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

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**Abstract.** In order to know the role of phytoplankton communities in the distribution pattern of alkaline phosphatase activity (APA), monthly investigations ~~was~~ were 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 phyla~~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 a broad spectrum phosphomonoesters (Kuenzler and Perras, 1965; Tanaka *et al*., 2008) and associate with cells surfaces of microbial organisms (Gonzalez *et al*.,1998) or freely-diffusible (Burns *et al*., 2013). Both

phytoplankton and bacteria can secrete extracellular APase which enables them to use organic P esters as a source of P for 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. The increase in APA can be attributed more to phytoplankton biomass than to the bacterial biomass. ~~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. (Li and Lei.,2008). Though many studies have been conducted to screen APase in different water bodies, little information could be obtained in the Three Gorges Reservoir (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. Based on the related researches focused on the complicated relationship between APA and phytoplankton

mentioned above, 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 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 area 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.

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*. 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. All samples were run in triplicate. In order to avoid the physiological and biological parameters changed dramatically, The water samples for APA test were filtered immediately after collection and strong oscillation in situ, the filters were put into a portable refrigerator at 0 $^oC$ and analyzed within 24 h. All water samples for the other parameters measurement were also stored in a portable refrigerator at 0 $^oC$ after collected and tested within 24 h. Concentrations of chlorophyll *a* (Chl *a*), total phosphorus (TP), soluble reactive phosphorus (SRP), chemical oxygen demand (COD) were analyzed after samples collected within 24h. Samples for quantitative phytoplankton analyses were fixed with neutral Lugol's solution, and concentrated after 48 h sedimentation (Utermohl, 1931).

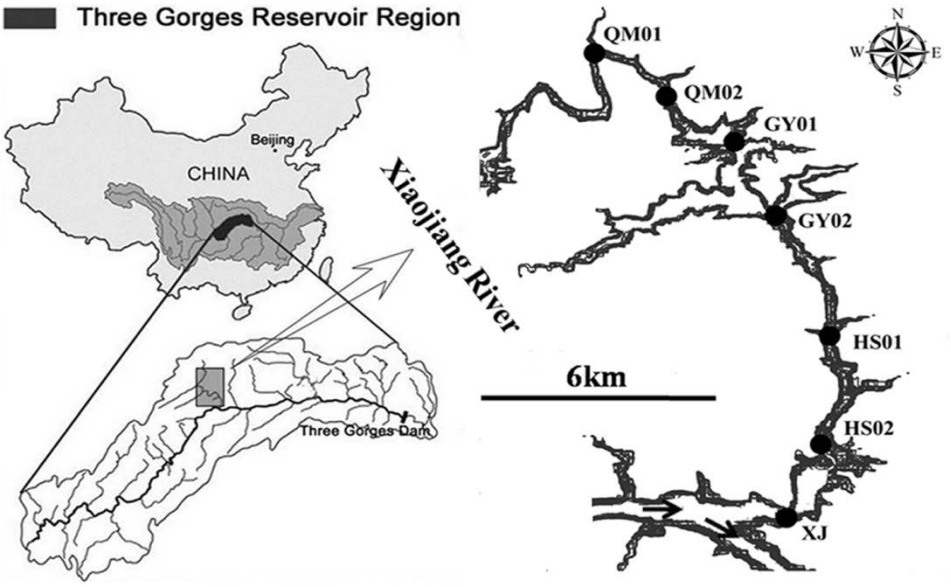



**Figure1.** Maps of the location of the Three Gorges Reservoir Region, and the sampling sites in the Xiaojiang River

## 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°C for 4h in the presence of 1ml 0.05M Tris-HCl buffer (pH=8.5) and 2ml 0.3mM p-nitronphenylphosphate (p-NPP) as substrate, 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 (the picoplankton/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 (Zhu *et al*, 2013). 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·$L^{-1}$·$min^{-1}$(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 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).

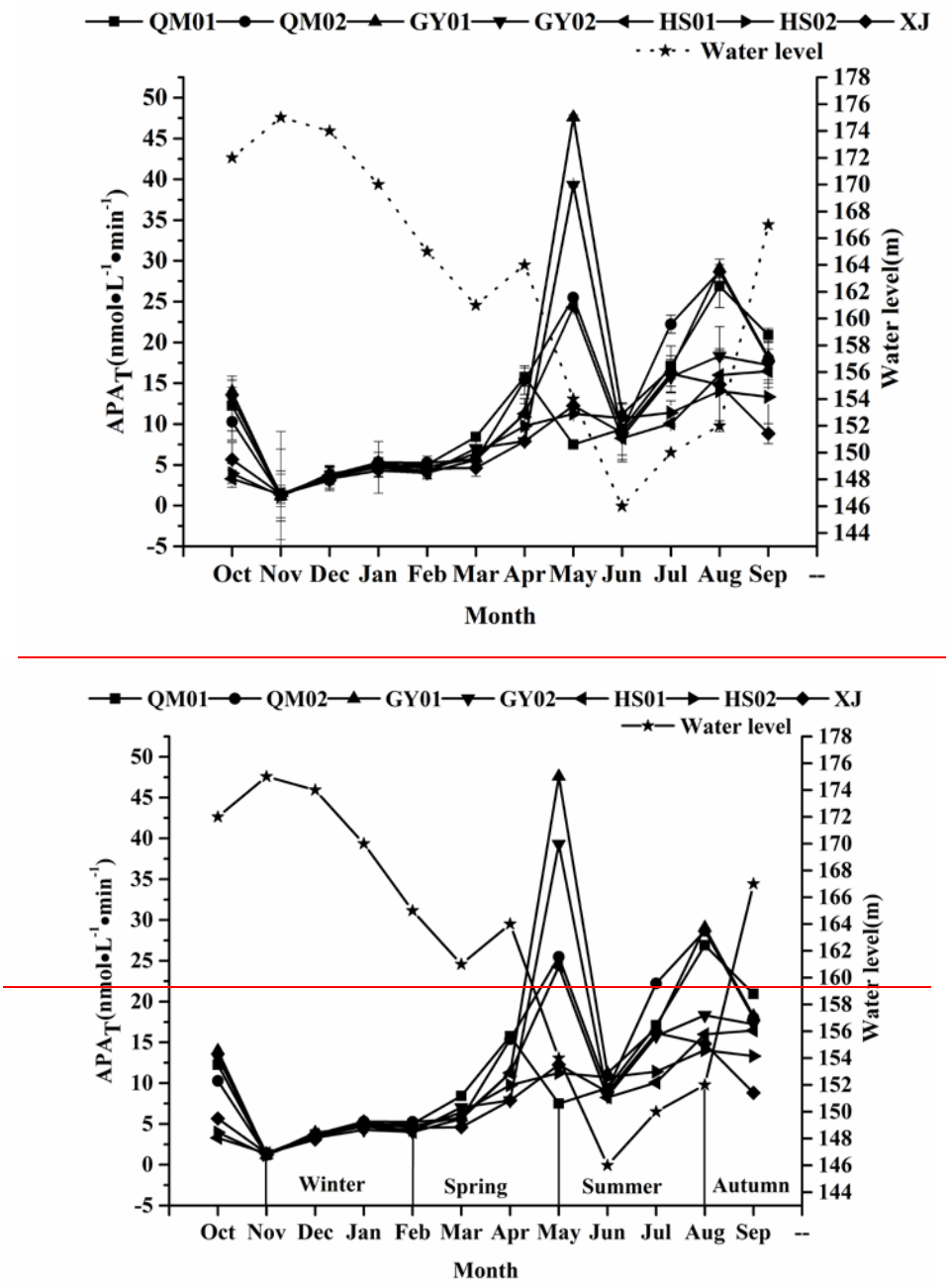



**Figure 2.** Seasonal variations in $APA_T$ concentrations in different sample sites and water level of the Xiaojiang River

## 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).

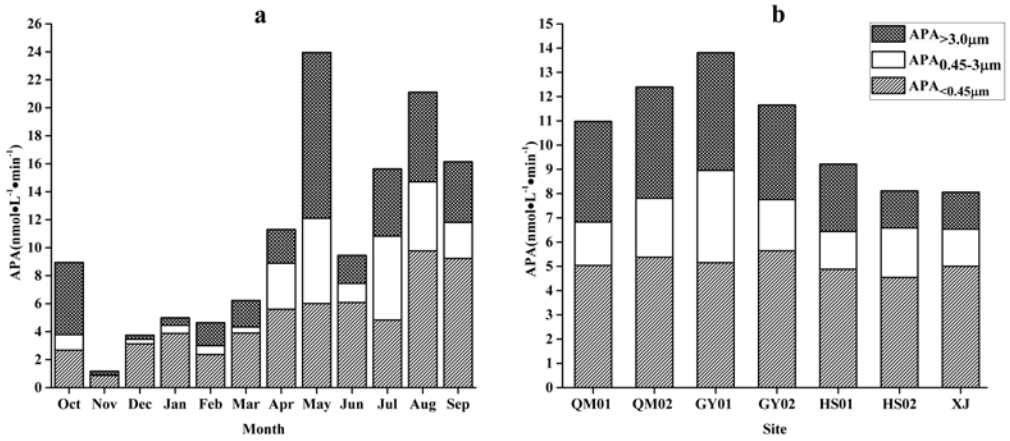



**Figure 3.** Seasonal (a) and spatial (b) variations of average size-fractionated APA in the Xiaojiang River. $APA_{>3.0\mu m}$: the alkaline phosphatase activity in algal fraction; $APA_{0.45\text{-}3.0\mu m}$: the alkaline phosphatase activity in bacterial fraction; $APA_{<0.45\mu m}$ : picoplankton/dissolved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

### 3.3 Phytoplankton communities

Bacillariophyta was the dominant group in winter and spring (72.7% in average, Fig.4) except cyanophyta are dominant in April. 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.

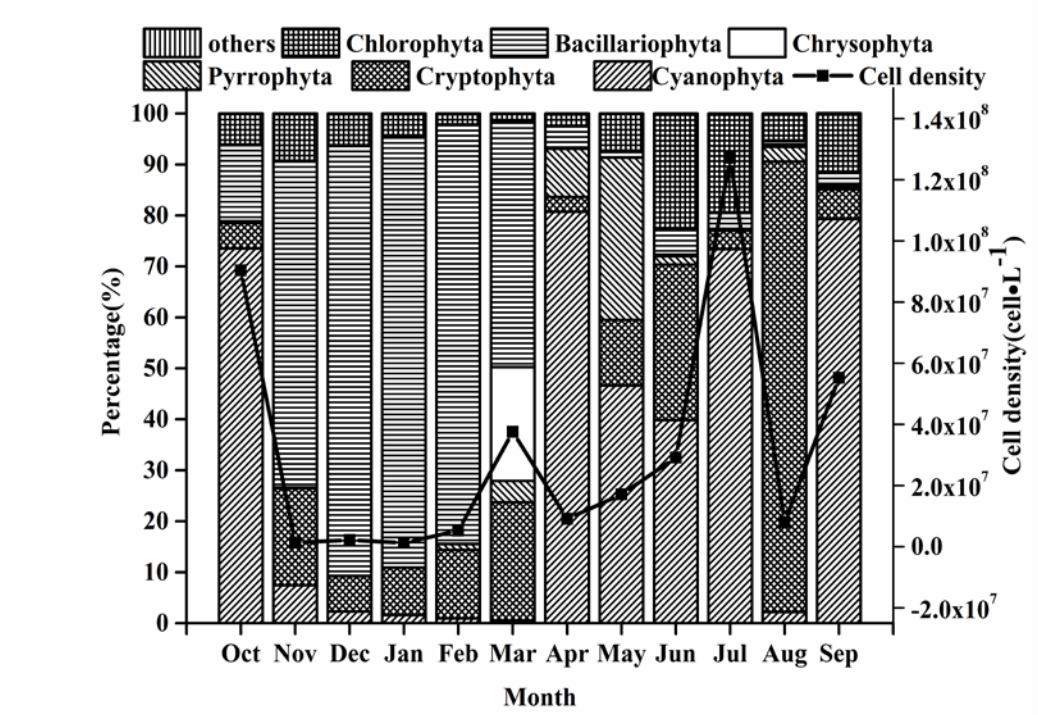
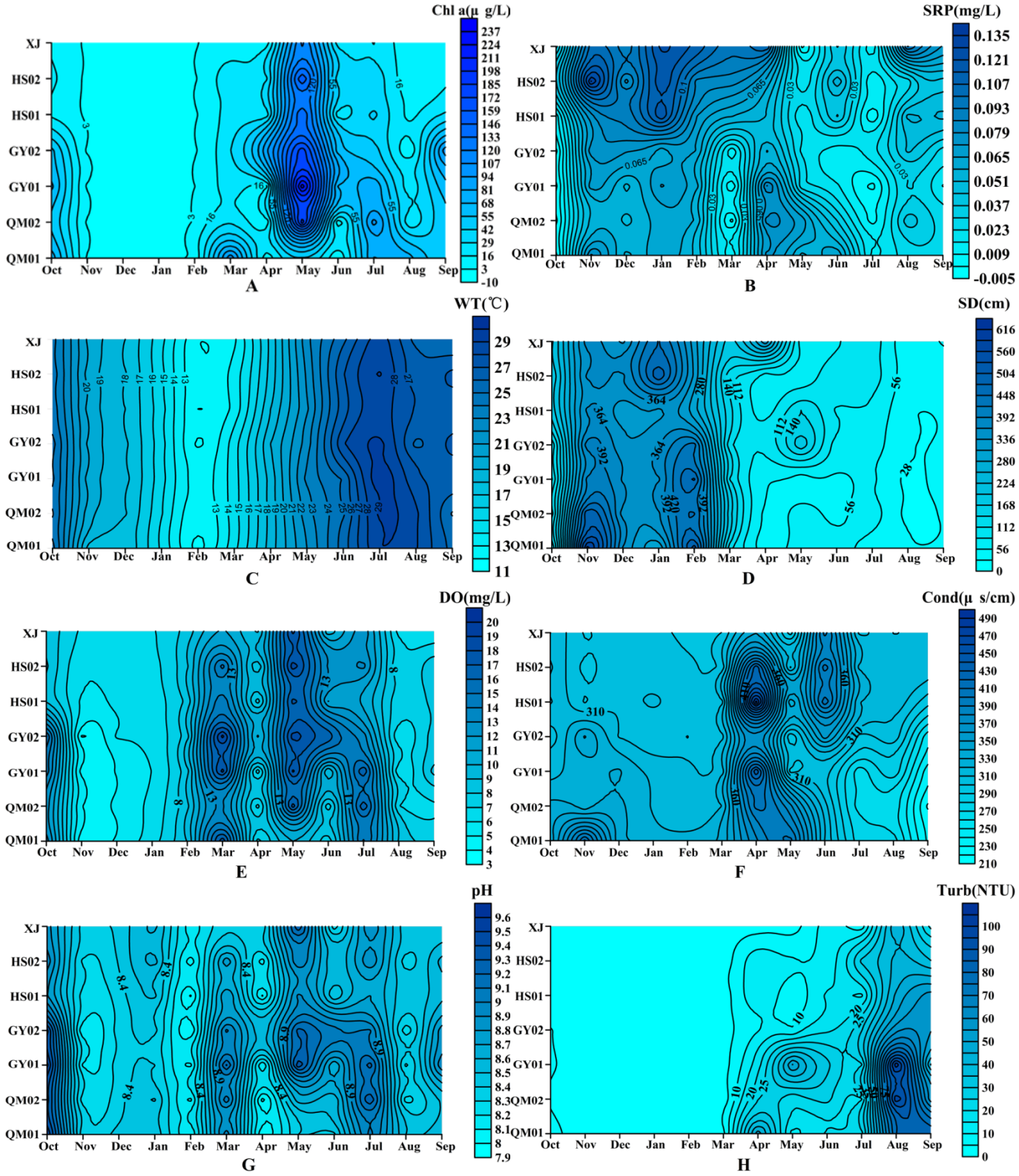



**Figure 4.** Seasonal variations of algal composition and algal cell density in the Xiaojiang River

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

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


Chl a(μ g/L)
237
224
211
198
185
172
159
146
133
120
107
94
81
68
55
42
29
16
3
-10
SRP(mg/L)
0.135
0.121
0.107
0.093
0.079
0.065
0.051
0.037
0.023
0.009
-0.005
WT(℃)
29
27
25
23
21
19
17
15
13
11
SD(cm)
616
560
504
448
392
336
280
224
168
112
56
0
DO(mg/L)
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
Cond(μ s/cm)
490
470
450
430
410
390
370
350
330
310
290
270
250
230
210
pH
9.6
9.5
9.4
9.3
9.2
9.1
9
8.9
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8
7.9
Turb(NTU)
100
90
80
70
60
50
40
30
20
10
0
XJ
HS02
HS01
GY02
GY01
QM02
QM01
Oct
Nov
Dec
Jan
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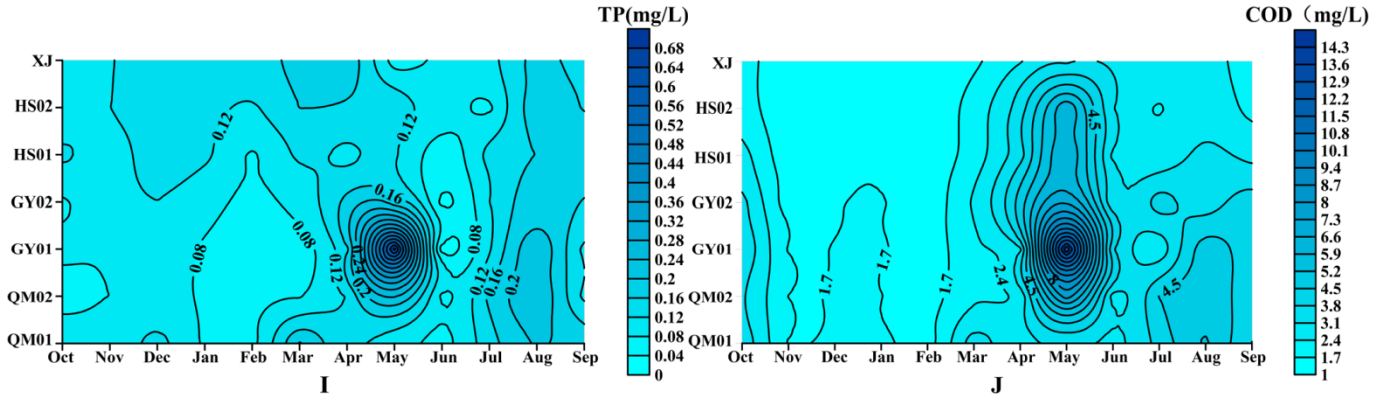



**Figure 5.** Temporal and spatial variations of A: chlorophyll *a* (Chl *a*) and other environmental parameters. B: soluble reactive phosphorus (SRP); C: water temperature (WT); D: transparency (SD); E: dissolved oxygen (DO); F: conductivity (Cond); G: pH; H: turbidity (Turb); I: total phosphorus (TP) 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~~,~~ and pH ~~and Chl *a*~~ were positively correlated with APA fractions. Cond., SD and WL were negatively correlated with APA.

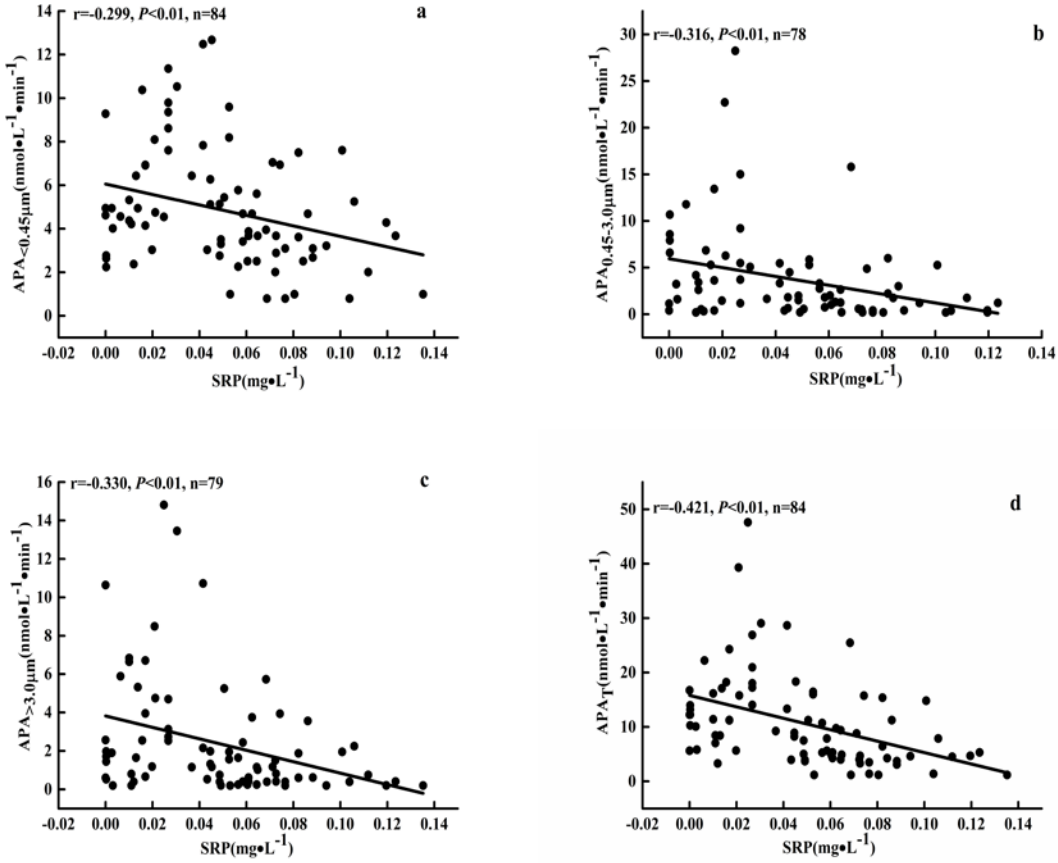



**Figure 6.** Relationship between soluble reactive phosphorus (SRP) concentrations and $APA_{<0.45\mu m}$ (a), $APA_{0.45\text{-}3\mu m}$ (b), $APA_{>3.0\mu m}$(c) and $APA_T$(d) in the Xiaojiang River

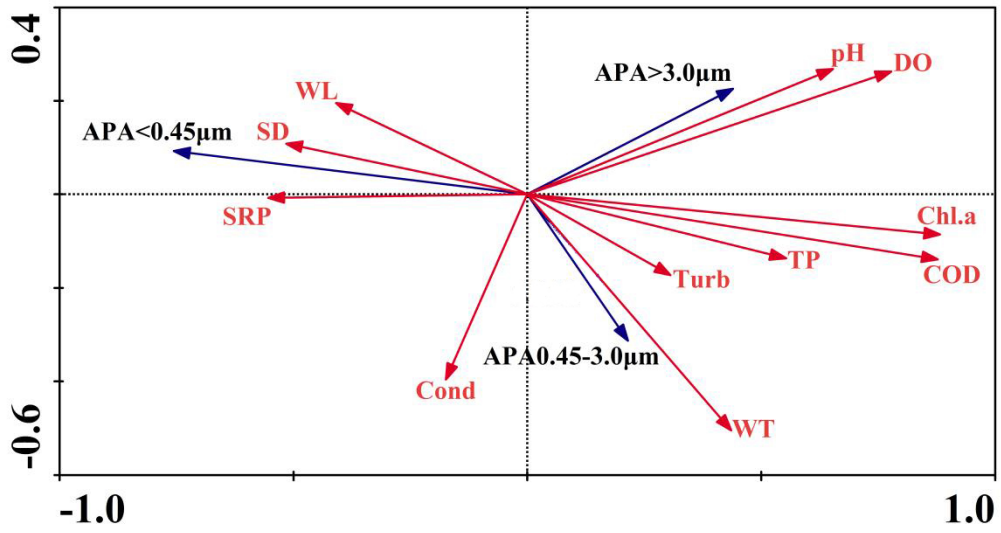
**Table 1.** Spearman correlations between APA and 10 environmental variables: water temperature (WT); ~~chlorophyll a (Chl a);~~ transparency (SD); dissolved oxygen (DO); conductivity (Cond); pH, turbidity (Turb); total phosphorus (TP); chemical oxygen demand (COD); water level (WL) in the Xiaojiang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



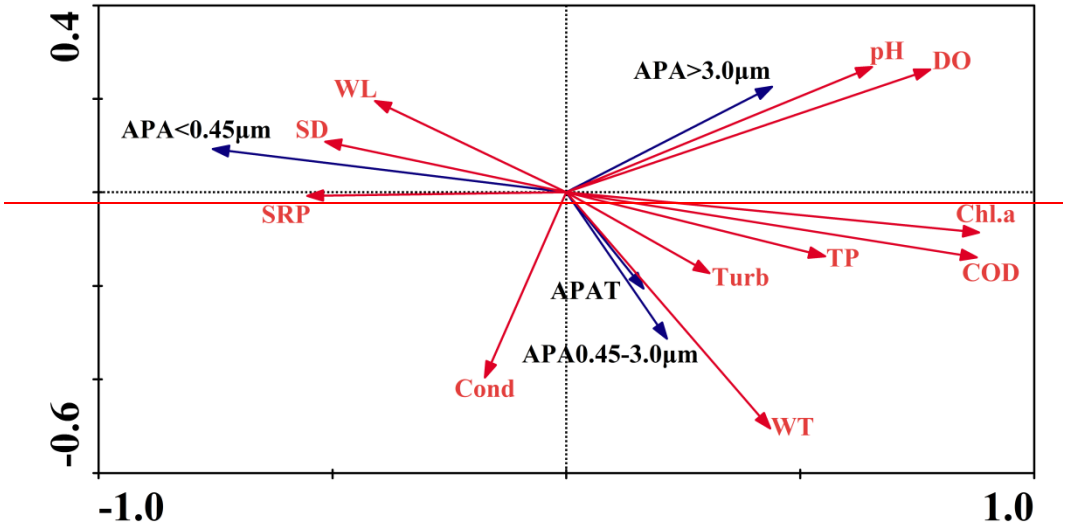




**Figure 7.** Biplot diagrams for RDA of the relationship between 11 environmental variables (red lines) and ~~APA$_T$,~~ APA$_{<0.45\mu m}$, APA$_{>3.0\mu m}$, APA$_{0.45\text{-}3\mu m}$ (blue lines.)

## 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).

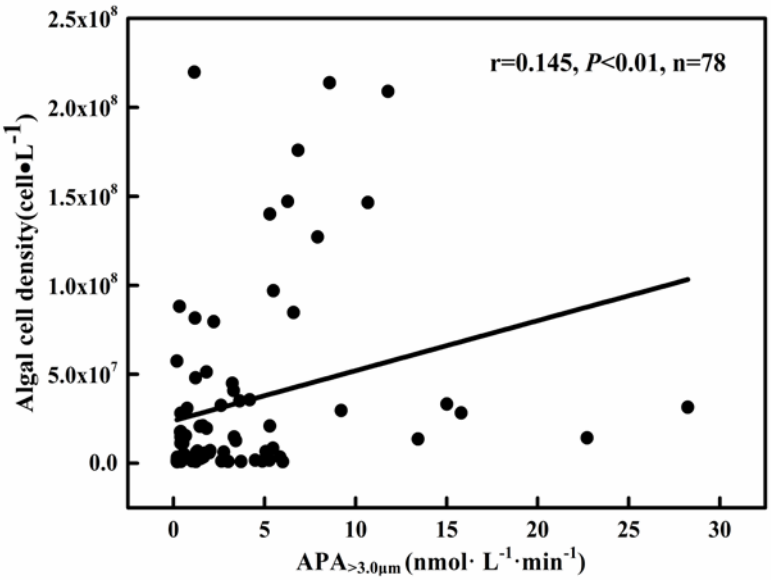



**Figure 8.** Relationships between $APA_{>3.0\mu m}$ and cell density in all sites

## 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). Though many (not all) phytoplankton cells have a host heterotrophic bacteria inhabiting or in close association with cells, and the overlapping size on the filter also influenced the final data, which making it difficult to assign the different size fractionation by filtration to individual cells alone, ~~The~~ it could be admitted that the coarser fraction ($APA_{>3.0\mu m}$), mainly from algae, was conventionally defined as "algal APA" (Liu *et al*., 2012) due to phytoplankton was the main contributor according to its size, biomass and physiological activity. 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 the picoplankton/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 picoplankton/dissolved APA ($APA_{<0.45\mu m}$) kept a relative stable and high level (53.4% of the $APA_T$). Some studies showed that the picoplankton/dissolved APA represents a significant part of the total activity. For example, Labry *et al* reported that picoplankton/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 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 was capsulated into reverse micellar media~~.~~, ~~Finally, they~~and it was 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).The results of Song *et al*. (2005) couldn't identify the exact origins form, the fraction < 0.45μm contains some pico-bacteria and some pico-phytoplankton and can't be called as the dissolved fraction. Here we changed it as the picoplankton/dissolved fraction. ~~It was deduced that phytoplankton acted as the main contributor of picoplankton/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 and the increased concentrations of SRP. 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). *Microcystis aeruginosa* was confirmed can also synthesize APase (Tan *et al*., 2012). It can explain that 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 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.

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