

## Strong seasonality in phytoplankton cell lysis in the NW Mediterranean littoral

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### Abstract

The seasonality of phytoplankton cell lysis rates in the NW Mediterranean coastal waters was examined based on measurements of the dissolved esterase activity between 1995 and 1998 in the Blanes Bay (NE Spain). The temporal variability of phytoplankton biomass and gross primary production (GPP) were characterized by a late winter bloom dominated by diatoms and high GPP in summer despite the low phytoplankton biomass in this season. The phytoplankton lysis rates were found to be strongly seasonal, being highest in summer (mean  $\pm$  SE =  $0.41 \pm 0.049$  d<sup>-1</sup>) when values as high as  $1.47$  d<sup>-1</sup> were attained. However, during the rest of the year, phytoplankton lysis rates remained low, particularly in winter ( $0.061 \pm 0.005$  d<sup>-1</sup>). There was a strong ( $R^2 = 0.71$ ,  $P < 0.00001$ ) positive relationship between monthly average lysis rates and water temperature. In addition, monthly average lysis rates followed gross primary production with a maximum correlation ( $r = 0.65$ ,  $P < 0.05$ ) at a lag time of 1–2 months, similar to the time span from bloom initiation to bloom collapse. These results identify phytoplankton cell lysis as an important route of phytoplankton carbon flow in Blanes Bay.

The elucidation of the fate of photosynthetic carbon in the planktonic ecosystem is a central component of the examination of marine food webs and their role in the carbon and nutrient budget in the sea (Forsberg 1985; Duarte and Cebrián 1996). Traditionally, sedimentation and grazing losses were considered to be the dominant fluxes (e.g., Walsh 1983). However, the findings of a strong coupling between phytoplankton and microbial heterotrophs (Pomeroy 1974; Cole et al. 1988) and the existence of a large, labile dissolved organic carbon (DOC) pool in surface waters (e.g., Carlson et al. 1994) have drawn considerable attention to the role of DOC release by phytoplankton as a major route of carbon transfer in marine food webs.

Release of DOC from phytoplankton was believed, however, to involve exudation by functional cells (Sharp 1977) or to result from grazing activity (i.e., sloppy feeding). Yet, recent evidence derived from C budgets in phytoplankton cultures (Biddanda and Benner 1997) and the use of enzymatic tracers (van Boekel et al. 1992; Brussard et al. 1995; Baldi et al. 1997; Agustí et al. 1998; Berges and Falkowski 1998) has pointed to cell lysis as a major process for DOC release from phytoplankton, whereas estimates in the North Sea indicate cell lysis to be a relatively unimportant loss process (Brussard et al. 1995). Recent reports assign a much greater role for phytoplankton cell lysis in the Mediterranean Sea during summer, where cell lysis is responsible for the development of mucilaginous masses in the Adriatic (Baldi et al. 1997) and for the loss of 50% of the summer gross primary production in the NW Mediterranean (Agustí et al. 1998). Whether the high phytoplankton lysis rates described

for the NW Mediterranean are sustained over the year or represent a summer phenomenon is, however, unknown. Cell lysis results from phytoplankton cell death, independent of its causes, so that cell lysis may be caused by a number of factors, including injury due to external factors, such as exposure to extreme growth conditions (e.g., photodamage, high temperature), virus infection, or programmed death (i.e., apoptosis) of stressed cells (cf. Agustí et al. 1998; Berges and Falkowski 1998; Kirchman 1999). Whatever the causes, phytoplankton lysis results in the release of the intracellular components to the surrounding seawater.

We describe here, based on a 3-yr study in Blanes Bay (NE Spain), the seasonality of phytoplankton cell lysis rates in the NW Mediterranean coastal waters. The Blanes Bay is a relatively shallow (<25 m), exposed bay with a considerable water exchange with the offshore waters. The seasonal patterns in planktonic processes reported for the Bay of Blanes have been found to be representative of seasonal processes at the basin (NW Mediterranean) scale (Duarte et al. 1999), so that any strong seasonal signal present in the Bay of Blanes is likely to represent a feature to be found elsewhere in the NW Mediterranean. We quantify cell lysis rates by the measurement of the dissolved esterase activity (van Boekel et al. 1993; Brussard et al. 1995; Agustí et al. 1998), calculate the phytoplankton carbon lost through this process, and compare it to the gross primary production rates in these waters. The estimates of cell lysis rates are based on the calculation of the release of intracellular esterases to the medium, which occurs following cell death and damage to the membranes conducive to cell death.

### Methods

The study was conducted in the Blanes Bay (NE Spain), NW Mediterranean Sea (41°39.90'N, 2°48.03'E). The Blanes Bay is an open and exposed bay, and its shallow waters are vertically mixed year round and receive little freshwater input during most of the year, except for irregular freshwater pulses during storms (Cebrián et al. 1996). Salinity ranges

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between 37 and 38‰ and is remarkably uniform over the year. Temperature ranges from 11°C (January to March) to 26°C (August to September). Phosphate and nitrate levels are generally low in summer (concentrations below 0.15 and 0.3  $\mu\text{M}$ , respectively), and net community production is low throughout the year, except during phytoplankton blooms (Satta et al. 1996a). The most important bloom during the year occurs in late winter (January–March cf. Duarte et al. 1999), when the community is dominated by centric diatoms, whereas lower phytoplankton biomass is reached during the summer, when the water column is poor in nutrients and the phytoplanktonic community is dominated by small cells (Mura et al. 1996a).

Surface seawater ( $\approx 0.5$  m) samples were collected at a fixed station (about 20 m depth) in Blanes Bay once a week from January 1995 to September 1997 and every 2 weeks from October 1997 to January 1998. The sampling frequency was higher (daily or three times a week) during the beginning of the sampling (January and February 1995). Water samples were collected from an outboard motor boat with clean 5-liter bottles, and samples were processed at the laboratory within 30 min to 1 h after sampling.

At the laboratory, 250 ml were filtered through a Whatman GF/F filter for fluorometric analysis of chlorophyll *a* (Chl *a*) concentration (Parsons et al. 1984). The filters were homogenized and kept refrigerated in the dark while pigments were extracted in 90% acetone for ca. 6 h. Fluorescence was then measured in a Turner Designs fluorometer. Chlorophyll *a* was transformed to carbon (Phyt<sub>C</sub>) by applying a factor of 50 mg C/mg Chl *a* (Harris 1986; Li et al. 1993).

Phytoplankton cell lysis rates were estimated from the quantification of dissolved esterase activity measured using the spectrofluorometric technique described by van Boekel et al. (1992), as modified by Agustí et al. (1998). Esterases are useful tracers of cell lysis because they are strictly cytoplasmatic and appear in the media as a result of cell breakage or membrane damage (e.g., Rotman and Papermaster 1966; van Boekel et al. 1992). In addition, their concentration in autotrophs is far greater than that in heterotrophs so that they provide a reliable tracer of phytoplankton cell contents (Agustí et al. 1998). This method quantifies the abundance of the enzyme from its activity at a constant temperature (20°C) in the presence of excess substrate. Nonspecific esterases cleave off the acetates of fluorescein di-acetate (FDA), which is added as a substrate, yielding the fluorescent compound fluorescein (e.g., Widholm 1972). Fluorescein accumulates in samples as a product of the reaction between esterases and FDA and increases with increasing FDA concentration according to Michaelis–Menten enzyme kinetics (e.g., Rotman and Papermaster 1966).

Dissolved esterase activity (EA, as FDA hydrolysis) was measured in four replicates for each sampling event. In short, 5 ml of water were filtered through 0.45  $\mu\text{m}$  Millipore Millex, and 50  $\mu\text{l}$  of EDTA and 50  $\mu\text{l}$  of FDA (Sigma) were added to the samples (to a final concentration of 0.02 and 0.2 mM, respectively) and mixed in a vortex mixer. After incubating the samples for 1 h at 20°C, the fluorescence emission was immediately measured in a Shimadzu RF-5000 spectrofluorometer at 451 nm and 510 nm excitation and

emission (10-nm bandwidth) wavelengths, respectively. Fluorescein production (nmol Fluorescein  $\text{L}^{-1} \text{h}^{-1}$ ) was calculated from the fluorescence development using a standard calibration curve determined by measuring the fluorescence of a range of fluorescein (Sigma) solutions (3 to 2,000 nmol Fluorescein  $\text{L}^{-1}$  of filtered seawater).

The particulate esterase activity (PEA) needed to estimate the lysis rate (see below) was calculated from the measured Chl *a* concentration (cf. van Boekel et al. 1992) using an average ratio of particulate esterase activity to Chl *a* of  $331 \pm 39$  nmol F ( $\mu\text{g Chl } a$ ) $^{-1} \text{h}^{-1}$  (Agustí et al. 1998).

Cell phytoplankton lysis rate ( $\mu_{\text{L}}$   $\text{d}^{-1}$ ) was calculated as the decrease in particulate esterase activity (PEA) with time (*t*) due to the production of dissolved EA during cell lysis and is calculated following the equation

$$\mu_{\text{L}} (\text{d}^{-1}) = \frac{\ln\left(\frac{\text{PEA}_t}{\text{PEA}_0}\right)}{t}, \quad (1)$$

where  $\text{PEA}_0$  represent the initial particulate esterase activity, estimated as described above, and  $\text{PEA}_t$  is the particulate esterase activity expected after a time interval *t* (i.e.,  $\text{PEA}_0$  minus the production of dissolved EA calculated over the time interval *t*).

The production of dissolved EA was derived by combining the measured dissolved EA activity with estimates of the rate of loss of the activity of the enzyme calculated in 10 experiments conducted in different seasons. Because esterases are intracellular enzymes, their release to the medium involves a severe change in ambient conditions (e.g., pH and exposure to oxidizing agents), which explains the general loss of activity of intracellular enzymes when released to seawater (Wetzel 1991). The experiments consisted of adding commercial esterases (porcine liver esterases, Sigma E-3128) to water samples from Blanes Bay, maintained at “in situ” temperature (12–21°C). The time series of loss of stability of EA was followed by sampling at increasing time intervals (e.g., 0, 1, 3, 6, 12, 24, and 36 h) to obtain an exponential decay curve. The rate of EA decrease ( $\mu(\text{loss})_{\text{EA}}$ ) was calculated from the changes in dissolved EA during time (*t*, hours) by fitting the exponential decay equation

$$\mu(\text{loss})_{\text{EA}} (\text{h}^{-1}) = \frac{\ln\left(\frac{\text{EA}_0}{\text{EA}_t}\right)}{t}. \quad (2)$$

$\mu(\text{loss})_{\text{EA}}$  was then used to calculate the half-life ( $T_{1/2}$ ) of the dissolved seawater–water as

$$T_{1/2} (\text{h}) = \ln(0.5)/\mu(\text{loss})_{\text{EA}}. \quad (3)$$

Assuming the dissolved EA to be in steady state at a time scale of a few hours, the loss and production rates of dissolved EA should be in balance (cf. Agustí et al. 1998). The assumption of steady state between release and breakdown of released esterases was supported by previous results (Agustí et al. 1998) and supported by the finding of strong diel cycles in DOC concentration in the Mediterranean (Gasol et al. 1998), as well as the observation that the mean

difference between dissolved esterase activity between consecutive days in this study was  $16 \pm 3\%$  ( $N = 32$ ), similar to the difference between replicated measurements. The production rate of dissolved EA ( $EA_{\text{Prod}}$  nmol F L<sup>-1</sup> h<sup>-1</sup>) in 1 d was then calculated from  $T_{1/2}$  (transformed to 24 h) and the values of dissolved EA in the water (EA) as

$$EA_{\text{Prod}} \text{ (d}^{-1}\text{)} = \frac{\frac{1}{2}EA}{T_{1/2}}, \quad (4)$$

and phytoplankton lysis rate ( $\mu_L$ ) was calculated using Eq. 1 by replacing  $PEA_t$  by  $PEA_0 - EA_{\text{Prod}}$ .

$$\mu_L \text{ (d}^{-1}\text{)} = \ln\left(\frac{PEA_0 - EA_{\text{Prod}}}{PEA_0}\right) \quad (5)$$

The estimates of cell lysis rates reported here rely on the assumption of a constant esterase:chlorophyll ratio to calculate the particulate esterase activity, which may not hold across seasons. We used error propagation techniques to estimate the uncertainty about the estimates of cell lysis rates derived from variation in the esterase to chlorophyll ratio and the error about the estimated half-life of dissolved esterase activity. The ratio of esterase to chlorophyll varies by a factor of two among species (Agustí et al. 1998), with the standard error of the mean being about 12% of the average value. This estimate of variability in the ratio was derived from the analysis of a limited set of phytoplankton species (Agustí et al. 1998) and needs to be verified by examination of a broader set of species. The error analysis was based on a bootstrap approach (Efron and Tibshirani 1986) based on the combination of values, randomly sampled from the normal distribution of the esterase half-life for the particular temperature with the mean and standard deviations derived from the regression between half-life and water temperature, and the mean and standard deviation of esterase:Chl *a*. This process was iterated 1,000 times, yielding an estimate of the mean error estimate due to these sources of  $13 \pm 0.1\%$ , on average, of the mean estimates of lysis rates derived here. This source of uncertainty, which is comparable to the variability among replicates, is of minor importance when compared to the >15-fold variation in rates encountered along the study.

Gross oxygen production (GPP) rates were calculated from oxygen variations after incubation of samples in "light" and "dark" bottles. At each sampling event, water samples were filtered through a 243- $\mu\text{m}$  mesh to remove macrozooplankton and carefully siphoned into 125-ml narrow-mouth Winkler bottles. Five replicate bottles were immediately used to determine the initial oxygen concentration, and incubations of dark and light bottles were carried out using five replicates each. These bottles were incubated for 20–24 h at surface sea temperature in an incubator. The relatively long incubation times, similar to those used for plankton-poor waters (e.g., Williams and Jenkinson 1982) were necessary to obtain reliable estimates of oxygen changes in these sparse plankton communities. Light bottles were incubated at 200  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Dissolved oxygen concentrations were analyzed using Winkler titration, as modified by Carritt and Carpenter (1966), with automatic

potentiometric end-point detection based on changes in Eh potential (Oudot et al. 1988) determined with a Mettler DL-21 Autotitrator. The coefficient of variation for replicated estimates of dissolved oxygen concentration was 0.4%. Gross production ( $\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ ) was calculated as the sum of the rate of change in oxygen concentration in light bottles, converted to daily (24-h) estimates by considering the length of the day and night periods for each sampling event, and that of oxygen consumption in dark bottles. We chose to measure GPP instead of net primary production as a basis to compare the importance of lysis as a loss process because of uncertainties as to the measurement of the net primary production.

## Results

The average Chl *a* concentration in Blanes Bay from January 1995 to January 1998 was  $0.76 \pm 0.05 \mu\text{g Chl } a \text{ L}^{-1}$  (mean  $\pm$  SE), but it was highly variable, ranging from a maximum of  $5.44 \mu\text{g Chl } a \text{ L}^{-1}$  (January 1996) to a minimum of  $0.06 \mu\text{g Chl } a \text{ L}^{-1}$  (August 1996). Phytoplankton Chl *a* in Blanes Bay showed a seasonal pattern (Fig. 1) that was characterized by the presence of an important bloom in late winter (generally in January–February) and a series of less important blooms occurring in June–July and in the fall (Fig. 1). The minimum Chl *a* concentration was observed in summer (August and September, Fig. 1). Despite an identifiable seasonality of phytoplankton Chl *a* concentration in Blanes Bay, there was high interannual variability as well (Fig. 1). The winter bloom of 1995 was characterized by a series of small blooms extending until the spring, in contrast with that observed during January of 1996, when the most important bloom in the series described was observed (Fig. 1). Similarly, there was interannual variability in the lowest phytoplankton biomass observed, and the summer of 1996 was the period when Chl *a* has reached the lowest values (Fig. 1).

Gross primary production (GPP) varied from 0.02 to 10.9  $\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$  during the period studied. Monthly averaged GPP values showed a clear seasonal pattern, showing the highest GPP during the summer (Fig. 2a). The difference in the magnitude of GPP between years was, however, small (Fig. 2a). The seasonal variability observed for GPP was in contrast with the pattern observed for phytoplankton biomass, since the periods of highest production in Blanes Bay (Fig. 2a) corresponded to the periods when phytoplankton biomass was minimal (Fig. 1). As a consequence, the turnover of phytoplankton carbon varied seasonally and was much higher during the summer than during the rest of the year, being lowest in winter (Fig. 2b). The fastest turnover was found during the summer of 1996, as a consequence of the low phytoplankton biomass found during this summer despite high gross primary production, comparable to those observed in 1995 and 1997 (Fig. 2a).

Dissolved esterase activity (as FDA hydrolysis rates, nmol F L<sup>-1</sup> h<sup>-1</sup>) showed a clear seasonal pattern characterized by low values in spring and fall and high values during the summer and in mid- to late winter (January–February, Fig. 3). This pattern differed greatly from that found for phyto-

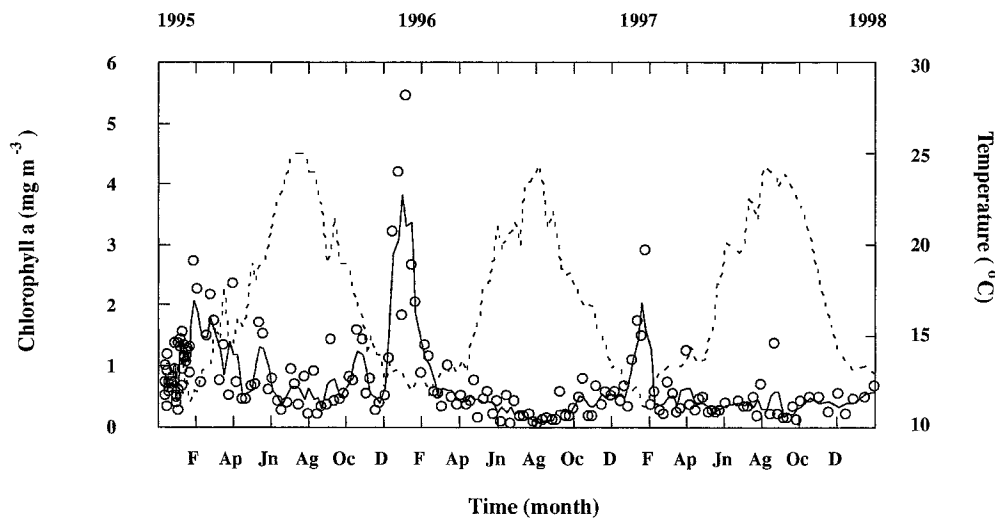


Fig. 1. Temporal evolution of water temperature (broken line) and Chl *a* concentration in Blanes Bay from January 1995 to January 1998. Open circles and solid line represent the individual measurements and the smoothed (three-point running average) trend in Chl *a* concentration, respectively.

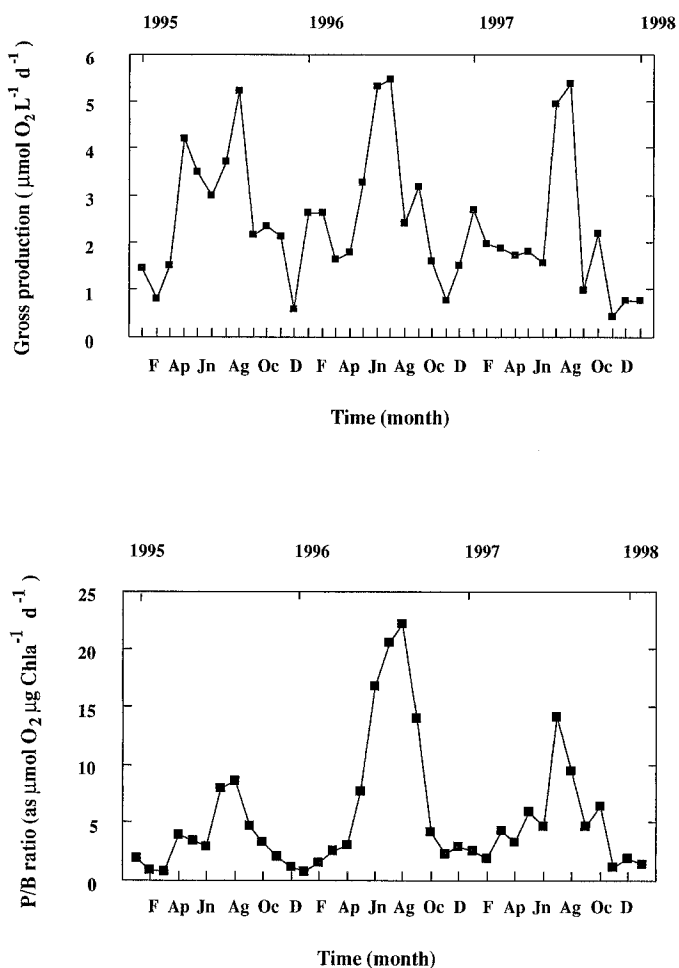


Fig. 2. Monthly average gross primary production (upper panel) derived from  $O_2$  evolution and phytoplankton P/B ratio (lower panel; GPP/phytoplankton Chl *a*) in Blanes Bay from 1995 to 1998.

plankton biomass (Fig. 1). The average value of dissolved esterase activity (DEA) in Blanes Bay was  $49.1 \text{ (nmol F L}^{-1} \text{ h}^{-1}\text{)}$ , ranging from  $17.9$  to  $137 \text{ nmol F L}^{-1} \text{ h}^{-1}$ , respectively. Interannual variability in DEA was also considerable in Blanes Bay, where the maximum values observed during the summer of 1996 were much lower than those reached during the summers of 1995 and 1997 (Fig. 3).

The activity of dissolved esterases decreased exponentially with time at rates that varied between  $-0.017$  and  $-0.05 \text{ h}^{-1}$ , indicating a variation in half-life of DEA of 40 to 14 h, respectively, over the temperature range examined ( $12$ – $21^\circ\text{C}$ ). Temperature explained 54% of the differences in the rate of loss of stability of dissolved EA among the experiments (Fig. 4), as described by the following relationship.

$$\mu(\text{loss})_{\text{EA}} \text{ (h}^{-1}\text{)} = -0.00248 \times T \text{ (}^\circ\text{C)} + 0.0092$$

$$R^2 = 0.54 \quad P < 0.009$$

This relationship was used to predict the half-life of DEA in Blanes Bay from the water temperature in situ needed to calculate mortality estimates. The particulate esterase activity in phytoplankton cells (PEA,  $\text{nmol F L}^{-1} \text{ h}^{-1}$ ) calculated from Chl *a* concentration was on average  $250 \pm 17$  (mean  $\pm$  SE)  $\text{nmol F L}^{-1} \text{ h}^{-1}$  and varied from 1.23 to  $1,800 \text{ nmol F L}^{-1} \text{ h}^{-1}$ .

Phytoplankton lysis rates in Blanes Bay ranged broadly from  $0.0083$  to  $1.47 \text{ d}^{-1}$  and averaged  $0.17 \pm 0.016 \text{ d}^{-1}$ . Phytoplankton lysis rates were strongly seasonal, showing the highest average values during the summer ( $0.41 \pm 0.049 \text{ d}^{-1}$ ; Fig. 5), and were lowest in mid winter. Episodes of high phytoplankton lysis rates were, however, observed during the fall although these were lower ( $0.15 \pm 0.018 \text{ d}^{-1}$ , mean  $\pm$  SE) than those observed in summer (Fig. 5). As a result, there was a strong ( $R^2 = 0.71$ ,  $P < 0.00001$ ) positive relationship between monthly average lysis rates and water temperature (Fig. 6). In addition, monthly average lysis rates followed monthly average GPP with a maximum correlation ( $r = 0.65$ ,  $P < 0.05$ ) at a lag time of 1–2 months (Fig. 7).

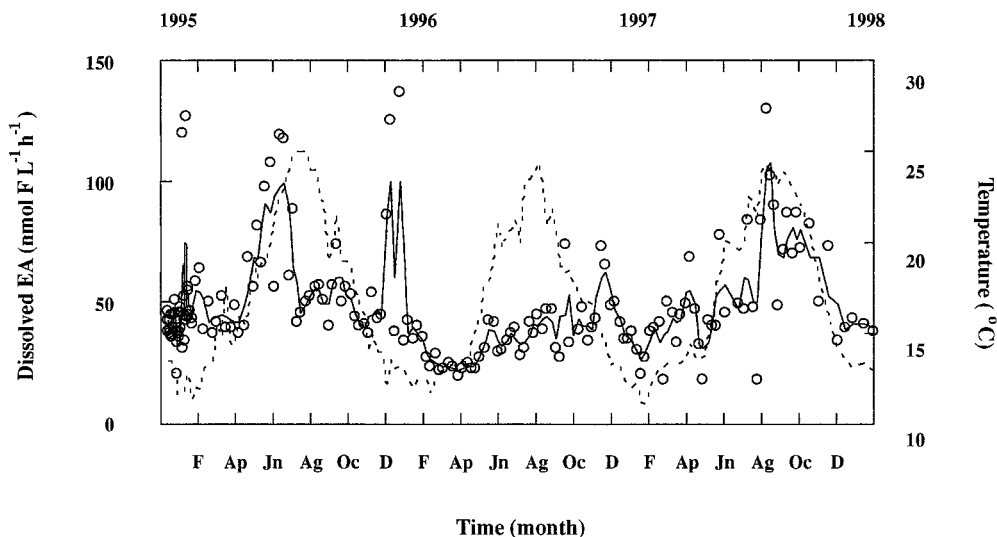


Fig. 3. Temporal evolution of dissolved esterase activity (DEA) in Blanes Bay. The broken line represents the water temperature, and open circles and the solid line represent the individual estimates and a smoothed trend in DEA, respectively.

## Discussion

The results presented confirm the importance of cell lysis as an important loss factor for phytoplankton in the NW Mediterranean coastal waters and demonstrate its importance to be highly seasonal. The average phytoplankton lysis rates found in summer were high, averaging  $0.41 \pm 0.049 \text{ d}^{-1}$ , but reaching values as high as  $1.47 \text{ d}^{-1}$ . However, during the rest of the year, phytoplankton lysis rates remained low, especially during the winter, when lysis rates averaged  $0.061 \pm 0.005 \text{ d}^{-1}$ . The high phytoplankton lysis rates observed during the summer add to recent results from the Adriatic Sea (Baldi et al. 1997) and the open NW Mediterranean Sea (Agustí et al. 1998), suggesting this to be a widespread phenomenon in the Mediterranean Sea. Although lysis rates

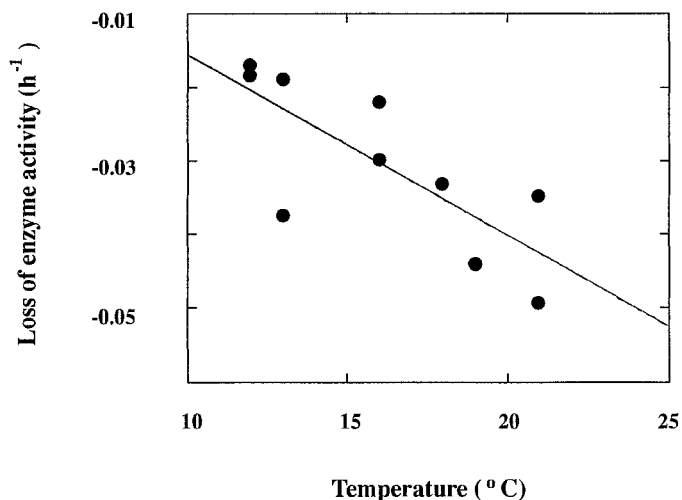


Fig. 4. The relationship between the rate of loss of activity of dissolved esterases and temperature in water samples from Blanes Bay. The solid line represents the fitted regression equation.

were positively correlated with water temperature, this does not necessarily imply a direct effect, for high temperature is associated to stress derived from excessive irradiance and UV levels and nutrient deficiency. The mean summer lysis rates reported here were, however, lower, than that reported for the stratified surface waters of the open NW Mediterranean, where lysis rates averaged  $0.86 \text{ d}^{-1}$  (Agustí et al. 1998). The comparison between lysis rates requires, however, consideration of the uncertainties associated with this method, the most important of which is likely to be the assumption of a constant esterase to chlorophyll ratio. We have, however, evaluated this source of uncertainty to introduce, on average, an uncertainty of 13% of the mean lysis rate estimates, which should not influence the comparisons. Moreover, the uncertainties associated with this assumption in the method compares favorably with those of other techniques to measure phytoplankton processes where no incubation or confinement of the community is involved, in that the method used here to estimate lysis rates is not invasive. In contrast, the need to enclose and incubate phytoplankton populations is an important source of uncertainties in other accepted rate estimates of phytoplankton processes (e.g., primary production).

The temporal variability of phytoplankton biomass and GPP found in Blanes Bay is in agreement with the seasonal patterns described previously for this Bay (cf. Mura et al. 1996a; Satta et al. 1996a,b; Duarte et al. 1998). The seasonal pattern found for phytoplankton lysis in Blanes Bay, characterized by important lysis rates during the summer, should be, therefore, related to the seasonal variability in phytoplankton growth conditions. The high temperature, irradiance, and low inorganic nutrients concentration (Cebrián et al. 1996; Duarte et al. 1998), together with the low phytoplankton biomass during the summer, should help explain the high lysis rates experienced by phytoplankton. These conditions are in contrast with the higher nutrient availability

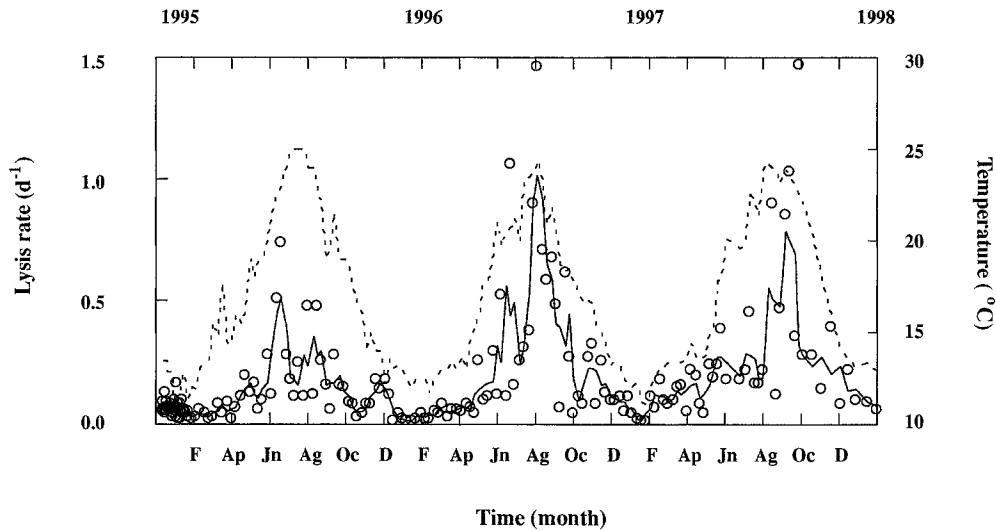


Fig. 5. The time evolution of phytoplankton lysis rates in Blanes Bay from January 1995 to January 1998. The broken line represents the water temperature. Open circles and the solid line represent single estimates and a smoothed curve of phytoplankton lysis rates, respectively. The error about the estimated lysis rates averages 13% about the estimated values.

during winter (Cebrián et al. 1996), which allows the development of higher phytoplankton biomass. The highest GPP rates were found in summer, despite the low phytoplankton biomass, resulting in high production/biomass (P/B) ratios during this season, indicative of fast phytoplankton gross growth rate, as expected from the positive relationship between P/B ratios and temperature in the Blanes Bay (Satta et al. 1996b). The high P/B ratios in summer indicate a fast turnover of the phytoplankton biomass, which, provided the low summer biomass, also implies high phytoplankton losses. The maximum growth rates of phytoplankton reported

for Blanes Bay varied from 1.5 d<sup>-1</sup> for summer *Synechococcus* (Agawin et al. 1998) to 1 d<sup>-1</sup> for diatoms in late winter (Mura et al. 1996b), suggesting the losses associated to lysis rates, which averaged 0.41 and 0.061 d<sup>-1</sup> in summer and winter, to be most important in summer. Grazing by zooplankton is, however, also far more important in summer (about 1 d<sup>-1</sup>) than in winter (<0.01 d<sup>-1</sup>, Duarte unpubl. data), so that phytoplankton experiences high losses in summer, although these are low compared to their maximum winter growth rates, allowing for the development of blooms in late winter (Duarte et al. 1999) and their subsequent export. Other sources of mortality, such as viral infections, remain low throughout the year (<2% of cells infected in the summer, Tuomi unpubl. data).

The estimates of lysis rates obtained are unconstrained, and their robustness can be further assessed by comparison to the measured photosynthetic rates. The observed GPP far

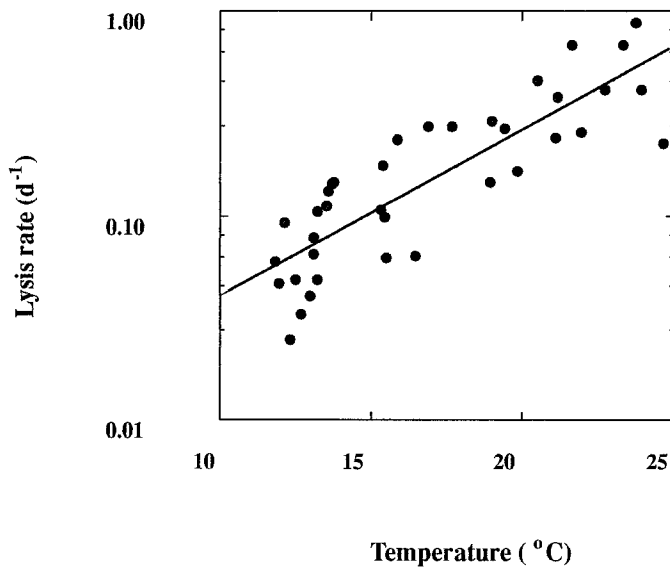


Fig. 6. The relationship between monthly average phytoplankton lysis rates and water temperature for Blanes Bay. The solid line represents the fitted regression equation. The error about the estimated lysis rates averages 13% about the estimated values.

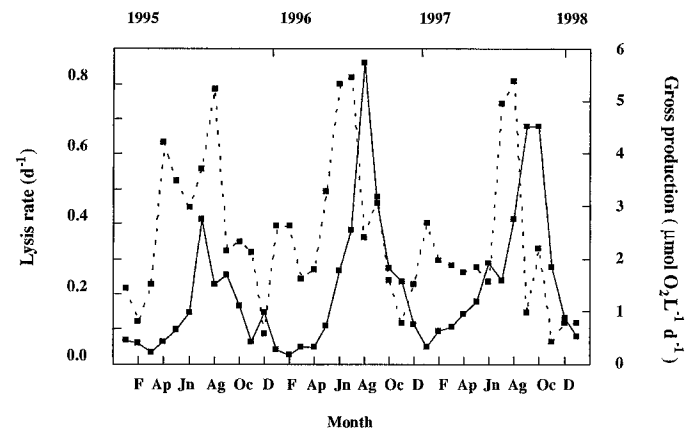


Fig. 7. Seasonal succession of monthly averaged phytoplankton lysis rates (solid line) and gross primary production (broken line) for Blanes Bay during 1995-1998.

exceeds the losses involved in cell lysis, so that cell lysis represented about  $\frac{1}{4}$  of the gross primary production, both in winter, when both lysis rates and GPP are low, and summer, when they are high. These results identify, therefore, phytoplankton cell lysis as an important route of loss of phytoplankton carbon in Blanes Bay, able to sustain a considerable bacterial production during the winter bloom as well as during the summer. Available estimates of bacterial production in Blanes Bay indicates values around  $0.7 \mu\text{g C L}^{-1} \text{d}^{-1}$  in winter (recalculated from del Giorgio et al. 1996) and  $4 \mu\text{g C L}^{-1} \text{d}^{-1}$  in summer (Gasol unpubl. data), which represent about 5% and 20% of the gross primary production and can be supported from the loss of primary production associated through cell lysis.

Small eukaryotes and cyanobacteria (*Synechococcus*) dominated the phytoplankton biomass during the summer, representing 60% of the phytoplanktonic biomass (Agustí unpubl. data), as described previously for the bay (Mura et al. 1996; Agawin et al. 1998). During the winter, however, diatoms (represented by the genera *Chaetoceros*, *Thalassiosira*, *Skeletonema*, and *Nitzschia*, among others) dominated the phytoplankton biomass. This parallel shift in the community from a dominance of diatoms in winter to pico and nanoplankton in summer and the parallel increase in the phytoplankton lysis rates in summer point to a possible association between high lysis rates and the dominance of small phytoplankters in oligotrophic waters that needs to be explored further.

The highest values of dissolved EA reported here for Blanes Bay were similar to those reported for the surface oceanic waters of the NW Mediterranean in summer (Agustí et al. 1998). The temporal variability of dissolved EA (as FDA hydrolysis) observed and the calculated rates of loss of activity of dissolved esterases indicate a significant dynamic of this enzyme in the surface waters of Blanes Bay. The experiments conducted to quantify the loss of activity of dissolved esterase with time in Blanes Bay indicated that temperature was an important factor reducing the enzyme activity once dissolved (explaining 54% of the rate of loss of DEA) and leading to a rapid decay of the enzyme activity with increasing temperature. Thus, the fact that the DEA in the water remains high in summer (half-life of 21 h) despite fast decay rates of DEA indicates high dynamics of DEA at short time scales (day).

Moreover, increased phytoplankton lysis rates followed increased GPP with a lag of 1–2 months, similar to that in the response of bacterial populations to phytoplankton blooms (Vaqué 1996; Duarte et al. 1998). This lag represents the intrinsic duration of bloom events, from the initial increase in GPP to bloom collapse in Blanes Bay (Prairie and Duarte 1996), thus implying that cell death plays an important role in bloom collapse and the subsequent release of DOC for bacterial use.

The results presented indicate a dominant contribution of phytoplankton cell lysis as a loss process for photosynthetic carbon in Blanes Bay. Cell lysis rates should lead to high DOC release and therefore be conducive to the development of the active microbial food web there (del Giorgio et al. 1996). Whether these patterns can be extrapolated elsewhere in the NW Mediterranean must be tested, but the seasonality

in Blanes Bay has been reported to provide an adequate paradigm of the NW Mediterranean littoral waters (Duarte et al. 1999). Indeed, the importance of lysis rates was even higher in the open NW Mediterranean Sea (Agustí et al. 1998), suggesting that high cell lysis rates may be a dominant route channeling the efficient transfer of phytoplankton C to the microbial food web that characterizes the oligotrophic ocean (Legendre and Rasoulzadegan 1995; Gasol et al. 1997). What the causes of such high phytoplankton lysis rates are, is at yet unknown. Possible candidates include viral infection, photodamage, environmental stress, or a deficient control of cell division under adverse environments (e.g., Bratbak et al. 1993; Parpais et al. 1996). Whatever the cause, it is evident that these effects are associated with environmental conditions, because lysis rates are strongly seasonal in the NW Mediterranean Sea and appear to increase from the more eutrophic North Sea (maximum rates  $0.3 \text{d}^{-1}$ , Brussaard et al. 1995) to the more oligotrophic open NW Mediterranean Sea ( $0.86 \text{d}^{-1}$ , Agustí et al. 1998).

The importance of phytoplankton lysis rates demonstrated for the NW Mediterranean (cf. Agustí et al. 1998) indicates that this process should be incorporated into models of carbon flow in the planktonic ecosystem. In particular, phytoplankton cell lysis provides a direct link between phytoplankton production and carbon supply to the microbial food web, which is consistent with the suggested importance of both of these processes in oligotrophic systems. Furthermore, the growing evidence that cell lysis plays an important role in regulating phytoplankton (van Boekel et al. 1993; Brussaard et al. 1995; Baldi et al. 1997; Agustí et al. 1998) and bacterioplankton (del Giorgio et al. 1996) populations, the dominant components of marine planktonic biomass (Gasol et al. 1997), clearly points out the need to measure, understand, and model cell lysis processes as a possible major driving factor in the dynamics of marine planktonic communities.

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