Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

# Phytoplankton communities determine the spatio-temporal heterogeneity of alkaline phosphatase activity: evidence from a tributary of the Three Gorges Reservoir

**Yijun Yuan[1,2], Yonghong Bi[1,*] and Zhengyu Hu[1]**

[1] Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, 430072, Wuhan, China

[2] University of Chinese Academy of Sciences, 100049, Beijing, China

*Corresponding author: E-mail address: biyh@ihb.ac.cn.
Tel/fax: +86 27 68780016.

**Abstract.** In order to know the role of phytoplankton communities in the distribution pattern of alkaline phosphatase activity (APA), monthly investigation was conducted in the Xiaojiang River, a tributary of the Three Gorges Reservoir (TGR). Different APA fractions ($APA_T$, $APA_{<0.45\mu m}$, $APA_{0.45\text{-}3\mu m}$ and $APA_{>3.0\mu m}$), environmental parameters, and phytoplankton communities were screened synchronously. Significant spatio-temporal differences of APA with the highest value in summer and the lowest in winter ($P<0.05$) were observed. The annual average $APA_T$ ranged from 7.78-14.03 nmol • $L^{-1}$ • $min^{-1}$ with the highest in the midstream and the lowest in the estuary. The dominant phytoplankton species in summer and winter were Cyanophyta and Bacillariophyta, respectively. The mean cell density in the midstream and in the estuary were $5.2\times10^7$ cell • $L^{-1}$ and $1.4\times10^7$ cell • $L^{-1}$, respectively. That $APA_{>3.0\mu m}$ were significantly higher than $APA_{0.45\text{-}3\mu m}$ indicated phytoplankton was the main contributor to alkaline phosphatase. Correlation analysis indicated the dominant species and cell density could determine the distribution pattern of APA. Turbidity (Turb), total phosphorus (TP), chemical oxygen demand (COD), water temperature (WT), pH and chlorophyll *a* (Chl *a*) were proved to be positively correlated with APA; soluble reactive phosphorus (SRP), conductivity (Cond), transparency (SD) and water level (WL) were negatively correlated with APA. It was concluded that spatio-temporal heterogeneity of APA determined by phytoplankton communities was related to water temperature and hydrodynamics.

## 1 Introduction

Alkaline phosphatase (APase) can hydrolyze broad spectrum phosphomonoesters (Kuenzler and Perras, 1965; Tanaka *et al*., 2008) and associate with cells surfaces of microbial organisms (Gonzalez *et al*.,1998). Both phytoplankton and bacteria can secrete extracellular APase which enables them to use organic P esters as a source of P for

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

compensation of P deficiency (Ivancic *et al*., 2009). The significant seasonal and regional variations of APA were found (Zhang *et al*.,2013). The inverse proportion of alkaline phosphatase activity (APA) to SRP concentration was summarized as "induction-repression" mechanism (Jansson *et al*., 1988). APase plays an important role in the aquatic phosphorus cycling.

Relationship between APA and phytoplankton has been paid more attention since 1960s (Perry, 1972; Kuenzler, 1965). Kalinowska tried to figure out the major contributor of APase through membrane filtration method (Kalinowska, 1997). Even if size fractionation by filtration is never completely absolute (i.e., overlapping size), it still provides useful insights on the major microorganisms possibly contributing to APA. Because of the higher biomass of phytoplankton than bacteria in the open ocean and coastal areas, the phytoplankton makes a bigger contribution to the hydrolysis of DOP to DIP (Nausch, 1998). Therefore, phytoplankton contributed greatly to APA production and was significantly influenced by P bioavailability. Production of extracellular phosphatases has been detected in many phytoplankton species (Rengefors *et al*., 2001; Cao *et al*., 2005; Strojsova *et al*., 2008). 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 (Strojsova and Vrba, 2009). Enzyme-labeled fluorescence (ELF) analysis revealed pronounced differences in the makeup of phytoplankton responsible for APA in San Francisco and Monterey bays (Nicholson *et al*., 2006). Though many studies have been conducted to screen APase in different water bodies, little information could be obtained in the Three Gorges Reservoir (TGR).

TGR is the biggest deep river-type reservoir in the world. More than 170 tributaries carrying runoff and bringing nutrients and pollutants into it, which affected the trophic status and resulted in phytoplankton blooms in many bays of the TGR. To date, little information of APA in the TGR and its tributaries could be found. Due to the complicated relationship between APA and ecological factors, it is necessary to screen the distribution pattern of APA in the TGR. Xiaojiang River is one of the tributaries in the TGR, which was suffered from phytoplankton blooms frequently like other tributaries; eutrophication in Xiaojiang River is very serious after the Three Gorges Dam (TGD)'s impoundment since 2003 (Li *et al*, 2009). In this study, Xiaojiang River was selected as the delegate of the tributary in the TGR, phytoplankton and APA in Xiaojiang River were screened. It was assumed that the phytoplankton community successions may lead to the spatio-temporal heterogeneity of alkaline phosphatase activity. In order to verify this hypothesis, monthly investigation was conducted, different APA fractions ($APA_T$, $APA_{<0.45\mu m}$, $APA_{0.45\text{-}3\mu m}$ and $APA_{>3.0\mu m}$), environmental parameters and phytoplankton communities were screened synchronously. The role of phytoplankton communities in the spatio-temporal heterogeneity of APA and its

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

influence factors in the Three Gorges Reservoir were demonstrated. The results of this study can help to know how APA production changes with phytoplankton communities' successions in TGR.

## 2 Materials and methods

### 2.1 Samples and sites

Xiaojiang River, a tributary of the TGR, originates from Kaixian, Chongqing Municipality with a length of 180 km and watershed area of 5172.5 $km^2$. It flows from north to south; entering into the TGR in Yunyang County. The distance from the estuary to the TGD is 248 km.

Surface water samples (0.5m) were collected with a Van Dorn sampler at seven sampling sites (XJ, HS02, HS01, GY02, GY01, QM02, QM01) (Fig.1) monthly from October 2013 to September 2014. Water temperature (WT), pH, dissolved oxygen (DO) and conductivity (Cond.) were measured using a YSI model Professional Plus multiparameter probe (USA); Transparency (SD) was measured with a Secchi disk; and turbidity (Turb.) was measured with a WGZ-B turbidmeter (XinRui, Shanghai). Water level (WL) was recorded by GPS *in situ*. Concentrations of chlorophyll *a* (Chl *a*), total phosphorus (TP), soluble reactive phosphorus (SRP), chemical oxygen demand (COD) were analyzed in 24 h. Samples for quantitative phytoplankton analyses were fixed with neutral Lugol's solution, and concentrated after 48 h sedimentation (Utermohl, 1931).

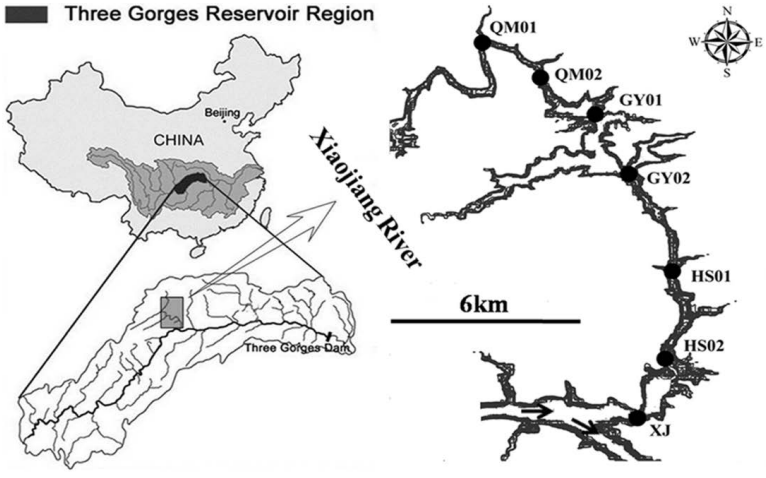



**Figure1.** Maps of the location of the Three Gorges Reservoir Region, and the sampling sites in the Xiaojiang River

### 2.2 Measurement of APA

APA was measured using a modified procedure (Gage and Gorham, 1985; Boon, 1989). A total of 2ml water samples were incubated at 37 ℃ for 4h in the pı Tris-HCl buffer (pH=8.5) and 2ml 0.3mM p-nitronphenylphosphate (p-NPP) as substrate,

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

subsequently, 0.1ml 0.1M NaOH was added into the mixture after 4h. The release of p-nitrophenol from p-nitronphenylphosphate was determined by absorbance at 410nm using a spectrophotometer (TU-1810), and APA was calculated in $nM \cdot L^{-1} \cdot min^{-1}$. APA was determined in unfiltered water ($APA_T$) and water samples filtered through 0.45 (dissolved alkaline phosphatase activity, $APA_{<0.45\mu m}$) and 3.0μm membrane filters ($APA_{<3.0\mu m}$). The activity in algal fraction ($APA_{>3.0\mu m}$) and in bacterial fraction ($APA_{0.45\text{-}3.0\mu m}$) were calculated as follows: $APA_{>3.0\mu m}= APA_T - APA_{<3.0\mu m}$, $APA_{0.45\text{-}3.0\mu m}= APA_{<3.0\mu m} - APA_{<0.45\mu m}$ (Chrost *et al*., 1984).

### 2.3 Measurement of SRP, Chl *a* , TP, COD and phytoplankton quantification

Water samples used for the Chl *a* measurement were filtered with Whatman GF/C filter, then the residuals on the filter were extracted using 90% acetone solution in the darkroom for 24 h at 4°C, and Chl *a* was analyzed spectrophotometrically (A.P.H.A, 1995). The concentrations of SRP were measured after all water samples were filtered through pre-washed filters (Whatman GF/C, glass microfiber filters).The concentrations of SRP, total phosphorus (TP) and chemical oxygen demand (COD) were analyzed according to the standard methods (A.P.H.A, 1995). Phytoplankton was quantified at 400× magnification with a light microscope (OLYMPUS BX41). The identification of phytoplankton species is according to Hu and Wei (Hu and Wei, 2006).

### 2.4 Statistical analysis

Statistical analysis was carried out using the SPSS 13.0 package. Variance analysis (one-way ANOVA) was used to compare the means of APA in different seasons and sampling sites. Non-parametric correlation (Spearman) analyses were employed for determining relationships among $APA_{<0.45\mu m}$, $APA_{0.45\text{-}3\mu m}$, $APA_{>3.0\mu m}$, $APA_T$ and the environmental factors. Detrended correspondence analysis (DCA) of the size-fractionated APA and environmental data was performed using CANOCO version 4.5 to determine whether linear or unimodal ordination methods should be applied. Before the analysis, the abiotic and biological data were transformed by log(x+1). Redundancy analysis (RDA) was performed to get an approximate ordering of the size-fractionated APA's optima for environmental variables. The significance of canonical axes and environmental variables to explain the variance of the size-fractionated APA was tested using Monte Carlo simulations with 499 permutations.

## 3. Results

### 3.1 $APA_T$ distribution pattern

The $APA_T$ ranged from 1.19-47.6 $nmol \cdot L^{-1} \cdot min^{-1}$(Fig.2). The lowest level of $APA_T$ was observed in winter. Besides, the average $APA_T$ in summer and autumn were significantly higher than in other seasons ($P<0.05$). Meanwhile, significant difference between summer

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

and autumn were not detected ($P$>0.05). The mean water level was high in winter(169.7±4.5 m) and low in summer(149.3±3.1 m), the variations of water level presented different trends with that of $APA_T$ at temporal scales.

The highest value of annual average $APA_T$ in GY01 and lowest in XJ were also showed in Fig.2. No difference of $APA_T$ among the seven sites was observed in winter and spring ($P$>0.05). The average $APA_T$ of GY01 in summer and autumn are significantly higher than those of HS02 and XJ ($P$<0.05).

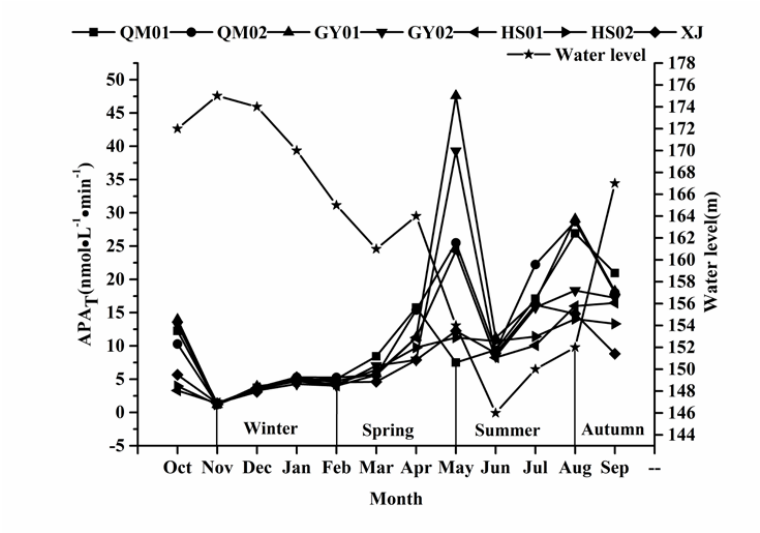



**Figure 2.** Seasonal variations in $APA_T$ concentrations in different sample sites and water level of the Xiaojiang River

### 3.2 Size-fractionation of APA

The average size-fractionated APA indicated that $APA_{<0.45\mu m}$ accounted for the major portion of $APA_T$, whereas the average $APA_{>3.0\mu m}$ are significantly higher than $APA_{0.45\text{-}3\mu m}$ ($P$<0.05) (Fig.3a). The average $APA_{>3.0\mu m}$ accounted for 28.1% of $APA_T$ and $APA_{0.45\text{-}3\mu m}$ accounted for 16.7%. In addition, the size-fractionated APA ($APA_{<0.45\mu m}$, $APA_{0.45\text{-}3\mu m}$ and $APA_{>3.0\mu m}$) in summer and autumn are significantly higher than those in winter ($P$<0.05).

At spatial scales, the average $APA_T$ consisted of 30.2% $APA_{>3.0\mu m}$ and 20.4% $APA_{0.45\text{-}3\mu m}$ in all sites. The $APA_{<0.45\mu m}$ kept a relatively stable and high level. Both $APA_{0.45\text{-}3\mu m}$ and $APA_{>3.0\mu m}$ in midstream (GY01) are higher than those in estuary (XJ) ($P$<0.05).

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

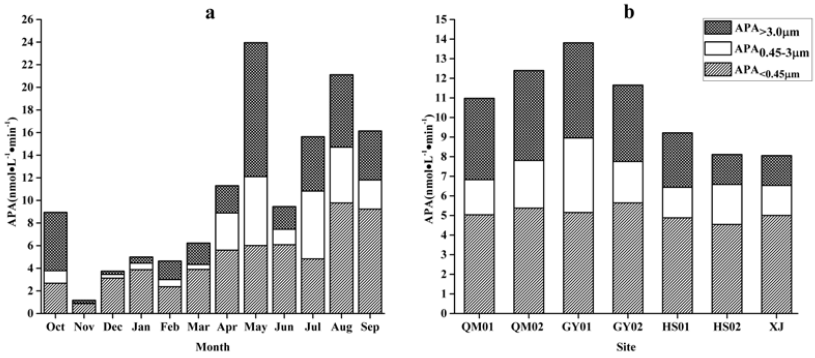



**Figure 3.** Seasonal (a) and spatial (b) variations of average size-fractionated APA in the Xiaojiang River. $APA_{>3.0\mu m}$: the alkaline phosphatase activity in algal fraction; $APA_{0.45\text{-}3.0\mu m}$: the alkaline phosphatase activity in bacterial fraction; $APA_{<0.45\mu m}$ : dissolved alkaline phosphatase activity

### 3.3 Phytoplankton communities

Bacillariophyta was the dominant group in winter and spring (72.7% in average, Fig.4). In summer and autumn, phytoplankton mainly consisted of Cyanophyta (65.6% in average) except the Cryptophyta accounted for 88.4% in August. The mean algal cell density was the highest in July 2014 ($1.27\times10^{8}$cell • $L^{-1}$), and the lowest in January 2014 ($1.3\times10^{6}$cell • $L^{-1}$). The cell density was higher in summer and autumn than in spring and winter.

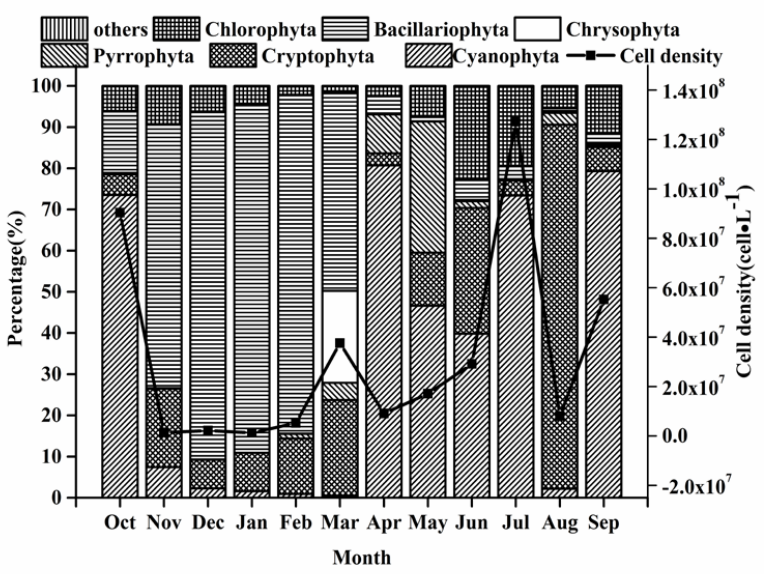



**Figure 4.** Seasonal variations of algal composition and algal cell density in the Xiaojiang River

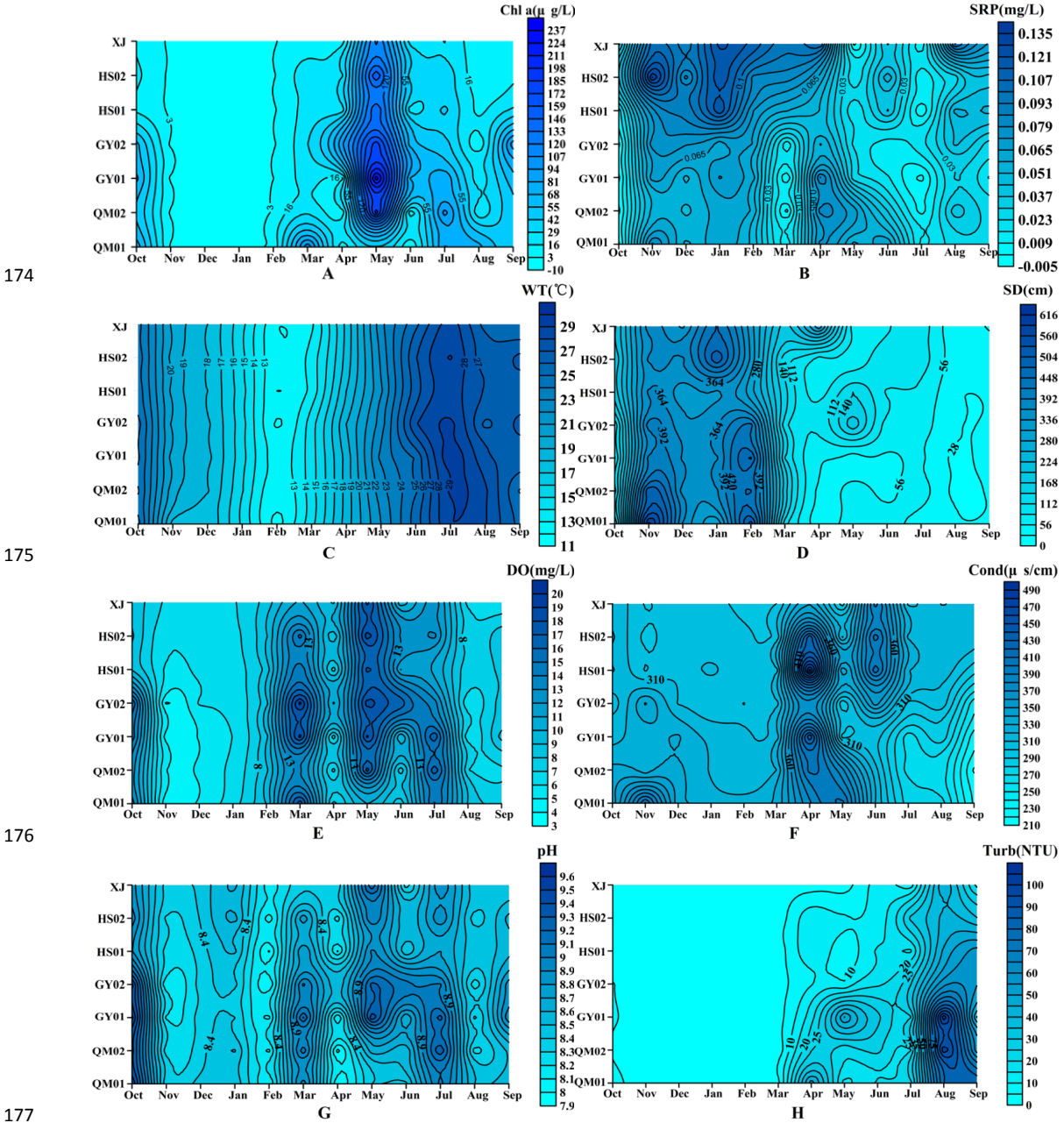
### 3.4 Spatio-temporal characteristics of Chl *a* and environmental parameters

Significant seasonal variations of Chl *a* and environmental parameters could be observed

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

(Fig.5). The values of Chl *a*, TP, COD in spring were apparently higher than the values of other seasons, because the river suffered a *Microcystis* sp. bloom in May, which also resulted in the minimum values of SRP and SD emerged. The levels of TP, COD, Chl *a*, WT, Turb, DO and pH stayed low in winter, contrary to the values of SRP and SD. The values of SRP fluctuated more frequently than other parameters in different seasons. The concentrations of SRP in estuary (XJ) were higher than in upstream. Chl *a* in estuary were higher than that in upstream in May and the values was higher in upstream in March.

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.



Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

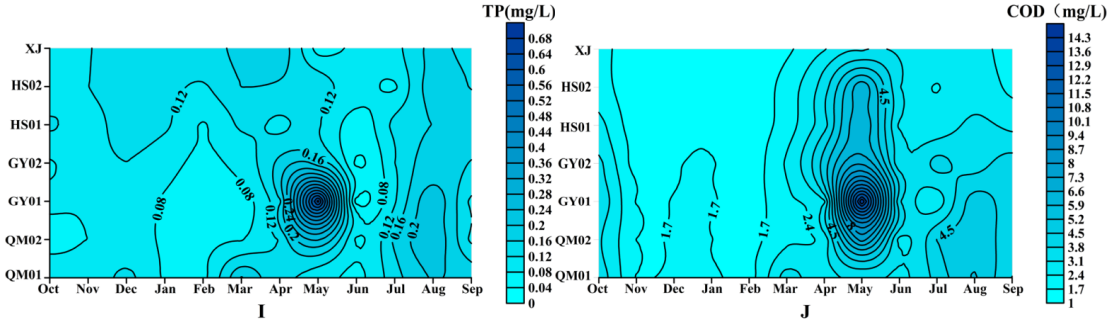



**Figure 5.** Temporal and spatial variations of A: chlorophyll *a* (Chl *a*) and other environmental parameters. B: soluble reactive phosphorus (SRP); C: water temperature (WT); D: transparency (SD); E: dissolved oxygen (DO); F: conductivity (Cond); G: pH; H: turbidity (Turb); I: total phosphorus (TP) and J: chemical oxygen demand (COD)

### 3.5 Relationships between APA and environmental parameters

SRP concentrations showed negative correlation to $APA_{<0.45\mu m}$ (Fig.6a), $APA_{0.45\text{-}3\mu m}$(Fig.6b), $APA_{>3.0\mu m}$(Fig.6c) and $APA_T$(Fig.6d). The Spearman correlations among environmental variables and $APA_{<0.45\mu m}$, $APA_{0.45\text{-}3\mu m}$, $APA_{>3.0\mu m}$ and $APA_T$ were presented in Table 1. Turb, TP, COD, WT, pH and Chl *a* were positively correlated with APA fractions. Cond., SD and WL were negatively correlated with APA.

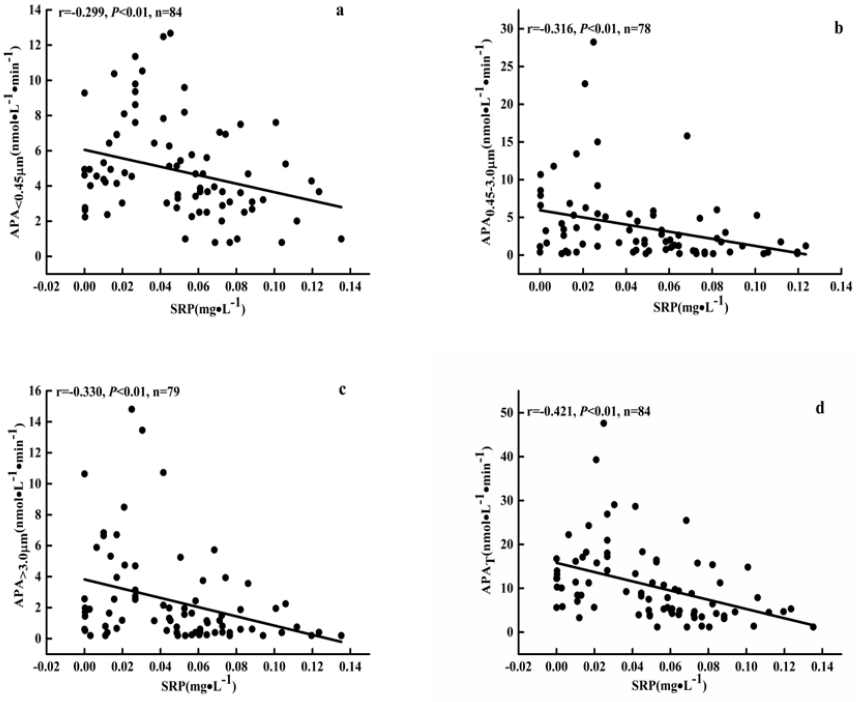



**Figure 6.** Relationship between soluble reactive phosphorus (SRP) concentrations and $APA_{<0.45\mu m}$ (a), $APA_{0.45\text{-}3\mu m}$ (b), $APA_{>3.0\mu m}$(c) and $APA_T$(d) in the Xiaojiang River

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

**Table 1.** Spearman correlations between APA and 10 environmental variables: water temperature (WT); chlorophyll a (Chl a); transparency (SD); dissolved oxygen (DO); conductivity (Cond); pH, turbidity (Turb); total phosphorus (TP); chemical oxygen demand (COD); water level (WL) in the Xiaojiang River

| | $APA_T$(n=84) | $APA_{<0.45\mu m}$(n=84) | $APA_{>3.0\mu m}$(n=78) | $APA_{0.45\text{-}3\mu m}$(n=79) |
|---|---|---|---|---|
| WT | 0.642** | 0.562** | 0.404** | 0.609** |
| Chl *a* | 0.749** | 0.469** | 0.564** | 0.637** |
| SD | -0.844** | -0.815** | -0.586** | -0.698** |
| DO | 0.478** | | 0.382** | 0.368** |
| Cond | -0.251* | | -0.256* | |
| pH | 0.405** | | 0.271* | 0.271* |
| Turb | 0.858** | 0.834** | 0.582** | 0.753** |
| TP | 0.388** | 0.357** | 0.346** | 0.413** |
| COD | 0.858** | 0.684** | 0.646** | 0.751** |
| WL | -0.678* | -0.699* | | -0.713** |

*$P<0.05$

**$P<0.01$

Redundancy analysis (RDA) was performed to analyze the relationship between environmental parameters and size-fractionated APA. The ordination diagrams of environmental variables and size-fractionated APA for axis 1 and axis 2 were shown in Fig.7. The Monte Carlo test revealed that the first canonical axis and all canonical axes were significant (F=25.932, P=0.002; F =3.086, P=0.002; 499 random permutation). For environmental variables and size-fractionated APA, all canonical axes cumulatively explained 83.3% of the variance in APA–environment relationships, and the first two canonical axes accounted for 26.5% and 31.5% of the variance separately. The first axis was positively correlated with Chl *a* (0.65), DO (0.57), COD (0.65) and negatively correlated with SRP (−0.41), SD (−0.38) and WL (−0.30). The second axis was mainly negatively correlated with Cond (−0.13) and WT (−0.16). $APA_{<0.45\mu m}$ and $APA_{>3.0\mu m}$ was the major portion of $APA_T$. $APA_T$, $APA_{>3.0\mu m}$ and $APA_{0.45\text{-}3\mu m}$ were located on the right-hand side of the biplot. They were correlated negatively with WL, SD, SRP and Cond, and positively with other parameters.

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

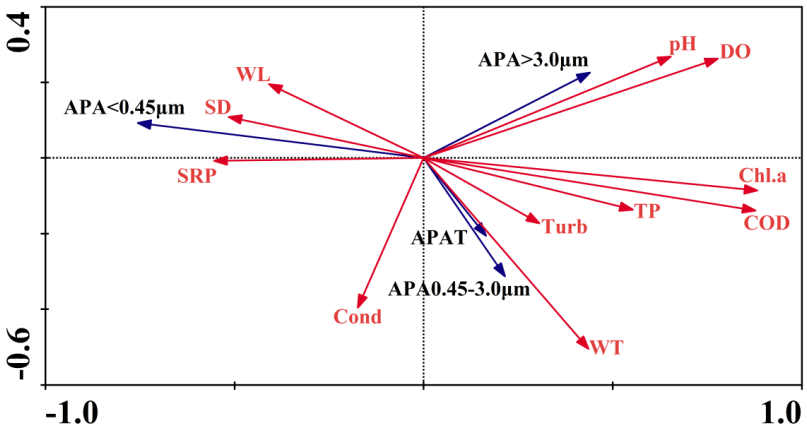



**Figure 7.** Biplot diagrams for RDA of the relationship between 11 environmental variables (red lines) and $APA_T$, $APA_{<0.45\mu m}$, $APA_{>3.0\mu m}$, $APA_{0.45\text{-}3\mu m}$ (blue lines.)

### 3.6 Relationships between $APA_{>3.0\mu m}$ and algal cell density

$APA_{>3.0\mu m}$ reached the highest in midstream (GY01) in May (28.24 nmol • $L^{-1}$ •$min^{-1}$), and undetectable in estuary (XJ) in December. Values ranged from 0.19-22.71 nmol • $L^{-1}$ • $min^{-1}$at the other sites. The mean cell density was the highest in midstream (GY02, $5.2\times10^7$cell • $L^{-1}$) and the lowest in estuary (XJ,$1.4\times10^7$cell • $L^{-1}$). A significant positive relationship was found between $APA_{>3.0\mu m}$ and cell density among all sites (Fig.8).

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

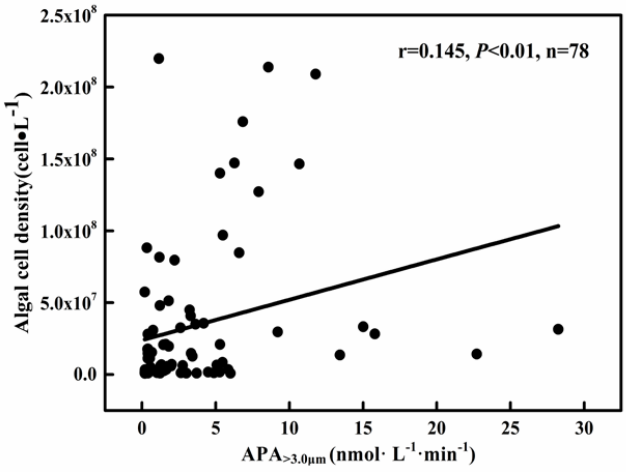



**Figure 8.** Relationships between $APA_{>3.0\mu m}$ and cell density in all sites

## 4. Discussion

APase has different sources, different kinds of bacteria, phytoplankton and zooplankton can excrete extracellular phosphatase (Davey *et al*., 2001). Specific APA was related to different phosphatase producing organisms. Phytoplankton associated phosphatase activity is considered as a phosphorus deficiency indicator (Rose and Axler, 1997). The coarser fraction ($APA_{>3.0\mu m}$), mainly from algae, was conventionally defined as "algal APA" (Liu *et al*., 2012). It was confirmed $APA_{>3.0\mu m}$ accounted for the major portion of total APA (55–87.9%) than $APA_{0.45\text{-}3\mu m}$ (Cao *et al*, 2010). It could be deduced that the phytoplankton was the major contributor of bulk APA based on the larger proportion of $APA_{>3.0\mu m}$(52.73%) than $APA_{0.45\text{-}3\mu m}$(21.09%) (Wang *et al*., 2015). In this investigation, $APA_{>3.0\mu m}$ contributed in average 28.1% in the $APA_T$, while bacterial APA accounted for 16.7%, APA in algal fraction ($APA_{>3.0\mu m}$) was also higher than that in bacterial fraction ($APA_{0.45\text{-}3\mu m}$). Therefore, the phytoplankton contributed greatly to APA production that was consistent with the observations in Wangyu River in China (Wang *et al*., 2015). Meanwhile, the dissolved APase ($APA_{<0.45\mu m}$) kept a relative stable and high level (53.4% of the $APA_T$). Some studies showed that the dissolved APase represents a significant part of the total activity. For example, Labry *et al* reported that dissolved APA represented 13% to 44% of $APA_T$ in the Bay of Biscay (on the French Atlantic coast) (Labry *et al*., 2005). Higher proportions were recorded in the northern Red Sea (42–74%) (Li *et al*., 1998). The dissolved APase can be liberated into the environment through the lysis of dead phytoplankton cells and from cells damaged by zooplankton grazing (Chrost, 1991). The high values may result from physical damage of cells by water current and zooplankton grazing on phytoplankton. Nevertheless, some study

Biogeosciences Discuss., doi:10.5194/bg-2016-455, 2016
Manuscript under review for journal Biogeosciences
Published: 12 December 2016
© Author(s) 2016. CC-BY 3.0 License.

found that the dissolved APA might origin from bacteria (Hoppe and Ullrich, 1999). In order to elucidate the origins of dissolved APA, Song *et al* microencapsulated the dissolved alkaline phosphatase into reverse micellar media. Finally, they proved that the different behaviors of dissolved phosphatase of surface and overlying water might be due to the different origins, with the former being algae and the latter being bacterial (Song *et al*.,2005). It was deduced that phytoplankton acted as the main contributor of dissolved APA in our research. Besides, the positive relationships between APA and the environmental parameters that have been treated as the indexes of the productivity and trophic status, such as Chl *a*, Turb and COD, and the negative relationship between APA and SD can also indicate that the phytoplankton is the main contributor of APA.

The introduction of Enzyme-labeled fluorescence (ELF) method can not only demonstrates the existence of extracellular APase, but also localize where they are (Rengefors *et al*., 2001). Different algal species showed significant different secreting ability of APase. Pyrrophyta, Bacillariophyta, and Chlorophyta can easily produce extracellular phosphatase as evidenced by ELFA labeling (Cao *et al*., 2010). In this study, phytoplankton communities were dominated by Bacillariophyta in winter. The low $APA_{>3.0\mu m}$ during this period may result from the low algal cell density of phytoplankton. Results in some shallow eutrophic lakes revealed that the species belonging to Pyrrophyta were regularly phosphatase-positive, while Bacillariophyceae were phosphatase negative except *Aulacoseira* sp. (Cao *et al*., 2009). Dinoflagellates were poor competitors for phosphate accumulation compared to diatoms; they have to excrete much more APase than diatom to hydrolyze DOP to satisfy their P demand, even when phosphate is adequate (Rengefors *et al*.,2003). In nutrient addition experiments, a higher percentage of dinoflagellates were identified with cell-specific APA than diatoms (Dyhrman *et al*., 2006). It can explain why the $APA_{>3.0\mu m}$ peaked in May when the Pyrrophyta subdominated the phytoplankton community. It was consistent with the results in Monterey Bay that dinoflagellates comprised only 14% of all cells counted and accounted for 78% of APase-producing cells examined (Nicholson *et al*.,2006). As the cell density of Cyanophyta increased in summer and autumn, the $APA_{>3.0\mu m}$ was also prompted. *Microcystis aeruginosa* was confirmed can also synthesize APase (Tan *et al*., 2012). The dominating of Cyanophyta during the summer and autumn resulted in the high amount of APA. The synchronous pattern of alkaline phosphatase activity and algal cells amount can also be found in Jialing River (Pu *et al*., 2014). The higher algal cell density in midstream than in estuary can also explain why the $APA_T$ was higher in midstream. It could be concluded that phytoplankton communities determined the level of $APA_{>3.0\mu m}$, which determined the significant seasonal and regional variations of $APA_T$ .

APA showed significant seasonal and regional variations, with lower value in inlet waters and higher value in the estuarine, and relatively low in winter and high in summer (Jansson *et*

© Author(s) 2016. CC-BY 3.0 License.

*al*.,1988). However, the distribution characteristics of APA in this study were not consistent strictly with the above mentioned. The $APA_T$ fluctuated frequently from spring to autumn. Relative stable level of $APA_T$ in winter can be seen in Figure 2. This phenomenon may result from the fluctuant water level of the TGR. For the sake of flood control and hydropower, the water level in the TGR is subjected to the specific management of the TGD and is meant to seasonally fluctuate between 145 and 175 m a.s.l. It has been demonstrated that the turbulence promoted the phytoplanktonic APA and accelerated the biogeochemical cycle of P in Lake Taihu (Zhou *et al*., 2016). This was consistent with our results that the high APA was present during the significant water level fluctuated period from spring to autumn. Meanwhile, it has been proved that the APA increased with water temperature (Healey and Hendzel, 1979; Huber and Kidby, 1984). The positive relationship between WT and APA in this study (Table.1) supports the conclusion that WT determined the APA through its effects on the phytoplankton seasonally and the direct influences on APase.

## 5. Conclusions

The size-fractionation of APA indicated that the phytoplankton contributed greatly to APA production and the spatio-temporal heterogeneity was the characteristics of APA distribution pattern. The phytoplankton communities with different dominant species and the algal cell density determined the significant seasonal and regional variations of $APA_T$. Water level and water temperature were also proved related to the APA's spatio-temporal variation.

*Acknowledgements.* This study has been jointly supported by the National Natural Science Foundation of China (No: 31123001) and the Science and Technology Research Project of China Three Gorges Corporation (No. CT-12-08-01). We would like to thank Dianbao Li and Yi Yang for valuable assistance during the field sampling. The help of Chunxiang Xia, Haiyu Niu and Wujuan Mi with the laboratory analysis is greatly appreciated.

## References

A.P.H.A.(1995) Standard methods for the examination of water and wastewater. 19th ed.American Public Health Association, Washington D.C.

Boon, P.I. (1989) Organic matter degradation and nutrient regeneration in Australian freshwaters.I: Methods for exoenzyme assays in turbid aquatic environments. *Archiv für Hydrobiologie*., **115**,339-359.

Cao, X., Song, C. and Zhou, Y. (2010) Limitations of using extracellular alkaline phosphatase activities as a general indicator for describing P deficiency of phytoplankton in Chinese shallow lakes. *J Appl Phycol*., **22**,33-41.

Cao, X., Song, C., Zhou, Y., Strojsova, A., Znachor, P., Zapomelova, E. and Vrba, J. (2009)

© Author(s) 2016. CC-BY 3.0 License.

Extracellular phosphatases produced by phytoplankton and other sources in shallow eutrophic lakes (Wuhan, China): taxon-specific versus bulk activity. *Limnology*., **10**, 95-104.

Cao, X., Strojsova, A., Znachor, P., ZApomelova, E., Liu, G., Vrba, J. and Zhou, Y. (2005) Detection of extracellular phosphatases in natural spring phytoplankton of a shallow eutrophic lake (Donghu, China). *Eur J Phycol.*, **40**,251–258

Chrost, R.J. (1991) Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Chróst RJ (ed) Microbial enzymes in aquatic environments. Springer, New York Berlin Heidelberg. p. 29.

Chrost, R.J., Siuda, W. and Halemejko, G. (1984) Longterm studies on alkaline phosphatase activity (APA) in a a lake with fish-aquaculture in relation to lake eutrophication and phosphorus cycle. *Archiv für Hydrobiologie*., **70**,1–32.

Davey, K.E., Kirby, R.R., Turley, C.M., Weightman，A.J. and Fry, J.C. (2001) Depth variation of bacterial extracellular enzyme activity and population diversity in the northeastern North Atlantic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography.*, **48**,1003-1017.

Dyhrman, S. T. and Ruttenberg, K. C. (2006) Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization. *Limnol Oceanogr.*, **51**,1381-1390.

Gage, M. and Gorham E. (1985) Alkaline phosphatase activity and cellular phosphorus as index of the phosphorus status of phytoplankton in Minnesota Lakes. *Freshw Biol.*, **15**,227-233.

Gonzalez-Gil, S., Keafer, B., Jovine, R. and Anderson, D.M. (1998) Detection and quantification of alkaline phosphatase in single cells of phosphorus-starved marine phytoplankton. *Mar Ecol Prog Ser.*, **164**,21-35.

Healey F.P. and Hendzel, L.L. (1979) Fluorometric measurement of alkaline phosphatase activity in algae. *Freshw Biol.*, **9**,429-439.

Hoppe, H.G. and Ullrich, S. (1999) Profiles orectoenzy~nesin the Indian Ocean: phenomena of phosphatase activity in the inesopelagic zone. *Aquat Microb Ecol.*, **19**, 139-148.

Hu, H. and Wei, Y. (2006) The freshwater algae of China-systematics, taxonomy and ecology. Science Press, Beijing.

Huber A.L. and Kidby D.K. (1984) An examination of the factors involved in determining phosphatase activities in estuarine water. 1: Analytical procedures. *Hydrobiologia.*, **111**,3-11.

Ivancic, I., Radic, T., Lyons, D.M. and Kraus, R. (2009) Alkaline phosphatase activity in relation to nutrient status in the northern Adriatic Sea. Mar Ecol Prog Ser., **378**,27-35.

© Author(s) 2016. CC-BY 3.0 License.

Jansson, M., Olsson, H. and Pettersson, K. (1988) Phosphatase-origin,characteristics and function in lakes. *Hydrobiologia.*, **170**,157-175.

Kalinowska, K. (1997) Eutrophication processes in a shallow, macrophyte dominated lake–alkaline-phosphatase activity in Lake Łuknajno (Poland). *Hydrobiologia*., **342**, 395-399.

Kuenzler, E. J. (1965) Glucose - 6 - phosphate utilization by marine algae1. *J Phycol.*,**1**, 156-164.

Kuenzler, E. J. and Perras, J. P. (1965) Phosphatases of marine algae. *Biol Bull.*, **128**,271-284.

Labry, C., Delmas, D. and Herbland, A. (2005) Phytoplankton and bacterial alkaline phosphatase activities in relation to phosphate and DOP availability within the Gironde plume waters (Bay of Biscay). *J Exp Mar Biol Ecol.*, **318**,213-225.

Li, H., Veldhuis, M. and Post, A.F. (1998) Alkaline phosphatase activities among planktonic communities in the northern Red Sea. *Mar Ecol Prog Ser.*, **173**,107-115.

Li, Z., Fang, F., Guo, J. and Tian, G.(2009) Spring algal bloom and nutrients characteristics in Xiaojiang River backwater area, Three Gorge Reservoir. *Journal of Lake Sciences.*, **21**,36-44.

Liu, H., Zhou, Y., Xiao, W., Ji, L., Cao, X. and Song, C. (2012) Shifting nutrient-mediated interactions between algae and bacteria in a microcosm: evidence from alkaline phosphatase assay. *Microbiological research*., **167**,292-298.

Nausch, M. (1998) Alkaline phosphatase activities and the relationship to inorganic phosphate in the Pomeranian Bight (southern Baltic Sea). *Aquat Microb Ecol.*, **16**,87-94.

Nicholson, D., Dyhrman, S., Chavez, F. and Paytan, A. (2006) Alkaline phosphatase activity in the phytoplankton communities of Monterey Bay and San Francisco Bay. *Limnol Oceanogr.*, **51**,874-883.

Perry, M. (1972) Alkaline phosphatase activity in subtropical Central North Pacific waters using a sensitive fluorometric method. *Mar Biol.*, **15**, 113-119.

Pu, P., Zhang, Z. and Wang, M. (2014) Seasonal variation and significance of alkaline phosphatase activity on algal blooming in Chongqing urban section of Jialing River. *Asian Journal of Chemistry.*, **26**, 6067-6072.

Rengefors, K., Pettersson, K., Blenckner, T. and Anderson D. M. (2001) Species-specific alkaline phosphatase activity in freshwater spring phytoplankton: Application of a novel method. *J Plankton Res.*, **23**,435-443.

Rengefors, K., Ruttenberg, K. C., Haupert, C. L., Taylor, C., Howes, B. L. and Anderson D.M. (2003) Experimental investigation of taxon-specific response of alkaline phosphatase activity in natural freshwater phytoplankton. *Limnol Oceanogr.*, **48**,

© Author(s) 2016. CC-BY 3.0 License.

1167–1175

Rose, C. and Axler, R. (1997) Uses of alkaline phosphatase activity in evaluating phytoplankton community phosphorus deficiency. *Hydrobiologia.*, **361**,145-156.

Song, C., Cao, X. and Li, J. (2005) Vertical variation in dissolved alkaline phosphatase activity in a shallow eutrophic lake determined in reverse micelles. *J Freshw Ecol.*, **20**,627-634.

Strojsova, A. and Vrba, J. (2009) Short-term variation in extracellular phosphatase activity: possible limitations for diagnosis of nutrient status in particular algal populations. *Aquat Ecol.*, **43**,19–25

Strojsova, A., Nedoma, J., Strojsova, M., Cao, X. and Vrba, J. (2008) The role of cell-surface-bound phosphatases in species competition within natural phytoplankton assemblage: an in situ experiment. *J Limnol.*, **67**,128–138

Tan, X., Ma, P., Song, L. and Zhang, Q. (2012) Physiological and Ultrastructural Responses of Microcystis aeruginosa to Different Phosphorus Concentrations. *Fresenius Environ Bull.*, **21**, 838-843.

Tanaka, T., Thingstad, T. F., Lovdal, T. and Riebesell, U. (2008) Availability of phosphate for phytoplankton and bacteria and of labile organic carbon for bacteria at different $pCO_2$ levels in a mesocosm study. *Biogeosciences.*, **5**,669–678.

Utermohl, VH. (1931) Neue Wege in der quantitativen Erfassung des Planktons. *Verh Int Ver Theor Angew Limnol.*, **5**,567–596.

Wang, P., Ren, L., Wang, C., Qian, J. and Hou, J. (2015) Presence and patterns of alkaline phosphatase activity and phosphorus cycling in natural riparian zones under changing nutrient conditions. *J Limnol.*, **74**, 155-168.

Zhang C, Cheng X, Wang J and Du Y. (2013) Spatial-temporal Variations of Alkaline Phosphatase Activity in the Kuilei Lake and Their Influencing Factors. *Chinese Journal of Applied and Environmental Biology.*, **19**,489-494.

Zhou, J., Qin, B., Casenave, C. and Han, X. (2016) Effects of turbulence on alkaline phosphatase activity of phytoplankton and bacterioplankton in Lake Taihu. *Hydrobiologia.*, **765**,197-207.