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Significance of phosphomonoesterase activity in the regeneration of phosphorus in a meso-eutrophic, P-limited reservoir

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Abstract

The significance of phosphomonoesterase (PMEase) activity in the regeneration of phosphorus was investigated in a phosphorus-limited reservoir during the growing season (April–October) using the artificial substrate *p*-nitrophenylphosphate (*p*NPP). Despite the relatively high concentration (up to 0.45 μmol l^{-1}) of soluble nonreactive phosphorus (SNP) in the reservoir and the high total PMEase activity (up to 1144 nmol *p*NP $l^{-1}h^{-1}$), the rate of phosphate release from SNP accounted for less than 0.6% of the direct orthophosphate uptake by the planktonic community. The high apparent affinity and maximum velocity for *p*NPP may underestimate the velocities of Pi release by PMEase. More critical studies using natural substrates are needed to assess the role of PMEase as Pi supplier for the planktonic community. PMEase activity was apparently not related to soluble or particulate phosphorus fractions, suggesting that PMEase activity may not be useful as an indicator of P-limitation, unless other phosphorus pools (e.g. intracellular phosphorus) are considered.

Keywords: Phosphomonoesterase activity; Phosphorus regeneration; Phosphorus limitation

1. Introduction

In the aquatic environment, bacteria and phytoplankton may obtain part of their total phosphorus requirements from phosphomonoesters (PME), which are a variable fraction of the dissolved organic phosphorus (DOP) pool. In terrestrial environments organic phosphorus is of great importance in supplying P for biological uptake (Harrison, 1987; Tarafdar and Claasen, 1988). Phosphate is released from PME after enzymatic hydrolysis by a phosphomonoesterase (PMEase) (Suzumura et al., 1998). Usually, the enzymatic activity is associated with the membrane surface (González-Gil et al., 1998), but the enzyme can also be released into the extracellular medium; in this case it has been termed soluble PMEase activity (Aaronson and Patni, 1976). PMEase activity may be inducible (Aaronson and Patni, 1976), that is synthesis of the enzyme is stimulated by the presence of substrate. In addition, the end product Pi may repress or inhibit the activity of PMEase (Boavida and Heath, 1984a; Wynne, 1987). However, some forms of PMEase are constitutive (Cembella et al., 1984) and their synthesis is independent of external substrate or product.

PME may be externalised by plankton through secretion or cell lysis. Additional allochthonous sources of PME are drainage, waste-water input and other degradation products of organic matter in the surrounding catchment (Herbes et al., 1975; Tarapchack and Moll, 1990). Also, bio-available forms of phosphorus can be transferred from sediments to overlying water when the sediment is disturbed and re-suspended (especially during overturn) or by diffusion and microbial processes (Boström et al., 1988). So far, the characterisation and quantification of PME has proven difficult, largely because of the low concentrations in natural waters (Suzumura et al., 1998).

Although PMEase may play an important role in the supply of phosphorus from PME, not only in terrestrial ecosystems (Spier and Ross, 1978) but also in freshwater ecosystems (especially those with low Pi and high phosphorus demand), the role and regulation of this enzyme in aquatic ecosystems remain unclear. For instance, Heath (1986); Boavida and Heath (1988) found that P regenerated by PMEase did not contribute significantly to the total P demand by the plankton community. In contrast, Chróst

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Table 1
Some limnological features of La Concepción reservoir. Values represent ranges obtained in monthly samples during 1990. Data were obtained from Gálvez (1990). TP: total phosphate. SRP: soluble reactive phosphorus. ND: concentration below detection limits

Variable	Units	Range of values
TP	μmol P l^{-1}	2.5–3.5
SRP	μmol P l^{-1}	ND-2
NO_3^-	μmol N l^{-1}	ND-60
Alkalinity	meq l^{-1}	3–4.5
Ca^{2+}	μmol Ca^{2+} l^{-1}	500–750
pH		7.5–9.0
Transparency	m	0.35–6.5
Temperature	°C	11–26
Chlorophyll *a*	μg l^{-1}	<1–100

(1988), Bentzen et al. (1992) and Hantke et al., 1996a concluded that PMEase activity is of substantial benefit for P nutrition of algae. In this context, a study by Hernández et al. (1996a) in a temperate mesotrophic lake suggested that PME satisfied a large percentage (>40%) of the total algal P demand, but did not contribute significantly to bacterial P demand. Probably, the disparity among results is due to the different methodological approaches used in these and other studies. Most of the studies that estimate PMEase activity in the field are based on the use of the artificial, colorimetric substrate *p*-nitrophenyl-phosphate (*p*NPP) (e.g. Boavida and Heath, 1988; Jamet et al., 1997) or on the artificial fluorimetric substrates (such as 4-methylumbelliferyl phosphate MUP) (e.g. Jansson et al., 1988; Rose and Axler, 1998). The simplicity of these methodologies and the low cost make MUP and *p*NPP the preferred substrates to measure PMEase activity. Nevertheless, higher affinities and lower velocities of the PMEase have been estimated when using radiolabelled natural substrates (Heath and Edinger, 1990; Bentzen et al., 1992; Hernández et al., 1996a). The radiometric methods permit use of "natural" substrates. However, the use of radiolabelled substrates to measure PMEase activity is hampered by its high cost and it is limited to relatively few labs.

Previous studies in La Concepción, a P-limited reservoir in southern Spain, have shown that the phosphate concentration is very low (<0.1 μmol l^{-1}) after the typical spring phytoplankton bloom and during summer (Fernández, 1986). However, the concentration of soluble nonreactive phosphorus (SNP) is relatively high, with typical values about 0.7 μmol l^{-1} (Fernández, 1986). Under these conditions any recycling mechanism of phosphorus, such as the regeneration of Pi by PMEase activity, will be of great importance for phytoplankton growth. The objective of this study was to assess the importance of PMEase activity (using *p*NPP as substrate) in the supply of the total Pi demand to the aquatic community of a reservoir.

2. Materials and methods

2.1. Study site

The study was carried out in La Concepción, a medium sized, meso-eutrophic and P-limited reservoir located in southern Spain (Istán, Málaga). At maximal capacity (61 hm^3), the surface area is approximately 2.1 km^2, with a maximum depth of 68 m. Some limnological characteristics of the reservoir are shown in Table 1. The sampling station was located at the deepest part of the reservoir, approximately at 50 m from the dam.

During the study, surface temperature ranged from 17°C in late April to 26°C during July and August. Bottom temperatures were constant at approx. 12°C. Stratification developed from May to October and a stable thermocline was formed around 10–18 m depth. Dissolved oxygen was not detected in the hypolimnion from June to October, when the overturn started.

The development of phytoplankton in this reservoir follows the general pattern described by Sommer et al. (1986) in the seasonal succession of planktonic organisms in freshwater. Three distinct periods can be regularly distinguished during the growing season: the spring bloom, a clear-water phase, and a late summer bloom.

2.2. Sampling programme and analyses

Sampling was carried out at approximately 14-d intervals from 24 April to 25 October 1993. Water samples were collected using a 5-l Van Dorn water sampler, from the surface and six additional depths, always including a near-bottom sample and the thermocline. Water was transferred to 1-l bottles that were previously washed and acid rinsed. Temperature, oxygen concentration and pH were recorded from the surface to the bottom at maximum intervals of 1 m with a water quality logger (YSI Grand, mod. 3800), which was calibrated before each use. For bacterial enumeration, 6 ml of water samples were immediately transferred to 10-ml sterile assay tubes and fixed with formaldehyde (2.5% final concentration). Tubes were kept in the dark at 4°C before analysis. Bacteria were counted using the acridine orange method (Hobbie et al., 1977). Between 5 and 10 random fields were counted, which always resulted in more than 300 bacterial counts, following the recommendations of Hobbie et al. (1977).

Water samples were transported to the laboratory (3 h from the sampling site) under ice and 0.5 l was filtered through a 0.22-μm nitrate cellulose filter. The filtrate was frozen at −20°C for later analysis of total soluble phosphorus (TSP) and soluble reactive phosphorus (SRP). For chlorophyll *a* (hereafter chl-*a*) analyses, 0.5 l of water was filtered through 0.7-μm glass fibre filters (Whatman GF/F). The chl-*a* was extracted from the material retained on filters with 90% (v/v) acetone after 24 h in the dark at 4°C. The

Table 2
Chlorophyll *a*, bacterial density and PMEase activitiy in different size fractions of the water of La Concepcion reservoir. Data were obtained at 3-m depth during two sampling days in 1993. Values represent mean ± SD ($n = 3$)

Date	Chl-*a* ($\mu g\ l^{-1}$)		Bacteria ($\times 10^6\ ml^{-1}$)		PMEase activity ($nmol\ l^{-1}\ h^{-}$)	
	>2.7 μm	2.7–0.7 μm	Unfiltered	<0.7 μm	<0.7 μm	<0.22 μm
23 March 93	8.4 ± 0.8	0.71 ± 0.09	0.9 ± 0.1	0.05 ± 0.01	53.4 ± 4.3	45.4 ± 5.2
6 April 93	12.5 ± 1.1	0.67 ± 0.1	1.1 ± 0.3	0.13 ± 0.05	67.1 ± 3.2	54.3 ± 7.2

concentration was estimated spectrophotometrically according to Talling and Driver (1963).

SRP was determined by the malachite green method (Fernández et al., 1985). TSP was determined after acid digestion in an autoclave (30 min) at 121°C (Mackereth et al., 1978). The difference in concentration of TSP and SRP was considered to be soluble nonreactive phosphorus (SNP). The total phosphorus (TP) was analysed as SRP following an acid digestion of unfiltered samples. Particulate phosphorus (PP) was estimated by subtracting the concentration of TSP from TP.

The enzymatically hydrolysable phosphorus concentration (PME) was measured in 0.7-μm filtrates according to Francko and Heath (1979). Unfortunately, the formation of a white–yellow precipitate at 37°C after the addition of 10^{-2} M Tris buffer at pH 9.0 interfered with the determination of the SRP by this procedure. This was probably because of the high content of $CaCO_3$ in the water of the reservoir. Therefore, the potential contribution of PME to DOP was inferred from previous studies in freshwater and data from a nearby catchment (Shan et al., 1994; Hernández et al., 2000).

2.3. PMEase activity assays

PMEase activity was assayed spectrophotometrically following a modification of the method of Reichardt et al. (1967), which uses *p*NPP as substrate. PMEase activity was measured as the linear increase in absorbance as the substrate was enzymatically hydrolysed, releasing the coloured product *p*-nitrophenol (*p*NP). The activity was measured in triplicate samples from each depth. Water samples were previously filtered through a nylon net (pore size 250 μm) to remove large zooplankton and size fractionated. A volume of 50 ml of water was passed through glass fibre filters (Whatman GF/D) with a pore size of 2.7 μm and a portion of the filtrate retained to measure PMEase activity. The remainder was filtered through 0.7-μm filter (Whatman GF/F). All filtrations were performed under a pressure of approx. 150 mbar. Thus PMEase activity was determined in untreated water samples, and in the 2.7 and 0.7-μm filtrates.

We assumed that the difference in PMEase activity between the untreated samples and the 2.7-μm filtrate gave an estimate of the algal PMEase activity while the difference between the 2.7 and 0.7-μm filtrates provided an estimate of the bacterial PMEase activity. The activity of free dissolved PMEase (= soluble) was determined in the 0.7-μm filtrate. These assumptions are based on preliminary experiments performed before the period of study (Table 2). On the two occasions investigated, <1% of the total chl-*a* was collected in the 2.7-μm filtrate, and between 6 and 12.4% of the total bacterial number was counted in the 0.7-μm filtrate. Finally, we observed that after consecutive filtration of a water sample through 0.7 and 0.22-μm filters, >80% of the activity determined in the 0.7-μm filtrate was found in the 0.22 μm filtrate.

For all PMEase activity assays 0.3 ml of filtered (<0.22 μm) 1 $mmol\ l^{-1}$ *p*NPP solution were added to 2.7 ml of water sample. Samples were incubated for 3 to 6 h at 25°C. The substrate concentration at the start of the assay (100 $\mu mol\ l^{-1}$) was high enough to saturate the enzyme. No buffer was added, as the water of the reservoir is highly buffered (alkalinity was always over 4.0 $meq\ l^{-1}$ at the surface; Table 1); previous experiments showed pH changes of less than 0.1 units during the period of incubation. Triplicate controls (distilled water plus *p*NPP) were run for each PMEase determination. Before the enzyme assays, the absorbance of each sample was read at 410 nm in 1-cm cuvettes. The increase in absorbance after the assays was read at the same wavelength. The extinction coefficient for *p*NP was corrected for the pH for each sample using an equation relating the extinction coefficient to pH (Hernández et al., 1996b), and PMEase activity was expressed as nmol pNP released $l^{-1}\ h^{-1}$. To calculate specific PMEase activity of algal biomass (chl-*a*), PMEase was expressed as nmol pNP released (μg chl-$a^{-1}\ h^{-1}$).

2.4. Kinetic parameters of PMEase activity

Kinetic parameters were investigated on two occasions in spring (April) and autumn (October). Water samples were incubated at *p*NPP concentrations ranging from 3 $\mu mol\ l^{-1}$ to 1 $mmol\ l^{-1}$ and PMEase activity was measured as described above. The kinetic parameters (apparent affinity ($S_{0.5}$) and maximum velocity (V_{max})) of the total PMEase (unfiltered water samples) were calculated by linear regression by the Eadie–Hofstee transformation (v versus v/s, v being the velocity and s the substrate concentration) of the Michaelis–Menten equation.

Phosphorus released by PMEase activity was calculated from the Michaelis–Menten equation using the kinetic parameters for PMEase activity and the assumed PME

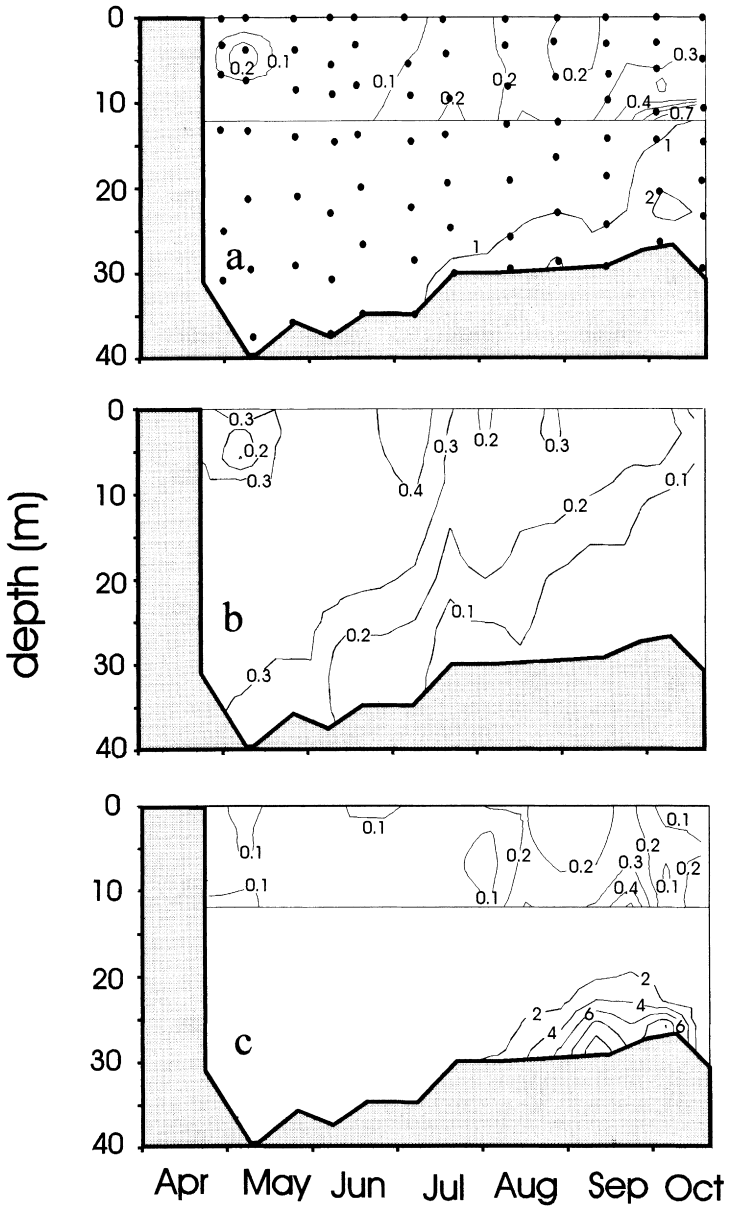

Fig. 1. Depth–time distribution of isopleths of (a) SRP (μmol l^{-1}), (b) SNP (μmol l^{-1}), and (c) PP (μmol l^{-1}) in La Concepcion reservoir. The shadowed area represents the reservoir bottom. Note also that sampling starts in late April. In (a) and (b) values in the upper 12 m are magnified.

concentration as:

$$v = ([\mathrm{PME}] \times V_{\max})/([\mathrm{PME}] + S_{0.5})$$

where v is the velocity of phosphorus released by PMEase activity (nmol pNP released $l^{-1}\,h^{-1}$), [PME] is the concentration of phosphomonoester (nmol l^{-1}), $V_{\max}$ is the apparent maximum velocity (nmol ^{-1}pNP released $l^{-1}\,h^{-1}$) and $S_{0.5}$ is the apparent affinity (nmol l^{-1}). The PME concentration was inferred from the SNP values, assuming a maximum potential percentage of 50% of SNP as substrate for PMEase (Hernández et al., 2000).

2.5. Kinetic parameters for phosphate uptake

The kinetic parameters of Pi uptake for the epilimnetic planktonic community were assumed to be the same as those estimated by Fernández et al. (1997). In their study, SRP values and phytoplanktonic species were similar to those observed during the period of the present study. According to these authors, the maximum velocity for Pi uptake and $S_{0.5}$ were 17.4 μmol P $l^{-1}\,h^{-1}$ and 3 μmol P l^{-1}, respectively during April, and 20.3 μmol P $l^{-1}\,h^{-1}$ and 3.6 μmol P l^{-1} during October. The velocities of Pi uptake were calculated following the Michaelis–Menten equation, using the SRP concentrations in the water and the kinetic

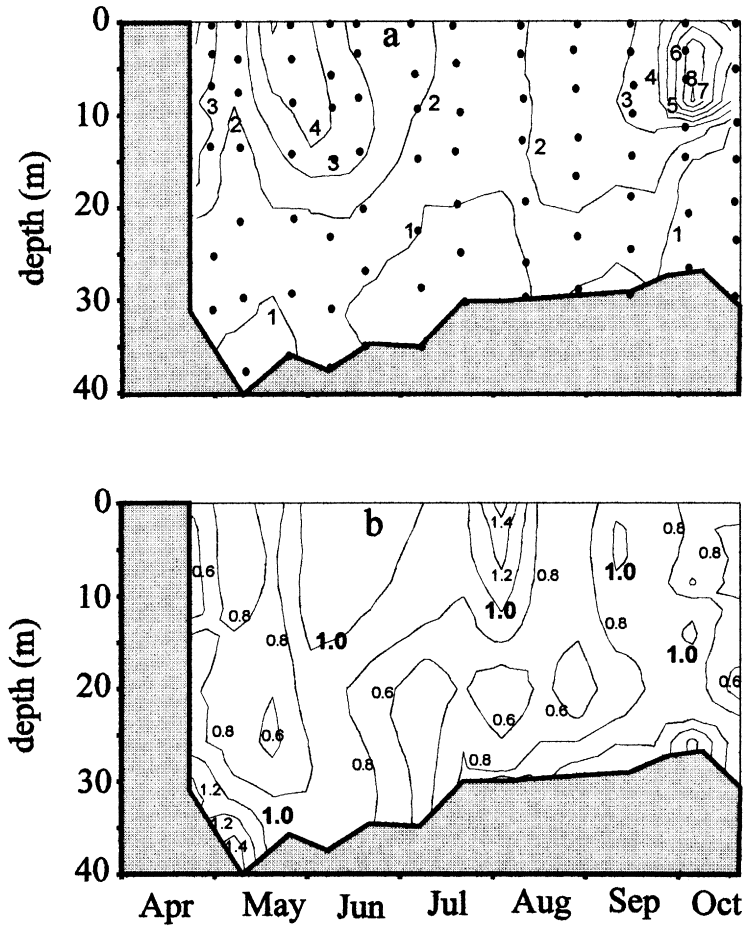

Fig. 2. Depth–time distribution of isopleths of (a) chlorophyll *a* ($\mu g\ l^{-1}$) and (b) bacterial density ($\times 10^6\ ml^{-1}$) in La Concepcion reservoir. Shadowed area as in Fig. 1.

parameters of Pi uptake estimated by Fernández et al. (1997), as follows:

$$v = ([SRP] \times V_{max})/([SRP] + S_{0.5})$$

where v is the velocity of phosphorus uptake (nmol P uptake $l^{-1}\ h^{-1}$), [SRP] is the concentration of soluble reactive phosphorus (nmol l^{-1}), V_{max} is the apparent maximum velocity for phosphorus uptake (nmol P uptake $l^{-1}\ h^{-1}$) and $S_{0.5}$ is the apparent affinity for the phosphorus uptake (nmol l^{-1}).

3. Results

3.1. Seasonal variation

The epilimnetic concentration of SRP at 12 m, just above the thermocline, ranged from 0.03 μmol l^{-1} during April and May to 0.8 μmol l^{-1} in early October (Fig. 1a). Below the thermocline, SRP increased up to 1.4 μmol l^{-1} when oxygen concentration was undetectable. SNP concentration was relatively high during spring with a maximum (0.45 μmol l^{-1}) in late June (Fig. 1b). During summer and early autumn, SNP gradually decreased in the water column. Overall, SNP concentration decreased with depth.

The epilimnetic PP concentrations, just above the thermocline, ranged from less than 0.01 μmol P l^{-1} in early May to 0.45 μmol l^{-1} in late September (Fig. 1c). Similar to SRP, the concentration of PP below the thermocline increased from July onwards, to a maximum of 16.1 μmol l^{-1} at the bottom in early September. The SNP in the epilimnion averaged 54% of total phosphorus, while SRP and PP represented 26 and 21%, respectively.

Chl-*a* concentrations in the epilimnion were relatively low (approx. 3 $\mu g\ l^{-1}$) at the beginning of the study (Fig. 2a) and increased slightly in late May and early June (up to 5.5 $\mu g\ l^{-1}$). Values decreased again during July, August and September and then peaked (up to 8.7 $\mu g\ l^{-1}$) in early October. During April and May, the most abundant phytoplanktonic groups were small Cryptomonads and pennate diatoms, especially *Fragillaria crotonensis*. In the late summer peak, the phytoplankton was dominated by the diatom *Melosira granulata* and the dinoflagellates *Peridinium cinctum*, and to a minor extent, *Ceratium hirundinella*.

No seasonal trends were apparent in bacterial numbers, and depth profiles were similar throughout the study period

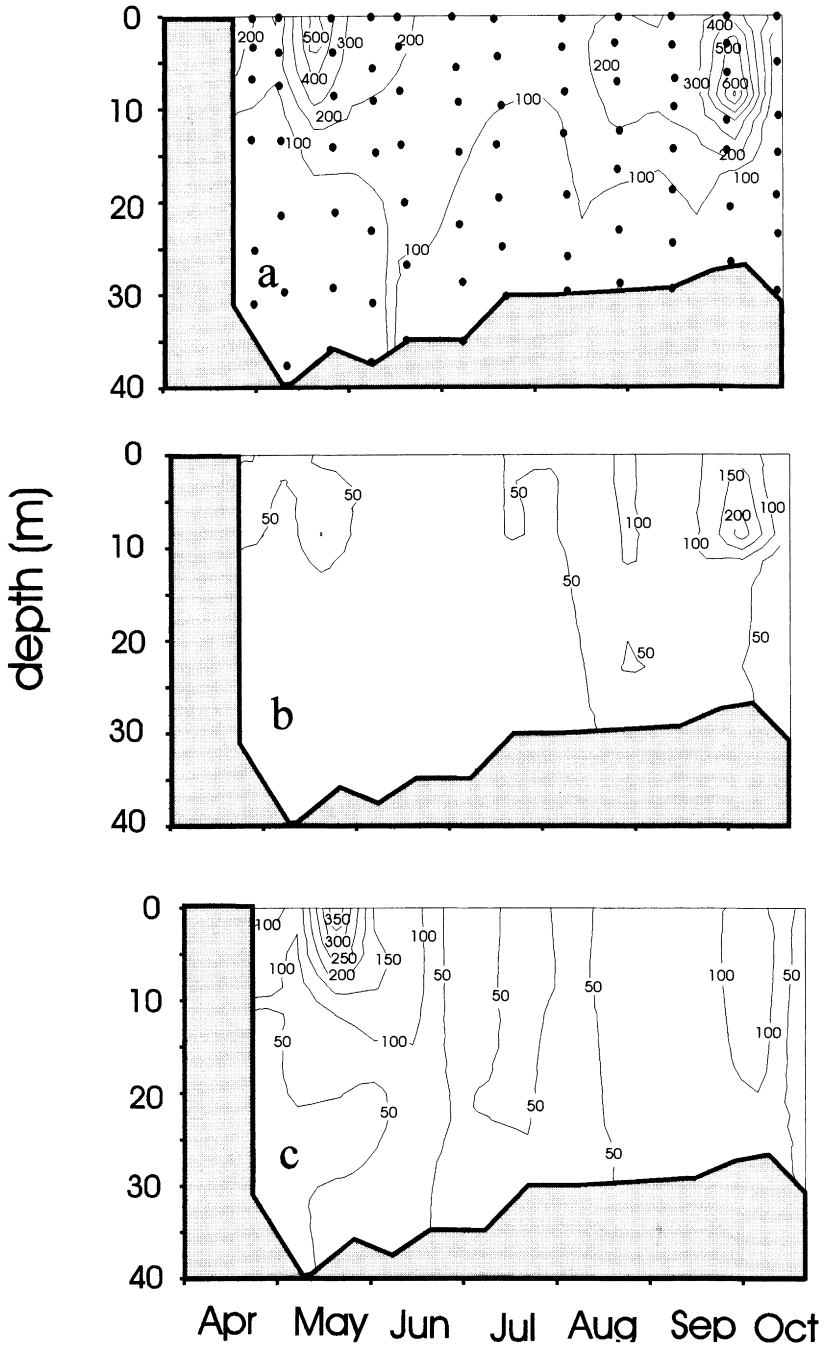

Fig. 3. Depth–time distribution of isopleths of (a) algal PMEase activity (nmol *p*NP l^{-1} h^{-}), (b) bacterial PMEase activity (nmol *p*NP l^{-1} h^{-}) and (c) dissolved PMEase activity (nmol *p*NP l^{-1} h^{-}) in La Concepcion reservoir. Values represent means ($n = 3$). The coefficient of variation was always lower than 15%. Shadowed area as in Fig. 1.

(Fig. 2b). Bacterial abundance was relatively high at the surface ($0.8–1.4 \times 10^6$ bacteria ml^{-1}) and peaked at the bottom (the overall maximum was 1.8×10^6 ml^{-1} in early May). The minimum values of bacterial abundance were observed just below the thermocline.

Algal PMEase activity (Fig. 3a) ranged from 5.4 nmol *p*NP l^{-1} h^{-} just above the bottom in late October to 758 nmol *p*NP l^{-1} h^{-} at 8 m in early October. Seasonally, values were relatively high in the middle of May (up to 677 nmol *p*NP l^{-1} h^{-}) and decreased during the summer. Usually the activities decreased with depth, especially below the thermocline. Bacterial PMEase activity (Fig. 3b) varied from below the detection limit to 254 nmol *p*NP l^{-1} h^{-} in early October. As for algal PMEase activity, bacterial PMEase activity was relatively high in mid-May and early October, whereas significantly lower values were observed during summer. Enzymatic activities clearly decreased with depth throughout the study.

Table 3
Percentages of the algal, bacterial and dissolved PMEase activities in the total PMEase activity. Minimum, maximum and mean percentages during the study period are shown

Size fraction	Epilimnion ($n = 55$)			Hypolimnion ($n = 27$)		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Algal	13	55	100	7	27	60
Bacterial	0	18	56	0.4	34	59
Dissolved	0	25	63	13	38	87

Dissolved PMEase activity (Fig. 3c) was highest in mid-May (up to 538 nmol *p*NP l^{-1} h^{-}), when SNP concentration was highest, and remained relatively low throughout the rest of the study. Although no vertical variations were evident, surface values of dissolved PMEase activity tended to be higher than bottom ones.

The contribution of algal, bacterial and dissolved PMEase activity to the total PMEase activity measured in the reservoir was highly variable during the study (Table 3). The contribution of algal PMEase was highest (100%) during the clear water phase (summer months) and, as expected, the mean activity related to this size fraction was highest in the epilimnion. Overall, the mean percentage contributions to total PMEase activity made by bacteria were greater near the bottom (34%) than in the epilimnion (18%). The percentage of dissolved PMEase was highest in May, when the SNP concentration was greatest.

The specific PMEase activity ranged from <32 nmol *p*NP (μg chl-a^{-1}) h^{-1}, below the thermocline, to 258 nmol *p*NP (μg chl-a^{-1}) h^{-1} in early October at 15 m depth (Fig. 4). In the epilimnion >70% of the samples showed activities lower than 100 nmol *p*NP (μg chl-a^{-1}) h^{-1}.

3.2. Relationship between PMEase activity and other variables

Fig. 5 shows the relationship between total PMEase activity and the concentration of the different phosphorus fractions in the epilimnion (SRP, SNP and PP). None of these fractions was linearly related to total PMEase activity. However, the highest activities were obtained at threshold concentrations of phosphate <0.4 μmol l^{-1} (Fig. 5a). A similar conclusion was reached when the algal, bacterial and dissolved PMEase activities were compared to the phosphorus fractions (data not shown). All maximum PMEase activities occurred between 0.2–0.4 μmol l^{-1} SNP, but PMEase did not increase at lower concentrations (Fig. 5b).

Chl-*a* concentration was linearly related to both total and algal PMEase activity (Fig. 6a). This biomass estimator explained, respectively, 50 and 53% of the variability in both PMEase activities in the epilimnion. In contrast, bacterial abundance was not related either to bacterial or total PMEase activity (Fig. 6b).

3.3. Phosphate uptake and phosphorus release by PMEase activity

The rate of P release by total PMEase activity was estimated using the concentration of SNP (50% of which was assumed to be PME), and the estimated kinetic parameters for the plankton community. The total PMEase activity showed typical Michaelis–Menten kinetics (Table 4). Although phytoplankton composition varied between the two sampling dates, the kinetic parameters were similar. The apparent maximum velocity (V_{max}) and the apparent half-saturation constant ($S_{0.5}$) were slightly higher in April than in October. Fig. 7a shows that the phosphorus regenerated by PMEase activity in the epilimnion ranged from

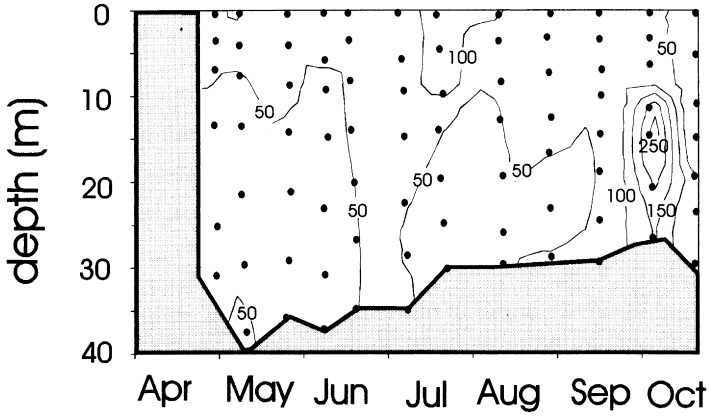

Fig. 4. Depth–time variations of isopleths of the specific PMEase acitivity (nmol *p*NP (μg chl-a^{-1}) h). Shadowed area as in Fig. 1.

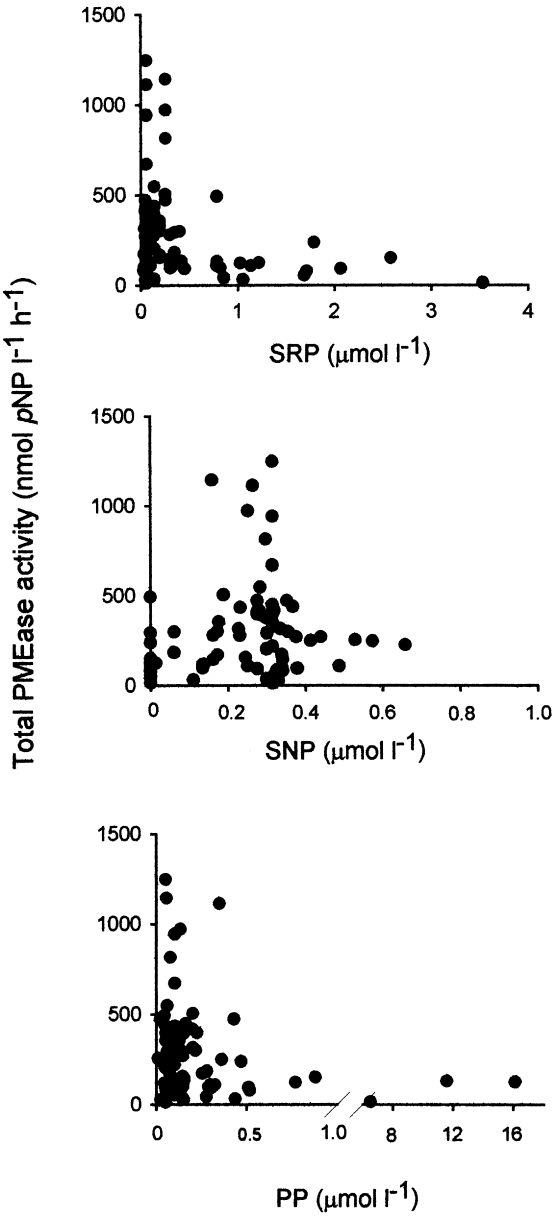

Fig. 5. Relationship between total PMEase activity (nmol *p*NP l^{-1} h^{-}) and (a) SRP, (b) SNP and (c) PP in the epilimnion.

negligible contributions to 2.5 nmol P l^{-1} h^{-}. Relatively high velocities were found in late May–early June (about 1.5 nmol P l^{-1} h^{-}). The velocities decreased to 0.4 nmol P l^{-1} h^{-} during July and early August and increased again in early October, where the maximum phosphate regeneration was found.

On the other hand, velocities for phosphate uptake (Fig. 7b) in the epilimnion peaked in early May (up to 1500 nmol P l^{-1} h^{-}) but were relatively low during June, July and August (Fig. 7b). The highest P uptake was measured in early October, just above the thermocline. These values coincided with the late summer chl-*a* peak and the highest epilimnetic SRP concentrations.

Rates of phosphorus release from PME and P uptake by the plankton community were compared to express the relative importance of PMEase activity to the total phosphate demand for the planktonic community (Fig. 7c). Throughout the of study, the Pi supplied by total PMEase activity in the epilimnion accounted for <1% of the phosphate taken up as SRP by the planktonic community.

4. Discussion

4.1. PMEase as indicator of P deficiency

In some previous studies, PMEase activity in freshwater ecosystems correlates inversely with external phosphorus fractions, as SRP (Chröst et al., 1984) or TP (Smith and Kalff, 1981). Based on these and other data, PMEase activity has been considered as an indicator of phosphorus deficiency (Gage and Gorham, 1985; Rose and Axler, 1998), i.e. the enzymatic activity is induced when external phosphate concentration is low, and repressed when phosphate concentration is high. However, the usefulness of PMEase activity as an indicator of P deficiency in aquatic ecosystems has been questioned by others (Cembella et al., 1984; Jansson et al., 1988). This is especially important if there are diurnal variations of PMEase activity (Huber and Hamel, 1985) or if there are potential zooplanktonic sources of the enzyme (Boavida and Heath, 1984b). In the present study, no linear relationship between PMEase activity (total, dissolved or attributable to algal or bacterial size-fractions) and external phosphorus fractions (SRP, SNP and PP) were observed (Fig. 5). However, it seems clear from Fig. 5 that total PMEase activity may be induced when external SRP and PP are low, and/or repressed at high SRP or PP. Nonetheless, there were many instances in which both PMEase activity and external phosphorus fractions were very low. In conclusion, our findings suggest an independence, on a seasonal basis, between the phosphorus fractions in the water and PMEase activities, especially at low concentrations. This is in agreement with previous reports from other freshwater ecosystems (Huber and Kidby 1984; Feuillade et al., 1990). Hino (1988) concluded that the enzymatic activity was probably more affected by the algal species present than by the SRP concentration.

There are several possible explanations for the absence of a correlation between PMEase activity and external phosphate. In many phytoplanktonic species, the induction of PMEase activity may depend on the internal phosphorus pool (Gage and Gorham, 1985; Pettersson, 1985). In addition, some algae produce both inducible and constitutive PMEase, which means that the enzymatic activity may be partially independent of the external phosphorus pool (Siuda 1984; Boavida and Heath 1984a). The independence between PMEase activity and SNP may also be due to the variable proportion of PME in the SNP pool; SNP includes both inorganic (e.g. associated with $CaCO_3$) and DOP. The proportion of PME in DOP is also highly variable, from a

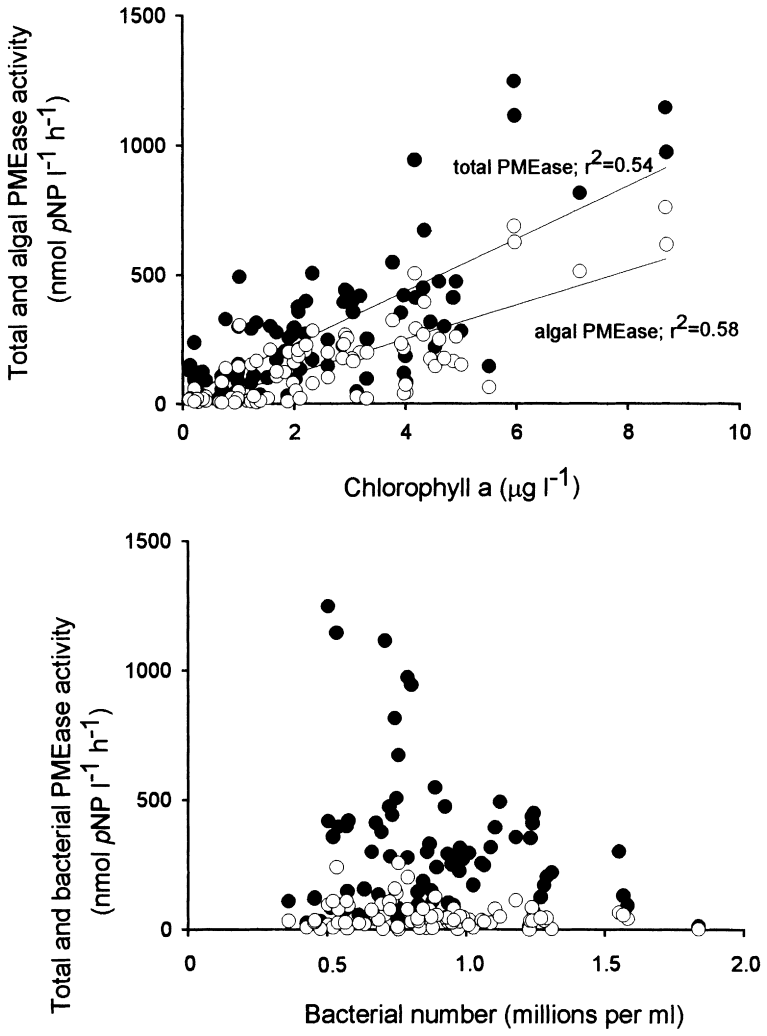

Fig. 6. (a) Relationship of total PMEase activity (full circles), and algal PMEase activity, (open circles) with chlorophyll *a*, (b) relationship of total PMEase activity (full circles), and bacterial PMEase activity (open circles) with bacterial density.

negligible contribution to more than 50% (Shan et al., 1994; Suzumura et al., 1998; Hernández et al., 2000). Finally, PP may also be composed of inorganic and organic P, a variable amount of which may represent non-living material.

The algal PMEase activity tended to co-vary with the concentration of chl-*a* in the epilimnetic waters (Fig. 6). However, 47% of the variation in enzymatic activity was not explained by this biomass estimator. Therefore, although this and other studies have shown significant correlations between PMEase activity and chlorophyll (e.g. Siuda, 1984), the use of chlorophyll to express specific PMEase activity has obvious limitations (Hernández et al., 1999). Chlorophyll *a* content of phytoplanktonic freshwater

Table 4
Apparent kinetic parameters of total PMEase activity for the planktonic community. Data of PMEase activity vs substrate concentration were fitted to a Michaelis–Menten equation. The significance of the regression is given by the coefficient of determination (R^2). The major phytoplanktonic taxa on each sampling day are indicated

Date	$S_{0.5}$ (μmol l^{-1})	V_{max} (μmol l^{-1} h^{-})	R^2	Major phytoplanktonic species
24 April 1993	28	1.74	0.97	*Cyclotella* spp., *Fragillaria crotonensis*, Crytomonads
4 October 1993	23	1.06	0.92	*Melosira granulata, Peridinium cinctum, Ceratium hirundinella*

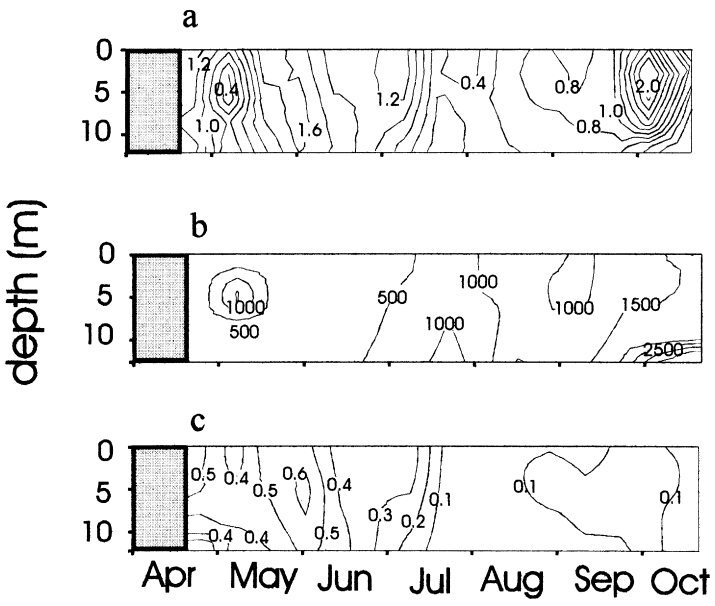

Fig. 7. Phosphorus released by total PMEase activity (nmol P l^{-1} h^{-}) in the epilimnetic waters of La Concepcion reservoir (a), phosphorus taken up by the plankton community (nmol P l^{-1} h^{-}) (b), and percentages of phosphorus released by PMEase activity related to the phosphate taken up by the plankton community (c). Note that sampling starts in late April.

organisms shows intra and inter-specific variations in response to a range of ecophysiological conditions, such as nutrient concentration or irradiance (Gálvez et al., 1988).

4.2. Significance of PMEase in phosphorus regeneration

According to the present study, the phosphorus that could be supplied by PMEase under natural conditions accounted for <1% of the phosphate taken up as SRP (Fig. 7c). This result was similar to that of Heath (1986) in several freshwater ecosystems and Boavida and Heath (1988) in East Twin Lake (Ohio), both using *p*NPP as substrate. In contrast, other studies (Chróst, 1988; Bentzen et al., 1992; Hantke et al., 1996a) concluded that PMEase activity is of substantial benefit as P supply for the planktonic community. Recently, Hernández et al., 1996a also found that PME may satisfy more than 40% of the total algal P demand.

Some of our assumptions might affect the conclusions regarding the significance of PMEase activity in the phosphate regeneration in La Concepción reservoir. The rates of phosphate supplied by PMEase activity were based on a maximum PME concentrations of SNP in the catchment area (Hernández et al., 2000) and the kinetic parameters for *p*NPP as a model substrate. According to the SNP concentrations, the maximum assumed PME concentration was lower than 0.25 μmol l^{-1}, and hence, the rate of P release by enzymatic activity seems to be controlled by the kinetic parameters. Compared to fluorimetric or radiolabelled substrates, studies using *p*NPP generally show higher apparent V_{max} and $S_{0.5}$. The *p*NPP concentration that gives the half-saturation constant is often more than 100 times the PME concentration in the water (e.g. Heath and Cooke, 1975; Heath, 1986; this study), and thus, there would be substantially less P release from PME. However, when PMEase activity is estimated using fluorimetric substrates, lower maximum rates and a higher affinity are usually measured (e.g. Berman et al., 1990; Hantke et al., 1996b; Vrba, pers. comm.). Although some studies have considered all these substrates as reliable models to estimate PMEase activity (Flint and Hopton, 1977), studies using radiolabelled natural substrates (Heath and Edinger, 1990; Bentzen et al., 1992; Hernández et al., 1996a) have shown that the affinity for these are usually much higher than for artificial substrates. Therefore, the use of *p*NPP may underestimate the significance of PMEase in the Pi regeneration (Hernández et al., 1996a).

The rates of P released from PME calculated in our study seems to be in the lower range of the values reported in other studies using *p*NPP as substrate (Heath, 1986; Boavida and Heath, 1988). In contrast, Hernández et al., 1996a calculated rates using ^{32}P-glucose-6-phosphate as substrate that are almost 20 times higher than those reported in our study.

An additional problem in assessing the rate of Pi regenerated by PMEase is the fact that kinetic parameters for both PMEase and phosphate uptake may change seasonally (Pettersson, 1980; Hantke et al., 1996b). This variability may reflect changes in phytoplankton biomass rather than variations in the kinetic characteristics of the enzymes. However, in the present study, changes in both apparent $S_{0.5}$ and V_{max} were low, despite marked differences in the algal community.

Our results suggest that the regeneration of phosphorus by the cleavage of PME does not account for more than 0.6% of the phosphorus uptake by the plankton community in the P-limited reservoir of La Concepción. Although this result agrees with previous reports (e.g. Boavida and Heath,

1988), we acknowledge that more complete studies are needed to assess the significance of PMEase activity in this reservoir. These should include the direct measurement of the PME concentration, measurements of P uptake by plankton, and comparisons of fluorimetric and natural substrates to estimate PMEase activity.

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