#### **RESEARCH ARTICLE**

# **Aquatic Sciences**



# **A study of bioavailable phosphorus in the infowing rivers of Lake Taihu, China**

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#### **Abstract**

Phosphorus (P) is a vital nutrient for algal growth. Aside from soluble reactive P (SRP), organic P (OP) is used by algae via alkaline phosphatase (AP) hydrolysis, which can play an important role in supplying P. Enzymatically-hydrolysable OP (EHP) can potentially be used as an indicator of bioavailability of P other than SRP in natural waters. We investigated the ecological signifcance of alkaline phosphatase activity (APA), EHP concentration and P turnover time in the infowing rivers of Lake Taihu (Taihu) during three hydrologic periods. Results indicated high SRP concentration and low SRP demand by algal suppressed APA in the inflowing rivers, the highest proportion of OP mineralization rate  $(v)$  to the maximum reaction velocity of AP ( $V_{max}$ ) is only 14.7%. P turnover time of the inflowing rivers was generally from 3 to 7 days and in exceptional cases, it could exceed 10 days. The high EHP reserve and the sufficient AP for OP mineralization render the rivers a signifcant source of utilizable OP, further exacerbating eutrophication of Taihu.

**Keywords** Eutrophication · Human sewage · Alkaline phosphatase activity · Organic phosphorus mineralization rate · Phosphorus turnover time · Hydrologic periods

## **Introduction**

Eutrophication is one of the most challenging environmental problems in nutrient-enriched waters (Qin [2009;](#page-9-0) Smith and Schindler [2009;](#page-9-1) Wang and Wang [2009;](#page-9-2) Zhu et al. [2015](#page-9-3); Schindler et al. [2016](#page-9-4)). It is generally accepted that key nutrient inputs include nitrogen (N) and phosphorus (P) (Hecky

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and Kilham [1988;](#page-8-0) Lowery [1998](#page-8-1); Gurkan et al. [2006;](#page-8-2) Bhagowati and Ahamad [2018](#page-8-3)). Phosphorus is an essential element for plant growth and its input has long been recognized as necessary to maintain primary production. P is also a vital nutrient stimulating problematic harmful cyanobacterial blooms and so its control is of prime importance in mitigating the global proliferation of such blooms in freshwater ecosystems (Daniel et al. [1998;](#page-8-4) Schindler and Vallentyne [2008](#page-9-5)).

P in water can be separated into numerous forms, such as total P (TP), total dissolved P (TDP), organic phosphorus (OP), molybdate reactive P or soluble reactive P (SRP), molybdate unreactive P (Xie et al. [2011\)](#page-9-6). Among the various forms of P, SRP is the most biologically available and readily usable for phytoplankton and bacteria (Cembella et al. [1982](#page-8-5); Kwon et al. [2011\)](#page-8-6). However, OP often represents the largest phosphorus pool in water and can greatly exceed SRP concentrations (Shun et al. [1994](#page-9-7); Oh et al. [2005;](#page-9-8) Lin et al. [2018\)](#page-8-7). OP may also be an important P storage for phytoplankton when depletion of SRP occurs in the surface water (Oh et al. [2002](#page-9-9); Kwon et al. [2011;](#page-8-6) Lin et al. [2018](#page-8-7)).

Microbial utilization of OP through enzymatic hydrolysis is one of the most important pathways in the P cycle (Cembella et al. [1982](#page-8-5); Suzumura et al. [1998](#page-9-10)). Alkaline phosphatase (AP) hydrolyzes OP to SRP under SRP depleted conditions (Kuenzler and Perras [1965](#page-8-8); Jin et al. [2006](#page-8-9); Xie et al. [2011;](#page-9-6) Li et al. [2015;](#page-8-10) Lin et al. [2018](#page-8-7)). Enzyme assays with soluble substrate showed that extracellular AP is important in providing P for phytoplankton growth, particularly during blooms induced by nutrient over-enrichment (Harke et al. [2012;](#page-8-11) Okubo et al. [2014](#page-9-11); Ivančić et al. [2016](#page-8-12)). Alkaline phosphatase-hydrolyzable OP or enzymatically-hydrolysable OP (EHP) have potential use as indicators of bioavailability of P other than SRP in natural waters (Shun et al. [1994](#page-9-7)), and the enzyme plays an important role in P supply (Zhang et al. [2007](#page-9-12)). The most common practice to evaluate OP utilization under P limited conditions is to determine alkaline phosphatase activity (APA) (Hoppe [2003;](#page-8-13) Ryzhakov and Stepanova [2016\)](#page-9-13).

Lake Taihu (Taihu) is the third largest freshwater lake in China, located in the Changjiang (Yangtze) Delta. It has a surface area of about  $2338 \text{ km}^2$  and mean depth of 1.9 m (Qin et al. [2007](#page-9-14)). Taihu serves as a drinking water source for approximately 20 million local inhabitants and plays an important role in the Jiangsu Province economy. However, in recent decades, cyanobacterial blooms occur regularly from late spring through fall throughout the lake (Chen et al. [2003](#page-8-14); Xu et al. [2015](#page-9-15)). P is the primary limiting nutrient in winter and spring in Taihu, while in summer and autumn N and P co-limit algal growth (Xu et al. [2010\)](#page-9-16). Controlling P supply is thus a critical step in reversing eutrophication and the restoration of the lake (Zhu et al. [2013\)](#page-9-17).

Infowing rivers are a key source of nutrient pollution in Taihu (Zhao et al. [2011\)](#page-9-18). Previous studies have shown that about 80% of the nutrients discharged to Taihu came from these rivers and surface runoff (Xie et al.  $2007$ ; Wang et al. [2011](#page-9-20)). Therefore, monitoring and controlling nutrient discharge from infowing rivers is a necessary component of efective lake management (Zhao et al. [2011;](#page-9-18) Wang and Bi [2016\)](#page-9-21).

Past research and development of water quality standards have focused on TP, TDP and SRP. However, few studies have investigated the contributions of EHP and APA to P cycling in rivers discharging to Taihu. In view of their potential ecological signifcance, we measured EHP, APA, OP mineralization rate (*v*) and P turnover time (*t*) in infowing rivers of Taihu during various hydrologic periods. The objective of the study was to evaluate the rate of P turnover, P bioavailability and mobility. These results could help show the impact of infowing rivers on Taihu's algal bloom potential.

# **Materials and methods**

#### **Sampling times and sites**

The rainy season in the Taihu Basin begins in April, with water levels usually reaching their peak in late July, while the lowest water levels occur between February and March every year, according to long-term hydrology data from the Lake Taihu Basin Authority of Ministry of Water Resources (<http://www.tba.gov.cn/channels/43.html>). Therefore, we chose February, May and August 2009 to represent low, moderate and high fow periods. Samples were collected from 21 to 25 February, from 24 to 26 May and from 26 to 28 August of 2009. Nine rivers discharging to Taihu (30°55′40″–31°32′58″N; 119°52′32″–120°36′10″E) were selected for this study. In1 (Zhihu port), In2 (Hengtang river) and In3 (Wangyu river) are polluted by industry and they are located on the north side of Taihu. Se4 (Xujiang river), Se5 (Zhi port) and Se6 (Cao port) are polluted by domestic sewage and they are located on the east side of Taihu. Ag7 (Zhongtang river), Ag8 (Changxing port) and Ag9 (Jiapu port) are heavily infuenced by agricultural pollution and they are located on the southwest side of Taihu (Fig. [1](#page-2-0)). Three sampling sites were set up along the upper, middle and lower reaches of each river, amounting to a total of 27 sample sites examined in our study. The downstream sampling sites were at the intersection of each river and Taihu.

#### **Sample collection and laboratory analysis**

Water samples were collected at 0.5 m below the surface. The transparency of water was measured by Secchi depth (SD). Water temperature, pH, dissolved oxygen (DO), turbidity and chlorophyll-*a* (Chl-*a*) were measured in situ using a Yellow Springs Instruments (YSI) 6600 V2 multisensor sonde. TP, TDP, SRP, EHP and APA were measured in the laboratory. TP and TDP were determined by persulfate digestion and then determined by spectrophotometry at 700 nm after reaction with molybdate, ascorbic acid, and tri-valent antimony (Jin and Tu [1990](#page-8-15)). A flow-injection system (Skalar Co., [http://www.skalar.com\)](http://www.skalar.com) was used to determine SRP concentrations in Whatman GF/F membrane-fltered water samples.

EHP was measured as follows: 1 mL 1.0 mol/L Tris bufer (pH 8.2) and 5 mL pure chloroform was added to 100 mL of initial water sample, with subsequent incubation at 30 °C for 5 days (Peters [1981;](#page-9-22) Chrost et al. [1986\)](#page-8-16). Subsamples were fltered on Whatman GF/F flters, followed by SRP concentration measurements using a flow-injection system (Skalar Co., [http://www.skalar.com\)](http://www.skalar.com). EHP concentration was calculated as the diference between the SRP concentration in the incubated subsample and the initial water sample. All samples were run in triplicate.

APA was determined by analyzing the production of *p*-nitrophenol (pNP) from model substrate *p*-nitrophenyl phosphate (pNPP) hydrolyzed by AP in the initial water samples (Berman [1970;](#page-8-17) Sayler et al. [1979](#page-9-23); Joner et al. [2000](#page-8-18)). In the assay, 1 mL 0.5 mol/L Tris butter (pH 8.4) and 2 mL millimolar pNPP were added to 2 mL of sample, followed by

rivers around Taihu and 27 sampling sites in the rivers

<span id="page-2-0"></span>

incubation at 30 °C for 6 h in the dark (Gao et al. [2006\)](#page-8-19). The absorbance at 410 nm was measured and enzymatic activity was calculated from absorbance using a standard curve based on pNP. Eight gradient millimolar pNPP concentrations ranging from 0.02 to 2.0 mmol/L were chosen to determine the following kinetic constants and APA was determined at the fnal substrate concentration of 1.0 mmol/L. All samples were run in triplicate.

It was found that the reaction rate was determined by enzyme concentration and substrate concentration if other conditions remained constant. If enzyme concentration remains unchanged, the reaction rate presents a complex process of change when substrate concentration is changed. The Michaelis–Menten equation is a velocity equation of the relationship between the initial enzymatic reaction rate and substrate concentration of the enzymatic reaction. It includes the enzymatic reaction rate and other factors, such as inhibitors. The affinity between enzyme and substrate as well as potential reaction rate of extracellular AP hydrolysis can be inferred by using the Michaelis–Menten equation (Tabatabai and Bremner [1971;](#page-9-24) Manzoni and Porporato [2009;](#page-9-25) Allison et al. [2010](#page-8-20); Wang and Post [2013](#page-9-26); Hui et al. [2013](#page-8-21)):

$$
v = \frac{V_{\text{max}}[S]}{K_m + [S]}
$$

where *v* (nmol/L/min) is OP mineralization rate;  $V_{max}$ (nmol/L/min) is the maximum reaction velocity of AP; *Km* (μmol/L) is the Michaelis constant; [*S*] (μmol/L) is substrate concentration. We used Lineweaver–Burk transformation  $\left(\frac{1}{v} = \frac{K_m}{V_{\text{max}}} \cdot \frac{1}{\left(S\right)} + \frac{1}{V_{\text{max}}} \right)$  of the Michaelis–Menten equation to calculate  $V_{max}$  and  $K_m$  (Zhou et al. [2004\)](#page-9-27).  $K_m$  and  $V_{max}$ were computed by linear regression analysis on the values obtained in the assay (Han and Srinivasan [1969](#page-8-22); Zhou et al. [2004;](#page-9-27) Xu et al. [2018](#page-9-28)). *v* was calculated by using  $K_m$ ,  $V_{max}$ combined with the EHP as substrate concentration. P turnover time *t* (min) was calculated with EHP and *v* (Larionova et al. [2007](#page-8-23); Tischer et al. [2015](#page-9-29)).

Mean value and standard deviation of the variation of the upper, middle and lower reaches of each river were all

calculated using Microsoft Excel 2007 software. Pearson correlation coefficients were determined with a Statistical Program for Social Sciences (SPSS) 16.0 software. Signifcance levels were reported as significant  $(0.01 \le p < 0.05)$ or highly significant  $(p < 0.01)$ .

# **Results**

## **Dynamic parameters (***Km***,** *Vmax***) of AP in the infowing rivers of Taihu**

Average  $K_m$  value of AP in the nine inflowing rivers ranged from 14 to 61 µmol/L during low flow period, from 19 to 52 µmol/L during moderate fow period and from 10 to 34  $\mu$ mol/L during high flow period. The highest  $K_m$  values of AP appeared in rivers polluted by domestic sewage (Se4, Se5, Se6) and the lowest  $K_m$  values of AP appeared in rivers polluted by agriculture (Ag7, Ag8, Ag9) during the various hydrologic periods. A comparison of average SRP concentrations of the three types of rivers (Gao et al. [2016](#page-8-24)) shows that SRP concentrations in the rivers polluted by domestic sewage (Se4, Se5, Se6) contained the highest SRP and rivers polluted by agriculture (Ag7, Ag8, Ag9) were the lowest.

 $K_m$  values of AP showed no significant difference  $(p=0.691)$  between the low flow period and the moderate flow period.  $K_m$  values of AP in the high flow period showed a significant difference with the low flow period  $(p=0.021)$ and with the moderate flow period  $(p=0.024)$ . The linearly dependent coefficients between  $K_m$  and SRP are 0.65, 0.60 and 0.54 during low, moderate and high flow periods. Statistical results showed there were no signifcant diferences in pH among the low, moderate and high flow periods  $(p \ge 0.068)$ .  $K_m$  values of AP were negatively dependent on temperature (linearly dependent coefficient  $r = -0.87$ ).

Average  $V_{max}$  of AP in the inflowing rivers ranged from 2.7 to 10.6 nmol/L/min during the low flow period, from 1.6 to 10.9 nmol/L/min during the moderate fow period and from 1.5 to 13.6 nmol/L/min during the high flow period. There was no significant difference in the  $V_{max}$  of AP among the low, moderate and high flow periods ( $p \ge 0.644$ ).

## **Organic P mineralization rate (***v***) in the infowing rivers of Taihu**

OP mineralization rate  $\nu$  in the inflowing rivers increased gradually from the low flow period to the high flow period. There was no signifcant diference between the low and moderate flow periods  $(p=0.289)$  as well as between the moderate and high flow periods  $(p=0.098)$ , but there was a significant difference between the low and high flow periods  $(p=0.032)$ . There was no significant difference among the three types of rivers  $(p>0.9)$ . It shows that OP mineralization rate *v* was much lower than  $V_{max}$  of AP in the inflowing rivers by comparing  $V_{max}$  of AP with  $\nu$  in the nine sampled rivers of Taihu. The highest proportion of  $v$  to  $V_{max}$  $(v/V_{max})$  is only 14.7%. The average  $v/V_{max}$  for rivers polluted by industry ranging from 2.6 to 12.5%, for rivers polluted by agriculture ranging from 0 to 11.3% and for rivers polluted by domestic sewage ranging from 1.3 to 14.7%. *v*/*Vmax* increased gradually from the low flow period to the high flow period, which means that the AP enzymatic hydrolysis reaction in the inflowing rivers increased from the low flow period to the high fow period.

*v* and  $V_{max}/K_m$  had similar variation trend during the low, moderate and high fow periods (Fig. [3\)](#page-5-0). The variation trends of EHP concentration and OP mineralization rate *v* in the infowing rivers were also similar (Fig. [4\)](#page-6-0), and OP mineralization rate increased with the increase of EHP concentration. The proportion of EHP to TP (EHP/TP) was nearly equal to the proportion of SRP to TP (SRP/TP) during the moderate flow period and EHP/TP was higher than SRP/TP during the high fow period in the three types of infowing rivers (Fig. [5\)](#page-6-1).

#### **P turnover time (***t***) in the infowing rivers of Taihu**

We measured P turnover time in the infowing rivers of Taihu and there were no significant differences among low, moderate and high flow periods ( $p \ge 0.167$ ), nor were there signifcant diferences among the three types of rivers  $(p \ge 0.08)$ . EHP concentrations in the inflowing rivers ranged from 0.011 to 0.113 mg/L and it generally needed half a week to a week (range 3–7 days) to be mineralized (Fig. [6](#page-7-0)).

## **Discussion**

## **Characteristics of AP in the infowing rivers of Taihu**

Michaelis constant  $K_m$  and  $V_{max}$  of AP are responsible for the major part of the phosphatase activity (Bogé et al. [2014](#page-8-25)).  $K<sub>m</sub>$  is used to measure the affinity between enzyme and substrate (Tischer et al.  $2015$ ). A smaller  $K<sub>m</sub>$  value represents a stronger affinity (Wang et al. [2006;](#page-9-30) Xiao et al. [2018](#page-9-31)). The temporal patterns showed that Affinity between AP and substrate in the infowing rivers during high fow period was higher than that during low and moderate fow periods. *Vmax* is used to characterize the intrinsic catalytic rate of the enzyme. When the substrate is combined completely with the enzyme, the maximum reaction velocity can be obtained.

In Fig. [2](#page-4-0)a–c it appears that  $K_m$  values of AP are also high when SRP concentrations are high. The variation characteristic of SRP concentrations and  $K<sub>m</sub>$  values in the nine inflowing rivers during low, moderate and high flow periods (Fig.  $2a-c$ ) indicates that affinity between AP and substrate is inversely related to SRP concentrations in the infowing rivers. Natural environmental conditions such as pH, substrate concentration and temperature could markedly afect the APA (Hui et al.  $2013$ ). However, there were no significant diferences in pH among the low, moderate and high flow periods and  $K<sub>m</sub>$  values of AP were negatively dependent on temperature indicated that the high water temperature (average 31  $^{\circ}$ C) during high flow period increased the affinity between AP and substrate in the infowing rivers. These



<span id="page-4-0"></span>**Fig. 2** Soluble reactive P (SRP) concentration and its variation with Michaelis constant  $(K_m)$  in the inflowing rivers of Taihu during **a** low, **b** moderate and **c** high flow periods

results were similar to Gershenson et al. [\(2009\)](#page-8-26) and Tan et al. [\(2018\)](#page-9-32).

Based on diferent habitats of Taihu, Lu et al. ([2009\)](#page-8-27) reported the  $K<sub>m</sub>$  values of AP in the grass type zone, Gonghu Bay zone, Zhushan Bay zone, Meiliang Bay zone, the central zone and estuary zone of the western bank were 20.50, 20.11, 15.70, 13.34, 11.75 and 9.17 µmol/L respectively. *Km* values of AP in Taihu were lower than those in the infowing rivers and it indicated that affinity between AP and substrate in Taihu was higher than that in the infowing rivers. *Vmax* of AP in Taihu in 2008 ranged from 7.70 to 26.9 nmol/L/min and the average  $V_{max}$  was 13.70 nmol/L/min (Lu et al. [2009](#page-8-27)). The average *Vmax* of AP in Taihu in 1999 was 191 nmol/L/ min (Gao et al. [2004\)](#page-8-28). The average *Vmax* of AP in Taihu in 2011 was 16.0 nmol/L/min under no turbulent condition (Ding et al. [2016](#page-8-29)). The *Vmax* of AP in Taihu in 2014 increased from 39 nmol/L/min under calm condition to 458 nmol/L/min under long-term moderate wind condition, averaging 184 nmol/L/min (Chao et al. [2017\)](#page-8-30). *Vmax* of AP in Taihu was far higher than that in the infowing rivers. Taihu is a typical shallow hyper-eutrophic lake, with an average depth of 1.9 m and it is disturbed by wind-induced wave most of time (Qin et al. [2007](#page-9-14)). Wind-induced wave disturbances can cause rapid sediment resuspension (Tammeorg et al. [2013\)](#page-9-33). APA and TP in sediment were resuspended in the water column with the sediment resuspension, adding P cycling rate and bio-available P (Chao et al. [2017\)](#page-8-30). As a result, algae largely grew and reproduced, accompanying high *Vmax* of AP.

AP is often repressed at high SRP concentrations and reactive at low SRP concentrations (Jansson et al. [1988](#page-8-31)). APA has therefore been used as a SRP defciency indicator in algae and in natural plankton populations. Previous studies indicate that phytoplankton and bacterioplankton began to synthesize AP to hydrolyze phosphate esters when SRP was depleted (Berman [1970;](#page-8-17) Chrost et al. [1986\)](#page-8-16). Previous work carried out on Taihu showed that APA was signifcantly increased when SRP concentrations were less than 0.020 mg/L and 0.020 mg/L is the SRP threshold to activate APA of phytoplankton (Gao et al. [2000](#page-8-32)). SRP concentrations were far higher than 0.020 mg/L in most of the infowing rivers (Fig. [2a](#page-4-0)–c) and APA was repressed in the rivers. However, SRP was only about 8% of TP and the average SRP concentration was 0.0137 mg/L in Taihu (Gao et al. [2004](#page-8-28)). As a result, *Vmax* of AP in Taihu was far higher than that in the infowing rivers.

Phytoplankton are infuenced by a wide range of factors including water temperature, water column stability, fushing rate, irradiance, and available nutrients (Havens et al. [2019](#page-8-33); Islam et al. [2013](#page-8-34)). Many types of algae fourish in lakes with poor water flow and a flush of water suppress blooms (Islam et al. [2013;](#page-8-34) Havens et al. [2019\)](#page-8-33). It has been documented that, on a global scale, cyanobacteria are favored by warmer water temperatures (Kosten et al. [2012\)](#page-8-35). However, water temperature of the nine infowing rivers had no signifcant diference with Taihu during low  $(p=0.274)$ , moderate  $(p=0.830)$ and high flow periods  $(p=0.425)$ . Water transparency of the nine infowing rivers had no signifcant diference with Taihu during low  $(p=0.812)$ , moderate  $(p=0.074)$  and high flow periods  $(p=0.826)$ . So, water temperature and irradiance were not the main causes for slow algal growth in the infowing rivers. Taihu is relative stable because of the water control gates (Wu and Kong [2009](#page-9-34)) and the water retention time of the lake is approximately 180 days (Xu et al. [2015](#page-9-15)). The water retention time of the infowing rivers were short because of their high flow velocity. Though the algal cells are in a thick (low Reynolds number) environment and cannot sense the movement of water, algae cannot make full use of SRP in the infowing rivers because of the short water retention time. Therefore, high flow velocity of the inflowing rivers indirectly affected the growth of algae. High flow velocity of rivers is not suitable for algae growth compared with the lake, so the demand for SRP by algae in the infowing rivers is lower than that in Taihu. This helps explain why *Vmax* of AP in Taihu was far higher than that in the infowing rivers. High SRP concentration and low demand for SRP by algae lead to *Vmax* of AP in the infowing rivers being lower than that in Taihu.

## **Impact of OP mineralization rate in the infowing rivers of Taihu**

 $K<sub>m</sub>$  and  $V<sub>max</sub>$  represent the characteristics of AP itself, and OP mineralization rate  $\nu$  is the actual reaction rate between AP and substrate EHP.  $\nu$  is affected by the catalytic efficiency of AP, substrate concentration and SRP concentration according to the reversible enzyme-driven reaction principle. However, the two catalytic property parameters  $K<sub>m</sub>$  and  $V<sub>max</sub>$  have different characteristics, the decrease of  $K_m$  and the increase of  $V_{max}$  means enhanced OP mineralization by simultaneously increasing the reaction velocity *v* and improving the affinity for the substrate. In order to analyze the relationship between OP mineralization rate *v* and catalytic efficiency of AP in the inflowing rivers,  $V_{max}/K_m$ is used here as a proxy for the catalytic efficiency of AP (Wang et al. [2018](#page-9-35)). Figure [3](#page-5-0) indicates that OP mineralization rate increases with the increase of AP catalytic efficiency. *v* accounts for only a small proportion of  $V_{max}$  and *v* can potentially increase. The results of Fig. [3](#page-5-0) indicate that AP in the inflowing rivers is sufficient for OP mineralization and AP is not the reason for the low OP mineralization rate.

Another possible controlling factor of *v* is substrate concentration. The substrate refers to phosphatase-hydrolyzable OP in our study, namely EHP. OP mineralization rate increased with the increase of EHP concentration (Fig. [4\)](#page-6-0) indicated that substrate concentration was enough for the



<span id="page-5-0"></span>**Fig. 3** The variation characteristic of organic P (OP) mineralization rate (*v*) and catalytic efficiency ( $V_{max}/K_m$ ) of alkaline phosphatase (AP) in the infowing rivers of Taihu during **a** low, **b** moderate and **c** high flow periods

enzyme-driven reaction and it was not the reason for the low OP mineralization rates.

The reason for the low OP mineralization rate  $\nu$  in the inflowing rivers was not due to the catalytic efficiency of AP or the substrate (EHP) concentration according to the above analysis, but rather the inhibition by the higher SRP concentration. Compared with Taihu, algae cannot make full use of SRP in the infowing rivers because of the short water retention time. As a result, SRP in rivers was utilized





<span id="page-6-0"></span>**Fig. 4** The variation characteristic of enzymatically-hydrolysable organic P (EHP) concentration and organic P (OP) mineralization rate (*v*) in the infowing rivers of Taihu during **a** low, **b** moderate and **c** high flow periods

slowly by algae and the existent higher SRP concentration inhibited the reversible enzyme-driven reaction. Thus, OP mineralization rate *v* in the infowing rivers was very low. The high EHP reserve (Fig.  $5$ ) and the sufficient AP for OP mineralization point to the infowing rivers as a potential threat to water quality in Taihu.

<span id="page-6-1"></span>**Fig. 5** The proportion of EHP to TP (EHP/TP) and the proportion of SRP to TP (SRP/TP) in the infowing rivers of Taihu during **a** low, **b** moderate and **c** high fow periods

# **The diference of P turnover time (***t***) between Taihu and its infowing rivers**

Halemejko and Chrost [\(1984](#page-8-36))'s study on Lake Glebokie in Poland found that phosphatases participated in the mineralization of approximately 70% OP in the spring seston after a 60 day decomposition period. During the summer, seston decomposition was accompanied by 90% mineralization of P after 30 days (Halemejko and Chrost [1984\)](#page-8-36). During autumn, after 60 days, 96% of the initial content of OP in the algae was mineralized (Halemejko and Chrost [1984\)](#page-8-36). Hudson et al. [\(1999\)](#page-8-37) measured phosphorus regeneration rates from 20 temperate freshwater lakes from three major physiographical regions of North America and found regeneration could release an amount of P equal to particulate P pool every 5 days (range 3.5–7 days). P turnover time was from 275.9 to 20.3 min in diferent ecotype sites of Taihu and the most rapid P turnover time was on the order of several minutes (Gao et al. [2006](#page-8-19)). P turnover time in the infowing rivers of Taihu was far longer than the P turnover time of Taihu (Fig. [6\)](#page-7-0). One reason was that Taihu had longer hydraulic residence times, more algal biomass and higher demand for SRP by algae than infowing rivers. The diference in structure and form of OP compounds might be another reason for the diference in P turnover time. A variety of soluble organic P compounds is present in the upper layer of water (Halemejko and Chrost [1984\)](#page-8-36). These compounds include phospholipids, phosphomononucleotides and their derivatives (Kleerekoper [1953;](#page-8-38) Lean and Nalewajko [1976\)](#page-8-39). Sugar phosphates and aminophosphoric acids contain very stable C–P bonds (Demain et al. [1965\)](#page-8-40). Phosphoric monoester or diesters contain easily hydrolysable P–O–C bonds (Halemejko and Chrost [1984\)](#page-8-36). Further studies will focus on the relationship between the specifc molecular structure of EHP and P turnover time in diferent types of infowing rivers.

## **Conclusions**

The affinity between AP and substrate is inversely related to SRP concentration in the inflowing rivers and the affinity in Taihu was higher than that in the infowing rivers. High SRP concentration and low SRP demand by algae suppressed APA in the infowing rivers. As a result, the highest proportion of *v* to  $V_{max}$  is only 14.7%. The EHP concentrations in the infowing rivers ranged from 0.011 to 0.113 mg/L and half a week to a week (range 3–7 days) was needed to mineralize it. In exceptional cases, the P turnover time could exceed 10 days. The high EHP reserve and the sufficient AP for OP mineralization render the infowing rivers a potential threat to water quality and cyanobacterial bloom formation in Taihu, especially during warm, maximum irradiance summer periods when maximum bloom potential exists.

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<span id="page-7-0"></span>**Fig. 6** Enzymatically-hydrolysable organic P (EHP) concentration and the corresponding P turnover time (*t*) in the infowing rivers of Taihu during **a** low, **b** moderate and **c** high fow periods

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#### **Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no confict of interest.

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