

Comparison of photosynthetic fluorescence characteristics of several submerged plants in Honghu Lake, China

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Abstract

Submerged plants are the pioneer species of eutrophic water remediation, and they are important for maintaining the health of aquatic ecosystem, while light is the main limiting factor for the growth of submerged plants. In this study, we measured the maximal quantum yields of photosystem II (F_v/F_m) and rapid light curves (RLCs) of five dominant submerged macrophytes in situ by using pulse-amplitude modulated fluorometer (Diving-PAM). Results revealed that *P. crispus* L. and *M. verticillatum* L. had the highest F_v/F_m value, all species' F_v/F_m are less than 0.8. In addition, the variation trends of F_v/F_m and F_v/F_m were same. All species showed statistically significant differences in α , while *P. crispus* L. and *M. verticillatum* L. showed the highest α value in the five species. And the variation trends of $rETR_m$ and E_k were basically the same. It indicated that *P. crispus* L. and *M. verticillatum* L., both of which had high photosynthetic efficiency, had excellent ability to withstand hard light. Compared five species, *P. crispus* L. and *M. verticillatum* L. had resistance capacity to hard light as well as faster photosynthetic rate, and *V. natans* (Lour.) Hara had higher resistance capacity to low light. Thus, when submerged plants are used for water restoration, *V. natans* (Lour.) Hara could be regarded as a pioneer species in eutrophication water restoration. *P. crispus* L. and *M. verticillatum* L. will have better effects when used in shallow water areas.

Keywords: Honghu Lake; submerged macrophytes; rapid light curve; quantum yield

Introduction

During the past several decades, most lakes around the world have been suffering from eutrophication (Smith and Schindler, 2009; Yang *et al.*, 2017). The causes of lake eutrophication are various: Under the combined interference of natural and man-made factors, a large amount of nutrients are imported into the lake, resulting in an increase in carbon, nitrogen, phosphorus and other source elements in the water body, which leads to an increase in phytoplankton biomass and a decrease in water transparency, gradually transforming it from a nutrient-poor lake to a nutrient-rich lake (Qin *et al.*, 2013; Bal *et al.*, 2017; Liao *et al.*, 2017). Lake eutrophication is the most serious water quality problem in the world, which directly causes the frequent occurrence of toxic algal blooms, the death of fish, the decline of biodiversity, the degradation of lake ecological structure and function and other related ecological problems (David *et al.*, 2008; Wang and Wang, 2009).

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Submerged plants are important structural shapers and functional maintainers of lake ecosystems. Using submerged plants to repair eutrophic lakes has the characteristics of low cost, low carbon and environmental protection, and is a truly sustainable management method (Zhang *et al.*, 2012). However, Light is one of the major factors limiting the growth of submerged macrophytes (Barko and Smart, 1981) and plays a decisive role in the growth and spatial distribution of submerged plants (Bornette and Puijalón, 2011). The decrease of water transparency caused by eutrophication directly leads to the deterioration of underwater light environment, which reduces the photosynthetic capacity of submerged plants, and then leads to the disappearance of a large number of submerged plants (Gudrun and Sara, 2011; Zhao *et al.*, 2018). Our aim is to find a pioneering species with strong photosynthetic capacity in low-light environments with low water transparency, so as to provide a theoretical basis for eutrophication management of shallow lakes.

V. natans (Lour.) Hara, *H. verticillata*, *M. verticillatum* L., *P. crispus* L. and *P. wrightii* Morong are dominant species of submerged plant communities in shallow lakes (water depth is around 150cm) in the middle and lower reaches of the Yangtze River with high pollution tolerance and high pollution absorption. In recent years, the studies on these submerged plants mainly focus on the absorption of nitrogen, phosphorus and heavy metals (Yang *et al.*, 2015; Zhang *et al.*, 2015; Xie *et al.*, 2016), mostly by laboratory simulation experiments.

Chlorophyll fluorescence dynamics technology is a rapid, simple, accurate and non-damaging measurement, which is an important means of detecting and analyzing the relationship between plant photosynthesis and the environment (Zhang, 1999; Küster and Altenburger, 2007; Liu *et al.*, 2010). At present, fluorescence technology has been widely used in the physiological and ecological research of terrestrial higher plants, as well as large seagrass and coral, but less applied in the physiological and ecological research of aquatic higher plants in fresh water (Lamote and Dunton, 2006; Belshe *et al.*, 2007; Hallik *et al.*, 2012; Wang *et al.*, 2015; Zhong *et al.*, 2018; Liu *et al.*, 2019). Existing studies on the fluorescence characteristics of higher aquatic plants in fresh water mostly focus on the comparative analysis of ecological factors on aquatic plant stress under experimental control conditions. However, studies on the comparison of photosynthetic fluorescence parameters of aquatic plants in natural habitats have rarely been reported (Hussain *et al.*, 2019). In this study, we measured main leaf fluorescence kinetics parameters and fast light response curve of five kinds of submerged plant by using in-situ detecting method, and compared their PSII main fluorescence parameters characteristics in natural water, aiming at providing a theoretical basis for remediation of lake eutrophication by submerged plants.

Materials and Methods

Site and conditions

Honghu Lake (N29°42'~29°58', E113°13'~113°29') is the largest lake in Hubei province, China, lying on the north shore of the Yangtze River middle reaches, which plays a significant role in water supply, flood storage and drainage, pollution self-purification, agricultural and fishery production in the Jiangnan Plain of the middle and lower reaches of the Yangtze River (Liu *et al.*, 2004). The vegetation division is part of the arctic plant region and the China-Japan forest plant subregion. From the shore to the center of the lake, there are wet plants (*Polygonum hydropiper* L. *et al.*), emergent plants (*Zizania latifolia* *et al.*), floating-leaved plants (*Trapa natans* *et al.*), submerged plants (*V. natans* (Lour.) Hara *et al.*) and other ecological types successively (Li, 1997).

Methods

Taking the dense growth of submerged plants in Honghu ravine as the sampling area, five common submerged plants: *V. natans* (Lour.) Hara, *H. verticillata*, *M. verticillatum* L., *P. crispus* L. and *P. wrightii* Morong (all these submerged plants were identified by Flora of China (Wu, 2007)) were selected as the research

objects, and their leaf green fluorescence parameters were determined in situ by Diving-PAM. We selected 3 samples from each plant in the lake area with a water depth of 150cm randomly, and 1-2 fully developed healthy leaves were selected for each plant.

F_v/F_m is PSII maximum photochemical quantum yield, they reflect the PSII intrinsic light energy conversion efficiency, which can be described by the equation:

$$F_v/F_m = (F_m - F_o) / F_m$$

where F_o is the minimal fluorescence (testing light is $0.15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); F_m represents the maximal fluorescence (saturation pulse light is $4000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

PSII photochemical effective quantum yield of photochemical reaction (F_v'/F_m') can be described by the equation:

$$F_v'/F_m' = (F_m' - F_o') / F_m'$$

where F_o' and F_m' represent the minimal and the maximal fluorescence under the condition of actinic light adaptation, respectively.

Then, actinic light was turned on at the intensity of 33, 104, 219, 342, 515, 708, 1042 and $1407 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. The fluorescence before turning on the saturation pulse was recorded as F_o , and the fluorescence measured after turning on the saturation pulse light was recorded as F_m' , then we got variable fluorescence (ΔF): $\Delta F = F_m' - F_o$, the effective quantum yield (Y) can be calculated:

$$Y = (F_m' - F_o) / F_m' = \Delta F / F_m'$$

Relative electron transport rate (rETR) was described by the equation (Ralph *et al.*, 1998):

$$\text{rETR} = Y \times \text{PAR} \times 0.5 \times 0.84$$

The least square method was used for the fast light curve fitting, and the equation we used is proposed by Platt (Platt *et al.*, 1980). and Ralph (Ralph and Gademann, 2005). The equation is as follows:

$$\text{rETR} = \text{rETR}_m (1 - e^{-\alpha \cdot \text{PAR} / \text{rETR}_m}) e^{-\beta \cdot \text{PAR} / \text{rETR}_m}$$

where, rETR_m is the maximum potential relative electron transfer rate in the absence of light inhibition, α is the initial slope of the rETR-PAR curve, reflecting the ability of plants to use light energy, β is the photoinhibition parameter. Thus, the half-saturated light intensity (E_k) is $E_k = \text{rETR}_m / \alpha$.

Statistical analysis

Microsoft Excel 2018 and IBM SPSS Statistics 22.0 were used for statistical analysis of all data, all diagrams were drawn by Origin 2018 and one-way ANOVA was used to compare the differences between different data groups.

Results

Comparison of light parameters of five submerged plants

F_v/F_m reflect the PSII intrinsic light energy conversion efficiency. PSII F_v/F_m of 5 plants were *V. natans* (Lour.) Hara respectively 0.603, *H. verticillata* 0.723, *M. verticillatum* L. 0.751, *P. crispus* L. 0.778, *P. wrightii* Morong 0.646 (Figure 1). Among them, the difference of F_v/F_m between *V. natans* (Lour.) Hara and the other four submerged plants reached a significant level ($P < 0.05$), as well as *P. wrightii* Morong, while the F_v/F_m of *V. natans* (Lour.) Hara was the smallest among the tested plants, followed by *P. wrightii* Morong. There is no significant difference in F_v/F_m between *H. verticillata* and *P. crispus* L. ($P < 0.05$), and the *P. crispus* L. showed higher F_v/F_m than *H. verticillata*, simultaneously. No significant differences in F_v/F_m between *M. verticillatum* L. and *H. verticillata* or *P. crispus* L. were observed.

F_v'/F_m' reflect open PSII reaction center original light trapping efficiency (Waldhoff *et al.*, 2002). PSII F_v'/F_m' of 5 plants were *V. natans* (Lour.) Hara respectively 0.423, *H. verticillata* 0.574, *M. verticillatum* L. 0.608, *P. crispus* L. 0.653 and *P. wrightii* Morong 0.493, respectively (Figure 1). Among them, the difference of F_v'/F_m' between *V. natans* (Lour.) Hara and other four plants reached a significant level ($P < 0.05$), as well as *P.*

crispus L. and *P. wrightii* Morong. Moreover, the F_v'/F_m' of *P. crispus* L. is the highest, *P. wrightii* Morong is the second, and *V. natans* (Lour.) Hara is the lowest. However, the differences of F_v'/F_m' between *H. verticillata* and *M. verticillatum* L. are not significant, and their F_v'/F_m' are lower than *P. crispus* L. and higher than *P. wrightii* Morong, significantly.

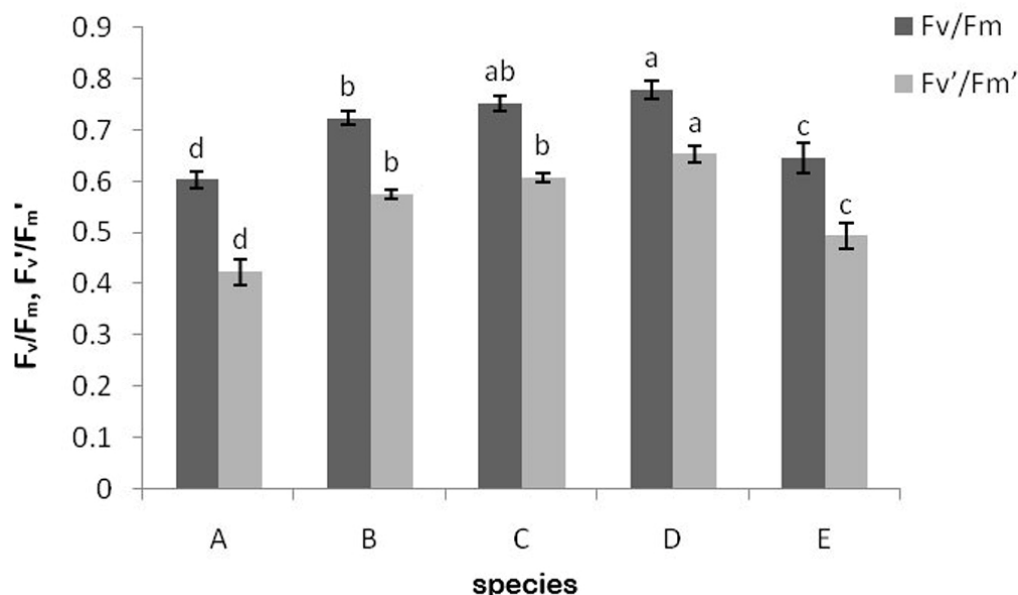


Figure 1. Comparative study on F_v/F_m and F_v'/F_m' of submerged macrophytes (A means *V. natans* (Lour.) Hara B means *H. verticillata* (L. f.) Royle C means *M. verticillatum* L. D means *P. crispus* L. E means *P. wrightii* Morong, the same as below. The different normal letters represent significant differences among species at 0.05 level, the same as below.)

Rapid light curve analysis of different submerged plants

As can be seen from Figure 2, rapid light curves (RLCs) of five common submerged plants show obvious changes, which can be fitted by exponential attenuation equation (Li *et al.*, 2008) ($R^2 > 0.998$, $n=9$). By analyzing RLCs, a series of parameters reflecting photosynthetic capacity can be obtained.

α is the initial slope of the rETR-*PAR* curve, reflecting the ability of plants to use light energy. α of five plants were respectively *V. natans* (Lour.) Hara 0.213, *H. verticillata* 0.373, *M. verticillatum* L. 0.348, *P. crispus* L. 0.487, and *P. wrightii* Morong 0.317 (Figure 3), among which, the difference of between *H. verticillata* and *M. verticillatum* L. was not significant, but the difference between *H. verticillata* and other three plants reached a significant level ($P < 0.05$), as well as *M. verticillatum* L. In addition, *P. crispus* L. had the highest α , *H. verticillata* and *M. verticillatum* L. are in the middle level, *P. wrightii* Morong is next, *V. natans* (Lour.) Hara is the lowest.

rETR_m is the maximum potential relative electron transfer rate of plants under no light inhibition. As shown in Figure 4, the rETR_m of the 5 plants were respectively *V. natans* (Lour.) Hara 12.3, *H. verticillata* 28.6, *M. verticillatum* L. 38.4, *P. crispus* L. 51.7, and *P. wrightii* Morong 16.5, Among them, the differences in rETR_m between each submerged plant reached a significant level ($P < 0.05$). This indicates that the maximum potential relative electron transfer rate of the five submerged plants during the period of no light inhibition is *P. crispus* L. > *M. verticillatum* L. > *H. verticillata* > *P. wrightii* Morong > and *V. natans* (Lour.) Hara.

Half-saturated light intensity (E_k) reflects the tolerance of plants to hard light. As shown in Figure 5, E_k of the five plants were respectively *V. natans* (Lour.) Hara 57.90, *H. verticillata* 76.63, *M. verticillatum* L. 110.35, *P. crispus* L. 106.15, and *P. wrightii* Morong 51.99, among them, the E_k differences between *P. crispus* L. and *M. verticillatum* L. were not significant, however, they reached a significant level with other 3 plants

respectively ($P < 0.05$). And these five submerged plants showed high light tolerance, *P. crispus* L. and *M. verticillatum* L. were the strongest, *H. verticillata* is in the middle level, *V. natans* (Lour.) Hara is next, *P. wrightii* Morong is the weakest.

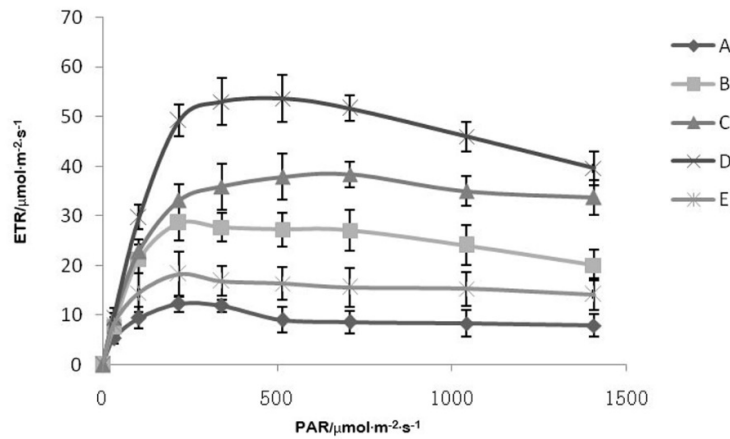


Figure 2. Comparative study on the rapid light curves of five submerged macrophytes

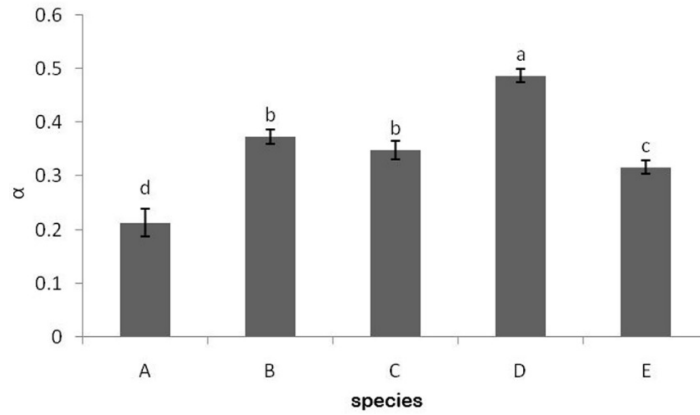


Figure 3. Comparative study on the α of five submerged macrophytes

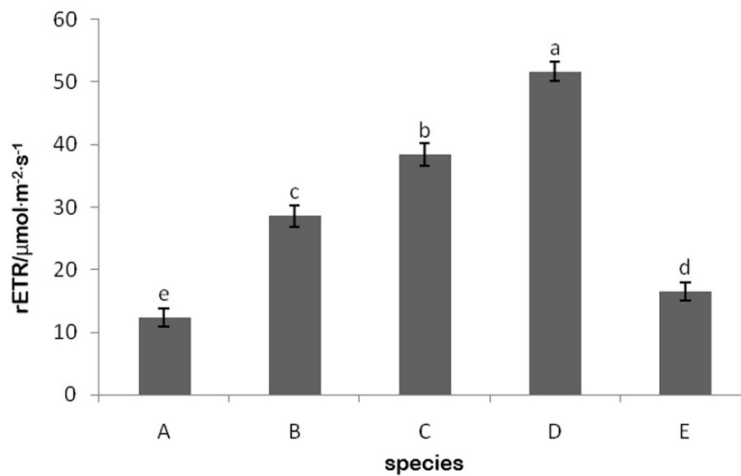


Figure 4. Comparative study on the rETR_m of five submerged macrophytes

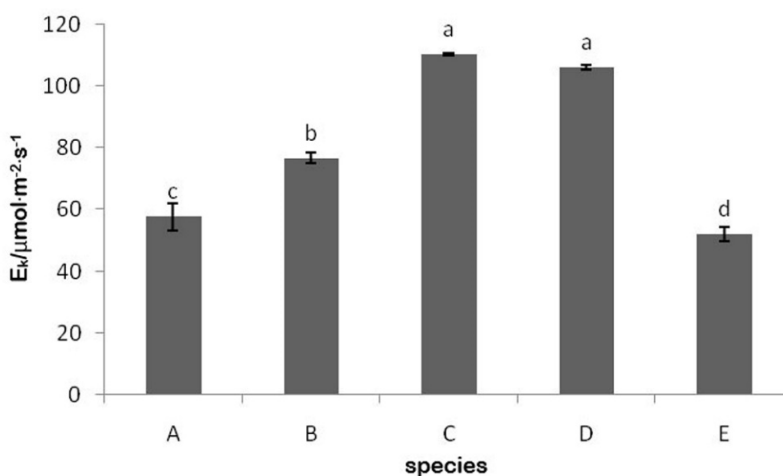


Figure 5. Comparative study on the E_k of five submerged macrophytes

Discussion

Submerged plants have the ability to self-regulate and adapt to the environment, they can adjust the distribution of resources in the plant body for adapting to the environment, which is often manifested as changes in the growth and physiological parameters (Li *et al.*, 2008). Photosynthetic fluorescence parameters in leaves closely relate to photosynthesis in plants and accurately reflect the actual situation of plant photosynthesis in certain circumstances (Guo and Tan, 2015).

F_v/F_m , the maximum quantum yield of PSII, reflects the potential light energy conversion efficiency of the PSII reaction center, and this efficiency is independent of species (Cao *et al.*, 2012; Cheng *et al.*, 2014). F_v/F_m changes little under non-stress conditions and measures approximately 0.83 in most higher plants; the value is significantly reduced in plants subjected to environmental stress. In the present study, F_v/F_m of 5 plants are less than 0.8, showing that the 5 kinds of submerged plants may be intimidated, meanwhile, outside stress may do some damage to the PSII reaction center of submerged plants (Jian *et al.*, 2016). While the variation trends of F_v/F_m' and F_v/F_m were same. At present, there have been reports on the effects of UV-B radiation, polycyclic aromatic hydrocarbon pollution and the attachment of *reticulosa* on F_v/F_m of aquatic plants (Marwood *et al.*, 2001; Li *et al.*, 2009) and the effects of other factors on F_v/F_m is seldom reported. Combined with the water quality in the sampling area, the most likely reason for the decrease of F_v/F_m value of submerged plants in the sampling area is the attached filamentous algae (Song *et al.*, 2009). As for the tolerance of F_v/F_m of different plants to stress factors, further studies are needed.

α , which reflects the level of the light-harvesting capacity of leaves, is associated with the light absorption coefficient of leaves and the light utilization efficiency of PSII. In this study, different species have different α values, *P. crispus* L. and *M. verticillatum* L. are relatively high in the five species. While the variation trends of $rETR_m$ and E_k were basically the same. *P. crispus* L. and *M. verticillatum* L., both of which have high photosynthetic efficiency, have excellent ability to withstand hard light. α of *V. natans* (Lour.) Hara is the lowest, it suggests that *V. natans* (Lour.) Hara has a strong ability to withstand weak light.

Previous report showed that with only 5% natural light intensity, *V. natans* (Lour.) Hara cell membrane permeability still had no obvious change, so it had a strong growth advantage in the bottom of natural water, which may be a pioneer species in the restoration of eutrophic water (Xiao *et al.*, 2006).

Conclusions

Different species have different characteristics of light and fluorescence. External stress may affect F_v/F_m of five submerged plants in Honghu Lake. The photosynthetic capacity of submerged plants is greatly affected by the light response curve, different species have different tolerances to light intensity. Among the five species, *V. natans* (Lour.) Hara has the strongest tolerance to low light and can be used as a pioneer in eutrophication restoration in shallow lakes. In summary, in the ecological restoration of shallow lakes 150 cm deep in the middle and lower reaches of the Yangtze River, the arrangement sequence of five submerged plants is *V. natans* (Lour.) Hara, *P. wrightii* Morong, *H. verticillata*, *M. verticillatum* L. and *P. crispus* L. from deep to shallow. In this study, the photosynthetic fluorescence characteristics of five submerged plants at 150 cm water depth were measured and analyzed, the comparison of photosynthetic fluorescence characteristics of various submerged plants under different water depth gradients in natural water need to be further studied.

Authors' Contributions

Conceptualization: YQZ, BHJ and LYY; Data curation: YQZ and BHJ; Form analysis: BHJ and LYY; Funding investigation acquisition: YQZ and BHJ; Methodology: YQZ and BHJ; Project administration: LYY; Resources: LYY; Software: YQZ; Supervision: YQZ; Validation: YQZ; Visualization: YQZ and BHJ; Writing-original draft: YQZ and BHJ; Writing-review and editing: YQZ, BHJ and LYY. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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