Flow cytometric observation of picophytoplankton community structure in the cascade reservoirs along the Wujiang River, SW China

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ABSTRACT

Picophytoplankton community structure has been seasonally investigated in the cascade reservoirs along the Wujiang River from April 2006 to January 2007. Besides picoeukaryotes, two groups of picocyanobacteria have also been detected by flow cytometry. One is a phycoerythrin-rich picocyanobacteria (PE-rich Pcy), the other is a red-fluorescing cells with lacking orange fluorescence and could be a phycocyanin-rich picocyanobacteria (PC-rich Pcy). The average abundances of PC-rich Pcy, PE-rich Pcy and picoeukaryotes were 103, 104 and 102 cells mL⁻¹, respectively. PE-rich Pcy was the dominant population but showed a reduction with eutrophication, and therefore the community structure of picophytoplankton transformed from dominant PE-rich Pcy to dominant PE-rich Pcy and PC-rich Pcy, which suggested they are excellent indicators for the change of trophic state. Picophytoplankton community structure also presented a seasonal variation, indicating the different response of each picophytoplankton group to water temperature.

Key words: phycoerythrin-rich picocyanobacteria, phycocyanin-rich picocyanobacteria, picoeukaryotes

1. INTRODUCTION

Picophytoplankton (0.2-2 μm; Sieburth et al. 1978) comprise prokaryotic picocyanobacteria and eukaryotic phototrophs. They are ubiquitous in both freshwater and marine ecosystem (Stockner 1988; Callieri 2007). Pico-cyanobacteria use phycobilisome as accessory pigments for photosynthetic light collection (Glazer 1982). According to phycobilisome composition, freshwater picocyanobacteria are divided into phycoerythrin (PE)-rich and phycocyanin (PC)-rich types (Pick 1991; Becker et al. 2002). Nowadays, it is well known that picophytoplankton abundance and biomass increase and its relative importance decreases with the increase of trophic state in freshwater systems (Szelag-Wasielewska 1997; Stockner et al. 2000; Bell & Kalff 2001; Callieri & Stockner 2002). Light and water temperature are also two important factors controlling picophytoplankton growth (Wehr 1993; Agawin et al. 2000; Wakabayashi & Ichise 2004).

Rivers are the chief carriers of dissolved and particulate matter from land to sea (e.g., Meybeck 1982; Ittekkot 1988). Damming on river alters its hydrological condition, material cycle and then transforms aquatic ecosystem from riverine type to limnological type (e.g., Humborg et al. 1997). Reservoirs created by dams are complex and dynamic ecosystems and it is important to understand how these ecosystems operate and respond to change for an efficient management (Wetzel 2001). Picophytoplankton is an important component in freshwater ecosystem (Callieri & Stockner 2002). They respond swiftly to environmental conditions and can be effectively used as early indicators of ecosystem change (e.g., Schallenberg & Burns 2001). Nowadays, few studies have conducted with picophytoplankton in the cascade reservoirs (e.g., Becker et al. 2002).

The Wujiang River is a major power source for China’s massive West-to-East Power Transmission Project. A series of reservoirs have been constructed for this project since 1971. We have seasonally investigated three groups of picophytoplankton (PC-rich Pcy, PE-rich Pcy, and picoeukaryotes) in these reservoirs. The aim of this study is to understand the succession of different picophytoplankton groups in these cascade reservoirs, and to discern the indicative function of picophytoplankton on environmental change.

2. METHODS

2.1. Study sites and sampling

The 1037-km-long Wujiang River is a southern tributary of the Changjiang River, and it has a runoff of 53.4 billion cubic meters with a fall of 2124 m. The river water chemistry is controlled by carbonate dissolution by both carbonic and sulfuric acid, and dominated by Ca2+, HCO3–, Mg2+ and SO42– (Han & Liu 2004).

Investigations were carried out at five reservoirs along the Wujiang River (Fig. 1). They are Wujiangdu Reservoir, Dongfeng Reservoir, Hongjiadu Reservoir, Yinzidu Reservoir, Puding Reservoir (Tab. 1). A total of 25 stations were investigated (Fig. 1.). At the fol-
Following stations, water samples were taken with 5 L Niskin bottles at different depths: A1, A4, and D1 at the depths of 0, 10, 20, 40, 60 m, A5 at the depths of 0, 10, 20, 40, 60, and 80 m, B3 at the depths of 0, 20, 40, 60 m, B4 and C4 at the depths of 0, 20, 40, 60, 80 m, E4 at the depths of 0, 5, 10, 20 m, E6 at the depths of 0, 5, 10, 20, 30 m, respectively. Release water samples were collected at stations A6, B5, C5, D2, and E7 and other water samples were obtained from surface water.

Sample collection was carried out in April, July, October, 2006 and January, 2007. Water samples for determining abundances of different picophytoplankton groups were filtered by 50 μm fabrics in order to remove the bigger impurities and were put aside in darkness for 15 min with paraformaldehyde; then immediately stored in liquid nitrogen till analysis in one month (Pan et al. 2005). Water temperature was measured in situ by a portable multi-parameter instrument (pIONneer 65). In autumn, immediately after collection, water samples were filtered through 0.22 μm membrane filters (Millipore) and cold stored in darkness for measuring the concentrations of PO$_4^{3-}$, NO$_3^-$ by automatic flow analyzer (Sans plus Systems, SKALAR). A small portion of each sample was stored for measuring total nitrogen and phosphorus (TN and TP), respectively. TP was determined spectrophotometrically (Unico UV-2000) using the molybdenum blue method after alkaline potassium persulfate digestion. TN was also analyzed spectrophotometrically (Unico UV-2000) after alkaline potassium persulfate digestion.

2.2. Analysis of picophytoplankton

Picophytoplankton samples were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an air-cooled argon laser (488 nm, 15 mW). Forward light scatter, side light scatter, orange fluorescence (585±21 nm) and red fluorescence (>650 nm) were recorded for each particle in the sam-
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ple, and the data obtained were processed with CELL-Quest™ software (Becton Dickinson, San Jose, CA, USA). Yellowish green fluorescent beads (1.002 μm) (Polysciences Inc., catalogue # 18660) were added to calibrate cell fluorescence emissions and light scatter signals. The picophytoplankton groups could be discriminated and enumerated according to their specific autofluorescence properties and light scatter differences (Collier 2000).

Statistical analysis of the data was done with the software SPSS (version 11.5; SPSS Inc.). Pearson’s correlation coefficient analysis was carried out.

3. RESULTS
3.1. Hydrographic conditions

Thermal stratification developed during spring and summer; water temperature began to mix at the end of autumn and mixed well during winter in the investigated reservoirs (Fig. 2). The average surface water temperatures were 17.7 °C in April, 26.2 °C in July, 20.9 °C in October, and 12.8 °C in January, respectively. There was clear vertical decrease in temperature with depth during spring and summer (e.g., 13.0-25.9 °C at station C4 in July), which was more pronounced in the larger reservoirs (i.e., in Wujiangdu Reservoir and Hongjiadu Reservoir; Tab. 1). The water column of Wujiangdu Reservoir was not thermally stratified at the depth above 60 meters (i.e., 20.4-21.2 °C). However, the thermal stratification in water column of other four reservoirs was still clear (e.g., 10.6-21.9 °C at station C4 in October). In January Puding Reservoir showed lower water temperature than other four reservoirs, probably due to its smallest reservoir capacity and highest storage level (Tab. 1). Release water came from the deep water of the reservoir. In those reservoirs in which thermal stratification was developed the temperatures of release water showed values lower than those of surface water.

![Fig. 2. Profile of temperature in the investigated reservoirs.](image-url)
The values of NO$_3^-$ and TN in these investigated reservoirs did not show any significant differences; however, the values of PO$_4^{3-}$ and TP in Wujiangdu Reservoir were about 10$^2$ and 10 times higher than that of other four reservoirs, respectively (Tab. 2), indicating that Wujiangdu Reservoir was hypereutrophic and the other four reservoirs were meso-eutrophic according to Vollenweider & Kerekes (1982).

### 3.2. Detection of Picocyanobacteria

A group of red-fluorescing picocyanobacteria with lacking orange fluorescence (phycoerythrin) has been detected by flow cytometry in water samples collected in the investigated reservoirs. They could be discriminated from phycoerythrin-rich picocyanobacteria (PE-rich Pcy) by their lack of orange fluorescence (Fig. 3a) and from picoeukaryotes by their smaller side light scatter and much lower red (chlorophyll) fluorescence (Fig. 3a, b, b2). These cells showed similar side light scatter to PE-rich Pcy (Fig. 3b, b1, b2), indicating both of them have similar cell size. They are probably a small phytoplankton with a phycocyanin-based light harvesting system which lacks phycoerythrin. They could be phycocyanin-rich picocyanobacteria (PC-rich Pcy). Further evidences are required to characterize these PC-rich Pcy (e.g., pigment characterization or molecular phylogenetics); however, they were not conducted in this study.

### 3.3. Distributions of different picophytoplankton groups

PE-rich Pcy, PC-rich Pcy and picoeukaryotes have been investigated in this study. The concentration of PC-rich Pcy was one order of magnitude higher than that of picoeukaryotes and one order of magnitude lower than that of PE-rich Pcy (Tab. 3). Picoeukaryotes showed comparable concentrations in different investi-
gated months; however, the concentration of PC-rich Pcy in July was one order of magnitude higher than that in other three investigated months, and the concentration of PE-rich Pcy in January was one order of magnitude lower than that in other three investigated months (Tab. 3), indicating they had asynchronous responses to water temperature.

Tab. 3. Abundances of picophytoplankton in the reservoirs. Average±SD; PC-rich Pcy, phycocyanin-rich picocyanobacteria; PE-rich Pcy, phycoerythrin-rich picocyanobacteria; Euk, picoeukaryotes.

<table>
<thead>
<tr>
<th>Month</th>
<th>Reservoir</th>
<th>PC-rich Pcy (10^3 cells mL)</th>
<th>PE-rich Pcy (10^3 cells mL)</th>
<th>Euk (10^2 cells mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>Wujiangdu</td>
<td>5.5 ± 7.7</td>
<td>5.2 ± 8.9</td>
<td>4.1 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>Dongfeng</td>
<td>1.9 ± 1.6</td>
<td>41.1 ± 63.3</td>
<td>1.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Hongjiadu</td>
<td>2.4 ± 2.1</td>
<td>27.3 ± 32.5</td>
<td>1.8 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Yinzidu</td>
<td>2.8 ± 3.2</td>
<td>26.7 ± 29.9</td>
<td>4.1 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Puding</td>
<td>11.4 ± 14.6</td>
<td>38.4 ± 55.0</td>
<td>5.2 ± 4.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5.3 ± 8.9</td>
<td>25.5 ± 44.4</td>
<td>3.5 ± 3.1</td>
</tr>
</tbody>
</table>

The concentrations of picoeukaryotes were similar among the investigated reservoirs (Fig. 4); however, PC-rich Pcy in Puding Reservoir (Fig. 5) and PE-rich Pcy in Wujiangdu Reservoir (Fig. 6) showed different concentrations from other four reservoirs (Tab. 3). This indicated they had different responses to trophic state of the investigated reservoirs.

The vertical distributions of three groups of picophytoplankton were similar in the investigated reservoirs (Fig. 4); however, PC-rich Pcy in Puding Reservoir (Fig. 5) and PE-rich Pcy in Wujiangdu Reservoir (Fig. 6) indicated they had different responses to trophic state of the investigated reservoirs.

4. DISCUSSION

4.1. Relationships between picophytoplankton and hydrographic factors

Each picophytoplankton group presented the lowest abundance in winter and showed a positive correlation between cell number and water temperature (Tab. 4), which indicated the influence of thermal stratification on their growths. However, the extent of temperature influencing PC-rich Pcy was more than that of PE-rich Pcy and picoeukaryotes, reflecting different responses of different picophytoplankton groups to temperature variations. Meanwhile, cell numbers of different picophytoplankton groups were all negatively correlated to depth. This may reflect the importance of light quality on their growth (e.g., Voros et al. 1998).

Nutrient supply is an important factor affecting the picophytoplankton distributions (Stockner & Shortreed 1994; Vrede et al. 1999). PE-rich Pcy exhibited a negative correlation with PO_4^{3-} in autumn in these reservoirs (Tab. 4), reflecting that high phosphate concentration decreases picocyanobacterial growth rates (e.g., Schallenberg & Burns 2001). However, high phosphate can not constrain the PE-rich Pcy growth at higher water temperature (23.1±4.7 °C) in summer, suggesting that there are multi-factors in controlling PE-rich Pcy abundance. Other factors such as grazing by ciliates (Chang et al. 2003) or other microzooplankton, viral infections and co-sedimentation with organic particles (Fuhrman 1999, Waite et al. 2000) may also affect the picophytoplankton distributions; however, they were not tested in this study.

Tab. 4. Relationships between picophytoplankton and temperature (n=226), depth (n=226), and PO_4^{3-} (n=60), respectively, in terms of the results of Pearson’s correlation coefficient. **, Correlation is significant at the 0.01 level (2-tailed); *, Correlation is significant at the 0.05 level (2-tailed).

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Depth</th>
<th>PO_4^{3-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-rich Pcy</td>
<td>.540**</td>
<td>-294**</td>
<td>-.015</td>
</tr>
<tr>
<td>PE-rich Pcy</td>
<td>.263**</td>
<td>-325**</td>
<td>-256*</td>
</tr>
<tr>
<td>Picoeukaryotes</td>
<td>.414**</td>
<td>-298**</td>
<td>-.132</td>
</tr>
</tbody>
</table>

4.2. Variations of different picophytoplankton groups in the cascade reservoirs

Variations in abundances of picophytoplankton reveal distinct abilities of different picophytoplankton groups to cope with different environmental factors (e.g., water temperature, light, and trophic state). PE-rich Pcy, PC-rich Pcy and picoeukaryotes presented different ratios of the cell numbers from each other (Fig. 7) due to different trophic state in these cascade reservoirs.

Damming on river can cause considerable reductions in nutrient loads owing to the removal of these nutrients in reservoir sediments. However, this removal might be overcompensated by anthropogenic trophic inputs downstream of the reservoir. This is the case of Wujiangdu Reservoir. Its high phosphorus concentration is due to fishery and excess phosphorus input by the Xifeng River, a southern tributary of the Wujiang River. For the cascade reservoirs, upriver reservoir receives more nutrient than downriver reservoir. This is the case of Puding Reservoir that shows a higher trophic state than Dongfeng Reservoir although both are impounded in 1989. Puding Reservoir is still more trophic than Hongjiadu Reservoir because of its longer running time although they are upriver reservoirs. The change of picophytoplankton community structure reflects these situations very well.
Fig. 4. Profile of picoeukaryotes in the investigated reservoirs.
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![Graph showing profile of phycocyanin-rich picocyanobacteria (PC-rich Pcy) in the investigated reservoirs.](image)

Fig. 5. Profile of phycocyanin-rich picocyanobacteria (PC-rich Pcy) in the investigated reservoirs.
Fig. 6. Profile of phycoerythrin-rich picocyanobacteria (PE-rich Pcy) in the investigated reservoirs.
Fig. 7. Picophytoplankton community structure in the investigated reservoirs. PC-rich Pcy, phycocyanin-rich picocyanobacteria; PE-rich Pcy, phycoerythrin-rich picocyanobacteria. A1-S is the water sample of A1 at surface; A1-20 is the water sample of A1 at the depth of 20 meters.
With eutrophication, the abundance of PE-rich Pcy decreased and that of PC-rich Pcy increased (Fig. 7). This is in agreement with the model presented by Voros et al. (1998) and with the results in different lakes (Callieri 1996). So, picophytoplankton community structure transformed from dominant PE-rich Pcy to dominant PE-rich Pcy and PC-rich Pcy (e.g., in Wujia-angdu Reservoir). Picoeukaryotes was a minor contribution to autotrophic picoplankton (<5%) (Weisse 1993; Padisak et al. 1997; Callieri 2007); and in this study the increase of picoeukaryotes with eutrophication (e.g., >10% in Wujia-angdu Reservoir) was found. The seasonal variation of picophytoplankton community structure also reflected different responses of autotrophic picoplankton to temperature. The investigation about autumn phytoplankton composition showed that Margalef diversity index and Shannon-Weiner index cannot discern trophic state of these five reservoirs (Wang et al. 2008); however, the change of picophytoplankton community structure can, suggesting they are excellent indicators for the change of trophic state.

5. CONCLUSIONS

Besides picoeukaryotes, two groups of picocyanobacteria have also been detected by flow cytometry. One is a phycoerythrin-rich picocyanobacteria (PE-rich Pcy), the other is a red-fluorescing cells with lacking blue light and its influence on picocyanobacterial types in lakes at different trophic levels. Mem. Ist. Ital. Idrobiol., 46: 1243-1248.


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