

Summary and overview of the CYCLOPS P addition Lagrangian experiment in the Eastern Mediterranean

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Abstract

CYCLOPS was a European Framework 5 program to further our understanding of phosphorus cycling in the Eastern Mediterranean. The core of CYCLOPS was a Lagrangian experiment in which buffered phosphoric acid was added to a $<4 \times 4$ km patch of water together with SF₆ as the inert tracer. The patch was followed for nine days in total. Results obtained prior to the experiment showed that the system was typically ultra-oligotrophic and P-starved with DON:DOP, PON:POP and DIN:DIP all having ratios greatly in excess of 16:1 in surface waters. To our surprise, we found that although the added phosphate was rapidly taken up by the microbial biota, there was a small but significant decrease in chlorophyll *a* and no increase in primary production, together with an increase in heterotrophic bacterial activity, ciliate numbers and in the gut fullness and egg numbers in the zooplankton community. A microcosm experiment carried out using within-patch and out-of-patch water showed that the phytoplankton community were N and P co-limited while the

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bacteria and micrograzers were P-limited. Thus this system tends to N and P co-limitation of phytoplankton productivity in summer possibly caused by bioavailable DIN being converted into non-bioavailable forms of DON.

On the basis of the data collected within the programme it was concluded that this behavior could be explained by three non-mutually exclusive processes described as (1) trophic by-pass in which the added phosphate gets directly to the grazing part of the predatory food chain from the heterotrophic bacteria bypassing the phytoplankton compartment phosphate, (2) trophic tunnelling in which phosphate is rapidly taken up by both phytoplankton and bacteria via rapid luxury consumption. This causes an immediate change in the phosphorus content but not the abundance of the prey organisms. The added P then “reappears” as responses at the predator level much more rapidly than expected, and (3) mixotrophic by-pass in which inorganic nutrients, including the added P, are taken up by mixotrophic ciliates directly, bypassing the phytoplankton. For details of the results of this study and the processes described, the readers are referred to the relevant papers within this volume.

The implications of these results for nutrient cycling in the Eastern Mediterranean are discussed. In particular it is noted that the efficient and rapid grazing observed in this study might explain why the system although impacted by anthropogenic nutrient input has shown little or no measurable change in microbial productivity since added nutrients are rapidly transferred out of the photic zone via the by-pass and tunnelling processes and are exported from the basin. It is also suggested that fish productivity is higher than has been suggested by conventional food chain models due to this grazing. Two possible reasons for the unusual P-starved nature of the basin are presented.

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1. Introduction

This manuscript presents an overview of the CYCLOPS program and in particular a synthesis of the results of the P-addition experiment. Here we describe briefly the initial background and aims of the project. The location of the study area and the conditions in the surface waters prior to the experiment are presented, followed by a brief description of the logistics of the Lagrangian experiment. The sampling and methods of all the experiments are summarized. After describing the physical conditions in the Cyprus Eddy during the experiment, the biological changes caused by addition are summarized together with those chemical changes that are not presented elsewhere in this volume. A synthesis of the overall meaning of all the CYCLOPS results is then given. This is followed by a broad overview of the implications of our new understandings of the controls on nutrient cycling in the Eastern Mediterranean for environmental studies in the Eastern Mediterranean and elsewhere.

2. The logical basis for the CYCLOPS experiment

The Mediterranean in general and the eastern basin in particular is one of the largest bodies of water in the world that is thought to be phosphorus-limited (Krom et al., 1991). This conclusion was based originally on the molar ratio of nitrate:pho-

sphate in the deep water of the Eastern Mediterranean, which is 29:1 (Kress and Herut, 2001; Krom et al., 1991, 2005) and far higher than that in the Western Mediterranean (23:1) and the Eastern North Atlantic (16:1). In the Eastern Mediterranean the winter phytoplankton bloom ceases as soon as the system runs out of phosphate supplied by winter mixing. Characteristically this happens when there is 300–1000 nmol kg⁻¹ of nitrate remaining in the surface waters (Kress and Herut, 2001; Krom et al., 1992). This nitrate has been shown to be isotopically unusually heavy (16–40‰ compared with 3–12‰ at depth; (Struck et al., 2001), which is characteristic of water in which a phytoplankton bloom developed but ceased before all the nitrate was taken up by biological productivity.

Additional biological evidence for P-limitation of the surface waters of the Eastern Mediterranean has been obtained from a series of observations on phytoplankton activity and specific microcosm experiments (Becacos-Kontos, 1977; Berland and Al, 1987; Berland et al., 1990; Bonin et al., 1989; Pojed and Kveder, 1977; Vukudin and Stojanski, 1976). Zohary and Robarts (1998) showed that in winter, bacteria from various sites in the Eastern Mediterranean were P-limited. However, all of this chemical and biological evidence was indirect. In the case of the biological enrichment experiments, they were subject to the typical problems associated with ‘bottle’ experiments, especially in a system with uniquely low phytoplankton biomass.

In those areas of the world's oceans that are HNLC (high-nutrient low-chlorophyll), it was found that the probable crucial nutrient (iron) is unusually low in the surface waters (Martin et al., 1994). Bottle experiments had suggested that the system was iron-limited and that an addition of iron would result in increased phytoplankton growth. However, to confirm this result and avoid the limitations/artifacts arising from in vivo incubations, a series of in situ Lagrangian iron addition experiments were planned and carried out (Boyd et al., 2004). These experiments involved the addition of the limiting nutrient plus SF₆ as an inert tracer to follow the patch (Law et al., 1998). The CYCLOPS experiment employed the same framework to address the impact of a P-addition in the Eastern Mediterranean.

In addition to the main Lagrangian experiment, a series of microcosm experiments were carried out to understand, under controlled conditions, some specific aspects of the nutrient cycling processes which were occurring in nearshore (Kress et al., 2005) and pelagic offshore systems (Herut et al., 2005; Zohary et al., 2005). As a result of these experiments we were able to provide not only the answers to these specific questions but also the extent to which such microcosms are able to mimic the natural ecosystem as represented by the Lagrangian addition experiment itself.

3. The Eastern Mediterranean as a vulnerable ecosystem

The Mediterranean in general and the Eastern Mediterranean in particular is ultra-oligotrophic with the lowest nutrient content in the world found in its deep waters as well as nutrients below conventional analytical detection limits in its surface waters. This is caused by its unusual anti-estuarine circulation. Nutrient-depleted surface waters flow into the basin at the straits of Gibraltar and Sicily, while intermediate waters containing significant amounts of dissolved inorganic nutrients are exported to the Western Mediterranean and the North Atlantic. However the basin is surrounded by land with a large population on the northern shores particularly and a major influx of tourists each year (Turley, 1999). This results in a high anthropogenic nutrient flux to the basin (Krom et al., 2004), which is probably increasing with time. It is reasonable to suggest that relatively small changes in the fluxes of nutrients might have a disproportionate effect on

the marine ecosystem structure in the region. It is thus crucial to understand the controls on nutrient cycling if we are to understand and even predict man induced changes to the system. Such changes could result from changing input from the coastal system including riverine fluxes and activities such as mariculture, aeolian input (dust) or even changes in circulation patterns.

Prior to the CYCLOPS experiment, the nature of the microbial community of the offshore Eastern Mediterranean was known only to a limited extent (Christaki et al., 2001; Li et al., 1993; Pitta et al., 2001; Robarts et al., 1996; Yacobi et al., 1995; Zohary et al., 1998; Zohary and Robarts, 1992). It was clear that the shallow waters (ca. 0–50 m) were dominated by nano and picoplankton, particularly the cyanobacterium *Synechococcus* and by heterotrophic bacteria (Krom et al., 2003). It also was known that there were rather small numbers of larger eukaryotic phytoplankton (Kimor et al., 1987). Initially a conceptual model developed by Thingstad and co-workers for the coastal Western Mediterranean and elsewhere (Thingstad and Rasoulzadegan, 1999) was used to describe the functioning of the microbial community. As a result of the CYCLOPS project this model was modified to reflect the new understandings of the functioning of the microbial community in the ultra-oligotrophic offshore part of the basin (Thingstad, 2005).

4. Description of the CYCLOPS program

In May 2000 a microcosm experiment was carried out in the National Institute of Oceanography, Haifa, Israel. The purpose of this experiment was to test analytical methodologies for use in the ultra-oligotrophic conditions found in the South-East Levantine basin and to conduct a preliminary, controlled examination of the response of Eastern Mediterranean surface water to nutrient additions. In this experiment nitrate, phosphate and glucose were added to microcosm bottles and the biological and chemical evolution was followed for five days. Preliminary interpretation of these data are presented in Thingstad et al. (2001). The seawater used in this experiment, although having been sampled from 30 km offshore, was actually taken from a plume of advected coastal water. Thus the results from this experiment provide valuable data on the contrasting responses of the coastal and pelagic microbial ecosystems to nutrient additions (Kress et al., 2005).

In May 2001, a pilot study for the Lagrangian P-addition experiment was carried out in the Cyprus Eddy. Considerable practical experience was gained in the execution of a phosphate-release experiment, although problems with power supply to the mapping equipment and detachment of the drifter buoy drogue during a storm on Day 2 limited the monitoring of the patch. Data from a Saharan dust storm that occurred during the 2001 experiment on May 11 are presented (Groom et al., 2005; Herut et al., 2005), but we focus

primarily on the results of the main experiment in May 2002.

5. Practical description of the May 2002 P-addition experiment

The P-addition experiment was undertaken on 17–27 May 2002 from the *R/V Aegaeo* in the center of the Cyprus Eddy (see Fig. 1). The phosphate solution was prepared prior to sailing on the quay at Limassol, Cyprus, by adding concentrated

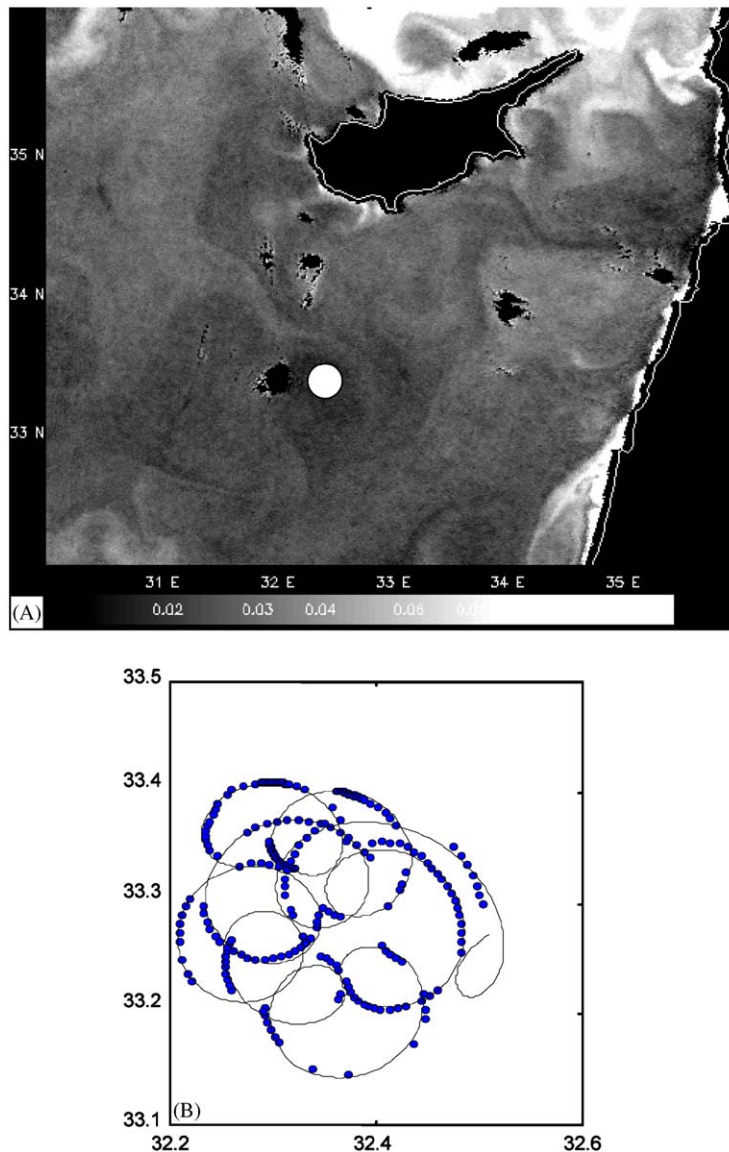


Fig. 1. (A) Ocean image of the Eastern Mediterranean with the white circle indicating the position of the Cyprus Eddy. (B) Plot of drifter buoy movement during the nine-day experiment. The dots are positions obtained from the buoy, and the solid line is a fit to these data. For more detail see Figs. 1, 2 and 3 of Law et al. (2005).

phosphoric acid to 500 kg of sodium bicarbonate in nutrient-depleted seawater in 6×3000 -l tanks. The final mean concentration of ~ 2.2 M dissolved inorganic orthophosphate was confirmed by direct measurement. SF_6 saturation was achieved using an in-line saturation unit in which surface water was continuously sprayed through an atmosphere of pure SF_6 . The SF_6 solution was mixed with the phosphate solution using a dual pumping system, and pumped into the wake of the ship to a depth of ~ 9 – 11 m in a 16-m surface mixed layer as described in Law et al. (2005). Approximately 39,000 mol of phosphate solution and 0.44 mol of SF_6 were added to the surface water. The release took 6.5 h at a ships speed of ~ 5 knots, and covered an area of $< 4 \times 4$ km. The mid-point of the release at 1200 GMT 17/5/02 was used as the reference Time zero (T_0) for the experiment.

The patch was mapped on a daily basis at a ship speed of 7–10 knots for periods of 8–11 h overnight. On every other day the ship moved to a minimum distance of 15 km from the patch center for vertical profiling at an OUT station. Water-column profiles were obtained by CTD hydrocast at both IN and OUT stations, with discrete SF_6 analysis on 350-ml water samples using a technique similar to the mapping, but with the inclusion of a vacuum-sparging step (Law et al., 1994). For further details on the practicalities of the Lagrangian experiment see Law et al. (2005).

6. Sampling and methods

Details of all the casts taken and the measurements made in each cast are given in Tables 1 and 2. A full listing of this information together with all the measurements made is available on the CYCLOPS website (www.earth.leeds.ac.uk/cyclops). The chemical and biological parameters determined during this study are given in Table 2. All of the methods for the data presented elsewhere in this volume are given in the relevant papers. The details of methods given below are only for the chemical evolution data, which are not presented elsewhere in this volume.

6.1. Sampling and methods for chemical changes

Details of the sampling and methods for chemical changes are presented in Krom et al. (2005). Briefly, dissolved oxygen was measured at sea using Carpenter–Winkler titration procedure (Carpenter,

1965) and a radiometer automatic titrator (TTT80), equipped with a dual platinum electrode, in the dead-stop end point mode. The precision was 0.3% as determined by analyzing replicate samples from the same Niskin bottle. Dissolved nutrients were determined on unmodified samples on-board ship within hours. A colorimetric segmented-flow analytical system, using a long path-length (2 m) liquid waveguide capillary cell (LWCC) as the detection flow-cell was used to measure for nanomolar phosphate, nitrite and nitrate. For those measurements of dissolved nutrients in the micromolar range a conventional 5-channel Technicon AAI, segmented flow autoanalyser, with techniques developed for optimal sensitivity (Woodward, 1994; Woodward and Rees, 2001) was used. Ammonium measurements were determined using a fluorimetric detection technique, following ammonia gas diffusion across a teflon membrane (Woodward and Rees, 2001). Samples for DOC and DON were analyzed by high-temperature combustion (Alvarez-Saldago and Miller, 1998; Spyres et al., 2000). A high-temperature combustion system that has a Shimadzu TOC-5000A infrared gas analyzer coupled to an Antek 7000 nitrogen-specific chemiluminescence detector was used for the simultaneous measurement of DOC and TDN. DOP_{UV} was determined as SRP after UV irradiation method using a 125 W Hg vapor lamp. Particulate organic carbon (POC) and nitrogen (PON) were analyzed on a Leeman Lab 440 CHN elemental analyzer. Measurement of particulate-P was by persulphate oxidation, modified after Koroleff (1976).

7. Results

7.1. Physical oceanography of the Cyprus Eddy during the CYCLOPS experiment

The CYCLOPS addition experiment was carried out as close as was possible to the center of the Cyprus warm-core Eddy. This was done for practical reasons. The Eastern Mediterranean is highly dynamic. The CYCLOPS experiment was limited by having to raise P concentration significantly and the only way to do this practically was to start with a small patch. By carrying out the experiment in the center of a warm-core eddy we made it easier to constrain and thus follow the patch over time.

The Cyprus Eddy (a.k.a. Shikmona Gyre) is a quasi-stationary warm-core feature situated south

Table 1
Cast list for all stations where the data are presented in this volume

Station	Date	Depth	In/Out	GMT	Local time	Cumulative time ^a	Days	Lat. ^b	Long. ^b	Purpose
2CYC 18	140502		Eddy (1)	13:35	15:35			3325.46	3206.86	Microbial and chem
2CYC 19	140502	200	Eddy (1)	15:10	17:10			3320.25	3217.47	Microbiol, rate
2CYC 20	140502		Eddy (1)	18:25	20:25			3320.31	3230.28	Microbial and chem
2CYC 21	140502		Eddy (1)	20:20	22:20			3309.80	3229.68	Microbial and chem
2CYC22	150502	200	Eddy (1)	12:50	14:50			3320.23	3217.35	Microbiol
2CYC23	150502		Eddy (1)	15:40	17:40			3324.85	3218.49	Dust microcosm
2CYC24	160502	250	Eddy (1)	09:15	11:15			3320.17	3217.60	Pre-addition microbial and chem
2CYC25	160502	150	Eddy (1)	20:45	22:45			3320.12	3217.73	Faecal pellets, mesozoopl.
2CYC26	170502	1600	Deep Eddy (1)	08:35	10:35			3320.10	3217.46	Deep chemical
2CYC27	180502	250	In (2)	09:00	11:00	21 h 0 m	1	3319.63	3220.41	Microbial and chem
2CYC30	180502	250	In (2)	17:18	19:18	29 h 18 m	1	3323.50	3220.73	Microbial and chem
2CYC31	180502	150	In (3)	21:45	23:45	33 h 45 m	1	3323.32	3225.32	Zooplankton cast
2CYC32	190502	250	In (2)	08:50	10:50	44 h 50 m	2	3317.82	3220.51	Microbial and chem
2CYC37	190502	150	In (3)	22:15	00:15		2	3319.06	3228.03	Zooplankton cast
2CYC38	200502	250	In (2)	08:24	10:24	68 h 24 m	3	3312.09	3224.84	Microbial and chem
2CYC39	200502	120	In (2)	10:30	12:30	70 h 30 m	3	3312.51	3223.26	Extra cast chem analysis (Kitidis)
2CYC40	200502	12	In (2)	12:25	14:25	72 h 25 m	3	3313.41	3222.45	Microcosm
2CYC41	200502	45	Out (1)	15:05	17:05			3303.91	3225.23	Microcosm
2CYC42	210502	150	In (3)	00:10	02:10	84 h 10 m	4	3314.20	3227.56	Zooplankton cast
2CYC43	210502	45	In (2)	08:20	10:20	92 h 20 m	4	3309.08	3222.22	Microbial and chem
2CYC44	210502	250	Out (1)	09:45	11:45			3302.45	3217.43	Microbial and chem
2CYC45	210502	120	In (2)	13:15	15:15	97 h 15 m	4	3312.37	3218.41	Micrograzing
2CYC47	220502	120	In (3)	00:15	02:15	108 h 15 m	5	3309.41	3222.56	SF ₆
2CYC48	220502	250	In (2)	08:55	10:55	116 h 55 m	5	3313.45	3215.43	Microbial and chem
2CYC49	220502	250	In (2)	11:00	13:00	119 h 0 m	5	3314.80	3215.22	Micrograzing
2CYC50	220502	1620	Deep outside eddy	15:35	17:35		5	3304.79	3150.33	Microbial and chem
2CYC51	230502	250	Out (1)	00:05	02:05			3316.37	3229.97	Zooplankton cast
2CYC53	230502	250	In (2)	09:00	11:00	141 h 0 m	6	3316.60	3212.97	Microbial and chem
2CYC54	230502	120	In (2)	11:00	13:00	143 h 0 m	6	3316.31	3212.83	Extra cast Partner 4
2CYC55	230502	45	Out (1)	14:20	16:20			3320.28	3204.40	Microbial and chem
2CYC56	240502	150	In (2)	00:15	02:15	156 h 15 m	7	3317.65	3216.88	Zooplankton cast
2CYC57	240502	20	In (3)	07:35	09:35	163 h 35 m	7	3316.74	3214.65	Micrograzing
2CYC58	240502	350	Deep In (2)	09:00	11:00	165 h 0 m	7	3317.37	3215.30	Microbial and chem
2CYC60	250502	150	Out (1)	00:20	02:20			3321.08	3211.12	Zooplankton cast
2CYC61	250502	20	In (3)	07:30	09:30	187 h 30 m	8	3316.89	3218.90	Micrograzing
2CYC62	250502	250	In (2)	08:45	10:45	188 h 45 m	8	3318.89	3220.48	Microbial and chem
2CYC63	250502	120	Out (1)	10:55	12:55			3319.79	3221.35	
2CYC64	250502	250	Out (1)	14:10	16:10			3330.17	3220.34	Microbial and chem
2CYC66	260502	180	In (2)	09:10	11:10	213 h 10 m	9	3316.73	3229.30	Microbial and chem
2CYC68	260502	2600	Deep outside Eddy	16:50	18:50			3343.44	3212.61	Microbial and chem

CTD measurements were made on all casts taken. Stations marked (1) were stations taken from the center of the Eddy but outside the patch. During the addition experiment these were the control stations. Stations marked (2) were stations within the patch where SF₆ was measured and detected. Stations marked (3) were stations in the patch but where SF₆ was not determined.

of Cyprus. It has been found at different times both to the east or to the west of the Eratosthenes seamount (Brenner et al., 1990). In winter there is deep mixing in the core of the eddy. The depth of this mixing depends on the severity of the winter and the history of the particular Cyprus Eddy. The eddy that was sampled in 1989 was mixed to a depth of almost 500 m (Krom et al., 1993); the eddy

sampled during the exceptionally cold winter of 1991/2 was mixed to ~550 m (Zohary et al., 1998). The Cyprus Eddy sampled in May 2001 was only isothermal to 300 m and that in May 2002 to 280 m. In May 2002 the isothermal isohaline layer was between 100 and 280 m (Fig. 2). Above this layer the salinity decreased to a minimum at 40 m. This layer is generally interpreted as a remnant of the Atlantic

Table 2
Chemical and biological measurements made on samples collected during the CYCLOPS cruise (May 2002)

Determination	Where samples were analysed principally	Principal reference in the volume in which these data are used
SF ₆ /phosphate Lagrangian experiment	On-board	Law et al., 2005
Dissolved nutrients (nitrate, nitrite, phosphate, ammonia, silicate)	On-board and in lab	This manuscript
Dissolved oxygen	On-board	This manuscript
UV labile dissolved organic phosphorus	In lab	Krom et al., 2005
Dissolved organic C and N	In lab	Krom et al., 2005
Chlorophyll- <i>a</i> (fluorescence)	On-board and in lab	Psarra et al., 2005; Zohary et al., 2005
Chlorophyll- <i>a</i> (HPLC)	In lab	Psarra et al., 2005; Zohary et al., 2005
Primary production (¹⁴ C)	In lab	Psarra et al., 2005
Phytoplankton and microzooplankton species counts	In lab	Pitta et al., 2005; Psarra et al., 2005
Mesozooplankton counts	In lab	Pasternak et al., 2005
Bacterial activity (leucine)	On-board	Herut et al., 2005; Kress et al., 2005; Pitta et al., 2005; Zohary et al., 2005
Picoplankton counts (flow cytometer)	In lab	Herut et al., 2005; Pitta et al., 2005; Psarra et al., 2005; Zohary et al., 2005
Bacterial abundance (epifluorescent microscopy)	In lab	Herut et al., 2005; Kress et al., 2005; Pitta et al., 2005; Psarra et al., 2005; Zohary et al., 2005
Microbial P cycling (³³ P)	On-board	Flaten et al., 2005; Herut et al., 2005; Kress et al., 2005; Zohary et al., 2005
Dust flux and composition	In lab	Carbo et al., 2005; Herut et al., 2005
<i>Other measurements</i>		
FRRF	On-board	Mantoura, unpublished data
N fixation (¹⁵ N)	In lab	Rees et al., in preparation
Photochemical production of inorganic phosphate and nitrogen	On-board	Kitidis et al., in preparation

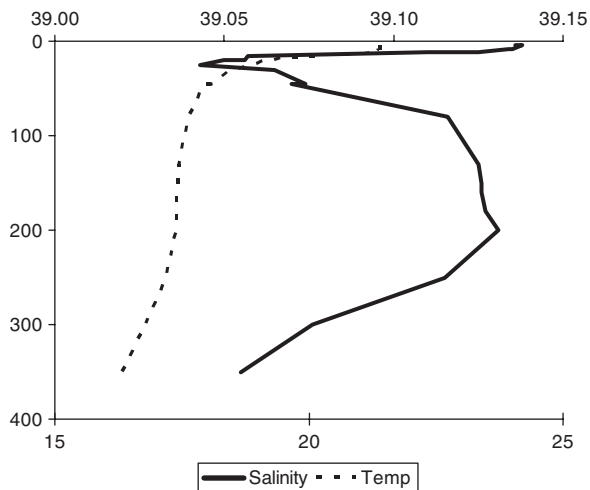


Fig. 2. Temperature and salinity vs. depth profile from Station 58 in the core of the Cyprus Eddy. Modified from (Krom et al., 2005).

surface waters. The phosphoric acid mixture was added at a depth of ~9–11 m into the surface mixed layer. The surface mixed layer varied somewhat

during this experiment increasing from 12 m immediately after the P addition on May 17 to 15 m after 36 h. SF₆ acted as a proxy for the added phosphate and biological response was examined primarily within the depth range of the tracer. There was a slight increase in depth of the SML to a maximum on May 23 (Day 6) of 20 m and then a decrease to 14 m at the end of the experiment on May 26 (Day 9; see Fig. 3; Law et al., 2005). The distribution of SF₆ and thus of the added phosphate in the patch was mainly in the upper 15 m though some SF₆ was observed as deeply as 30 m on Days 5–6. It is for this reason that biological and chemical measurements, designed to show the changes induced by the patch, were concentrated in the upper 30 m of the water column.

The May 2002 experiment was carried out very close to the center of the eddy (Zodiatis et al., 2005). During the nine days of the experiment the patch was tracked by a drogued buoy. This buoy rotated one complete revolution every 24 h (see Fig. 1 and Law et al., 2005). The center precessed about a secondary center with a periodicity of 10 days.

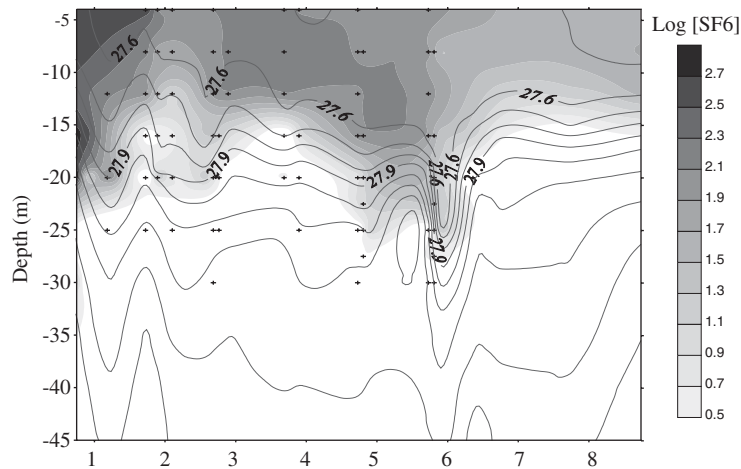


Fig. 3. Contour depth plots of $\log [\text{SF}_6]$ (color bar) with sample depths indicated (crosses) and overlain by σ_t contour lines (see Law et al., 2005).

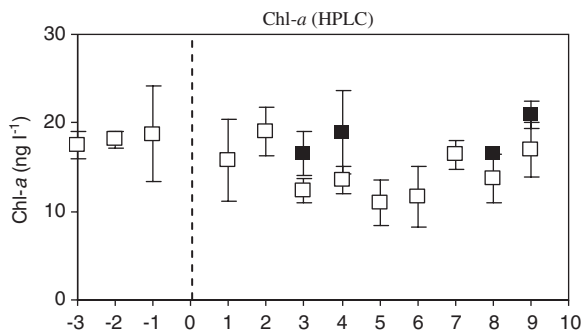


Fig. 4. Plot of chlorophyll *a* determined by HPLC vs time. OUT stations are marked with solid markers and IN stations with an empty marker. Data points represent means of all measurements between 0 and 20 m (usually $N = 5$) in each cast \pm SD. The dashed line indicates the day of the P-addition (17/5/02). For a more complete data set including chlorophyll *a* determined by other complimentary methods see Fig. 4 in Psarra et al. (2005).

7.2. Chemical and biological characteristics of the eddy before the experiment

Immediately prior to the CYCLOPS addition experiment the ultra-oligotrophic status of the surface waters was confirmed. Both nitrate and phosphate were below the detection limit of conventional nutrient analysis and close to or at the detection limit even for nanomolar technology. Chlorophyll concentrations and daily primary productivity were the low values typical of this system (see Fig. 4 and Psarra et al., 2005). Pigment analysis of size-fractionated material indicated that

picoplankton ($< 2 \mu\text{m}$ fraction) and primarily cyanobacteria dominated the phytoplankton (Psarra et al., 2005).

In this study we carried out the first systematic dissolved and particulate organic matter measurements in the Eastern Mediterranean. The results showed that the surface waters contained $65\text{--}100 \mu\text{M}$ of DOC, $3\text{--}11 \mu\text{M}$ of DON and $\sim 50 \text{ nM}$ of UV-oxidizable DOP (Krom et al., 2005). The DON:DOP ratio was estimated to be $\sim 50:1$, while the measured PON:POP was $27\text{--}32:1$. This together with the measured nitrate:phosphate ratio in deep water of $25\text{--}28:1$ shows that there was no chemical reservoir of nutrients with an N:P ratio close to the Redfield ratio of $16:1$.

Despite the low P-content in biomass (Krom et al., 2005) turnover-time for orthophosphate was low for a marine system (see Fig. 5 and Flaten et al., 2005). Combining biomass and turnover-time we got a conservative estimate of the affinity constant (Flaten et al., 2005), consistent with that expected for diffusion limited orthophosphate uptake (Thingstad and Rassoulzadegan, 1999). For this system with a primary production of $\text{ca. } 35 \mu\text{mol C m}^{-3} \text{ day}^{-1}$ and a bacterial production of $\text{ca. } 5 \mu\text{mol C m}^{-3} \text{ h}^{-1}$, stoichiometric conversion using C:P-ratios of 106 and 50 for phytoplankton and bacteria, respectively (Moutin et al., 2002), gives an estimated total P-demand of the bacteria and phytoplankton community together of $\text{ca. } 0.11 \mu\text{mol-P m}^{-3} \text{ h}^{-1}$. This is approximately one order of magnitude below the measured $1.6 \pm 0.2 \mu\text{mol-P m}^{-3} \text{ h}^{-1}$ maximum potential for

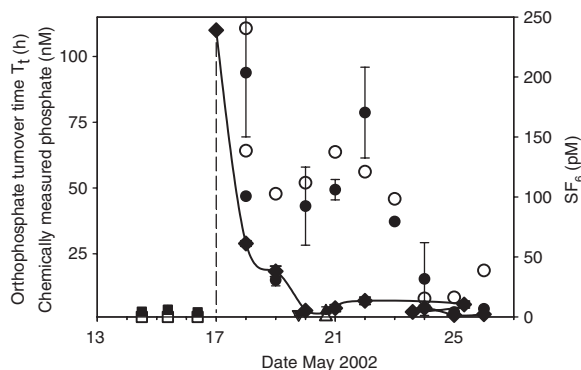


Fig. 5. The change in SF₆-concentrations (○), measured soluble reactive phosphorous (SRP) (◆), and orthophosphate turnover-time before (■) inside (●), outside (△), and below (□) the patch. Vertical dashed line indicates the time of experimental P-release. Error bars indicate SE of 4 samples taken inside the 0–16 m layer (see Flaten et al., 2005 for more details).

phosphate uptake measured in the unperturbed system (Fig. 5), indicating a huge reserve potential for P-uptake. Taken together, these observations showed the P-starved nature of the surface ecosystem at the time of the addition experiment.

7.3. Dilution kinetics of the patch

Once the fertilizer was added to the surface water it expanded rapidly. From dilution of the SF₆-tracer, Law et al. (2005) calculated a dilution rate that indicated the added P would dilute to background levels 7–8 days after the release, as confirmed by nanonutrient measurements. This scenario was confirmed by the measured orthophosphate turnover time, which increased more than 20-fold upon addition, returning to values < 10 h one week after addition (Flaten et al., 2005).

7.4. Biological responses to the P-addition

Probably the most surprising single result of this study was that after the phosphorus addition of the limiting nutrient there was a decrease in chlorophyll *a* in the patch compared to control stations outside the patch and to stations both before and after the addition. Details of the nature and amount of the decrease are given in Psarra et al. (2005). This decrease was in contrast to our expectations before the experiment, which was that chlorophyll should increase.

If all of the assayed 230 nM excess-N had been assimilated by *Synechococcus* sp, and assuming a N:Chl *a* = 0.31 nm N ng⁻¹ Chl *a* for P-replete *Synechococcus* sp. (Bertilsson et al., 2003; Jeffrey et al., 1999), this would correspond to a ca. 40-fold increase in chlorophyll in the absence of dilution and grazing (from 18 to 741 μg Chl m⁻³). The system returned to background chlorophyll levels after ca 1 week as the patch was mixed with outside water. With the precision in the data, we cannot give any exact value for the net difference between chlorophyll growth (μ) and loss rate (γ) induced by the known dilution of the patch, but a rough estimate suggest a negative post-addition net growth $\mu - \gamma \approx -0.6 \text{ day}^{-1}$ (Thingstad et al., 2005). Some of this change may have been caused by a reduction in phytoplankton growth rates (μ , day⁻¹), estimated to be ca. 1.0 day⁻¹ prior to the addition, reducing to 0.7 day⁻¹ after 4 days (Psarra et al., 2005), indicating an increase in grazing and other loss terms from ~1.0 day⁻¹ to ~1.3 day⁻¹. Our explanation for this response was that the phytoplankton were N and P co-limited at the time of the addition experiment. This was confirmed by an on-deck microcosm experiment where ammonium was added to water collected in- and out-side the patch. A phytoplankton bloom was initiated in NH₄-enriched patch water, but not in NH₄-enriched water collected outside the patch (Zohary et al., 2005). This showed that despite the clear indications of P-starvation and excess-N, the system was co-limited by N and P for phytoplankton.

Unlike the phytoplankton, there was a significant increase in bacterial production rate while there was no clear trend in bacterial numbers (see Psarra et al., 2005; Pitta et al., 2005 for details). It is likely that the bacteria were able to access part of the large DON pool in the system and thus were simple P-limited. It is suggested that the bioassayed excess-N was the pool of N available only to heterotrophic bacteria (Thingstad and Mantoura, 2005).

Despite the decrease in phytoplankton chlorophyll after the addition, the particulate-P (Fig. 6) increased already at the first sampling after addition, while at the same time there was an order-of-magnitude drop in the maximum potential for orthophosphate uptake (see Flaten et al., 2005). This is the pattern that would be expected for phytoplankton and heterotrophic bacteria rapidly increasing their internal cell quota for P and reducing their maximum uptake capacity to the level required for growth.

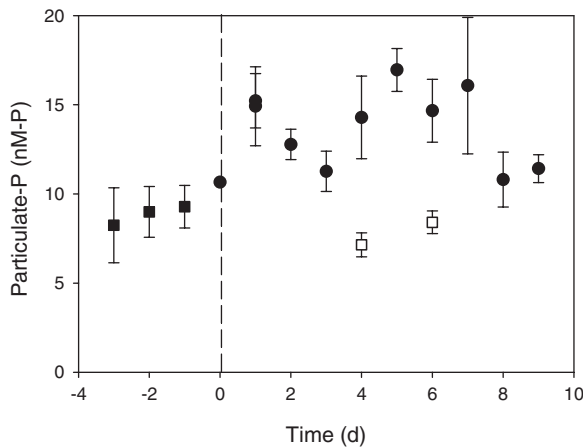


Fig. 6. Particulate-P averaged over depths ≤ 16 m for samples taken before (filled squares), inside (filled circles) or outside (open squares) the experimental patch. Error bars indicate the standard error of determinations at 4 depths (for more details see Flaten et al., 2005).

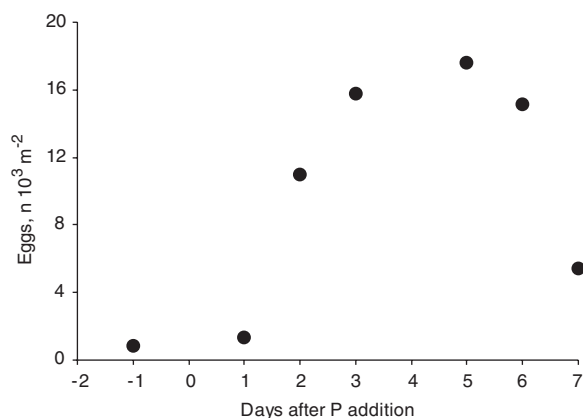


Fig. 7. Abundance of copepods eggs (ind. m^{-2}) in the upper 150 m layer in the center of the experimental P-enriched patch before (Day-1) and after P-release. See Fig. 4 in Pasternak et al. (2005).

There was also a rapid and significant increase in micrograzers and micro- and probably also macro-grazing activity in the patch (see Pitta et al., 2005; Pasternak et al., 2005). This included a statistically significant response in ciliate abundance peaking on the first day after addition (Pitta et al., 2005). There was also a clear increase in copepod egg abundance in the water column (Pasternak et al., 2005). For other grazing responses see Pitta et al. (2005) and Pasternak et al. (2005) (Fig. 7).

7.5. Chemical changes during the addition experiment

Prior to the P-addition, phosphate, nitrate and nitrite were at or close to the limit of detection ($< 1\text{--}2 \text{ nM}$) throughout the upper 60 m (Fig. 8). Both nitrate and phosphate increased at the deep chlorophyll maximum (DCM), which represents the top of the seasonal nutricline that forms in the eddy at the end of deep winter mixing in March–April (Krom et al., 1992). Nitrite also increased at the DCM as part of maximum found at all stations at this depth while ammonia-N was constant throughout the upper 150 m at 60–80 nM (Krom et al., 2005).

Immediately after the P-addition (Day 0.9) phosphate increased to 25–30 nM in the upper 20 m and up to 80 nM at 30 m (Law et al., 2005; Fig. 5). There was an increase in nitrate within the patch of 20 nM at the surface, decreasing to background levels (2.2 nM) at 45 m. At the same time ammonia-N increased to 90–130 nM in the patch water (0–30 m). This increase in ammonia-N occurred throughout the upper 150 m to 90–105 nM. Nitrite levels remained unchanged. On day 1.9, the phosphate decreased within the patch to 10–20 nM. Ammonia-N was still elevated with levels of 80–140 nM in the patch and 80–90 nM below the patch to 150 m. Nitrate (0–5 nM) and nitrite levels ($< 1 \text{ nM}$) were at or close to limits of detection to the top of the DCM. By Day 2.8, phosphate was at background levels ($< 2\text{--}3 \text{ nM}$) and remained at low levels for the remainder of the experiment (Law et al., 2005). There was an increase in nitrite at the bottom of the patch (16 and 20 m) on Day 3.8 to 14 and 21 nM (Fig. 8). This peak decreased the next day to 3–5 nM and by Day 7.8 had disappeared entirely (Fig. 9). As the nitrite values decreased, nitrate values increased to 10–30 nM in the patch, decreasing to background values by Day 7.8. During this period, ammonia-N decreased dramatically from values of 80–90 nM on Day 4 to 30–40 nM on Day 5. These low values were found throughout the upper 150 m. The ammonia-N concentration gradually increased back towards initial values of 50–65 nM by Day 7.8.

Fig. 10 shows the simultaneous changes in the OUT stations during this period. There were no significant changes in phosphate, nitrate or nitrite during the sampling period either within the upper 20–30 m (the depth of the patch) or below that

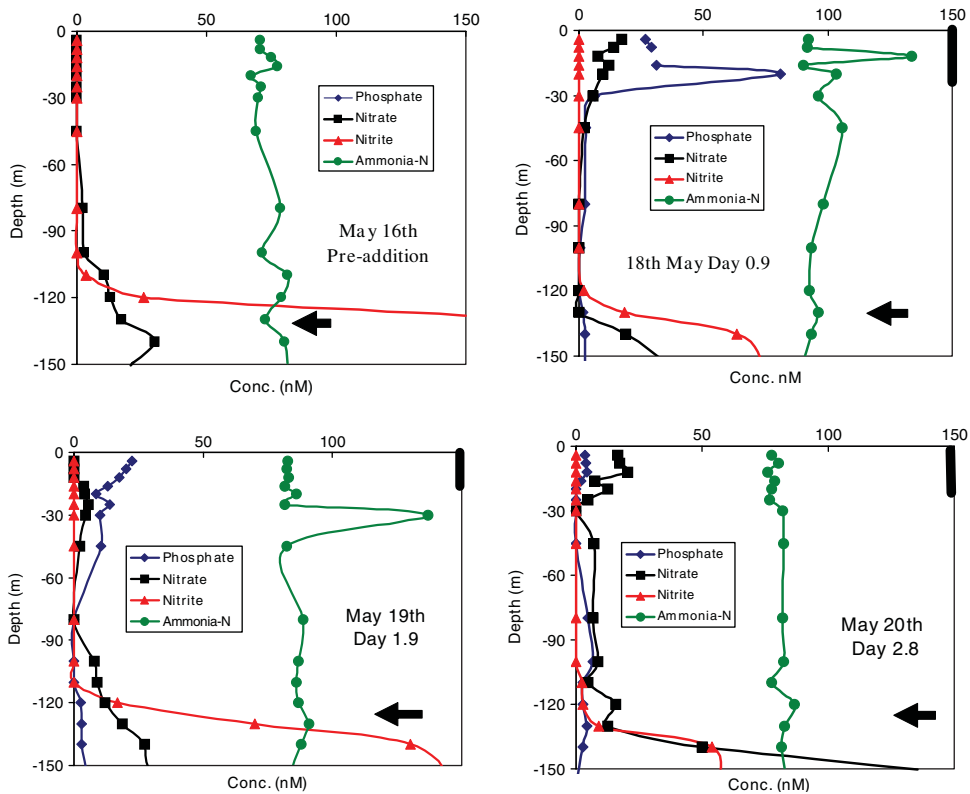


Fig. 8. Changes in dissolved nutrients in the IN Patch stations prior to the addition on May 17 and for the subsequent three days (Day 0.9, 1.9 and 2.8). Data are shown to a depth of 150 m though SF₆ as a measure of the extent of the patch itself was confined to the upper 30 m.

depth to 150 m. Ammonia-N did show some measurable changes with a small increase in ammonia from 60–80 nM on May 16th, the day before the addition, to 80–90 nM on Day 4. There was a decrease to 50 nM on Day 6 followed by an increase to pre-addition levels two days later. These changes showed the same trend as that within the patch but smaller in magnitude.

When the dissolved oxygen values in the patch (0–30 m) were compared with those for the control stations, it was found that the patch water had on average ~2% less dissolved oxygen within it compared to similar depth water out of the patch (Fig. 11).

These results confirm the importance of heterotrophic processes as the major response of the system to P-addition. There were small but measurable increases in ammonia and decreases in dissolved oxygen in the patch water. Indeed the increase in ammonia occurred deeper in the water column, possibly caused by the diurnal migration of zooplankton and other larger grazers.

The formation of a nitrite maximum at the base of the patch suggests a rapid nitrification response to the addition of this extra ammonia-N to the system.

8. Discussion

8.1. Understanding the response of the microbial community to the P-addition

It is suggested that there are three, not mutually exclusive, mechanisms that could explain the observed increase in bacterial activity and positive predator responses, together with a decrease in phytoplankton biomass. One could be termed a “trophic bypass” mechanism. In this process, the added phosphate gets directly to the grazing part of the predatory food chain from the heterotrophic bacteria bypassing the phytoplankton compartment (Fig. 12). The second process is a “trophic tunneling” mechanism. Here phosphate is rapidly taken up by both phytoplankton and bacteria via rapid

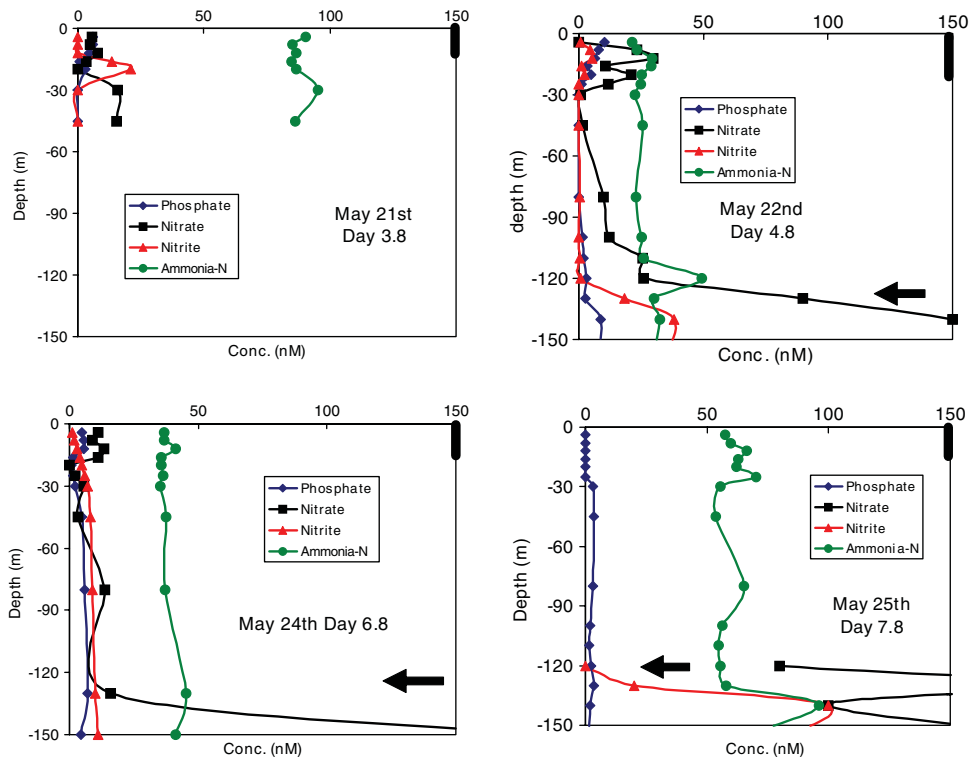


Fig. 9. Changes in dissolved nutrients in the IN Patch stations from Day 3.8 to Day 7.8. Data are shown to a depth of 150 m though the Patch itself was confined to the upper 30 m.

luxury consumption. This causes an immediate change in the phosphorus content but not the abundance of the prey organisms. If there are important metabolic processes of the predators such as cell division or egg production that are P-limited, the added P would then “reappear” as responses at the predator level much more rapidly than expected from conventional models that transport P through nutrient-prey-predator successions with fixed element stoichiometry. The third mechanism is “mixotrophic bypass”. In this mechanism, inorganic nutrients, including the added P, are taken up by mixotrophic ciliates directly, bypassing the phytoplankton. Support for this mechanism, which is described in more detail in (Pitta et al., 2005) is the increase in the biomass of mixotrophic ciliates by 50% after the P-addition.

The increased bacterial activity as a result of the P-addition provides evidence in support of the “bypass” mechanism. In this system, which is dominated by pico-sized phytoplankton species, one would expect predator successions from heterotrophic bacteria to rapidly reach predators that are also able to prey on phytoplankton. This bypass

mechanism is in agreement with previous observations indicating P-limitation of heterotrophic bacteria in the Eastern Mediterranean (Zohary and Robarts, 1998). It also implies that there is a degradable component of the large DOC pool (65–100 μM) measured by us (Krom et al., 2005). The bypass route would be enhanced if heterotrophic bacteria have better access than phytoplankton to the pool of bioavailable excess-N. Indeed we speculate that the excess-N detected in the APA bioassays (Thingstad and Mantoura, 2005) was available in the form of DON e.g. proteins and amino acids, which according to the traditional view is more available to heterotrophic bacteria than to phytoplankton. However, it is difficult to reconcile this “bypass” mechanism as the only trophic transport of P with the observation that the ciliate and copepod responses occur before any apparent increase in bacterial production.

The presence of a flexible biomass stoichiometry was evident from the rapid increase in particulate-P (Fig. 6) believed to be caused by luxury uptake of added phosphate into the phytoplankton and bacteria (see Pitta et al., 2005, for details). The

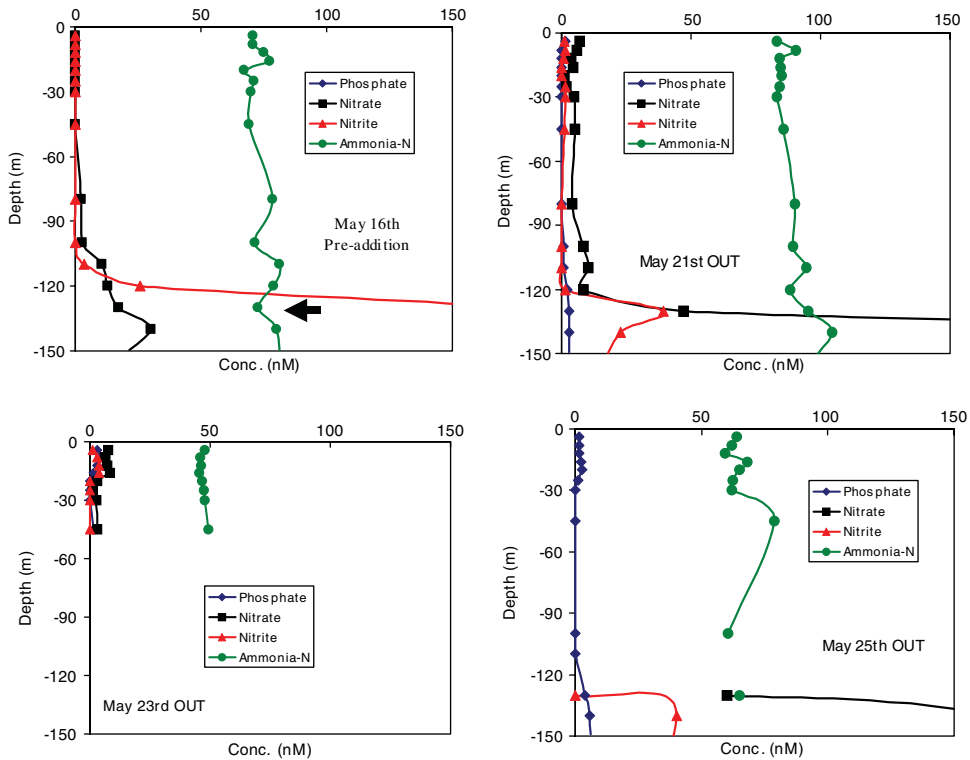


Fig. 10. Changes in dissolved nutrients in the OUT of patch stations. Stations shown are the station sampled prior to the addition (May 16) and three control stations (May 21, 23 and 25). Data are shown to a depth of 150m though the Patch itself was confined to the upper 30 m.

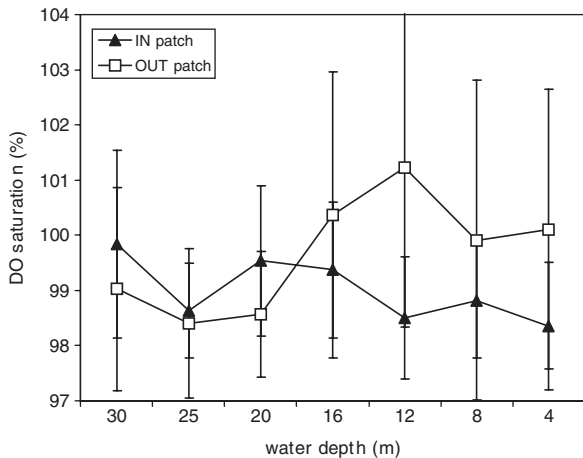


Fig. 11. The difference in dissolved oxygen (% saturation) within the patch water compared with data collected from out of the patch control stations.

amount of P transported per prey into the predatory food chains after the addition presumably must have increased accordingly. It is thus tempting to adapt the limnologist’s hypothesis of P-starved

mesozooplankton (Elser et al., 2001). We therefore suggest that a change in food quality (P-content), rather than in food quantity (abundance), was the main signal initiating cell division in ciliates and egg production in copepods shortly after the addition. Implicit in such a scenario is that zooplankton use the time between nutrient pulses to accumulate energy, carbon and nitrogen, being ready for a rapid response once their prey becomes P-rich. In addition it was suggested that mixotrophic by-pass occurred in which phosphate was transferred directly to higher trophic levels via uptake into mixotrophic ciliates (see Pitta et al., 2005 for more details).

8.2. The nature of nutrient limitation of phytoplankton productivity in the offshore Eastern Mediterranean

There is strong evidence that the system is conventionally P-limited in winter at the time of the annual phytoplankton bloom. However by the time of the addition, both the phosphate and nitrate concentrations in the SML were below the detection limit (~2 nM) of the nanomolar technique used

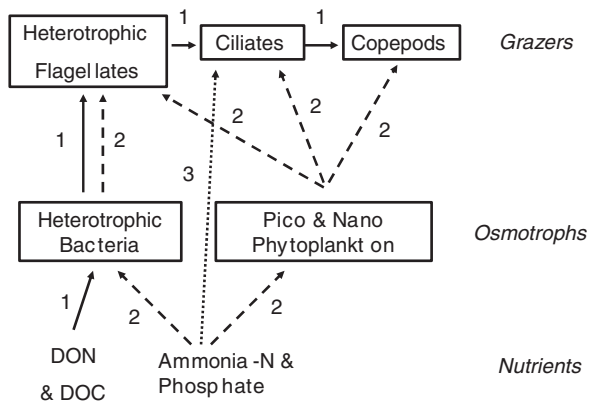


Fig. 12. Idealized model of the P-flow through the lower part of the pelagic food web. We suggest that the added-P can be transported to the level of grazers through “bypass” where heterotrophic bacteria can produce biomass due to access to the DON pool (process 1 solid arrows), or by “tunnelling” where luxury consumption increases the cell quota of P in osmotrophs, thus changing the quality, but not the quantity of prey for P-limited predators (process 2, broken arrows) or by mixotrophic bypass (process 3). Remineralization process omitted for clarity. (See Thingstad, 2005; Pitta et al., 2005 for more details of these processes.)

(Krom et al., 2005). Both the response of the phytoplankton to the Lagrangian P-addition and the results of the on-board microcosm experiment suggest that by May, phytoplankton productivity was N and P co-limited. It has been known previously that if oceanic surface waters have excess P, then they will relax towards co-limitation of the phytoplankton by N-fixation (Tyrell, 1999). In this study we have found that this system which is P-limited with excess N at the end of the annual phytoplankton bloom also relaxes towards N and P co-limitation for the phytoplankton community. It is likely that nitrate has been taken up by the microbial community in spring and then excreted after grazing as both ammonia-N and DON, a proportion of the excreted-N is in a form which is no longer immediately available to the phytoplankton community though the observed P-limitation of the bacteria implies that it is available to heterotrophic bacteria (Thingstad et al., 2005).

Although our interpretation requires a much more elaborate conceptual model of the pelagic food web than needed to explain the phytoplankton bloom as the main response to iron additions in HNLC-areas, no fundamentally new biological mechanisms are proposed. Most aspects of this new conceptual framework can be tested experi-

mentally using much simpler designs than the Lagrangian experiment used here. This is particularly true since we have shown that the microbial responses in the on-board microcosm experiments were very similar to those in the Lagrangian addition (Zohary et al., 2005). In this proposed interpretation, the counterintuitive combination of a decrease in chlorophyll with a positive zooplankton response is a function of the oligotrophic nature of the system, the P-starvation, and that there is a pool of excess-N that is preferentially available to heterotrophic bacteria. The biological response is thus linked to the particular biogeochemical features of the post-bloom Eastern Mediterranean ecosystem.

For many purposes, alternative microbial food chains and flexible stoichiometry may be sophistications that can be disregarded in biogeochemical models. If our interpretation is correct, however, these biological mechanisms are central in determining how nutrient pulses affect Eastern Mediterranean productivity and biogeochemistry. With LNLC being the dominant mode in the world's surface oceans, an increasing awareness of P-limited regions in the world's oceans (Ammerman et al., 2003) and an increasing trend towards high N:P in atmospheric inputs (Jickells, 2002), the results also seem relevant both to large areas of today's oceans and to global change.

8.3. Possible implications for the functioning and long-term sustainability of the Eastern Mediterranean ecosystem

It is perhaps somewhat surprising that despite the large and increasing human population situated around the basin, the offshore ecosystem has remained highly ultra-oligotrophic. The fundamental reason for this is that this region seems to have an efficient buffer system in which added nutrients are exported from the basin via the intermediate water at Sicily. A contributory factor to this buffer mechanism is the rapid and efficient transfer of input nutrients by the zooplankton community out of the surface layers to the DCM and below. The largest input of nutrients to the offshore system is via atmospheric input which supply 30% of the phosphate and 60% of the nitrogen (Krom et al., 2004). Many of these nutrients are supplied in short term pulses in Saharan dust storms or in winter rain events. The rain events take place in winter when there is deep winter mixing and nutrients are

immediately mixed down to depths of 100–200 m. Detailed analysis of the May 2001 dust storm showed that although this dust storm did result in a quantitative increase in chlorophyll in the surface waters, the chlorophyll produced was grazed back to background within 24–48 h (Herut et al., 2005). Once this pulse reaches the zooplankton community, which are known to migrate on a diurnal basis to the DCM, the pulse would be rapidly transferred with it and excreted either as waste products of the zooplankton themselves or when they are preyed upon by higher animals, thus taking the nutrients down to the top of the Levantine Intermediate Water, which is present below ~100 m in this system.

In a study on fish larval recruitment, (Walline, 1987) observed that there seemed to be significantly more fish larvae present in the Eastern Mediterranean (off the Israeli shore) than would be predicted from the observed primary productivity using the relationship between primary productivity and fish catch suggested by (Ryther, 1969). Subsequent estimates have suggested that this ratio for the open oligotrophic ocean should be reduced from a ratio of greater than 100,000 (Ryther, 1969) to 8500 (Table 3; Pauly and Christensen, 1995).

Nonetheless these ratios are substantially greater than recent estimates of the ratio of primary productivity to fish catches in the region (70–400; Caddy et al., 1995; Stergiou et al., 1997). One important contributory factor is the fact that fishery catches in the Eastern Mediterranean combine both coastal and offshore fisheries and thus it is not possible to determine a simple ‘open-ocean’ value. However, it is suggested from the CYCLOPS study that primary production is transferred rapidly up

the food chain, and thus it may not be reasonable to make such calculations, which are necessary to determine the sustainability of fishery in the region, without more detailed understandings of the food web interactions such as those developed in this study.

It is clear from the results of this study that the pelagic microbial ecosystem is fundamentally different from that of the coastal system. This was shown by the results of the 2000 microcosm experiments in Haifa, which involved coastal water (Kress et al., 2005), and those undertaken in 2001 and 2002, which used strictly pelagic water (Zohary et al., 2005). The principle difference was that the offshore system has only ~1 diatom cell/ml (Psarra et al., 2005) in May as opposed to tens/ml in the coastal water (Kress et al., 2005). Disappearance of the niche for diatoms in oligotrophic systems is the predicted behavior of the food web structure suggested by Thingstad and Rassoulzadegan (1999), where diatoms can only co-exist with their supposedly superior nano- and pico-sized phytoplankton competitors when the system’s nutrient content is high. When both ammonia and phosphate were added to this system there was a rapid increase in *Synechococcus* while *Prochlorococcus* disappeared, but only a small change in the eukaryote community (Psarra et al., 2005). There was also no change in the silicate content of the water. By contrast when these nutrients were added to the coastal ecosystem, there was a significant increase in the larger phytoplankton and a measured decrease in silicate (Kress et al., 2005). There is also evidence that this difference might be important from the silicate profile offshore, which shows a clear depletion of silicate in offshore waters,

Table 3
The relationship between primary productivity and fish catches for the Eastern Mediterranean

Primary productivity (g Cm ⁻² y ⁻¹)	Fish catch (including discards) (g fish m ⁻² y ⁻¹)	PP/fish catch ratio	Location	Reference
110 ^a	0.55–1.56	70–200	Levant	Caddy et al., 1995
110 ^a	0.25–0.78	140–400	S Aegean/NW Levant	Stergiou et al., 1997
103	0.012	8500	Open ocean	Pauly and Christensen, 1995
310	2.9	110	Tropical coastal	Pauly and Christensen, 1995
		10 ⁶	Open ocean	Ryther, 1969

^aThe value for primary productivity in the Eastern Mediterranean is derived from measurements at the OUT-of-eddy stations in this study (Psarra et al., 2005). Previous estimates of primary productivity in the Cretan Sea (60–80 g Cm⁻²y⁻¹; Psarra et al., 2000) would result in an even lower ratio.

and yet there is no measured change in silicate in any of the CYCLOPS experiments nor have such changes been observed in seasonal studies of nutrients in the offshore basin (Kress and Herut, 2001). This implies that this profile must be advected from the coastal ecosystem where significant populations of diatoms do exist. It is thus clear that it is important to understand in more detail the functioning of these two different ecosystems (nearshore vs. pelagic) if we are to understand the fate of added nutrients to this potentially fragile marine ecosystem. Thus future studies are needed to understand how the coastal microbial community functions and what the exchange processes are between the coastal system and the offshore system studied in the CYCLOPS program.

There are two radically different hypotheses which have been put forward to explain why the Eastern Mediterranean is P-starved and P-limited. The most widespread hypothesis is that there is extensive N₂-fixation across the basin (Bethoux et al., 2002; Ribera D'alcala et al., 2003; Sachs and Repeta, 1999; Sarmiento et al., 1988), which results in the high N:P ratio observed in inorganic nutrients in the deep water (Kress and Herut, 2001) and by implication in all the other nutrient phases as found in Krom et al. (2005). Pantoja et al. (2002) have calculated on the basis of ¹⁵N content of nitrate that ~90% of the nitrate in the basin is supplied by N₂-fixation. Recently Krom et al. (2004) have suggested that the P-starved nature of the system is due to the N:P ratio of all the external inputs of nutrients to the system being far in excess of 16:1. The unique anti-estuarine circulation of the system results in low export of organic matter to the deep water and sediments, and thus there is insufficient nitrate reduction in the system to reduce the N:P ratio to 16:1 as is observed in other oceans worldwide. It has been suggested that the amount of N₂-fixation that occurs is limited by the iron supply (Berman-Franks et al., 2001). Since the Eastern Mediterranean has one of the highest inputs of dust anywhere in the world (Guerzoni et al., 1999), which results in an unusually high dissolved iron content in surface waters (Statham and Hart, 2005), this is the ideal location to test this hypothesis. If N₂-fixation requires only iron and sunlight, then the Eastern Mediterranean should have one of the highest N₂-fixation rate in the world's oceans. If on the other hand N₂-fixation also requires an excess of phosphorus, as has been suggested by Mills et al. (2004), Tyrell (1999) and others, then the P-starved

nature of the system would suggest that there should be very low N₂-fixation rates. Further research is needed to show whether N₂-fixation is an important process in the offshore Eastern Mediterranean.

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