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Preliminary results from a Lagrangian phosphate addition experiment in the Eastern Mediterranean (CYCLOPS)

P. Carbo[1], M. Krom[1], T. F. Thingstad[2], G. Fonnes[2], E. Skjoldal[2], N. Kress[3], T. Zohary[3], B. Herut[3], C. Law[4], M. Woodward[4], F. Mantoura[4], M. Liddicoat[4], F. Rassoulzadegan[5], T. Tanaka[5], A. Tselepides[6], P. Pitta[6], S. Psarra[6], T. Polychronaki[6], P. Drakopoulos[6], P. Wassmann[7], C. Riser[7], G. Zodiatis[8].

1 — School of Earth Sciences, Leeds University, Leeds UK.
2 — Department of Microbiology, University of Bergen, Bergen, Norway.
3 — National Institute of Oceanography, IOLR, Haifa, Israel.
4 — Plymouth Marine Lab., Plymouth, UK.
5 — University Pierre & Marie Curie, Villefranche-sur-mer, France.
6 — Institute of Marine Biology of Crete, Heraklion, Greece.
7 — Norwegian College of Fisheries Science, University of Tromso, Tromso, Norway.
8 — Oceanography Section, Department of Fisheries and Marine Research, Nicosia, Cyprus.

Abstract

Phosphate and SF_6 (an inert tracer) were injected into the upper mixed layer in an area of 7.5 km^2. 7.5 hours after the injection the chemically determined orthophosphate had reduced to half its initial value and after 36 hours to <5nM. At the same time there was an increased turn-over time of biologically available orthophosphate of more than one order of magnitude, suggesting that the phosphate had been removed by biological uptake. Primary productivity and phytoplankton biomass showed a nutrient-starved autotrophic community, unaffected by the phosphorus addition in terms of biomass or productivity, their changes being rather attributed to small-scale spatio-temporal variability. There were complex changes in the detailed functioning of the microbial ecosystem which are being evaluated. There was a large reservoir of DOP in the photic zone (50-400 nM), with 15-25 nM of particulate P and <5 nM of DIP. Only 17% of the DOP was UV-labile. It is now hypothesized that DOP may be important in providing the P substrate needed to remove excess nitrate from the surface waters between winter and early summer and thus drive the system towards co-limitation.

Keywords Phosphate, limitation, Eastern Mediterranean, DOP

Introduction

An in-situ Lagrangian phosphate addition experiment was carried out at the core of the Cyprus Eddy, in May 2001. The design of this experiment was based on the successful IRONEX and SOIREE experiment to determine whether the East Central Pacific and Southern Oceans were iron limited (Boyd and Law, 2001). It has been shown that the Eastern Mediterranean is P-limited on the basis of chemical determinations (Krom et al., 1991). The aim of the CYCLOPS experiment was to determine whether the Eastern Mediterranean was P limited in May by determining how the natural microbial ecosystem responds to a single addition of phosphate. A Lagrangian experiment was carried out in which phosphate and an inert tracer (SF6) were added to a patch of water and the chemical and biological response to that addition were followed.

Methodology

The Lagrangian experiment was carried out in the center of the Cyprus anticyclonic eddy, SE Levantine Basin, by the R/V Aegaeo between 5-17 of May 2001. Physical data was gathered using a Sea Bird Electronics (SBE911plus) CTD profiler, furnished with oxygen and fluorometer sensors. Water samples were taken using a multi-sampler/Rosette system (General Oceanics). Samples were subsequently analysed for nutrients and biological parameters at stations outside and inside the area of phosphate addition (referred to in the text as OUT stations and IN stations respectively). The details of the chemical and biological analyses are described in Table 1.

Phosphate addition experiment

Approximately 3.2 tonnes of phosphate were added with 0.29 mol SF_6 to a 30m surface mixed layer over an area of ~7.5 km^2 on 11/05/01. Conservative dilution would have resulted in a mean dissolved inorganic phosphate (DIP) concentration of ~103 nmol/l in the patch (compared with undetectable DIP outside the patch), and an average SF_6 concentration of ~1230 fmol/l (~1600 times the background level). SF_6 results from samples taken from a transect 7.5 hours after the addition showed that the patch surface area had increased by a factor of 2.3 (Figure 1). The mean IN patch dissol-

ved inorganic phosphorus (DIP) concentration at that time was 22 nmol/l, half that of the expected value. The disparity between the SF_6 concentration and DIP indicated that ~50% of the DIP had been lost or taken up by biological activity. At this time electrical and other problems resulted in SF_6 being tracked only by discrete sampling. A second IN station was sampled 36 hours after the addition using discrete samples collected by CTD. The drifter buoy and SF_6 patch remained closely associated for the first two days, until the drogue was lost from the drifter buoy during a storm. After searching for the patch for 2 days, it was found again. The first Box survey (14/05/01) of 15 stations revealed a maximum SF_6 concentration of 36 fmol/l, indicating a 15-fold dilution of the SF_6 tracer since the first transect three days previously. A 2nd Box survey of 12 stations confirmed this, with some evidence of vertical heterogeneity in the SF_6 tracer distribution. The patch had moved at horizontal speed of approximately 39 km/d which was much faster than expected and greater than the velocity determined in the Southern Ocean (Boyd and Law, 2001).

Results

Nutrients

The background concentrations of phosphate and nitrate at the OUT stations in the upper 100 m were below detection limit for the conventional automated method (<10nM) for all samples. An improved method for P determination, the 'nanomolar waveguide system', was put to a successful field trial on-board ship. Results from this system (detection limit 1nM) confirmed that natural water column DIP concentrations were <5 nmol/l to the depth of the nutricline. Silicic acid concentrations in the upper 100 m were 1.11 ± 0.25 µM.

Phosphate measured within the patch rapidly declined over time. After 7.5 hours, the mean IN patch dissolved inorganic phosphorus (DIP) concentration was 22 nmol/l (Figure 1), and after 36 hours, a maximum DIP concentration of 0.02 µM was found at 15 m depth with most samples at or below the analytical limit of detection. The next measurement was 5 days after the P addition (due to technical problems and weather conditions), when P concentration had fallen to background levels. There was no evidence of a change in other nutrient concentration as a result of the P addition.

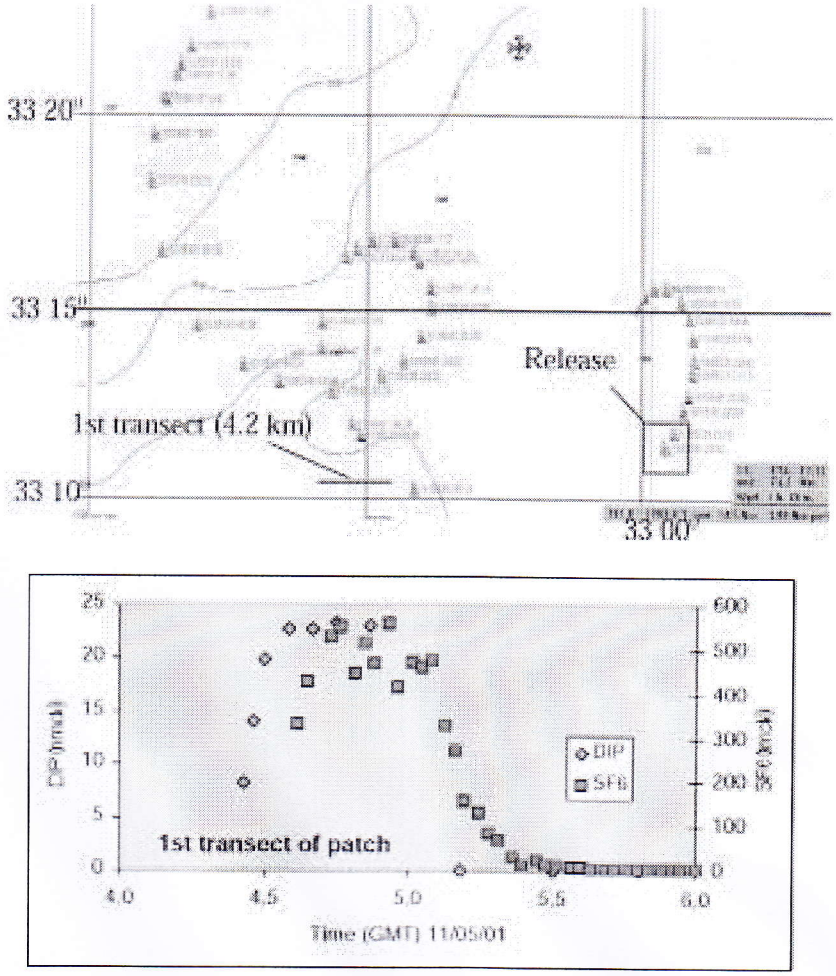

Fig. 1 : Figure showing the position of the Phosphate/SF_6 patch as located by the position of the drogued bouy. The inset shows the measured concentration of phosphate (DIP) and insert tracer (SF_6) transect 7.5 hours after the release. It is estimated that ~50% of the DIP had been removed by biological uptake during this period.

Dissolved organic-P (DOP), particulate P and dissolved organic carbon (DOC)

The acid persulphate technique gave fairly high values for total-P in the top 30m (200-400 nM-P). Particulate-P accounts for only a small fraction of the total-P (15-25 nM) (Figure 2a). UV-labile DOP was determined separately on a number of depth profiles. There was a significant pool of UV-labile DOP (~50 nM) in the surface waters (Figure 2b). Since inorganic-P was below detection limit in the euphotic zone, the total-P determined by both methods was essentially all DOP. There was no apparent difference bet-

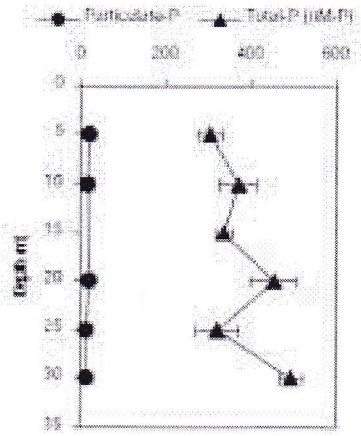

Fig. 2a : Total-P (-DOP) and particulate-P concentrations.

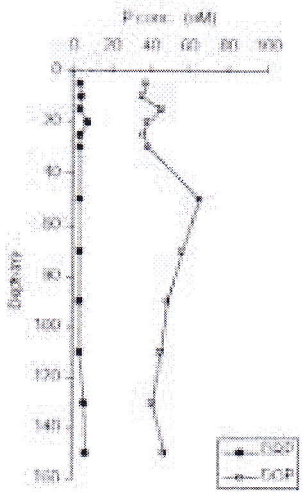

Fig. 2b : UV-labile DOP - IN station, 5 days after P addition.

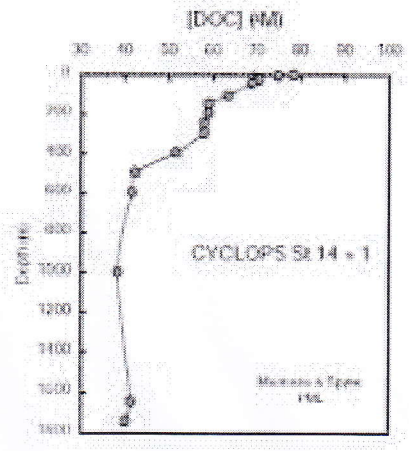

Fig. 3a : DOC depth profile of the CYCLOPS Eddy.

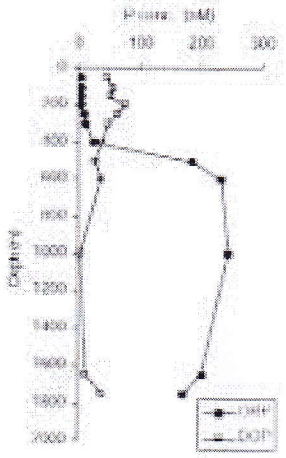

Fig. 3b : Dissolved reactive P (DRP) and UV-labile DOP.

ween DOP measured at an OUT stn (4 days after addition) and an IN stn (5 days after addition).

Dissolved organic carbon (DOC) and UV-labile DOP were also determined on profile to 1750m. DOC ranged from 80 µmM at the surface to 40 µmM from surface to 500 m depth. From 500 m to 1750 m it was fairly constant, at approximately 40 µmM (Figure 3a). DOP decreased from 50 nM in the photic zone to a minimum of <5 nM at 1000 m (Figure 3b) The-

re was an increase in the DOP and a decrease in the DOC which corresponds to the new Levantine Deep water formed in the Aegean.

PO_4 turnover time and particle size distribution (15m depth)

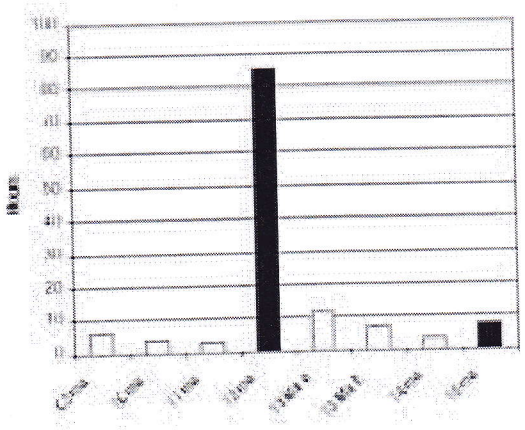

Fig. 4 : Turnover-time for PO4 in samples from 15m depth. IN stations are shown in black (12th and 15th May).

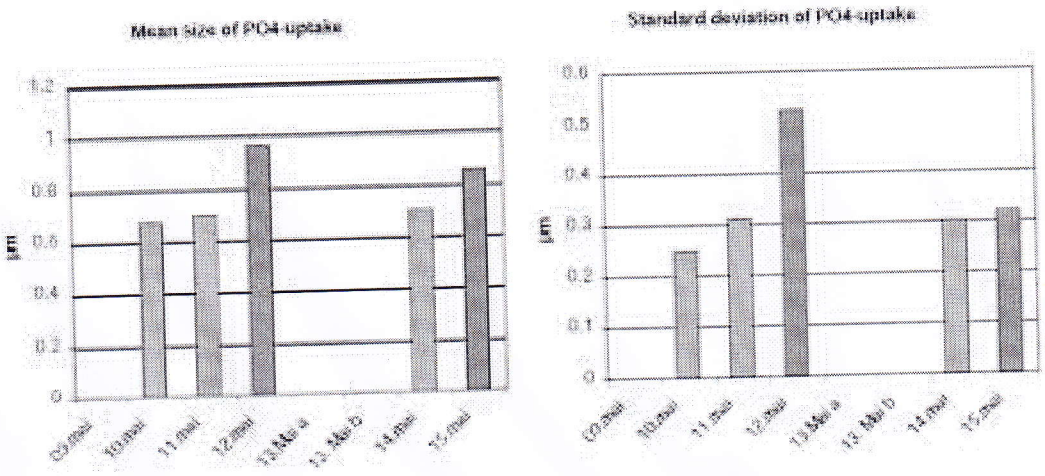

Fig. 5a & 5b : Shift in mean size of organisms involved in P uptake. IN stations are shown in dark grey (12th and 15th May).

Only half of the phosphate originally injected was detected as dissolved inorganic phosphate 7.5 hours after release and had fallen to below detection limits (<5nM) by 36 hours after release. At the same time (12/05/01) there was an increased turnover time of biologically available orthophos-

phate of more than one order of magnitude (from <10 h to nearly 100 h). (Figure 4) This suggests that the phosphate had been removed by biological uptake. The uptake corresponds to an approximate doubling of the existing particulate-P. Phosphate uptake as a function of size was very close to normal distribution, with a mean of 0.7 um (Figure 5a), indicating a dominance of uptake in picoautotrophs. Phosphate uptake was shifted into slightly larger organisms as a result of the P addition (Figure 5b). This is consistent with the expectation from previous bottle experiments that the addition of phosphate removed P-starvation in the microbial population (Thingstad et al. 2001) particularly within the larger organisms in the microbial population.

Chlorophyll a

During the period of the experiment the water column was stratified, with a mixed 30 m upper layer and a seasonal thermocline established between 30 and 70 m. Total chlorophyll *a* averaged 0.03 μg l^{-1} in the surface mixed layer (0-30 m). It remained very low down to 70 m. The deep chlorophyll maximum (DCM) extended between 90 and 120 m and had a chlorophyll *a* content of 0.213 ±0.07 μg l^{-1}. It was always situated under the thermocline while in most cases (5/8) it was located at 110 m. Chlorophyll *a* remained almost constant over the 5 days of the experiment. The dominance of picoplankton in the surface layer was evident in the IN stations, while

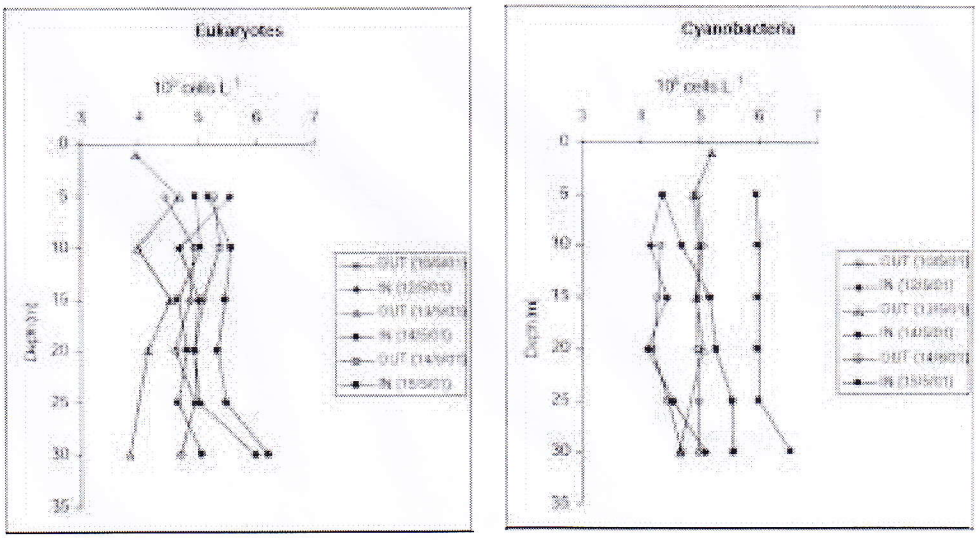

Fig. 6a & 6b : The abundance of eukaryotic and cyanobacterial cells in the surface water (0 – 40 m) of the Cyprus Eddy before and after the addition of phosphorus, at IN and OUT stations.

no differences can be seen for either total, pico or nano chl *a* concentrations between the first and the subsequent days ($p>0.05$).

Photosynthetic picoplankton

There was no distinct difference in the abundance of eukaryotic phytoplankton cells between samples taken inside vs. outside the patch (Figure 6a). For unicellular picoplanktonic cyanobacteria, however, it was evident that the highest values coincided with the sites of highest bacterial activity and high SF_6 values (Figure 6b).

Phytoplankton

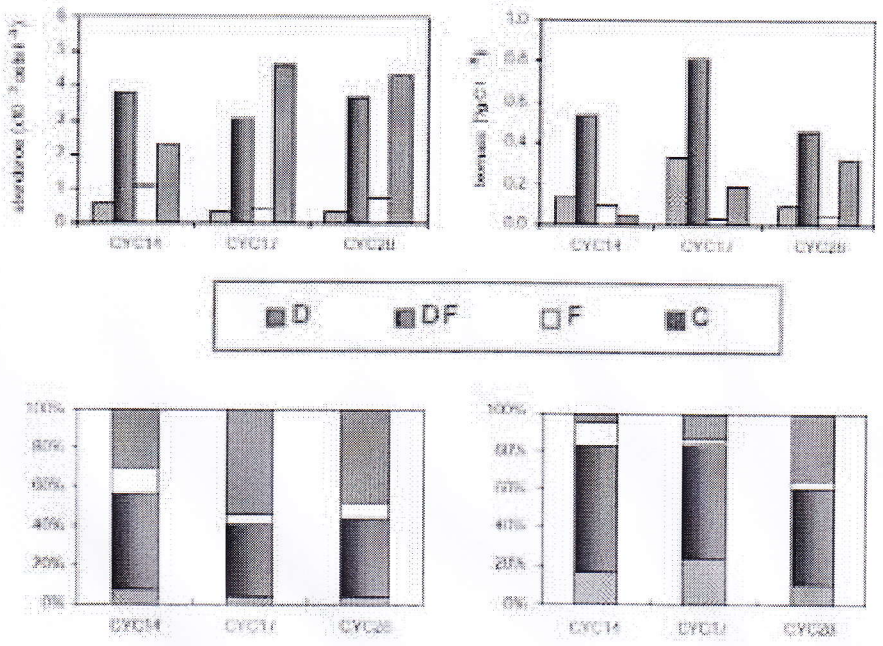

Fig. 7 : Mean (upper panel) and relative (lower panel) contribution of phytoplankton groups in abundance and biomass, during one OUT (CYC14) and two IN (CYC17 and CYC28) primary production casts (D : diatoms, DF : dinoflagellates, F : flagellates, C : coccolithoforids).

Interestingly, the most pronounced variation in the micro-phytoplanktonic community (only IN stations, Figure 7) was the relative contribution of each group. Dinoflagellates were the most important group all over the experiment, both in terms of abundance (66-39 %) and biomass (67-52%). Diatoms and flagellates shared the rest of proportions but within the first two

days from the addition and until the end of the experiment they were replaced by coccolithophorids in terms of abundance whereas in terms of biomass coccolithophorids shared proportions with diatoms.

Heterotrophic nanoflagellate (HNF) and autotrophic nanoflagellate (ANF) biomass

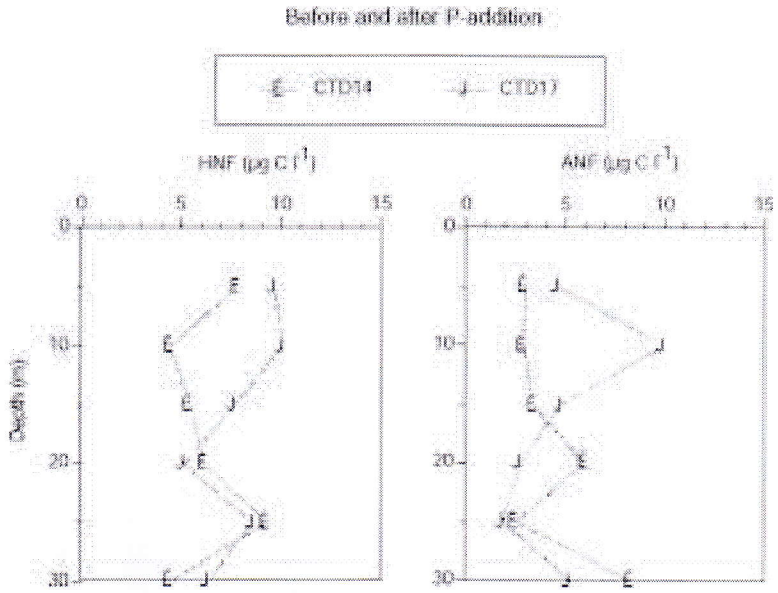

Fig. 8 : HNF and ANF biomass OUT stn (CTD14) and IN stn (CTD17).

The biomass of HNF and ANF ranged from 4 to 10 and from 2 to 10 µg C l^{-1}, respectively, in the IN and OUT stations in the upper 30 m (Figure 8). Both biomasses appeared to increase after P addition at 5, 10 and 15 m, especially at 10 m depth. HNF increased by a factor of 2 and ANF by a factor of 3. Below 20 m, there was either no change, or a slight decrease, as a result of P addition.

Ciliates

Ciliate abundance ranged from 82 to 223 cells l^{-1} in the upper 30 m ; these extremely low values are not unexpected for the region. Both abundance and biomass decreased sharply down to 30 m and more smoothly down to 150 m (Figure 9). No differences were found between the IN and OUT stations, in terms of profiles and integrated values or when abundance, biomass or species composition of ciliates were considered.

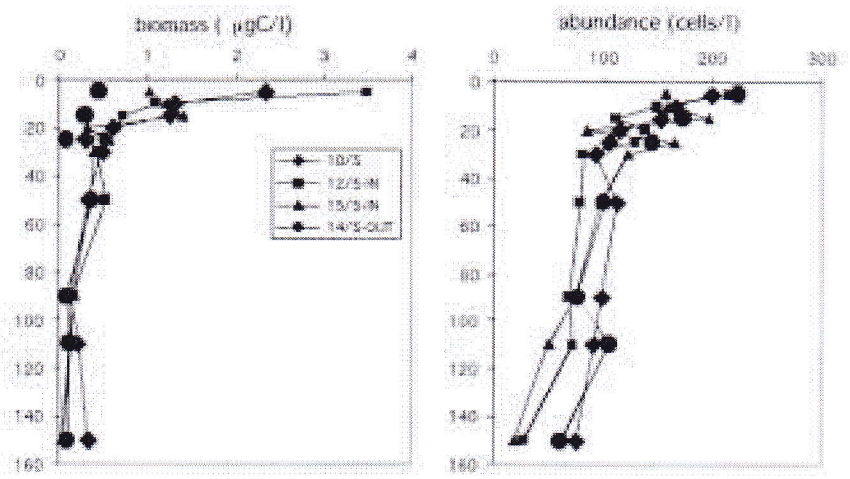

Fig. 9 : Vertical distribution of ciliate biomass and abundance before and after the P-release.

Heterotrophic bacteria

Bacterial numbers in the surface water (upper 40 m) of the eddy at the OUT station ranged from 0.7 x 10^5 (NFG 15, 25 m depth) to 2.2 x 10^5 cells ml^{-1} (CYC 21, 1 m) with an average of 1.35 x 10^5. These are extremely low abundances for natural populations of heterotrophic bacteria, even for the ultra-oligotrophic Levantine Basin. A response to the P fertilization was not detected in bacterial numbers. There was no indication of higher or lower bacterial counts in the upper waters (0 – 30 m) of the IN stations as opposed to the OUT stations. If anything, the few highest values, > 2 x 10^5 cells ml^{-1}, were all recorded outside the patch.

Particle volumes and size spectra

Total particle volume (0.5-50 µm) ranged from 5x10^4 to 2.3x10^5 μm^3 ml^{-1} and decreased greatly at 5 and 10 m after P addition.

Primary production

Total primary production decreased significantly over the 2 days following P addition ($p<0.01$) and had decreased even further by 5 days after addition ($p<0.0001$; Table 2). Although biomass remained almost constant, primary production gradually decreased over the 5 days, as a result of a reduced assimilation index of the phytoplankton populations involved.

Bacterial activity

In most sampling stations and depths there was no distinct difference in bacterial activity between samples taken inside vs. outside the patch. However, the few exceptionally high values were all for samples taken from inside the patch, and corresponding with relatively high SF_6 values. Expressing the same data as bacterial specific activity, i.e. activity per bacterial cell (pmol Leucine $(10^6$ cells$)^{-1}$ h^{-1}) gave similar results : the highest specific activity took place at the same sites of highest total bacterial activity.

Discussion

There was a rapid uptake of phosphate in the patch after addition with 50% being lost from the water column within 7.5 hours and values close to background being found after 36 hours. There was evidence from the turnover time that this loss from the water column was due to biological uptake. The uptake seemed to be concentrated in the larger sized organisms. The other biological data showed a balanced but starved autotrophic community in the area, which did not seem to be affected by the phosphorus addition in terms of phytoplankton biomass or productivity. Other biological parameters showed a complex response to the addition with changes that were often difficult to see above the natural variability within the system. Thus there were a few exceptionally high values of bacterial activity in the patch but no change in bacterial numbers. Heterotrophic and autotrophic nanoflagellates increased at some depths within the patch as did unicellular picoplanktonic cyanobacteria. Ciliates and eukaryotic phytoplankton were unchanged. It is difficult to create a coherant story regarding the microbial response to P addition because the patch was lost from 36 hours for a further 2.5 days and when it was found again it was very highly diluted. Further work is being carried out to understand better the complex changes in the detailed functioning of the microbial ecosystem.

It is well known that the Eastern Mediterranean is extremely oligotrophic and with levels of DIP close to or at analytical limits of detection throughout most of the year (Kress and Herut, 2001). However, the data obtained during this experiment showed that there was a considerable DOP pool in the photic zone. Most of this DOP pool was refractory to UV light and presumably also to biological activity. The UV-labile pool was only ~17% of the total DOP concentration compared to more than 51% found in the oligo-

trophic North Pacific (Karl and Yanagi,1997). It is suggested that the atypically low percentage of UV-labile DOP in Eastern Mediterranean waters may be due to the ultra-clear water, which holds the world record Secchi disk depth (Berman *et al.*, 1985). This may result in the UV labile DOP being broken down in-situ to DIP, which is then available for microbial uptake. Confirmation of these preliminary results will be sought during the 2002 cruise to the same area. It is speculated that this may be the mechanism whereby the system switches from P limitation in winter, when there is excess free nitrate left in the surface waters, and the situation in May, when the system appears to N&P co-limited.

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Table 1 : Methods

Parameter	Method	Reference
SF_6	Automated In-line/off-line cryogenic trapping/ECD-GC analysis	Law et al, 1994 ; 1998
Nutrients (on board)	In-line/off-line 5 channel Technicon AAII, segmented flow autoanalyser	Woodward ; 1994
Nanomolar phosphate (on board)	Segmented flow, long path-length liquid waveguide technology	
Nutrients (frozen)	In-line/off-line 5 channel Technicon AAII, segmented flow autoanalyser	Kress and Herut, 2001
UV labile dissolved organic phosphorus (DOP)	UV irradiation	Armstrong and Tibbits (1968)
Total phosphorus (Total-P) and particulate P	Acid persulphate method	Koroleff, 1983
Dissolved organic carbon (DOC)	High Temperature Catalytic Oxidation	
Pigment analysis	High Performance Liquid Chromatography	
Eukaryotic phytoplankton and prochlorophytes	Flow cytometry	
Autotrophic and heterotrophic nanoflagellates (ANF and HNF)	Stained with DAPI and counted under an epifluorescence microscope Biomass was estimated by assuming constant cell volumes and a volume to carbon conversion factor	
Primary production	^{14}C *in situ* technique	Steemann Nielsen, 1952
Chlorophyll *a*	TURNER TD 700 fluorometer	Yentsch & Menzel, 1963
Size-fractionation of PP and Chl *a*	Parallel filtration on 0.2, 0.6 and 2.0 µm polycarbonate filters (Poretics, Ø 47 mm) with the 0.2-2 µm fraction representing the pico-phytoplankton and > 2 µ m the nano- and micro-phytoplankton.	
Phytoplankton	Taxonomic determinations of nano- and micro-phytoplankton (> 5 m cell size) using inverted microscopy and cell counts (species structure) followed by calculations of biomass descriptors.	Utermöhl, 1958
Ciliates	Inverted microscopy ; distinguished into size-classes, trophic modes (auto-, hetero-, mixotrophs) and major taxonomic groups (oligotrichs, choreotrichs, tintinnids). Biomass, was estimated by calculating the biovolume and applying the appropriate conversion factor.	

Table 2 : Primary production and Chlorophyll-*a* (integrated over the 0-30 m).

Location of cast (IN or OUT)	PP (mg C $m^{-2}h^{-1}$)	Chl-*a* ($\mu g.l^{-1}$)
IN (before P addition)	3.5	0.7
IN (36 hours after P addition)	3.0	0.7
IN (5 days after P addition)	2.3	0.7
OUT	2.8	0.9