Phytoplankton pigment biogeochemistry across the Iberian shelf

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ABSTRACT: *The distribution of chlorophyll and carotenoid pigments has been studied in depth profiles and surface transects across the NW Iberian shelf break in a synergetic contribution to both OMEX WP I and WP II activities. Samples have been collected from four WP II cruises (OMEX CD105, June 1997;OMEX CD110, January 1998; OMEX Poseidon P237, February - March, 1998;Belgica BG9815) and from one WP I cruise (CD114, August, 1998). Analysis of all samples has been completed and the bulk of data has been banked with BODC facilitating chemotaxonomic interpretation; use in calibration of in situ optical and fluorometric sensors and development of ocean colour remote sensing algorithms. In this report we present selected surface water pigment data and its use in describing the Lagrangian evolution and inter-seasonal differences in phyto-biomass and its composition on and across the NW Iberian shelf.*

INTRODUCTION: In oceanography, chlorophyll *a* (CHL*a*) has long been recognised as a unique molecular marker of phytoplankton biomass. Traditionally the distribution of CHL*a* has been studied by spectrophotometry and fluorometry (*e.g.*, Lorenzen, 1967, Holm-Hansen *et al.*, 1965). However, these methods suffer from inaccuracies associated with spectral interferences from chlorophyll *b* (CHL*b*), carotenoids and CHL*a*-degradation products (*e.g.*, chlorophyllides, phaeophytins and phaeophorbides) which may occur during senescence, grazing, sedimentation, and re-suspension of phytoplankton. The use of high performance liquid chromatography (HPLC) allows both a more accurate estimate of CHL*a* and the rapid separation and quantification of up to 50 additional chloropigments and carotenoids in extracts of marine plankton (Jeffrey *et al.*, 1997).

Many of these chlorophyll and carotenoid pigments exhibit strong chemotaxonomic associations, which may be used to oceanographically map the distribution of phytoplankton assemblages. A summary of our current understanding of pigment chemotaxonomy is presented in *Table 1*. For example, 19'-hexanoyloxyfucoxanthin has been found to be a biomarker of prymnesiophytes (Wright and Jeffrey, 1987) including coccolithophores and *Phaeocystis* spp., while fucoxanthin has been used as a marker for diatoms (Barlow *et al.*, 1993).

The analysis of phytoplankton pigments by HPLC and the exploitation of the chemotaxonomic relationships summarised in *Table 1* provides us with incisive information on the taxonomic composition of the phytoplankton community as well as the biomass abundance.

Studies of phytoplankton pigments within OMEX II-II WP I and WP II are highly complimentary, the principle objectives being:

- To investigate chlorophyll and carotenoid pigment distribution, production, sedimentation and degradation across the NW Iberian shelf and shelf break in order to understand the dynamics of plankton production and in relation to the hydrography of the region.
- Undertake surface pigment mapping for ground truthing remotely sensed ocean colour satellite data.
- Provision of data for intercalibration studies and for calibration of *in situ* optical and fluorometric sensors

STATUS OF ACTIVITIES: Samples have been collected from four WP II cruises and from one WP I cruise to data (Table 2). Analysis of chlorophyll and carotenoid pigments in samples from all cruises has been completed. Data from all cruises (with the present exception of *CD114*) has been reprocessed, quality controlled and banked with BODC (including intercalibration data). This data may be utilised in chemotaxonomic interpretation of phyto-biomass and its composition; use in

calibration of *in situ* optical and fluorometric sensors and development of ocean colour remote sensing algorithms. Overall, targets within the both WP I and WP II are currently being met.
In this report we present the methodology used in our approach and present selected data describing Lagrangian (*CD114*) and interseasonal (*CD105 vs. CD110*) changes in biomass abundance and composition along and across the NW Iberian shelf.

METHODOLOGY: **Sample collection and analysis**: In general, seawater samples were collected from CTD water bottles on station and from the non-toxic supplies whilst on passage or in survey mode. Phytoplankton were harvested by filtering 1000 - 4000-ml samples through 25 mm GF/F filters using vacuum filtration. Pigments were extracted from the filters into 90% acetone or 100% methanol with the aid of ultrasonication. Extracts were centrifuged or filtered to remove debris and then analysed for a range of chlorophyll, carotenoids and phaeopigments by reverse phase HPLC as follows:
Extracts were held at 2°C in an autosampler unit, and vortex mixed with ammonium acetate buffer (1:1 v/v) before injection. Pigments were separated on a C-8 column using a binary mobile phase system with linear gradient (Barlow *et al*., 1997). Pigments and phaeopigments were detected by absorbance at 440 nm and 667 nm respectively using diode array detection. Pigment identity was secured by co-elution with authentic pigments (VKI, Denmark) and confirmed through spectral correlation with standard UV-visible spectra (300-750 nm). Pigments were quantified with respect to a *canthaxanthin* internal standard *via* relative response factors, whilst phaeopigments were quantified using response ratios.
Pigment chemotaxonomy: The use of pigment chemotaxonomy in the characterisation of phytoplankton biomass composition has expanded greatly over the last decade, largely due to advances in HPLC methodologies. HPLC also allows the study of fragile (*e.g.*, flagellates) and sub-micron (*e.g.*, prochlorophytes) species which are often missed in microscopic enumeration of phytoplankton. However, the information gained from pigment data, is largely qualitative and more recently attempts have been made to obtain quantitative estimates of algal class abundances from pigment data (see Mackey *et al*., 1997). We have adopted a hybrid approach using elements of multiple linear regression analysis (MLR, Gieskes *et al*., 1988) and CHEMTAX (Mackey *et al*., 1996, 1997) to obtain quantitative estimates of phytoplankton class abundances from HPLC measurements of pigments.

PRELIMINARY RESULTS AND DISCUSSION: Pigments detected in the OMEX grid included chlorophylls *a*, *b*, c_1, c_2, c_3 and a wide range of chemotaxonomic carotenoids. Of these, we used the biomarkers carotenoids, peridinin (PER), 19'-butanoyloxyfucoxanthin (BUT), fucoxanthin (FUC), 19'-hexanoyloxyfucoxanthin (HEX), alloxanthin (ALLO), zeaxanthin (ZEA) and chlorophyll *b* (CHLb) to indicate the presence and abundances of dinoflagellates, chrysophytes, diatoms, prymnesiophytes, cryptophytes, cyanobacteria and chlorophytes respectively (*Table 1*).
1. *CD114* Lagrangian Experiments: Leg 1 of *CD114* tracked movement of a patch of newly upwelled water southward along the shelf. The temporal evolution of CHL*a* and the relative contributions of key classes of phytoplankton to this CHL*a* are shown in Figure 1. It may be seen that CHL*a* concentrations increased rapidly during the initial phase of Leg 1 and that this increase was largely due to proliferation of chlorophytes, prymnesiophytes and cryptophytes. While CHL*a* concentrations varied significantly during the latter stages of this study the relative importance of the contributing classes of phytoplankton remained relatively constant with diatoms, prymnesiophytes, cryptophytes and chlorophytes contributing ~ 90 % of the total CHL*a* (Figure 1).
Leg 2 of *CD114*, also carried out within a Lagrangian framework, tracked the progression of a cool water filament that extended off the shelf into the ocean. During the experimental phase of Leg 2 (Figure 1), levels of CHL*a* remained consistently below 250 ngl^{-1}. In the first half part of the study a relatively mixed and stable population of phytoplankton were present with prymnesiophytes and chlorophytes being the dominant classes, together contributing ~ 55 % of the measured CHL*a*. During the second half, the contribution of CHL*a* from eukaryotes declined and accordingly the prokaryotic component of the biomass increased from 10 to 46 % with progression of the filament offshore.

2. Inter-seasonal Comparison; *CD105* (summer) *vs. CD110* (winter): From the summer *CD105* and winter *CD110* cruise data presented in Figure 2 it can be seen that levels of CHL*a* in Iberian Shelf waters contrast sharply with those measured across the shelf break during both seasons. These patterns appear to be primarily related to nutrient abundances (data not shown). During summer a clear transition in the dominant classes of phytoplankton was observed across the shelf (Figure 2). On shelf (station P100) diatoms were calculated to contribute ~ 40 % of measured CHL*a* while offshore (P2000) prokaryotes were calculated to contribute > 50% of total CHL*a*. In addition to this transition from large eukaryotes to prokaryotes, certain classes appear to be most important in intermediate regions or regimes *e.g.*, prymnesiophytes contributed 42 and 32 % of the CHL*a* at P200 and P1000 stations respectively.
During winter, no clear transition in biomass composition was observed. Prymnesiophytes (10-32 % CHL*a*) chlorophytes (19-31 %), cryptophytes (19-34 %) and diatoms (11-21 %) were the most important classes. In contrast to summer, during winter prokaryotes contributed a maximum of only 7 % of measured CHL*a* during winter (P2800).

CONCLUSION: Studies of chemotaxonomic pigments are significantly contributing to our understanding of the temporal and spatial variability in distribution, abundance and composition of phytoplankton biomass in the OMEX II-II study region.

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Table 1. *Chlorophyll and Carotenoid Chemotaxonomy of Phytoplankton: ● : major pigment ; · : minor or variably occurring pigment.*

	Cyano-bacteria	Prochlo-rophytes	Diatoms	Prymne-siophytes	Chloro-phytes	Prasino-phytes	Dinofla-gellates	Pelago-phytes	Crypto-phytes
Chlorophylls									
Chlorophyll *a* (CHL*a*)	●		●	●	●	●	●	●	●
Divinyl CHL*a*		●							
Chlorophyll *b* (CHL*b*)					●	●			
Divinyl CHL*b*		●							
CHLc_1			●						
CHLc_2			●	●			●	●	●
CHLc_3				●				●	
Carotenoids									
Fucoxanthin			●	·				·	
19'-Hexanoyloxy-fucoxanthin				●					
19'-Butanoyloxy-fucoxanthin				·				●	
Peridinin							●		
Dinoxanthin							·		
Diadinoxanthin			·	·			·		
Diatoxanthin			·	·			·		
Prasinoxanthin						●			
Zeaxanthin	●	●							
Lutein					●				
Alloxanthin									●
β carotene	●	●	●	●	●	●	●	●	●

Table 2*. Summary of sample collection and analysis for phytoplankton pigments during OMEX II-II.*

OMEX cruise (work-package)	Date	Samples	Analysis	Data reprocessing	QC + data banking
CD105 **(WP II)**	*June 1997*	*330 - vertical profiles + underway*	√	√	√
CD110 **(WP II)**	*January 1998*	*> 80 - vertical profiles + underway*	√	√	√
Poseidon *P237-1* (WP II)	*February – March 1998*	*> 100 - vertical profiles + underway*	√	√	√
Belgica *BG9815* (WP I)	*June - July 1998*	*20 - underway only*	√	√	√
CD114 **(WP I)**	*July - August 1998*	*250 - vertical profiles and underway*	√	√	

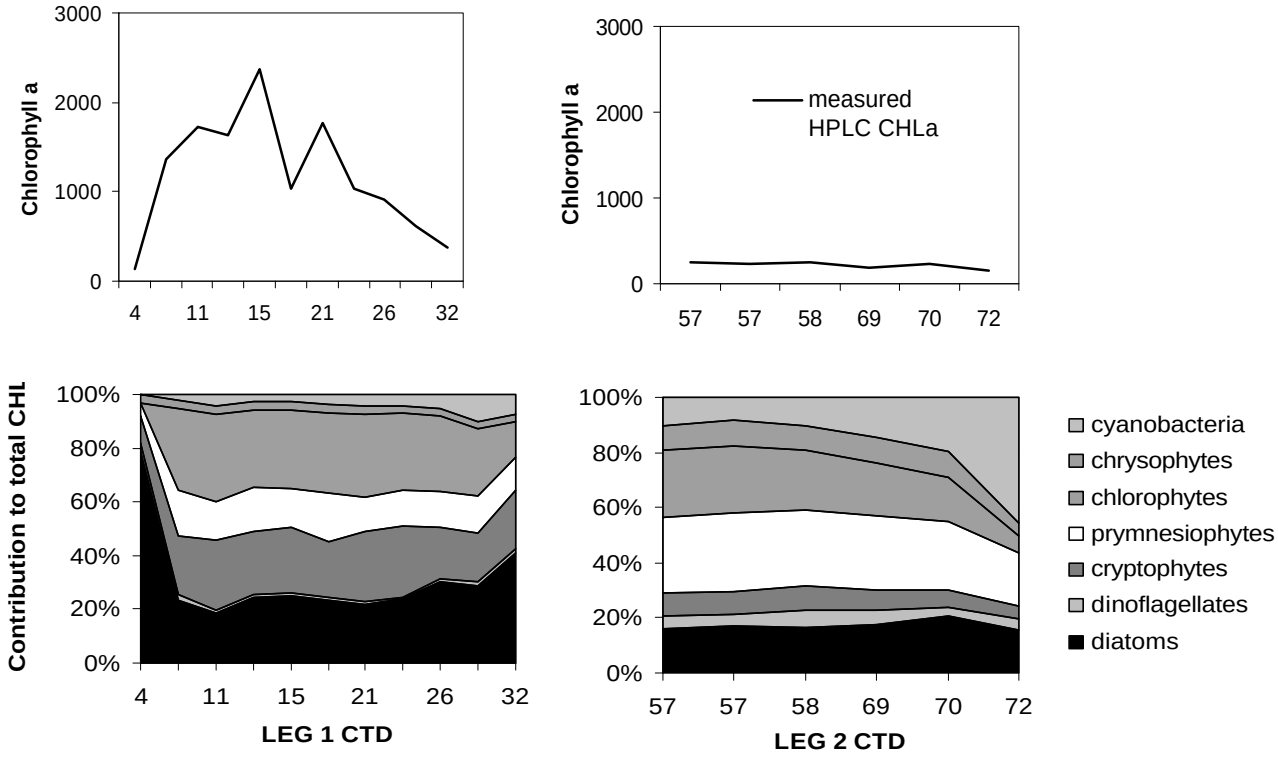

Figure 1. Evolution of surface CHL*a* and biomass composition during Leg 1 (left) anf Leg 2 (right) of *CD114* Lagrangian studies

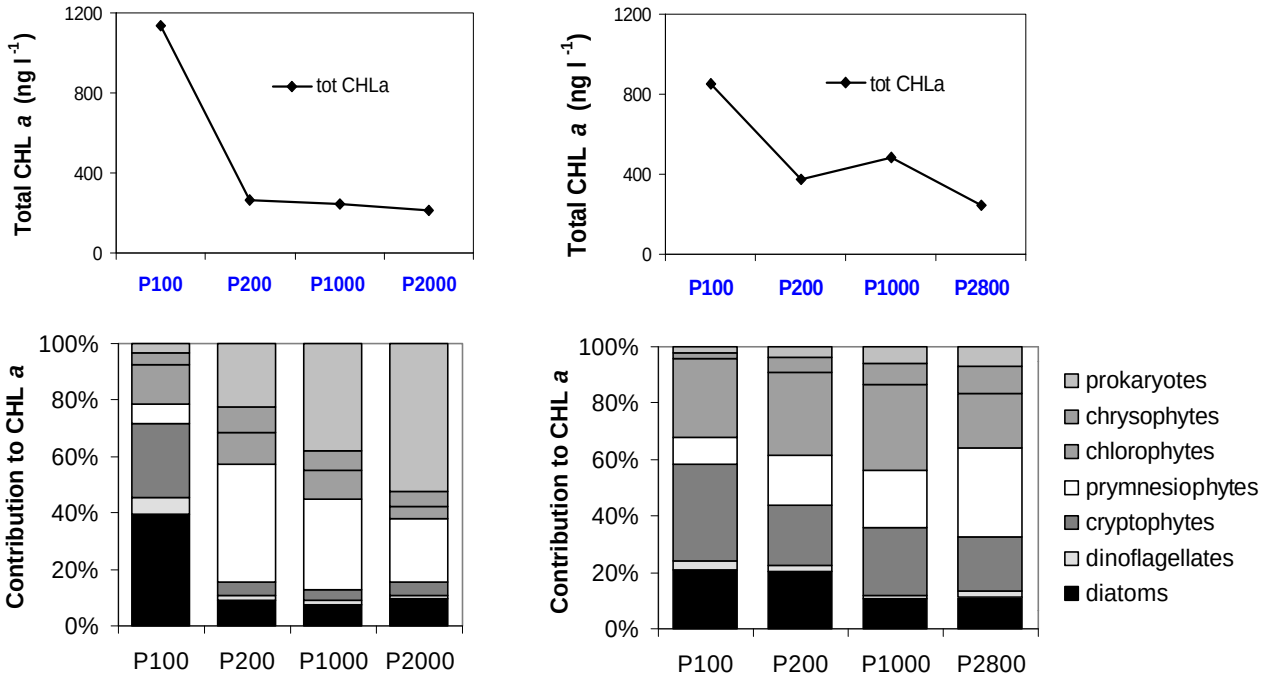

Figure 2. Comparison of CHL*a* concentrations and biomass composition along OMEX 'P' line during summer (*CD105*, left) and winter (*CD110*, right).