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Vertical partitioning of phosphate uptake among picoplankton groups in the P-depleted Mediterranean Sea

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Abstract

Radiolabeled orthophosphate (P_i) incubations coupled with cell sorting were conducted in the Mediterranean Sea to assess the contribution of picoplanktonic groups to total P_i uptake and to potentially explain their spatial distribution. Under natural P_i concentrations (P-deficient and stratified conditions during the survey), total P_i uptake was dominated in the surface by heterotrophic prokaryotes (Hprok; up to 82 %) and shifted to a cyanobacterial dominance around the deep chlorophyll maximum depth. These experiments were completed with concentration bioassays at 4 stations to determine P_i uptake kinetic constants (maximum cell-normalized uptake rates V_{max} and the half-saturation constant plus natural concentration $K + S_n$) in picophytoeukaryotes (Pic), *Synechococcus* (Syn), *Prochlorococcus* (Proc) and Hprok. Syn cells were the most efficient in the uptake of P_i at the cellular level at saturating concentration (100 nmol L^{-1}). Indeed, Syn displayed the highest V_{max} (up to 132 amol P $cell^{-1}$ h^{-1}) compared to other groups like Hprok (V_{max} up to 2 amol P $cell^{-1}$ h^{-1}), and the lowest $K + S_n$. This suggests that they could be quickly reactive to a pulsed supply of P_i. Hprok and Proc cells seemed more adapted to take up P_i at low concentrations, with low $K + S_n$ values (2.9 to 22.7 nmol P L^{-1}), conferring them a nutritional advantage under P-deficient conditions. Such characteristics may explain the successful year-long coexistence of osmotrophic autotrophs and heterotrophs in the Mediterranean Sea and their vertical distribution in the photic zone.

1 Introduction

Understanding nutrient uptake strategies in microorganisms is a necessity to predict their biogeochemical response to environmental changes. Heterotrophic (Hprok) and phototrophic prokaryotes (cyanobacteria) dominate the planktonic biomass in oligotrophic areas of the surface ocean and account for most of the carbon fluxes through the microbial loop (Azam et al., 1983). The Sargasso and the Mediterranean

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Sea seasonally undergo phosphate (P_i) deficiency relative to living organisms demand, with nanomolar concentrations of P_i and P_i turnover times as low as minutes or hours (McLaughlin et al., 2013; Moutin et al., 2002; Sebastián et al., 2012; Thingstad et al., 1998; Wu et al., 2000). Phosphate nutrition is preferentially achieved by harvesting orthophosphate (P_i) in most osmotrophs, but recent studies show that dissolved organic phosphorus (DOP) can be a significant source of P as well, particularly in its most labile forms like ATP (e.g. Björkman et al., 2012; Casey et al., 2009; Duhamel et al., 2012; Glibert et al., 2004; Lomas et al., 2010; Sebastián et al., 2012; Fu et al., 2006; Björkman and Karl, 1994). It is now accepted that P_i uptake capacities of a microbial community are mostly influenced by the degree of P_i limitation in the environment. Along with an increased stratification and oligotrophication of the surface ocean, a widely spread size-shift in the structure of phytoplankton communities is expected, from nano- and micro- eukaryotes to pico-sized cells, as observed in the North Pacific Subtropical Gyre by Church et al. (2002) and Karl et al. (2001). How phytoplankton and bacteria share P resources when they are poorly available has been debated for over 30 years. The existence of different P_i acquisition systems in microorganisms was highlighted in studies where Hprok systems were found to be saturating at much lower P_i concentrations than phytoeukaryotes (> 3 µm: Currie et al., 1986; Currie and Kalff, 1984). Some eukaryotes may develop mixotrophic capabilities and realize a significant fraction of their P from preying on P-richer prokaryotes (Christaki et al., 1999; Hartmann et al., 2011) and from DOP hydrolysis induced by ectoenzymes (ATPases, alkaline phosphatase, e.g. Webb, 1992). Eukaryotic phytoplankton may compensate their low affinity for the substrate at low concentrations (high Km) with high P_i storage capacity (Cotner and Biddanda, 2002) compared to prokaryotes and a more efficient growth mechanism, i.e. a low half-saturation constant for growth (Ks, Rhee, 1973). P_i uptake by microbes in natural environments has largely been assessed using size fractionation. Studies concur on the high contribution of the small size fractions (< 0.8, < 1, < 2 or < 3 µm) to P_i uptake fluxes (Björkman and Karl, 1994; Currie et al., 1986; Moutin et al., 2002; Tanaka et al., 2003; Thingstad et al., 1998,

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1993). This contribution generally increases in aquatic systems with short P_i turnover times and increases in low-P_i systems after P amendments, emphasizing the idea that heterotrophic prokaryotes are high competitors in P-limited areas (e.g. Björkman et al., 2012; Currie et al., 1986; Drakare, 2002; Labry et al., 2002). However, size fractionation offers a limited resolution of microbial processes, especially in oligotrophic environments where osmotrophs are small and where taxonomic and functional types overlap in size. The development of combined radiolabeling techniques and cell sorting by flow cytometry has improved the level of resolution to study P_i uptake strategies in heterotrophic and phototrophic microbes, as shown by at least 6 articles published since 2007 (Casey et al., 2009; Michelou et al., 2011; Talarmin et al., 2011; Zubkov et al., 2007; Björkman et al., 2012; Duhamel et al., 2012).

When looking into the contribution of picoplanktonic groups to total P_i uptake, prokaryotes are better competitors than eukaryotes, and among prokaryotes, taxon-specific uptake rates of P_i are significantly higher for cyanobacteria compared to Hprok. Nevertheless, the comparison among those studies is not always possible, due to methodological differences (fixation, inducing P_i leakage) or to different targeted groups. Finally, only one study suggests the adaptation of microorganisms to P amendments (Björkman et al., 2012), with concentration kinetics experiments providing useful insights into their coexistence in P-deficient environments.

The present study investigates the contribution of sorted picoplankton groups to total P_i uptake flux in the P_i-depleted surface waters of the stratified Mediterranean Sea (down to 200 m). An estimation of kinetic constants (maximum P_i uptake rate-V_{max}, and the sum of the half-saturation constant plus P_i natural concentration $K + S_n$) of $^{33}P_i$-radiolabeled orthophosphate uptake in sorted Hprok, cyanobacterial and picophytopanktonic cells is also provided to compare competitive abilities of the groups at different depths and under different ambient P_i concentrations. In spite of the amount of phosphorus-related studies conducted in the Mediterranean Sea, such experiments have never been carried out in this system. Our results contribute to

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further understand how prokaryotic autotrophs and heterotrophs share P_i resources when the phytoplankton biomass is dominated by prokaryotes.

2 Material and methods

2.1 Study sites and collection

An east–west transect across the Mediterranean Sea was realized during the BOUM cruise (Biogeochemistry from Oligotrophic to Ultraoligotrophic Mediterranean) on the French R/V *L'Atalante* from 16 June to 20 July 2008 (Fig. 1). Samples were collected using 12 L Niskin bottles mounted on a rosette equipped with a conductivity–temperature–density (CTD) profiler and sensors for pressure, oxygen, photosynthetically available radiation (PAR), and chlorophyll fluorescence. P_i uptake and turnover time measurements reported in this study were fully processed onboard. Total chlorophyll *a* (TChl *a*) concentrations were determined as described in Crombet et al. (2011). Critical depths and integrated nutrient concentrations were calculated as described in Moutin and Prieur (2012). P_i turnover times were measured at each of the 33 stations while taxon-specific processes were assessed at 3 long duration stations (St. A, B, C) and at 4 short duration stations (St. 5, 9, 21, and 25) along the euphotic zone.

2.2 Enumeration of phototrophs and heterotrophs using flow cytometry

Triplicated aliquots of 1.8 mL were sampled and fixed with a 2 % (w/v) formaldehyde solution, stored for at least 30 min at room temperature, flash frozen in liquid nitrogen and then stored at −80 °C until further processing onshore within 6 months. Samples were thawed an hour before analysis and 1 µm yellow–green beads (Fluoresbrite, Polysciences) were systematically added as a standard. The sheath fluid was filtered (< 0.2 µm) seawater and analyses were conducted with the software Cell Quest Pro. Microbes were enumerated using a FACScan flow cytometer (BD Biosciences)

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equipped with an air-cool argon laser (488 nm, 15 mW). The red fluorescence signal of the chlorophyll *a* was collected on a long-pass filter (> 650 nm) to identify phytoplankton groups including *Prochlorococcus* and pico-phytoeukaryotes, according to Marie et al. (2000). *Synechococcus* cells were discriminated from other phototrophs using their orange fluorescence signal (585/21 nm).

Samples for the enumeration of heterotrophic prokaryotes were stained with SYBRGreen I (Invitrogen–Molecular Probes) at 0.025 % (vol/vol) final concentration for 15 min at room temperature in the dark. Counts were performed using a FACSCalibur flow cytometer (BD Biosciences) equipped with an air-cooled argon laser (488 nm, 15 mW). Stained bacterial cells, were discriminated and enumerated according to their right-angle light scatter (SSC) and green fluorescence of the nucleic acid dye (530/30 nm). Autotrophic prokaryotes were discarded from this analysis based on their red fluorescence.

2.3 Sample preparation for P_i uptake measurements

Acid-washed and sample-rinsed Nalgene bottles were filled with 10 mL of seawater samples. Preliminary measurements were conducted at the beginning of the cruise to adjust the incubation conditions so that P_i uptake was linear with time. The methodology and calculations employed for bulk and cell-normalized, taxon-specific P_i uptake rates were based on the protocol by Talarmin et al. (2011). Briefly, a radioactive working solution was freshly prepared on board by diluting a carrier-free [^{33}P]-H_3PO_4 mother solution (Perkin Elmer, USA, 569.5×10^{12} Bq mmol^{-1}) in 0.2 µm-filtered milliQ water, and added to the samples. $^{33}P_i$ incorporation was stopped by adding an excess amount (0.1 mM final concentration) of a cold P_i solution. Samples were split in 3 aliquots of 5 mL (for bulk-whole water-P_i uptake measurements), 3.5 mL (for sorting of phototrophic groups) and 1.5 mL (for sorting of Hprok cells) mL and kept in the dark at +4 °C until further processing (< 1 h for the 5 mL subsamples, 10 min to 6 h for the others). Blank samples were added 0.1 mmol L^{-1} (final concentration) of cold P_i 15 min prior to radiolabeling and processed like the other samples. Blank values represented

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on average 5.0 to below 13 % of regular values estimated for bulk (n = 11) and sorted groups (n = 36), respectively. The radioactivity was counted within 5 h after addition of the scintillation cocktail using an embarked Packard LS 1600 liquid scintillation counter.

2.3.1 In situ P_i uptake rates

At St. A, 9, 21 and 25, samples were collected at several depths in order to assess the vertical distribution of the taxon-specific P_i uptake under ambient concentrations of P_i. Samples were spiked with a final concentration of 20 pmol L^{-1} of the ^{33}P-orthophoshate working solution and incubated for 15 min.

2.3.2 Concentration kinetics of P_i uptake

Concentration kinetics experiments were conducted 15 m above the depth of the deep chlorophyll maximum (DCM) at St. A, B and C by adding increasing quantities of a cold KH_2PO_4 solution (0–100 nmol L^{-1} added concentration, Sa). Samples were spiked with the radioactive working solution 15 min later, with an activity of 0.34 MBq per sample, i.e. a final radioactive P_i concentration of 60 pmol L^{-1} (Sa*), and incubated for 45 min. Bulk P_i uptake rates, bulk P_i turnover times, bulk and taxon-specific kinetic constants V_{max} (maximum velocity of P_i uptake) and $K + S_n$ (K being the half-saturation constant and S_n the natural P_i concentration, their sum being determined as the intersection of the plotted line with the x axis), were based on the work by Thingstad et al. (1993). Such estimations were obtained using the linear regression of P_i turnover time vs. added P_i concentrations (Sa, considering Sa* negligible).

2.3.3 Bulk P_i uptake measurements

The 5 mL aliquots were gently filtered on a 0.2 µm polycarbonate membrane superimposing a GF/D filter soaked with a cold P_i solution without rinsing. A 5 s increase of the vacuum pressure ended the filtration to remove non-incorporated P_i.

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Filters were then placed in scintillation vials with 5 mL of Phase Combining System scintillation cocktail (PCS, GE Healthcare).

2.3.4 Flow sorting of labeled picoplankton groups

Other aliquots were processed by flow sorting on an embarked FACSAria cell sorter (BD Biosciences) equipped with two lasers: a 488 nm (13 mW, Coherent, Sapphire Solid State) and a 633 nm (11 mW, JDS Uniphase air-cooled HeNe), using the same detection strategy as for enumeration of phototrophic and heterotrophic cells. The sheath fluid was 0.2 µm filtered seawater. The instrument was controlled by a computer equipped with the FACSDiva software set on the four-way sorting 0/32/0 purity mode. Re-sorting of sorted samples was conducted randomly to check for the purity of the sorts, which reached 98 %. Sorted cells were collected in 2 mL microcentrifuge tubes where PCS scintillation cocktail was added up to a final volume of 2 mL.

Experiments were replicated on one occasion, and per cell P_i uptake rates varied by less than 4 % in prokaryotic sorts and by 11 % in Pic ($n = 4$).

2.4 Soluble reactive phosphorus (SRP)

SRP was measured at the nanomolar level according to the Rimmelin and Moutin procedure (Rimmelin and Moutin, 2005), derived from the initial MAGIC method proposed by Karl and Tien (1992), using 250 mL triplicates per depth. The detection limit of this technique was around 5 nmol L^{-1}.

2.5 Statistical analyses

Averaged values are reported as mean ± one standard deviation (SD). The Michaelis–Menten model was fitted to some of the data points on a plot of the total P_i concentration ($S = S_a + S_n$) vs. P_i uptake rate, once V_{max} and $K + S_n$ constants had been determined and when a significant correlation was found ($p < 0.05$).

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3 Results

3.1 Environmental and biological conditions

A westward shoaling of the DCM (Table 1) and nutricline depths (120 to 50 m and 200 to 50 m, respectively) was observed along the transect. Total chlorophyll *a* concentrations integrated over 150 m were up to twice as high in the western basin (e.g. St. 25) compared to the Levantine basin (e.g. St. 9; Table 1), emphasizing the strong P_i-deficiency over the euphotic zone at most of the stations sampled.

SRP concentrations ranged 2–379 nmol L^{-1}, varying with depth and location (Table 2). The highest maximum surface values were measured in the Western Basin (St. 21 and 25), possibly due to mesoscale variability. However, short P_i turnover times (0.8 to 10 h) were estimated (Fig. 2), revealing a high turnover of P_i in the surface communities along the whole transect at surface depths.

3.2 Enumeration of microbial groups

Hprok cells were the most abundant group, and varied little over sampled stations and depths, with an average of $3.9 \pm 0.3 \times 10^5$ cells mL^{-1} ($n = 24$), with extreme values of 2.7×10^5 cells mL^{-1} and 10.2×10^5 cells mL^{-1} (Fig. 3). Abundances of Proc cells varied between undetected levels to 1.4×10^5 cells mL^{-1}. Their pigment signature was too weak to be detected in surface waters without staining, therefore many experiments are missing data for Proc cells below 50 m. Syn cells were the most abundant at the coastal station 25 (7.7×10^4 cells mL^{-1} at 40 m) and the least abundant in the deep euphotic zone (130 m St. A, 1.4×10^5 cells mL^{-1}; Fig. 3). Finally, Pic cells were the most abundant in the Western Basin, ranging 230 cells mL^{-1} up to 0.3×10^5 cells mL^{-1}.

3.3 Bulk P_i uptake

P_i uptake rates ranged between 0.03 and 21.6 nmol P L^{-1} h^{-1} and varied by a factor of 10 along the water column at stations with the lower turnover times (9 and 21), while

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rates could be multiplied by 200 from one depth to the other at St. 25 and A (Fig. 4). No significant correlation was found between P_i uptake rates and SRP concentrations.

3.4 Taxon-specific P_i uptake

When all available, summed contributions of the 4 sorted groups to P_i uptake under ambient P_i concentrations reached 25 to 110 % of the bulk signal. At the 4 stations, a higher contribution (up to 82.5 % at St. A; Fig. 4) of Hprok cells was observed, with taxon-specific uptake rates between 0.74 and 27.75 amol P $cell^{-1}$ h^{-1} (Table 1) between surface and 25 or 50 m. In the vicinity of the DCM, cyanobacterial cells were the major contributors to bulk signal (up to 72 % at St. A; Fig. 4), mostly Proc cells in open sea samples (Fig. 3a and c). Syn cells contributed the most to bulk signal (53 %) closer to the coast, where they reached the highest abundance measured during the cruise (7.7×10^5 cells mL^{-1}; Fig. 3). At St. A, taxon-specific P_i uptake rates were the highest for picoeukaryotes, and consistently high for all sorted groups (Table 1). Over all experiments, taxon-specific uptake rates ranged 2.6–402, 3.6–40.0, 8.4–134.8, and $0.05–36.1 \times 10^{-18}$ mol P $cell^{-1}$ h^{-1} for Syn, Proc, Pic, and Hprok, respectively (Table 1).

3.5 Kinetics of the P_i uptake

The bulk community exhibited a wide range of maximum P_i uptake rates (V_{max}, 0.2–4 nmol L^{-1} h^{-1}), about 20 times lower at St. A compared to the other sites (Fig. 5; Table 2). Signals measured at St. B and C in sorted Pic cells during the isotope dilution experiments were not significant (less than twice the blank values). Unfortunately, technical problems prevented Hprok cells from being processed at St. A in a reasonable delay after the cold chase. At St. A, B and C, the highest V_{max} value was obtained for cyanobacterial populations (1.8 nmol L^{-1} h^{-1}; Table 2). Taxon-specific maximum uptake rates (V^c_{max}) were higher for Syn (35 and 132 amol $cell^{-1}$ h^{-1}) compared to the other groups (0.2–21 amol $cell^{-1}$ h^{-1}) at the most P_i-depleted stations (B and C), but V^c_{max} was higher in sorted Pic cells at St. A (Table 2). Estimations of the $K + S_n$ constant were

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3 to 6.5 times higher for Syn cells than for other groups, while Proc and Hprok values at St. C were comparable. In most cases, the ascendant part of the fitted Michaelis–Memten curve did not coincide with our data because P_i uptake rates were already approximating the V_{max} value in the lowest added P_i concentrations (Proc and Hprok at St. B and C; Fig. 5). Out of 9 successful sorts, 5 groups exhibited a subsaturated P_i uptake under natural conditions (V^{exp}/V_{max}): Syn at St. A, B and C (13, 58 and 37 %, respectively), Proc and Pic at St. A (44 and 86 %, respectively).

4 Discussion

4.1 General considerations and methodological caveats

Consistently low P_i turnover times (< 10 h) were estimated in surface samples from the eastern basin, which is congruent with previous studies (Flaten et al., 2005; Moutin et al., 2002). These conditions were favorable to use short-term incubations with trace concentrations of radiolabeled P_i. The embarked equipment allowed to perform all experiments on live samples with a minimum of manipulations, within a time window where signal loss was minimal (Talarmin et al., 2011).

It is important to point out that the detection limit for SRP measurements was close to the ambient concentrations, hence a potential overestimation of the latter and of P_i uptake rates calculated using Sn. This caveat would not affect the main results about the relative capabilities of sorted groups in a given sample to take up P_i, as the same P_i concentration was multiplied by the turnover rates (h^{-1}) of each sorted group or bulk community for a given depth. Our inability to detect *Prochlorococcus* in most surface samples cut back any generalizations regarding this group, in spite of significant signals measured deeper in the euphotic zone. The added contributions of the 3 or 4 sorted groups did not necessarily add up to 100 %. Analytical error associated with per cell rates is about 10 % and error on cell abundances around 5 %. Furthermore, viruses and mixotrophic bacterivorous ciliates or nanoflagellates, potentially by-passing nutrients to

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the highest trophic levels of the microbial loop (Krom et al., 2005; Paffenhöfer et al., 2007), could also contribute to bulk P_i uptake fluxes. Attached bacteria, excluded from our cell sorting experiments might also represent a significant contributor to the bulk P_i uptake, if their specific activity is higher than that of free-living bacteria.

4.2 Taxon-specific P_i uptake rates

In half of the samples for which Syn and Pic were sorted, taxon-specific P_i uptake rates were higher in Syn cells than in Pic cells, and in all samples, Hprok-specific rates were consistently lower compared to cyanobacteria. Considering the importance of converting per cell rates to per volume rates (Casey et al., 2009), our results would still highlight the major role of cyanobacteria in P_i uptake fluxes as outcompetitors of phytoeukaryotes under the lowest ambient SRP concentrations.

4.2.1 Contribution of the sorted groups to total P_i uptake

Down to about 25 m above the DCM, at the 4 stations sampled for depth profiles, the main contributors to P_i uptake fluxes were the heterotrophic prokaryotes. Michelou et al. (2011) found dominance of bulk P_i uptake by Hprok in the Sargasso Sea, in surface and DCM layers. These authors determined that cyanobacteria (Proc + Syn) contributed to less than 10 % of the P_i uptake fluxes. These contributions were a result of (1) low Syn abundances (on average 2×10^4 cells mL^{-1}), although this group exhibited 2 to 5-fold higher taxon-specific uptake rates than Proc and Hprok and intermediate Proc abundances (on average 2.8×10^5 cells mL^{-1}), combined with (2) lower cell specific rates for Proc (0.4 amol P $cell^{-1}$ h^{-1}). In contrast, we found in the Mediterranean Sea a higher contribution of cyanobacteria close to the DCM, mostly due to abundances higher than those observed in the North Atlantic at the time of the cruise (Michelou et al., 2011).

The companion paper of these experiments published earlier by Casey et al. (2009) found no difference in the contribution of Proc and Syn to P_i utilization. *Prochlorococcus*

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contributed to 45 % of the bulk P_i uptake in the North Atlantic (Zubkov et al., 2007). In the North Pacific Subtropical Gyre, Pro and Hprok cells did not reveal different contributions to total P_i uptake fluxes, despite taxon-specific rates 3-fold higher for Proc than for Hprok (Björkman et al., 2012). At the SOLA station of the eastern Mediterranean Sea (Banyuls sur mer, France), Syn contributed the most to the total P_i uptake (35 %) among 3 sorted groups, while Pic and Hprok showed low and non-significantly different contributions (Talarmin et al., 2011). For the first time, our results suggest that the uptake of P_i at an offshore oligotrophic location was not dominated by a single group of microbes in the upper euphotic zone and that *Prochlorococcus* cells significantly contributed to microbial P_i cycling in the Mediterranean Sea. Our findings are comparable to measurements carried out in the North Atlantic where Syn and Pro contributed up to 7 and 45 % to bulk P_i uptake, respectively (Zubkov et al., 2007).

4.2.2 P_i uptake kinetics

Maximum P_i uptake rates of the bulk community were 2 to 40 times higher than any of the V_{max} estimated for sorted groups, while bulk $K + S_n$ values were included in the range measured for sorted groups. This shows that the community is globally well adapted to low P_i concentrations but has the potential to take up P_i much faster. Unsorted groups (larger protists) may not have a significant biomass or contribute highly to bulk P_i uptake fluxes, but they may have the ability to store large amounts of P_i in case of upwelled or deposited inputs.

Among the sorted groups, a continuum of P_i uptake capabilities was observed in the Mediterranean Sea. Per cell data suggested a higher maximum uptake rate for Pic (only 1 sample) than Syn, followed by Proc and Hprok cells, respectively. Also, observed Syn P_i uptake was not saturated (i.e. $V_{exp} < V_{max}$, data not shown), which reinforces the potential of *Synechococcus* as an organism able to respond rapidly and significantly to pulsed P_i events (Moutin et al., 2002). The lack of replication in the experiments did not allow statistical comparison of the kinetic constants. Our experiments indicated comparable uptake ability under low P_i concentrations in Proc and Hprok cells. During

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the cruise, cell volume was not estimated, in spite of its importance in the diffusion limitation model (Vadstein and Olsen, 1989). Per cell P_i uptake rates converted in per volume P_i uptake rates showed opposite trends, with cyanobacteria harvesting P_i more efficiently than larger phytoplankton (Casey et al., 2009). This is consistent with a recent study showing that the theoretical surface-limited growth rate does not apply to cells with a volume below 40 μm^3 (Dao, 2013), due to a poor cellular machinery compared to the absorbing capability (surface).

4.3 Ecological and biogeochemical implications

The vertical partitioning of P_i uptake detected in the present study may show that P_i concentrations in low P_i environments are a major factor explaining the distribution of osmotrophs, due to their different nutrient uptake strategies. The concept of competition for limited bioavailability of P_i may actually be closer to a more common occupation of different ecological niches where one or more limiting resources regulate the biodiversity. In the surface where P_i concentrations are the lowest, only organisms with the lowest $K + S_n$ can utilize P_i efficiently, i.e. Proc and Hprok cells. It is likely that the cyanobacterial contribution to P_i uptake decreased below the DCM because of light limitation (Duhamel et al., 2012), while Hprok in the subsurface layers might have been carbon limited. More details about such limitations could be provided by linking phylogenetic diversity of cyanobacteria to their P_i uptake performances, as shown for different taxa of heterotrophic prokaryotes in mesocosms from the Mediterranean Sea (Sebastián et al., 2012). In most studies coupling flow sorting with radiolabeled techniques, the biomass of sorted groups was not evaluated. Recently, Casey et al. (2013) showed that carbon biomass of various taxa was the only variable linking changes in phytoplankton community structure and interannual trends in carbon export fluxes and elemental stoichiometry.

Additional data on the utilization of dissolved organic phosphorus across the Mediterranean Sea would be necessary to establish P_i requirements for the most abundant microbes. It would also allow to estimate C : P uptake ratios in order to

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compare them to the eastward increasing C : P export ratios (from 40.8 : 1 at St. A to 212 : 1 at St. C, cf. Moutin and Prieur, 2012). N_2 fixation in the Mediterranean sustained up to 35 % of the new production in the Western Basins, while it was negligible in the Eastern Basin (Bonnet et al., 2011). This further supports the fact that P cycling is the key to establishing accurate biogeochemical budgets in the Mediterranean Sea.

5 Conclusions

P_i uptake kinetic constants and P_i uptake fluxes through the microbial communities of the stratified Mediterranean Sea were estimated along a gradient of oligotrophy. Our findings indicated that the spatial distribution of heterotrophic and photo-autotrophic picoplankters in this P_i-deficient environment was partly attributable to their respective capabilities to take up P_i. Heterotrophic prokaryotes dominated P_i uptake fluxes in the surface layers, where the lowest P_i turnover times were detected, which was consistent with their low V^{max} and low half-saturation constant. Their abundance did not decrease drastically with depth, however P_i uptake fluxes in the subsurface, above the DCM layer, were dominated by *Prochlorococcus* offshore and *Synechococcus* closer to the coast. Presumably, carbon limitation of bacterial activity reduced the capabilities of heterotrophic prokaryotes to take up P_i, while cyanobacteria would be able to acquire carbon via photosynthesis. While a few taxon-specific P_i uptake rates from various areas were published in the past 7 years, the vertical partitioning of P_i uptake fluxes among sorted picoplankton groups was shown for the first time in our study. The iteration of such experiments in other areas of the world ocean would contribute to tease apart the roles of nutrient limitation and biodiversity in P_i uptake fluxes.

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Table 1. Environmental context of the experiments: sampled depth (m), depth of the deep chlorophyll maximum (DCM_d), 0–150 m integrated Chl *a* concentrations, range of SRP concentrations measured at those stations between 0 and 150 m, and per cell rates of P_i uptake measured in sorted groups *Synechococcus* (Syn), *Prochlorococcus* (Proc), picoeukaryotes (Pic) and heterotrophic prokaryotes (Hprok) measured under ambient concentrations.

Station	depth (m)	DCMd (m)	Chl-*a* int (mg C m^{-2})	[SRP] (nM)	Cell-specific P_i uptake rate (amol P $cell^{-1}$ h^{-1})			
					Syn	Proc	Pic	Hprok
C	100	108	25.10	12.78	49.81	17.46	NA	1.18
9	5	128	16.20	20.22	58.73	NA	NA	27.75
9	50			17.56	14.01	NA	NA	0.74
9	75			12.44	17.64	NA	NA	7.65
9	105			23.11	41.70	35.53	NA	7.13
9	120			13.56	46.51	39.94	NA	13.53
5	50	114	24.70	12.67	42.96	13.04	NA	0.44
B	100	141	21.20	8.59	24.30	20.30	NA	36.14
21	5	87	22.40	9.60	401.58	NA	NA	3.59
21	50			13.13	9.60	12.68	NA	2.61
21	70			14.39	9.01	2.19	8.43	0.18
21	85			80.00	23.46	5.77	49.38	0.73
A	6	88	24.70	10.61	24.09	NA	8.63	11.47
A	13			5.81	10.68	NA	3.98	5.81
A	25			6.31	5.77	NA	3.29	3.17
A	75			8.33	8.59	11.75	11.66	1.82
A	90			8.33	13.10	9.46	33.67	0.40
A	100			16.41	25.14	1.58	7.94	0.11
A	110			24.24	5.00	1.46	7.27	NA
A	130			20.20	5.66	3.63	3.77	0.07
25	5	51	38.50	17.33	68.66	NA	44.93	8.66
25	25			18.00	48.69	37.22	55.52	16.20
25	40			18.00	27.67	30.22	66.96	1.34
25	50			18.67	48.98	NA	134.80	0.76
25	60			19.11	2.59	NA	40.88	0.05

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Table 2. P_i uptake kinetic characteristics of the bulk community and sorted groups: V_{max} is the theoretical volumetric maximum uptake rate of a group, V^c_{max} is the cell-normalized maximum uptake rate, and $K + S_n$ is the theoretical sum of the half saturation constant for uptake and the natural [P_i]. LD: below the limit of detection (< 3 blank values). NA: data not available.

			V^c_{max} (amol P cell^{-1} h^{-1})				V_{max} (nmol P L^{-1} h^{-1})					$K + S_n$ (nmol P L^{-1})				
St.	z (m)	T_{Pi} (h)	Syn	Proc	Pic	Hprok	Bulk	Syn	Proc	Pic	Hprok	Bulk	Syn	Proc	Pic	Hprok
C	100	3.9	132	21	LD	2	4.0	1.8	1.1	LD	0.6	15.3	22.9	6.7	LD	6.3
B	100	1.3	35	6	LD	0.2	3.9	0.08	0.46	LD	0.09	5.2	18.8	5.9	LD	2.9
A	90	370.9	12	1	99	NA	0.2	0.03	0.02	0.02	NA	62.6	128.4	22.7	27.7	NA

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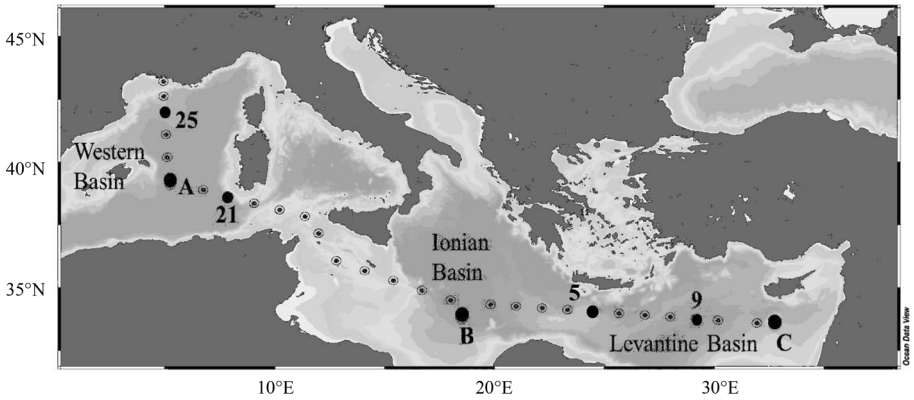

Figure 1. Map of the Mediterranean Sea with locations of sampling sites during the BOUM cruise transect. Numbered and lettered stations denote locations where our experiments were conducted.

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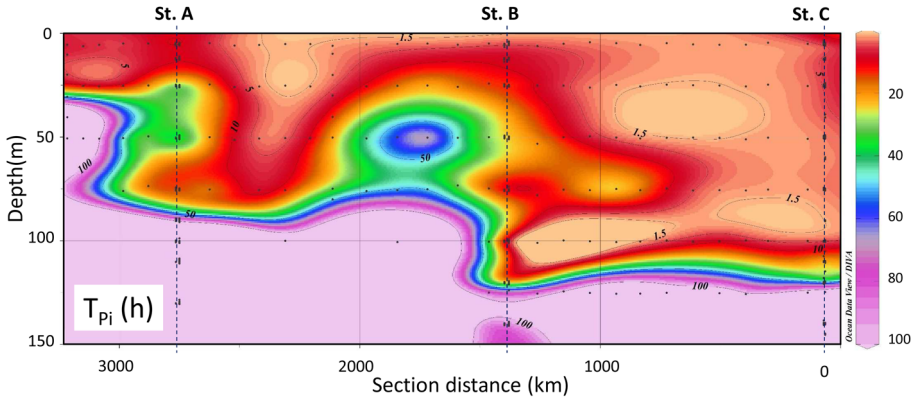

Figure 2. Phosphate turnover times of the bulk community (> 0.2 µm) over the BOUM cruise transect.

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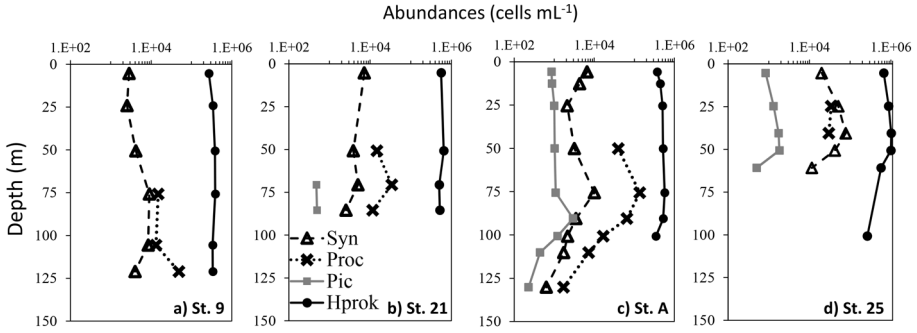

Figure 3. Abundances of *Synechococcus* (Syn), *Prochlorococcus* (Proc), picophytoeukaryotes (Pic), heterotrophic prokaryotes (Hprok) at the depths of sorted experiments at stations 9, 21, A and 25.

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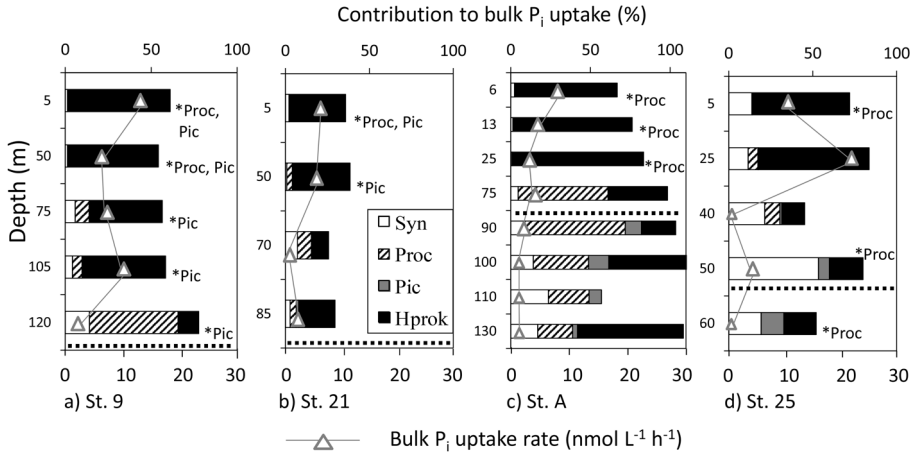

Figure 4. Cumulated contributions of picoplankton groups (superimposed bars for: *Synechococcus Syn*, *Prochlorococcus Proc*, picophytoeukaryotes *Pic*, heterotrophic prokaryotes *Hprok*, superimposed histograms) to the bulk P_i uptake (upper scale, 100 % = measured bulk P_i uptake rate) and bulk P_i uptake rates (lower scale, white triangles) along the euphotic water column at stations 9, 21, A and 25. Dotted horizontal lines mark the deep chlorophyll maximum depth. Beware of the different vertical scales.

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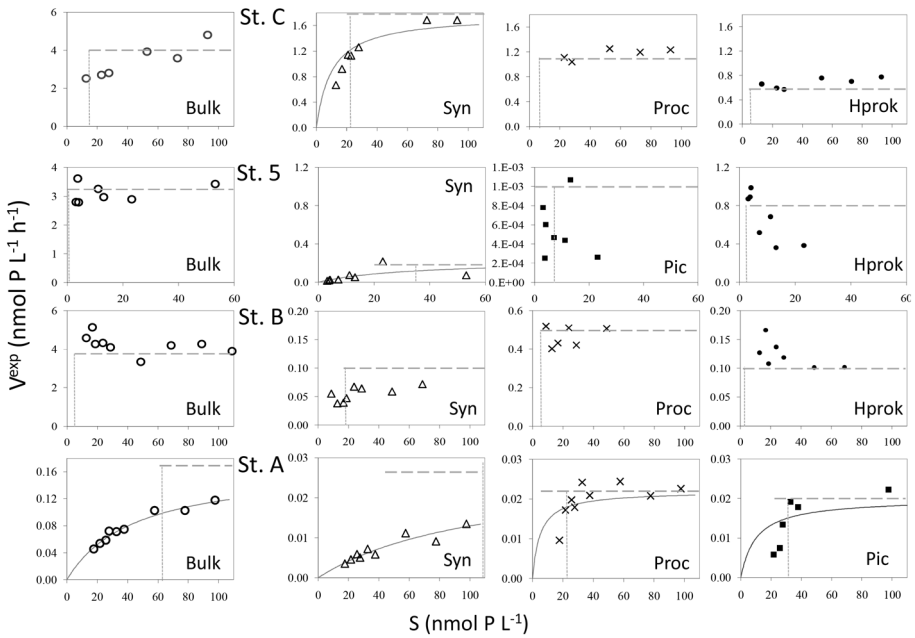

Figure 5. Bulk and taxon-specific P_i uptake rates (V^{exp}) at increasing P_i concentrations (in situ + added) in sorted *Synechococcus* (Syn), *Prochlorococcus* (Proc), picophytoeukaryotes (Pic) and heterotrophic prokaryotes (Hprok) at stations where concentration bioassays were conducted. Straight horizontal and vertical lines represent computed V_{max} and $K + S_n$, respectively. If a significant correlation ($p < 0.05$) between measured P_i uptake rates and those calculated from the Michaelis–Menten model, the uptake curve was fitted to the data.

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