ELSEVIER

Deep-Sea Research II 52 (2005) 3011–3023

DEEP-SEA RESEARCH
PART II

www.elsevier.com/locate/dsr2

P-limited bacteria but N and P co-limited phytoplankton in the Eastern Mediterranean—a microcosm experiment

Tamar Zohary[a,*], Barak Herut[b], Michael D. Krom[c], R. Fauzi C. Mantoura[d], Paraskevi Pitta[e], Stella Psarra[e], Ferreidoun Rassoulzadegan[f], Noga Stambler[g], Tsuneo Tanaka[f], T. Frede Thingstad[h], E. Malcolm S. Woodward[i]

[a]*Israel Oceanographic Limnological Research (IOLR), The Kinneret Limnological Laboratory, PO Box 447 Migdal 14950, Israel*
[b]*IOLR—Oceanographic Research Institute, Tel Shikmona, Haifa, Israel*
[c]*School of Earth Sciences & Earth and Biosphere Institute, Leeds University, Leeds, UK*
[d]*Marine Ecology Laboratory, International Atomic Energy Authority, Monaco*
[e]*Hellenic Centre for Marine Research, Heraklion, Crete, Greece*
[f]*Station Zoologique, Villefranche-sur-mer, France*
[g]*Bar Ilan University, Israel*
[h]*Department of Microbiology, University of Bergen, Bergen, Norway*
[i]*Plymouth Marine Laboratory, Prospect Place, Plymouth, UK*

Received 19 October 2004; accepted 16 August 2005
Available online 28 October 2005

Abstract

An on-board microcosm experiment was set up to test the hypothesis that the observed lack of phytoplankton biomass increase response to a mesoscale in situ P-enrichment experiment in the P-limited Eastern Mediterranean (Krom et al., 2005a) was a consequence of co-limitation by P and N availability in this ultraoligotrophic environment. Six microcosms were filled with subsurface seawater (ambient DIN: 90–100 nM) taken from inside a P-enriched patch (IN), which in the absence of biological activity would have had ca. 22 nM of PO_4^{-3}. Another six microcosms were filled with unfertilized (<2 nM PO_4^{-3}) subsurface seawater from outside the patch (OUT). The bottles were either supplemented with 1600 nM NH_4^+ or not, incubated on-deck and subsampled daily, or at the first and last (fourth) day of the experiment, for a suite of biological parameters. The addition of N to OUT water did not induce cell abundance increases in either the phototrophic or heterotrophic sides of the food chain, in line with previous assessments that the Eastern Mediterranean is not purely N-limited. The IN and OUT treatments, to which no NH_4^+ was added, mimicked the behavior of the in situ experiment, with an order of magnitude higher bacterial production of IN vs. OUT water, but no noticeable phytoplankton response. The addition of N to IN water, previously exposed to P, led to substantial responses of the entire microbial community, including 4 to 80-fold increases in chlorophyll, other pigments, bacterial activity, and the abundance of ciliates—relative to IN water to which N was not added. The ca. 10-fold increase in chlorophyll within 4 days was mostly due to a major increase in both abundance ($\times 4$) and fluorescence per cell ($\times 17$) of *Synechococcus*, whereas *Prochlorococcus* disappeared. These changes were accompanied by removal from the water of 570 nM of the added NH_4^+, equivalent to 570/22 or N : P ratio of 26 : 1, similar to the ratio measured for POM in the area. Possibly, non-Redfield ratios were maintained, still leaving by day 4 some 1100 nM of N that could not be used due to the lack of P. These results support our hypothesis that the lack of response of phytoplankton to the mesoscale P-enrichment was due to their concurrent N-starvation, i.e. N and

*Corresponding author. Tel.: +972 4 6721444x205; fax: +972 4 6724627.
E-mail address: tamarz@ocean.org.il (T. Zohary).

0967-0645/$ - see front matter
doi:10.1016/j.dsr2.2005.08.011

P co-limitation. In contrast, bacteria could grow when only P was added, implying pure P-limitation. Thus, the heterotrophic and autotrophic components of the same aquatic community experienced different limitations.

Keywords: *Synechococcus*; *Prochlorococcus*; Heterotrophic bacteria; Ciliates; P-limitation; Eastern Mediterranean

1. Introduction

An experimental way to demonstrate nutrient limitation in aquatic systems is to add the suspected limiting nutrient and demonstrate growth beyond that recorded for an unfertilized control. In most cases, either N or P will be limiting to phytoplankton growth (Tilman, 1977). However, in ultra-oligotrophic systems like the Eastern Mediterranean (Yacobi et al., 1995), where the ambient concentrations of both dissolved inorganic N and P are exceptionally low (Krom et al., 2003), co-limitation or near co-limitation is likely, i.e. that the addition of the primary limiting nutrient will immediately lead to limitation by the other, and substantial growth will occur only when both nutrients are added.

The Eastern Mediterranean is unusual in that its deep waters are characterized by high molar N : P ratios of ~28 : 1 (Krom et al., 1991; Kress et al., 2003), indicative of P-limitation. There is direct evidence that the Eastern Mediterranean is P-limited in winter (Zohary and Robarts, 1998), the season when phytoplankton biomass is highest (Krom et al., 2003). At the end of the bloom around March–April (Groom et al., 2005), while all of the phosphate has been consumed, there is measurable nitrate (~0.3 μM) remaining in the water column (Kress and Herut, 2001). However by early summer, when the CYCLOPS experiment took place, there is no longer any measurable nitrate present in the surface waters (Kress and Herut, 2001; Krom et al., 2005b), and the evidence for continued P-limitation is weaker. We have used an opportunity of a Lagrangian in situ P addition experiment conducted as part of the CYCLOPS program (Krom et al., 2005a) also to explore an alternative possibility of concurrent P and N limitations on phytoplankton and bacterial production in summer.

In the in situ P-addition experiment H_3PO_4 combined with SF_6 tracer were injected into the surface water of the Cyprus Eddy in the Eastern Mediterranean over a 16-km^2 area on 17 May 2002, creating a patch of P-enriched water with maximum concentrations of ca. 110 nM over 20 m depth (Law et al., 2005). The temporal dynamics of the chemical environment and the biota inside the P-enriched patch were then followed over the next nine days and compared to the dynamics in a control site outside the patch. Uptake and dispersal processes led to a rapid decline of the measured PO_4 concentration inside the patch (Law et al., 2005). Three days after the PO_4 addition, when the waters used in this microcosm experiment were sampled, the PO_4 concentration calculated from SF_6 concentrations in the patch was 22 nM while the measured concentration in this water was below detection limits (<2 nM), essentially the same as the background concentration outside the patch. The difference of ~20 nM was considered to be the amount of P taken up by the microbial community as a result of the P addition. This P uptake was confirmed by changes in P turnover times and increases in particulate P concentrations (Flaten et al., 2005). While some significant differences in the response of various food-chain components to the addition of P were reported (Pitta et al., 2005; Flaten et al., 2005; Pasternak et al., 2005), the most expected response of increase in phytoplankton biomass did not take place (Psarra et al., 2005).

Using a smaller scale, on-board microcosm experiment, we examined the hypothesis that the addition of NH_4^+, a readily available form of nitrogen, to Eastern Mediterranean surface water that have previously been supplemented with P, will lead to an increase in phytoplankton biomass, suggesting N and P co-limitation, or close to co-limitation, of phytoplankton growth.

2. Methods

2.1. Experimental set up and sampling

Sea water from 8 m depth was collected at 14:00 on 20 May 2002, three days after the in situ P addition, from within the patch, pre-filtered through 200 μm mesh to exclude large predators, and used to fill six replicate 8-l low-density polyethylene Nalgene bottles. Another identical six bottles were filled