Phytoplankton pigment results from the PROSOPE cruise

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Sampling

Three types of sampling devices were used during the cruise (the codes correspond to the pigments file only):

- 12 l rosette bottles (code: CTD samples).
- A diaphragm pump (code: PMP samples) collected water at different depths between CTD casts 10 and 11.
- During transit the outlet of the ship's thermo-salinometer provided filtered water (200 µm) from a depth of 3 m (code: THERMO samples).

Storage

Volumes ranging between 1 l and 5.6 l were collected, depending upon the concentration of particles. They were then filtered through 0.7 µm GF/F filters. These filters were either frozen in liquid nitrogen for analysis at the laboratory, or stored at -20°C until extraction and analysis on board (most samples collected at midday were stored in liquid nitrogen).

Extraction and analysis

Extraction was done in 3 ml of methanol, according to the procedure described by Vidussi *et al*., 1996.
The HPLC system comprises

- a *Hewlett Packard* "Chemstation for LC" software (A.06.03)
- a *Thermoquest* Autosampler (AS 3000)
- a *Hewlett Packard* degasser (HP 1100)
- a *Hewlett Packard* binary pump (HP 1100)
- two detectors:
  - A *Hewlett Packard* diode array detector (HP 1100) with measurements at 440 nm (for carotenoids and chlorophylls) and at 667 nm for pheopigments.
  - A *Thermoquest* fluorimeter (AS 3000)

The analytical method, based on a gradient between a Methanol – Ammonium Acetate mixture (70:30) and a 100% Methanol solution (solvent A and solvent B respectively), is similar to that described by Vidussi *et al*. (1996). Nevertheless, there have been some modifications to this method in order to separate certain peaks and increase sensitivity:

- Flow rate = 0.5 ml/min
- Reverse phase chromatographic column (RP-C8), internal diameter: 3 mm (Reference: Hypersil MOS.3µm)
- Gradient (*minutes*; % solvent A; % solvent B) : (*0*; 80; 20), (*4*; 50; 50), (*18*; 0; 100), (*22*; 0; 100).

Description of the data

- Column 2: Times are given in Universal Time (U.T)
- Column 3: Local Time is equal to the U.T. plus the figure given in this column.

❖ Station (Column 6) : see map

This column indicates the geographical situation of the sample
UPW#1 to UPW#4: Moroccan upwelling (long station)
MIO#1 to MIO#5: Ionian Sea (long station)
DYF#1 to DYF#5: DyFAMed site (long station)
ST#1 toST#9: short stations

❖ Column 7: CTD number, THERMO number or PMP number.

❖ Column 8: Bottle number (for CTD samples).

❖ Column 9: Sampling depths in metres.

❖ Flags (Column 10): This column indicates the analytical conditions, in particular concerning the configuration of the automatic sampler. Three cases are found:

- *Thalassa* : analysis at sea
- *LABO1* : laboratory analysis 1
- *LABO2* : laboratory analysis 2

For *Thalassa* and *LABO1*, a 250 µl preparation syringe was used. For *LABO2*, this was replaced by a 1 ml syringe, thus allowing an improvement of the detection limits of different pigments as compared to those for *LABO1* and *Thalassa* (see Table 1):

Table 1: Detection limits (in $mg.m^{-3}$) for different pigments according to the analytical conditions.

Pigments	*Thalassa* and *LABO1*	*LABO2*
Chlorophylls *c*	~ 0.003	~ 0.001
Carotenoids	~ 0.002	~ 0.001
Chlorophyll *a*	~ 0.001	< 0.001
pheopigments	~ 0.001	~ 0.001

Note that the detection of Chlorophylls *c* was not optimal for *Thalassa* and *LABO1*. Consequently, the concentrations of these compounds should only be considered as semi-quantitative.

❖ Calibration

Two calibrations were carried out according to the configuration of the automatic sampler. The first was applied to the *Thalassa* and *LABO1* data (June 1999), the second to the *LABO2* data (December 1999). These calibrations provided HPLC response factors for Peridinin, 19'-Butanoyloxyfucoxanthin, Fucoxanthin, 19'-Hexanoyloxyfucoxanthin, Alloxanthin, Zeaxanthin, Chlorophyll *b* and Chlorophyll *a* (standards provided by the International Agency for ^{14}C Determination, Denmark).

The response factors for DV Chl *a* and DV Chl *b* were computed (i) knowing the specific extinction coefficients of Chl *a* (or Chl *b*), (ii) taking into account the absorption of Chl *a* and DV Chl *a* (or Chl *b* and DV Chl *b*) at 440 nm when the spectra of both pigments are normalised at their red maxima and (iii) considering that both pigments have the same molar absorption coefficient at this red maximum.

As for the remaining pigments, their specific extinction coefficients were either derived from previous calibrations or from literature (Jeffrey *et al*., 1997).

❖ Pigments

The following pigments, listed in the same order as their retention times, were identified spectrally and quantified in relation to the peak area. **The concentrations are given in mg.m^{-3}**.

Mg-2,4 DVP	Mg 2,4- Divinyl Pheoporphyrin a_5 monomethyl ester
Chl c-like1	Chlorophyll *c* –like:
Chl c3	Chlorophyll c_3
Chl c1+c2	Chlorophyll c_1+c_2[1]
Peri	Peridinin
19'-BF	19'-Butanoyloxyfucoxanthin
Fuco	Fucoxanthin
Prasino	Prasinoxanthin
19'-HF	19'-Hexanoyloxyfucoxanthin
Viola	Violaxanthin
Neo	Neoxanthin
Diadino	Diadinoxanthin
Allo	Alloxanthin
Diato	Diatoxanthin
Zea	Zeaxanthin
Lut	Lutein
Total Chl b	Sum of Chlorophyll *b*, Chlorophyll *b*-like and Divinyl Chlorophyll *b* [2]
DV Chl a	Divinyl Chlorophyll *a*
Total Chl a allomers	Sum of Chlorophyll *a* allomers
Chl a	Chlorophyll *a*
Total Chl a epimers	Sum of Chlorophyll *a* epimers
a-Car	α Caroten
b-Car	β Caroten
Phide a	Pheophorbide *a*

1: Chl c1+c2: As Chlorophyll c_1 and Chlorophyll c_2 co-elute, they were first identified spectrally before being quantified then added up.
2: Total Chl b: As Chlorophyll *b* and Divinyl Chlorophyll *b* literally co-elute, they were first identified spectrally, then quantified with their respective extinction coefficients and finally added up.

❖ Remarks concerning the data processing:

- *Use of internal standard (β-apo-8'-carotenal):* this allowed the pigment concentrations to be corrected relative to internal standard (IS) variations. A linear relationship was established between the uncorrected and the corrected values (excluding CTD002 data), and is characterised by the following equation:

$$y = 1.1218\ x - 0.0097$$

with y : corrected concentrations and x : uncorrected concentrations

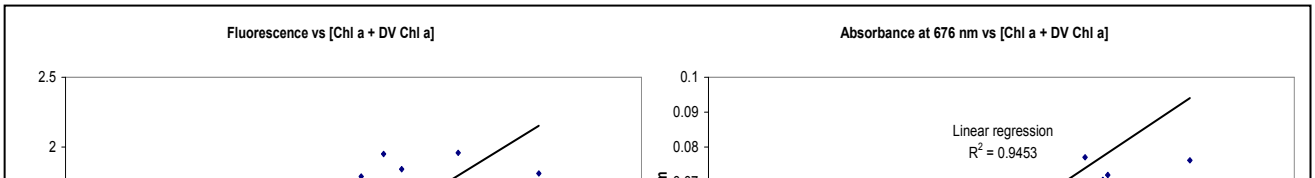
As CTD 002 data presented non-homogenous IS concentrations, its pigment concentrations were calculated by applying the above equation to the uncorrected concentrations.

- The fluorimeter being more sensitive than the DAD, a relationship between the two types of measurements was developed in order to provide chlorophyll *a* concentrations which were below the detection limits of the DAD 440 nm signal. The same procedure was carried out for the determination of Pheophorbide *a* concentrations

- In order to verify the order of the bottles, detect any analytical errors or confusions and validate the results, two comparisons were made:

  - The Chlorophyll *a* + Divinyl Chlorophyll *a* concentrations were compared to *absorbance* measurements at 676 nm. These were performed, immediately after filtration, by a *Li-Cor* spectroradiometer (LI-1800) on the same filter as that used for pigment determination.

  - The Chlorophyll *a* + Divinyl Chlorophyll *a* concentrations were compared to *fluorescence* values provided by the *Chelsea* fluorimeter mounted on the rosette.



- CTD 073 comprises results from both *LABO1* conditions (bottles 7,13,15,17,21) and LABO2 conditions (bottles 1,3,5,9,11). CTD 069 also presents a composite between *LABO2* (bottles 7,8,9,10,11,18,19,20,21) and *LABO1* (bottle 16) results.

References

Jeffrey S.W., Mantoura R.F.C. and Wright S.W., 1997. Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO publishing. 661 pp.

Vidussi F., Claustre H., Bustillos-Guzman J., Cailliau C. and Marty J.C., 1996. Determination of chlorophylls and carotenoids of marine phytoplankton: separation of chlorophyll a from divinyl-chlorophyll a and zeaxanthin from lutein. *Journal of Plankton Research*, **18**(12), 2377-2382.