

Estimating the carbon flux through main phytoplankton groups in the northwestern Mediterranean

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Abstract

We performed dilution experiments and primary production measurements in surface waters during a cruise in the northwestern Mediterranean Sea in June 2000 to quantify the carbon (C) flux through phytoplankton groups that can be distinguished by their pigment markers. Pigments in the dilution experiments were measured by high-pressure liquid chromatography (HPLC). Pigment per cell and synthesis rates of photoprotectant versus light-harvesting pigments indicated that cells did not undergo photoacclimation during the 24-h dilution experiments. Mean chlorophyll *a* (Chl *a*) concentration was 0.128 mg m⁻³, and mean primary production was 4.99 mg of C m⁻³ d⁻¹. Prymnesiophyceae contributed 51% of the total Chl *a*, green algae 21%, diatoms 13%, cyanobacteria 6%, and Pelagophyceae and Dinophyceae 4% each. Size fractionation showed that 68% of total phytoplankton were <5 μm. A mean growth rate of 0.89 d⁻¹ was completely offset by a mean grazing rate of 1.01 d⁻¹. Growth and mortality were also balanced for the individual phytoplankton groups, with highest rates for diatoms and lowest for green algae. Despite the high growth rates measured, nutrient-amended incubations showed that these rates were nutrient limited, although to a different extent for each group. Green algae experienced the most severe limitation and cyanobacteria the least. The comparison of C incorporation rates and Chl *a* synthesis rates permitted the estimation of a C:Chl *a* ratio of 47 (g:g) for newly produced organic matter in the study area. This value, corrected for the different degree of nutrient limitation, was used to estimate the C flow through the different phytoplankton groups. Because of their high growth rates, diatoms played a disproportionate role in the C flux compared to their biomass contribution.

Phytoplankton is the gate for the incorporation of inorganic carbon (C) into organic matter in pelagic ecosystems. Standing stocks of phytoplankton components in systems that are not subject to strong physical disturbances remain stable at least in the scale of days or weeks (Zubkov et al. 2000). If we take into account that phytoplankton metabolism in surface waters is highly dynamic, with doubling times close to 1 d (Furnas 1990; Calbet and Landry 2004), it becomes evident that this apparent stability should be driven by a fine balance between growth and losses. Sedimentation, viral infection, and autolysis are loss processes that might become significant at certain periods (Agustí and

Duarte 2000; Becquevort and Smith 2001; Brussaard 2004). However, grazing is arguably the main mechanism that consumes the organic matter produced by phytoplankton (Banse 1994). Therefore, production and grazing condition most of the C flux through phytoplankton.

A rather puzzling characteristic of aquatic habitats is the coexistence of diverse phytoplankton groups with differing degrees of abundance and growth. Phytoplankton growth rates are admittedly different among species or taxonomic groups (Furnas 1990), even under the same environmental conditions (Burkill et al. 1987; Strom and Welschmeyer 1991; Latasa et al. 1997). Fast and slow grazing rates for fast and slow growing phytoplankton groups have been repeatedly observed (McManus and Ederington-Cantrell 1992; Latasa et al. 1997; Stelfox-Widdicombe et al. 2000), maintaining fairly constant the relative biomass contribution of the different groups. In spite of its importance for our understanding of the C fluxes in the ecosystem, little is known about the role of different taxonomic groups, especially of pico- and nano-eukaryotes. The reason for this lack of knowledge is due, in part, to the difficulties in measuring *in situ* C fixation for individual taxa. Pigment labeling could provide this information, but it has been seldom applied (Goericke 1998). Moreover, pigment labeling will only provide estimates of C incorporation into phytoplankton taxa, but it will not give information on the fate of that C.

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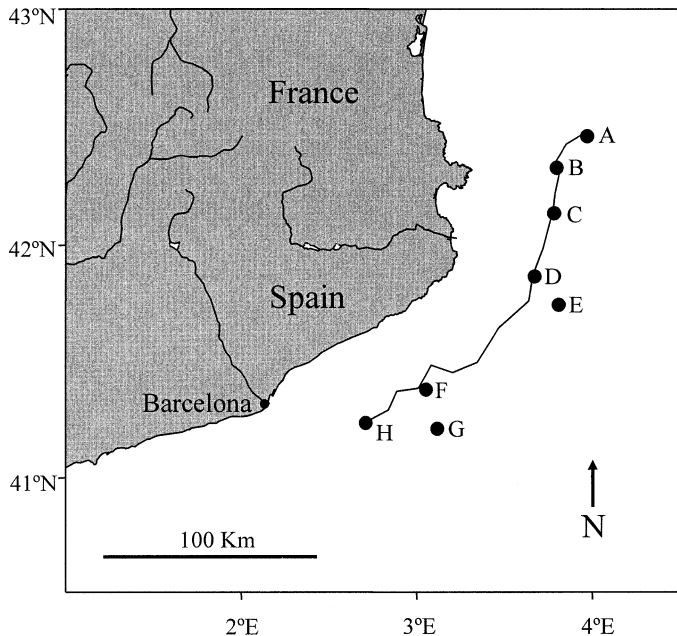


Fig. 1. Map of the area where the eight dilution experiments were performed. Primary production experiments were performed at stations A, D, E, G, and H. The line follows the path of three drifters.

In this study, we quantified the incorporation of C and its losses via grazing for different phytoplankton groups that can be distinguished by their pigment markers. We carried out dilution experiments and measured pigment concentration to estimate stocks, synthesis, and destruction rates of chlorophyll *a* (Chl *a*) allocated to each phytoplankton group. In parallel, we made estimates of primary production. Combining these four parameters, we were able to quantify the C incorporated and lost for each phytoplankton group. In addition, we addressed a series of methodological problems that can jeopardize these estimates.

Materials and methods

The experiments reported were carried out during Aro2000, a 10-d cruise that took place in the northwestern Mediterranean in June 2000 on board the RV *García del Cid*. Three drifters launched at the continental shelf edge of the Gulf of Lion were followed up in a southwestern direction along a path of >200 km of a relatively low salinity area influenced by the Rhône river outflow (Fig. 1). Nine transects, perpendicular to the drifters' direction, were performed with a total of 73 stations sampled. Salinity, temperature, and fluorescence of the water column were measured at all stations with a CTD (conductivity-temperature-depth instrument).

Growth and grazing were estimated in eight dilution experiments (A–H), two of them (E and G) on the oceanic side of the drifters' path (Fig. 1). The experiments were performed with water from 4- to 8-m depth following the method described in Latasa et al. (1997). The initial seawater was retrieved with a 30-liter Niskin bottle equipped with a 10-mm-diameter spigot, which allowed a gentler exit of water

than the standard 4-mm spigot. Whole seawater was diluted, in duplicate, to 75%, 50%, and 25% with gravity-filtered seawater (0.2- μm -pore-size Gelman Suporcap) and directly dispensed into 2-liter polycarbonate bottles. The dilution series plus two undiluted bottles were spiked with Guillard's f/2 nutrient medium to a final added concentration of 3 $\mu\text{mol L}^{-1}$ of NO_3 , 0.12 $\mu\text{mol L}^{-1}$ of PO_4 , and 0.36 $\mu\text{mol L}^{-1}$ of SiO_4 . NH_4 was additionally added in a concentration of 3 $\mu\text{mol L}^{-1}$. Three more bottles with undiluted water without nutrients added were included in each experiment. Samples were incubated on-deck in a Plexiglas incubator protected with a blue screen to mimic the intensity and quality of surface-water irradiance. Temperature was controlled with on-line running surface seawater. All material, including measurement cylinders, funnels, silicone tubing, and incubation bottles, was thoroughly cleaned with 5% HCl and rinsed with Milli-Q water. Initial samples were taken in triplicates. After 24 h, final samples were taken for high-pressure liquid chromatographic (HPLC) analysis of pigments and flow cytometry.

For flow cytometry, 2-ml samples were fixed with a mixture of paraformaldehyde and glutaraldehyde to a final concentration of 1% and 0.05%, respectively, and frozen in liquid nitrogen. Cyanobacteria consisted of *Synechococcus*. Their concentration and optical properties were estimated following the procedures recommended in Gasol and del Giorgio (2000). Fluorescence per cell was normalized to yellow-green fluorescence of latex beads (Polysciences, Inc.), used as the internal reference, and was expressed in relative units. Cell volume was estimated by the right-angle light scatter.

For pigment quantification, 2 liters of water was pressure filtered (0.3 atm) through glass fiber filters (Whatman GF/F), which were subsequently folded and wrapped in aluminum foil and frozen in liquid nitrogen. For size fractionation studies, sampled seawater was serially passed through inline filter holders equipped with an initial polycarbonate filter (Poretics, 5- μm pore size) and a final glass fiber filter (GF/F Whatman, 0.7- μm nominal pore size). These samples were obtained at the same stations of dilution experiments but from different casts. Pigments were extracted by soaking the filter in 3 ml of 90% acetone overnight at 4°C. The tube with the filter in acetone was then shaken in an ice-cooled Vibrogen IV cell mill (Edmund-Bühler) with 1-mm-diameter glass beads for 10 min. The extract was centrifuged at 4°C, and the supernatant was transferred to an Eppendorf vial. One milliliter of clear extract was mixed with 0.2 ml of H_2O , and 150 μl was injected into the chromatographic system. Pigment HPLC analysis was performed following the protocol described in Latasa et al. (2001).

The composition and dynamics of major groups were studied using pigment markers as proxies. Their contribution to bulk Chl *a* was quantified using Chemtax, a computer program to estimate the biomass of phytoplankton groups from pigments (Mackey et al. 1996). Chl c_2 , peridinin, 19'-butanoyloxyfucoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, diadinoxanthin, zeaxanthin, and Chl *b* constituted the matrix to estimate the contribution of green algae, Dinophyceae, Prymnesiophyceae, Pelagophyceae, cyanobacteria, and diatoms to total Chl *a* stock (Table 1). Violaxanthin,

Table 1. Chemtax input and output pigment to Chl *a* ratios.

		Chl <i>c</i> ₂	Per	But	Fuco	Hex	Ddx	Zeax	Chl <i>b</i>
Input	Green algae	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.346
	Dinophyceae	0.357	0.679	0.000	0.000	0.000	0.236	0.000	0.000
	Prymnesiophyceae	0.133	0.000	0.000	0.027	0.514	0.252	0.000	0.000
	Pelagophyceae	0.390	0.000	1.452	0.599	0.000	0.160	0.000	0.000
	Cyanobacteria	0.000	0.000	0.000	0.000	0.000	0.000	0.845	0.000
	Diatoms	0.206	0.000	0.000	0.440	0.000	0.207	0.000	0.000
Output	Green algae	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.248
	Dinophyceae	0.357	0.679	0.000	0.000	0.000	0.250	0.000	0.000
	Prymnesiophyceae	0.154	0.000	0.000	0.026	0.476	0.040	0.000	0.000
	Pelagophyceae	0.390	0.000	1.452	0.572	0.000	0.160	0.000	0.000
	Cyanobacteria	0.000	0.000	0.000	0.000	0.000	0.000	0.845	0.000
	Diatoms	0.233	0.000	0.000	0.554	0.000	0.373	0.000	0.000

prasinolanthin, and lutein, pigments that could aid in distinguishing some Prasinophyceae and Chlorophyceae within the green algae group, were always near or below the detection limit; therefore, Chl *b* was attributed to green algae and not to Chlorophyceae or Prasinophyceae in particular. Pigment ratios used as seed values (input matrix) were selected from literature values. The output pigment matrix (Table 1) was obtained after running Chemtax consecutively twice. Previous experience had shown us that Chemtax produces more accurate results when operated this way. Chemtax was applied to both initial and final samples of the incubations to obtain the pigment concentration allocated to each phytoplankton group at the beginning (P_0) and end (P_f) of each experiment.

To obtain reliable results, Chemtax should be applied to a data set where pigment ratios within the different groups do not change. Pigment ratios are mostly affected by irradiance, nutrients, and, to a minor extent, species composition (Falkowski and Raven 1997; Goericke and Montoya 1998). All the samples included in this study originated from 4–8-m depth, where irradiances were saturating, and the nutrient field did not experience substantial changes (Vidal pers. comm.). Because of the constancy in the physical and chemical environment, we argue that pigment variations within taxa were minimal and that it was not justified to split the database in different “pigment subgroups.” Therefore, Chemtax was applied to the entire pigment database.

The overall percent Chl *a* corresponding to the <5- μ m size fraction of each phytoplankton group (%Chl *a*_{<5 μ m group}) was estimated as follows:

$$\% \text{Chl } a_{<5\mu\text{m group}} = \frac{100 \times \sum_{i=1}^8 \text{Chl } a_{<5\mu\text{m group}}}{\sum_{i=1}^8 \text{Chl } a_{\text{total group}}}$$

where, for each phytoplankton group, $\sum \text{Chl } a_{<5\mu\text{m group}}$ and $\sum \text{Chl } a_{\text{total group}}$ are, respectively, the sum of the Chl *a* in the <5- μ m fraction and the total Chl *a* corresponding to the group, for the eight stations considered.

Apparent growth rates (k) were estimated from $k = \ln(P_f/P_0) \times t^{-1}$, where P_f and P_0 are final and initial concentrations of pigment assigned by Chemtax, and t is the du-

ration of the experiment (1 d). In the experiments with nutrient additions, the calculated intrinsic growth rate (μ_n) corresponds to the y-axis intercept of the linear regression of k on dilution. The grazing rate (g) is the slope of the regression. The intrinsic growth rate without nutrient amendments (μ) is the addition of the apparent growth rate of the undiluted samples (the net growth) plus the grazing rate (g). A basic assumption of these experiments is that grazing is not affected by nutrient addition.

A simple nutrient limiting index (N_L) was calculated as $N_L = \mu : \mu_n$ (Landry et al. 1995, 1998).

The dynamics of Chl *a* production and destruction, as a concentration of Chl *a* synthesized or grazed per unit time, were estimated for each group following the equations of Frost (1972):

$$\text{Chl } a_{\text{synthesized}} = \frac{\mu \times \text{Chl } a_0 \times [e^{(\mu-g)t} - 1]}{\mu - g} \quad (1)$$

$$\text{Chl } a_{\text{grazed(destroyed)}} = \frac{g \times \text{Chl } a_0 \times [e^{(\mu-g)t} - 1]}{\mu - g} \quad (2)$$

Chl a_0 is the initial concentration of Chl *a* (assigned by Chemtax), and μ and g are the Chl *a*-based intrinsic growth and grazing rates.

Primary production of water samples from the same location and depths as those used for five of the dilution experiments (A, D, E, G, and H) was estimated by photosynthesis-irradiance (P-E) experiments of ¹⁴C incorporation, as described in more detail in Morán and Estrada (2001). Surface water was retrieved with a Niskin bottle attached to a rosette. Samples (70 ml) were introduced in tissue culture bottles (12 light plus 1 dark), inoculated with 3.7×10^5 Bq (10 μ Ci) of ¹⁴C-bicarbonate, placed in a closed linear incubator under an irradiance gradient of 1 to ~2,000 μ mol photons $\text{m}^{-2} \text{s}^{-1}$, and kept at in situ temperature with circulating seawater. After 2 h of incubation starting around midday (± 1 h), the content was filtered through Whatman GF/F filters. Filters were placed in vials and exposed for 12 h to HCl fumes to eliminate inorganic ¹⁴C. Subsequently, the filters were left to dry before the addition of 4.5 ml of Ready Safe liquid scintillation cocktail. The radioactivity in the filters was counted with a Beckman LS6000 liquid scintillation counter. Dark bottle values were subtracted from light ones.

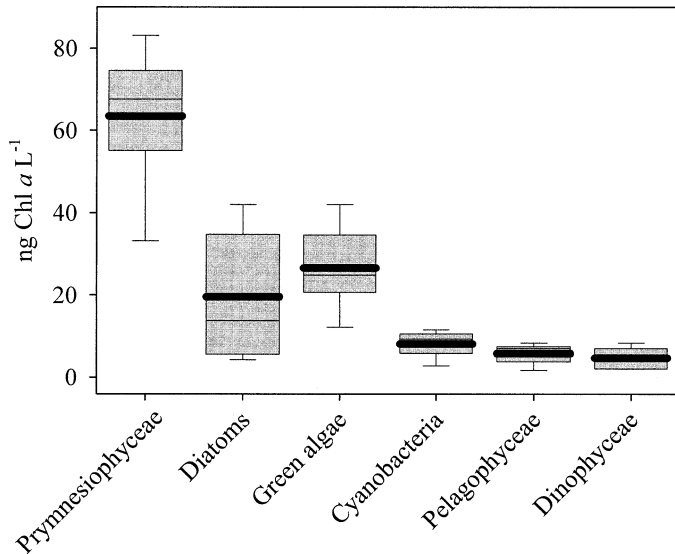


Fig. 2. Chl *a* concentration from initial samples distributed in the different phytoplankton groups as allocated by Chemtax. Thick and thin horizontal lines within the box (comprising 25 and 75 percentiles) represent mean and median, respectively. Error bars indicate 5 and 95 percentiles.

Since no photoinhibition was observed, photosynthetic parameters were obtained using the model of Webb et al. (1974).

Photosynthetic parameters (α^B and P_m^B), hourly averages of in situ photosynthetic active radiation (PAR), and initial Chl *a* measurements were used to estimate primary production rates during the light period. To compare C incorporation derived from ^{14}C primary production with those from 24-h dilution experiments, daily primary production should consider phytoplankton respiration during the dark period. Dark losses of photosynthate due to phytoplankton respiration are proposed to be $\sim 20\%$ of light production for equal (12 h) day and night lengths (Marra and Barber 2004). Since day length during the sampling period was 15 h, we subtracted 12% from the primary production value during the light period to obtain daily net primary production.

Results

Composition of phytoplankton—The hydrographic conditions of the area corresponded to those of a summer stratification period, with a well-developed deep chlorophyll maximum located at 50–60-m depth. The data presented in this study correspond to 4–8-m surface waters. The concentration of major pigments was similar in the initial samples of the eight dilution experiments (Fig. 2), with only relatively higher concentrations of fucoxanthin in experiments A, B, and C (data not shown). This lack of variability was due, probably, to the stable environmental conditions experienced by the original samples. The average Chl *a* concentration in these samples, taken from 4–8-m depth, was 0.128 mg m^{-3} ($\pm 0.047 \text{ SD}$, $n = 8$), a value that is typical for the area and time of the year (Estrada et al. 1993).

The amount of Chl *a* assigned to each group (Fig. 2) in-

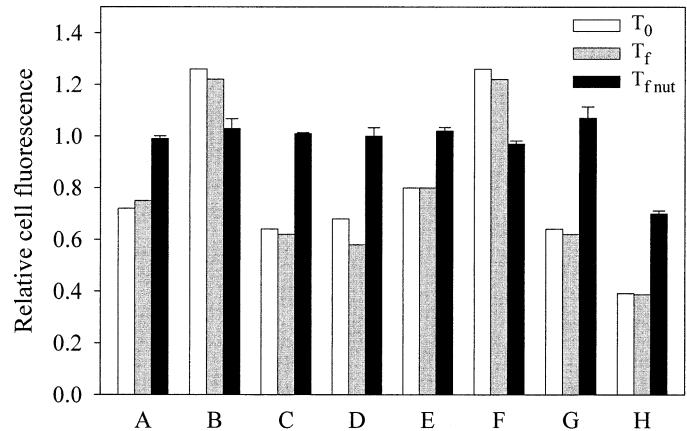


Fig. 3. Red fluorescence (Chl *a*) of *Synechococcus* at times initial (T_0) and final (T_f) with and without nutrient addition.

dicated that the phytoplankton were dominated by Prymnesiophyceae (49%), green algae (21%), and diatoms (15%). Cyanobacteria (6.3%), Pelagophyceae (4.5%), and Dinophyceae (3.7%) were also present. Diatoms showed the largest variability. Optical microscopy observation of surface samples from the stations corresponding to the experiments (Arin unpubl. data) indicated that the most common diatom was *Thalassionema nitzschioides*, with the occasional presence of small *Chaetoceros* spp., *Pseudo-nitzschia* spp., and *Rhizosolenia* spp. Total diatom concentrations ranged from 100 to 850 cells L^{-1} , with the exception of station A, which presented a population of senescent *Chaetoceros* spp. Dinophyceae and coccolithophorid (Prymnesiophyceae) concentrations ranged, respectively, from 1,500 to 5,000 cells L^{-1} and from 10,000 to 19,000 cells L^{-1} .

Size fractionation showed that 68% of total phytoplankton were $< 5 \mu\text{m}$. Most Dinophyceae and diatoms were in the large fraction, with 22% and 41% of their assigned Chl *a* $< 5 \mu\text{m}$. The next group in size was Prymnesiophyceae, with 77% of their Chl *a* $< 5 \mu\text{m}$. Green algae, Pelagophyceae, and cyanobacteria had 86%, 89%, and 98% of their biomass $< 5 \mu\text{m}$.

Photoacclimation in the dilution experiments—Photoacclimation is a serious concern in incubation experiments, especially when measuring pigments, since it is mainly reflected in changes in the amount of pigment per cell. In our experiments, the chlorophyll fluorescence per *Synechococcus* cell, measured by flow cytometry, did not show statistical differences ($p = 0.68$, paired *t*-test) between the initial and final populations. The large increase of this parameter after nutrient addition in most experiments proves its sensitivity and confirms that *Synechococcus* cells did not undergo photoacclimation during our incubations (Fig. 3).

For most phytoplankton groups, which cannot be distinguished by flow cytometry, pigment per cell cannot always be measured in field samples. An alternative is to observe changes in ratios between light-harvesting and light-protecting pigments. When the irradiance provided during the experimental period is higher than that experienced previously by the algae, a faster synthesis of photoprotectant pigment

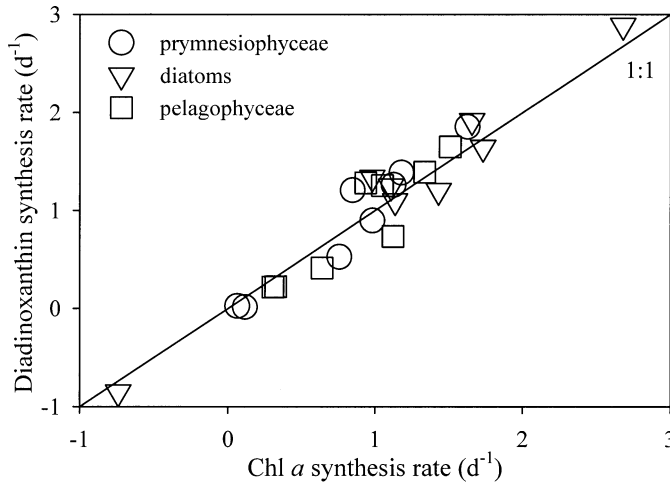


Fig. 4. Chl *a* and diadinoxanthin synthesis rates for Pymnesiophyceae, Pelagophyceae, and diatoms. The 1:1 line is drawn.

should be expected, and vice versa. In this study, we have compared the diadinoxanthin (light protecting) and Chl *a* (light harvesting) synthesis rates during the experiments for three phytoplankton groups: Pymnesiophyceae, Pelagophyceae, and diatoms. The synthesis rates of diadinoxanthin for each group were estimated from the assignment of the diadinoxanthin pool with Chemtax in an identical manner as for Chl *a* (see *Materials and methods*). For all three groups, the 95% confidence intervals of the regression between light-protectant and light-harvesting pigments included the values of 1 and 0 for the slope and the intercept, respectively (Fig. 4), indicating a balanced synthesis of both kinds of pigments and, therefore, a balanced growth. The most deviating points, corresponding to diatoms, belonged to experiments E and G with fucoxanthin concentrations around 2–3 ng L⁻¹, very low values that hindered a correct estimation of diatom biomass by Chemtax (Mackey et al. 1996).

Phytoplankton growth and mortality rates in the dilution experiments—Growth (μ) and grazing (g) rates of phytoplankton remained within a relatively narrow range in spite of some deviating points such as the growth rate for experiment G (Table 2). Growth and grazing were basically balanced, showing a clear coupling between total phytoplankton growth and mortality ($r = 0.80$, $p = 0.18$, $n = 8$). Average

Table 2. Instantaneous growth rates with and without nutrient addition (μ_n and μ , d⁻¹), grazing (g), and net growth ($\mu_{net} = \mu - g$) rates (d⁻¹) for phytoplankton (measured as Chl *a* in all the experiments).

Experiment	μ_n	μ	g	μ_{net}
A	1.672	1.066	1.308	-0.242
B	0.888	0.534	0.423	0.112
C	1.501	0.990	1.076	-0.086
D	1.151	0.699	1.117	-0.417
E	2.323	1.506	1.309	0.197
F	1.671	0.867	1.074	-0.207
G	1.014	0.159	0.636	-0.477
H	1.976	1.328	1.130	0.198
Mean	1.524	0.893	1.009	-0.115

growth rate was 0.89 d⁻¹ (± 0.43 SD, $n = 8$), and grazing rate was 1.01 d⁻¹ (± 0.34 SD, $n = 8$). As a result, the net growth rate of phytoplankton was slightly negative (-0.11 d⁻¹). Focusing on the dynamics of the Chl *a* assigned with Chemtax to the different phytoplankton groups, diatoms exhibited the highest growth rates, followed by cyanobacteria, Pelagophyceae, Pymnesiophyceae, and Dinophyceae; green algae showed the slowest growth rates (Table 3). Group-specific mortality due to grazing mirrored the differences in growth throughout the different experiments and, in fact, growth and grazing rates were never statistically different ($p > 0.05$, t -test) for any group (Fig. 5). The net growth rate of the different groups was slightly negative or very close to zero, with slight positive values only for cyanobacteria and Dinophyceae.

The daily integrated Chl *a* synthesized and grazed for the different phytoplankton groups showed that diatoms and cyanobacteria turned over their standing stock biomass in ca. 1 d (Table 3). In contrast, green algae presented the slowest turnover. Since production is a function of growth rates and biomass, Pymnesiophyceae were synthesizing half of the total Chl *a* biomass, diatoms about 25%, green algae and cyanobacteria almost 10% each, and Pelagophyceae and Dinophyceae the rest. All this newly synthesized biomass underwent a strong grazing, with green algae and Pymnesiophyceae presenting negative net integrated biomass changes (Table 3), although these were not statistically different from zero ($p = 0.20$ and 0.07, respectively, one-sample t -test, two-tailed p value).

Table 3. Standing stock (ng L⁻¹), instantaneous synthesis and destruction rates (d⁻¹), and daily integrated synthesis and destruction (ng L⁻¹) of Chl *a* (as derived from Eqs. 1 and 2) for each phytoplankton group (means from $n = 8$ experiments \pm SD).

	Biomass (ng Chl <i>a</i> L ⁻¹)	Instantaneous Chl <i>a</i> synthesis rates (d ⁻¹)	Instantaneous Chl <i>a</i> grazing rates (d ⁻¹)	Daily integrated Chl <i>a</i> synthesis (ng Chl <i>a</i> L ⁻¹)	Daily integrated Chl <i>a</i> grazed (ng Chl <i>a</i> L ⁻¹)
Green algae	26.5 \pm 10.9	0.41 \pm 0.11	0.57 \pm 0.34	9.83 \pm 4.97	14.5 \pm 9.82
Dinophyceae	4.74 \pm 2.70	0.70 \pm 0.23	0.69 \pm 0.23	3.33 \pm 2.24	3.07 \pm 1.37
Pymnesiophyceae	63.4 \pm 18.4	0.84 \pm 0.55	1.01 \pm 0.53	46.8 \pm 30.1	56.6 \pm 32.7
Pelagophyceae	5.80 \pm 2.64	0.87 \pm 0.47	0.87 \pm 0.21	4.85 \pm 3.03	4.89 \pm 2.46
Cyanobacteria	8.14 \pm 3.43	0.90 \pm 0.50	0.78 \pm 0.38	9.40 \pm 7.68	7.49 \pm 5.16
Diatoms	19.6 \pm 15.9	1.32 \pm 0.44	1.34 \pm 0.56	25.6 \pm 21.6	26.0 \pm 22.4
Total phytoplankton	128 \pm 46.9	0.89 \pm 0.43	1.01 \pm 0.34	97.8 \pm 48.2	111 \pm 50.1

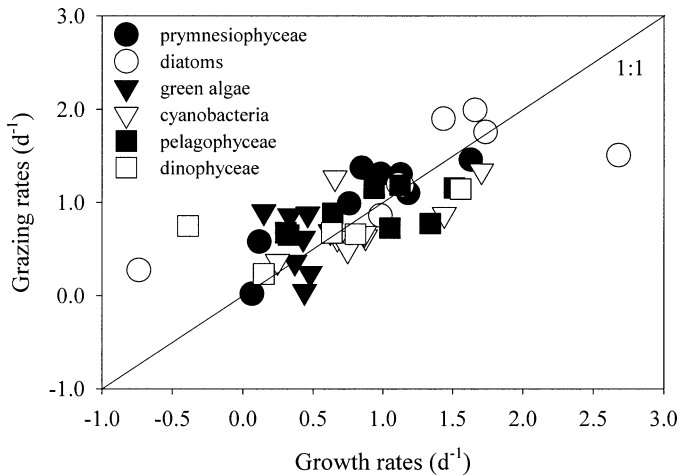


Fig. 5. Growth and grazing rates for all eight experiments and six phytoplankton groups. The 1:1 line is drawn.

We have compared the growth rates estimated using both the Chl *a* assigned by Chemtax and the main pigment marker associated with the different algal groups (Fig. 6). Appreciable differences were found only for diatoms in experiments E and G, with initial fucoxanthin concentrations of 2.1 and 3.0 ng L⁻¹, which, as noted above, probably led to errors in the Chl *a* assigned by Chemtax to this group.

The effect of nutrient addition was very evident in the growth rates of all experiments. Theoretically, it could have resulted in a nutrient-stimulated growth rate (μ_n) of 1.52 ± 0.49 d⁻¹ (Table 2). However, flow cytometric observations (Fig. 3) showed that, for *Synechococcus*, an important part of this apparently increased growth rate was a consequence of a rapid synthesis of pigments that was not balanced with a corresponding increase in cell volume, used as a proxy of cell C (data not shown). A similar result has been observed in the iron (Fe)-limited waters of the Equatorial Pacific after Fe addition (Cavender-Bares et al. 1999). Here, all the groups responded with a disproportionate increase in Chl *a* per cell when nutrient replete. Therefore, it is very likely that the same reaction occurred for the rest of the groups at our study site. This observation also shows that this effect should be taken into account in the interpretation of pigment results that originated from experimental procedures implicating incubations, such as nutrient “bioassays” or dilution experiments. The nutrient-limiting index ($N_L = \mu : \mu_n$) showed that green algae and Prymnesiophyceae experienced the strongest nutrient limitation and that cyanobacteria experienced the lowest, with the rest of the groups showing N_L values close to that of bulk phytoplankton (Table 4).

Primary production, phytoplankton growth rates, and the C:Chl a ratio—Mean \pm SD Chl *a*-normalized maximum photosynthetic rate (P_m^B) in the *P-E* experiments was 3.48 ± 2.02 mg of C mg Chl *a*⁻¹ h⁻¹. Highest values were found in experiments E and H, with 5.95 and 5.42 mg C mg Chl *a*⁻¹ h⁻¹, respectively. The initial slope of the *P-E* curve or maximum Chl *a*-normalized light utilization coefficient (α^B) was less variable than P_m^B , with a mean value of 0.0044 ± 0.0008 mg of C mg Chl *a*⁻¹ h⁻¹ ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$)⁻¹, which

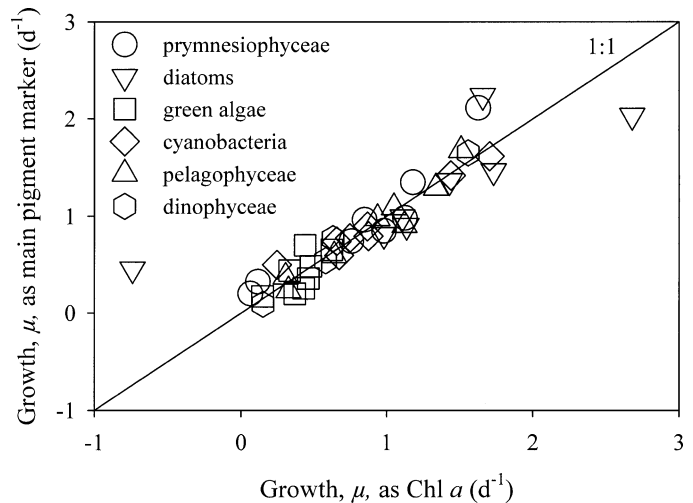


Fig. 6. Comparison of pigment-specific growth rates of the different groups estimated from Chemtax-derived Chl *a* and from major pigment markers. The 1:1 line is drawn.

can be considered low (Behrenfeld et al. 2004). Daily primary production derived from photosynthetic parameters showed a variation very much correlated with Chl *a* biomass ($r = 0.93$, $p = 0.021$, $n = 5$).

Combining ¹⁴C-derived daily primary production and Chl *a*-based growth rates, we were able to derive two estimates of the C:Chl *a* ratio of the newly synthesized organic matter for the five stations with coinciding *P-E* curves and dilution experiments. Both estimates compare the integrated primary production and the integrated synthesis of Chl *a* calculated from Eq. 1, but they differ in the period of time used to obtain the integrated values: estimate 1 considered 24 h, corresponding to the dilution experiment period, and estimate 2 considered 2 h, corresponding to the ¹⁴C incubation period. Model II regression of assimilated C on synthesized Chl *a* assigned significant slopes of 52 ± 18 and 47 ± 10 for the 24- and 2-h calculations, respectively (Fig. 7). An additional estimate of a C:Chl *a* value of 47 ± 10 was obtained after comparing C assimilation rates with instantaneous Chl *a* synthesis rates, expressed as ng (C or Chl *a*) L⁻¹ d⁻¹ (see Discussion and Fig. 7). Although these numbers appear rather low for surface populations, they are in the upper limit of the values reported for an adjacent area in May 1989 by Delgado et al. (1992) using C estimates derived from microscopic observation of surface phytoplankton volumes.

Table 4. Nutrient limitation factor ($N_L = \mu : \mu_n$) estimated for the different phytoplankton groups.

	N_L
Green algae	0.466
Dinophyceae	0.564
Prymnesiophyceae	0.485
Pelagophyceae	0.575
Cyanobacteria	0.817
Diatoms	0.601
Total phytoplankton	0.563

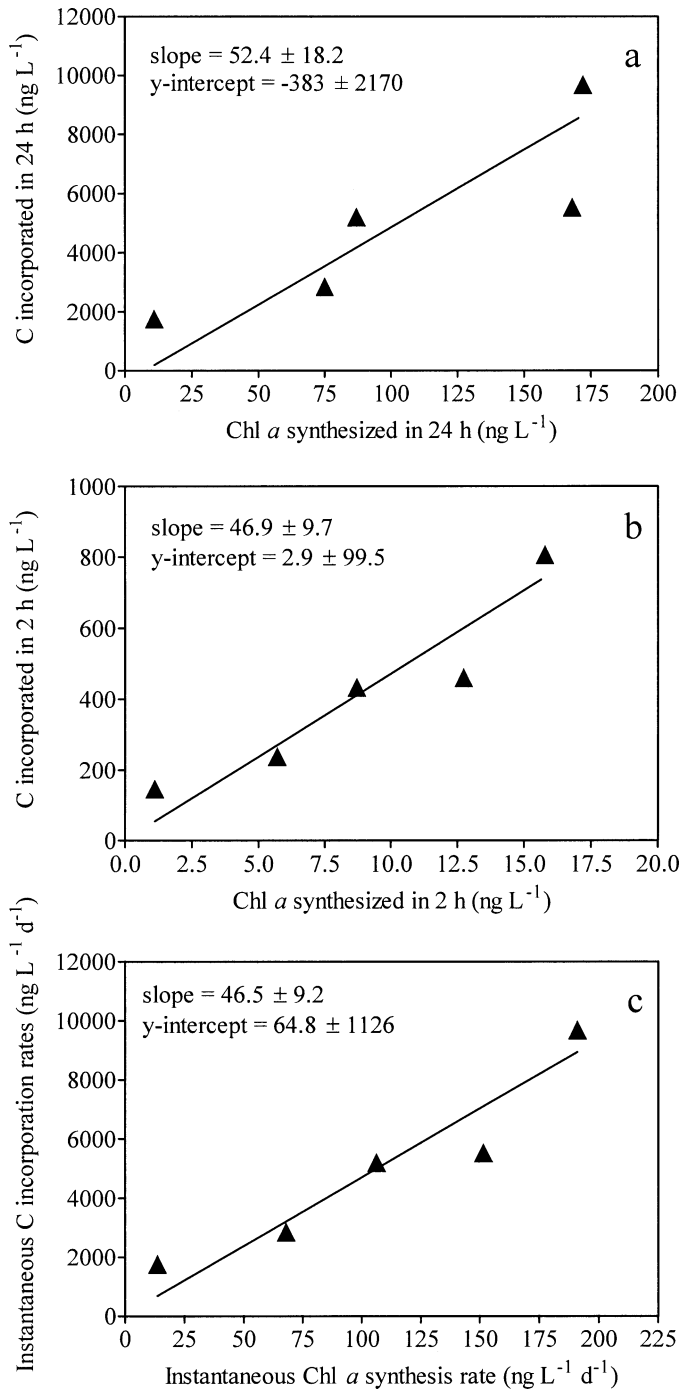


Fig. 7. Relationship between C incorporated and Chl *a* synthesized. (a) and (b) consider the synthesis of Chl *a* integrated for 24- and 2-h periods, taking into account the measured rates of growth and grazing and therefore changes in biomass throughout the incubation periods. (c) Represents instantaneous synthesis rates on a 1-d basis.

The C flowing through the individual phytoplankton groups—The obtained C:Chl *a* ratios could be used to estimate the C dynamics of total phytoplankton by directly converting the Chl *a* that is synthesized and destroyed into C equivalents (Table 3). However, this approach should be

used with more caution for separate phytoplankton groups because these were nutrient limited to a different extent, i.e., nutrient addition had a stronger effect on some groups than on others, and therefore, their C:Chl *a* (or Chl *a*:C) ratios might respond differently (Table 4). This problem can be approached after making some assumptions by estimating the C:Chl *a* for each group as a function of the nutrient-limiting factor (N_L). Laws and Bannister (1980) showed a positive linear relationship between growth rate and Chl *a*:C ratio for a single species, and Geider et al. (1998) also modeled a relationship that can be considered linear within a wide range of growth rates. This linearity implies that the change in growth rate is proportional to the change in the corresponding Chl *a*:C ratio. Since N_L is proportional to the relative increase in growth rate after nutrient addition, N_L could be related to the Chl *a*:C ratio. To account for the different extent of nutrient limitation, we normalized the nutrient-limiting factor (N_L) from each group to that of total phytoplankton, $N_{L\text{group}}:N_{L\text{total-phytoplankton}}$. The calculated ratios were 0.83 and 0.86 for green algae and Prymnesiophyceae; 1.00, 1.02, and 1.07 for Dinophyceae, Pelagophyceae, and diatoms; and 1.45 for cyanobacteria. We assume that these values represent the degree of nutrient limitation of each group relative to total phytoplankton and that the nutrient-replete growth rate (μ_n) should correspond to an “optimal” Chl *a*:C ratio common for all phytoplankton groups. Then, for each group, we multiplied the Chl *a*:C ratio from total phytoplankton by the $N_{L\text{group}}:N_{L\text{total-phytoplankton}}$ ratio to estimate the group-specific Chl *a*:C values. These values are considered corrected for the degree of nutrient limitation and will be used to estimate the C dynamics of each group. As a common Chl *a*:C ratio for nutrient-replete total phytoplankton, we used the value 1/47 (g:g), the inverse value of the slope of the model II regression (which is symmetric for both axes) between instantaneous rate estimates (Fig. 7c, see Discussion). The resulting C:Chl *a* ratios for each phytoplankton group were 57 and 55 for green algae and Prymnesiophyceae; 47, 46, and 44 for Dinophyceae, Pelagophyceae, and diatoms; and 32 for cyanobacteria.

Since the growth rates were more similar among groups than their biomass, the C dynamics were largely driven by biomass, with Prymnesiophyceae dominating both biomass and C dynamics in the study area (Fig. 8). The fastest and slowest growing groups, diatoms and green algae, were the more deviating points from this pattern, with a relatively overrated and underrated contribution to the C flow relative to their contribution to biomass.

Discussion

The main objective of this work was to show the importance of the different phytoplankton groups in the flux of C in the ecosystem. We could achieve this goal by combining a suite of approaches and measurements. By using pigment markers and Chemtax together with the dilution experiments, we were able (1) to quantify the contribution of the different phytoplankton groups relative to Chl *a* stock; (2) to check for imbalanced growth and pigment synthesis because of photoacclimation; and (3) to quantify the dynamics

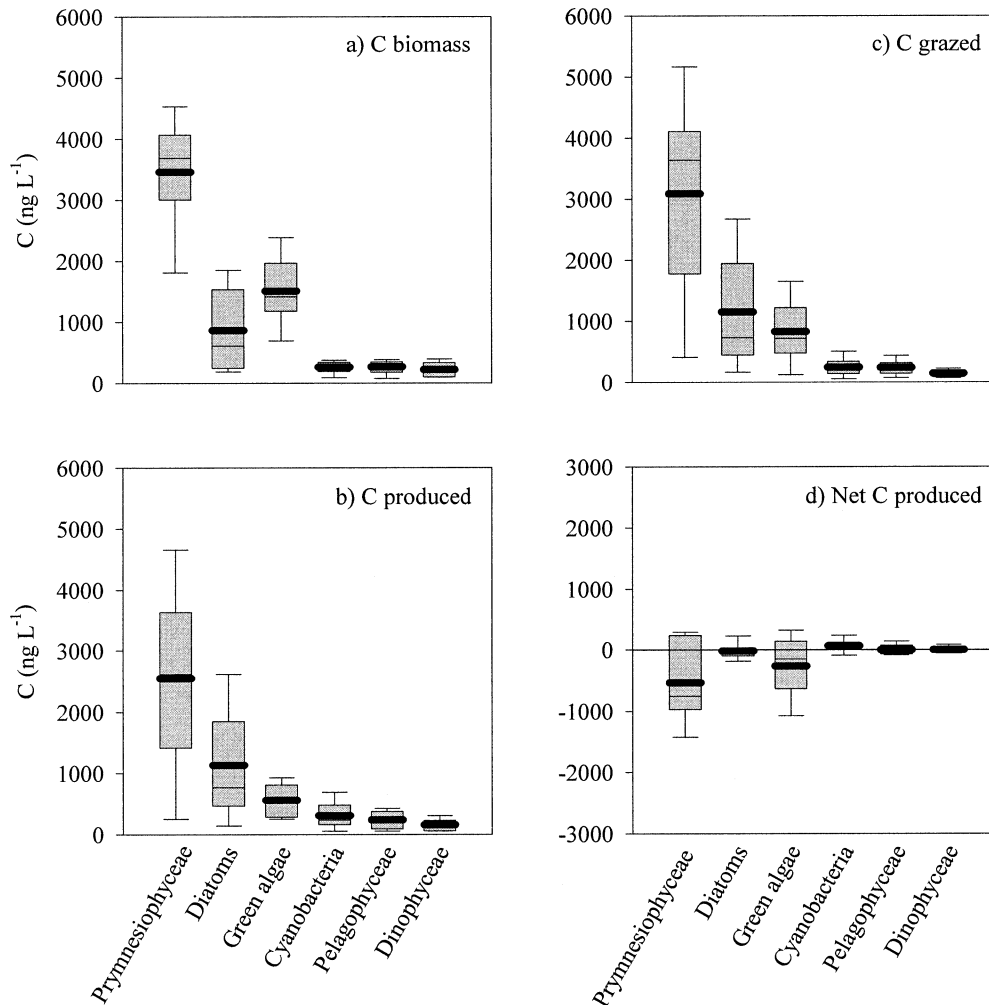


Fig. 8. (a) C biomass and daily integrated (b) synthesis, (c) grazing, and (d) net accumulation of C for the eight experiments. Symbols as in Fig. 2.

of growth and grazing. Finally, the combination of C incorporation and Chl *a* synthesis allowed calculating a C : Chl *a* ratio of newly produced organic matter that was used to estimate the C dynamics for the individual phytoplankton groups.

A dynamic system with relatively high phytoplankton growth rates—In our study site, the nutrient-limited phytoplankton were growing at about one division per day (μ , Table 2). High growth rates have been reported in nutrient-depleted waters of the subtropical Pacific and Atlantic Oceans (Vaulot et al. 1995; Lessard and Murrell 1998; Quevedo and Anadón 2001). These systems are dominated by photosynthetic prokaryotes, which should show advantage in obtaining nutrients from nutrient-poor waters (Chisholm 1992). In agreement, the phytoplankton in our oligotrophic system were dominated (68% in terms of Chl *a*) by phytoplankton $<5 \mu\text{m}$. However, these small-sized phytoplankton were mainly made up by eukaryotes, a significant difference from permanently oligotrophic waters where prokaryotes dominate.

This view of high growth rates in poor oceanic areas has

been challenged by Marañón et al. (2003), who measured low phytoplankton growth rates in both oligotrophic gyres of the Atlantic Ocean, where *Prochlorophyceae* are dominant. The open-ocean region studied by Marañón et al. (2003) is more nutrient deprived than our study area. In samples close to or below the detection limit of classical methods of nutrient measurements, phytoplankton is highly dependent on nutrient fluxes and probably very sensitive to their chemical form (Moore et al. 2002; Berg et al. 2003). We argue that the so-called low-nutrient areas should be separated between those corresponding to permanent low-nutrient regimes and those subjected to episodic nutrient pulses. An example of different phytoplankton composition in apparently similar nutrient-poor waters can be observed between the time series off Bermuda, with a yearly spring bloom and a transient but significant presence of *Synechococcus* under low-nutrient concentrations, and Hawaii, permanently stratified and dominated by *Prochlorococcus* (Cavender-Bares et al. 2001). It is obvious that *Synechococcus* is using the strong capacity of planktonic systems to retain nutrients via local recycling processes. The significant presence of diatoms in our study area supports the idea that high phy-

toplankton growth rates like those we reported need at least temporal inputs of nutrients that might fuel them for extended time periods. It also indicates that ours was a transient state from high to low phytoplankton biomass. High Chl *a* concentrations in the studied area during this period of the year are related to the Rhône river outflow (maximum in May after spring ice melting) that fosters a phytoplankton bloom readily visible with satellite imagery. The senescent population of *Chaetoceros* spp. diatoms at station A was probably residual of that bloom.

Diverse behavior of phytoplankton groups—The taxonomic differences of phytoplankton have not been usually incorporated in plankton modeling. Instead, size has become a relevant parameter in this type of approach to investigate the ecological and biogeochemical role of phytoplankton (Legendre and Le Fèvre 1995; Legendre and Rassoulzadegan 1996; Armstrong 1999). In our study, diatoms and cyanobacteria, representing probably the two extremes of the phytoplankton size spectrum, showed the highest growth rates. Diatoms are usually the phytoplankton with the fastest growth in a wide range of trophic conditions (Burkill et al. 1987; Strom and Welschmeyer 1991; Latasa et al. 1997), and they also dominate the large-sized phytoplankton (Latasa and Bidigare 1998). In our study, the most important groups in a sequence from small to large size were cyanobacteria, green algae, Prymnesiophyceae, and diatoms. The mean growth rate estimated for each group was independent of this size progression, indicating that size cannot always be associated with growth rates. This observation contradicts the classic view in oligotrophic areas where small cells should present faster growth rates because of their competitive advantage in nutrient uptake.

Nutrient addition showed that all phytoplankton groups were nutrient limited. Cyanobacteria seemed to undergo the least stress, as expressed by the N_L index (Table 4), a result that was expected because of the supposedly competitive edge in nutrient acquisition of high surface:volume ratio cells (Chisholm 1992). However, the most stressed cells according to our proposed index were the green algae, widely dominated by very small cells (86% in $<5 \mu\text{m}$). On the basis of physical constraints, size can be considered a first proxy of physiological processes related to surface:volume ratios, such as diffusive nutrient uptake or incoming irradiance. However, active transport of substances (Flynn 2002), adaptation to different irradiance regimes (Moore et al. 1998), and metabolic activity spent in nongrowing activities (Buitenhuis et al. 1999) are processes with an impact on the final capacity to generate growth that are not necessarily linked to size.

Strong and selective coupling between growth and grazing—The differential biomass contribution of phytoplankton groups found in our study indicates that Prymnesiophyceae and green algae must have reached a preferential net accumulation of biomass over the rest of the taxa, a situation that was leveling off according to the negative net growth measured for those two dominant groups. During our study, the phytoplankton stock was grazed more rapidly than produced. Even diatoms were actively grazed down by the microzoo-

plankton in spite of their large size ($\sim 60\% >5 \mu\text{m}$), as already observed in other systems (Strom et al. 2001). Negative net growth because of microzooplankton grazing has been reported for small data sets or individual experiments (Lessard and Murrell 1998; Fileman and Burkill 2001; Stelfox-Widdicombe et al. 2004), although microzooplankton grazing consumes overall about two-thirds of phytoplankton production (Strom et al. 2001; Verity et al. 2002; Calbet and Landry 2004). These results should not be regarded as contradictory. Under the absence of new inputs of nutrients into the euphotic zone, losses should be close to or slightly higher than production. Therefore, a gradual decline in phytoplankton biomass should be expected. A loss mechanism such as sedimentation is ignored in dilution experiments, but under the oligotrophic conditions found in our study area, sedimentation is considered a minor factor. Losses due to autolysis or viral infection are included in dilution experiments as not-expressed growth, i.e., their induced mortality is independent of the dilution and is not accounted in the grazing term but causes the intrinsic growth rates to be underestimated.

The groups making up the bulk of the pigment biomass and especially the Prymnesiophyceae were the less reactive groups of the phytoplankton in terms of growth and grazing. In this sense, Prymnesiophyceae and green algae were responsible for the main losses only because they had the highest biomass, not because they underwent higher specific grazing rates. This lack of a positive correlation between grazing rates and biomass has already been shown for natural samples (Strom and Welschmeyer 1991; Verity et al. 2002). While there might be physiological reasons for different growth efficiencies among groups (Chan 1978), it is less clear why the microzooplankton seem to avoid slow growing groups. Indications have been documented that zooplankton preferentially graze on nutrient-rich phytoplankton because of their higher nutritional value (Stoecker et al. 1986; John and Davidson 2001). In our case, the comparison of nutrient-added and nonamended incubations showed that all groups were limited by nutrients at least in terms of pigment synthesis (Table 4). In addition, we could classify the degree of limitation according to our N_L ratio. No significant ($p > 0.05$) relationship was found between the degree of nutrient limitation and the grazing rates, either for the entire database or for each phytoplankton group. Although our simple parameter to establish the degree of nutrient limitation did not permit us to establish clearly whether grazing responds to different nutritional status of the prey or to intrinsic growth rates of the prey per se, the much better correlation with growth than with nutrient limitation seems to indicate that the intrinsic growth rate plays a more decisive role for prey selection. The way the predators can detect the prey growth rate independently from nutritional status is an unresolved question, but possible mechanisms are reviewed by Strom (2002).

C: Chl a and the C flowing through the different phytoplankton groups—The comparison between primary production estimated from ^{14}C incubations and phytoplankton growth rates estimated from dilution experiments is not straightforward (Moigis 2000). The significance of phyto-

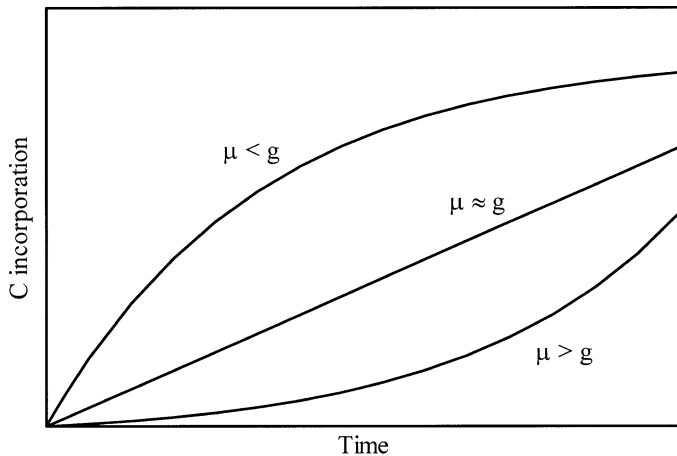


Fig. 9. Simulated time dynamics of C incorporation according to the balance between growth, μ , and grazing, g . Scales are different for the different curves to emphasize their shape.

plankton production obtained from ^{14}C incubations in terms of net or gross rates has been widely discussed in the past, e.g., in relation with growth and grazing effects, length of the incubation, or role of respiration of recent photosynthate (Williams and Le Fèvre 1996; Laws et al. 2000). A recent work by Marra (2002) suggests that the ^{14}C technique approximates net production on most occasions. In theory, the C uptake estimates derived from our 2-h ^{14}C incubations should include the organic ^{14}C incorporated into the microzooplankton that grazed the “hot” phytoplankton minus the ^{14}C lost by extracellular release of organic matter and respiration by the phytoplankton minus the portion of ^{14}C ingested that might be excreted and respired by the microzooplankton. This last loss could be ignored in our experiments because of the short incubation period. On the other hand, the intrinsic growth rate (μ) calculated from dilution experiments estimates the net change in biomass (Chl a) when grazing is absent. Therefore, the intrinsic growth rate estimated from dilution experiments includes all the C flowing through phytoplankton minus that which is extracellularly released and respired for 1 d. When comparing both methods as applied in this study, the rate of C incorporation derived from 2-h ^{14}C incubations should be similar to the intrinsic C synthesis estimated from dilution experiments.

A discussion of the impact of grazing on ^{14}C experiments has been recently taking place (Banse 2002; Marra 2003). In an answer to Banse, Marra questioned the importance of grazing in all situations. Intuitively, grazing seems to be incompatible with the frequent observation that ^{14}C uptake is linear over periods of hours (Marra 2002; Marra and Barber 2004). According to these authors, the explanation for this linearity is because most or all C respired is refixed in photosynthesis. However, a linear C assimilation curve can also be obtained if the initial biomass remains constant due to dynamic interactions with other components of the trophic web. It is true that Eq. 1 includes an exponential function of time, but the kinetics of C incorporation depend on the net balance between growth and grazing (Fig. 9). Since algal biomass remains quite stable in many systems because of

balanced growth and losses, the linearity of C uptake cannot be used as an argument to deduce the importance of grazing.

In our system, with similar values for growth and grazing rates, C incorporation rates and Chl a synthesis rates integrated for 2 and 24 h should render similar linear relationships. This is confirmed by the comparison of the 2- and 24-h estimates of C incorporation and Chl a synthesis (Fig. 7). However, there is a better correlation for the 2-h estimates than for the 24-h estimates ($r = 0.94$ vs. $r = 0.86$). The reason for this difference is because there were slight changes in biomass during incubation (because of slight disequilibrium between growth and grazing), which were not accounted for in our estimates of C assimilation. The largest discrepancy arises from a single experiment (experiment G, Table 2), where growth and grazing were the least coupled, and changes in biomass during the experiment became the most pronounced. Since calculations of C production derived from ^{14}C uptake experiments typically assume that the initial biomass remains constant, we suggest a comparison of C production rates with instantaneous Chl a synthesis rates (Chl $a_{\text{synthesized}}$) by applying the formula $\text{Chl } a_{\text{synthesized}} = \text{Chl } a_0 \times \mu$, thus removing any changes in phytoplankton biomass during incubation. The correlation obtained for instantaneous rates is even better than for 2-h integrals ($r = 0.95$, Fig. 7c). This approach has already been used to estimate intrinsic growth rates from primary production and Chl a -derived C stocks (Arin et al. 2002). Comparison of instantaneous Chl a synthesis rates derived from dilution experiments with C production rates that is based on ^{14}C uptake allows an estimation of C:Chl a ratios (Gallegos and Vant 1996; Obayashi and Tanoue 2002) while skipping the direct measurements of phytoplankton C (Fileman and Burkill 2001; Brown et al. 2003; Stelfox-Widdicombe et al. 2004). On the basis of our results, we propose instantaneous rates as used in Fig. 7c as the best approximation to calculate C:Chl a ratios. It should be noted, however, that this approach assumes a biomass steady state and should not be used to study the C dynamics at time scales for which this assumption does not hold true. In this case, Eqs. 1 and 2 would give a better representation of the C fluxes in the system.

In summary, this study has shown that the differences in C flux were generally a function of biomass, because the variability was larger in biomass (C.V. = 105%) than in growth rates (C.V. = 35%) among the six phytoplankton groups studied. Prymnesiophyceae and green algae channeled most of the autotrophic C, with diatoms playing a disproportionate role compared to their biomass because of their high growth and grazing rates. The size-fractionation study indicated that the overall size of the individual groups was not a good indicator of their ecological behavior. As noted by Chisholm (1992), “the ecological differences between the taxonomic groups appear to override the influence of size on growth potential.” In this study, we have provided data exposing important ecological information on the C flux of phytoplankton at the class level. Existing models are normally based on the response of the whole, or sometimes size-defined, phytoplankton community. Our results should be a step toward the incorporation of taxonomic entities, from class to species or strains, into ecological and biogeochemical models.

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