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Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus*?

Abstract—Experimental work during a cruise along a W–E transect in the Mediterranean Sea suggests that (1) orthophosphate concentrations in the upper photic zone show a decreasing trend from the west to the east reaching levels well below 1 nM and (2) microorganisms in the 0.6–2-μm size fraction, probably *Synechococcus*, have, in addition to high affinity for orthophosphate, significantly higher maximum uptake rates than heterotrophic bacteria or eukaryotic algae. These specific advantages concerning orthophosphate uptake at low (<5 nM) as well as at relatively high (5–25 nM) concentrations could explain both general *Synechococcus* abundance in P-depleted environments and transient blooms of this species in the open ocean where episodic orthophosphate nanopulse events are likely to occur.

Recent work has shown that dissolved mineral phosphate concentrations are well below the classical colorimetric detection limit of 30 nM in several oligotrophic oceanic provinces (Karl et al. 1997; Wu et al. 2000; Moutin and Raimbault 2002). It has been proposed that primary production (Karl et al. 1997; Wu et al. 2000; Sañudo-Wilhelmy et al. 2001) as well as bacterial production (Thingstad and Rassoulzadegan 1999; Van Wambeke et al. 2002) are controlled by the availability of phosphate. There is thus need for a better understanding of the photic P cycle in marine systems. Most primary production in oligotrophic environments is realized by picoplanktonic (<2 μm) unicellular cyanobacteria. *Synechococcus* is virtually ubiquitous in all marine environments (Partensky et al. 1999) and was found to be the most abundant part of the phytoplankton in surface waters of the Mediterranean Sea during summer (Vaulot et al. 1996). Transient blooms of *Synechococcus* have also been observed in the open ocean (Glover et al. 1988; Morel 1997). The causes of such cyanobacterial predominance remain to be elucidated (Morel 1997).

The Mediterranean Sea presents an interesting gradient of nutrient distribution toward its eastern part (Krom et al. 1991; Moutin and Raimbault 2002) because of the exchange of Atlantic and Mediterranean water at the strait of Gibraltar. This particular feature led us to study phosphate availability in the upper surface water as well as phosphate uptake kinetics from planktonic organisms living under oligotrophic and ultraoligotrophic conditions.

This work was conducted during the PROSOPE (PROductivité des Systèmes Océaniques PElagiques) cruise (Fig. 1) in the Mediterranean Sea (September 1999). Samples were taken 10–15 m deep using 12-liter Niskin bottles to initiate measurements of (1) primary production (PP) and (2) bacterial production (BP) rates (*see* Moutin and Raimbault [2002] for detailed protocols of the ^{14}C method and Van Wambeke et al. [2002] for the ^{3}H-leucine method) and (3) orthophosphate turnover time (days), which corresponds to the ratio between concentration (nM) and uptake (nM d^{-1}).

Turnover time for bioavailable orthophosphate (T_{PO_4}) was measured twice in 10-ml samples incubated with 18.5 kBq (0.5 μCi) carrier-free $^{33}PO_4$ (Amersham BF1003) in polycarbonate vials using an on-deck incubator. Incubations (15–30 min) were stopped by a 100-μl addition of 10 mM nonradioactive KH_2PO_4 (cold chase). Filtrations were performed in less than 1 h on 0.2-μm (25-mm diameter) Poretics polycarbonate filters. Radioactivity on filters (cpm) was measured by scintillation liquid counting and T_{PO_4} was calculated from the equation

$$T_{PO_4} = -t/\ln[1 - (R_f - R_b)/R_t]$$

where R_f, R_b, and R_t are the radioactivity of the filter, the blank (fixed with ca. 100 μl of 2 g L^{-1} $HgCl_2$), and the total tracer added to the sample, respectively. Turnover times measured every 3 h during 24 h at Sta. 7 indicated no significant differences between light and dark measurements.

Isotope dilution curves were determined by adding 2.5, 5, 10, and 25 nM cold orthophosphate to additional subsamples and measuring uptake as above on 0.2-, 0.6-, and 2-μm polycarbonate filters. Linear regression of T_{PO_4} versus added concentration of cold orthophosphate allowed the estimation for each size fraction (0.2–0.6 μm, 0.6–2 μm and >2 μm) of the terms $K_s + [PO_4]$ and V_{max} (Thingstad et al. 1993), where K_s is the half saturation constant for uptake, $[PO_4]$ is the natural concentration of biologically available orthophosphate, and V_{max} is the maximum uptake rate. The mean coefficient of determination was 0.964 (SD = 0.037, n = 21). In addition, T_{PO_4} at natural phosphate concentration was estimated from the y-axis intercept (no orthophosphate addition).

Biologically available orthophosphate $[PO_4]$ was estimated from T_{PO_4} (d). Total PO_4 uptake was derived from carbon PP (nM d^{-1}) and carbon BP (nM d^{-1}) taking a C : P ratio of 106 and 50, respectively.

$$V \text{ (nM d}^{-1}) = \text{PP}/106 + \text{BP}/50$$

$[PO_4]$ (nM) is then determined from the turnover time.

$$[PO_4] = T_{PO_4}[\text{PP}/106 + \text{BP}/50]$$

Chlorophyll *a* (Chl *a*) and particulate phosphate were determined by serial filtration of 1-liter samples through 47-mm Poretics polycarbonate filters (0.2, 0.6, 2 μm) and using Sartorius polyester separators. Chl *a* concentration was determined with a Turner Design 10-AU-005-CE fluorometer with optical configurations optimized to produce maximum sensitivity for Chl *a* (Welschmeyer 1994) and using methanol extraction. Particulate phosphate was determined using a persulfate wet-oxidation method (Pujo-Pay and Raimbault 1994). Polyester separators were cleaned by preliminary wet-oxidation followed by a rinse with milliQ water.

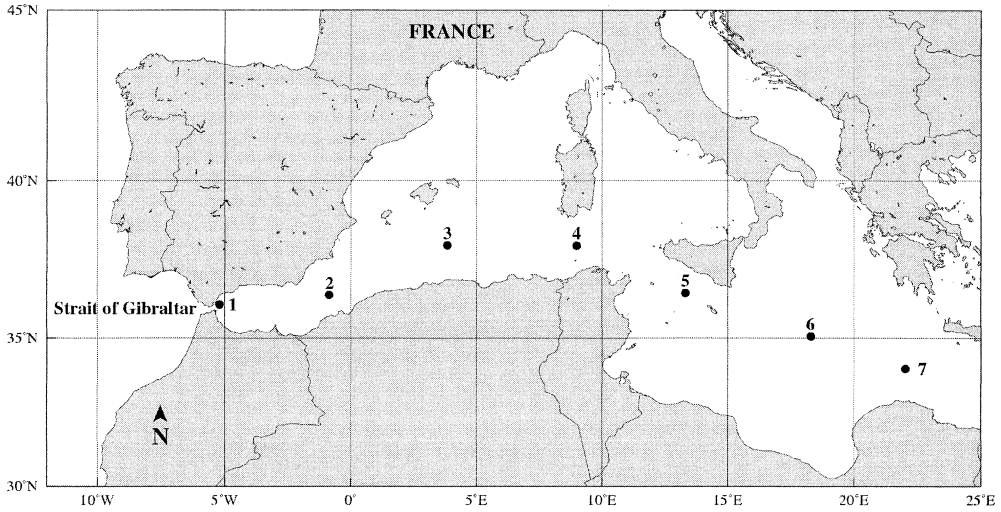

Fig. 1. Station locations during the PROSOPE cruise transect in the Mediterranean Sea (September 1999).

Picoplankton abundance was determined by flow cytometry according to Marie et al. (1997) on each sample and on the <0.6-μm size fraction. Bacterial abundance was determined by epifluorescence microscopy (Van Wambeke et al. 2002) on the same fractions. The latter data were converted to carbon biomass using the following conversion factors (53, 250, 2108, and 20 fg C cell^{-1} for *Prochlorococcus*, *Synechococcus*, picoeukaryote, and heterotrophic bacteria respectively; Campbell et al. 1997). Percentages of picoplanktonic carbon biomass (Table 1) were obtained from each population in the <0.6- and >0.6-μm size fractions by dividing the carbon biomass of each population in one size fraction by the sum of the estimated carbon biomass of all measurable picoplanktonic populations in that size fraction.

Maximum specific uptake rates (V_{max}) were calculated by dividing the maximum uptake rates by particulate phosphate concentration; for the >2-μm size fraction, rates were divided by half of the particulate phosphate concentration to take into account P in zooplankton (Thingstad and Rassoulzadegan 1999). Affinity (α) was calculated from $f/(T_{PO_4}B)$ where f is the fraction of total incorporation going into one size fraction, T_{PO_4} is the turnover time for orthophosphate, and B is the biomass obtained from particulate phosphate in that fraction (Thingstad and Rassoulzadegan 1999). The latter expression does not depend on the estimation of $[PO_4]$. The parameter α is a more useful and reliable parameter than K_s to apply to studies on nutrient competition between species because α is a true estimate of uptake rate (V) at low substrate concentration ($V = \alpha$[substrate]). Because V_{max} corresponds to a constant uptake at high concentrations ($V = V_{max}$), the representation using α and V_{max} describes the two extremes of nutritional conditions for uptake.

Biological available orthophosphate—Surface (10–15 m) orthophosphate concentrations varied from 3 nM near the strait of Gibraltar to 0.2 nM in the Ionian Sea (Table 2). When, in the surface waters (<30 m) of the eastern North Atlantic (40°N, 20°W), the soluble reactive phosphate (SRP) concentrations were measurable with the classical chemical method (Tréguer and LeCorre 1975), bioavailable PO_4 estimated by our method corresponded well with chemically measured SRP (Fig. 2).

The concentrations calculated for bioavailable PO_4 in the Mediterranean Sea (range indicated in Fig. 2) are lower than the detection limit of the chemical method, even when using the magnesium-induced coprecipitation (MAGIC) procedure (Karl and Tien 1992). Our values are consistent with those recently proposed in both lakes (Hudson et al. 2000) and marine waters (Wu et al. 2000). Hudson et al. (2000) measured uptake and regeneration using radio-labeled P and deduced $[PO_4]$ assuming an equilibrium between uptake and regeneration, which is also implicit in our calculation. Wu et al. (2000) increased the volume of seawater processed in the MAGIC procedure. It is also consistent with the 0.8-nM concentration estimated by Thingstad et al. (1996) in Villefranche Bay, NW Mediterranean Sea, based also on turnover time and rate, but obtaining the rate from loss of radioactivity from the particulate fraction following a cold chase.

The method proposed here is easy to use because primary and bacterial production rates are regularly measured during oceanographic cruises.

Assuming that dissolved organic phosphate compounds are hydrolyzed outside the cells (Thingstad et al. 1996), the phosphate required to create new biomass is then taken up in the form of orthophosphate only. Our calculation is based on typical biomass C : P ratios, assuming ratios of 24-h net uptake rates and of biomass composition to be equal, which is true as far as uptake can be coupled with growth. There are data from oceanic studies to substantiate that the chemical composition of oceanic phytoplankton typically is in the Redfield proportions (Goldman 1986). Values from 75 : 1 to 150 : 1 for the molar C : P ratio are consistent with the concept of a uniform chemical composition (Goldman et al. 1979). In heterotrophic bacteria, a lower ratio is generally measured because of their higher content of P. Fagerbakke et al. (1996) found a ''typical'' mean C : P ratio for bacteria of 50 : 1 within a range of 29–65, a value similar to the one found by Goldman et al. (1987) (i.e., 45 : 1). Minimum and

Table 1. Picoplankton and heterotrophic bacteria abundance and carbon biomass estimated using conversion factors (Campbell et al. 1997) for the total, <0.6-μm, and >0.6-μm size fractions. nd, no data; nm, not measurable.

	Heterotrophic bacteria				*Prochlorococcus*				*Synechococcus*				Picoeukaryotes				Picoplankton C biomass (μg C L^{-1})	
	10^5 cell ml^{-1}		% C biomass		10^3 cell ml^{-1}		% C biomass		10^3 cell ml^{-1}		% C biomass		10^2 cell ml^{-1}		% C biomass			
Sta.	Total	<0.6	Total	<0.6	>0.6	Total	<0.6	>0.6	Total	<0.6	>0.6	Total	<0.6	>0.6	Total	<0.6	>0.6	Total	<0.6	>0.6	<0.6	>0.6
1	10.5	0.9	50	91	9	25.0	7.5	3	2	4	61.6	1.9	36	2	70	21.9	4.7	11	5	17	21	21
2	7.5	0.9	44	90	9	30.7	15.4	5	6	4	56.1	1.6	41	3	69	17.9	1.0	11	1	18	15	20
3	7.5	0.7	59	85	14	48.1	26.4	10	9	12	23.5	2.1	23	3	57	9.6	2.4	8	3	16	16	9
4	5.8	0.9	67	90	27	15.1	9.5	5	5	5	15.1	0.9	22	2	55	5.8	1.7	7	3	14	11	6
5	6.5	0.7	72	93	25	26.2	12.7	8	5	12	15.1	0.7	21	1	62	nd	nd	0	0	0	12	6
6	4.4	0.7	68	94	29	nm	nm	0	0	0	10.2	0.8	20	3	46	7.0	1.1	12	3	25	8	5
7	4.5	0.9	71	87	42	nm	nm	0	0	0	10.5	0.4	21	1	56	5.2	4.6	9	12	3	8	5

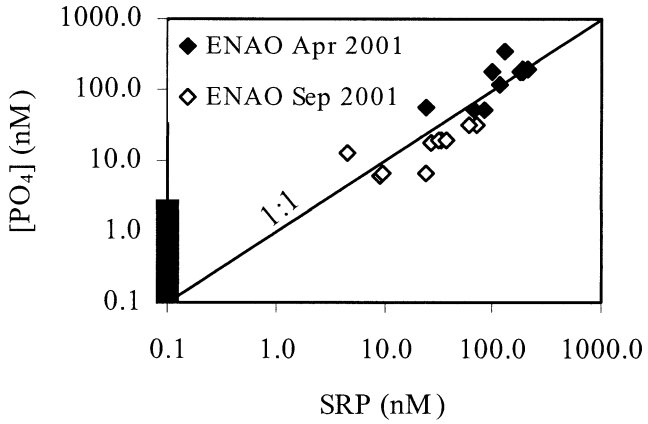

Fig. 2. Orthophosphate concentrations, [PO_4], estimated from turnover time measurements versus soluble reactive phosphate (SRP) concentrations in surface samples (<30 m) of the eastern North Atlantic (40°N, 20°W): [PO_4] = 1.098 SRP with r^2 = 0.68 (n = 21). The vertical bar represents the range of [PO_4] estimated from turnover time measurements (0.1–3 nM) in the upper Mediterranean Sea (PROSOPE cruise, September 1999), which is below the detection limit of SRP using the classical chemical method. y and x-axis scales are logarithmic.

maximum estimates of orthophosphate concentrations were calculated using the following C : P ratios for algae and heterotrophic bacteria: 150 : 1 to 65 : 1 for the upper range and 75 : 1 to 29 : 1 for the lower range. For example, such a dramatic change in C : P ratios would lead to [PO_4] estimates at Sta. 7 in the range 0.19–0.40 nM, which in any case are well below 1 nM.

Increase of oligotrophy from west to east in the upper photic zone of the Mediterranean Sea at the end of the summer season might be related to the low availability of orthophosphate (0.1–3 nM). The microbial P cycle in the photic zone is sustained by fast recycling of very low orthophosphate concentrations.

Biomass, PO_4 uptake kinetics, and affinity constants—Biomass in terms of Chl *a* or particulate phosphate in the upper photic zone (Fig. 3) shows a gradient from the Strait of Gibraltar to the Ionian Sea. This general trend is also observed for heterotrophic bacteria and picoplankton abundance, as well as for bacterial and primary production rates (Table 2). The largest amount of Chl *a* was found in the 0.6–2-μm size fraction (except at Sta. 6, which probably had a filtration problem), and 58–72% of primary production was realized by organisms <2 μm in size. Heterotrophic bacteria represented between 87 and 94% of the picoplanktonic C biomass in the <0.6-μm size fraction, whereas *Synechococcus* represented between 46 and 70% of the picoplanktonic C biomass in the >0.6-μm size fraction (Table 1). *Synechococcus* represented, at maximum, 3% of the C biomass in the <0.6-μm size fraction, whereas heterotrophic bacteria represented up to 42% of the >0.6-μm size fraction. *Prochlorococcus*, when detectable by fluorescence, was approximately equally abundant in the two size fractions and never accounted for more than 12% of the picoplanktonic C biomass in the >0.6-μm size fraction. At present, there are no

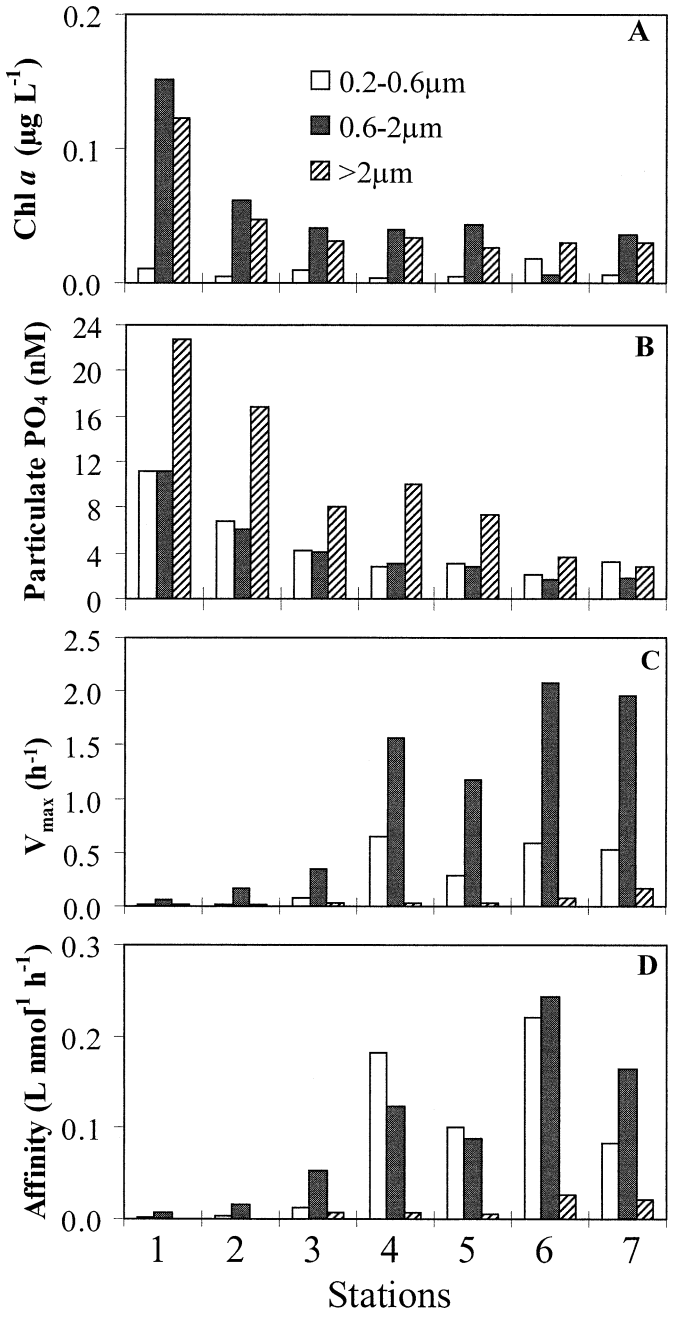

Fig. 3. Biomass and kinetic parameters for PO_4 uptake from different size fractions sampled along a west–east transect in the Mediterranean Sea (5°W–22°E). (A) Chl *a*; (B) particulate phosphate; (C) maximum uptake rate of phosphate (V_{max}); (D) phosphate affinity (α).

available direct estimates of carbon per cell for these microbial groups in the Mediterranean Sea. The estimation of carbon biomass from cell numbers is therefore subject to uncertainty. Even when using 10 fg C cell^{-1} as a lower limit for heterotrophic bacteria and 136 fg C cell^{-1} as an upper limit for *Prochlorococcus* (F. Partensky, pers. comm.), most picoplanktonic C biomass was represented by heterotrophic bacteria in the <0.6-μm size fraction and by *Synechococcus* in the >0.6-μm size fraction.

Values of V_{max} and α constants for PO_4 uptake showed large spatial variation (Fig. 3). There was an increasing trend in affinity from west to east for the three size fractions studied, thus suggesting a corresponding decreasing trend in orthophosphate availability. Although affinity increased in all size fractions, organisms in the 0.2–0.6-μm size fraction, mainly heterotrophic bacteria, showed the largest increase in affinity when orthophosphate availability was low ($[PO_4] <$ 0.5 nM). This pattern suggests an increased competitive advantage in PO_4 uptake at low orthophosphate concentration for heterotrophic bacteria and, therefore, might explain the higher bacterial biomass relative to other picoplanktonic groups in the eastern Mediterranean Sea (Table 1).

The relatively high affinities measured in the 0.6–2-μm size fraction can probably be attributed to *Synechococcus.* With regard to P acquisition, both biochemical and genetic data have demonstrated the presence of a high-affinity PO_4 uptake system comparable to that found in heterotrophic bacteria for marine *Synechococcus* spp. (Scanlan and Wilson 1999). Heterotrophic bacteria and cyanobacteria (mainly *Synechococcus*) are the dominant populations in the upper layer of the Mediterranean Sea, which may be related to their high affinities for phosphate.

Higher V_{max} values were obtained for the 0.6–2-μm size fraction at all sampled stations, both in the western and in the eastern Mediterranean Sea, suggesting that *Synechococcus* has an advantage in PO_4 uptake when orthophosphate concentration increases. Thus, compared to heterotrophic bacteria, *Synechococcus* cyanobacteria seem to be well adapted to exploit episodic orthophosphate pulses. They might obtain part of their required phosphate from pulses produced by wind-induced turbulence events providing phosphate from the deep layer (Andersen and Prieur 2000), from atmospheric deposits (Herut et al. 1999), or from microscale nutrient patches produced as by-products of planktonic heterotrophic metabolism (McCarthy and Goldman 1979). Whatever the source of PO_4, the increasing orthophosphate concentration, which could significantly enhance PO_4 fluxes through cyanobacteria, might not be detectable using classical chemical analysis. In a recent review, Vadstein (2000) indicated that cyanobacteria have a typical maximum specific cell phosphorus-based uptake rate (U_m) that is three times higher than that of typical bacteria, whereas the U_m of green algae is three times lower than the typical value for bacteria. Although the latter experimental studies were carried out with freshwater cyanobacteria and high concentrations of phosphate, which are very different from those encountered in our study, Vadstein's (2000) findings could also explain cyanobacteria superiority in PO_4 uptake when competing for pulsed supply.

The relative abundance of *Synechococcus* in marine environments, as well as transient blooms already observed in the open ocean (Glover et al. 1988, Morel 1997), might be related to a high potential PO_4 uptake capacity relative to other photosynthetic and heterotrophic microorganisms. *Prochlorococcus* are small cyanobacteria that, in theory, on the basis of size alone, could outcompete *Synechococcus* (Thingstad and Rassoulzadegan 1999). However, their potential role in PO_4 uptake competition could not be shown in Mediterranean Sea surface waters where they are only a small part of the C biomass. Further work is required to

Table 2. Salinity; primary and heterotrophic bacterial carbon production rates; turnover time (T_{PO4} mean and SD); and orthophosphate concentration, [PO_4], in the upper layer (10–15 m).

Sta.		1	2	3	4	5	6	7
Salinity	(psu)	36.70	36.79	36.77	37.87	37.94	38.65	38.90
Primary production	(nM d^{-1})	491	443	250	257	137	124	252
Bacterial production	(nM d^{-1})	222	149	130	70	88	60	58
T_{PO4}								
Mean	(h)	9.1	7.8	3.3	1.1	1.8	1.1	1.6
SD	(h)	1.2	0.2	1.0	0.1	0.2	0.2	
[PO_4]	(nM)	3.11	2.33	0.51	0.18	0.24	0.10	0.26

study PO_4 uptake competition between *Prochlorococcus* and *Synechococcus.*

The maximum affinity constants estimated in our study were around 0.25 L (nmol P)$^{-1}$ h^{-1} and can be compared to the theoretical maximum value obtained for diffusion limitation. This can be expressed as $\alpha^{max} = 3D/\sigma r^2$, where D is the diffusion rate for PO_4, σ is the internal P concentration in the cell, and r the cell radius. Following the calculations of Thingstad and Rassoulzadegan (1999), α^{max} for an 0.9-μm cell ($r = 0.45$ μm) and Redfield composition should be ~0.23 L (nmol P)$^{-1}$ h^{-1}. Our estimates are thus consistent with the idea that *Synechococcus* cells in the eastern Mediterranean Sea live in an environment with a concentration of bioavailable phosphate close to what would imply diffusion limitation of the cells.

Other cyanobacteria, such as the P-limited cyanobacterium *Trichodesmium*, which has been shown to fuel up to half of the new production in the subtropical North Pacific Ocean (Karl et al. 1997), could be better PO_4 competitors than eukaryotic algae.

Besides the debate on whether nitrate or phosphate is the proximate or ultimate nutrient limiting marine productivity, both views (N- or P-controlled C cycle) need to be improved by taking into account dominant populations, particularly cyanobacteria, for a better understanding of marine life and the global carbon cycle.

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