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Responses of coastal osmotrophic planktonic communities to simulated events of turbulence and nutrient load throughout a year

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A year-long series of monthly experiments with laboratory enclosures were conducted with water from Blanes Bay (NW Mediterranean) to analyse the change in the short-time response of the osmotrophic planktonic community to simulated turbulence and nutrient input events. Both experimental factors triggered a relative increase of biomass in the enclosures, in terms of chlorophyll a, bacteria and particulate organic matter. Ratios of particulate organic nitrogen to phosphorus became lower in the water than in the sediment, although turbulence partially smoothed out this difference. Initial physico-chemical conditions significantly influenced the short-time responses to experimental forcing. The response to turbulence, in terms of chlorophyll a, was maximum in spring. The response to nutrient enrichment was found to be seasonal, and was correlated with photoperiod and temperature, and also in situ nitrate and silicate concentrations and Secchi depth, which are proxies of recent inputs of nutrients resulting from episodes of resuspension and river discharge. This study shows robust qualitative regularities in the response of the osmotrophic planktonic community to episodes of turbulence and nutrient enrichment, with quantitative variability throughout the year, depending mostly on the recent record of hydrodynamic forcing.

Featured Article

INTRODUCTION

The dynamics of plankton in coastal areas is related to the variability in external energy supply (Margalef, 1978). In the upper layer of the ocean, the input of mechanical energy is due to many different processes, such as winds, tides, surface wave breaking or Langmuir circulations (Gargett, 1989). This energy fuels turbulence that manifests across a wide range of spatio-temporal scales, from those of energy input to those of dissipation as heat. As a consequence, the effects of turbulence on planktonic organisms operate at different scales (e.g. Kiørboe, 1993; Marrasé *et al.*, 1997).

At the largest scales of energy input, on the order of tens of metres to hundreds of kilometres, turbulence is a key factor in explaining the distribution of phytoplankton with respect to their resources, namely nutrients and light (e.g. Sverdrup, 1953; Huisman *et al.*, 2002). Mixing enhances the diffusion of nutrients from nutrient-rich bottom layers, and may alter the light regime experienced by the phytoplankton cells. Large eddies can also keep planktonic organisms in suspension within the mixed layer (Ross, 2006), favouring those cells with high settling velocities, such as diatoms and large non-motile cells in general, over those that may control their position into the water column by swimming, as is the case for dinoflagellates. In coastal waters, the input of

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external energy may be associated with nutrient loading, because stormy weather, which translates into strong winds and high waves, often implies rains that produce episodes of terrestrial runoff. In shallow coastal waters turbulence can, in addition, increase the flux of nutrients from the sediments (Dade, 1993).

These large scale motions break into progressively smaller eddies without significant loss of kinetic energy. As eddies get smaller, viscosity becomes more important, until they reach the Kolmogorov length scale, where the viscous forces equal the inertial ones. Below this scale, energy starts to be dissipated as heat, eddies are no longer formed, and the fluid velocity field is characterized by a laminar shear, changing randomly in direction and intensity. Kolmogorov scales typically range from centimetre to millimetre. At these small scales and below, the direct effects of turbulence on planktonic organisms have been seen to derive from four main processes: (i) an increase in uptake rate of nutrients (Lazier and Mann, 1989; Karp-Boss *et al.*, 1996; Peters *et al.*, 2006), (ii) an increase in the encounter rates between particles (Rothschild and Osborn, 1988) potentially leading to an enhancement of grazing rates, mating and particle aggregation; (iii) an increase in sedimentation rates due to increased aggregation (Kiørboe, 1997) or to enhanced settling velocities (Ruiz *et al.*, 2004); and (iv) an alteration of a variety of physiological processes (reviewed by Berdalet and Estrada, 2005).

All these influences of turbulence, both at large and small scales, operating on plankton individuals, can lead to changes at the ecosystem level. These include changes in the species composition of the community (Margalef, 1978; Estrada *et al.*, 1987), in its size structure (Arin *et al.*, 2002; Malits *et al.*, 2004; Cózar and Echevarría, 2005) and elemental stoichiometry (Maar *et al.*, 2002), and even in ecosystem metabolism (Alcaraz *et al.*, 2002; Peters *et al.*, 2002).

Coastal areas are boundaries between land, sea and atmosphere, and therefore are subjected to a particularly high variability in physical forcing. As a result, turbulence and its associated nutrient enrichment occur in an episodic and unpredictable manner. For example, the influence of land and its topography can considerably alter the spatial distribution of winds and turbulence (Guadayol and Peters, 2006). Because the effects of turbulence and nutrient enrichment are highly species-specific (Peters and Marrasé, 2000; Sullivan and Swift, 2003; Piehler *et al.*, 2004; Berdalet *et al.*, 2007) and size-dependent (Karp-Boss *et al.*, 1996), the response of the pelagic ecosystem to these episodic perturbations depends on the initial composition and structure of the planktonic community (Estrada *et al.*, 1987). We hypothesize that, as a consequence, the response to such disturbances will change throughout the seasonal cycle following changes in the planktonic community. To test this hypothesis, we assessed the short-time reactivity to turbulence and to nutrient additions of different planktonic assemblages during an annual cycle. We monitored the responses in terms of the biomass of the osmotrophic planktonic community to simulated events of turbulence and nutrient load in a series of monthly laboratory experiments performed between June 2003 and June 2004 in a coastal area (Blanes Bay, NW Mediterranean).

METHOD

Experimental design

From June 2003 to June 2004, a monthly experiment was performed (Table I). Subsurface water was sampled at the Blanes Bay Microbial Observatory (41°40′N, 2°48′E, 800-m offshore, 20–24-m depth, Alonso-Sáez *et al.*, 2008). Blanes Bay is an open, shallow oligotrophic bay located 65 km north of Barcelona, between the submarine Blanes Canyon to the north and the mouth of the river La Tordera to the south. Its bottom is sandy and has a relatively steep slope (∼2%, Vaqué *et al.*, 1997). Sampling was always conducted around 11 a.m. (local time) from a small boat. Measurements of surface water temperature, with a mercury thermometer, and Secchi disk depth were conducted *in situ*. Meteorological data were acquired from the nearby station of Malgrat de Mar (Catalan Meteorological Service, http://www.meteocat.net), located at 41°38′57″N 2°45′8″E.

Sample water for the experiments was screened *in situ* through a 200 μm nylon mesh to remove mesozooplankton and brought to the laboratory in plastic carboys within 2 h. In the laboratory, water was distributed into 15 L cylindrical transparent methacrylate containers with an inner diameter of 24.2 cm. The basic experimental design consisted of six containers, three of them subjected to natural levels of turbulence (see below), and the other three kept still. Of each set of three containers, two received an addition of nutrients and one was left as control.

Experiments lasted for 3 days and were conducted in a temperature-controlled chamber set at the *in situ* water temperature. We used a combination of cool-white and gro-lux fluorescent lamps, placed vertically in parallel to the long axis of the cylindrical containers to ensure a uniform distribution of light in all enclosures. Measured light intensity inside the experimental containers was 225 μmol photons $m^{-2} s^{-1}$, which is around the

Table I: Experimental dates and initial water conditions

Experiment number	Date	Temperature (°C)	Salinity	Secchi depth (m)	Stratification	Photo period (h)	Chl *a* (μg L^{-1})	NO_3^- (μmol L^{-1})	NO_2^- (μmol L^{-1})	NH_4^+ (μmol L^{-1})	SiO_2 (μmol L^{-1})	PO_4^{3-} (μmol L^{-1})
1	25 June 2003	25.0	37.9	16.0	Yes	14.95	0.29	0.25	0.05	0.22	1.80	0.04
2	14 July 2003	25.0	37.2	17.5	Yes	14.69	0.43	0.08	0.06	0.41	1.33	0.03
3	04 August 2003	25.0	37.8	23.8	Yes	14.06	0.31	0.01	0.07	0.46	1.49	0.02
4	16 September 2003	23.0	38.7	18.5	No	12.05	0.28	0.24	0.05	0.48	2.08	0.10
5	21 October 2003	18.0	37.5	3.5	No	10.34	0.44	4.43	0.16	0.40	7.33	0.02
6	25 November 2003	16.0	37.6	9.0	No	9.23	1.08	0.86	0.22	0.29	1.18	0.19
7	16 December 2003	14.5	36.1	6.5	No	9.03	3.93	3.70	0.58	0.32	5.93	0.14
8	26 January 2004	14.0	38.6	14.0	No	9.60	1.06	1.38	0.20	0.09	1.69	0.19
9	23 February 2004	12.9	37.9	7.0	No	10.70	1.04	1.61	0.25	0.07	1.19	0.13
10	22 March 2004	12.8	37.9	12.0	No	12.1	1.13	2.42	0.25	0.09	2.63	0.22
11	19 April 2004	12.6	36.6	6.0	No	14.54	1.36	2.90	0.20	0.14	3.76	0.12
12	25 May 2004	15.4	37.7	9.0	Yes	14.71	0.71	0.68	0.15	0.05	0.11	0.18
13	28 June 2004	21.1	37.6	14.0	Yes	14.92	1.29	0.25	0.07	0.04	0.07	0.13

saturating irradiance reported for this system for most of the year (200 μmol photons $m^{-2}\,s^{-1}$, Satta *et al.*, 1996). Such a light level is normal in this Mediterranean system, even in winter and with relatively high turbidity (Blanes Bay Microbial Observatory, unpublished data). The light:dark cycle was adjusted to match the *in situ* conditions.

Nutrient enrichment

Additions of inorganic nutrients were made to follow molar Redfield ratios (Si:N:P = 15:16:1). NO_3^- was added at 4 μmol L^{-1}, PO_4^{3-} at 0.25 μmol L^{-1}, and SiO_2 at 4 μmol L^{-1}. The addition of SiO_2 declined during the annual series as there was precipitation in the stock solution, until it reached 0.3 μmol L^{-1} in experiment 9 (February 2003). From there on, a new stock solution was used. Metals were prepared as in the f/2 medium and added maintaining their proportion to nitrate. These nutrient concentrations are well below the maxima found in this area (Blanes Bay Microbial Observatory time series), which are 7.1 μmol L^{-1} for NO_3^-, 1.0 for PO_4^{3-} and 7.6 for SiO_2. After addition, all containers were gently stirred with a sterile plastic pipette to favour their homogenization. Samples for determination of inorganic nutrient concentration were taken from each container about 2 h after the addition. In experiment 6 (November 2003), nutrients were mistakenly added to all containers.

Turbulence generation

In order to experimentally simulate the effects of turbulent events, we used an oscillating grid system. This experimental setup has been traditionally used as a means to generate isotropic small-scale turbulence in studies of plankton dynamics (Peters and Redondo, 1997; Sanford, 1997) and of sediment resuspension (Hopfinger and Toly, 1976). By using oscillating grids, we could generate small-scale turbulence and at the same time increase the proportion of cells kept in suspension, thus simulating a turbulent event both at the small and at the large scales.

The oscillating grid system used is described in Peters *et al.* (Peters *et al.*, 2002). Stroke length and frequency of oscillation were set at 13 cm and 2.5 rpm, respectively. The average turbulent kinetic energy dissipation rate obtained with these settings, estimated applying equations in Peters and Gross (Peters and Gross, 1994), was $O(10^{-2})$ $cm^2 s^{-3}$. As water volume was reduced owing to sampling during the experiment, the frequency of grid oscillation and the stroke length were slightly adjusted to maintain the initial dissipation rate,

using equations in Peters and Gross (Peters and Gross, 1994). Measurements done *a posteriori* with an acoustic Doppler velocimeter with these conditions (Guadayol *et al.*, in press) gave an integrated dissipation rate within the region delimited by the movement of the grid of $O(10^{-3})$ $cm^2 s^{-3}$. Thus, depending on the method used, our estimates of the generated turbulence were in the range 10^{-3}–10^{-2} cm^2 s^{-3}.

Sampling procedure and analyses

Between 1.5 and 2 L of water was taken daily at 10 a.m. local time from each container by siphoning water through an autoclaved, milliQ rinsed, glass tube. Samples were taken for determination of inorganic nutrients, particulate organic matter, total nitrogen (TN) and phosphorus (TP), chlorophyll *a* concentration (Chl *a*), bacteria, cyanobacteria and microphytoplankton. Samples for plastidic and heterotrophic nanoflagellates (PNF and HNF) were taken from the initial water and the last day. On the last day, additional sampling for several of the parameters (Chl *a*, particulate organic matter, TN, TP and microphytoplankton) was performed to estimate the sedimented biomass. All containers were gently stirred with a sterile pipette until the settled material was completely resuspended, and then the sampling was performed as above.

Samples for the estimation of dissolved inorganic nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and SiO_2) were obtained in acid-rinsed polyethylene tubes and kept frozen at −20°C. Analyses were performed with an Alliance Evolution II autoanalyzer following methods in Hansen and Koroleff (Hansen and Koroleff, 1999) with minor modifications.

Chlorophyll *a* concentration was determined fluorometrically (Yentsch and Menzel, 1963) with a Turner Designs fluorometer. Subsamples of 20 mL were filtered through Whatman GF/F filters, which were immediately immersed in 6.5 mL of 90% acetone, and kept at 4°C and dark for 24 h, to allow chlorophyll extraction.

For the determination of particulate organic matter, 200–500 mL of water was filtered through precombusted (450°C, 4 h) glass fibre filters (Whatman GF/F), immediately frozen in liquid nitrogen and then stored at −80°C. For each sample, two filters were processed to estimate, respectively, the particulate organic carbon and nitrogen (POC and PON), and the particulate organic phosphorus (POP). Before analysis in a Carlo-Erba CHN analyser, the POC/PON filters were thawed in an HCl^- saturated atmosphere for 48 h to remove carbonates, and dried at 80°C for 24 h. POP was determined following the oxidation (120°C, 30 min) of the filter in acidic persulphate and subsequent colorimetric analysis of dissolved phosphate (Hansen and Koroleff, 1999).

Samples for TP and TN where kept in acid-rinsed polyethylene tubes at −20°C. TP was determined by wet oxidation using the same procedure as for POP. TN was determined after persulphate oxidation following Hansen and Koroleff (Hansen and Koroleff, 1999).

Abundances of bacteria and cyanobacteria (*Prochlorococcus spp.* and *Synechococcus spp.*) were determined by means of flow cytometry following methods in Olson *et al.* (Olson *et al.*, 1993) and Gasol and del Giorgio (Gasol and del Giorgio, 2000) as described in Peters *et al.* (Peters *et al.*, 2002). Bacterial average cell volume (B, μm^3) was estimated using the calibration function provided by Gasol and del Giorgio (Gasol and del Giorgio, 2000):

$$B = 7.5 \cdot 10^{-3} + 1.1 \cdot 10^{-1} \left(\frac{F_{\text{bacteria}}}{F_{\text{beads}}} \right) \qquad (1)$$

where F_{bacteria} is the mean value of the green fluorescence attributed to bacteria, and F_{beads} is the mean green fluorescence of the yellow-green 1 μm Polysciences latex beads used as standard in this study.

Average volume for cyanobacteria was estimated assuming a spherical shape. We used a value of 1 μm for *Synechococcus spp.* (Agawin *et al.*, 1998), and a mean diameter of 0.7 μm for *Prochlorococcus spp.* (Vaulot *et al.*, 1990).

Samples for heterotrophic nanoflagellate (HNF) and plastidic nanoflagellate (PNF) were fixed with 1.5% (final concentration) glutaraldehyde, and stained before 24 h with DAPI (4′,6′diamidino-2-phenylindole) and then filtered through black polycarbonate filters of 0.8 μm pore size. Filters were kept at −20°C before counting with an epifluorescence microscope (Porter and Feig, 1980). Organisms were classified in 4 size classes: <4, 4–8, 8–16 and >16 μm. Cellular volume was estimated from the mean size of each class assuming a spherical shape.

Samples for microphytoplankton were fixed with formalin-hexamine (0.4% final concentration) and kept at 4°C until analysis. Aliquots of 12–50 mL were transferred to sedimentation chambers, and left undisturbed for 24 h. Microphytoplankton was identified and counted with an inverted microscope at ×100 and ×400 magnifications (Utermöhl, 1958). Dominant phytoplankton was identified at least to the genus level. Width and length of cells were measured for each cell or for each colony or chain, and the number of cells

per aggregate was also counted. When possible, volume of each cell was calculated applying the formula provided by Hillebrand *et al.* (Hillebrand *et al.*, 1999). In radially asymmetrical cells, the closest two-parameter geometrical shape was assumed. This may have caused a bias in the estimation of biovolumes, especially when radially asymmetrical cells were dominant.

Calculations and statistical analyses

The responses of the phytoplankton to the experimental factors were quantified from the Chl *a* values of the water column as follows. We assumed that within the short temporal frame of the experiments, the increase in Chl *a* in response to the experimental factors could be adequately modelled by an exponential function. Estimations of the net growth rate of Chl *a* in the water column for each container were thus calculated by adjusting an exponential model to the experimental values of chlorophyll *a* concentration from daily samplings. The adjustment was made by non-linear least square estimation. The difference between the apparent net growth rate of turbulent and still treatments was considered as a proxy of the net response of the phytoplankton biomass to turbulence. Similarly, the difference in growth rates between enriched and control treatments was considered a proxy of the response to nutrient additions.

Sedimentation rates were derived from TP measurements. It was assumed that unlike nitrogen, which is abundant in the atmosphere and may be exchanged through the air–water interface, phosphorus had no extraneous supply or sink in the containers (Maar *et al.*, 2002). Therefore, the amount of TP in each container was independent of any physical, chemical or biological processes, except for the sampling. Thus, the decay in the concentration of TP in the water column was due to sedimentation dynamics only. The calculation of net sedimentation rates was done with an exponential model of water column TP concentration against time from Day 0 to Day 3. Although this model assumes that particles remain homogenously distributed in the container, which may be unrealistic under still conditions, it was the model that best fits the experimental data in all cases. The adjustment was done by non-linear least square estimation. Sedimentation rates could only be reliably calculated for nutrient enriched containers, because differences in TP from day to day in the non-enriched containers were too close to the sensitivity of the method of analysis.

Osmotrophic plankton biomass-size spectra were calculated adjusting a Pareto type I statistical distribution (Vidondo *et al.*, 1997) to the concentration and volume data for phytoplankton and bacterioplankton:

$$\mathrm{pdf}(s) = cks^{-(c+1)} \quad (2)$$

where pdf stands for probability density function, s is size, and c and k are the shape and scale parameters, respectively. The c parameter is equivalent to the negative slope of the normalized biomass-size spectrum (Vidondo *et al.*, 1997). Thus, large values of c indicate dominance of small organisms, whereas low values are indicative of a relatively higher abundance of large organisms. Items used were the free living cells and aggregates or colonies, based on epifluorescence microscopy counts for PNF < 16 μm in diameter and Utermöhl counts for larger flagellates.

Analysis of covariance (ANCOVA) and analysis of variance (ANOVA) were performed to assess the effect of the two experimental factors, i.e. turbulence (TUR) and nutrient enrichment (NUT) on several of the sampled parameters. Further, the relevance of the initial conditions was represented by the experiment number (experiment, EXP). The ANCOVA was performed with all the available data, using as continuous predictor the sampling time (TIME), i.e. the time of microcosm sampling since the beginning of the experiment (time = 0). For each parameter, the data for the ANCOVA were standardized by dividing the values at different sampling times by the initial values of each experiment, and linearized by applying the natural logarithm. The time 0 data were excluded from the analyses of covariance. For simple comparisons, two-tailed Student's *t*-tests were performed. Degrees of covariation were estimated through the non-parametric Spearman's rank correlation coefficient (r_s). Unless otherwise indicated, level of significance for all statistical analyses was set to 0.05.

RESULTS

Initial conditions

A detailed description of the annual cycle in Blanes Bay, including meteorological, hydrographical and water column data, is given elsewhere (Guadayol *et al.*, in press). Briefly, during summer of 2003, river discharge from the nearby river La Tordera, and thus the input of nutrients from terrestrial runoff, were low because of anomalously high air temperatures, unusual atmospheric stability and reduced precipitation (Trenberth *et al.*, 2007). In addition, weak winds and

low wave heights may have resulted in low nutrient resuspension from the sediments. As a consequence, during the first part of the experimental series, between June and September 2003, *in situ* dissolved inorganic nutrient concentrations were very low. With autumn came the typical Mediterranean episodes of intense rain and subsequent increases in river flow, which drove the system to less oligotrophic conditions. Initial water conditions for the experiments are given in Table I. The water column was thoroughly mixed between October 2003 and April 2004. In summer, a shallow thermocline appeared at 2–3 m (Vila-Costa *et al.*, 2007). Salinity variations were due to river discharge events and possibly to the intrusion of saline water from the submarine Blanes Canyon (Masó and Tintoré, 1991).

In situ chlorophyll *a* concentration tended to increase throughout the study year (Table I). The maximum concentration (3.93 $\mu g\,L^{-1}$) was found in December 2003, after an episode of high river discharge occurred 2 weeks before, and a prolonged period of high significant wave heights that reached values of almost 5 m. Likely, some resuspension had taken place associated with the preceding stormy conditions. During the sampling for the experiment (hereafter referred as "Exp") 6 in November 2003, a large amount of the brown alga *Codium sp.* was observed on the beach and macrophyte detritus were seen under the microscope. The meteorological records in the previous weeks, however, do not indicate any large storm that could have resulted in resuspension.

The dynamics of both NO_3^- and SiO_2 (Table I) were strongly linked to terrestrial runoff and more weakly to resuspension. They showed three major peaks: in sampling for Exp 5 (October 2003), Exp 7 (December 2003) and Exp 11 (April 2004). These peaks followed periods of high waves and/or river discharges (data not shown). In the last two experiments, coinciding with a persistent diatom bloom of *Chaetoceros spp.*, silicate concentrations were at their minimum (0.07 $\mu mol\,L^{-1}$). In contrast, PO_4^{3-} was apparently not related either to terrestrial runoff or to sediment resuspension driven by waves or wind, but increased throughout the period of study, from values close to the analytical detection limit in summer 2003 (between 0.02 and 0.03 $\mu mol\,L^{-1}$) to maximum values in winter and spring 2004. As a consequence of these different dynamics of dissolved inorganic N and P, the N:P ratios (mol:mol, Table I) showed a wide range, from less than 3 in June 2004 to more than 300 in October 2003.

In summer 2003, phytoplankton was dominated by small microorganisms, mainly cyanobacteria and autotrophic nanoflagellates, whereas the contribution of diatoms in terms of carbon was only around 10%. For the remainder of the series, from October 2003 to June 2004, diatoms were clearly dominant except in December, when there was a peak in autotrophic nanoflagellates. In the last 3 months, there was a persistent dominance of *Chaetoceros spp.*, which reached abundances of more than 1000 cells mL^{-1}.

General trends in the water column

Both experimental factors, that is, nutrient addition and turbulence, had a significant effect on all biomass parameters monitored during the 13 experiments (ANCOVA, Table II). Nevertheless, the factor that explained the most variability in all dependent parameters, except in POP, was the experiment number (EXP), which represents the initial conditions. As expected, in all cases but for Exp 7 (December 2003), Chl *a* increased after addition of nutrients (Fig. 1, see also Supplementary online colour version). This increase adjusted well to an exponential curve, and no decay was observed before the experiment was terminated. In the non-enriched treatments, Chl *a* either increased or remained stationary, except again in Exp 7, and in Exps 12 and 13. The observed accumulation of Chl *a* in the water column implies that, for this parameter, sedimentation rates were lower than the growth rates.

Bacterial abundance also responded positively to nutrient addition and turbulence (ANCOVA Table II, Fig. 2, see also Supplementary online colour version), although the response was weaker than that of Chl *a*. In most experiments, the concentration increased during the 1st and 2nd day and decreased before the end of the experiment.

Diatom abundance in the water column increased in the enriched treatments in Exps 1–4 (data not shown). In those experiments, the silicate additions were close to the expected concentrations (see Methods) and the initial diatom abundances were lower than in the subsequent tests. Consistently, the slope of the biomass-size spectrum (*c*) decreased from the initial sample to Day 3. In Exps 5–13, however, the diatom abundance in the seston decreased in all treatments. In these cases, the *c* parameter increased over time in each treatment, which indicates that smallest organisms became relatively more important by the end of the experiments. Considering the whole annual series of tests, the slopes of the size spectra showed no significant change due to turbulence or nutrient addition from Day 0 to Day 3 (ANOVA).

The PO_4^{3-} concentration measured 2 h following the experimental addition was always lower than expected from nutrient addition (0.17 ± 0.05 $\mu mol\,L^{-1}$ average $\pm$ SD, $n = 13$). This indicates a high uptake rate of P by

Table II: Summary of ANCOVAs

		Parameter									
		Chl *a*		Bact		POC		PON		POP	
		$n = 297$		$n = 280$		$n = 178$		$n = 187$		$n = 297$	
		Adj. $R^2 = 0.74$		Adj. $R^2 = 0.61$		Adj. $R^2 = 0.71$		Adj. $R^2 = 0.68$		Adj. $R^2 = 0.91$	
		df = 48		df = 48		df = 44		df = 48		df = 48	
Treatment	df	SS	*P*-value	SS	*P*-value	SS	*P*-value	SS	*P*-value	SS	*P*-value
Intercept	1	0.65	0.057	13.17	*	3.38	*	0.32	0.057	13.60	*
TIME	1	15.84	*	0.79	0.008	6.70	*	5.02	*	0.06	0.236
EXP	11	76.55	*	25.13	*	16.23	*	15.30	*	24.75	*
NUT	1	32.52	*	17.14	*	6.77	*	11.81	*	70.20	*
TUR	1	4.12	*	0.72	0.012	1.76	*	1.19	*	0.57	*
EXP × NUT	11	7.31	*	3.68	*	1.59	0.037	1.28	0.208	10.81	*
EXP × TUR	11	1.11	0.853	0.06	0.999	0.63	0.623	0.74	0.661	0.40	0.620
NUT × TUR	1	0.03	0.664	0.03	0.588	0.01	0.670	0.02	0.642	0.00	0.945
EXP × NUT × TUR	11	0.71	0.968	0.20	0.999	0.33	0.933	0.48	0.899	0.14	0.988
Error		44.00		26.11		10.32		12.00		10.97	

All dependent variables were standardized dividing by the initial values, and linearized by taking natural logarithm. Continuous predictor is TIME, the experimental sampling time. TIME = 0 were excluded from the analysis. EXP represents the experiments. Exp 6 was excluded from the analyses because there were not unenriched treatments. Also, POC water column data for Exp 2 were not available. NUT (nutrient enrichment) and TUR (turbulence) are treated as on–off variables. Degrees of freedom (df) and *P*-values are shown.
* *P*-value < 0.001.

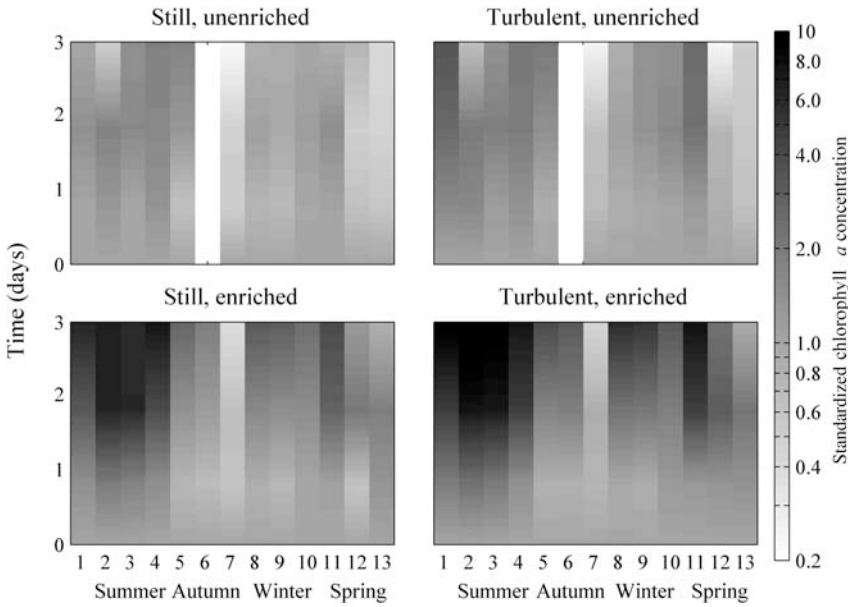

Fig. 1. Temporal evolution of chlorophyll *a* concentration (*Y*-axis) for each experiment (*X*-axis). The different subplots show the four different experimental conditions. Data were standardized by dividing each value on time (*t*) by the initial values of each experiment. Note that the colour-scale is in logarithmic mode. Values between samplings were obtained by linearly interpolating in a grid of 1-h resolution. The white bar corresponds to Exp 6, where controls received also nutrients, and were excluded for the analyses. A colour version of this figure is available online.

the initial communities. The NO_3^-:PO_4^{3-} ratio increased over time in the enriched treatments of all experiments from June 2003 to March 2004, except for the still treatment of Exp 2 in July 2003, where it decreased. Further, it decreased in the last three experiments. Coherently, the SiO_2:NO_3^- ratio only increased in Exp 2 and in the last four experiments, suggesting a faster uptake of nitrogen than of silicate. Thus, the natural communities of the sampled system were apparently limited by P for most of the year, and by N between

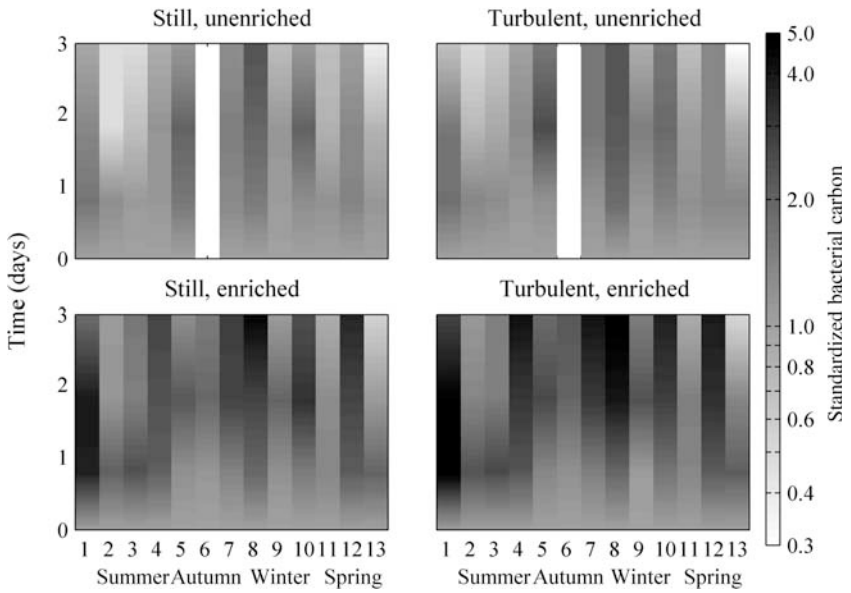

Fig. 2. Temporal evolution of bacterial abundance (*Y*-axis) for each experiment (*X*-axis). The different subplots show the four different experimental conditions. Data were standardized by dividing each value on time (*t*) by the initial values of each experiment. Note that the colour-scale is in logarithmic mode. Values between samplings were obtained by linearly interpolating in a grid of 1-h resolution. The white bar corresponds to Exp 6, where controls received also nutrients, and were excluded for the analyses. A colour version of this figure is available online.

April and June 2004, when diatom concentrations were highest and the initial NO_3^-:PO_4^{3-} ratio was already low, and in July 2003.

The fraction of organic P that contributed to the total particulate forms (i.e. POP/TP) increased in the nutrient enriched treatments. Changes in this ratio throughout the year were most conspicuous when comparing the first sampling of each experiment, ca. 16 h after the addition of nutrients (Fig. 3). At this time, POP/TP ratios were within a relatively narrow range in the control containers (Fig. 3A), whereas in the fertilized treatments (Fig. 3B) showed a marked seasonal pattern, being maximum in summer experiments, and minimum in winter. In fact, they were found to be positively correlated to the photoperiod ($r_s = 0.75$, *P-value* = 0.005 for still treatments, $r_s = 0.73$, *P-value* = 0.006 for turbulent treatments). In subsequent days, this pattern was smoothed and eventually disappeared.

Variability in the responses

The responses of phytoplankton biomass to the experimental factors were assessed as the difference between treatment and control in chlorophyll *a* net growth rate in the water column (Fig. 4). Both experimental factors triggered positive responses throughout the year, although the response to nutrient addition was higher than that to turbulence. In order to explore relationships with initial water conditions, we looked for correlations between these responses and initial conditions (Table III).

No significant relationship was found between the response of phytoplankton biomass to turbulence and any of the initial conditions (Table III), except for a weak correlation with photoperiod. However, there were significant differences between the seasons, whereas no differences were found due to enrichment in the response to turbulence (ANOVA, Adj. $R^2 = 0.28$, *P-value* = 0.028). The *post hoc* Tukey analysis showed the response in spring to be higher than in summer (*P-value* = 0.027) and in autumn (*P-value* = 0.075).

The response to nutrient addition was positively although weakly correlated to both photoperiod and temperature, and more strongly correlated to Secchi depth (Table III). It was strongly and negatively correlated to the concentration of dissolved inorganic N (particularly to NO_3^-) and to SiO_2. An ANOVA (Adj. $R^2 = 0.48$, *P-value* = 0.006) revealed that differences in the response to nutrients among seasons were also significant, whereas no differences were detected between still and turbulent treatments. The *post hoc* Tukey analysis showed that response in autumn was lower than in spring and summer. Levene's test indicated that the data had no homogeneous variance, but the non-parametric Kruskal–Wallis test ($H_{(3,24)} = 12.98$, *P-value* = 0.005) confirmed the results of the ANOVA.

Sedimentation

After resuspension, all biomass parameters (Chl *a* and particulate organic matter) were significantly higher in

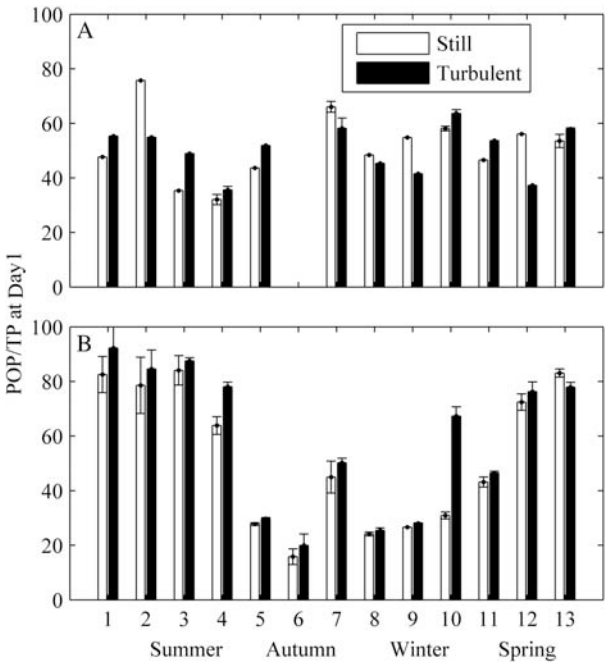

Fig. 3. Contribution of the organic phosphorus fraction to the total phosphorus particulate forms (POP/TP, in %), estimated 16 h since the nutrient addition at the beginning of the experiment in the control treatments (**A**) and in the nutrient enriched treatments (**B**). Error bars represent standard deviations of the replicates. White bars correspond to the still microcosms and the black ones to those exposed to turbulence.

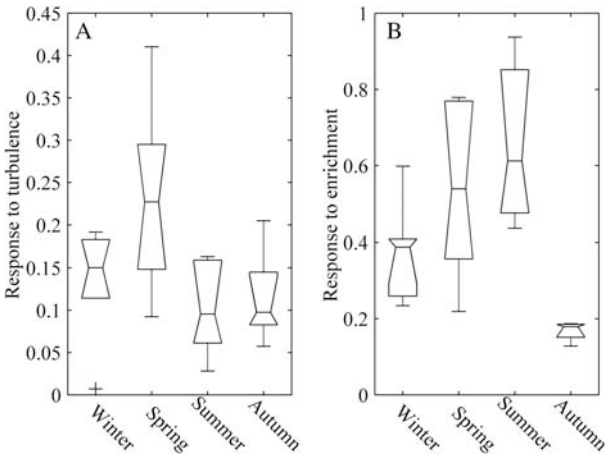

Fig. 4. Boxplot of the responses to turbulence (**A**) and to nutrient enrichment (**B**) grouped by seasons. Responses were calculated as differences in exponential growth rates calculated from chlorophyll *a* concentration in the water column (see text). Boxes have lines at the lower quartile, median and upper quartile values. Whiskers show the minimum and maximum values of the group. Cross represents an outlier.

Table III: Spearman's correlation coefficients of initial conditions with the mean response to turbulence and nutrient-enrichment

	Response to turbulence		Response to enrichment	
	r	*P*-value	*r*	*P*-value
Photoperiod	0.48*	0.097*	0.58*	0.052*
Daily irradiance	0.42	0.157	0.73**	0.010**
Temperature	−0.12	0.692	0.55*	0.064*
Secchi depth	−0.07	0.816	0.72**	0.008**
Salinity	−0.05	0.865	0.11	0.744
Size spectra slope	0.04	0.906	−0.28	0.379
Chl *a*	−0.09	0.779	−0.27	0.404
Bact	−0.07	0.830	0.06	0.850
$NO_3^- + NO_2^- + NH_4^+$	−0.22	0.470	−0.84**	0.001**
PO_4^{3-}	0.15	0.630	−0.14	0.667
SiO_2	−0.34	0.263	−0.76**	0.007**

*P-value < 0.100, **P-value < 0.050.

enriched treatments than in controls (ANOVA, Table IV). The same holds in turbulent enrichments, except for POC. In Fig. 5, the chlorophyll values estimated in the turbulent treatments are plotted against the values in the still ones, considering the results of the 13 experiments, but grouped for each sampling day (1–3) and after resuspension. The Spearman's correlations between turbulent and still for each day's dataset were all significant ($r_s > 0.92$; *P-value* < 0.001). The slope of the best fit linear regression model ($y=ax$) between still and turbulent treatments increased during the experiment, from 1 at the initial time, to ca. 1.6 on Day 3, and then it decreased back to ca. 1.1 after resuspension (Fig. 6). This pattern, observed for Chl *a*, was also seen for POC and PON, and less clearly for TN (Fig. 6). On the other hand, the slopes corresponding to the POP and TP values were close to 1 for all sampling times, and even lower after resuspension. This indicates that POC and PON were accumulating in the sediment at a higher rate under still conditions, whereas POP showed no difference due to turbulence. Thus, the PON:POP ratio (or POC:POP) in the water column should be relatively higher under turbulent conditions. The PON:POP and POC:POP ratios should also be higher in the sediment than in the water column. An ANOVA with the ratio PON:POP of Day 3 revealed differences between sediment and water column and between enriched and control treatments. A *post hoc* Tukey test confirmed that those ratios were higher in the sediment. However, the differences between water column and sediment were significant only in still conditions, not under turbulence. Thus, turbulence smoothed the differences in PON:POP ratio between water column and sediment.

Net sedimentation rates calculated from TP ranged between ca. 0.09 and 0.25 day^{-1}. Only rates for

Table IV: Summary of ANOVAs with data after resuspension of settled material

		Parameter							
		Chl *a*		POC		PON		POP	
		$n = 90$		$n = 60$		$n = 60$		$n = 90$	
		Adj. $R^2 = 0.98$		Adj. $R^2 = 0.94$		Adj. $R^2 = 0.98$		Adj. $R^2 = 0.99$	
		df = 47		df = 47		df = 47		df = 47	
Treatment	df	SS	*P*-value	SS	*P*-value	SS	*P*-value	SS	*P*-value
Intercept	1	129.43	*	57.49	*	58.11	*	57.57	*
EXP	11	54.13	*	9.82	*	11.78	*	7.81	*
NUT	1	27.38	*	5.57	*	7.43	*	24.88	*
TUR	1	0.79	*	0.02	0.310	0.12	0.002	0.08	0.002
EXP × NUT	11	3.73	*	1.51	0.001	0.74	*	3.03	*
EXP × TUR	11	0.81	*	0.20	0.570	0.15	0.174	0.32	*
NUT × TUR	1	0.00	0.916	0.04	0.172	0.05	0.031	0.01	0.258
EXP × NUT × TUR	11	0.28	0.200	0.13	0.817	0.24	0.046	0.16	0.056
Error	12	0.74		0.24		0.09		0.31	

EXP represents the 13 experiments. NUT (nutrient enrichment) and TUR (turbulence) are treated as on–off variables. Exp 6 was excluded from the analyses because there were not unenriched treatments. Degrees of freedom (df) and *P*-values are shown.
*P-value < 0.001.

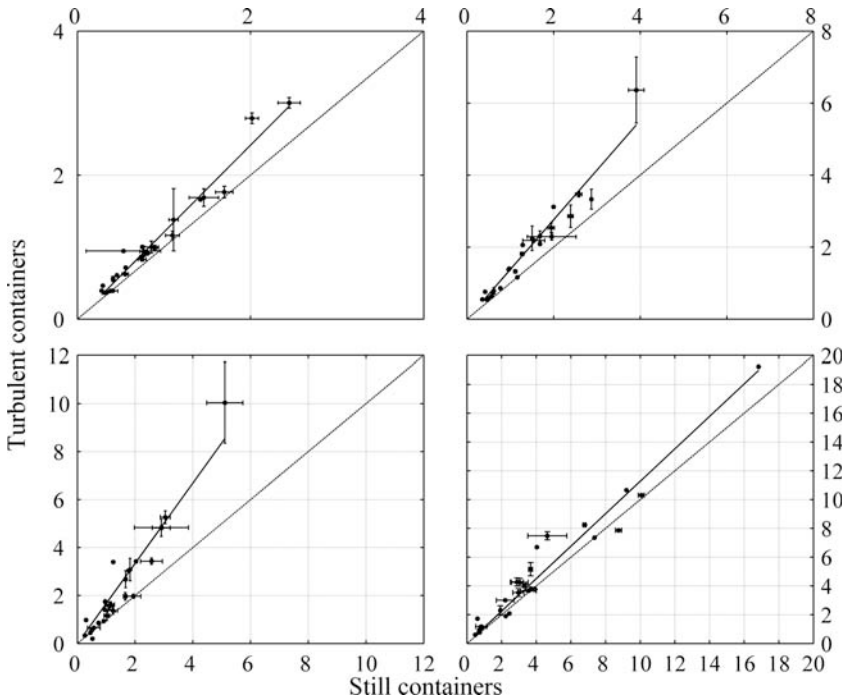

Fig. 5. Chlorophyll *a* values estimated in the turbulent treatments (*Y*-axis) plotted against the values in the still ones, considering the results of the 13 experiments, but grouped for each sampling day (1–3) and after resuspension. Vertical and horizontal bars are standard deviations between replicates. Continuous lines show the best linear regression fit model, of the form $y = ax$, where y is the value of the parameter in the turbulent treatment, and x is the value in the still one. Discontinuous lines represent the 1:1 ratio, plotted as reference.

enriched containers were calculated, as in unenriched containers the differences between initial and final TP concentrations were very small, and an exponential decay was often not clearly observed. In the turbulent treatments, net sedimentation rates were significantly lower (paired $t_{12} = 3.22$, $P\text{-value} = 0.007$), although differences in rates between still and turbulent treatments were relatively small (0.03 day^{-1} on average).

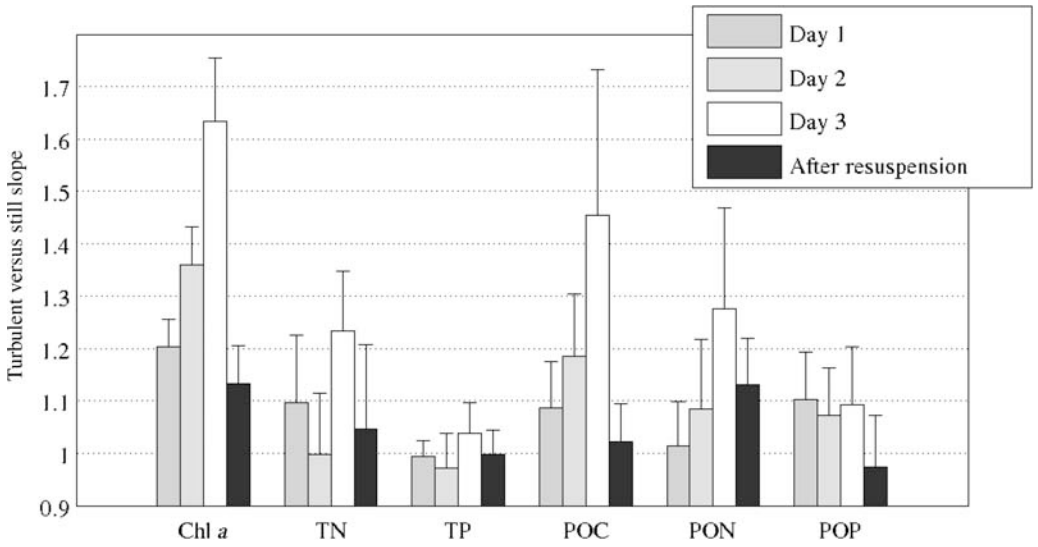

Fig. 6. Slopes of the best fit regression models of the form $y = ax$, where y is the value of the parameter in the turbulent treatment, and x is the value in the still one. Vertical bars represent the confidence intervals at 95%.

Data on phytoplankton abundance after resuspension were not available for all experiments, only for Exp 4, and for Exps 6 to 13. Even so, differences in abundance before and after resuspension were highly significant for the main phytoplankton groups, that is for diatoms (paired $t_{35} = 5.61$, *P-value* < 0.001), coccolithophorids (paired $t_{35} = 6.73$, *P-value* < 0.001) and dinoflagellates (paired $t_{35} = 2.41$, *P-value* $= 0.021$). The most conspicuous differences were found in diatom abundances, which in many cases were larger after resuspension by more than one order of magnitude.

DISCUSSION

Experimental versus natural conditions

Our experimental setup was designed to simulate the effects of a turbulent event of standard intensity and duration in a coastal zone. Therefore, we used turbulence levels and nutrient enrichments within the range of conditions that could be reasonably found in the Blanes Bay coastal ecosystem. Our estimates of the experimental turbulence were in the range 10^{-3}–10^{-2} cm^2 s^{-3}. These levels of dissipation could be generated at 1 m depth (below the wave-affected surface layer, Stips *et al.*, 2005) by wind speeds between 2 and 5 m s^{-1} (calculated from equation 5 in MacKenzie and Leggett, 1993). In this zone, the average frequency of wind events with a mean intensity of 5 m s^{-1} and a minimum duration of 3 days is of 1.5 season^{-1} (calculated applying equation 8 in Guadayol and Peters, 2006, using parameters estimated from the nearby meteorological station of Malgrat de Mar between years 1990 and 1997). Unlike other zones of the Catalan coast, spring is the season with the highest frequency of low intensity wind events, such as the ones simulated in this study. High intensity events are more frequent in autumn (Guadayol and Peters, 2006).

Similar studies with natural osmotrophic communities from coastal waters of the NW Mediterranean (e.g. Arin *et al.*, 2002; Malits *et al.*, 2004) did not consider the seasonal variability, and used higher turbulence intensities (0.018 to 0.055 cm^2 s^{-3}) and longer experiments (4–8 days). Despite the realistic moderate forcings of the present study, the effect of both turbulence and nutrient enrichment on chlorophyll and particulate organic matter production was found to be highly significant (Tables II and IV).

Performing a time series of microcosm experiments throughout a seasonal period offers additional challenges compared to single experiments. In order to isolate the effects of the experimental factors, all other parameters should be held constant across the experiments. However, temperature and photoperiod, which vary seasonally and with potentially large influence on system metabolism at mid-latitudes, were adapted to *in situ* values. Other initial conditions such as light intensity and quality were held constant across experiments. These parameters change at very short time scales, and there was no way to be certain that conditions at the time of sampling were representative of a particular month. On the other hand, we did not want to reproduce or study the effect of the rapid variability

in light intensity and quality (through changing cloud cover for instance). Therefore, we chose a constant light level, low enough as to be very common in these Mediterranean waters even in winter, but high enough as to be saturating for primary producers (Satta *et al.*, 1996). In this way, we ensured that all phytoplankton communities would be growing at the maximum rate allowed by the nutrient and turbulent conditions, which were the factors under consideration.

Another factor that has to be taken into account in microcosm experiments is the size of the enclosures, which poses a limit on which organisms can be studied. The abundance of any given taxon depends on the size of the organisms and on the taxonomic level. The estimates of abundance of scarce organisms can lead to biased conclusions, because of large estimation errors and low statistical power. To avoid these, we have restricted our analyses to general biomass indexes (like chlorophyll *a* and bacterial carbon concentrations, or particulate organic matter), and to the dominant plankton groups (such as diatoms). On the other hand, we did not specifically examine the dynamics of relatively large and/or scarce organisms (such as ciliates, copepod nauplii or dinoflagellates).

Common trends

Turbulence significantly increased Chl *a* within the experimental system at all times of the year, when considering only the water column (ANCOVA, Table II, Fig. 1), and also after including resuspended sediment (ANOVA, Table IV, Fig. 6). The mechanisms for this enhanced production in the water column may include some or all of the following: (i) increased nutrient flux towards large osmotrophs (Karp-Boss *et al.*, 1996; Peters *et al.*, 2006), (ii) differential sedimentation, (iii) differential grazing (Peters *et al.*, 1998, 2002), and (iv) increased growth rate of phytoplankton. Although we did not measure grazing losses (iii), they are not likely to play a major role in the dynamics of microphytoplankton in this experimental setup. First, because at these low levels, turbulence is more likely to have a positive than a negative effect on grazing rates through the enhancement of encounter rates. Therefore, the expected outcome, if any, would be a negative indirect effect on net phytoplankton growth. Second, because larger predators of large osmotrophs, if present, were mostly screened out by a 200 μm nylon mesh at the beginning of the experiment. Third, because the duration of the incubations was too short to allow any significant copepod development from earlier stages. Regarding the phytoplankton growth rate (iv), certain phytoplankton species have been seen to grow better in experiments with cultures, especially under moderate turbulence conditions like those used here (e.g. Sullivan and Swift, 2003; Havskum *et al.*, 2005; Havskum and Hansen, 2006). This enhancement has been attributed to the increased gas exchange which avoids excessively high pH (Havskum and Hansen, 2006). Although aeration may play a role in the present study, the concentrations of autotrophic biomass reached were much lower than in those experiments, and pH did not noticeably increase as a result of turbulence (data not shown). Thus, the most likely mechanisms for the observed increase of phytoplankton biomass under turbulent conditions are the increase of nutrient flux (i) and the decrease of net sedimentation (ii).

The decrease in diatom concentration during most of the experiments, as well as its increase after resuspension on Day 3, suggest that at the relatively low turbulent levels used in this study, sedimentation–resuspension dynamics is playing a major role. The decrease after resuspension in the turbulent versus still slopes shown in Fig. 6 for several parameters can only be explained by different sedimentation–resuspension dynamics in still and turbulent conditions. The robustness of this relationship suggests that these dynamics are, within the experimental containers, fairly constant throughout the year, regardless of different size structures and physiological states of the planktonic community. However, even though the slope is less steep after resuspension, its deviation from the 1:1 line (Figs 5 and 6) implies a higher biomass production in the turbulent containers, as already seen from ANOVAs. Once large cells are either kept in suspension and/or resuspended to a higher degree owing to turbulence, the increase in nutrient flux towards cells can keep populations growing and/or in a better physiological state.

Bacteria also showed a significant response to turbulence and enrichment, although this was low compared to that of phytoplankton (Fig. 2). Their growth did rarely exhibit an exponential pattern. Most often they increased in number at the beginning of the experiment, and then stabilized or decreased. This pattern is commonly observed in this kind of experiments (e.g. Arin *et al.*, 2002; Malits *et al.*, 2004). In longer experiments (at least 8 days), a second peak of bacterial concentration following the chlorophyll *a* maximum is commonly observed. Such a post-bloom phase peak is triggered by the release of dissolved organic material from phytoplankton and their predators (Malits *et al.*, 2004; Pinhassi *et al.*, 2004). In these situations, the observed effects on bacterial concentration during this phase can be a consequence of higher phytoplankton growth, rather than of any direct influence of turbulence or of nutrient addition. However, given the

duration of our experiments, the significant response to the experimental factors is more likely to reflect immediate effects in bacterial populations. Since bacteria are smaller, sedimentation–resuspension dynamics are much less important than for phytoplankton (e.g. Peters *et al.*, 1998), and observed changes in abundance are likely to reflect small-scale turbulence effects.

The natural levels of turbulence used in this study should in theory not directly affect bacterial uptake rates of substrates of low molecular weight (Peters *et al.*, 1998; Malits *et al.*, 2004). This is because the increase in uptake rates due to small-scale turbulence depends on the size of the organism (Karp-Boss *et al.*, 1996). The consistent positive response to turbulence observed in this study is most likely due to indirect effects through changes in trophic web interactions, as suggested by Peters and co-authors (Peters *et al.*, 1998; Peters *et al.*, 2002). According to these studies, under turbulent conditions phagotrophic flagellates increase their grazing pressure on cyanobacteria and small autotrophic flagellates, which are more nutritious, reducing as a consequence their pressure on bacteria.

POP showed a different behaviour compared to PON and POC. In the first place, particulate organic matter was richer in P in the water column than in the sediment. This suggests that either both PON and POC are sedimenting at a higher net rate than POP, or P has a shorter residence time in the sediment. In this respect, it is known that organic P is remineralized more rapidly than C both in the dissolved (Clark *et al.*, 1998) and in the particulate fractions (Faul *et al.*, 2005).

Moreover, the ratios between biomass parameters in still and in turbulent treatments throughout the experiment (Fig. 6) indicate a different response to turbulence of P with respect to that of N and of C in the particulate fraction. We did not directly measure sedimentation of particulate organic matter but some insight can be gained from budget analysis. Net sedimentation rates of TP were higher in still than in turbulent containers, but the differences were very small (0.03 day^{-1} on average), when compared with the differences between chlorophyll *a* growth rates of the two treatments (0.15 day^{-1} on average). Furthermore, neither TP nor POP showed any difference at any sampling time between still and turbulent treatments, which also indicates a low effect of turbulence in P net sedimentation rates. In contrast, the ratios of POC and PON between turbulent and still treatments were increasing throughout the experiments, and then returned to values close to 1 after resuspension (Fig. 6), indicating differences in net sedimentation rates due to turbulence.

As a consequence, POC and PON were higher in the water under turbulent conditions. The outcome is that turbulence relatively increased the PON:POP ratio in the water column, decreasing differences with the sediment. Similarly, Maar *et al.* (Maar *et al.*, 2002) found higher PON:POP, POC:POP and living C:POP ratios under turbulent conditions after 4 days of experimentation. They interpreted this finding as an effect of small-scale turbulence on turnover rates of P, which they found to be higher under turbulent conditions as predicted from theory (Karp-Boss *et al.*, 1996). In a mesocosm study, Eppley *et al.* (Eppley *et al.*, 1978) found N:P utilization ratios to be lower in stirred than in unstirred enclosures, implying also a higher turnover rate for P under turbulent conditions. These authors concluded that differences in utilization ratios were likely due to different dynamics of the water column. However, this study may be not comparable to ours, since turbulence was introduced daily for a short time with the aim of avoiding sedimentation and promoting resuspension of large cells.

Sedimentation–resuspension dynamics can also add to the interpretation. Small particles, such as bacteria and cyanobacteria, settle at a slower rate than larger phytoplankton. In fact, no differences in sedimented bacteria can be observed between still and turbulent treatments, even at the higher levels of turbulence used in other studies (Peters *et al.*, 1998). Thus, if there is differential sedimentation of nano- and microphytoplankton due to turbulence but not of bacteria, the percentage of bacteria in the water is expected to be higher in still than in turbulent conditions. Bacteria have N:P ratios of around 10:1 (Fagerbakke *et al.*, 1996, and references therein), lower than phytoplankton. Consequently, an increase in the proportion of bacteria in the water can partly explain the decrease of PON:POP in the water column, particularly under still conditions. However, this hypothesis is not supported by the percentage of heterotrophic bacteria in our experiments, which was found not to be significantly affected by turbulence (ANCOVA, data not shown). Furthermore, Arin *et al.* (Arin *et al.*, 2002) found lower percentages of heterotrophic C under turbulent conditions.

In the field, some increases in biomass associated with turbulent episodes are not related to enhanced growth rates, but to resuspension from the sediment of benthic autotrophic flagellates (Garstecki *et al.*, 2002), which may not contribute to the primary production in the water column, and may rapidly settle back when turbulence falls below a critical value. This could explain the dynamics found in Exp 7, which were very different from the other experiments. The observed decline in biomass parameters was likely caused by sedimentation. The initial chlorophyll *a* was unusually high for this system, and the phytoplankton community was

dominated by autotrophic nanoflagellates. This coincided with the absolute maximum in NO_2^-, and also with relative maxima in NO_3^-, NH_4^+ and SiO_2 concentrations (Table I). This anomalous behaviour can be explained by previous meteorological conditions. During the first half of the month, a steady wind from the NE blew, and the significant wave height reached values of almost 5 m (Guadayol *et al.*, in press). This probably led to an intense resuspension event, detected by a minimum in the Secchi disk record, which explains the maxima in Chl *a* and nutrients observed and the tendency to sink during the experiment.

Temporal dynamics of the responses

Our aim was to evaluate the role of the initial phytoplankton composition over the year on the short-time responses to experimental factors assessed in terms of bulk biomass parameters such as chlorophyll *a* concentration. We did not seek to detect changes in terms of phytoplankton taxonomic composition. In long-time enclosure experiments (e.g. 20 days), it is common to find that during the first stages (e.g. 6 days) the response to experimental factors in terms of chlorophyll *a* is mainly due to diatoms (Estrada *et al.*, 1987; Estrada *et al.*, 1996). Other phytoplankton groups, such as haptophytes, dinoflagellates and nanoflagellates, become dominant in later stages of the succession. Large diatoms are particularly favoured by turbulence, because of their life strategy (Margalef, 1978; Smetacek, 1985) and size (Peters *et al.*, 2006). Thus, the absolute and relative amount of diatoms should be reasonably good predictors of the capability of the system to quickly respond to turbulence and nutrient enrichments. However, in the present study, the responses in terms of chlorophyll *a* growth rate were not found to be significantly related neither to the proportion and abundance of diatoms, nor to the size structure of the community (Table III). On the other hand, responses to experimental factors, particularly to nutrient fertilization, were related to initial physicochemical conditions.

This apparent minor role of diatoms could be caused by a low Si concentration of enrichments, which could limit diatom growth in some of the experiments, or stimulate sedimentation (Bienfang *et al.*, 1982). However, the Si:N ratio in the water column either did not change throughout the experiments or increased, suggesting no general limitation by silica. Then, why is the response to turbulence not related to the initial community, as was hypothesized? In particular, why did diatom-dominated communities not respond more strongly? Two complementary explanations can be put forward.

The first explanation is related to the realistic intensity of the experimental factors, which was lower than in many similar experiments (e.g. Estrada *et al.*, 1996; Arin *et al.*, 2002; Cózar and Echevarría, 2005). Under such moderate levels of turbulence, sedimentation seems to play an important role. The abundance of diatoms decreases in all experiments but the first four, in which diatom net growth rate seems to be overcoming net sedimentation rate. Diatoms, even if they benefit from the small-scale turbulence generated, may not remain in the water column long enough to show a significant advantage in terms of biomass.

The second explanation is that the time scale used in these experiments was very short. Turbulence increases the uptake rate of solutes, and this enhancement is higher the larger the osmotroph (Lazier and Mann, 1989; Karp-Boss *et al.*, 1996; Peters *et al.*, 2006). However, this benefit is relative, and the smallest osmotrophs still have the highest bulk uptake and growth rates. The advantage of diatoms becomes important when compared to similar-sized osmotrophs, like dinoflagellates. Microphytoplankton blooms are hypothesized to occur because grazing control on smallest organisms is stronger, and occurs at shorter time scales than on large osmotrophs (Kiørboe, 1993; Thingstad, 1998; Irigoien *et al.*, 2005; Kiørboe, 2008). In this series of experiments, the size-spectrum slopes increase in all experiments and treatments except in the first four. This shows that most of the observed response in chlorophyll *a* in the water column must be attributed to picophytoplankton. Although diatoms are able to bloom in 3 days (e.g. Estrada *et al.*, 1996), picophytoplankton is still better suited to take advantage of the moderate nutrient forcing.

It was the purpose of our study to adjust the intensity and duration of experimental factors to realistic forcings found in Blanes Bay. Such moderate episodic events of turbulence and/or nutrient fertilization indeed affect the trophic status of the ecosystem, and can therefore influence the successional pattern at a seasonal time scale. However, the initial response to these events seems to be independent of the stage of the succession, or more precisely, the smallest fraction is responsible for the initial response regardless of the community composition and structure. Blooms of microphytoplankton, and particularly of diatoms, may require more time and/or stronger perturbations to develop. The differences in response due to initial community composition are usually detected after several days (Estrada *et al.*, 1987), or under more intense forcing (Estrada *et al.*, 1996). Diatom growth is often detected in similar experiments, provided that nutrient enrichment and turbulence are high enough (e.g. Oviatt *et al.*, 1981; Estrada

et al., 1987; Petersen *et al.*, 1998; Arin *et al.*, 2002; Cózar and Echevarría, 2005). Diatom blooms may be seemingly expected to develop only when the perturbation surpasses a certain threshold of intensity, which is likely dependent on the system and the season. This is relevant in scenarios of increasing input of nutrients in coastal zones due to anthropogenic environmental perturbations (Vollenweider *et al.*, 1996), and of higher frequency and intensity of extreme hydrological phenomena (Trenberth *et al.*, 2007). Even though the open ocean water column stability is expected to increase due to global warming (e.g. Sarmiento *et al.*, 2004; Falkowski and Oliver, 2007), more intense and frequent forcing events, especially in coastal areas, may translate into a higher system production, including diatoms (Peters, 2008). Thus, determining the thresholds of duration and intensity necessary for the development of diatom blooms becomes important for predicting the fate of the new production after a perturbation.

The maximum responses of Chl *a* to turbulence throughout the year were found in spring (Fig. 4A), coinciding with the period of maximum frequency of moderate wind events (Guadayol and Peters, 2006). Nevertheless, this does not imply a better adaptation of the community to turbulence. For example in Exp 1 (spring 2003), the response was the maximum of the whole series, but wind speeds and waves, at the time of sampling and several days before, were unusually low (Guadayol *et al.*, in press). Moreover, although the frequency of moderate wind events is lowest in autumn in this particular area of the Catalan coast (Guadayol and Peters, 2006), the significant wave height in 2003 during this season was maximal, reaching over 4 m. The size structure of the community and its composition did not explain the changes in the responses either. These were not found to be directly related to any seasonal parameter, except for a weak relationship with the photoperiod (Table III). Thus, the response of the plankton community to turbulence has been seen to be independent of other external factors.

In contrast, the response to nutrient enrichment was more dependent on initial conditions. It showed a moderate seasonal component, being positively correlated with the photoperiod and daily irradiance, as well as with water temperature (Table III). A similar pattern is observed in the POP/TP ratio after 1 day of nutrient enrichment (Fig. 3B), which was also correlated with the photoperiod. Additionally, in the analysis of covariance (Table II), the interaction EXP × NUT was found to be significant (except for PON), indicating a different response to nutrient enrichment throughout the year. These seasonal patterns are not related to the size structure of the community, which did not follow a seasonal curve because in summer 2004 diatoms (*Chaetoceros* spp.) were dominant and the slopes of the size spectra were low.

Correlations with seasonal parameters, such as temperature, photoperiod or irradiance, suggest that the changes throughout the year in the responses, especially to enrichments, could be due to the differences in conditions of growth not related to the experimental factors. Particularly, we would expect that any environmental change with a positive effect on phytoplankton growth, such as nutrient enrichment or turbulence, will be magnified by the number of hours to grow, i.e. by the photoperiod. Simply, given that there are some differences in specific growth rates due to experimental factors, the divergences in terms of absolute biomass between the treatments will be larger as phytoplankton have more time to grow. In a similar way, temperature may play a role since metabolic rates are exponentially related to temperature (e.g. Brown *et al.*, 2004). One might suggest that this seasonal pattern could also be explained by photoinhibition during winter or at least by the presence in summer of phytoplankton better adapted to high levels of light. However, the experimental light intensity (225 $\mu E\ m^{-2}\ s^{-1}$) is common for this Mediterranean system even in winter (Blanes Bay Microbial Observatory, unpublished data).

The influence of other parameters, not directly related to seasonality, on the pattern of variability of the responses to nutrients cannot be ruled out. Actually, the strongest correlations were found with Secchi depth, and with N and Si concentrations. The fact that the response was inversely related to the initial concentrations of NO_3^- and SiO_2 but not of PO_4^{3-}, suggests that there was a general limitation either by N and/or by Si. However, this is a system generally found to be limited by P (Lucea *et al.*, 2005; Pinhassi *et al.*, 2006). Furthermore, the N:P utilization ratios in the enriched treatments were above the Redfield ratio only in 4 of the 13 experiments (data not shown), and the NO_3^-:PO_4^{3-} ratio similarly increased in the water column of the enriched treatments in most of the experiments. The concentration of PO_4^{3-} in the water, due to its high turnover rates (Benitez-Nelson, 2000, and references therein), may be a poor estimator of the P dynamics in the water column (Pomeroy, 1960; Hecky and Kilham, 1988), and therefore of any systemic limitation by this element. The high turnover rates, together with the luxury consumption, make the PO_4^{3-} signal after an enrichment episode highly ephemeral. Thus, the measured concentration of inorganic phosphorus after 2 h of addition is always lower than expected. In contrast, both NO_3^- and SiO_2, as well as the Secchi

depth, are good indicators of recent events of terrestrial runoff and/or wave induced resuspension of sediments, and have therefore highly episodic dynamics in this coastal system. The correlation they show with the response to nutrient enrichment could reflect the importance of nutrient input history. Response to nutrient enrichment is lower if the system has recently come through a nutrient fertilization event, whereas it is larger when the community is nutrient starved, whatever the limiting element. For example, in October 2003, after a strong river discharge event, bacterioplankton was not limited by phosphorus (Pinhassi *et al.*, 2006), despite an N:P ratio much higher than the Redfield ratio.

CONCLUSIONS

Although laboratory experiments are only approximations that cannot fully reproduce the complexity of environmental variability, ecologically relevant information can be obtained from these studies. The response of the community to laboratory turbulence is quantitatively variable in time, but does show very robust qualitative regularities.

Under the moderate, "realistic" experimental conditions used in this study, both nutrient addition and turbulence triggered a highly significant increase in chlorophyll *a* concentration. In the case of turbulence, these increments could be due to direct effects of small-scale turbulence or to altered sedimentation–resuspension dynamics under turbulent flow, as expected in natural conditions as well. The dynamics of POC and PON differed from that of POP, indicating different sedimentation rates and/or different turnover rates. In general, POC and PON were accumulated in the sediment at a higher net rate. Turbulence slightly modified this pattern by relatively increasing the N:P ratio of the particulate organic matter in the water column.

There were considerable changes in the phytoplankton response to experimental factors throughout the year, but on the short-time scales considered, no relationship was apparent between these responses and the initial phytoplankton composition and size structure. The changes in the response to nutrient fertilization were related to seasonal forcings, namely photoperiod and temperature, and also to the recent availability of nutrients, whereas the response to turbulence was found to be only weakly seasonal. The input of nutrients from runoff in coastal zones has been increasing during the last century due to anthropogenic environmental perturbations (Vollenweider *et al.*, 1996). The frequency and intensity of extreme hydrological phenomena is predicted to increase during the next decades (Trenberth *et al.*, 2007). The ability of the plankton community to respond to these forcings is of importance to understand the flux of carbon in coastal systems. The results of this study suggest that the capacity of phytoplankton to react quickly to episodic nutrient events depends on the trophic state of the system. We found that the maximum responses when the frequency of nutrient inputs was lower. In contrast, phytoplankton response to turbulence seems to be independent of the frequency of turbulent episodes.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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REFERENCES

Agawin, N. S. R., Duarte, C. M. and Agustí, S. (1998) Growth and abundance of *Synechococcus sp.* in a Mediterranean Bay: seasonality and relationship with temperature. *Mar. Ecol. Prog. Ser.*, **170**, 45–53.

Alcaraz, M., Marrasé, C., Peters, F. *et al*. (2002) Effects of turbulence conditions on the balance between production and respiration in marine planktonic communities. *Mar. Ecol. Prog. Ser.*, **242**, 63–71.

Alonso-Sáez, L., Vázquez-Domínguez, E., Cardelús, C. *et al*. (2008) Factors controlling the year-round variability in carbon flux through bacteria in a coastal marine system. *Ecosystems*, **11**, 397–409.

Arin, L., Marrasé, C., Maar, M. *et al*. (2002) Combined effects of nutrients and small-scale turbulence in a microcosm experiment. I. Dynamics and size distribution of osmotrophic plankton. *Aquat. Microb. Ecol.*, **29**, 51–61.

Benitez-Nelson, C. R. (2000) The biogeochemical cycling of phosphorus in marine systems. *Earth-Sci. Rev.*, **51**, 109–135.

Berdalet, E. and Estrada, M. (2005) Effects of small-scale turbulence on the physiological functioning of marine microalgae. In Subba Rao, D. V.(ed.), *Algal Cultures, Analogues of Blooms and Applications*. Science Publishers, Enfield, NH, USA, pp. 459–500.

Berdalet, E., Peters, F., Koumandou, V. L. *et al*. (2007) Species-specific physiological response of dinoflagellates to quantified small-scale turbulence. *J. Phycol.*, **43**, 965–977.

Bienfang, P. K., Harrison, P. J. and Quarmby, L. M. (1982) Sinking rate response to depletion of nitrate, phosphate and silicate in 4 marine diatoms. *Mar. Biol.*, **67**, 295–302.

Brown, J. H., Gillooly, J. F., Allen, A. P. *et al*. (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1789.

Clark, L. L., Ingall, E. D. and Benner, R. (1998) Marine phosphorus is selectively remineralized. *Nature*, **393**, 426.

Cózar, A. and Echevarría, F. (2005) Size structure of the planktonic community in microcosms with different levels of turbulence. *Sci. Mar.*, **69**, 187–197.

Dade, W. B. (1993) Near bed turbulence and hydrodynamic control of diffusional mass-transfer at the sea-floor. *Limnol. Oceanogr.*, **38**, 52–69.

Eppley, R. W., Koeller, P. and Wallace, G. T., Jr (1978) Stirring influences the phytoplankton species composition within enclosed columns of coastal sea water. *J. Exp. Mar. Biol. Ecol.*, **32**, 219–239.

Estrada, M., Alcaraz, M. and Marrasé, C. (1987) Effects of turbulence on the composition of phytoplankton assemblages in marine microcosms. *Mar. Ecol. Prog. Ser.*, **38**, 267–281.

Estrada, M., Berdalet, E., Marrasé, C. *et al*. (1996) Effect of different nutrient combinations on phytoplankton development in microcosms. In Yasumoto, T., Oshima, Y. and Fukuyo, Y. (eds), *Harmful and Toxic Algal Blooms*. Intergovernmental Oceanographic Comission of UNESCO, pp. 297–300.

Fagerbakke, K. M., Heldal, M. and Norland, S. (1996) Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. *Aquat. Microb. Ecol.*, **10**, 15–27.

Falkowski, P. G. and Oliver, M. J. (2007) Mix and match: how climate selects phytoplankton. *Nat. Rev. Microbiol.*, **5**, 813–819.

Faul, K. L., Paytan, A. and Delaney, M. L. (2005) Phosphorus distribution in sinking oceanic particulate matter. *Mar. Chem.*, **97**, 307–333.

Gargett, A. E. (1989) Ocean turbulence. *Annu. Rev. Fluid Mech.*, **21**, 419–451.

Garstecki, T., Wickham, S. A. and Arndt, H. (2002) Effects of experimental sediment ressuspension on a coastal planktonic microbial food web. *Estuarine Coastal Shelf Sci.*, **55**, 751–762.

Gasol, J. M. and del Giorgio, P. A. (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Sci. Mar.*, **64**, 197–224.

Guadayol, Ò. and Peters, F. (2006) Analysis of wind events in a coastal area: a tool for assessing turbulence variability for studies on plankton. *Sci. Mar.*, **70**, 9–20.

Guadayol, Ò., Peters, F., Marrasé, C. *et al*. (in press) Episodic meteorological and nutrient load events as drivers of coastal planktonic ecosystem dynamics: a time series analysis. *Mar. Ecol.-Prog. Ser.* doi:10.3354/meps07939.

Guadayol, Ò., Peters, F., Stiansen, J. E. *et al*. (in press) Evaluation of oscillating grids and orbital shakers as means to generate isotropic and homogeneous small-scale turbulence in laboratory enclosures commonly used in plankton studies. *Limnol. Oceanogr. Meth.*

Hansen, H. P. and Koroleff, F. (1999) Determination of nutrients. In Grasshoff, K., Kremling, K. and Ehrhardt, M. (eds), *Methods of Seawater Analyses*. Wiley-Vch, pp. 161–228.

Havskum, H. and Hansen, P. J. (2006) Net growth of the bloom-forming dinoflagellate *Heterocapsa triquetra* and pH: why turbulence matters. *Aquat. Microb. Ecol.*, **42**, 55–62.

Havskum, H., Hansen, P. J. and Berdalet, E. (2005) Effect of turbulence on sedimentation and net population growth of the dinoflagellate *Ceratium tripos* and interactions with its predator, *Fragilidium subglobosum*. *Limnol. Oceanogr.*, **50**, 1543–1551.

Hecky, R. E. and Kilham, P. (1988) Nutrient limitation of phytoplankton in fresh-water and marine environments—a review of recent-evidence on the effects of enrichment. *Limnol. Oceanogr.*, **33**, 796–822.

Hillebrand, H., Durselen, C. D., Kirschtel, D. *et al*. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Hopfinger, E. J. and Toly, J. A. (1976) Spatially decaying turbulence and its relation to mixing across density interfaces. *J. Fluid Mech.*, **78**, 175–188.

Huisman, J., Arrayás, M., Ebert, U. *et al*. (2002) How do sinking phytoplankton species manage to persist? *Am. Nat.*, **159**, 245–254.

Irigoien, X., Flynn, K. and Harris, R. (2005) Phytoplankton blooms: a "loophole" in microzooplankton grazing impact? *J. Plankton Res.*, **27**, 313–321.

Karp-Boss, L., Boss, E. and Jumars, P. A. (1996) Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanogr. Mar. Biol. Annu. Rev.*, **34**, 71–107.

Kiørboe, T. (1993) Turbulence, phytoplankton cell size, and the structure of pelagic food webs. *Adv. Mar. Biol.*, **29**, 1–72.

Kiørboe, T. (1997) Small-scale turbulence, marine snow formation, and planktivorous feeding. In Marrasé, C., Saiz, E. and Redondo, J. M. (eds), *Lectures on Plankton and Turbulence. Sci. Mar.*, **61**(Suppl. 1), 141–158.

Kiørboe, T. (2008) *A Mechanistic Approach to Plankton Ecology*. Princeton University Press, Princeton, USA.

Lazier, J. R. N. and Mann, K. H. (1989) Turbulence and the diffusive layers around small organisms. *Deep-Sea Res.*, **36**, 1721–1733.

Lucea, A., Duarte, C. M., Agustí, S. *et al*. (2005) Nutrient dynamics and ecosystem metabolism in the Bay of Blanes (NW Mediterranean). *Biogeochemistry*, **73**, 303–323.

Maar, M., Arin, L., Simó, R. *et al*. (2002) Combined effects of nutrients and small-scale turbulence in a microcosm experiment. II. Dynamics of organic matter and phosphorus. *Aquat. Microb. Ecol.*, **29**, 63–72.

MacKenzie, B. R. and Leggett, W. C. (1993) Wind-based models for estimating the dissipation rates of turbulent energy in aquatic environments: empirical comparisons. *Mar. Ecol. Prog Ser.*, **94**, 207–216.

Malits, A., Peters, F., Bayer-Giraldi, M. *et al.* (2004) Effects of small-scale turbulence on bacteria: a matter of size. *Microb. Ecol.*, **48**, 287–299.

Margalef, R. (1978) Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanol. Acta*, **1**, 493–509.

Marrasé, C., Saiz, E. and Redondo, J. M. (eds) (1997) Lectures on plankton and turbulence. *Sci. Mar.*, **61**(Suppl. 1), 1–238.

Masó, M. and Tintoré, J. (1991) Variability of the shelf water off the northeast Spanish coast. *J. Mar. Syst.*, **1**, 441–450.

Olson, R. J., Zettler, E. R. and DuRand, M. D. (1993) Phytoplankton analysis using flow cytometry. In Kemp, P. F., Sherr, B. F., Sherr, E. B., Cole, J. J. *et al.*(eds), *Handbook of methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton, pp. 163–186.

Oviatt, C. A., Hunt, C. D., Vargo, G. A. *et al.* (1981) Simulation of a storm event in marine microcosms. *J. Mar. Res.*, **39**, 605–626.

Peters, F. (2008) Diatoms in a future ocean—stirring it up. *Nat. Rev. Micro.*, **6**, 407.

Peters, F. and Gross, T. (1994) Increased grazing rates of microplankton in response to small-scale turbulence. *Mar. Ecol. Prog. Ser.*, **115**, 299–307.

Peters, F. and Redondo, J. M. (1997) Turbulence generation and measurement: application to studies on plankton. In Marrasé, C., Saiz, E. and Redondo, J. M. (eds), *Lectures on Plankton and Turbulence. Sci. Mar.*, **61**(Suppl. 1), 205–228.

Peters, F. and Marrasé, C. (2000) Effects of turbulence on plankton: an overview of experimental evidence and some theoretical considerations. *Mar. Ecol. Prog. Ser.*, **205**, 291–306.

Peters, F., Marrasé, C., Gasol, J. M. *et al.* (1998) Effects of turbulence on bacterial growth mediated through food web interactions. *Mar. Ecol. Prog. Ser.*, **172**, 293–303.

Peters, F., Marrasé, C., Havskum, H. *et al.* (2002) Turbulence and the microbial food web: effects on bacterial losses to predation and on community structure. *J. Plankton Res.*, **24**, 321–331.

Peters, F., Arin, L., Marrasé, C. *et al.* (2006) Effects of small-scale turbulence on the growth of two diatoms of different size in a phosphorus-limited medium. *J. Mar. Syst.*, **61**, 134–148.

Petersen, J. E., Sanford, L. P. and Kemp, W. M. (1998) Coastal plankton responses to turbulent mixing in experimental ecosystems. *Mar. Ecol. Prog. Ser.*, **171**, 23–41.

Piehler, M., Twomey, L., Hall, N. *et al.* (2004) Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA. *Estuarine Coastal Shelf Sci.*, **61**, 197–209.

Pinhassi, J., Sala, M. M., Havskum, H. *et al.* (2004) Changes in Bacterioplankton composition under different phytoplankton regimens. *Appl. Environ. Microbiol.*, **70**, 6753–6766.

Pinhassi, J., Gomez-Consarnau, L., Alonso-Saez, L. *et al.* (2006) Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW Mediterranean Sea. *Aquat. Microb. Ecol.*, **44**, 241–252.

Pomeroy, L. R. (1960) Residence time of dissolved phosphate in natural waters. *Science*, **131**, 1731–1732.

Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identification and enumeration of bacteria and blue-green algae. *Limnol. Oceanogr.*, **25**, 943–948.

Ross, O. N. (2006) Particles in motion: how turbulence affects plankton sedimentation from an oceanic mixed layer. *Geophys. Res. Lett.*, **33**, L10609.

Rothschild, B. J. and Osborn, T. R. (1988) Small-scale turbulence and plankton contact rates. *J. Plankton Res.*, **10**, 465–474.

Ruiz, J., Macías, D. and Peters, F. (2004) Turbulence increases the average settling velocity of phytoplankton cells. *Proc. Natl Acad. Sci. USA*, **101**, 17720–17724.

Sanford, L. P. (1997) Turbulent mixing in experimental ecosystem studies. *Mar. Ecol. Prog. Ser.*, **161**, 265–293.

Sarmiento, J. L., Slater, R., Barber, R. *et al.* (2004) Response of ocean ecosystems to climate warming. *Global Biogeochem. Cycles*, **18**, GB3003–GB3003.

Satta, M. P., Agustí, S., Mura, M. P. *et al.* (1996) Microplankton respiration and net community metabolism in a bay on the NW Mediterranean coast. *Aquat. Microb. Ecol.*, **10**, 165–172.

Smetacek, V. S. (1985) Role of sinking in diatom life-history cycles—ecological, evolutionary and geological significance. *Mar. Biol.*, **84**, 239–251.

Stips, A., Burchard, H., Bolding, K. *et al.* (2005) Measurement and simulation of viscous dissipation in the wave affected surface layer. *Deep-Sea Res. Part II-Top. Stud. Oceanogr.*, **52**, 1133–1155.

Sullivan, J. M. and Swift, E. (2003) Effects of small-scale turbulence on net growth rate and size of ten species of marine dinoflagellates. *J. Phycol.*, **39**, 83–94.

Sverdrup, H. U. (1953) On the conditions for the vernal blooming of phytoplankton. *J. Cons. Perm. Int. Exp. Mer.*, **18**, 287–295.

Thingstad, T. F. (1998) A theoretical approach to structuring mechanisms in the pelagic food web. *Hydrobiologia*, **363**, 59–72.

Trenberth, K. E., Jones, P. D., Ambenje, P. *et al.* (2007) Observations: surface and atmospheric climate change. In Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L. *et al.*(eds), *Climate Change 2007: The Physical Science Basis. Contribution of working group I to the fourth assessment of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 235–336.

Utermöhl, H. (1958) Zur vervollkommung der quatitativen Phytoplankton-Methodik. *Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **9**, 1–39.

Vaqué, D., Blough, H. A. and Duarte, C. M. (1997) Dynamics of ciliate abundance, biomass and community composition in an oligotrophic coastal environment (NW Mediterranean). *Aquat. Microb. Ecol.*, **12**, 71–83.

Vaulot, D., Partensky, F., Neveux, J. *et al.* (1990) Winter presence of prochlorophytes in surface waters on the NorthWestern Mediterranean-sea. *Limnol. Oceanogr.*, **35**, 1156–1164.

Vila-Costa, M., Pinhassi, J., Alonso, C. *et al.* (2007) An annual cycle of dimethylsulfoniopropionate-sulfur and leucine assimilating bacterioplankton in the coastal NW Mediterranean. *Environ. Microbiol.*, **9**, 2451–2463.

Vidondo, B., Prairie, Y. T., Blanco, J. M. *et al.* (1997) Some aspects of the analysis of size spectra in aquatic ecology. *Limnol. Oceanogr.*, **42**, 184–192.

Vollenweider, R. A., Rinaldi, A., Viviani, R. *et al.* (1996) Assessment of the state of eutrophication in the Mediterranean Sea. *MAP technical report Series*, Vol. 106. UNEP, Athens, pp. 1–449.

Yentsch, C. S. and Menzel, D. W. (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-sea Res.*, **10**, 221–231.