

Light Availability May Control Extracellular Phosphatase Production in Turbid Environments

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Abstract Extracellular phosphatase production by phytoplankton was investigated in the moderately eutrophic Lipno reservoir, Czech Republic during 2009 and 2010. We hypothesized that production of extracellular phosphatases is an additional mechanism of phosphorus acquisition enabling producers to survive rather than to dominate the phytoplankton. Hence, we examined the relationship between light availability and phosphatase production, as light plays an important role in polymictic environments. Bulk phosphatase activity was measured using a common fluorometric assay, and the production of phosphatases was studied using the Fluorescently Labelled Enzyme Activity technique, which enabled direct microscopic detection of phosphatase-positive cells. In total, 29 taxa of phytoplankton were identified during both years. Only 17 taxa from the total number of 29 showed production of extracellular phosphatases. Species dominating the phytoplankton rarely produced extracellular phosphatases. In contrast, taxa exhibiting phosphatase activity were present in low biomass in the phytoplankton assemblage. Moreover, there was a significant relationship between the proportion of phosphatase positive species in samples and the $Z_{eu}:Z_{mix}$ ratio (a proxy of light availability). A laboratory experiment with different light intensities confirmed the influence of light on production of phosphatases. Our seasonal study confirmed that extracellular phosphatase production is common in low-abundance populations but not in dominant taxa of the phytoplankton. It also suggested the importance of sufficient light conditions for the production of extracellular phosphatases.

Introduction

Phytoplankton composition, biomass, and productivity are strongly determined by synergistic effects of availability of nutrients and light conditions [1]. The most commonly limiting nutrient in most temperate freshwaters is phosphorus (P), although the P requirement of phytoplankton is relatively small [2, 3]. Phytoplankton can take up only one of the forms of dissolved P in water—orthophosphate. Another important form of P, dissolved organic phosphorus, is not directly bioavailable. However, it may serve as an alternative P source for species able to produce phosphatases [4].

These enzymes hydrolyze various phosphorus-containing dissolved organic compounds into bioavailable orthophosphate [5]. Expression of alkaline phosphatases is regulated by the intracellular P content [6] rather than by external P concentration, since cells have different cellular P-requirements. Moreover, some cells are able to accumulate and store P beyond immediate metabolic needs in a process termed “luxury uptake” [7].

For direct detection of phosphatase activity at a single-cell level, the Fluorescently Labelled Enzyme Activity assay (FLEA, in earlier papers called ELF technique) has been exploited [8–10]. Enzymatic hydrolysis of the soluble fluorogenic substrate, ELF[®]97 phosphate, results in the precipitation of an insoluble fluorescent product, ELF alcohol, at or near the site of the phosphatase activity [11]. Its fluorescent signal is bright and stable, allowing quantification by image cytometry [9].

Production of extracellular phosphatases has been detected in many freshwater and marine phytoplankton species (e.g. [12–17]). Various taxa are exhibiting differences in the presence, localization and labelling pattern of phosphatases. Both seasonal and short-term variations also have been detected in enzyme activity of phytoplankton [15, 18].

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Štrojsová et al. [16] showed that production of extracellular phosphatases may be an advantage for algal and cyanobacterial producers in competition for resources when intracellular reserves or ambient P are scarce. Producers of phosphatases are able to cleave P from dissolved organic phosphorus, and therefore survive and accomplish more cell divisions. On the other hand, Novotná et al. [19] documented a reduced phosphatase production in algal populations under severe P and light limitation (nutrient and energy shortage). Additional evidence comes from Cao et al. [17], who observed species producing phosphatases under excessive P concentrations in a polymictic eutrophic lake. An overall puzzle concerning extracellular phosphatases is that active species rarely dominate a phytoplankton assemblage [13, 14, 16, 19].

Much research has been devoted to investigation of the relationships between both external and internal concentrations of P and phosphatase production. Much less is known about the effects of light, which is the principal energy source for phytoplankton. There are some indications that light availability may have an effect on phosphatase production, e.g., Novotná et al. [19] showed that the production of extracellular phosphatases in a natural dinoflagellate population was likely influenced by light. Also Nedoma et al. [20] reported a coincidence of high phosphatase activity with favourable light conditions in a dimictic reservoir. Despite these contributions, the role of light availability for phosphatase production by phytoplankton still remains largely unknown.

The present study aims to test the hypothesis that production of extracellular phosphatases is an additional mechanism of P acquisition enabling producers to survive rather than to dominate the phytoplankton. Moreover, in order to fill the gap in knowledge, we focused on the effect of light availability on phosphatase production in a polymictic environment.

Methods

Site Description

The study was conducted in the shallow moderately eutrophic Lipno reservoir located in South Bohemia, Czech Republic (726 m a.s.l.; 48°39'17"N, 14°9'47"E for the locality Frymburk). Lipno reservoir was built as the uppermost part of the Vltava River cascade of reservoirs for hydropower production, flood protection and recreation purposes. The reservoir has an area of 46.5 km², a volume of 306 × 10⁶ m³, and a shoreline length of 115 km. Maximal depth is 22 m and the average depth is 6.6 m. The reservoir is dimictic; however, strong thermal stratification is a rare phenomenon [21] as it has its main axis in the northwest to southeast direction, which corresponds also to the prevailing wind directions [22]. Furthermore, the middle portion of the reservoir has the

characteristic of a wide but shallow valley exposed to winds, thus, it could be considered polymictic. Our sampling site at the Frymburk profile is located in this part of the reservoir. The downstream segment towards the dam has the character of a canyon with steep slopes along its shores.

Sampling

Sampling was performed monthly from April to October in 2009 and 2010 (7 samples every year) in co-operation with the Vltava River Basin Authority, the organization managing the reservoir and performing regular monitoring. The mixing depth (Z_{mix}) was estimated according to individual vertical temperature profiles measured using submersible probe with fine scale measurement at 0.2 m intervals (FluoroProbe, bbe-Moldaence, Kiel, Germany) as the depth where the maximum rate of temperature decrease was identified. The euphotic layer (Z_{eu}) was estimated as twice the Secchi depth and the $Z_{\text{eu}}:Z_{\text{mix}}$ ratio was calculated as a proxy of light availability [23]. Integrated epilimnion samples were taken from the depth of 0–4 m and used for further analyses.

Unfiltered subsamples were taken for the isolation of clonal cultures of phytoplankton. Splits from these samples preserved with Lugol's solution and stored in the dark were used for phytoplankton composition and biomass determination. Species were enumerated employing the Utermöhl method [24] on the inverted microscope (Olympus IMT-2). Mean algal cell dimensions were obtained for biomass calculation using the approximation of cell morphology to regular geometric shapes.

Soluble reactive P (SRP) concentrations were determined spectrophotometrically [25], total dissolved P (TDP) was determined by inductively coupled plasma mass spectrometry [26, 27].

Experimental Conditions

To obtain clonal cultures of phytoplankton, the glass-capillary technique was used as described by Zapomělová et al. [28]. All isolates that were tested for phosphatase production were deposited in the culture collection of the Biology Centre of AS CR, Institute of Hydrobiology, České Budějovice, Czech Republic. They were cultured in liquid WC medium [29] under stable culture conditions (21 °C, 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity, spectral range approximately 400–700 nm, 16 h:8 h light/dark cycle). Diatoms were maintained in WC medium enriched with silica. In order to closely investigate the relationship between light availability and phosphatase production we performed experiments with different light conditions. A strain of *Dolichospermum circinale* 26 LIP09/VII was chosen because of its ability to produce phosphatases both in culture and in nature. The culture from exponential phase was further incubated in a batch experiment under several light

intensities: 2, 100, 200, 680, and 830 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, at 20 °C in liquid WC medium [29]. The experiment lasted four days and samples for inspecting phosphatase activity (presence/absence using FLEA assay, see below) were taken in 24-h intervals.

Phosphatase Assays

Bulk phosphatase activity (PA_B) in the whole sample and in the 2- μm filtrate (Poretics membrane filters) was examined in natural samples with the common spectrofluorimetric method [30] using 4-methylumbelliferyl phosphate (MUFPP, Glycosynth, UK). Duplicates of water samples were buffered with Tris/HCl buffer (pH 7.5; 10 mmol L^{-1} final concentration), supplemented with MUFPP (100 $\mu\text{mol L}^{-1}$ final concentration), and incubated for 2 h at 20 °C. The incubation was terminated by HgCl_2 addition (4 mmol L^{-1} final concentrations). Parallel duplicates supplemented with MUFPP and HgCl_2 were always incubated as blanks [31]. Prior to fluorescence reading, an alkaline solution (40 mmol L^{-1} of NaOH with 8 mmol L^{-1} of EDTA- Na_2 final concentrations [32]) was added. Fluorescence was measured using a spectrofluorometer (Spekol 11 with M4 FC 520, Zeiss, Germany; exCitation at 365 nm and emission 465 nm) and corrected for the blank. Bulk phytoplankton activity, i.e. the enzyme activity associated with $>2 \mu\text{m}$ particles, was then calculated as the difference between the total activity and activity in the $<2 \mu\text{m}$ fraction.

Presence or absence of phosphatase activity (binary metric) in the samples was detected using the modified FLEA assay [9]. Water samples (4.5 mL) were incubated with ELF[®]97 phosphate (ELFP, Molecular Probes; 20 $\mu\text{mol L}^{-1}$ final concentration) at the room temperature. All samples were buffered with Tris/HCl buffer (10 mmol L^{-1} final concentration) at pH 7.5 to ensure ELF alcohol (ELFA) precipitation [10]. After a 3-h incubation, samples were prefixed with the HgCl_2 solution (see above) and filtered over mild vacuum ($<20 \text{ kPa}$) through a membrane filter (2 μm pore size, Pragopore). Filters with retained plankton were inspected for the presence of ELFA precipitates with an epifluorescence microscope (Nikon i90, Japan) using the UV-excitation filter set (excitation/emission, 360–370 nm/ $>420 \text{ nm}$). Any cell with a clear ELFA precipitate, whatever its number, fluorescence intensity, and size, was defined as positively labelled (active). A species was considered phosphatase-active when at least one cell of this species in a sample was positively labelled.

Statistical Analyses

Software Prism 5 (GraphPad Software Inc., La Jolla, USA) was used for Student's t tests, simple linear regression and correlations. The same software was used to test differences in phosphatase activity among light treatments. We employed

one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. The arcsine transformation of data was performed prior to ANOVA analysis.

Results

Seasonal Development

TDP concentrations in the reservoir were high at all times (Fig. 1) with annual averages 10.3 $\mu\text{g}\cdot\text{L}^{-1}$ (range 8.0–13.0 $\mu\text{g}\cdot\text{L}^{-1}$) in 2009 and 9.7 $\mu\text{g}\cdot\text{L}^{-1}$ (8.0–13.0 $\mu\text{g}\cdot\text{L}^{-1}$) in 2010. In contrast, SRP concentrations with annual averages of 1.0 $\mu\text{g}\cdot\text{L}^{-1}$ (range 0.3–2.3 $\mu\text{g}\cdot\text{L}^{-1}$) in 2009 and 3.8 $\mu\text{g}\cdot\text{L}^{-1}$ (1.7–7.0 $\mu\text{g}\cdot\text{L}^{-1}$) in 2010 were generally low (Fig. 1). The $Z_{\text{eu}}:Z_{\text{mix}}$ ratio was a value usually less than 1.0 (range 0.2–1.7, Fig. 2), suggesting certain light insufficiency lasting for most of the studied periods.

Fractionation of bulk extracellular phosphatase activity revealed that the PA_B associated with the phytoplankton ($>2 \mu\text{m}$) accounted for most of the total PA_B during both years (Fig 2). The phytoplankton PA_B and total phytoplankton biomass showed significant positive correlation (Pearson's $r=0.51$, $p<0.05$, $R^2=0.27$), whereas there was no significant correlation between the total PA_B and SRP or TDP concentration.

Seasonal changes in total phytoplankton biomass showed a different pattern between the years studied (Figs. 2 and 3). In 2009, the biomass remained low and stable during the whole period studied; while in 2010, there was a noticeable peak in August. Bacillariophyceae (*Aulacoseira italica*, *Melosira varians*) and Cryptophyceae (*Cryptomonas* spp., *Plagioselmis minuta*) were the most abundant species in

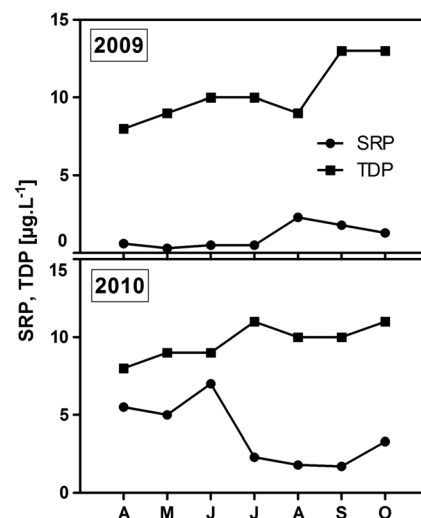


Fig. 1 Seasonal development of soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP)

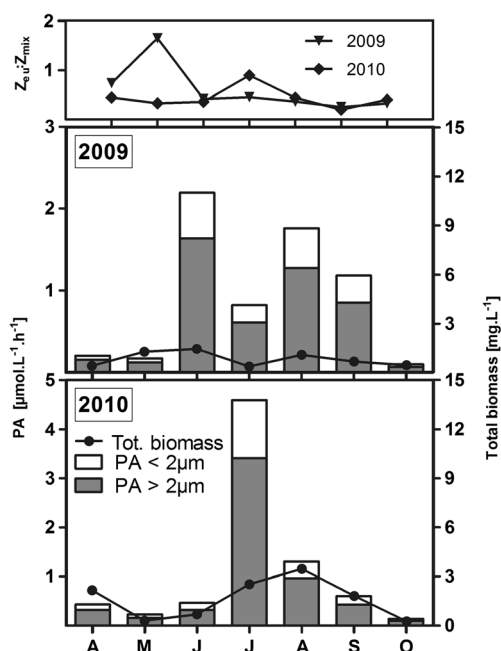


Fig. 2 Seasonal development of the proxy of light availability (Z_{eu}, Z_{mix}), bulk phosphatase activity (PA_B) in either fraction ($PA_B < 2 \mu m$; $PA_B > 2 \mu m$), and total biomass of phytoplankton in 2009 and 2010

spring and autumn, while Cyanobacteria, mostly *Dolichospermum* spp., dominated in summer during both years studied.

Dynamics of Phosphatase Activity on the Species Level

In total, 29 taxa of phytoplankton were identified during both years (Table 1). Of these, 17 taxa showed production of extracellular phosphatase in the reservoir and/or in cultures. ELFA-labelled cells in natural phytoplankton were detected in 12 taxa (Table 2) in three taxonomic groups—Bacillariophyceae, Chlorophyceae, and Cyanobacteria. Production of extracellular phosphatase occurred during the whole period studied (April–October) in both years. The taxa

Fragilaria crotonensis, *Staurastrum planctonicum*, and *Dictyosphaerium* sp. produced the enzyme only in one year (2009), while other species (e.g., *Woronichinia naegeliana*) were active during both years (Table 2). Generally, not all of the cells of a given active population or even of a single colony were ELFA-labelled, except for the green alga *Ankyra ancora*, which expressed enzymatic activity for the whole population whenever present in the phytoplankton.

Most of the taxa from the phytoplankton inspected for ELFA labelling never expressed extracellular phosphatase in natural conditions of Lipno reservoir. However, when the ability to produce the enzyme was tested on monoclonal cultures obtained from the reservoir, some species showed enzymatic activity though they were inactive in nature (e.g., *Dolichospermum* cf. *flos-aque*, *Pediastrum duplex*, Table 1). Species dominating the phytoplankton (i.e., accounting for >10 % of total biomass) produced extracellular phosphatases only rarely during the studied years. *W. naegeliana* and *D. crassum* accounted for 25 % and 13 % of total phytoplankton biomass in July 2009, respectively, whereas *A. italica* and *Cuspidothrix issatschenkoi* for 16 % and 13 % in September 2009, respectively. Other species, which exhibited phosphatase activity, were present in lower (<10 %) biomass in the phytoplankton (Table 2). Moreover, a number of those species produced phosphatases when their contribution to the total biomass was low but they showed little activity when their biomass became a significant part of the phytoplankton. This pattern was particularly apparent and significant in the diatoms *A. italica* and *M. varians* (Tables 2 and 3). The cyanobacteria *D. crassum*, *D. circinale*, and *D. lemmermannii* indicated the same phenomenon, although not so clearly. By contrast, the cyanobacterium *W. naegeliana* was active regardless of the proportion of its biomass to the total biomass (Table 2).

As most species rarely exhibited phosphatase activity, we used a parameter showing the ratio of active species to all inspected species present in samples. It indicated the

Fig. 3 Seasonal development and composition of phytoplankton biomass of 12 taxa exhibiting phosphatase activity. “Others” summarize biomass of all other taxa present in a particular sample

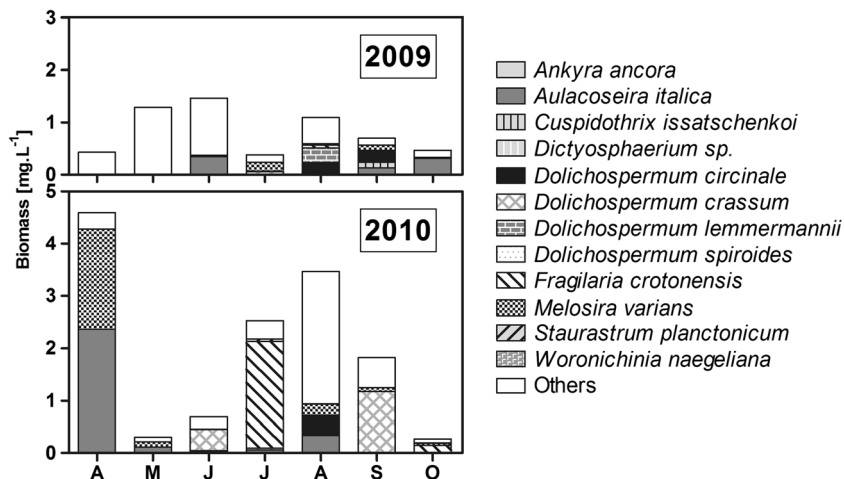


Table 1 List of all taxa from Lipno reservoir, both phytoplankton samples and culture isolates, inspected for phosphatase activity in both years: –, never ELFA-labelled; +, ELFA-labelled; 0, no isolates available

Taxon	Phytoplankton	Culture
Bacillariophyceae		
<i>Asterionella formosa</i>	–	0
<i>Aulacoseira italica</i>	+	0
<i>Diatoma vulgare</i>	–	0
<i>Fragilaria crotonensis</i>	+	0
<i>Melosira varians</i>	+	0
Chlorophyceae		
<i>Ankyra ancora</i>	+	0
<i>Dictyosphaerium</i> sp.	+	–
<i>Dictyosphaerium tetrachotomum</i>	–	0
<i>Eudorina elegans</i>	–	+
<i>Kirchmeliella lunaris</i>	–	–
<i>Monoraphidium contortum</i>	–	0
<i>Planctosphaeria</i> cf. <i>gelatinosa</i>	–	–
<i>Pediastrum duplex</i>	–	+
<i>Scenedesmus</i> sp.	–	0
Cyanobacteria		
<i>Aphanizomenon flos-aquae</i>	–	+
<i>Cuspidothrix issatschenkoi</i>	+	0
<i>Dolichospermum circinale</i>	+	+
<i>Dolichospermum crassum</i>	+	+
<i>Dolichospermum</i> cf. <i>flos-aque</i>	–	+
<i>Dolichospermum lemmermannii</i>	+	–
<i>Dolichospermum spiroides</i>	+	0
<i>Merismopedia punctata</i>	–	0
<i>Microcystis</i> cf. <i>aeruginosa</i>	–	–
<i>Microcystis ichthyoblabe</i>	–	–
<i>Pseudanabaena</i> cf. <i>mucicola</i>	–	–
<i>Woronichinia naegeliana</i>	+	0
Trebouxiophyceae		
<i>Koliella longiseta</i>	–	0
Zygnemophyceae		
<i>Staurastrum planctonicum</i>	+	0
<i>Staurastrum</i> sp.	–	+

proportion of phosphatase positive species. This parameter was significantly related to the $Z_{cu}:Z_{mix}$ ratio (linear regression, $F=5.485$, $R^2=0.31$, $p<0.05$; Fig. 4), a proxy of light availability.

Similar relationship between light intensity and the percentage of cells producing phosphatases in a sample was clearly apparent also in a laboratory experiment (Fig. 5). The percentage of active cells (producing phosphatases) was significantly higher under high light intensity (680 and 830 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) than under lower light intensity (2 and 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at every sampling time. Moreover, the

increase in proportion of active cells was more rapid in high light treatments (Fig. 5).

Discussion

We found large variability in phosphatase production among different taxa and also within the populations of single species. Even the ELFA-labelling in an active population did not show homogenous distribution and the overall frequency of positive cells in one population was usually low. This result is in agreement with many studies employing the FLEA assay on samples from natural environments [14, 17, 33, 34] and supports the hypotheses that individual cells differ in physiological state, and that the need for P is variable in the population [6, 14, 35].

A green alga *Ankyra ancora* appeared to be an exception as it exhibited a strong labelling whenever present in the Lipno reservoir samples. ELFA labelling of almost the entire population was reported previously from another reservoir in the Czech Republic [14, 15]. A possible explanation of this phenomenon could be a very weak red autofluorescence of chlorophyll *a* in *Ankyra* cells and thus inactive cells may be hardly detectable with epifluorescent microscopy. Štrojsová and Vrba [18], however, documented the cells of *A. ancora* without any activity from the Římov reservoir.

One would expect the production of extracellular phosphatases is an advantage for phototrophic producers in the competition for phosphorus in low P environments and consequently they should proliferate and dominate the phytoplankton biomass. Our results, however, contradict this hypothesis. In our study phosphatase activity and high biomass were rarely coupled (Table 2). Also Štrojsová et al. [14] reported only few species, which were ELFA-labelled when they dominated the phytoplankton in their three-year study. Besides, the authors suggested that phosphatase activity of the dominant *Staurastrum planctonicum* was of bacterial origin and the algal cells were in a bad condition. On the other hand, Cao et al. [17] reported a dinoflagellate bloom with phosphatase activity from polymictic Lake Donghu, China, and Novotná et al. [19] measured high species-specific phosphatase activity of dominant dinoflagellate populations in acidified mountain lakes in the Bohemian Forest, Czech Republic. In our study, the taxa which exhibited phosphatase activity were usually present in low biomass in the reservoir phytoplankton (Table 2). Moreover, a number of species produced phosphatase when their proportion in total biomass was low, whereas they did not show phosphatase activity when they were a large fraction of the total biomass (Table 2).

It is evident from the previous studies performed on freshwater and marine plankton [13–15, 36, 37] that relatively less abundant species tend to be phosphatase positive. This is an

Table 2 Summary of phosphatase producing phytoplankton in Lipno reservoir

Taxon	2009							2010						
	A	M	J	J	A	S	O	A	M	J	J	A	S	O
<i>Ankyra ancora</i>	0.1	0.1	0.1	2.2	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0
<i>Aulacoseira italica</i>	1.6	0.1	24	0	0.3	16	65	50	38	0	2.3	9.7	0	0
<i>Cuspidothrix issatschenkoi</i>	0	0	0	0.1	0	13	0.1	0	0	0	0	0	0	0
<i>Dictyosphaerium</i> sp.	0	0	1.4	0.3	0.1	0	0.9	0	0	0	0	0	0	0.52
<i>Dolichospermum circinale</i>	0	0	0	0	21	3.1	0	0	0	6.7	0	11	0	4.7
<i>Dolichospermum crassum</i>	0	0	0	13.2	0	0.1	0	0	0.1	58	1.4	0	65	0
<i>Dolichospermum lemmermannii</i>	0.3	0.1	0.1	0.1	25	0.1	0.1	0	0	0	0	0	0	0
<i>Dolichospermum spiroides</i>	0	0	0	3.3	0	0.1	0	0	0	0	0.1	0	0	0
<i>Fragilaria crotonensis</i>	0	0	0	1.1	5.8	0.4	0.1	0	0	0	80	0.1	0.1	50
<i>Melosira varians</i>	0.5	0.1	0.1	34	1.4	12.1	5.1	42	34	0	0	6.3	3.9	14
<i>Staurastrum planctonicum</i>	0	0	0	1	0.3	0	0	0	0	0	1.7	0	0	0
<i>Woronichinia naegeliana</i>	0.3	0.1	0.1	0.1	25	0.1	0.1	0.1	0.1	1.1	0.1	0.3	0.2	1.4

Species exhibiting phosphatase activity are in bold. Numbers denote contribution (%) of a given species to the total phytoplankton biomass; 0—taxon was absent in a sample

interesting phenomenon suggesting that the production of phosphatase is the strategy to survive in phytoplankton assemblage rather than to dominate it. This is in accordance with conclusions of Rengefors et al. [13] and Štrojsová et al. [14], who stated that the production of phosphatases is not necessary for success in phytoplankton competition and it is rather an additional mechanism of P acquisition under P-limitation. Also Ranhofer et al. [36] reported that relatively less abundant species were ELFA-labelled, indicating that utilization of organic-bound P may not be of broad scale importance to marine phytoplankton growth in Winyah Bay, USA.

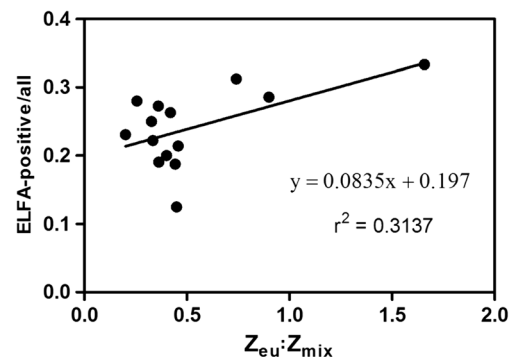
Table 3 Differences between mean contribution (%) of a given species to the total phytoplankton biomass when producing (ELFA positive) or not producing (ELFA negative) phosphatases

Species	ELFA positive	ELFA negative	<i>t</i> test
<i>Ankyra ancora</i>	0.26	–	–
<i>Aulacoseira italica</i>	4.06	37.3	<0.05
<i>Cuspidothrix issatschenkoi</i>	4.40	–	–
<i>Dictyosphaerium</i> sp.	1.40	0.46	–
<i>Dolichospermum circinale</i>	4.83	16.0	NS
<i>Dolichospermum crassum</i>	4.90	41.0	NS
<i>Dolichospermum lemmermannii</i>	0.15	8.40	NS
<i>Dolichospermum spiroides</i>	1.17	–	–
<i>Fragilaria crotonensis</i>	1.10	19.5	–
<i>Melosira varians</i>	2.05	25.0	<0.01
<i>Staurastrum planctonicum</i>	1.70	0.65	–
<i>Woronichinia naegeliana</i>	2.08	–	–

Student's *t* tests, NS not significant

Species differ in their ability to store and utilize phosphorus [38], so that at the same ambient P concentrations species exhibit different degrees of P limitation [6]. In other words, phosphatase production is likely related to specific P-utilization needs of species or individual cells [39, 40]. Although we provided here some evidence that phosphatase production was a survival strategy rather than a competitive dominance strategy, the final confirmation should be an object of further study, since we did not investigate individual P-thresholds for the species identified in this study.

We measured two P forms in Lipno reservoir. TDP overestimate P bioavailability [41]; however, higher concentrations of TDP (Fig. 1) showed a pool of P partially available for producers of phosphatases. In contrast, low SRP concentration indicated possible P limitation of phytoplankton (Fig. 1). These conditions should enhance phosphatase production, but the majority of species did not produce them

**Fig. 4** Linear regression of the proxy of light availability ($Z_{eu}:Z_{mix}$) and the ratio of producing species (ELFA positive) to all the species present in samples

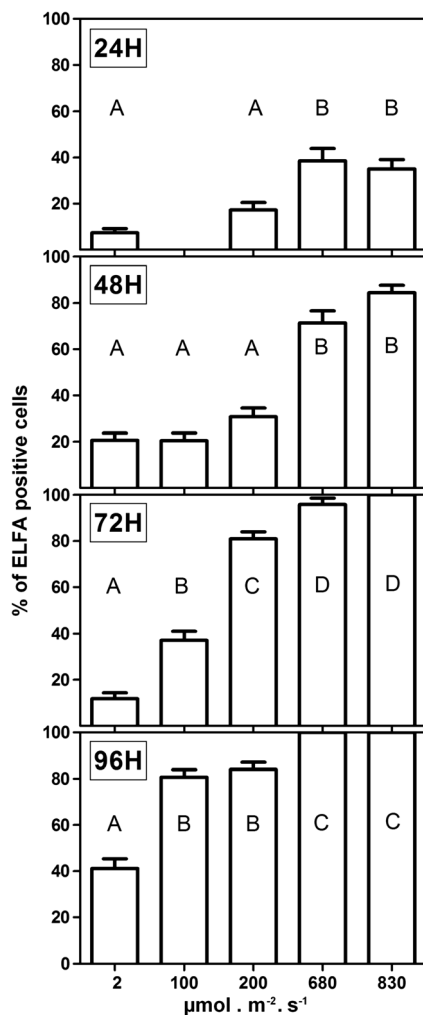


Fig. 5 The relationship between light intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and the proportion (%) of ELFA positive cells (producing phosphatases) in a culture of *Dolichospermum circinale* 26 LIP09/VII. Differences in the percentage of cells showing phosphatase activity was tested (ANOVA with Tukey's multiple comparison test). Same letters indicate the treatments that are not significantly different ($p < 0.05$) within the given time. The sample from 100 μmol treatment in 24H was omitted due to damage of investigated cells

(Table 1). Our data suggest that, besides P concentrations, light availability was an important factor.

Our study showed the importance of sufficient light conditions for the production of extracellular phosphatases as recently suggested by Novotná et al. [19]. The $Z_{\text{eu}}:Z_{\text{mix}}$ ratio indicated almost permanent light limitation [23] at the Frymberk profile, which could lead to the observed low frequency of active species in the populations due to insufficient supply of light energy. Moreover, this low light availability might either impair P acquisition or reduce P demand. Novotná et al. [19] showed that optimal light conditions connected with high P demand resulted in enhanced species-specific PA. The significant positive relationship between the proxy of light availability and number of active species in this

study could indicate that both P and light were co-limiting factors [42, 43] controlling expression of extracellular phosphatases. Our experiment with the batch culture of *D. circinale* suggested that sufficient light intensity caused not only apparently higher growth rate and biomass (not shown), but likely also faster P depletion, which may induce earlier and higher phosphatase activity compared to low light intensity (Fig. 5). On the other hand, the reduced growth under light limitation led to slower P uptake and/or lower P need (sufficient cell quota for P) that neither required nor allowed the production of extracellular phosphatases. Our results suggested that light and P could complement each other, with light facilitating the production of phosphatases and thus the acquisition of P. We therefore argue that light conditions in turbid environments may be still sufficient for autotrophic growth but insufficient for phosphatase production. Such conditions may favour other competition strategies, such as mixotrophy (both phagotrophs and mixotrophs). This phenomenon should be considered and explored in further studies.

Our seasonal study suggests that extracellular phosphatases production is rather common in low-abundant populations but not in the dominant taxa of phytoplankton. Generally, this study indicates that production of extracellular phosphatases is not a competitive advantage in the phytoplankton of turbid environments. In mesotrophic or even humic, polymictic water bodies, such as Lipno reservoir, there are other mechanisms (e.g. light availability), which may be more important in structuring the phytoplankton.

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