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Carbon biomass, and gross growth rates as estimated from ^{14}C pigment labelling, during photoacclimation in *Prochlorococcus* CCMP 1378

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ABSTRACT: The ^{14}C labelling of chlorophylls and carotenoids is increasingly used to evaluate phytoplanktonic biomass and growth rates in oceanic systems. Rigorous testing of the technique in the laboratory, however, is necessary prior to its application in the field. A Mediterranean clone of *Prochlorococcus*, a photosynthetic prokaryote which is an important component of the autotrophic biomass in oligotrophic environments, was subjected to shifts in light intensity. Particulate organic carbon (POC) was monitored by CHN analysis, pigments by HPLC and *Prochlorococcus* and heterotrophic bacteria concentrations by flow cytometry. Using a combination of HPLC and on-line radioactivity detection, ^{14}C labelling kinetics of divinyl-chlorophyll *a* (Dv-chl *a*) and zeaxanthin were followed. *Prochlorococcus* changed its Dv-chl *a* content markedly in response to change in light intensity, but not its zeaxanthin content, which remained nearly constant around 1.07 fg cell^{-1} regardless of the irradiance. Pigment synthesis rates were correctly estimated from their ^{14}C incorporation rates whatever the light level. From POC measurements and cell concentrations, the *Prochlorococcus* carbon content was estimated to be 49 fg C cell^{-1}. Moreover, under both constant and shifted (high to low and vice versa) light conditions, *Prochlorococcus* growth rate (as computed from variations in cell density) was much better estimated from zeaxanthin than from Dv-chl *a* labelling rates.

KEY WORDS: *Prochlorococcus* · Photoacclimation · Pigment synthesis rates · Carbon content · ^{14}C pigment labelling method

INTRODUCTION

The estimation of dissolved inorganic carbon fixation by phytoplankton is of prime importance in understanding global biogeochemical cycles in the oceans. Carbon fixation is generally expressed in terms of the primary production rate (g C m^{-2} d^{-1}). However, a more meaningful variable is the growth rate (d^{-1}), which, for carbon, can be computed for each depth as the ratio of the primary production rate (g C m^{-3} d^{-1}) to the phytoplankton carbon concentration (g C m^{-3}). *In situ*, however, the determination of phytoplankton growth rate is not easy due to uncertainties in evaluating both carbon biomass and primary production (Peterson 1977, Furnas 1990, Gieskes 1991). The assessment of phytoplankton carbon in oceanic waters is difficult because only particulate carbon can be easily measured and it also includes phytoplankton detrital material and heterotrophic biomass (Redalje & Laws 1981, Welschmeyer & Lorenzen 1984). Several methods have been developed to estimate autotrophic carbon from cell size and volume (e.g. Verity et al. 1992), but in general they are not very precise. Primary production estimates have also improved in the last decade with the advent of ultra-clean incubation methods (Fitzwater et al. 1982, Lohrenz et al. 1992). Interpretation of the

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measurements is, however, closely linked to the duration of incubation. Long incubations include not only fixation terms but also loss terms (respiration, excretion, grazing) and therefore provide net estimates of primary production rates, while short incubations, which result in lower loss terms, provide gross rates (Carpenter & Lively 1980, Smith & Platt 1984, Welschmeyer & Lorenzen 1985). Gross rather than net parameters are essential for the understanding of phytoplankton dynamics: they reflect the phytoplankton capacity to fix carbon in direct response to changes in environmental factors such as light intensity, temperature, nutrients and trace element supply (Geider 1987, Buma et al. 1991). The ^{14}C chlorophyll *a* (chl *a*) labelling method is a useful tool to estimate phytoplankton gross growth rates and phytoplankton carbon in the field (Redalje & Laws 1981, Welschmeyer & Lorenzen 1984). One of its basic hypotheses is that the rate of ^{14}C chl *a* labelling, in contrast to that of ^{14}C cellular labelling, is not affected by the loss processes mentioned above (Welschmeyer & Lorenzen 1984).

Another factor that complicates the interpretation of carbon fixation rates is that, in natural communities, not all species respond in the same way to variations in environmental conditions, in particular light intensity and nutrient concentrations (Furnas 1990, Gieskes et al. 1993). The introduction of HPLC (high performance liquid chromatography) for the determination of chlorophyll and carotenoid pigments (Mantoura & Llewellyn 1983) allows the contribution of various phytoplankton groups to be separated from the total autotrophic biomass (Gieskes 1991, Claustre 1994, Claustre et al. 1994b) Gieskes & Kraay (1989) proposed extending the original ^{14}C chl *a* labelling method to all pigments in order to infer the specific gross growth rates of individual taxonomic groups in mixed populations. This approach requires appropriate laboratory calibrations prior to extensive applications at sea. In particular, the ^{14}C labelling of a diagnostic chlorophyll or carotenoid has to be compared to ^{14}C labelling of the whole cell in monospecific cultures under various light conditions. Goericke & Welschmeyer (1992a, b) showed that, for time scales shorter than 24 h and for various eukaryotes and for cyanobacteria, although labelling of pigments and of the total carbon pool were not linearly related, the use of a non-linear model could explain most of the observed variance.

Oceanic prochlorophytes (*Prochlorococcus*) are small prokaryotes (0.6 to 0.8 µm in diameter) discovered less than 10 yr ago by flow cytometry (Chisholm et al. 1988). Their pigment composition is now precisely established and includes: zeaxanthin, chlorophyll *c*-like, divinyl-chlorophylls *a* and *b* (Dv-chl *a* and Dv-chl *b*) and α-carotene (Chisholm et al. 1991, Goericke & Repeta 1992). Since the discovery of *Prochlorococcus*, several studies have emphasized the importance of their contribution to the autotrophic biomass in the oligotrophic ocean (Li et al. 1992, Campbell et al. 1994, Claustre & Marty 1995). Their vertical distribution in the water column is governed by light, water stratification, and nutrient supply (Olson et al. 1990, Vaulot et al. 1990). In permanently stratified sub-tropical waters (e.g. off Hawaii; Campbell & Vaulot 1993), *Prochlorococcus* dominates both at the surface and at the depth of the deep chlorophyll maximum (DCM). In waters that are only stratified in summer and undergo deep mixing in winter such as the northern Sargasso Sea, *Prochlorococcus* is preferentially found near subsurface in winter, and around the DCM in summer, at light levels corresponding to 10 to 0.1% of those at the surface (Olson et al. 1990). The contribution of *Prochlorococcus* to primary production still remains poorly documented (see however Goericke & Welschmeyer 1993b, Li 1994, Vaulot et al. 1995) Labelling of *Prochlorococcus* pigments could help solve this problem. To validate this approach, a Mediterranean strain of *Prochlorococcus* was grown under 2 different light intensities corresponding roughly to 1 and 10% of 24 h average transmitted surface light intensities in oceanic waters where *Prochlorococcus* are found (Claustre & Marty 1995). Low to high and high to low light shift experiments were performed. Both Dv-chl *a* and zeaxanthin, which play a photosynthetic and a non-photosynthetic role in *Prochlorococcus*, respectively (Partensky et al. 1993, Moore et al. 1995), were labelled and their activity monitored using an on-line radioactivity monitor coupled to the HPLC. The different rates obtained from population growth, carbon uptake and pigment labelling were compared and found to differ significantly under some of the conditions tested.

MATERIAL AND METHODS

Culture conditions and experimental set-up. The *Prochlorococcus* clone used in this study was originally isolated from surface waters of the Mediterranean Sea in winter (clone CCMP 1378, obtained from the Bigelow Laboratory for Ocean Sciences, ME, USA). Cultures were grown at 21 ± 1°C, in a modified K/10 medium (Keller et al. 1987), consisting of 50 µM NH_4Cl, 10 µM Glycero-PO_4 and trace metals as in K/10-Cu with 10^{-8} M $NiCl_2$ and 10^{-8} M H_2SeO_3 added. Prior to the experiment, 4 cultures in 4 l polycarbonate flasks (Nalgene) were acclimated during 2 wk to continuous blue light, 2 at low intensity (L = 7 µmol quanta m^{-2} s^{-1}) and 2 at 8-times higher intensity (H = 56 µmol quanta m^{-2} s^{-1}). At time t = 0, 2 of the 4 cultures were switched, respectively, from high to low (HL) and from

low to high (LH) intensities. The other 2 cultures were maintained at their initial intensity as controls (HH and LL). At the time of the light shift, cultures were inoculated with a $NaH^{14}CO_3$ solution (final concentration 1 mCi l^{-1}, Amersham). Biomass parameters and kinetics of carbon and pigment labelling were monitored for 50 h with varying frequencies (8 samples during the first 24 h and 3 during the following 26 h).

***Prochlorococcus* and bacteria concentrations.** Samples were preserved with paraformaldehyde 0.5%, frozen in liquid nitrogen and stored at −80°C until further analysis (modification from Vaulot et al. 1989). Prior to analysis, samples were thawed at room temperature, diluted 1:100 to prevent coincidence while counting, and stained with 1 µg ml^{-1} of Hoechst 33342 for 30 min (Monger & Landry 1993). Cell concentrations were determined by running a fixed volume of sample on a Coulter EPICS 541 flow cytometer equipped with a 5 W argon laser (Coherent) set for UV emission (353–357 nm). The following parameters were measured: right angle scatter (a function of cell size), chlorophyll fluorescence (long-pass 670 nm filter) and Hoechst 33342-DNA fluorescence (480 nm band-pass filter). Bacteria were easily discriminated from *Prochlorococcus* by their lack of chlorophyll fluorescence (Monger & Landry 1993). Absolute concentrations were determined using custom-designed software CYTOPC (Vaulot 1989) running on an IBM-PC compatible computer.

Total carbon measurements. Particulate organic carbon (POC) was measured from triplicate 50 ml samples filtered onto precombusted (6 h at 500°C) 25 mm Whatman GF/F filters, and stored in liquid nitrogen. Filters were kept about 24 h in a drying room before carbon determination by combustion in a LECO 900 CHN Analyzer standardized with ethylene-diaminetetraacetic acid (EDTA). Dissolved inorganic carbon (DIC) was measured on a total organic carbon analyzer from 5 ml samples fixed with 10 µl of a mercuric chloride saturated solution.

Pigment pools. For pigment quantification, 15 ml samples were filtered onto 25 mm Whatman GF/F filters stored in liquid nitrogen. Each filter was sonicated in 5 ml of 90% acetone. The description of the HPLC system and of the quantification procedure has been presented earlier (Morel et al. 1993, Claustre et al. 1994a). Dv-chl *a* and chl *a*, and divinyl-pheophytin *a* (Dv-phe *a*) and pheophytin *a* (phe *a*) were assumed to have the same molar extinction coefficient at the red peak. Calibration of the detector was therefore performed using monovinyl-compounds and led to extinction coefficients at 440 nm in the HPLC solvent of 105.6 and 43.4 l g^{-1} cm^{-1} for Dv-chl *a* and Dv-phe *a*, respectively. The extinction coefficient for zeaxanthin under the same conditions was 253.2 l g^{-1} cm^{-1}.

Carbon and pigment activities. The POC activity was obtained from triplicate 10 ml samples filtered onto 25 mm Whatman GF/F. Filters were put in scintillation vials with 500 µl of a 1 N HCl solution and set for 2 h on a shaking plate before adding 10 ml of Aquasol 2. DIC activity was obtained from 50 µl samples put in scintillation vials with 50 µl ethanolamine, 500 µl distilled water and 10 ml Aquasol 2. POC and DIC activities were both measured with a Packard Tri Carb 4000 scintillation counter.

For the determination of pigment specific activities (raw data were not presented here), 50 to 200 ml samples were filtered onto 47 mm Whatman GF/F filters and stored in liquid nitrogen. Pigments were extracted in 4 ml of 100% acetone. The presence of coeluting lipids, extracted by acetone and not detected by spectrophotometry in the visible range, may lead to an overestimate of growth rates by increasing the radioactive signal (Gieskes & Kraay 1989, Goericke 1992, Riemann et al. 1993). The determination of specific radioactivities requires, therefore, purification of the pigment extract (Gieskes et al. 1993), which involves successive liquid-liquid partitions with a solvent mix of hexane, acetone, methanol and distilled water (detailed protocol in Fig. 1). Two aliquots were isolated and injected: a purified non-acidified extract and a purified acidified extract. Measurements of both ^{14}C activities and concentrations of the pigments were made concurrently with 2 on-line monitors: a Milton Roy LDC spectrophotometer (440 nm) for pigment quantification and an EGG Berthold radiomonitor with a GT-200 solid scintillator cell for the measurement of pigment activities. Gradient flow in the radiomonitor-HPLC system consisted of 3 mixing solvents: to the 2 mixed solvents normally used in HPLC (Morel et al. 1993, Claustre et al. 1994a), a third mixed solvent (80% acetone + 20% methanol) was added to ensure, at the end of the run, the rinsing of the HPLC column and GT-200 cell from unpolar labelled contaminants. Calibration of the radiomonitor was done by the direct injection of a sample originating from the cultures, whose radioactivity was first measured with a Packard Tri Carb 4000 scintillation counter The combination of the 2 monitors allows: (1) an easier and more rapid setting-up of the purification protocol (Fig. 1) since contamination can be evaluated in quasi real-time; (2) a more rapid analysis without manipulation, which minimizes the risk of loss or degradation.

A possible way to prove that pigments are radiochemically pure is to calculate, after cells are grown for more than 8 generations, the relative specific activities, which should be no larger than 1 (Goericke 1992). However, in this study, the purity of pigments was tested by comparing the different chromatograms obtained (Fig. 2). The radio-chromatogram resulting from

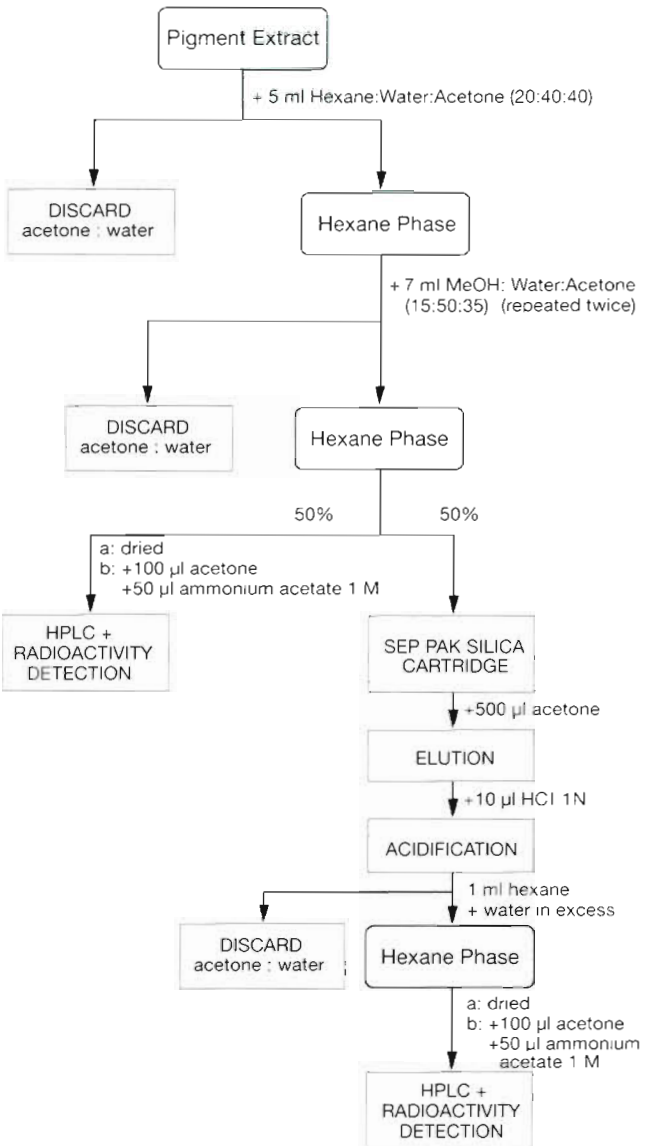

Fig. 1. Purification protocol of labelled pigments from labelled lipid contaminants

a non-purified extract (Fig. 2A) indicates contamination by labelled lipids. In the case of purified non-acidified extracts, the peaks in the radio-chromatogram obtained (Fig. 2B) visually attest to the purity of zeaxanthin and α-carotene. This was not the case for Dv-chl *b*, and purity of Dv-chl *a* is uncertain. The radio-chromatogram after acidification of the extract (Fig. 2C) suggested that Dv-phe *a* and divinyl-pheophytin *b* (Dv-phe *b*) were pure. Specific activities of divinyl-pheophytins are expected to equal those of their parent divinyl-chlorophylls, because the 2 molecules have the same carbon atom number Before acidification, the purified extract was passed through an normal phase silica cartridge (Sep Pak) in order to eliminate α-carotene which coelutes with Dv-phe *a* (compare Fig 2B, C). The identity of Dv-chl *a* and Dv-phe *a* specific activities for all samples (data not shown) suggests that Dv-chl *a* was probably radiochemically pure (Fig. 2B). Admittedly, we do not have such a control to demonstrate the purity of zeaxanthin. However, growth rates calculated here from these specific activities are generally lower than those calculated from variations in cell numbers, suggesting that no excess radiolabelling was measured. *Prochlorococcus* contains also Dv-chl *b* and α-carotene but labelling of these pigments was below the level of detection at every light condition for Dv-chl *b* and for shifted light conditions for α-carotene.

Pigment synthesis rates. Pigment synthesis rates were calculated in 2 different ways, from the variations in pigment concentrations (Eq. 1 in Table 1) and from ^{14}C incorporation into pigments. In the latter case, a 2-pool model, the F-P model, was used following Goericke & Welschmeyer (1992a, b, 1993a) to describe the flow of ^{14}C from the DIC to the pigment and to its nearest precursor The F-P model is described quantitatively by Eq. (2) in Table 1. This model takes into consideration the turnover rates of the pigment precursors. When the precursor turnover rates are infinitely high, and the pigment turnover is zero, then the F-P model reduces to a 1-pool model (Eq. 3 in Table 1), the P model, described by Welschmeyer & Lorenzen (1984) (e.g. Goericke & Welschmeyer 1992a, b, 1993a). The concepts, equations and supporting experimentations are fully described by Goericke & Welschmeyer (1992a, b, 1993a).

RESULTS

Biomass parameters

At the beginning of the light shift experiment, cell density at time *t*, $N_{(t)}$, was roughly the same for each pre-conditioned light intensity [$N_{(0)}$ = 10 × 10^6 cells ml^{-1} for LL and 13 × 10^6 cells ml^{-1} for HH; Fig 3]. After 48 h, cultures at high light intensity reached cellular densities about 3 times higher than cultures at low light intensity. Zeaxanthin concentrations were also 3 times higher at high than at low light. Dv-chl *a* concentrations increased in all cases, especially in the HH case (4-fold; Fig. 3). Dv-chl *a* concentration per cell was relatively constant for non-shifted cultures with average values of 1.5 and 4.1 fg Dv-chl *a* $cell^{-1}$ for LL and HH, respectively (Fig. 4A). In contrast, cell Dv-chl *a* content changed inversely to the shift in light condition. Changes were more rapid in the case of shift-up. The zeaxanthin cell content varied slightly from 0.92 fg zeaxanthin $cell^{-1}$ in HH and in HL exper-

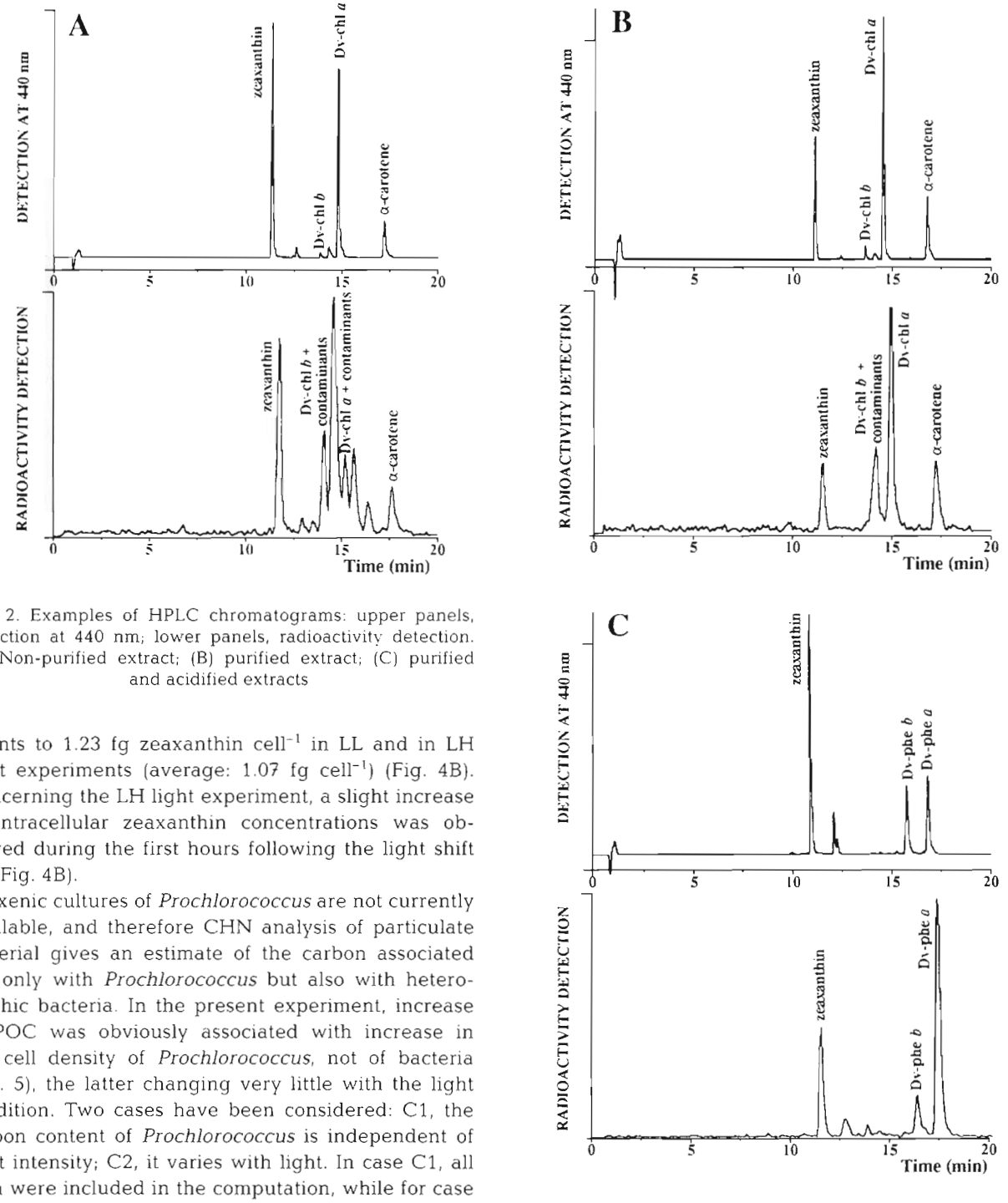

Fig 2. Examples of HPLC chromatograms: upper panels, detection at 440 nm; lower panels, radioactivity detection. (A) Non-purified extract; (B) purified extract; (C) purified and acidified extracts

iments to 1.23 fg zeaxanthin cell^{-1} in LL and in LH light experiments (average: 1.07 fg cell^{-1}) (Fig. 4B). Concerning the LH light experiment, a slight increase in intracellular zeaxanthin concentrations was observed during the first hours following the light shift up (Fig. 4B).

Axenic cultures of *Prochlorococcus* are not currently available, and therefore CHN analysis of particulate material gives an estimate of the carbon associated not only with *Prochlorococcus* but also with heterotrophic bacteria. In the present experiment, increase in POC was obviously associated with increase in the cell density of *Prochlorococcus*, not of bacteria (Fig. 5), the latter changing very little with the light condition. Two cases have been considered: C1, the carbon content of *Prochlorococcus* is independent of light intensity; C2, it varies with light. In case C1, all data were included in the computation, while for case C2, only data from non-shifted cultures or from shifted cultures after 24 h were included. The carbon content of *Prochlorococcus* and bacteria was then obtained by multiple regression for each case (Table 2). The case C2 did not lead to statistically different carbon contents at low and high light, and therefore it is considered in the following that case C1 holds true and that a single value of 49 fg C cell^{-1} can be used for all light levels.

This important bacterial contamination prevented us from calculating the carbon biomass of *Prochlorococcus* sp. Indeed, bacteria may have incorporated labelled carbon (Carpenter & Lively 1980, Laws 1984), and thus we cannot attribute the ^{14}C-fixation into total POC solely to activity of *Prochlorococcus marinus*, which is a necessary condition to calculate its carbon biomass (see Eqs. 9 & 10 in Welschmeyer & Lorenzen 1984).

Table 1. Definitions of the different variables used this study. *i*: zeaxanthin or Dv-chl *a*

Symbol	Unit	Definition
$POC(t)$	µg C l^{-1}	Total particulate organic carbon present at time t
$N(t)$	µg C l^{-1}	*Prochlorococcus* cell density at time t
$\mu_{cell}(t)$	d^{-1}	Average *Prochlorococcus* growth rate: $N(t) = N(0)$ exp$[\mu_{cell}(t)$ $t]$
$\mu_i(t)$	d^{-1}	Average i synthesis rate calculated from variations in i concentrations: $C_i(t) = C_i(0)$ exp$[\mu_i(t)$ $t]$, where $C_i(t)$ (µg l^{-1}) is concentration of pigment i at t (1)
$^{\cdot}\mu_{carbon}(t)$	d^{-1}	Average ^{14}C incorporation rate into POC
$^{\cdot}\mu_i(t)$	d^{-1}	Average i synthesis rate calculated from ^{14}C labelling of pigment i (see models below)
k_p		Pigment synthesis rate-normalized turnover rate of the pigment
k_f		Pigment synthesis rate-normalized turnover rate of pigment precursor
$L_i(t)$	Dimensionless	Relative specific activity of i at time t
P model:		$L_i(t) = 1 - \exp[-{}^{\cdot}\mu_i(t) \cdot t]$ (2)
F-P model:		$L_i(t) = 1 - \frac{1+k_f}{k_f - k_p} \cdot \exp[-(1+k_p) \cdot {}^{\cdot}\mu_i(t) \cdot t] - \frac{1+k_p}{k_p - k_f} \cdot \exp[-(1+k_f) \cdot {}^{\cdot}\mu_i(t) \cdot t]$ (3)

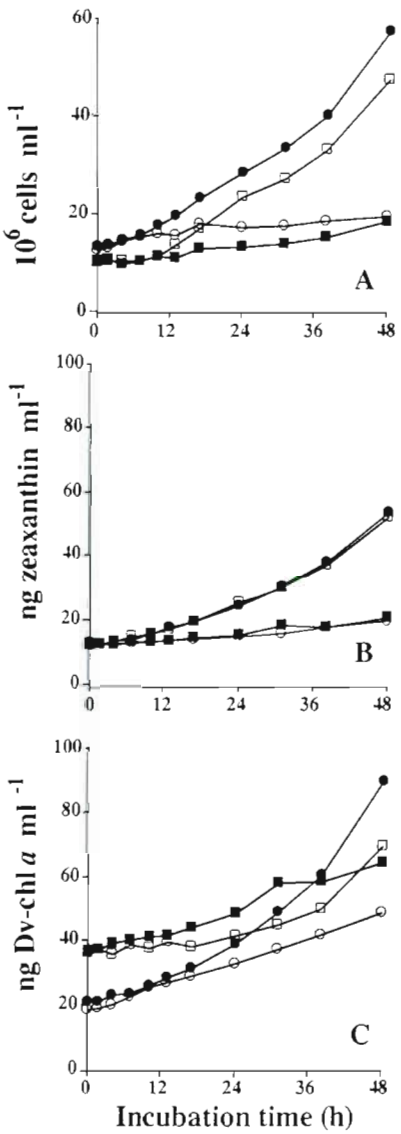

Fig. 3. (A) Cell density, (B) zeaxanthin concentration and (C) Dv-chl *a* concentration during growth of *Prochlorococcus* CCMP 1378 under various light conditions. (●) Constant high light (HH: 56 µmol quanta m^{-2} s^{-1}); (■) constant low light (LL: 7 µmol quanta m^{-2} s^{-1}); (○) high to low light shift (HL: 56 to 7 µmol quanta m^{-2} s^{-1}); (□) low to high light shift (LH: 7 to 56 µmol quanta m^{-2} s^{-1})

Pigment synthesis rates

Pigment synthesis rates (μ) were calculated from variations in zeaxanthin [$\mu_{zea}(t)$] and in Dv-chl *a* [$\mu_{dva}(t)$] concentrations at time t (Fig. 6). In LL and HH experiments $\mu_{zea}(t)$ respectively averaged 0.26 and 0.64 d^{-1} and was similar to $\mu_{dva}(t)$ (0.28 and 0.60 d^{-1}, respectively). After 24 h, values of $\mu_{zea}(t)$ were equivalent to values found in constant light in shifted experiments. In contrast, after 24 h, values of $\mu_{dva}(t)$ varied from 0.13 to 0.31 d^{-1} in LH and from 0.57 to 0.47 d^{-1} in HL experiments.

Pigment synthesis rates were also calculated from ^{14}C incorporation ($^{\cdot}\mu$) into zeaxanthin [$^{\cdot}\mu_{zea}(t)$] and into Dv-chl *a* [$^{\cdot}\mu_{dva}(t)$]. No values are given for the beginning of the incubation since specific activities measured were below the level of detection. For each light condition, we calculated the turnover rates of zeaxanthin and Dv-chl *a* (k_p), and of their precursors (k_f), all normalized to their respective pigment synthesis rates, by non-linear fitting (Table 3). In all experiments, the turnover rates of zeaxanthin and Dv-chl *a* (k_p) were always very low (Table 3), probably not significantly different from zero (there were not enough data to gauge the standard error for the estimate of k_p). In the constant light and LH experiments, the turnover rates of both zeaxanthin and Dv-chl *a* precursors (k_f) were very large (Table 3). The F-P model thus reduced to the simpler P model, and growth rates were calculated from Eq. (1) in Table 1. In the HL experiments, k_f was low for both zeaxanthin and Dv-chl *a*, and therefore the F-P

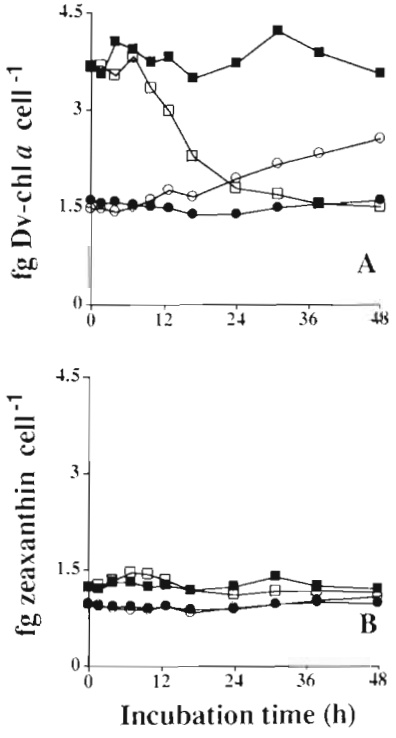

Fig. 4. (A) Dv-chl *a* and (B) zeaxanthin cellular content in *Prochlorococcus*. (●) Constant high light (HH: 56 µmol quanta m^{-2} s^{-1}); (■) constant low light (LL: 7 µmol quanta m^{-2} s^{-1}); (○) high to low light shift (HL: 56 to 7 µmol quanta m^{-2} s^{-1}); (□) low to high light shift (LH: 7 to 56 µmol quanta m^{-2} s^{-1})

model was relevant. In the HL case, growth rates were calculated by solving the following equations numerically for $\mu_i(t)$, from the relative ^{14}C-specific activities of zeaxanthin (Eq. 3) and of Dv-chl *a* (Eq. 4) (see Table 2):

$$L_i(t) = 1 - 1.11 \exp[-1.01 \cdot \mu_i(t) \cdot t] + 0.11 \exp[-10.62 \cdot \mu_i(t) \cdot t] \quad (3)$$

$$L_i(t) = 1 - 3.81 \exp[-1.01 \cdot \mu_i(t) \cdot t] + 2.81 \exp[-1.37 \cdot \mu_i(t) \cdot t] \quad (4)$$

Pigment synthesis rates calculated from variations in concentrations and from ^{14}C incorporation were compared for each light condition (Fig. 6). In LL, HH and LH experiments, $\mu_{zea}(t)$ and $\mu_{zea}(t)$ were equivalent after 24 h. In HL experiments, $\mu_{zea}(t)$ calculated with the F-P model equaled $\mu_{zea}(t)$, in contrast to the value from the P model that was 40% smaller. In constant light conditions, $\mu_{dva}(t)$ underestimated $\mu_{dva}(t)$ by at about 20%. In LH conditions, $\mu_{dva}(t)$ and $\mu_{dva}(t)$ were equivalent. In HL experiments, $\mu_{dva}(t)$ calculated from the P model underestimated $\mu_{dva}(t)$ by 80%, while $\mu_{dva}(t)$ calculated from F-P model equaled $\mu_{dva}(t)$. Results for 13, 24 and 48 h are summarized in Table 4

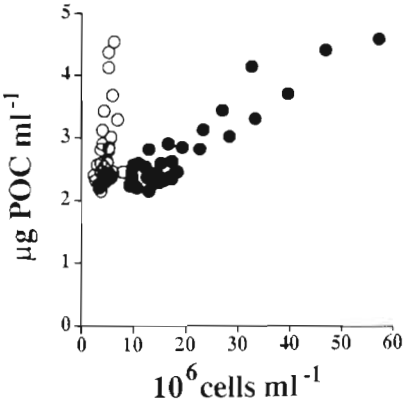

Fig. 5. Relationship between particulate organic carbon, (●) *Prochlorococcus* and (○) heterotrophic bacteria densities for the 4 cultures of *Prochlorococcus* CCMP 1378

Growth rates and pigment synthesis rates

Growth rates were calculated for each light condition from variations in the number of cells [$\mu_{cell}(t)$] (Fig. 6). After 24 h, μ_{cell} depended only on the current irradiance and not on the preconditioning irradiance. Under constant light, the ^{14}C cellular incorporation rates [$\mu_{carbon}(t)$] were 50 and 20% higher than $\mu_{cell}(t)$ for LL and HH, respectively (Fig. 6). In LH conditions, $\mu_{carbon}(t)$ was more than 100% higher than $\mu_{cell}(t)$. In HL conditions, conversely, $\mu_{carbon}(t)$ was 20 to 50% lower than $\mu_{cell}(t)$ and they became equivalent after 48 h.

The kinetics of $\mu_{dva}(t)$ and $\mu_{cell}(t)$ were similar under constant light conditions but they were radically different for light shifts (Fig. 6). After 24 h, $\mu_{dva}(t)$, as calculated from the F-P model, exceeded $\mu_{cell}(t)$ by 85% in HL conditions, and this excess decreased to 45% after 48 h. In LH conditions, $\mu_{dva}(t)$ was up to 5 times smaller than $\mu_{cell}(t)$. In contrast, after 24 h incubation and for all light conditions, the kinetics of $\mu_{zea}(t)$ and of $\mu_{cell}(t)$ were very comparable and differences never exceeded 20% (Fig. 6). However, for light shift conditions, and before 24 h, $\mu_{zea}(t)$ and $\mu_{cell}(t)$ were very different, especially in LH experiments. Pigment synthesis rates after 24 h, as calculated from pigment labelling rates, were comparable to *Prochlorococcus* growth rates as calculated from cell density (Fig. 7).

Table 2. Carbon content of *Prochlorococcus* (C_{Pro}) and of heterotrophic bacteria (C_{bact}) as estimated from POC and cell density measurements using multiple regressions. C1: carbon is independent of light ; C2: carbon content varies with light

	Light condition	C_{Pro} (fg C $cell^{-1}$)	C_{bact} (fg C $cell^{-1}$)	r^2	n
C1	All data	49 ± 9	334 ± 36	0.969	39
C2	LL and HL (t > 24 h)	65 ± 67	311 ± 213	0.959	14
	HH and LH (t > 24 h)	48 ± 10	321 ± 50	0.984	15

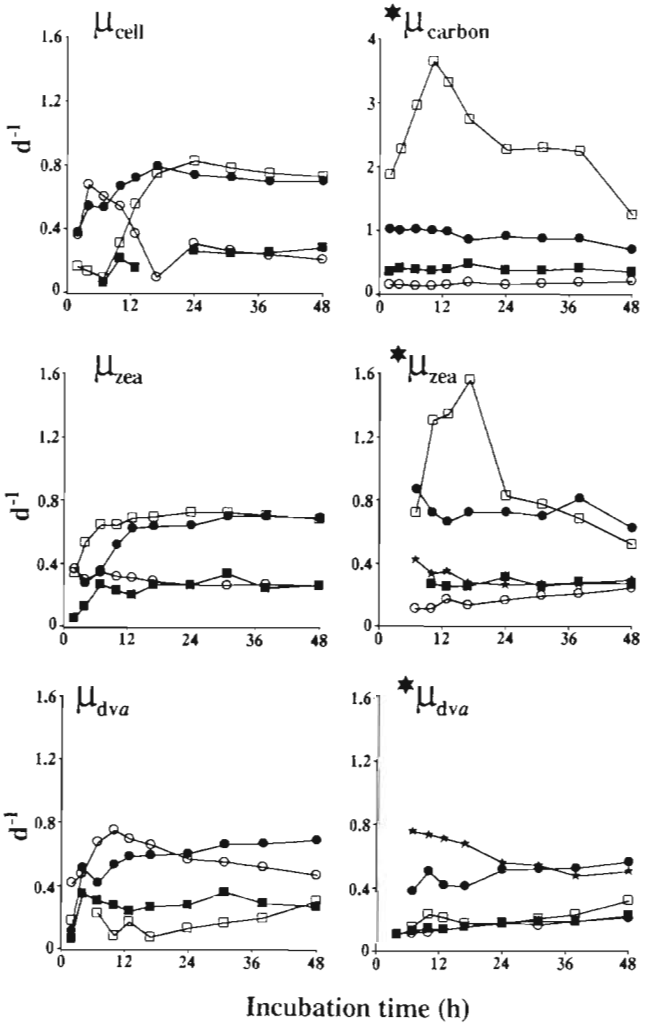

Fig. 6. *Prochlorococcus* growth rate as estimated from variations in cell density (μ_{cell}), from ^{14}C incorporation into POC (μ_{carbon}), from variations in zeaxanthin and in Dv-chl *a* concentrations (μ_{zea}, μ_{dva}) and from ^{14}C incorporation into zeaxanthin and Dv-chl *a* as P modeled (μ_{zea}, μ_{dva}) in LL, HH, and LH experiments, and P and F-P modeled (μ_{zea}, μ_{dva}) in HL experiments. (●) Constant high light (HH: 56 µmol quanta m^{-2} s^{-1}); (■) constant low light (LL: 7 µmol quanta m^{-2} s^{-1}); (○) if P modeled and (★) if F-P modeled: high to low light shift (HL: 56 to 7 µmol quanta m^{-2} s^{-1}); (□) low to high light shift (LH: 7 to 56 µmol quanta m^{-2} s^{-1})

Table 3. Turnover rates of zeaxanthin and Dv-chl *a* (k_p) and of their precursors (k_l) estimated from ^{14}C pigment labelling as F-P modeled (in Goericke & Welschmeyer 1993a). inf.: large turnover rate. Definitions in Table 1

	Zeaxanthin			Dv-chl *a*		
	k_l	k_p	n	k_l	k_p	n
LL	inf	−0.02	7	inf.	−0.38	9
HH	inf	+0.09	8	inf.	−0.20	8
LH	inf.	−0.03 (if t > 24 h)	8	inf.	+0.14 (if t > 24 h)	8
HL	9.62	+0.01	8	0.37	+0.01	8

DISCUSSION

Prochlorococcus pigment content and synthesis during photoacclimation

In the ocean, the pigment content of a phytoplankton population reflects not only its acclimation to growth at the prevailing light conditions at the depth of sampling, but also its past light history. Light level variations induce changes in intracellular pigment concentrations and turnover rates. Such changes are pigment-specific; e.g. photosynthetic pigment pools decrease and increase, respectively, when light intensity increases (Riper et al. 1979, Geider 1987, Kohata & Watanabe 1989, Goericke & Welschmeyer 1992a). Field observations suggest that *Prochlorococcus* has a large pigment plasticity and can photoacclimate to broad ranges of irradiance (Veldhuis & Kraay 1990, Gieskes 1991) Until now, laboratory studies on the metabolism of *Prochlorococcus* (Morel et al. 1993, Partensky et al. 1993) have been principally conducted under constant light conditions. In response to an increase in irradiance, *Prochlorococcus* Dv-chl *a* content decreased from 4.2 to 1.2 fg $cell^{-1}$ for a surface Mediterranean strain and from 2.8 to 1.1 fg $cell^{-1}$ for a deep-water Sargasso Sea strain (Partensky et al. 1993). In the present study, conducted on a subclone of the *Prochlorococcus* Mediterranean Sea strain, the range for cellular Dv-chl *a* content (4.0 to 1.5 fg $cell^{-1}$) agrees with this earlier study. Concerning zeaxanthin, the results are less clear. The earlier study observed that it increased from 0.65 to 0.94 fg $cell^{-1}$ in the Mediterranean Sea strain, but that it did not exhibit a clear tendency in the Sargasso Sea strain, varying between 0.53 and 0.83 fg $cell^{-1}$ (Partensky et al. 1993). In our experiments, zeaxanthin was relatively constant at 1.07 ± 0.14 fg $cell^{-1}$, i.e. falling slightly outside of these previous ranges. In natural populations from the sub-tropical North Atlantic, the *Prochlorococcus* zeaxanthin content was estimated to be constant but higher (1.97 fg $cell^{-1}$; Veldhuis & Kraay 1990). *In situ*, estimates of *Prochlorococcus* zeaxanthin content are uncertain because *Synechococcus* also contains zeaxanthin.

The dynamic aspects of photoacclimation were investigated from the analysis of variations of both cellular content and labelling of zeaxanthin and Dv-chl *a* after light shift (Fig. 6). During the shift-up experiment, rates computed from pigment change and from pigment labelling of Dv-chl *a* were both very low. This suggests that Dv-chl *a* synthesis was shut down as light increased. The concomitant increase in cell division induced a decrease in the

Table 4. Comparison of average growth rates (d^{-1}) of *Prochlorococcus* [$\mu_{cell}(t)$], and zeaxanthin and Dv-chl *a* synthesis rates [$\mu_{zea}(t)$ and $\mu_{dva}(t)$, respectively] (d^{-1}) under each light condition for different incubation times. $\mu_{carbon}(t)$: average ^{14}C incorporation rate into POC; $\mu_{zea}(t)$ and $\mu_{dva}(t)$: pigment synthesis rate calculated from ^{14}C incorporation into zeaxanthin and Dv-chl *a*, respectively

Light	Time (h)	$\mu_{cell}(t)$	$\mu_{carbon}(t)$	$\mu_{zea}(t)$	$\mu_{zea}(t)$		$\mu_{dva}(t)$	$\mu_{dva}(t)$	
					P model	F-P model		P model	F-P model
LL	13	0.15	0.38	0.19	0.25		0.24	0.13	
	24	0.26	0.37	0.26	0.31		0.28	0.18	
	48	0.29	0.35	0.27	0.27		0.27	0.22	
HH	13	0.72	0.97	0.62	0.66		0.58	0.41	
	24	0.74	0.90	0.64	0.71		0.60	0.51	
	48	0.70	0.70	0.69	0.62		0.70	0.48	
LH	13	0.56	3.30	0.69	1.34		0.17	–	
	24	0.83	2.25	0.72	0.82		0.13	0.21	
	48	0.73	1.24	0.69	0.51		0.31	0.28	
HL	13	0.37	0.14	0.31	0.17	0.34	0.69	0.13	0.71
	24	0.31	0.15	0.26	0.16	0.25	0.57	0.17	0.55
	48	0.21	0.19	0.26	0.25	0.29	0.47	0.17	0.50

Dv-chl *a* cell content through a dilution effect (Goericke & Welschmeyer 1992a). However, Dv-chl *a* was probably not actively degraded, since this would have led to a decrease in total Dv-chl *a* concentration which was not observed (Fig. 3). In contrast, zeaxanthin rates computed from changes in pigment concentration and from labelling largely differed during the first 17 h after shift-up. The zeaxanthin specific activity (data not shown) increased as expected during the first 17 h, but then decreased (by 15%) until 24 h, to increase again beyond 24 h. This is quite difficult to reconcile with known physiological mechanisms. We refer to the probable existence of 2 cellular zeaxanthin pools with different localisations and functions (A. Post & J.-C. Thomas pers. comm.). These 2 pools of zeaxanthin may have different synthesis rates in response to changes in light intensity. Immediately following shift-up, one pool (pool A) may be rapidly synthesized from external labelled carbon, leading to an increase in zeaxanthin specific activity, while the other pool (pool B) is synthesized from internal non-labelled carbon. Then synthesis of pool A may stop or largely decrease while synthesis of pool B goes on from unlabelled carbon resulting in a diminished specific activity. Finally, after this phase of photoacclimation, synthesis is normally from external labelled carbon. Further experiments are necessary before a definitive interpretation can be made.

While Dv-chl *a* obviously plays a photosynthetic role in *Prochlorococcus*, conclusions concerning the precise role of zeaxanthin for the cell are more difficult to make. Increasing evidence is found in the literature suggesting that zeaxanthin acts as a photoprotective pigment in higher plants through the xanthophyll cycle (Demmig et al. 1987), and in cyanobacteria (Pearl et al. 1983, Gieskes & Kraay 1986, Kana et al. 1988). In the latter case, while cellular content of zeaxanthin has been shown to remain constant over a wide range of white fluorescent growth irradiance (Kana et al. 1988), it has been shown to vary according to the light quality, being higher if cells are exposed to blue-green illumination (Bidigare et al. 1989). From our study, conducted under blue light conditions, we conclude a non-

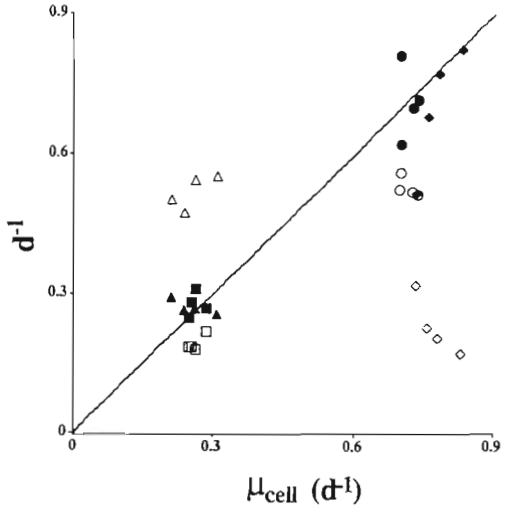

Fig. 7. Comparison of *Prochlorococcus* growth rate estimated after 24 h of incubation from variations in cell density (μ_{cell}) with zeaxanthin and Dv-chl *a* synthesis rates as calculated from ^{14}C zeaxanthin and Dv-chl *a* labelling in LL (7 µmol quanta m^{-2} s^{-1}), in HH (56 µmol quanta m^{-2} s^{-1}), in LH (7 to 56 µmol quanta m^{-2} s^{-1}) and in HL (56 to 7 µmol quanta m^{-2} s^{-1}) light experiments. In LL: (■) μ_{zeaP}; (□) μ_{dvaP}; in HH: (●) μ_{zeaP}; (○) μ_{dvaP}; in LH: (◆) μ_{zeaP}; (◇) μ_{dvaP}; and in HL: (▲) μ_{zeaFP}; (△) μ_{dvaFP}

photosynthetic rather than a photoprotective role of zeaxanthin for *Prochlorococcus*, as has been previously suggested (Moore et al. 1995). The non-photosynthetic role of zeaxanthin was here revealed more distinctly from labelling rates rather than from the change in cellular contents, since the latter did not change significantly with light conditions.

Evaluation of *Prochlorococcus* specific gross growth rate from ^{14}C pigment labelling

The *Prochlorococcus* carbon content of 49 fg C cell^{-1} computed in this paper from cell counts and POC measurements is very close to those estimated by previous authors on the basis of cell size and conversion factors from the literature (59 fg C cell^{-1}, Li et al. 1992; 53 fg C cell^{-1}, Campbell et al. 1994). In contrast, Veldhuis & Kraay (1990) found a somewhat higher value (124 fg C cell^{-1}) using different conversion factors. The apparent lack of influence of light intensity on *Prochlorococcus* cell carbon content that we observed is consistent with observations by Kana & Glibert (1987a) for *Synechococcus*.

The carbon that is assimilated through photosynthesis is subsequently redistributed within the cell to participate in the synthesis of different molecules (in particular pigments), leading ultimately to cell division. The ^{14}C-fixation in total POC was due to metabolical activity of *Prochlorococcus* sp. and of bacteria. Nevertheless, we assume that the large discrepancy observed during the first 24 h in the shift-up experiment (LH) between variations in cell density and POC labelling rates (Fig. 6) indicates that the incorporation of inorganic labelled carbon in *Prochlorococcus marinus* does not immediately result in cell division. It corresponds rather to an increase in the synthesis of carbon compounds, not only pigments, but also probably soluble sugars (Claustre & Gostan 1987). This temporal decoupling between cell growth and cell division explains why cell labelling cannot always be used adequately to assess phytoplankton population gross growth rate, particularly when phytoplankton photoacclimate and during short incubation experiments (Carpenter & Lively 1980, Smith & Platt 1984, Welschmeyer & Lorenzen 1985).

The use of the ^{14}C pigment labelling method to estimate phytoplankton growth rate requires that pigment synthesis and population growth are both in balance throughout the labelling period (Goericke & Welschmeyer 1992a, b, 1993a, Gieskes et al. 1993). The differences observed between $\mu_{zea}(t)$, $\mu_{dva}(t)$ and $\mu_{cell}(t)$ for the light control (HH and LL), at the beginning of each light experiment (Fig. 6), may suggest 'unbalanced' growth of *Prochlorococcus* sp. We believe that such discrepancies more likely resulted from the difficulties in estimating growth rates over very short time intervals. The imprecision in pigment quantification and in cell density determination affects the evaluation of the growth rates in a more significant manner (simply as a result of calculation) at the beginning than at the end of the incubation experiments.

CONCLUSIONS

In the present experiments, cell division rates were quite close to those estimated from zeaxanthin labelling data after 24 h, and this was true whatever the light conditions. On a shorter time scale, zeaxanthin labelling reflected the photoacclimation metabolism of the cell rather than cell division. In constant light, the Dv-chl *a* synthesis rate was quite equivalent to the *Prochlorococcus* growth rates. In contrast, the 2 rates were not in agreement following a light shift. While the Dv-chl *a* synthesis rate largely underestimated the *Prochlorococcus* growth rates during shift-up, the Dv-chl *a* synthesis rate exceeded the *Prochlorococcus* growth rates during shift-down. These discrepancies decreased as incubation time increased but, in contrast to zeaxanthin, 24 h were not sufficient to balance Dv-chl *a* synthesis with cell division.

In constant light and after shift-up, the 2-component F-P model (Goericke & Welschmeyer 1993a) was reduced to the simpler 1-component P model, since the turnover rate of pigment precursors was nil, while for shift-down the latter was not true and the F-P model was required. A basic hypothesis of the F-P model is that new pigment synthesis is made at the expense of external dissolved carbon. However, cells exposed to decreased light intensities may compensate by synthesizing Dv-chl *a* from internal carbon pools not yet labelled (Welschmeyer & Lorenzen 1984, Goericke & Welschmeyer 1992a, 1993a). When this is the case, Dv-chl *a* synthesis rates calculated from pigment labelling rates and from concentration variations necessarily differ.

In summary, zeaxanthin synthesis and *Prochlorococcus* growth rates were balanced in all cases after 24 h incubations. In contrast, Dv-chl *a* synthesis and growth were balanced but only under constant light conditions. Thus, we propose that zeaxanthin may be a better biomarker of *Prochlorococcus* growth rates than Dv-chl *a* for field applications.

Implications for field applications

The Mediterranean strain of *Prochlorococcus* used in this study is probably representative of populations encountered in various oceanic regimes on the basis of

genetic (Palenik 1994) and immunological (L. Campbell pers. comm.) evidence. Therefore, the present dataset is relevant to photosynthetic and photoacclimation processes of *Prochlorococcus* in the field. The range of rates in our study overlapped those measured in oceanic waters such as those obtained from 9 h zeaxanthin labelling under high irradiance in eastern Indonesian waters (0.7 to 0.8 d^{-1}; Gieskes & Kraay 1989) or from 24 h Dv-chl *a* labelling in the oligotrophic Sargasso Sea (0.1 d^{-1} at the bottom of the euphotic zone to 0.3 d^{-1} near the surface; Goericke & Welschmeyer 1993b).

The question of the incubation duration to be chosen at sea has recently been reconsidered (Mingelbier et al. 1994). 24 h dawn-to-dawn incubation was recommended by JGOFS (1988) for estimating the daily rate of primary production using the classical ^{14}C method, in order to allow comparisons among sampling areas and periods (Mingelbier et al. 1994). In field studies, when applying the ^{14}C pigment labelling method, incubation length varied according to authors. When short incubation times were applied (9 to 12 h), authors assumed that physiological equilibrium, ensuring that labelling rates are the same for every cell carbon compound, takes a few hours in the field, since cells are not exposed to light changes as radical as those in culture (Gieskes & Kraay 1989, Riemann et al. 1993). Nevertheless, variations of irradiance during the day may force phytoplankton to photoadapt (Goericke 1992, Goericke & Welschmeyer 1993a). From our experiment, it was clear that when phytoplankton photoadapt the time to obtain equivalent pigment synthesis and cellular growth rates differed according to the light shift. Thus, we recommend at-sea incubations last 24 h, since they will smooth out diel phenomena and include the whole photocycle (Goericke 1992, Goericke & Welschmeyer 1993a, Vaulot et al. 1995).

As pointed out above, zeaxanthin rather than Dv-chl *a* labelling may be more appropriate to assess *Prochlorococcus* growth rate, especially when one suspects that photoacclimation takes place in the water column (e.g. during a wind-induced mixing period; Bustillos-Gùzman et al. 1995). However, in highly stratified waters, estimation of *Prochlorococcus* growth rates from Dv-chl *a* labelling may be adequate. Dv-chl *a* labelling may in fact often be the only valid approach since zeaxanthin is present both in *Prochlorococcus* and *Synechococcus* (Kana & Glibert 1987a, b, Kana et al. 1988). As these 2 genera very often cooccur in oligo- and mesotrophic waters (Li et al. 1992, Campbell et al. 1994, Claustre & Marty 1995), zeaxanthin labelling would yield estimates of the average growth rate of photosynthetic prokaryotes (Veldhuis & Kraay 1990) while Dv-chl *a* labelling would yield growth rates of *Prochlorococcus* only. An alternative method to the ^{14}C pigment labelling is flow cytometry sorting of ^{14}C labelled cells (Li 1994). The association of the 2 methods for natural populations would allow comparison of the rates of carbon incorporation and those of pigment labelling. Discrepancies between these different rates could reveal whether photoacclimation phenomena occur in the field.

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