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Measured photophysiological parameters used as tools to estimate vertical water movements in the coastal Mediterranean

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Aspects of phytoplankton photophysiology were investigated in a coastal area of the Mediterranean Sea (Gulf of Naples) using high performance liquid chromatography pigment analysis, flow cytometric cellular fluorescence information and in vivo pump and probe measurements. Discrete sampling, continuous profiles and on-board kinetic experiments were used to estimate photoacclimation kinetics and to identify the time scales of these reactions in phytoplankton. Based on the relationship between the depth of the euphotic zone and the mean chlorophyll concentration, the area could be classified as Case 1 water, although, oligotrophy was not reflected in phytoplankton photosynthetic efficiencies, suggesting that it represented a transient feature induced by an eddy, originating offshore. Fronts at the edge of the eddy exhibited different photophysiological features, in which the phytoplankton showed greater physiological stress, brought about by the convergent motion within the front. Kinetic experiments allowed identification of time scales for photoacclimation. Using photoprotective pigments as tracers we estimated vertical mixing velocities in the upper layer of 0.07 cm s^{-1} and from this we inferred kinetic coefficients for different photophysiological processes in the mixed layer as well as significant threshold values for photoacclimation rates to be 4% h^{-1}. From the threshold value, the vertical eddy diffusivity in the area at the time of sampling was estimated as 1.75×10^{-2} m^2 s^{-1}, a high value for vertical mixing.

INTRODUCTION

Understanding the variability in organic carbon fluxes in the oceans requires knowledge of how local physical forcing affects organism concentrations and their physiological characteristics. Mesoscale dynamics of phytoplankton generally correspond to changes in hydrodynamic conditions as a result, for example, of transition between different water masses, eddies, fronts or instability waves, whose effects overlap with seasonal oscillations and large-scale climatological cycles (Harris, 1984; Robinson and Golnaraghi, 1994). These physical structures are of considerable importance for fisheries and carbon budgets at larger scales (Claustre, 1994; Townsend *et al.*, 1994).

A full mechanistic understanding of the factors that control phytoplankton production in the oceans still eludes ecologists, partly because phytoplankton physiological responses are dependent on the past history of each individual cell (Chisholm *et al.*, 1986).

The uncertainty about the relationship between phytoplankton dynamics and hydrodynamics at mesoscales mainly results from difficulties in conducting studies with high resolution. Such constraints may be overcome for spatial studies (once physical features such as eddies have been identified), from synoptic ship or satellite observations. However, the conversion of ocean colour data into productivity or ecophysiology of phytoplankton is still limited by the lack of accurate quantitative knowledge of the processes influencing phytoplankton physiology (Cullen *et al.*, 1997; Millie *et al.*, 1997; Stuart *et al.*, 2000).

An integrated approach coupling information on phytoplankton taxonomy and physiology is therefore necessary in order to gather key ecological information on the reactions of algae to the physical environment. Pigment chemotaxonomy, coupled with flow cytometry, is very useful, since these analyses can characterize phytoplankton composition, as well as provide information concerning phytoplankton physiological status and

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trophic interactions (Millie *et al.*, 1993; Veldhuis and Kraay, 2000). Variable fluorescence, indeed, is a very useful parameter from which to estimate the photosynthetic performance of phytoplankton communities in real time, when submersible probes are used (Greene *et al.*, 1992, 1994; Falkowski and Kolber, 1993, 1995; Behrenfeld and Falkowski, 1997b). The ability to measure photophysiological parameters (such as variable fluorescence or photoprotective pigments), indicative of photosynthesis responses, represents a major advance in oceanography, and their use is becoming increasingly widespread [e.g. (Greene *et al.*, 1994; Falkowski and Kolber, 1995; Behrenfeld *et al.*, 1996; Strutton *et al.*, 1997)].

From these parameters, equations can be formulated to estimate quantitatively both phytoplankton composition and the relevant physiological processes involved in light-dependent processes such as photoprotection (Behrenfeld and Falkowski, 1997a; Sakshaug *et al.*, 1997; Bouman *et al.*, 2000b; Stuart *et al.*, 2000). Regional *in situ* relationships between photophysiological parameters and physical variables also need to be determined to provide a better determination of ocean colour products, since they affect the variability of the bio-optical characteristics of phytoplankton communities (Bouman *et al.*, 2000a; Stuart *et al.*, 2000). For instance, photoprotective pigments may significantly contribute to total *in vivo* pigment absorption, as seen in culture experiments (Fujiki and Taguchi, 2001). For this information to be translated to interpret *in situ* patterns, measurements at the right scales are needed (Lewis *et al.*, 1984; Bouman *et al.*, 2000a; Stuart *et al.*, 2000).

In addition, photoacclimative responses, when coupled to information on their kinetics, give hints on the past light history of algal cells, and from this, estimates of their vertical displacement along the water column (due to mixing) can be inferred [e.g. (Falkowski, 1983; Lewis *et al.*, 1984; Cullen and Lewis, 1988; Claustre *et al.*, 1994; Dusenberry *et al.*, 1999, 2001)]. In this study, *in situ* photoacclimative responses as well as *in situ* incubation experiments are used as a tool to estimate the mixing velocity and vertical eddy diffusivity in the upper layer of the water column (Lewis *et al.*, 1984). To our knowledge, this is the first report of such estimations drawn from real *in situ* data (i.e. without using a physical–biological coupled model). We believe that the approach used is promising for a better understanding of *in situ* photoacclimative processes, and their impact on primary productivity variability, as well as for their more accurate modelling. Estimates of physical processes from biological parameters represent a useful tool to obtain information that is very hard to retrieve by other means.

This study was conducted in the Gulf of Naples, representing a coastal area of the Mediterranean Sea, which in general is characterized by hydrographic cycles (Nival and Corre, 1976), and as such are pertinent case studies where mesoscale sampling can be applied to examine the relationship between phytoplankton dynamics and hydrological forcing (Casotti *et al.*, 2000).

The present study investigated phytoplankton photophysiology as a function of water mass distribution, using *in situ* fluorescence coupled with pigment analysis and flow cytometry at five depths at each of 18 stations, in 0–70 m depth in the Gulf of Naples. The specific aim was to identify which phytoplankton physiological features could be associated with specific hydrographic conditions and derive equations to describe these relationships that could be used at a regional scale to feed mathematical models of plankton dynamics of the study area. By examining light-related parameters in relation to the physical variability, we provide insights into the factors controlling phytoplankton biomass and ecophysiological reactions in coastal areas. In turn, the kinetics of photoacclimation and the temporal scales of photorelated parameters have been employed to use the characteristics of phytoplankton as monitors of the physical environment (Harris, 1984).

METHOD

Sampling

Eighteen stations were sampled along three coast-to-offshore transects on November 9 and 10, 1995 (Figure 1). Transect 1 includes stations from 1 to 8, transect 2 has stations from 9 to 14 and transect 3 has stations from 14 to 18. Continuous profiles of temperature, salinity and density were acquired with a CTD probe (SBE 911+; Seabird Electronics, Bellevue, WA, USA). Water

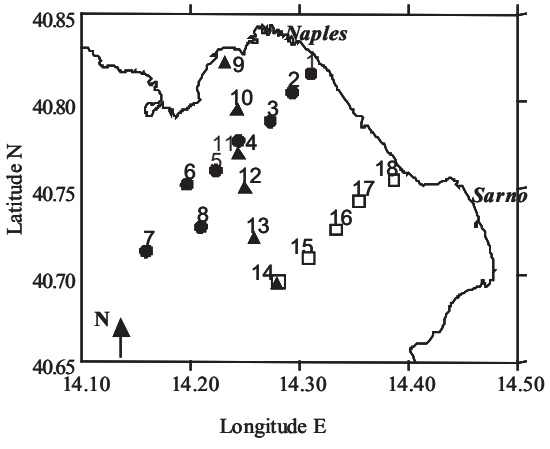

Fig. 1. Gulf of Naples (Italy). Sampling site and stations. Transect 1: stations 1 to 8 (●); Transect 2: stations 9 to 14 (▲); Transect 3: stations 14 to 18 (□).

samples were taken from 2, 10, 20, 40 and 70 m with a rosette sampler equipped with 12 Niskin bottles, for nutrient concentrations, pigments and ultraplankton counts and fluorescence by flow cytometry.

Light and fluorescence

Light [photosynthetically active radiation (PAR), 2π, W m^{-2}] and fluorescence (r.u.) profiles along the water column (from 0 to 50 m) were taken using the 'Prim-Prod' 1.08 submersible probe [EcoMonitor 1993 (Antal *et al.*, 2001)]. The Prim-Prod allows the measurement of chlorophyll fluorescence yield and it operates in pump-and-probe mode (Kolber and Falkowski,1993). F_0 is measured after a weak probe flash while F_m is measured following a saturating flash that closes all available photosynthetic reaction centres. The duration of saturating flashes is too short to allow multiple turnover of reaction centres, therefore producing an instantaneous estimate of F_m. F_m is recorded by the probe flash 0.05 ms after the saturating flash. Further details on the functioning of the Prim-Prod are presented elsewhere (Antal *et al.*, 2001). The so-called 'variable fluorescence' (F_v) was calculated as $F_v = F_m - F_0$, and the F_v/F_m ratio was considered as the maximum change in the quantum yield of fluorescence ($\Delta\phi_m$). $\Delta\phi_m$ represents an estimate of the photosynthetic energy conversion efficiency (Falkowski and Kolber, 1993) and can be quantitatively related to photosynthetic efficiency (Genty *et al.*, 1989; Kiefer and Reynolds, 1992).

At each station, from the light profile a vertical attenuation coefficient (K, m^{-1}) was estimated between 1 and 10 m depth.

Pigments

Two litre samples were filtered onto Whatman GF/F glass-fibre filters and immediately stored at $-20°C$. High performance liquid chromatography (HPLC) analyses were performed within 2 weeks of collection according to a slight modification of the protocol of Brunet *et al.*, described in Casotti *et al.* (Brunet *et al.*, 1993; Casotti *et al.*, 2000). Determination and quantification of single pigments were realized using chlorophyll and carotenoid standards obtained from the Water Quality Institute, International Agency for ^{14}C Determination, Denmark.

Absorption spectra reconstruction

Whole cell absorption spectra were reconstructed using the method of Bidigare *et al.* (Bidigare *et al.*, 1990), using the following equation :

$$a_{ph}(\lambda) = \Sigma a_i(\lambda) C_i \quad (1)$$

where, $a_{ph}(\lambda)$ is the absorption coefficient of phytoplankton at wavelength λ, $a_i(\lambda)$ is the *in vivo* specific absorption coefficient of the ith pigment at wavelength λ, and C_i is the concentration of the ith pigment (mg m^{-3}). Before reconstruction, pigments were grouped in five categories: (a) chlorophyll (Chl) *a* and equivalents (Chl *a* allomers), (b) Chl *b*, (c) Chl *c*, (d) photosynthetically active carotenoids (pac) and (e) non-photosynthetic carotenoids (npc, including diadinoxanthin, diatoxanthin, α and β carotenes and zeaxanthin).

From the Chl *a*-normalized spectra, average (between 400 and 700 nm) absorption coefficients of phytoplankton ($\tilde{a}_p$, m^2 mg Chl a^{-1}) were estimated. The same coefficients without the contribution of npc were also calculated, and will be referred to as $\tilde{a}_p^*$ (m^2 mg Chl a^{-1}) (Lindley *et al.*, 1995). This last coefficient, $\tilde{a}_p^*$, represents the absorption of light which is funnelled into the reaction centres of the photosystems and is therefore available to photosynthesis. The ratio of the two absorption coefficients, $\tilde{a}_p^*/\tilde{a}_p$, is considered to be an estimate of the degree of photoprotection performed by algae (Lindley *et al.*, 1995).

As already stressed elsewhere (Lindley *et al.*, 1995), this spectral reconstruction technique does not take into account the contribution of debris to absorption, or the package effect, especially occurring in large cells (Morel and Bricaud, 1981; Nelson *et al.*, 1993). This limits its application to homogeneous phytoplankton populations distributed within a small size spectrum (Stuart *et al.*, 1998), which was generally the case in this study(Casotti *et al.*, 2000).

On-board kinetic photoacclimation experiments

Sea water from 20 m depth at station 11 (10% of incident surface light), was taken at dusk and incubated on deck in a clear 20 l polycarbonate container. The container was screened with neutral density plastic sheet (Lee Filter, USA), which allowed 90% of incident light through. One litre samples were taken every hour starting the following dawn for 11 hours throughout the whole light period. Variations in concentrations of specific xanthophylls, normalized by Chl *a*, were used to calculate kinetic coefficients for adaptation to the increase and decrease of irradiance during the natural daylight cycle (Falkowski, 1983; Claustre *et al.*,1994). Such xanthophylls were the diatoxanthin (Dt) and the diadinoxanthin (Dd), known to be involved in photoprotective reactions in chromophyte algae (Demers *et al.*, 1991; Brunet *et al.*, 1993). The kinetic coefficient K (t^{-1}) was estimated from the equation:

$$X = (X_0 - X_\infty)\exp(-Kt) + X_\infty \quad (2)$$

where X is the photodependent parameter [Dt/Chl *a*, Dd/Chl *a* or Dt/(Dt + Dd) ratios], X_0 is the initial value,

X_∞ is the final value, t is time (in hours) and K is the first-order kinetic coefficient (h^{-1}) (Falkowski, 1983; Claustre *et al.*, 1994). The equation was calculated for the increasing light period and for the decreasing light period separately.

We would like to stress that such incubation experiments can be usefully applied to *in situ* data only when sampling starts at dawn, and when the phytoplankton subjected to the shift originates from low levels of irradiance, so that interferences from photoinhibition, photoprotection, or photo stress are avoided.

Flow cytometry

Samples were fixed for 15 min with glutaraldehyde 0.5% final concentration, frozen in liquid nitrogen and stored at −80°C for later analysis (Vaulot *et al.*, 1989). Thawed samples were analyzed using a FACScalibur flow cytometer (Becton Dickinson), equipped with a standard laser and filter set and using 0.22 μm filtered sea water as the sheath fluid. Fluorescent beads with a diameter of 0.97 μm (Polysciences) were added into each sample, and all parameters were normalized to their values and expressed as relative units (r.u.). Data were analysed using CellQuest software (Becton-Dickinson). Further details are indicated in Casotti *et al.* (Casotti *et al.*, 2000).

RESULTS

Physical features, light and phytoplankton

Sampling took place in the Gulf of Naples (Italy) in November 1995, during the transition from autumn to winter. The transition from coastal to offshore waters was very gradual. In the central area, an anticyclonic eddy, generated by the general circulation of the Tyrrhenian Sea, was observed. The eddy, which had an internal deformation radius calculated to be 8 km (Casotti *et al.*, 2000), was characterized by higher temperature and salinity values (Figure 2a–d). In the vertical plane, a mixed layer occupied the upper 30 m of the area sampled, and a stratification, between 40 and 60 m, separated the upper water masses, including the eddy, from less saline water, identified as Modified Atlantic Water. The existence of water masses with different origins and characteristics was evident from phytoplankton ecophysiological markers. A more detailed description of water masses and phytoplankton and bacterial distribution is reported in Casotti *et al.* (Casotti *et al.*, 2000).

Light (PAR, μmol m^{-2} s^{-1}) penetrated deeper in the eddy than in the other water masses (stations 13, 14 and 15) because of the lower suspended matter content, as indicated by the lower attenuation coefficient (K, Figure 2e,f). The front at the edge of the eddy was marked by lower PAR values (attenuation coefficient values higher at stations 12 and 16 relative to stations 13, 14 and 15, Figure 2e,f), and an accumulation of phytoplankton at the 20 m depth of station 12 (Figure 3a).

Phytoplankton biomass (Chl *a*) ranged from 0.10 to 1.60 μg l^{-1}, and decreased from the coast to offshore. The lowest values were observed in the eddy (stations 13, 14 and 15; Figures 3a and 3b), where the depth of the euphotic layer [1% of surface irradiation (I_0)] was deeper (55 m, data not shown). A significant negative relationship between the depth of the euphotic layer and the mean Chl *a* concentrations was found as:

$$Z_{eu} = 36.447 C^{-0.356} \quad (3)$$

where Z_{eu} is the euphotic depth (m) and C is the mean chlorophyll concentration in the euphotic layer (μg l^{-1}) (Figure 4, $r^2 = 0.62$, $n = 16$). Stations 1 and 2 were excluded from the regression analysis because of their high enrichment, caused by the proximity of water outfalls from the coast. Discrepancies with Morel's equation for Case 1 waters (Morel, 1988) were small (36.45 versus 38 and −0.356 versus −0.428). We concluded that at the time of sampling the Gulf of Naples lay within the definition of Case 1 waters as represented by its optical characteristics.

Photoprotective pigments

Surface diatoxanthin/Chl *a* (Dt/Chl *a*) was higher in the eddy (average of 0.0055) relative to the other stations (0.0044), which may have been because of higher light penetration (Figures 5a,b and 6). The highest value (0.0300) was recorded at station 12 at 20 m depth, associated with an accumulation of Chl *a* (Figures 6a and 3a). When this value was subtracted from the Dt/Chl *a* value measured at surrounding stations (mean of 0.0012), the excess of Dt/Chl *a* was 0.0288. In contrast to Dt/Chl *a*, diadinoxanthin/Chl *a* (Dd/Chl *a*) did not present differences between the eddy and the other stations (Figure 5c,d). The correlation between the Dt/Chl *a* and the Dd/Chl *a* ratios was significant (Spearman correlation, $r^2 = 0.45$, $n = 65$), but only Dt/Chl *a* was significantly correlated to light (I, Spearman correlation, $r^2 = 0.57$, $n = 65$).

A non-linear relationship was found between Dt/(Dt + Dd) and light (Figure 7a), and followed equation (4):

$$\mathrm{Ln}[\mathrm{Dt}/(\mathrm{Dt}+\mathrm{Dd})] = -3.3840 + 0.0028I - 1.6240 \times 10^{-6} I^2 \quad (4)$$

where $r^2 = 0.70$, $n = 30$.

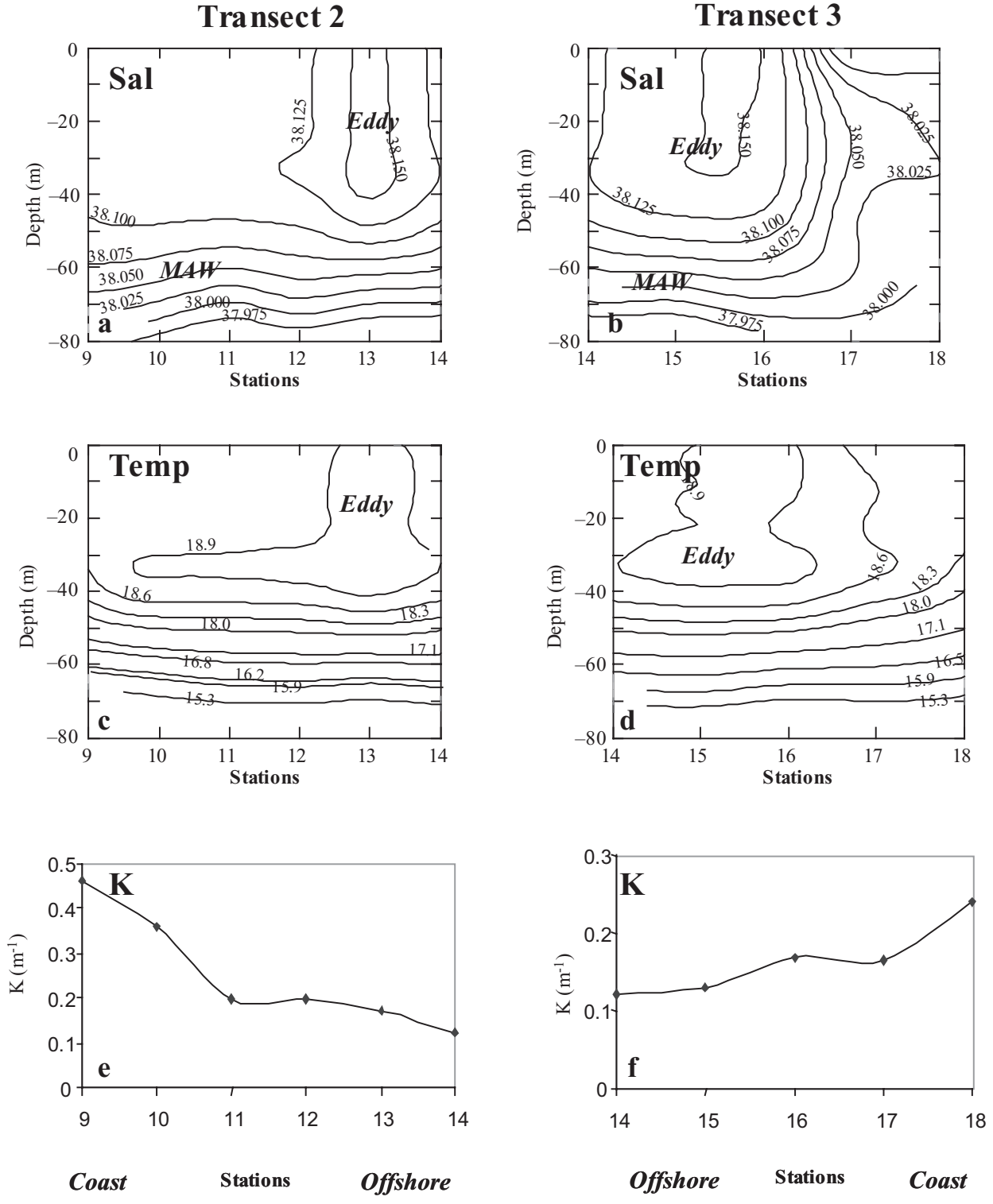

Fig. 2. Salinity (p.s.u., **a** and **b**), temperature (°C, **c** and **d**) and attenuation coefficient (K, m^{-1}, **e** and **f**) distribution along transects 2 (**a**, **c**, **e**) and 3 (**b**, **d**, **f**). Note the change of scale for K (m^{-1}) between the graphs (**e**) and (**f**).

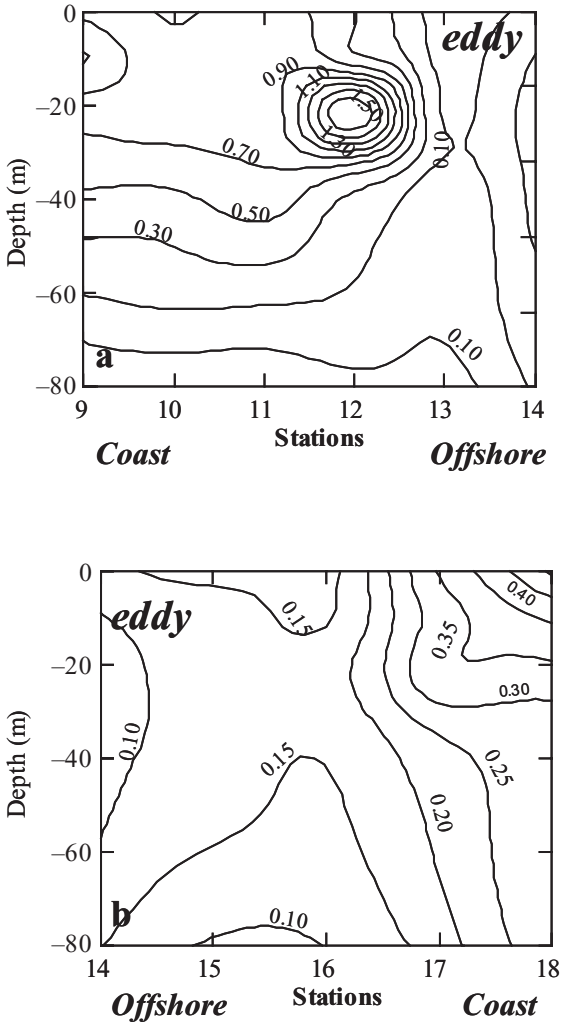

Fig. 3. Chl *a* concentration (μg l^{-1}) along transects 2 (**a**) and 3 (**b**).

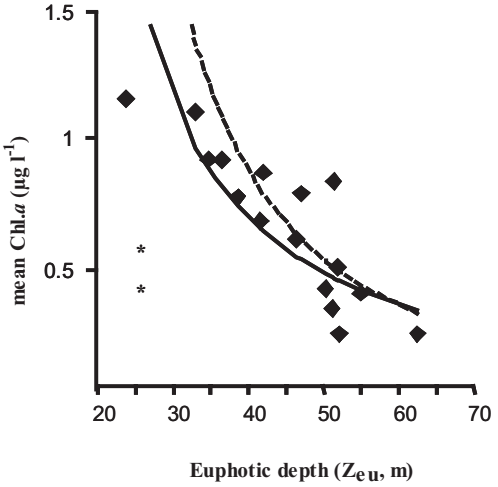

Fig. 4. Regression between mean Chl *a* concentration within the euphotic layer (C = Chl *a* in mg m^{-3}) and depth of the euphotic layer (Z_{eu}, m): $Z_{eu} = 36.45C^{-0.356}$ ($r^2 = 0.62$, $n = 16$). Stations 1 and 2 (*) were excluded from the regression, showing different biological patterns because their total depth was shallower than 40 m. The dashed line corresponds to the equation for Case 1 waters (Morel, 1988).

In addition, Dt/(Dt + Dd) was correlated with F_v/F_m as follows (Figure 7b):

$$\mathrm{Ln}[\mathrm{Dt}/(\mathrm{Dt}+\mathrm{Dd})] = -2.6920 + 1.3060(F_v/F_m) - 5.0760(F_v/F_m)^2 \quad (5)$$

where $r^2 = 0.57$, $n = 30$. Zero values of Dt/(Dt + Dd) were excluded and only values from the euphotic layer were considered.

Zeaxanthin, the photoprotective pigment in green algae, and also a taxonomic marker pigment of prokaryotic phytoplankton, was not correlated with irradiance, with any other photoprotective pigment ($P > 0.05$, $n = 65$), nor with violaxanthin, the epoxyded xanthophyll of the photoregulated cycle of green algae (Demming-Adams, 1990). It was, however, positively correlated with Chl *a* ($r^2 = 0.75$, $n = 65$), and for these reasons, in this study it has been considered only as a taxonomic marker of prokaryotic phytoplankton (Casotti *et al.*, 2000) and not as a marker of photoprotection of green algae. Although we have no direct evidence (i.e. counts) that green algae were not present in our samples, Chl *b* concentration was always very low, and this has been interpreted as an indication of a reduced contribution of these algae to total Chl *a* biomass.

Absorption coefficients

The Chl *a*-normalized average absorption coefficient of phytoplankton ($\tilde{a}_p$) was significantly lower at coastal stations and higher offshore ($P < 0.01$, data not shown). Values were not significantly different at the different depths within the surface mixed layer (mean of 0.030 m^2 mg Chl a^{-1}, $P > 0.05$), but were significantly lower ($P < 0.01$, $n = 15$) at 70 m.

The Chl *a*-normalized average absorption coefficient of phytoplankton without the contribution of photoprotective pigments ($\tilde{a}_p$*) showed homogeneous values (Figure 8a) in the surface layer [mean (0.026 m^2 mg Chl a^{-1}) between 2 and 20 m]. Higher values were found at 40 m [mean of (0.031 m^2 mg Chl a^{-1}), $P < 0.01$, $n = 15$] and lower at 70 m [mean (0.023 m^2 mg Chl a^{-1}), $P < 0.01$, $n = 15$]. A low value (0.020 m^2 mg Chl a^{-1}) was found at 20 m at station 12 (frontal region), where phytoplankton biomass rich in Dt accumulated (Figure 6a).

Values of $\tilde{a}_p^*/\tilde{a}_p$ were lowest in the eddy, where light penetration, and therefore photoprotection, was higher (Figure 8b). None of the absorption coefficient values was significantly related to light.

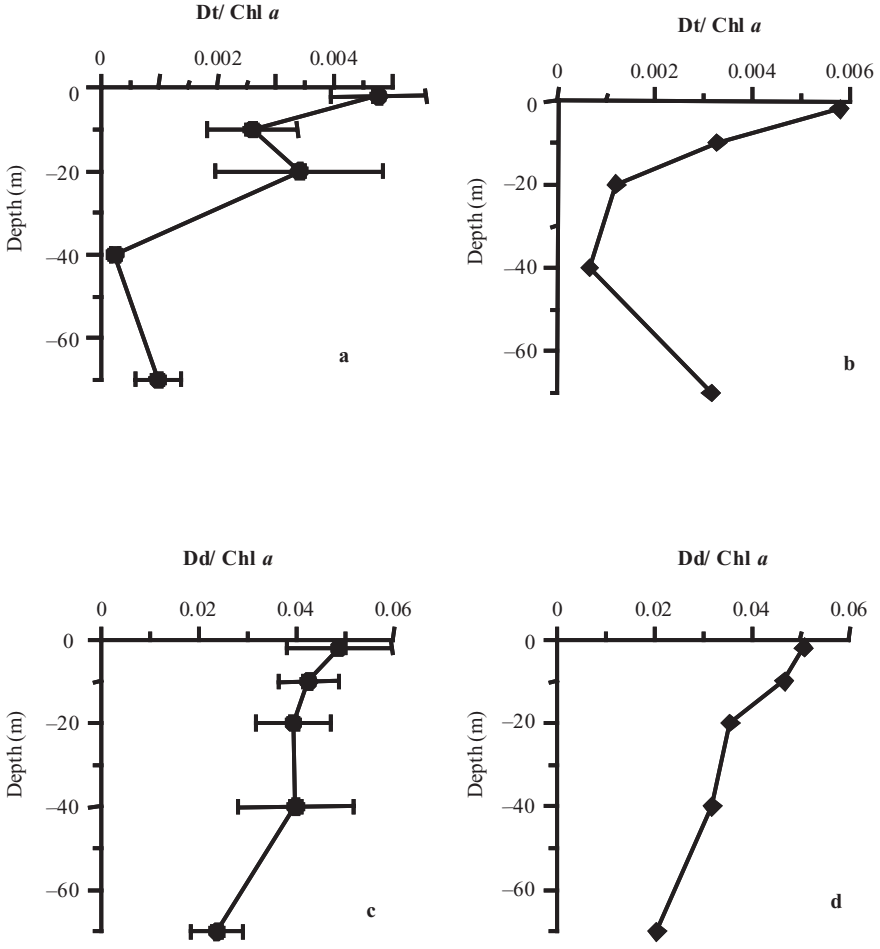

Fig. 5. Mean vertical profiles through the water column of (**a**) Dt/Chl *a* (all stations except the eddy, $n = 15$), (**b**) Dt/Chl *a* in the eddy ($n = 3$), (**c**) Dd/Chl *a* (all stations except the eddy, $n = 15$) and (**d**) Dd/Chl *a* in the eddy ($n = 3$). Error bars are ± 1 standard deviation.

Photosynthetic efficiency

F_v/F_m values ranged from 0.09 to 0.57 at the surface, and the lowest values were observed in the eddy (Table I). The total average was 0.38 (± 0.15 SD, $n = 69$).

Values increased with depth from 2 m (0.23 ± 0.16) to 40 m (0.53 ± 0.06) and were significantly different at each depth (Student's *t*-test, $P < 0.001$, $n = 18$). Moreover, coefficients of variation of F_v/F_m decreased from 2 (0.64) to 40 m (0.11) and variances were significantly different between depths (Fisher–Snedecor test, $P < 0.01$).

The largest difference per metre was found between 2 and 10 m (variation of 0.0160 m^{-1}), while the smallest differences were between 10 and 20 m or 20 and 40 m (variations of 0.0059 and 0.0054 m^{-1}, respectively), suggesting a stronger photoacclimation response in the upper 10 m layer than below, in relation to the stronger decrease of light with depth in the same layer.

Mean F_v/F_m in the surface layer was significantly different ($P < 0.05$, Table I) in the eddy with respect to surrounding waters, with lower values in the eddy, suggesting a high-light 'stress' of phytoplankton in this ecosystem.

F_v/F_m and irradiance were related according to the following equation (Figure 7c):

$$\mathrm{Ln}(F_v/F_m) = -0.37 + 0.1309\ \mathrm{Ln}(I) - 0.0396[\mathrm{Ln}(I)]^2 \quad (6)$$

$r^2 = 0.82$, $n = 60$; where I is irradiance.

Photoacclimation and mixing estimates

To investigate the dynamics of phytoplankton photoacclimation mechanisms, kinetic coefficients (k, h^{-1}) were calculated for photoprotective pigment ratios, Dt/Chl *a*, Dt/(Dt + Dd) and Dd/Chl *a*, for either increase or decrease of natural light during the day using equation (2) (Figure 9), as measured during the incubation experiment. We assumed that the changes and the kinetics in photoprotective parameters observed in phytoplankton

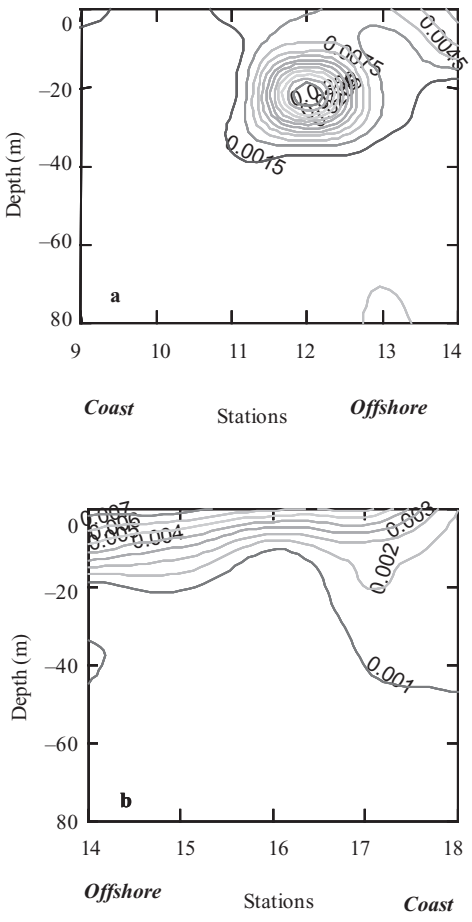

Fig. 6. Dt/Chl *a* distribution along transects 2 (**a**) and 3 (**b**).

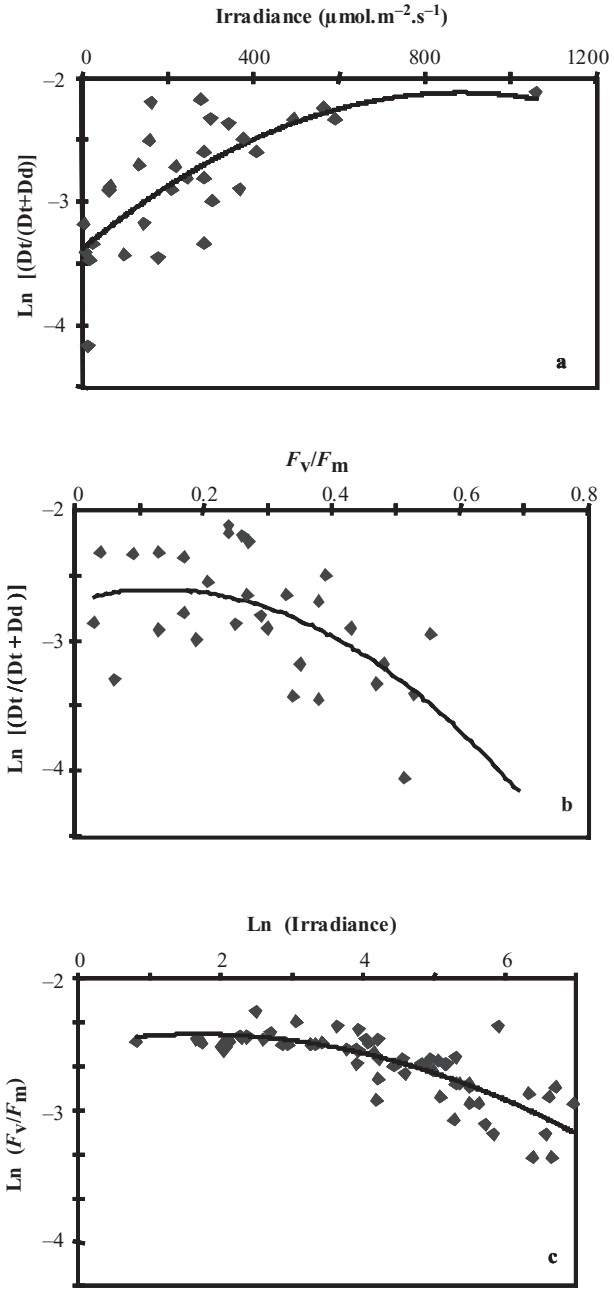

Fig. 7. Relationships between: (**a**) Dt/(Dt + Dd) ratio and irradiance ($r^2 = 0.70$, $n = 30$), (**b**) Dt/(Dt + Dd) ratio and F_v/F_m ratio ($r^2 = 0.57$, $n = 30$) and (**c**) F_v/F_m ratio and irradiance ($r^2 = 0.82$, $n = 60$). Standard errors of each constant estimates (a, b, and c corresponding to the following general equation: $a + bX + cX^2$) are: (**a**) 0.16, 0.0010 and 0.0000010, respectively; (**b**) 0.32, 2.36 and 3.85, respectively; and (**c**) 0.28, 0.09 and 0.016, respectively.

shifted from 10 to 90% incident light (incubation experiment) were representative of the changes of the *in situ* phytoplankton subjected to the same shift in light intensity as a consequence of mixing. The calculated *k* values were therefore applied to samples taken at the same relative light levels (i.e. between 20 and 2 m depths) as the incubation.

First-order kinetics was assumed, following Falkowski and Claustre *et al.* (Falkowski, 1983; Claustre *et al.*, 1994). Values of the kinetic coefficients are reported in Table II. Mean rates were higher for Dt/(Dt + Dd), and higher for increasing light. Dt/Chl *a* and Dt/(Dt + Dd) changed faster than Dd/Chl *a* when light was increasing, whereas the opposite was true when light was decreasing.

The *k* value for Dd/Chl *a* (0.465) was in agreement with Claustre *et al.* (Claustre *et al.*, 1994) who measured a *k* of 0.5 h^{-1} for an increase in irradiance from 3 to 50% of surface irradiance. Further comparison is not possible because the *in situ* kinetic coefficients of photoprotective pigments are poorly documented in the literature.

Photodependent parameters, such as photoprotective pigments or F_v/F_m were not homogeneously distributed along the water column (e.g. Figure 5; Table I), suggesting that the turbulent motion inside the mixed layer was slower than the phytoplankton adaptation velocity.

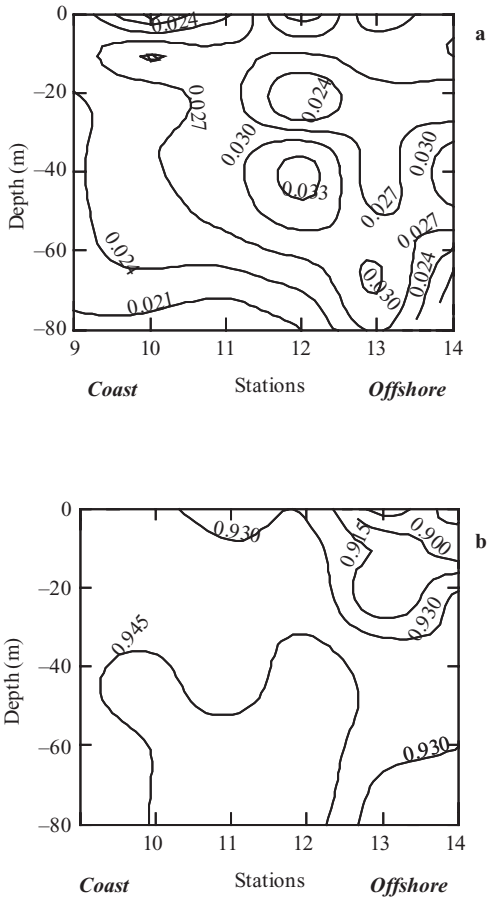

Fig. 8. $\tilde{a}_p$* coefficient (**a**) and $\tilde{a}_p$*/$\tilde{a}_p$ ratio (**b**) distribution along transect 2.

However, the vertical distribution of these photodependent parameters did not follow an exponential decrease, as would be expected if only light intensity adaptation were present. Therefore, phytoplankton was not fully adapted to the present light along the water column, and this is suggested to be a consequence of vertical displacement as induced by mixing. As a consequence,

Table I: Average F_v/F_m values and SD at 2, 10 and 20 m depths at all stations, except the eddy (all water masses, n = 15 for each depth) and in the eddy (eddy, n = 3 for each depth)

Depth (m)	F_v/F_m all water masses	F_v/F_m eddy
2	0.23 ± 0.15	0.18 ± 0.07
10	0.38 ± 0.10	0.29 ± 0.08
20	0.44 ± 0.07	0.37 ± 0.03
Mean (2–20)	0.35 ± 0.14*	0.29 ± 0.09*

*Significant difference ($P < 0.05$) between these two mean values.

the following equation could be employed, using kinetic coefficients from the incubation experiments (Table II) to estimate vertical velocities of phytoplankton cells within the mixed layer:

$$T = (1/-K)\mathrm{Ln}[(X_t - X_\infty)/(X_0 - X_\infty)] \qquad (7)$$

where T is the time (h) necessary to change from X_0 (initial value, i.e. at 20 m) to X_t (value at time t, at 2 m). X is the photodependent parameter [Dt/Chl *a*, Dd/Chl *a* or Dt/(Dt + Dd) ratios] and X_∞ is the final value of the incubation experiment [see equation (2)].

From this equation, the time necessary for the changes from X_0 to X_t value (photodependent parameter X at times 0 and t, respectively) could be estimated, and, from this the mixing velocity was calculated by dividing the depth of the mixed layer (in our case 20 m) by the time.

The estimated time of mixing lay between 5 h [using Dt/(Dt + Dd)] and 10 h (using Dd/Chl *a*), with an average value of 7 h, which is exactly the time estimated using Dt/Chl *a*. It is noteworthy that mean values were used, so as to eliminate the variability of photorelated parameters during the natural diel light oscillation.

Using this average value, the mixing velocity was estimated as 0.07 cm s^{-1}, which suggests that phytoplankton

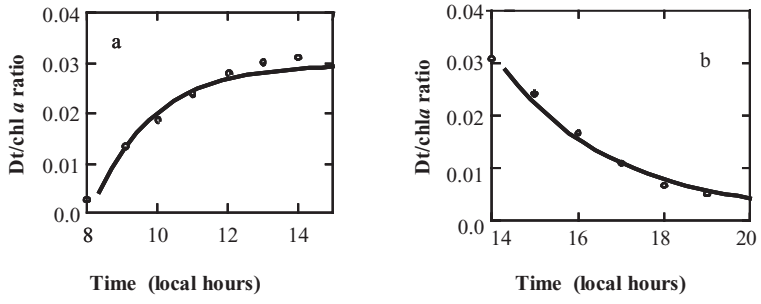

Fig. 9. Examples of xanthophyll pigment distribution to light changes during the on-board incubation experiment: temporal distribution of Dt/Chl *a* (**a**) during the increasing light period (sunset to 14:00 h) and (**b**) during the decreasing light period (14:00 to 19:00 h). The lines represent the fit of the data with equation (2) (see text); (**a**) Dt/Chl $a = 0.037 \exp(-0.59t) + 0.033$ ($r^2 = 0.93$); (**b**) Dt/Chl $a = 0.031 \exp(-0.38t) + 0.001$ ($r^2 = 0.99$).

Table II: First-order kinetic coefficients (k, h^{-1}) for Dt/Chl a, Dt/(Dt + Dd) and Dd/Chl a from on-board kinetic experiments (see text)

	Dt/Chl *a*	Dt/(Dt + Dd)	Dd/Chl *a*
LL to HL	0.590	0.610	0.660
HL to LL	0.380	0.550	0.270
Mean	0.485	0.580	0.465

LL, low light; HL, high light. All statistical relationships are significant at $P < 0.01$.

cells could be transported from 20 to 2 m depth in 7 h and from 10 to 2 m in 3.1 h.

Assuming mixing between the two depths and using the result of the velocity calculation, we estimated percentage changes per hour of other photodependent parameters between 2 and 20 m relative to their initial values (considered as their mean value at 20 m, Tables IIIa and b). Dt/(Dt + Dd) changed the fastest, followed by Dt/Chl *a*, F_v/F_m, F_o/Chl *a*, Dd/Chl *a*, and zeaxanthin/Chl *a*, red fluorescence of *Synechococcus* and *Prochlorococcus* and phytoplankton absorption coefficients.

DISCUSSION

The values of F_v/F_m measured during this study were similar to values measured by Olaizola *et al.* in the north-eastern Atlantic Ocean (Olaizola *et al.*, 1996), but higher than values reported from other oligotrophic areas, such as the Equatorial Pacific [0.25–0.45 (Greene *et al.*, 1992)], or a subtropical gyre in the Atlantic Ocean [0.40 (Falkowski and Kolber, 1993)]. The highest values of F_v/F_m measured (0.64) were very close to the highest measured value [0.65 (Kolber and Falkowski, 1993)] and very close to values measured at an upwelling north-western African coastal station (Falkowski and Kolber, 1993). No correlation was found between nutrients (concentrations and/or ratios) and photosynthetic parameters (e.g. F_v/F_m). Therefore, despite the oligotrophic feature at the time of sampling, phytoplankton appeared not to be nutrient-limited [except in the eddy, (Casotti *et al.*, 2000)], and light and hydrodynamics appeared to be the main factors controlling photosynthesis. Similar observations were reported for the oligotrophic north Atlantic Ocean, in which there was higher heterogeneity in water mass composition and trophic status than would be expected in oligotrophic areas (Olaizola *et al.*, 1996). Our data suggest that oligotrophy was only a transient feature of the study area. The general circulation patterns of the Tyrrhenian Sea strongly influence the optical characteristics of the study area, which could be considered as Case 1 water (Morel, 1988) at the time of sampling, as shown by equation (3). Although no data are available on the temporal occurrence of such events, they have been reported throughout the year, and strongly characterize both the size spectrum of phytoplankton and its average primary production (Zingone *et al.*, 1995).

Photoacclimation and water mass characteristics

Photoprotection presented different patterns as related to water masses. Higher photoprotection was observed in the more transparent waters of the eddy, where 35% of incident surface light was measured at 10 m, compared

*Table III: Percentage variation of photodependent parameters, as calculated using a mixing estimate of 0.07 cm s^{-1} derived from coefficients calculated from the incubation experiments and equation (7). (**a**) Values for all stations except those in the eddy (n = 15), (**b**) values for stations in the eddy (n = 3)*

% variation	Dt/(Dd+Dt)	Dt/Chl *a*	F_v/F_m	F_0/Chl *a*	Dd/Chl *a*	RedSyn	Zea/Chl *a*	RedPro	$\tilde{a}_p$	$\tilde{a}_p$*	$\tilde{a}_p$*/$\tilde{a}_p$
(**a**) All stations except eddy											
% variation in 7 h	66.00	55.00	45.00	30.00	22.00	11.30	8.20	7.60	3.00	0.30	3.00
% variation h^{-1}	9.45*	7.85*	6.45	4.30	4.15	3.75*	1.20*	1.08	0.45*	0.04*	0.45
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	ns	ns	ns	ns	ns	ns
(**b**) Stations in the eddy											
% variation in 7 h	78.00	66.00	49.00	33.00	30.00	9.00	20.00	12.20	7.50	5.90	2.10
% variation h^{-1}	11.15*	9.45*	7.00	4.70	4.28	1.30*	2.85*	1.75	1.10*	0.85*	0.30

Abbreviations as defined in the text. RedSyn, red fluorescence of *Synechococcus* chlorophyll; RedPro, red fluorescence of *Prochlorococcus* chlorophyll; Zea, zeaxanthin. *P* indicates the significance of comparison tests of means (Student's *t*-test, $n = 15$) between 2 and 20 m mean values. ns, non-significant difference ($P > 0.05$). * indicates that differences between parts (**a**) and (**b**) are significant ($P < 0.05$).

with coastal stations, where only 23% of incident surface light reaches the same depth.

Low values of $\tilde{a}_p^*/\tilde{a}_p$ in the sub-surface (Figure 7b) were caused by photoprotective pigments (diadinoxanthin and diatoxanthin), which can strongly influence the total *in vivo* absorption of phytoplankton (Lindley *et al.*, 1995; Stuart *et al.*, 1998) and also have an effect on the quantum yield of photosynthetic carbon fixation (Babin *et al.*, 1996). Fujiki and Taguhi (2001) showed that the relative contribution of diadinoxanthin plus diatoxanthin to the total *in vivo* absorption (400–700 nm) of cultured diatoms ranged between 5 and 30%, depending on the species composition and light intensity, and that there was an inverse relationship between the amount of (Dd + Dt) and quantum yield of growth (Fujiki and Taguhi, 2001). Therefore, photoprotective reactions may account for a relevant part of the observed variability of optical and photosynthetic properties at sea, and must be taken into account when modelling primary productivity from Chl *a* data.

In the transition layer, where ~1% of incident light was measured, higher values of $\tilde{a}_p$ and $\tilde{a}_p^*$ were estimated, relative to the surface, similar to observations in other coastal waters (Hoepffner and Sathyendranath, 1992). However, values of absorption are likely to be overestimated, due to a higher proportion of diatoms and prymnesiophytes (Casotti *et al.*, 2000), for which the package effect can cause a decrease in efficiency of light absorption (Nelson *et al.*, 1993).

At depth (70 m), the low values of $\tilde{a}_p$ and $\tilde{a}_p^*$ confirm previous observations by Hoepffner and Sathyendranath (Hoepffner and Sathyendranath, 1992) and were probably a result of the composition of the phytoplankton community, with a higher proportion of small cells, such as prokaryotic phytoplankton, as well as pelagophytes in the Modified Atlantic Waters (Casotti *et al.*, 2000).

Fronts delimiting the eddy exhibited different photophysiological features at different stations. Presence and accumulation of phytoplankton biomass rich in Dt at 20 m at station 12 (probably of surface origin), indicates trapping of phytoplankton inside the convergent front. The trapped biomass accounted for 1.39 μg l^{-1}, as estimated by subtracting the value observed from that expected at 20 m at station 12. The latter has been calculated from fitting the vertical profile of Chl *a* at station 12 and at the other surrounding stations, allowing the Chl *a* concentration at 20 m to be estimated assuming no accumulation. This estimate was based on the very similar vertical profiles of Chl *a* between station 12 and the surrounding stations (except at 20 m where biomass accumulated at station 12). The excess value is quite consistent and indicates that dynamic processes within fronts can be relevant for phytoplankton dynamics at the mesoscale.

The process of accumulation occurred some time prior to sampling since cells were in the process of acclimating, since the F_v/F_m values were not significantly different to nearby stations at the same depth, and were consistent with the expected exponential increase with depth (data not shown). At the same time, the excess in Dt/Chl *a* was extremely high (0.0290), indicating a pigment response to environmental stress, as also observed by other authors (Claustre *et al.*, 1994).

At station 16, also located at the edge of the eddy, no accumulation was observed at depth, and lower light penetration may be caused by accumulation of particulate matter, probably trapped by the front at the surface (Yoder *et al.*, 1993).

The difference between stations 12 and 16 could be the result of the greater distance of station 16 relative to the convergence area, or to downward phytoplankton transport being slower. We cannot exclude the possibility that the convergence occurred on only one side of the eddy. The dynamics of vertical transport within fronts can be very complicated and variable in space and time (Barth, 1994).

Kinetic coefficients and mixing

For all parameters, there was a stronger physiological response in the eddy (IIIb) than in surrounding waters (Table IIIa), probably as a result of the higher light penetration (Olaizola *et al.*, 1996), or limiting nutrients (Claustre *et al.*, 1994; Casotti *et al.*, 2000).

It was also evident that the different photophysiological parameters examined reacted with different time scales, and therefore could be used as indicators of different physical features.

Our results showed that parameters showing a variation of $<4\%$ h^{-1} did not show significant differences between depths of 2 and 20 m (Table IIIa), suggesting that this was the lower threshold for photoacclimation rates of phytoplankton cells.

Using this threshold value, we estimated the vertical diffusivity coefficient (Lewis *et al.*, 1984; Cullen and Lewis, 1988). These authors distinguish two situations (photoacclimation-dominated and turbulent-mixing-dominated), based on the magnitudes of two dimensionless factors ($Kv/l^2\gamma$ and Kl), representing the turbulent mixing and photoacclimation, respectively. An intermediate situation would be represented by:

$$Kv/l^2\gamma = Kl \tag{8}$$

where Kv is the vertical eddy diffusivity coefficient, l is the depth of the mixed layer, γ is the rate of change of the photoadaptive parameter and K is the attenuation coefficient [from (Lewis *et al.*, 1984); M. R. Lewis, personal communication].

Since the mean attenuation coefficient (K) estimated from our data was 0.20 m^{-1} and the depth of the mixed layer (l) was 20 m, the dimensionless Kl could be calculated as equal to 4. In this case, equation (8) becomes:

$$K\mathrm{v}/l^2\gamma = 4 \tag{9}$$

and

$$K\mathrm{v} = 1.75 \times 10^{-2}\mathrm{m}^2\mathrm{s}^{-1} \tag{10}$$

where $l = 20$ m and $\gamma = 0.04$ h^{-1} (4% h^{-1}), suggesting that the hydrographic conditions were associated with enhanced levels of vertical mixing. The estimated value of vertical eddy diffusivity is relatively high when compared with other oceanic regions (Denman and Gargett, 1983; Lewis *et al.*, 1984; Dusenberry *et al.*, 1999). However, we can expect higher dynamics in the water column in winter in coastal areas when compared with more stable areas or stratified water columns, such as those examined so far.

The information on the vertical distribution of several photoadaptive parameters may therefore be used as tracers of the physical environment, and this information is particularly valuable, since these values are very hard to measure by other means (Cullen and Lewis, 1988).

In conclusion, despite the regional value of the data presented, the approach of using photodependent biological parameters to infer characteristics and dynamics of the upper layer of the ocean appears promising and it represents a valuable tool to characterize the ecophysiological variability of coastal systems.

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