Combined effects of nutrients and small-scale turbulence in a microcosm experiment.

II. Dynamics of organic matter and phosphorus

Marie Maar^{1,*}, Laura Arin², Rafel Simó², Maria-Montserrat Sala², Francesc Peters²,
Cèlia Marrasé²

¹National Environmental Research Institute, Frederiksborgvej 399, 4000 Roskilde, Denmark

²Institut de Ciències del Mar (CMIMA-CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

ABSTRACT: In the oligotrophic sea, phytoplankton and bacteria compete for nutrients. Turbulence changes the outcome of this competition by means of an increase in the nutrient flux to cells by the shear fields, which is cell-size dependent. This effect is insignificant for small cells such as natural bacteria. The hypothesis is that turbulence will increase the phytoplankton competition-capability for nutrients and reduce the organic matter utilisation by bacteria. Consequently, the composition of particulate organic matter should change. To test this hypothesis, we studied the response of natural plankton communities to turbulence enclosed in 15 l microcosms. We evaluated the response in terms of the ratio of heterotrophic:total biomass and the stoichiometry of particulate organic matter. Results under turbulent and still conditions were compared in 3 nutrient-induced conditions: nitrogen surplus (N, with initial addition of an excess of nitrogen, N:P ratio = 160), nitrogen:phosphorus ratio balanced (NP, with initial addition of nitrogen and phosphorus as Redfield ratio, N:P ratio = 16) and control (C, no nutrient addition). In N and NP conditions, turbulence decreased the heterotrophic:total biomass ratio up to 2-fold, and induced changes in the stoichiometry of the particulate organic matter. We found higher values of carbon:phosphorus and nitrogen:phosphorus ratios in turbulent than in still treatments. The magnitude of these responses to turbulence depended on the induced nutrient conditions. In the control microcosms, we found the maximum differences of carbon:phosphorus ratio between turbulence and still treatments. In terms of biomass, the response to turbulence was clear in the enriched conditions and insignificant in the control microcosms.

KEY WORDS: Small-scale turbulence · Phosphorus · Particulate organic matter stoichiometry · Microcosms

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

In the oligotrophic NW Mediterranean Sea, phosphorus (P) has been suggested to be the limiting nutrient for phytoplankton (Margalef 1997, Thingstad et al. 1998) as well as for heterotrophic bacteria (Thingstad et al. 1993, 1998, Zweifel et al. 1993, Sala et al. 2002). The outcome of the competition for nutrients between

bacteria and different sizes of phytoplankton has implications for the structure and function of the pelagic food web. If small cells dominate the uptake of nutrients, the 'microbial' food web will be favoured with respect to the 'classical' food chain (Legendre & Le Fèvre 1991). Under P-limiting conditions, the ability of a cell to compete with others will depend, in principle, on its affinity constant for P. Theoretically, the affinity constant (α) for orthophosphate can be calculated from the physical law of diffusion (Thingstad 1998):

*E-mail: mam@dmu.dk

$$\alpha = 3 D \sigma^{-1} r^{-2} \quad (1)$$

where D is the diffusion coefficient for phosphate ($\sim 4.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), σ is the volume specific P content ($\mu\text{mol P cm}^{-3}$) and r is the cell radius (cm). Due to their size, bacteria, pico- and nanophytoplankton are expected to out-compete larger phytoplankton species under nutrient limitation (see Table 1). However, the relative advantage of the smaller hetero- and autotrophic components can be reduced in the presence of turbulent mixing. It has been suggested that small-scale turbulence will favour large non-motile phytoplankton cells by keeping them suspended in the euphotic zone of the water column and by replacing the nutrient-depleted water around them (Pasciak & Gavis 1974, Lazier & Mann 1989, Kjørboe 1993, Karp-Boss et al. 1996).

Studies of nutrient uptake and phytoplankton growth under turbulence are scarce. Pasciak & Gavis (1975) found a Michaelis-Menten type relationship between nitrate-uptake rate and shear rate for the large diatom *Ditylum brightwellii* at low nitrate concentrations. Savidge (1981) observed increased growth and uptake of the diatom *Phaedactylum tricornutum* when agitated under P-limited conditions. In natural waters, where other components of plankton are present, turbulence can change the relative importance of autotrophs to heterotrophic bacteria (Arin et al. 2002, this issue). These 2 biomass compartments have different nutrient:carbon demands. Thus, we speculate that turbulence could change the dynamics of nutrient concentrations. The availability of nutrients has been shown to affect the stoichiometry of organic matter. Guildford & Hecky (2000) found higher carbon:phosphorus ratios in P-limiting conditions. Thomas et al. (1999) found deviations of the Redfield ratio associated with a high contribution of recycling nutrients. In this paper, we examine the dynamics of nutrients in relation to the response of plankton to turbulence in experiments with Mediterranean coastal waters. As P is often the limiting nutrient in these waters, we studied the variations of P in the particulate (POP) and dis-

solved (DOP) organic forms, as well as the soluble reactive phosphorus (SRP) variations. We hypothesise that small-scale turbulence will affect P turnover-rate and plankton structure by favouring large osmotrophic organisms. We also expected these effects on plankton to be reflected in the quality of particulate organic matter. To test these hypotheses, we enclosed natural plankton communities in 15 l microcosms and studied their response to turbulence under different N:P ratio conditions.

MATERIALS AND METHODS

The microcosm experiment. Sea water was collected ca. 1 km offshore of the Catalan coast north of Barcelona (Spain). The sub-surface water was screened through a 150 μm Nytex mesh to remove mesozooplankton and transported in clean 50 l containers. In the laboratory, water was distributed in twelve 15 l cylindrical containers (made of transparent Plexiglas, 24.2 cm inner diameter and 34.5 cm depth). The enclosures were divided into 3 series: controls with no nutrient addition (C), enriched with a nitrogen-surplus addition (N) and enriched following the nitrogen:phosphorus Redfield ratio (NP), and incubated under still (S) and turbulent (T) conditions (Table 2). Each combination of treatments had 2 replicates. Nutrients were added at the beginning of the experiment according to Table 2. In N and NP containers, silicate and metals were also added. The metal solution was added in the same proportion to nitrate as in f/2 medium (Guillard 1975). The experiment was carried out in an environmental chamber at $15 \pm 1^\circ\text{C}$ under a 12:12 h light:dark cycle and at light irradiance of $225 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ within the containers. Small-scale turbulence was generated by a system of vertically oscillating grids as described in Arin et al. (2002). The turbulent kinetic energy dissipation rate (ϵ) was estimated as $0.055 \text{ cm}^2 \text{ s}^{-3}$.

Inorganic and organic nutrients. Inorganic nutrients, namely soluble reactive phosphorus (SRP- PO_4),

Table 1. The theoretical affinity constant estimated for different organisms from the physical law of diffusion (Eq. 1) by assuming spherical shapes. The cell carbon contents were calculated as $0.35 \text{ pg C } \mu\text{m}^{-3}$ for bacteria (Bjørnsen 1986), $0.433 \times (\mu\text{m}^3)^{0.863} \text{ pg C cell}^{-1}$ for nanoflagellates (Verity et al. 1992) and $0.109 \times (\mu\text{m}^3)^{0.991} \text{ pg C cell}^{-1}$ for diatoms (Montagnes et al. 1994). Carbon contents were converted to phosphorus by a ratio of 50 and 106 for bacteria and phytoplankton, respectively. The literature values of the affinities are from a review by Vadstein & Olsen (1989)

Organism	Radius (μm)	Volume specific P content ($\mu\text{mol P cm}^{-3}$)	Theoretical affinity ($\text{l } \mu\text{mol P}^{-1} \text{ h}^{-1}$)	Literature affinity ($\text{l } \mu\text{mol P}^{-1} \text{ h}^{-1}$)
Bacteria	0.25–0.75	583	16–142	30–146
Pigmented nanoflagellates	1–3	515–590	1.1–8.9	2–17
Diatoms	3–10	170–174	0.3–3.3	0.9–10

Table 2. Summary of the treatments (each treatment has 2 replicates) with the initial added nutrient concentrations (μM). No nutrients were added in the control containers (SC and TC). Dissolved inorganic nitrogen (DIN) and silicate (Si) concentrations (μM ; mean of 2 replicate microcosms $\pm$ SE) on Days 0 and 4 in each treatment, and the osmotrophic biomass increase:dissolved inorganic nitrogen consumed (OBI:DINC) and diatom biomass increase:Si consumed (DBI:SiC) ratios (both in $\mu\text{M}:\mu\text{M}$) from Day 0 to 4. Due to the low inorganic nutrient concentrations and a poor increase in biomass these ratios were not calculated in the control containers. (S = still, C = control, T = turbulent, N = N-surplus, NP = Redfield ratio)

Treatment	Initial nutrient addition			DIN		OBI:DINC	SiO ₃		DBI:SiC
	PO ₄	NO ₃	SiO ₃	Day 0	Day 4		Day 0	Day 4	
SC	–	–	–	0.24 $\pm$ 0.01	0.23 $\pm$ 0.04	–	0.37	0.51 $\pm$ 0.03	–
TC	–	–	–	0.36 $\pm$ 0.07	0.16 $\pm$ 0.005	–	0.25 $\pm$ 0.005	0.25 $\pm$ 0.04	–
SN	0.1	16	30	21.35 $\pm$ 1.71	14.36 $\pm$ 0.55	0.49 $\pm$ 0.29	35.97 $\pm$ 1.32	25.88 $\pm$ 0.63	0.07 $\pm$ 0.07
TN	0.1	16	30	19.58 $\pm$ 0.50	13.44 $\pm$ 0.54	5.35 $\pm$ 0.42	33.88 $\pm$ 0.22	21.65 $\pm$ 0.03	1.38 $\pm$ 0.17
SNP	1.0	16	30	18.72 $\pm$ 0.17	11.04 $\pm$ 0.07	2.82 $\pm$ 0.46	33.44 $\pm$ 0.33	22.83 $\pm$ 0.05	0.13
TNP	1.0	16	30	18.97 $\pm$ 0.26	1.62 $\pm$ 0.76	5.02 $\pm$ 0.31	33.00 $\pm$ 0.22	19.34 $\pm$ 1.02	4.63 $\pm$ 0.84

NO₃, NO₂, NH₄ and SiO₃, were determined daily in all the containers, with an Alliance Evolution II auto-analyzer following standard procedures as described in Grasshoff (1983). Particulate and dissolved organic phosphorus and nitrogen were determined by wet oxidation into SRP and NO₃. Analyses were done in 1 series of replicates (A) daily from Day 0 to 7 except for Day 5. Subsamples of 250 ml were taken in polyethylene bottles and filtered through pre-combusted GF/F filters under gentle vacuum (<50 mm Hg). The filters were stored in tinfoil envelopes at -20°C . Simultaneous determination of particulate organic phosphorus and nitrogen (POP, PON) was achieved following the persulphate oxidation procedures of Pujo-Pay & Raimbault (1994). In brief, 20 ml Milli-Q water and 2.5 ml persulphate + boric acid reagent were added to the filter in glass vials and autoclaved at 120°C for 30 min. Then, samples were centrifuged to remove filter remains and analysed for PO₄ and NO₃ + NO₂ in the autoanalyser as above. The standard error of analysis was generally within 10%. The same wet oxidation method was also applied to the total dissolved P (TDP) determination in the filtrates (Koroleff 1983, Valderama 1995). In glass vials, 30 ml aliquots were mixed with 4 ml of the oxidant reagent, autoclaved under the same conditions as for POP and autoanalysed. Dissolved organic P (DOP) was calculated by subtracting SRP from TDP. Standard error of analysis replicates for DOP was generally within 25%.

Sedimented phosphorus. To gain a more complete picture of P dynamics, sedimented particulate phosphorus had to be determined. Accumulation of biomass on the bottom was evident in several of the containers; however, we did not sample and measure it directly as we wanted to avoid disturbing the system. Thus, sedimented POP was estimated as follows. Assuming that P could not be lost from the container because its air-water exchange through volatile forms is insignificant, the value of the P burden for each

treatment was fixed as the concentration of total P on Day 0 right after the addition of nutrients. Then, sedimented POP at Day i was estimated by subtracting the measured P pools in the water column from the total P burden, i.e. sedimented POP _{i} = P burden – (PO₄ + DOP + POP) _{i} .

Phosphate turnover rate. The turnover rate K (h^{-1}) of phosphate was measured by adding radioactively labelled orthophosphate to subsamples from 1 series of replicates (A) of each treatment on Days 0 to 7 (except Day 5). K is defined as the speed of exchange of a phosphate concentration C by a community of uptake rate U :

$$K = UC^{-1} \quad (2)$$

Uptake of radiolabelled orthophosphate was measured by adding 100 μl carrier-free $^{33}\text{PO}_4^{3-}$ (Amersham) to 10 ml subsamples in scintillation vials to give a final radioactive concentration of 2×10^5 to 4×10^5 dpm ml^{-1} . From each microcosm, 3 or 4 subsamples were incubated for between 5 min and 5 h depending on the expected turnover rate. Temperature and light irradiance was similar to the experimental containers. Incubations were terminated by adding a cold chase of 100 μl 0.01 M K₂HPO₄ to the vials. Subsamples of 3.3 ml were filtered through 25 mm polycarbonate filters of 0.2 μm . To minimise cell breakage, filters were supported by Whatman GF/F glass fibre filters saturated with 0.1 M K₂HPO₄. Suction was applied and increased gradually until all samples had passed through the 0.2 μm filters and then increased to a maximum pump capacity of 0.7 bar until the filters were dry. Blanks were determined each day by adding the cold chase to the subsample before the isotope. The filters were transferred to polyethylene scintillation vials (Zinssner) and counted in 7 ml Ultima-Gold XR in a Beckmann liquid scintillation counter. The exact start concentration of the added $^{33}\text{PO}_4^{3-}$ was determined by transferring 100 μl subsample to each of 3 plastic vials

with 7 ml scintillation fluid. Turnover rate of phosphate K was calculated from the equation:

$$K = -\ln(1-f) t^{-1} \quad (3)$$

where t is the incubation time and f the fraction of added isotope retained on the $0.2 \mu\text{m}$ filters corrected for blanks. The radioactivity of blanks was below 4000 dpm and the signal:blank ratio was higher than 6.2 in C and N containers and higher than 2.5 in NP containers.

Plankton biomass. Phytoplankton biomass was monitored by daily chlorophyll a (chl a) measurements and converted to carbon biomass using a factor of 30

(Strickland 1960). Chl a was analysed fluorometrically (Yentsch & Menzel 1963) after extraction in 90 % acetone. In addition, phytoplankton biomass was also estimated by their group composition. On Days 0, 4 and 8, abundances were determined for the diatoms, dinoflagellates and coccolithophorids using an inverted microscope (Utermöhl 1958), for the autotrophic picoeukaryotes, *Synechococcus* and *Prochlorococcus*, by flow cytometry (Gasol & del Giorgio 2000) and for the autotrophic nanoflagellates by epifluorescence microscopy (Porter & Feig 1980). Carbon contents were estimated from the following conversion factors: diatoms, dinoflagellates and coccolithophorids, $\text{pg C cell}^{-1} = 0.109 \times (\mu\text{m}^3)^{0.991}$ (Montagnes et al. 1994); *Synechococcus*, $0.357 \text{ pg C } \mu\text{m}^{-3}$ (Bjørnsen 1986, Kana & Gilbert 1987, Verity et al. 1992); picoeukaryotic and autotrophic nanoflagellates, $\text{pg C cell}^{-1} = 0.433 \times (\mu\text{m}^3)^{0.863}$ (Verity et al. 1992); and *Prochlorococcus*, $0.133 \text{ pg C } \mu\text{m}^{-3}$ (Simon & Azam 1989).

The heterotrophic components of the plankton biomass (bacteria + heterotrophic nanoflagellates + ciliates) were also estimated on Days 0, 4 and 8. Abundances were determined for heterotrophic bacteria by flow cytometry (Gasol & del Giorgio 2000), for heterotrophic nanoflagellates using epifluorescence microscopy (Porter & Feig 1980) and for ciliates using an inverted microscope (Utermöhl 1958). Bacterial biomass was estimated using a carbon conversion factor of $0.35 \text{ pg C } \mu\text{m}^{-3}$ (Bjørnsen 1986). The same conversion factor was used for autotrophic nanoflagellate organisms. For ciliates, carbon conversion factors of $0.2 \text{ pg C } \mu\text{m}^3$ (Putt & Stoecker 1989) and $0.053 \text{ pg C } \mu\text{m}^3$ (Verity & Langdon 1984) were used for non tintinnids and tintinnids, respectively. More details on plankton counts and biomass calculations are reported in Arin et al. (2002).

On Day 8, one replicate of the TN and TNP containers was sampled because the other replicate was left for another experiment. In this paper, the term 'osmotrophic biomass' is used in reference to phytoplankton plus bacterial biomass in terms of carbon, while the term 'living carbon' refers to the whole plankton community. The osmotrophic biomass increase (OBI) was calculated

Fig. 1. The mass balance of phosphorus with time expressed as particulate organic phosphorus (POP), the estimated sedimented phosphorus (Sed), inorganic phosphate (SRP-PO_4) and dissolved organic phosphorus (DOP). (S = still, T = turbulent, C = control, N = N-surplus, NP = Redfield ratio). Mean of 2 replicate microcosms per treatment $\pm$ SE for SRP-PO_4 values. Analytical means $\pm$ SE for POP (below the value) and DOP. Error transmissions are shown for the calculated sedimented values (above the value)

during the first 4 d of the experiment, taking into account the estimated sedimented fraction. To estimate the OBI, the biomass of osmotrophs on Day 0 is subtracted from the biomass on Day 4 (biomass in the column water + sedimented biomass). As we only measured the sedimented biomass of osmotrophs on Day 8, we estimated the sedimented biomass on Day 4 in 2 ways by considering that: (1) sedimentation was constant over the time course of the experiment; and (2) the sedimentation across the experiment was proportional to the biomass in the column. As we did not find any significant differences between both estimations, we used the former one to calculate the OBI.

Statistical analyses. All statistical analyses were conducted using the SYSTAT software package. Analysis of covariance (ANCOVA) was applied to different variables using the times of sampling as the covariate. Statistical significance was considered as $p \leq 0.05$ for each of the independent variables (presence or absence of turbulence; nutrient addition treatment). Data are usually presented as means and standard errors of the replicates of each experimental treatment. For organic P and N compounds as well as for P turnover data, the errors are related to the analytical replicates as we only sampled 1 container of each condition for those variables.

RESULTS

Phosphorus dynamics

The dynamics of phosphorus in the control containers (C) behaved similarly over time (Fig. 1). The suspended particulate fraction (POP) was very low and decreased slightly from 0.1 to 0.04 μM between Days 1 and 6. The phosphate concentration varied between 0.02 and 0.13 μM and DOP decreased from 0.08 to 0 μM . In the N series, the suspended biomass increased 1 d after the addition of nutrients (0.11 ± 0.02 and 0.16 ± 0.01 μM in SN and TN, respectively). Sedimentation took place mainly in SN with a final settled biomass on Day 6 of 0.11 μM that corresponds to 55 % of total (suspended + settled) biomass. There were no significant differences in the phosphate concentration (0.04 to 0.10 μM) and DOP (< 0.13 μM) between the turbulent and still containers. In the NP series, the biomass increased considerably due to the enrichment. The suspended biomass peaked on Day 4 with 0.32 and 0.65 μM in SNP and TNP, respectively, and was followed by a small decrease. Sedimentation was, however, important in both containers, where the estimated settled biomass on Day 6 was 0.56 and 0.62 μM in SNP and TNP, respectively. The higher biomass build-up in TNP resulted in faster depletion of phos-

phate than in SNP. Phosphate concentrations on Day 6 were below detection limit in TNP and 0.31 μM in SNP.

Initial P turnover rate before nutrient additions was 1.1 h^{-1} (Fig. 2). In general, we found higher values of P turnover rate in turbulent treatments (ANCOVA, $p = 0.069$), especially in the NP containers. In the C and N series, the turnover rate varied between 0.2 and 1.9 h^{-1} . In the NP series, the turnover rate increased with decreasing P concentrations and was strongest in TNP (from 0.005 to 1.7 h^{-1} between Days 1 and 6).

Plankton biomass. The experiment starts with a phytoplankton biomass of around $30 \mu\text{g C l}^{-1}$ (Fig. 3). A slight increase of this biomass was observed in the control containers at the beginning of the experiment

Fig. 2. The turnover rate (h^{-1}) of phosphate during the experiment. Symbols are the mean of 3 to 4 analytical replicates $\pm$ SE. (S = still, T = turbulent, C = control, N = N-surplus, NP = Redfield ratio). Note logarithmic scale in the NP treatment graph

Fig. 3. Phytoplankton and osmotrophic biomass ($\mu\text{g C l}^{-1}$) during the experiment and percentage of heterotrophic biomass to total biomass on Days 0, 4 and 8. (S = still, T = turbulent, C = control, N = N-surplus, NP = Redfield ratio). Mean of 2 replicate microcosms per treatment $\pm$ SE. No replicates for TN and TNP were available on Day 8

which then decreased to around $1.2 \mu\text{g C l}^{-1}$. No significant differences were observed between the still and turbulent containers for this nutrient condition over the course of the experiment.

In the enriched enclosures (N and NP), phytoplankton biomass was significantly higher with turbulence

Table 3. Diatom biomass ($\mu\text{g C l}^{-1}$) and diatom mean volume per cell ($\mu\text{m}^3 \text{ cell}^{-1}$) and mean surface:volume ratio (mean of 2 replicate microcosms $\pm$ SE) on Day 4 for the still (S) and turbulent (T) containers of the C (control), N (N-surplus) and NP (Redfield ratio) treatments

	Biomass ($\mu\text{g C l}^{-1}$)	Mean volume ($\mu\text{m}^3 \text{ cell}^{-1}$)	Mean surface: volume ratio
SC	2.6 ± 0.2	647 ± 43	1.04 ± 0.035
TC	4.4 ± 0.4	646 ± 135	1.00 ± 0.070
SN	44 ± 13	644 ± 61	1.04 ± 0.025
TN	243 ± 29	1892 ± 109	0.51 ± 0.005
SNP	55 ± 0.2	669 ± 45	0.98 ± 0.015
TNP	787 ± 69	1688 ± 55	0.47 ± 0.020

($p < 0.001$). In the TN containers, an increase of phytoplankton was observed during the experiment (up to $304 \mu\text{g C l}^{-1}$ on Day 8), while in TNP phytoplankton biomass peaked on Day 4 (with $571 \pm 32 \mu\text{g C l}^{-1}$) and was followed by an abrupt decrease until Day 8. In SN and SNP, phytoplankton biomass increased to a maximum of 110 ± 75 and $125 \pm 6.3 \mu\text{g C l}^{-1}$, respectively, by the end of the experiment on Day 8. On Day 4, for all nutrient conditions, diatom biomass (in terms of carbon) was higher in the turbulent than in the still treatments (Table 3).

Osmotrophic biomass shows practically the same pattern as phytoplankton biomass (Fig. 3). The contribution of bacteria to total osmotrophic biomass was, on average, higher in the control (45%) than in the nutrient enriched containers (20%).

The percentage of heterotrophic to total biomass in control containers increased during the experiment to ca. 80% (Fig. 3). A lower contribution of heterotrophs was observed in N and NP containers. Heterotrophic biomass was lower than 20% on Day 4 in the TNP treat-

Fig. 4. Particulate organic nitrogen:particulate organic phosphorus (PON:POP) ratio during the experiment. Symbols are the mean of 3 analytical replicates $\pm$ SE (S = still, T = turbulence, C = control, N = N-surplus, NP = Redfield ratio)

ments and on Day 8 in the TN treatments, coinciding with the highest values of phytoplankton biomass. For each nutrient condition, a higher percentage of heterotrophic:total biomass was observed in the still than in turbulent containers ($p \leq 0.05$, ANOVA considering Days 4 and 8 as levels of a categorical variable), except in the controls where similar values were found (Fig. 3).

Particulate organic matter composition and nutrient consumption

In conditions without nutrient additions, the ratio PON:POP showed significantly higher values in turbulent than in still treatments throughout the experiment. In the nutrient added conditions (N and NP), the ratio

showed, in general, higher values with turbulence after Day 4; although when analysing the whole data set, the differences between still and turbulent treatments were not significant (Fig. 4). We found significant differences from Day 4 to 8 (ANCOVA, $p < 0.01$).

In the N and NP conditions, OBI in relation to inorganic phosphate consumed (IPC) was significantly higher in turbulent containers than in the still ones (Fig. 5a; t -test: $p = 0.006$ for N and $p = 0.05$ for NP). Higher values of this ratio were observed in TN than in TNP (12%, $p = 0.006$) while this was not so clear in SN compared to SNP (1%, $p = 0.09$). In addition, for each nutrient condition, OBI in relation to dissolved inorganic nitrogen consumed (DINC) was higher in turbulent than in still treatments (Table 2; t -test: $p = 0.01$ and $p = 0.06$ for the N and NP conditions, respectively). The diatom biomass, as well as the diatom biomass increase (DBI) in relation to the silicate consumed (SiC), was significantly higher with turbulence for the N ($p = 0.02$) and NP ($p = 0.03$) conditions (Table 2). The same ten-

Fig. 5. (a) Osmotrophic biomass increase (μ M carbon) versus phosphate consumed (μ M) from Day 0 to 4. Note the 2 replicates of each treatment. (b) Living carbon:particulate organic phosphorus (Living C:POP) on Days 0 and 4. Lines indicate the value of the Redfield ratio. (S = still, T = turbulence, C = control, N = N-surplus, NP = Redfield ratio)

dency was observed in the living C:POP ratio for Day 4 (Fig. 5b) which was higher in turbulent than in still containers (t -test: $p = 0.03$) and within the turbulent treatments, it was higher in the non-enriched container (C).

DISCUSSION

Despite working with natural communities, the replicates for the different treatments behaved alike. We found no significant differences in nutrient, chl *a* concentration or bacterial abundance between replicates. The good replicability in similar microcosm experiments has already been found in previous experimental studies (Marrasé et al. 1989, Peters et al. 2002). This argues in favour of the appropriateness of the experimental set-up used here to examine 'cause-effect' relationships, when the initial water manipulations are carefully done.

The response to turbulence was different depending on the nutrient conditions. Without nutrient addition (C containers), it was very difficult to attribute changes in biomass to turbulence. Previous experiments also encountered difficulties in seeing an effect owing to turbulence under low nutrient concentrations (Petersen et al. 1998, Peters et al. 2002). The nutrient levels in the experiments conducted by Peters et al. (2002) were similar to those found in our control conditions. They found a slightly higher proportion of heterotrophs under turbulence and an increase of heterotrophy after enclosing the communities in all the treatments, except for the ones where nutrients were added. While in our case, turbulence did not induce a significant increase in heterotrophy in the non-enriched conditions, we did find an increase of heterotrophy towards the end of the experiment. This increase of heterotrophy probably reflects a dominance of recycling and regenerated production.

A different temporal trend of the trophic status of the system was found when nutrients were added. The addition of nutrients induced a phytoplankton increase during the first period of the experiment in all the containers, characterised by a decrease in heterotrophy (Fig. 3). After Day 4, phytoplankton biomass changed little (SN and SNP containers) or decreased abruptly (TNP containers) and an increase in the proportion of heterotrophs was observed in these treatments, except in the TN containers (see below). Thus, for plankton and nutrient dynamics, we detected 2 phases during the experiment. The first phase was fuelled by new production (phytoplankton absorbing nutrient additions) and the second phase (post-bloom) was driven by regenerated production. A specific response was found in turbulent treatments with N-surplus (TN), where the photosynthetic organisms were growing

slowly and the system did not get to the post-bloom phase during the time course of the experiment. This, together with high P turnover rate (from 1.8 to 0.5 h^{-1} during the first 4 d), seems to indicate a faster P recycling in N-surplus conditions favouring autotrophic growth. Other experiments performed with Mediterranean water also found an anomalous phytoplankton production with N-surplus in comparison with P-surplus additions (Granéli et al. 1999). These authors also attributed the exceptional phytoplankton growth to a fast recycling of P. Superimposed to these succession patterns induced by nutrient additions. We found that both nutrients and turbulence influenced the dynamics of plankton.

Changes over time in inorganic nutrients (Si, N and P) indicate a faster nutrient decrease under turbulence (Table 2). This faster consumption matched a faster increase of osmotrophic biomass (Fig. 3). In addition, the increase of osmotrophic biomass with respect to nutrient consumption during this period was clearly different in turbulent and still conditions. The ratio of osmotrophic biomass increase to inorganic nitrogen and phosphorus consumed (OBI:INC and OBI:IPC, respectively) was significantly higher in turbulent conditions (Table 2, Fig. 5a). As sedimented biomass was taken into account to calculate OBI, we can not attribute the higher values of the ratio in turbulent treatments to differences in sedimentation or resuspension of osmotrophic biomass between treatments. In addition, when we compare the ratio living C:POP in the water column, we also find higher values under turbulent conditions (Fig. 5b). These higher values can not be explained by the differences in the proportion of heterotrophs or diatoms to the total biomass. This is because the controls (without nutrient additions) show the highest differences in living C:POP and no or low differences in the proportion of heterotrophs or diatoms between turbulent and still conditions (Table 3, Fig. 3). A possible explanation for the high living C:POP in turbulent conditions is that P was used faster, biomass increased faster and the community reached a P-deficient situation earlier than in still water. In this limiting situation, carbon assimilation and cell division were not affected equally by the limitation. This interpretation is supported by the fact that the values of PON:POP were higher in turbulent treatments compared to still ones after Day 4 (Fig. 4), and also by the higher values of P turnover rate found in turbulent treatments. Although in principle we expect turnover rate to increase with biomass, we did not find this trend. Actually, P turnover rate and biomass were not significantly correlated, indicating that the faster turnover rate was a response to a combination of factors not linearly associated to biomass.

When comparing the results across different nutrient conditions, both the OBI:IPC and living C:POP ratios were higher in the microcosms without P additions. These results agree with Guildford & Hecky (2000), who compiled data from different systems. They found the highest values of the POC:POP ratio (up to 5-fold Redfield ratio) in systems where the total P (TP) was lower than 0.5 μM . In our study, the conditions with no or low P additions account for a TP of less than 0.3 μM , finding the highest values of the living C:POP ratio. It may be that under P deficiency, cell division decreases or stops while photosynthesis (carbon assimilation) continues. Since these 2 activities have different P demands (Margalef 1997), the stoichiometry of biomass would change. Guildford & Hecky (2000) found not only the highest values of the POC:POP ratio in systems with low TP but also the highest variability. For systems with less than 0.5 μM of TP, they found values of the POC:POP ratio that ranged from close to 100 to more than 600. In our study, the largest differences in the living C:POP ratio between turbulence and still treatments were found in the microcosms where no or low amounts of P were added and TP was <0.3 μM . Our results indicate that 1 possible cause of the high POC:POP ratio variability in natural systems could be the variability in hydrodynamic conditions.

We also found that the increase in diatom biomass with respect to Si consumed (DBI:SiC, Table 2) was higher in turbulent treatments. The mean diatom cell volume was larger under turbulence and therefore the mean surface:volume ratio was lower (Table 3). This could explain the lower demand for Si compared to carbon (mol:mol). These results, together with the aforementioned differences in P and N dynamics, indicate that the amount of carbon assimilated for 1 unit of nutrient (P, N or Si) was higher in turbulent conditions and that the stoichiometry of the particulate matter was affected by mixing. It has been shown that nutrient limitation influences the stoichiometry of organic matter and the POC:POP and PON:POP ratios have already been used as indicators of nutrient limitations. Our observations highlight turbulence as a cause of variability in the stoichiometry of organic matter.

The values of the living C:POP ratio found in turbulent treatments had important deviations from Redfield ratios, especially in non-enriched conditions (2.1 times higher than the Redfield ratio), while in still treatments, these values were close to or lower than Redfield. In terms of carbon fluxes, biological factors such as species' composition can influence carbon sedimentation rates. Our results showed larger cell sizes and a higher proportion of diatoms under turbulence (Table 3). This can favour sedimentation and therefore carbon sequestration, once the kinetic energy decays. Therefore, during mixing episodes, carbon assimila-

tion could be underestimated if hydrodynamic factors are not considered. In our experiment, we had only 2 constant hydrodynamic conditions: still and turbulent ($\epsilon = 0.055 \text{ cm}^2 \text{ s}^{-3}$). In nature, turbulent environments are the norm and mixing events are episodic. Therefore, further experiments varying the level and the duration of the exposure to turbulence will be necessary to elucidate the linearity of the response of the particulate organic matter. Caution should be taken in extrapolating the results of this study to all natural scenarios since we suspect that the response of the particulate organic matter will depend on the characteristics of mixing phenomena (depth of the mixing layer, duration and the intensity of the mixing event, etc.). Nevertheless, this finding might be important for the construction of models with biogeochemical fluxes and indicates that hydrodynamic variables like turbulence should be examined when looking for trends in carbon and nutrient fluxes across systems. The significance of our results is that they provide evidence of the link between mechanical energy and geochemical fluxes through biological interactions.

Acknowledgements. We thank Mercedes Castaño for laboratory assistance, Roser Ventosa for nutrient analysis and Oscar Guadayol for turbulence intensity calculation for the NW Mediterranean. Further, we are grateful to Frede Thingstad for introducing the ^{33}P -method and to Michael Olesen for fruitful discussions. The comments and suggestions by 3 anonymous reviewers greatly improved previous versions of the manuscript. Aisling Metcalfe was of great help editing the English. This study was supported by European Community (MEDEA:MAS3-CT95-0016, NTAP:EVK3-CT-2000-00022) and Spanish CICYT (MAR 98-0854). M.M. acknowledges the Knud Højgaards fond, Denmark. This is the ELOISE Contribution No. 278/40.

LITERATURE CITED

- Arin L, Marrasé C, Maar M, Peters F, Sala MM, Alcaraz M (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
- Bjørnsen PK (1986) Automatic determination of bacterioplankton biomass by image analysis. *Appl Environ Microbiol* 51:1199–1204
- Gasol JM, del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Sci Mar* 64(2):197–224
- Granéli E, Carlsson P, Turner JT, Tester PA, Béchemin C, Dawson R, Funari E (1999) Effects of N:P:Si ratios and zooplankton grazing on phytoplankton communities in the northern Adriatic Sea. I. Nutrients, phytoplankton biomass, and polysaccharide production. *Aquat Microb Ecol* 18:37–54
- Grasshoff K, Ehrhardt M, Kremling K (1983) *Methods of seawater analysis*, 2nd edn. Verlag Chemie, Weinheim
- Guildford SJ, Hecky RE (2000) Total nitrogen, total phospho-

- rus, and nutrient limitation in lakes and oceans: is there a common relationship? *Limnol Oceanogr* 45:1213–1223
- Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith W, Chanley MH (eds) Culture of marine invertebrates. Plenum Publishing, New York, p 26–60
- Kana T, Gilbert PM (1987) Effect on irradiance up to 2000 $\mu\text{E m}^{-2} \text{s}^{-2}$ on marine *Synechococcus* WH 7803-I. Growth: pigmentation, and cell composition. *Deep-Sea Res* 34:479–516
- Karp-Boss L, Boss E, Jumars PA (1996) Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanogr Mar Biol Annu Rev* 34:71–107
- Kjørboe T (1993) Turbulence, phytoplankton cell size, and the structure of pelagic food webs. *Adv Mar Biol* 29:1–72
- Koroleff F (1983) Determination of total nitrogen and phosphorus. In: Grasshoff K, Ehrhardt M, Kremling K (eds) Methods for seawater analysis, 2nd edn. Verlag Chemie, Weinheim, p 162–173
- Lazier JRN, Mann KH (1989) Turbulence and the diffusive layer around small organisms. *Deep-Sea Res* 36: 1721–1733
- Legendre L, Le Fèvre J (1991) From individual plankton cells to pelagic marine ecosystems and to global biogeochemical cycles. In: Demers S (ed) NATO ASI Series, Vol G27. Particle analysis in oceanography. Springer-Verlag, Berlin, p 261–300
- Margalef R (1997) Our biosphere. In: Kinne O (ed) Excellence in ecology. Ecology Institute, Oldendorf/Luhe
- Marrasé C, Duarte CM, Vaqué D (1989) Succession patterns of phytoplankton blooms: directionality and influence of algal cell size. *Mar Biol* 102:43–48
- Montagnes DJ, Berges SJA, Harrison PJ, Taylor FJR (1994) Estimating carbon, nitrogen, protein and chl *a* from volume in marine phytoplankton. *Limnol Oceanogr* 39: 1044–1060
- Pasciak WJ, Gavis J (1974) Transport limitations of nutrient uptake in phytoplankton. *Limnol Oceanogr* 19(6):881–888
- Pasciak WJ, Gavis J (1975) Transport limited nutrient uptake rates in *Ditylum brightwellii*. *Limnol Oceanogr* 20: 604–617
- Peters F, Marrasé C, Havskum H, Rassoulzadegan F, Dolan J, Alcaraz M, Gasol JM (2002) Turbulence and the microbial food web: effects on bacterial losses to predation and on community structure. *J Plankton Res* 24:321–331
- Petersen JE, Sanford LP, Kemp WM (1998) Coastal plankton responses to turbulence mixing in experimental ecosystem. *Mar Ecol Prog Ser* 171:23–41
- Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25:943–948
- Pujo-Pay M, Raimbault P (1994) Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. *Mar Ecol Prog Ser* 105:203–207
- Putt M, Stoecker DK (1989) An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. *Limnol Oceanogr* 34: 1097–1103
- Sala MM, Peters F, Gasol JM, Pedrós-Alió C, Marrasé C, Vaqué D (2002) Seasonal and spatial variations in the nutrient limitation of bacterioplankton growth in the northwestern Mediterranean. *Aquat Microb Ecol* 27: 47–56
- Savidge G (1981) Studies of the effects of small-scale turbulence on phytoplankton. *J Mar Biol Assoc UK* 61:477–488
- Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* 51:201–213
- Strickland JDH (1960) Measuring the production of marine phytoplankton. *Bull Fish Res Board Can* 122:1–172
- Thingstad TF (1998) A theoretical approach to structuring mechanisms in the pelagic food web. *Hydrobiologia* 363: 59–72
- Thingstad TF, Skjoldal E, Böhne RA (1993) Phosphorus cycling and algal-bacterial competition in Sandsfjord, western Norway. *Mar Ecol Prog Ser* 99:239–259
- Thingstad TF, Zweifel UL, Rassoulzadegan F (1998) P-limitation of heterotrophic bacteria and phytoplankton in the northwest Mediterranean. *Limnol Oceanogr* 43(1):88–94
- Thomas H, Ittekkot V, Osterroht C, Schneider B (1999) Preferential recycling of nutrients—the ocean's way to increase new production and to pass nutrient limitation? *Limnol Oceanogr* 44(8):1999–2004
- Utermöhl H (1958) Zur Vervollkommenung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor Angew Limnol* 9:1–38
- Vadstein O, Olsen Y (1989) Chemical composition and phosphate uptake kinetics of limnetic bacterial communities cultured in chemostats under phosphorus limitation. *Limnol Oceanogr* 34:939–946
- Valderrama JC (1995) Methods of nutrient analysis. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) Manual on harmful marine microalgae. IOC Manuals and Guides No. 33. UNESCO, Paris, p 251–268
- Verity PG, Langdon C (1984) Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. *J Plankton Res* 6:859–868
- Verity PG, Robertson CY, Tronzo CR, Andrews MG, Nelson JR, Sieracki ME (1992) Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol Oceanogr* 37:1434–1446
- Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chl and phaeophytin by fluorescence. *Deep-Sea Res* 10:221–231
- Zweifel UL, Norrman B, Hagström Å (1993) Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. *Mar Ecol Prog Ser* 101:23–32