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Early spring phosphorus limitation of primary productivity in a NW Mediterranean coastal zone (Gulf of Lions)

Frédéric Diaz[1,*], Patrick Raimbault[1], Benyahia Boudjellal[2], Nicole Garcia[1], Thierry Moutin[1]

[1]Laboratoire d'Océanographie et de Biogéochimie (UMR-CNRS 6535), Centre d'Océanologie de Marseille, Campus de Luminy, 13288 Marseille cedex 09, France
[2]Institut des Sciences de la Mer et de l'Aménagement du Littoral, Staoueli, Algeria

ABSTRACT: Evidence of phosphorus limitation of algal C- and N-uptake in a NW Mediterranean coastal area (Gulf of Lions) was obtained from a field survey of inorganic and organic N, P and C and from bioassays carried out during the late winter-early spring 1998. Dissolved inorganic nitrogen ($\Sigma DIN = NO_3 + NO_2 + NH_4$) and phosphorus (DIP) distributions showed a clear DIP depletion in the inorganic fractions available for primary production. While below the 150 m depth, the mean ΣDIN to DIP ratio was close to the typical Mediterranean ratio of 22, while values found in the upper layer (0 to 150 m) were about 3 times higher (68.4:1 on average). In this upper layer, N:P (19.9:1) and C:P (159.7:1) ratios in the particulate organic matter were higher than the Redfield ratio and also indicated P depletion in this fraction. In the dissolved organic pool, P depletion was higher than in the particulate organic pool, since the mean C:N:P ratios were 1674:75:1 in the photic layer. Dissolved organic forms of C and N represented the bulk (ca 94 and 86%, respectively) of the total organic matter, while ca 31% of the organic P was in particulate fraction. The apparent imbalance between N and P in the inorganic fraction was partly attributed to an imbalance in the corresponding nutrient utilization by the phytoplanktonic community, and partly due to the influence of the Rhone River. Additions of small amounts of DIP to surface samples led (1) to a decrease in C-uptake (≅30%) during the first 24 h incubation, (2) to a rapid increase in chlorophyll biomass and (3) to stimulate nitrate uptake (≅60%), suggesting DIP limitation of new production and of algal biomass during the spring 1998 in the Gulf of Lions.

KEY WORDS: Nutrients · Phosphorus limitation · Primary productivity · NW Mediterranean

INTRODUCTION

For a long time, the Mediterranean Sea has been known as an oligotrophic area with lower nutrient concentrations than the adjacent Atlantic Ocean (McGill 1961). If one assumes that N-limited Atlantic water flows through the Gibraltar Strait and that *P* is recycled faster than N, then one could expect N limitation in the photic layer of the Mediterranean Sea. Numerous data, however, show a higher nitrate to phosphate ratio in Mediterranean waters than that of 16:1 measured in the Atlantic Ocean (Redfield et al. 1963). The Mediterranean ratio is generally recognized to be higher than 20:1 (McGill 1969), implying a trend of Mediterranean waters to be P-depleted relative to N (Krom et al. 1991). High inorganic N:P ratios were measured in the eastern basin (Krom et al. 1991) and in the Adriatic Sea (Vukadin & Stojanski 1976), as well as in the Ionian and Ligurian Seas (McGill 1965). The same dissolved inorganic phosphorus (DIP) depletion was observed in deep waters at several places in the Mediterranean Sea (Miller et al. 1970, Spencer 1983, Coste et al. 1984, Raimbault & Coste 1990). The review of Berland et al.

*E-mail: diaz@com.univ-mrs.fr

(1980) led to the conclusion that the Mediterranean Sea is different than most other major ocean basins in that P, relative to other macro-nutrients, seems to play an essential role in phytoplankton limitation. P limitation of primary productivity has been demonstrated by nutrient enrichment and bioassay experiments in both the eastern (MacIsaac & Dugdale 1972) and northwestern Mediterranean basins for the whole year (Devèze 1959, Muñoz & San Feliu 1972, Jacques et al. 1973a) or for a part of the year (Ballester et al. 1967, Berland et al. 1973, Fiala et al. 1976). In this latter case, N and iron were then found to play a key role during other times of the year. Hence, Owens et al. (1989) even concluded that N limitation was more probable than P limitation in the western Mediterranean. More recently, phytoplankton and heterotrophic bacteria communities were found to be P-limited in the NW basin during summer, which has crucial implications for the dynamics of dissolved organic carbon (DOC) in the photic layer (Zweifel et al. 1993, Thingstad et al. 1998). These latter 2 studies confirmed the theoretical model of Thingstad et al. (1997), in which both phytoplankton and bacteria compete for mineral nutrients associated with a predator control of bacteria biomass allowing DOC to accumulate in summer. Thus, the summer functioning of the microbial food web under P limitation is now well understood, but studies of potential P limitation of phytoplankton or even of heterotrophic bacteria have been scarce for the late winter-early spring in the NW basin. There is no *a priori* reason to assume ambient nutrient depletion during this period of the year, since strong winter vertical mixing of the water column in this area (Millot 1990) usually allows the supply of sufficient nutrients to the photic layer to initiate the spring bloom a few weeks later (Lefèvre et al. 1997).

However, we herein report a peculiar situation concerning P depletion in the dissolved inorganic pool, as well as in the particulate and dissolved organic matter, in the late winter-early spring 1998 over the Gulf of Lions. These results of P limitation are supported by the results of bioassay experiments.

MATERIALS AND METHODS

Sample collection. This study was performed during the MOOGLI 1 Cruise (MOdélisation et Observation du Golfe du LIon) on the RV 'L'Atalante' from March 15 to April 2, 1998 (defined as the early spring period) within the framework of the European Metro-Med and French PNOC (Programme National d'Océanographie Côtière) programs. Hydrological measurements were conducted at 20 stations located within <200 m depth and beyond the continental shelf of the Gulf of Lions in the NW Mediterranean Sea (Fig. 1). Samples for nutrient analysis and for total and particulate organic matter measurements were collected at 12 depths between 5 and 400 m, using a rosette system with 12 l Niskin® bottles. Profiles of photosynthetically available radiation (PAR) were obtained with an irradiance profiling sensor (QSP-200L, Biospherical® Instruments) fixed on the rosette system. Conductivity, temperature and depth-oxygen measurements were made with a conductivity-temperature-depth-oxygen profiling system (CTDO Seabird®, model 911+). Samples for chlorophyll and productivity measurements were collected only at the surface (5 m depth) at most of the stations during a second cast of sampling (about 72 h after the first set). During this second set, nutrients were again determined (at the surface only).

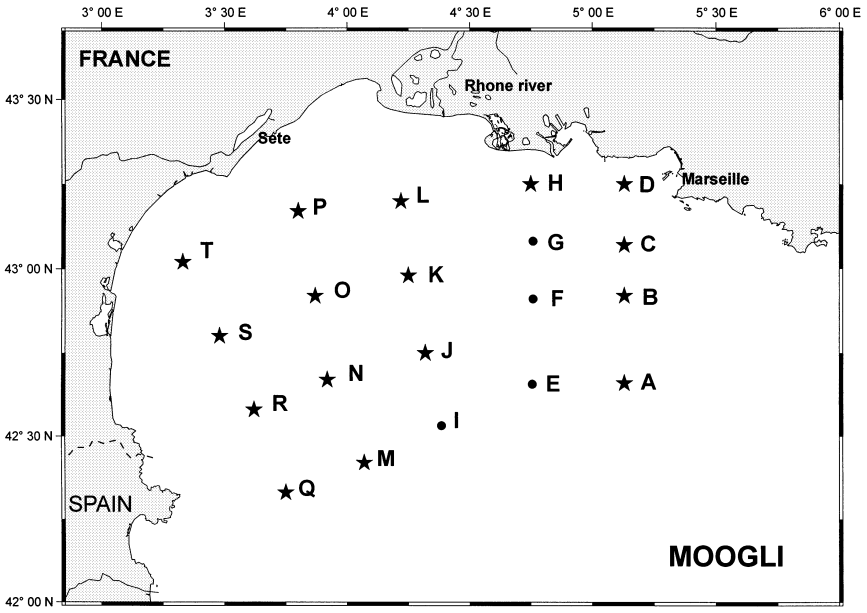

Fig. 1. Station locations during the MOOGLI 1 Cruise (March 14 to 30, 1998). (★) Stations were also sampled for productivity experiments

Nutrient analysis and chlorophyll measurements. Samples for ambient nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and DIP concentrations were collected from each depth in polyethylene flasks and immediately analyzed using a Technicon Auto-Analyser® II (precision: ± 0.030 µM) according to the working procedures of Tréguer & Le Corre (1975). Baseline was obtained from a sample of deionized water (Milli-Q® system). The detection limit (2 SD of the blank) was

0.050 µM for NO_3^- + NO_2^-, and NH_4^+ and 0.015 for DIP. NH_4^+ concentrations were lower than the detection limit of the procedure (<0.050 µM) for most of the samples. ΣDIN was defined as NO_3^- + NO_2^- + NH_4^+, with NO_3^- content representing 67 to 100% of ΣDIN. Samples for ambient silicate [$Si(OH)_4$] concentrations were collected on some occasions and frozen at –20°C until laboratory analyses. At the laboratory, $Si(OH)_4$ contents were manually determined by spectrophotometry (detection limit of 0.050 µM) according to the working procedure of Mullin & Riley (1955). Samples (250 ml) for chlorophyll *a* (chl *a*) were collected at the surface and immediately filtered onto baked (450°C for 24 h) Whatman® glass-fiber filters (GF/F) (25 mm in diameter). Chl *a* concentrations were determined by fluorometry (Turner Designs®, model 10.005R) using the methanol extraction procedure (Raimbault et al. 1988).

Particulate organic matter determinations. Samples for particulate organic matter (POM) determinations were collected in acid-cleaned 0.6 l polycarbonate (PC) bottles (Nalgene®) and immediately filtered on baked Whatman® GF/F filters. Filters were then frozen at –20°C until laboratory analysis. Particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) were simultaneously determined by a wet-oxidation method using sealed vials as outlined by Raimbault et al. (1999a).

Total organic matter determinations. 40 ml of seawater sample for total organic matter (TOM) determinations were directly collected in 50 ml acid-cleaned Pyrex bottles (Duran Schott®) and fitted with Teflon-lined screw caps. Immediately after collection, samples were poisoned with 100 µl of mercuric chloride (final concentration 20 µg ml^{-1}) for preservation until laboratory analysis. Total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) were simultaneously determined by persulfate wet-oxidation according to Raimbault et al. (1999b). Concentrations of dissolved organic matter (DOM) were computed by removing the POM fraction and inorganic forms of N and P from the total matter pool.

Nitrate and carbon uptake experiments. The ^{15}N-tracer method was used to measure nitrate uptake (Dugdale & Goering 1967). Surface samples were collected in acid-cleaned 0.6 l PC bottles (Nalgene®) and spiked with $Na^{15}NO_3$ (99.9 atom% ^{15}N). Mean $^{15}NO_3^-$ enrichment was 11.3% (±6.8%) of the corresponding ambient NO_3^- concentration. Samples were incubated from dawn to dawn under simulated *in situ* conditions for 24 h in a deck incubator cooled with sea surface water at 50% of surface irradiance. Samples were then gently vacuum-filtered (<100 mm Hg) through a baked GF/F and filters were immediately dried at 60°C. At the laboratory, filters containing PON were analyzed for ^{15}N content using a continuous-flow method (Europa Scientific) in which Dumas combustion (Roboprep-CN) is linked in-line to a triple collector mass spectrometer (Tracer mass) via a capillary interface based on the design of Preston & Owens (1983). Mass-spectrometric signals were used to determine ^{15}N abundance and total nitrogen mass of samples. Absolute NO_3^- uptake rates (µM d^{-1}) were computed according to Dugdale & Wilkerson (1986).

C-uptake was determined at the surface using the ^{14}C-tracer technique (Steeman-Nielsen 1952) with the experimental protocol of Fitzwater et al. (1982). Acid-cleaned (0.5 N, HCl) 0.32 l PC bottles were filled with water sampled with Niskin® bottles and were incubated under the same conditions as in the ^{15}N experiments. After the bottles were filled, 0.25 ml of a ^{14}C working solution (20 µCi as $NaH^{14}CO_3$) was added to each bottle. Before incubation, total added ^{14}C activity was assayed on a 0.25 ml aliquot collected in a glass scintillation vial containing 0.25 ml of ethanolamine. At the laboratory, 10 ml of a liquid scintillation cocktail (Ultima Gold®) and 1 ml of Milli-Q® water were added to these vials before counting. At each station, additional samples were inoculated with the tracer and immediately filtered to determine blanks. At the end of incubation, samples were filtered onto Whatman® GF/F filters at <100 mm Hg. Filters were placed into scintillation vials and wet with 0.25 ml HCl (0.5 N). At the laboratory, these vials were dried at 60°C for 24 h and the liquid scintillation cocktail was then added to the dried filters before counting. Counting was performed on a Packard® Tri-carb 2100TR scintillation counter. C-uptake rates (µM d^{-1}) were calculated from the equation proposed by Platt & Sathyendranath (1993). Dissolved inorganic carbon (DIC) concentration was assumed to be 25 000 mg m^{-3} (Robinson & Williams 1989).

Phosphorus addition experiments. In parallel to productivity samples, additional samples were collected at each station and spiked with DIP. These were then treated under the same conditions as non-DIP-enriched samples (controls). DIP additions were fixed to 0.060 µM, leading to a mean ΣDIN:DIP ratio of 17.3 ± 12 in the bioassays (see Table 2).

RESULTS

Temperature distribution in the water column showed a typical winter structure, with a large mixed layer down to 50 m and even to 100–150 m (Stns I and R) with values ranging between 12.5 and 13.5°C in the upper layer (data not shown). Most of the sampled sites presented salinity values ranging between 37.80 and

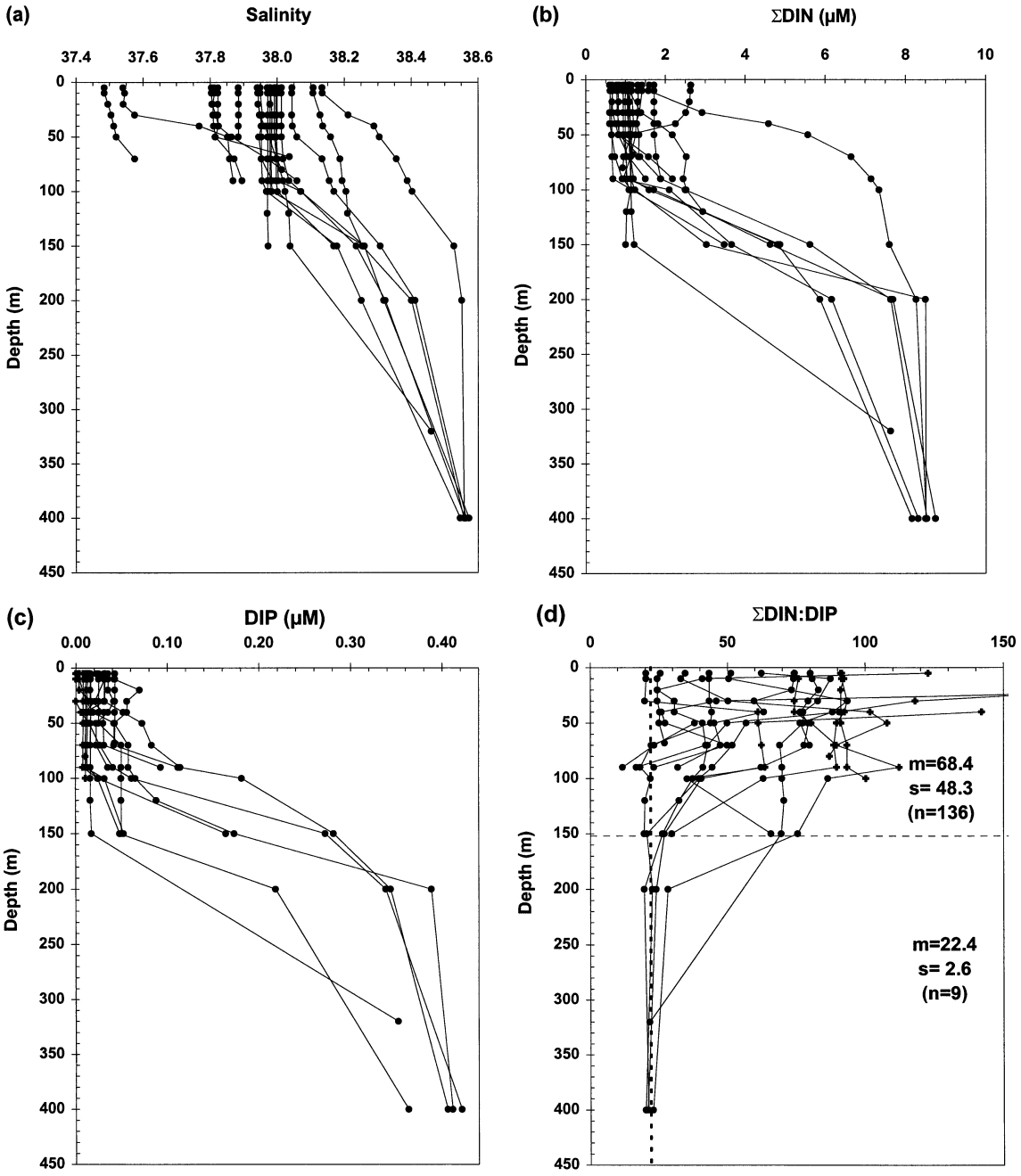

Fig. 2. (a) Salinity, (b) ΣDIN (NO_3^- + NO_2^- + NH_4^+), (c) phosphate (DIP) and (d) ΣDIN:DIP molar ratio versus depth at the 20 stations of the MOOGLI 1 Cruise. The vertical dashed line in (d) indicates the value of the NO_3^-:DIP ratio (=22) reported by McGill (1969) for the Mediterranean area. Mean (m), standard deviation (σ) and the number of data (n) of NO_3^-:DIP ratios were computed for 2 distinct layers (0 to 150 and >150 m). (+) Data representing or based on DIP concentrations below the detection limit

38.05 in the mixed layer, except at 2 stations (Stns K and L) which showed a marked coastal influence (salinity <37.55). Two other offshore stations (Stns E and I) were less mixed and showed high salinity values (>38.10) up to the surface (Fig. 2a). Below the mixed layer, salinity values increased up to 38.55 and were characteristic of Mediterranean Levantine Intermediate Water (LIW).

ΣDIN concentrations in the mixed layer (Fig. 2b) were 0.50 to 1.80 μM (except at Stn K, 2.50 μM) while those of DIP were <0.040 μM (Fig. 2c) and often below the detection limit in the photic layer (0 to 60 m). Pro-

files of $Si(OH)_4$ concentrations (data not shown) paralleled those of ΣDIN and indicated surface content of 1 to 2 µM. According to the sampling strategy, nitracline and phosphacline started generally at the same depth. Below the surface mixed layer, DIP and ΣDIN concentrations were similar and increased to about 0.401 (±0.026, n = 4) and 8.52 (±0.17, n = 4) µM, respectively at 400 m. Values (20 to 271) and patterns for the ΣDIN:DIP ratio (Fig. 2d) were relatively variable in the upper 150 m: shallow areas (Stns H and L) had generally a uniform pattern while offshore areas (Stns I and M) showed the highest ratios at the surface. Below 150 m, the ΣDIN:DIP ratio always decreased and reached a value of 22.4 ± 2.6, which is close to the typical ratio (22 to 21:1) given for the Mediterranean Sea (McGill 1969). On pooling all the data of the ΣDIN:DIP ratio from the 0 to 150 m layer, the arithmetic mean ratio was ca 68 ± 48 and was much higher than McGill's (1969) value. Linear regression (model II, Sokal & Rohlf 1995) of DIP versus ΣDIN from the surface to 150 m gave a calculated slope of 31.34 and an intercept of 0.53 µM that was significant ($p < 0.001$) (Fig. 3). This confirmed (1) the relative DIP depletion in most of the 0 to 150 m samples, and (2) DIP exhaustion at ΣDIN concentrations <ca 0.50 µM. The PON:POP and POC:POP ratios (Table 1, Fig. 4) seemed to confirm P depletion in POM in the 0 to 100 m layer. However, several profiles (for C:P and N:P ratios especially) were slightly lower than the Redfield (1958) ratios (Fig. 4). The mean POC:PON ratio (8.2 ± 2.0) was higher than the Redfield (1958) standard value of 6.63 (Table 1, Fig. 4). Considering the dissolved organic pool, C:P and N:P ratios were greater than in the particulate organic pool (Table 1). Particulate organic forms of C and N corresponded to only ca 6 and 14% of the respective total organic forms (Table 1) while ca 31% of the organic phosphorus pool was in particulate form.

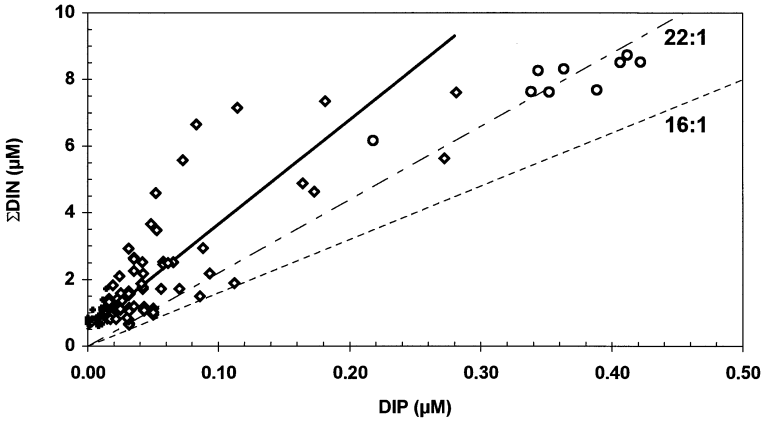

Fig. 3. ΣDIN versus DIP relationship. (◇) From 0 to 150 m layer; (+) points based on the DIP data below the detection limit; (○) from the 200 to 400 m layer. Solid line represents the linear regression (model II, Sokal & Rohlf 1995: ΣDIN = 31.34 DIP + 0.53, $r^2 = 0.647$, n = 136) of data from the 0 to 150 m layer. Dashed and dashed-dotted lines indicate the 16:1 (global ocean) and 22:1 (Mediterranean waters) ratios, respectively

During the second sampling, DIP concentrations were below the detection limit of the methodology, except at 3 stations (Stns H, L and K with values close to the detection limit), while ΣDIN ranged between 0.26 and 3.43 µM (Table 2). ΣDIN:DIP ratios at these stations were much higher (>32) than the 22:1 Mediterranean ratio. Chl *a* concentrations ranged between 0.37 and 3.14 mg m^{-3}. It is worthwhile to note that the highest chl *a* concentrations were found at Stns H, K and L, where DIP was still detectable (Table 2). C- and NO_3^- uptake rates ranged between 0.51 and 5.78 µM d^{-1} and between 0.019 and 0.62 µM d^{-1}, respectively

Table 1. Mean (±SD) particulate (POM) and dissolved (DOM) organic matter in terms of carbon (POC and DOC), nitrogen (PON and DON) and phosphorus (POP and DOP) along with corresponding molar ratios obtained from all depths at all stations

	POC	PON	POP	C:N	C:P	N:P
POM (µM)						
Mean (±SD)	5.5 (±1.9)	0.69 (±0.24)	0.036 (±0.014)	8.2 (±2.0)	159.7 (±47.2)	19.9 (±5.0)
Range	1.9–10.4	0.22–1.48	0.010–0.064	3.8–13.9	88.6–323.9	10.5–31.5
n				153	152	152
	DOC	DON	DOP			
DOM (µM)						
Mean (±SD)	85.9 (±13.6)	4.32 (±0.42)	0.079 (±0.021)	19.8 (±3.0)	1139.8 (±390.8)	56.5 (±15.9)
Range	48.9–106.6	3.41–5.52	0.027–0.147	11.5–27.7	490.6–3111.1	31.4–150.2
n				134	132	132

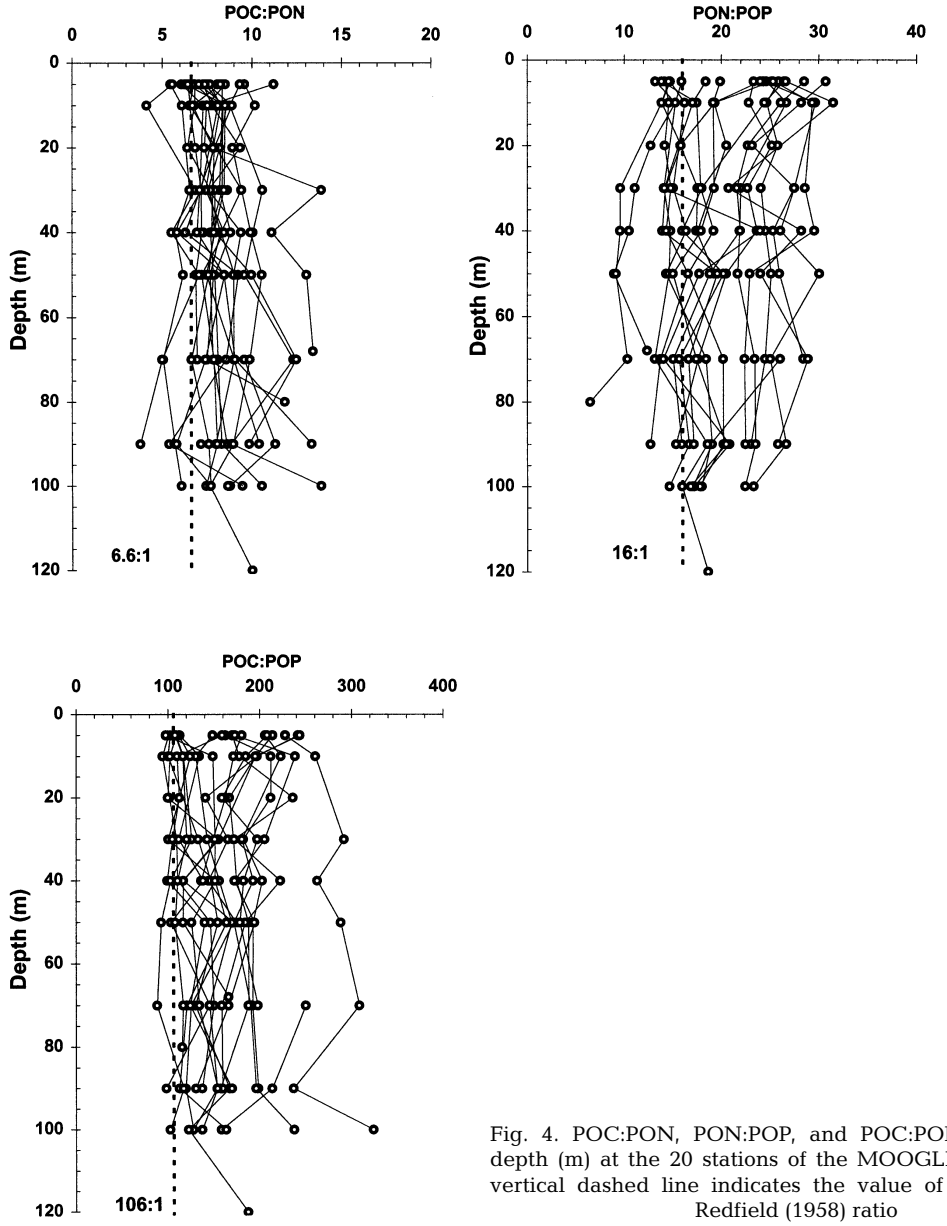

Fig. 4. POC:PON, PON:POP, and POC:POP ratios versus depth (m) at the 20 stations of the MOOGLI 1 Cruise. The vertical dashed line indicates the value of the respective Redfield (1958) ratio

(Table 3). As for chl *a* concentrations, the highest productivity rates were measured at the stations where DIP concentrations were higher than the detection limit. C-uptake rates measured in the DIP-spiked samples were systematically lower than those obtained from the corresponding control, except at 3 stations, 2 of which (Stns H and L) were not initially DIP-depleted. Excluding the values of these latter stations, the mean decrease in C-uptake rate was ca 29% in the DIP-spiked samples. However, this latter decrease is not significant (*t*-test, $p > 0.15$). In contrast, DIP additions resulted in a large enhancement of NO_3^- uptake at most of the sampled stations. Omitting the 3 stations (Stns D, N and R) where an enhancement was not observed, NO_3^- uptake averaged ca 60% greater in DIP treatments relative to controls. A sample collected at Stn K was spiked with 0.150 µM P and changes in dissolved nutrient concentrations (ΣDIN, DIP), NO_3^- and C-uptake were then monitored for 3 d (Fig. 5). At the end of the first daylight incubation (+12 h), the decrease in ΣDIN (Fig. 5a) was low (ca 6% and ca 14% in the control and in the DIP-spiked sample, respectively). During this period, the decrease in DIP was significant (ca 40 to 50%) in both the control and the DIP-spiked sample (Fig. 5b). As for the other stations, the C-uptake rate was lower (by ca 34%) in the DIP-spiked

Table 2. Hydrological data collected at the surface (5 m) during the second set of sampling (~72 h after the first one). ΣDIN:DIP* is the ΣDIN:DIP ratio in the DIP-spiked productivity samples. DIP additions were 0.060 µM except at Stn K (0.150 µM). bdl = below detection limit

Stn	Chl (mg m^{-1})	ΣDIN (µM)	DIP (µM)	ΣDIN: DIP	ΣDIN: DIP*
A	1.22	0.65	bdl	–	10.5
B	0.37	0.26	bdl	–	4.2
C	0.73	0.38	bdl	–	6.1
D	0.66	0.42	bdl	–	6.7
H	3.14	3.43	0.036	94.9	54.8
J	0.47	0.55	bdl	–	8.8
K	2.45	1.63	0.050	32.7	8.2
L	1.39	1.02	0.018	56.0	16.3
M	0.99	1.82	bdl	–	13.1
N	0.69	0.71	bdl	–	11.3
O	0.58	1.09	bdl	–	17.5
P	0.60	1.45	bdl	–	23.2
Q	0.76	1 39	bdl	–	22.3
R	0.52	1.32	bdl	–	21.1
S	0.68	1.40	bdl	–	17.6
T	0.69	1.07	bdl	–	17.1

sample than in the control (Fig. 5c) whereas a large enhancement of NO_3^- utilization (ca 60%) was observed in the DIP-spiked sample (Fig. 5d) relative to the control. During the first dark period, the NO_3^- decrease was ca 2.3 times higher in the DIP-spiked sample than in the control (Fig. 5a). Parallel to the NO_3^- decrease, dark DIP utilization markedly occurred in the DIP-spiked sample while DIP concentration remained stable in the control (Fig. 5b). About 60 and 30% of the remaining NO_3^- and DIP, respectively, were consumed during the second period of daylight in the DIP-spiked sample, in contrast to the control where nutrient utilization remained close to zero (Fig. 5a,b). At the end of this second daylight period, C- and NO_3^- uptake were much higher, by a factor of ca 2.5, in the DIP-spiked sample relative to the control (Fig. 5c,d). A decrease in nutrients continued in the DIP-spiked sample during the second night period and NO_3^- and DIP concentrations were respectively <0.100 and <0.030 µM after 48 h incubation (Fig. 5a,b). At the end of the experiment (60 h incubation), the DIP-spiked sample was NO_3^- depleted while the control still contained 0.80 µM NO_3^-. At the same time, C- and NO_3^- uptake were respectively higher by a factor of ca 1.4 and 2.5 in the DIP-enriched sample than in the control (Fig. 5c,d). In the latter experiment, DIP addition led to a continuous increase (Fig. 5e) in chl *a* content (from 2.50 to 5.35 mg m^{-3}) while the chl *a* content in the control decreased from the end of the second daylight period (from 3.30 to 2.70 mg m^{-3}).

DISCUSSION

Previous studies performed at the same period as that of the present work, i.e. in late March (Coste et al. 1972, Coste et al. 1977), reported generally higher concentrations of both DIP (3 to 8 times higher) and ΣDIN

Table 3. NO_3^- (ρNO_3^-) and C-uptake rates measured at the surface (5 m) during the second set of sampling in the control and in the DIP-spiked sample (+DIP) for 24 h incubations. PE: photosynthetic efficiency in the control. % change: difference (in %) between uptake rates measured in the DIP-spiked sample and the control. Means of the % change were computed omitting the underlined values. Values from Stn K are rates obtained from 12 h incubations. nd: no data

Stn	ρNO_3 (µM d^{-1}) Control	+DIP	% change	C-uptake (µM d^{-1}) Control	+DIP	% change	PE (µmol C µg chl a^{-1} d^{-1}) Control
A	0.12	0.19	62	1.22	0.84	–31	1.00
B	0.02	0.04	94	0.51	0.47	–8	1.40
C	0.09	0.11	26	1.21	0.97	–19	1.65
D	0.05	0.05	–10	0.58	0.87	49	0.89
H	0.62	1.14	84	5.78	8.93	54	1.84
J	nd	nd		0.88	0.74	–16	1.89
K	0.28	0.44	57	5.07	3.59	–29	2.08
L	0.10	0.17	76	1.94	2.27	17	1.40
M	0.11	0.14	32	1.90	1.54	–19	1.92
N	0.08	0.07	–11	0.90	0.81	–10	1.31
O	0.03	0.04	24	0.73	0.43	–41	1.25
P	0.06	0.09	67	0.99	0.54	–46	1.65
Q	0.07	0.11	53	1.28	1.08	–16	1.69
R	0.06	0.05	–15	0.83	0.42	–49	1.59
S	0.03	0.05	42	1.28	0.88	–31	1.89
T	0.02	0.04	83	1.18	0.49	–59	1.72
Mean			58			–29	1.57

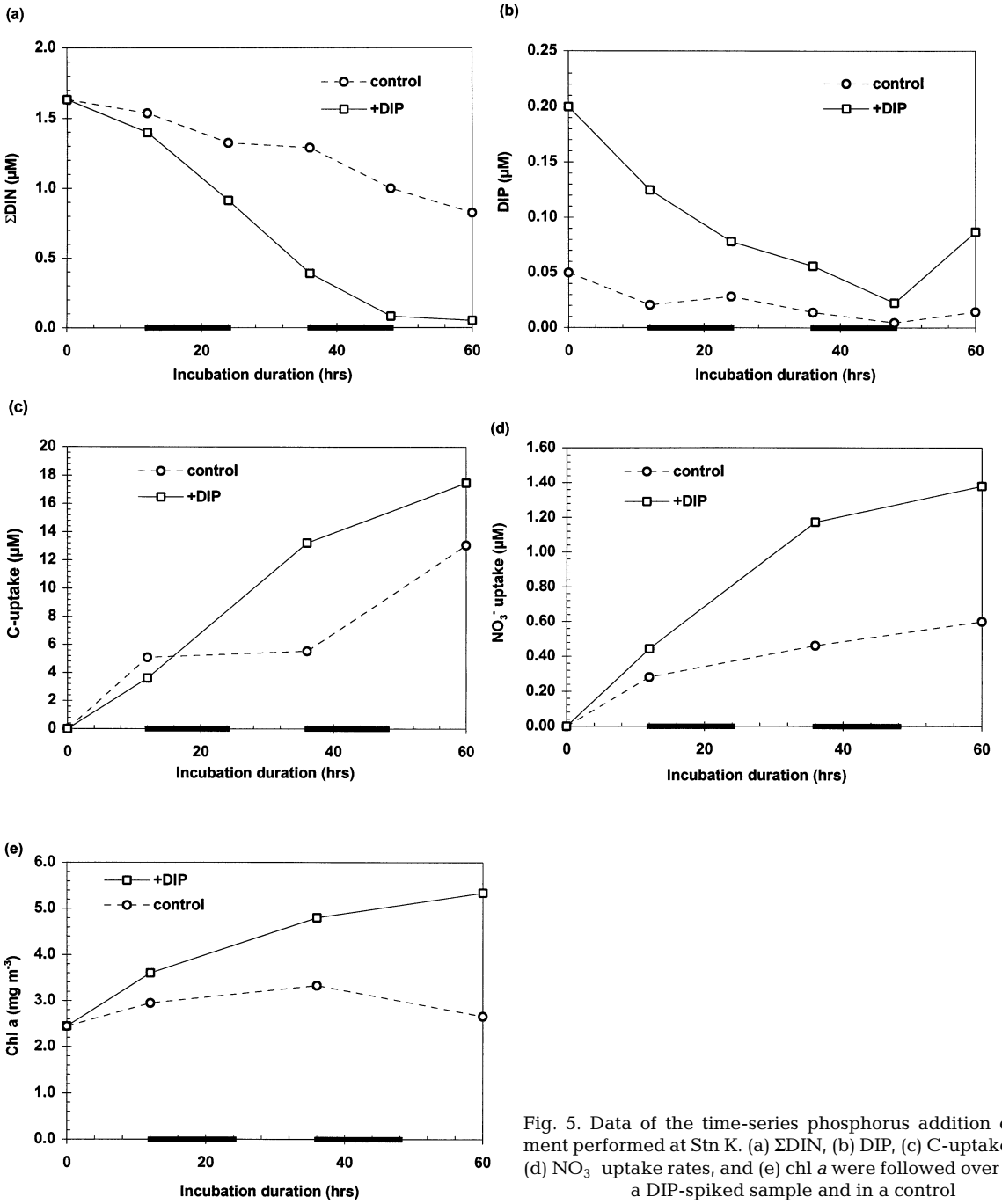

Fig. 5. Data of the time-series phosphorus addition experiment performed at Stn K. (a) ΣDIN, (b) DIP, (c) C-uptake rates, (d) NO_3^- uptake rates, and (e) chl *a* were followed over 60 h in a DIP-spiked sample and in a control

(1 to 6 times higher) in the photic layer. Thus, nutrient stocks in the upper layer appeared to be rather low during our experiment. Earlier studies demonstrated that the nutrient distribution in the Gulf of Lions at the end of winter is directly dependent on previous mixing of the water column (Coste et al. 1972, Lefèvre et al. 1997). This latter process is induced by the strong stress of northwesterly winds (Gascard 1978) and is highly variable over the year, thus resulting in high variability of nutrient supply to the upper layer. Earlier time-series studies on the evolution of N and P stocks in the Gulf of Lions also confirmed the trend of the inter-annual variability of nutrient availability in the early spring (Conan 1996). Therefore, a weak winter mixing of the water column could explain the general low nutrient content of the photic layer during our survey. But this was not likely, since the thickness of the mixed layer (50 to 100 m) was similar to that generally

observed at the end of winter in the area (Coste et al. 1972). Nevertheless, the ΣDIN:DIP ratio remained much higher than the reference value of 22:1 for Mediterranean areas and concentrations of ΣDIN were still significant in the upper layer even when DIP was exhausted (Fig. 3). This observation clearly suggests that P availability was insufficient to meet phytoplankton demand in the whole photic zone. High ΣDIN:DIP ratios may be explained by one or several early phytoplankton blooms which could have occurred before the survey. Let us consider a typical deep Mediterranean water with a ΣDIN:DIP ratio of 22:1 (i.e. DIP ≡ 0.41 µM and NO_3^- ≡ 8.5 µM) reaching the photic layer during winter mixing. Nutrient utilization by phytoplankton with typical N:P requirements of 16:1 (Redfield 1958) would then increase ΣDIN:DIP ratios (Fig. 6: see 'theoretical removal') to very high values (>150), with final significant NO_3^- contents of ca 2 µM, while DIP concentrations would become <0.015 µM. Our data closely fit the curve of 'theoretical' removal (Fig. 6) even if the observed high ratios (>150) cannot be firmly proved experimentally and should be regarded with caution, since conventional methods for measuring DIP do not allow for accurate detection of DIP content below 0.015 µM. Thus, our model suggests that early phytoplankton blooms were probably the cause of such high N:P ratios in the dissolved inorganic fraction of the photic layer. Some other observations further strengthen our hypothesis. Owens et al. (1989) reported low nitrate to phosphate ratios (2.0 to 22.7) in January 1989, i.e. just after the first winter mixing but before the typical bloom period. Jacques et al. (1973b) reported chl *a* concentrations up to 0.50 mg m^{-3} in the Gulf of Lions at this period of the year and Morel & André (1991) concluded from remote-sensed data (CZCS images) that the algae crop does not collapse during winter. On some occasions, we also observed (unpubl. data) high chl *a* contents (up to 0.80 mg m^{-3}) and surface primary production rates (up to 1.6 µM d^{-1}) during mid-January or February in the Gulf of Lions. Further examination of Fig. 6 showed that most of the ΣDIN:DIP ratios for low DIP contents were located below the theoretical curve, indicating an actual gap between the data and the model. In fact, it is likely that another process could result in faster P recycling relative to N recycling. Some other very high ΣDIN:DIP ratios (>50) located at the top of the nitracline immediately below the chlorophyll maximum have been observed in the eastern Mediterranean Basin (Krom et al. 1991) as well as in the western Basin (Raimbault & Coste 1990). In these cases, the high ΣDIN:DIP ratios could be attributed to a phosphacline deeper than the nitracline, suggesting uncompleted NO_3^- utilization by phytoplankton due to the lack of DIP at the bottom of the photic layer. As demonstrated in the present work,

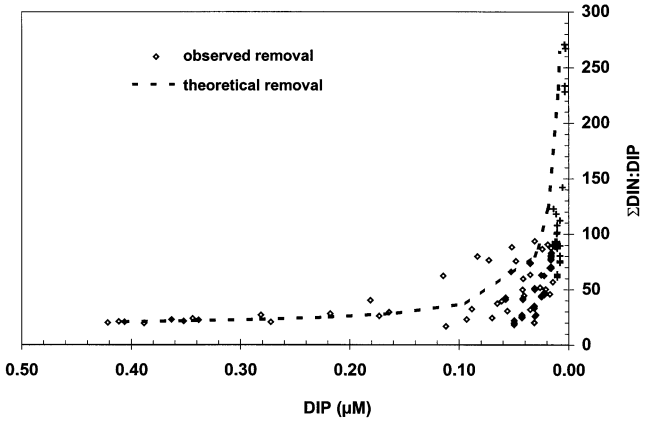

Fig. 6. NO_3^-:DIP versus DIP relationship. (◇) Data; (+) ratios obtained from DIP data below the detection limit. Dashed line represents the theoretical curve of phytoplankton DIP utilization with corresponding NO_3^-:DIP ratios in upwelled deep Mediterranean water (DIN:DIP = 22, and DIP = 0.41 µM). The typical Redfield (1958) ratio of N:P requirements (16:1) for phytoplankton was used in the model

such observations may be primarily attributed to the depletion in P relative to N (1:22) in deep Mediterranean waters (Fig. 6). Several suggestions were made to explain the high N:P ratio of deep Mediterranean waters, including fast N-fixation rates in coastal areas (Béthoux & Copin-Montegut 1986) and selective phosphate precipitation due to input of iron-containing dust from the Sahara (Krom et al. 1991).

In our study area, the imbalance between N and P observed in the surface waters might be also maintained by the Rhone influence. It has been recently emphasized (Moutin et al. 1998) that the N discharge of the Rhone River is mainly in the form of NO_3^- while a large part (almost half) of P discharge is in particulate form. According to these findings, the large excess of N relative to P (as orthophosphate) in the dissolved inorganic pool led to a mean ΣDIN:DIP of ca 70. Considering the organic matter discharges, a mean TN:TP ratio of only ca 15 (Moutin et al. 1998) is measured but the major part of the organic forms do not become available for phytoplankton due to the precipitation and/or rapid sedimentation processes near the Rhone mouth (Martin et al. 1989). Thus, due to the Rhone River inputs, waters in the Gulf of Lions seem to have a N surplus relative to P in the inorganic fraction available for primary production. Several other coastal areas heavily influenced by freshwater inputs, such as the northern Baltic (Lignell at al. 1992), Norwegian fjords (Thingstad et al. 1993), the Bay of Biscay (Herbland et al. 1998) and coastal waters of China (Harrison et al. 1990) have been demonstrated to be P- rather than N-limited. In this context, further studies are now required to prove whether, as recently suggested by

Tyrell (1999) for the global ocean, the external Rhone inputs could control in the long term the balance between N and P in the entire Mediterranean Sea.

The existence of nutrient limitation is generally difficult to assert, and low or undetectable chemical measurements of orthophosphate do not necessarily indicate phytoplankton limitation or deficiency (Thingstad et al. 1998). This is because phytoplankton vary greatly in their requirements for N and P (Rhee & Gotham 1980), and some communities can increase their biomass with inorganic N:P ratios that are very different from the Redfield (1958) ratio (Ryther & Dunstan 1971, Terry et al. 1985). In addition, phytoplankton populations, although under 'systemic' (biomass) limitation (Paasche & Erga 1988, Cullen 1991) in oligotrophic areas, grow at rates close to their maximum growth rates (Goldman et al. 1979). The growth rate limitation has been termed 'physiological' by Paasche & Erga (1988). Our results support both types of limitation, particularly during the second set of sampling. According to Thingstad & Rassoulzadegan (1995), 'systemic' nutrient limitation would be demonstrated by a (temporary or permanent) increase in biomass following the addition of the limiting nutrient to the system. Our time-course experiment, for which an increase in chl *a* was observed over more than 48 h in the DIP-spiked sample relative to the control (Fig. 5e), may provide evidence for such a limitation. In addition, stations with measurable DIP contents (Table 2) showed higher chl *a* concentrations than those with a DIP content below the detection limit. Although our DIP-enrichment experiments cannot unequivocally prove a 'physiological' limitation of the phytoplankton standing stock, one may nevertheless suggest (as outlined in Herbland et al. 1998) a growth rate limitation from the following fact. The mean photosynthetic efficiency (Table 3) was 1.57 µmol C µg chl a^{-1} d^{-1} and would be equivalent to a growth rate of 0.38 d^{-1}, i.e. a 0.54 doubling d^{-1} (assuming a phytoplankton carbon-to-chlorophyll ratio of 50, Banse 1977). If the theoretical maximum growth rate is calculated according to Eppley's equation (Eppley 1972), the maximum expected growth rate in these waters would be 1.94 doublings d^{-1}, at an average temperature of 13.10°C. Though Eppley's equation was obtained from laboratory cultures, this computation would indicate that phytoplankton species in our area may have grown at rates much lower (i.e. ca 28%) than their expected maximum rates. Goldman et al. (1979) demonstrated that high growth rates lead to typical C:N:P ratios of 106:16:1, whatever the medium composition in terms of inorganic ratios is, whereas low growth rates imply that C:N:P ratios in the phytoplankton are greatly influenced by the medium composition. Our possibly low growth rates may therefore explain the P depletion and the high C:N ratio observed in the particulate organic matter in some samples (C:N:P ratios of 160:20:1; Table 1, Fig. 4). Our bioassay and time-series experiments provide further evidence for P limitation of the encountered phytoplankton communities. The non-activation of C-uptake rates observed during the first 24 h in parallel to the nitrate-uptake enhancement in the DIP-spiked sample is a typical response of autotroph organisms to the addition of a limiting inorganic nutrient. In daylight, C fixation (i.e. the carbohydrate synthesis) is temporarily reduced or even suppressed to the benefit of nutrient (both NO_3^- and DIP) uptake (Table 3, Fig. 5) due to competition for photosynthetic reductant between CO_2 and N assimilation (Paasche 1971, Falkowski & Stone 1975, Slawyk & Collos 1982). Examination of the time-course results indicates that after 24 h, phytoplankton is back to a balanced growth situation and C-uptake appears to be significantly stimulated by a factor of 1.5 to 2.5. In the DIP-spiked sample, rapid removal (–40 to –50%, Fig. 5b) of DIP occurred from the first daylight period, while a high decrease in ΣDIN (–40%, Fig. 5a) was only observed from the first dark period. This pattern of significant NO_3^- uptake after a delay of 12 h following DIP addition has been already observed in the oligotrophic area of the tropical Atlantic (Raimbault & Pujo-Pay 1993). The addition of the limiting DIP probably first induced a reduction in the NO_3^- consumption because of the redirection of the photosynthetically derived energy to DIP uptake during the first daylight period, as has been previously shown in some laboratory studies (Terry 1982a,b). It is worthwhile to note that the acceleration of nitrate consumption occurred during the dark period following the DIP addition (Fig. 5b). One explanation for that latter feature might be that algae were almost balanced between N and P deficiency and that they were likely P replete at the end of the first daylight period; as soon as the algae got some P, they took up N to balance P uptake from the first dark period. This is further supported by the observation that some C:P and N:P ratios were close to the Redfield (1958) ratios (Table 1, Fig. 4). However, one should be aware of the potential role of heterotrophic bacteria to compete successfully with algae for dissolved inorganic nutrients (Currie & Kalff 1984, Hoch & Kirchman 1995); this feature is particularly obvious in the NW Mediterranean Sea, where P limitation of both heterotrophic bacteria and phytoplankton communities during summer has already been suggested (Thingstad & Rassoulzadegan 1995, Thingstad et al. 1998). Although we cannot firmly prove from our experiments whether heterotrophic bacteria were P-limited or not during the MOOGLI experiment, the potential role heterotrophic bacteria played in the present work in dark NO_3^- uptake cannot be ruled out.

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