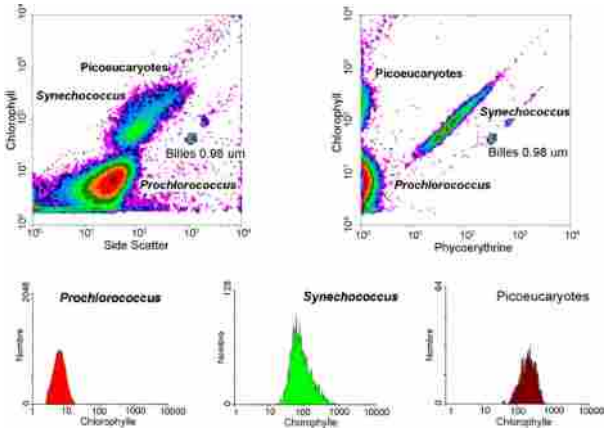
Croucher Course, Hongkong

July 2025

Course 1: How advances in biotechnology has driven our knowledge on marine picocyanobacteria? An inside story







Frédéric Partensky

Station Biologique de Roscoff

CNRS & Sorbonne University

Dept Adaptation & Diversity in the Marine Environment (UMR 7144)

Ecology of Marine Plankton (EcoMaP) Team

Roscoff, France

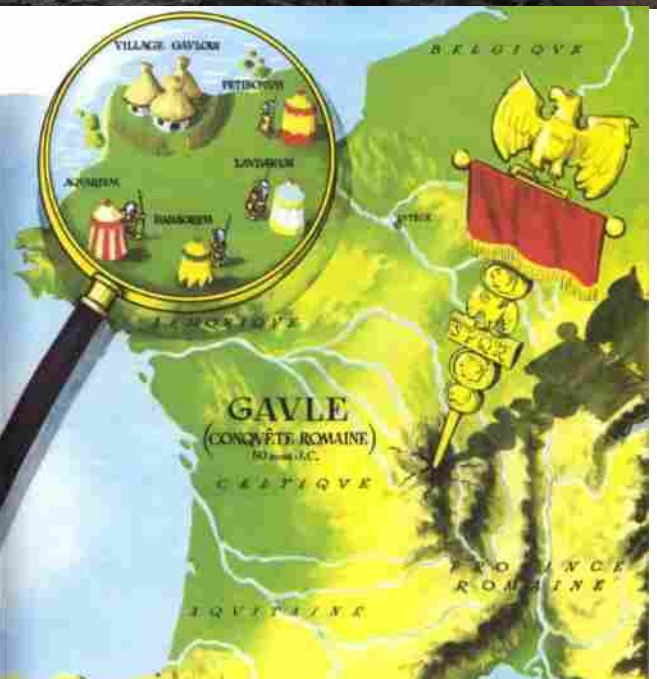
CNRS UPMC Station Biologique Roscoff

cnrs

SCIENCES SORBONNE UNIVERSITÉ

Station Biologique, Roscoff, Brittany, France





About 350 m from Asterix's village

2 Research units:



& Diversity

Biology of M

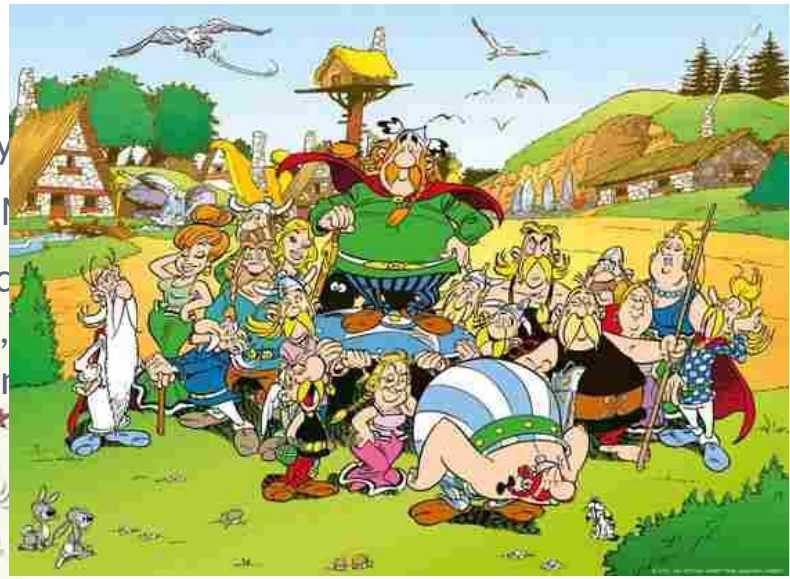
and servic

collection,

cytometry, DNA sequencin

spectrometry, etc.





Station Biologique, Roscoff, Brittany, France



The SBR was founded in 1872 by Henri de Lacaze-Duthiers
Professor of zoology at Sorbonne University in Paris.

He also founded the Banyuls station near the spanish border.



Station Biologique, Roscoff, Brittany, France



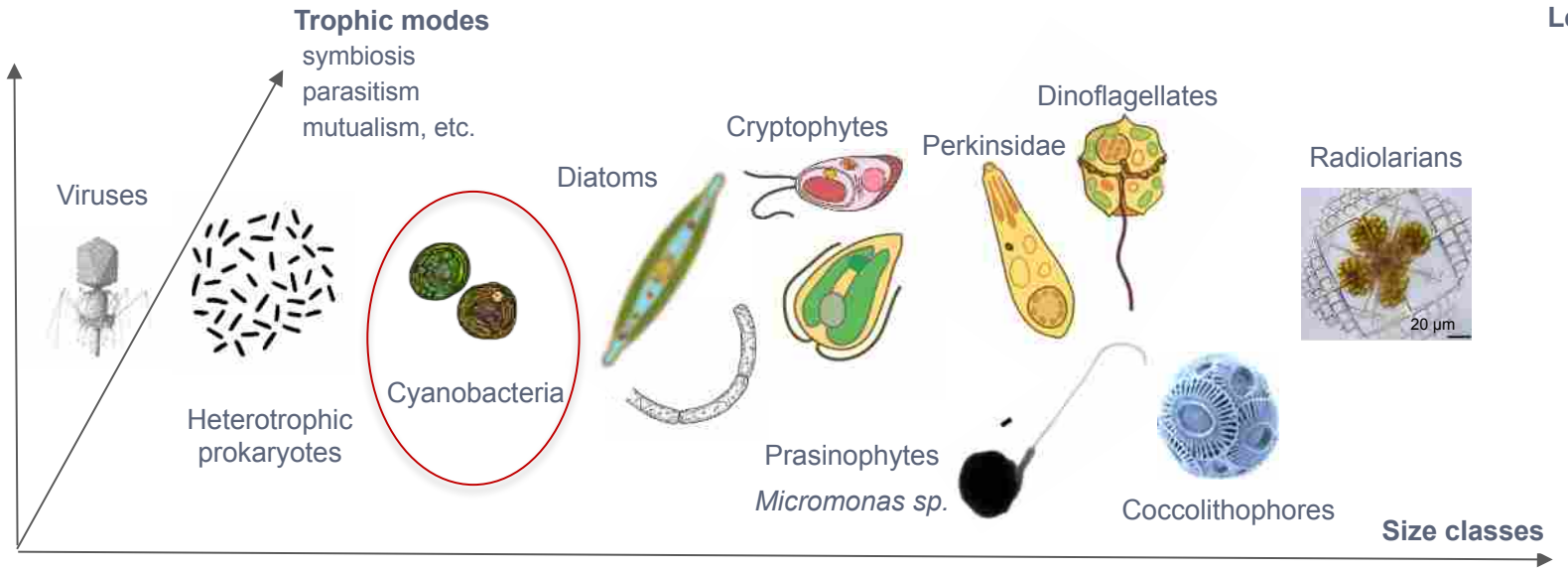




Station Biologique de Roscoff
1872—2022

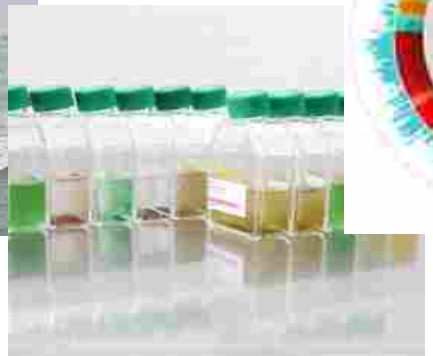


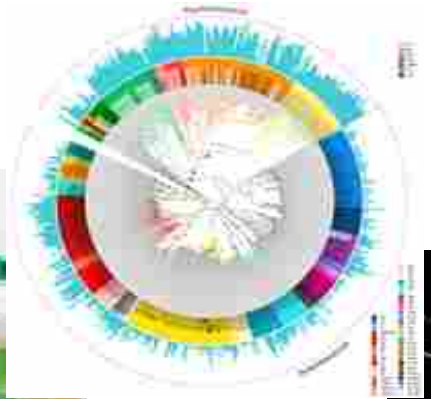
The Ecology of Marine Plankton (ECOMAP) Team

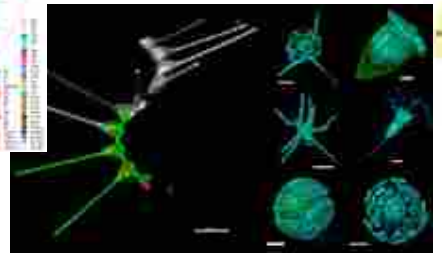


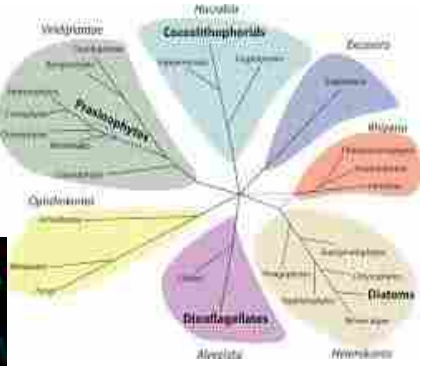
The ECOMAP team studies the diversity, ecology, physiology and evolution of marine planktonic organisms and their interactions across various scales from viruses to radiolarians and from the molecule to the global Ocean.











The Ecology of Marine Plankton (ECOMAP) Team

ECOLOGY OF MARINE PLANKTON (ECOMAP)

PERMANENT STAFF

Laurence GARCZAREK



DR1 CNRS
Genetic and functional diversity of marine cyanobacteria

Frédéric PARTENSKY



DR1 CNRS
Function and evolution of cyanobacterial light-harvesting complexes

Christophe SIX



MC SU
Adaptation of phytoplankton to temperature

Juliana BERNARDES



MC SU
Machine learning in marine ecology

POST-DOCS, PhD STUDENTS & TEMPORARY STAFF

Morgane RATIN



IR CNRS
Mol. biology & genetics of cyanobacteria
Security & safety

Francesco MATTEI



Post-Doc
Global modelling of cyanobacteria distribution

Emile FAURE



Post-Doc
Metagenomics of marine picocyanobacteria

Perrine KERGOAT



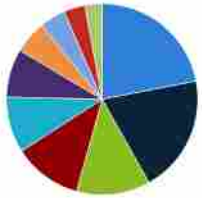
PhD student
Niche adaptation of marine cyanobacteria



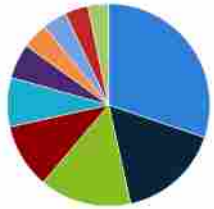
Roscoff RCC Culture Collection

12 000 entries
>450 Synech.

CLASSES



GENUS







Croucher course 1 outline

I. 1979-1999: The golden age of fluorescence techniques

II. 1996-2003: Characterization of the original light- harvesting complex of *Prochlorococcus*

III. 1998: The advent of the molecular ecology of picocyanobacteria

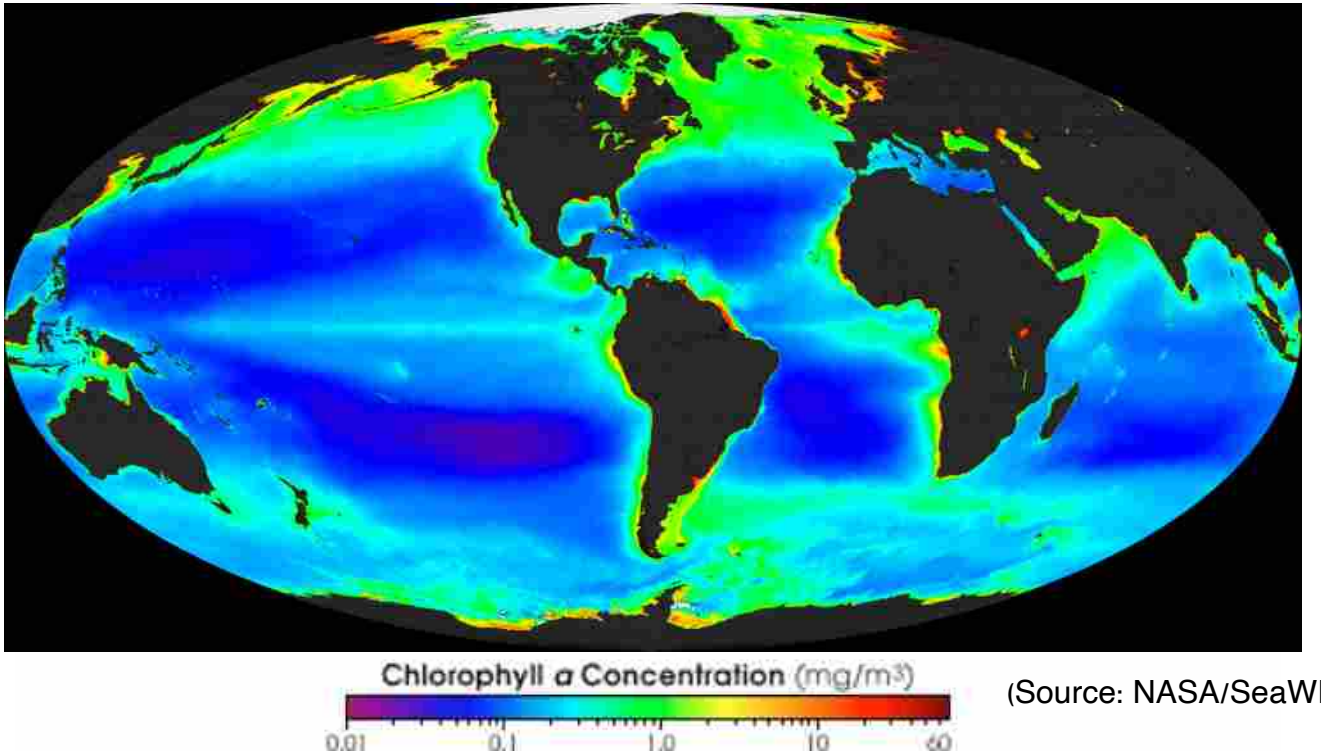
IV. 2003-on: The omics era: an introduction

V. Optional : Cyanophages

Course 2: Using omics to decipher niche adaptation mechanisms in marine picocyanobacteria

Course 3: A multi-scale study of *Synechococcus* pigment types: From genes to the global Ocean

Oceanic gyres were long thought as being “biological deserts”…



(Source: NASA/SeaWIFS)

Only in the late 70’s came the first evidences that these zones were populated by small-sized phytoplankton (<2-3 µm) called “picophytoplankton”.

Among these, *Synechococcus and Prochlorococcus* proved to be the two most abundant photosynthetic organisms on Earth and major contributors to oceanic primary production.

- *Marine Synechococcus official* discovery : 1979
- *Prochlorococcus official* discovery : 1988

Epifluorescence Microscopy of natural samples (+ Culturing)



Nature January

JOHN B. WATERBURY
STANLEY W. WATSON
ROBERT R. L. GUILLARD
LARRY E. BRAND

Department of Biology,
Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543

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 cyano-

Synechococcus Nägeli, 1849

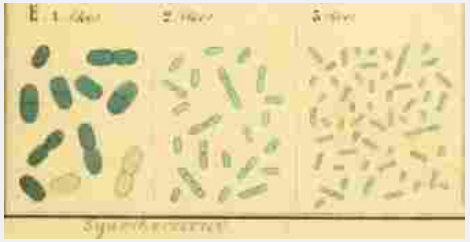
Greek: *synechos*, in succession, and *kokkos*, granule

Holotype species: *Synechococcus elongatus* (Nägeli) Nägeli

Publication details: Nägeli, 1849: 56

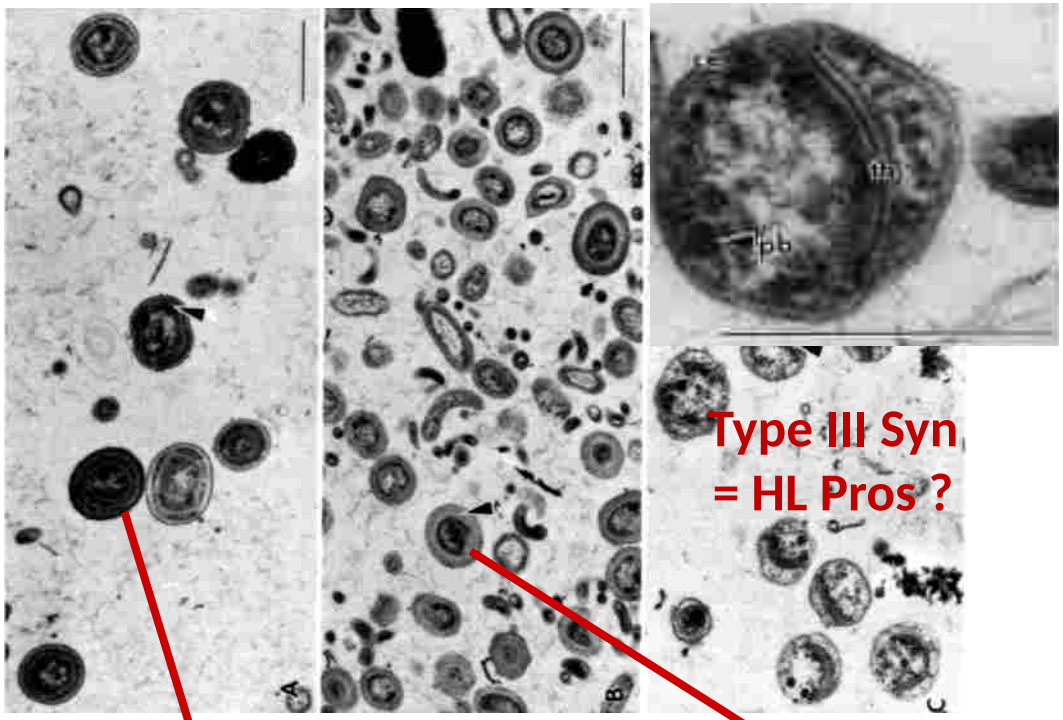
Original publication and holotype designation: Nägeli, C. (1849).





"Genera of unicellular algae, physiologically and systematically processed."
In: New memoirs of the General Swiss Society for Natural Sciences."

Transmission Electron Microscopy on natural samples



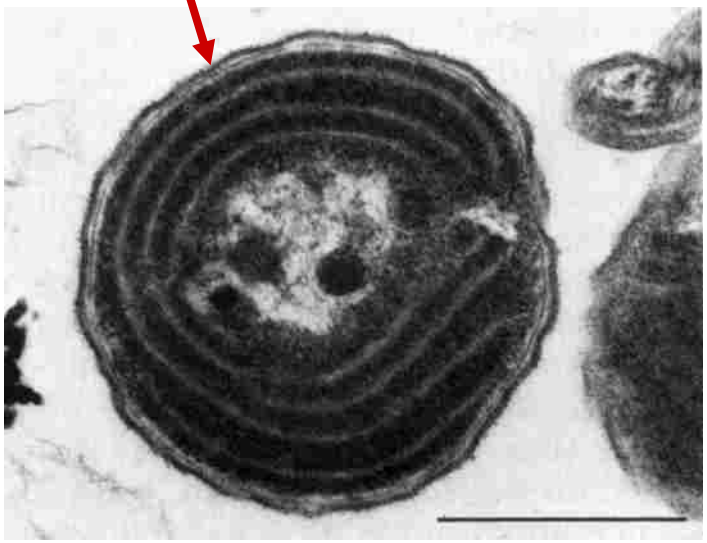
LIMNOLOGY AND OCEANOGRAPHY
ASLO
Association for the Sciences of Limnology and Oceanography

Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass1

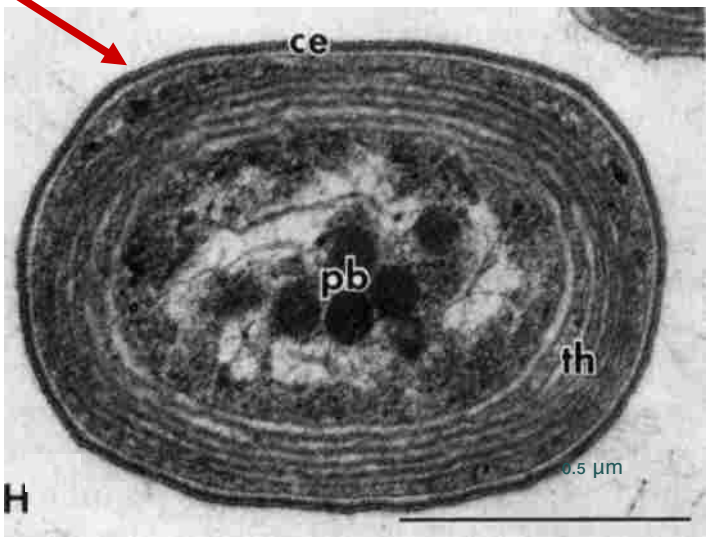
Paul W. Johnson, John McN. Sieburth

First published: September 1979 | https://doi.org/10.4319/lo.1979.24.5.0928

In the photic zone of the open sea, procaryotic cells with an ultrastructure typical of **chroococcoid cyanobacteria** and similar sized populations of autofluorescent bacteria (**0.5–1.0 × 1.0 µm**) were observed at concentrations usually between **10^3–10^4 cells per ml**. The ubiquitous ultrastructural type was an **orange-autofluorescing, phycoerythrin-containing cyanobacterium amenable to culture**. Two **other morphological types** which have not been cultured occur sporadically in larger concentrations, one apparently favoring deeper water. [..]. These cells may play a significant role in oceanic primary productivity.



Type I *Synechococcus*



“Type II

Type II cells, 0.7 x 0.9 pm also have **peripheral thylakoids, but more closely spaced**, [..]. These **type II cells occur only in oceanic samples and primarily at 100 m, where they can dominate the picoplankton.**

Johnson & Sieburth 1979

Quantitative HPLC pigment analyses of natural seawater samples

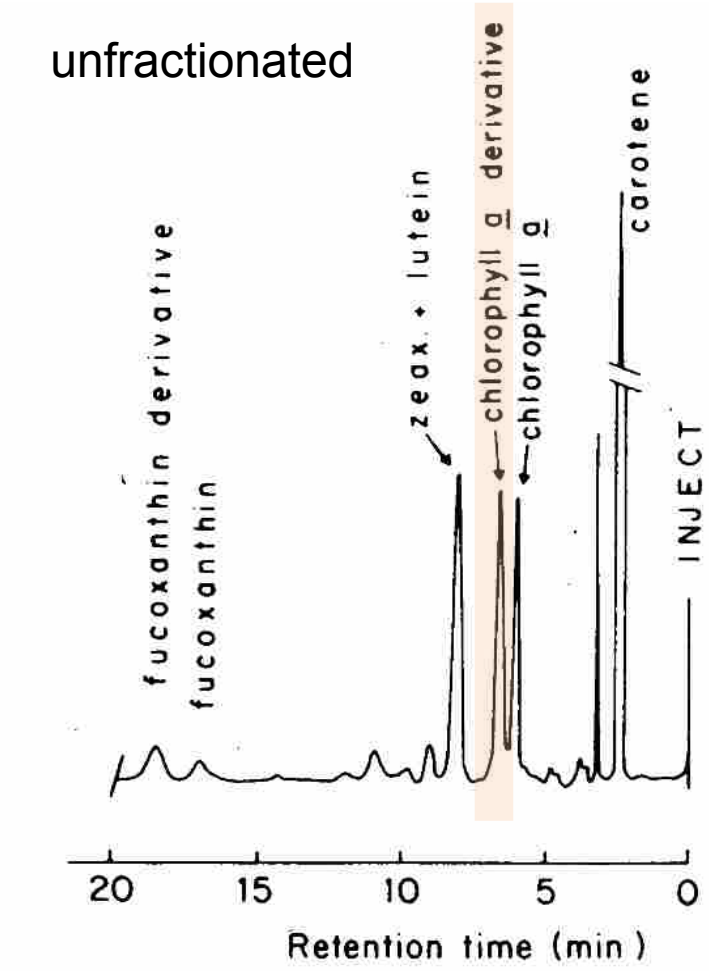
LIMNOLOGY AND OCEANOGRAPHY
ASLO Association for the Sciences of Limnology and Oceanography

Unknown chlorophyll a derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis1

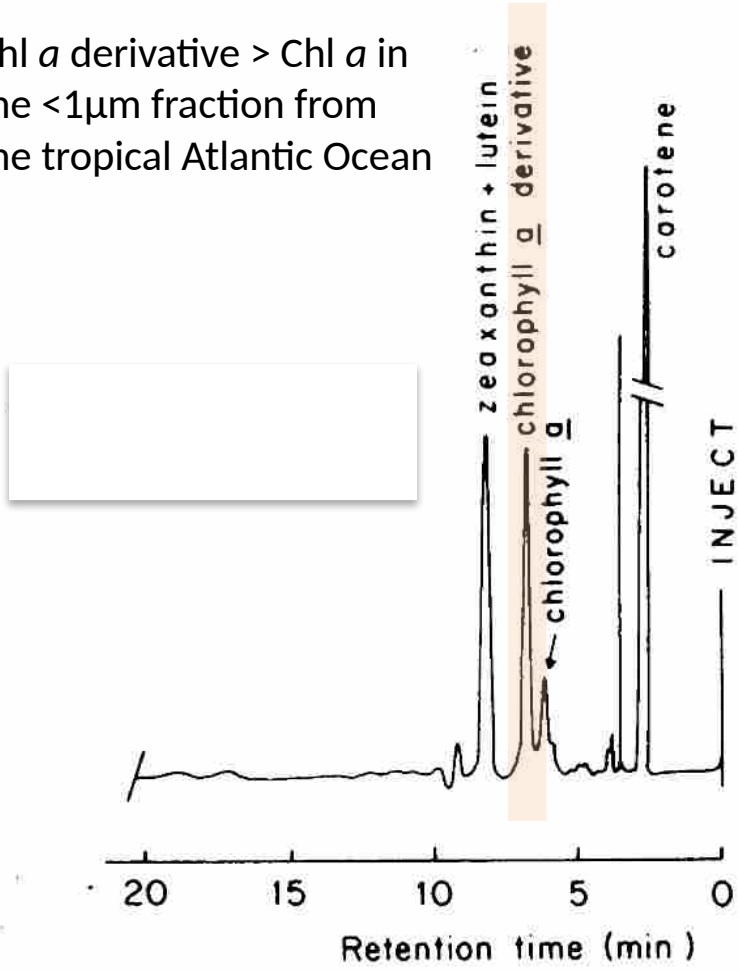
Winfried W. Gieskes, Gijsbert W. Kraay

July 1983 | https://doi.org/10.4319/lo.1983.28.4.0757

Unknown Chl *a* derivative associated with particles <1 µm which contain zeaxanthin as the major carotenoid. Although these tiny particles may be detrital, the coccoid cyanobacteria typically found in large numbers in open ocean waters are of similar size.



Chl *a* derivative > Chl *a* in the <1µm fraction from the tropical Atlantic Ocean



Gieskes and Kraay 1983

Quantitative HPLC pigment analyses of natural seawater samples

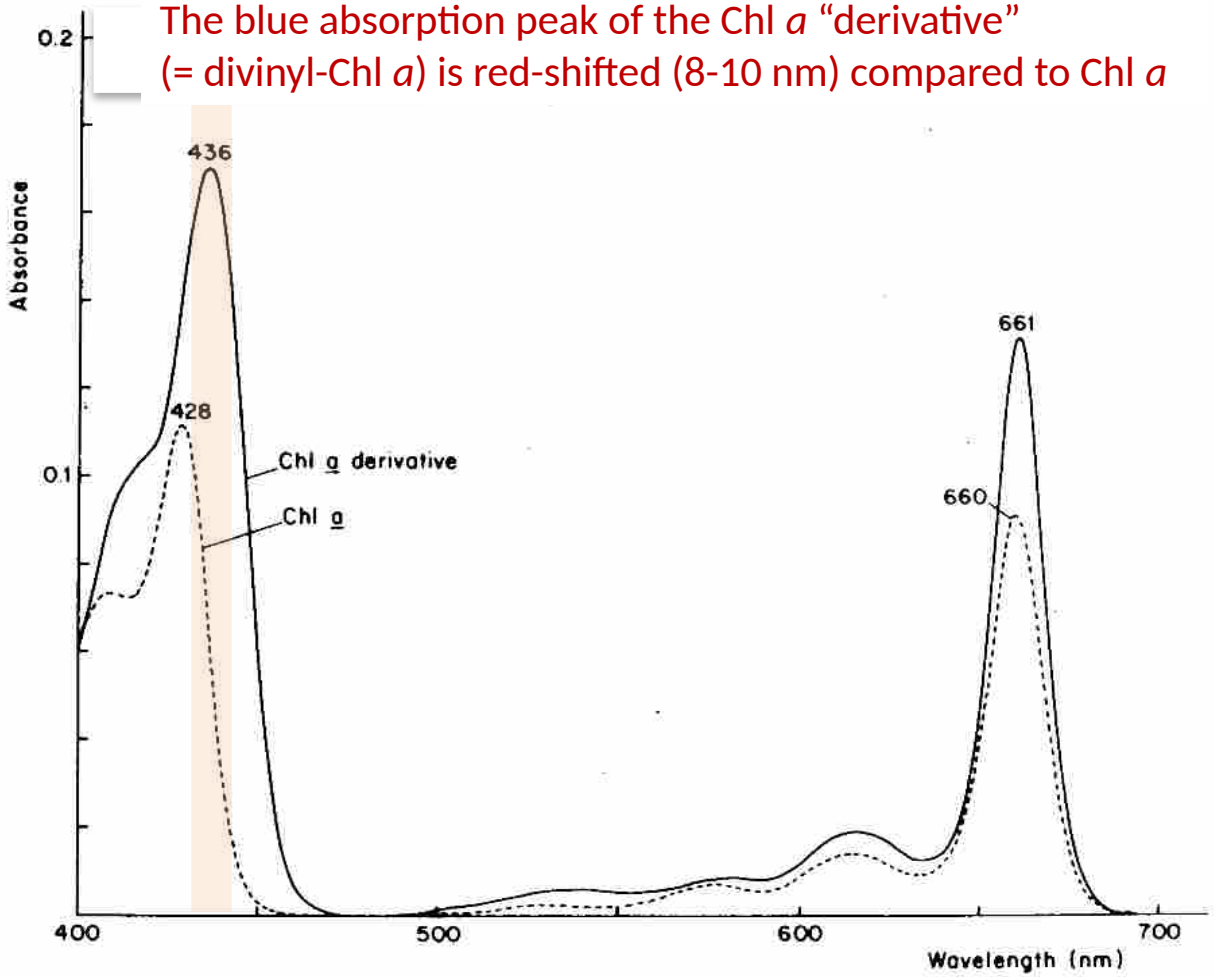
LIMNOLOGY AND OCEANOGRAPHY
ASLO Association for the Sciences of Limnology and Oceanography

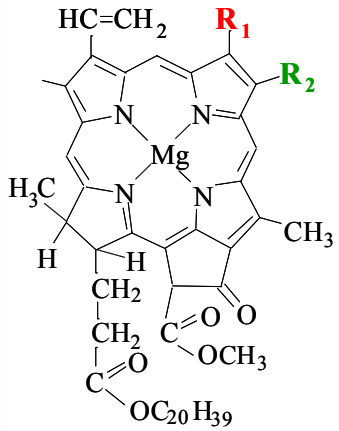
Unknown chlorophyll a derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis1

Winfried W. Gieskes, Gijsbert W. Kraay

First published: July 1983 | https://doi.org/10.4319/lo.1983.28.4.0757 | Citations: 108

The blue absorption peak of the Chl *a* "derivative"
(= divinyl-Chl *a*) is red-shifted (8-10 nm) compared to Chl *a*





Chl	R_1	R_2
a_1	CH_3	$CH_2\ CH_3$
a_2	CH_3	$CH\ CH_2$
b_1	CHO	$CH_2\ CH_3$
b_2	CHO	$CH\ CH_2$

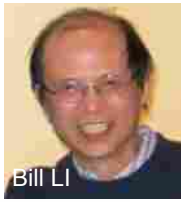
Gieskes and Kraay 1983

$^{14}CO_2$ measurements on size-fractioned seawater samples

In the early 80's, some investigators still claimed that Chl-containing particles in the picoplankton size range were not intact viable cells but fragments or degradation products of larger cells (chloroplasts). *Synechococcus* was known but not its contribution to primary production.

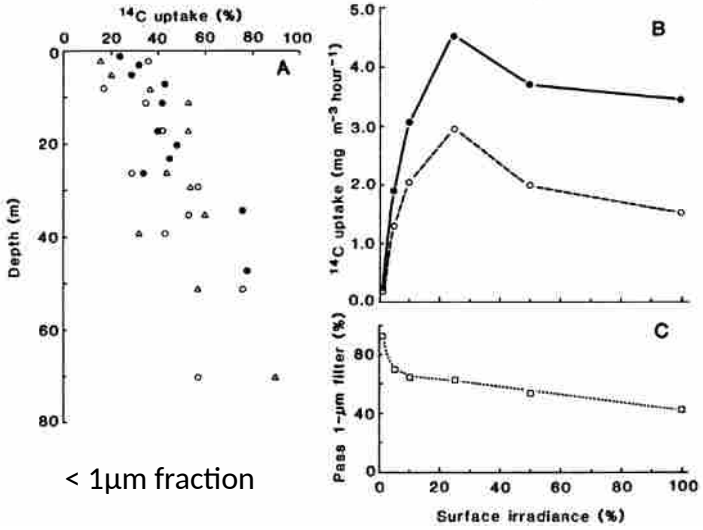
SCIENCE, VOL. 219
21 JANUARY 1983

W. K. W. Li, D. V. Subba Rao
W. G. Harrison, J. C. Smith
J. Cullen, B. Irwin, T. Platt
Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, Nova Scotia B2Y 4A2 Canada



Autotrophic Picoplankton in the Tropical Ocean

Abstract. *In phytoplankton of the eastern tropical Pacific Ocean from 25 to 90 percent of the biomass (measured as chlorophyll a) and 20 to 80 percent of the inorganic carbon fixation were attributable to particles that could pass a screen with a 1-micrometer pore diameter. Evidence is presented that these are indeed autotrophic cells and not cell fragments.*



< 1µm fraction

Li et al. Science 1983

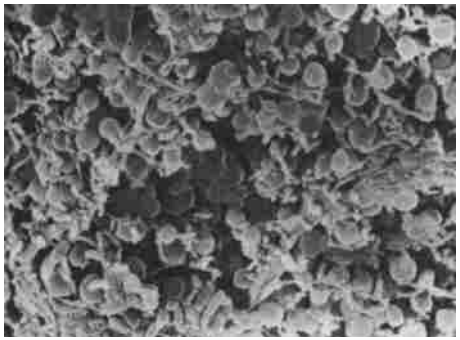
NATURE VOL. 301 24 FEBRUARY 1983

Photosynthesis of picoplankton in the oligotrophic ocean



Trevor Platt, D. V. Subba Rao & Brian Irwin

Marine Ecology Laboratory, Bedford Institute of Oceanography, Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2



Picophytoplankton supplies about 60% of total primary production at chlorophyll maximum in the open ocean

Fig. 1 Scanning electron micrograph of natural phytoplankton sample showing coccoid cells. Scale bar, 10 µm.

Table 1 Photosynthesis parameters for natural plankton assemblages from the chlorophyll maximum on Mid-Atlantic Ridge around latitude 36° N

	α (P^B_m/I)		P^B_m (mg C (mg Chl a)$^{-1}$ h^{-1})		I_k (W m^{-2})		I_m (W m^{-2})		I_b (W m^{-2})		B (mg^{-3})	
Station	<1 µm	>1 µm	<1 µm	>1 µm	<1 µm	>1 µm	<1 µm	>1 µm	<1 µm	>1 µm	<1 µm	>1 µm
8	0.059	0.015	0.72	0.27	12	19	35	57	67	150	0.18	0.24
9	0.025	0.014	0.25	0.24	9.9	16	35	53	180	190	0.21	0.26
10	0.057	0.036	0.78	0.50	14	14	43	46	120	190	0.18	0.19
11	0.072	0.030	0.50	0.37	6.9	13	23	43	100	190	0.21	0.22
12	0.084	0.049	0.70	0.92	8.3	19	30	64	170	280	0.25	0.41
13	0.078	0.059	0.67	0.62	8.7	11	30	37	130	190	0.23	0.26
14	0.077	0.032	0.77	0.41	10	13	32	43	100	200	0.14	0.24
15	0.079	0.039	0.75	0.63	9.5	16	34	55	180	250	0.32	0.38
17	0.088	0.040	1.0	0.56	12	14	39	47	140	200	0.26	0.22
18	0.10	0.040	0.76	0.38	7.6	9.5	26	39	120	360	0.27	0.39
19	0.10	0.068	0.57	0.48	5.8	7.1	19	27	81	190	0.23	0.28
Mean	0.074	0.038	0.68	0.49	9.5	14	31	46	130	220	0.22	0.28

α Is the initial slope; P^B_m is the assimilation number; I_k, I_m and I_b are irradiances and B is the chlorophyll biomass. All samples were separated by differential screening after incubation.

Platt et al. Nature 1983

Unveiling pigment diversity within *Synechococcus*

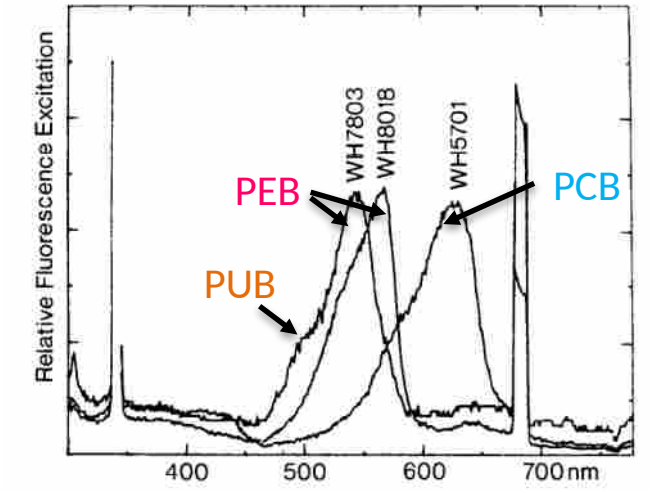
Limnol. Oceanogr., 30(6), 1985, 1303–1315

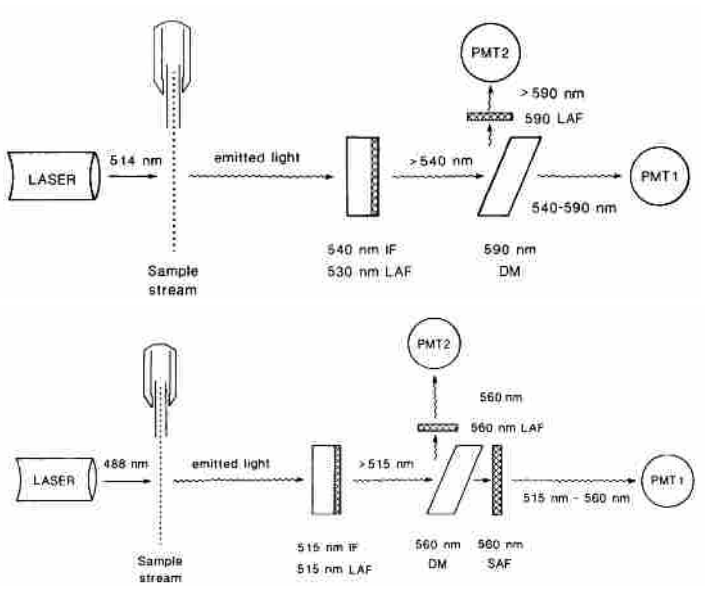
Discrimination between types of pigments in marine *Synechococcus* spp. by scanning spectroscopy, epifluorescence microscopy, and flow cytometry[1]

A. Michelle Wood[2]

P. K. Horan *D. A. Phinney* *J. B. Waterbury*
K. Muirhead *C. M. Yentsch*







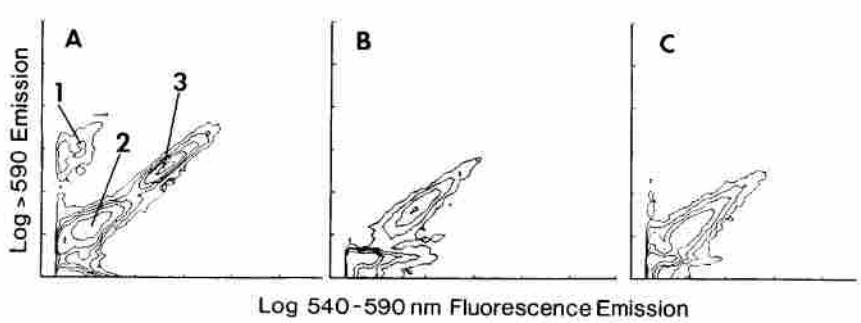


Fig. 8. As Fig. 6, but in the <10-μm fraction of three natural populations: A—the surface water of Ferry Reach, a coastal lagoon in St. George, Bermuda (note trimodal population structure); B—the interstitial waters in sediment adjacent to mangrove roots at St. George, Bermuda; C—open water about 5 m below the surface in 10 m of water off the NW coast of Bermuda. Either 100,000 (A) or 25,000 (B, C) cells were analyzed. Contour intervals—5, 20, 100, and 250 cells; laser line—514 nm; laser power—1,300 mW (A) or 500 mW (B, C).

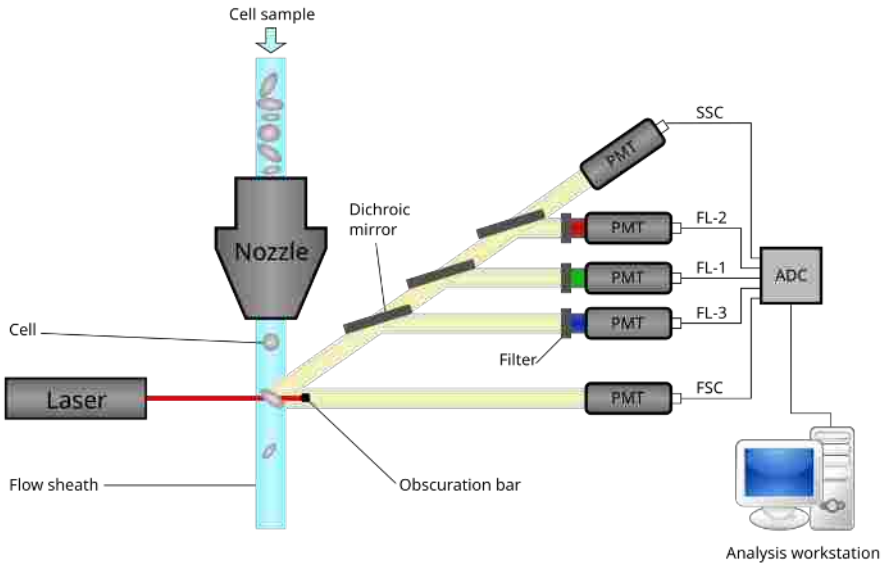
3 *Syn*. pigment types :

- Phycoerythrin-lacking (phycocyanin only) strains
- PE composed of only phycoerythrobilin (type 2 PE)
- PE composed of phycoerythrobilin and phycourobilin (type 1 PE)

Actually much more pigment diversity, see course 3

Flow cytometry has a lot of potential for studying *Syn*. at sea and picophytoplankton at large!

Flow cytometry at sea: They did it!

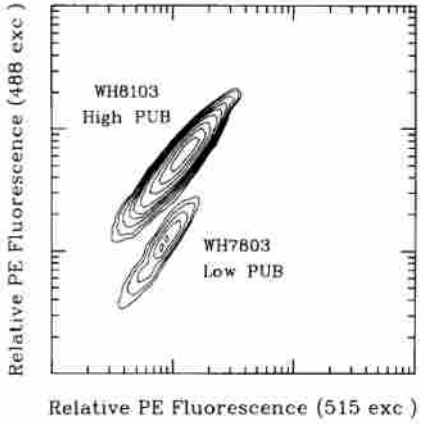




Deep-Sea Research, Vol. 35, No. 3, pp. 425–440, 1988.

Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry

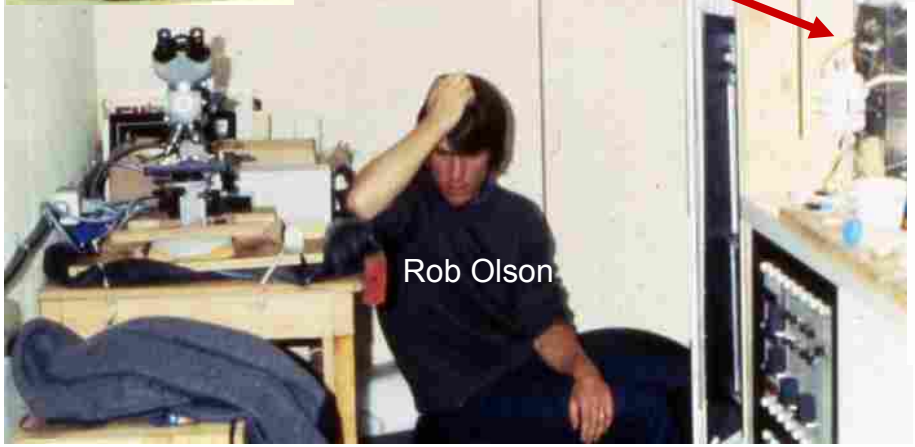
R. J. Olson,* S. W. Chisholm,† E. R. Zettler* and E. V. Armbrust†

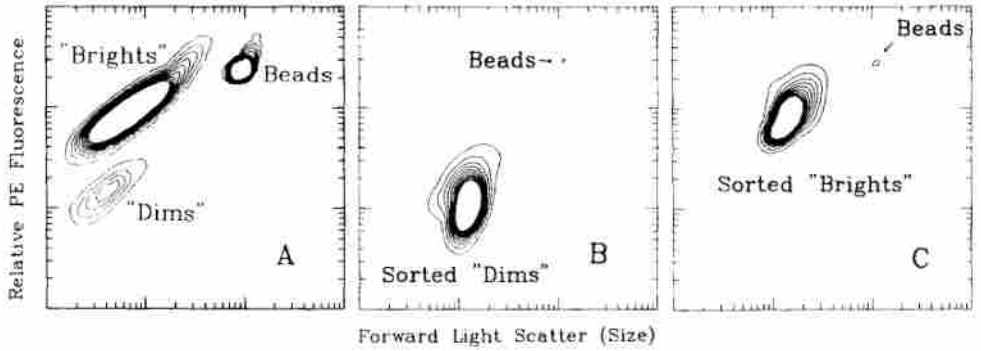






Coulter EPICS V





Blue (488 nm) is more efficiently absorbed by PUB than PEB thus high-PUB cells fluoresce more than low-PUB cells for a given amount of phycoerythrin.

Flow cytometry at sea

Limnol. Oceanogr., 35(1), 1990, 45–58

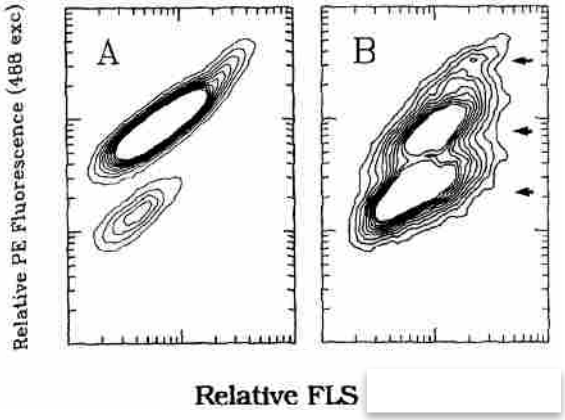
Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans

Robert J. Olson
Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Sallie W. Chisholm
48-425 Ralph M. Parsons Laboratory, Massachusetts Institute of Technology, Cambridge 02139

Erik R. Zettler
Woods Hole Oceanographic Institution

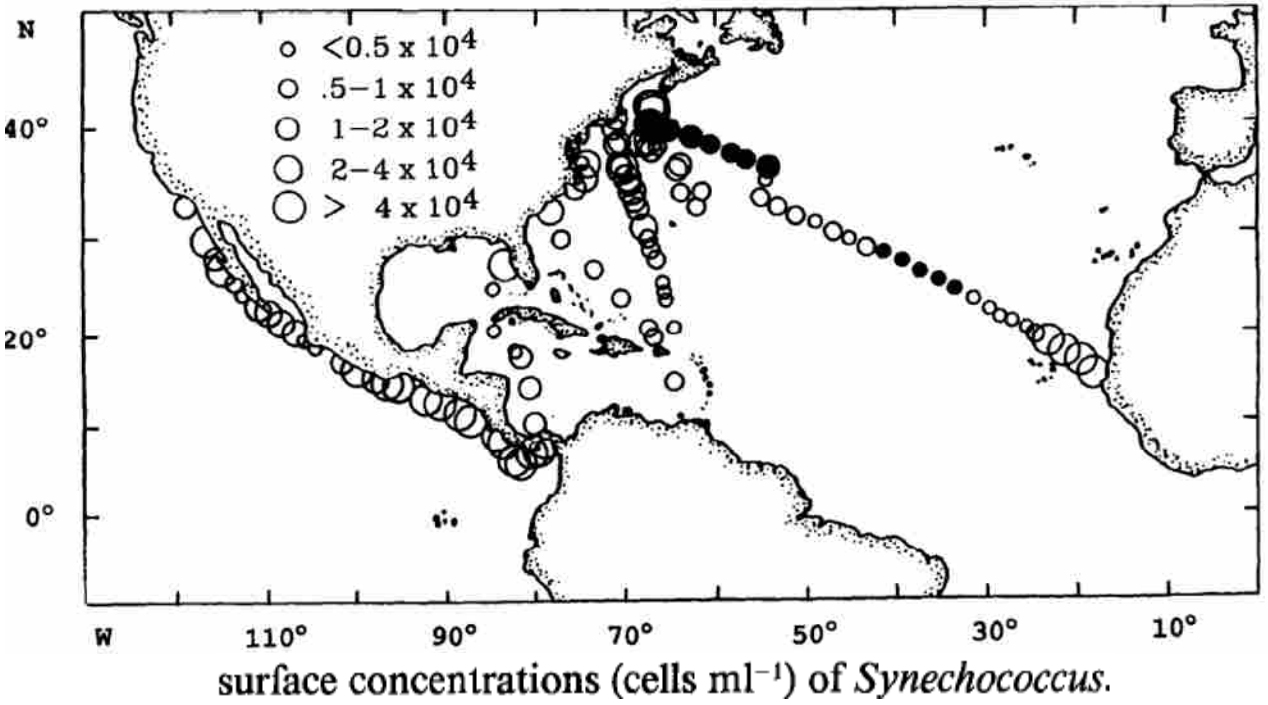
E. Virginia Armbrust
Massachusetts Institute of Technology



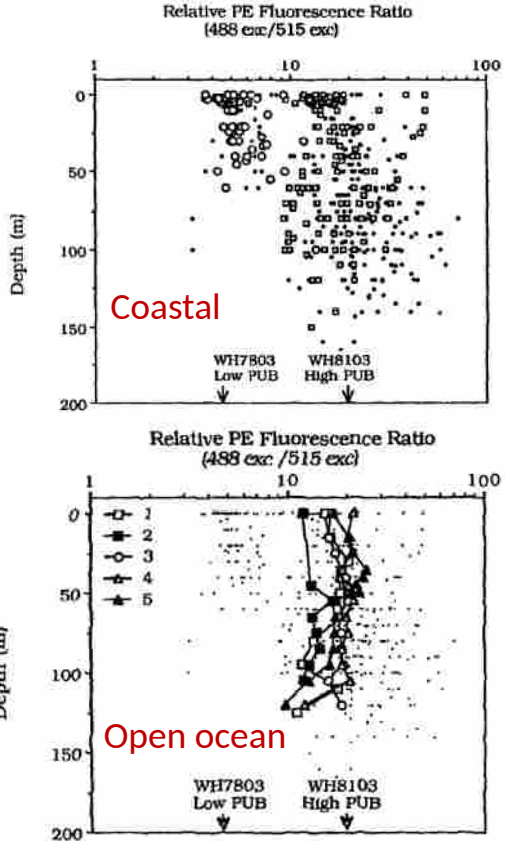
Syn. ubiquitous but much less abundant in oligotrophic waters than nearshore.

Co-occurring low and high-PUB *Syn*. populations at many near-coastal stations.

Only high PUB cells offshore at all depths.

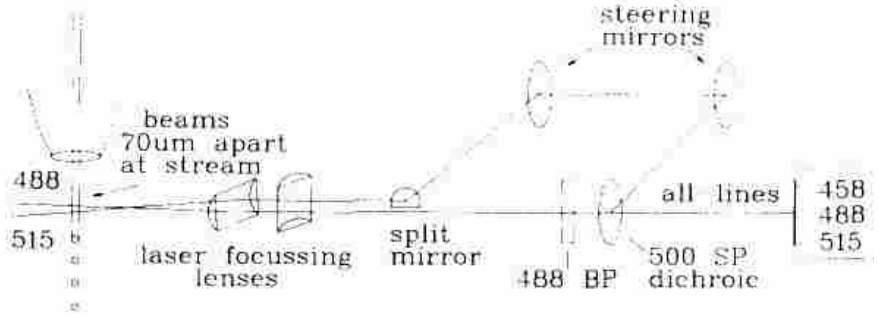


surface concentrations (cells ml^{-1}) of *Synechococcus*.



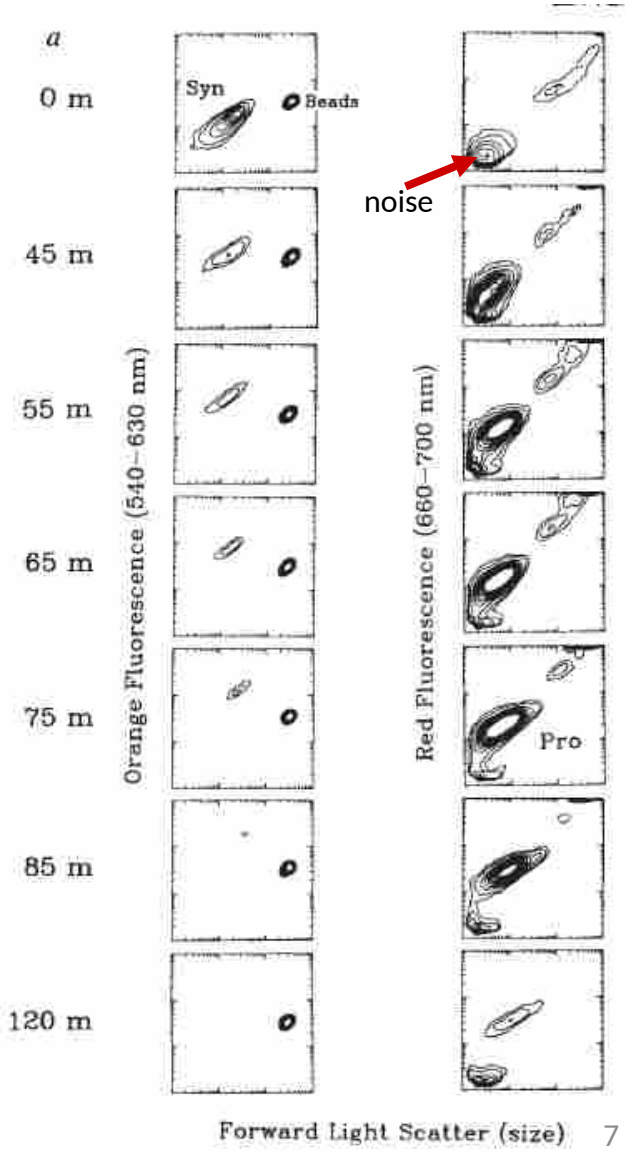
Flow cytometry at sea

"Little greens" emerging from background noise (first observed in 1985)









… and again TEM and HPLC

LETTERS TO NATURE NATURE VOL. 334 28 JULY 1988

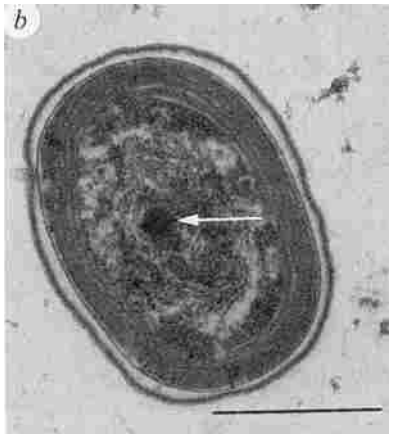
A novel free-living prochlorophyte abundant in the oceanic euphotic zone

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

The recent discovery of photosynthetic picoplankton has changed our understanding of marine food webs[1]. Both prokaryotic[2,3] and eukaryotic[4,5] species occur in most of the world's oceans and account for a significant proportion of global productivity[6]. Using shipboard flow cytometry, we have identified a new group of picoplankters which are extremely abundant, and barely visible using traditional microscopic techniques. These cells are smaller than the coccoid cyanobacteria and reach concentrations greater than 10^5 cells ml^{-1} in the deep euphotic zone. They fluoresce red and contain a divinyl chlorophyll *a*-like pigment, as well as chlorophyll *b*, α-carotene, and zeaxanthin. This unusual combination of pigments, and a distinctive prokaryotic ultrastructure, suggests that these picoplankters are free-living relatives of *Prochloron*[7]. They differ from previously reported prochlorophytes—the putative ancestors of the chloroplasts of higher plants—in that they contain α-carotene rather than β-carotene and contain a divinyl chlorophyll *a*-like pigment as the dominant chlorophyll.





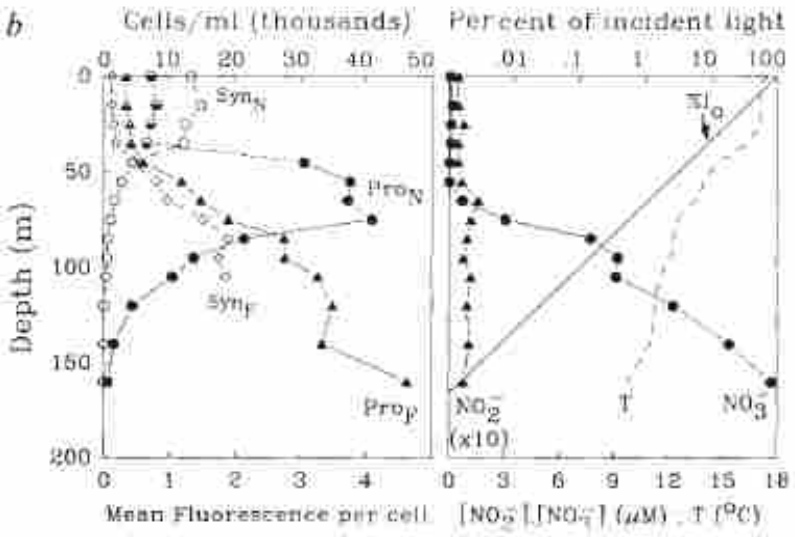


Table 1 Concentrations of pigments in the prochlorophytes and their absorption maxima (A_{max}) compared to those reported in the literature. The samples were from 100-m depth in the Gulf Stream (38°09.5′ N, 66°50.8′ W; 19 September 1987)

Pigment	Concentration* (fg cell⁻¹)	A_{max}† this study (nm)	A_{max} reported (nm)	Reference
Chlorophyll *a*	trace‡	—	428, 660	22, 23
Pheophytin *a*	—	408, 667‡	408, 667	22, 23
Divinyl chlorophyll *a*	2.15‡	—	436, 661	22, 23
Divinyl pheophytin *a*	—	417, 667‡	417, 667	22, 23
Chlorophyll *b*	2.31	457, 646	455, 645	17
Zeaxanthin	0.69§	(429), 450, 478	(425), 450, 478	17
α-Carotene	0.29	(423), 444, 475	423, 444, 473	28

Besides div-Chl a, the tiny, red-fluorescing cells contain "Chl *b*" and lack phycoerythrin, like *Prochloron* and *Prochlorothrix*.

=> unnamed "prochlorophyte"

Chisholm et al. Nature 1988

… and again TEM and HPLC

LETTERS TO NATURE NATURE VOL. 334 28 JULY 1988

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

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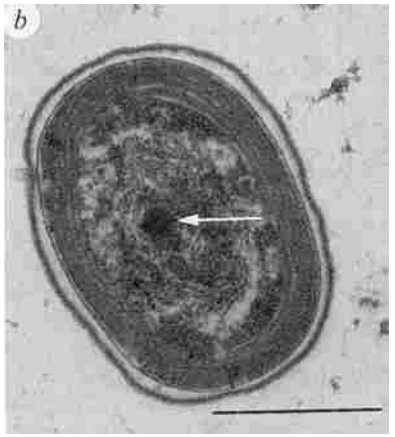
48-425 Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
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=> unnamed "prochlorophyte"

Chisholm et al. Nature 1988





This discovery and many following achievements earned Penny Chisholm numerous awards:

1992 American Academy of Arts and Sciences fellowship

2003 Member of the United States National Academy of Sciences

2010 Alexander Agassiz Medal

2012 Ruth Patrick Award from the Association for the Sciences of Limnology and Oceanography

2013 National Medal of Science (awarded by President Barack Obama)

2013 Ramon Margalef Prize in Ecology

2019 Crafoord Prize in Biosciences (equivalent to Nobel Prize)

Penny Chisholm is certainly one of the most awarded marine biologists of all times!

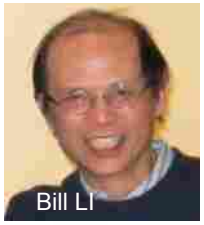
Flow cytometry (and microscopy) at sea : Not far behind!

Deep-Sea Research, Vol. 35, No. 9, pp. 1615–1638, 1988.

Vertical distribution of North Atlantic ultraphytoplankton: analysis by flow cytometry and epifluorescence microscopy

WILLIAM K. W. LI* and A. MICHELLE WOOD†

Abstract—The vertical distribution of numerically abundant ultraphytoplankton at four stations in the central North Atlantic was established using a bench-top mercury-arc lamp flow cytometer and epifluorescence microscopy. Cyanobacteria were identified on the basis of phycoerythrin fluorescence. Red-fluorescing cells (presumably eukaryotic algae) were categorized on the basis of Coulter volume and/or size and number of chloroplasts. The data revealed a diverse community of red fluorescent cell types, including a population of very abundant (but unidentified) cells that frequently were more numerous and slightly smaller than the cyanobacteria. While eukaryotic picoplankton have been reported previously, the overwhelming numerical dominance of these cells in some cases (>90% of total) has not been emphasized.

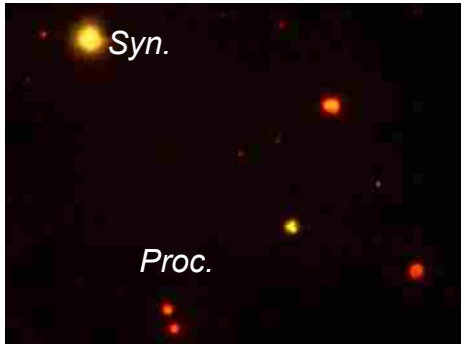


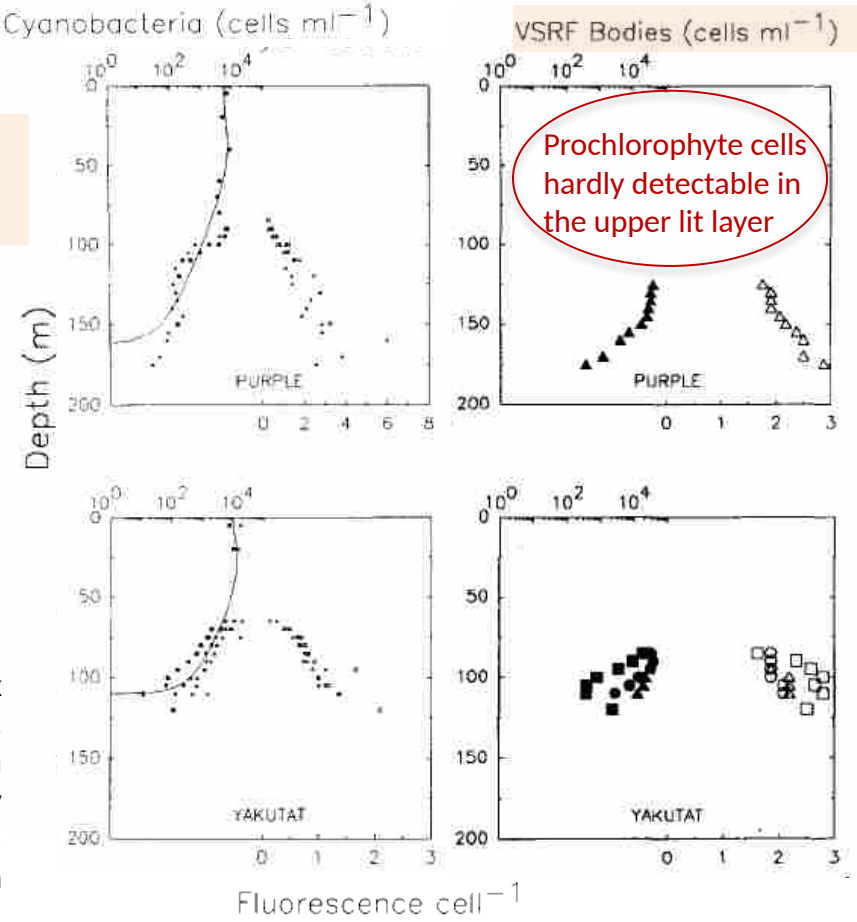


FACS Analyser Becton Dickinson



CSS *Hudson* cruise 87-022 in the central North Atlantic Ocean in **June-July 1987**





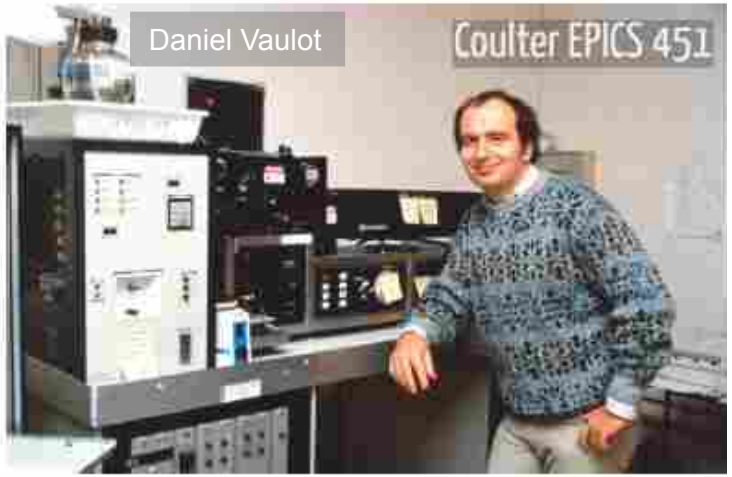
"A group of non-phycoerythrin-containing cells (or particles) with very weak red fluorescence was often numerically dominant over other picoplankters. These very small red fluorescing bodies (VSRF bodies) filtered through 1 µm but not through 0.4 µm. They were visible under epifluorescence microscopy as small, faint red spots but only when slides were freshly prepared and viewed within a few minutes after sample filtration after the viewer had been completely acclimated to darkness.

These VSRF bodies likely correspond to the novel free-living prochlorophyte described by Chisholm et al. (1988)."

Li & Wood 1988

Flow cytometry at sea : Not far behind!

Cruise CHLOMAX - 1987





C. R. Acad. Sci. Paris, t. 308, Série III, p. 9-14, 1989

Océanographie biologique/*Biological Oceanography*

Green photosynthetic bacteria associated with the deep chlorophyll maximum of the Sargasso Sea

Jacques NEVEUX, Daniel VAULOT, Claude COURTIES and Eri FUKAI

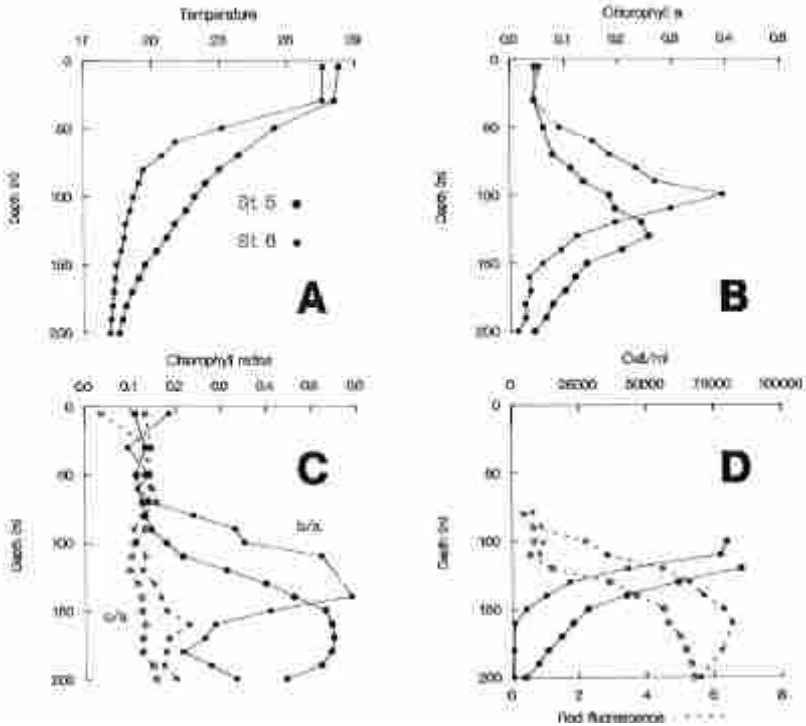
Abstract — Very small and very abundant red-fluorescing cells were detected with ship-board flow cytometry in the deep chlorophyll maximum of the Sargasso Sea. Their concentration decreased from a maximum of 10^5 cell. ml^{-1} at 100 m to fewer than 200 cell. ml^{-1} at 250 m. They had a size of less than 0.8 µm and contained photosynthetic pigments similar to eukaryotic chlorophylls *a* and *b*, with the exception of a slight shift in the fluorescence spectra. Although, key informations on these cells are still lacking, it is very likely that they are prokaryotes and play a fundamental role in the formation and maintenance of the deep chlorophyll maximum of oligotrophic oceans.



Gyrodinium aureolum







Neveux et al. 1989

Flow cytometry at sea : Not far behind!





LIMNOLOGY AND OCEANOGRAPHY
ASLO
Association for the Sciences of Limnology and Oceanography

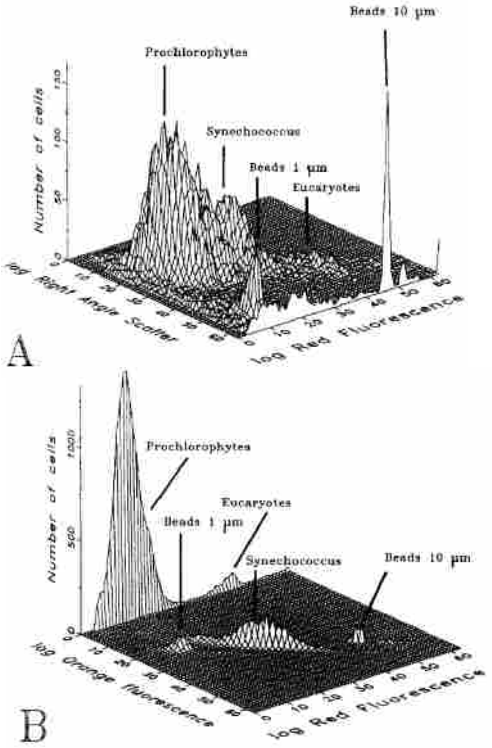
Winter presence of prochlorophytes in surface waters of the northwestern Mediterranean Sea

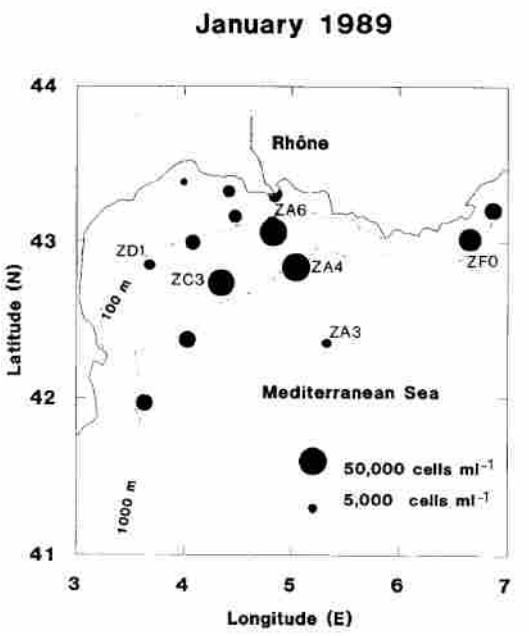
Daniel Vaulot, FrÉdÉric Partensky, Jacques Neveux, R. Fauzi C. Mantoura, Carole A. Llewellyn

First published: July 1990 | https://doi.org/10.4319/lo.1990.35.5.1156 | Citations: 119

Cells with characteristics similar to prochlorophytes were detected by flow cytometry during winter in the surface waters of the northwestern Mediterranean Sea and the low-salinity dilution zone of the Rhône River. **Maximum abundances reached 50,000 cells ml^{-1} at the surface** and were only slightly lower than those observed previously at the bottom of the euphotic zone in the Atlantic and Pacific Oceans.

=> Cells detected in mixed waters with temperature was as low as 13°C!)





Vaulot et al. 1990

Flow cytometry and HPLC combined at sea

MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser.
Vol. 68: 121–127, 1990

Vertical distribution and pigment composition of a picoplanktonic prochlorophyte in the subtropical North Atlantic: a combined study of HPLC-analysis of pigments and flow cytometry

Marcel J. W. Veldhuis, Gijsbert W. Kraay

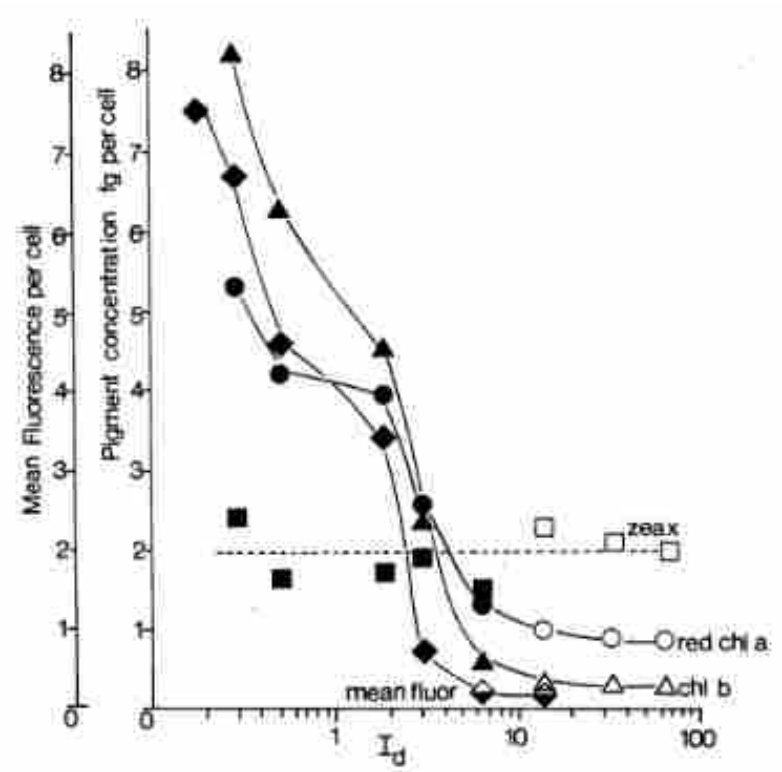


[Div-chl a] per cell increased from 0.91 fg cell^{-1} in surface to 5.4 fg cell^{-1} at the base of the euphotic zone

[Chl b] from 0.44 fg cell-1 to 8.2 fg cell^{-1}

[Zeaxanthin] almost constant with depth (1.97 ± 0.15 fg cell^{-1}).

A linear relationship was found between the sum of 'red-shifted' chlorophyll *a* and chlorophyll *b* and the mean red fluorescence signal of the *Proc*. cells.



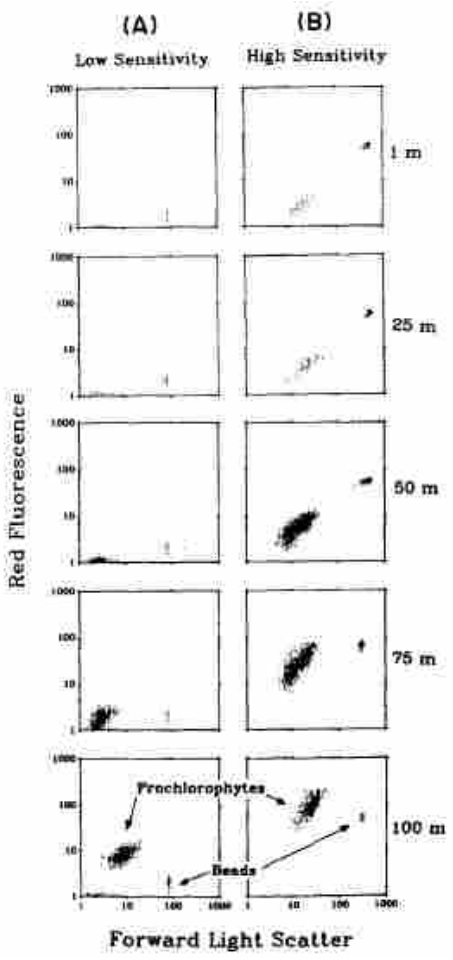
Flow cytometry at sea: improving the signal for detecting *Prochlorococcus*?

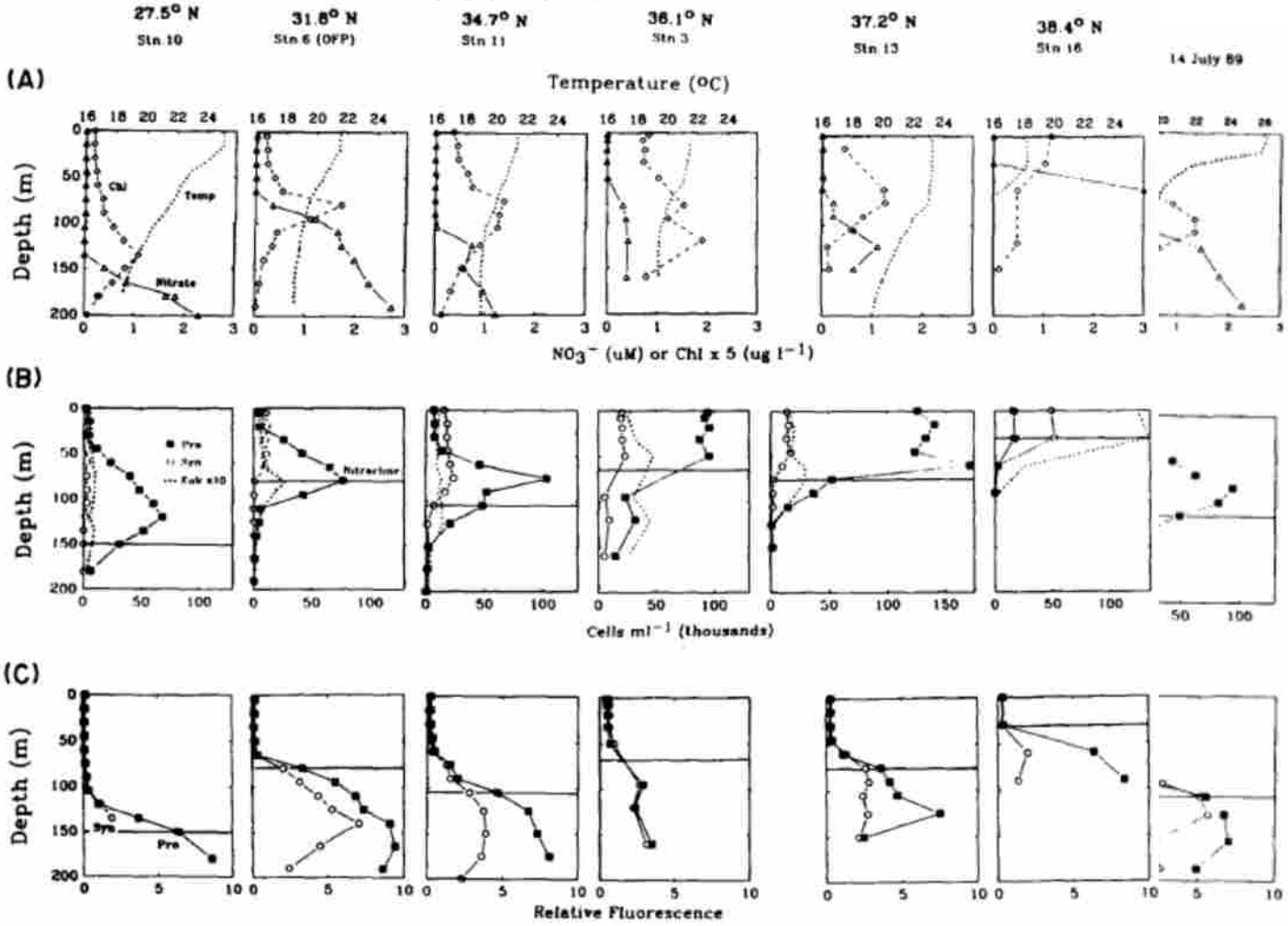
Deep-Sea Research, Vol. 37, No. 6, pp. 1033–1051, 1990.

Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean

ROBERT J. OLSON,* SALLIE W. CHISHOLM,† ERIK R. ZETTLER,*
MARK A. ALTABET* and JEFFREY A. DUSENBERRY*†







Culturing, TEM and HPLC: When the “little greens” finally got a name!

Arch Microbiol (1992) 157: 297–300

Archives of Microbiology

© Springer-Verlag 1992

Short communications

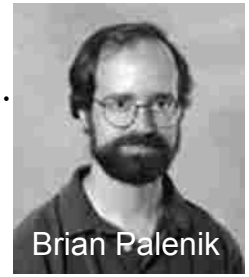
Prochlorococcus marinus* nov. gen. nov. sp.: an oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b

Sallie W. Chisholm[1], Sheila L. Frankel[1], Ralf Goericke[2], Robert J. Olson[2], Brian Palenik[1,2,3], John B. Waterbury[2], Lisa West-Johnsrud[1], and Erik R. Zettler[2]



Formal description of *P. marinus*

Prochlorococcus nov. gen. :
Prochlorococcus [Pro'chlorococ"cus.
Gr. prefix *pro*, before (primitive);
Gr. adj; *chloros*, green;
Latine: *coccus*, berry.
= Primitive green sphere].



“Two strains of *P. marinus* are in culture; both strains are clonal but neither is bacteria-free. The strains were isolated from the Sargasso Sea (CCMP1375 *= SS120, a clone of LG for “little green”*) in May 1988 (by B. Palenik) and the Mediterranean Sea (CCMP1375 *= MED4*) in Jan. 1989 (by D. Vaulot and F. Partensky).”

One additional isolate [*NATL1*] has been isolated from the N. Atlantic [*by FP*] using the same procedures.”
Protocol: Gravity filtration through 0.6 µm filter, serial dilutions…

LIMNOLOGY AND OCEANOGRAPHY

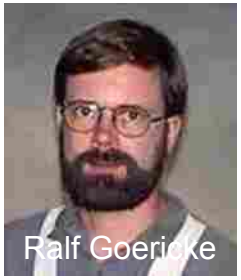
ASLO Association for the Sciences of Limnology and Oceanography

The pigments of Prochlorococcus marinus: The presence of divinylchlorophyll a and b in a marine procaryote

Goericke Ralf, Daniel J. Repeta

March 1992 | https://doi.org/10.4319/lo.1992.37.2.0425

Pigments of *P. marinus* :
divinyl-chlorophyll *a* and *b*,
zeaxanthin, *α*-carotene,
‘unknown carotenoid’, Mg
3,8 divinyl-pheoporphyrin.



Per Stickforth Arch Microbiol (2003) 179 : 409–415

Deep Sea Research Part I: Oceanographic Research Papers

Volume 40, Issues 11–12, November–December 1993, Pages 2283-2294

The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea ☆

Ralf Goericke * †, Nicholas A. Welschmeyer * ‡

=> Divinyl-Chls *a* and *b* are truly specific of *Prochlorococcus*, so one can determine precisely the contribution of *Prochlorococcus* to total Chl *a* and *b* in the field.

1990: Start of the metabarcoding revolution : Identifying non-cultured bacteria using PCR-amplified 16S rRNA from field samples

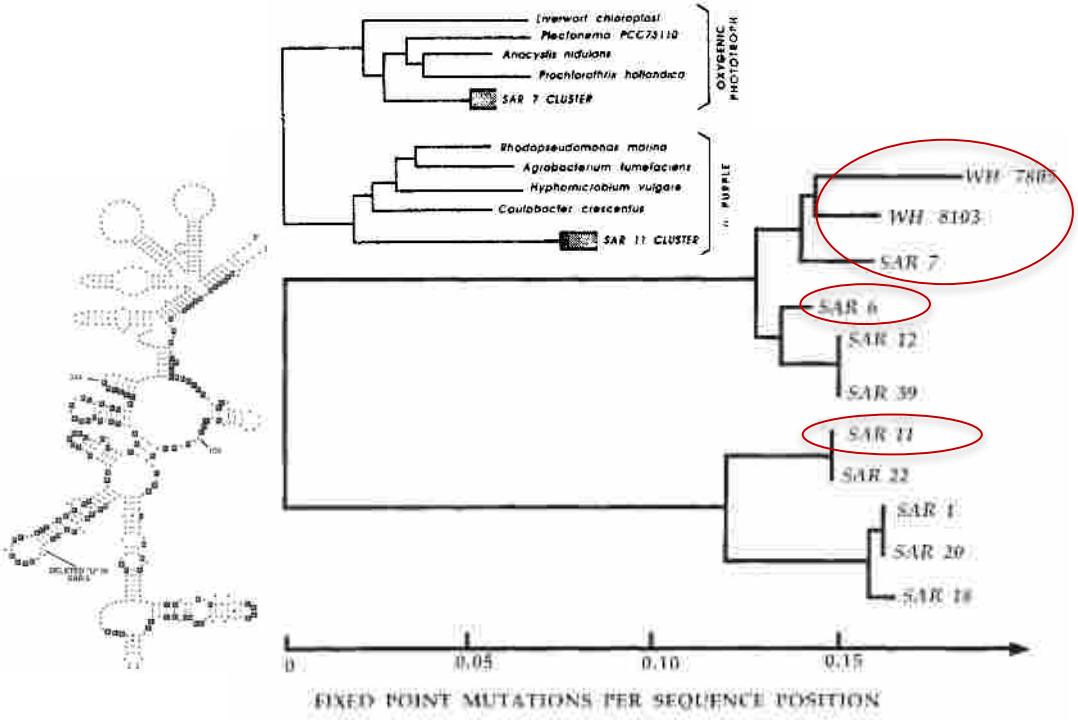
NATURE · VOL 345 · 3 MAY 1990

Genetic diversity in Sargasso Sea bacterioplankton

Stephen J. Giovannoni, Theresa B. Britschgi, Craig L. Moyer & Katharine G. Field

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331, USA

species[1–3]. We have phylogenetically analysed clone libraries of eubacterial 16S ribosomal RNA genes amplified from natural populations of Sargasso Sea picoplankton by the polymerase chain reaction[4]. The analysis indicates the presence of a novel microbial group, the SAR11 cluster, which appears to be a significant component of this oligotrophic bacterioplankton community. A second cluster of lineages related to the oxygenic phototrophs—cyanobacteria, prochlorophytes and chloroplasts—was also observed.



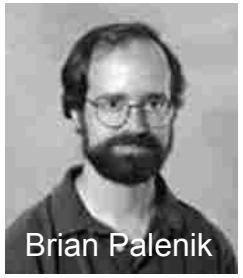
Paper famous for the discovery of the alpha-bacterial group « SAR 11 » which later proved to be the most abundant bacterial group in the Ocean. Now called « Order Pelagibacterales ».
Type species = *Candidatus* Pelagibacter ubique (communis?) HTCC1062

Also reports the first 16S rRNA sequences from natural populations of *Syn.* (SAR7) … and *Proc.* (SAR6) unidentified by then because there was no reference sequences.

Yet molecular ecology studies specifically targeting picocyanobacteria only started in 1998…

Giovannoni et al. 1990, Nature

Molecular biology: Prochlorophytes are *not* a valid taxonomic group


Brian Palenik

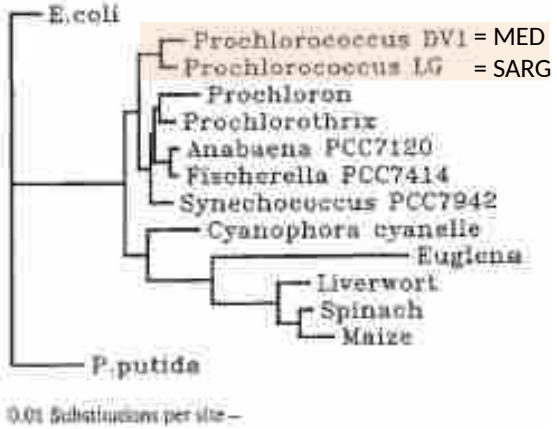
NATURE · VOL 355 · 16 JANUARY 1992

Multiple evolutionary origins of prochlorophytes, the chlorophyll *b*-containing prokaryotes

B. Palenik & R. Haselkorn

Molecular Genetics and Cell Biology, University of Chicago, 920 E. 58th Street, Chicago, Illinois 60637, USA

PROCHLOROPHYTES are prokaryotes that carry out oxygenic photosynthesis using chlorophylls *a* and *b*, but lack phycobiliproteins as light-harvesting pigments[1]. These characteristics distinguish them from cyanobacteria, which contain phycobiliproteins, but no chlorophyll *b*. Three prochlorophyte genera have been described: *Prochloron*[1–3], *Prochlorothrix*[4] and *Prochlorococcus*[5,6]. The prochlorophytes share their pigment characteristics with green plant and euglenoid chloroplasts, which has led to a debate on whether these chloroplasts may have arisen from an endosymbiotic prochlorophyte rather than a cyanobacterium[2,7]. Molecular sequence data, including those presented here based on a fragment of the rpoC1 gene encoding a subunit of DNA-dependent RNA polymerase, indicate that the known prochlorophyte lineages do not include the direct ancestor of chloroplasts[8–11]. We also show that the prochlorophytes are a highly diverged polyphyletic group. Thus the use of chlorophyll *b* as a light-harvesting pigment has developed independently several times in evolution. Similar conclusions have been reached in parallel studies using 16S ribosomal RNA sequences[12].



rpoC1

LETTERS TO NATURE

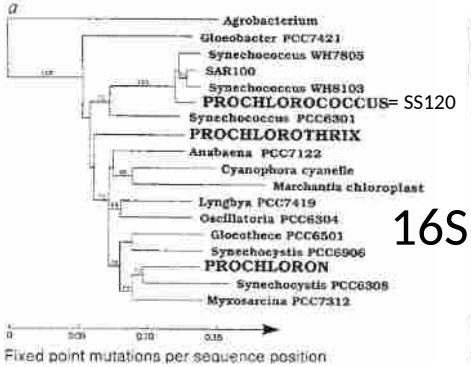
Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation


Ena

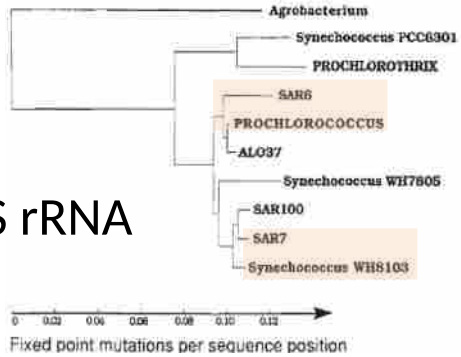
Ena Urbach, Deborah L. Robertson* & Sallie W. Chisholm

Ralph M. Parsons Laboratory, 48-425 Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
* Department of Molecular Genetics and Cellular Biology, University of Chicago, Chicago, Illinois 60637, USA

THE taxonomic group Prochlorales (Lewin 1977) Burger-Wiersma, Stal and Mur 1989 was established to accommodate a set of prokaryotic oxygenic phototrophs which, like plant, green algal and euglenoid chloroplasts, contain chlorophyll *b* instead of phycobiliproteins. Prochlorophytes were originally proposed (with concomitant scepticism[1–3]) to be a monophyletic group sharing a common ancestry with these 'green' chloroplasts[4–6]. Results from molecular sequence phylogenies, however, have suggested that *Prochlorothrix hollandica*[7] is not on a lineage that leads to plastids[8–12]. Our results from 16S ribosomal RNA sequence comparisons, which include new sequences from the marine picoplankter *Prochlorococcus marinus*[13,14] and the *Lissoclinum patella* symbiont *Prochloron* sp.[15], indicate that prochlorophytes are polyphyletic within the cyanobacterial radiation, and suggest that none of the known species is specifically related to chloroplasts. This implies that the three prochlorophytes and the green chloroplast ancestor acquired chlorophyll *b* and its associated structural proteins in convergent evolutionary events. We report further that the 16S rRNA gene sequence from *Prochlorococcus* is very similar to those of open ocean *Synechococcus* strains (marine cluster A (ref. 16)), and to a family of 16S rRNA genes shotgun-cloned from plankton in the north Atlantic and Pacific Oceans[17–19].



16S rRNA



Comparative physiology of *Prochlorococcus* strains

Plant Physiol. (1993) 101: 285–296

Photoacclimation of *Prochlorococcus* sp. (Prochlorophyta) Strains Isolated from the North Atlantic and the Mediterranean Sea

Frédéric Partensky*[1,2], Nicolas Hoepffner[3], William K. W. Li, Osvaldo Ulloa, and Daniel Vaulot

Biological Oceanography Division, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, B2Y 4A

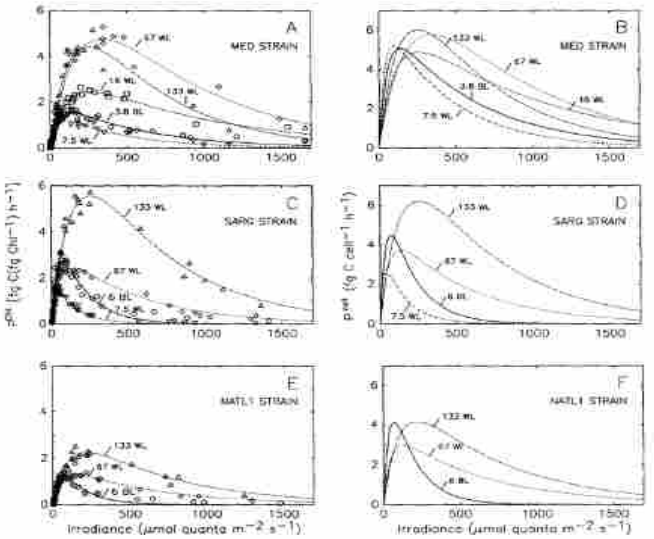


Figure 5. P versus I curves for *Prochlorococcus* strains grown under different WL or BL irradiances (μmol quanta m^{-2} s^{-1}). A and B, MED strain; C and D, SARG strain; E and F, NATL1 strain. A and C, Normalized per Chl; B and D, Normalized per cell. For readability, only fitted curves have been represented for graphs on a per cell basis (B and D).

Strain	Light Color	I_k	Photosynthetic Parameters									a^*_{Chl}	Φ_{CO_2}
			α^{Chl}	P^{Chl}_m	P^{Chl}_s	α^{cell}	P^{cell}_m	P^{cell}_s	$P_m:P_s$	I_m	I_h		
MED	Blue	3.8	0.036 ± 0.002	1.53	NA*	0.119	5.07	NA	NA	141	549	0.013	0.065
	White	7.5	0.041 ± 0.002	1.22	0.28	0.174	5.19	1.19	4.36	100	425	0.011	0.084
	White	16.0	0.031 ± 0.001	2.47	0.46	0.062	4.91	0.91	5.37	258	922	0.012	0.059
	White	67.0	0.041 ± 0.003	4.78	2.20	0.050	5.78	2.66	2.17	341	730	0.023	0.040
	White	133.0	0.054 ± 0.003	4.31	3.86	0.073	5.85	5.24	1.12	225	371	0.020	0.064
SARG	Blue	6.0	0.111 ± 0.007	2.68	NA	0.186	4.49	NA	NA	72	176	0.026	0.097
	White	7.5	0.118 ± 0.009	1.39	0.68	0.234	2.76	1.35	2.04	39	145	0.032	0.084
	White	67.0	0.060 ± 0.003	2.47	2.12	0.091	3.76	3.22	1.17	135	512	0.021	0.065
	White	133.0	0.065 ± 0.003	5.58	4.79	0.073	6.23	5.35	1.16	253	569	0.024	0.062
NATL1	Blue	6.0	0.049 ± 0.003	1.18	NA	0.172	4.13	NA	NA	71	154	0.013	0.087
	White	67.0	0.037 ± 0.002	1.36	1.20	0.082	3.02	2.66	1.13	127	603	0.015	0.056
	White	133.0	0.032 ± 0.002	2.27	2.08	0.059	4.17	3.82	1.09	219	636	0.014	0.052

3-6 fg C cell^{-1} h^{-1}

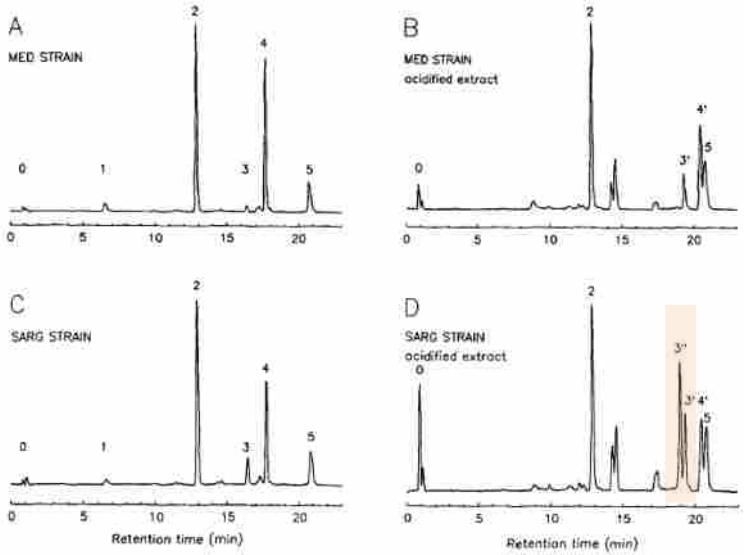


Figure 1. HPLC chromatograms for MED and SARG strains grown at 133 μmol quanta m^{-2} s^{-1}. A and C, Chromatograms for nonacidified samples. B and C, Chromatograms after acidification with 0.1 N HCl. 1, Chl *c*-like pigment; 2, zeaxanthin; 3, total Chl *b* (DV-Chl *b* plus Chl *b*-like pigment); 3', DV-phaeophytin *b*; 3'', phaeophytin *b*-like pigment; 4, DV-Chl *a*; 4', DV-phaeophytin *a*; 5, α-carotene. Other minor peaks have not been firmly identified (see "Results").

Strain	Light Color	I_k	Pigment Ratios				
			Chl *c*-like: DV-Chl *a*	Chl *c*-like: DV-Chl *a* + *b*	Zeaxanthin: DV-Chl *a*	α-Carotene: DV-Chl *a*	DV-Chl *b*: Total Chl *b**
							%
MED	Blue	1.3	0.035	0.031	0.421	0.239	100.0
	Blue	3.8	0.035	0.031	0.411	0.233	100.0
	White	7.5	0.039	0.034	0.348	0.210	100.0
	White	16.0	0.031	0.028	0.600	0.238	100.0
	White	67.0	0.035	0.032	0.842	0.246	100.0
	White	133.0	0.041	0.038	0.749	0.215	100.0
SARG	Blue	6.0	0.070	0.040	0.496	0.469	95.3
	White	1.0	0.102	0.039	0.447	0.584	99.6
	White	7.5	0.086	0.039	0.426	0.503	95.5
	White	67.0	0.044	0.027	0.813	0.444	72.0
	White	133.0	0.036	0.024	1.171	0.364	40.5
NATL1	Blue	6.0	0.100	0.053	0.351	0.391	86.9
	White	7.5	0.120	0.062	0.318	0.368	94.1
	White	67.0	0.072	0.045	0.607	0.193	33.5
	White	133.0	0.065	0.046	0.872	0.128	16.8



=> Possible *chromatic* acclimation process?

Optical properties of *Prochlorococcus and Synechococcus* strains

Journal of Marine Research, 51, 617–649, 1993

Prochlorococcus and _Synechococcus_: A comparative study of their optical properties in relation to their size and pigmentation

by André Morel[1], Yu-Hwan Ahn[1,2], Frédéric Partensky[3], Daniel Vaulot[3] and Hervé Claustre[1]

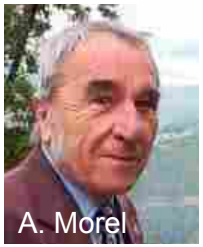


Table 4. Geometrical and optical cross sections (μm²), and optical efficiency *Q*-factors for the *Prochlorococcus* and *Synechococcus* strains (note that $\sigma_{a,b} = \sigma_g Q_{a,b}$). $\tilde{b}_b$ and b_b^* are the backscattering ratios (dimensionless) and the Chl-specific backscattering coefficients (m² mg⁻¹), respectively.

Strains	D_2 [μm]	σ_g [μm²]	Q_a, Q_b (blue max.)		σ_a (blue max.)	Q_b (500 nm)	σ_b (500 nm)	$Q_{bb}\cdot10^3$ (500 nm)	$\tilde{b}_b\cdot10^3$ (500 nm)	$b_b^*\cdot10^3$ (blue max.)
PROCHLOROCOCCUS										
MED (1)	0.58	0.264	0.370	0.173	0.098	0.166	0.041			
MED (2)	0.59	0.273	0.314	0.152	0.086	0.148	0.040	—	—	—
MED (3)	0.59	0.273	0.231	0.164	0.063	0.155	0.039	1.068	6.875	0.098
NATL1 (1)	0.70	0.385	0.186	0.137	0.072	0.165	0.074	—	—	—
NATL1 (2)	0.62	0.302	0.209	0.146	0.063	0.155	0.047	—	—	—
NATL1 (3)	0.73	0.419	0.327	0.211	0.137	0.218	0.091	—	—	—
								—		
SARG (1)	0.71	0.400	0.213	0.174	0.085	0.168	0.067	—	—	
SARG (2)	0.71	0.400	0.253	0.171	0.101	0.176	0.070	0.759	4.318	0.092
SYNECHOCOCCUS										
ROS04 (H)	1.05	0.866	0.118	0.448	0.102	0.376	0.326	0.414	1.101	0.311
ROS04 (L)	1.17	1.075	0.118	0.475	0.127	0.383	0.411	0.444	1.158	0.332
MAX01 (H)	0.88	0.608	0.106	0.362	0.064	0.310	0.188	0.446	1.441	0.275
MAX01 (L)	0.99	0.770	0.104	0.361	0.080	0.307	0.236	0.638	2.078	0.384
MAX41 (L)	0.92	0.665	0.122	0.320	0.081	0.278	0.185	—	—	—
DC2 (L)	1.14	1.015	0.116	0.517	0.118	0.421	0.427	0.414	0.984	0.198

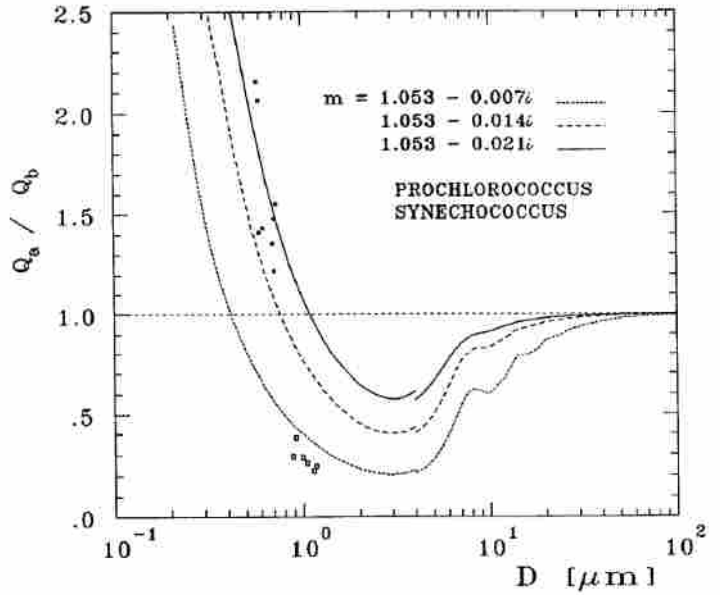


Figure 11. Ratio of efficiency factor for absorption to the efficiency factor for scattering, plotted as a function of the diameter *D*. The theoretical curves are computed (van de Hulst approximation for $D > 4$ μm and exact Mie solution for $D < 4$ μm) for the three values of the complex index of refraction as indicated.

The probability for photons to be absorbed by a *Proc.* cell exceeds that of being scattered while all phytoplankters, including *Synechococcus*, are always more efficient at scattering than capturing light.

Absorption cross sections (σ_a) of *Proc.* and *Syn.* nearly the same (ca. 0.1 μm²) in the blue.
=> advantage for *Proc.*, as it can achieve a higher photosynthetic rate per unit of biomass.

Morel et al. 1993

The absorption capacity of *Proc.* cells is only about 20% less that of the same amount of pigments in solution.
=> Almost no 'package effect' (pigment auto-shading) ≠ larger phytoplanktonic cells. This possibly contributes to Proc. successful colonization of the base of the upper lit layer.

Comparative physiology of *Prochlorococcus* and *Synechococcus* strains

Vol. 116: 259–275, 1995

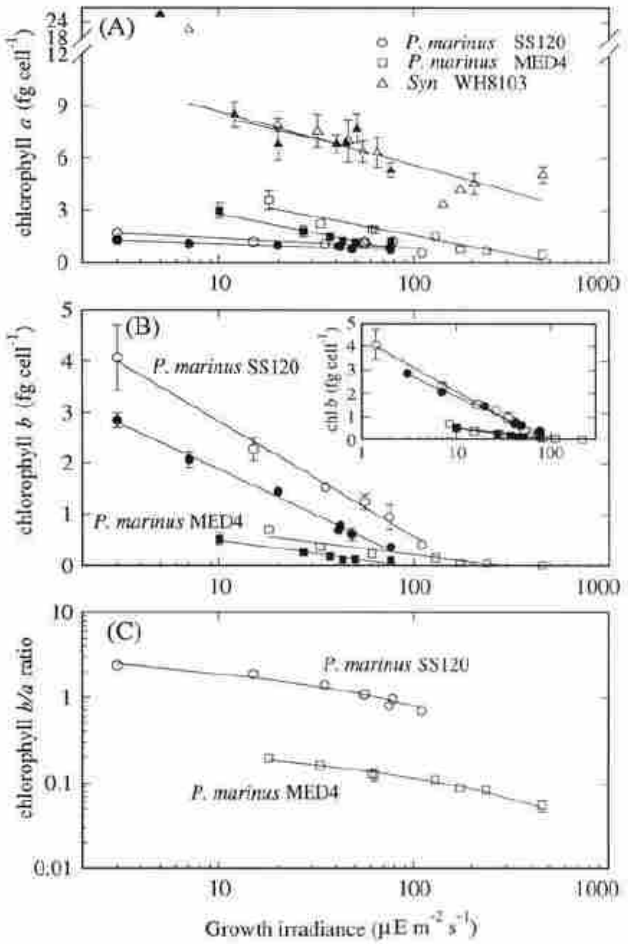
MARINE ECOLOGY PROGRESS SERIES
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Comparative physiology of *Synechococcus* and *Prochlorococcus*: influence of light and temperature on growth, pigments, fluorescence and absorptive properties

Lisa R. Moore[1], Ralf Goericke[2], Sallie W. Chisholm[1]

[1]Ralf M. Parsons Laboratory 48-425, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
[2]Marine Life Research Group, Scripps Institution of Oceanography, La Jolla, California 92093-0218, USA





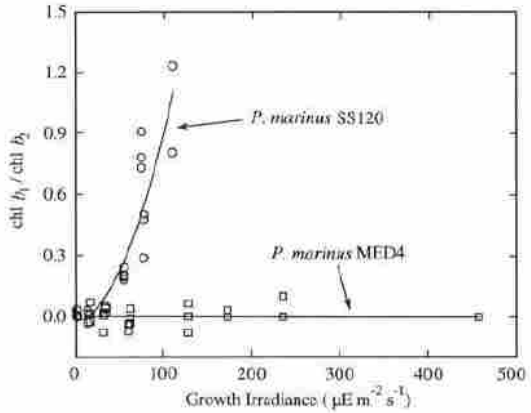


Fig. 6. Ratio of chl b_1 to chl b_2 in *Prochlorococcus marinus* SS120 (○) and *P. marinus* MED4 (□) grown under white light

Chromatic acclimation confirmed for SS120 using HPLC on non-acidified samples

MED4 is high-light adapted
SS120 is low-light adapted

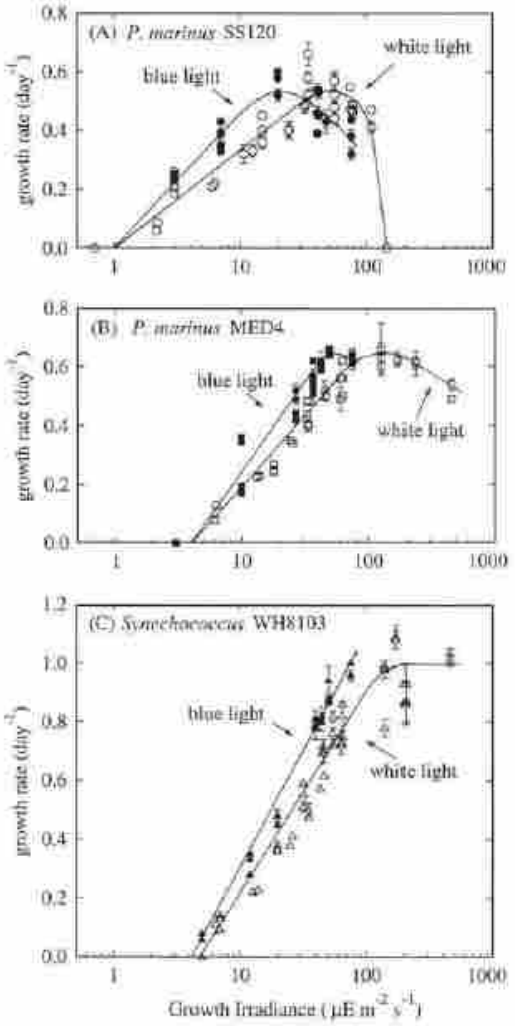


Fig. 4. Growth rate (± SD) as a function of both white and blue light irradiance. Cultures were maintained at 24.5°C under a 14 h light:10 h dark regime.

Moore et al. 1995

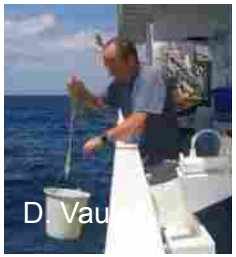
Flow cytometry at sea: discovery of distinct *Prochlorococcus* "phototypes"

Deep-Sea Research I, Vol. 40, No. 10, pp. 2043–2060, 1993

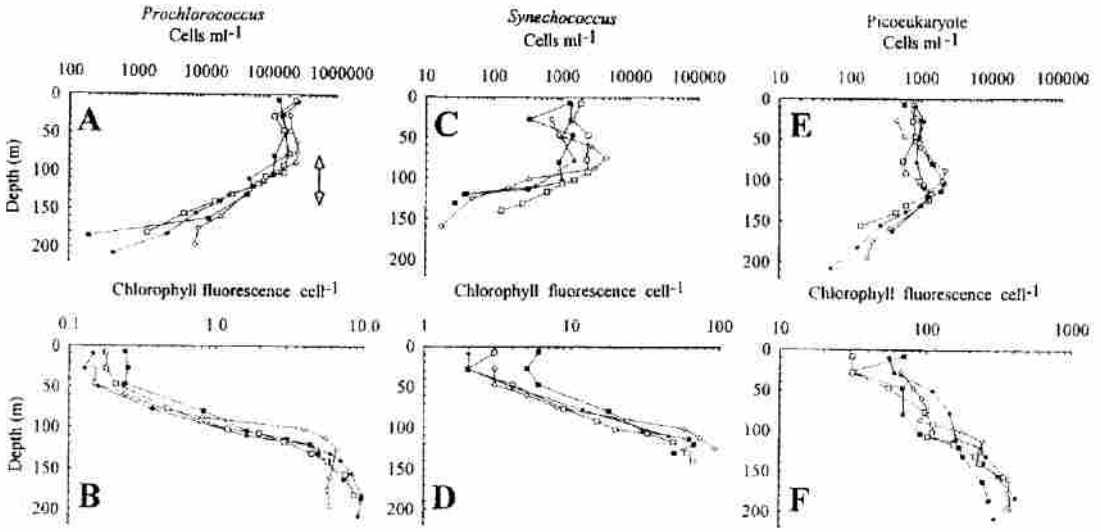
Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA)

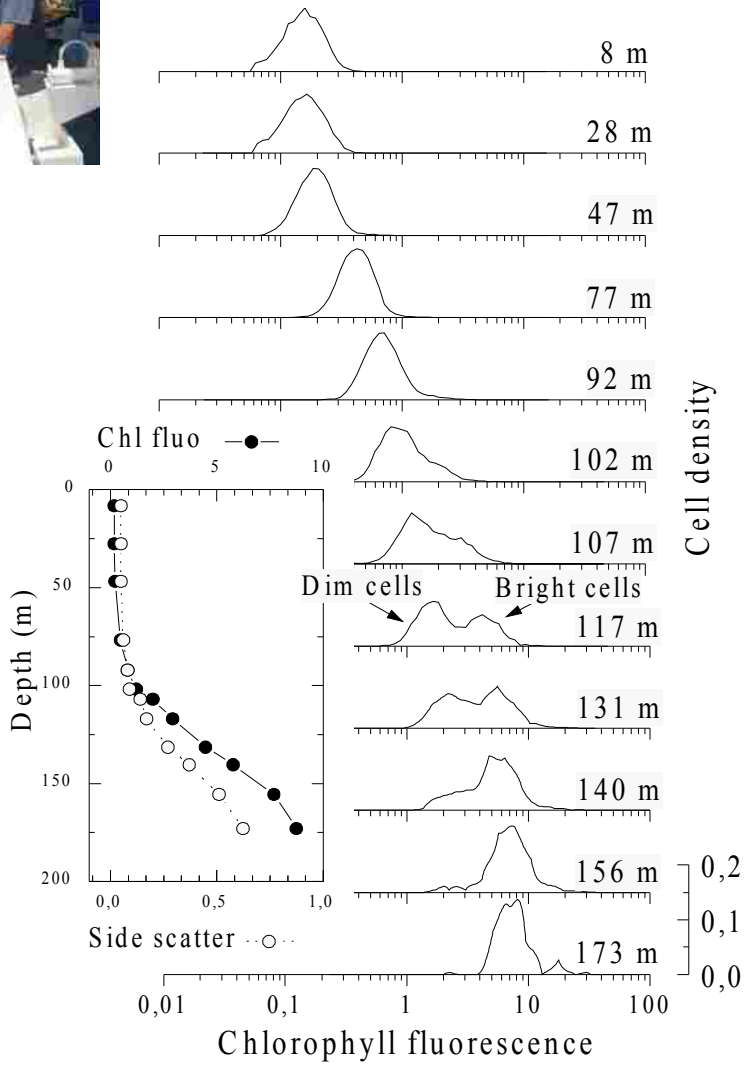
LISA CAMPBELL* and DANIEL VAULOT†





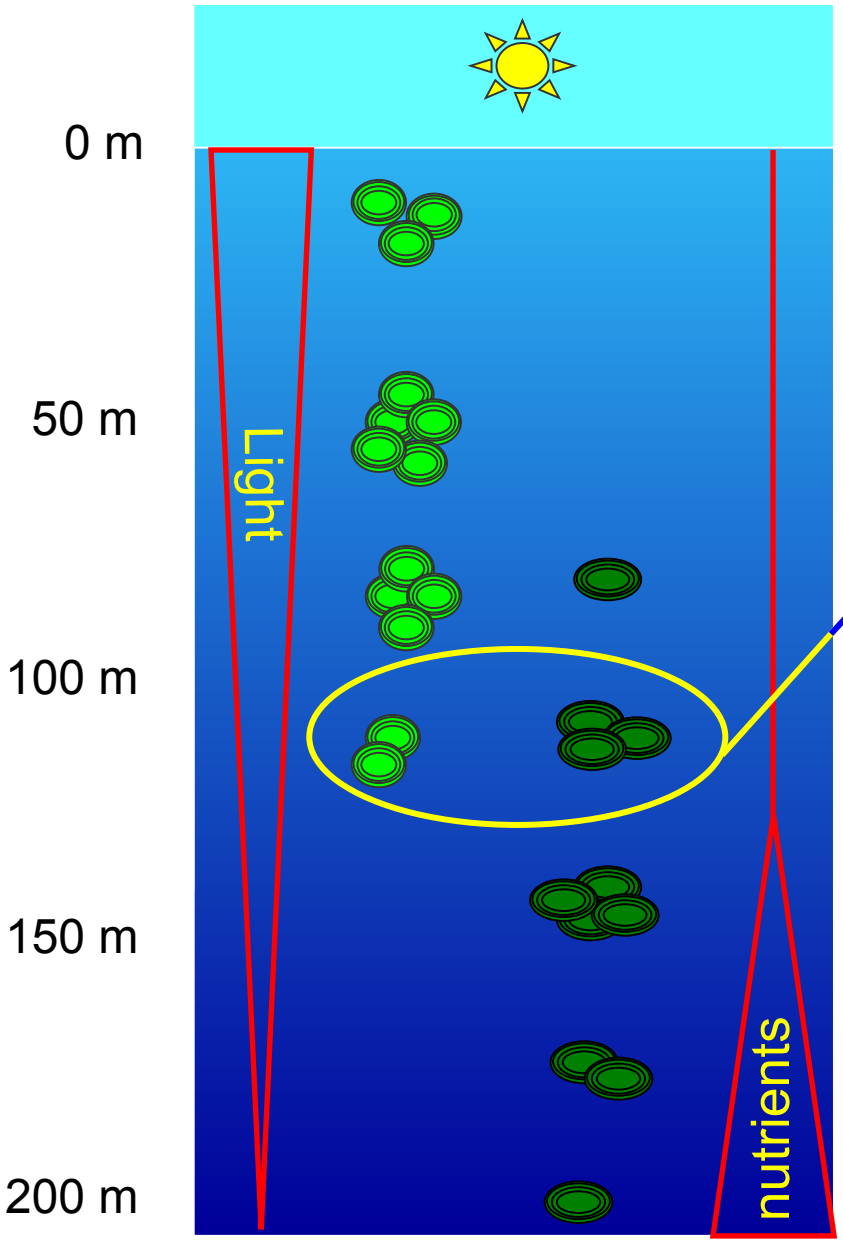
Abstract—The structure of the picoplankton community in the subtropical Pacific was examined on four depth profiles, one from each season, sampled at the Hawaii Ocean Time-series station ALOHA (22°45′N, 158°W). Three cell populations were discriminated by flow cytometry: *Prochlorococcus* prochlorophytes, *Synechococcus* cyanobacteria, and picoeukaryotes. *Prochlorococcus* were the most abundant component (maximum *ca* 2×10^5 cells ml^{-1}). Unlike previous reports, their concentration was almost constant down to roughly 100 m, with a slight maximum at the surface or near the chlorophyll maximum. Cellular chlorophyll fluorescence increased 50-fold between surface and deep populations. One distinguishing feature of the community off Hawaii was the co-occurrence near the chlorophyll maximum of at least two distinct *Prochlorococcus* populations with different chlorophyll and DNA contents. Throughout the year, *Synechococcus* abundance was two orders of magnitude lower and there was no seasonal alternation between *Prochlorococcus* and *Synechococcus*, as observed in the northern Sargasso Sea. *Synechococcus* populations did not extend below 120 m and were dominated by high phycourobilin cell types.





=> Evidence of the occurrence of two *Prochlororococcus* phototypes with low or high red fluorescence

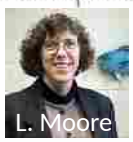
Flow cytometric cell sorting + culturing of *Prochlorococcus* phototypes



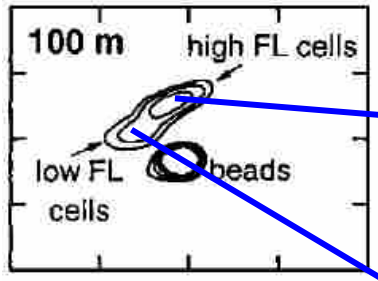
NATURE | VOL 393 | 4 JUNE 1998

Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes

Lisa R. Moore*†, Gabrielle Rocap*†‡§ & Sallie W. Chisholm*‡







Cell sorting

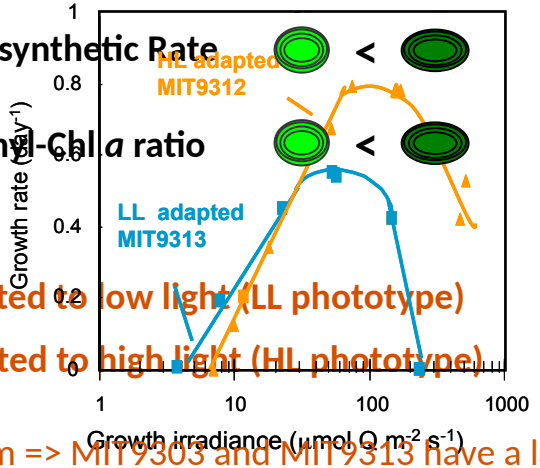
Culturing

MIT9303
MIT9313

MIT9302
MIT9312

Differences between strains & :

- Growth irradiance maximum >
- Maximum Photosynthetic Rate <
- Total Chl *b* / divinyl-Chl *a* ratio <



Strains adapted to low light (LL phototype)

Strains adapted to high light (HL phototype)

No filtration on 0.6 µm => MIT9303 and MIT9313 have a larger cell size than previous LL isolates (SS120 and NATL1-2)

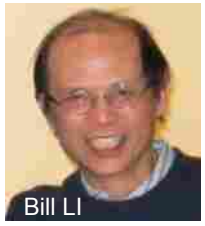
Moore et al. 1998, *Nature*

On-board incubations with $^{14}CO_2$ followed by flow cytometric cell sorting

Limnol. Oceanogr., 39(1), 1994, 169–175

Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting

William K.W. Li



Abstract—A partitioning of ultraphytoplankton primary production among prochlorophytes, cyanobacteria, and eucaryotic algae was made by shipboard flow cytometric sorting of ^{14}C-labeled cells. Aggregate primary production was derived from the sum, over all three ultraplankton groups, of the product of cell abundance and cell-specific rate of ^{14}C uptake which ranged from 0.03 to 4 fg C cell^{-1} h^{-1} for prochlorophytes and 0.2 to 10 fg C cell^{-1} h^{-1} for cyanobacteria.

Table 1. Primary production of three North Atlantic ultraphytoplankton samples.

Sta.	Location	z (m)	$\%I_0$*	Chl (μg liter^{-1})	Group†	n, (cells liter^{-1})	p, (fg C cell^{-1} h^{-1})	P,‡	% agg
14	32°39.5′N, 26°39.2′W	60	4	0.21	PRO	1.62×10^{8}	0.27	44.2	57
					CYA	2.35×10^{6}	0.82	1.9	2
					LEUC	1.02×10^{6}	24.09	24.5	32
					sum			70.6	91
					agg			77.4	
20	29°00.1′N, 19°16.3′W	60	2	0.15	PRO	1.57×10^{8}	0.03	4.6	16
					CYA	3.34×10^{6}	0.19	0.6	2
					LEUC	1.24×10^{6}	17.93	22.3	79
					sum			27.5	97
					agg			28.3	
30	31°13.9′N, 10°48.0′W	1	91	0.37	PRO	6.63×10^{7}	0.81	53.6	11
					CYA	1.27×10^{7}	7.68	97.8	20
					SEUC	8.49×10^{6}	7.32	62.1	13
					LEUC	1.25×10^{6}	193.16	241.1	49
					sum			454.5	92
					agg			493.4	

* Irradiance as percent of intensity at sea surface.
† PRO—prochlorophytes; CYA—cyanobacteria; SEUC—small eucaryotic ultraphytoplankton; LEUC—large eucaryotic ultraphytoplankton; sum—PRO+CYA+SEUC+LEUC; agg—aggregate from filtration of bulk sample ≤3 ml.
‡ $P = (n \cdot p)10^{-6}$, ng C liter^{-1} h^{-1}.

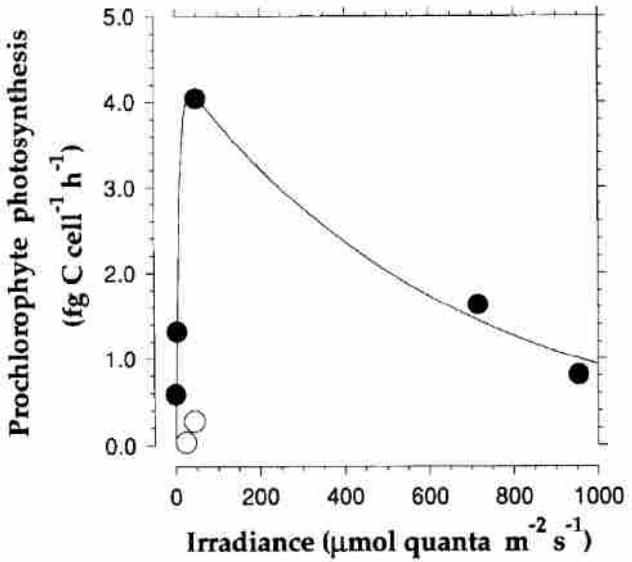


Fig. 4. Prochlorophyte cellular photosynthesis (fg C cell^{-1} h^{-1}) as a function of average irradiance experienced over 8 h in simulated in situ incubations. Moroccan upwelling station (●) and deep (60 m) open-ocean stations (○).

. The maximum cellular rate of photosynthesis recorded here (4 fg C cell^{-1} h^{-1}) is very similar to those reported by Partensky et al. (1993) for *Prochlorococcus* clones cultured in the laboratory (3–6 fg C cell^{-1} h^{-1}).

The dominant primary producer was not necessarily the numerically dominant (*Proc.*) nor necessarily the group with the highest cell-specific rate of ^{14}C uptake (eukaryotes).

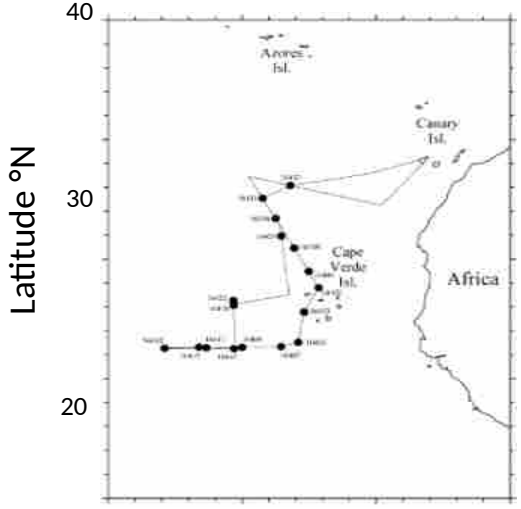
On-board incubations with $^{14}CO_2$ followed by flow cytometric cell sorting

The ISME Journal (2010) 4, 1180–1192

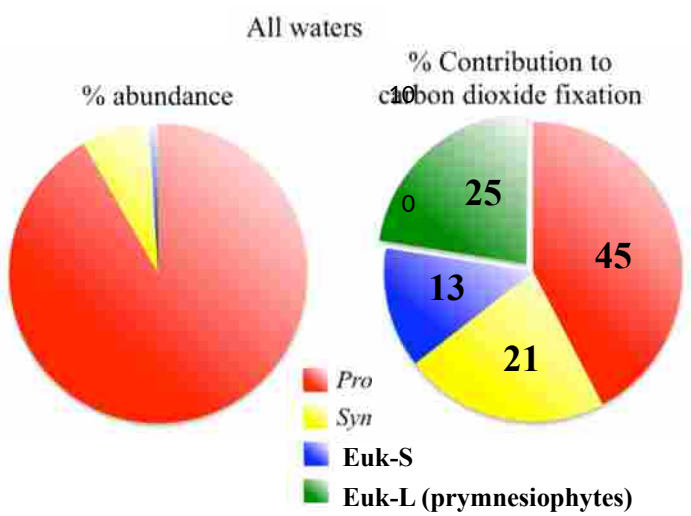
Significant CO_2 fixation by small prymnesiophytes in the subtropical and tropical northeast Atlantic Ocean

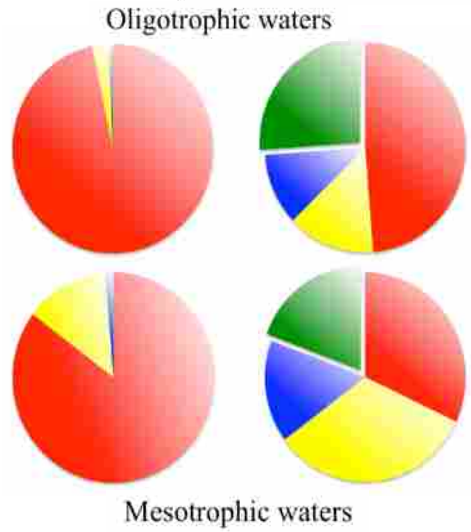
Ludwig Jardillier[1,3], Mikhail V Zubkov[2], John Pearman[1] and David J Scanlan[1]





Station		Contribution to CO_2 fixation (%)				
		PRO	SYN	EUK	PE2	PE3
	16396 A	52	7	40		
	16398 A	84	13	23		
	16405 B	66	7	25	7	18
	16407 A	45	15	37		
	16418 A	40	8	27	6	25
	16422 B	41	7	27	4	35
	16425 B	36	6	25		
	16429 B	26	20	47	12	25
	16431 B	34	17	28	16	21
	16432 B	53	16	26	15	15
	16400 A	28	35	32		
	16402 A	17	38	27		
	16403 A	19	29	29	15	18
	16409 A	35	34	28	12	17
	16411 B	39	29	31	15	12
	16413 B	34	27	37	18	28
	16415 B	21	31	39	13	14
	16417 A	34	29	28		
mean	**oligotrophic waters**	48	12	31	10	23
	mesotrophic waters	28	32	31	14	18
S.D.	**oligotrophic waters**	17	5	8	5	7
	mesotrophic waters	8	4	5	2	6





Jardillier et al., ISME J (2010)

Slide courtesy of DJ Scanlan

Flow cytometry + on-board incubations of seawater with a growth inhibitor

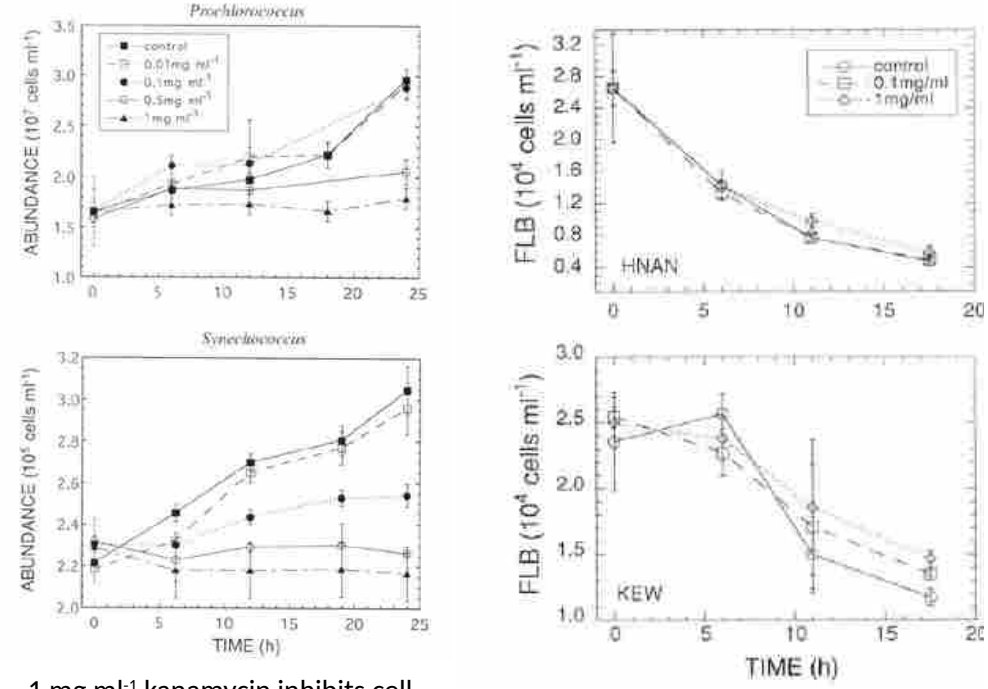
Vol. 1.3: 277–287, 1995

MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser.

Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique

Hongbin Liu, Lisa Campbell, Michael R. Landry

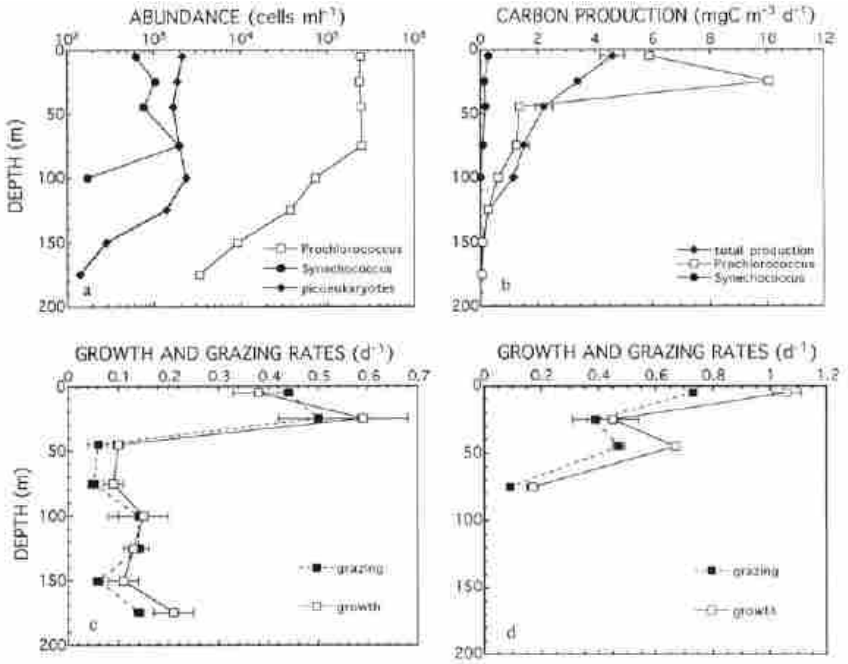
Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, Hawaii 96822, USA



1 mg ml^{-1} kanamycin inhibits cell division of *Proc.* and *Syn.*
A 24 h (dawn to dusk) incubation period is critical for obtaining an unbiased growth rate because of diel cycles of DNA synthesis and growth.

Limited effect of kanamycin on protozoan grazing

Station ALOHA



Growth rate *Proc.* = 0.4-0.5 day^{-1} (0.65 div./day) in surface
Growth rate *Syn.* = 1.0 day-1 (1.45 div./day) in surface
Mortality rates : 20-116% of growth rate for *Proc.*
43-87% for *Syn.*

⇒ Growth generally exceeded grazing by protozoans
(Yet predation by viruses wasn't assessed)

Estimated integrated production:
382 mg C.m^{-2} for *Proc.* vs. 14.6 mg C.m^{-2} for *Syn.*

Flow cytometry and DNA staining of *Prochlorococcus* cells

Science 9 Jun 1995 • Vol 268, Issue 5216 • pp. 1480-1482

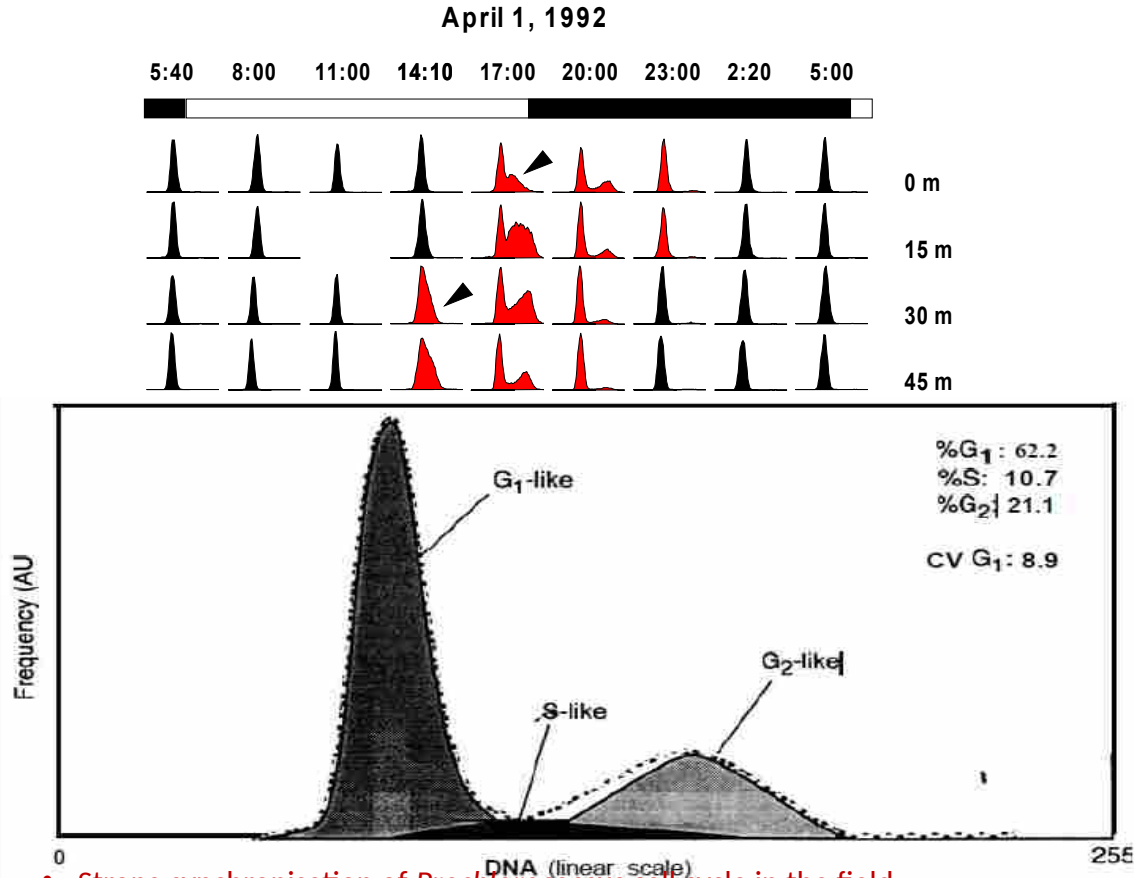
Growth of *Prochlorococcus*, a Photosynthetic Prokaryote, in the Equatorial Pacific Ocean

Daniel Vaulot,* Dominique Marie, Robert J. Olson, Sallie W. Chisholm



$$\mu_{cc} = \frac{1}{n \cdot t_{S+G_2}} \sum_{i=1}^{n} \ln[1 + f_{S+G_2}(i)]$$

n = number of samples taken during a 24-h period and $f_{S+G2}(i)$ the fraction of cells in S+G2 for sample i deconvoluted from individual DNA distributions.
T_{S+G2} estimated as 6 h from the surface down to 75 m and then increased up to 12 h at 125 m.



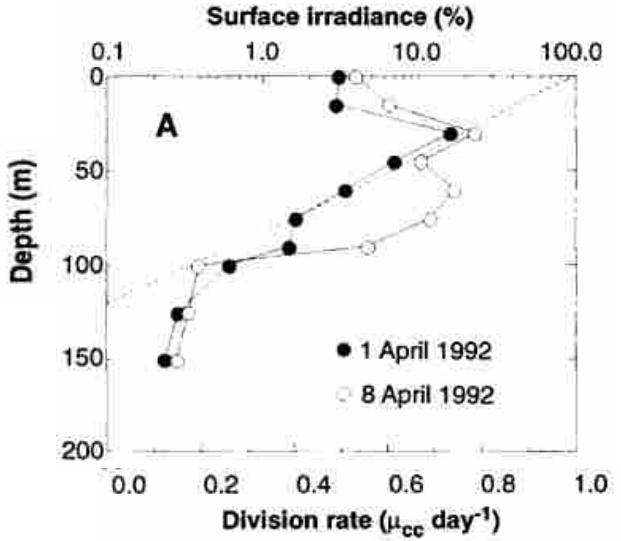


Fig. 4. *Prochlorococcus* division rate (μ_{cc} day^{-1}) estimated from cell cycle phase variation

Max. growth about 0.69 day^{-1} (= 1 div./day) at 30 m
Estimated *Proc.* production = 174-498 mg C.m^{-2}/day accounting for 5-19% total gross primary production at the equator.

- Strong synchronisation of *Prochlorococcus* cell cycle in the field
- Timing varies with depth

=> DNA replication delayed in surface waters is possibly due to UV radiations

Flow cytometry and DNA staining of *Prochlorococcus* cells

Kolowrat et al. BMC Microbiology 2010, 10:204
http://www.biomedcentral.com/1471-2180/10/204

BMC Microbiology

RESEARCH ARTICLE Open Access

Ultraviolet stress delays chromosome replication in light/dark synchronized cells of the marine cyanobacterium *Prochlorococcus marinus* PCC9511

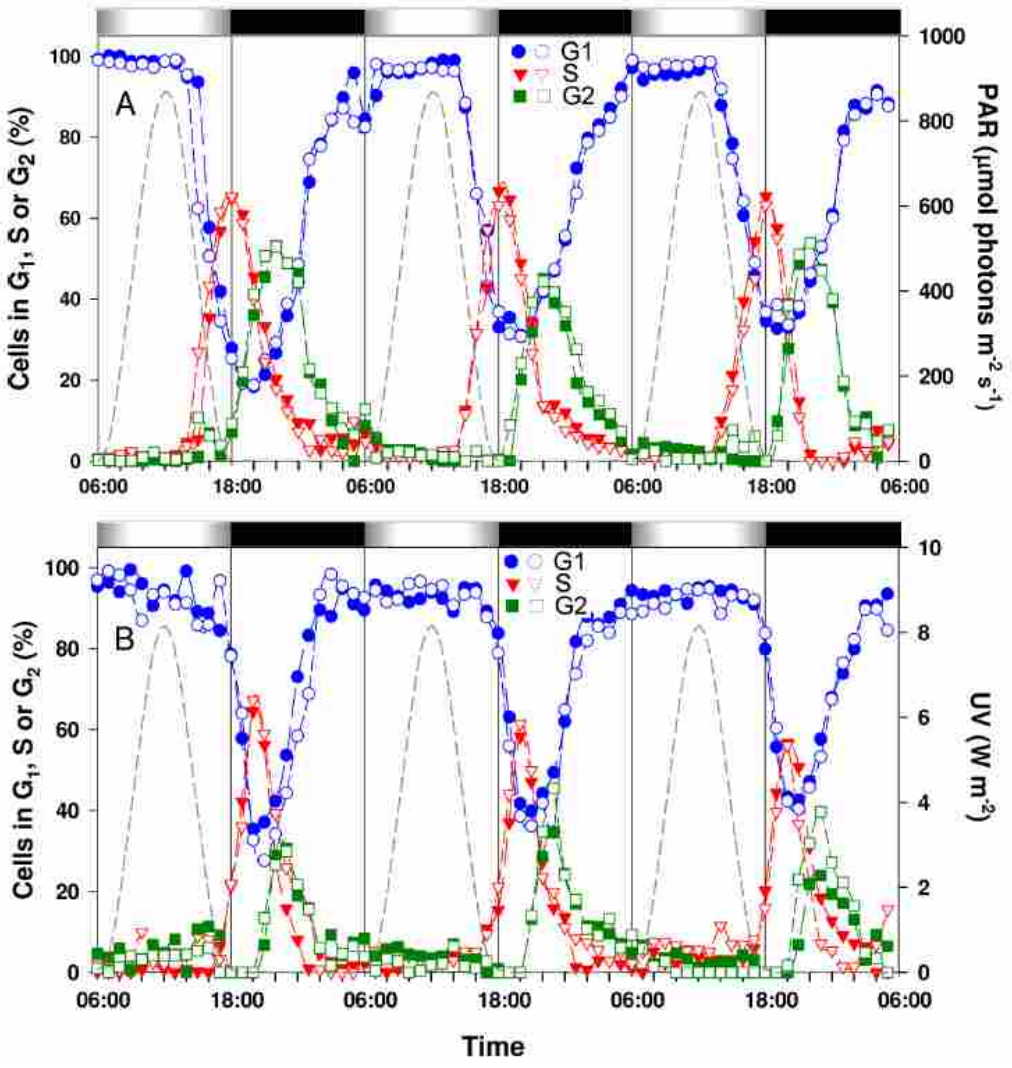
Christian Kolowrat[1,2], Frédéric Partensky[1,2*], Daniella Mella-Flores[1,2], Gildas Le Corguillé[1,3], Christophe Boutte[1,2], Nicolas Blot[1,2,4], Morgane Ratin[1,2], Martial Ferréol[5], Xavier Lecomte[1,2], Prisdillia Gourvil[1,2], Jean-François Lennon[1], David M Kehoe[6], Laurence Garczarek[1,2]

=> Lab experiments confirmed that natural doses of UV-A+B caused a delay in DNA replication.

=> Delay occurs the same day if UV switched on in the morning.

Growth parameters*	Batch Cultures	
	HL	HL+UV
μ_{cc} (d^{-1})	0.67 ± 0.05	0.68 ± 0.03
μ_{nb} (d^{-1})	0.60 ± 0.13	0.62 ± 0.11
T_{G1} (h)	16.8 ± 1.6	18.4 ± 0.8
T_S (h)	4.03 ± 0.30	3.47 ± 0.28
T_{G2} (h)	3.97 ± 0.30	2.53 ± 0.28
S_i	32.4 ± 2.2	24.6 ± 1.1

Growth rates calculated using the μ_{cc} method about 10% higher than those calculated from the actual change in cell number (μ_{nb}).



Developing standardized protocols for analyzing pico's by FCM



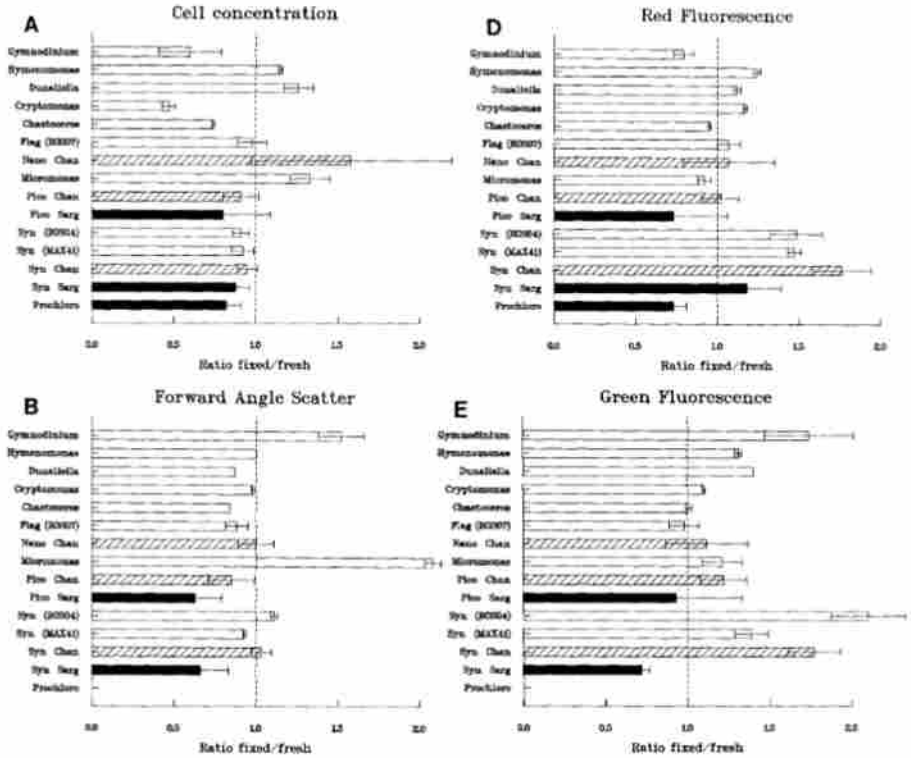
Cytometry PART A
Journal of Quantitative Cell Science
ISAC
Heterogeneity in Fragility and other Biochemical and Biophysical Properties | Free Access

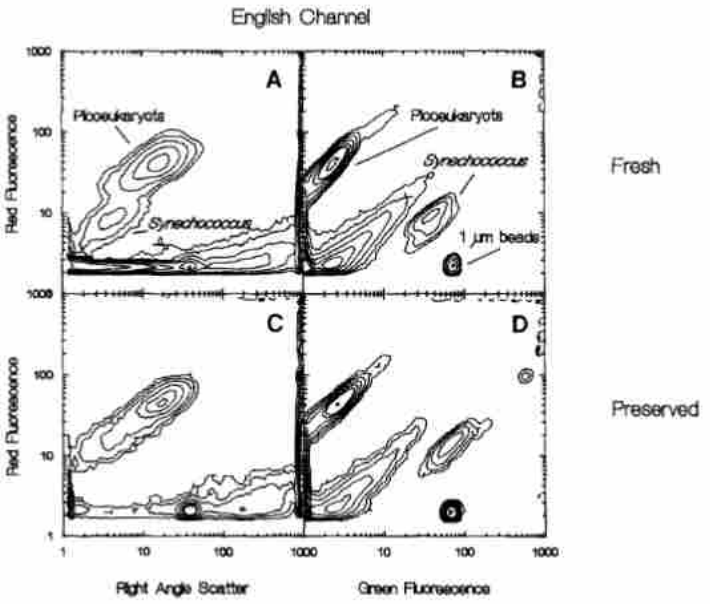
A simple method to preserve oceanic phytoplankton for flow cytometric analyses

D. Vaulot, C. Courties, F. Partensky

First published: September 1989 | https://doi.org/10.1002/cyto.990100519

A simple method was developed to preserve marine phytoplankton populations so that delayed flow cytometric analyses could be performed. The method consisted of immediate fixation with 1% glutaraldehyde (final concentration) followed by storage in liquid nitrogen. The





Vaulot et al. 1989

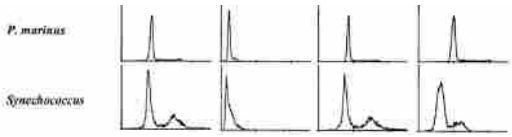
Developing standardized protocols for analyzing pico's by FCM



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1996, p. 1649–1655 Vol. 62, No. 5
Copyright © 1996, American Society for Microbiology

Application of the Novel Nucleic Acid Dyes YOYO-1, YO-PRO-1, and PicoGreen for Flow Cytometric Analysis of Marine Prokaryotes

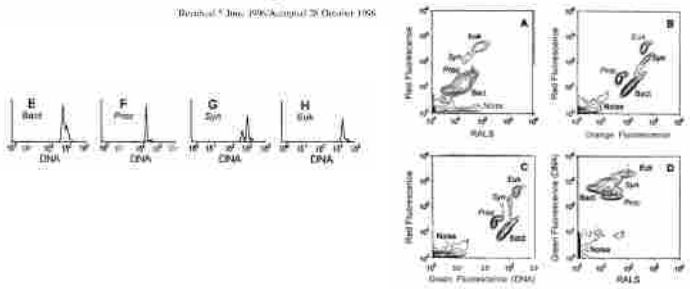
DOMINIQUE MARIE,* DANIEL VAULOT, AND FRÉDÉRIC PARTENSKY



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1997, p. 186–193
Copyright © 1997, American Society for Microbiology

Enumeration and Cell Cycle Analysis of Natural Populations of Marine Picoplankton by Flow Cytometry Using the Nucleic Acid Stain SYBR Green I

DOMINIQUE MARIE,* FRÉDÉRIC PARTENSKY, STÉPHAN JACQUET, AND DANIEL VAULOT



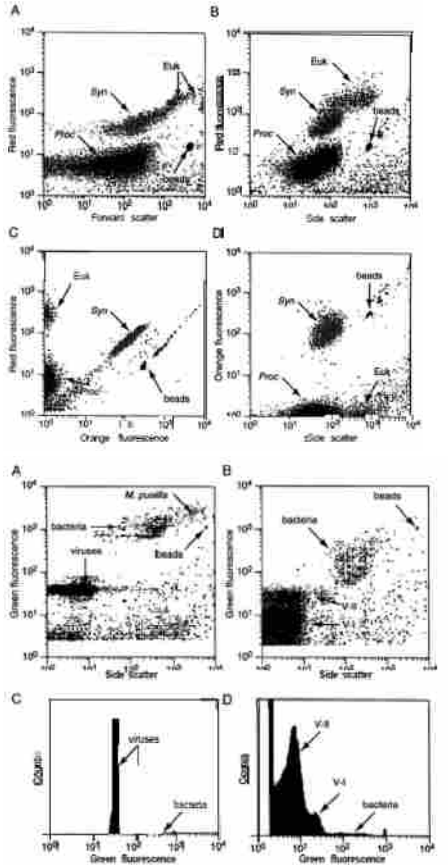
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1999, p. 45–52
0099-2240/99/$04.00+0
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Enumeration of Marine Viruses in Culture and Natural Samples by Flow Cytometry

DOMINIQUE MARIE,[1]* CORINA P. D. BRUSSAARD,[2] RUNAR THYRHAUG,[2] GUNNAR BRATBAK,[2] AND DANIEL VAULOT[1]

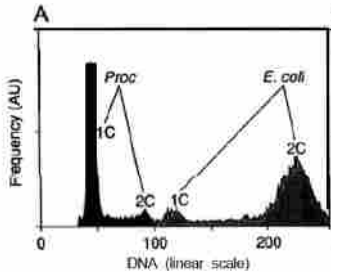
Current Protocols in Cytometry
Supplement 10 New Topics October 1999

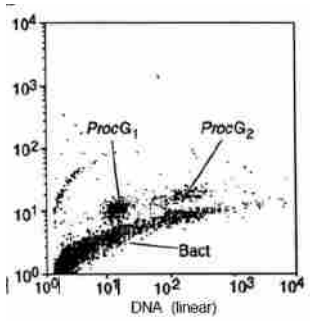
Enumeration of Phytoplankton, Bacteria, and Viruses in Marine Samples



Supplement 11 New Topics January 2000

DNA/RNA Analysis of Phytoplankton by Flow Cytometry







Section 5 – Chapter 7

Flow Cytometry Analysis of Marine Picoplankton

D. MARIE, N. SIMON, L. GUILLOU, F. PARTENSKY, AND D. VAULOT

Spatial variability of picocyanobacterial distribution

Deep-Sea Research I, Vol. 43, No. 8, pp. 1191–1213, 1996

Vertical structure of picophytoplankton at different trophic sites of the tropical northeastern Atlantic Ocean

FRÉDÉRIC PARTENSKY,* JEAN BLANCHOT,†
FRANÇOIS LANTOINE,‡ JACQUES NEVEUX‡
and DOMINIQUE MARIE*

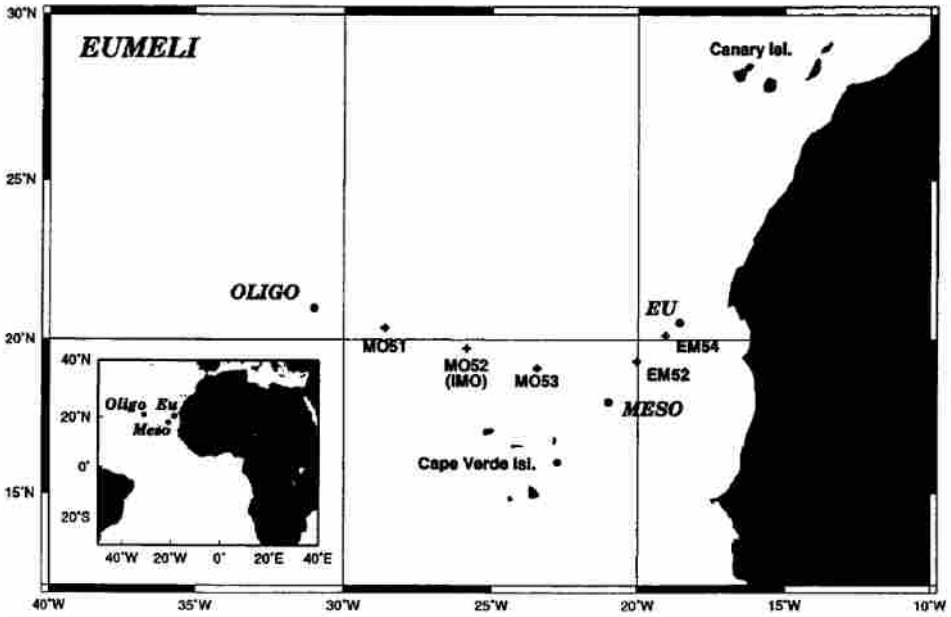
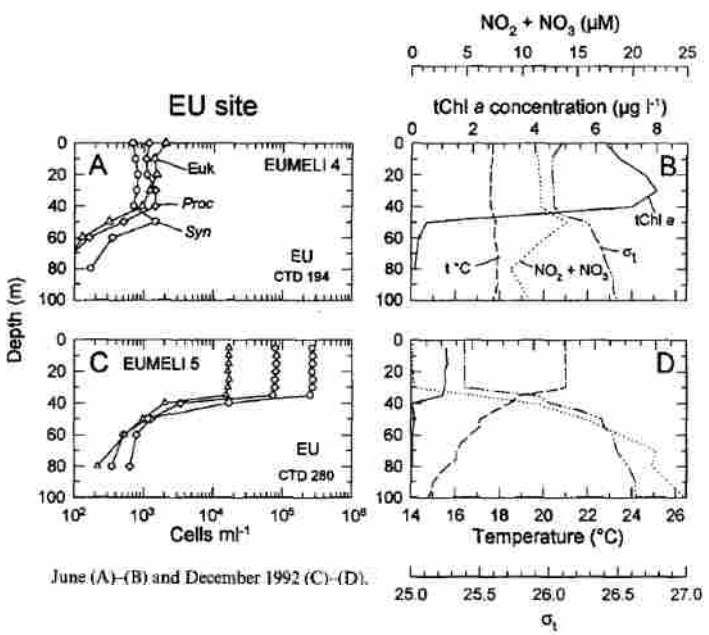
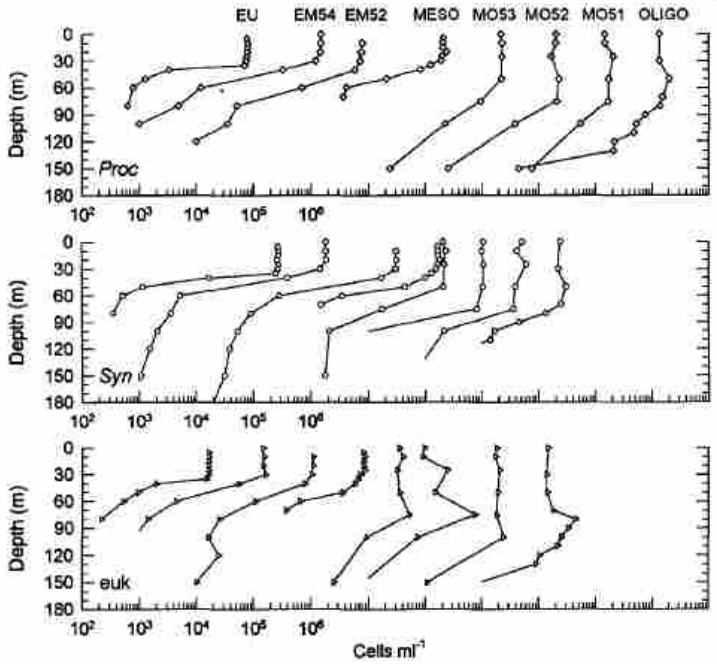


Fig. 1. Map showing the sites sampled during the EUMELI 5 cruise (December 1992). MESO, OLIGO and IMO (equivalent to MO 52) sites were also sampled at EUMELI 3 (October 1991), and EU, MESO and OLIGO sites were occupied during EUMELI 4 (June 1992).



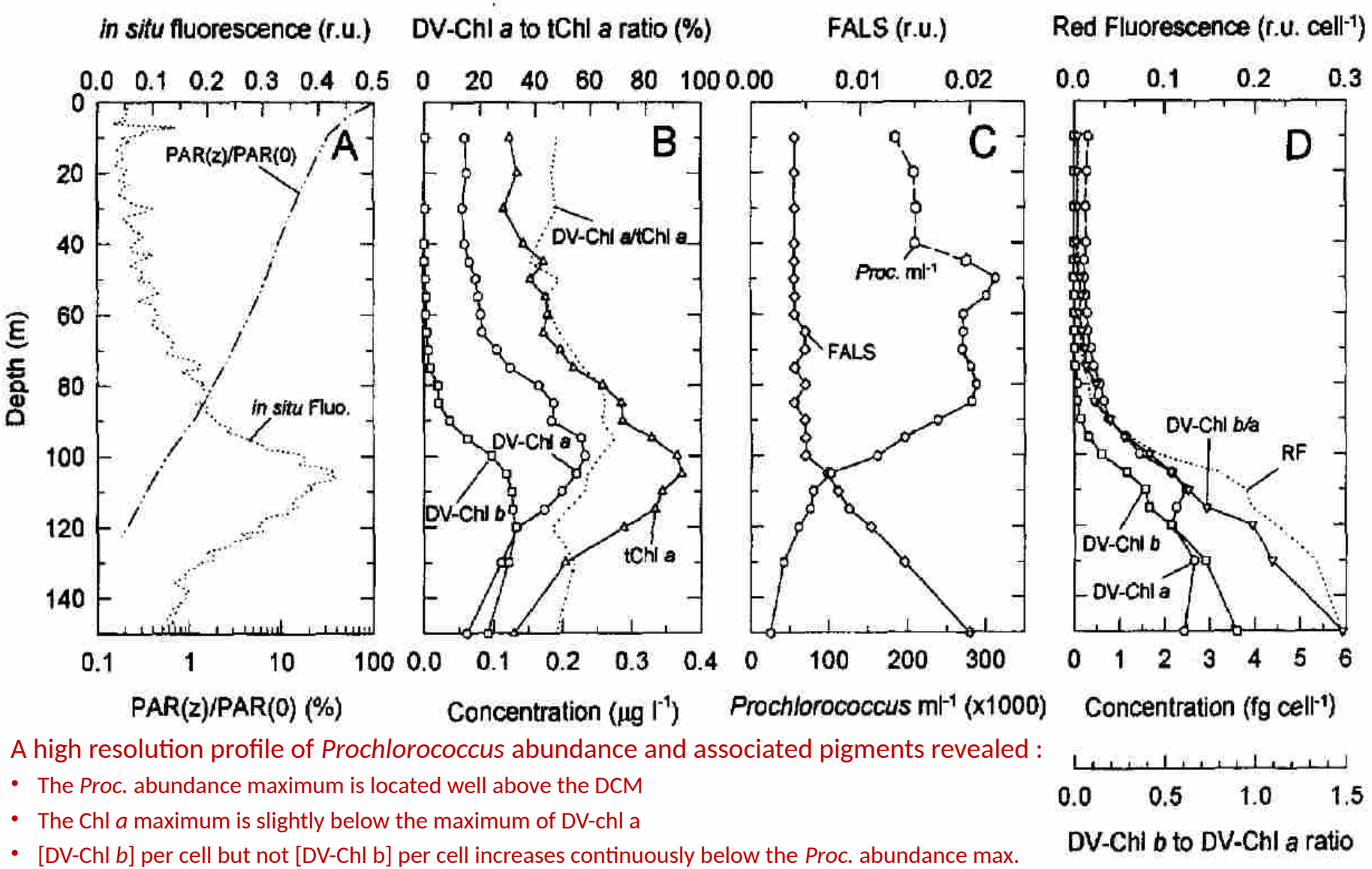
June (A)–(B) and December 1992 (C)–(D).



Partensky et al., 1996, *Deep Sea Research*

Vertical variations of *Prochlorococcus* pigment content

Sta. OLIGO, Oct. 1991, N Atlantic 21°N 31°W



A high resolution profile of *Prochlorococcus* abundance and associated pigments revealed :

- The *Proc.* abundance maximum is located well above the DCM
- The Chl *a* maximum is slightly below the maximum of DV-chl a
- [DV-Chl *b*] per cell but not [DV-Chl b] per cell increases continuously below the *Proc.* abundance max.
- The DV-Chl *a* / Chl *a* ratio varies little with depth.

Partensky et al., 1996, *Deep Sea Research*

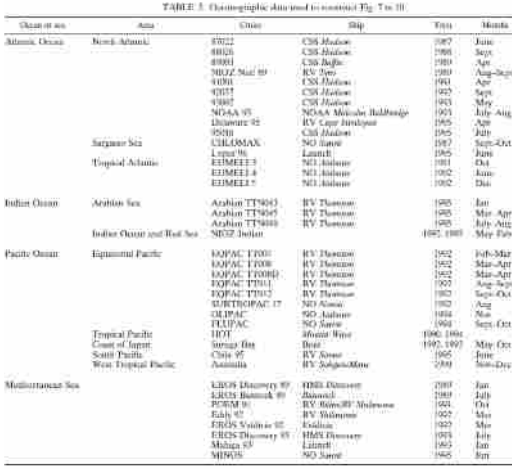
Global biogeography of picocyanobacteria

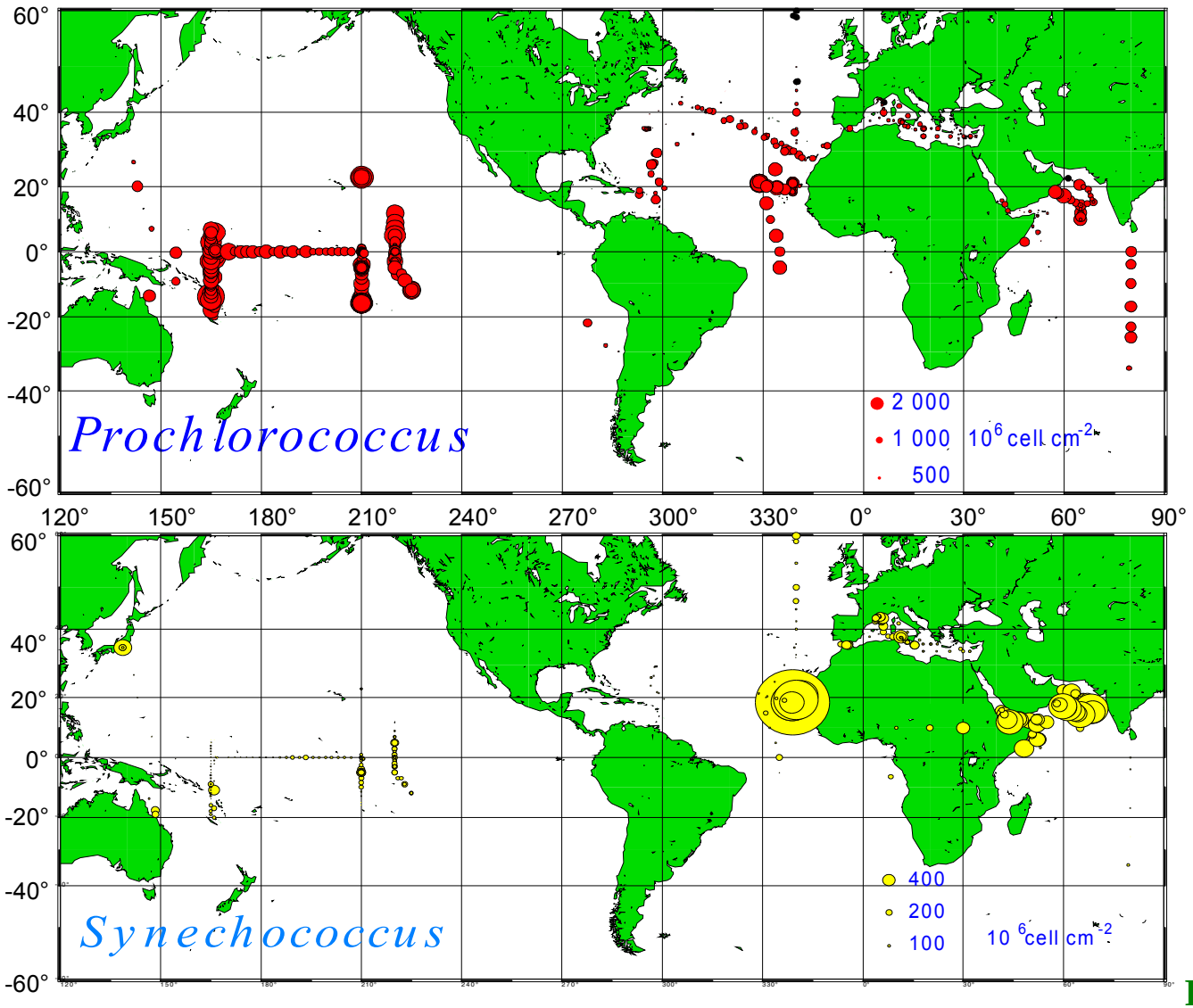
MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Mar. 1999, p. 106–127

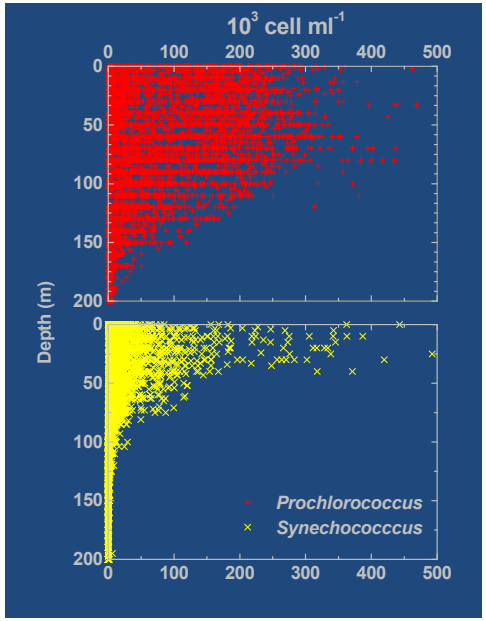
Prochlorococcus, a Marine Photosynthetic Prokaryote of Global Significance

F. PARTENSKY,[1]* W. R. HESS,[2] AND D. VAULOT[1]

Global maps from 38 oceanographic cruises

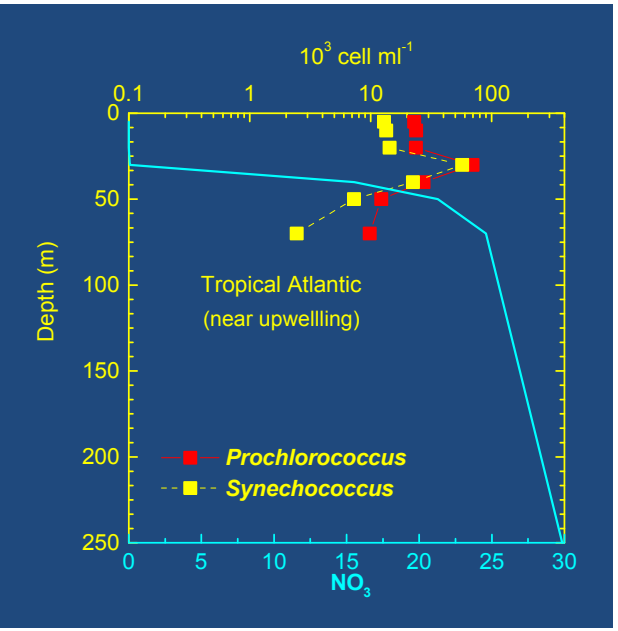


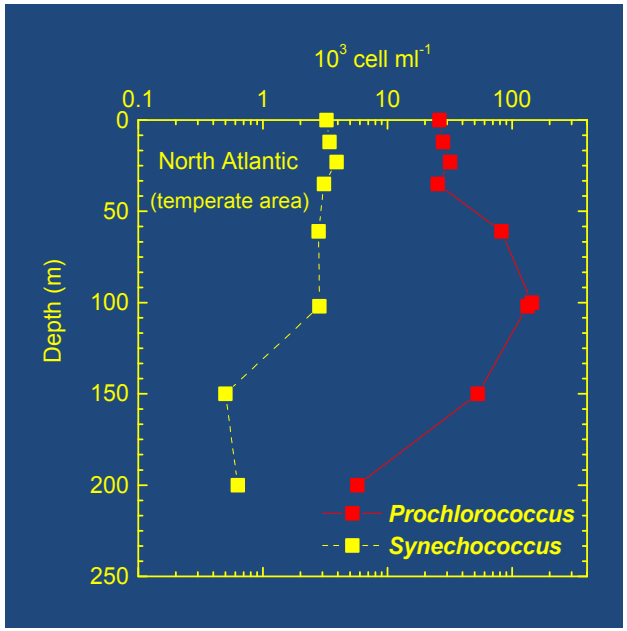


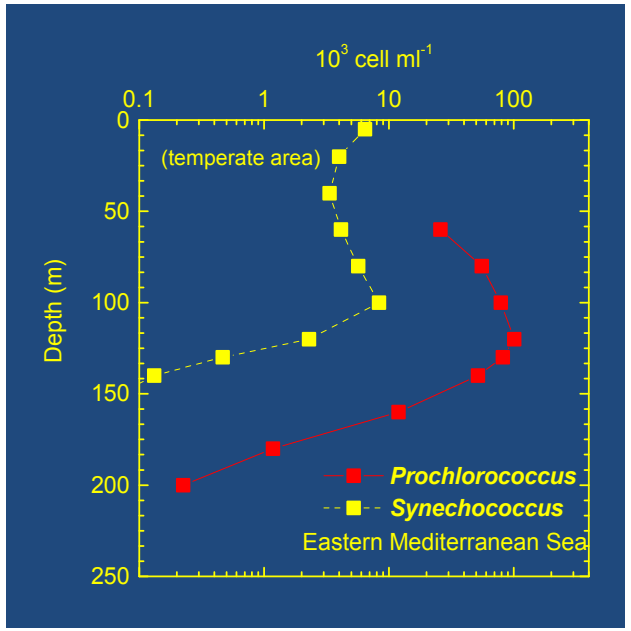


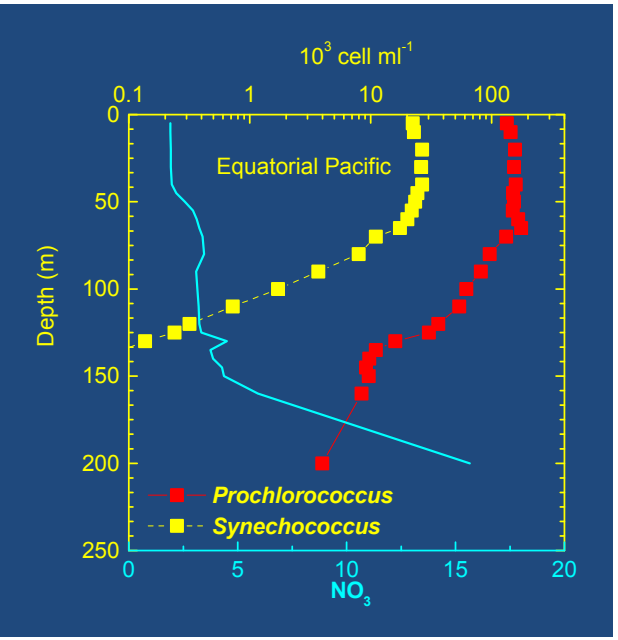
Partensky et al., 1999, MMBR

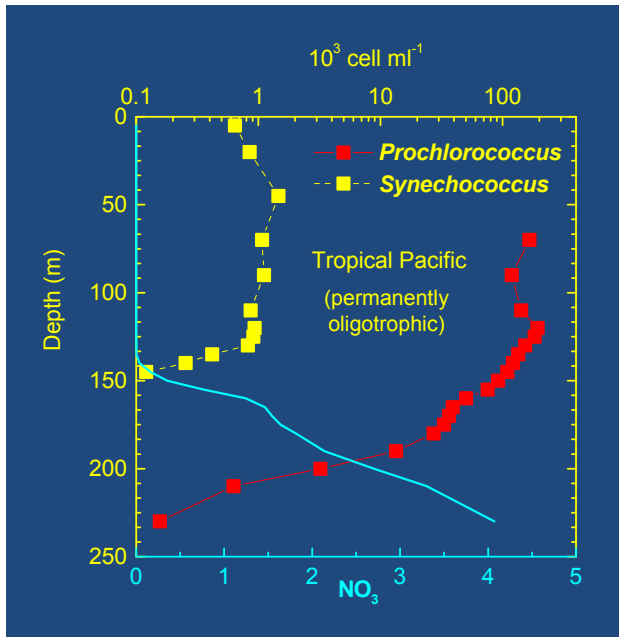
Global biogeography of picocyanobacteria











⇒ [Syn] / [Proc] ratio
varying from 1:1 in nutrient-rich waters
to 1:100 in tropical oligotrophic waters

Seasonal succession of marine picocyanobacteria

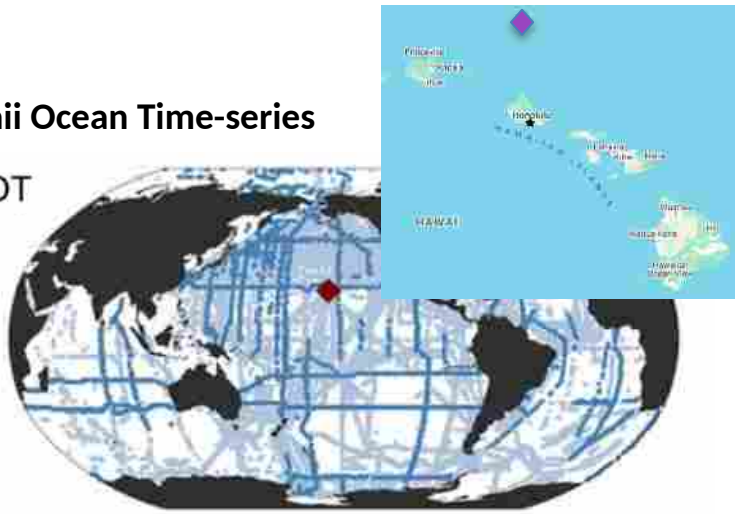
Deep-Sea Research I, Vol. 44, No. 2. pp. 167-192, 1997

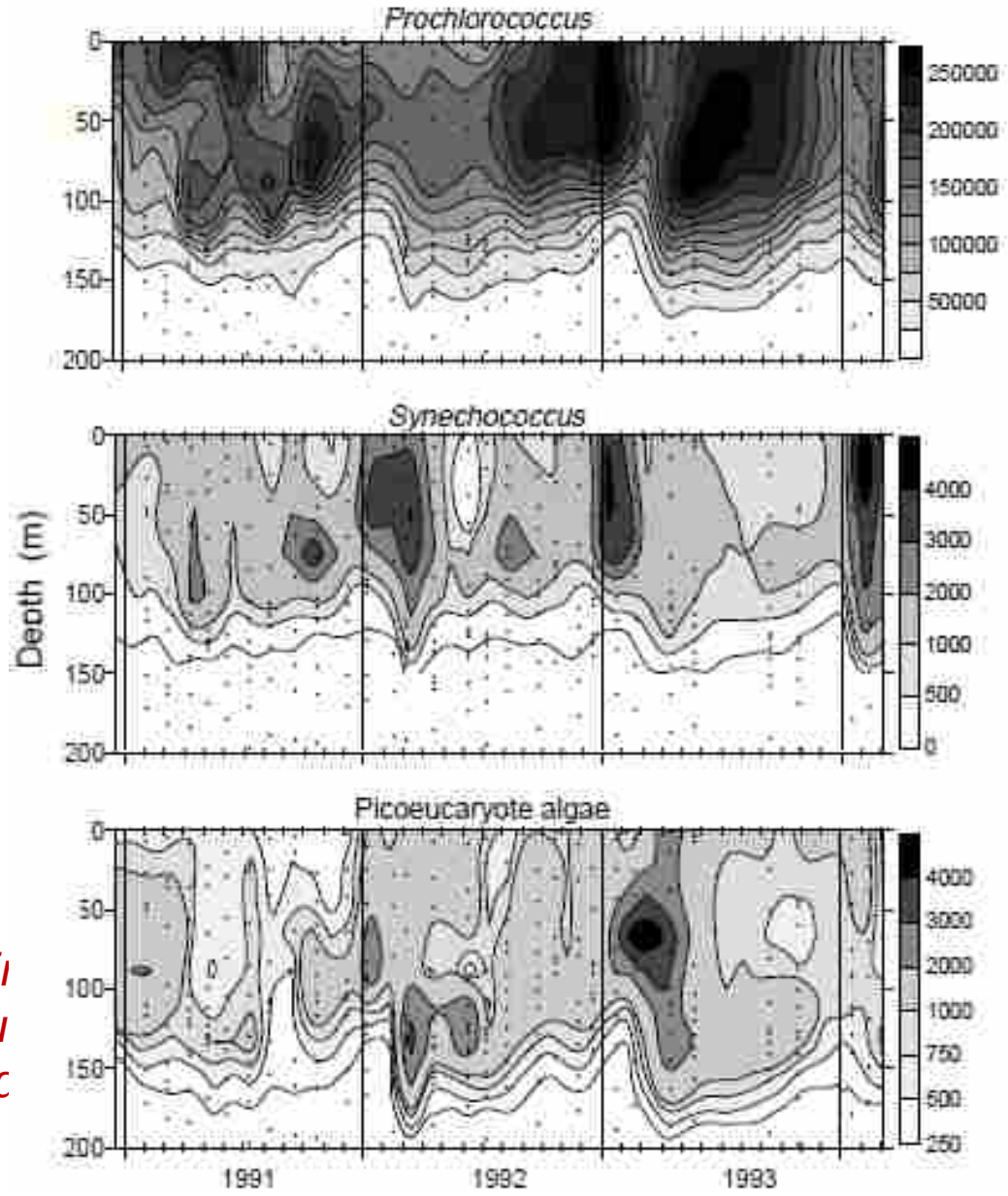
Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991-1994 ENSO event

LISA CAMPBELL, *‡ HONGBIN LIU,* HECTOR A. NOLLA* and DANIEL VAULOT†

Hawaii Ocean Time-series

HOT





Syn. peaked in winter, picoeukaryotes i spring, and Proc. during summer/fall bu seasonality not very marked (only 2-fol variations for Proc. and 3-4-fold for Syn.).

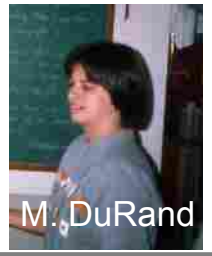
Campbell et al., 1997

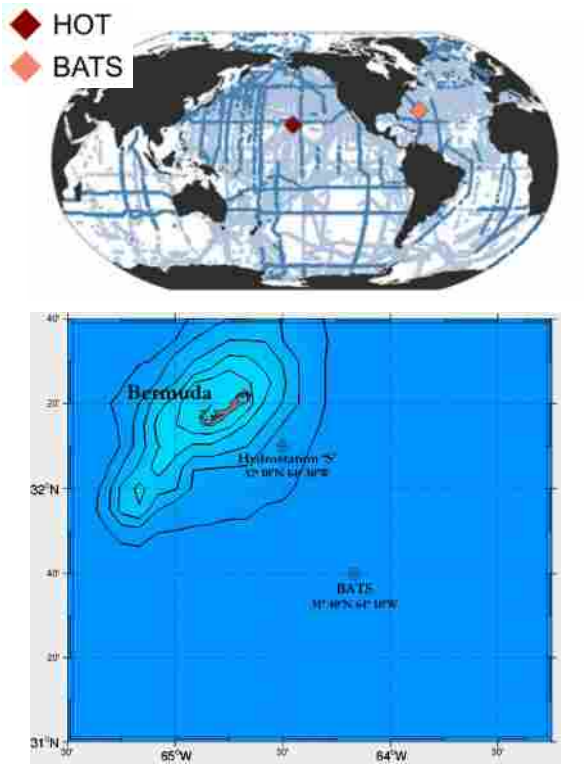
Seasonal succession of marine picocyanobacteria

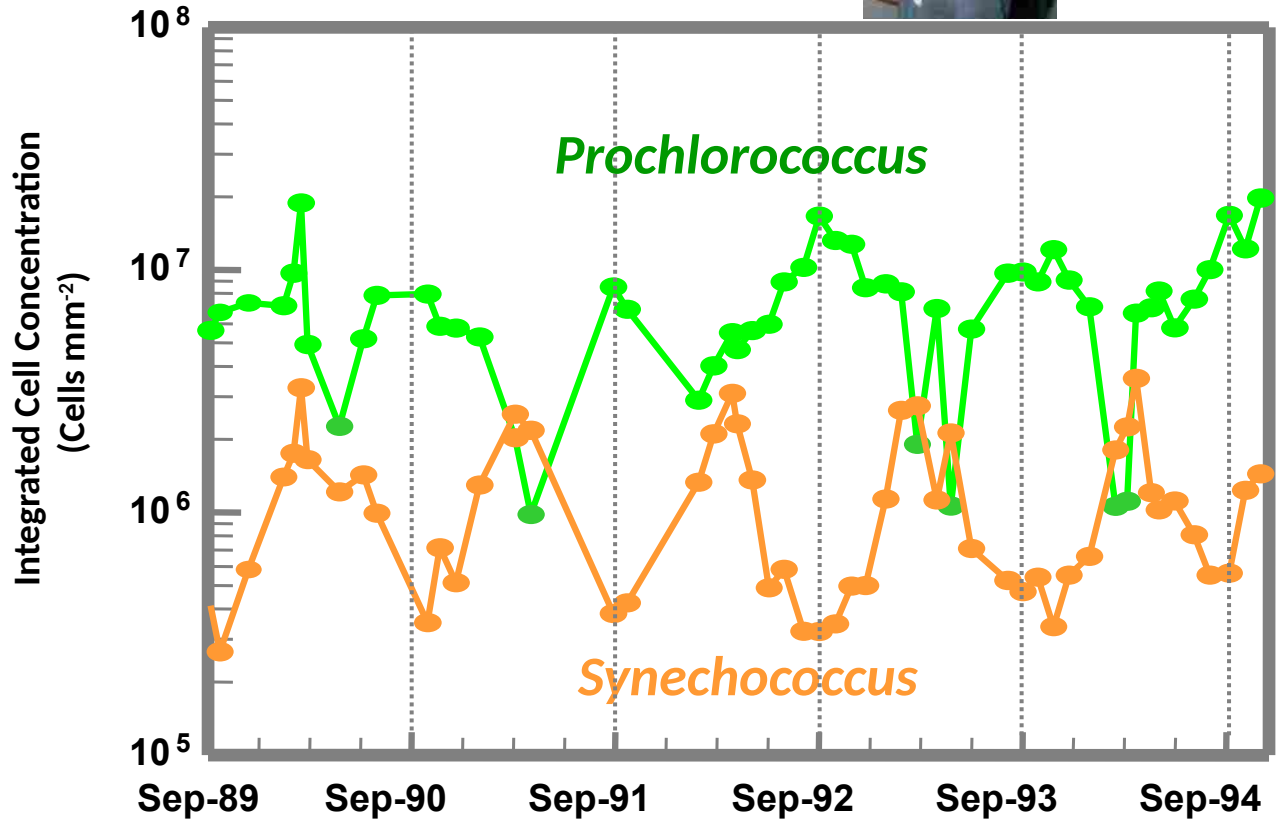
Deep-Sea Research II 48 (2001) 1983–2003

Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea

Michele D. DuRand[a,b,*], Robert J. Olson[a], Sallie W. Chisholm[c]







Syn. peaked during the spring bloom when the water column was deeply mixed and nutrients high in surface waters while *Proc. peaked* in the summer and fall with a deeper sub-surface maximum than *Syn.*

DuRand et al., 2001

Seasonal succession of marine picocyanobacteria

Ocean time-series reveals recurring seasonal patterns of virioplankton dynamics in the northwestern Sargasso Sea

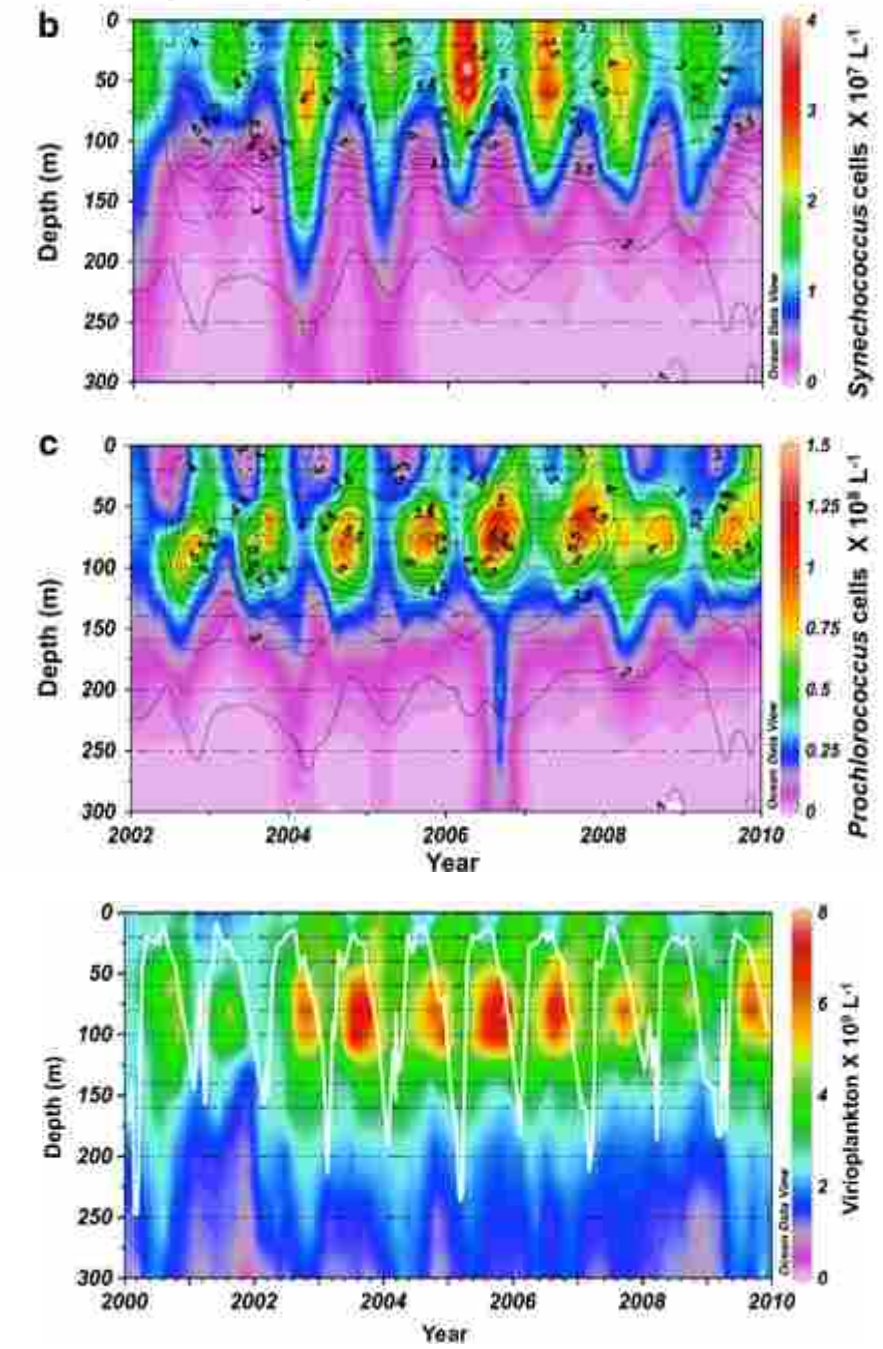
Rachel J Parsons, Mya Breitbart, Michael W Lomas & Craig A Carlson

The ISME Journal **6**, 273–284 (2012)

Syn. abundance maxima developed in the spring within the mixed layer shortly after water column stratification.

Proc. abundance maxima developed between 60 and 100 m during periods of water column stratification.

Distribution patterns of *Proc*. and virioplankton were tightly coupled over depth and through time.



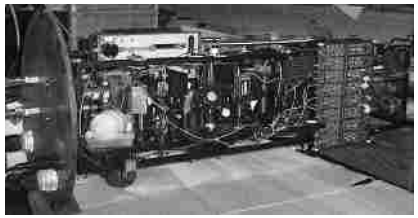
Seasonal succession of marine picocyanobacterial using an automated submersible flow cytometer: the FlowCytobot

SCIENCE
21 OCTOBER 2016 • VOL 354 ISSUE 6310

Physiological and ecological drivers of early spring blooms of a coastal phytoplankter

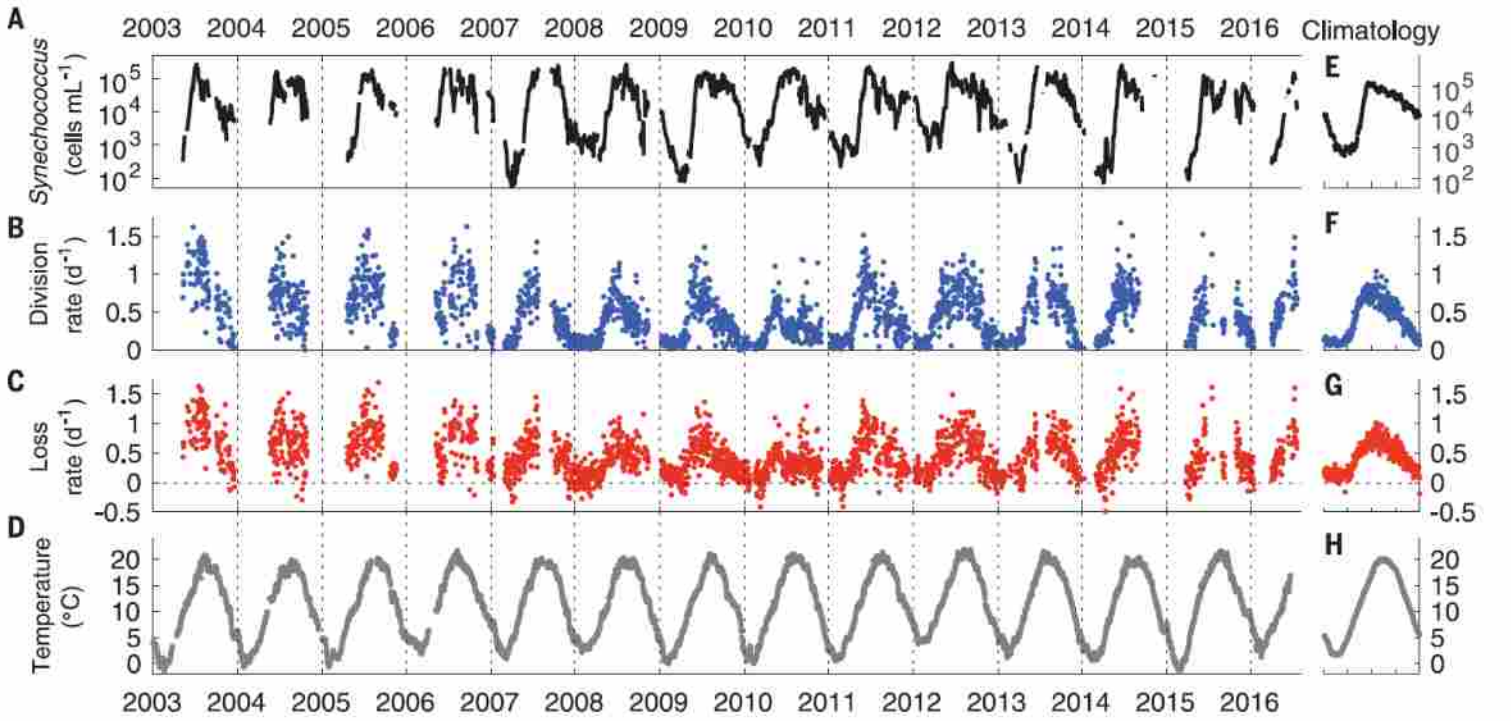
Kristen R. Hunter-Cevera,[1] Michael G. Neubert,[1] Robert J. Olson,[1] Andrew R. Solow,[2] Alexi Shalapyonok,[1] Heidi M. Sosik[1]*





A regular spring bloom in coastal waters off Woods Hole, USA is driven by increases in *Synechococcus* cell division rates in response to warmer water temperature and slight imbalance in loss rate.

Earlier spring blooms in the period 2003-2012 correlate with warmer temperatures.



Croucher course 1 outline

I. 1979-1999: The golden age of fluorescence techniques

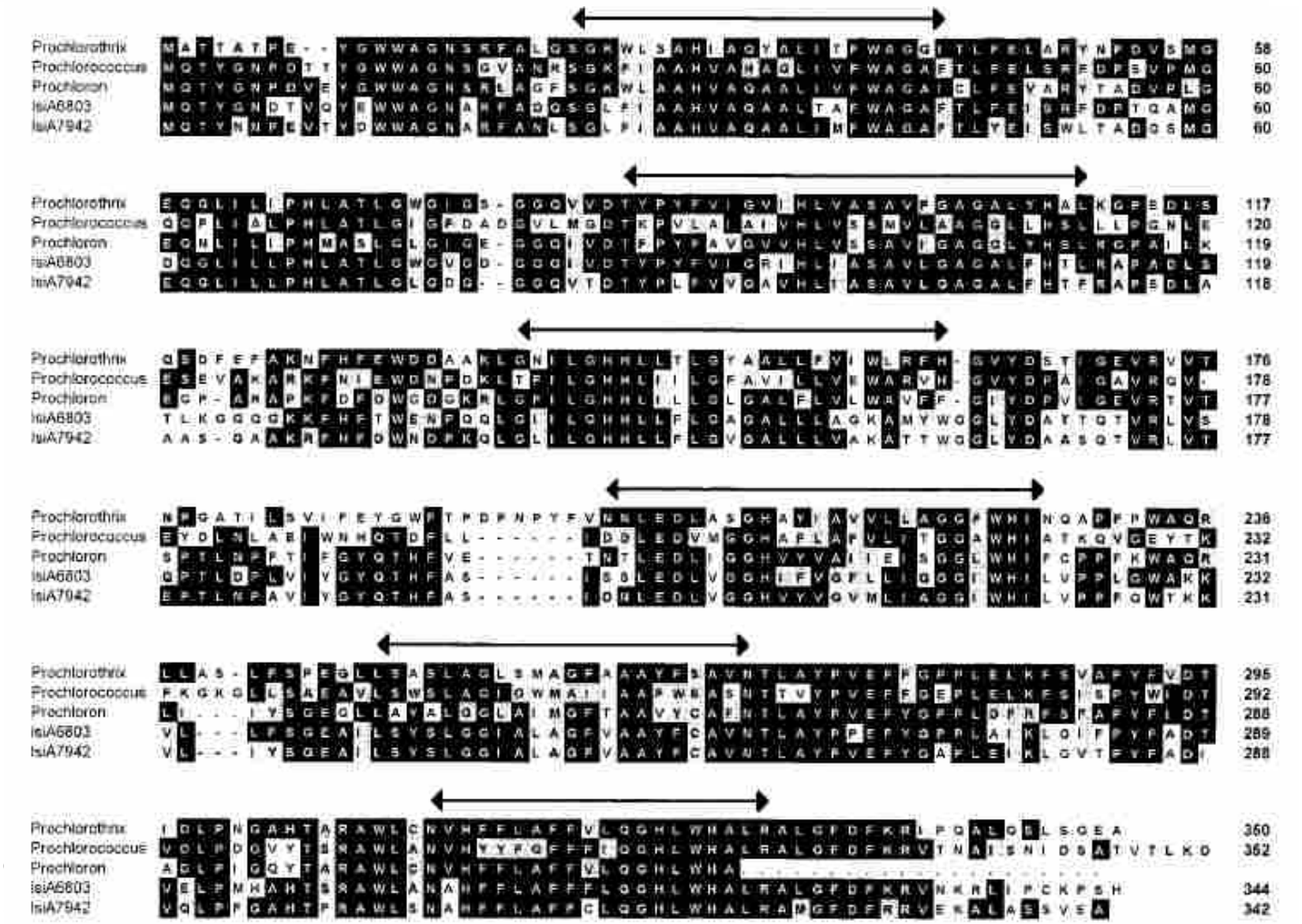
II. 1996-2003: Characterization of the original light- harvesting complex of *Prochlorococcus*

III. 1998: The advent of the molecular ecology of picocyanobacteria

IV. 2003-on: The omics era: an introduction

Identification of *Prochlorococcus* Light-Harvesting Complexes

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 15244–15248, December 1996



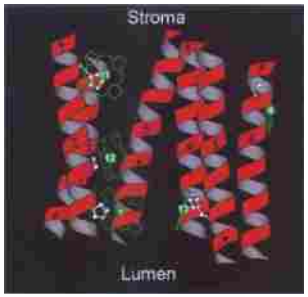
Identification of *Prochlorococcus* Light-Harvesting Complexes

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 15244–15248, December 1996

Independent evolution of the prochlorophyte and green plant chlorophyll *a*/*b* light-harvesting proteins

J. La Roche*†, G. W. M. van der Staay†‡§, F. Partensky§, A. Ducret¶, R. Aebersold¶, R. Li‖, S. S. Golden‖, R. G. Hiller**, P. M. Wrench**, A. W. D. Larkum††, and B. R. Green‡,‡‡





Chl a/*b* antenna named « Pcb » for « prochlorophyte Chl *a/b* protein »

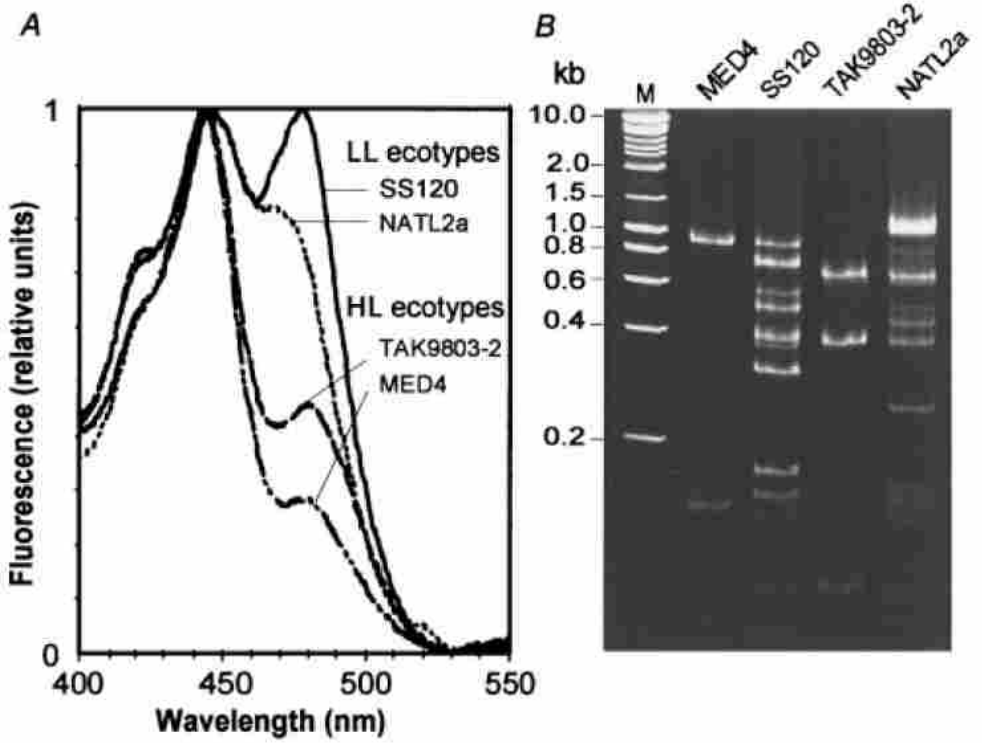
Pcb and IsiA related to the PSII antenna PsbC (= CP43), all having 6 transmembrane helices, and much more distantly to plant or algal LHC than have 3 helices.

Characterization of *Prochlorococcus* Light-Harvesting Complexes

PNAS | April 11, 2000 | vol. 97 | no. 8 | 4099

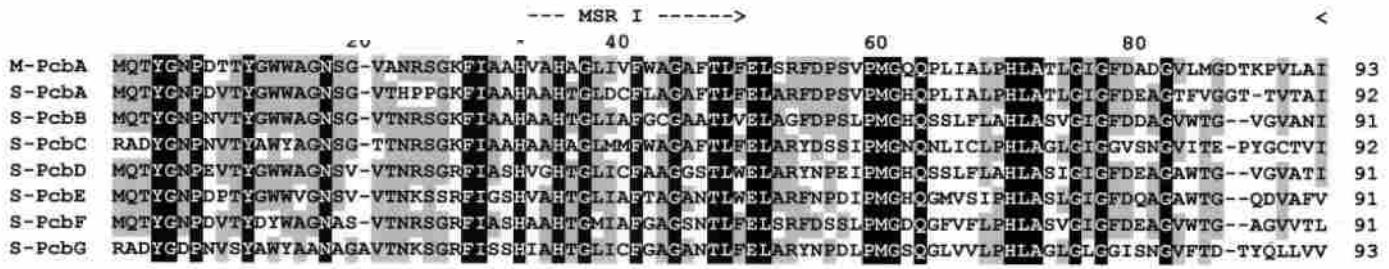
Multiplication of antenna genes as a major adaptation to low light in a marine prokaryote

Laurence Garczarek*, Wolfgang R. Hess†, Julia Holtzendorff†, Georg W. M. van der Staay*‡, and Frédéric Partensky*§



The *Proc. LL* strain SS120 possesses a gene family of 7 transcribed genes encoding different Pcb copies *(1 missed!)* while the HL strain MED4 has a single *pcb* gene, which were successfully cloned and sequenced.

+Evidence that the the LL strain NATL2A also has many *pcb* copies while the HL strain TAK9802 has 2.

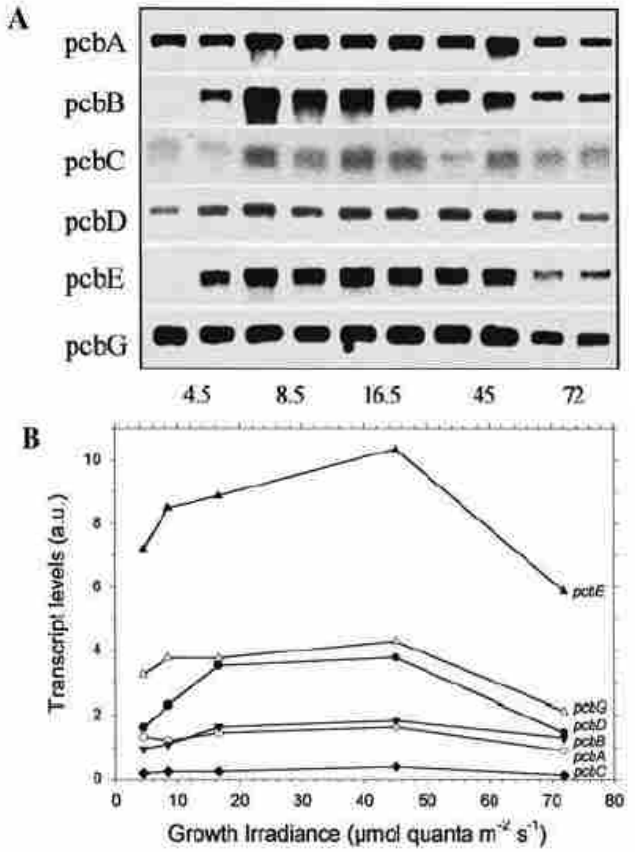


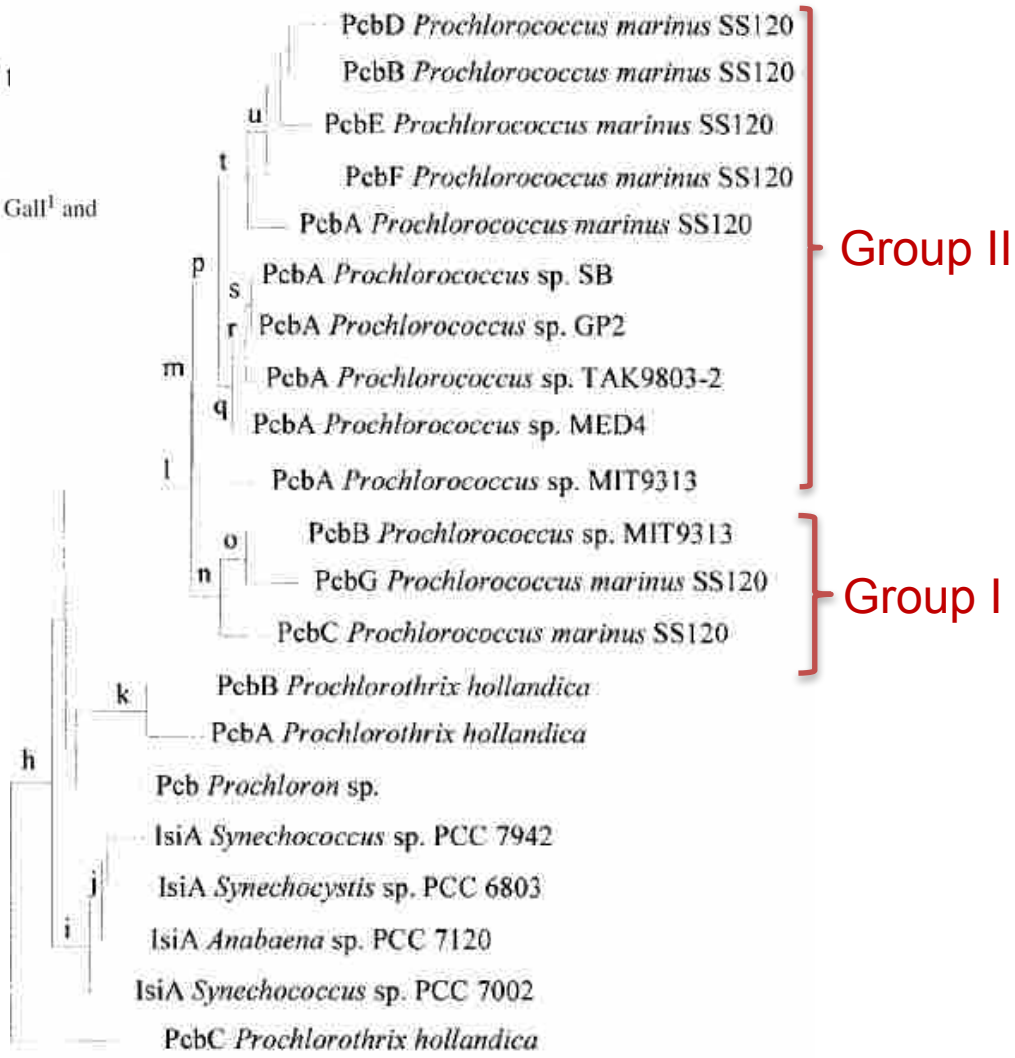
Characterization of *Prochlorococcus* Light-Harvesting Complexes

Plant Molecular Biology **46:** 683–693, 2001.

Expression and phylogeny of the multiple antenna genes of l low-light-adapted strain *Prochlorococcus marinus* SS120 (Oxyphotobacteria)

Laurence Garczarek[1], Georg W. M. van der Staay[1,3], Wolfgang R. Hess[2], Florence Le Gall[1] and Frédéric Partensky[1,*]





At least 6 *pcb genes* of SS120 are differentially expressed with irradiance

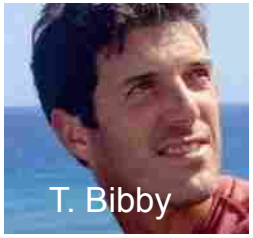
SS120 has two phylogenetically distinct groups of *pcb* genes : possibly PSI and PSII-associated?

Ultrastructure of *Prochlorococcus* Light-Harvesting Complexes

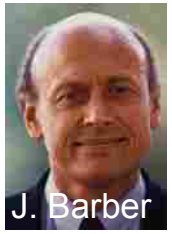
Nature **412**, 743–745 (2001) | 16 August 2001

Iron deficiency induces the formation of an antenna ring around trimeric photosystem I in cyanobacteria

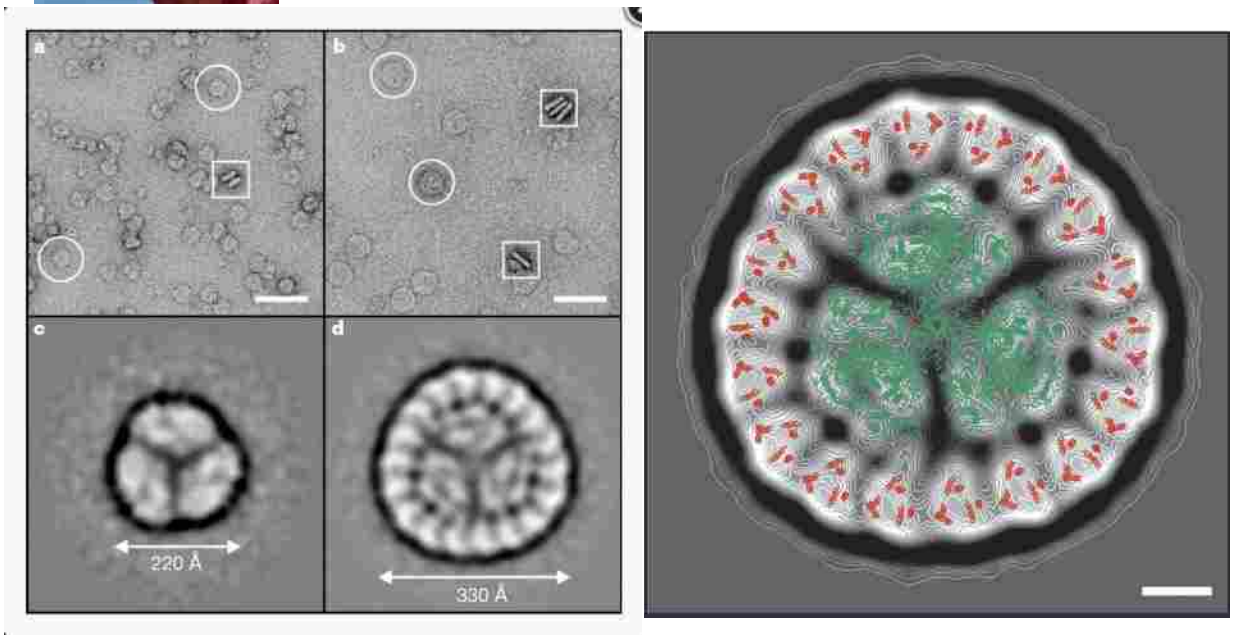
Thomas S. Bibby, Jon Nield & James Barber







Same configuration for *Proc*. SS120 Pcb antenna as for *Synechocystis* IsiA



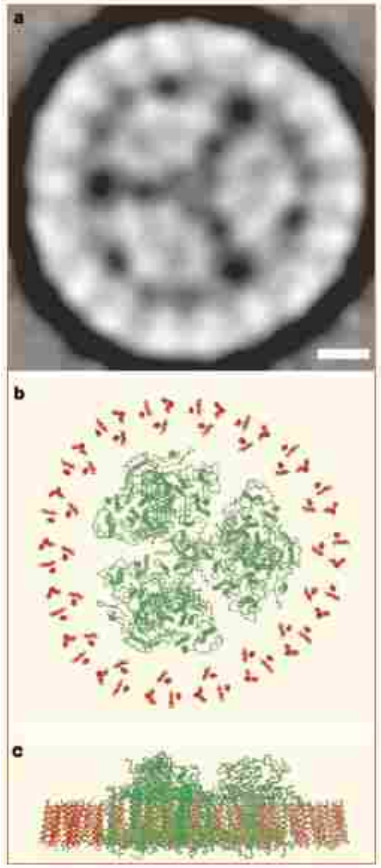
NATURE | VOL 413 | 11 OCTOBER 2001

brief communications

Oxyphotobacteria

Antenna ring around photosystem I

T. S. Bibby*, J. Nield*, F. Partensky†, J. Barber*



IsiA (=CP43') from *Synechocystis* PCC 6803 associates with photosystem I (PSI) to form a complex that consists of a ring of 18 molecules around a PSI trimer.

Ultrastructure of *Prochlorococcus* Light-Harvesting Complexes

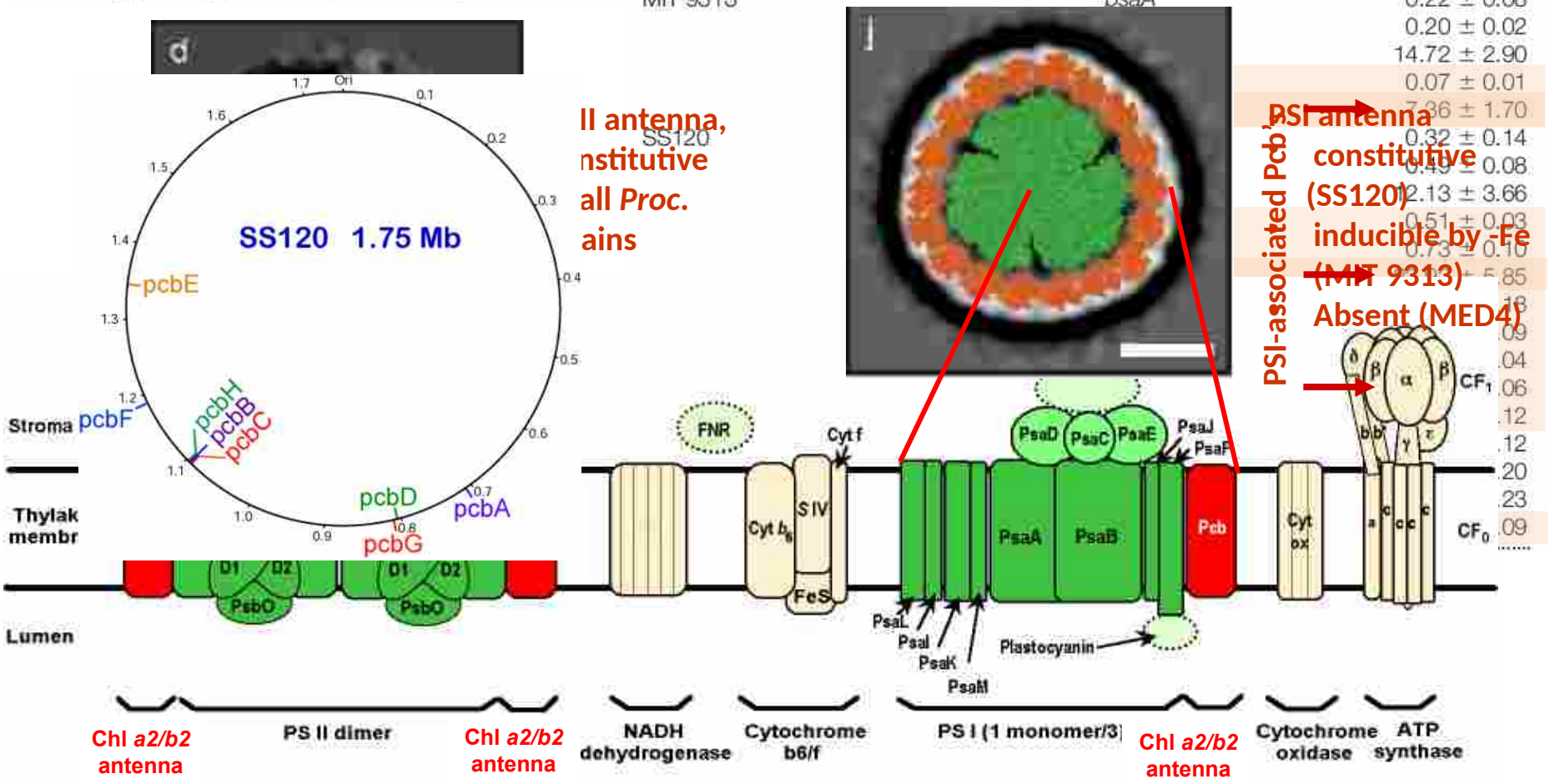
NATURE | VOL 424 | 28 AUGUST 2003 |

Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem

T. S. Bibby[1], I. Mary[2], J. Nield[1], F. Partensky[2] & J. Barber[1]

Table 1 Quantitative RT–PCR analyses of the expression of the indicated genes under +Fe and −Fe conditions

Prochlorococcus strain	Gene	Induction ratio (±s.d.)
MIT 9313	*psaA*	0.22 ± 0.08
		0.20 ± 0.02
		14.72 ± 2.90
		0.07 ± 0.01
		7.36 ± 1.70
		0.32 ± 0.14
		0.49 ± 0.08
		12.13 ± 3.66
		0.51 ± 0.03
		0.73 ± 0.10
		5.85



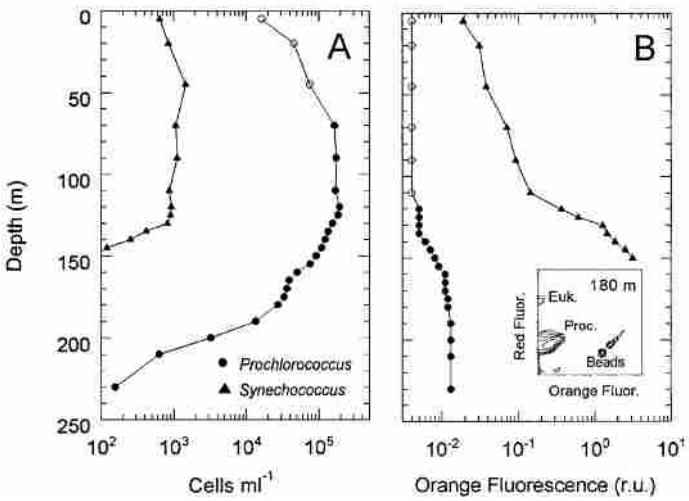
PSI- then PSII-associated Pcbs have arisen from multiple events of gene duplication and divergence from an ancestral *isiA* gene

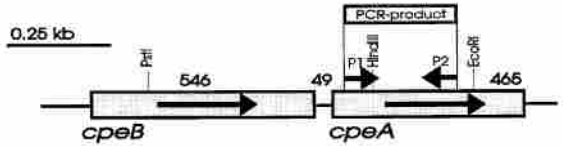
Bibby et al., 2003 *Nature*

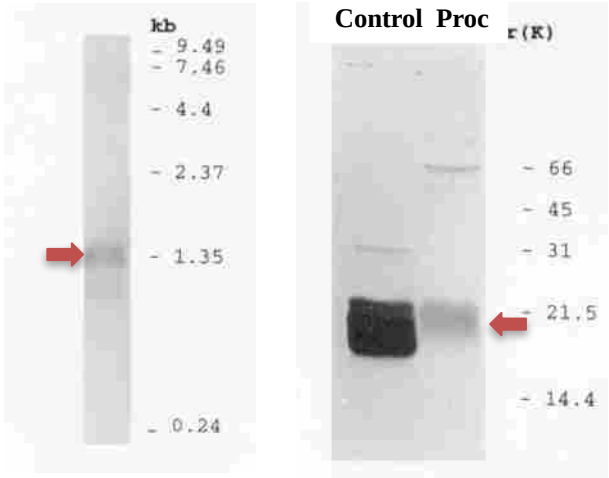
Unveiling the presence of phycoerythrin in *Prochlorococcus*

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 11126–11130, October 1996

WOLFGANG R. HESS*†, FRÉDÉRIC PARTENSKY‡, GEORG W. M. VAN DER STAAY‡, JOSÉ M. GARCIA-FERNANDEZ‡, THOMAS BÖRNER*, AND DANIEL VAULOT‡

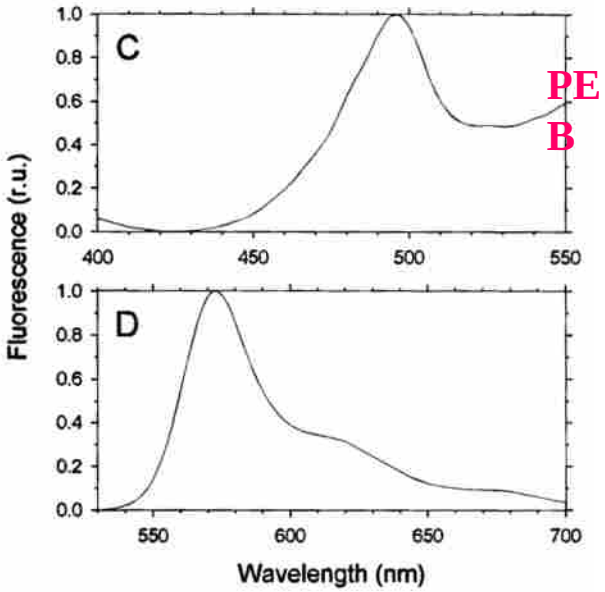






Hess et al., 1996

PUB

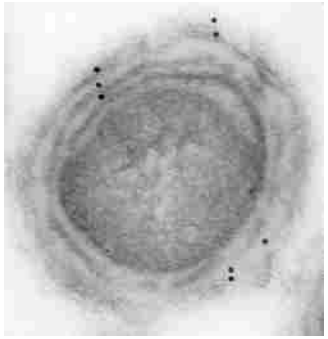


PE B

The *cpeBA* operon is expressed and translated into PE in SS120

PE contributes for only 2.6 % to light absorption at 495 nm.

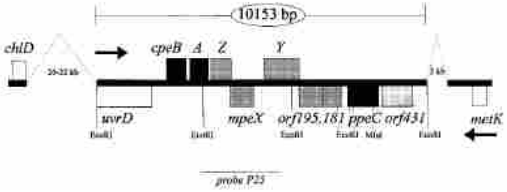
=> Light sensor?



Plant Molecular Biology **40**: 507–521, 1999.

Phycoerythrins of the oxyphotobacterium *Prochlorococcus marinus* are associated to the thylakoid membrane and are encoded by a single large gene cluster

Wolfgang R. Hess[1,*], Claudia Steglich, Christiane Lichtlé[2] and Frédéric Partensky[3]



Croucher course 1 outline

I. 1979-1999: The golden age of fluorescence techniques

II. 1996-2003: Characterization of the original light- harvesting complex of *Prochlorococcus*

III. 1998: The advent of the molecular ecology of picocyanobacteria

IV. 2003-on: The omics era: an introduction

Molecular ecology approaches for studying picocyanobacteria

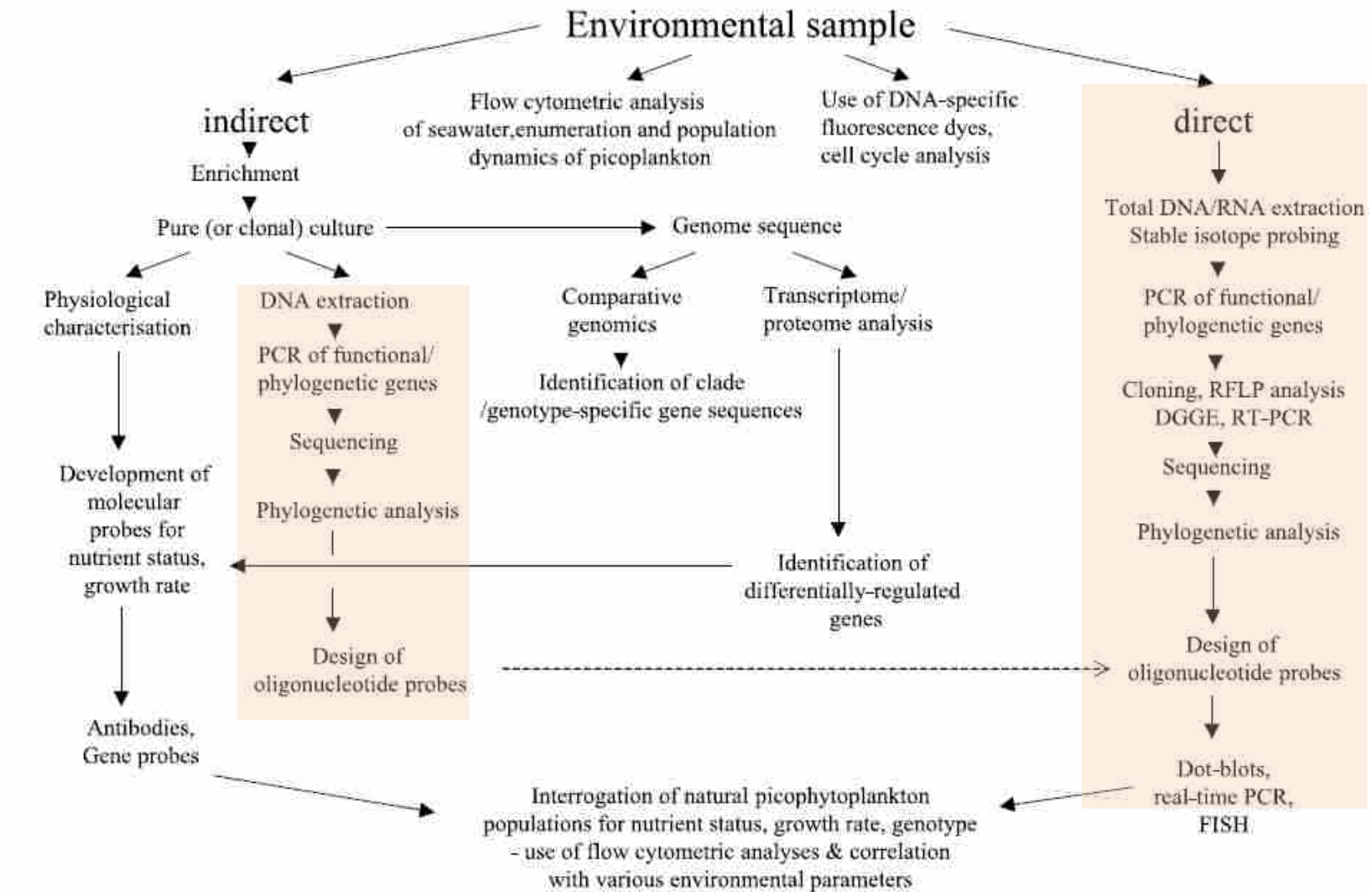
FEMS Microbiology Ecology 40 (2002) 1–12

Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*

David J. Scanlan *, Nyree J. West







Molecular ecology approaches : DGGE

Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA

G Muyzer, E C de Waal, A G Uitterlinden

Applied and Environmental Microbiology®

Volume 59: Issue 3
/aem.59.3.695-700.1993



Environmental Microbiology (2004) 6(5), 528–534

The use of DGGE analyses to explore eastern Mediterranean and Red Sea marine picophytoplankton assemblages

Gil Zeidner and Oded Béjà*



DGGE analyses using the photosynthetic gene *psbA*

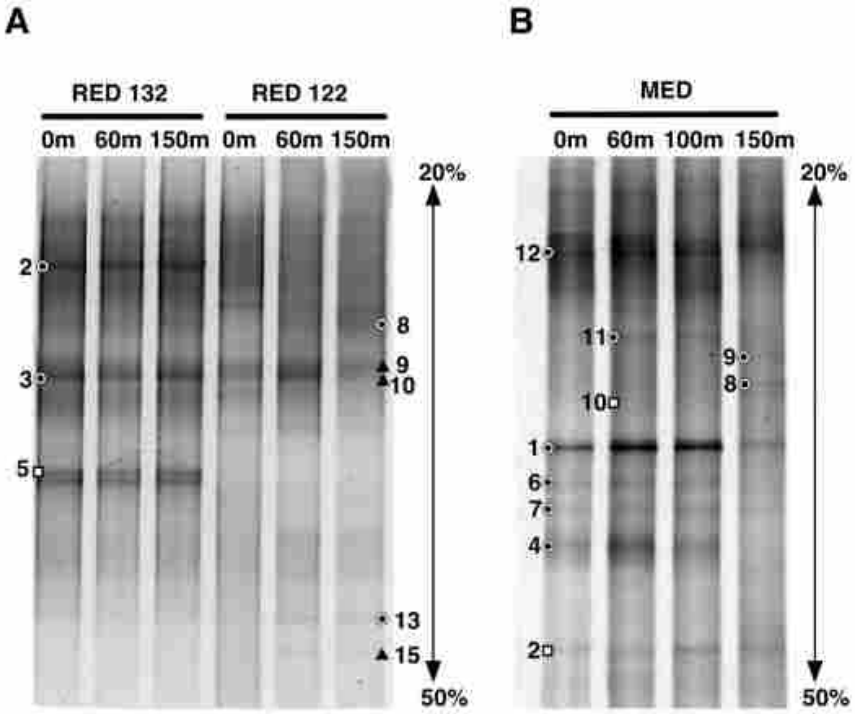


Fig. 3. DGGE gel of *psbA* amplicons from the Red and Mediterranean Seas.
A. Red Sea samples from station A (122) and a station next to the Gulf of Suez (132).
B. Mediterranean Sea samples from station H06. Bands collected for sequencing are marked with a number, and the square markings represent *psbA* bands coming from *Synechococcus*; circles, *Prochlorococcus*; and triangles, picoeukaryotic algae.

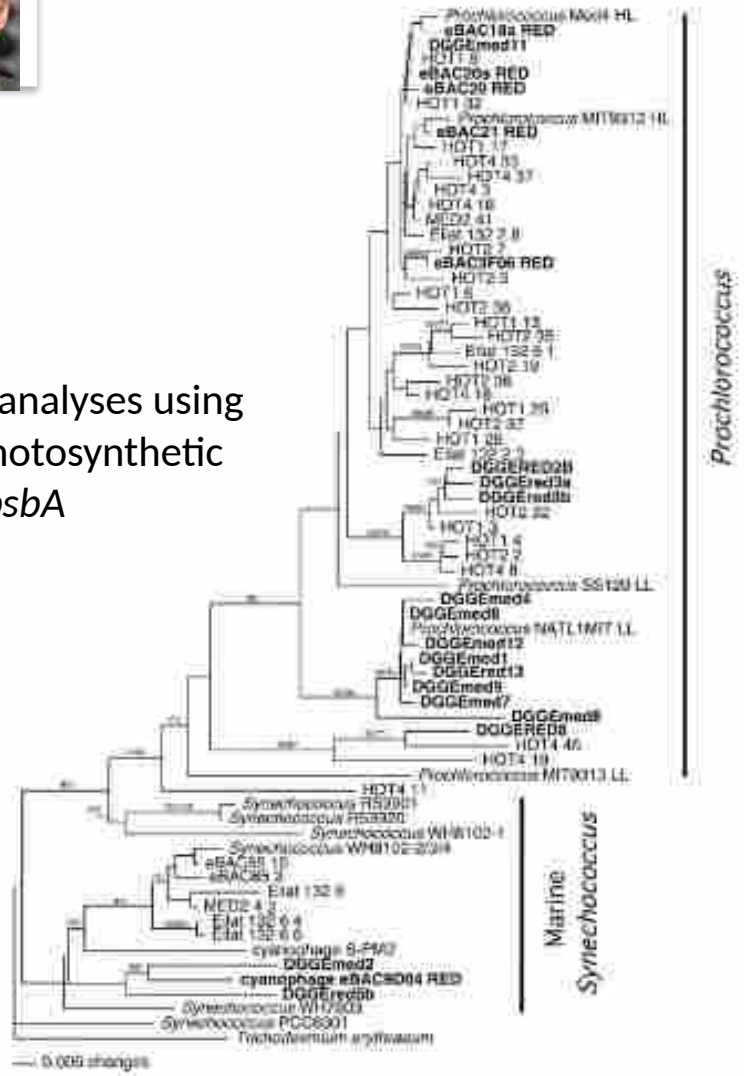


Fig. 4. Phylogenetic relationships of PsbA proteins. Evolutionary distances for the PsbA proteins were determined from an alignment of 320 amino acid positions using neighbour-joining analysis (Thompson *et al.*, 1997).
A. Cyanobacteria PsbA sequences.

Molecular ecology approaches : RFLP

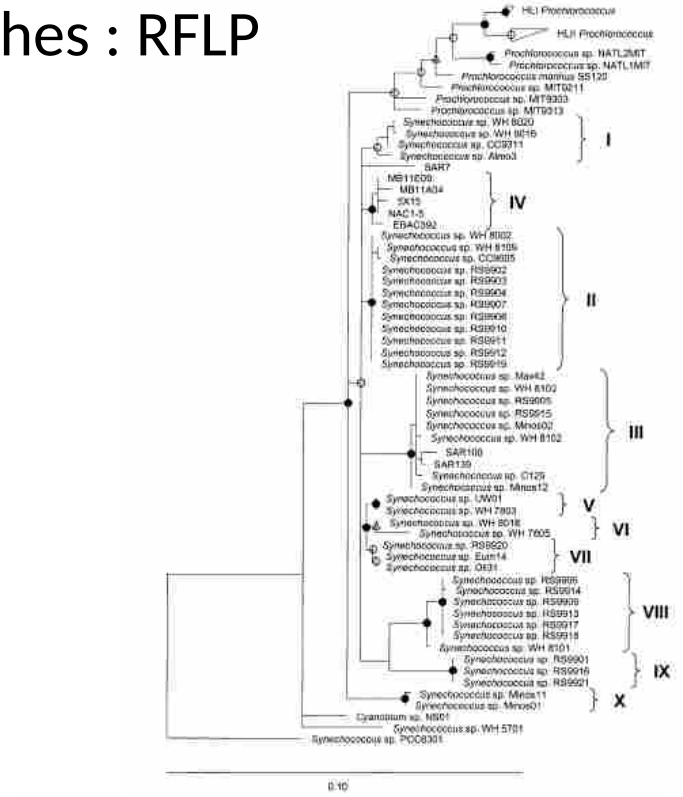
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2003, p. 2430–2443 Vol. 69, No. 5

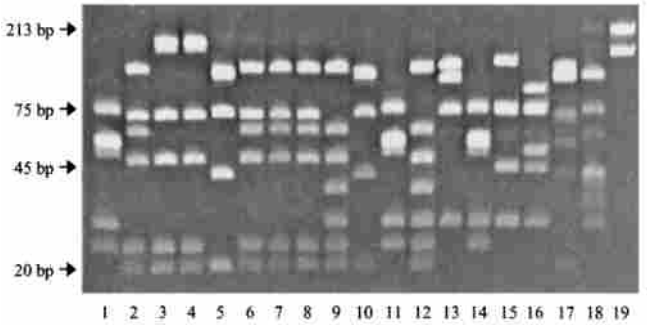
Clade-Specific 16S Ribosomal DNA Oligonucleotides Reveal the Predominance of a Single Marine *Synechococcus* Clade throughout a Stratified Water Column in the Red Sea

Nicholas J. Fuller,[1] Dominique Marie,[2] Frédéric Partensky,[2] Daniel Vaulot,[2] Anton F. Post,[3] and David J. Scanlan[1]*

TABLE 1. Origin and characteristics of *Synechococcus* strains used in this study

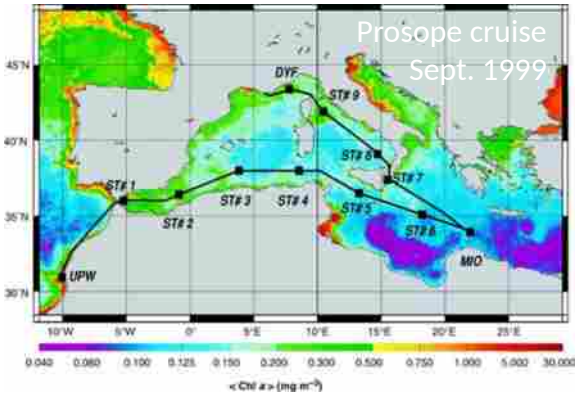
Clade no.	RCC no.[a]	Strain	Site of isolation	Depth (m)	Isolation date[b]	PUB/PEB ratio	Motility[c]	N requirement(s)	Obligately marine[d]	Isolating scientist(s)
I	43	Almo3	Mediterranean Sea 36°11′N, 1°51′W	0	1-5-91	0.4	−		+	F. Partensky, this study
I		CC9311[a,d]	California current	95	1993	1.16		NO_3^-		B. Palenik
I		WH 8016[a,e]	Woods Hole, Mass.		6-80	0.40		NO_3^-, NH_4^+		J. Waterbury
I		WH 8020[a,e]	Sargasso Sea 38°41′N, 69°19′W	50	26-6-80	0.78		NO_3^-, NH_4^+	+	J. Waterbury
II		CC9605[a,d]	California current		1996			NO_3^-		B. Palenik
II	541	RS9902[a]	Gulf of Aqaba 29°28′N, 34°55′E	1	20-3-99	1.9	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	542	RS9903[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	11-5-99	2.3	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	543	RS9904[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	14-6-99	2.1	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	546	RS9907[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	23-8-99	0.5	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	547	RS9908[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	7-9-99	0.5	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	549	RS9910[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	7-9-99	0.5	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	550	RS9911[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	11-5-99	1.0	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	551	RS9912[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	23-8-99	0.5	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II	558	RS9919[a]	Gulf of Aqaba 29°28′N, 34°55′E	50	22-11-99	1.9	−	NO_3^-, NH_4^+	+	N. Fuller, this study
II		WH 8002[a,e]	Gulf of Mexico 19°45′N, 92°25′W		15-4-80	0.48		NO_3^-, NH_4^+	+	J. Waterbury
II		WH 8109[a,e]	Sargasso Sea 39°29′N, 70°28′W		6-81	0.89		NO_3^-, NH_4^+		J. Waterbury
III		C129[a,c]	Gulf of Aqaba 29°28′N, 34°55′E	20	9-93	0.9[e]	+			D. Lindell and A. Post
III	30	Max42	Sargasso Sea 26°18′N, 63°26′W	120	9-10-87	1.4	−		+	D. Vaulot and C. Courties, this study
III	62	Minos12	Mediterranean Sea 34°0′N, 18°0′E	20	19-6-96	1.8	−		+	D. Vaulot, this study
III	311	Minos02[a]	Mediterranean Sea 39°10′N, 6°10′E	15	26-5-96	1.6	−		+	F. Partensky, this study
III	544	RS9905[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	18-7-99	1.3	−	NO_3^-, NH_4^+	+	N. Fuller, this study
III	554	RS9915[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	18-10-99	0.8	−	NO_3^-, NH_4^+	+	N. Fuller, this study
III		WH 8102[a,b,e]	Tropical Atlantic 22°30′N, 65°36′W		15-3-81	1.90	+	NO_3^-, NH_4^+	+	J. Waterbury
III		WH 8103[a,b,e]	Sargasso Sea 28°30′N, 67°24′N		17-3-81	1.3	+	NO_3^-, NH_4^+	+	J. Waterbury
V		UW01[a]				0.5	−	NO_3^-	+	K. Peeters
V		WH 7803[a,b,e]	Sargasso Sea 33°45′N, 67°30′W		1-7-78	0.5	−	NO_3^-, NH_4^+		J. Waterbury
VI		WH 7805[a,b,e]	Sargasso Sea 33°45′N, 67°30′W		30-6-78	No PUB		NO_3^-, NH_4^+	+	J. Waterbury
VI		WH8018[a,b,e]	Woods Hole, Mass.		6-80	No PUB	−	NO_3^-, NH_4^+	+	J. Waterbury
VII	37	Eum14	Tropical Atlantic 21°2′N, 31°8′W	105	1-10-91	2.2	−		+	F. Partensky, this study
VII	44	Oli31	Equatorial Pacific 5°60′S, 150°0′W	70	11-11-94	1.7	−		+	D. Vaulot, this study
VII		RS9920[a]	Gulf of Aqaba 29°28′N, 34°55′E	150	22-11-99	1.7	−	NO_3^-, NH_4^+		N. Fuller, this study
VIII	545	RS9906[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	23-8-99	NA[e]	−	NO_3^-, NH_4^+	−	N. Fuller, this study
VIII	548	RS9909[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	7-9-99	NA	−	NO_3^-, NH_4^+	−	N. Fuller, this study
VIII	552	RS9913[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	7-9-99	NA	−	NH_4^+	−	N. Fuller, this study
VIII	553	RS9914[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	18-10-99	NA	−	NH_4^+	−	N. Fuller, this study
VIII	556	RS9917[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	22-11-99	NA	−	NH_4^+	−	N. Fuller, this study
VIII	557	RS9918[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	22-11-99	NA	−	NO_3^-, NH_4^+	−	N. Fuller, this study
VIII		WH 8101[a,b,e]	Woods Hole, Mass.		1981	NA		NO_3^-, NH_4^+	−	J. Waterbury
IX	540	RS9901[a]	Gulf of Aqaba 29°28′N, 34°55′E	1	20-3-99	0.7	−	NO_3^-, NH_4^+	+	N. Fuller, this study
IX	555	RS9916[a]	Gulf of Aqaba 29°28′N, 34°55′E	10	22-11-99	0.7	−	NO_3^-, NH_4^+	+	N. Fuller, this study
IX	559	RS9921[a]	Gulf of Aqaba 29°28′N, 34°55′E	150	22-11-99	0.7	−	NO_3^-, NH_4^+	+	N. Fuller, this study
X	61	Minos11	Mediterranean Sea 34°0′N, 18°0′E	20	19-6-96	0.7	−		+	D. Vaulot, this study
X	309	Minos01[a]	Mediterranean Sea 39°10′N, 6°10′E	15	26-5-96	0.7	−		+	F. Partensky, this study
		NS01[a]	Central North Sea			NA	−	NO_3^-	−	G. Nieuwland
		WH 5701[a,e]	Long Island Sound		1957	NA		NO_3^-, NH_4^+	−	J. Waterbury





RFLP analysis was used here for rapid genetic screening of initial *Syn*. isolates by using the *ntcA* gene, which encodes the single, core global N regulator, amplified with general marine picocyanobacterial primers.

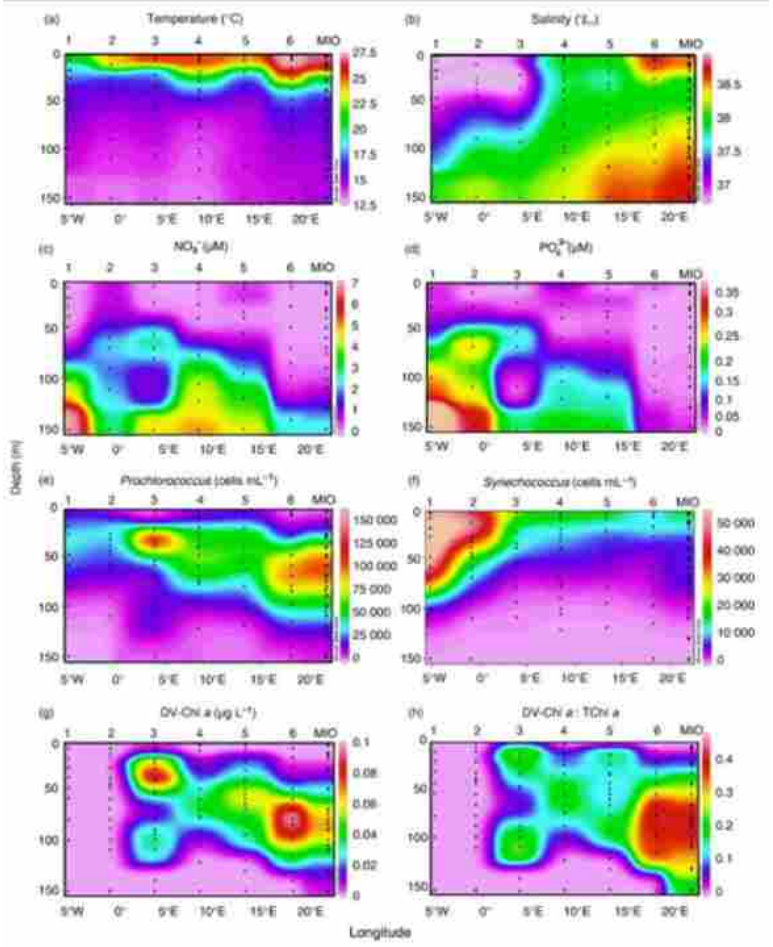
Molecular ecology approaches : RFLP



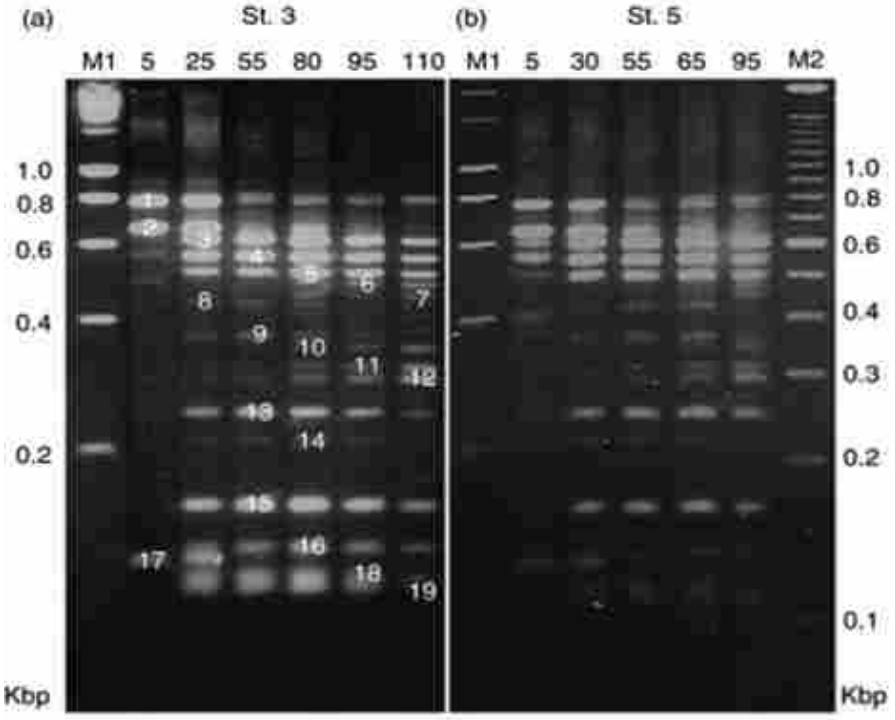
FEMS Microbiology Ecology, Volume 60, Issue 2, May 2007, Pages 189–206,

High vertical and low horizontal diversity of *Prochlorococcus* ecotypes in the Mediterranean Sea in summer

Laurence Garczarek ✉ , Alexis Dufresne , Sylvie Rousvoal , Nyree J. West , Sophie Mazard , Dominique Marie , Hervé Claustre , Patrick Raimbault , Anton F. Post , David J. Scanlan , Frédéric Partensky



A RFLP analysis of *pcb* gene amplicons digested by HaeIII revealed at least four genetically different *Proc*. ecotypes, occupying distinct but overlapping light niches in the photic zone. At comparable depths, similar banding patterns were observed throughout the sampled area, suggesting a horizontal homogenization of ecotypes. Yet, band sequencing revealed a large genetic microdiversity within those ecotypes.



Molecular ecological approaches : Barcoding and metabarcoding

DNA extracted from cultures or environmental samples



PCR amplification of selected marker (16S, ITS, *rpoC1*, …)

Cut DNA with restriction enzymes

Chromosomal DNA Fragment To Be Cloned

Vector DNA

Join vector and DNA

Plasmid 4 kb

Bacteriophage 20 kb

Cosmid 40 kb

BAC 200 kb

N x

Recombinant DNA Molecule

Insertion into *E. coli*

N x

Recombinant DNA Molecule

Bacterial Chromosome

Plating

Picking of recombinant bacteria

Sequencing, quality checking

Phylogenetic analysis

Identification of Operational taxonomic units (OTUs)

Phylogeny of Marine Picocyanobacterial Strains (16S rRNA)

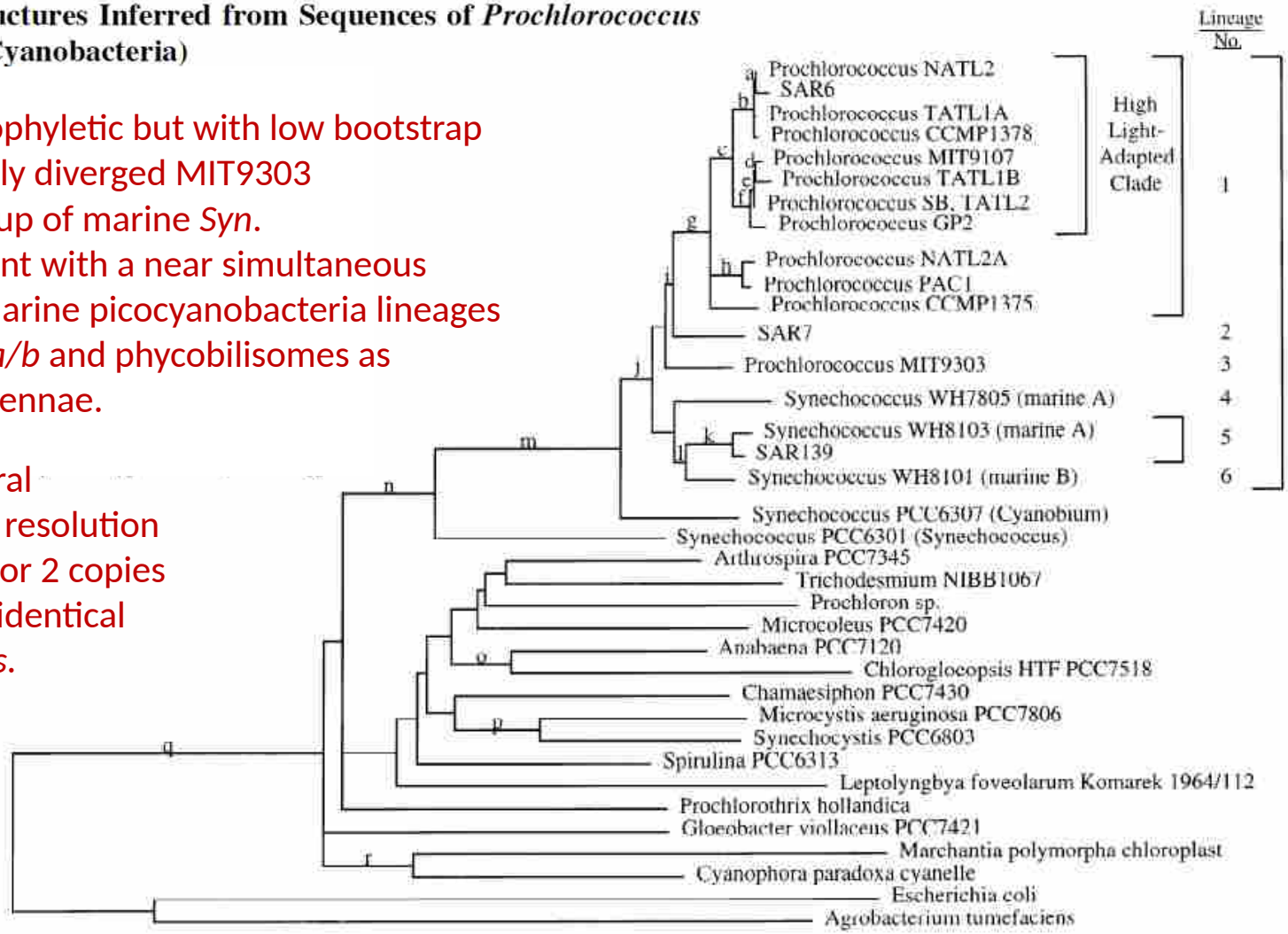
J Mol Evol (1998) 46:188–201

Ena Urbach,[1] David J. Scanlan,[2] Daniel L. Distel,[3] John B. Waterbury,[3] Sallie W. Chisholm[1]

Rapid Diversification of Marine Picophytoplankton with Dissimilar Light-Harvesting Structures Inferred from Sequences of *Prochlorococcus* and *Synechococcus* (Cyanobacteria)

Proc. lineage monophyletic but with low bootstrap support for the early diverged MIT9303
Proc. is a sister group of marine *Syn*.
=> Pattern consistent with a near simultaneous diversification of marine picocyanobacteria lineages having divinyl-Chl *a/b* and phycobilisomes as photosynthetic antennae.

16S rRNA has several drawbacks: it lacks resolution and is present in 1 or 2 copies in *Proc.* and 1 to 3 identical copies in most *Syns*.



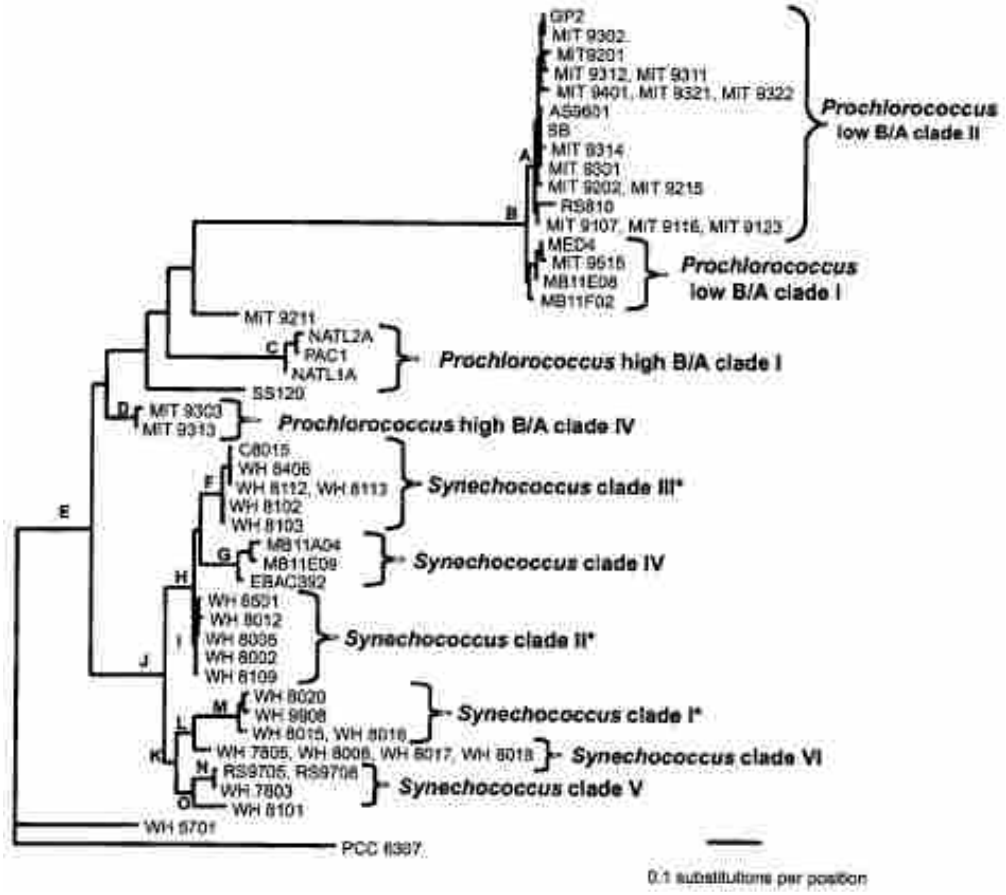
16S rRNA

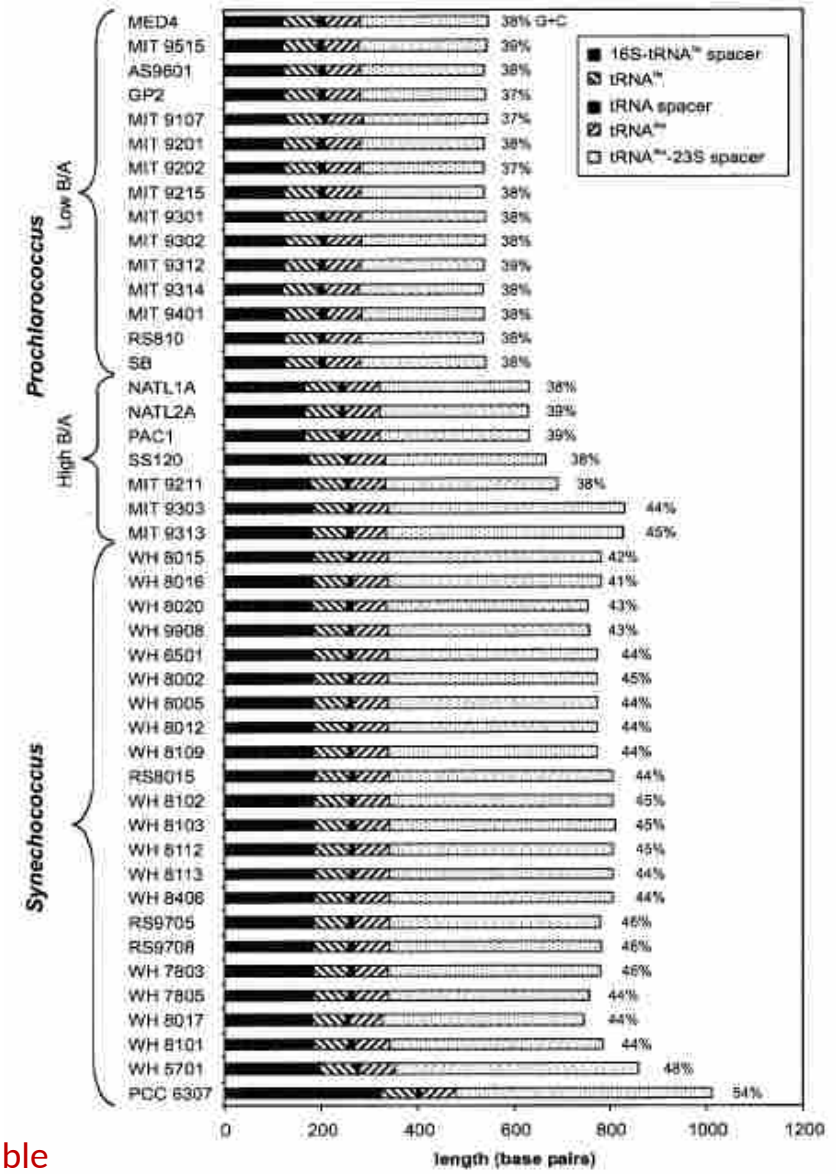
Phylogeny of Marine Picocyanobacterial Strains (ITS)

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2002, p. 1180–1191 Vol. 68, No. 3

Resolution of *Prochlorococcus* and *Synechococcus* Ecotypes by Using 16S-23S Ribosomal DNA Internal Transcribed Spacer Sequences

Gabrielle Rocap,[1]† Daniel L. Distel,[2] John B. Waterbury,[3] and Sallie W. Chisholm[4]





ITS has a much better resolution than 16S and in this study was able to distinguish 6 clades in both *Proc.* and *Syn.*

Yet, ITS length varies in length especially among *Syn.*, making it hard to align and inconvenient for large datasets.

Metabarcoding of Marine Picocyanobacteria in the Field (*rpoC1*)

Nature **396**, 226–228 (1998)

Niche adaptation in ocean cyanobacteria

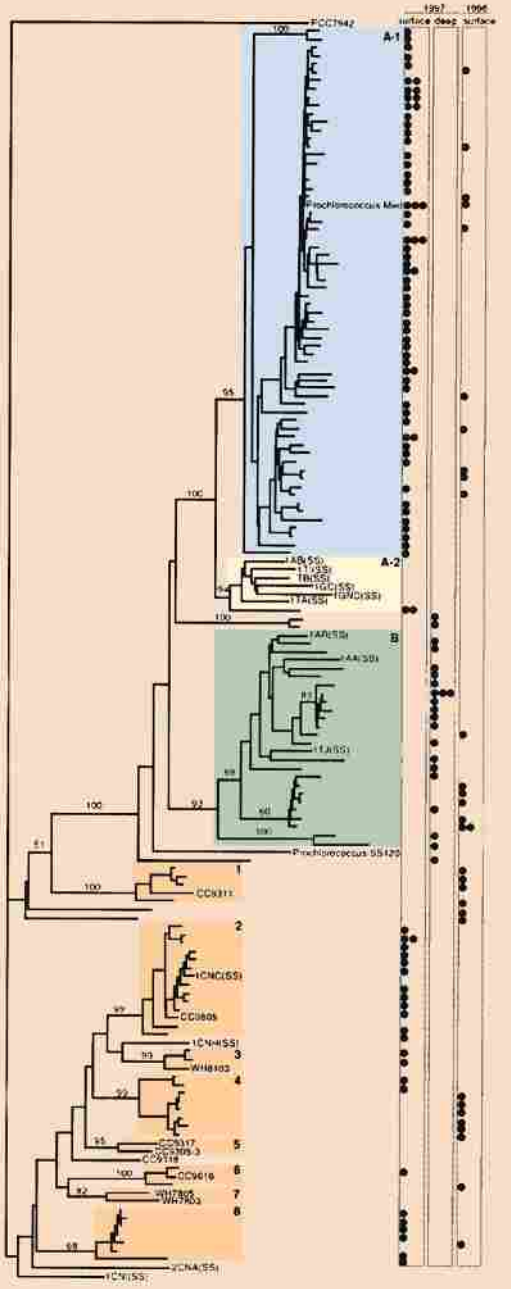
Michael J. Ferris & Brian Palenik

The 1st metabarcoding analysis dedicated to picocyanobacteria.

Tree based on PCR-amplified 270 nt fragments of the cyanobacterial DNA-dependent RNA polymerase C1 gene (*rpoC1*) from communities of bacteria inhabiting oligotrophic sites in the Pacific Ocean, off the coast of California and showed the exceptionally high genetic diversity occurring within field populations of both *Proc*. and *Syn*.

Introduced in 1992 by B. Palenik, *rpoC1* is a high resolution single core gene marker with sufficient variability to enable design of cyanobacteria-specific primers.

rpoC1 has been used in all picocyanobacteria diversity studies from this group (except the last one: Harding et al. 2025 Env Microbiol) and a few other groups.



Metabarcoding of Marine Picocyanobacteria in the Field (*petB*)

Environmental Microbiology (2012) 14(2), 372–386

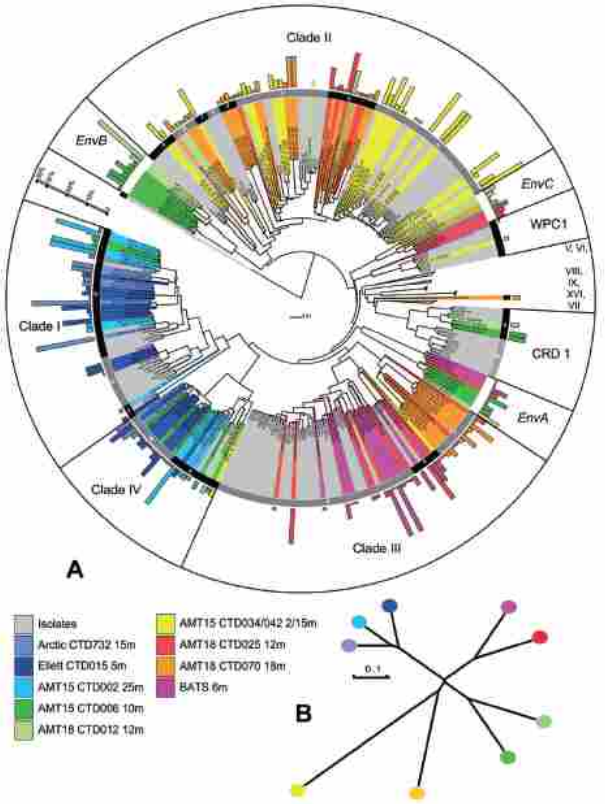
Multi-locus sequence analysis, taxonomic resolution and biogeography of marine *Synechococcus*

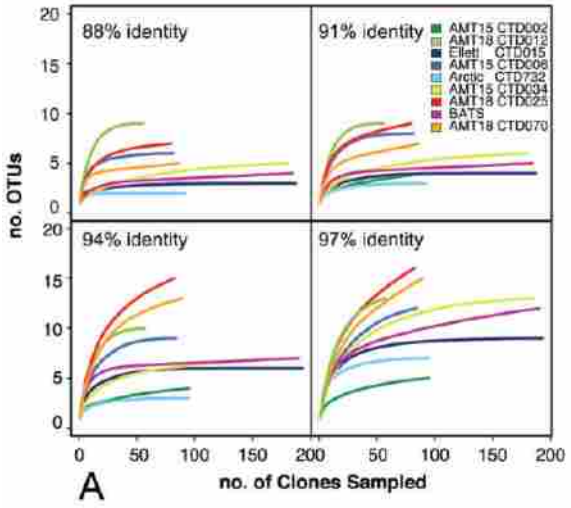
Sophie Mazard,* Martin Ostrowski,† Frédéric Partensky[2,3] and David J. Scanlan**

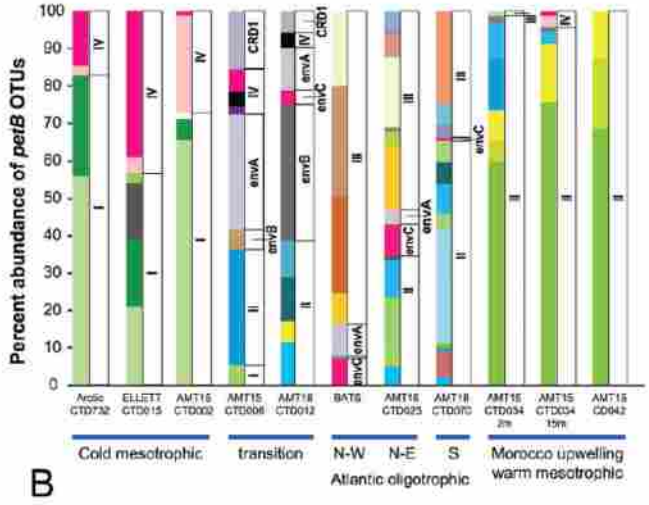
D. Scanlan's group developed its own high resolution, single core <u>*photosynthetic*</u> marker gene : *petB*, encoding the b_6 subunit of the cytochrome b_6/f complex.

This study highlighted the occurrence of several <u>within-clade</u> genotypes of *Syn*. that may represent 'ecotypes' adapted to distinct ecological niches.

=> Till then, *petB* has been used by Dave's and our own group for all further fine-scale diversity studies (see Course 2).







petB clone libraries were prepared from contrasting oceanic sites and analysed by RFLP analysis. The distinct RFLP types were counted and one or more representative of each type was sequenced.

At the level of 94% petB identity, rarefaction curves demonstrate that the *Syn*. population is well sampled so this is the cut-off retained to define OTU's.

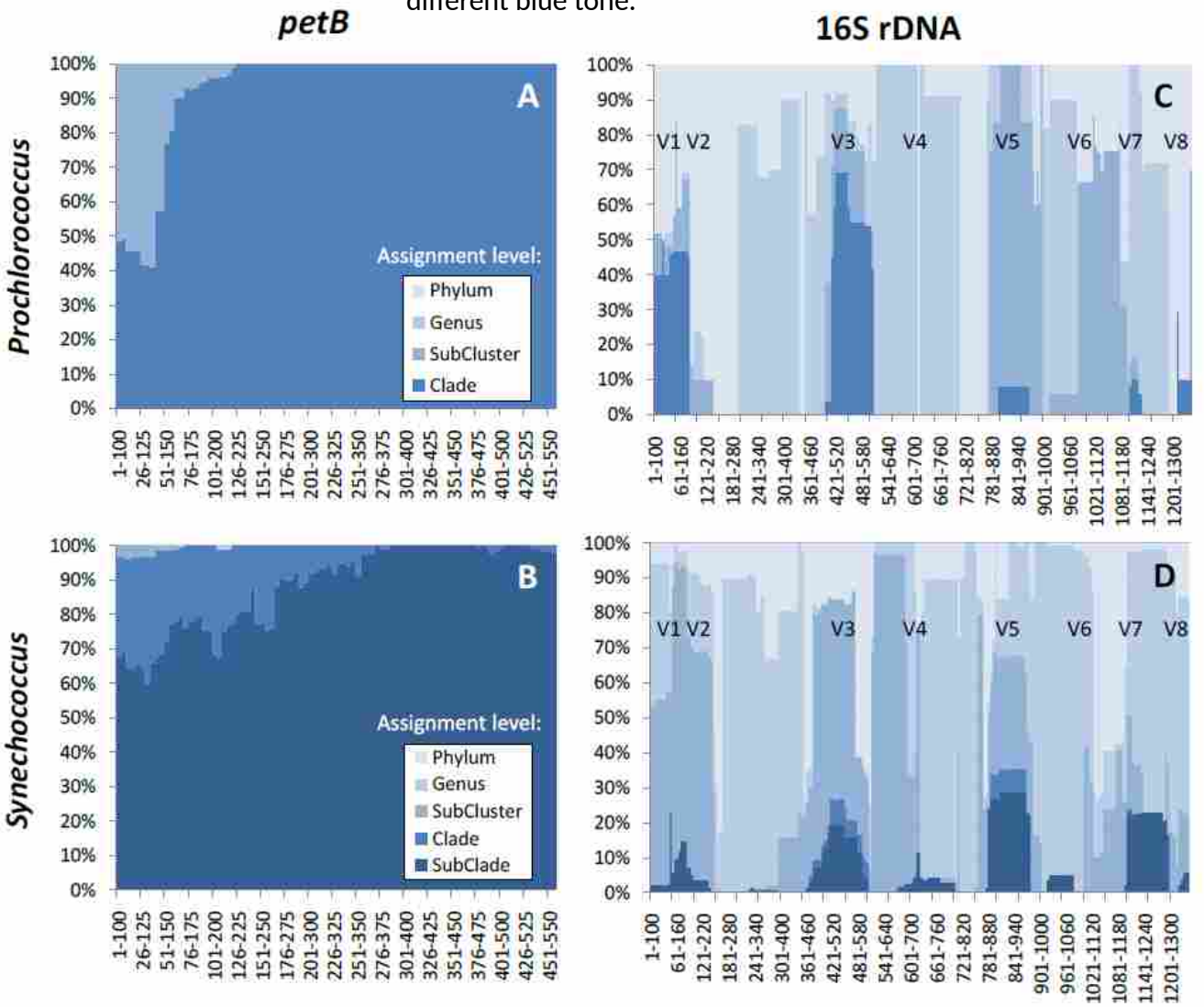
Comparison of taxonomic resolution between 16S rRNA and *petB*

PNAS June 2, 2016 | 113 (24) E3365-E3374

Delineating ecologically significant taxonomic units from global patterns of marine picocyanobacteria

Gregory K Farrant [1], Hugo Doré [1], Francisco M Cornejo-Castillo [2], Frédéric Partensky [1], Morgane Ratin [1], Martin Ostrowski [3], Frances D Pitt [4], Patrick Wincker [5], David J Scanlan [4], Daniele Iudicone [6], Silvia G Acinas [2], Laurence Garczarek [7]

Variation of the assignment ability of each individual 100 bp gene fragment along the sequence of *petB* (A-B) and 16S rRNA (C-D) genes using reference databases for *Proc.* (A,C) or *Syn.* (B-D). Simulated reads were generated by 100 bp sliding windows along the marker sequences and the lowest taxonomic level at which they could be assigned is shown by a different blue tone.



Molecular ecological approaches : Dot blot Hybridization

Environmental DNA sample

↓

PCR with 16S rDNA primers specific of cyanobacteria

↓

Immobilize 30 ng of product on membrane (in triplicate)

↓

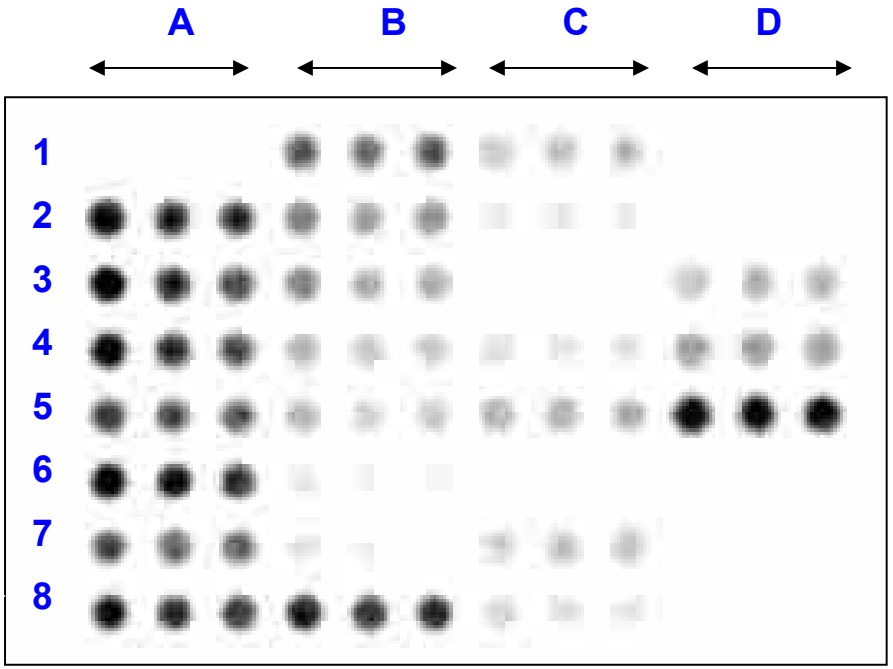
Hybridize ^{32}P-labelled probes specific of *Synechococcus* clades

↘

Calculate relative abundance of *Synechococcus* clades in the environment (% relative hybridization)

→

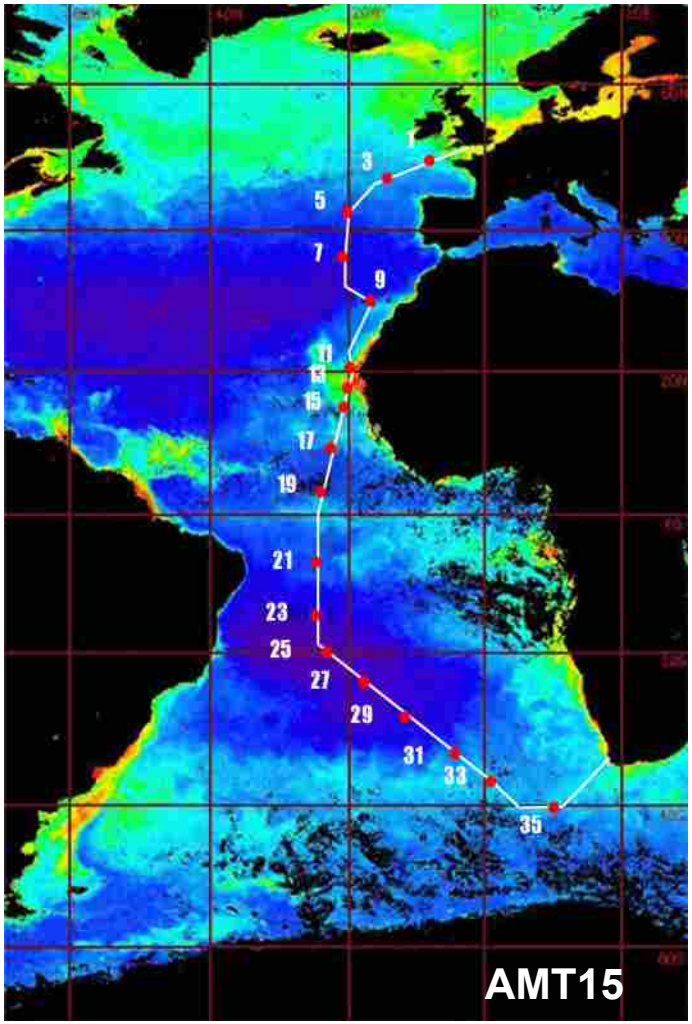
Detect quantity of specifically bound probe



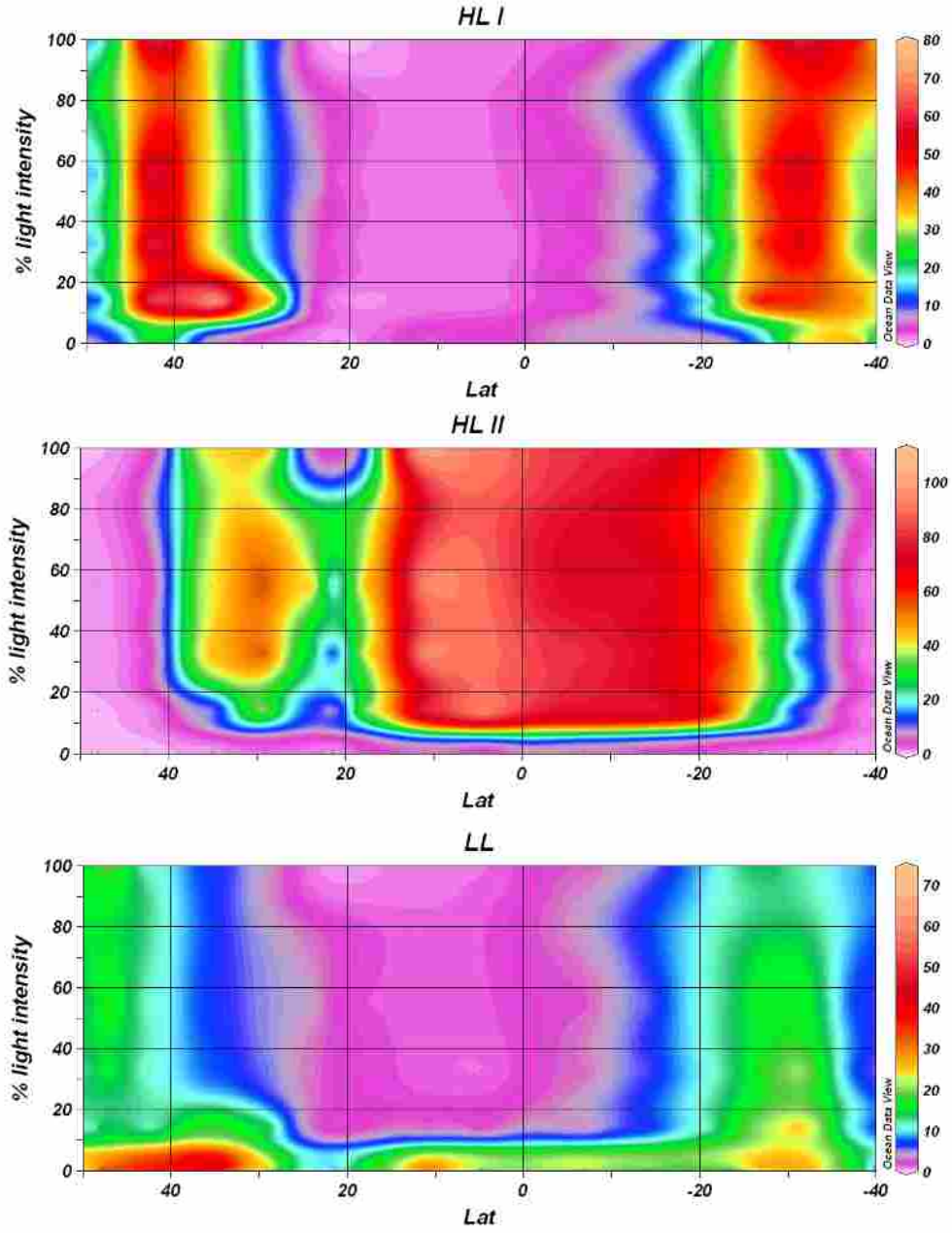
Slide courtesy of DJ Scanlan

Molecular ecological approaches : Dot blot Hybridization

Atlantic gyres



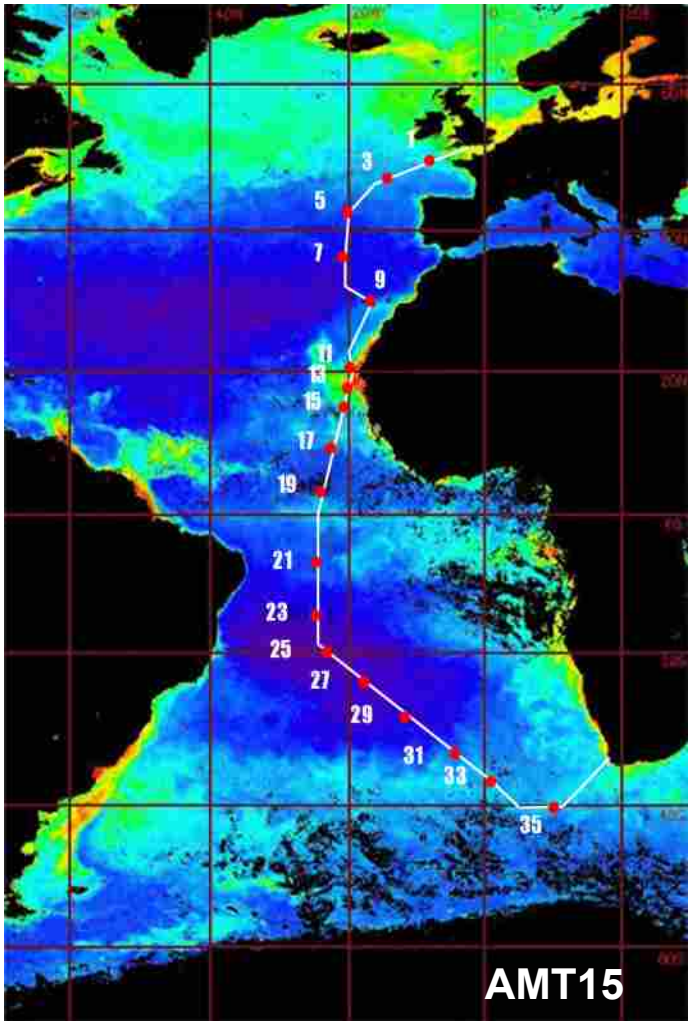
Prochlorococcus



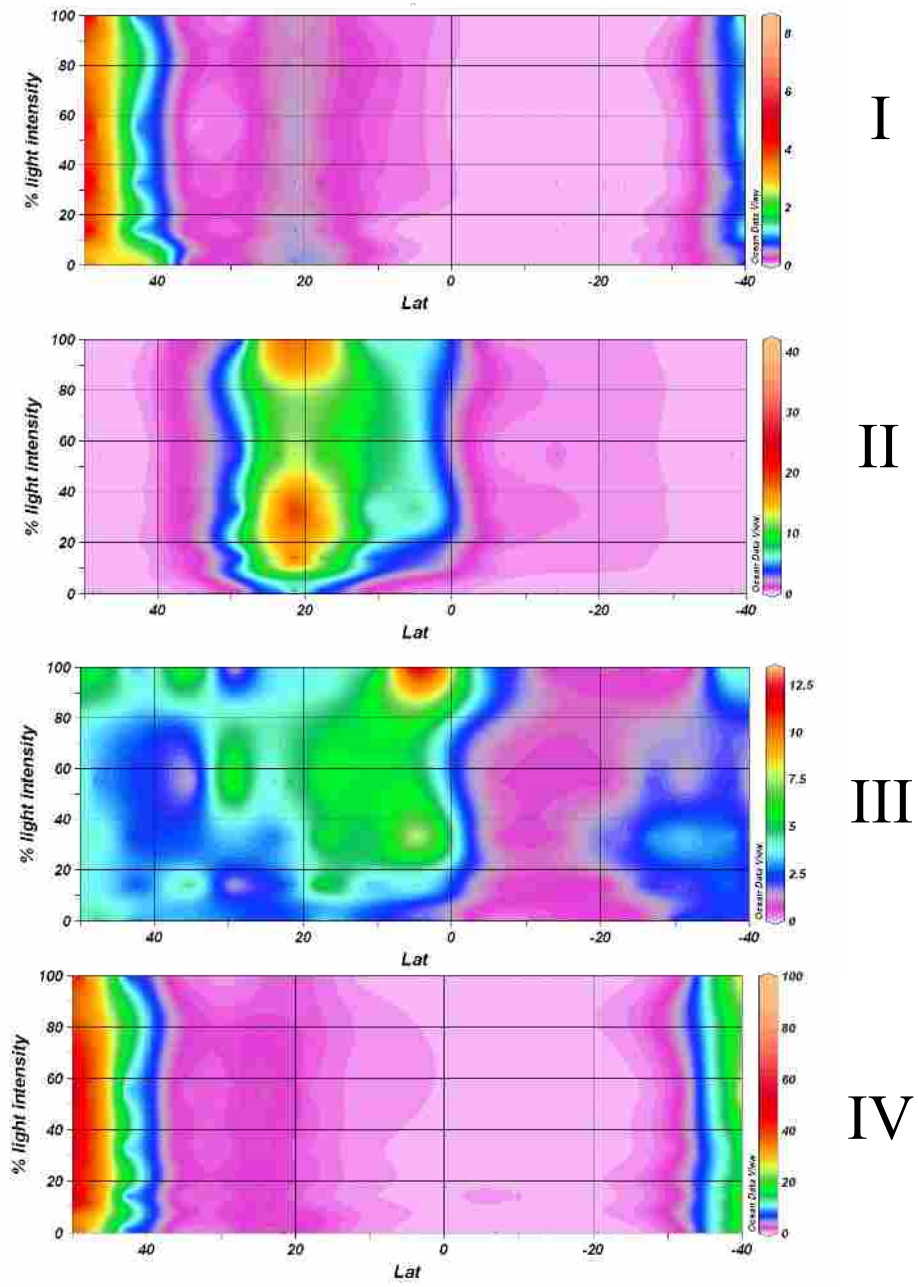
Zwirglmaier et al. 2007 *Environ. Microbiol.*

Molecular ecological approaches : Dot blot Hybridization

Atlantic gyres



Synechococcus



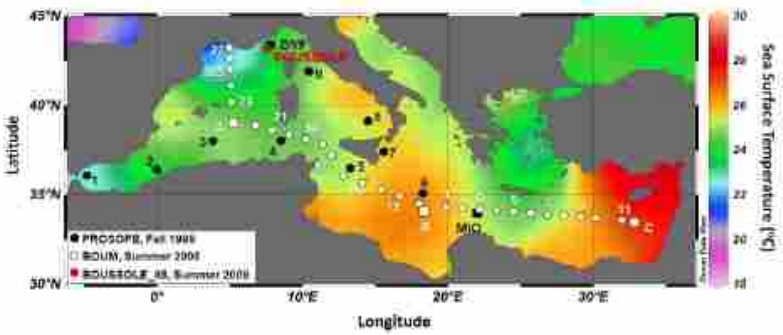
Zwirglmaier et al. 2007, *Environ. Microbiol.*

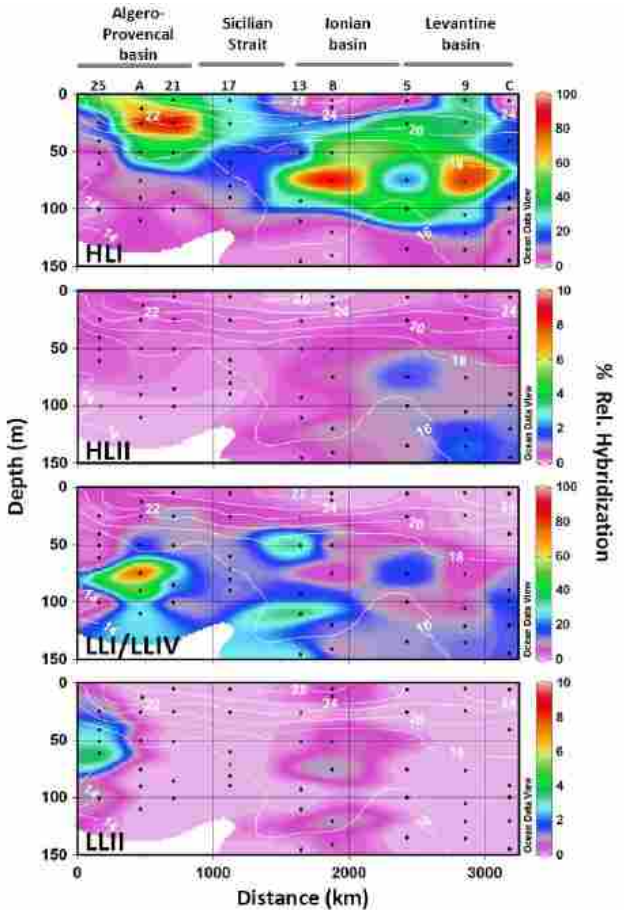
Molecular ecological approaches : Dot blot Hybridization

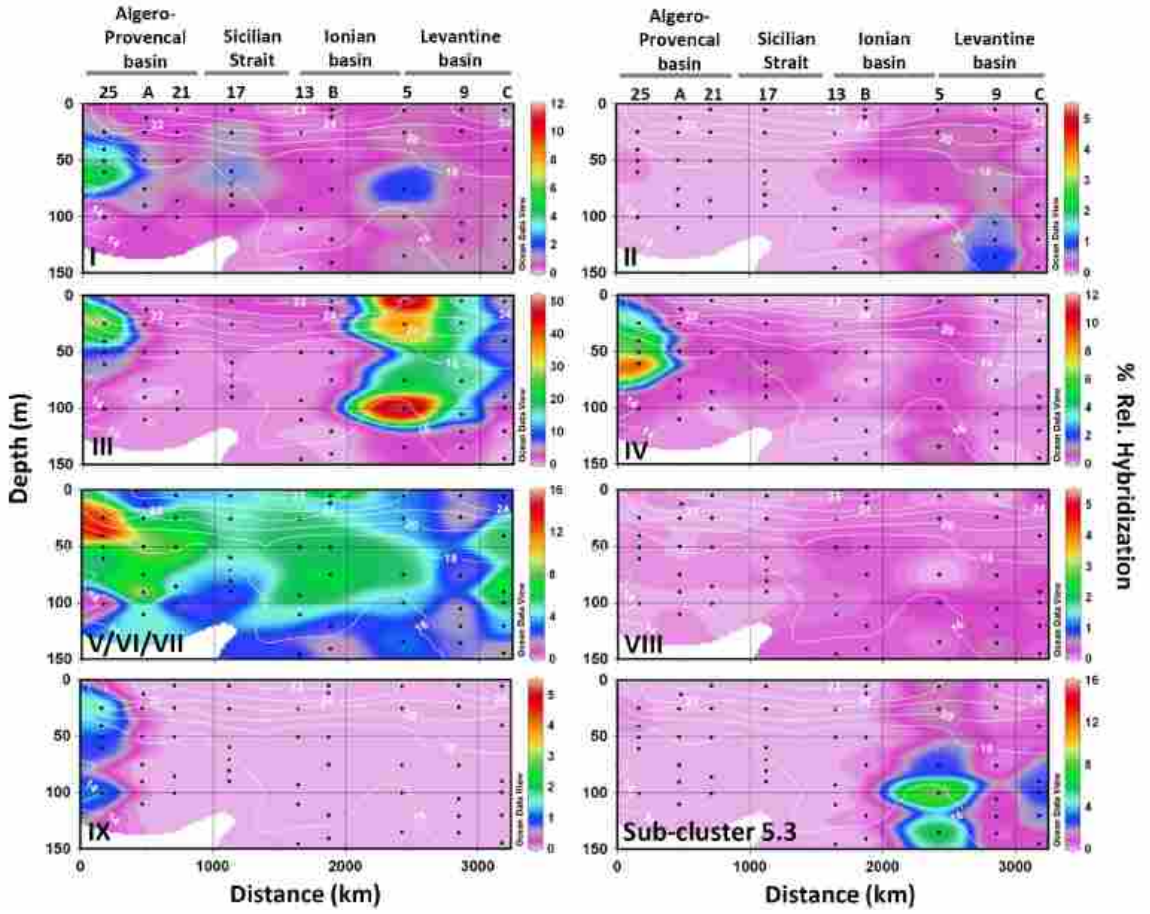
Biogeosciences, 8, 2785–2804, 2011

Is the distribution of *Prochlorococcus* and *Synechococcus* ecotypes in the Mediterranean Sea affected by global warming?

D. Mella-Flores[1,2,*], S. Mazard[3,4,*], F. Humily[1,2], F. Partensky[1,2], F. Mahé[1,2], L. Bariat[5], C. Courties[5], D. Marie[1,2], J. Ras[6], R. Mauriac[7], C. Jeanthon[1,2], E. Mahdi Bendif[1,2], M. Ostrowski[3,4], D. J. Scanlan[3], and L. Garczarek[1,2]







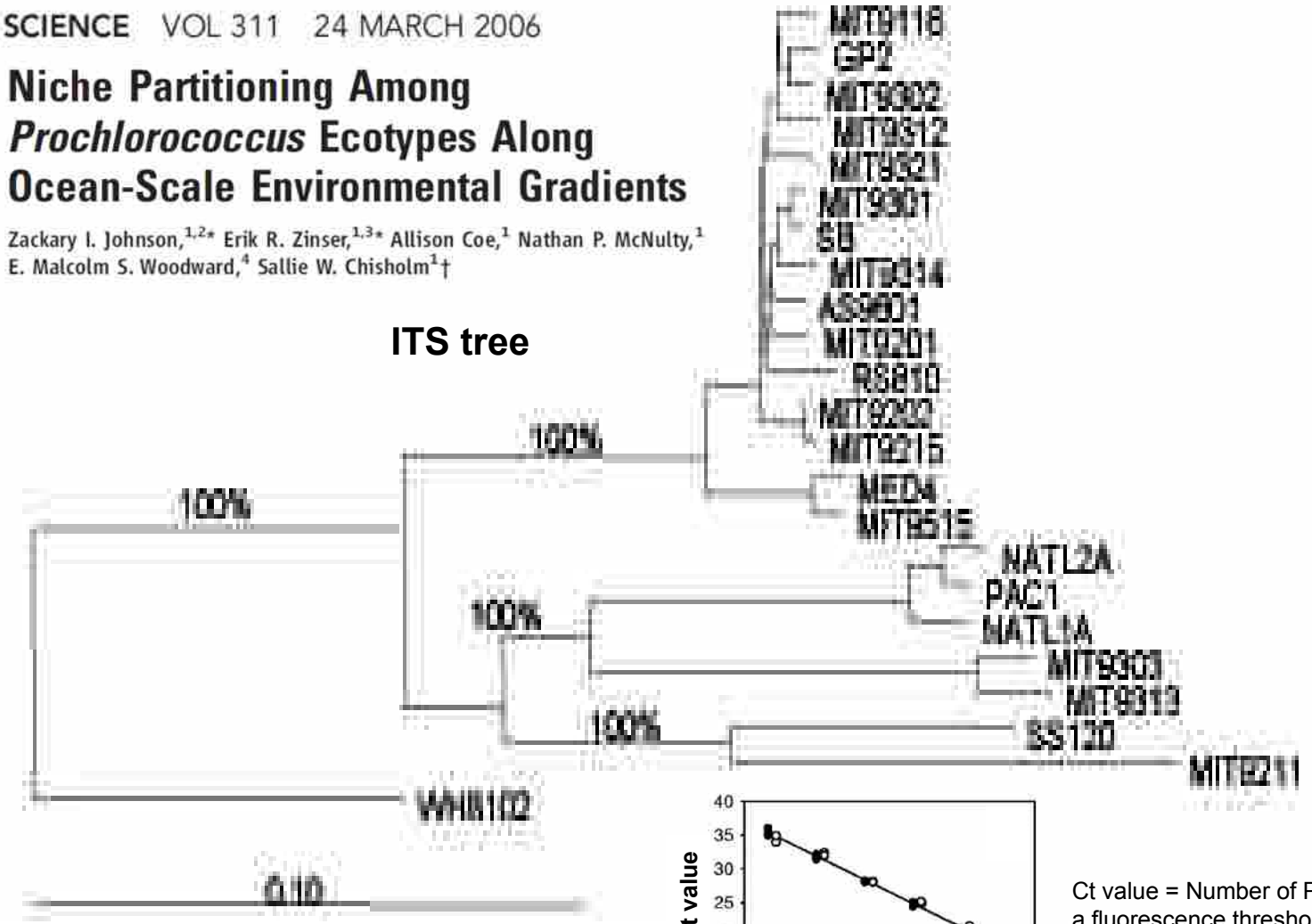
Molecular ecological approaches : Quantification of *Prochlorococcus* ecotypes by Real Time PCR

SCIENCE VOL 311 24 MARCH 2006

Niche Partitioning Among *Prochlorococcus* Ecotypes Along Ocean-Scale Environmental Gradients

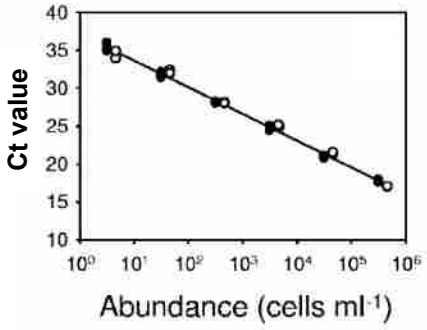
Zackary I. Johnson,[1,2]* Erik R. Zinser,[1,3]* Allison Coe,[1] Nathan P. McNulty,[1] E. Malcolm S. Woodward,[4] Sallie W. Chisholm[1]†

ITS tree



Probes designed for each ecotype

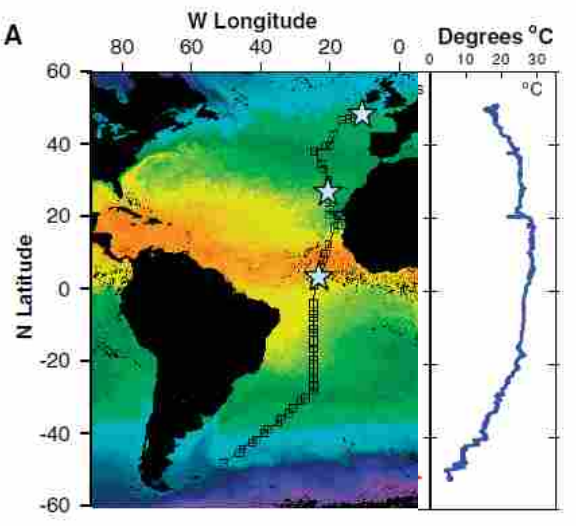




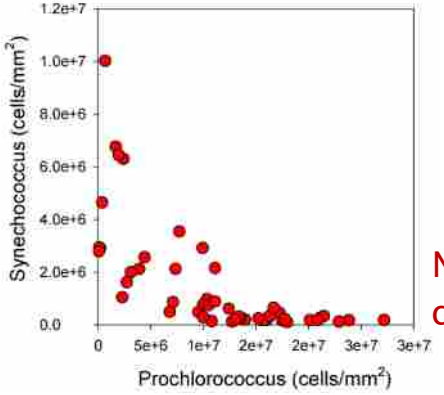
Ct value = Number of PCR cycles necessary to reach a fluorescence threshold crossing all amplification plots in the middle of the exponential phase of amplification.

Molecular ecological approaches : Quantification of *Prochlorococcus* ecotypes by Real Time PCR

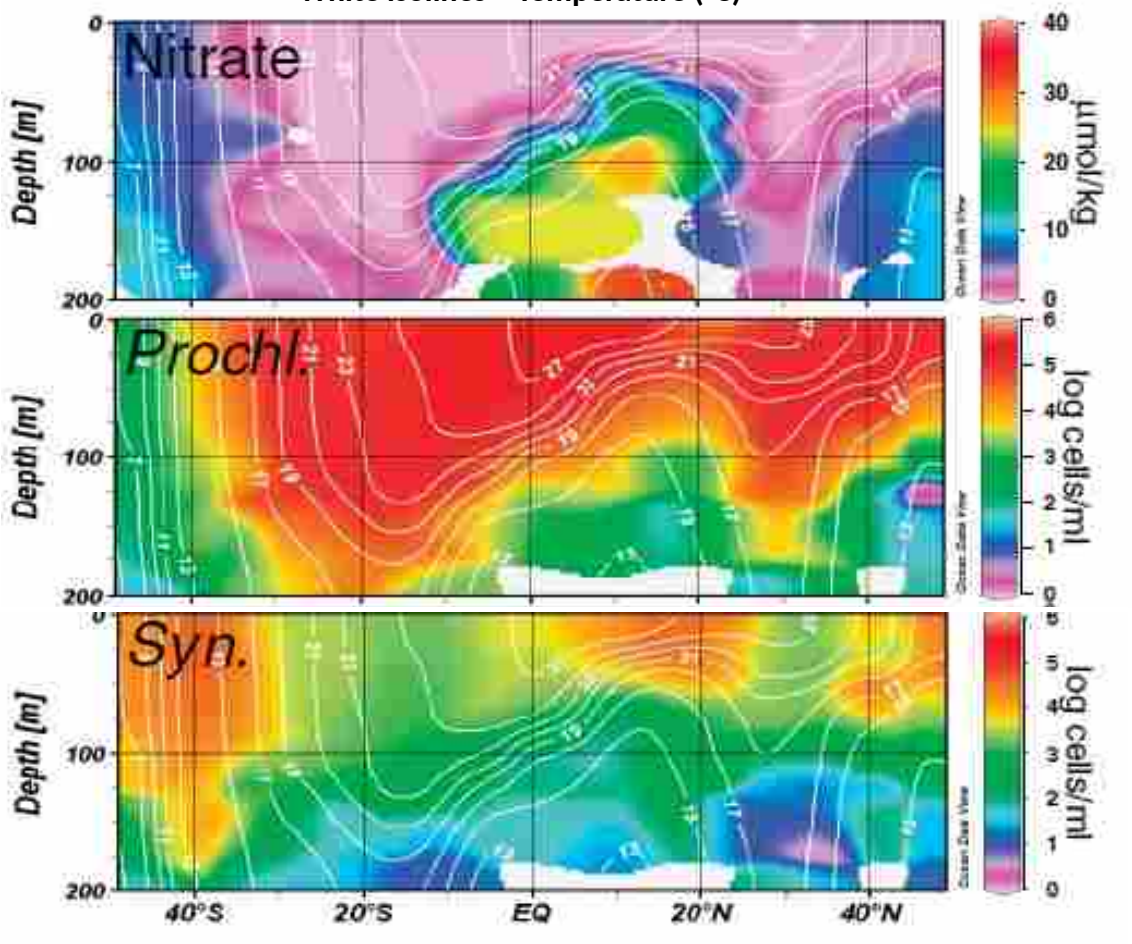
AMT13 cruise Sept-Oct 2003



Δ surface Temperature along the transect: 5-29 °C



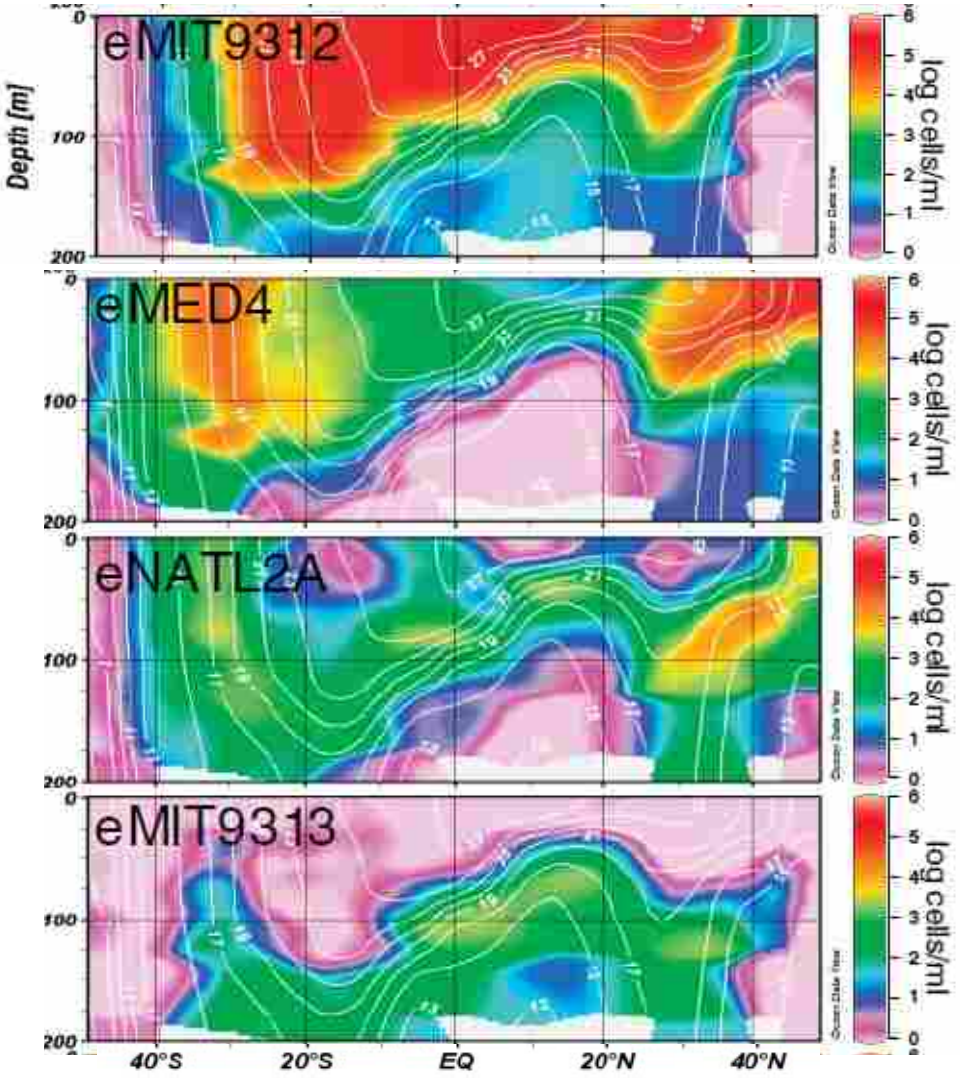
White isolines = Temperature (°C)



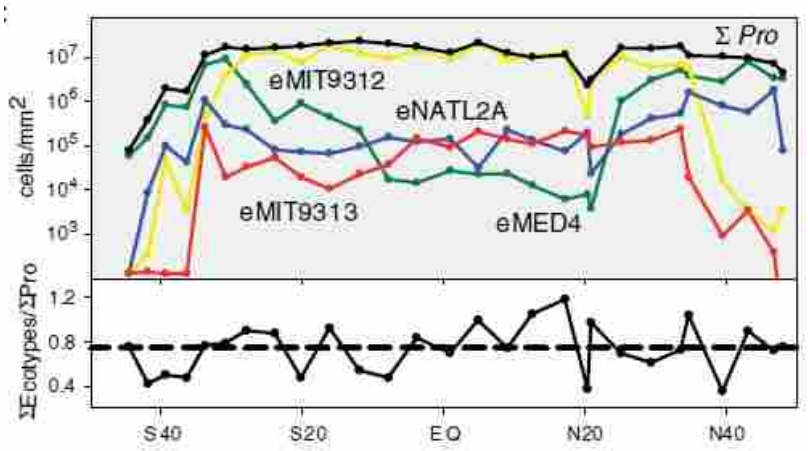
Mutual exclusion *of Proc.* and *Syn.* at high concentrations.

Johnson et al. 2006 *Science*

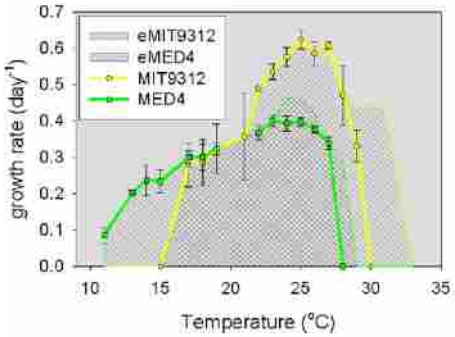
Molecular ecological approaches : Quantification of *Prochlorococcus* ecotypes by Real Time PCR: Spatial distribution



Johnson et al. 2006 *Science*



eMIT9312 (HLII) largely dominate in the upper layer in (sub)tropical areas and eMED4 (HLI) in temperate waters.



eNATL2 (LLI) occur at intermediate depth and can reach the surface when the water is mixed.

eMIT9313 (LLIV) dominate in the deep layer

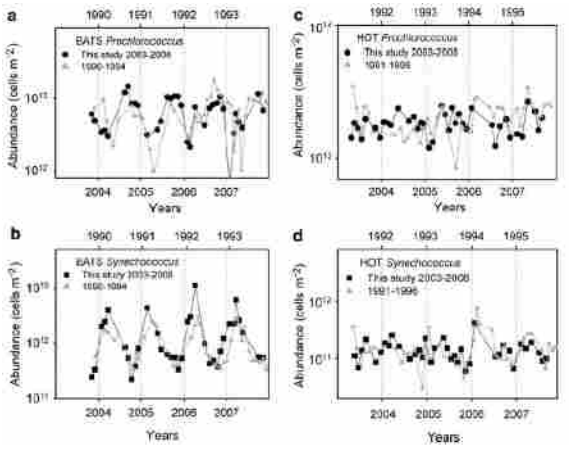
eSS120 (LLII) and eMIT9211 (LLIII) virtually absent: possible problem of probe design.

Molecular ecological approaches : Quantification of *Prochlorococcus* ecotypes by Real Time PCR: Seasonal variations

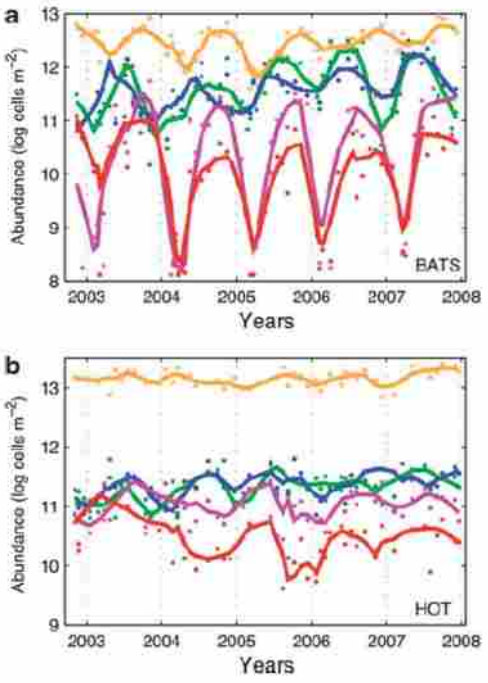
The ISME Journal (2010), 1–13

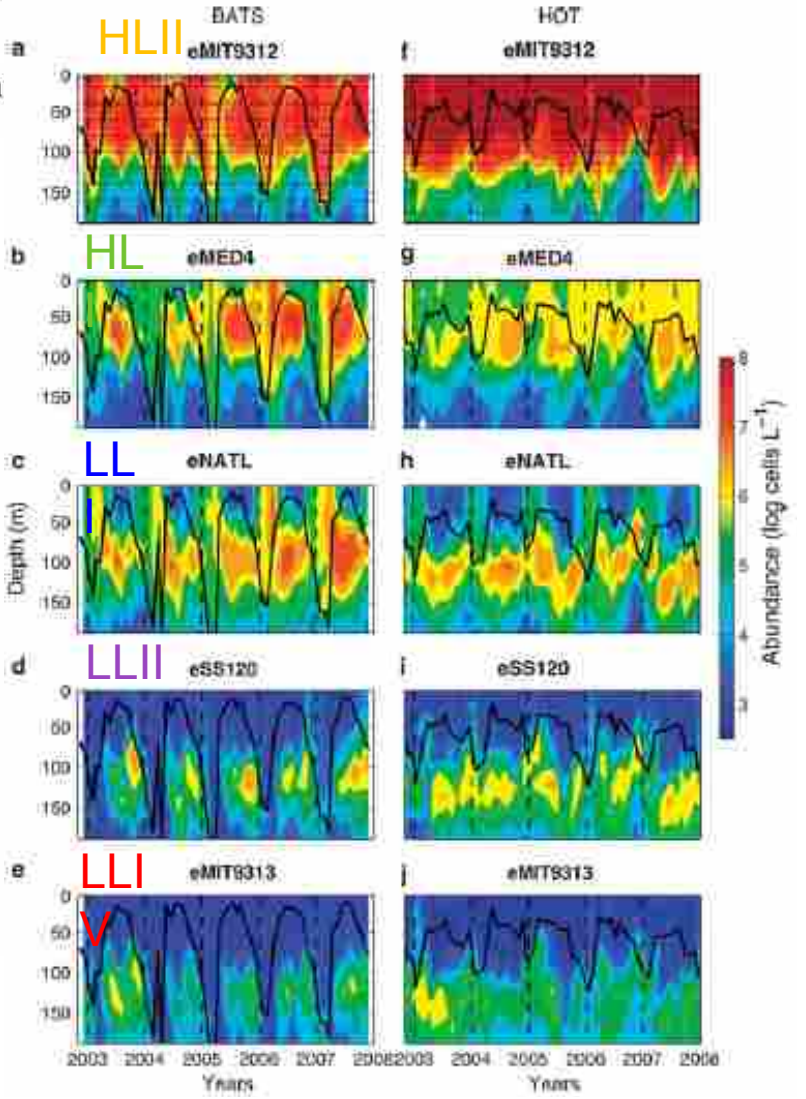
Temporal dynamics of *Prochlorococcus* ecotypes in the Atlantic and Pacific oceans

Rex R. Malmstrom[1], Allison Coe[1], Gregory C. Kettler[2], Adam C Martin
Jorge Frias-Lopez[1,4], Erik R Zinser[1,5] and Sallie W Chisholm[1]



HLII HLI LLI LLII LLIV



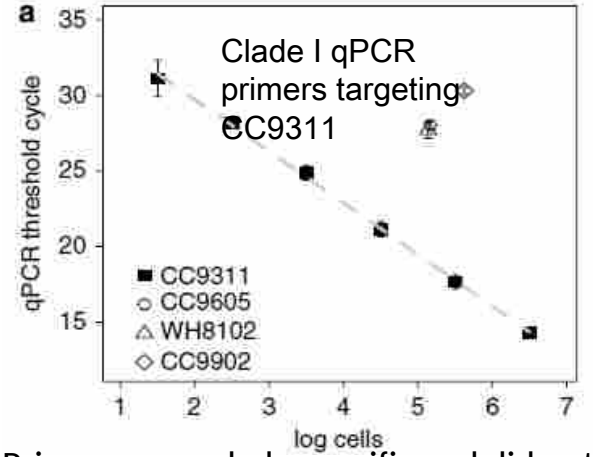


Molecular ecological approaches : Quantification of *Synechococcus* ecotypes by Real Time PCR: Seasonal variations

The ISME Journal (2009), 1–13

Temporal variation of *Synechococcus* clades at a coastal Pacific Ocean monitoring site

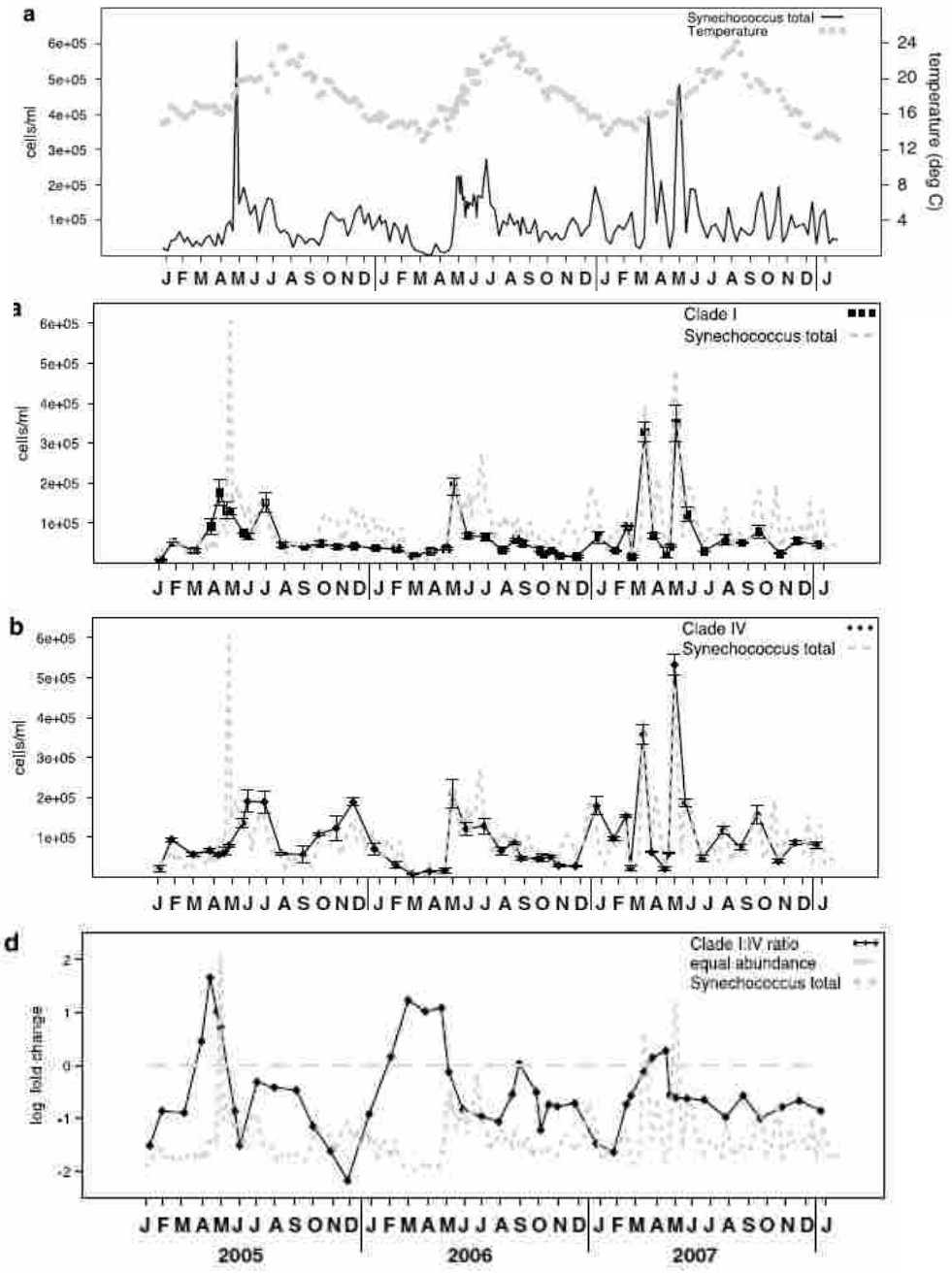
Vera Tai and Brian Palenik



Primers were clade specific and did not amplify DNA from any non-target strain.

Clades I and IV co-dominant throughout the time series and their relative abundance followed a seasonal cycle. Clade IV was typically more abundant, but clade I dominated during periods just before the annual spring bloom of *Syn*.

Clades II and III were absent during spring and early summer, but appeared at low abundances in late summer and winter.



Prochlorococcus and *Synechococcus* : The first genomes !

In **April 2003**, the **human genome** consortium announced that it had generated an essentially complete genome sequence, but the sequencing phase actually stopped a couple of years earlier, leaving hundreds of vacant sequencers …

letters to nature

NATURE | VOL 424 | 28 AUGUST 2003 |

The genome of a motile marine *Synechococcus*

B. Palenik[1], B. Brahamsha[1], F. W. Larimer[2,3], M. Land[2,3], L. Hauser[2,3], P. Chain[3,4], J. Lamerdin[3,4], W. Regala[3,4], E. E. Allen[1,5], J. McCarren[1], I. Paulsen[3], A. Dufresne[6], F. Partensky[6], E. A. Webb[7] & J. Waterbury[7]

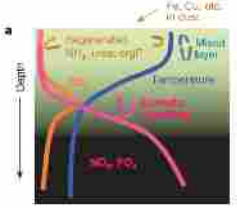
Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem

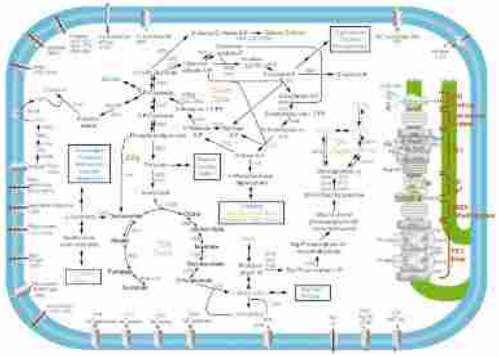
T. S. Bibby[1], I. Mary[2], J. Nield[1], F. Partensky[2] & J. Barber[1]

Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation

Gabrielle Rocap[1], Frank W. Larimer[2,3], Jane Lamerdin[3], Stephanie Malfatti[3], Patrick Chain[3,4], Nathan A. Ahlgren[1], Andrae Arellano[5], Maureen Coleman[2], Loren Hauser[2,3], Wolfgang R. Hess[6]*, Zackary I. Johnson[5], Miriam Land[2,3], Debbie Lindell[5], Anton F. Post[10], Warren Regala[3], Manesh Shah[2,3], Stephanie L. Shaw[6]*, Claudia Steglich[6], Matthew B. Sullivan[7], Claire S. Ting[8], Andrew Tolonen[7], Eric A. Webb[11], Erik R. Zinser[5] & Sallie W. Chisholm[5,8]

'Not just the genomes but the genomes in context. We are trying to understand how the genome content reflects the environment in which the different *Proc.* live' Penny Chisholm





10020–10025 | PNAS | August 19, 2003 | vol. 100 | no. 17

Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome

Alexis Dufresne*, Marcel Salanoubat†, Frédéric Partensky*‡, François Artiguenave†, Ilka M. Axmann§, Valérie Barbe†, Simone Duprat†, Michael Y. Galperin¶, Eugene V. Koonin¶, Florence Le Gall*, Kira S. Makarova¶, Martin Ostrowski‖, Sophie Oztas†, Catherine Robert†, Igor B. Rogozin¶, David J. Scanlan‖, Nicole Tandeau de Marsac**, Jean Weissenbach†, Patrick Wincker†, Yuri I. Wolf¶, and Wolfgang R. Hess§††

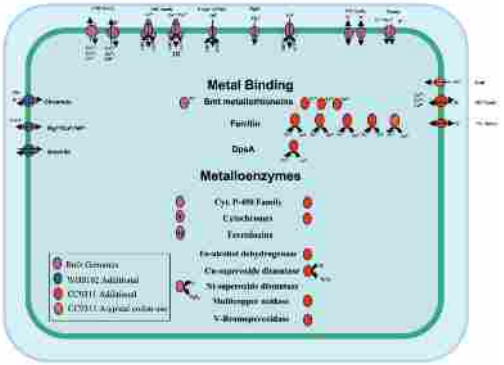
Prochlorococcus and *Synechococcus* : more and more genomes !

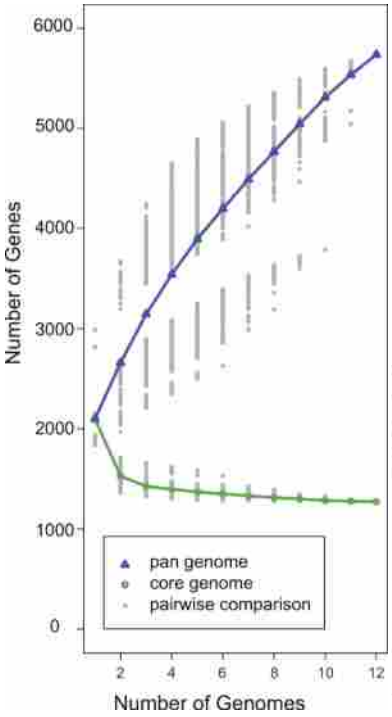
PNAS | September 5, 2006 | vol. 103 | no. 36 | 13555–13559

Genome sequence of *Synechococcus* CC9311: Insights into adaptation to a coastal environment

Brian Palenik*, Qinghu Ren†, Chris L. Dupont*, Garry S. Myers†, John F. Heidelberg†, Jonathan H. Badger†, Ramana Madupu†, William C. Nelson†, Lauren M. Brinkac†, Robert J. Dodson†, A. Scott Durkin†, Sean C. Daugherty†, Stephen A. Sullivan†, Hoda Khouri†, Yasmin Mohamoud†, Rebecca Halpin†, and Ian T. Paulsen†‡

1 more *Synechococcus* genome in 2006 …





PLOS Genetics

December 2007 | Volume 3 | Issue 12 | e231

Patterns and Implications of Gene Gain and Loss in the Evolution of *Prochlorococcus*

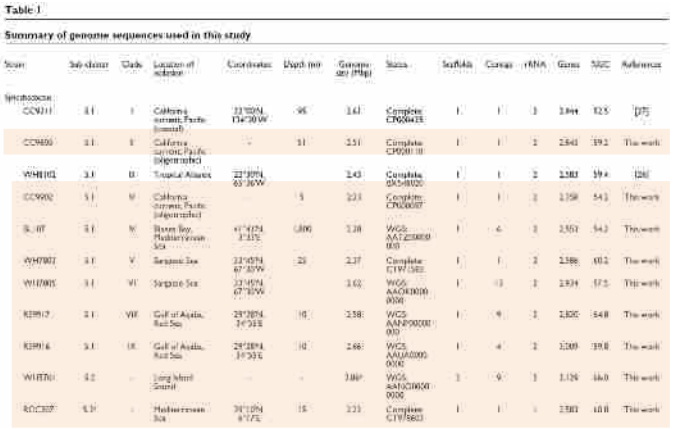
Gregory C Kettler, Adam C Martiny, Katherine Huang, Jeremy Zucker, Maureen L Coleman, Sebastien Rodrigue, Feng Chen, Alla Lapidus, Steven Ferriera, Justin Johnson, Claudia Steglich, George M Church, Paul Richardson, Sallie W Chisholm

8 more *Prochlorococcus* genomes in 2007…

Table 1. General Characteristics of the *Prochlorococcus* and *Synechococcus* Isolates Used in This Study

Cyanobacterium	Isolate	Light Adaptation	Length (bp)	GC %	Number of Genes*	Isolation Depth	Region	Date	Accession Number	Reference
Prochlorococcus	MED4	HL(I)	1,657,990	30.8	1,929	5m	Med. Sea	Jan. 1989	BX548174	[7,38]
	MIT9515†	HL(I)	1,704,176	30.8	1,908	15m	Eq. Pacific	Jun. 1995	CP000552	[18]
	MIT9301†	HL(II)	1,642,773	31.4	1,907	90m	Sargasso Sea	Jul. 1993	CP000576	[18]
	AS9601†	HL(II)	1,669,886	31.3	1,926	50m	Arabian Sea	Nov. 1995	CP000551	[21]
	MIT9215†	HL(II)	1,738,790	31.1	1,989	5m	Eq. Pacific	Oct. 1992	CP000825	[19]
	MIT9312	HL(II)	1,708,204	31.2	1,962	135m	Gulf Stream	Jul. 1993	CP000111	[4,60]
	NATL1A†	LL(I)	1,864,731	35.1	2,201	30m	N. Atlantic	Apr. 1990	CP000553	[20]
	NATL2A†	LL(I)	1,842,899	35	2,158	10m	N. Atlantic	Apr. 1990	CP000095	[22]
	SS120	LL(II)	1,751,080	36.4	1,925	120m	Sargasso Sea	May 1988	AE017126	[8,26]
	MIT9211†	LL(III)	1,688,963	38	1,855	83m	Eq. Pacific	Apr. 1992	CP000878	[19]
	MIT9303†	LL(IV)	2,682,807	50.3	3,022	100m	Sargasso Sea	Jul. 1992	CP000554	[4]
	MIT9313	LL(IV)	2,410,873	50.7	2,843	135m	Gulf Stream	Jul. 1992	BX548175	[4,27]
Synechococcus	CC9311	Syn.	2,606,748	52.5	3017	95m	Calif. Current	1993	CP000435	[9]
	CC9902	Syn.	2,234,828	54.2	2504	5m	Calif. Current	1999	CP000097	Palenik, unpublished data
	WH8102	Syn.	2,434,428	59.4	2782		Sargasso Sea	Mar. 1981	BX548020	[2,36]
	CC9605	Syn.	2,510,659	59.2	2991	51m	Calif. Current	1996	CP000110	Palenik, unpublished data

As of today, >2000 *Proc.* & *Syn.* genomes are available in public databases!



… and 9 more *Syn.* genomes in 2008.

Genome Biology 2008, **9**:R90

Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria

Alexis Dufresne*†, Martin Ostrowski*‡, David J Scanlan‡, Laurence Garczarek*, Sophie Mazard‡, Brian P Palenik§, Ian T Paulsen¶, Nicole Tandeau de Marsac¥, Patrick Wincker#, Carole Dossat#, Steve Ferriera**, Justin Johnson**, Anton F Post††, Wolfgang R Hess‡‡ and Frédéric Partensky*

Croucher course 1 outline

I. 1979-1999: The golden age of fluorescence techniques

II. 1996-2003: Characterization of the original light- harvesting complex of *Prochlorococcus*

III. 1998: The advent of the molecular ecology of picocyanobacteria

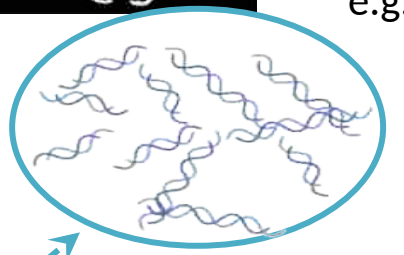
IV. 2003-on: The omics era: an introduction

The Metagenomic



METABARCODE (Tags)

e.g. 16S, *rpoC1*, *petB*

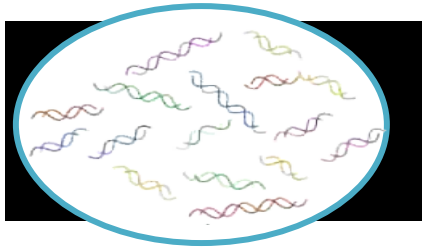


PCR amplification
& sequencing

METAGENOME
METATRANSCRIPTOME



DNA/RNA
extraction

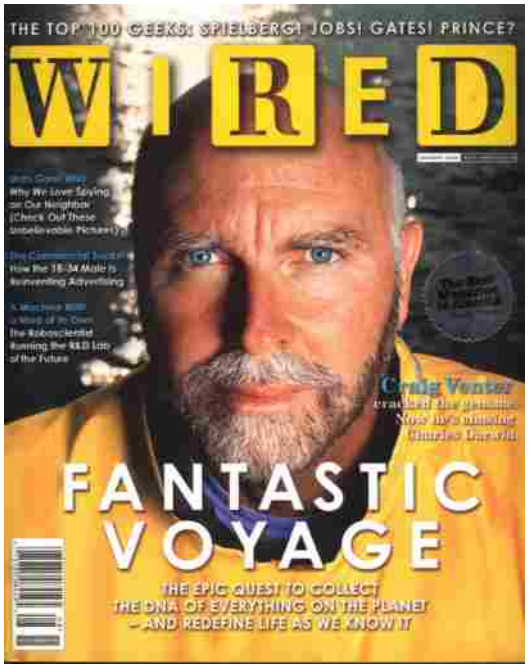


Complete
sequencing

Any gene from any (fairly abundant) organism

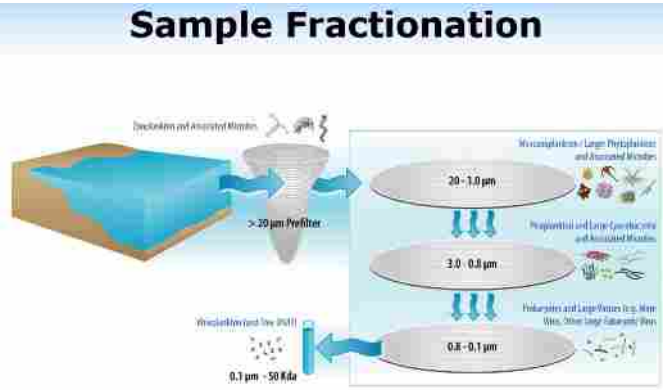
Microbial community

The metagenomics revolution was boosted by two major expeditions





After a pilot project of shotgun metagenomics sequencing at the BATS site in 2003, J. Craig Venter, inspired by the voyages of Darwin on the H.M.S. Beagle, launched the Sorcerer II Global Ocean Sampling (GOS) Expedition, which circumnavigated the globe for several years (2003-5, 2007, then 2009-10), sampling surface waters.



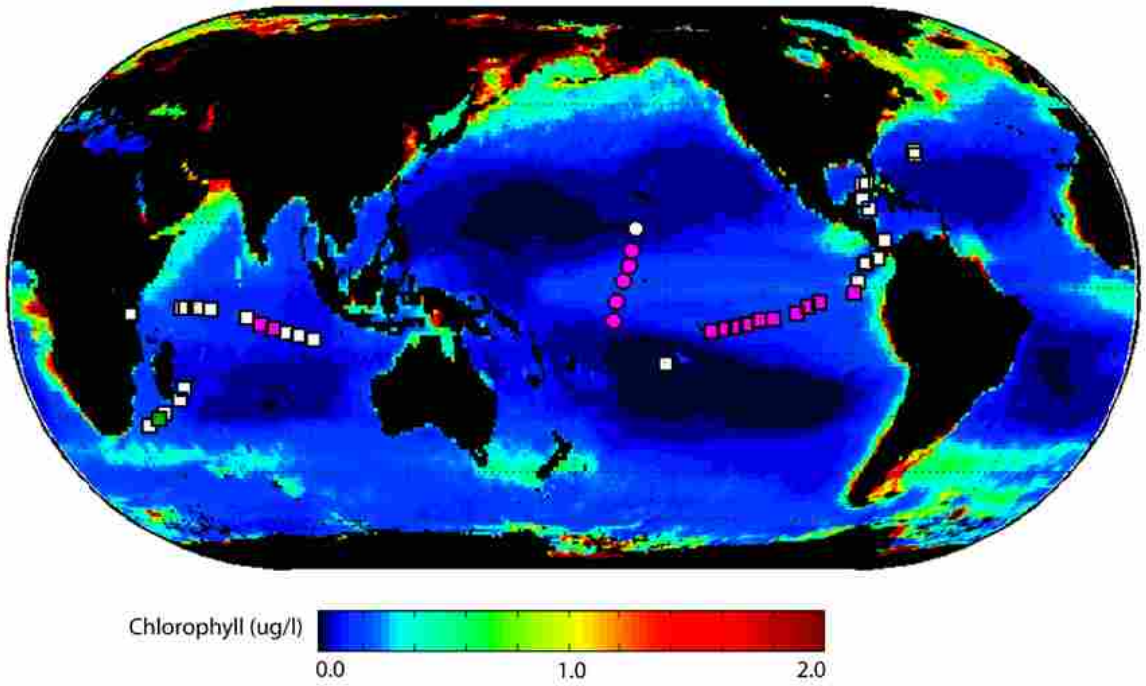


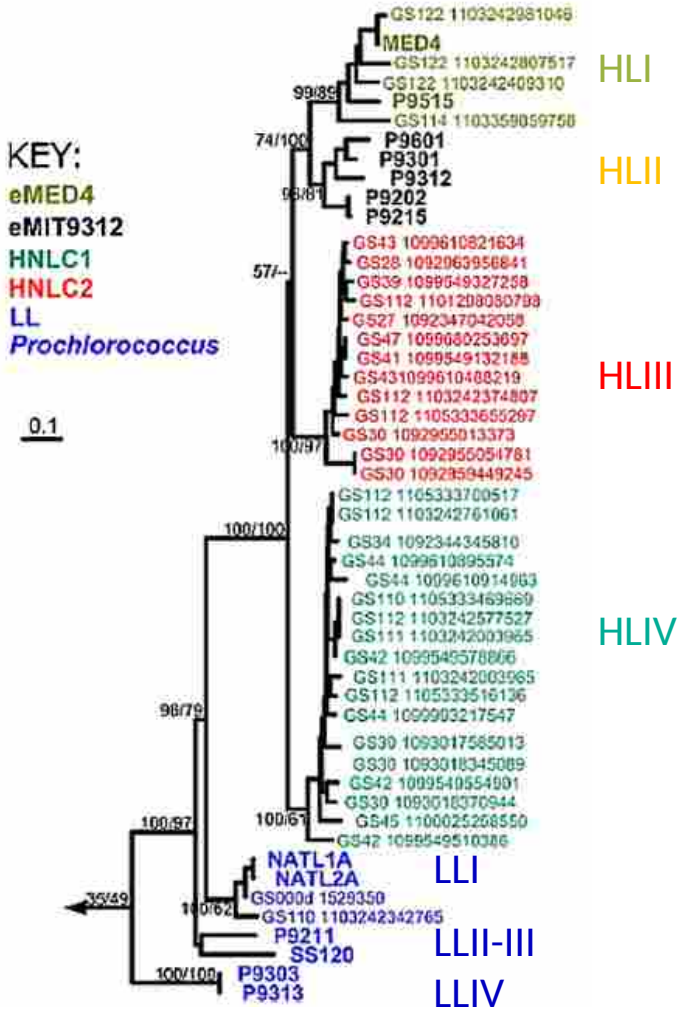
Unveiling uncultured *Prochlorococcus* clades adapted to low-iron areas

16184–16189 | PNAS | September 14, 2010 | vol. 107 | no. 37

Characterization of *Prochlorococcus* clades from iron-depleted oceanic regions

Douglas B. Rusch[a], Adam C. Martiny[b,c], Christopher L. Dupont[d], Aaron L. Halpern[a,1], and J. Craig Venter[d,2]

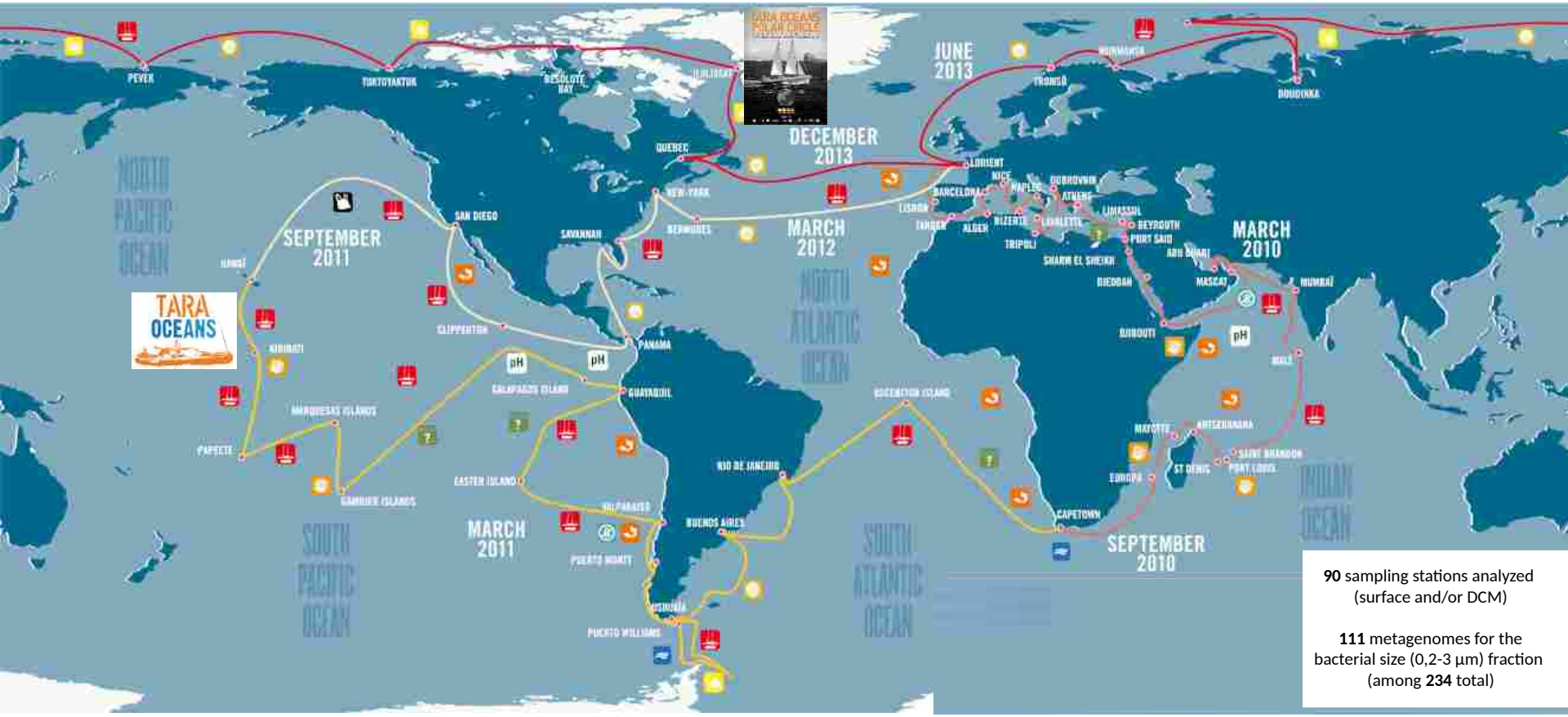




One metagenome-assembled genome (MAG) was constructed for each clade providing some evidence of adaptation of these clades to low-Fe areas :

reduction of Fe requirement by lowering the number of Fe-containing proteins.

The metagenomics revolution was boosted by two major expeditions



90 sampling stations analyzed (surface and/or DCM)

111 metagenomes for the bacterial size (0,2-3 µm) fraction (among **234** total)



The 2nd global circum-navigation was initiated by Eric Karsenti It was held on board the 36-m long *Tara* schooner lent by cloth designer agnès b. and her son Etienne Bourgois, who founded the *Tara* Foundation. The expedition which mobilised over 100 scientists was split in two parts:

- *Tara* Oceans (2010-12)
- *Tara* Polar circle (2013)



The metagenomics revolution was boosted by two major expeditions

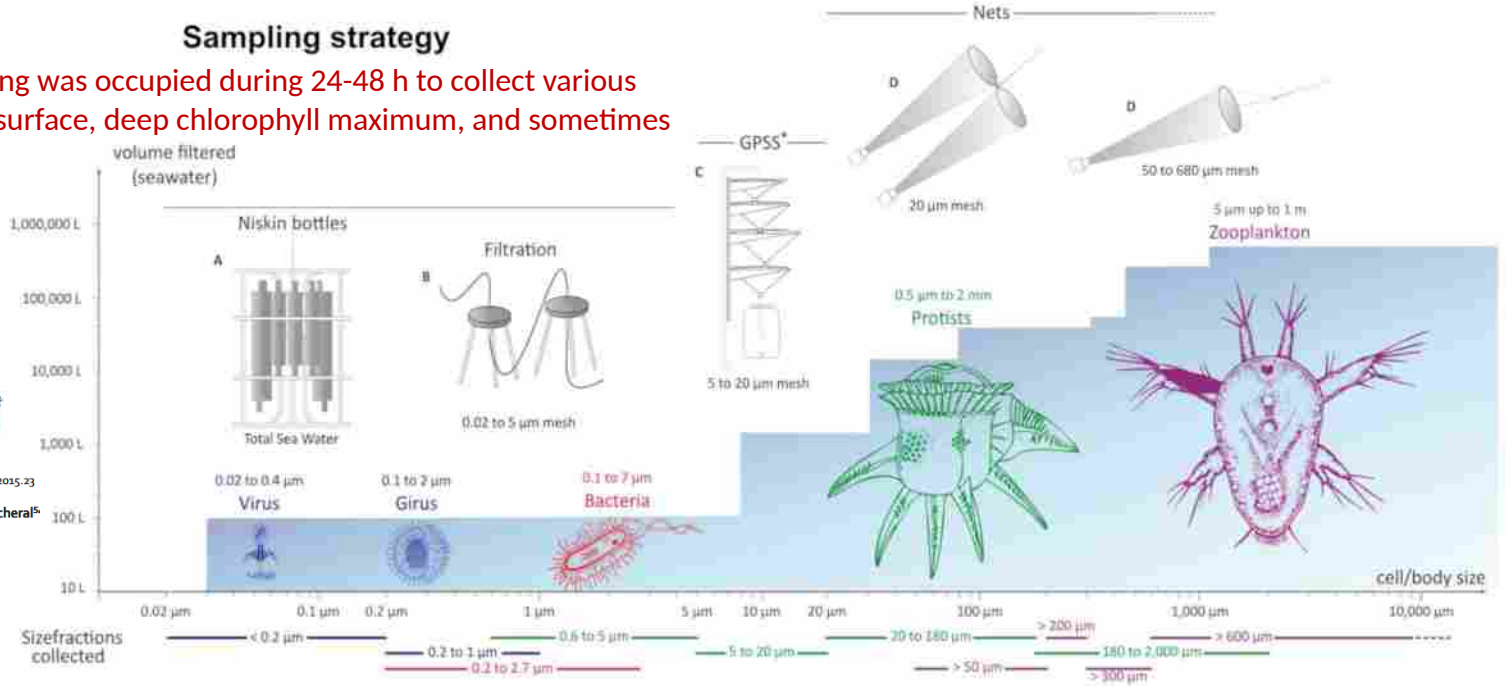
Each station sampling was occupied during 24-48 h to collect various size fractions from surface, deep chlorophyll maximum, and sometimes mesopelagic zone.

TARA OCEANS

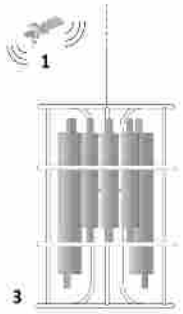
Open science resources for the discovery and analysis of *Tara* Oceans data

SCIENTIFIC DATA | 2:150023 | DOI: 10.1038/sdata.2015.23

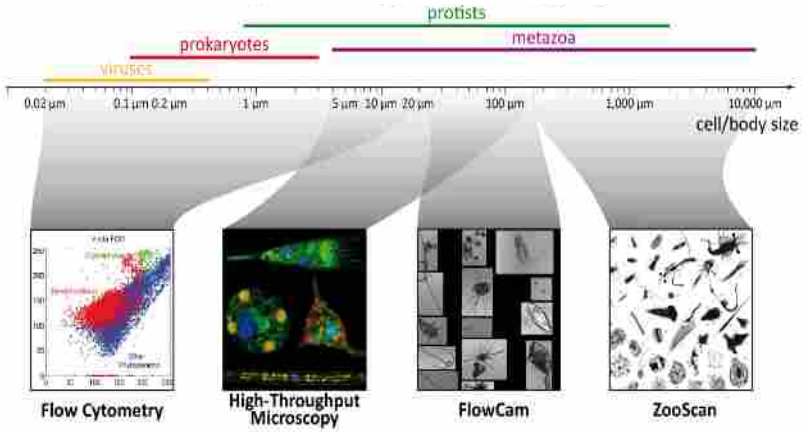
Stéphane Pesant[1,2], Fabrice Not[3,4], Marc Picheral[5] & *Tara* Oceans Consortium Coordinators[†]



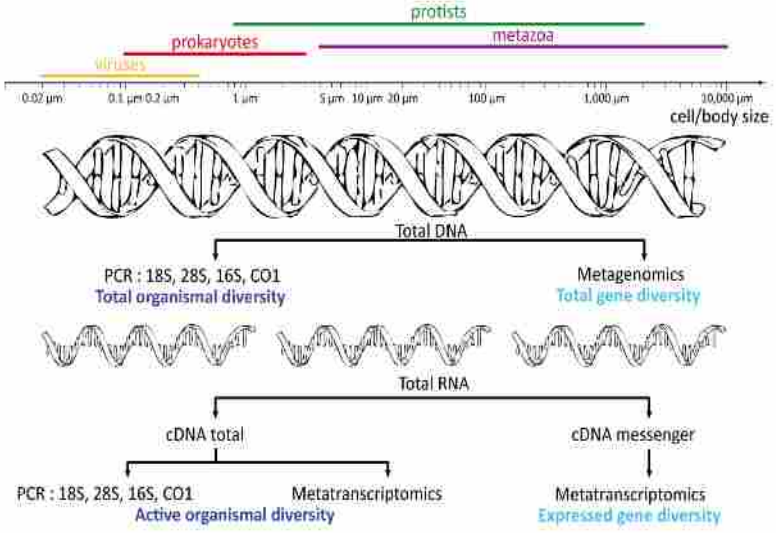
Physico-chemistry



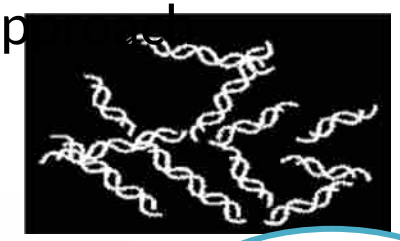
High Throughput Imaging



High Throughput Sequencing



The miTags ap



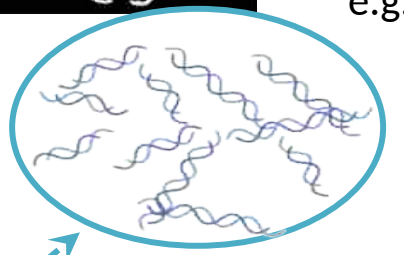
METABARCODE (Tags)

e.g. 16S, *rpoC1*, *petB*...

environmental microbiology Volume 16, Issue 9

Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities

Ramiro Logares, Shinichi Sunagawa, Guillem Salazar, Francisco M. Cornejo-Castillo, Isabel Ferrera, Hugo Sarmento, Pascal Hingamp, Hiroyuki Ogata, Colomban de Vargas, Gipsi Lima-Mendez, Jeroen Raes, Julie Poulain, Olivier Jaillon, Patrick Wincker, Stefanie Kandels-Lewis, Eric Karsenti, Peer Bork, Silvia G. Acinas



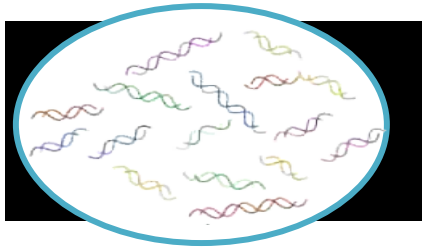
PCR amplification & sequencing



Microbial community

RNA/DNA extraction

METAGENOME
METATRANSCRIPTOME



Complete sequencing

in silico extraction of marker genes

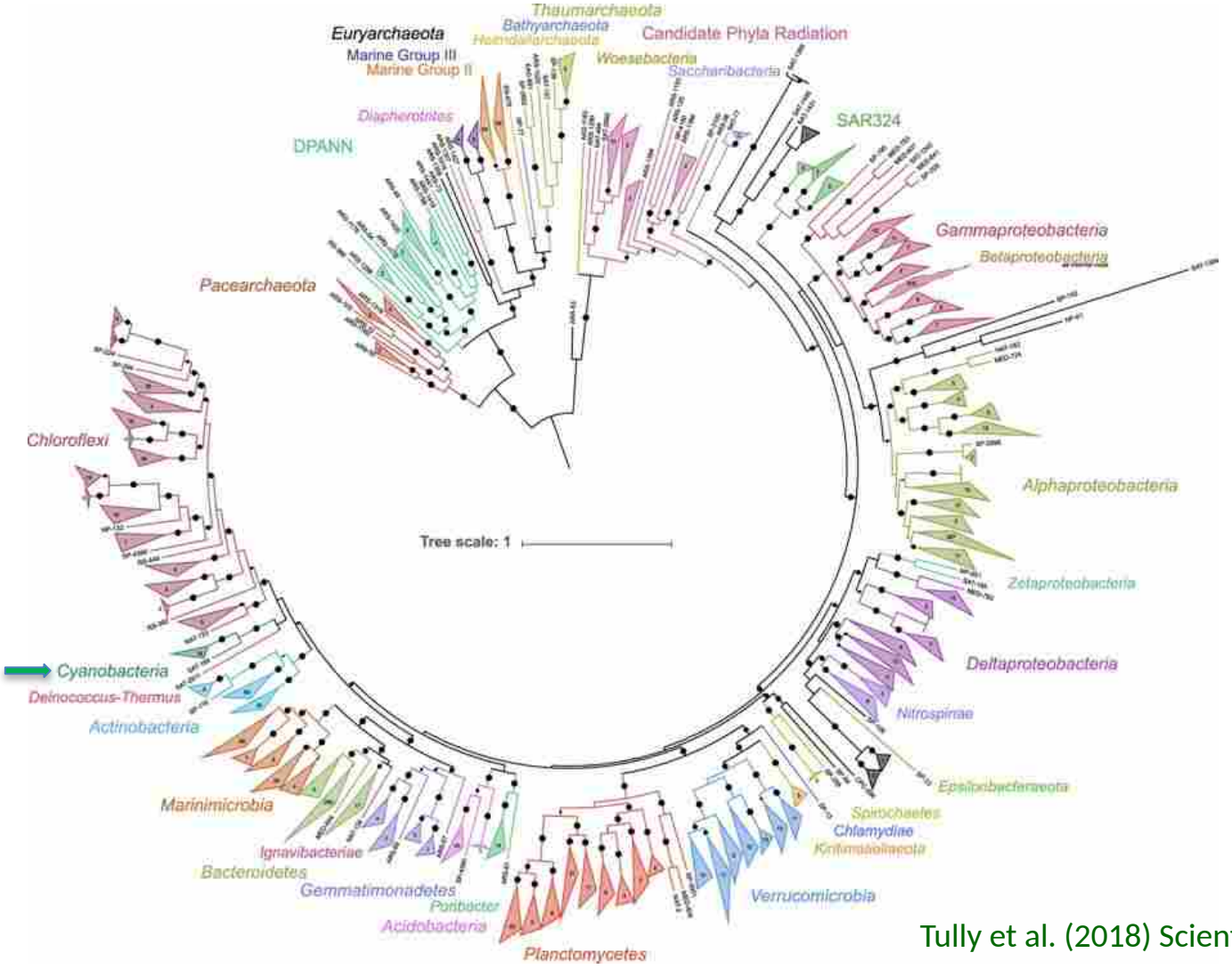


miTags

e.g., 16S rRNA, *rpoC1*, *petB*

=> No PCR bias: more reliable quantitatively than metabarcoding

The *Tara* Oceans metagenomic dataset: Generation of MAG's



Tully et al. (2018) Scientific data

Generation of 2,631 draft genomes (MAG's) of Bacteria and Archaeae with an estimated completion of ≥50% (1,491 draft genomes >70% complete; 603 genomes > 90% complete, including 16 cyanobacteria).

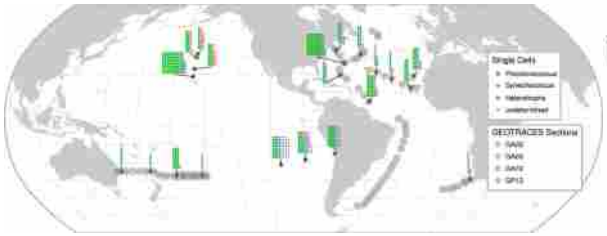
Many more *Proc*. and *Syn*. MAGs have been assembled since then…

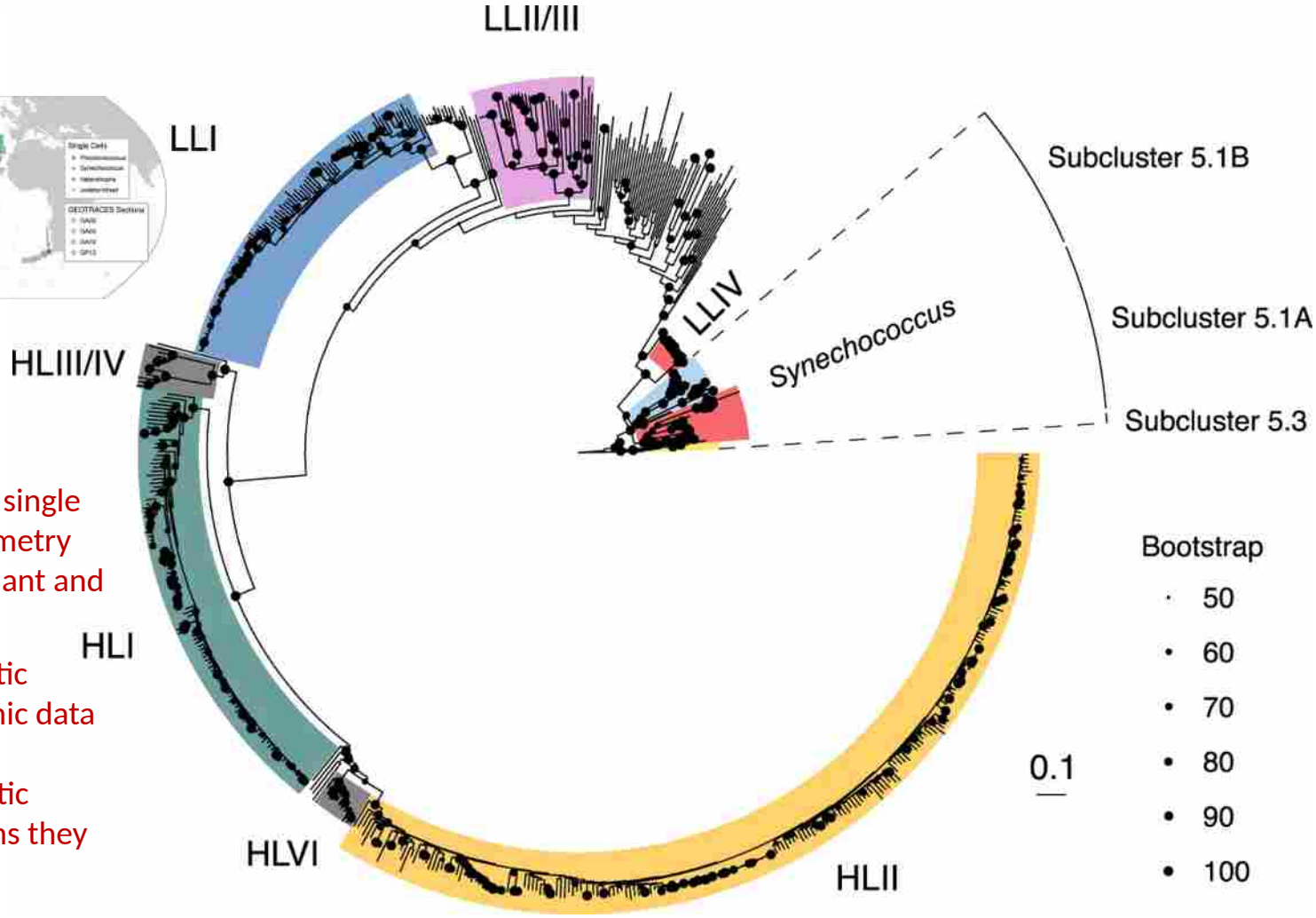
More Picocyanobacterial Genomes from the Field: The SAG approach

scientific **data** **5**, 180154 (2018).

Single cell genomes of *Prochlorococcus*, *Synechococcus*, and sympatric microbes from diverse marine environments

Paul M. Berube, Steven J. Biller, Thomas Hackl, Shane L. Hogle, Brandon M. Satinsky, Jamie W. Becker, Rogier Braakman, Sara B. Collins, Libusha Kelly, Jessie Berta-Thompson, Allison Coe, Kristin Bergauer, Heather A. Bouman, Thomas J. Browning, Daniele De Corte, Christel Hassler, Yotam Hulata, Jeremy E. Jacquot, Elizabeth W. Maas, Thomas Reinthaler, Eva Sintes, Taichi Yokokawa, Debbie Lindell, Ramunas Stepanauskas & Sallie W. Chisholm



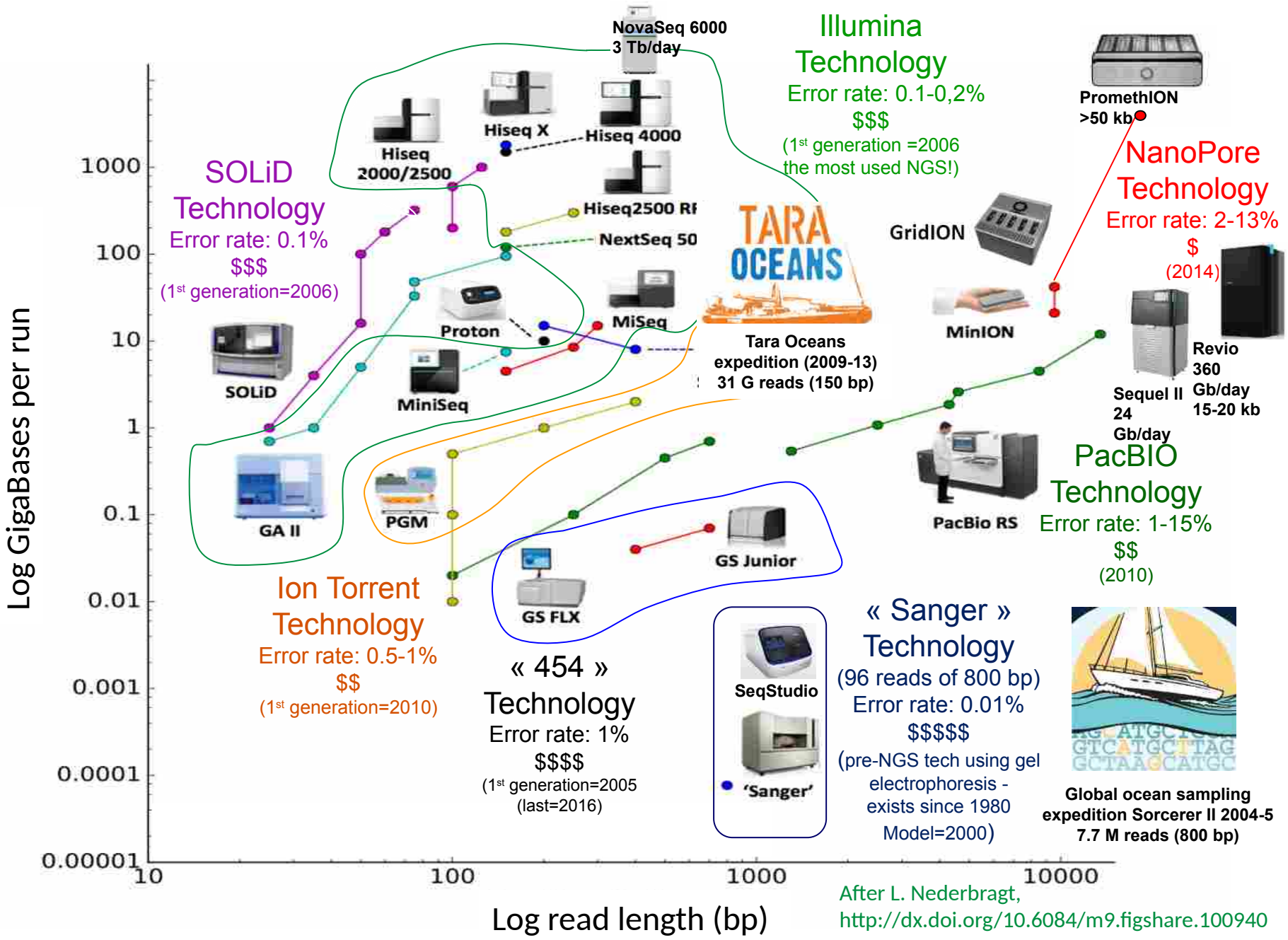


Genomes obtained from single cells sorted by flow cytometry allows to get both abundant and rare taxa.

⇒ useful for phylogenetic anchoring of metagenomic data sets and expanding our knowledge of phylogenetic lineages and the functions they harbor.

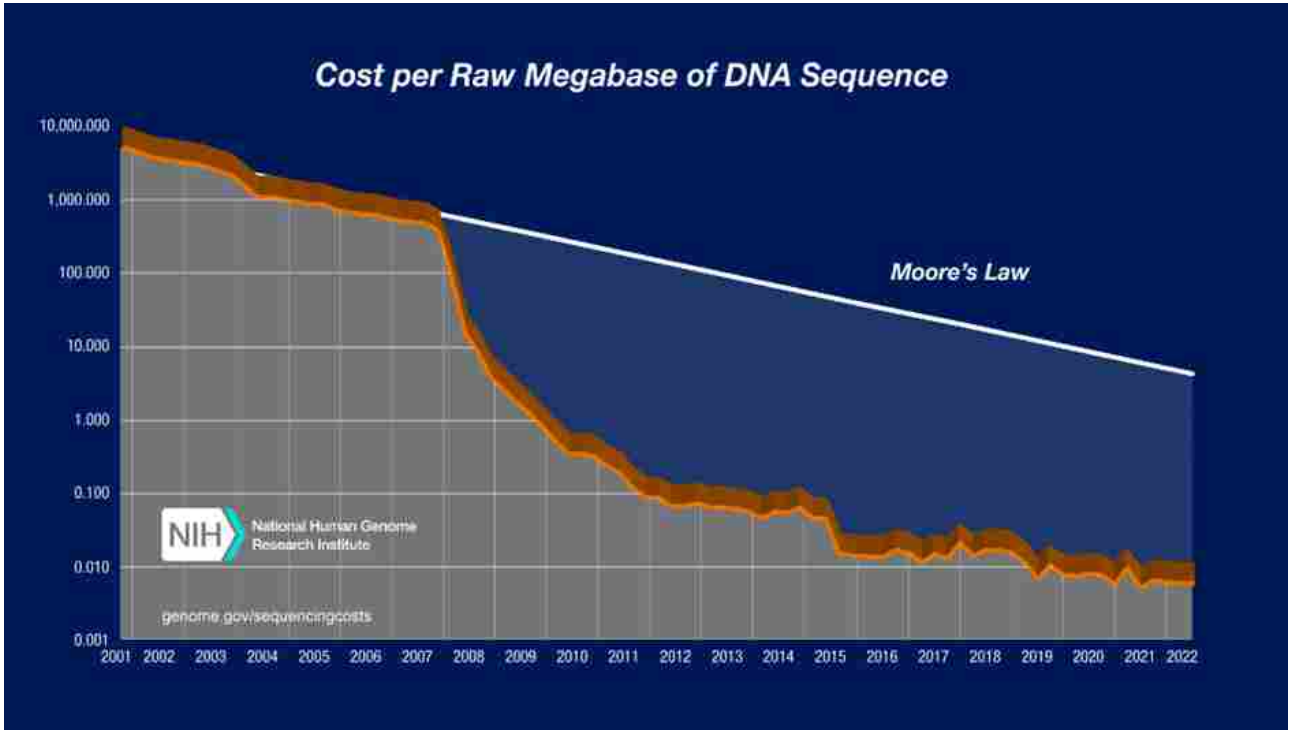
Phylogeny from 66 isolate genomes and 489 SAGs for *Proc.*, and 27 isolate genomes and 50 SAG's for *Syn*.

The Next Generation Sequencing Revolution



After L. Nederbragt,
http://dx.doi.org/10.6084/m9.figshare.100940

The Next Generation Sequencing Revolution



Since 2008, the cost of sequencing is falling >>2 faster as the cost of computing, so that the cost of a genome has now more to do with data analysis than with sequencing.

It has now become simpler and less expensive to sequence a whole genome than a few individual genes from a given organism.

=> Paradigm shift in approaches to be used to answer questions about the molecular ecology of picocyanobacteria.

My next two talks will show you examples of what information can be retrieved from integrating biology, physiology, ecology and omics of marine picocyanobacteria, with a particular focus on *Syn*.

Croucher course 1 outline

I. 1979-1999: The golden age of fluorescence techniques

II. 1996-2003: Characterization of the original light- harvesting complex of *Prochlorococcus*

III. 1998: The advent of the molecular ecology of picocyanobacteria

IV. 2003-on: The omics era: an introduction

V. Optional: Cyanophages



Acknowledgments

SBR

ECOMAP Team

Laurence Garczarek
Morgane Ratin

Daniella Mella-Flores
Hugo Doré
Ulysse Guyet
Gregory Farrant

Bioinformatics Core Facility

Mark Hoebeke
Jukka Siltanen
Erwan Corre
Loraine Guéguen

Other collaborators

David J. Scanlan (Warwick Univ., UK)
Wolfgang Hess (Freiburg Univ.)
Julie LaRoche
Jim Barber
And many others…

Funding

ANR
European Union FP5
Genoscope

Thanks for your attention!

Questions ?

COMING NEXT

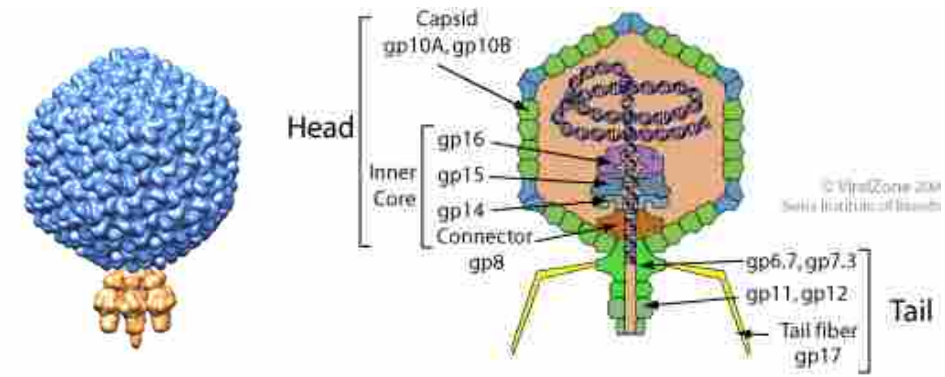
Course 2: Using omics to decipher niche adaptation mechanisms in marine picocyanobacteria

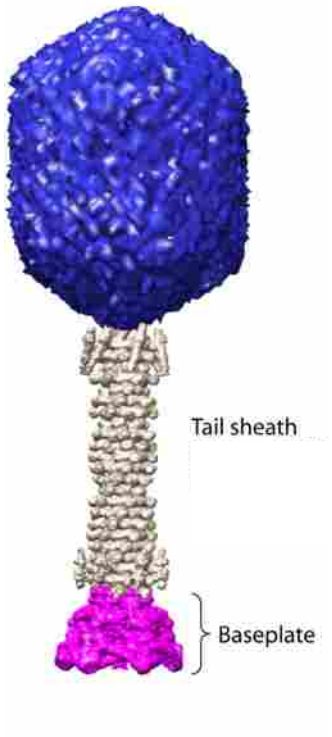
Course 3: A multi-scale study of *Synechococcus* pigment types: From genes to the global Ocean

Bacterial Viruses = (Bacterio)Phages

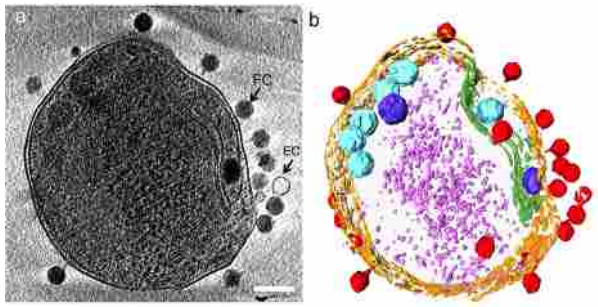
All (cyano)phages infesting *Prochlorococcus* and *Synechococcus* cells isolated to date belong to only one order (Caudovirales) and three families:

- Podoviridae: short tail
- Myoviridae: contractile tail
- Siphoviridae: long, non-contractile tail









Prochlorococcus MED4 cell infected by the podovirus P-SSP7

Veesler & Cambillau
Microbiol. Mol. Biol. Rev. (2011)

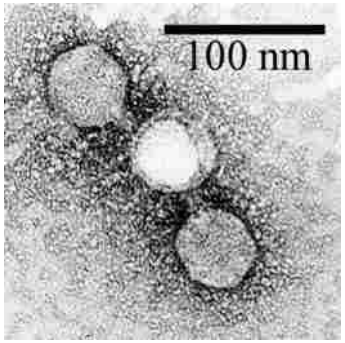
Picocyanobacteria have Phages with various Specificity

Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*

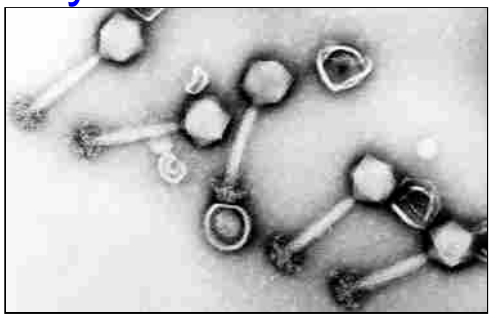
Matthew B. Sullivan[1], John B. Waterbury[3] & Sallie W. Chisholm[2]

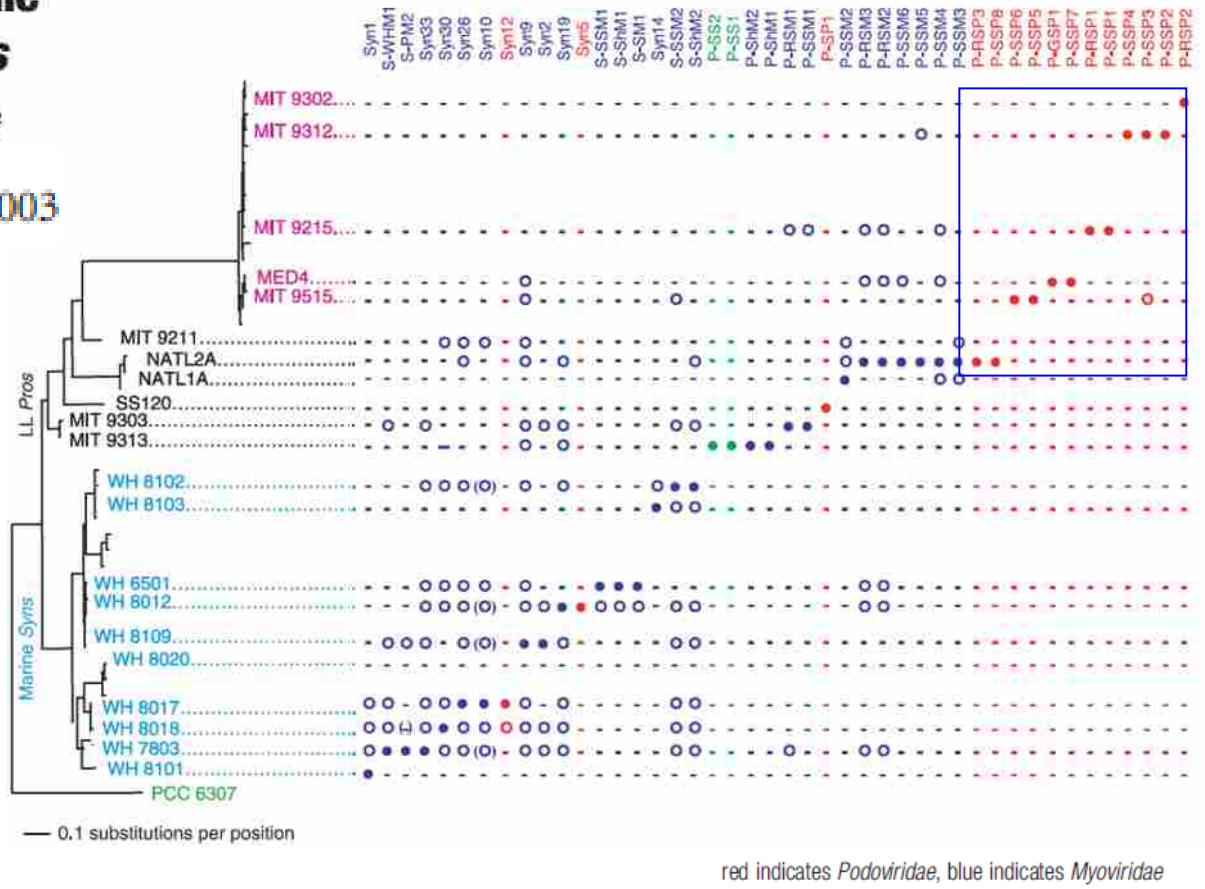
NATURE | VOL 424 | 28 AUGUST 2003

Podoviridae



Myoviridae

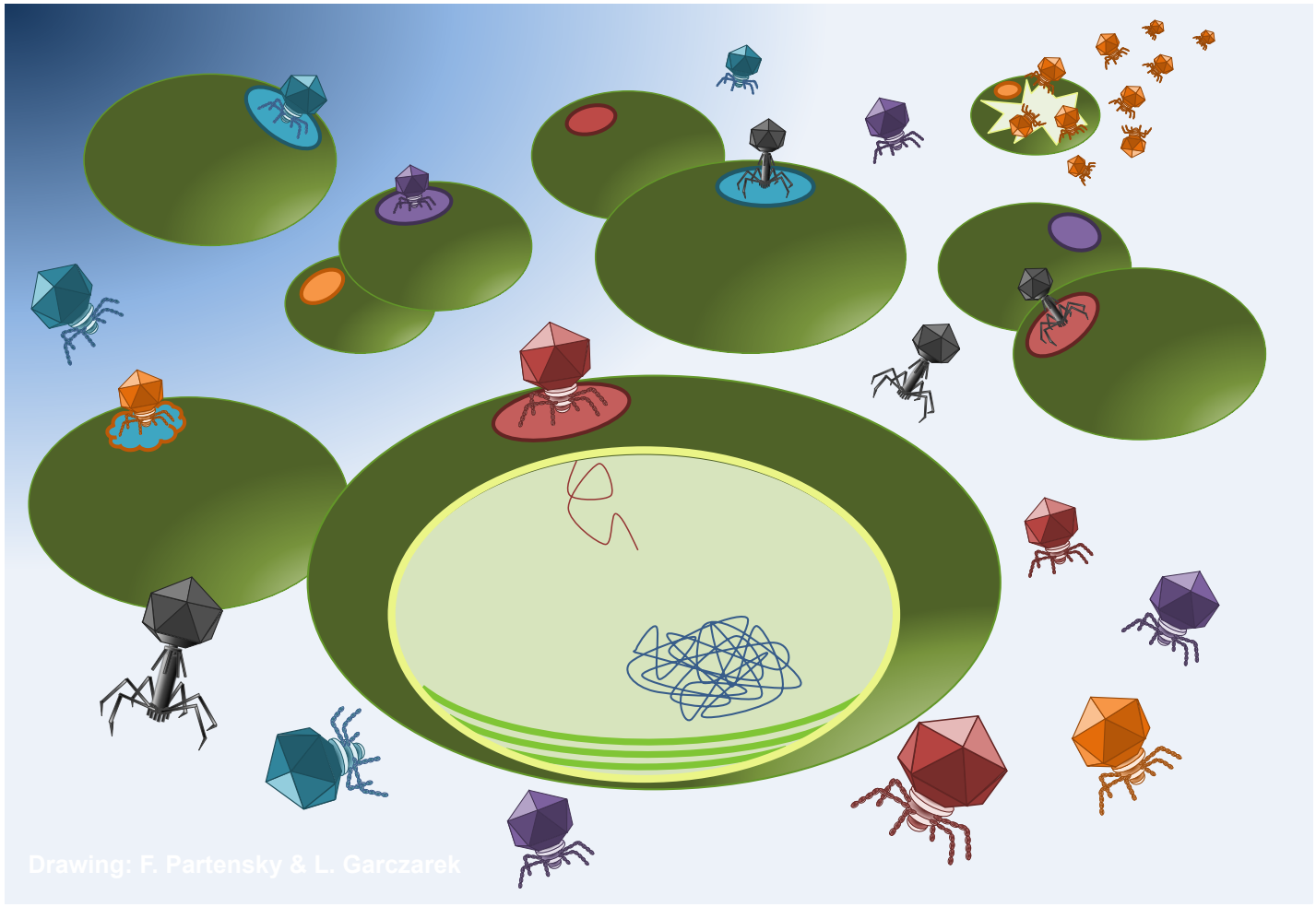




red indicates *Podoviridae*, blue indicates *Myoviridae*

- Some phages (Podoviridae) are strain-specific
- Others (Myoviridae) can infect marine *Synechococcus* as well as with HL- and LL-adapted *Prochlorococcus* isolates

Arms Race between Phages and their Hosts



Prochlorococcus cells can resist viral infection by inactivating genes involved in virus attachment…

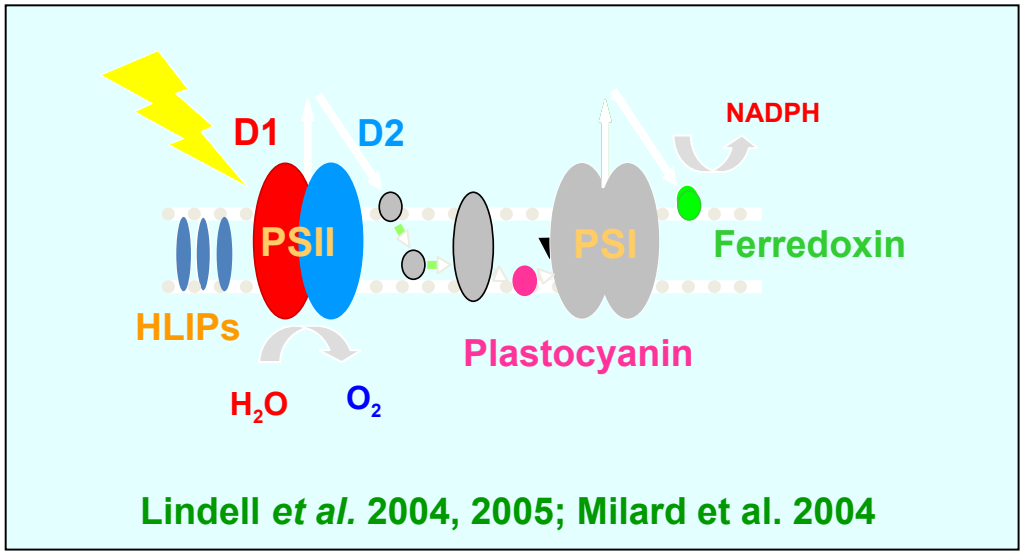
… but this may affect cell fitness (lower growth) or favor infection by other viruses

ARTICLE

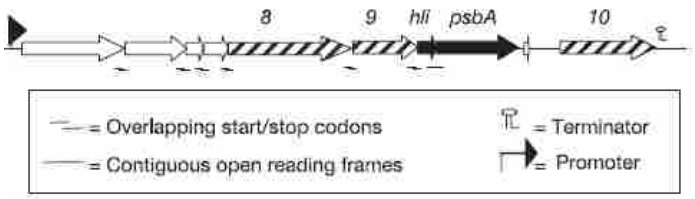
Genomic island variability facilitates *Prochlorococcus*–virus coexistence

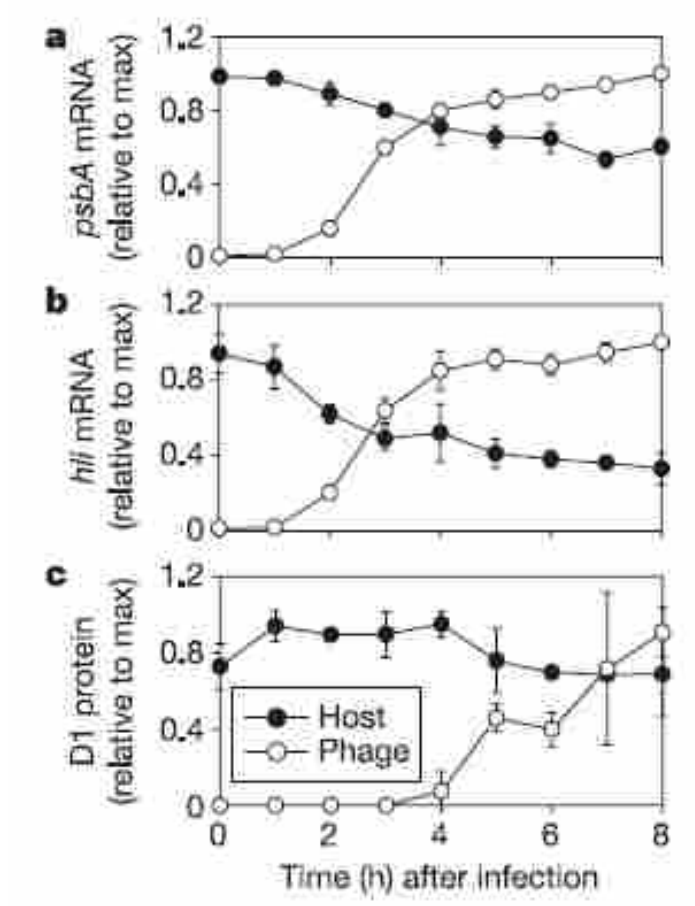
Avrani *et al.* 2011 Nature

Cyanophages contain Photosynthetic Genes !



Lindell *et al.* 2004, 2005; Milard et al. 2004

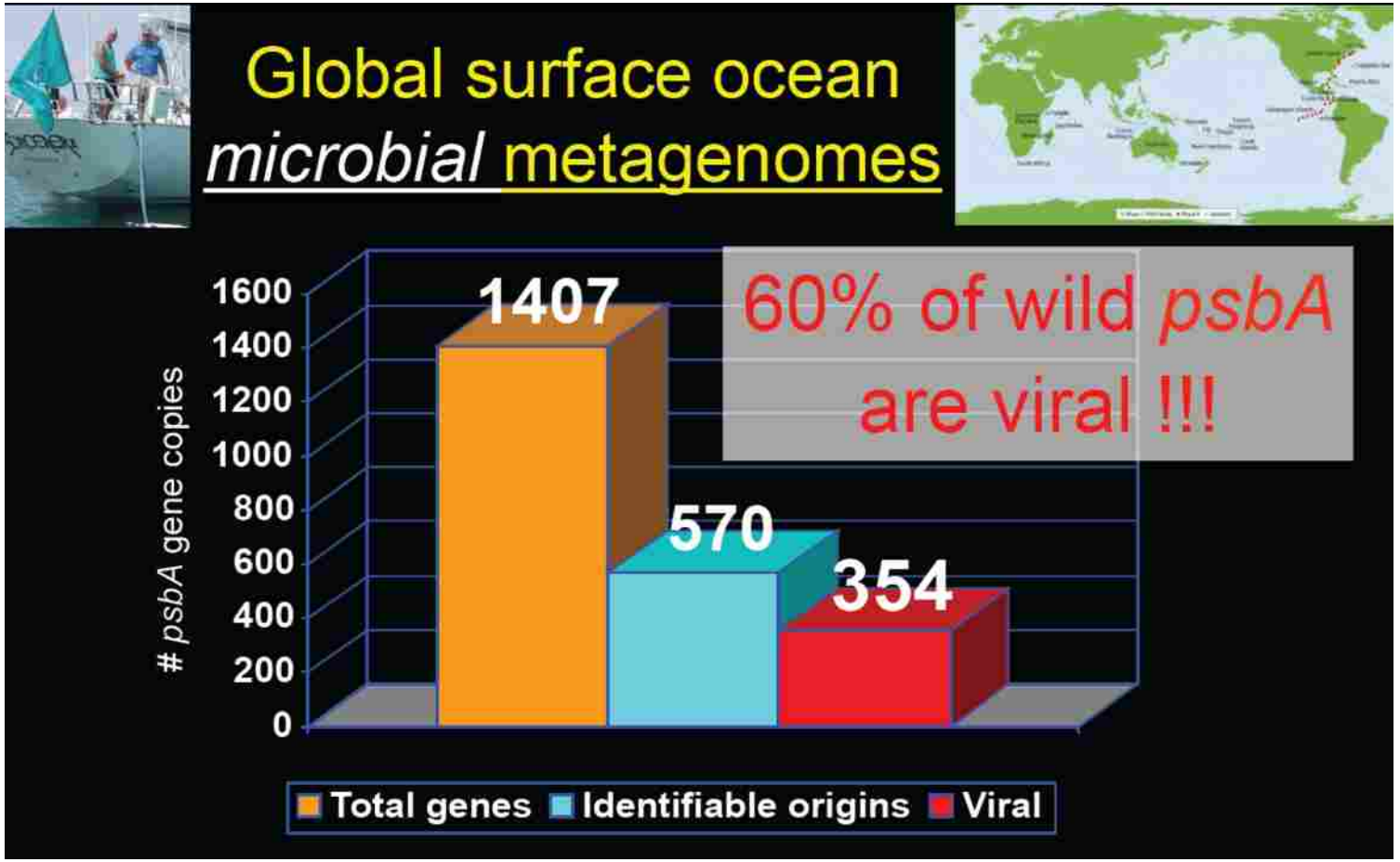




Lindell *et al.* 2005 Nature

- Many photosynthetic genes can be found in phages but the most frequent is *psbA*, encoding the D1 core protein of photosystem II.
- *psbA* from the phage is expressed during infection in order to maintain the photosynthetic machinery (high turnover D1 protein) long enough for the lytic cycle to be completed

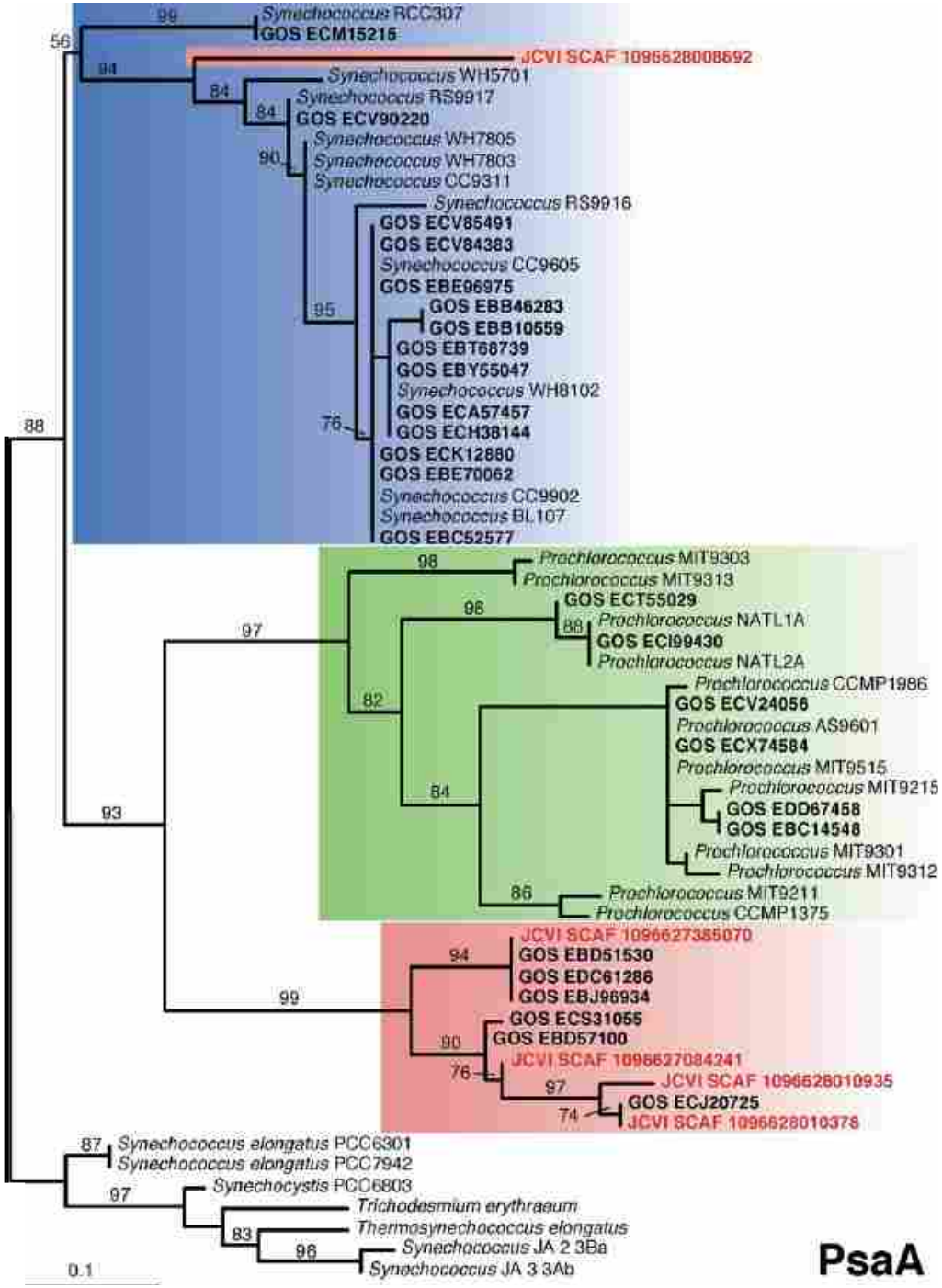
Cyanophages contain Photosynthetic Genes



Sharon *et al.* 2007 ISME Journal

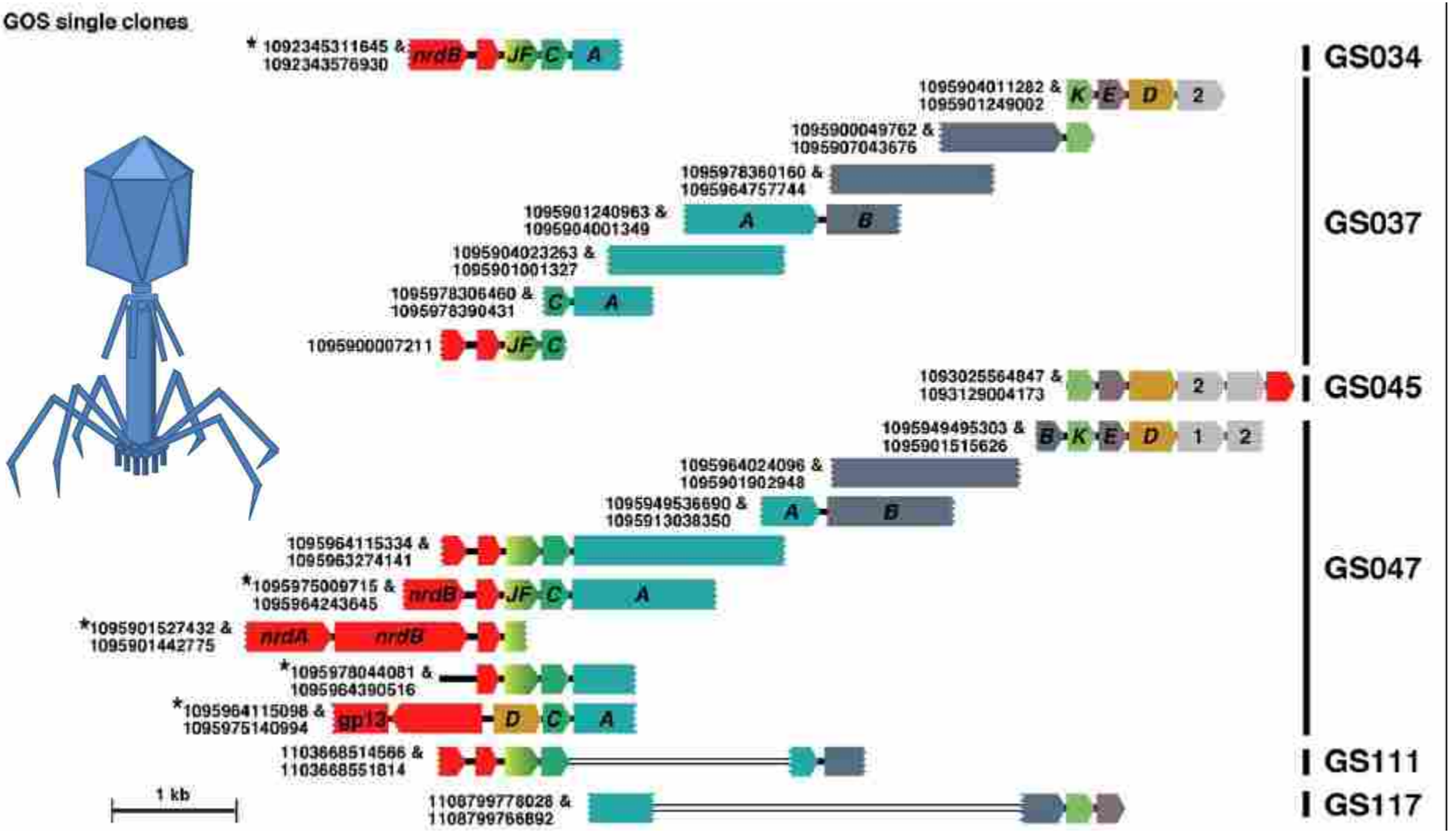
Phylogeny of PsaA (PSI Reaction Center)

PsaA sequences from phages form a separate branch within the marine picocyanobacteria



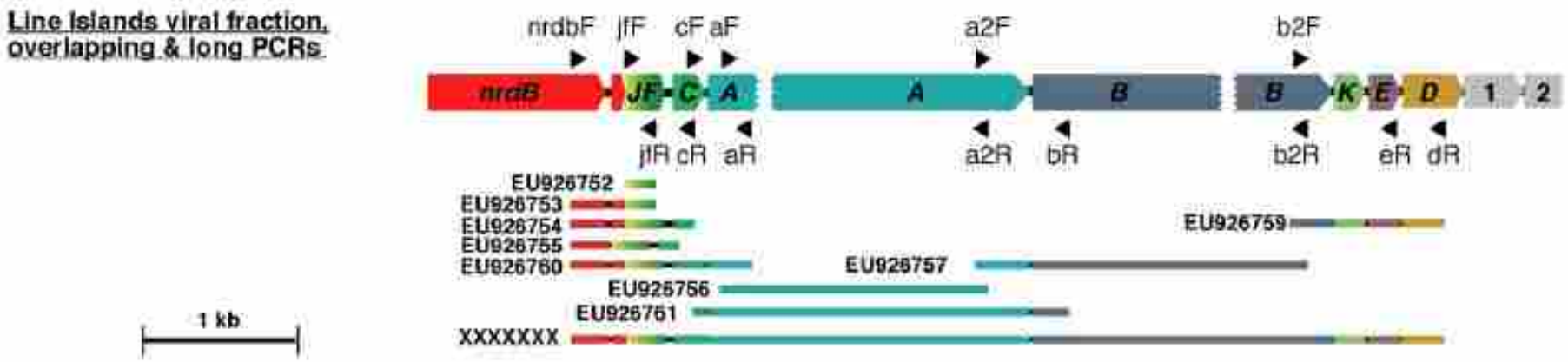
Sharon *et al.* 2009 Nature

Some “Scaffolds” (Sequence Assemblages) Contain several PSI Genes from Phages



Sharon *et al.* 2009 Nature

The Definitive Proof

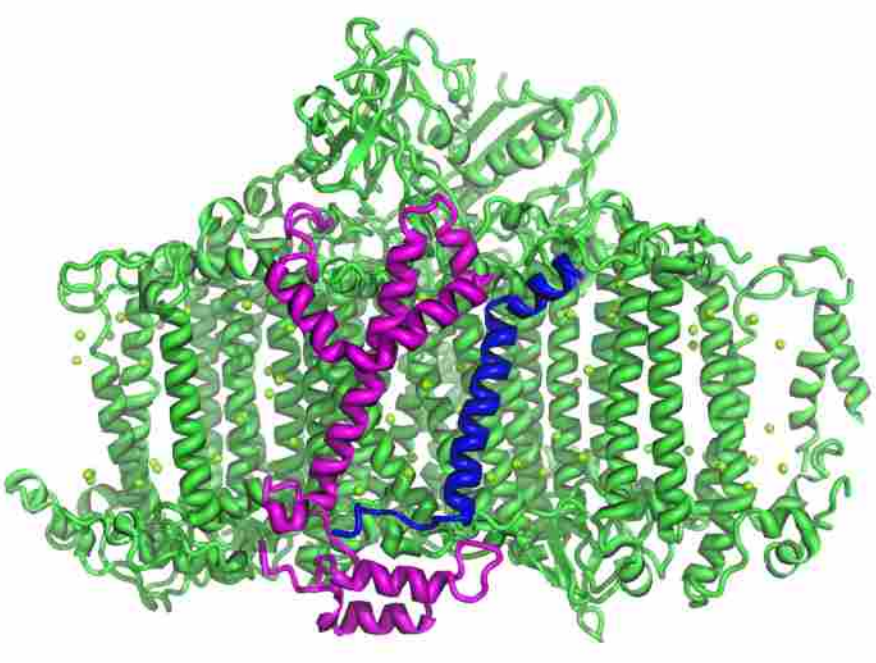


- A **unique** gene arrangement since these 7 PSI genes are never found in a cluster in cyanobacterial genomes (except *psaA-B* and *psaJ-F*)

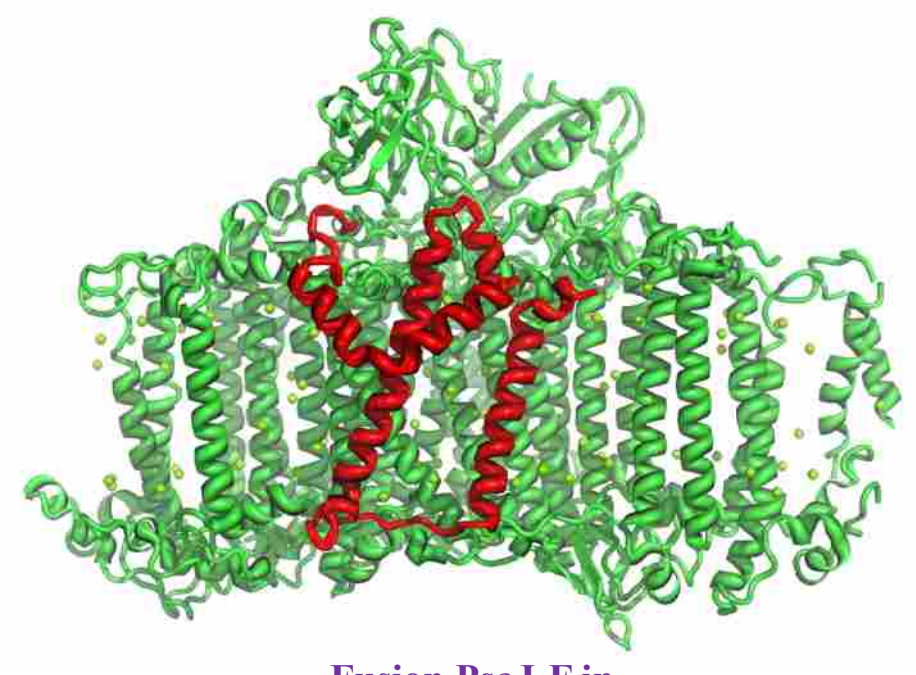


A viral Photosystem I !

- This 7 gene set is sufficient to synthesize a complete **monomeric** PSI



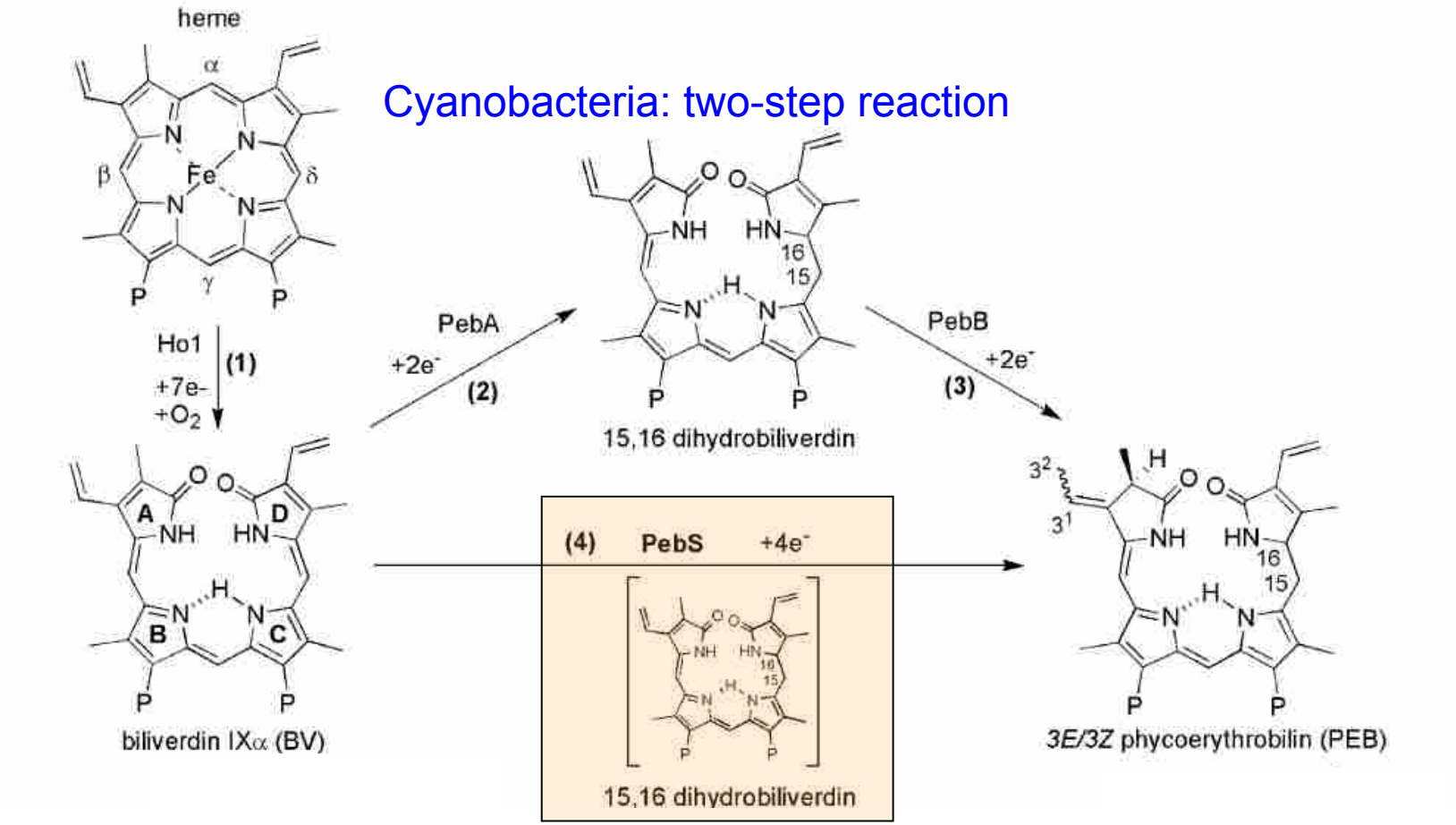
PsaF (purple) & PsaJ (blue) in cyanobacteria



Fusion PsaJ-F in 'viral' PSI

- The fusion of *psaJ-F* genes implies that the 'viral' PSI presents a structural alteration at the level of the docking site of plastocyanin, which could allow other electron donors to bind (ex: cytochromes) => energy coming from respiration?

Bioengineering by Cyanophages: A novel phycobilin biosynthesis gene



Cyanobacteria: two-step reaction

Phages: one-step reaction

Dammeyer *et al.* 2008 Curr. Biol.

Acknowledgments

SBR

ECOMAP Team

Laurence Garczarek
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Hugo Doré
Ulysse Guyet
Gregory Farrant

Bioinformatics Core Facility

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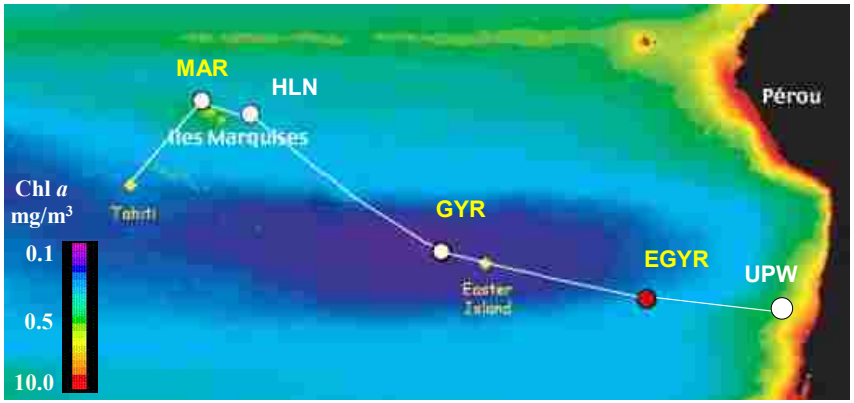
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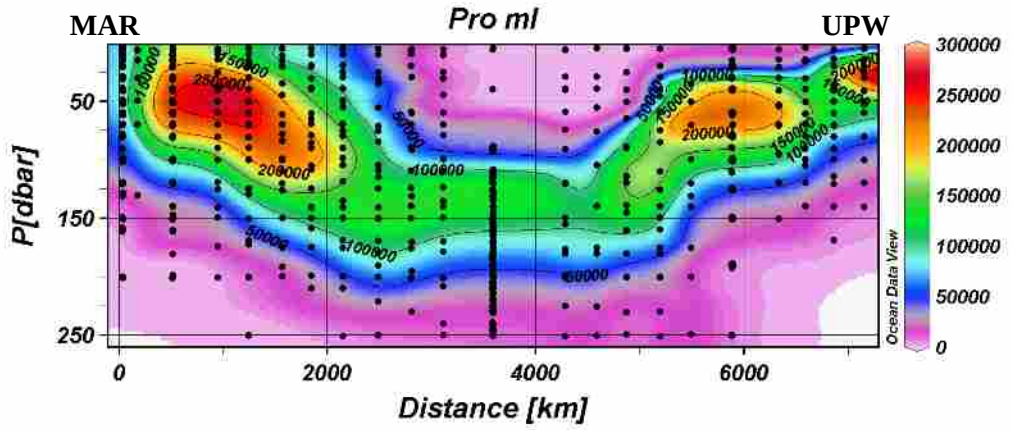
Biogeosciences, 4, 837–852, 2007

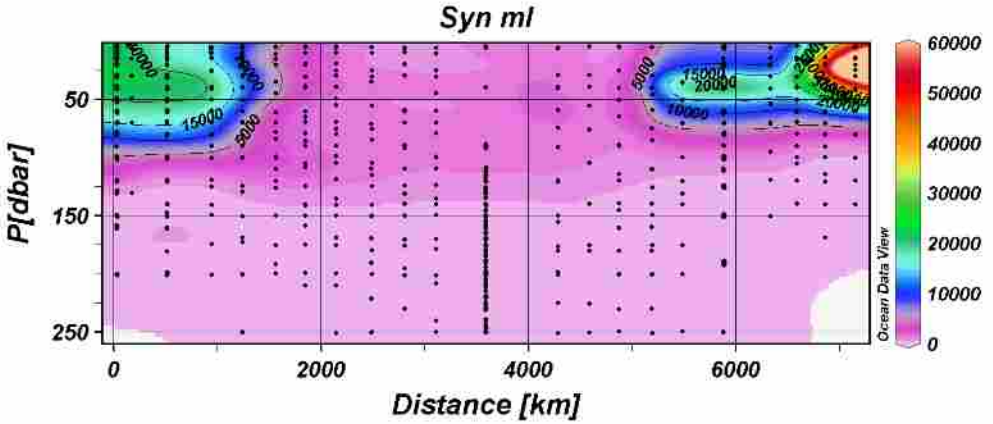
Contribution of picoplankton to the total particulate organic carbon concentration in the eastern South Pacific

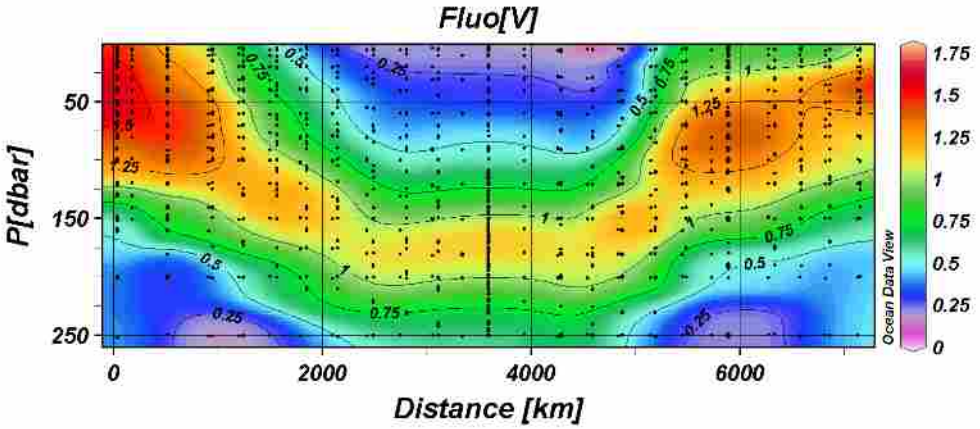
C. Grob[1,2,3], O. Ulloa[2], H. Claustre[3], Y. Huot[3], G. Alarcón[2], and D. Marie[4]











Grob et al. 2008, *Environ. Microbiol.*