5/3/96

MARINE PHYCOBILIPROTEINS

Present in Cyanobacteria ~ 1.0 μm (coccoid)
Cryptomonads 5 - 15 μm
Red algae intertidal / seaweed
subtidal

Accessory pigments to photosynthesis.

Not soluble in organic solvents
not readily analyzed by std pigment methods

Research in oceanography:
20 yrs behind chl a
10 yrs behind other accessory pigments

Pioneer work done by Charlie Yentsch
+ Dave Phinney

Active in 480 - 640 nm
480 - 570, in the ocean

- pigment diversity
- pigment concentration + distribution in coastal waters
- remote sensing of phycoerythrin.

CRYPTOMONAD PHYCOBILIPROTEINS

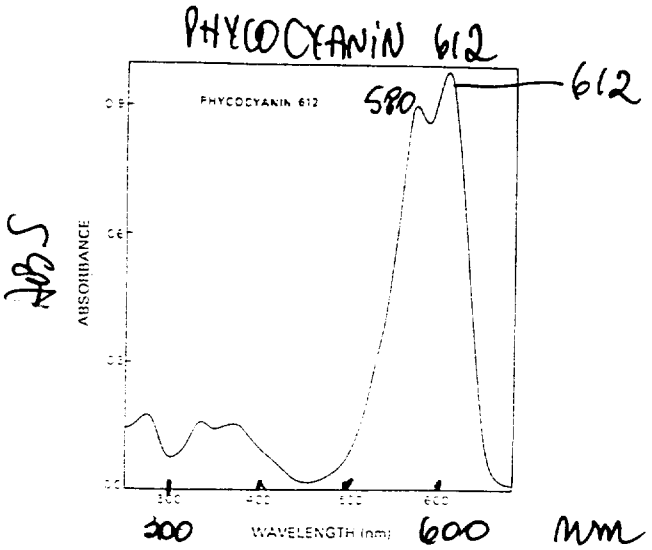


FIGURE 8. Absorption spectrum of phycocyanin 612 (*Hemiselmis virescens*). The sample is in pH 6.0, 0.1-ionic strength sodium phosphate buffer at room temperature. The band at 575 nm contains a major cryptoviolin contribution, and the band at 612 nm is largely phycocyanobilin.

IN CHLOROPLAST MEMBRANE

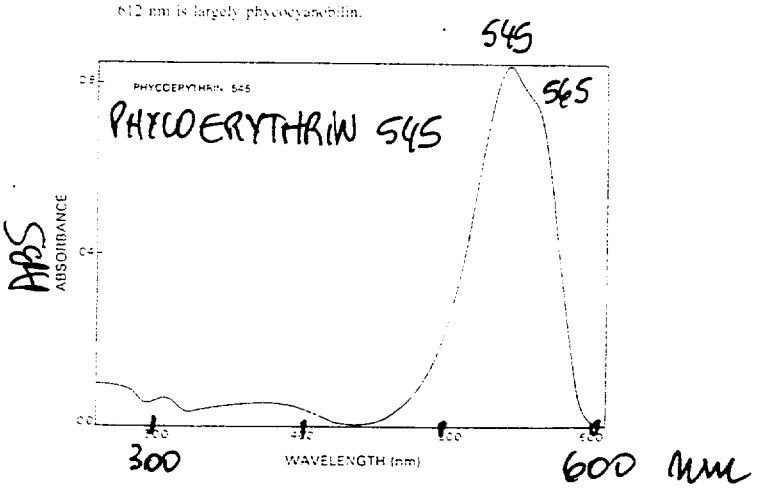


FIGURE 9. Absorption spectrum of phycoerythrin 545 (*Rhodomonas lens*). The sample is in pH 6.0, 0.1-ionic strength sodium phosphate buffer at room temperature.

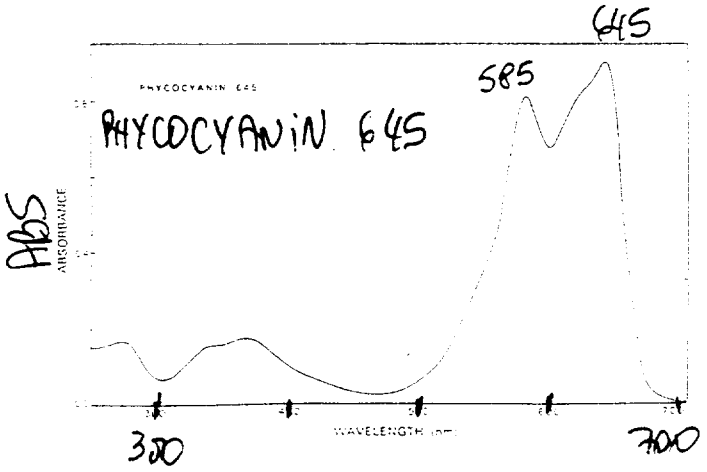
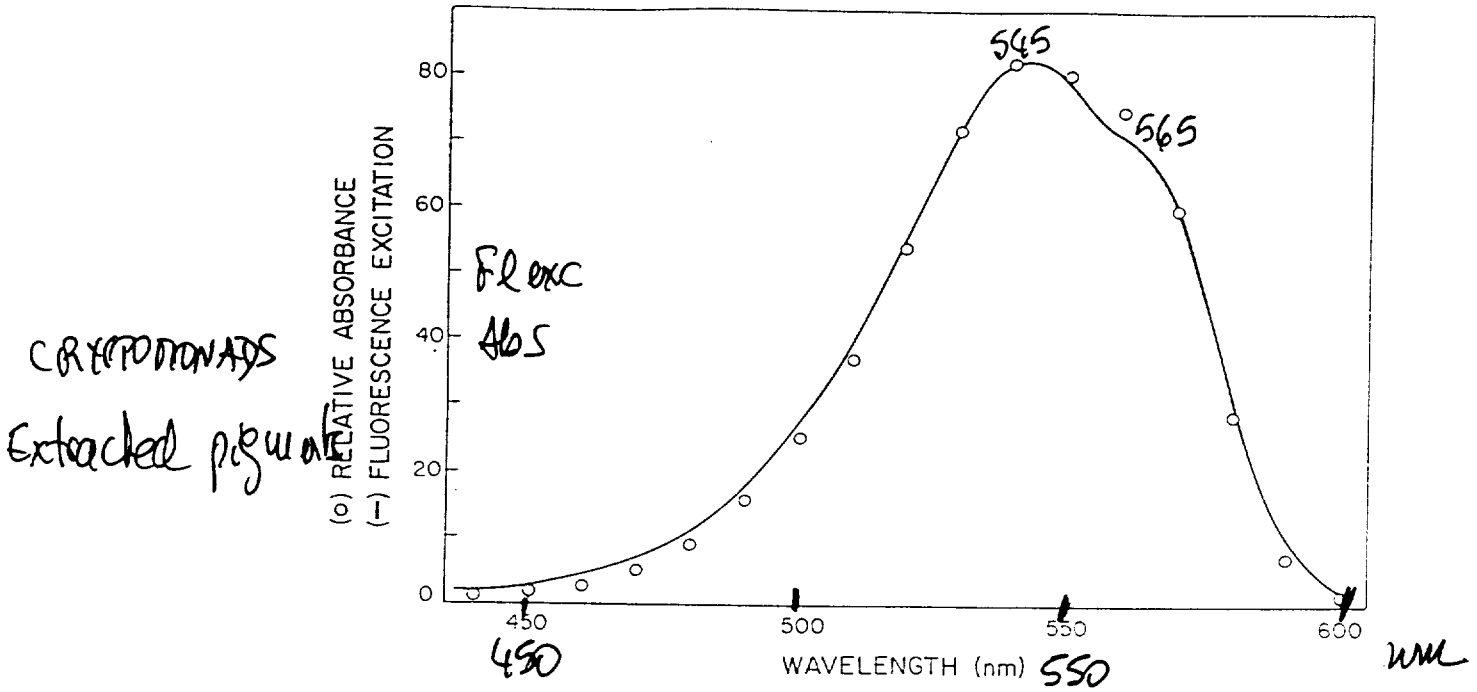


FIGURE 7. Absorption spectrum of phycocyanin 645 (*Chroomonas* sp.). The sample is in [illegible]

McColl + Guard-Friar (1987)



CRYPTOMONADS
Extracted pigment

FIGURE 6. Fluorescence excitation and absorption spectrum of phycoerythrin 545 (*Rhodomonas lens*). Excitation spectrum was fully corrected for instrumentation factors.

Phycobiliproteins

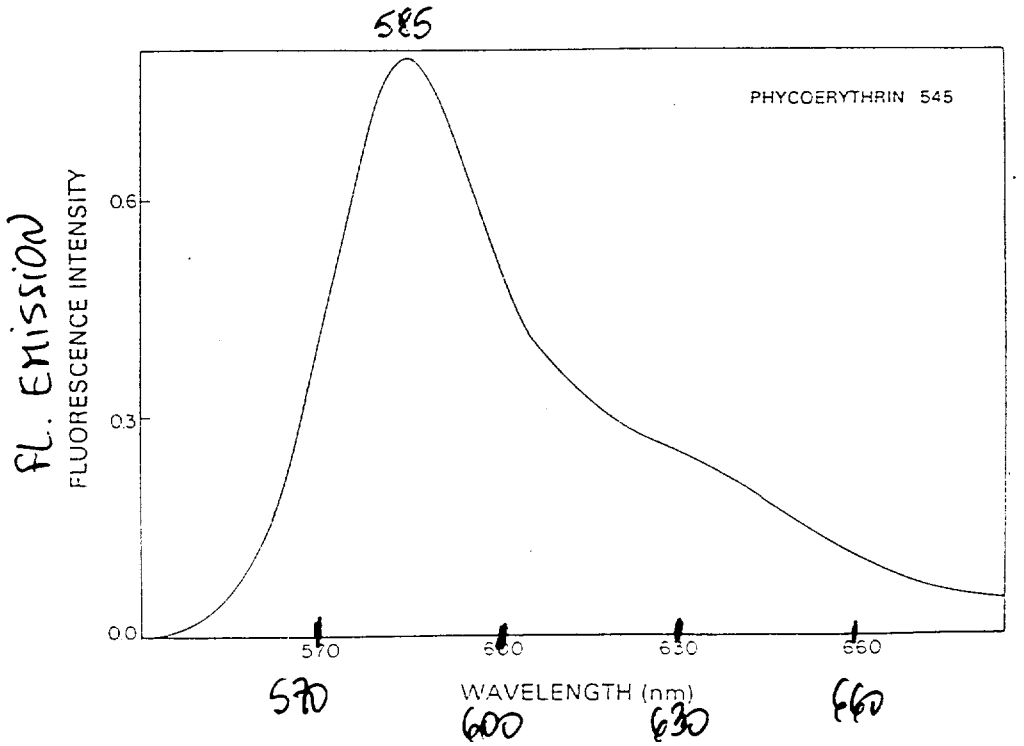


FIGURE 10. Fluorescence emission spectrum (fully corrected) of phycoerythrin 545 (*Rhodomonas lens*). The sample has an absorption of 0.05 at 545 nm in a 1-cm light path. The emission spectrum was taken on a sample in pH 6.0, 0.1-ionic strength sodium phosphate buffer at room temperature. Excitation of various wavelengths throughout the visible absorption band gave identical emission bands. Picosecond time-resolved fluorescence kinetics studies have demonstrated, however, that the emission is not completely homogenous. A small amount of fluorescence leakage does occur from higher-energy "sensitizing" chromophores.

CYANOBACTERIA

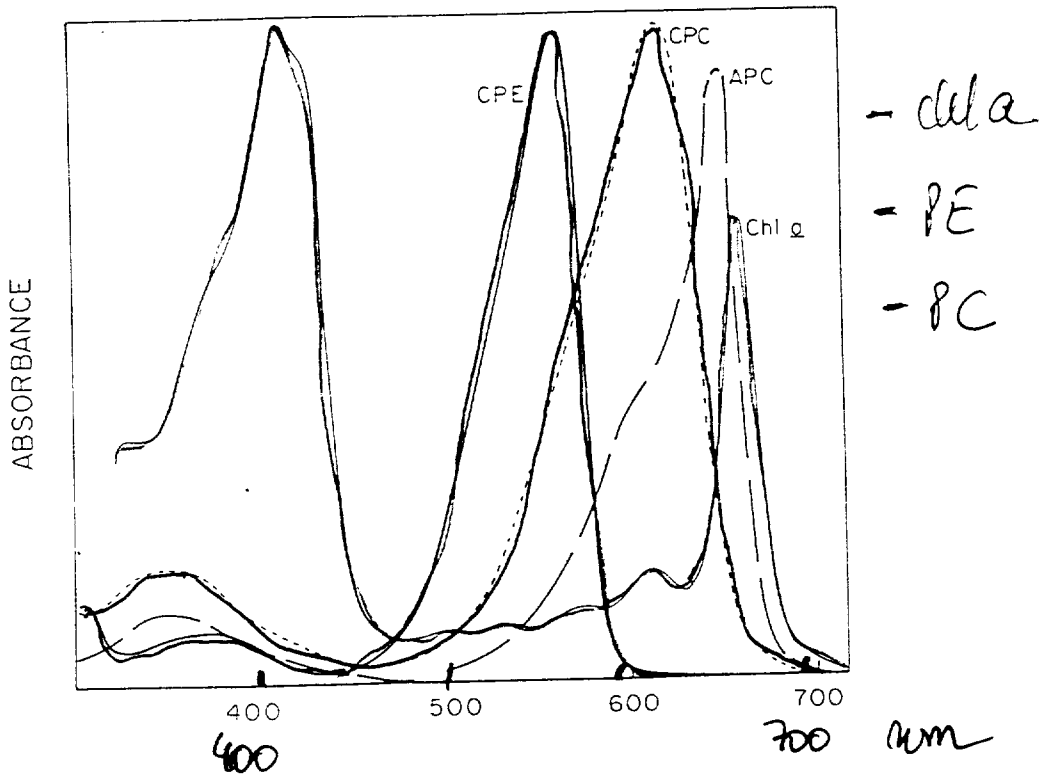
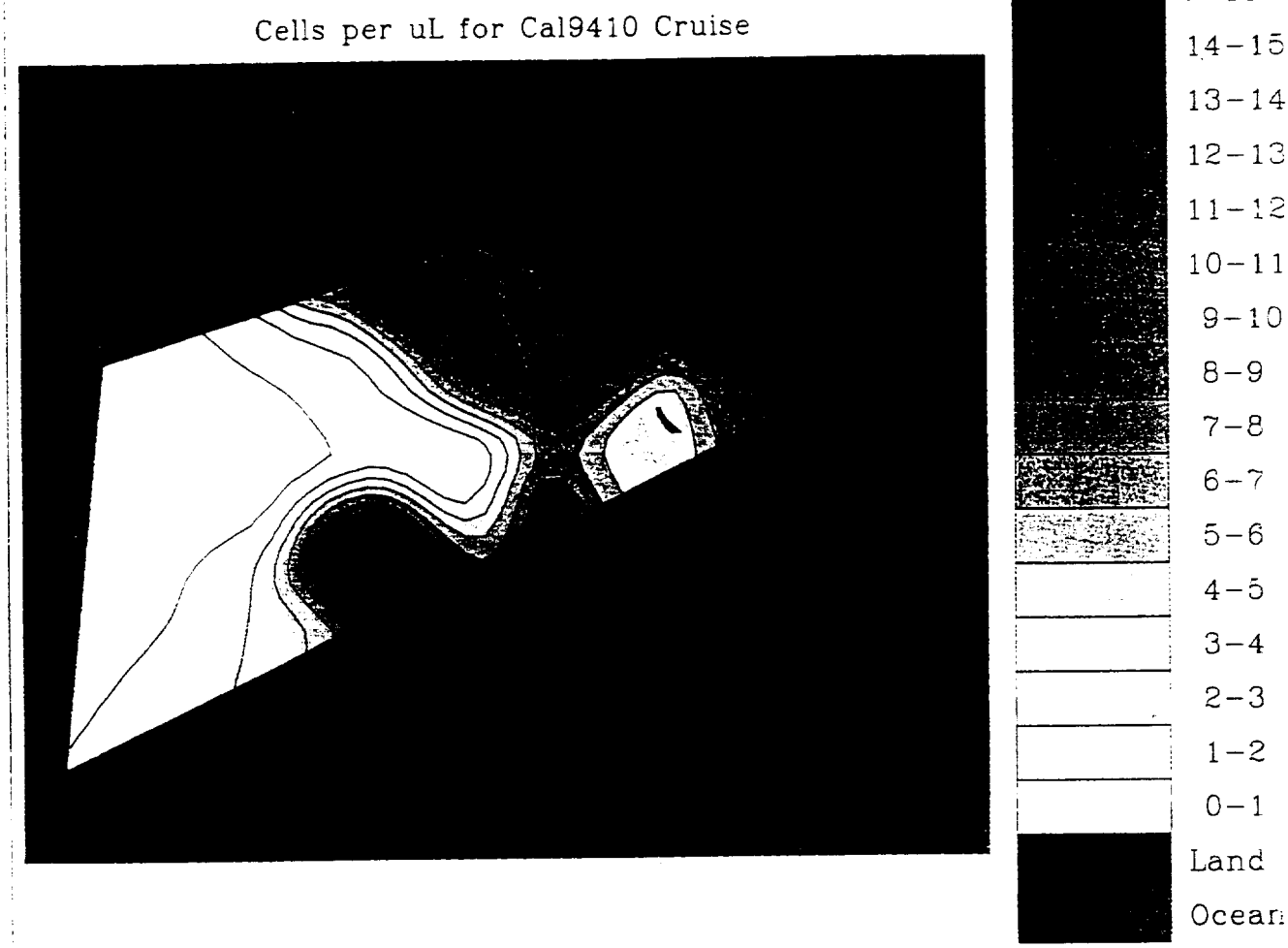


FIGURE 1. Absorption spectra of allophycocyanin (APC), C-phycocyanin (CPC), C-phycoerythrin (CPE), and chlorophyll (Chl *a*). Chlorophyll *a* is dissolved in acetone, and the purified biliproteins are in pH 6 to 7 sodium phosphate buffer.

(in ribosomes)

CALCOFI data: 7 cruises (94 + 95)
4 cruise / yr.

CALCOFI OCT 94
CYANOBACTERIA ABUNDANCE
at SURFACE



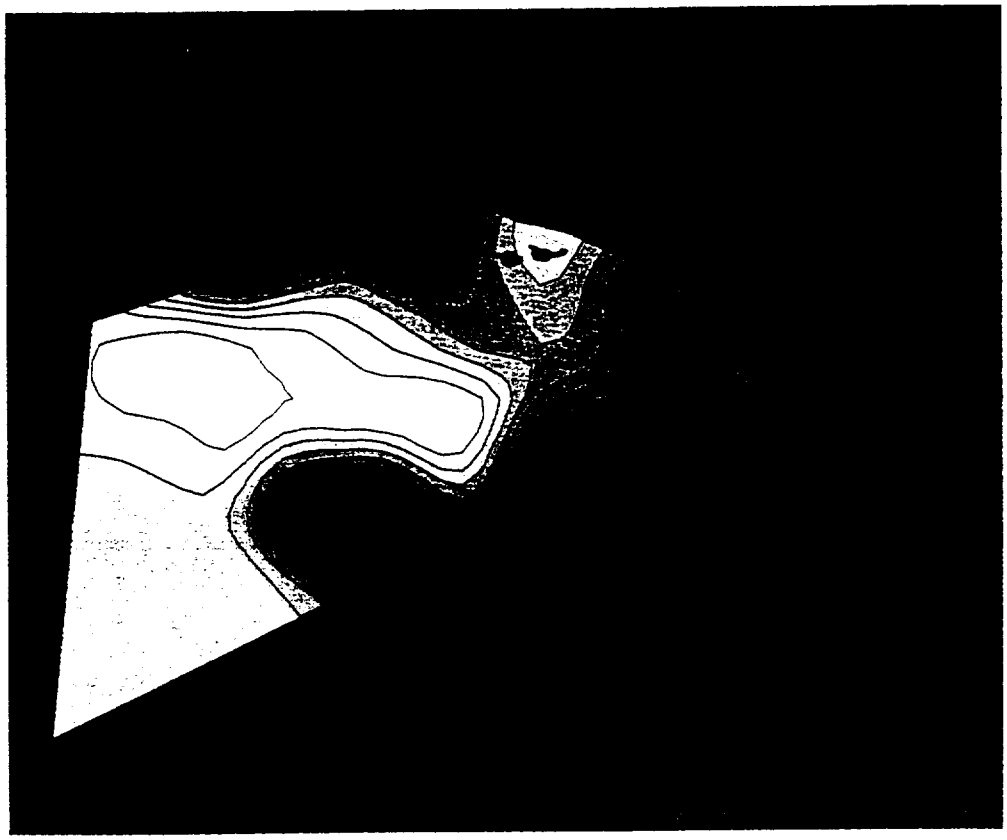
Vernet + Iturriaga (submitted)

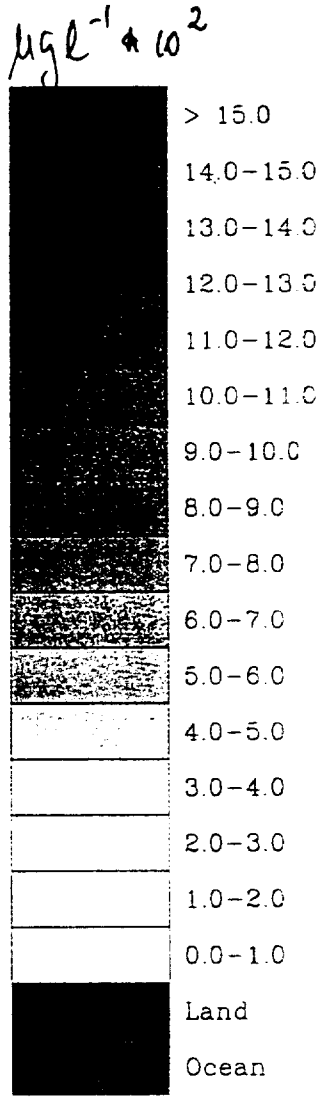
fig 3

CALCOFI OCT 94

PHYCOERYTHRIN 543

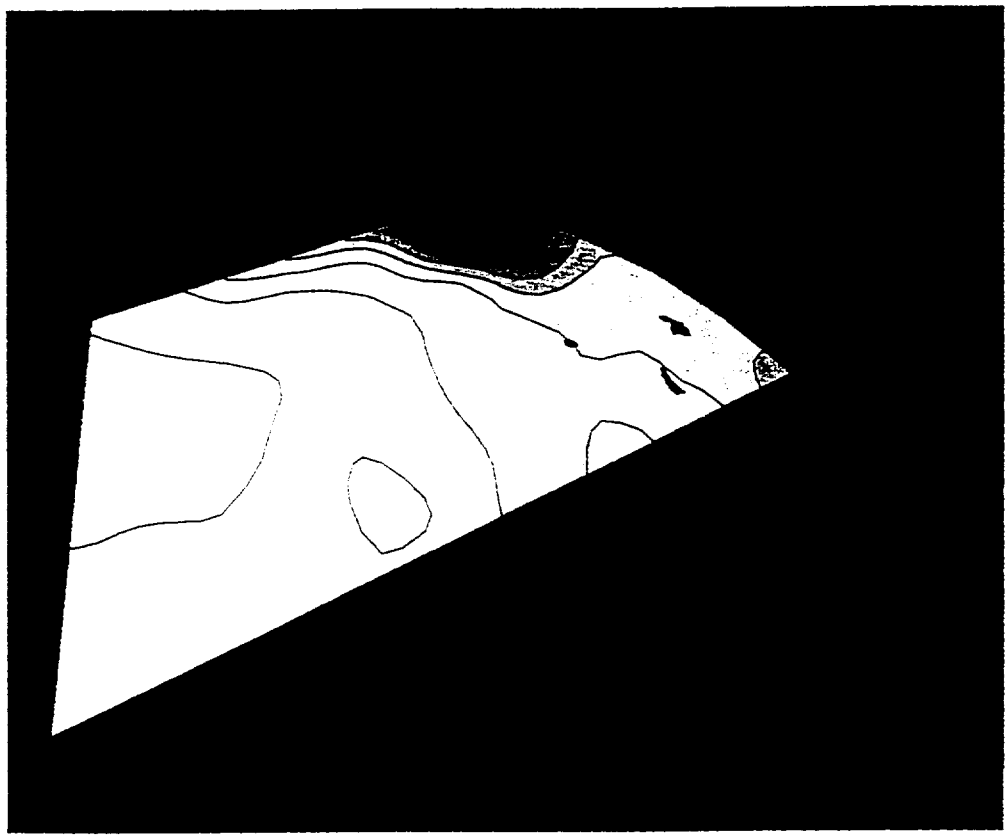
PEB (ug/L X100) for Cal9410 Cruise

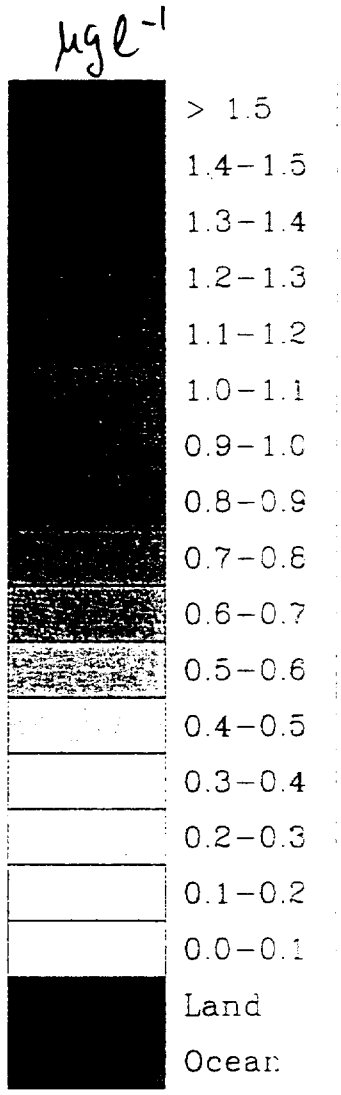




CALCOFI OCT 94
CHLOROPHYLL CONCENTRATION

Chlorophyll a [ug per L] for Cal9410 Cruise





CALCOFI 1994 Seasonal variability

Table 1. Surface chlorophyll *a*, phycoerythrin concentrations and cyanobacteria abundance for 1994 at the California Current sampled during the CalCOFI cruises. Data presented as average + standard deviation and range of values encountered in parenthesis.

Date	Phycoerythrin ($\mu g\ l^{-1}$)	Cyanobacteria 10^{-6} (cell l^{-1})	PEB cell^{-1} (fg cell^{-1})	Chl *a* + Phaeo ($\mu g\ l^{-1}$)
January	0.092 ± 0.123 (0.0037-0.425)	12.0 ± 10.6 (0.088-56.0)	13.7 ± 18.6 (4.08-51.3)	0.56 ± 0.68 → AVG ± STD (0.13 - 1.91) → range
March/April	0.042 ± 0.088 (0.0019-0.512)	10.15 ± 9.68 (0.088-36.3)	4.52 ± 4.43 (0.29-20.8)	0.55 ± 1.34 (0.08 - 7.23)
October	0.084 ± 0.120 (0.0017-0.236)	16.24 ± 14.17 (2.15-52.8)	6.08 ± 7.27 (0.82-25.4)	0.49 ± 1.18 (0.08 - 5.75)
Annual Average	0.069 ± 0.11	12.79 ± 3.12	8.1 ± 4.91	0.54 ± 1.09

Cell carbon can be calculated from
cell size
cell abundance

Verret + Iturriaga (unpublished data)

COAST OF SOUTHERN CALIFORNIA
VERTICAL VARIABILITY

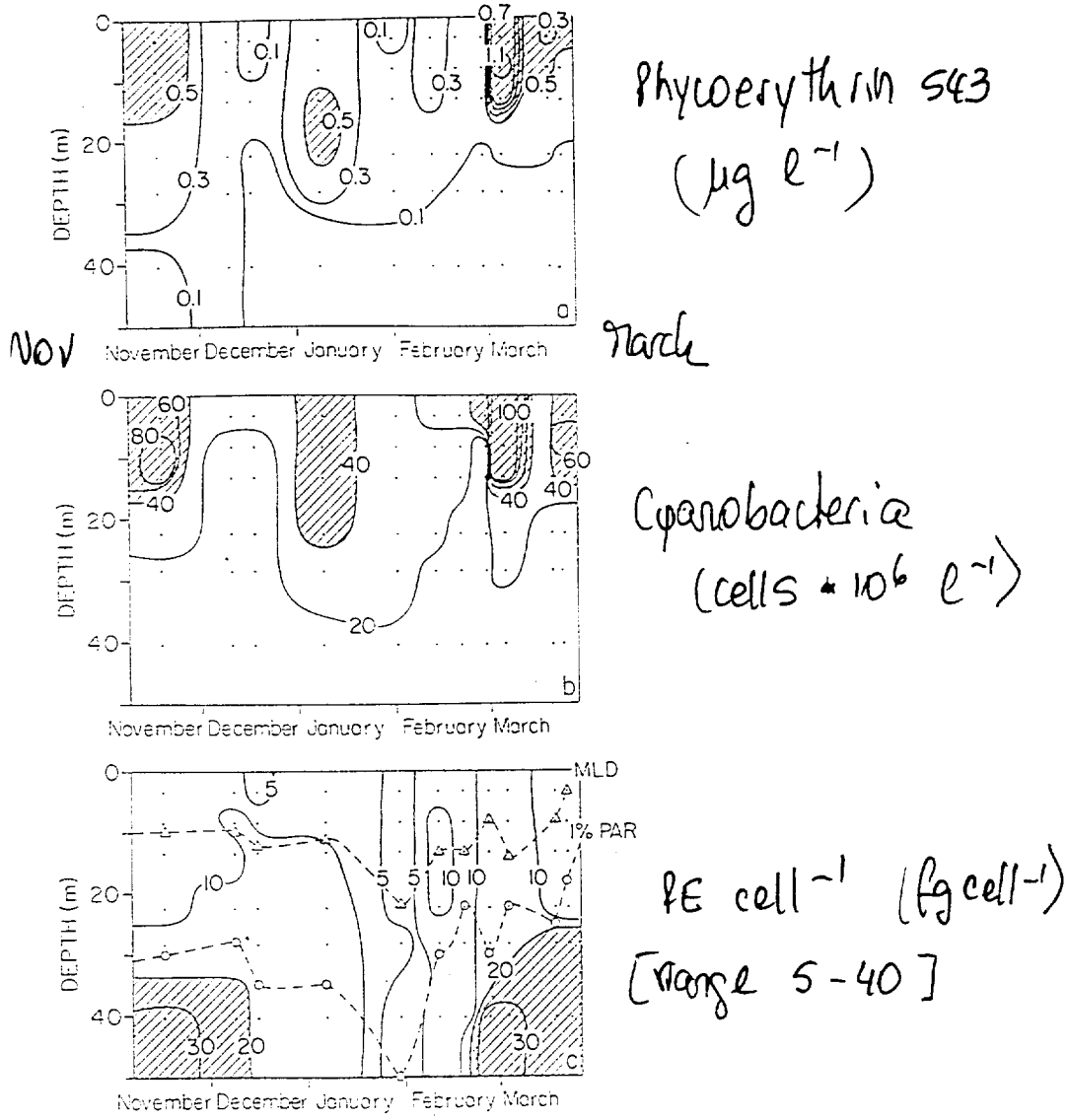


Fig. 3. Contours of field concentrations of *Synechococcus*-type cells and their phycobiliproteins: (a) concentration of extracted phycoerythrin-543 (PE-543, μg l^{-1}); (b) distribution of *Synechococcus* cell concentration ($\times 10^6$ cells l^{-1}); (c) estimated PE-543:cell for *Synechococcus* spp. ($\times 10^{-9}$ μg PE $cell^{-1}$). (△) Depth of the mixed layer (MLD); (○) 1 % isolume for PAR

Vernet et al (1990)

HOW TO MEASURE PE < Abs / Fe properties

Coccoid Cyanobacteria phycoerythrins

PUB = phycourobilin
PEB = phycoerythrobilin

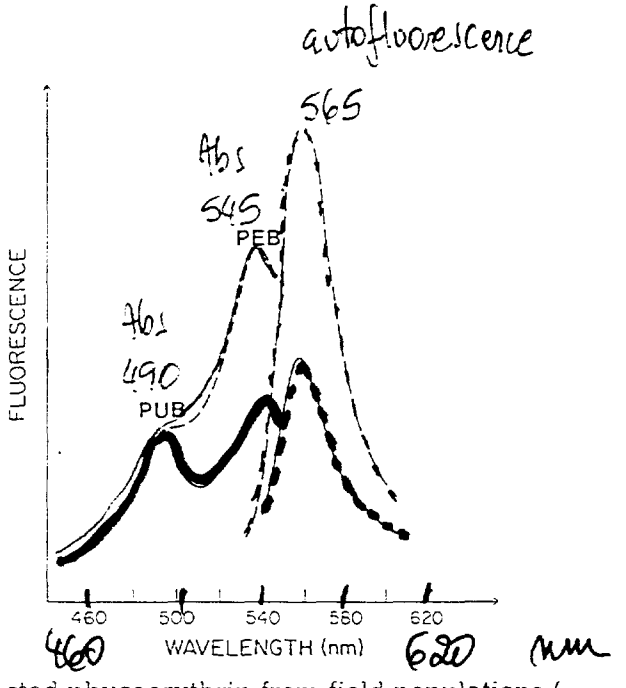


Fig. 2. Extracted phycoerythrin from field populations (———) and from *Synechococcus* clone WH7803 (– – –) excitation (460 to 540 nm) and emission (530 to 600 nm) spectra in Trizma base and sodium EDTA buffer, pH 7. For emission spectra the excitation wavelength was 520 nm and for excitation spectra the emission wavelength was 562 nm. Fluorescence in arbitrary units. Excitation peaks at 495 and 543 nm correspond to absorption by phycourobilin (PUB) and phycoerythrobilin (PEB) respectively

nm

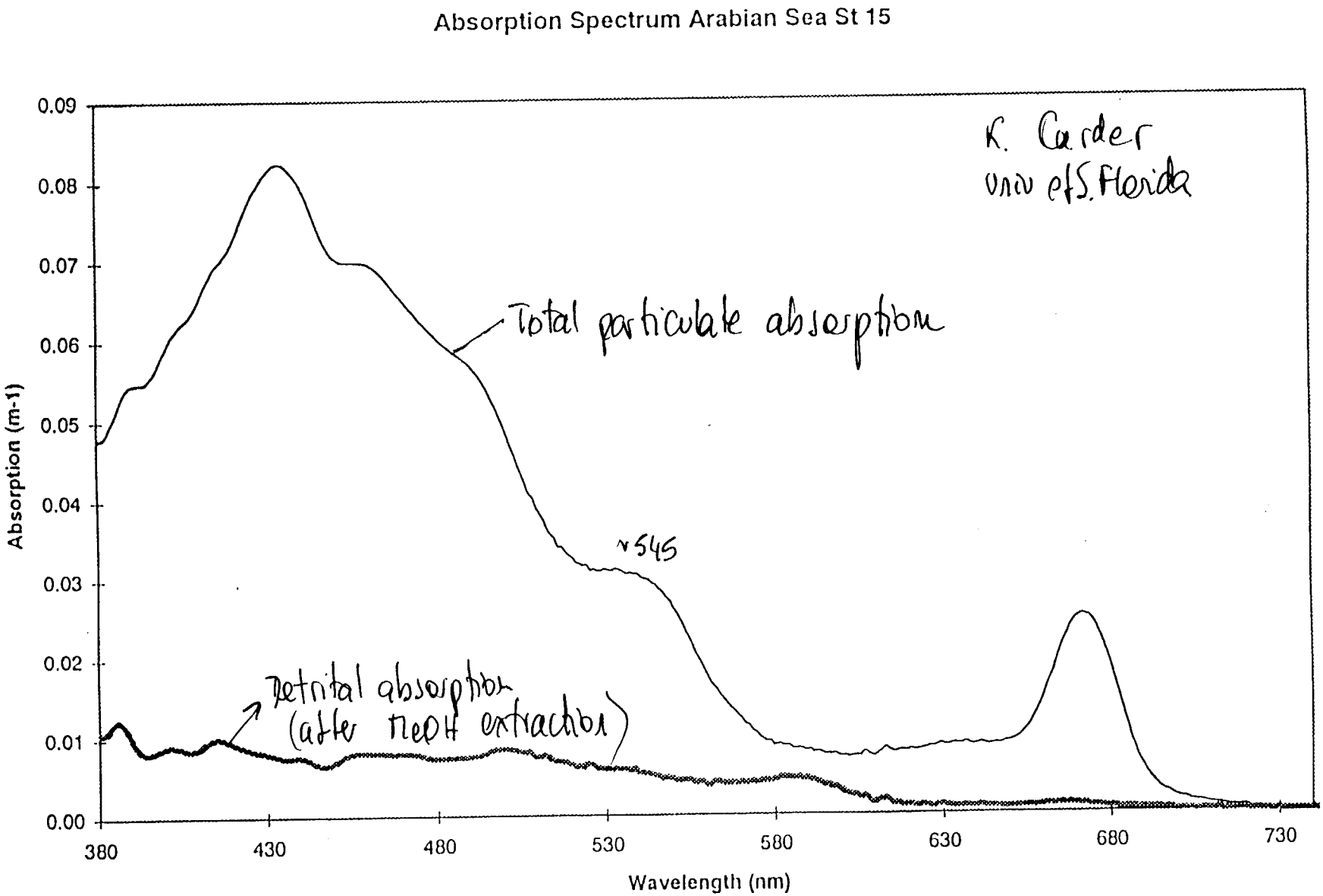
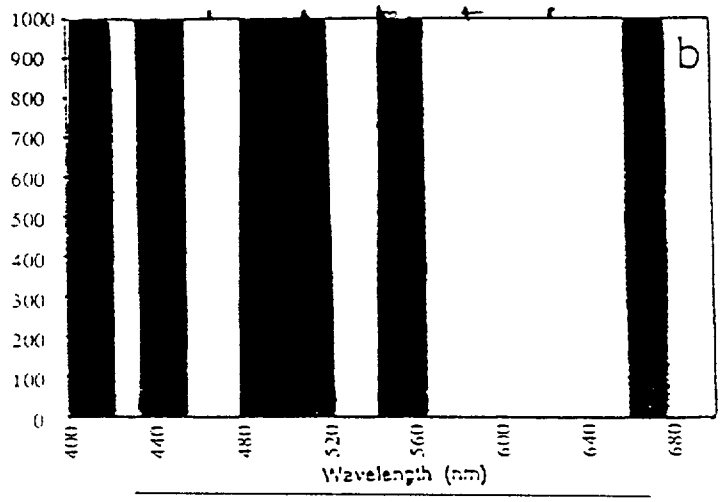


Figure 4.- Absorption spectra of phytoplankton in the Arabian Sea showing absorption shoulder at 545 nm corresponding to phycoerythrin absorption: total absorption (thin line), detrital absorption (thick line). Courtesy of Dr. K. Carder, University of South Florida.

Remote sensing of phycobiliproteins:

seawifs bands



400 520 660 nm

Remote sensing of phycobiliproteins

- Absorption properties
  - 480 nm, PUB → overlap w/ most carotenoids
  - 545 nm, PEB → possible in bloom areas
    - Northern Arabian Sea
    - North Atl
    - W coast Antarctic Peninsula (cryptomonads)
  - combination of several λ. (Sathyendranath et al., 1994)
- Fluorescence properties
  - autofluorescence of PEB at 565-570 nm

Pl f = (cyano abundance,
PEB / cell,
quantum yield of fluorescence)

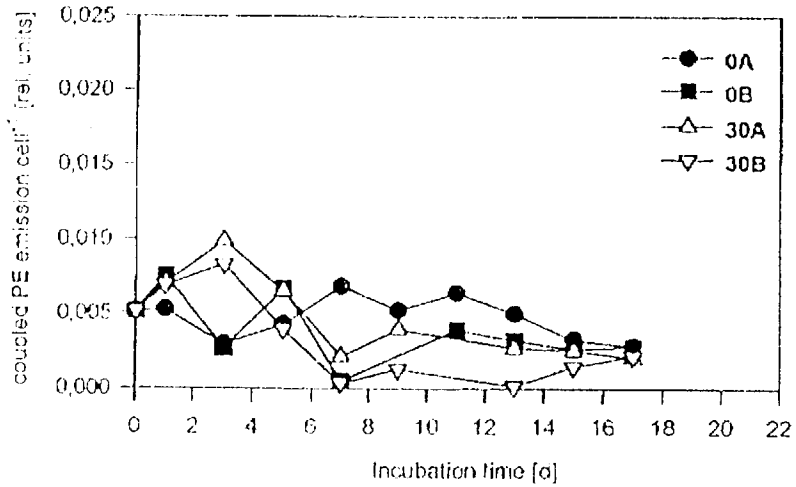
- Work to do:
  (1) Relationship between water-column optics + PE abundance
  CalCOFI '94 + '95

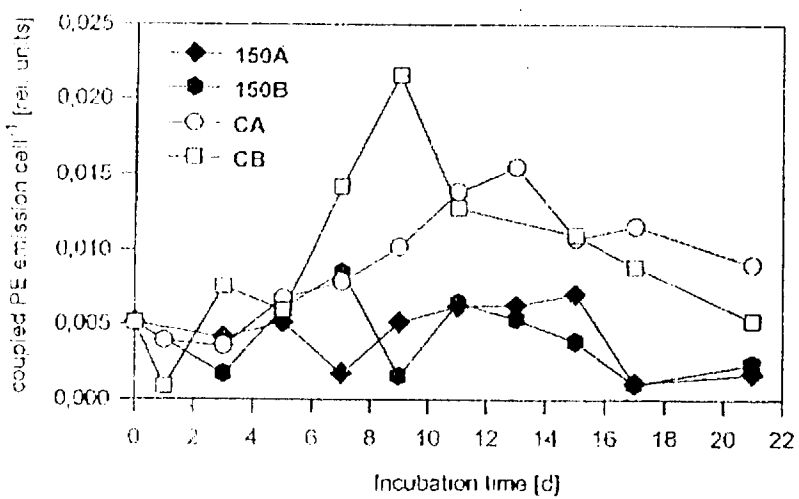
  (2) fluorescence yield / cell. (quantify)
  - lab
  - field

PE fluorescence emission / cell

coupled PE

(Δ after - before glycerol)



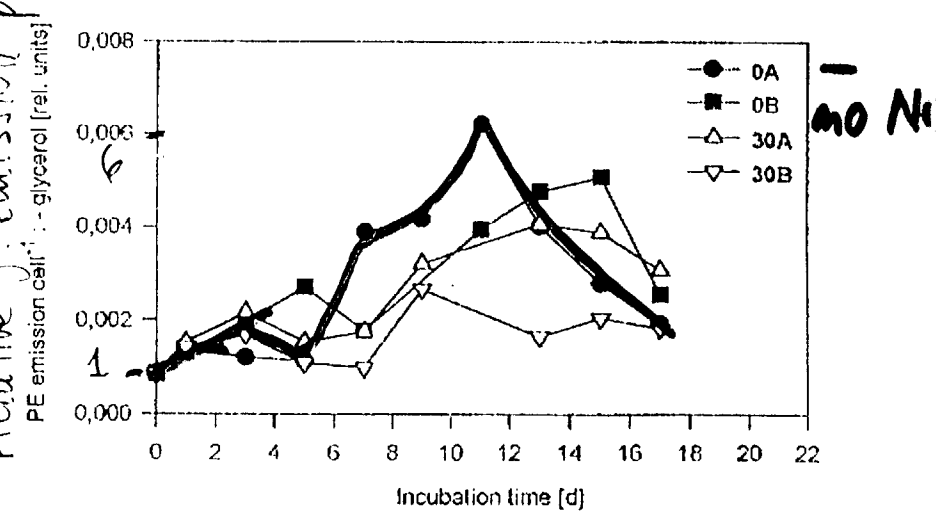


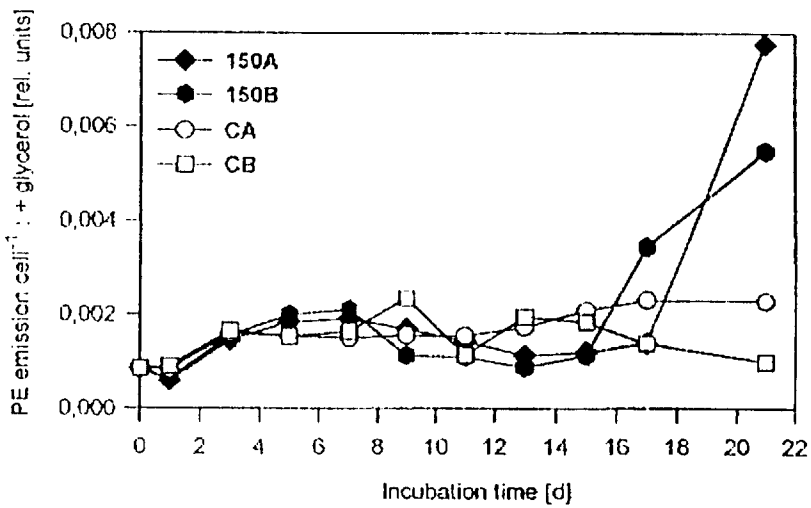
PE fluorescence emission / cell

uncoupled PE ≙ emission before gly

Relative fl. emission per cell

Ym 565 nm





Vernet + Detmer (in prep)

Need to calibrate fluorescence em.

Figure 3.- Surface phycoerythrin (PE) concentration in the CalCOFI grid during April 1994, in 10^2 mg m^{-3}. Pigments estimated by *in vitro* fluorescence emission on a fosfate buffer at pH 7 (Vernet et al., 1990).

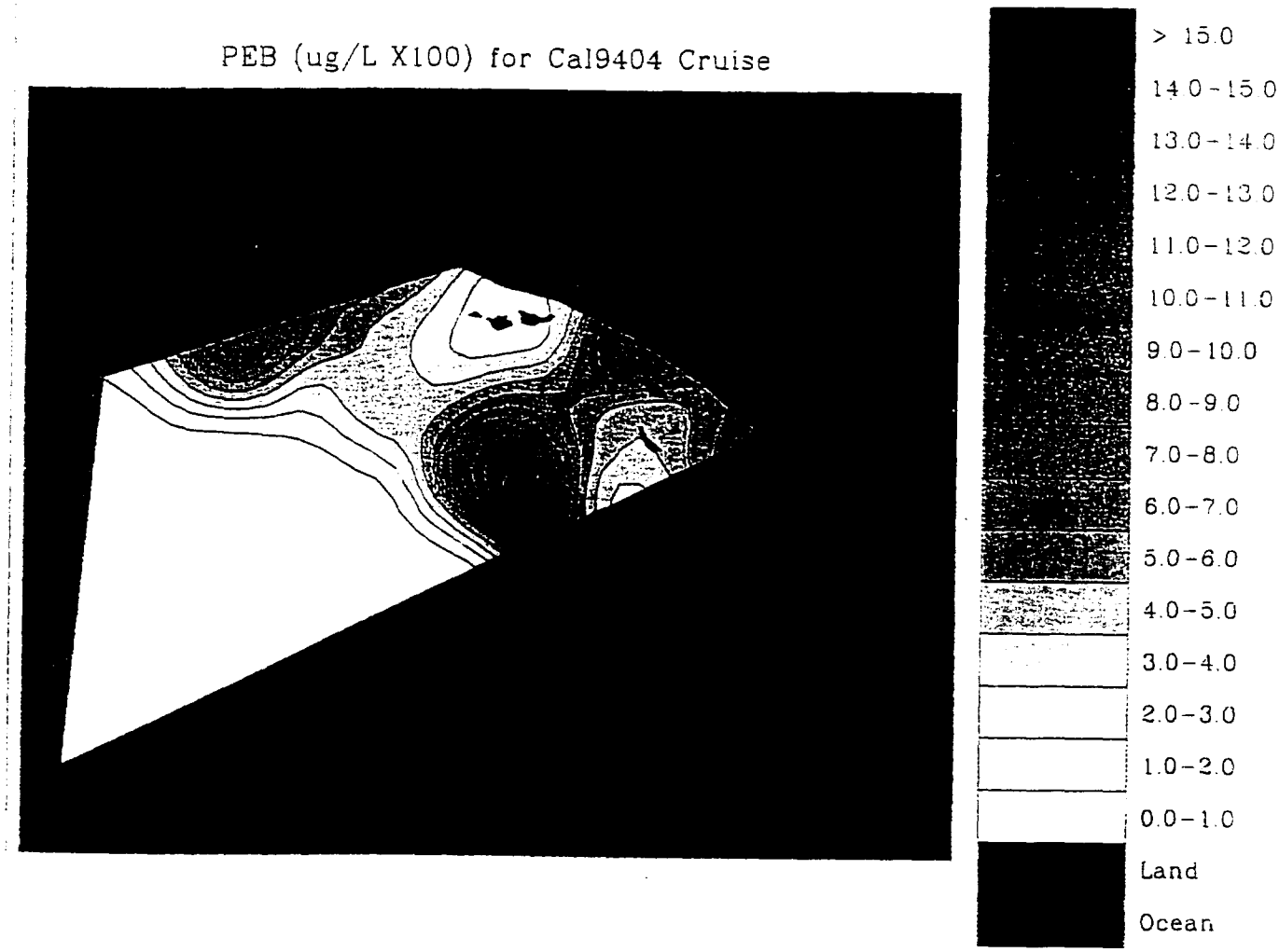


Figure 2.- Surface distribution of cyanobacteria abundance [illegible] grid during April 1994, in cells ml^{-1}. Concentrations varied from 2.15 to 52.[illegible] cells ml^{-1}. Cells counted on a fluorescence microscope, in collaboration with Ms. Tiffany M[illegible], Scripps Institution of Oceanography.

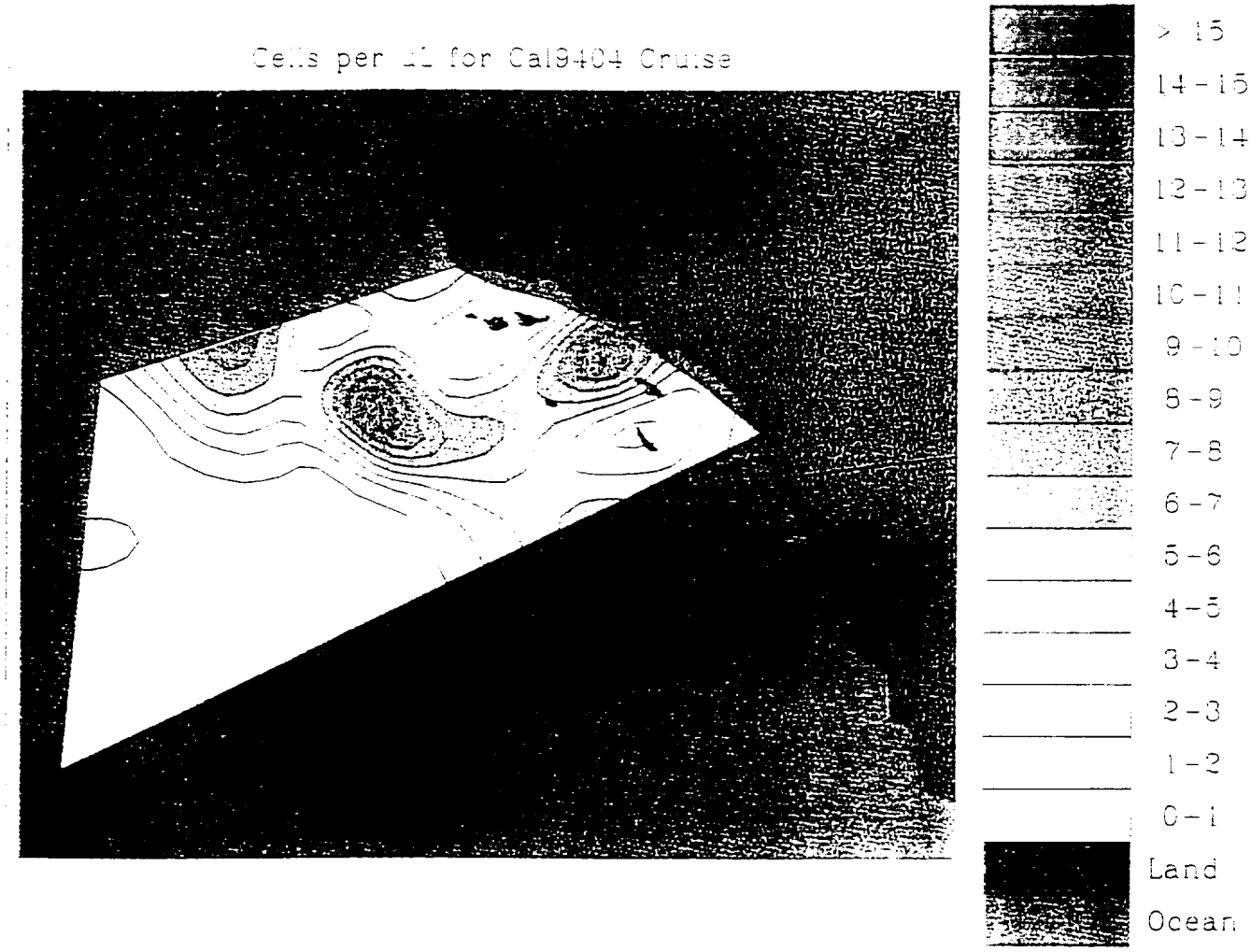
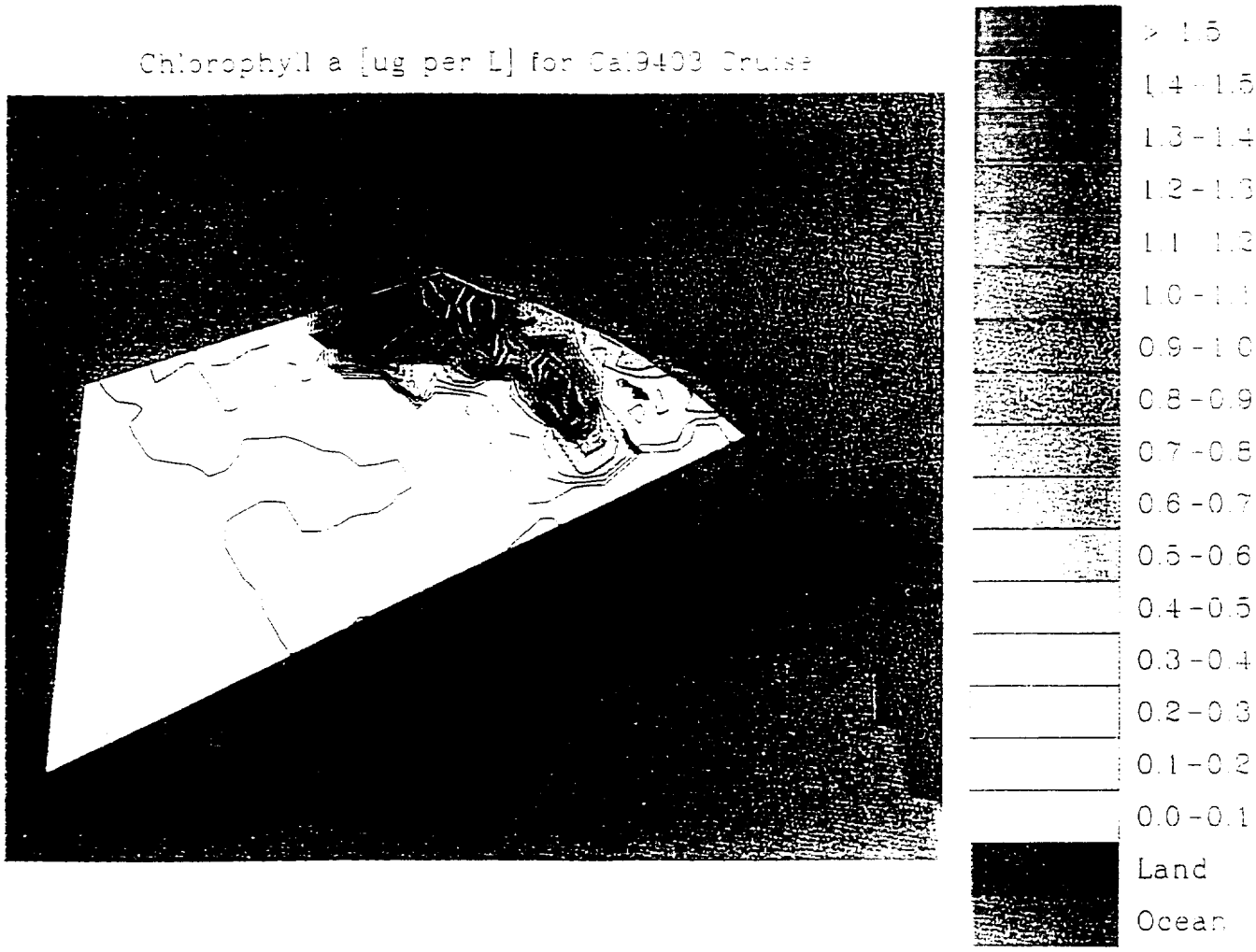


Figure 1.- Surface chlorophyll *a* distribution in the CalCOFI grid [illegible] April 1994. Concentrations vary from 0.08 to 5.75 mg m^{-3}. Pigments extracted [illegible] acetone and measured on a Turner Designs fluorometer.



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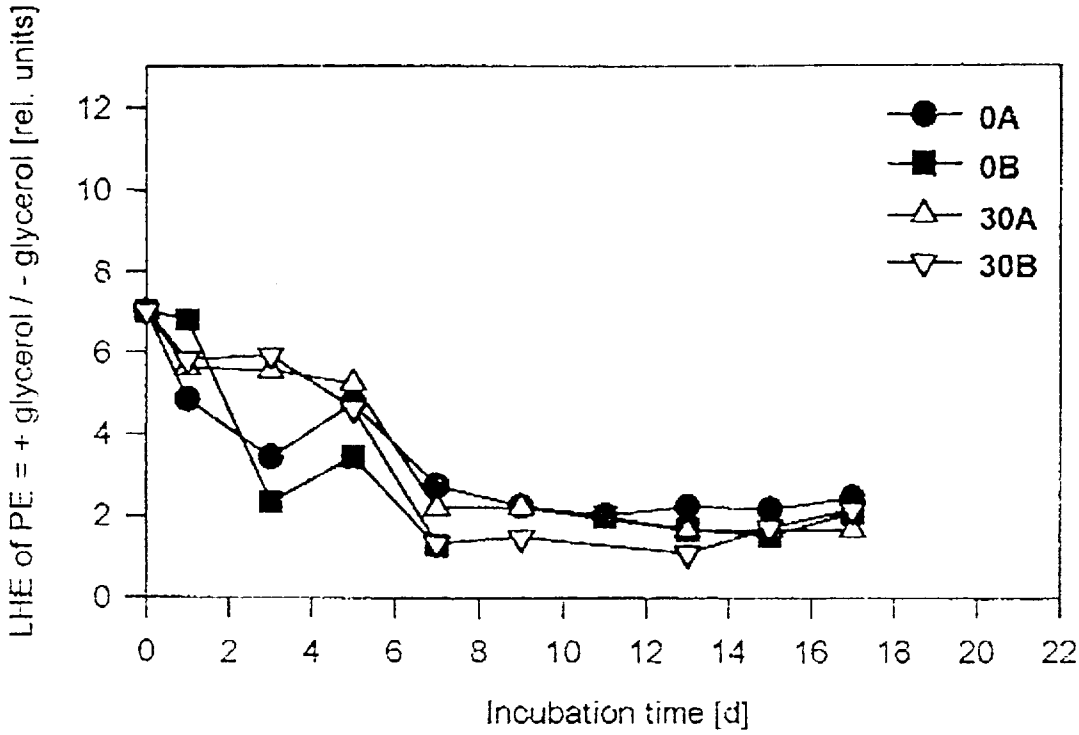
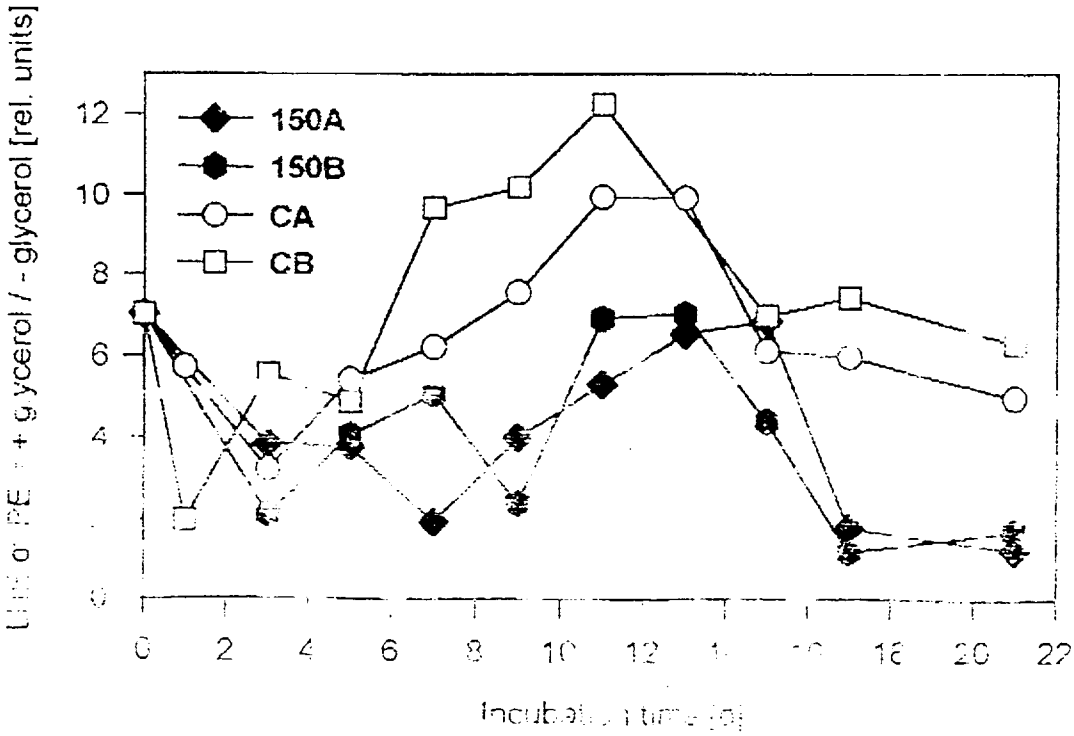
Annual Average	0.069 ± 0.11	12.79 ± 3.12	8.1 ± 4.91	0.54 ± 1.09

Table 1 summarizes the similarities and differences between cyanobacteria and cryptomonads (Gantt, 1979; Glazer, 1988; Hill and Rowan, 1989):

	CYANOBACTERIA	CRYPTOMONADS
Temperature	tropical temperate	tropical temperate polar
Distribution	coastal open ocean	coastal
Phycobili-somes	present	absent
Phycobili-protein number	all 3 pigments	only 1 of the pigments
Phycobili-protein type	phycourobilin phycoerythrin phycocyanin allophycocyanin	 phycoerythrin type I phycoerythrin type II phycoerythrin type III phycocyanin type I phycocyanin type II
Absorption maxima *in vivo*	PUB: 493 nm PEB: 545 nm PC: 645 nm	PE I: 545 nm PE II: 555 nm PE III: 566 nm PC I: 585, 645 nm PC II: 588, 615 nm
Fluorescence emission maxima *in vivo*	PUB: none PEB: 565 nm PC: 665 nm	PE type I: 585 nm PE type II: 580 nm PE type III: 617 nm PC I: 655 nm PC II: 637 nm
Auto-fluorescence	575 nm	580 nm
Microscopic enumeration	FL.EM. 575 nm (yellow)	FL.EM. 575 nm (yellow)
Cell size	0.5-1.5 um	2-20 um
Flash fluorometer	yes	yes
Flow-through		

+/- Ratio in LHE of PE

5/30/95 Ignore previous plot from 2/27/95