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 WPI and WPII activities. To date samples have been collected from three (WP II) cruises: OMEX CD105 (June 1997),OMEX CD 110 (January 1998), OMEX Poseidon (February - March, 1998). Whilst analysis of samples from CD110 and Poseidon cruises is presently underway, analysis of samples from CD105 has been completed. Data has been quality controlled and banked with BODC facilitating chemotaxonomic interpretation; use in calibration of in situ optical and fluorimetric sensors and development of ocean colour remote sensing algorithms and. In this report we present preliminary chlorophyll and carotenoid data and initial chemotaxonomic observations from OMEX CD105.

INTRODUCTION

The evolution of chlorophylls and carotenoids as the key light harvesting pigments in plant and bacterial photosynthesis profoundly altered the biogeochemical cycles of C, N and O in the oceans, on land and in the atmosphere and initiated the evolution of eukaryotic food chains. In the oceans, the photosynthetic pigments, particularly chlorophyll *a* (Chl *a*) have long been recognised as unique molecular markers of phytoplankton biomass. Whilst the distribution of Chl *a* has traditionally been studied by spectrophotometry and fluorimetry (e.g. Lorenzen, 1967, Holm-Hansen *et al*, 1965) these methods suffer from inaccuracies associated with spectral interferences from chlorohyll *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. However, 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 other 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 (Bjornland and Liaaen-Jensen, 1989; Wright and Jeffrey, 1987) including coccolithophores (e.g. *Emiliania huxleyi*, *Gephyrocapsa oceanica*) and *Phaeocystis* spp. while fucoxanthin has been used as a marker for diatoms (Barlow *et al.* 1993) and 19'-butanoyloxyfucoxanthin appears to be mainly associated with chrysophytes and pelagophytes (Bjornland and Liaaen-Jensen, 1989).

The analysis of phytoplankton pigments by high performance liquid chromatography (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. In addition, chlorophyll degradation products can be used as indicators of transformation processes such as grazing and bacterial degradation that contribute significantly to the turnover of phytoplankton carbon and subsequent sedimentation processes (Barlow *et al*. 1993b).

Our activities within WPI and WPII are highly complimentary principle, 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 associated organic matter transformation in relation to the hydrography of the region.

- Undertake surface pigment and mapping for ground truthing remotely sensed ocean colour satellite data.
- Provision of data for intercalibration studies and for the calibration of *in situ* optical and fluorimetric sensors

STATUS OF ACTIVITIES

Samples have been collected from three (WP II) cruises to date (Table 2). Analysis of chlorophyll and carotenoid pigments in samples from CD105 has been completed. Data has been quality controlled and banked with BODC (including intercalibration data) facilitating chemotaxonomic interpretation; use in calibration of *in situ* optical and fluorimetric sensors and development of ocean colour remote sensing algorithms and. Analysis of samples and data from CD110 and Poseidon cruises is presently underway**.** Data generated is directly relevent to both WPI and WPII objectives. All targets within the programme are currently being met.

In this report we present the methodology used in our approach and preliminary results and initial discussion for data resulting from participation in CD105.

METHODOLOGY

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 with the aid of ultrasonication. Extracts were centrifuged and / or filtered through Teflon syringe filters to remove debris. Extracts were then analysed for a range of chlorophyll, carotenoids and pheopigments 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 (methanol / 1.0 M ammonium acetate ; 100% methanol; Barlow *et al*., 1997). Pigments and pheopigments 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 pheopigments were quantified using response ratios. As well as the resolution of key chemotaxonomic chlorophyll and carotenoid pigments, baseline separation of mono- and divinyl chlorophyll *a*, and lutein and zeaxanthin, and partial separation of mono- and divinyl chlorophyll *b* was achieved in a total analysis time of less than 30 min.

PRELIMINARY RESULTS AND DISCUSSION

Pigments detected in the OMEX grid included chlorophylls *a*, *b*, c_1c_2, c_3, and a wide range of chemotaxonomic carotenoids. We used the accessory biomarkers carotenoids, peridinin (PER), 19'-butanoyloxyfucoxanthin (BUT), fucoxanthin (FUC), 19'-hexanoyloxyfucoxanthin (HEX), alloxanthin (ALLO), zeaxanthin (ZEA) and chlorophyll *b* (CHLb) to indicate the presence of dinoflagellates, pelagophytes, diatoms, prymnesiophytes, cryptophytes, cyanobacteria and green flagellates respectively (*Table 1*).

Biomass

Vertical profiles of CHL*a* for the N, P and S stations in the OMEX grid are presented in Figure 1. It may be seen that deeper waters off the shelf are characterised by a well-defined deep CHL maximum (DCM; e.g. Figure 1; Stations N3300, P1000, S2000, CHL*a* ~ 70m). Progression on-shelf is accompanied by a general shallowing in the CHL*a* maximum zone: either with conservation of the

characteristic DCM (e.g. Figure 1 station S200; CHL*a* max ~ 60m), or less commonly with transition to a surface CHL*a* maximum (SCM; e.g. Figure 1; station p100). Outside coastal stations in which a SCM was observed, surface CHL*a* concentrations were typically below 100 ng/l.

Through integrating CHL*a* depth profile data it is possible to assess the integrated biomass abundance in the upper water column: such treatment of data collected on the OMEX N, P and S series stations is presented in Figure 3. It may be seen (Figures 1 and 2) that whilst there are significant changes in the composition of the phytoplankton assemblages studied and in their distributions in the upper water column (e.g. Figure 2, P series), that integrated biomass remains relatively stable across the shelf break (Figure 3).

Eukaryotic phytoplankton

19'-Hexanoyloxyfucoxanthin dominated the accessory pigment budget in this study, both in surface waters and throughout the upper water column. Concentrations of HEX reached a maximum of 960 ng/l in association with the sub-surface CHL*a* max at S200. These high concentrations of HEX, accompanied by substantial levels of the secondary accessory pigment CHLc3, indicate prymnesiophytes to be the dominant microalgae group throughout the transect and re-iterates the importance of this algal group in this region. Other biomarker pigments that detected in significant concentrations included zeaxanthin, peridinin and fucoxanthin (Figure 3) and chlorophyll b, indicating cyanobacteria, dinoflagellates, diatoms and green algae to make important contributions to contribute to the phyto-biomass.

Exceptions to the dominance of HEX may be observed at Station P100 for example (Figure 3). Here FUC was the dominant accessory pigment (HEX : FUC >3 : 1). At this station high concentrations of FUC and PER indicate large eukarytotes to dominate the phyto-biomass.

Prokaryotic phytoplankton

Concentrations of ZEA, the primary marker pigment for cyanobacteria, were greatest offshore and at the southern extreme of the OMEX grid. In this region, concentrations of ZEA were greatest in surface waters and at 60 - 70 m where the total picoplanktonic biomass (cyanobacteria + prochlorophytes) was greatest.

In depth profiles, dvCHL*a* contributed a mean of 13 % of total CHL*a* (dvCHL*a* + CHL*a*, %). The maximum contribution of dvCHL*a* to total CHL*a* was found to be 44 % (station T200). These ratios are within the ranges of 25-40 % determined in the sub-tropical northwest Atlantic by Goericke and Repeta (1993) and 11-40 % in the northwestern Mediterranean by Barlow *et al.*, (1997).

In general highest abundances of dvCHL*a* were observed at the base of the euphotic zone indicating that prochlorophytes are better adapted to lower light conditions than other green algae and cyanobacteria. Prochlorophytes use divinyl CHL*a* and *b*, red shifted by 5 nM with respect to their mono- vinyl analogues to increase absorption of blue light, the only spectral region of solar irradience available at depth in the ocean (Glover *et al*., 1986). The observation that dvCHL*a* and *b* rapidly disappear upon transfer to shallower depth is a further indication of the adaptation of prochlorophytes to life at depth (Gieskes, 1991).

CONCLUSIONS

Solid progress has been made toward establishing a phytoplankton data set which will facilite a chemotaxonomic assessment of the spatial and temporal variability in phytoplankton abundance and composition in the OMEX study region (Table 2). Pigments are key biogeochemical parameters providing important interdisciplinary links within the OMEX community. There is significant potential

within the framework of OMEX for pigment data to be used in the assessment of phytoplankton production, microzooplankton grazing and vertical transport in the coming year. Data will also contribute to the development of ocean colour remote sensing algorithms; correlation studies with pCO2 and in studies of the pelagic cycling of carbon.

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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*		●							
Chl c_1			●						
Chl c_2			●	●			●	●	●
Chl c_3				●				●	
Phyt-Chl c_2				•					
Carotenoids									
Fucoxanthin			●	•				•	
19'-Hexanoyloxy-fucoxanthin				●					
19'-Butanoyloxy-fucoxanthin				•				●	
Peridinin							●		
Dinoxanthin							•		
Diadinoxanthin			•	•			•		
Diatoxanthin			•	•			•		
Prasinoxanthin						●			
Zeaxanthin	●	●							
Lutein					●				
Alloxanthin									●
β,β carotene	●		●	●	●	●	●	●	●
β,ε-carotene		●			•				

Table 2. Summarised phytoplankton pigment chemotaxonomy sample collection program within OMEX II-II (WPI and WPII).

Period	**Ship**	**Sampling strategy**
June 1997	*Charles Darwin (CD105)*	330 samples - 41 vertical profiles + underway (includes intercalibration exercise with Belgica).
February - March, 1998	*Charles Darwin (CD110)*	80 samples - 6 vertical profiles + underway
February - March, 1998	*Poseidon*	>100 samples - 12 vertical profiles.
July - August, 1998	*Charles Darwin (CD114 Leg 1 and Leg II)*	*Proposed - CTD + underway in series drift experiments*
August, 1998	*Spanish charter of Russian vessel*	*Proposed - CTD + underway in mapping OMEX grid*

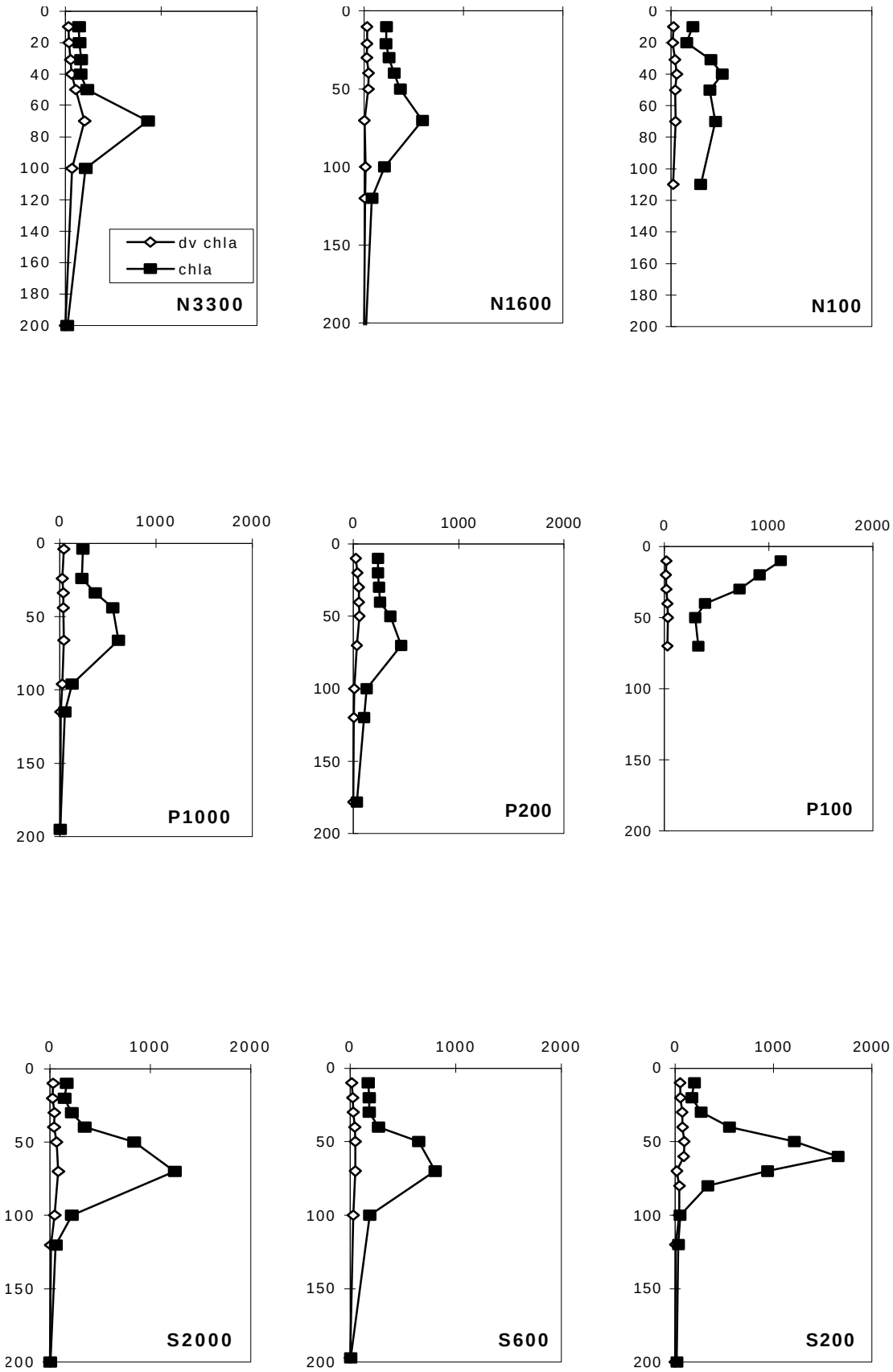

Figure 1. Depth profiles of chlorophyll a (CHLa) and divinyl CHLa (dv CHLa) at OMEX N, P and S stations during June 1997 (CD105)

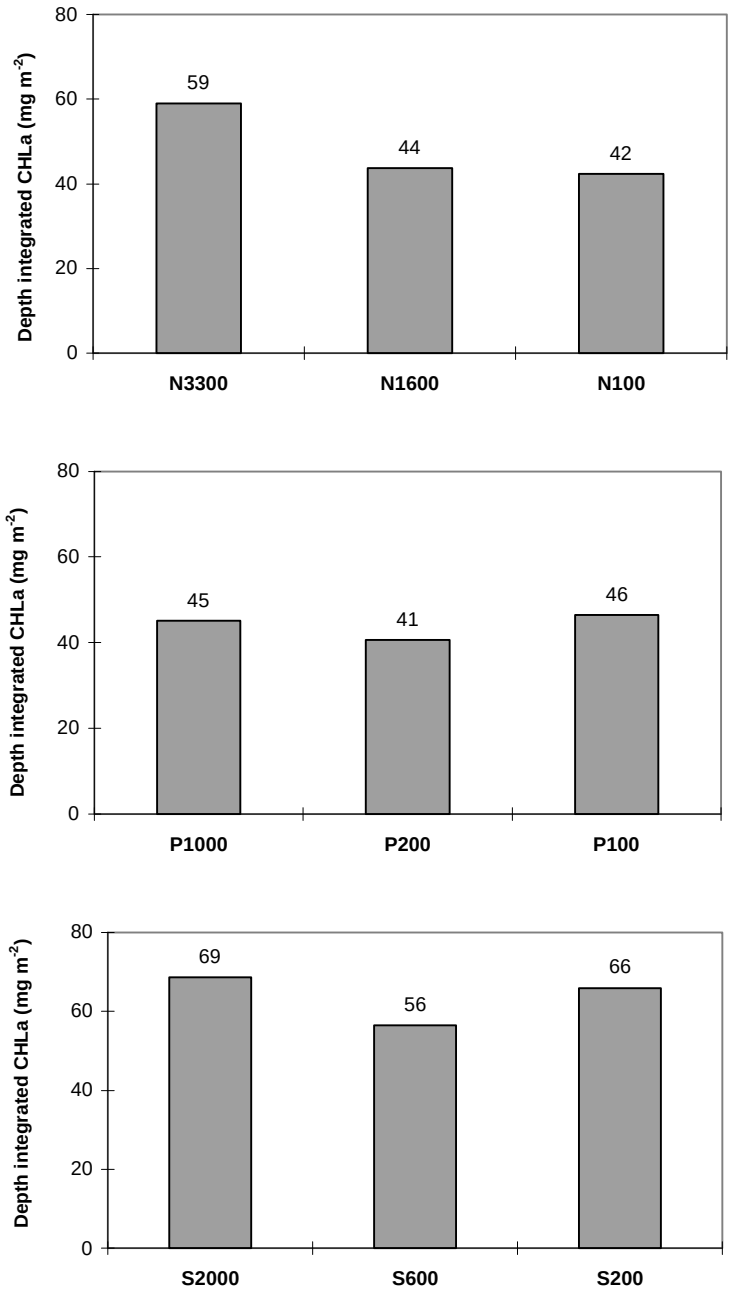

Figure 2. Depth integrated (0 - 200m) chlorophyll a concentrations at OMEX N, P and S stations in January 1997 (CD 105).

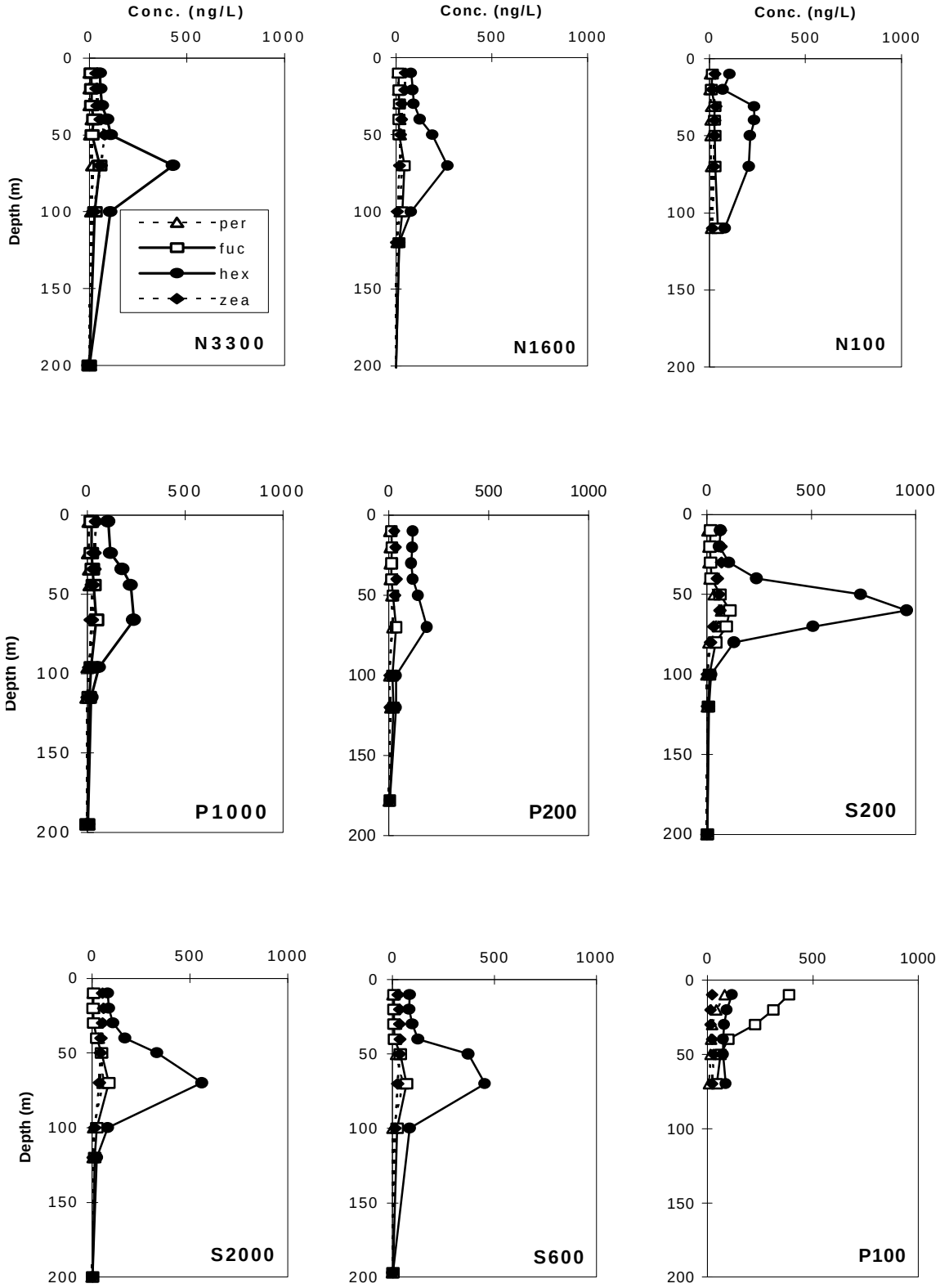

Figure 3. Depth profiles of the key accessory carotenoids peridinin (PER), fucoxanthin (FUC), 19'-hexanoyloxyfucoxanthin (HEX) and zeaxanthin (ZEA) at OMEX N, P and S stations during June 1997 (CD105).