Method for HPLC pigments analysis

Water for pigment analysis (2 liters) was filtrated on 25 mm Whatman GF/F glass fiber filters. The filters were frozen and analyzed by HPLC within 3 months. Filters were ground and sonicated in 3-ml methanol (HPLC grade) under dim light conditions. The method used until 1993 was derived from that of Mantoura and Llewellyn (1983). The general procedure for HPLC pigment analysis, identification and quantification has been described (Claustre *et al.*, 1994 a,b). With the separation system used (RP-C18), a partial resolution of divinyl-chlorophyll *a* (DV Chl *a*) from chlorophyll *a* (Chl *a*) has been achieved. For samples from 1994 and later, the separation method used (RP-C8) is described in Vidussi *et al.* (1996), and the resolution between DV Chl *a* and Chl *a* has been complete.

The continuity of the set of data was obtained by the utilization of an internal standard (β-apocarotenal) added to each sample in the extraction solvent. The possible effect of the change of analytical method was tested by analyzing the same samples with the two procedures. The agreement between the two methods was good (+- 5%) of the same order than the agreement between 2 analyses of the same sample using the same method. A special attention was given to the quantification of DV Chl *a*, which is fully resolved from Chl *a* in the second part of the experiment. Although partial, the separation of these two compounds in the first phase of our study was sufficient for a good matching of the data from the two methods (equivalent to other pigments) except for low concentrations of DV Chl *a* (below 5 ng l^{-1}) not detected in the first method.

Results are reported in terms of Chl *a*, divinyl-chlorophyll *a* (DV Chl *a*) and Total Chl *a* (TChl *a* = Chl *a* + DV Chl *a*). Chlorophyll *b* (Chl *b*) and divinyl-chlorophyll *b* (DV Chl *b*) not resolved with the first separation method, and partially resolved by the new one, are presented together as TChl *b*. Lutein and zeaxanthin were partially resolved using the method of Vidussi *et al.* (1996), but data are presented as the sum of the two compounds. The lutein was only occasionally detected and always at very low levels with respect to zeaxanthin. Then the couple lutein-zeaxanthin can be considered as essentially zeaxanthin.

Chlorophylls and carotenoids were detected and quantified by absorbance at 440 nm. Identification of pigments was performed by comparison of on-line collected absorption spectra with those of a library of spectra established from standards and reference cultures obtained from the Villefranche sur mer culture collection. The standard carotenoids used for the calibration of the HPLC [peridinin (peri), alloxanthin (allo), fucoxanthin (fuco), zeaxanthin (zea), 19'-hexanoyloxyfucoxanthin (19'HF), 19'-butanoyloxyfucoxanthin

(19'BF)] were provided by R. Bidigare as part of a JGOFS intercalibration exercise. Chlorophyll a and chlorophyll b were from Sigma Chemical Co. Diode Array detection was achieved on selected samples until 1993 (Waters 991) and on all samples since 1994 (HP 1100).

A range of phytoplankton pigments has been detected, in order to characterize different phytoplankton groups. A recent review of taxonomic pigments can be found in Jeffrey (1997). Divinyl-chlorophyll *a* is the typical marker of prochlorophytes whereas Chl *a* is the universal descriptor of other phytoplankton taxa. Fucoxanthin (Fuco) characterizes diatoms and peridinin (peri) dinoflagellates. Nano- and pico-flagellates containing chlorophyll *c* are characterized by 19'-hexanoyloxyfucoxanthin (19'HF, prymnesiophytes) and by 19'-butanoyloxyfucoxanthin (19'BF, chrysophytes and pelagophytes). Zeaxanthin (Zea) is the marker of cyanobacteria but it is also present in prochlorophytes.

Contour maps were obtained using Surfer program (Golden software Inc.) and Kriging method.

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