FINAL REPORT

CONTRIBUTION OF PHYCOERYTHRIN-CONTAINING PHYTOPLANKTON TO REMOTELY SENSED SIGNALS IN THE OCEAN"

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The purpose of this project was to evaluate the importance of phycoerythrin-containing phytoplankton, in particular coccoid cyanobacteria, to remote sensing. We proposed to estimate cyanobacteria abundance and pigmentation and their relationship to water-column optics. We have estimated the contribution of cyanobacteria to scattering and backscattering in both open ocean (Sargasso Sea) and coastal waters (western coast of North Atlantic and the California Current). Sampling and data processing is finished and we are now publishing the results from the funding period. Relationship between water column optics and phycoerythrin concentration and algorithm development are being carried out in collaboration with Drs. B.G. Mitchell and M. Kahru.

Phycoerythrin (PE) is present only in two phytoplankton groups (Cyanobacteria and Cryptomonads). PE is similar to chlorophyll a: it is able to absorb light and to fluoresce in vivo in the visible region. Our research has been focussed to inquire the possibilities of determining PE with in situ optics, in order to produce maps of PE concentrations and distribution. Little is known about the contribution of phycobiliproteins to reflectance spectra despite information

regarding their role as primary producers and the ability of cryptomonads to form blooms. Phycobiliproteins can affect the reflectance ratios by their absorption (centered at 490nm for Phycourobilin (PUB) and 540nm for PEB) properties and their autofluorescence, which is centered at 565-575 nm. Both of these pigments may affect SeaWiFS band centered at 555nm.

Major findings under this project:

(1) We have successfully measured phycoerythrin concentration from cyanobacteria and crytomonads from field populations based on aqueous extracts.

(2) Cyanobacteria from open waters, growing at high irradiance and low nutrient concentration, have on average an order of magnitude less phycoerythrin content than cells in coastal waters (MS submitted).

(3) Detection of variability of cyanobacteria populations (as indicated by their fluorescence properties) in 2 patterns: an off-shore on-shore gradient, as previously published, and also variability within coastal waters.

(4) This is the first study where field analysis of phycoerythrin are performed on a mesoscale effort and with seasons variability.

(5) Extracted phycobiliproteins from cyanobacteria showed a phycoerythrin fluorescence emission with a chromophore emitting at about 610 nm (phycocyanin).

(6) Development of continuous flow phycoerythrin fluorescence at 570 nm.

Major accomplishments during the grant period:

I. Publications:

Iturriaga, R., J. Berwald, and G.J. Sonek (1996): "A new technique for the determination of spectral reflectance of individual and bulk particulate suspended matter in natural water samples". SPIE Proc. Vol. 2963: 455-640.

Vernet, M., and R. Iturriaga. "Contribution of cyanobacteria to optical properties of surface waters in the western North Atlantic the spring of 1993". Marine Ecology Progress Series (submitted).

Iturriaga, R., J. Berwald, S. Gallegos and M. Vernet: "Spectral reflectance of Yellow Sea phytoplankton cells, detrital matter and minerals and their specific contribution to total reflectance". (Applied Optics, to be submitted on July 1998).

Vernet, M., A. Detmer, A., and R. Iturriaga" Response of cyanobacteria to nitrate limitation: growth, pigmentation and fluorescence properties" (To be submitted to Marine Ecology Progress Series, March 1998).

II. Presentations at Conferences

R. Iturriaga and M. Vernet (1994)." Surface distribution of Phycoerythrin-containing phytoplankton in the North Atlantic estimated by pigment analysis. Ocean Sciences AGU/ASLO. San Diego.

T. Nguyen, E. Frame, M. Vernet, R. Iturriaga, T. Moisan, J. Collier, G. Toledo, B. Palenik, and B.G. Mitchell (1998). "Synechococcus spp. cyanobacteria cell numbers and pigment concentrations in the California Current. Ocean Sciences AGU/ASLO San Diego.

III. Field observations:.

a) **North Atlantic:**

A manuscript on the abundance of phycoerythrin (PE), a pigment characteristic picoplanktonic cyanobacteria was determined on surface samples on a transect from Bermuda to Cape Henlopen in March 1993. In tropical waters east of the Gulf Stream, phycourobilin and phycoerythrobilin were present in similar concentrations (the absorption maxima for these chromophores is 488-493 nm and 543-545 nm). To the west of the Gulf Stream boundary, phycoerythrobilin (PEB) became relatively more abundant than phycourobilin even though overall concentrations of this pigment decreased. The contribution of picoplanktonic cyanobacteria to total scattering at 550 nm was high in the Sargasso Sea (average 9.4%) and low in coastal waters (average 0.2%). Contribution to total backscattering was maximum at 575 nm when PE fluorescence was included in the backscattering efficiency factor. Overall, backscattering of cyanobacteria followed the gradient in cell abundance which was an order of magnitude higher in open waters.

Scattering: Average contribution of surface cyanobacteria (picoplankton, average 1.0 μm diameter) to total scattering in temperate coastal waters in the California Current varies between 3.4 $\pm$ 3.3 %, with a range between 0.3% to 14%. These values are within the range estimated by Stramski and Kiefer (1991) for cyanobacteria (3% to 40% of the total scattering) and similar to estimates of the contribution of larger cells to total scattering. These values are representative of low to high cyanobacteria abundance in these waters, with cell concentrations varying by 3 orders of magnitude, from $0.09 * 10^9$ to $56 * 10^9$ cells m^{-3}.

Backscattering: As for all phytoplankton, the contribution of cyanobacteria to total backscattering was small. An average of 1.2% in the Sargasso Sea and 0.035% in the coast of Delaware. For the California Coast, we estimate a yearly average of 0.5% $\pm$ 0.5% and a range from 0.05% to 1.9%.

b) **California Current System:**

Analysis of the 1994 data showed that in the California current system average surface cyanobacteria abundance is 13.09 $\pm$ 11.6 10^9 cells m^{-3} with a range of 3 orders of magnitude, from 0.09 10^9 to 54 10^9 cell m^{-3}. Cellular phycoerythrin content is 6.14 $\pm$ 8.36 fg $cell^{-1}$, with values varying from 0.29 to 51.3 fg $cell^{-1}$. The phycoerythrin was mainly dominated by phycoerythrobilin, with absorption maximum at 545 nm and emission at 564-565 nm. In addition, we observed a seasonal variability in cellular pigmentation (phycoerythrin per cell) with lower values in spring and higher in fall and winter (see Table 1).

The contribution of picoplanktonic cyanobacteria to scattering and backscattering was determined at the main spectral absorption and fluorescence wavelengths specific of this phytoplankton group (493 and 545 nm for absorption and 575 nm for emission). Overall values for cyanobacteria scattering (b) at 493 (PEB -rich type) fluctuated from 0.002 to 0.006 m^{-1}, with average scattering by cyanobacteria in the region of 4.9 $\pm$ 4.4 10^{-3} m^{-1} and backscattering (bb) from 1.2 * 10^{-5} to 7 * 10^{-6} m^{-1}. At 550 and 575 nm, values followed similar patterns. However, when backscattering values at 575 nm included phycoerythrin cell fluorescence (Morel et al., 1993), the backscattering estimate increased by a factor of 3 (~ 1.3 * 10^{-5} to 3.3 * 10^{-5}). Scattering and backscattering values followed the seasonal pattern of cyanobacteria abundance and PEB per cell. When picoplanktonic cyanobacteria scattering is calculated as a percentage of the total scattering, which is estimated from (chlorophyll *a* + phaeopigment) concentrations (Gordon and Morel, 1983; Morel, 1988), the contribution of cyanobateria range from 0.2% to 14% in surface waters at 550 nm, with average value of 3.4% $\pm$ 3.4% (Table 2). The contribution of cyanobacteria to total backscattering at 550 nm is 0.5% $\pm$ 0.5% (Table 2). As shown above, we expect this contribution to be higher at 575 nm due to phycoerythrin cell fluorescence.

Spatial variability of surface chlorophyll *a*, cyanobacteria abundance and phycoerythrin concentration are shown in Figs 1, 2 & 3. Mesoscale variability is high. The data presented here is for October 1994 and it is representative of other times of the year as well. In this particular cruise, phytoplankton was concentrated on the NE corner of the CalCOFI grid, off Concepcion Point (Fig. 1). Cyanobacteria abundance does not follow chl *a* and presents 3 areas of high abundance (Fig. 2). Similar mesoscale features are seen in PE distribution (Fig. 3).

The range of values in cell abundance observed in the California Current are representative of coastal waters. Earlier studies in the area report that picoplanktonic cyanobacteria undergo major changes in cell abundance (1.4 to 116 * 10^9 cell m^{-3}), as well as in phycobiliptotein pigments (2 to 40 fg PE per cell) (Vernet et al., 1990). Such values are similar to fluctuations observed in the North Atlantic (Iturriaga and Marra, 1988; Olson et al., 1990; Morel et al., 1993; Li, 1994) but lower than some blooms observed in the spring in the North Atlantic (May 1990, R. Iturriaga, unpublished data) and the Arabian Sea (A. Detmer, Univ. of Kiel, unpublished data).

A model indicated that inclusion of PE fluorescence emission as a source in spectral backscattering of the ocean may be relevant (Morel et al. 1993). Emission maximum for PE was centered at 565 nm, overlapping the 555-nm band in SeaWIFS. This is a potential source of

interference for the 555 nm band which can be estimated if we have phycobiliprotein (PE) abundance and fluorescence yield.

Data collection on the cruises include samples for the estimation of cyanobacteria cell abundance and cell size, phycoerythrin and chlorophyll concentrations, continuous measurement of phycoerythrin emission at 565 nm, water column optics as measured by a MER-8010 for the SeaWIFS relevant bands by B.G. Mitchell, and other enviromental variables collected by the Marine Life Research Group at Scripps Institution of Oceanography (nutrients, surface oxygen, and total primary production). In addition, cyanobacteria abundance and fluorescence per cell were measured by cytofluorometry by J. Collins (SIO).

IV. Instrumentation development:

An *in situ* flow-through fluorometer to estimate phycoerythrin containing phytoplankton has been developed and was tested in the field.

A modified *in situ* flow-through fluorometer for phycoerythrin fluorescence at 570 nm by phytoplankton has been tested in all cruises. The set-up is similar to the continuous record of chlorophyll *a* fluorescence. We have changed the configuration to include a photomultiplier sensitive in the green region of the spectrum and filters to match the maxima of absorption (493 nm and 545 nm) and fluorescence emission (565-575 nm) of marine cyanobacteria. A second modification of the illumination system permitted to achieve better sensitivity and resolution in continuous sampling (Figure 4). This improved version was successfully tested in 1996 in CalCOFI cruise 9602. With this instrument we will estimate horizontal picoplankton abundance in continuous surface mapping and relate to chlorophyll *a* fluorescence (685 nm) used as an estimate of total phytoplankton abundance.

Recommendations for Future Research

We believe the optical signals for cyanobacteria are best suited for partitioning of the active and passive remote sensing of phytoplankton into taxonomic groups. Several lines of research will provide improved tools for this objective:

(1) Groundtruthing of the phycoerythrin absorption and fluorescence properties, and algorithm for MODIS-type sensors, in collaboration with F. Hoge and R. Swift, Wallops Island, Goddard Flight Space Center.

(2) Study of the influence of cyanobacteria scattering and backscattering on remote sensed signals in open ocean and coastal environments, in particular case II waters, in order to improve ocean color algorithms.

(3) Include the remote sensing of cryptomonads' phycoerythrin, different from the cyanobacteria PE.

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STRAMSKI, D. and D.A. KIEFER (1991) Light scattering by microorganisms in the open ocean. Progr. Oceanogr., **28**, 343-383.

VERNET, M., B.G. MITCHELL and O. HOLM-HANSEN (1990) Adaptation of *Synechococcus in situ* determined by variability in intracellular phycoerythrin-543 at a coastal station off the Southern California coast, USA. Mar. Ecol. Progr. Ser., **63**, 9-16.

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 (0.13 - 1.91)
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

Table 2.- Contribution to scattering and backscattering by cyanobacteria at 550 nm. Total scattering and backscattering estimated from (chlorophyll a + phaeopigment) concentrations according to Gordon and Morel (1983) and Morel (1988). Estimated from the California Current on 1994 on the CalCOFI grid. Data presented as average + standard deviation and range of values encountered in parenthesis.

Date	Scattering (m^{-1})			Backscattering (m^{-1})		
	Total	Cyano $* 10^3$	%Cyano	Total $* 10^3$	Cyano $* 10^5$	%Cyano
January	0.23 ± 0.13 (0.08 - 0.44)	6.6 ± 2.8 (3.1 - 11.7)	2.96% ± 1.6% (0.4% - 10.9%)	3.2 ± 1.1 (1.7 - 4.7)	1.42 ± 0.61 (0.6 - 2.5)	0.48% ± 0.23% (0.23% - 0.79%)
March/April	0.16 ± 0.19 (0.06 - 1.2)	3.8 ± 3.6 (0.33 - 13.6)	2.58% ± 3.03% (0.3 - 14.1)	2.1 ± 1.4 (1.1 + 7.8)	0.81 ± 0.77 (0.06 - 2.7)	0.36% ± 0.41% (0.05% - 1.8%)
October	0.16 ± 0.17 (0.06 - 0.88)	6.1 ± 5.3 (0.99 - 10.8)	4.49% ± 3.7% (1.1% - 5.1%)	2.1 ± 1.2 (1.0 - 7.3)	1.3 ± 1.13 (0.1 - 4.2)	0.49% ± 0.54% (0.1% - 1.9%)
Yearly Average	0.17 ± 0.17	4.9 ± 4.4	3.4% ± 3.3%	2.2 ± 1.3	1.05 ± 0.93	0.49% ± 0.47%

FIGURE LEGENDS

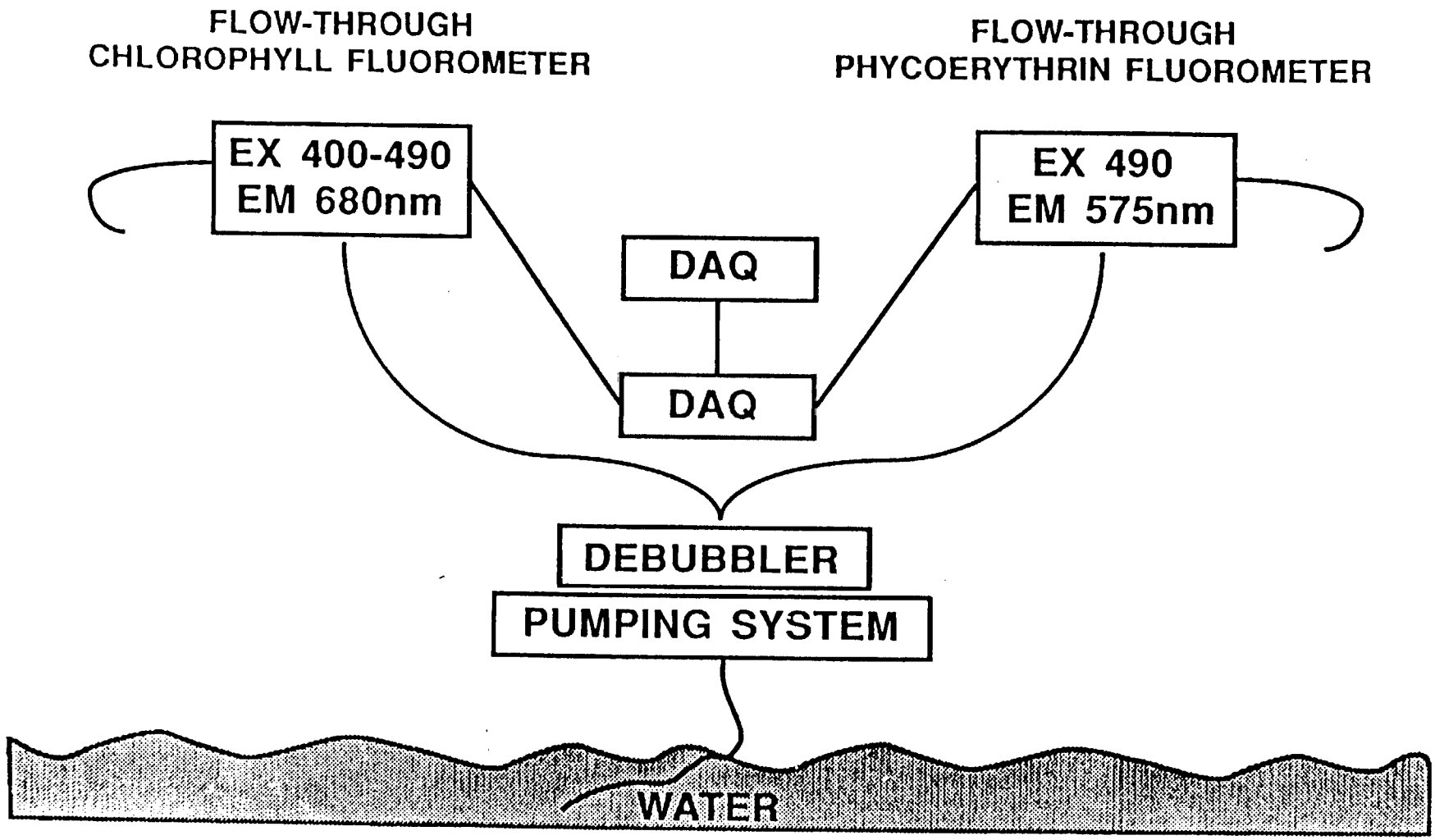
Figure 1 - Surface chlorophyll *a* distribution in the CalCOFI grid during October 1994 . Concentrations vary from 0.08 to 5.75 mg m^{-3}. Pigments extracted in 90% acetone and measured on a Turner Designs fluorometer.

Figure 2 - Surface distribution of cyanobacteria abundance in the CalCOFI grid during October 1994, in cells μl^{-1}. Concentrations varied from 2.15 to 52.8 cells μl^{-1}. Cells counted on a fluorescence microscope, in collaboration with Ms. Tiffany Moisan, Scripps Institution of Oceanography.

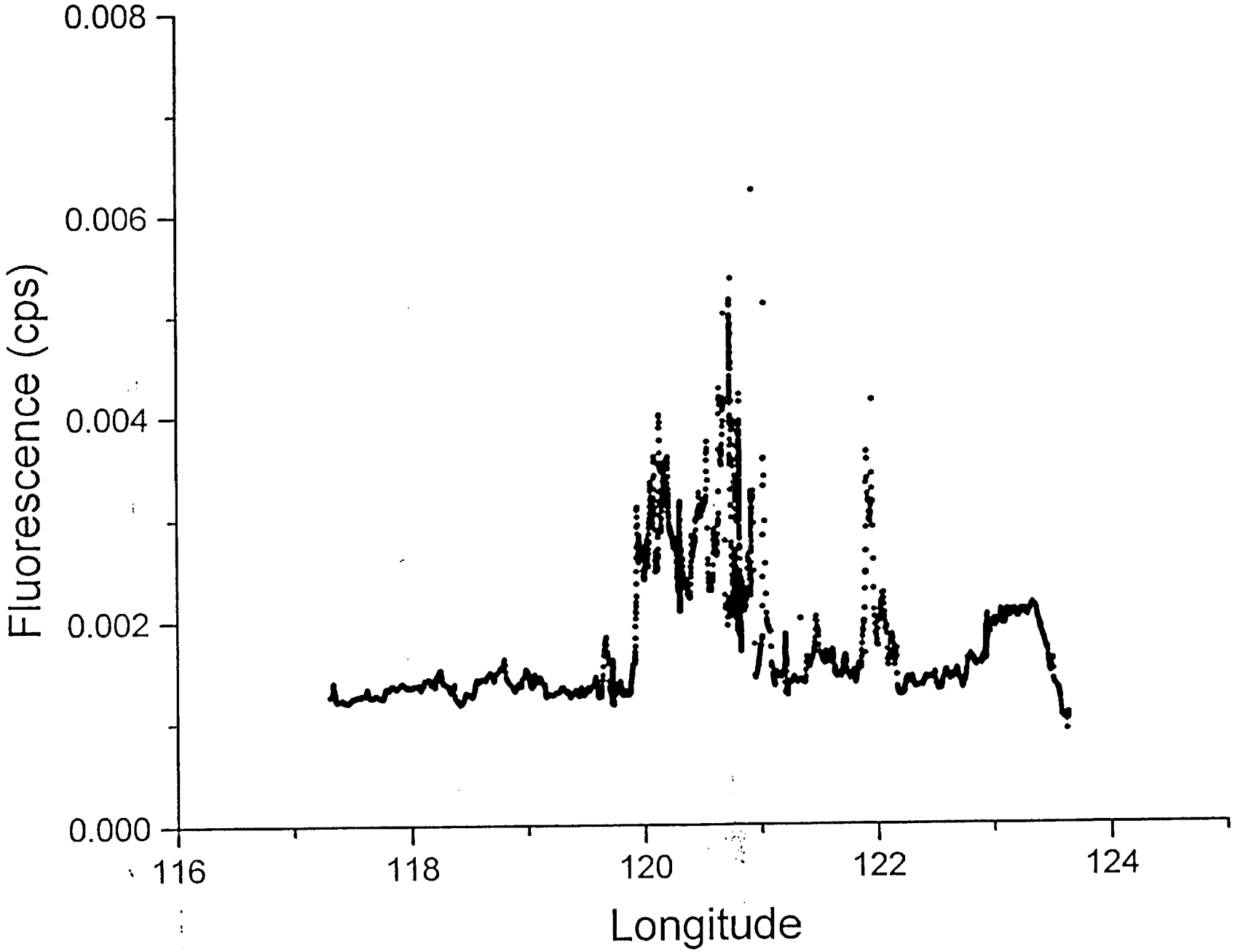
Figure 3 - Surface phycoerythrin (PE) concentration in the CalCOFI grid during October 1994, in 10^2 mg m^{-3}. Pigments estimated by *in vitro* fluorescence emission on a phosphate buffer at pH 7 (Vernet et al., 1990).

Figure 4 – Configuration of the flow-through filter fluorometer for determination of phycoerythrin fluorescence *in vivo*.

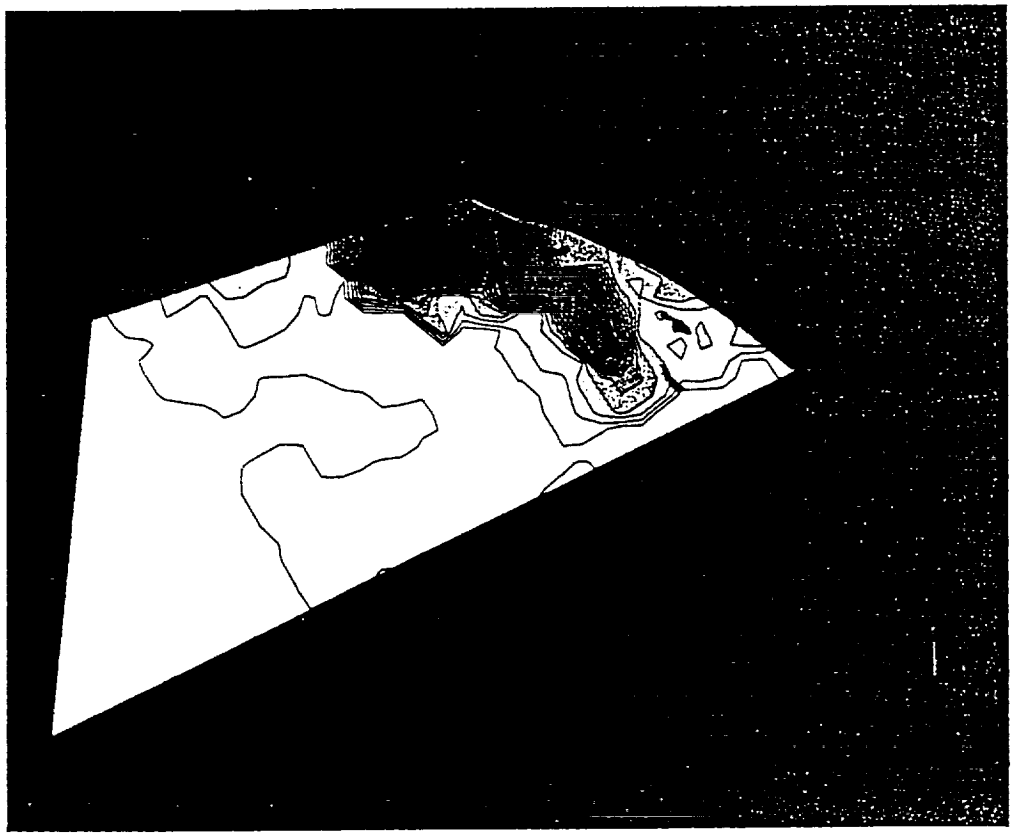
Figure 5 – Trace of fluorescence emission at 570 nm indicating spatial variability of phycoerythrin containing cyanobacteria during February 1996 in the California Current System on an onshore-offshore transect.

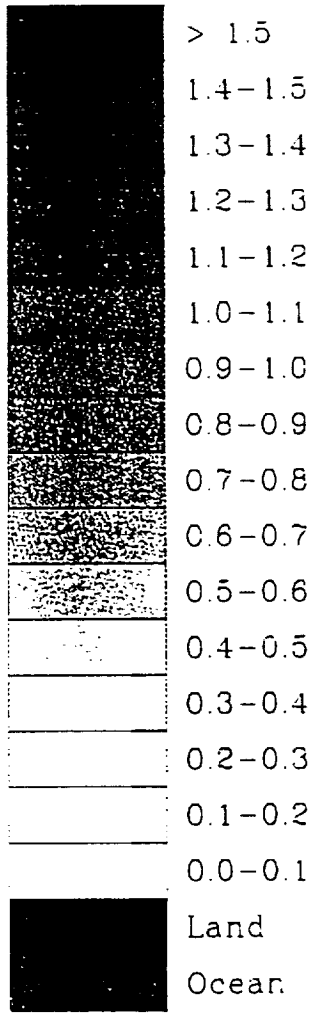

FLOW-THROUGH
CHLOROPHYLL FLUOROMETER
FLOW-THROUGH
PHYCOERYTHRIN FLUOROMETER
EX 400-490
EM 680nm
EX 490
EM 575nm
DAQ
DAQ
DEBUBBLER
PUMPING SYSTEM
WATER

CALCOFI - 0296
Phycoerythrin Fluorescence

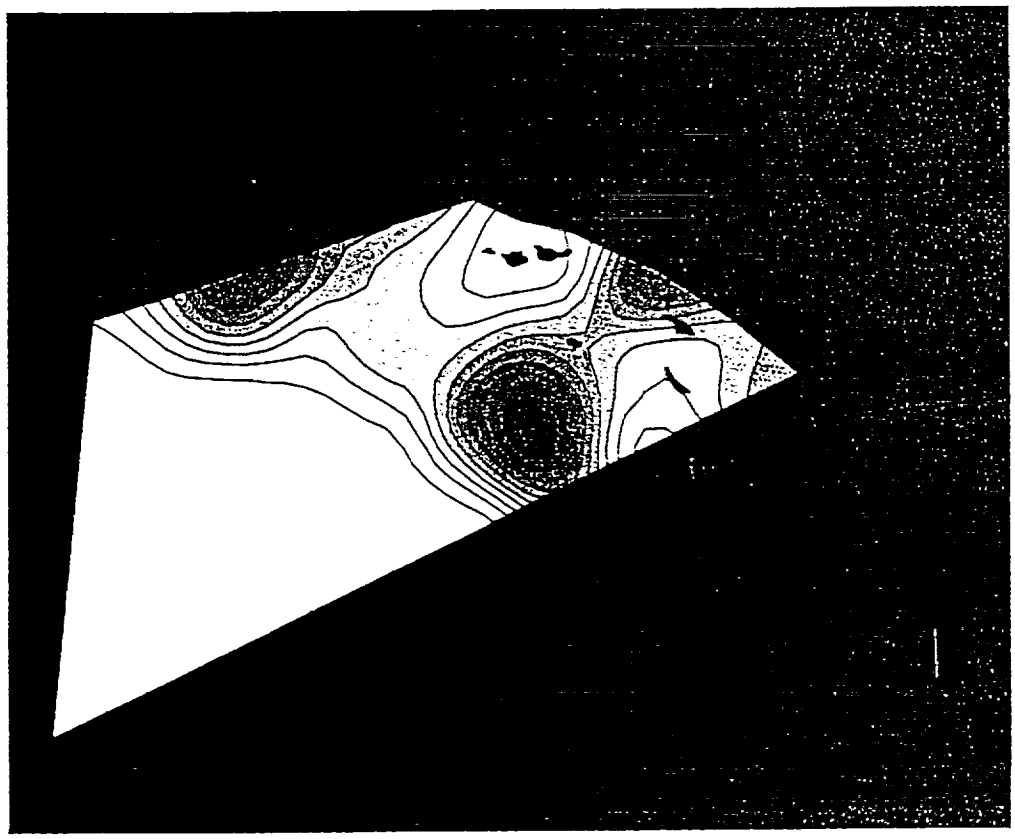


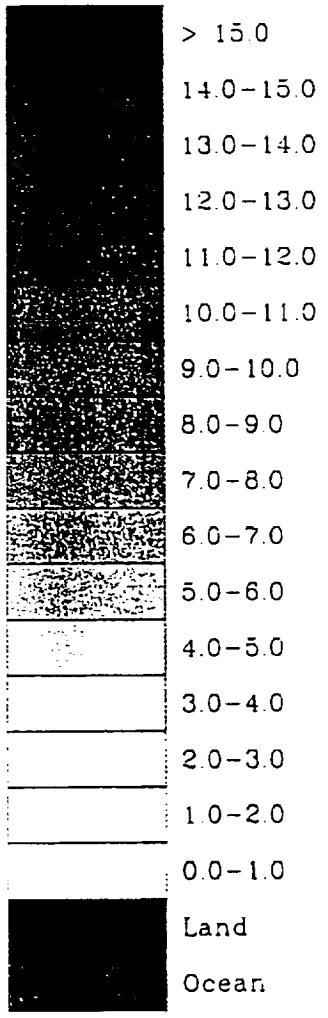
Chlorophyll a [ug per L] for Cal9403 Cruise

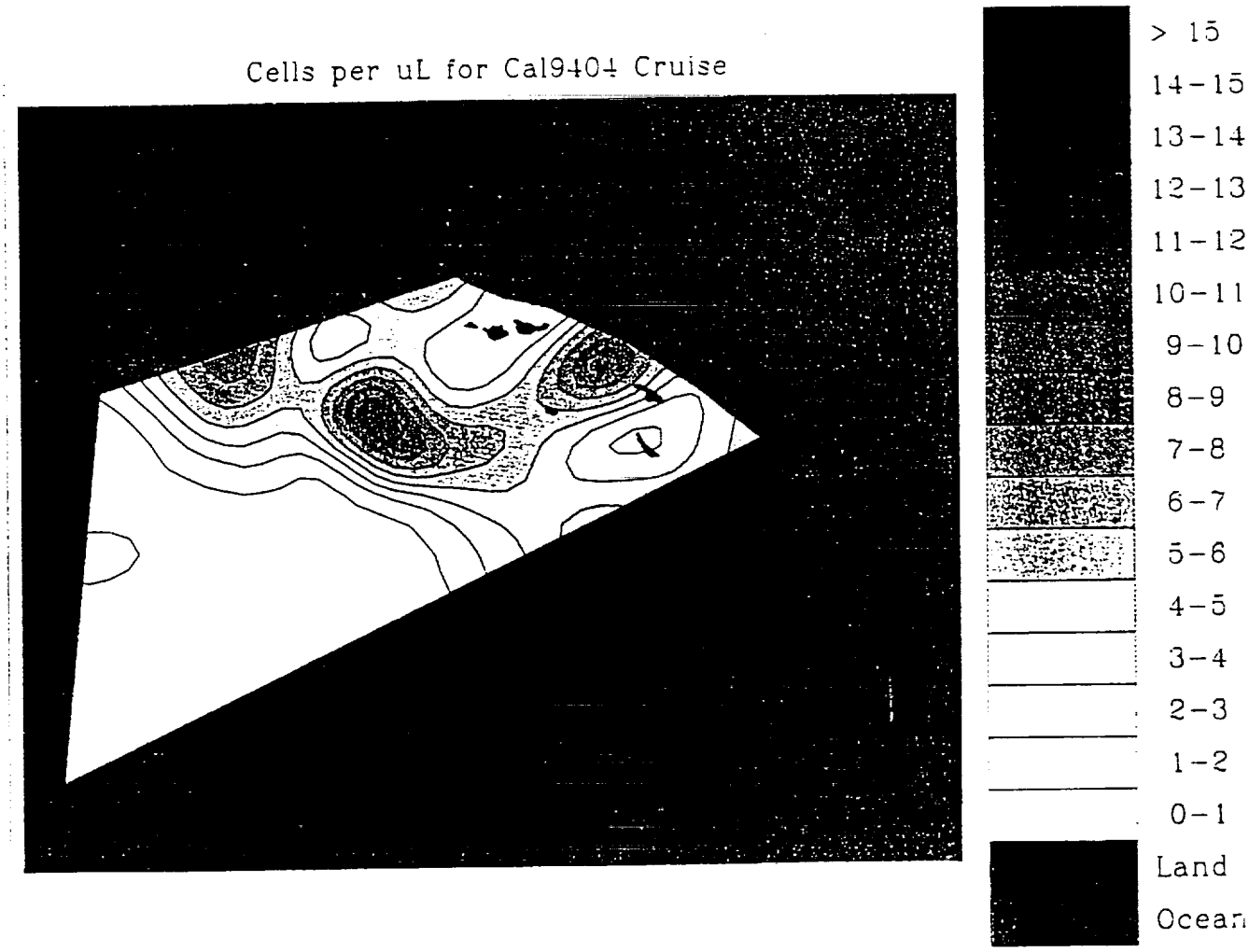




PEB (ug/L X100) for Cal9404 Cruise






Cells per uL for Cal9404 Cruise
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14-15
13-14
12-13
11-12
10-11
9-10
8-9
7-8
6-7
5-6
4-5
3-4
2-3
1-2
0-1
Land
Ocean