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Effects of Water Turbidity on the Photosynthetic Characteristics of *Myriophyllum spicatum* L.

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Abstract: Mature *Myriophyllum spicatum* L. were planted in ponds with different turbidities (30 NTU, 60 NTU, 90 NTU), which were made by sand particles less than 100 μm . After two months growth, measurements of chlorophyll parameters were made on leaves near stems by a pulse-amplitude fluorometer (Diving-PAM) to investigate the effects of water turbidity on the photosynthetic characteristics of *Myriophyllum spicatum* L. The F_v/F_m and F_0 of *Myriophyllum spicatum* L. in three turbid ponds were not significantly affected by water turbidity. The qP and $\Delta F_v/F_m'$ of plants in pond B and C (30 and 60 NTU) were not significantly decreased when compared with control, while in pond D (90 NTU) they were apparently reduced ($p < 0.05$). Moreover, the values of qN in all four ponds were rather low (≤ 0.1). This indicates that water turbidity can't obviously affect the function of PSII of *Myriophyllum spicatum* L. and most light energy are still used to drive photosynthesis while less are dissipated as heat. The diurnal variation extents of $rETR$ in three turbid ponds were significantly smaller than that in control, indicating that the photosynthesis of plants in turbid ponds was significantly decreased with increasing water turbidity. The diurnal variation of F_v/F_m exhibited a gentle V-shape, which indicates *Myriophyllum spicatum* L. could endure higher irradiance. In ponds whose turbidity were higher than 60 NTU, the recovering speed of F_v/F_m was slower than that in control, but the difference wasn't significant, which demonstrates that water turbidity showed some impact on the anti-photoinhibition ability and recovery capacity of *Myriophyllum spicatum* L., but the impact was not significant. The value of $rETR_{max}$ was decreased with increasing water turbidity, showing that photosynthesis capacity of the plants was apparently decreased.

Key words: *Myriophyllum spicatum* L., water turbidity, photosynthetic characteristics

INTRODUCTION

Various suspended components of water turbidity can affect the productivity of submerged macrophytes (Korschgen *et al.*, 1997). Suspended substances in water can physically block the penetration of irradiance through the water column and greatly lower light availability to submerged plants and inhibit their growth (You and Song, 1995; Noriko *et al.*, 2003). Zhang *et al.* (2006) have found that suspended substances is one of the important factors controlling the reconstruction of submerged macrophytes. However, submerged macrophytes play an important role in forming a clear macrophyte-dominated state in shallow lakes instead of a turbid phytoplankton-dominated state (Scheffer, 1998) by several mechanisms, such as competing with algae for nutrients, reducing the resuspension of the sediment or providing refuge for zooplankton (Torben *et al.*, 2003; Mariana *et al.*, 2003). Therefore submerged macrophytes has been widely used

in lake restoration research. Generally, the suspended substances in turbid water could be divided into two major groups: one is organic matter and algae debris which usually exist in the eutrophic lake; the other is inorganic suspended solids like sands which appear in some lakes connected to rivers. Recent research always focuses on the former one, while the latter one has not so far been studied, especially its effect on submerged macrophytes.

When investigating the effect of light intensity on submerged macrophytes, plant morphology and its biomass are two major values (Korschgen *et al.*, 1997), although iodimetric analysis (Su *et al.*, 2004) and oxygen measurement system (Ben *et al.*, 2002) are also introduced in some articles. However, all these methods only can be used on the detached leaves and can't directly and truly reflect the photosynthetic process of submerged macrophytes. In the recent years, the introduction of a number of highly user-friendly (and portable) chlorophyll

fluorometer can well solve this problem. The DIVING-PAM Underwater Fluorometer is one of them, which has been widely used for studying *in situ* photosynthesis in underwater plants under natural conditions (Beer *et al.*, 1998). The technique is rapid, extremely sensitive and non-intrusive and can be performed on intact, attached leaves (Anthony and Christa, 1999). The operation of the tool is very easy and valuable information may be obtained within seconds. For these reasons Diving-PAM fluorometer has become an important tool in study of photosynthesis, in particular the functioning of PSII (Schreiber *et al.*, 1995).

In China, some lakes and streams connected with rivers have lost submerged vegetation and species diversity. There are only some emerging macrophytes like reeds and *Zizania latifolia* growing along the bank. The major reason is high water turbidity lowers light availability to submerged plants and reduces their growth. Therefore, the essential step to restore and revegetate the submerged plant is investigating the effect of water turbidity on its growth. *Myriophyllum spicatum* L., is a submerged aquatic herb with branching leafy shoots and finely dissected whorls of leaves (Aiken *et al.* 1979). It is popularly used in lake revegetation programs (Zhu *et al.*, 2002; Keskinan *et al.*, 2003; Lauridsen *et al.*, 2003; Sivaci *et al.*, 2004) not only because it can grow well in eutrophic lakes, but also because it is effective in reducing eutrophication (especially for N and P; Song *et al.*, 1997; Zhou *et al.*, 2000) and inhibiting blue-green algae (Nakai *et al.*, 2000; He *et al.*, 2002). However, its growth in turbid water rich in suspended solids has not been well studied. In this present study, the aim was to investigate the effect of water turbidity on the photosynthetic characteristics of *Myriophyllum spicatum* L. by the tool of Diving-PAM.

MATERIALS AND METHODS

Plant material: In this study, the plants of *Myriophyllum spicatum* L. were collected from ponds along Yangtse River located in the Liuhe area of Nanjing city. Twenty days before the experiment, plants in similar development level were selected and planted in the experimental ponds. The sediment in the ponds was 0.3 m in depth.

Experiments design: All experiments were conducted from July 11, 2005 to September 14, 2005 in four outdoor enclosure ponds, located in Jiangsu Key Lab of Environmental Change and Ecological Construction. All ponds were 9.4 m in length and 1.1 m in width. The depths of water were 1.3 m. According to the water turbidity in the lower region of Yangtse River, the turbidities in

3 ponds (B, C and D) were respectively adjusted to 30, 60 and 90 NTU (Nephelometric Turbidity Units), while one pond was left as control (A). During the experiment, a re-circulation system was implemented among 4 ponds by pumps and the velocity of water was set about 1.5 mm sec⁻¹. Every pond was evenly settled into 50 plants of *Myriophyllum spicatum* L. Before the experiment started, the average height of plants is about 1.0 m. Two months later, 10 mature plants of *Myriophyllum spicatum* L. in every pond were randomly selected and measurements of fluorescence parameters were made.

Sands preparation: Sands used in the study were totally collected from lower region of Yangtse River, located in city Zhenjiang of Jingsu. Then sands were solved in water and filtered by an iron grid (mesh diameter 100 μm). After 2 h, the upper part of filtrate, the suspended sands used in our study, were isolated and analyzed. The turbidity of water was measured with a HACH 2100P Portable turbidimeter.

Chlorophyll fluorescence measurements: All chlorophyll fluorescence measurements were performed using a Diving-PAM (PAM 2000: H. Waltz GmbH, Effeltrich, Germany) and all data were collected using a WinControl program (Waltz GmbH, Effeltrich, Germany). Ten mature plants of *Myriophyllum spicatum* L. in each pond were randomly selected and measurements were made on upper (adaxial) surface of leaves, which had been pre-darkened for 10 min. All data were reported as mean±SE. Then the data from three turbid ponds were respectively compared with the one from control. A software of SPSS 11.5 was used to examine significant difference among them and the criterion of significance was set at p<0.05. Measurements included: basic fluorescence upon exposure to weak light after dark adaptation (F₀), basic fluorescence after light adaptation (F₀'), maximum fluorescence (F_m), maximum fluorescence yield of a light adapted leaf exposed to a pulse of saturating light (F_m') and steady state fluorescence of a light adapted leaf (F). These measurements were used to determine quenching and electron transport rate. The potential maximal efficiency of PSII (F_v/F_m) of dark adapted leaves was calculated as F_v/F_m = (F_m-F₀)/F_m. The effective quantum Yield of PSII (ΔF_v/F_m') was calculated as Yield (ΔF_v/F_m') = (F_m' -F_t)/F_m' (Schreiber *et al.*, 1997). Photochemical quenching (qP) was calculated as qP = (F_m' -F_t)/(F_m' -F₀'). Nonphotochemical quenching (qN), which refers to the nonradiative dissipation of energy, was calculated as qN = (F_m-F_m')/(F_m-F₀). Relative electron transport rate (rETR) was calculated as rETR = Yield×PAR×0.84×0.5 (Schreiber *et al.*, 1997). The whole

process of chlorophyll fluorescence measurements were carried out as described previously (Kate and Giles, 2000) from 7:00 to 8:00 am. Initially, a measuring light ($0.15 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$) was switched on, giving a measure of the F_0 (minimal) level of fluorescence. A 0.8 sec saturating-pulse light ($4000 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$) was then applied, allowing the measurement of F_m . Following on this, a 10 sec actinic light ($30 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$) was applied and at appropriate interval, further saturating light was applied. From each of these, F_m' and F_v can be measured.

Diurnal variation of photosynthetic rate measurements:

The experiment was carried out every two hours during the day (from 7:00 am to 17:00 pm). Ten mature plants of *Myriophyllum spicatum* L. were randomly selected and the measurements were performed by the Diving-PAM (PAM 2000: H. Waltz GmbH, Effeltrich, Germany). Initially, the measures of F_0 and F_m were carried out as described previously. Then a 0.8 sec saturating-pulse light ($4000 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$) was applied to give measurements of F_m' and F_v . Upon this, the value of rETR and F_v/F_m could be calculated.

Rapid Light Curving (RLC) measurement: 10 mature plants of *Myriophyllum spicatum* L. were randomly selected in every pond and measurements of RLCs were performed by the Diving-PAM (PAM 2000: H. Waltz GmbH, Effeltrich, Germany). The whole process of RLCs measurements were carried out as described previously from 7:00 to 8:00 am. Initially, the leaves were pre-darkened for 10 sec. Then the leaf clip was removed and a measuring light ($0.15 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$) was switched on, giving a measure of the F_0 (minimal) level of fluorescence. A 0.8 sec saturating-pulse light ($4000 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$) was then applied, allowing the

measurement of F_m . Following on this, a series of 10 sec actinic lights (17, 49, 104, 176, 248, 342, 506, $684 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$) was applied and at appropriate interval, further saturating light was applied. From each of these, F_m' and F_v can be measured. According to $\text{ETR} = \text{Yield} \times \text{PAR} \times 0.84 \times 0.5$, weight mean values of ETR were obtained and then the RLCs could be visualized (Peter and Rolf, 2005).

RESULTS

Response of F_v/F_m and F_0 to increasing water turbidity:

F_v/F_m is a parameter, which measures the intrinsic (or maximum) efficiency of PSII (i.e., the quantum efficiency if PSII centres were essentially all open). Dark-adapted values of F_v/F_m reflect the potential quantum efficiency of PSII and are used as a sensitive indicator of plant photosynthetic performance (Björkman and Demmig, 1987; Johnson *et al.*, 1993). After dark-adaptation, the values of F_v/F_m leaves in pond B, C and D (30, 60 and 90 NTU) were measured by Diving-PAM. They were respectively decreased by 99.95, 97.69 and 97.23% compared with control (Fig. 1), but the decreases were not significant ($p > 0.05$).

F_0 , the yield of fluorescence in the absence of an actinic (photosynthetic) light when PSII reaction centres are totally open, gives information about the efficiency of photochemical quenching and by extension, the performance of PSII. The elevated level of non-photochemical energy dissipation within the antennae matrix can possibly result in the decrease of F_0 , while the photodamage and reversible inactivation to PSII reaction center can cause the increase of F_0 . The value of F_0 in pond B, C and D (30, 60 and 90) NTU, were respectively increased by 11.3, 15.8 and 21.0%, when compared with the control (Fig. 1), but the increases were not significant ($p > 0.05$).

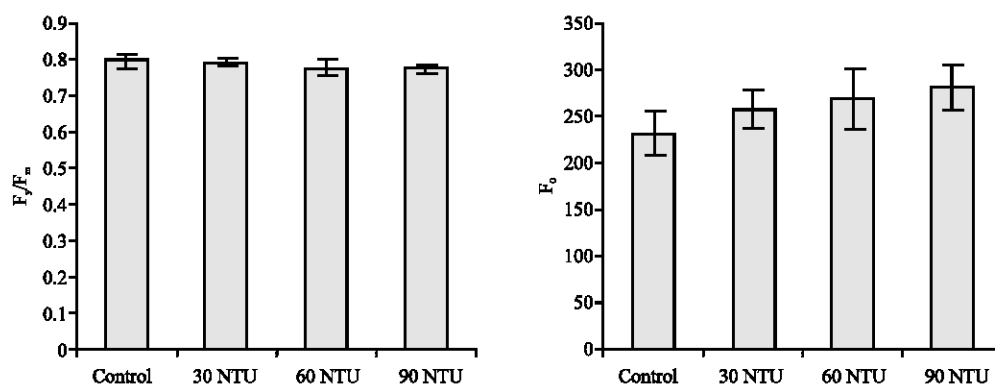


Fig. 1: F_v/F_m and F_0 of *Myriophyllum spicatum* L. in different turbidity ponds. Values are mean \pm SE ($n = 10$), n is number of plants

Response of $\Delta F_v/F_m'$, qP, qN and ETR to increasing water turbidity:

The photosynthetic performance of a plant under light exposing can be reflected by some fluorescence parameters, such as effective quantum Yield of PSII ($\Delta F_v/F_m'$), photochemical quenching (qP), non-photochemical quenching (qN) and relative electron transport rate (rETR). Since the light intensity is greatly diminished by the suspended solids in turbid water, the real irradiance the underwater leaf can get is usually lower than the irradiance on the surface water. Therefore, because the surface water irradiance at 7:00 am was about $30 \mu\text{mol photo m}^{-2} \text{sec}^{-1}$, the leaf of *Myriophyllum spicatum* L. was exposed to the actinic light ($30 \mu\text{mol photo m}^{-2} \text{sec}^{-1}$) for 10 sec in our experiment and then the measurements of fluorescence parameters were made.

$\Delta F_v/F_m'$, this parameter measures the photochemical efficiency of PS II, which refers to the proportion of light absorbed by chlorophyll associated with PS II that is used in photochemistry. Compared with the control pond, the values of $\Delta F_v/F_m'$ in pond B, C and D (30, 60 and 90 NTU) were respectively decreased by 0.3, 3.5 and 4.1% (Fig. 2A), but the decreases were not significant ($p > 0.05$).

qP, the parameter of photochemical quenching, gives an indication of the proportion of PSII reaction centers

that are open (Kate and Giles, 2000). A decrease in qP is due to closure of reaction centers and less quantum yield of carbon fixation, resulting from a saturation of photosynthesis by light. The values of qP in pond B, C and D (30, 60 and 90 NTU) were reduced by 3.0, 4.1 and 5.3% when compared with control (Fig. 2B). The decline achieved significant level in pond D (90 NTU) ($p < 0.05$).

qN, linearly related to heat dissipation, can measure changes in heat dissipation. Any change in qN gives information about change in the efficiency of heat dissipation, which occur as a result either of processes that protect the leaf from light-induced damage or of the damage itself (Kate and Giles, 2000). In all four ponds, although the values of qN were increased with increasing water turbidity (control < 30 < 60 < 90 NTU), they were all lower than 0.1, which implies that all plants showed limited development of non-photochemical quenching (Fig. 2C).

The Electron Transport Rate (ETR) was found to be closely related to the photosynthetic activity when measured by oxygen evolution or CO_2 uptake (Beer *et al.*, 1998), which is expressed as μmol of electrons per $\text{m}^{-2} \text{s}^{-1}$. Relative ETR is an approximation of the rate of electrons pumped through the photosynthetic chain (Beer *et al.*, 2001), which is calculated as $\text{rETR} =$

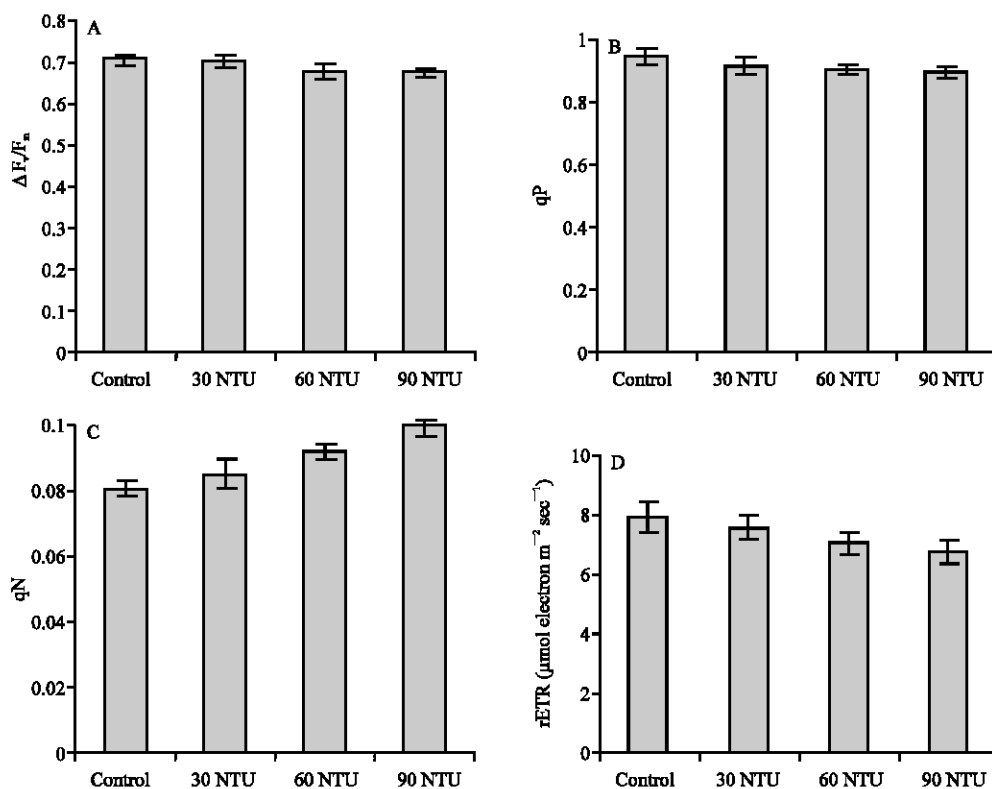


Fig. 2: Yield, qN, qP and rETR of *Myriophyllum spicatum* L. in different turbidity ponds. Values are mean±SE (n = 10), n is number of plants

Yield \times PAR \times 0.84 \times 0.5 (Schreiber *et al.*, 1997). In the experiment, the values of rETR in turbid ponds were all lower than the one in control. Statistical analysis demonstrates that water turbidity had significant effect on the plants in pond D (90 NTU) ($p < 0.05$), but no significant effect on the other two turbid ponds (30 and 60 NTU) was observed (Fig. 2D).

Response of diurnal variation of rETR and F_v/F_m to increasing water turbidity:

To further investigate the effect of water turbidity on the PSII properties of *Myriophyllum spicatum* L., the diurnal variation of rETR and F_v/F_m in all four ponds were determined with Diving-PAM. The diurnal variation of irradiance, rETR and F_v/F_m were plotted as dotted curves. At 7:00 am, the irradiance on the surface water gradually increased, reached an elevated level at 11:00 am, achieved a zenith at 13:00 pm and then gradually decreased (Fig. 3). The curves of diurnal rETR variation exhibited similar shapes to the curves of diurnal irradiance variation. During the period from 9:00 am to 15:00 pm, the values of rETR were decreased with the increasing water turbidity (control $>$ 30 $>$ 60 $>$ 90 NTU) and the differences of rETR between the three turbid ponds and the control pond were significant ($p < 0.05$).

In contrast, the diurnal F_v/F_m variation exhibited flat V-shaped curves, which were almost opposite shapes to the curves of diurnal irradiance variation (Fig. 4). The diurnal variation of F_v/F_m in pond B (30 NTU) was not significant different with the one in control pond ($p > 0.05$). Similar to control, its lowest value of F_v/F_m occurred at 13:00 pm, but at 17:00 pm its F_v/F_m could recover to 96.5% of the one at 7:00 am, showing a higher capacity for F_v/F_m recovery. In pond C and D (60 and 90 NTU), the differences between diurnal F_v/F_m variation and the control were enlarged when compared with pond B (30 NTU), but still not significant ($p > 0.05$). Moreover, the recovery of F_v/F_m in the two turbid ponds was slower than that in pond B (30 NTU), that is, at 17:00 pm the value of F_v/F_m respectively recovered to 95.0 and 92.9% when compared with their own initial value at 7:00 am.

Response of Rapid Light Curves (RLCs) to increasing water turbidity:

Light curves is another useful parameter to measure and describe the acclimation of the photosynthetic apparatus PSII to a range of water turbidity. In comparison to traditional light curves, a Rapid Light Curve (RLC) measures the effective quantum yield as a function of irradiance. It can provide a reliable assessment of photosynthetic activity, by integrating the leaf's ability to tolerate light fluctuation, as well as

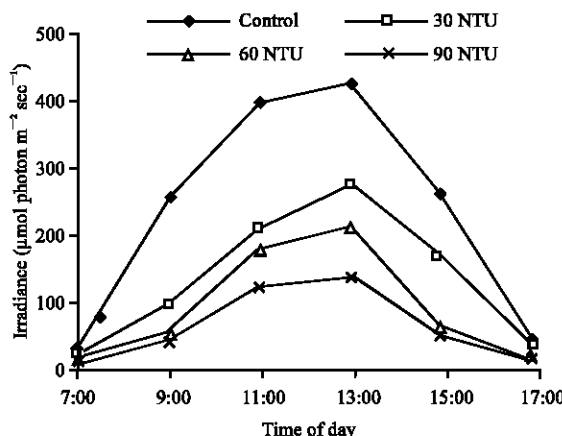


Fig. 3: Diurnal variation of irradiance in different turbidity ponds

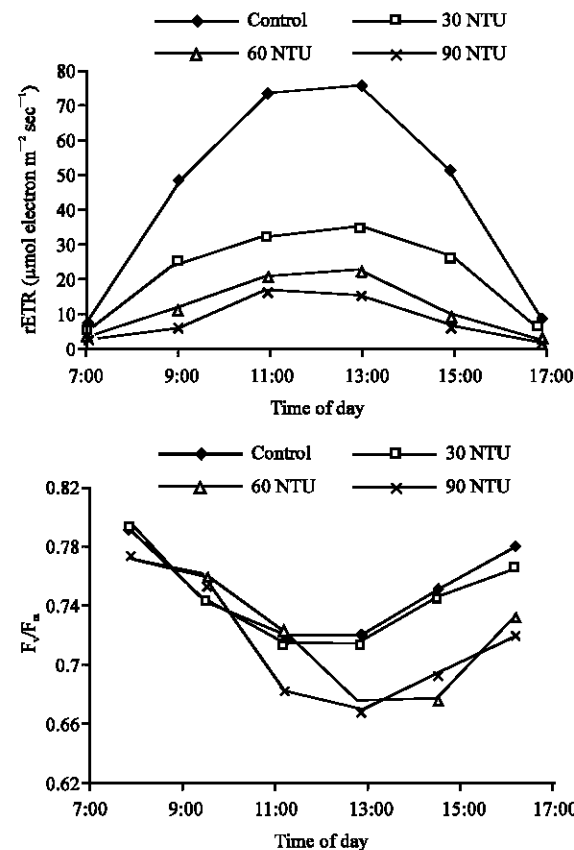


Fig. 4: Diurnal variation of rETR and F_v/F_m of *Myriophyllum spicatum* L. in different turbidity ponds. Values are mean \pm SE (n = 10), n is number of plants

reflecting its immediate short-term light history (Schreiber *et al.*, 1997). Figure 5 showed four average RLCs from four experimental ponds. Increasing water

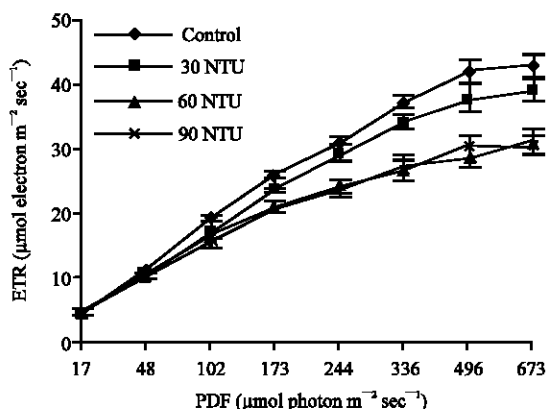


Fig. 5: Rapid-light curves (RLCs) of *Myriophyllum spicatum* L. in different turbidity ponds. Values are mean \pm SE (n = 10), n is number of plants

turbidity can not affect the minimum saturation irradiance (E_k) (Fig. 5). In four ponds (from A to D), the values of E_k were all $673 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$. The value of $rETR_{\text{max}}$ however, was affected by water turbidity. In control pond, the value of $rETR_{\text{max}}$ was $42.08 \mu\text{mol electron m}^{-2} \text{sec}^{-1}$, significantly higher than those in three turbid ponds (30, 60 and 90 NTU), whose $rETR_{\text{max}}$ were $37.71 \mu\text{mol electron m}^{-2} \text{sec}^{-1}$, $28.75 \mu\text{mol electron m}^{-2} \text{sec}^{-1}$ and $30.51 \mu\text{mol electron m}^{-2} \text{sec}^{-1}$, respectively. And with the increase of irradiance, the values of $rETR$ in three turbid ponds became more significant different with control. Additionally, the difference of $rETR$ between pond C and D (60 and 90 NTU) was not apparent, but the values of $rETR$ in the two ponds were profoundly lower than that in pond B (30 NTU).

DISCUSSION

Water turbidity caused by suspended particulates not only can physically block the penetration of irradiance through the water column, but also may be harmful to submerged macrophytes when deposited on leaf surfaces by reducing light transmission to photosynthetically active leaf surfaces and possibly altering gas and nutrient exchange (Korschgen *et al.*, 1997), which finally would lead to the decrease of photosynthetic capability and the damage to photosynthetic apparatus. After planted in different turbidity ponds for two-month, there were many suspended sands deposited on the leaf of *Myriophyllum spicatum* L. However, present study revealed that water turbidity may have caused a degree of photodamage to the leaf of *Myriophyllum spicatum* L., but can't apparently affect its photosynthetic apparatus-PSII, because the F_0 and F_v/F_m of dark-adapted leaf in the three turbid ponds didn't show significant difference with control.

Over the past 17 years, the measurement of the chlorophyll a fluorescence has proven to be a powerful method of assessing the properties of the PSII which is the most sensitive component of photosynthetic apparatus within intact leaves (Schreiber, 2004). Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be re-emitted as light-chlorophyll fluorescence (Kate and Giles, 2000). Yet, the total amount of chlorophyll fluorescence is very small (only 1 or 2% of total light absorbed) and most energy are used in photochemistry process and heat dissipation. The effective quantum yield of PSII ($\Delta F_v/F_m'$), photochemical quenching (qP) and non-photochemical quenching parameters (qN and NPQ) are some coefficients, describing the relative influence of the energy dissipation pathways. qP and $\Delta F_v/F_m'$ can give a measure of photochemistry, while qN and NPQ are both measures of the energy flow into heat (Peter and Rolf, 2005). An increase of qP and $\Delta F_v/F_m'$ indicates more light energy are used in photochemical process, while an increase of qN and NPQ indicates more energy are dissipated as heat. In our study, the fluorescence parameters of *Myriophyllum spicatum* L. were measured under a $30 \mu\text{mol photon m}^{-2} \text{sec}^{-1}$ actinic illumination. Results showed that in pond B and C (30 and 60 NTU), the value of qP, $\Delta F_v/F_m'$ and ETR were not significantly decreased, but the values of qN were rather low. This indicates that water turbidity can't obviously affect the function of PSII of *Myriophyllum spicatum* L. and most light energy are still used to drive photosynthesis while less are dissipated as heat.

Generally, the photosynthetic process of plant is closely related with the diurnal variation of irradiance. In turbid water rich in suspended sands, the irradiance under water is clearly decreased with increasing water turbidities. In the experiment, the diurnal variation curves of $rETR$ showed that the value of $rETR$ in all four ponds were initially increased with the increasing irradiance, reached a maximum at noon and then gradually decreased. However, compared with control, the values of $rETR$ in three turbid ponds were significantly decreased and were decreased with increasing water turbidity. This indicates that water turbidity showed significant effect on the photosynthetic process of *Myriophyllum spicatum* L. and the diurnal photosynthetic capacity of plants was decreased with increasing water turbidities. In contrast, the diurnal variation of F_v/F_m displayed almost opposite behavior to the diurnal variation of $rETR$. But the slope of the V-shape diurnal F_v/F_m variation curves was quite gentle, indicating that although high irradiance did induce

photoinhibition on the leaves of *Myriophyllum spicatum* L., but the plant's tolerant ability and recovery capacity to photoinhibition were not significantly affected.

Rapid light curves (RLCs) can provide detailed information on the saturation characteristics of electron transport, as well as the overall photosynthetic performance of a plant. Results of RLCs demonstrate that under high irradiance (higher than 100 $\mu\text{mol photo m}^{-2} \text{sec}^{-1}$), the relative electron transport rate (rETR) of *Myriophyllum spicatum* L., including the value of rETR_{max} was significantly decreased with increasing water turbidity, while its minimum saturation irradiance (E_k) was not affected. This implies that water turbidity can profoundly decreased the photosynthetic acclimation ability of *Myriophyllum spicatum* L. to light fluctuation and under higher irradiance the effect became more apparently with increasing water turbidity. However, its capacity to resist photoinhibition was not significantly affected.

In conclusion, results presented here show that mature *Myriophyllum spicatum* L. is a submerged species which could tolerate high water turbidity caused by suspended solids. It can grow well in turbid water whose turbidity is lower than 30 NTU (including 30 NTU). However, growing in water whose turbidity is higher than 60 NTU (including 60 NTU), its whole photosynthesis is dramatically reduced with increasing water turbidity, but no significant impact on its capacity to resist photoinhibition is observed under high irradiance. Therefore, *Myriophyllum spicatum* L. is a good aquatic species when applying revegetation and restoration in some lakes or streams connected with rivers. Obviously, further studies are needed to demonstrate the effect of water turbidity on the growth and development of *Myriophyllum spicatum* L. at a larger time and special scale.

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