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Growth and grazing rate dynamics of major phytoplankton groups in an oligotrophic coastal site

Andrés Gutiérrez-Rodríguez [a,*], Mikel Latasa [b,1], Renate Scharek [b], Ramon Massana [a], Gemma Vila [c], Josep M. Gasol [a]

[a] Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta 37-49, E-08003 Barcelona, Spain
[b] Centro Oceanográfico de Gijón (IEO), Avda. Príncipe de Asturias 70 bis, E-33213 Gijón, Spain
[c] Departament d'Ecologia, University of Barcelona (UB), Diagonal 645, E-08028 Barcelona, Spain

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ABSTRACT

There has been more attention to phytoplankton dynamics in nutrient-rich waters than in oligotrophic ones thus requiring the need to study the dynamics and responses in oligotrophic waters. Accordingly, phytoplankton community in Blanes Bay was overall dominated by *Prymnesiophyceae*, remarkably constant throughout the year (31 ± 13% Total chlorophyll *a*, Tchl *a*) and *Bacillariophyta* with a more episodic appearance (20 ± 23% Tchl *a*). *Prasinophyceae* and *Synechococcus* contribution became substantial in winter (*Prasinophyceae* = 30% Tchl *a*) and summer (*Synechococcus* = 35% Tchl *a*). Phytoplankton growth and grazing mortality rates for major groups were estimated by dilution experiments in combination with high pressure liquid chromatography and flow cytometry carried out monthly over two years. Growth rates of total phytoplankton (range = 0.30–1.91 d^{-1}) were significantly higher in spring and summer ($\mu > 1.3$ d^{-1}) than in autumn and winter ($\mu \sim 0.65$ d^{-1}) and showed a weak dependence on temperature but a significant positive correlation with day length. Microzooplankton grazing (range = 0.03–1.4 d^{-1}) was closely coupled to phytoplankton growth. Grazing represented the main process for loss of phytoplankton, removing 60 ± 34% (±SD) of daily primary production and 70 ± 48% of Tchl *a* stock. Chl*a* synthesis was highest during the *Bacillarophyceae*-dominated spring bloom (Chl a_{synt} = 2.3 ± 1.6 μg Chl *a* L^{-1} d^{-1}) and lowest during the following post-bloom conditions dominated by *Prymnesiophyceae* (Chl a_{synt} = 0.23 ± 0.08 μg Chl *a* L^{-1} d^{-1}). This variability was smoothed when expressed in carbon equivalents mainly due to the opposite dynamics of C:chl *a* (range = 11–135) and chl *a* concentration (range = 0.07–2.0 μg chl *a* L^{-1}). *Bacillariophyta* and *Synechococcus* contribution to C fluxes was higher than to biomass because of their fast-growth rate. The opposite was true for *Prymnesiophyceae*.

1. Introduction

Phytoplankton is the dominant primary producer in the ocean, and is responsible for roughly half of global organic carbon production (Field et al., 1998). This phytoplankton production can follow different pathways summarized in processes which lead to either export out of or remineralization within the euphotic zone. Hydrodynamics determines phytoplankton growth and mortality rates, and ultimately, community structure (Margalef, 1978; Cullen et al., 2002; Smetacek et al., 2004) and phytoplankton production (e.g. Michaels and Silver, 1988; Boyd and Doney, 2002). Highly stratified and nutrient-depleted systems tend to be dominated by small cells embedded in a complex microbial food web with multiple trophic levels. These systems are characterized by a close coupling between primary producers and grazers that efficiently recycle organic matter within the euphotic zone precluding high export rates. On the contrary, large phytoplankton cells typically dominate well-mixed and nutrient-rich water columns. The lower susceptibility of larger cells to microzooplankton grazing favours the export of organic matter to higher trophic levels (Ryther, 1969; Smetacek et al., 2004) and to sediments as rapidly sinking mesozooplankton faecal pellets or massive sinking events (Riegman et al., 1993).

* Corresponding author. Present address: Integrative Oceanography Division, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093, USA.
E-mail address: andresgutierrez@ucsd.edu (A. Gutiérrez-Rodríguez).
[1] Present address: Centro Oceanográfico de Gijón (IEO), Avda. Príncipe de Asturias 70 bis, E-33213 Gijón, Spain.

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In addition to community size structure, phytoplankton taxonomic composition constitutes a key factor for biogeochemical fluxes because of the specific chemical reactions mediated by particular groups (Le Quere et al., 2005; Hood et al., 2006). Taxonomic affiliation also determines important ecological properties of phytoplankton such as growth rate (Chisholm, 1992; Latasa et al., 1997; Goericke, 2002) or grazing mortality (Gaul and Antia, 2001; Strom et al., 2007; Olson et al., 2008). These properties constitute the proximate control on phytoplankton dynamics and carbon fluxes. Group-specific approaches to phytoplankton carbon fluxes, either based on size (Tilstone et al., 1999; Serret et al., 2001; Teira et al., 2001; Cermeño et al., 2006) or taxonomic criteria (Worden et al., 2004; Latasa et al., 2005; Morán, 2007), have provided valuable insights into the link between community composition and function. However, there is a limited number of these studies and as yet no comprehensive understanding of this connection.

To further explore this potential linkage we adopted a pigment-based approach to assess the relative contribution of major phytoplankton groups to carbon dynamics thriving under different environmental and biological conditions. The seasonality of Blanes Bay hydrodynamics offered the opportunity for assessing this in a temperate coastal oligotrophic site exhibiting strong seasonal physico-chemical variability (Lucea et al., 2005) accompanied by shifts in phytoplankton (Mura et al., 1996; Guadayol et al., 2009a), bacterial (Schauer et al., 2003; Alonso-sáez et al., 2007) and zooplankton (Calbet et al., 2001) taxonomic composition. We carried out monthly dilution experiments in combination with high pressure liquid chromatography (HPLC), flow cytometry (FCM) and microscopy during two years in order to estimate phytoplankton group-specific growth, grazing rates and associated carbon dynamics under different hydrodynamic conditions. Correlation and cluster analysis were then applied to explore potential links between environmental conditions, phytoplankton community composition and ecosystem function.

2. Material and methods

2.1. Study area and sampling

We conducted our study at the Blanes Bay Microbial Observatory (NW Mediterranean http://www.icm.csic.es/bio/projects/icmicrobis/bbmo/). The site is 800 m offshore and has a sand bottom at 20 m depth. Surface water for experiments was collected by sinking acid and milliQ-rinsed 20 L polycarbonate containers into the water as gently as possible to minimize shear and bubbles. The carboys were immediately covered with black bags to prevent cells from experiencing light shock. Full experiments were conducted at monthly intervals between December 2003 and December 2005. Pilot experiments were carried out in July, September and October of 2003. Six additional experiments were conducted evenly distributed from December 2006 to October 2007. Water temperature was measured with a mercury thermometer at the surface. Salinity was measured using a YSI 556 MPS probe. Sampling ended before 11:00 a.m. and transport to the laboratory was completed within the next 1.5 h.

2.2. Experimental set up and sample analysis

Growth and grazing rates were determined by the seawater dilution technique (Landry and Hassett, 1982), following modifications introduced by Landry et al. (1995a) and Gutiérrez-Rodríguez et al. (2010). Incubation bottles were filled with the appropriate filtered seawater (FSW) quantities to reach 90, 80, 70, 60, 50 and 40% in 0.63 L polycarbonate bottles. Three non-diluted replicates were also incubated. FSW was obtained by gravity filtration of natural seawater through a 0.22 μm pore-size cartridge (Pall-Gelman Suporcap). Whole seawater (WSW) was gently added using a silicone tube with its end submerged to avoid bubbles and starting from unamended bottles. We nutrient amended this 9-bottle series with F/2 media, ammonium and urea to a final nitrogen concentration of 7.5 μM following a 3:1:1 M ratio. Glucose was also added to a final concentration of 1.0 μmol L^{-1}. Three more non-diluted bottles without nutrient amendment were incubated as controls. Initial values for each incubation bottle were estimated from three replicates subsampled directly from the 20 L carboy and the corresponding theoretical dilution factor. All containers and silicone tubes were soaked overnight with 5% HCl and thoroughly rinsed with distilled water (4 times) and MilliQ water (2 times). The silicone tubing was further rinsed with particle free seawater and natural seawater just before filling the bottles. The Pall-Gelman Suporcap cartridges filled with 0.01% HCl solution were kept at 4 °C between experiments, and rinsed with ~10 L of distilled water and 2 L of natural seawater just before each experiment.

For experiments conducted between 2003 and 2005, the bottles were incubated for 24 h in a laboratory incubator set to the ambient temperature of the original sample. The daily light/dark cycle was adjusted to the length of the day. Irradiance was 320 μmol photon m^2 s^{-1}, assumed initially to be saturating for phytoplankton sampled from surface water without causing photoinhibition. The validity of this assumption was tested for each experiment following changes in cell pigment content with flow cytometry (see below). Light-saturation parameters of the photosynthesis vs. irradiance curve (E_k) averaged 327, 366, 640 and 320 μmol photon m^{-2} s^{-1} for winter, spring, summer and autumn, respectively (Cardelús pers. comm.). For the 6 experiments carried out in 2006 and 2007, the bottles were incubated under natural solar irradiance in a plexiglass incubator covered with a blue screen that mimicked in situ surface irradiance. The incubator was coupled to a continuous supply of surface water that controlled temperature within a 1 °C. After 24 h, final samples were taken for HPLC pigment quantification and FCM. Subsampling, storage and subsequent analysis for FCM and HPLC followed the method described in Latasa et al. (2005).

For pigment quantification, 0.5 L were filtered onto 25 mm glass fibre filters (Whatman GF/F) with low vacuum (0.2 atm) to prevent cells from breaking. These filters were folded, blotted dry, wrapped in aluminium foil and frozen at −80 °C until their analysis by HPLC. Pigment extraction was performed following the procedures of Wright and Jeffrey, 1997. Pigment analysis was performed following the method of Zapata et al. (2000) with the modifications described by Latasa et al. (2001). For flow cytometry, 2 ml of sample were fixed with P + G (Paraformaldehyde 1% and glutaraldehyde 0.05% final concentration) kept at room temperature in the dark for 15–20 min and frozen at −80 °C until subsequent analysis in the FCM (Gasol and Del Giorgio, 2000).

In the dilution experiments carried out during 2003, 2006 and 2007, total phytoplankton dynamics were assessed from changes in chlorophyll concentration determined from 90% acetone extracts of GF/F filters read with a Turner model fluorometer (Turner Designs 10A, Sunnyvale, California). Filters were submerged in 6 ml acetone (90%) inside centrifuge polypropylene tubes filled with 1 mm diameter glass spheres beads (0.5 ml approx.) and subsequently kept 24 h at −20 °C. Next day, samples were grinded by means of a Vibrogen IV cell mill (Edmund-Bühler) for 5 min. The milling chamber where samples were crushed was filled with ice in order to maintain low temperature. The samples were then centrifuged for 1 min at 4 °C and 4000 r.p.m., material from the walls of the tube removed and centrifuged for another 9 min. The soluble phase was carefully transferred with a plastic Pasteur pipette from the centrifuge tube to glass tubes before reading it in a Turner fluorometer.

2.3. Data analysis

Phytoplankton apparent growth rates (k) were estimated from changes in pigment concentrations. Chl *a*, Fucoxanthin, 19′-hexanoyloxyfucoxanthin, chl *b*, zeaxanthin and 19′-butanoyloxyfucoxanthin were used as diagnostic pigments for bulk phytoplankton, *Bacillariophyta*, *Prymnesiophyceae*, *Prasinophyceae*, *Synechococcus* and *Pelagophyceae* respectively (Latasa et al., 2005). Exponential growth and loss processes were assumed for the populations studied (Landry and Hassett, 1982)

$$k = \left(\frac{1}{t}\right)\ln\left[\frac{N_t}{N_0 \times D}\right] \quad (1)$$

where N_t and N_0 are final and initial pigment marker, D is the proportion of WSW and t is the duration of the experiment. The Chemtax computer programme (Mackey et al., 1996) was applied to the initial samples of the dilution experiments following the procedure described in Latasa (2007) to assess the proportion of Tchl *a* assigned to main phytoplankton groups.

The intrinsic growth rate for nutrient amended bottles (μ_n) was estimated as the intercept of the linear regression (model I) between apparent growth rates and dilution factor (fraction of whole seawater) following the "three point" rationale of Gallegos (1989) as described in Gutiérrez-Rodríguez et al. (2010). Grazing rate (m) was obtained from the difference between the intrinsic growth rate and net growth rate measured in non-diluted nutrient amended bottles ($m = \mu_n - \mu_{n,\mathrm{net}}$). Net growth rate ($\mu_{\mathrm{net}}$) will refer to k in the non-diluted bottles, while k will refer to apparent growth rate in the dilution treatments. Intrinsic growth rate at ambient nutrient levels (μ_0) was assessed as the sum of net growth rate for non-diluted non-nutrient enriched treatments and grazing rate ($\mu_0 = \mu_{\mathrm{net}} + m$). The nutrient limitation index was assessed as $N_L = \mu_0/\mu_n$ (Landry et al., 1995b, 1998). Chl *a* synthesis and destruction rates (μg chl *a* L^{-1} d^{-1}) were estimated according to the following equations (modified from Landry et al., 2000 based on Frost, 1972):

$$\text{Chl } a \text{ synthesis rate} = \frac{\mu_0 N_0\left(e^{(\mu_o - m)t} - 1\right)}{\mu_0 - m} \quad (2)$$

$$\text{Chl } a \text{ destruction rate} = \frac{m N_0\left(e^{(\mu_0 - m)t} - 1\right)}{\mu_0 - m} \quad (3)$$

A Photoacclimation index (*Phi*) was assessed from FCM samples for each experiment following the procedure described in Gutiérrez-Rodríguez et al. (2010). FL3/SSC ratio of picoeukaryote population was estimated for the initial (R_i) and final (R_f) samples. The average ratio of R_f/R_i in non-diluted unamended triplicates (Phi_{light}) and amended triplicates ($Phi_{\mathrm{light+nut}}$) quantified the effect of irradiance solely and the combined effect of both nutrient addition and irradiance, respectively, on photoacclimation during the incubation.

This index was then included in Eq. (1) to account for changes in cell pigment content derived from photoacclimation processes in nutrient amended and not amended incubations (Eqs. (4) and (5)).

$$k = \left(\frac{1}{t}\right)\ln\left[\frac{N_t}{N_0 \times D \times Phi_{\mathrm{nut}}}\right] \quad (4)$$

$$k = \left(\frac{1}{t}\right)\ln\left[\frac{N_t}{N_0 \times D \times Phi_{\mathrm{nonut}}}\right] \quad (5)$$

Synechococcus growth and grazing rate estimates were based on changes in zeaxanthin. Given its structural role (Kana et al., 1988) we assumed that changes in this pigment solely reflected changes in carbon biomass (Moore et al., 1995; Six et al., 2004).

2.4. Phytoplankton carbon (PhytoC)

Samples for phytoplankton counts of field samples collected at time zero of the experiments were fixed with formaldehyde buffered with hexamine (0.6% final concentration). Cell identification was carried out to the genus and, when possible, to the species level, with an inverted microscope equipped with bright-field and phase-contrast objectives (total magnifications from 100× to 400×) and a graduated ocular using the Utermöhl method. Biovolume and carbon of phytoplankton cells >5 μm were estimated according to Mender-Deuer and Lessard (2000), Mender-Deuer et al. (2001) and Stoecker et al. (1991). The biovolume of *Synechococcus* and phototrophic nanoflagellates (PNF < 5 μm) was estimated from epifluorescence counts while flow cytometry counts were used for *Prochlorococcus*. We assumed spherical shapes for the three groups. Biovolumes were converted to cell carbon using 226 fg C μm^{-3} and 265 fg C μm^{-3} as conversion factors for *Prochlorococcus* and *Synechococcus*, respectively (Bertilsson et al., 2003) and the expression $C = 0.433\ V^{0.863}$ for PNF (Verity et al., 1992). Samples for epifluorescence microscopy were fixed with ice-cold glutaraldehyde (1% final concentration), stained with 4-6-diamidino-2-phenylindole (DAPI, 5 μg ml^{-1} final concentration) and filtered onto 0.6 μm pore-size black polycarbonate filters. Filters were mounted on a slide with low fluorescence oil and counted usually the same day using an Olympus BX61 microscope. Protists (unicellular eukaryotes) were discriminated from prokaryotes and counted by standard epifluorescence microscopy based on their blue fluorescence under UV excitation due to the nucleus (Caron, 1983). Total protists counts were separated into two categories, aplastidic (presumably heterotrophic) and plastidic (presumably autotrophic) cells, based on the absence or presence of chlorophyll red fluorescence under blue light excitation. Moreover, organisms were classified into several size classes (≤2 μm, 3 μm, 4 μm, 5 μm) after visual measurements using a graduated ocular.

2.5. C:chl a and carbon fluxes

Phytoplankton C:chl *a* ratio was calculated from estimates of chl *a* and PhytoC for samples collected during 2003–2004. These ratios were used to transform bulk phytoplankton chl *a*-based dynamics into carbon units. C:chl *a* of the major phytoplankton groups were estimated following the approach described in Gutiérrez-Rodríguez et al. (2010). This method considers the degree of nutrient limitation of the different phytoplankton groups to adjust their C:chl *a* based on the linear relation between nutrient limitation and chl *a*:C ratio (e.g. Laws and Bannister, 1980; Geider et al., 1998). The group-specific C:chl *a* was obtained as the inverse of their chl *a*:C ratio and was employed to transform chl *a* fluxes into carbon equivalents.

3. Results

3.1. Physico-chemical conditions and phytoplankton community composition

Surface water temperature followed a seasonal pattern with minimum values during winter (~12 °C) and maximum during summer, (~25 °C, Fig. 1A). Concentration of inorganic N (Nitrate + Nitrite + ammonium) and phosphorus followed parallel trends with highest values during autumn and winter and lowest during summer (Fig. 1B). Inorganic N variability (range = 0.09–6.96 μmol L^{-1}) was related mainly to terrestrial runoff rather than wave or wind mediated sediment re-suspension, while phosphate variability (range = 0.1–0.42 μmol L^{-1}) was not statistically influenced by any of these factors (Guadayol et al., 2009b). Chl *a* concentration (range = 0.07–2.0 μg chl *a* L^{-1}) averaged 0.54 μg chl

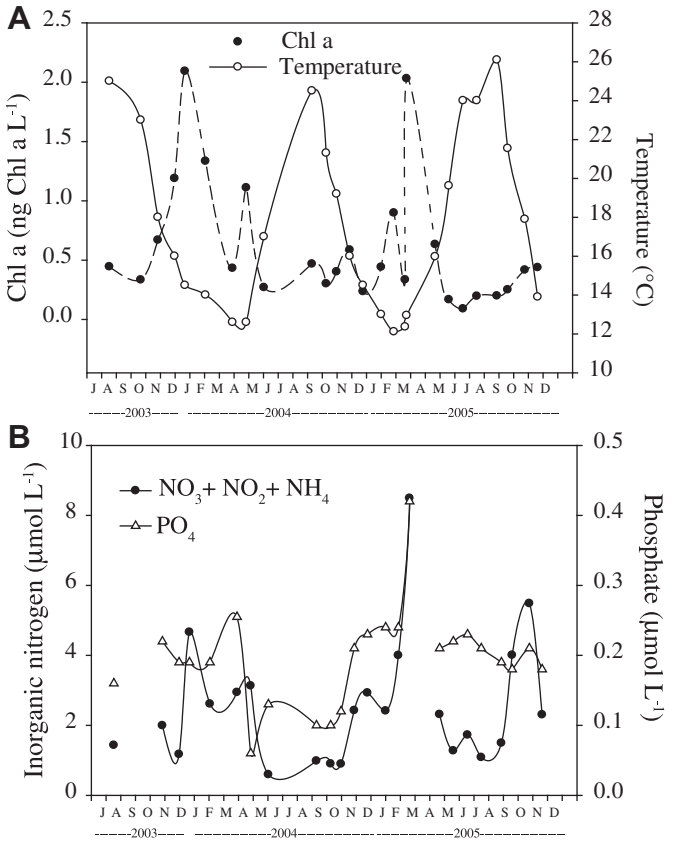

Fig. 1. (A) Seasonal changes in temperature (°C) and total chl a concentration (µg chl a L^{-1}) (B) inorganic nitrogen and phosphate (µmol L^{-1}) during the period of sampling.

a L^{-1} and described the same temporal trend as inorganic nutrients with higher values between November and April, when temperature remained below 16 °C (Fig. 1A).

On a year-round basis *Prymnesiophyceae* and *Bacillariophyta* were quantitatively the most important groups (31% and 20% of Tchl *a*, respectively) followed by *Synechococcus* (14%), *Prasinophyceae* (13%) *and Pelagophyceae* (8%) (Table 1). The relative contribution of *Dinophyceae* and *Cryptophyceae* together was 12%, but because we did not obtain reliable growth and grazing rate estimates for these two groups we did not include them in the subsequent analyses.

Prymnesiophyceae relative contribution to Tchl *a* was 31% on average and remained relatively constant over the year (Coefficient of variation = 43%), with minimum values during early spring and highest when the stratified conditions prevail and daily solar radiation doses are highest in Blanes Bay from May to September (Vila-Costa et al., 2007; Vallina and Simó, 2007). *Bacillariophyta*

Table 1
Year-round average chl *a* biomass of major phytoplankton groups and their relative contribution to total chl *a*. Error in brackets are the Standard Deviation. CV is the coefficient of variation, $n = 30$. (nd): Not detected.

Phytoplankton group	ng chl *a* L^{-1}	% chl *a*	Range (%)	CV (%)
Prymnesiophyceae	117 (±85)	31 (±13)	9.6–64	43
Bacillariophyta	107 (±142)	20 (±23)	0.0–73	115
Prasinophyceae	60 (±59)	13 (±11)	0.8–35	88
Synechococcus	33 (±36)	16 (±19)	nd–65	119
Pelagophyceae	33 (±24)	8.0 (±4.0)	1.6–17	50
Procholorococcus	6.4 (±6.8)	1.9 (±2.4)	nd–10	128
Dinophyceae	19 (±61)	2.7 (±3.1)	0.0–13	115
Cryptophyceae	76 (±240)	9.3 (±9.4)	0.2–52	100

concentration was much more variable (CV = 115%): it was highest during spring (70% of Tchl *a*) and reached a minimum during summer (<5%) before increasing again in late autumn (median = 49% of Tchl *a*, range = 17–65, $n = 5$). *Prasinophycecae* concentration and relative abundance remained low during spring and summer, increasing in late autumn and peaking in winter, usually in January and February (median = 31% of Tchl *a*, range = 19–35, $n = 4$). *Synechococcus* followed the opposite trend reaching highest abundance in summer (median = 34%, range = 10–65%, $n = 9$) while they were barely present during the rest of the year. *Pelagophyceae* contribution remained at low levels during most of the year with a slight increase during autumn (Fig. 2).

Cluster analysis of phytoplankton groups' abundances revealed different phytoplankton assemblages that succeeded each other following the different physico-chemical conditions throughout the year (Fig. 3). During early winter, *Prasinophyceae* along with either *Prymnesiophyceae* (cluster V) or *Bacillariophyta* (cluster II) dominated the community. *Bacillariophyta* clearly dominated community following the onset of the spring bloom in April and May (herein referred as Spring Bloom community, cluster I) while *Prymnesiophyceae* dominated the lower chl *a* stock following the decay of the bloom (herein referred as Post-bloom community, cluster IV). *Synechococcus* concentration rose to co-dominate summer chl *a* stock with *Prymnesiophyceae* (cluster III) during the stratification conditions set from June to September (Vila-Costa et al., 2007). *Prochlorococcus*, virtually absent during most of the year, presented maximum concentrations (10% Tchl *a*) in September and October. During late autumn (November–December), there was a secondary phytoplankton bloom largely driven by *Bacillariophyta*, which took over the previous *Synechococcus* and *Prochlorococcus* populations to end up dominating the community (cluster I). Flow cytometric observations confirmed this temporal sequence for picoeukaryotes (mainly *Prasinophyceae*) dominating in winter, *Synechococcus* in summer, and *Prochlorococcus* being only relevant in late summer although surviving through winter (details not presented).

3.2. Phytoplankton growth and microzooplankton grazing rates

Growth and grazing rates from all 31 dilution experiments are summarized in Table 2. Results from experiments carried out in 69, 83, 158, 295, 349 and 350 day of the year (which represents 20% of the total) were excluded from our analyses due to random apparent growth rates in the dilution series or high negative intrinsic growth rates (Table 2).

3.2.1. Phytoplankton growth rate

Growth rate of total phytoplankton (μ_0) averaged 0.97 ± 0.46 (SD) and ranged between 0.30 and 1.91 d^{-1} (Table 2). This variation followed a clear seasonal pattern with phytoplankton growing faster during spring and summer (>1.3 d^{-1}) than during fall and winter (~0.65 d^{-1}, ANOVA, $p = 0.0003$, Fig. 4). All phytoplankton groups exhibited this temporal trend but differences between seasons were statistically significant only for *Bacillariophyta* ($p = 0.032$) and *Prymnesiophyceae* ($p = 0.035$, Fig. 4). Overall, *Synechococcus* and *Bacillariophyta* grew systematically faster than the whole community (paired *t*-test, $p < 0.05$, S1). The opposite was true for *Prymnesiophyceae* that showed lowest growth rates and tended to grow slower than the whole community, yet in this case differences were not significant (paired *t*-test, $p = 0.25$, S1).

Growth rates were strongly correlated with day length for total phytoplankton (r-pearson = 0.729, $p < 0.0003$) and for all groups except for *Prasinophyceae*. The growth rate of bulk phytoplankton was not significantly correlated to temperature (r-pearson = 0.292, $p = 0.21$), although this correlation turned out to be positive and significant for *Prymnesiophyceae* (r-pearson = 0.543, $p < 0.05$). The

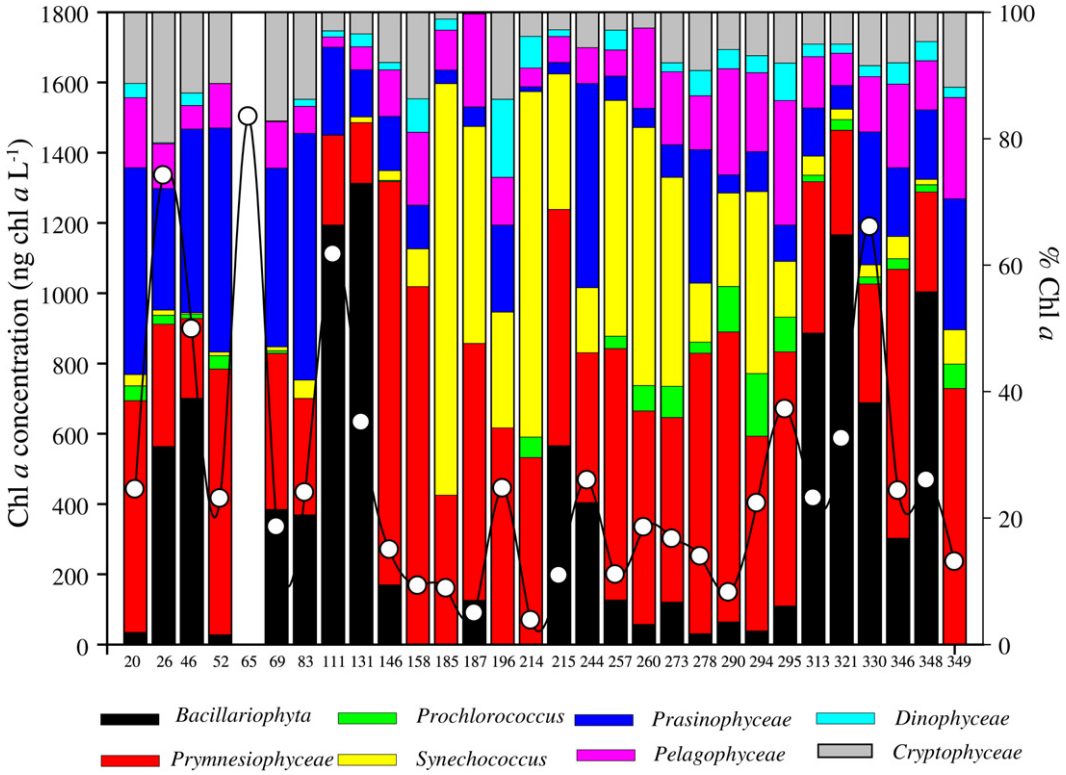

Fig. 2. Relative contribution of main algae groups to total Chl a, and concentration of total Chl a (μg L^{-1}, white dots). Experiments carried out in different years are disposed following an ordinal date scale in order to stress the recurrent phytoplankton community structure at different periods of the year.

growth rate of total phytoplankton and that of main phytoplankton groups remained uncorrelated with the concentration of inorganic nutrients (data not shown).

3.2.2. Microzooplankton grazing rates (*m*)

Microzooplankton grazing rates (*m*) ranged from 0.03 to 1.4 d^{-1} (Table 2). They followed the same temporal pattern as phytoplankton growth, being higher during spring and summer (~0.75 d^{-1}) than during fall and winter (~0.35 d^{-1}, ANOVA $p = 0.021$, Fig. 4). Both variables were strongly correlated (r-pearson = 0.730, $p < 0.0001$). Grazing mortality rates of major phytoplankton groups followed the same seasonal dynamics as those observed for total phytoplankton (Fig. 4) and mirrored group-specific's growth rates. *Bacillariophyta* were grazed at higher rates than the total phytoplankton community (paired *t*-test, $p < 0.0001$). Grazing rates on *Prymnesiophyceae* were the lowest among group-specific rates and systematically lower than the rates on the whole phytoplankton community (paired *t*-test, $p < 0.01$, S1).

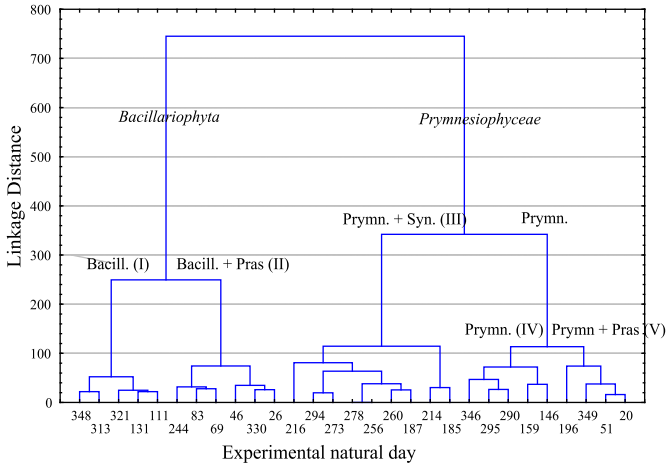

Fig. 3. Dendrogram classification (Ward's method, Manhattan distances) according to the contribution of the different phytoplankton groups to total Chl a assigned by Chemtax. Prymnesiophyceae (Prymn), Synechococcus (Syn), Prasinophyceae (Pras). Number of cases corresponds to the day of the sampling since Jan 1st. (cluster ordination as referred throughout the text).

Microzooplankton grazing removed daily ~70% of the phytoplankton stock. This average concealed a clear seasonal pattern. During spring and summer, the amount of phytoplankton grazed daily was equivalent to the standing stock (Daily turnover rate, % *Ps* ~ 100% d^{-1}), while during autumn and winter, grazing only accounted for half or less of the stock (ANOVA, $p = 0.0055$, Table 2). Overall, *Synechococcus* and *Bacillariophyta* were the most reactive groups and their stocks were turned over more than once per day. *Prymnesiophyceae* was the least reactive group and its stock was renewed every two days (Table 3).

On average, more than half of the daily phytoplankton primary production ($m/\mu_0 = 0.60 \pm 0.35$) was consumed by microzooplankton, but it varied within a wide range over the study period (range = 0.04–1.54, Table 2). Among specific groups, *Prymnesiophyceae* exhibited the lowest grazing pressure ($m/\mu_0 = 0.38 \pm 0.23$, $n = 13$), *Pelagophyceae* the highest ($m/\mu_0 = 0.67 \pm 0.17$, $n = 9$), and *Bacillariophyta*, *Synechococcus* and *Prasinophyceae* intermediate values (Table 3).

3.3. Phytoplankton chl a- and C-fluxes

Chl *a* daily synthesis and consumption rates were highest in spring (Chl *a* synthesis = 1.3 ± 1.5 μg chl *a* L^{-1} d^{-1}), minimum in summer and autumn (Chl *a* synthesis = 0.36 ± 0.18 and 0.34 ± 0.20 μg chl *a* L^{-1} d^{-1}, respectively) and intermediate in winter

Table 2
The nutrient amended (μ_n) and unamended growth rates (μ_0), grazing rates (m) and the p-value and coefficient of determination (R^2) of the model I linear regression from where the rates were estimated. Natural day as in Table 1. The nutrient limitation index (μ_0/μ_n) of phytoplankton growth. The proportion of daily primary production consumed by microzooplankton grazing (m/μ_0), the daily turnover rate of the phytoplankton stock (*%Ps*). *refers to non-valid dilution experiments.

Date	Natural day	μ_0 (d^{-1})	m (d^{-1})	μ_n (d^{-1})	R^2	p-value	m/μ_0	μ_0/μ_n	*%Ps* (%d^{-1})
2003/07/15	196	1.46 ± 0.06	1.43 ± 0.05	1.50 ± 0.04	0.92	0.002	0.98	0.98	145
2003/09/16	260	0.87 ± 0.05	0.24 ± 0.04	0.96 ± 0.07	0.732	0.007	0.28	0.9	34
2003/10/10	295*	0.49 ± 0.07	0.09 ± 0.06	0.55 ± 0.10	0.148	0.558	–	–	–
2003/11/25	330	0.42 ± 0.07	0.65 ± 0.05	0.36 ± 0.06	0.941	0.03	1.54	1.16	58
2003/12/16	350*	0.96 ± 0.19	0.56 ± 0.10	0.66 ± 0.08	0.898	<0.001	–	–	–
2004/01/26	26	0.63 ± 0.07	0.47 ± 0.07	0.54 ± 0.04	0.972	0.014	0.74	1.16	51
2004/03/23	83*	0.69 ± 0.30	0.04 ± 0.30	0.72 ± 0.30	0.208	0.441	–	–	–
2004/04/20	111	1.74 ± 0.11	0.66 ± 0.11	1.70 ± 0.10	0.709	0.002	0.38	1.02	119
2004/05/26	146	1.03 ± 0.16	0.96 ± 0.09	1.10 ± 0.16	0.721	0.056	0.93	0.94	100
2004/09/01	244	0.80 ± 0.11	0.32 ± 0.09	1.14 ± 0.06	0.628	<0.01	0.41	0.7	41
2004/09/29	273	1.78 ± 0.13	1.39 ± 0.11	1.92 ± 0.11	0.961	<0.0001	0.78	0.93	170
2004/10/21	294	0.30 ± 0.6	0.31 ± 0.06	0.56 ± 0.05	0.785	0.019	1.04	0.53	31
2004/11/17	321	0.92 ± 0.07	0.36 ± 0.07	0.95 ± 0.05	0.717	0.004	0.38	0.98	48
2004/12/14	349*	0.36 ± 0.06	0.05 ± 0.058	0.24 ± 0.05	0.077	0.438	–	–	–
2005/01/20	20	0.59 ± 0.06	0.15 ± 0.04	0.68 ± 0.038	0.492	0.024	0.26	0.87	19
2005/02/15	46	0.82 ± 0.09	0.20 ± 0.07	0.90 ± 0.06	0.507	0.048	0.25	0.92	28
2005/03/09	69*	0.66 ± 0.06	−0.04 ± 0.04	0.73 ± 0.04	0.21	0.266	–	–	–
2005/05/10	131	1.64 ± 0.12	1.40 ± 0.12	1.75 ± 0.09	0.945	<0.0001	0.85	0.94	158
2005/06/07	158*	0.76 ± 0.12	−0.03 ± 0.11	0.52 ± 0.11	0.006	0.859	–	–	–
2005/07/05	187	1.91 ± 0.05	1.04 ± 0.032	1.93 ± 0.03	0.991	<0.0001	0.55	0.99	166
2005/08/03	215	1.06 ± 0.07	0.57 ± 0.05	1.40 ± 0.03	0.959	<0.0001	0.54	0.76	74
2005/09/13	257	1.16 ± 0.11	0.37 ± 0.05	1.57 ± 0.05	0.952	<0.0001	0.32	0.74	57
2005/10/05	278	0.77 ± 0.05	0.35 ± 0.02	0.82 ± 0.02	0.46	0.208	0.46	0.94	44
2005/11/09	313	0.65 ± 0.06	0.03 ± 0.05	0.74 ± 0.04	0.025	0.709	0.04	0.88	4
2005/12/13	348	0.81 ± 0.107	0.42 ± 0.06	0.84 ± 0.06	0.779	0.004	0.52	0.96	51
2006/12/11	346	0.37 ± 0.06	0.26 ± 0.06	0.41 ± 0.05	0.674	<0.01	0.71	0.88	27
2007/02/21	51	0.58 ± 0.12	0.20 ± 0.04	0.86±	0.700	<0.01	0.35	0.67	25
2007/03/05	65	0.68 ± 0.05	0.76 ± 0.05	0.60 ± 0.04	0.788	<0.0001	1.12	1.15	73
2007/07/03	185	0.90 ± 0.07	0.62 ± 0.06	1.14 ± 0.06	0.931	<0.01	0.69	0.79	72
2007/08/01	214	1.53 ± 0.10	0.69 ± 0.10	1.45 ± 0.07	0.862	<0.01	0.45	1.06	108
2007/10/16	290	0.88 ± 0.12	0.38 ± 0.08	1.26 ± 0.07	0.722	0.04	0.44	0.7	50

(0.71 ± 0.37 μg chl *a* L^{-1} d^{-1}). C:chl *a* ratios ranged between 11 and 135 (g:g) over the 2003–2004 period. Minimum and maximum C:chl *a* were observed during the *Bacillariophyta* dominated-spring bloom recorded in April 2004 and during late spring post-bloom stratified conditions dominated by *Prymnesiophyceae*, respectively. Most values fell between 40 and 63 (25 and 75% percentile, respectively). Phytoplankton carbon synthesis ranged from 7.0 to 46 μg C L^{-1} d^{-1}, averaging 33 ± 11 μg C L^{-1} d^{-1}. The mean carbon grazed ranged between similar values (7.2–36 μg C L^{-1} d^{-1}) but was lower on average (18 ± 12 μg C L^{-1} d^{-1}). Carbon synthesis and consumption were highest during winter and spring, lowest in autumn and intermediate during summer (Fig. 5).

On a year-round average, *Bacillariophyta* and *Prymnesiophyceae* were the major contributors to carbon fluxes, followed by *Prasinophyceae*, *Synechococcus* and *Pelagophyceae* (Fig. 5). In winter, *Bacillariophyta and Prasinophyceae* dominated phytoplankton carbon synthesis (30 and 28%, respectively). *Bacillariophyta* synthesized most of the phytoplankton carbon during the spring bloom (75%) and *Prymnesiophyceae* during the post-bloom conditions in the spring. During the oligotrophic conditions in summer, *Prymnesiophyceae* (40%) and *Synechococcus* (29%) became responsible for most of the carbon production. *Bacillariophyta* dominated community carbon production during autumn (Fig. 5).

4. Discussion

4.1. Environmental conditions and phytoplankton community composition

Phytoplankton composition followed a clear seasonal pattern consistent with previous observations in the area and current knowledge of phytoplankton group environmental preferences. For example, *Bacillariophyta* were abundant during winter and peaked in early spring and autumn (Margalef and Castellví, 1967; Estrada, 1985; Mura et al., 1996; Marty et al., 2002) coinciding with the onset of spring stratification and the erosion of the summer thermocline in this area (Vila-Costa et al., 2007). *Synechococcus* was most abundant in summer (Agawin et al., 1998) when well-stratified and oligotrophic conditions are typically found (Alonso-sáez et al., 2008; Boras et al., 2009). Cluster analysis of the temporal distribution of phytoplankton groups allowed identifying recurrent phytoplankton associations succeeding throughout (Fig. 3). Despite the exploratory nature of this analysis some remarks can be made about the environmental preferences of these clusters. The main branches of the cluster separate communities dominated by either *Bacillariophyta* or *Prymnesiophyceae*, the overall dominant groups (Fig. 3). Within *Bacillariophyta*-dominated communities two subclusters were distinguished based on whether *Bacillariophyta* alone (i.e. represent more than 50% of chl *a* stock, Cluster I) or together with *Prasinophyceae* (Cluster II) were dominant. Cluster I comprised the spring 'entrainment' and the autumn 'deentrainment' bloom conditions (Cullen et al., 2002) under which *Bacillariophyta* dominated because of their competitive advantage in high nutrient and moderately mixed water conditions (Sarthou et al., 2005). The *Bacillariophyta–Prasinophyceae* association prevailed during the coldest period of the year (January-to-March), in agreement with the affinity of *Prasinophyceae* for strong mixing conditions reported in other temperate coastal (Bustillos-Guzmán et al., 1995; Rodriguez et al., 2003, 2006; Anderson et al., 2008) and open waters (Marty et al., 2002; Latasa et al., 2010). Interestingly, the same association was observed (exp 329, November 2003) after a sharp peak in wind energy flux and significant wave height recorded at the end of

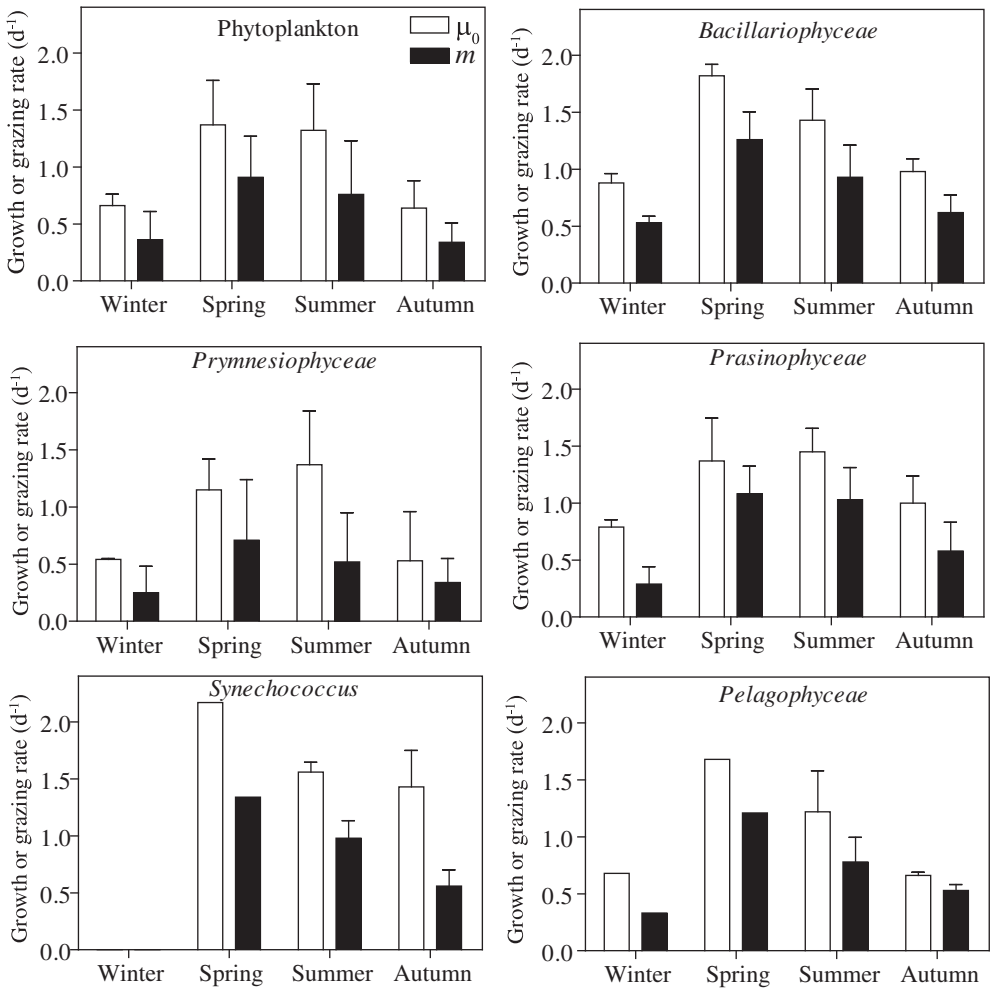

Fig. 4. Total phytoplankton and main phytoplankton groups' mean growth ($\mu 0$, d^{-1}) and grazing rates (m, d^{-1}) for spring, summer, autumn and winter. The error bars represent the standard deviation.

October 2003 (Guadayol et al., 2009b) probably lead to active mixing of the water column.

The *Prymnesiophyceae–Prasinophyceae* cluster (Cluster V) observed during early winter was generally associated to lower chl *a* levels than those recorded during the dominance of the contemporaneous *Bacillariophyta–Prasinophyceae* assemblage. The difference between the two assemblages appears as a response to the decay in the concentration of *Bacillariophyta* rather than an increase of *Prymnesiophyceae*. In fact, *Prymnesiophyceae* dominated phytoplankton assemblage (Cluster IV) during both transition periods following the decline of *Bacillariophyta* and *Synechococcus* after the spring bloom and summer period, respectively. Notably, *Prymnesiophyceae* were present throughout the year at relatively high concentrations and constituted the background of the autotrophic community in Blanes Bay (Fig. 2) upon which other groups build under suitable conditions.

Table 3
Group-specific average of growth rate (μ_0), grazing rate (m), the proportion of daily primary production consumed by microzooplankton grazing daily (m/μ_0), and the percentage of stock consumed daily (%*Ps*). The number in brackets refers to the number of experiments included in the statistics.

	μ_0	m	m/μ_0	%*Ps* (%d^{-1})
Phytoplankton	1.0 ± 0.46 (25)	0.57 ± 0.40	0.60 ± 0.34	68 ± 52
Bacillariophyta	1.3 ± 0.52 (16)	0.82 ± 0.52	0.62 ± 0.22	101 ± 58
Prymnesiophyceae	1.0 ± 0.44 (13)	0.42 ± 0.39	0.38 ± 0.27	56 ± 49
Prasinophyceae	1.2 ± 0.53 (16)	0.72 ± 0.60	0.56 ± 0.29	85 ± 60
Synechococcus	1.5 ± 0.33 (9)	0.86 ± 0.37	0.53 ± 0.13	120 ± 53
Pelagophyceae	1.0 ± 0.60 (9)	0.70 ± 0.36	0.67 ± 0.17	90 ± 14

4.2. Phytoplankton growth dynamics

Phytoplankton growth rate varied seasonally, reaching higher values during spring and summer than during fall and winter (Fig. 4), a seasonal trend consistent with previous studies in the North Pacific East and West coast (Strom et al., 2001; Kim et al., 2007). Further inspection reveals differences in the group-specific dynamics within the general seasonal signal. *Bacillariophyta* and *Synechococcus* tended to grow systematically faster than *Prymnesiophyceae* (Furnas, 1990; Strom and Welschmeyer, 1991; Latasa et al., 1997). However, intra-group variability was higher than inter-group variability suggesting that community composition played a minor role compared to environmental factors in determining the observed seasonality in phytoplankton growth rates.

Higher temperature and regenerated nutrients favour picophytoplankton abundance and contribution to primary production (Agawin et al., 2000; Murrell and Lores, 2004; Gaulke et al., 2010). Our findings are consistent with these observations. For instance, *Synechococcus* abundance ($r = 0.71$, $p < 0.01$) and its contribution to

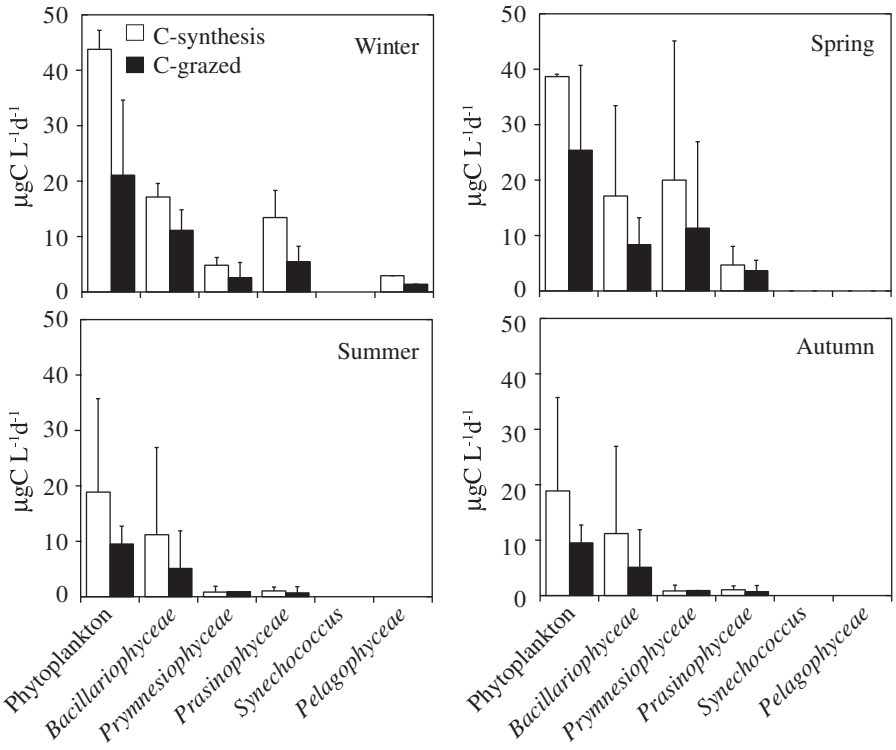

Fig. 5. Total phytoplankton and main phytoplankton groups' carbon synthesis (white bars) and carbon consumption by microzooplankton (black bars) mean values for winter, spring, summer and autumn. C flows are expressed in µg C L^{-1} d^{-1}. The error bars represent the standard deviation.

autotrophic carbon production ($r = 0.81$, $p < 0.05$) increased with temperature. Interestingly, the positive relation of *Synechococcus* abundance and primary production with temperature was not observed for growth rates ($r = 0.07$). A similar pattern emerges from data collected in a temperate eutrophic estuary (Wetz et al., 2011). Weltz et al. showed that the chl *a* fraction <3 µm was positively correlated with temperature ($r = 0.51$, $p < 0.001$), however the growth rates for this same fraction were not ($r = 0.31$, $p = 0.44$, Wetz et al., 2011). Unlike other studies (Agawin et al., 1998; Strom et al., 2001; Kim et al., 2007) the growth of total phytoplankton in Blanes Bay showed a weak dependence on temperature (r-pearson = 0.29, $p = 0.21$). Only the growth of *Prymnesiophyceae* showed a significant increase with temperature ($r = 0.54$, $p < 0.05$). However, the weak response for the rest of the groups released dependence of phytoplankton growth rates on temperature (Juhl and Murrell, 2005). The uncoupling between growth rate and biomass response to temperature highlights the role of grazing in shaping picophytoplankton abundance variations.

In the other hand, day length explained a large fraction of the variability in phytoplankton growth rates observed year-round ($r = 0.73$, $n = 24$). Day length strongly covariate with temperature ($r = 0.54$, $p < 0.05$) and is highly correlated to light intensity and dosage, i.e. shorter day length coincide with lower irradiance per hour and integration per day. Morán and Estrada (2005) observed a strong covariation between the maximum photosynthetic rates (P^B_m) and total daily irradiance along a seasonal cycle in the northwestern Mediterranean. The authors argued that changes in C:chl *a* ratio could explain this covariation. They pointed out that despite the known influence of nutrient status on C:chl *a* ratios (Geider et al., 1998), non significant correlation was observed between inorganic nutrient concentration and P^B_m and concluded that "… photosynthetic parameters seem to be greatly and coherently determined by incident PAR". Our results, showing a strong correlation between growth rate and day length and the lack of clear relation between the former and inorganic nutrient concentration, suggest that irradiance might have more influence than nutrient concentration not only on photosynthesis (Tilstone et al., 2003; Smyth et al., 2004; Morán and Estrada, 2005) but also on phytoplankton growth.

4.3. Microzooplankton grazing and its relationship phytoplankton growth

Grazing rates followed the seasonal trend exhibited by growth rates, but differences between periods were less marked (Fig. 4). The strong correlation found between growth and grazing ($r = 0.73$, $p < 0.0001$) is often reported in studies using the dilution technique (Strom and Welschmeyer, 1991, 2007; Latasa et al., 2005; McManus et al., 2007; Chen et al., 2009). The potential influence of methodological artefacts in this correlation was evaluated elsewhere (Gutiérrez-Rodríguez et al., 2009) confirming the ecological nature of the observed relation between growth and grazing dynamics. The factors regulating microzooplankton grazing rates in the sea are still poorly understood (Strom et al., 2007). Temperature affects different variables such as maximal growth rates or the clearance rate of heterotrophic protists (Rose and Caron, 2007; Unrein et al., 2007). However, grazing rates were not apparently affected by temperature in our study (r-pearson = 0.22, $p = 0.37$) suggesting that other factors masked the physiological response of microzooplankton to temperature.

The proportion of phytoplankton growth removed by microzooplankton constituted the major loss for phytoplankton production (60% of PP) in agreement with the globally estimated 60% for coastal ecosystems (Calbet and Landry, 2004). Perhaps, more interesting than the year-round average, is the analysis of the large variability observed (4%–154%). It is assumed that the

coupling between microzooplankton grazing and growth tighter and grazing impact higher in recycling systems where small phytoplankton dominate the community than in export systems dominated by larger cells (Smetacek, 1999). However, the high grazing activity during the spring bloom of 2005 (exp. 131) along with the similar m/μ_0 observed for the small-cell community of *Prymnesiophyceae–Synechococcus* (0.58) and the year-round average for bulk phytoplankton (0.56), support the emerging view that microzooplankton grazing can be substantial in phytoplankton communities dominated by large cells (Sherr and Sherr, 2007; Landry et al., 2008; Gutiérrez-Rodríguez et al., 2010). A seasonal study by Paranjape (1990) on the Grand Bank (Newfounland, Canada) also found lower grazing rates for the flagellate-dominated nanophytoplankton community in summer compared to grazing rates in spring *Bacillariophyta*-dominated community.

The fate of the small phytoplankton dominated production escaping microzooplankton control during summer is unclear. The concentration of Appendicularia reported during this season in Blanes Bay (Calbet et al., 2001) and their high feeding rates on picophytoplankton (Gorsky et al., 1999; Scheinberg et al., 2005) make this group a potential candidate for consuming this net production. Phytoplankton cell lysis, particularly high during the summer (Agustí and Duarte, 2000), is another important loss term for phytoplankton production that one could invoke but this mortality term is already accounted for in the intrinsic growth rates estimated by the dilution technique (Latasa et al., 2005). Alternatively, UV radiation, revealed as a substantial source of mortality in surface oligotrophic waters (Llabrés and Agustí, 2006), could channel part of the measured net autotrophic production into the microbial loop.

4.4. C:chl a and C-fluxes

Carbon fluxes through major phytoplankton groups were estimated from their carbon biomass, growth and grazing rates. Differences in group-specific biomass were larger than differences in rates, and thus, the proportion of total carbon flow channelled by different groups could be largely anticipated from their contribution to biomass. Beyond this general trend, the contribution of *Bacillariophyta* and *Synechococcus* to fluxes was higher than to biomass due to their fast-growth nature, while the opposite was true for slow-growing *Prymnesiophyceae*.

Chl *a* fluxes associated to the *Bacillariophyta*-dominated spring bloom cluster were on average 3- and 10-fold higher than those observed when *Bacillariophyta–Prasinophyceae* and *Prymnesiophyceae–Synechococcus* dominated in winter and summer, respectively. Interestingly, these differences were largely smoothed when fluxes were transformed to carbon equivalents. A more detailed analysis of environmental conditions along with the variables included in the C-fluxes calculations (chl *a*, C:chl *a*, growth and grazing rates), offers some insights into carbon dynamics associated to major phytoplankton assemblages occurring throughout the year. For example, the *Bacillariophyta–Prasinophyceae* assemblage dominating in winter (Cluster II) presented typically low growth rates possibly due to low irradiance conditions (intense mixing and low incident irradiance). However, C:chl *a* moderate ratios and high chl a concentration resulted in moderate carbon synthesis rates ($\sim$30 μg C L^{-1} d^{-1}). *Bacillariophyta* dominated the community biomass during early-spring and autumn (Fig. 2), but the proportion of primary production escaping from microzooplankton grazing was highly variable. For instance, it was much higher in the 2005 bloom compared to that of 2004 (m/μ_0 = 0.38 and 0.85, respectively). In autumn, phytoplankton growth rates (0.80 ± 0.14 d^{-1}) were close to the annual average in contrast to grazing rates that were the lowest of the year allowing a large fraction of production to escape from herbivorous grazing pressure. These observations highlight the importance of grazing in determining the magnitude and direction of biomass change of phytoplankton (Behrenfeld, 2010; Wetz et al., 2011), a fact frequently overlooked in the interpretations of ocean community dynamics (Banse, 1994).

High turnover of autotrophic communities has been proposed to sustain the relatively high heterotrophic:autotrophic biomass ratio observed in oligotrophic systems (Odum, 1971; Gasol et al., 1997). This view has been recently challenged by Marañón (2005), who based on an extensive analysis of chl *a*-normalized photosynthesis and C:chl *a* values from the literature, concluded that phytoplankton growth in subtropical gyres is low and nutrient limited (Marañón, 2005). In Blanes Bay, the *Prymnesiophyceae–Synechococcus* association dominated the autotrophic community during summer oligotrophic conditions, in agreement with observations in open ocean systems of temperate latitudes during the summer (e.g. Siegel et al., 1990; Latasa et al., 2010). The high growth and grazing rates associated to the *Prymnesiophyceae–Synechococcus* community suggest that oligotrophic systems are better conceptualized as consumer regulated systems where the autotrophic pool is rapidly renovated by means of a tight and efficient coupling between grazers and primary producers (Goldman et al., 1979; Banse, 1994; Gasol et al., 1997).

5. Conclusions

Phytoplankton composition exhibited a clear seasonal variability consistent with previous observations in temperate latitudes. Moreover, different phytoplankton associations consistently developed under specific physico-chemical conditions and their appearance could be link to particular environmental conditions. Phytoplankton growth also followed a strong seasonality being higher during spring and summer than during autumn and winter. Day length seemed to be the main factor controlling growth rate variability with temperature playing a minor role. Microzooplankton grazing rates were strongly correlated with phytoplankton growth rates at both community and class level. Different rate patterns could be assigned to the main phytoplankton groups. Dominance of fast-growing diatoms species during nutrient-rich early spring conditions and that of *Synechococcus* during summer stratified conditions boosted phytoplankton growth rate in this period of the year. However, the variability introduced by the taxonomic composition of phytoplankton was of second order compared to that created by abiotic factors. Day length for instance, explained a large fraction of phytoplankton growth rate variability. Finally, we described different carbon flow patterns for the major phytoplankton associations observed in this system. The consistent link between these associations and physico-chemical conditions along with the carbon flow patterns described for each community type increases our ability to characterize the function of different associations thriving under defined environmental conditions.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ecss.2011.08.008.

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