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Alkaline phosphatase activities in the central Atlantic Ocean indicate large areas with phosphorus deficiency

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ABSTRACT: The activity of the enzyme alkaline phosphatase (APA) was studied along 2 latitudinal transects in the central Atlantic (28° S to 28° N) and compared to the distribution of nutrient concentrations, planktonic biomass and other variables reflecting community P status. Using 3-0-methyl fluorescein phosphate (MF-P), APA was measured fluorometrically in 2 size fractions (<0.8 and <150 µm) and in the dissolved fraction (i.e. that fraction passing through 0.2 µm filters). Significant APA ($p < 0.05$) was recorded over extensive areas of the central Atlantic, ranging from 4 to 50 nmol MF-P l^{-1} h^{-1}. Most activity arose from the free dissolved fraction, followed by the <0.8 µm size fraction. The relatively low APA in the central Atlantic is due to the low biomass that characterises most of the area covered by the transects. Nevertheless, the specific activity per unit biomass was high in both the north and south Atlantic subtropical gyres in this area. Along the transects, APA displayed an inverse relationship to the calculated upward turbulent nutrient fluxes. The results support the existence of persistent P-limited conditions in much of the central Atlantic Ocean. This is consistent with stoichiometric data and estimated rate processes and fluxes in the area, indicating that P regeneration from dissolved organic phosphorus may play an important role in the central Atlantic, allowing efficient utilisation of P in the biogenic layer and avoiding P loss through the diffusive vertical flux of dissolved organic matter that preferentially removes C and N.

KEY WORDS: Alkaline phosphatase activity · Atlantic Ocean · Phosphorus deficiency · Enzyme activity

INTRODUCTION

Nutrient availability has been considered the primary factor limiting marine primary production. The earlier belief that nitrogen (Ryther & Dunstan 1971, Codispoti 1989) or phosphorus (Redfield 1958, Broecker 1982) were the elements most often limiting net organic production in the ocean has been gradually replaced by a more comprehensive view that also assigns an important role to silica and to trace elements, and which takes into account changes in the controlling factors at different spatial and temporal scales (Tyrrell 1999, Falkowski 2000, Pahlow & Riebesell 2000, Tréguer & Pondaven 2000, Karl 2002).

Analyses of nitrogen budgets have provided evidence of net nitrogen losses in parts of the Indian Ocean and the Pacific Ocean (Gruber & Sarmiento 1997). Nevertheless, the biological fixation of dinitrogen may protect marine ecosystems from N limitation. The recent finding of active nitrogenase expression in nanoplanktonic cyanobacteria (Zehr et al. 2001) has increased our knowledge on the conditions necessary for balanced nitrogen cycles in the ocean (Karl 2000, Fuhrman & Capone 2001), leading to more likely scenarios of P limitation (Sañudo-Wilhelmy et al. 2001, Mulholland et al. 2002). Anthropogenic N inputs, both riverine and atmospheric, have doubled the nitrogen inputs to the ocean over the last century (Prospero &

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Savoie 1989, Prospero et al. 1996), further increasing the potential for P limitation. These increased inputs, along with changes in the dominance of diazotropic organisms, probably account for reported changes from N- to P-control in the central Pacific (Karl et al. 2001). P deficiency has been reported for the Sargasso Sea (Cotner et al. 1997, Wu et al. 2000) and the Mediterranean Sea (Krom et al. 1991, Thingstad et al. 1998, Zohary & Robarts 1998). However, it is likely to occur in many other oceanic areas also, as meta-analyses of nutrient-limitation assays show that N limitation is most probable in polluted or impacted ecosystems, while pristine unpolluted marine waters are often P-limited (Downing et al. 1999). Hence, P may play a more prominent role as a limiting nutrient than hitherto recognised.

P limitation may also derive from peculiarities of its cycling within the ecosystem. Whereas dissolved organic matter contains large quantities of the nitrogen present in the upper ocean layers (Guildford & Hecky 2000, Bronk 2002), it is depleted in phosphorus, which turns over very rapidly (e.g. Cañellas et al. 2000, Karl & Björkman 2002). Enzymatic release of phosphate from dissolved organic phosphorus (DOP) is the mandatory initial step for DOP utilisation by planktonic organisms (Jansson et al. 1988). Hence, P nutrition in P-depleted waters may be closely dependent on the availability of enzymes to hydrolyse dissolved organic compounds. Alkaline phosphatase (AP) catalyses the hydrolysis of a wide variety of phosphomonoesters (Shan et al. 1994, Karl & Yanagi 1997). It is widespread among most components of the biota in aquatic systems, and has been found in bacteria, phytoplankton and zooplankton. Although constitutive enzymes are common in both bacteria and algae (i.e. algal acid phosphatase seems to be mostly constitutive), AP is to a large extent a repressible enzyme that is mainly produced during periods of pronounced phosphorus deficiency. AP synthesis has been related to the depletion of an internal-cell phosphorus pool that is switched on when inorganic phosphate is in low supply (Jansson et al. 1988). Conversely, high levels of inorganic phosphate often repress phosphatase synthesis (Cembella et al. 1984). In addition, phosphate is a frequent inhibitor that competes with phosphate esters for active sites on the phosphatases (Jansson et al. 1988). Consequently, AP activity (APA) and (especially) particulate APA have been related to the degree of inorganic phosphate deficiency (Healey & Hendzel 1980, Nausch 1993, Li et al. 1998, Zohary & Robarts 1998). Nevertheless, the use of APA as an indicator of phosphorus deficiency is not exempt from criticism. This has been partly related to the difficulty of exactly specifying the origin of free dissolved APA (i.e. arising from the excreta of algae and zooplankton, losses from dying or aged cells, and external inputs). Irrespective of origin, AP facilitates the breakdown of P from organic molecules which constitute an important P pool in the oligotrophic ocean, where phosphate concentrations are low (Vidal et al. 1999, Karl & Björkman 2002).

Despite the suggestion of P deficiency in the Atlantic (Pahlow & Riebesell 2000, Wu et al. 2000, Sañudo-Wilhelmy et al. 2001, Karl 2002), APA has only been reported for the Sargasso Sea (Cotner et al. 1997, Guildford & Hecky 2000), and there is a paucity of experimental data on nutrient limitation over extensive areas of the ocean. Here we provide the first data set on the extent of APA in the central Atlantic. We determined the activity of the enzyme AP along a transect in the central Atlantic (28° S to 28° N), encompassing the oligotrophic subtropical gyres and the relatively more productive equatorial zone (Agustí & Duarte 1999, Planas et al. 1999). Low inorganic nutrient concentrations and low plankton biomass prevail throughout most of the studied area, with the contribution of dissolved organic nitrogen and phosphorus to the total nutrient pool increasing in the most oligotrophic areas (Vidal et al. 1999). In particular, the rates of production of DOP are high in the central Atlantic (Cañellas et al. 2000), whilst the DOP pools are small (Vidal et al. 1999), suggesting fast P recycling. Plankton community structure changes along the transect, with picoplanktonic bacteria and cyanobacteria dominating the most oligotrophic areas (Agustí et al. 2001). We, therefore, hypothesise that APA plays an important role in P availability in the central Atlantic, with organisms in the <0.8 µm size class contributing a large portion of the APA to ultraoligotrophic waters of the subtropical gyres.

MATERIALS AND METHODS

The study was conducted as a part of the 'Latitude I' and 'Latitude II' cruises of the Spanish research vessel 'Hespérides', which examined the latitudinal changes affecting planktonic production in the central Atlantic. The cruises were performed between 17 March and 15 April 1995 (Latitude I) and between 21 October and 16 November 1995 (Latitude II). A total of 14 and 27 stations between 28.09° S and 27.98° N were sampled for alkaline phosphatase determinations on the Latitude I and Latitude II cruises, respectively. Water samples for APA determinations were collected from 5 m depth by 12 l Niskin bottles mounted on a Rosette frame fitted with a CTD (MARK III or MARK V Sea Bird). At a few stations we also measured APA at various depths in the mixed layer. Large particles and mesozooplankton were excluded with a 150 µm Nitex

mesh during transfer of the seawater into 0.25 l glass, sterilised Pyrex flasks. Samples for AP determinations were stored in dim light and temperatures near those of the collection site. Measurements were completed within 3 to 5 h of sample collection. APA was determined in untreated samples and in samples, which had been gently filtered through 0.22 µm filters (Millipore SLGS025). This fraction is referred to as 'free dissolved APA'. During the Latitude II cruise, we also determined the APA of water samples that had passed through 0.8 µm filters (Poretics). Filters were washed with distilled water and with the sample prior to collection of the filtrate, and all the material was autoclaved before use to minimise contamination with microbial phosphatase.

APA was measured fluorometrically using 3-0-methyl fluorescein phosphate (MF-P) (Perry 1972). The substrate was added to a final concentration of 10 µM in the sample. According to Healey & Hendzel (1979), who recorded Michaelis constant (K_m) values of 0.2 to 4 µM in an extensive study on APA kinetics, this concentration saturates activity of the enzyme in a variety of situations, since they found no consistent difference with either season or species composition in their samples. In addition, K_m decreases in conditions of pronounced phosphorus deficiency (Pettersson 1980). Accordingly, the concentration of substrate used was assumed to measure maximum or near maximum activity at most stations. Samples were incubated in a thermostatic bath at 35°C. This temperature initiates the highest APA response by natural plankton (total minus dissolved APA) exposed to temperature variations between 20 and 45°C (with little change of activity between 35 and 45°C; Healey & Hendzel 1979). The response of particulate APA to temperature differs little among natural populations sampled from 8 to 21°C, with APA at 35°C being 1.4 times higher than that at 30°C (Healey & Hendzel 1979). Surface (5 m depth) temperature was from 18 to 29°C during both 'Latitude' cruises. Use of 35°C as a standard temperature for APA measurements ensures the highest rates with the smallest chance of inhibition of the reaction during the short time required for measurements (Healey & Hendzel 1979). Therefore, the temperature and the concentration of the substrate analogue used in this study ensured the highest possible activity of the enzyme. In addition, comparisons are more realistic under uniform conditions of temperature and substrate concentration, yielding the highest and most stable activity than under variable conditions with a resultant variation in activity (Healey & Hendzel 1979). The incubations were carried out in dim light for a period of 1.5 h, with the fluorescence of subsamples being measured at regular intervals of 5 to 15 min. The linear increase in fluorescence, derived from the conversion of the substrate analogue to the highly fluorescent 3-0-methyl fluorescein (MF), was measured with a Hitachi spectrofluorometer (excitation at 365 nm and emission at 460 nm) equipped with a temperature-stabilised sample support. Calibration curves were made with MF standards. As MF-P has relative high background fluorescence, 2 to 3 controls were included in each APA assay. Controls were prepared with sterile seawater and incubated in the same conditions as the samples. The increase in fluorescence of each sample was compared to that of the controls included in the same APA assay to test for the significance of the fluorescence increases over the background levels measured in controls. We use the equation of Sokal & Rohlf (1986) to test the significance of the differences between the slopes of samples and controls. Where significant differences were found ($p < 0.05$), APA was calculated from the difference between the slopes of samples and controls. APA contributed by organisms of the <0.8 µm size class was calculated as the difference between the slopes of the 0.8 and 0.2 µm filtrates, and that of particles in the >0.8 µm size class as the difference between the slope for the whole sample and that of the 0.8 µm filtrate. Standard errors (SEM) were obtained from the relationship $(A \pm a) - (B \pm b) = (A - B) \pm \sqrt{(a^2 + b^2)}$, where A and B are the slopes of the sample and the control, respectively, and a and b are the standard errors of A and B, respectively (Karl & Yanagi 1997). The amount of specific APA was calculated from the total activity divided by chlorophyll *a* (chl *a*), particulate carbon or phosphorus, which were used as proxies of microplankton biomass. The purpose of using total APA normalised to chl *a* was to relate the total activity, independent of origin (algae or bacteria), to the biomass of the autotrophic microplankton. We used total APA instead of particulate APA to compute specific activity since: (1) dissolved APA made a significant and variable contribution to total APA; (2) the conditions which determine the release of the enzyme to the environment are not completely understood; (3) at most stations, dissolved AP could be of local origin, and thus should be included as part of the organism enzyme production; and (4) most literature data are computed from total APA. This approach has the disadvantage that it assigns dissolved AP, which retains its activity for some time (even periods of months; Li et al. 1998), to current biomass, thus integrating the enzyme production for some unknown period of time. APA was expressed as nmol MF-P l^{-1} h^{-1}.

Chlorophyll *a* concentration was determined fluorometrically, as described by Agustí & Duarte (1999), and the concentrations of dissolved inorganic (PO_4^{3-}, NO_3^- + NO_2^-) and organic (DON and DOP) nutrients were measured spectrophotometrically, following the standard methods of Grasshoff et al. (1999) as described in

Vidal et al. (1999). Samples for particulate organic carbon, nitrogen, and phosphorus determinations (POC, PON and POP) were collected on precombusted (450°C, 4 h) Whatman GF/F filters and immediately frozen (–20°C). POC and PON were analysed in a Carlo-Erba CHN analyser after removing the inorganic carbon with HCl fumes and then drying at 60°C. POP was determined after the wet oxidation of filters, following the same procedure as for DOP, with the subsequent analysis of the dissolved phosphate (Grasshoff et al. 1999). Bacterial carbon and bacterial growth rates were computed from standard epifluorescence counts of DAPI-stained particles during cruise Latitude I, and from flow cytometric counts of Syto13-stained samples during the second cruise, when we could separate prochlorophytes from chemotrophic bacteria. Abundance was converted to carbon using an average C content of 15 fg C bacterial cell^{-1} (Lee & Fuhrman 1987). Bacterial production was computed from the uptake of tritiated leucine converted to carbon with empirically derived conversion factors (Agustí et al. 2001). Bacterial growth rates (μ) were computed from bacterial production (BP) and bacterial biomass (BBM) as $\mu = \ln(1 + {}^{BP}/_{BBM})$.

The mixing layer depth (or thermocline depth) was defined as the depth at which maximum stratification occurs (i.e. the maximum Brunt-Väisälä buoyancy frequency, N^2) and was obtained from the vertical gradient in density computed from CTD data. The horizontal velocity was measured using a VM150 narrow-band acoustic Doppler current profiler (RD Instruments). These data and the Brunt-Väisälä buoyancy frequency estimates were combined to obtain the coefficient of vertical diffusivity following the procedure described in Planas et al. (1999) and Vidal et al. (1999). The turbulent flux of nutrients through the thermocline was then calculated, assuming Fickian transport, as the product between that coefficient and the gradient in nutrient concentrations across the thermocline (Lewis et al. 1986).

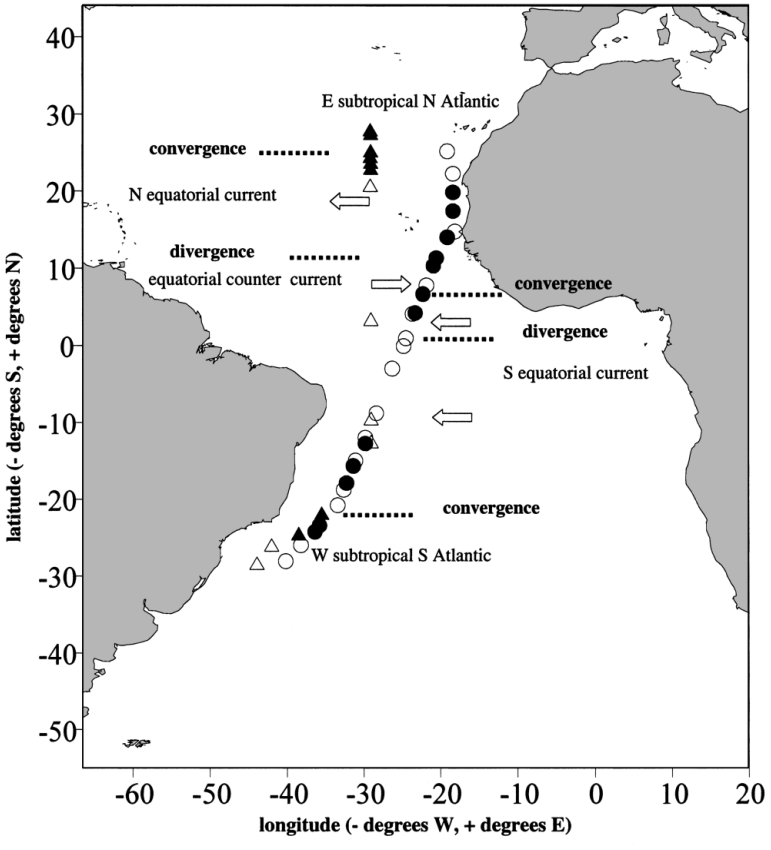

Fig. 1. Positions of stations, showing stations with significant surface (5 m) alkaline phosphatase activity (APA) during the Latitude I (triangles) and Latitude II (circles) cruises across the central Atlantic. Surface currents and the main features of the Ekman transport are also indicated. Open and filled symbols: undetectable and significant APA, respectively.

RESULTS

The 2 cruises spanned the western portion of the south Atlantic subtropical gyre, the equatorial current system, the waters off the NW African coast and the southeastern extreme of the north Atlantic subtropical gyre (Fig. 1). The surface mixing layer was deep at both extremes of the study area (the E subtropical N Atlantic and W subtropical S Atlantic, during Latitude I and Latitude II cruises, respectively), where it exceeded 150 m in thickness (Fig. 2a). Shallower mixing layers of about 40 m depth were found between 10° S and 15° N, corresponding to the equatorial current system, and also off the NW African coast, where the density gradients were sharpest, indicating the occurrence of upwelling. The Ekman transports in the equatorial region determine the upwelling of waters to the south of the equator (the equatorial divergence), which results from the south-east trade winds and the position of the intertropical convergence zone. Another permanent feature in the region is a zone of convergence at about 4° N, where the waters transported with the south-east trades and those linked to the deflection of equatorial counter-current (between 5 and 10° N) meet. A second upwelling area, at the northern extreme of the equatorial counter-current (10° N), has been related to water moving away in response to the north-east trade winds.

Surface (5 m depth) phosphate concentrations were below 0.1 μM in the northern extreme of the studied transect and in the south subtropical gyre

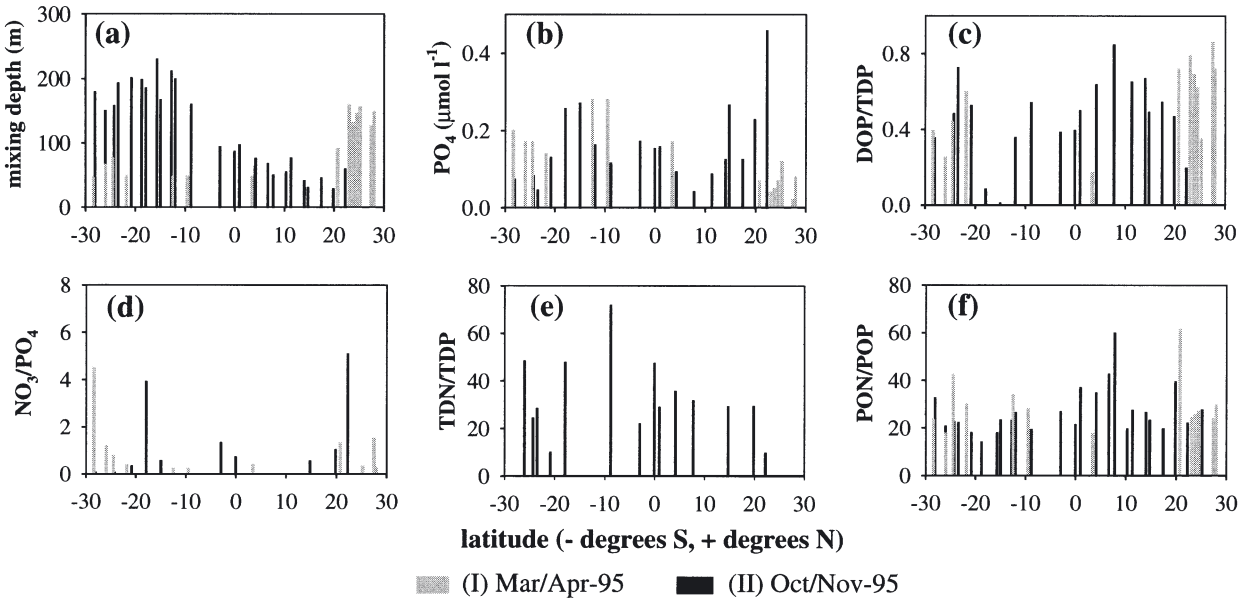

Fig. 2. Latitudinal variation in (a) mixing depth, (b) dissolved inorganic phosphate, (c) ratio of dissolved organic phosphorus to total dissolved phosphorus (DOP:TDP), (d) ratio of dissolved nitrate to phosphate, (e) ratio of total dissolved nitrogen to phosphorus (TDN:TDP), and (f) ratio of nitrogen to phosphorus in the particulate fraction (PON:POP), from surface (5 m depth) water samples collected from the central Atlantic during the Latitude I and Latitude II cruises

(during Latitude II cruise) and increased towards the equator, with the highest concentrations in the upwelling area off NW Africa (Fig. 2b). Dissolved organic phosphorus was measured throughout the study area, and contributed between 20 and 80% to the total dissolved pool (Fig. 2c, and Vidal et al. 1999). Surface nitrate concentrations were below 0.5 µM at most stations, with nitrate to phosphate ratios largely below the conventional Redfield value (Fig. 2d). The DON and DOP fractions increased the total dissolved nitrogen:dissolved phosphorus (TDN:TDP) ratio often above 20:1, similar to the ratio in the particulate fraction (Fig. 2e,f). PON:POP ratios were close to or even higher than 20:1 at nearly all stations (Fig. 2f).

We found significant APA over extensive areas of the central Atlantic (57 and 44% of the 14 and 27 stations sampled during the Latitude I and Latitude II cruises, respectively; Fig. 1). APA was detected at the southeast end of the north Atlantic subtropical gyre, with a local maximum of about 20 nmol MF-P l^{-1} h^{-1} recorded between 24° N, 29° W and 28° N, 29° W, in the region of the subtropical convergence, during the Latitude I cruise (Fig. 3a). APA was below 10 nmol MF-P l^{-1} h^{-1} southwards (between 20° N, 18° W and 10° N, 20° W, off the NW African coast) and towards the equator, along the Latitude II track. APA could not be detected in the region of the south equatorial current between 3° N and 13° S, during either of the 2 cruises (Fig. 1). APA reached a maximum of 48 nmol MF-P l^{-1} h^{-1} at 13° S and then decreased southwards in the western portion of the south Atlantic subtropical gyre (Fig. 3a).

Dissolved APA was found at all the stations (Latitude I cruise) and at 5 of the 12 stations of the Latitude II cruise, where we found significant enzyme activity (Fig. 3b) contributing from 40% to almost the entire activity. In particular, dissolved APA was very high in the cores of both the north and south subtropical convergence, where it accounted for most of the activity. The particulate fraction between 0.2 and 0.8 µm accounted for 40 to 90% of the total activity in 6 of 10 stations (mostly between 10 and 20° N and S), with significant APA on the Latitude II track for which we have fractionated data (Fig. 3c). The particulate fraction >0.8 µm contributed between 20 and 50% of the total activity at 4 stations and for almost all the observed activity at 11° N, in the upwelling region associated with the north equatorial counter-current (Fig. 3d).

The chl *a* (as a proxy of microplankton biomass), particulate carbon and phosphorus contents were low at both extremes of the studied transect, with higher values off the NW African coast (Fig. 4a–c). The relatively low phytoplankton biomass in both the north and south subtropical gyres combined with the APA measurements resulted in high specific APA, above 200 nmol MF-P (µg chl *a*)$^{-1}$ h^{-1} (Fig. 5a). In comparison, the relative high biomass in the upwelling area off the NW African coast resulted in a lower specific APA of about 44 nmol MF-P (µg chl *a*)$^{-1}$ h^{-1} (Fig. 5a). Likewise, the high APA found in the particulate fraction <0.8 µm corresponded to stations with moderate bacterial

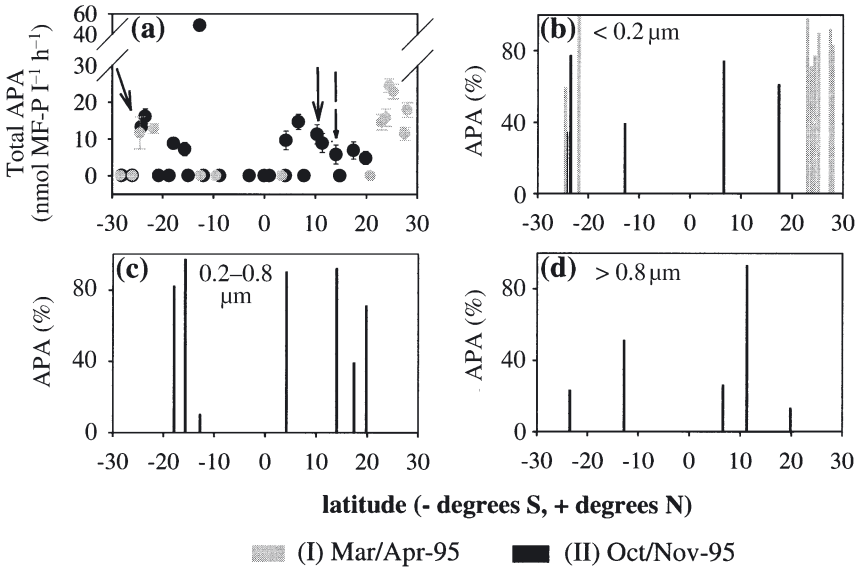

Fig. 3. Latitudinal variation in (a) alkaline phosphatase activity (APA), and in (b–d) contribution (%) of size fractions to total activity, where (b), (c) and (d) show dissolved APA, particulate APA in the 0.2–0.8 µm fraction and particulate APA in the >0.8 µm fraction, respectively. Water samples for APA determinations were collected from 5 m depth in the central Atlantic during the Latitude I and Latitude II cruises. Size-fractionated APA was determined only on the Oct/Nov-95 cruise. Error bars = ± SEM; solid and dashed arrows indicate stations lacking data for size-fractionated and dissolved APA, respectively

carbon and relative low bacterial growth rates (below 0.3 d^{-1}) (Fig. 4d,e). The distribution of specific APA along the transect displayed an inverse relationship to the calculated upward turbulent phosphate flux, which showed very low rates at both extremes of the studied transect and maximum values in the equatorial region (Fig. 5).

Vertical profiles of total APA showed significant activity at different depths in the mixed layer (Fig. 6), even at stations with undetectable surface activity.

DISCUSSION

We observed significant activity of the enzyme alkaline phosphatase in both the north and south Atlantic subtropical gyres, in waters off the NW African coast and in some locations in the tropical north Atlantic (Fig. 1). APA has also been reported for other open-ocean sites such as the subtropical central north Pacific (Perry 1972), the Sargasso Sea (Cotner et al. 1997, Guildford & Hecky 2000), the Indian Ocean (Hoppe & Ullrich 1999) and the Mediterranean Sea (Thingstad et al. 1998), suggesting that utilisation of P from DOP through APA is probably a characteristic feature of the oligotrophic ocean. The APA found in our study (between 4 and 50 nmol MF-P l^{-1} h^{-1}) is comparable to that reported by Li et al. (1998) and Thingstad et al. (1998) for some stations in the Gulf of Aqaba (Red Sea) and the NW Mediterranean, respectively. Nevertheless, our values are somewhat higher than those found in the central north Pacific, Indian, Sargasso and eastern Mediterranean seas (Perry 1972, Cotner et al. 1997, Zohary & Robarts 1998, Hoppe & Ullrich 1999). With the exception of the study of Li et al. (1998), in which the samples were incubated at 37°C, the remainder of the studies listed in Appendix 1 (available at www.int-res.com/journals/suppl/vidal_appendix.pdf) derive from APA

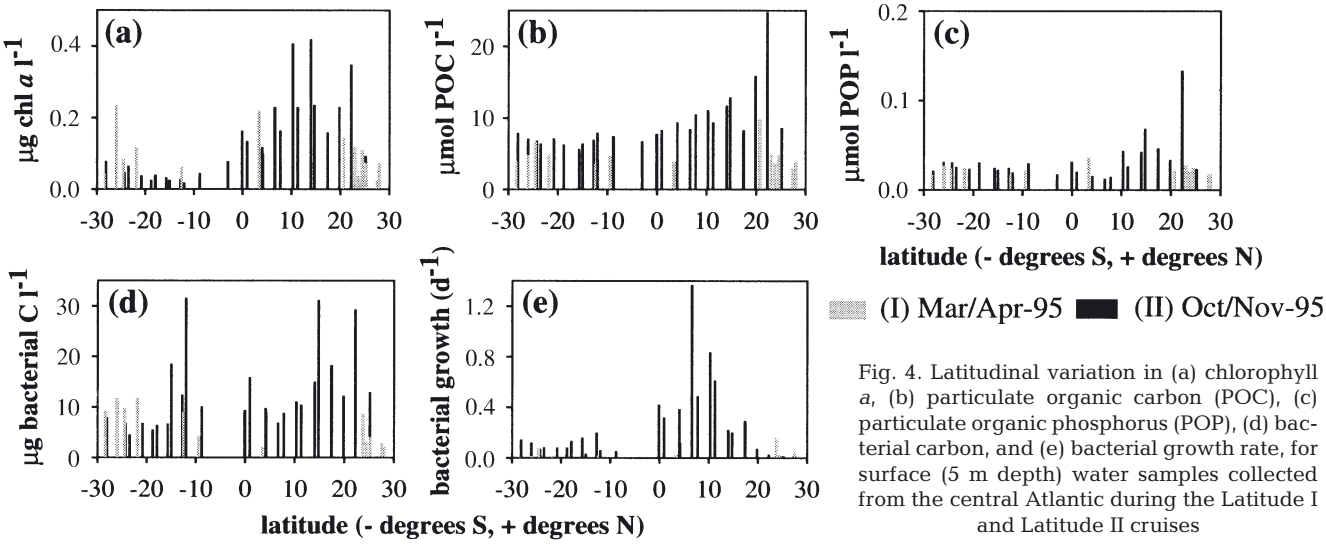

Fig. 4. Latitudinal variation in (a) chlorophyll *a*, (b) particulate organic carbon (POC), (c) particulate organic phosphorus (POP), (d) bacterial carbon, and (e) bacterial growth rate, for surface (5 m depth) water samples collected from the central Atlantic during the Latitude I and Latitude II cruises

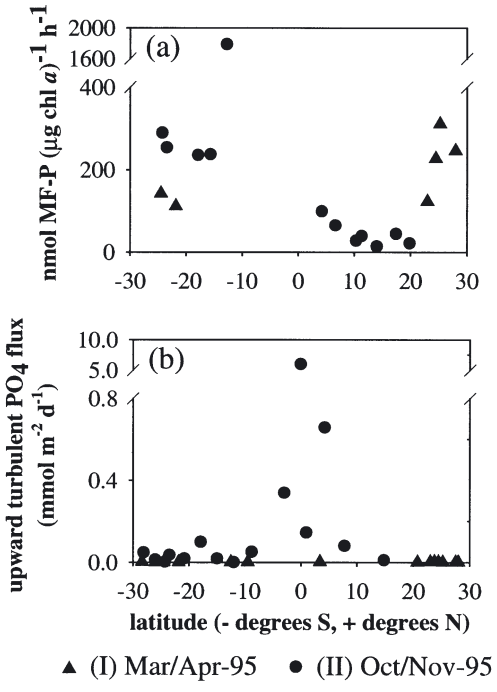

Fig. 5. Latitudinal variation in (a) specific alkaline phosphatase activity (APA) normalised to chlorophyll *a*, and (b) upward turbulent phosphate flux in the central Atlantic. MF-P: 3-0-methyl fluorescein phosphate

assays performed at the '*in situ*' temperature of the samples. According to Healey & Hendzel (1979), this determines APA below its maximum. We used an incubation temperature of 35°C for both transects, thus testing for the highest activity of the enzyme. This may explain, in part, why we found somewhat higher APA than recorded in other studies. We exclude background fluorescence arising from spontaneous hydrolysis of the substrate analogue as the cause of our slightly higher APA values, since although the substrate used (3-0-methyl fluorescein phosphate, MF-P) has a relatively high fluorescence, this was measured and accounted for (see 'Materials and methods'). In addition, the affinity of the enzyme for the substrate can differ depending on the structure of the organic part of the molecule (Jansson et al. 1988). This may lead to differences in the APA measured with different substrate analogues whenever these were not at saturating concentrations in the samples. The concentration of MF-P used here has been found to support saturated or near saturated rates of phosphatase activities in both cultured algae and natural populations (Healey & Hendzel 1979). APA is usually assayed at its maximal potential activity, thus reducing the differences associated with the use of various substrate analogues.

Taking into account the microplankton biomass allows better comparisons between APA at different locations. Although APA can be found in both the particulate and dissolved phase, it is usual to normalise total activity with reference to some surrogate of biomass (see 'Materials and methods'). Chlorophyll *a* has been the most used proxy of biomass (Appendix 1), despite the fact that autotrophic organisms are not the only contributors to APA and that some part of the activity may originate from bacteria and zooplankton. APA correlates with biomass surrogates such as chlorophyll when phosphate concentrations are above a certain threshold (Nausch 1993), so that some part of the variability recorded in Appendix 1 arises from differences in biomass. Hence, the relatively low APA observed in central Atlantic resulted in a higher specific APA when normalised to chl *a* concentrations (Appendix 1). Moreover, the low biomass at both extremes of the transect studied (E subtropical N Atlantic and W subtropical S Atlantic, Fig. 4), resulted in a high specific APA (Fig. 5a). If the differences among the different locations are valid (taking into account the various incubation temperatures of the APA assays in studies listed in Appendix 1), this would indicate that APA in the subtropical Atlantic is even

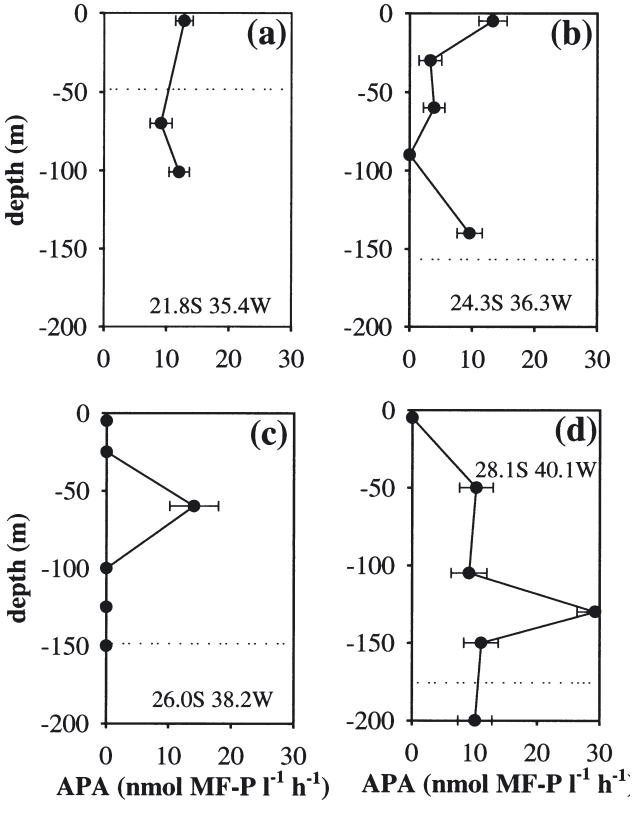

Fig. 6. Depth profiles of alkaline phosphatase activity (APA) in the central Atlantic. (a) Latitude I cruise, (b–d) Latitude II cruises. Error bars = ±SEM; dotted lines indicate depth of mixed layer

higher than that in the Sargasso Sea and in the western Mediterranean in summer (Cotner et al. 1997, Thingstad et al. 1998) being exceeded only by that in the northern Gulf of Aqaba, (northern Red Sea; Li et al. 1998) (Appendix 1). Specific APA from 0.24 to 1.2 nmol MF-P (µmol C)$^{-1}$ h^{-1}, expressed in terms of particulate organic carbon, has been found to indicate moderate P limitation in freshwaters, while values higher than 1.2 nmol suggest severe P limitation (Healey & Hendzel 1979). Hence, the specific APA found at both extremes of the north and south Atlantic subtropical gyres (between 1.8 and 7.1 nmol MF-P [µmol C]$^{-1}$ h^{-1}, Appendix 1) could indicate severe P limitation in those regions. This is consistent with data available for the western subtropical north Atlantic gyre (Mulholland et al. 2002), thereby sustaining the hypothesis of a prevalent high level of P stress in the subtropical north Atlantic. The APA off the NW African coast and in the tropical north Atlantic coincided with a relatively high biomass (Fig. 4), resulting in low specific activities of about 44 ± 11 nmol MF-P (µg chl *a*)$^{-1}$ h^{-1} (Fig. 5a), indicating relatively low P deficiency in this region. APA was absent from the southern extreme of the equatorial current system between 3° N and 13° S during both the Latitude I and Latitude II cruises (Fig. 1); this has been associated with shallow mixing layers and the upwelling of nutrient-rich waters. Indeed, the upward turbulent phosphate flux was high in this region, with undetectable APA, which could be linked to repression of the synthesis of the enzyme or to inhibition of its active sites as phosphate availability increased. Similar APA was found in the region around 24° S during the 2 cruises, indicating that P utilisation from DOP was a consistent feature of the plankton system in this region.

Inverse relationships between APA and phosphate have been frequently reported (Cembella et al. 1984, Jansson et al. 1988, Nausch 1993). However, the phosphate threshold for this regulatory mechanism may vary among ecosystems (Healey & Hendzel 1980, Chróst 1991), with presumably very low phosphate concentrations required in the oligotrophic ocean. The presence of APA in the central Atlantic was associated with relatively low concentrations and inputs of nutrients to the mixed layer (Figs. 2b & 5b), although these trends did not yield a significant correlation between these properties. Low phosphate concentrations are not necessarily accompanied by detectable APA (Jansson et al. 1988), possibly due to stored P or to a lag in the induction or activation of the enzyme's synthesis (Perry 1972). Indeed, AP is very stable, with turnover times exceeding the average generation time of most phytoplankton species (Liu et al. 1995). AP is released to the environment (where it may be detected as free dissolved activity) through either cell lysis or excretion, and maintains its activity over extended periods. Colloidal surfaces are centrally involved in the longevity and activity of extracellular enzymes in soils (Burns 1978), and may be important in aquatic systems as well (Boon 1993). Consequently, APA and, in particular, dissolved APA may reflect past episodes of P-deficiency, thus integrating the inorganic P history of the plankton community over long periods of up to 1.5 mo (Chróst 1991, Cotner et al. 1997, Li et al. 1998).

We can exclude cell breakage during filtration as the origin of dissolved phosphatase, since filtration was very careful and, in such case, dissolved APA would have occurred preferentially at the stations with the highest cell density. On the contrary, dissolved APA was most abundant in the oligothrophic stations and was absent from most (high-biomass) stations off the NW African coast. APA in the free dissolved fraction dominated the activity in the stations of the subtropical gyre with the highest specific APA (Figs. 3b & 5a), suggesting a reflection of strong P deficiency in the past, causing low microbial biomass and particle-bound APA. This is consistent with the high uptake rates and short turnover time of phosphate in the western part of the south subtropical gyre during the same Latitude II cruise (Cañellas et al. 2000), with values characteristic of P-deficient environments (Dolan et al. 1995, Cotner et al. 1997, Zohary & Robarts 1998). Dissolved phosphatases have been found to dominate the total enzyme activity in highly oligotrophic environments of both lakes and marine waters (Hantke et al. 1996, Li et al. 1998), and it has been suggested that this indicates the prevalence of P deficiency.

Our finding of APA in the central Atlantic confers a central role to dissolved organic molecules in the nutrition of microbial populations. A substantial part of the activity of the enzyme was associated with particles smaller than 0.8 µm (Fig. 3c), as would be expected from the dominance of picoplanktonic bacteria and cyanobacteria in the oligotrophic ocean (Fuhrman et al. 1989, Gasol et al. 1997) and their characteristic low C:P ratios (Chrzanowski & Kyle 1996, Heldal et al. 1996). In addition, phosphorus bound to organic molecules can be hydrolysed through an additional contribution from bacterial 5′–nucleotidase, which is not suppressed in the presence of phosphate (Ammerman & Azam 1985). With few exceptions (see Fig. 6), we tested only the presence of surface (5 m depth) APA, whereas APA may also be frequent at depth (Appendix 1). Our finding of APA at various depths within and outside the mixed layer, even at stations with undetectable surface activity, suggest that APA may be more frequent than hitherto recognised.

The presence of APA in the central Atlantic contrasts with the low nitrate availability of the surface mixing layer, with undetectable nitrate concentrations (below

0.02 µM) at most stations and low nitrate to phosphate ratios (Fig. 2d), which could suggest nitrogen limitation of plankton biomass and growth. However, N:P ratios were above the Redfield ratio when ammonium and dissolved organic nitrogen (DON) were also considered (Fig. 2e), although a variable unknown fraction of DON may be refractory (Vidal et al. 1999). The high TDN:TDP ratios observed in this study agree with those reported for the subtropical Sargasso Sea (Wu et al. 2000). High N:P ratios, above 20:1, also characterise the particulate pool (Fig. 2f), indicating nitrogen-sufficient conditions in the central Atlantic, probably reflecting the use of other nitrogen pools in addition to nitrate (Planas et al. 1999). Moreover, the nitrate uptake rates at these stations far exceeded the upward diffusive nitrate supply, further indicating additional, possibly atmospheric, nitrogen inputs (Planas et al. 1999). Similar conclusions were reached on the basis of the observation that downward DON fluxes matched or even exceeded upward nitrate inputs (Vidal et al. 1999). Therefore, the high N to P ratios observed in the total dissolved and particulate nutrient pool as well as the nitrogen fluxes estimates for the central Atlantic suggest the presence of additional N inputs, probably through N_2 fixation (Lipschultz & Owens 1996) and atmospheric deposition. Populations of diazotrophic organisms, in particular *Trichodesmium* spp., have frequently been reported for the central Atlantic (Carpenter 1983, Lipschultz & Owens 1996, Mulholland et al. 2002), and were also abundant off the north African coast in this study (S. Agustí unpubl. data). Indeed, high abundances of *Trichodesmium* spp. have recurrently been observed in the years 1995 to 1999 between the equator and 15° N, close to 20° W in the eastern Atlantic (Tyrell et al. 2003), i.e. at the same positions at which we found APA. Recent studies have extended the ability to fix N_2 to small nanoplankton (Zehr et al. 2001). Higher N_2 fixation rates in the central Atlantic than in the Pacific Ocean have been explained by higher iron availability in the Atlantic, which is supplied with Sahara dust (Carpenter 1983, Gruber & Sarmiento 1997). Under these conditions, N_2 fixation is limited by the availability of phosphorus (Sañudo-Wilhelmy et al. 2001), which could account for the presence of the AP enzyme in part of our study area.

The probable utilisation of DOP in the central Atlantic, which may be derived from the presence of AP, helps to explain the fast DOP turnover inferred from the high production rates of DOP (Cañellas et al. 2000) relative to the small DOP accumulation in the mixed layer (Vidal et al. 1999). Net production rates of DOP accounted, on average, for 6 ± 2 nmol l^{-1} h^{-1} during the autumn cruise (Cañellas et al. 2000), and the highest potential DOP hydrolysis that could be derived from APA was 13 nmol MF-P l^{-1} h^{-1} during the same cruise (Fig. 3a). Although APA does not measure DOP hydrolysis, comparisons between both estimates are illustrative of the probably fast DOP turnover in the central Atlantic. In this case, fast DOP recycling should allow an efficient use of P in the biogenic layer, while more refractory DON accumulates in (and is subsequently exported from) the mixed layer (Vidal et al. 1999). Higher biological and chemical lability of DOP than DON has been suggested in some studies (Smith et al. 1986, Abell et al. 2000). Hence, differences in the lability of dissolved organic compounds containing P and N might lead to uncoupled biogeochemical fluxes and to differential controls on N and P availability in the ocean (Jackson & Williams 1985, Smith et al. 1986, Wu et al. 2000). N_2 fixation provides the mechanism to cope with a progressive decrease in labile available nitrogen, while AP activity alleviates P deficiency.

In summary, our observation of APA in the central Atlantic and the high contribution of the dissolved fraction to total activity support the existence of persistent P-deficiency in most of the central Atlantic. This is consistent with stoichiometric factors and estimates of rate processes and elemental fluxes for the same dates and locations (Planas et al. 1999, Vidal et al. 1999, Cañellas et al. 2000), which point to excess N concentrations in the central Atlantic in agreement with previous studies (Gruber & Sarmiento 1997, Pahlow & Riebesell 2000). More severe P deficiency in the north Atlantic than in the north Pacific has also been recently suggested (Wu et al. 2000, Sañudo-Wilhelmy et al. 2001, Karl 2002). Thus, P regeneration from DOP, as implied from the observed APA, may play a pivotal role in the tropical and subtropical Atlantic, allowing an efficient use of P in the biogenic layer, and avoiding P loss from the mixed layer through the diffusive DOM flux that preferentially removes C and N.

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