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Diel changes in the alkaline phosphatase activity of bacteria and phytoplankton in the hypereutrophic Villerest reservoir (Roanne, France)

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Key words: reservoir, phytoplankton, bacteria, alkaline phosphatase activity, diel

Abstract

The diel changes of the size fractionated alkaline phosphatase activity (APA) were studied in relation to several abiotic and biotic factors in Villerest reservoir (located on the Loire river, near the city of Roanne, France), bihourly during two days in July 1992. The APA measured in this work exceeded considerably those reported in the literature, suggesting that dissolved mineral phosphorus was not available to microorganisms. At 1 m, the APA was primarily due to bacteria which actively assimilated organic *P* compounds released by photosynthetic algal metabolism. At 5, 10 and 20 m, the APA was predominantly algal. The high concentrations in SRP (soluble reactive phosphorus) would indicate that orthophosphates were not bioavailable. The reverse (*i.e.* availability to phytoplankton) would have resulted in undetectable levels of $P\text{-}PO_4^{3-}$ due to the massive proliferation of algae in Villerest reservoir.

Introduction

The Villerest reservoir located near the city of Roanne (France) is strongly affected by the process of eutrophication (Aleya *et al.*, 1993). In particular, several tributaries flowing into the reservoir carry a high load of suspended and dissolved matter chiefly nitrogen and phosphorus resulting from agricultural runoff and urban and industrial activities. Furthermore, for decades, phosphorus has been clearly demonstrated to contribute to the eutrophication of aquatic ecosystems (Vollenweider, 1968; Schindler & Fee, 1974). Several dephosphatization plants of tributaries have been constructed to limit the phosphorus inputs into Villerest reservoir. However, since the dephosphatization of wastes leads to the precipitation of the sole mineral phosphorus, this process would favour the development of many organisms (primarily algae and bacteria) able to hydrolyse organic phosphorus compounds through the phosphatase enzymes. In addition, close relationships have been reported between the phosphatase activity on the one hand and both orthophosphate deficiency (Healey, 1973) and the trophic status of the studied lakes (Olsson, 1990). The phosphatase activity has been shown to vary with the taxonomic composition of either the phytoplanktonic (Rivkin & Swift, 1979) or bacterial (Chrost *et al.*, 1986) communities. The cell surface enzyme phosphatase of both bacteria and algae can be released in solution passively or as a result of zooplankton grazing (Jansson *et al.*, 1988). Also, Berman (1970) found the phytoplankton to be a major contributer to total alkaline phosphatase activity (APA) whereas Stewart & Wetzel (1982) attributed the whole APA to bacteria.

Despite the growing information on the long term coupling between the APA and several environmental factors (e.g., Currie *et al.*, 1986; Valstein *et al.*, 1988; Cotner & Wetzel, 1992), little is known about their diel changes. The purpose of this work was thus to investigate the 48 hours diel variations of the size fractionated potential APA coupled with several abiotic and biotic parameters in the hypereutrophic Villerest reservoir.

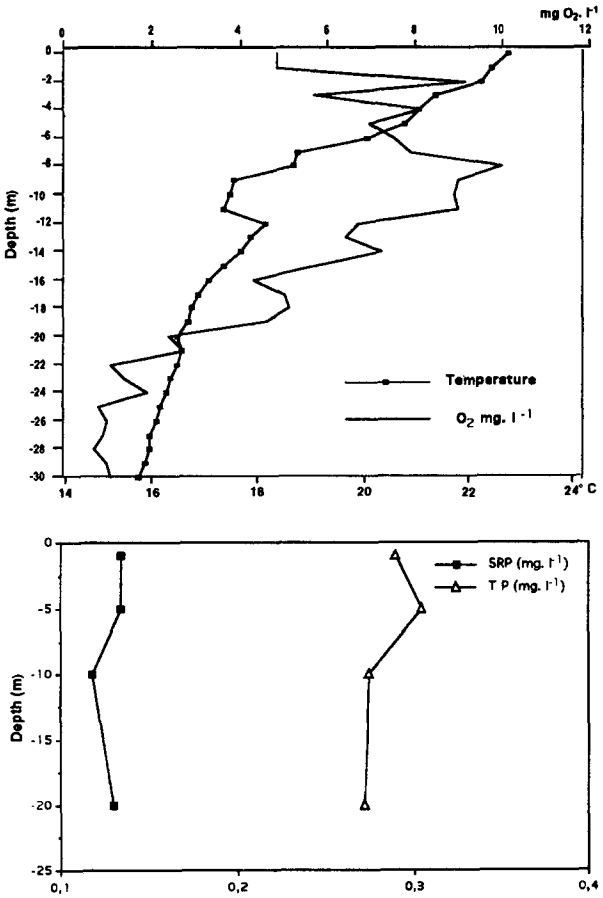

Fig. 1. Changes in temperature and oxygen levels (above) and spatial-temporal distribution of TP (total phosphorus) SRP (soluble reactive phosphorus) concentrations (below).

Materials and methods

Study site

The Villerest reservoir is located on the Loire river, 4 kilometers upstream from the city of Roanne (France). This reservoir was formed by the construction of a dam on the Loire river in 1984 by the EPALA (Public Department of the Management of the River Loire and its Tributaries). It's a polymictic and hypereutrophic lake (Aleya *et al.*, 1993). Its morphometric characteristics are listed in Table 1.

Samplings

Samples were collected with a Van Dorn bottle in the pelagic zone near the dam at 1, 5, 10 and 20 m.

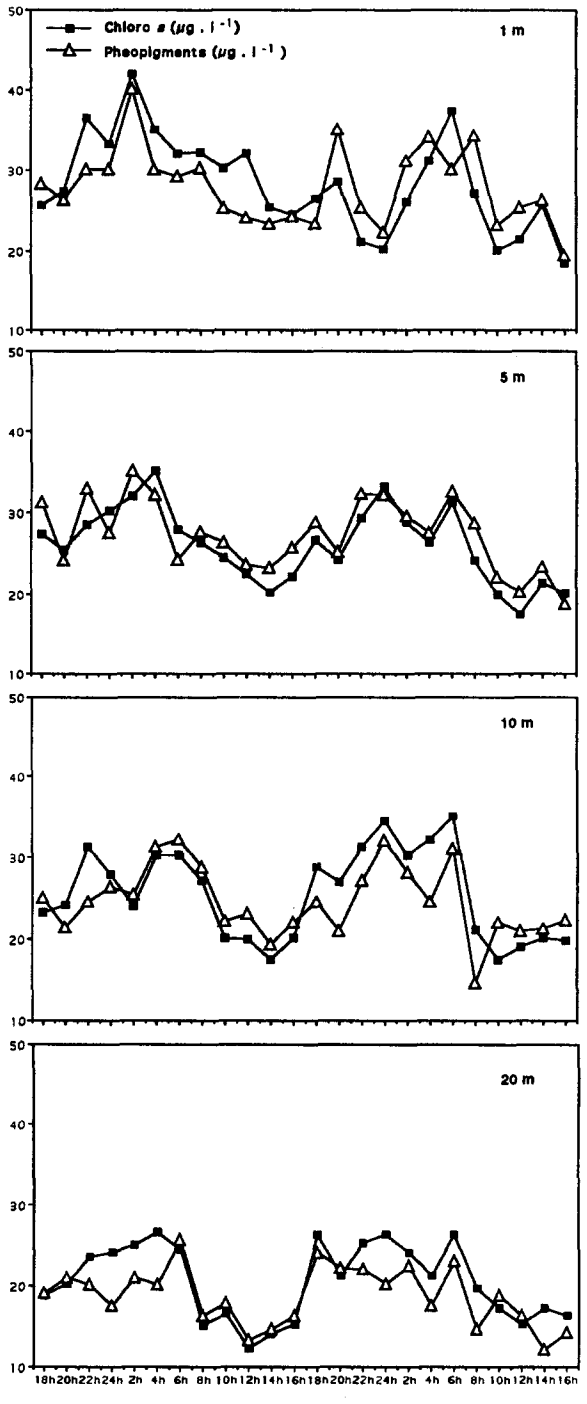

Fig. 2. Diel changes in chlorophyll *a* and pheopigment concentrations.

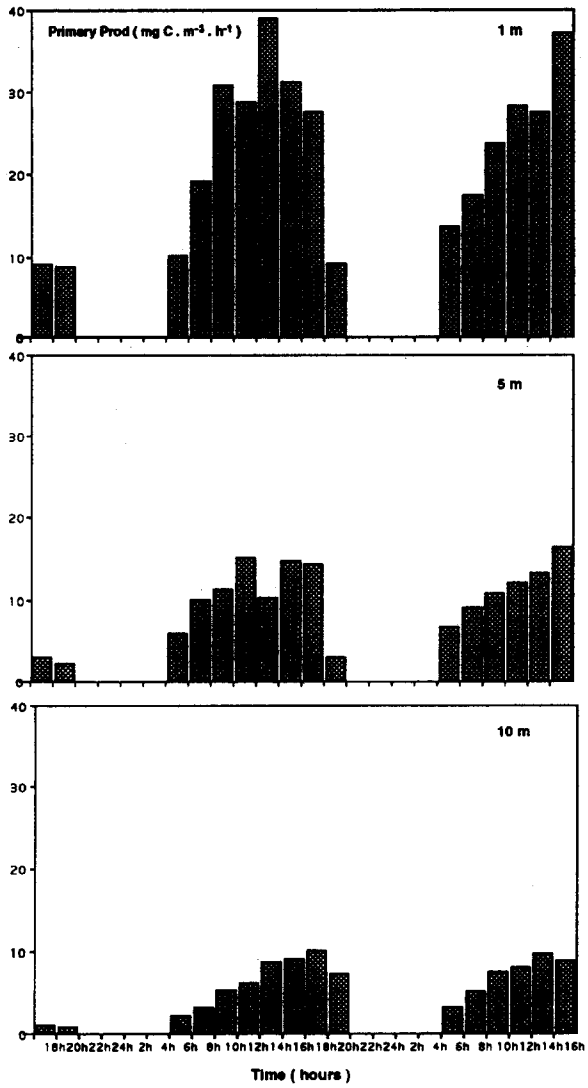

Fig. 3. Diel changes in the photosynthetic activity.

Table 1. Morphometric characteristics of Villerest reservoir.

Volume	128 10^6 m^3
Maximal depth	40 m
Length	40 km
Catchment basin	6520 km^2
Lake surface	30 km^2
Residence time	3 months

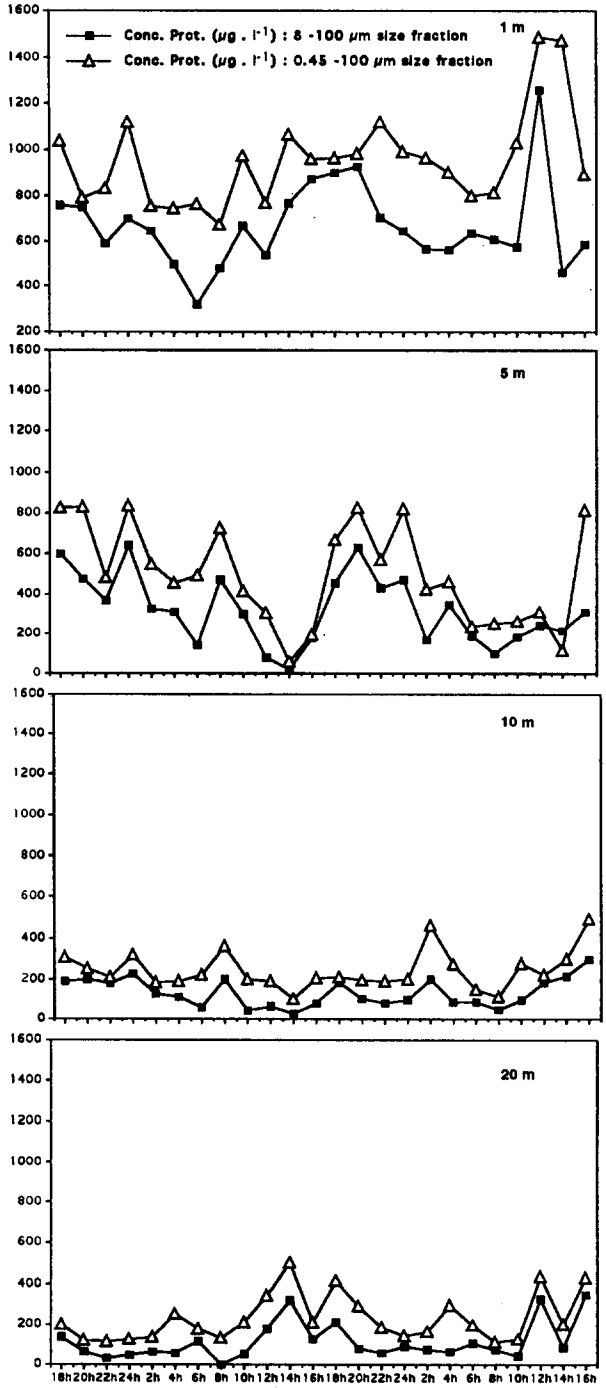

Fig. 4. Diel changes in particulate protein concentrations.

Samplings were made bihourly, started at 18.00 hours (3/7/92) and ended at 16.00 hours (5/7/92). The water was immediately passed through a 100 μm mesh nylon filter to remove the macrozooplankton.

Physical and chemical parameters

The variables: O_2, pH, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$ (soluble reactive phosphorus = SRP), Total *P*, Total N were assessed by standard methods reported elsewhere (Golterman *et al*, 1978). Incident light radiations (350–780 nm) were assessed continuously by an AANDEREA light detector connected to an integrator placed close to the lake shore.

Biovolumes, chlorophyll a, proteins

Algal cells were counted with an inverted microscope by Utermöhl's (1958) method modified by Legendre & Watt (1971–1972). Biovolumes were estimated from cell dimensions according to Lohman (1908). Chlorophyll *a* was extracted according to SCOR-UNESCO (1966) as detailed in Aleya & Devaux (1989). Particulate protein concentrations were determined according to the Lowry *et al.* (1951) method with an albumin (BSA) standard.

Potential alkaline phosphatase activity

Samples (100 ml) were filtered under low vacuum in the field on 8 μm and 0.45 μm Millipore filters. A dissolved fraction was obtained after a filtration on 0.22 μm Millipore filter. The filters and the filtrate were placed into hemolysis tubes containing a solution of Tris-HCl buffer 0.1 M, Mg^{++} 0.001M (pH = 8.5) and 1 mg ml^{-1} of p-Nitrophenyl Phosphate (pNPP). After incubation (6 hours at 37 °C), samples were analysed spectrophotometrically at 410 nm (Reichardt *et al.*, 1967). The results of potential alkaline phosphatase activity (APA) were expressed as mmol p-Nitrophenol (PNP) liberated h^{-1}.

Primary production

Primary production was measured *in situ* by $^{14}CO_2$-incorporation (Steemann-Nielsen, 1977). We used two clear and one dark bottle (100 ml) at each depth, inoculated with 0.5 ml $NaHC^{14}CO_3$ (92.5 Kbq of ^{14}C). Sampled depths were 1, 5 and 10 m.

Results and discussion

Physical-chemical characteristics

The physical-chemical characteristics of lake water varied only slightly during our diel experiments. The distribution of temperature indicated a thermocline between depths 6 and 9 m. In addition, oxygen concentrations were low (Fig. 1).

Concentrations in SRP and TP are summarized in Fig. 1. Either SRP or TP showed little variation.

Phytoplankton, chlorophyll a and primary production

Concentrations in chlorophyll *a* ranged between 19.36 and 40.12 $\mu g\ l^{-1}$. The highest value was recorded at the surface coinciding with an important development of the Cryptophyceae *Crytomonas ovata* (specific biomass: 20 mg l^{-1}). Overall chlorophyll *a* levels were high at depth where the Bacillariophyceae *Cyclotella* sp. prevailed. Values were also high during the night. Pheopigments behaved similarly to chlorophyll *a* concentrations indicating that a large fraction of phytoplankton cells was senescent (Fig. 2). Primary production varied from 0 to 38 mg C $m^{-3}\ h^{-1}$ at subsurface, *i.e.* 1 m (Fig. 3).

Particulate protein concentrations

Concentrations of particulate proteins ranged between 20 and 1262 $\mu g\ l^{-1}$ (size fraction: 8–100 μm) and between 58 and 1486 $\mu g\ l^{-1}$ (size fraction: 0.45–100 μm) (Fig. 4). These concentrations did not show clear diel variations as was noted for chlorophyll *a*.

- At 1 m, low protein concentrations were measured late at night (4 to 6 h). They increased over the day to reach a maximum of 929 $\mu g\ l^{-1}$ (size fraction: 8–100 μm), 1120 $\mu g\ l^{-1}$ (size fraction: 0.45–100 μm) between 16 h and 22 h on 4 July. Other peaks were recorded: 1262 $\mu g\ l^{-1}$ (size fraction: 8–100 μm), 1484 $\mu g\ l^{-1}$ (size fraction: 0.45–100 μm) at 12 h on 5 July.

On the other hand, incident irradiances (Fig. 5) peaked between 9 and 10 h on 4 July and between 11 and 12 h on 5 July. This suggests that the high light intensities have limited protein synthesis. This is in agreement with previous observations made from trophically different lakes (Ganf *et al.*, 1986; Amblard & Bourdier, 1990; Aleya, 1991, 1992) indicating that at high photon irradiance the cell metabolism switches

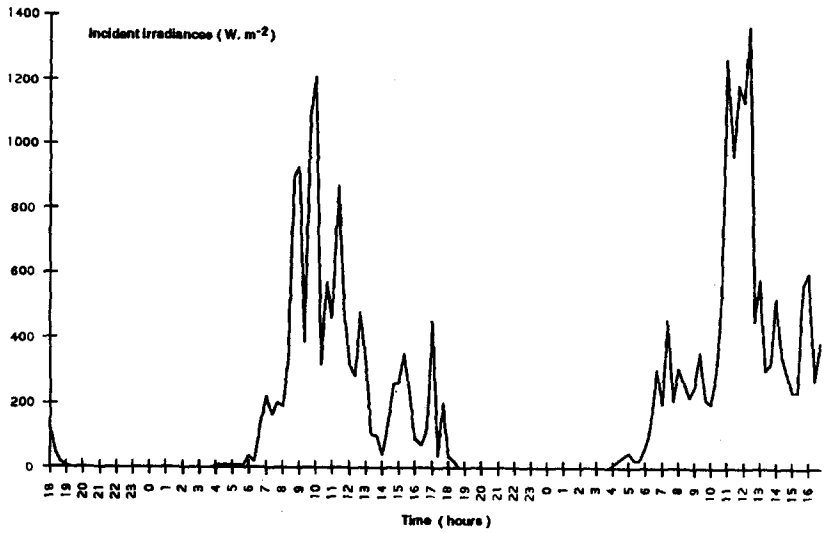

Fig. 5. Diel distribution of incident irradiances measured continuously.

towards an accumulation of reserve products such as carbohydrates.

- At 5 m, although we measured low protein concentrations late at night, the whole diel distribution was somewhat different with a minimum recorded at 12 h.
- At 10 m, protein concentrations changed very slightly indicating an accumulation in the metalimnion of decaying cells.
- At 20 m, an increase in protein concentrations was recorded at 12 h. This would suggest that a fraction of algae was healthy. Also, the decrease of light intensities with depth (due to a decrease of water transparency) would suggest that cells have an endogenous rhythm as reported by Tilzer (1973).

Obviously, photon fluxes impacted on primary production. This was particularly clear at 1 m since light intensities measured on 4 July at 10 h and 5 July at 12 h were correlated, resulting in a saturation of the photosynthetic activity (Fig. 5).

Alkaline phosphatase activity (APA)

Dissolved APA

In this work, we did not detect a dissolved APA. This would indicate that the phosphatases remained attached to both bacteria and algae.

Particulate APA

The gross APA levels were very high. The values ranged between 0.005 and 0.797 and between 0.045 and 1.22 mmol. PNP liberated h^{-1} for size fraction 8–100 μm and 0.45–100 μm, respectively (Fig. 6). The results from the particulate APA indicate the following:

- At 1 m, although the APA of the fraction 0.45–100 μm was not significantly ($p>0.05$) higher than that of fraction 8–100 μm, it suggests that bacteria and/or picoplankton (phototrophic or autotrophic) contributed at least partially to the total measured APA. However, the examination of the distribution of oxygen concentrations indicated that the bulk of the APA is due to heterotrophs, probably bacteria.

Also at this depth, the APA peaked at 18 h, on 4 July and at (6–8 h) on 5 July. This observation would suggest a coupling with primary production which depends on light levels. Indeed, a saturation was recorded at midday, on 4 July and another at the sunset, on 5 July.

At 5 m, the lowest APA were measured late night, over midday, but at 10 and 20 m, the levels did not change much. The APA of both fractions were quite similar indicating low bacterial contribution to the total enzymatic activity.

Specific alkaline phosphatase activity

The results from the specific activities depended on the biomass descriptor used. The values varied from 0.218 to 7.04 μmol. PNP liberated h^{-1} when the biomass is based on chlorophyll *a* estimation. The lowest levels were measured late night, and the highest specific

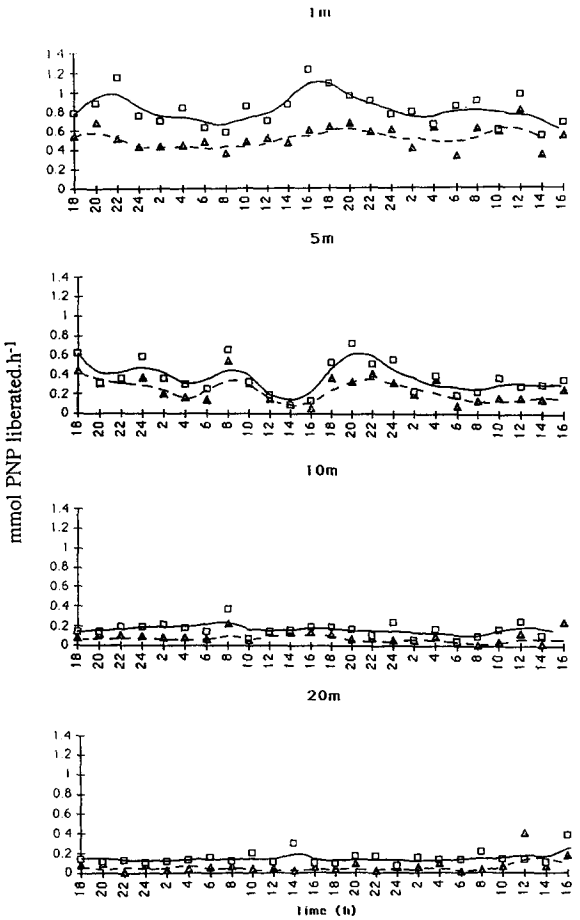

Fig. 6. Diel changes in alkaline phosphatase activity (APA) (expressed as gross and smooth concentrations) of 8–100 μm (—) and 0.45–100 μm (- - -) size fractions.

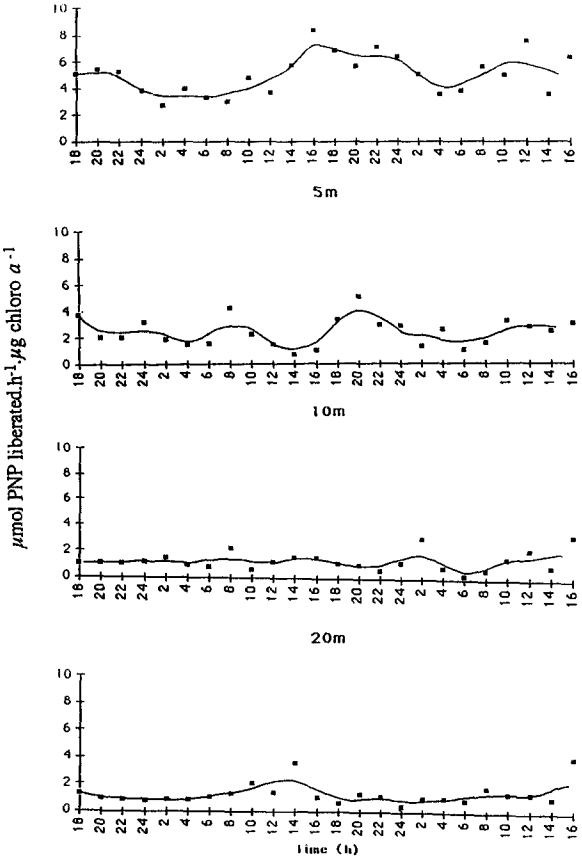

Fig. 7. Diel distribution of the alkaline phosphatase specific activity (expressed as smooth APA/Chlorophyll *a*) relative to fraction 0.45–100 μm.

activities were recorded at 1 m, at midday and over sunset at 5 m early and late in the day. At 10 and 20 m the diel changes of the specific activity were less important than those observed in the euphotic zone (Fig. 7).

Expressed per unit of proteins, the specific activities were considerably higher than those reported in the literature. The lowest diel variations of this parameter were observed in our study suggesting that the synthesis of phosphatases is made in the whole pool of proteins. The positive significant correlation ($r^2 = 0.814$, 22 df.) found between both parameters verified our assumption (Fig. 8).

Conclusion

The APA measured in the hypereutrophic Villerest reservoir exceeded considerably those reported in the literature (Koltz, 1985; Berman *et al.*, 1990).

Several authors have demonstrated that the APA is negatively correlated to concentrations in P-PO_4^{3-} and to internal *P* reserves (Gage & Gorham, 1985; Huber & Kidby, 1985). On the other hand, Berman (1970) argued that the APA measured were attributable more to total *P* than to SRP concentrations. However, since our investigation showed only slight changes of SRP and TP which persisted high, two assumptions can be proposed to explain the high levels of APA:

1. The dissolved mineral phosphorus (*i.e.* SRP) would not be available to microorganisms. Our suggestion is supported by observations made by Francko & Heath (1979), Murphy *et al.* (1983) who stated that in rich organic *P* compound conditions, P-

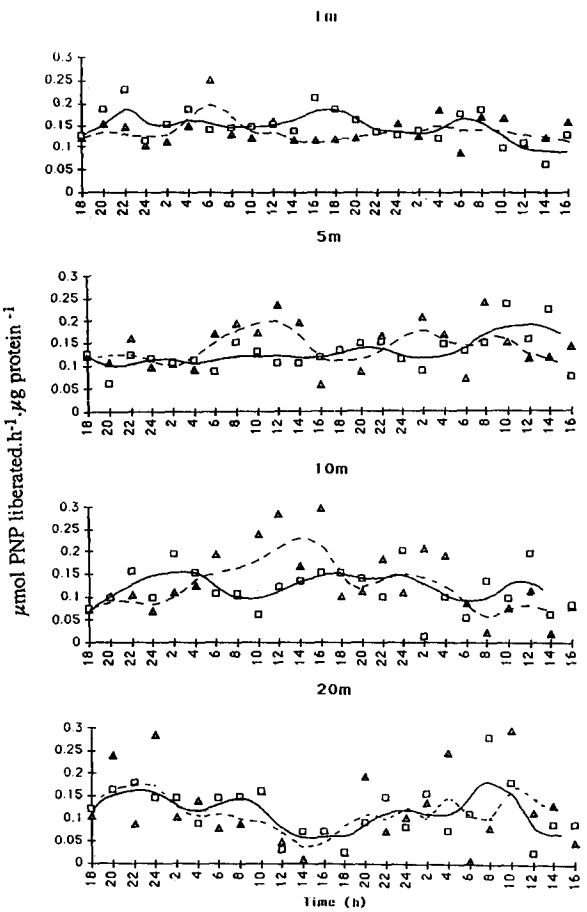

Fig. 8. Diel distribution of the alkaline phosphatase specific activity (expressed as smooth APA/proteins) relative to 8–100 μm (—) and 0.45–100 μm (- - -) size fractions.

PO_4^{3-} would not be bioavailable. Microorganisms must thus cleave inorganic phosphate from organic *P* compounds. This process, obviously, requires an intense production of alkaline phosphatases. A detailed examination of *P* species in Villerest reservoir is needed to clarify our hypothesis.

2. Phytoplankton consisted primarily of decaying cells. According to Grusky & Aaronson (1969), the unhealthy status of the cells is translated into high APA regardless of the concentrations in SRP. Also, Wynne (1981) reported increased APA concomitantly with cell multiplication.

Furthermore, at 1 m the bacterioplankton contributed significantly to total APA. In addition, the APA and protein concentrations showed similar distribution patterns throughout the water column. Clearly, this would indicate a simultaneous synthesis of both compounds. Conversely, the APA and protein concentrations were not correlated to chlorophyll *a* concentrations. This questions the ecophysiological significance of biomass descriptors used in this work.

To summarize, at 1 m, the APA was primarily due to bacteria which actively assimilated organic *P* compounds released by photosynthetic algal metabolism. At 5, 10 and 20 m, the APA was predominantly algal. The high concentrations in SRP would indicate that orthophosphates were not bioavailable. The reverse (*i.e.* availability to phytoplankton) would have resulted in undetectable levels of $P\text{-}PO_4^{3-}$ due to the massive proliferation of algae in Villerest reservoir.

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