#### NUTRIENTS, PHYTOPLANKTON AND PHOTOSYNTHESIS-IRRADIANCE RELATIONSHIPS

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# ABSTRACT

The 'Instituto de Investigaciones Marinas' (IIM) has participated in two WP2 cruises: BG9714-C (summer '97) and CD110-B (winter '98). Phytoplankton identification and counts and photosynthesis-irradiance (P-E) relationships were determined in summer and winter, whereas nutrients were mapped only during the winter. Poleward slope flows of warm and salty subtropical water were found during both cruises. Stratification during the summer contrasted with homogenisation during the winter in the upper water column. Cyanobacteria numbers were low and homogeneously distributed in the upper mixed layer (~125m deep) during the winter poleward. By contrast, they were more abundant in summer, with maximum numbers in the upper 20m. P-E curve parameters were also affected by the contrasting stratification regimes in summer and winter. Phytoplankton response to the light conditions was homogeneous in the winter mixed layer with low light saturation parameters, whilst summer phytoplankton at the bottom of the photic layer showed photoinhibition. Nutrient concentrations in the winter mixed layer were low, as expected for the subtropical waters carried north by the poleward flow.

# **INTRODUCTION**

Northerly winds usually blow between April and September at the latitudes of the NW Iberian area (Wooster et al. 1976, Fraga 1981), provoking upwelling of cold, nutrient-rich subsurface water. On the contrary, southerly winds prevail during the rest of the year, favouring downwelling. One of the most striking features during the winter is the presence of warm and salty subtropical water carried northwards along the slope (Frouin et al. 1990, Haynes and Barton, 1990). Considering this general pattern in the hydrographic field, the objectives of IIM within the framework of WP2 are to map the meso-scale variability of nutrients and phytoplankton in the NW Iberian shelf-edge during two cruises studying: 1) summer upwelling filaments and fronts; and 2) winter poleward slope currents and associated slope shallow water eddies. To cover these objectives, IIM has participated in the R/V BELGICA cruise BG9714-C (21 to 30 June 1997) and the RRS Charles Darwin cruise CD110-B (5 to 16 January 1998), collecting phytoplankton samples for posterior analysis in the laboratory and analysing nutrients during the winter cruise.

One of the major commitments of the OMEXII-II project is to deliver primary production rates derived from satellite imagery. Although photosynthesis-irradiance (P-E) relationships are essential to achieve this objective, data are scarce in the study area zone —mainly during winter. In addition, P-E relationships for the different phytoplankton populations at any hydrographic situation are commonly used as input parameters for the different coupled models that must be developed by other partners in the project. Therefore, IIM took the opportunity of participating in the aforementioned cruises to study the phytoplankton response to the light conditions.

## METHODS

## Nutrients

Analyses of 5-nutrient salts (ammonium, nitrite, nitrate, phosphate and silicate) were performed on board during cruise CD110B. Samples were directly drawn from the Niskin bottles into 50 ml polyethylene containers, and preserved at 4 °C until subsequent analysis on board. Nutrients were determined colorimetrically with an 'Alpkem Corporation' auto-analyser (Perstorp Analytical, Wisonwille, USA), working under the principle of segmented flow analysis. The signals from the five colorimeters were recorded in chart paper, and peak heights were measured manually.

#### Phytoplankton

Microplankton was sampled during cruises BG9714-C and CD110-B. Samples were drawn directly from the Niskin bottles into 100 ml Pyrex flasks and preserved in Lugol's iodine. Microplankton counting and identification is in progress. Samples are sedimented in composite sedimentation chambers. The sediment volume (25-100 ml) depends on the chlorophyll concentration of the sample. When possible, organisms are identified to the species level. Two transects at x400 and x250 are used to count the small species. The larger forms are counted from the whole slide at x100 magnification.

Autotrophic pico- ( $\leq 2\mu$ m) and nanoplankton (between 2 and 20 µm) as well as cyanobacteria were also sampled during the two cruises. Counting was performed by epifluorescence microscopy in water samples fixed with formalin (4% final concentration) on 0.2µm black Millipore polycarbonate filters.

## Photosynthesis-Irradiance (P-E) relationships

Photosynthetic parameters during both cruises were estimated using photosynthesis-irradiance (P-E) curves. Fourteen sub-samples from each sampled depth were pipetted into 75 ml Corning tissue culture flasks and inoculated with  $1.85 \times 10^5$  Bq (5 µCi) NaH<sup>14</sup>CO<sub>3</sub>. Then they were placed in linear incubators and maintained at *in situ* temperature using a water bath and pump. Osram tungsten halogen lamps with dichroic reflector and a Deco glass cover (50 W, 12 V) illuminated the front side of the incubators. The irradiance in each cell was measured using a Li-Cor cosine sensor Li-190SA. The last bottle in the incubators was used as a control and was covered with aluminium foil to measure carbon fixation in the dark. After 2-3 hours of incubation, the suspended material was filtered through 25 mm Whatrman GF/F glass fibre filters at a pressure of < 20 cm Hg and the filters were then exposed to HCl vapours for 12 hours. DPM were determined using a liquid scintillation counter with quench correction by the external standard method and a stored quench curve of known activity. The P-E data were fitted using the model of Web et al. (1974) when photoinhibition was not observed:

$$P_{z}^{B} = P_{m}^{B} \cdot \left[1 - \exp\left(-\boldsymbol{a}^{B} \cdot \boldsymbol{E}/P_{m}^{B}\right)\right]$$

or Platt et al. (1980) when photoinhibition was present:

$$P_{z}^{B} = P_{s}^{B} \cdot \left[1 - \exp\left(-\boldsymbol{a}^{B} \cdot \boldsymbol{E}/\boldsymbol{P}_{s}^{B}\right)\right] \cdot \exp\left(-\boldsymbol{b}^{B} \cdot \boldsymbol{E}/\boldsymbol{P}_{s}^{B}\right)$$

to obtain the broadband photosynthetic parameters  $P_m^B$ , light-saturated chlorophyll-specific rate of photosynthesis;  $\mathbf{a}^B$ , light-limited slope of the P-E curves; and  $E_k = P_m^B / \mathbf{a}^B$ , light saturation parameter.

#### Phytoplankton absorption spectra

Phytoplankton absorption spectra were measured during both cruises using a single beam spectrophotometer. Seawater volumes from 0.25 to 1 litre were filtered onto 25mm GF/F glass fibber filters depending on the concentration of suspended material. Glass-fibber filters wetted on prefiltered 0.22µm seawater were used as blanks. After readings, the filter pigment contents were extracted with absolute methanol during 30-60 minutes and optical densities of decolourised filters were measured again. Phytoplankton and detritus absorption coefficients were calculated according to Kishino et al. (1985) after correction for pathlength amplification factor (Arbones et al. 1996).

Phytoplankton absorption coefficients and water column spectral light profiles measured during each cruise will be used in the near future to estimate the spectral dependency of the light limited slopes of P-E curves as well as primary production as a function of the spectral light field.

#### **RESULTS AND DISCUSSION**

Despite the cruises were conducted to obtain representative pictures of contrasting hydrographic situations (summer upwelling during BG9714-C and winter poleward during CD110-B), two poleward slope currents were observed. Therefore, we have here the opportunity to compare contrasting summer and winter polewards. This is especially relevant for the case of the photosynthetic parameters, because of the lack of *in situ* observations during the wintertime.

## Tasks 4.1 and 4.2 Nutrient Oceanography and Conserved Nutrient Tracers

The most striking hydrographic structure during CD110-B was a tongue of subtropical warm (>15°C) and salty (>35.9 pss) water all along the NW Iberian margin. This is the signature of the recurrent poleward flow observed during the winter months in the study area (Frouin et al. 1990, Haynes and Barton, 1990), caused by the large scale climatology of the NE Atlantic (Wooster et al. 1976, Bakun and Nelson 1991).

Full-depth nutrient profiles at three stations along the OMEX-II 'line P' (zonal transect at  $42^{\circ}$  40'N) are presented in Figure 1. Station P2800 (in the ocean end of the line) is characterised by an upper mixed layer ~125m deep, where nutrients are homogeneously distributed. Nutrient levels in this layer are low (1.8 µmol kg<sup>-1</sup> nitrate, 0.15 µmol kg<sup>-1</sup> phosphate and 1.3 µmol kg<sup>-1</sup> silicate), as expected when water of subtropical origin arrives to our latitudes carried in poleward flows (Castro et al. 1997). Nitrate, phosphate and silicate increases sharply below the upper mixed layer. The observed concentrations are within the ranges for the water masses in the area (Pérez et al. 1993): Eastern North Atlantic Central Water (ENACW), Mediterranean Overflow Water (MOW), Labrador Sea Water (LSW) and North Atlantic Deep Water (NADW). A relative minimum of the three nutrients is observed in the domains of the MOW (800-1300m). The high silicate levels in the bottom sample (36 µmol kg<sup>-1</sup>) indicates the presence of NADW, since this water mass mixes with Antarctic Bottom Water in the Western North Atlantic and then enters the Eastern basin across the Vema Fracture Zone.

Nutrient profiles at station P200 (in the outer shelf) indicates the presence there of the homogeneous upper mixed layer associated to the poleward flow of subtropical water. Nutrient levels are similar to those observed at station P2800. Below the mixed layer, nitrate, phosphate and silicate increased sharply to the bottom.

The poleward flow confines continental waters outwelled from the Rías Baixas (four large indentations in the Northwestern Iberian coast) in the inner shelf. Nutrient profiles at station P100 contrast with stations P200 and P2800. Nitrate, phosphate and silicate are maximum in the ~20m deep surface mixed layer. Nutrient levels are 10  $\mu$ mol kg<sup>-1</sup> nitrate, 0.33  $\mu$ mol kg<sup>-1</sup> phosphate and 12  $\mu$ mol kg<sup>-1</sup> silicate. Salinity in the surface layer was <33 pss —much lower than in the poleward domain—indicating that high nutrient concentrations are associated to the continental load. Nutrient levels decreased sharply down to ~70m and a homogeneous bottom layer 30 meters thick is observed. Salinity, temperature and nutrients in the bottom layer are the same than in the poleward, suggesting that the continental water outwelled form the rías lie on the poleward flowing subtropical water.

The different hydrographic domains observed during the cruise also affect nitrite distributions. Concentrations >0.70  $\mu$ mol kg<sup>-1</sup> are observed in the surface mixed layer of continental water at station P100, whereas nitrite levels are much lower in the poleward domain (>0.15  $\mu$ mol kg<sup>-1</sup>). However, it is interesting to note that nitrite in the oceanic waters below the upper mixed layer is negligible (<0.02  $\mu$ mol kg<sup>-1</sup>). This nitrite excess could be associated with light limitation to primary production at our latitudes. Under these conditions phytoplankton is able to uptake and reduces nitrate to nitrite. However, conversion of nitrite to ammonium requires much more energy and reduction power, which could be limited under low light conditions. In this case, the nitrite accumulated into living cells needs to be exuded (Blasco 1971)—leading to the observed nitrite excess.

Finally, ammonium levels (not shown) have been very low ( $<0.1 \mu$ mol kg<sup>-1</sup>) during cruise and there was not significant differences between continental and oceanic waters.

# Task 8.1 Spatial and Temporal Distribution of Phytoplankton Biomass, Species, Pigments and Their Remote Sensing

Microplankton counts are still in course, because this is a large time-consuming technique. However, a quick view of data already available from the cruises allow us to deduce that microplakton populations during the two sampling periods were typical of the warm and salty waters in poleward currents (Castro et al. 1997). Microplankton abundance was much lower than during upwelling events and populations were mainly composed of small heterotrophic forms such as small flagellates, small naked dinoflagellates and a conspicuous population of oligotrichous ciliates.

Phytoplankton counts from epifluorescence microscopy (already finished) show relevant information on the smaller autotrophic forms during two poleward events. Figure 2 show the profiles of cyanobacteria and autotrophic pico- and nanoplankton at two stations located at the shelf-edge (P200 and O2P26) and the slope (P1000 and O2P27) during both cruises. The homogeneous profiles of pico- and nanophytoplankton (low numbers) and cyanobacteria (high numbers,  $10^3$  cells ml<sup>-1</sup>) during CD100-B reflected the existing winter mixed layer, ~125m deep. The abundance and distributions of pico- and nanophytoplankton during BG9714-C paralleled CD110-B. Conversely, cyanobacteria showed a quite different profile. They were more abundant (>10<sup>4</sup> cells ml<sup>-1</sup>) in the upper 20 m at the station closer to shore (O2P26), dropping sharply to  $10^3$  cells ml<sup>-1</sup> at 60 m. This indicates that surface waters of the summer poleward are more stratified than during the winter poleward. Offshore (at station O2P27) cyanobacteria abundance was lower, but even higher than during the winter. Then, we can conclude that autotrophic phytoplankton during summer poleward is mainly composed by cyanobacteria.

# Task 8.3 Parameterisation of Primary Production: Photosynthesis-irradiance relationships

The ranges and mean values of the light limited slope  $(a^{B})$  of P-E relationships are comparable during both cruises (Table 1). Most striking difference appears in the light saturated chlorophyll-specific rate  $(P_{m}^{B})$ , with higher means and ranges in summer. Consequently, summer phytoplankton shows a higher light saturation parameter (E<sub>k</sub>). The low light adaptation of the winter samples is reflected on the extremely low E<sub>k</sub> values  $(42 \pm 12 \,\mu\text{mol m}^{-2} \,\text{s}^{-1})$ .

Looking in more detail the P-E relationships (Figures 3 and 4) we can observe that there are lower  $P_m^B$  and  $a^B$  values in the samples from 60-70 m depth than in those from the surface (Figure 3). This fact indicates that the phytoplankton from deeper samples is low light adapted during the summer. The photoinhibition response found in P-E curves of these deeper samples also indicate that phytoplankton populations are receiving low irradiance continuously. On the contrary, the absence of photinhibition during the winter in the whole water column (Figure 4) indicates that the low light adaptation of the winter populations is caused by a well-mixed water column, which precludes that phytoplankton can receive a constant light (Figueiras et al. 1994).

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Table 1. Photosynthetic parameters during cruises BG9714-C and CD110-B. $a^{B}$ , light-limited slope;
$P_m^B$ , light-saturated chlorophyll-specific rate of photosynthesis; $E_k$ , light saturation parameter.

Photosynthesis Parameters		BG 9714-C	CD 110-B
		N = 55	n = 24
$a^{B}$ mg C (mgChl) <sup>-1</sup> h <sup>-1</sup> (µmol m <sup>-2</sup> s <sup>-1</sup> ) <sup>-1</sup>	Range	0.092 - 0.013	0.079 - 0.013
	Mean	$0.043 \pm 0.079$	$0.038\pm0.016$
$P_m^B \operatorname{mgC} (\operatorname{mg chl})^{-1} \operatorname{h}^{-1}$	Range	10.09 - 0.90	2.13 - 0.44
	Mean	$3.57 \pm 2.12$	$1.47\pm0.39$
$E_{k}$ (µmol m <sup>-2</sup> s <sup>-1</sup> )	Range	471 - 20	70 - 18
	Mean	$137 \pm 98$	42 ± 12



Fig. 1. Full-depth nitrite, nitrate, phosphate and silicate profiles at stns P 100, P 200 and P 2800 during cruise CD 110-B



Fig. 2. Cyanobacteria and pico- and nanophytoplankton abundance (cells ml<sup>-1</sup> x 10<sup>4</sup>) during cruises CD110-B and BG9714-C in two stations located at the shelf-edge (P200, O2P26) and at the slope (P1000, O2P27)



Fig. 3 Photosynthesis-irradiance relationships at the surface and at the bottom of the photic layer at two stations during the summer cruise BG9714-C. Stn. O2P26 shelf-edge, Stn. O2 P27 slope.



Fig. 4 Photosynthesis-irradiance relationships at the surface and at the bottom of the photic layer at two stations during the winter cruise CD110-B. Stn P200 shelf-edge. Stn P1000 slope