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A comparison of the growth and photosynthetic response of *Vallisneria natans* (Lour.) Hara to a long-term water depth gradient under flowing and static water

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ABSTRACT

In a mesocosm experiment, the growth and photosynthetic responses of *Vallisneria natans* (Lour.) Hara was studied monthly in different water depths under flowing and static water. Water depth showed a significant effect on the shoot length, below-ground: above-ground biomass, and total biomass, while water velocity showed only a significant effect on the total biomass. In addition, total biomass and shoot length at 45, 75 and 105 cm was higher in the flowing treatment than that in the static treatment, which suggested that total biomass and shoot length are promoted by water flow to some extent. All of the investigated photosynthetic showed different changes with different months. Water depth exhibited significant effects on the maximum photosynthetic efficiency F_v/F_m , the maximum electron transport rate $rETR_{max}$, Chl_a , $Chl_a + b$ and Chl_a/b , while water velocity showed only significant effects on Chl_a , $Chl_a + b$, ETR_{max} . The rapid light response curves varied differently with the time periods. In October, the time-course of slow chlorophyll *a* fluorescence induction curves, F_m peak, in the flowing treatment in 45, 75 and 105 cm is higher than that in the static treatment. All the results demonstrated that the differences between flowing and static water resulted in the different life strategy.

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Fluorescence; biomass; macrophytes; productivity; water depth; flowing and static water; F_v/F_m

Introduction

Vallisneria natans (Lour.) Hara, a perennial submerged clonal plant with a wide geographical range, grows predominantly in still water such as lakes and ponds, or flowing water in streams, creeks and rivers (Xu, Hu, Deng, Zhu, Zhou, and Liu 2016) where it is rooted in mud, silt or mixed sediment of silt, sand and gravel. *V. natans* also plays an important role in the maintenance and stabilization of freshwater ecosystems, such as providing food for waterfowl, nursery habitat for fishes and substrate for invertebrates and purifying water quality (Li et al. 2005; Wu et al. 2009). Therefore, it is used frequently to restore freshwater ecosystems in China (Xie et al. 2007). *V. natans* is widely distributed in shallow water and its area and density are generally lower when distributed in deep water. Cao et al (2014) have found that the suitable water depth for restoring *V. natans* is in the range of 100–140 cm in the region with high water transparency. In habitat such as rivers, flowing water creates the water movement, while

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wind and artificial perturbation-induced waves generate turbulence in lakes (Schutten et al. 2005). In such habitats, mechanical stress can result from exposure to water flow. Such stresses result ultimately in negative effects on plant growth (Power 1996; Crossley et al. 2002). On the other hand, water flow can have an indirect positive effect on plant growth. Moderate water motion can increase the flow of chemicals across the boundary layer (Koch 1994; Hurd 2000), thus favoring metabolic processes and, potentially, plant growth (Thomas et al. 2000; Stewart and Carpenter 2003).

Additionally, many studies reported that water depth is the most important environmental factor influencing water transparency and hence survival and distribution of submerged plants varies with depth (Milne et al. 2006; Cao et al. 2012; Zhu et al. 2012). Macrophytes are able to grow only at water depths less than the maximum depth of macrophytes. Therefore, determining the optimal light requirement threshold to enable macrophyte growth is essential for macrophyte recovery and aquatic system management (Collier et al. 2012). Thus, water depth is a major environmental factor influencing the growth, reproduction and distribution of submerged macrophytes (Strand and Weisner 2001; Wang et al. 2016; Xu, Hu, Deng, Zhu, and Li 2016). Submerged macrophytes exhibit high phenotypic plasticity in response to increasing water depth. Morphological characteristics, such as plant length, ramet number, internodal length and branch number may react to water depth (Zhu et al. 2012; Reckendorfer et al. 2013). Such adaptations are influenced by different photosynthetic efficiencies at low light intensities (Yang et al. 2004; Eusebio Malheiro et al. 2013).

It is known that for submerged aquatic macrophytes in general, moderate water flow improves leaf uptake of nutrients as well as uptake of dissolved inorganic carbon and oxygen (Smith and Walker 1980; Larkum et al. 1989; Stevens and Hurd 1997). Although plastic responses to water depth or water flow often have been reported for many aquatic plants (Atapaththu and Asaeda 2015; Baastrup-Spohr et al. 2016), there is a lack of information relating plasticity in photosynthetic response of submerged plants to both water depth and water flow. Chlorophyll fluorescence is strongly connected with plant photosynthesis and for this reason it has been central to understanding the fundamental mechanisms of photosynthesis, the responses of plants to environmental change and ecological diversity. In fact, chlorophyll fluorescence from plants is a detectable signal which can be measured at some distance from the studied sample in a non-intrusive way (Schreiber et al. 1995; Cerovic et al. 2002). The main purpose of this study was to compare the effects of water depth on the growth and photosynthetic properties of *V. natans* under flowing and static water, and to examine the interactions between water flow, water depth and growth.

Methods

Plant materials

Seedlings of *V. natans* were collected from the Plant Experiment Station of Wuhan Botanical Garden, CAS, and pre-cultured in tap water for acclimation. The sediment was collected from East Lake, a highly eutrophic lake in Hubei Province, China. (total nitrogen (TN) = 3.894 ± 0.152 mg/g DW, total phosphorus (TP) = 1.357 ± 0.028 mg/g DW, and organic matter content = $7.032 \pm 0.194\%$, mean \pm SD, $N = 6$) and placed into pots (20 cm in diameter, 15 cm high) as the planting substrate.

Experimental setup

The experiment was conducted in outdoor ponds. An engine outside of the raceways was installed and drove a clear-water pump to create water flow. The power of the engine was adjusted to produce demanded water velocities. Water flow was measured just above the sediment surface of each water depth utilizing a warm bead thermistor flow meter. The velocity (0.3 m/s) used was comparable to

the velocities found within *Vallisneria* plants in running water (0.01–0.1 m/s). The velocity in the flowing ponds was controlled by the inlet valve and power of the pump. The measurement of water velocity within macrophyte patches was measured by a hand-held and portable acoustic Doppler velocimeter. The experiment lasted five months and was replicated three times.

Pond experiment

The seedlings were cultivated in 60 cm water depth. A week later, a total of 72 young seedlings with similar size (3–4 leaves, <5 cm root, 30 cm long) were selected and transplanted individually to 24 small plastic trays (20 cm diameter × 20 cm high) filled with 5.5 kg sediment. Three seedlings were planted in each small plastic tray in a triangle arrangement. The small plastic trays were then placed into the eight ponds. After transplanting the plants, the pond was fully filled with tap water. There were four water depths (45, 75, 105 and 135 cm) and two water velocities (0 and 0.3 m/s) used in this experiment in a random design.

Harvest and plant morphology

At harvest, plants were dug out by hand with care to collect as many roots belonging to plants as possible and cleaned with tap water. The plants' fresh weight was recorded. Plants were separated into two parts: below-ground and above-ground. After drying at 80 °C for 72 h to constant weight, the two parts were weighed to measure biomass production. Plant shoot length was calculated as the average shoot length.

Measurements of chlorophyll (Chl) content and water chemistry

The total chlorophyll was extracted from 200 mg fresh apical shoots in 10 mL of 90% acetone in the dark for 48 h at 25 °C. Then the supernatant was used to measure the absorbance with a spectrophotometer (Daojin uv-1800, Japan) at 645 nm and 663 nm, respectively. The chlorophyll (*a* and *b*) content was calculated by the equations of Arnon (1949) and expressed in mg of pigments per g of fresh weight. Physicochemical analyses were carried out at each site on each sampling date: temperature (Thermo ORION 5-STAR, US), light intensity (ZDS-10, Shanghai), pH, dissolved oxygen (DO) and conductivity were measured once at 1500 h every three days.

Chlorophyll fluorescence

Light response curves of chlorophyll *a* fluorescence were performed simultaneously with a Dual-PAM-100 measuring system (Walz-Effeltrich, Germany) in detached, new, fully expanded leaves collected from the top of the plant. Leaves were immediately dark adapted for 5 min (to obtain open reaction centers (RCs)). A saturating pulse was applied to obtain the maximal fluorescence (F_m) and then leaves were exposed for 5 min at each photosynthetic photon flux density (PPFD) (0, 27, 58, 131, 221, 344, 435, 665, 1033 and 1957 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in order to obtain steady state readings. All the measurements were performed at room temperature. Recordings and calculations were performed with the Dual-PAM 1.7 data analyses and control software, and the data were retrieved from the software output (Walz-Effeltrich, Germany).

Statistical analyses

A fixed-model two-way ANOVA was used to evaluate effects of water depth and water flow on biomass, below-ground: above-ground biomass and shoot length, while repeated measures ANOVA was used to evaluate the pigment and chlorophyll fluorescence characteristics. Multiple comparisons

Table 1. Water chemical conditions during the experiment.

Parameter	Static	Flowing
	Mean \pm SD	Mean \pm SD
pH	7.69 \pm 0.003	8.75 \pm 0.005
TSS (mg/L)	16.31 \pm 0.01	15.58 \pm 0.01
TN	0.32 \pm 0.05	0.49 \pm 0.078
TP	0.06 \pm 0.01	0.083 \pm 0.014
DO (mg/L)	6.32 \pm 0.023	7.64 \pm 0.034
Chla (μ g/L)	1.53 \pm 0.47	2.78 \pm 0.53
EC (μ S/cm)	198.0 \pm 0.31	254.0 \pm 0.57
TDS	760.2 \pm 1.34	756.2 \pm 1.04
WT (cm)	150 \pm 0.01	82.8 \pm 33.12
ORP (mV)	-136.2 \pm 2.1	-98.2 \pm 1.5
Turbidity (NTU)	1.95 \pm 0.09	8.23 \pm 0.31

of means of were performed using Duncan's test at the 0.05 significance level. All plant data were log-transformed and met assumptions of normality prior to analyses, and homogeneity was tested using Levene's test. The statistical package SPSS 22.0 was utilized for all analyses.

Results

Results of environmental factors during the experiment

Table 1 shows the environmental parameters such as pH, total suspended solids (TSS), DO, chlorophyll *a* (Chla), electrical conductivity (EC), total dissolved solids (TDS), water transparency (WT), oxidation–reduction potential (ORP) and turbidity during the experiment. The parameters, pH, DO, Chla, EC, ORP and turbidity in the static water were lower than that in the flowing water (*t* test, *df* = 28, *p* < 0.05), while SD was higher (*t* test, *df* = 28, *p* < 0.05).

Figure 1 shows the water temperature and irradiance during the experiment. The water temperature in the flowing water was higher than that in the static water; additionally, the water temperature generally exhibited no obvious change in the flowing water from 45 to 135 cm water depth.

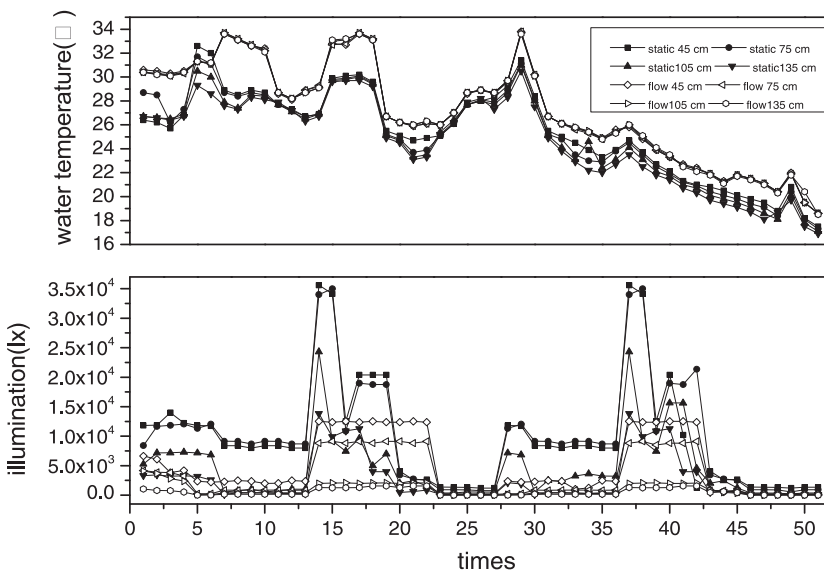


Figure 1. The water temperature and illumination of the sampling point of different water depths during the experiment.

The water temperature showed a progressive decrease in the static water from 45 to 135 cm water depth. Also, the irradiance in each pond showed a progressive decrease from 45 to 135 cm, but the irradiance in the static water was higher than that in the flowing water.

Plant growth and morphology

Total biomass, the ratios of below-ground: above-ground biomass and shoot length of *V. natans* in the experiment are shown in Figure 2. *V. natans* showed a significant increase in biomass with increasing water depth in the static treatment; Total biomass was significantly lower in the static water than in the flowing water (7–27 g per plant versus 3–13 g per plant). Total biomass increased from 3 to 13 g per plant in the static water with increasing water depth from 45 to 135 cm, indicating that plant growth was positively related to water depth. Statistical analysis showed that biomass accumulation was significantly affected by both the water depth and water velocity (Table 2, $p < 0.01$).

The ratios of below-ground: above-ground biomass of *V. natans* were significantly affected by water depth (Table 2), but not significantly by water velocity (Table 2, $p > 0.05$). Low water depth usually resulted in a high root: shoot ratio. Of the four water depth treatments, the ratios of below-ground: above-ground biomass were the highest in 45 cm in the flowing treatment (1.11 ± 0.23).

The shoot length of *V. natans* responded positively to water depth in the static water. At the end of the experiment, compared to the initial length (20 cm), the shoot length of *V. natans* increased greatly, according to the final shoot average length of *V. natans*, the order from long to short in the static treatments was: 135 cm (74.5 ± 5.92 cm) > 105 cm (62 ± 2.83 cm) > 75 cm (54.5 ± 4.43 cm) > 45 cm (20.5 ± 0.58 cm). In the static water, the length of *V. natans* increased about 3.4 times, while the length of *V. natans* increased about 2.1 times in the flowing water.

Pigment analysis

All the investigated pigment indices Chl a , Chl b , Chl $a + b$ and Chl a/b showed different changes with different months, water depth showed significant effects on Chl a , Chl $a + b$, Chl a/b , while water velocity showed only significant effects on Chl a , Chl $a + b$ (Table 3). The Chl a , Chl b , Chl a/b and total Chl concentrations are presented in Figure 3. In the static treatment, Chl a concentrations were 0.17 ± 0.04 , 0.21 ± 0.05 , 0.20 ± 0.02 and 0.27 ± 0.01 mg/g FW for 45, 75, 105 and 135 cm, respectively, in October, which were much lower than in June, July, August and September. Differently, in the flowing water, the Chl a concentrations in August were lower than other months. Analogously, in the flowing water, the Chl b was 0.06 ± 0.01 , 0.07 ± 0.02 , 0.05 ± 0.01 and 0.06 ± 0.01 mg/g FW for 45, 75, 105 and 135 cm, respectively, in September, which was obviously lower than other months. In October, total Chl concentrations in the static water were 0.23 ± 0.06 , 0.27 ± 0.07 , 0.24 ± 0.03 and 0.35 ± 0.02 mg/g FW for 45, 75, 105 and 135 cm, respectively, which were obviously lower than other months. The concentration tendencies of Chl a , Chl b and total Chl in both the static water and the flowing water were all the same; however, in October, Chl a/b ratio in the static water were 2.52 ± 0.27 , 4.07 ± 0.15 , 4.94 ± 0.71 and 3.58 ± 0.33 for 45, 75, 105 and 135 cm, which were obviously lower than the corresponding water depth in the flowing water (3.30 ± 0.47 , 7.16 ± 0.97 , 6.98 ± 0.41 , 5.12 ± 0.73). Different from the result above, Chl a/b ratio in the static water in October except in the 45 cm was significantly higher than other months.

The maximum photosynthetic efficiency (F_v/F_m) and $rETR_{max}$

The maximum photosynthetic efficiency (F_v/F_m) and the maximum electron transport rate ($rETR_{max}$) showed different changes with different months, and water depth had significant effects on F_v/F_m and $rETR_{max}$. In contrast, water velocity showed only significant effects on ETR_{max} (Table 3). Figure 4 shows the differences in F_v/F_m of *V. natans* in the static and flowing treatments. F_v/F_m in the static water were 0.62 ± 0.05 , 0.60 ± 0.02 , 0.64 ± 0.05 , 0.69 ± 0.06 and 0.63 ± 0.13 in 45 cm for

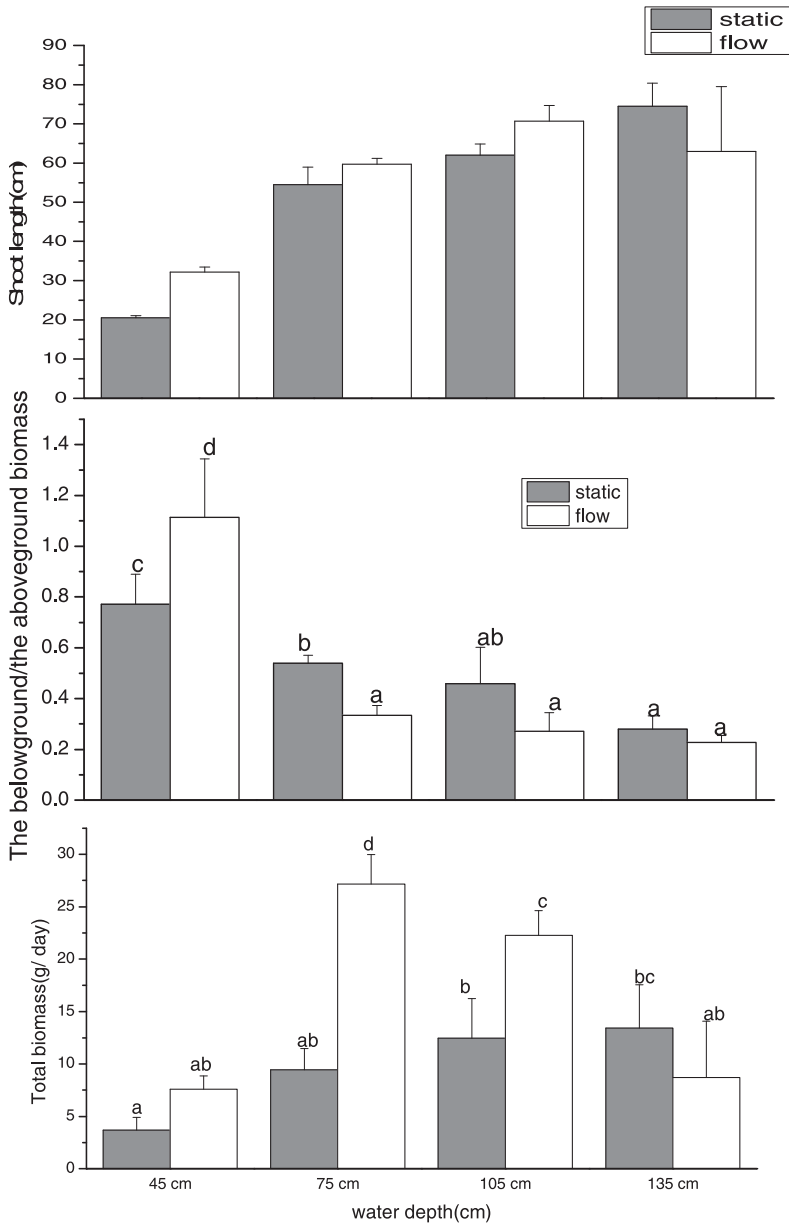


Figure 2. Total biomass, the ratios of below-ground: above-ground biomass and shoot length (cm) (means \pm SD) of *Vallisneria natans* grown in the experiment treatments.

Table 2. *F*-values and significance of two-way ANOVA of the effects of water depth and water velocity on total biomass, below-ground: above-ground biomass and shoot length of *V. natans*.

Dependent variable	Water depth (<i>D</i>)	Water velocity (<i>V</i>)	<i>D</i> \times <i>V</i>
Total biomass	15.27***	18.32***	8.58***
Below-ground: above-ground biomass	17.47***	1.304	1.69
Shoot length	78.48***	2.36	5.13**

*** $p < 0.001$.

** $p < 0.01$.

Table 3. F-values and significance of repeated measures ANOVA results showing water depth and flowing water effects on Chla, Chlb, Chla + b, Chla/b, F_v/F_m and ETR_{max} of *V. natans* from June to October during a five-month growth period.

Dependent variable	Month (M)	Water depth (D)	Water velocity (V)	$D \times V$	$M \times D$	$M \times V$	$M \times V \times D$
Chla	78.08***	8.22**	14.52**	12.46***	2.95**	110.27***	2.55*
Chlb	145.11***	0.92	0.20	10.62***	3.62**	55.92***	2.57*
Chla + b	100.79***	5.91**	8.05*	13.54***	2.35*	102.24***	2.58*
Chla/b	7.01***	3.77*	0.21	2.71	5.28***	28.39***	6.19***
F_v/F_m	26.46***	24.41***	0.73	0.443	2.89*	3.26*	1.56
ETR_{max}	26.85***	15.11***	10.66**	3.01	6.45***	14.66***	2.43*

*** $p < 0.001$.
 ** $p < 0.01$.
 * $p < 0.05$.

June, July, August, September and October, respectively, which showed little change, but in the flowing water F_v/F_m in 45 cm was 0.59 ± 0.06 , 0.48 ± 0.03 and 0.59 ± 0.04 for June, July and August, respectively, which was obviously lower than that in September (0.72 ± 0.02) and October (0.75 ± 0.02). Overall, F_v/F_m in 45 and 75 cm were lower than that in 105 and 135 cm. Additionally, F_v/F_m in June and July < October and September < August.

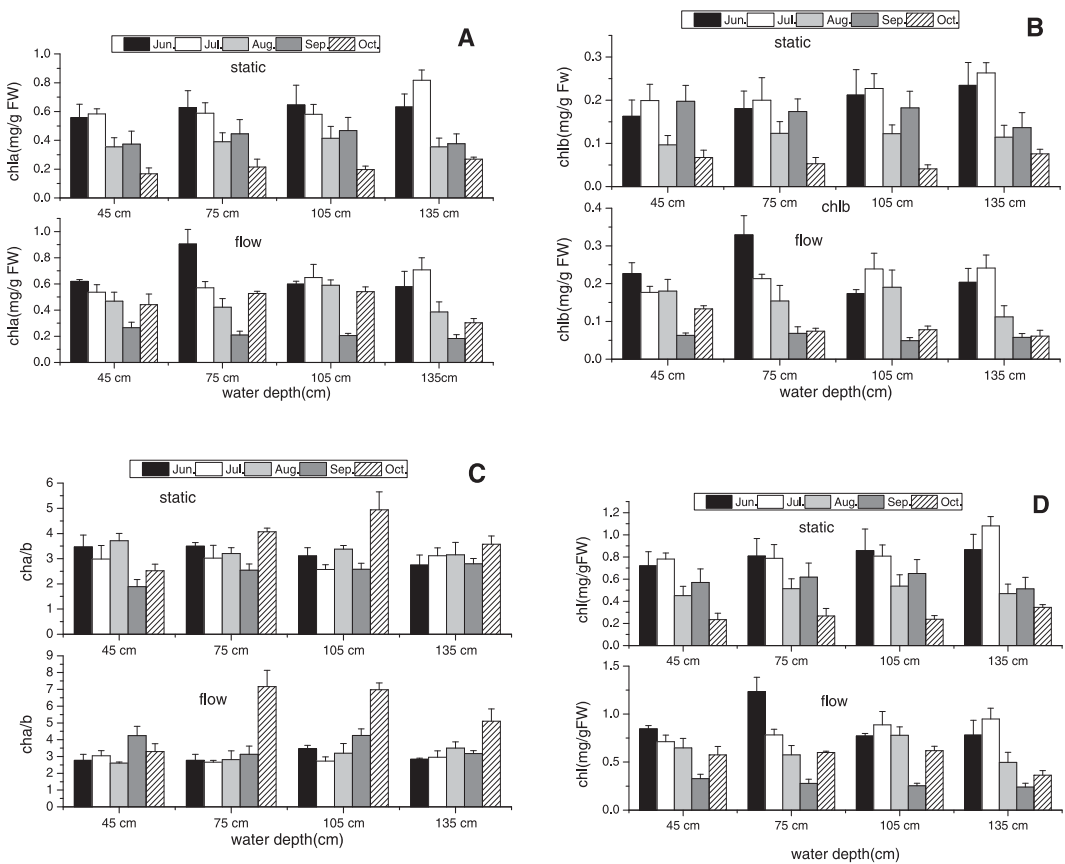


Figure 3. Chlorophyll content in *Vallisneria natans*. Chlorophyll concentrations were evaluated, on a fresh weight basis, in different water depths at static and flowing water treatment, over a period of five months. (A) Molar concentration of chlorophyll a. (B) Molar concentration of chlorophyll b. (C) Molar concentration of chlorophyll a + b. (D) Chlorophyll a to b molar ratio. Values are means with standard deviations ($n = 3$).

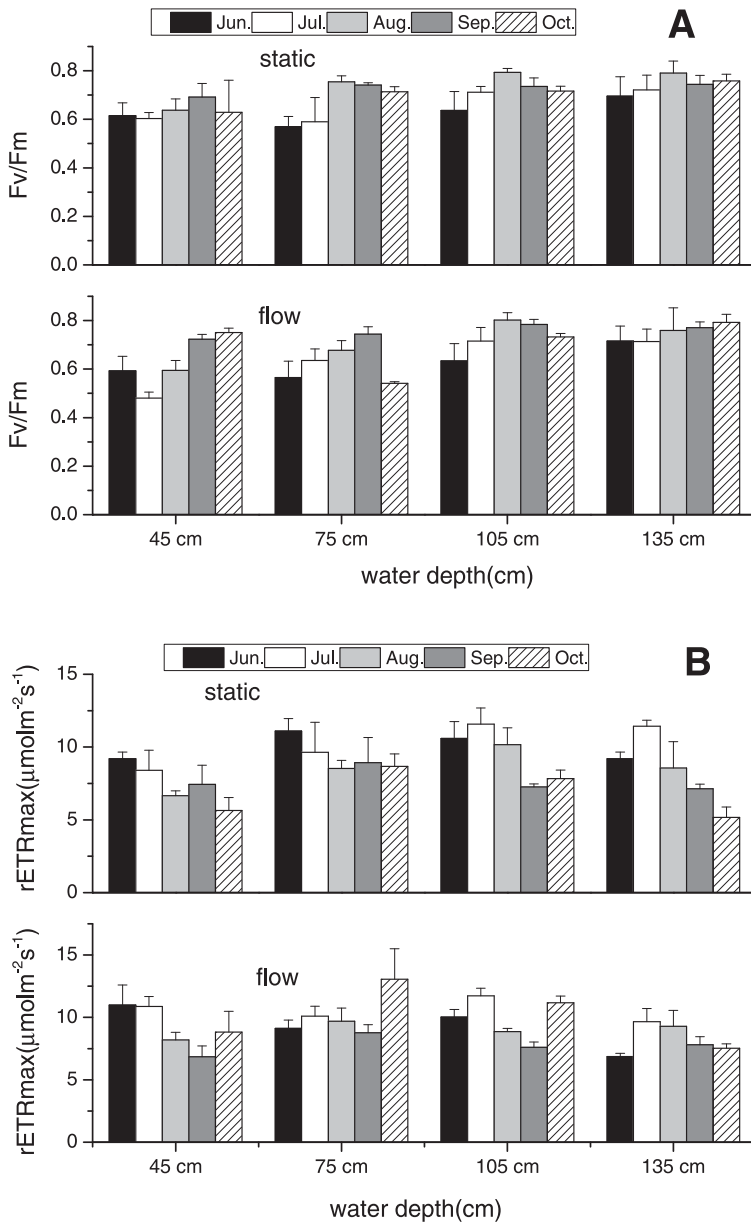


Figure 4. The maximum photosynthetic efficiency (F_v/F_m) and $rETR_{max}$ of *Vallisneria natans* during the experiment. Values are means \pm SD ($n = 3$).

In the static water, $rETR_{max}$ ($5.17 \pm 0.71 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was the lowest in 135 cm in October and the highest in 105 cm in July ($11.57 \pm 1.11 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). In the flowing water, $rETR_{max}$ was the highest ($13.07 \pm 2.43 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in 75 cm in October and the lowest ($6.83 \pm 0.87 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in 45 cm in September. Overall, $rETR_{max}$ in 45 and 135 cm were lower than that in 75 and 105 cm. Additionally, $rETR_{max}$ in September < August and October < July < June.

Rapid light response curves (RLCs) of *V. natans*

A further conclusion can be drawn based on the results of the RLCs. The RLCs are similar to the traditional oxygen-based photosynthesis irradiance (PeI) curve (Figure 5). When the intensity of the light limits *V. natans*, we see a linear rise, and the RLCs become a plateau as the photosynthetic pathway becomes limited.

Influence of water depth on the plant photosynthesis is also reflected in the ability to respond to the light levels. In the light response curve, the change of low light intensity phase curve is of important significance. Rapid light response curve (RLC) is the electron transfer rate curves of light intensity, rapid determination of light response curve can determine the actual photochemical efficiency of *V. natans*. If the light is not excessive, electron transport rate (ETR) and photosynthetic active radiation (PAR) is linear. When the light is excessive, the incidence of PAR and ETR is no longer linear, with a lower linear relationship between estimated values. Finally, ETR reaches saturation, which represents the photosynthetic electron transfer ability that is dependent on the physiological status and environmental factors. Figure 5 shows that the rapid kinetic curves of *V. natans*, the photosynthetic electron transfer rate increases with the increase of light intensity through the electron transfer of Photosystems II (PSII). When the light intensity increases to a certain value, the ETR does not increase, and shows a stable or slightly downward trend. In June, at 45 cm, when the PAR was 65–107 $\mu\text{mol m}^{-2}\text{s}^{-1}$, the electron transfer rate was no longer increasing. In 105 cm, when the PAR was 107–178 $\mu\text{mol m}^{-2}\text{s}^{-1}$, the electron transfer rate reached the maximum value. In June, in the same water treatment group, the photosynthetic electron transport capacity varied in 45, 75, 105 and 135 cm water depth, of which electronic transfer ability in 45 cm was the strongest, the electron transfer ability in 135 cm was relatively weak, significantly lower than that of other water depths. The ETR is significantly related to net photosynthetic rate of the plant, the dynamic changes of photosynthetic rate are consistent with the ETR. As a result, in June, photosynthetic capacity of *V. natans* is relatively strong in 75 cm, while photosynthetic capability of *V. natans* is weak in 135 cm. In July, both in the static water and flowing water, the electron transfer capacity is the strongest in 105 cm. In August, in the flowing water the photosynthetic capacity in 45 cm is the strongest, while

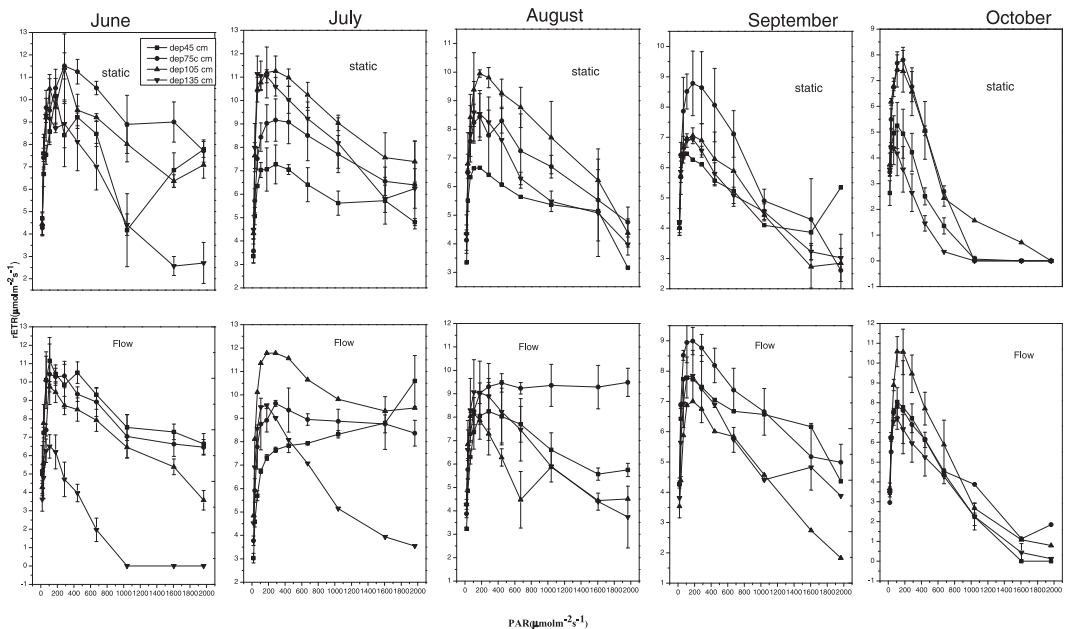


Figure 5. Mean relative electron transport rate (rETR) of rapid light response curves (RLCs) of *Vallisneria natans*. Values are means \pm SD ($n = 3$).

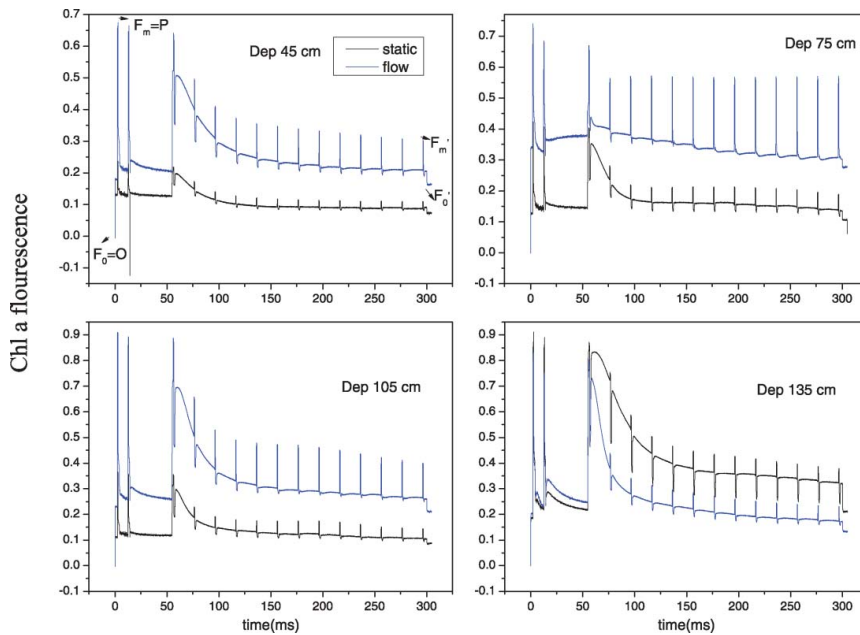


Figure 6. Time-course of slow chlorophyll *a* fluorescence induction (PAM) of *Vallisneria natans* in October.

in the static water the photosynthetic capacity in 105 cm is the strongest. In September, in the static water and the flowing water, the electron transfer capacity shows no significant difference.

At the end of the experiment, time-course of slow chlorophyll *a* fluorescence induction curves of *V. natans* exhibits different curves in different water depths in October. In 45, 75 and 105 cm, F_m peak in the flowing treatment is higher than that in the static treatment, while in 135 cm the curve trend is opposite to the results above (Figure 6). With the decline of temperature and light, plant decayed, and the electron transfer capacity decreased significantly in both the static water and the flowing water.

Discussion

This study compared the growth and photosynthetic responses of *V. natans* to a different water depth gradient under flow and static water. Water depth can affect the underwater temperature, nutrients, DO, etc. which is the synthesis of multiple factors (He et al. 2009). Water depth has an important effect on the intensity of light into the water, and greater water depth can reduce the intensity of light absorbed and utilized by submerged plants in water. The intensity of light decreases, which is not conducive to the germination, growth and distribution of submerged plants. The underwater light environment in aquatic systems, including the intensity and spectral composition, varies greatly with water depth (Zhang et al. 2012; Shi et al. 2014). At the same time, because of water power disturbance, *V. natans* in the depths of 45, 75, 105 and 135 cm under the static water experienced more different water temperatures, much longer periods of low temperatures, and more intense illumination than *V. natans* under the flowing water during the experiment. Additionally, in static water, there were much Spirogyra adhering on the leaves of *V. natans*. Indeed, metabolic processes may be reduced in standing water by limited chemical flow across the boundary layer and under these conditions. Flowing water can improve leaf uptake of nutrients as well as dissolved inorganic carbon, and oxygen. Moreover, while at medium velocities, growth and photosynthesis of submerged macrophytes are usually promoted and there is a positive correlation between biomass and velocity (Madsen and Sondergaard 1983; Chambers et al. 1991; Madsen et al. 1993).

Contrarily, constraints resulting from hydrodynamic stress contribute to modifications in shape of leaves, spatial organization of plant biomass, type of clonal growth and offspring production (Ferreiro et al. 2013).

Growth and morphology of *V. natans* adapting to the environment

Previous studies emphasize that morphological adaptations, such as reduction in the leaf number, are the result of aquatic plant response to hydrodynamic stress (Puijalón et al. 2007; Rooney et al. 2013). Morphological adaptations reducing damage risk have also been identified in plants exposed to mechanical stress. In this study, in the static water, the shoot length of *V. natans* increasing greater, about 3.4 times, while in the flowing water is relatively small, about 2.1 times. The ratios of below-ground: above-ground biomass in the 45 cm in the flowing water was higher than that in the static water. This result was consistent with that a size reduction to leaves exposed to flow, together with an increased allocation to below-ground organs (Idestam-Almquist and Kautsky 1995; Bagger and Madsen 2004), would result in weak forces and a greater anchoring effectiveness, thus reduce the uprooting risk (Crook and Ennos 1996; Niklas 1998; Puijalón et al. 2005). In the 45, 75 and 105 cm, the plant biomass was higher in the flowing treatment than that in the static treatment, which demonstrated that shifts in biomass allocation reflect an adaptive response to water flow. The results are similar to those from Puijalón et al. (2007); specifically, that water flow leads to increased clonal growth and biomass production and decrease in body size. It seems that this life strategy – a balance in flowing water – enhances the ability to survive.

Pigment content changes of *V. natans* adapting to the environment

Chl a is the main component of the photosynthetic RC complex, and the photosynthetic pigments that perform energy conversion. Depending on its protein environment, Chl a functions either as a light harvester, or as a redox participant in the primary charge separation in the RCs of PSII and Photosystem I (PSI) (Clegg and Govindjee 2010; Renger 2010). Chl b is the main composition of light-harvesting proteins complex, whose primary role is absorbing and transferring solar energy.

Measurements of the pigment concentrations reveal pronounced differences between the static and flowing water. It has been demonstrated that when leaves are exposed to relatively low-intensity illumination, the Chl concentration rises and the Chl a/b ratio declines (Lakshmi Praba et al. 2011). Chl a/b ratio values in the flowing water were higher than that in the static water, which illustrates that the water movement was of certain influence on the synthesis of chlorophyll, and further promoted the photosynthesis level of the plant, and slowed down the leaf senescence of *V. natans*.

The increase of Chl b promotes the content of light-harvesting complex proteins, while the number of grana and grana lamella could also be promoted (Anderson et al. 1973). Therefore, it could be concluded that the high content of pigment was an adaptation to the relatively low-intensity illumination, which could also explain the low value of the Chl $a/Chl b$ ratio. Woolhouse argued that as the leaf senescence, content of chlorophyll gradually declined, and chlorophyll a fell faster than chlorophyll b , so chlorophyll a/b was available as the leaf senescence index (Woolhouse 1974). The *V. natans* in static water were exposed to relatively higher levels of illumination, and thus Chl content was the most important for achieving the maximum potential photosynthesis (Lambers et al. 2008).

Responses of PSII to environmental changes

Measurements of Chl a fluorescence provide information on photosynthesis, including the energy absorption, distribution and utilization (Phinney and Cucci 1989; Zhang et al. 2007). The parameters F_v/F_m and $rETR_{max}$ were both analyzed in order to evaluate the changes in the photosynthetic activity of *V. natans* in the static and flowing treatments. The parameter $rETR_{max}$ expresses the maximum photosynthetic capacity, obtained when the photosynthetic rate is limited by the activity of

the electron transport chain or Calvin cycle enzymes (Ralph and Gademann 2005; Serôdio et al. 2006).

The maximum quantum yield of PSII, as measured by F_v/F_m , has been widely used as a potential indicator of environmental and chemical stress-induced perturbations in the photosynthetic apparatus (Schreiber et al. 2007). F_v/F_m of *V. natans* was approximately 0.73 s, which is similar to that recorded for other *Vallisneria* spp. (e.g. Hulsen et al. 2002). Overall, the parameters F_v/F_m in 45 and 75 cm were lower than that in 105 and 135 cm. The results of the chlorophyll fluorescence investigation could further demonstrate the different physiological conditions of *V. natans* in the experiment. In 45 cm, it is likely that the flowing water can improve the plant growth compared to static water. This greater growth may be due to the improved foliar uptake of nutrients and/or dissolved inorganic carbon. Water depth is an integrative index, however, does not affect plant growth in isolation, but instead affects plant growth in conjunction with sediment and other factors such as light intensity, water pressure and stability of environmental factors, wave and velocity of flowing (Maurer and Zedler 2002; Xu et al. 2011). Such adaptations are favored by different photosynthetic efficiency at low light intensities (Eusebio Malheiro et al. 2013), as changes in biomass allocation and morphology can enhance performance and optimize resource acquisition (Vretare et al. 2001). Light drives photosynthesis, leading to the production of oxygen and carbohydrates required for plant growth (Ralph et al. 2007; Liu et al. 2016). The study showed that the value of F_v/F_m in 45 cm in the static water was significantly higher than that in the flowing water, which suggested that the *V. natans* had relatively greater photosynthetic efficiency in the flowing water. Based on the analysis, chlorophyll content in *V. natans* decreased, the largest photochemical efficiency and electron transfer capacity decreased subsequently, resulting in a decline in photosynthetic efficiency. The result for F_v/F_m was similar to the findings relating to the concentrations of Chla, Chlb and total Chl. Therefore, in comparison with the *V. natans* in the flowing water, it can be proposed tentatively that the *V. natans* might experience greater damage from the environment, which could reduce their relative photosynthetic efficiency. The RLCs could reflect a similar result with regard to the fluorescence induction kinetics. There were significant differences between the plant in the static and flowing water in terms of the photochemical and non-photochemical quantum yields of PSII.

Conclusions

In conclusion, our experimental results proved that *V. natans* can respond to different long-term water depths under flowing and static water by morphological adaptations and changes of the physiological characteristics, and so on. Additionally, changes of physiological indexes in *V. natans* such as chlorophyll content and photosynthetic characteristics make plant morphology and biomass change obviously. Our results demonstrated that water depth and water velocity could be considered in the recovery of the submersed macrophytes in shallow lakes. Hence, we conclude that the results of this study could provide a potential theoretical basis reference for restoration and rehabilitation of submerged macrophytes.

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References

- Anderson JM, Goodchild D, Boardman N. 1973. Composition of the photosystems and chloroplast structure in extreme shade plants. *Biochim Biophys Acta BBA Bioenerg.* 325:573–585.
- Arnold DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24(1):1–15.
- Atapaththu KSS, Asaeda T. 2015. Growth and stress responses of Nuttall's water weed *Elodea nuttallii* (Planch) St. John to water movements. *Hydrobiologia.* 747(1):217–233.
- Baastrup-Spohr L, Møller CL, Sand-Jensen K. 2016. Water-level fluctuations affect sediment properties, carbon flux and growth of the isoetid *Littorella uniflora* in oligotrophic lakes. *Freshwater Biol.* 61:301–315.
- Bagger J, Madsen TV. 2004. Morphological acclimation of aquatic *Littorella uniflora* to sediment CO₂ concentration and wave exposure. *Funct Ecol.* 18:946–951.
- Cao J, Wang Y, Zhu Z. 2012. Growth response of the submerged macrophyte *Myriophyllum spicatum* to sediment nutrient levels and water-level fluctuations. *Aquat Biol.* 17:295–303.
- Cao Y, Zhang SJ, Liu YY, Guo ZC, Chen BX. 2014. Effects of water gradient on seedlings growth and biomass of *Vallisneria spiralis*. *Ecol Environ.* 23(8):1332–1337.
- Chambers PA, Prepas EE, Hamilton HR, Bothwell ML. 1991. Current velocity and its effect on aquatic macrophytes in flowing waters. *Ecol Appl.* 1(3):249–257.
- Cerovic ZG, Ounis A, Cartelat A, Latouche G, Goulas Y, Meyer S, Moya I. 2002. The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves. *Plant Cell Environ.* 25:1663–1676.
- Clegg RM, Govindjee MS. 2010. From Förster resonance energy transfer to coherent resonance energy transfer and back. *Proceedings of the SPIE 7561, Optical Biopsy VII, 75610C*; Feb. 22.
- Collier C, Waycott M, McKenzie L. 2012. Light thresholds derived from seagrass loss in the coastal zone of the northern Great Barrier Reef, Australia. *Ecol Indic.* 23:211–219.
- Crook MJ, Ennos AR. 1996. Mechanical differences between free-standing and supported wheat plants *Triticum aestivum* L. *Ann Bot.* 77:197–202.
- Crossley M, Dennison W, Williams R, Wearing A. 2002. The interaction of water flow and nutrients on aquatic plant growth. *Hydrobiologia.* 489(1–3):63–70.

- Eusebio Malheiro AC, Jahns P, Hussner A. 2013. CO₂ availability rather than light and temperature determines growth and phenotypical responses in submerged *Myriophyllum aquaticum*. *Aquat Bot.* 110:31–37.
- Ferreiro N, Giorgi A, Feijoo C. 2013. Effects of macrophyte architecture and leaf shape complexity on structural parameters of the epiphytic algal community in a Pampean stream. *Aquat Ecol.* 47:389–401.
- He W, Wang GX, Yang WB, Chen QM, Lu YC. 2009. Growth response of *Potamogeton crispus* to water depth gradient. *Chin J Ecol.* 28(7):1224–1228.
- Hulsen K, Minne V, Lootens P, Vandecasteele P, Hofte M. 2002. A chlorophyll a fluorescence-based *Lemna minor* bioassays to monitor microbial degradation of nanomolar to micromolar concentrations of linuron. *Environ Microbiol.* 4:327–337.
- Hurd CL. 2000. Water motion, marine macroalgal physiology, and production. *J Phycol.* 36:453–472.
- Idestam-Almquist J, Kautsky L. 1995. Plastic responses in morphology of *Potamogeton pectinatus* L. to sediment and above-sediment conditions at two sites in the northern Baltic proper. *Aquat Bot.* 52:205–216.
- Koch EW. 1994. Hydrodynamics, diffusion-boundary layers and photosynthesis of the seagrasses *Thalassia testudinum* and *Cymodocea nodosa*. *Mar Biol.* 118:767–776.
- Lakshmi Praba M, Vanangamudi M, Thandapani V. 2011. Effects of low light on yield and physiological attributes of rice. *Int Rice Res Notes.* 29:71–73.
- Lambers H, Chapin FS, Pons TL. 2008. Plant water relations. In: *Plant physiological ecology*. New York (NY): Springer; p. 163–223.
- Larkum AWD, Roberts G, Kuo J, Strother S. 1989. Gaseous movement in seagrasses. In: Larkum AWD, McComb AJ, Shepherd SA, editors. *Biology of seagrasses: a treatise on the biology of seagrasses with special reference to the Australasian region*. Amsterdam: Elsevier; p. 686–722.
- Li ZQ, Dan Y, Tu MH. 2005. Seed germination of three species of *Vallisneria* (Hydrocharitaceae), and the effects of freshwater microalgae. *Hydrobiologia.* 544:11–18.
- Liu XH, Zhang YL, Shi K, Lin JF, Zhou YQ, Qin BQ. 2016. Determining critical light and hydrologic conditions for macrophyte presence in a large shallow lake: the ratio of euphotic depth to water depth. *Ecol Indic.* 71:317–326.
- Madsen TV, Sand-Jensen K, Beer S. 1993. Comparison of photosynthetic performance and carboxylation in a range of aquatic macrophytes of different growth form. *Aquat Bot.* 44:373–384.
- Madsen TV, Sondergaard M. 1983. The effects of current velocity on the photosynthesis of *Callitriche stagnalis* Scop. *Aquat Bot.* 15:187–193.
- Maurer DA, Zedler JB. 2002. Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. *Oecologia.* 131:279–288.
- Milne JM, Murphy KJ, Thomaz SM. 2006. Morphological variation in *Eichhornia azurea* (Kunth) and *Eichhornia crassipes* (Mart.) Solms in relation to aquatic vegetation type and the environment in the floodplain of the Rio Paraná, Brazil. *Hydrobiologia.* 570:19–25.
- Niklas KJ. 1998. Effects of vibration on mechanical properties and biomass allocation pattern of *Capsella bursa-pastoris* (Cruciferae). *Ann Bot.* 82:147–156.
- Phinney DA, Cucci TL. 1989. Flowing cytometry and phytoplankton. *Cytometry.* 10(5):511–521.
- Power P. 1996. Effects of current velocity and substrate composition on growth of Texas wildrice (*Zizania texana*). *Aquat Bot.* 55:199–204.
- Puijalón S, Bornette G, Sagnes P. 2005. Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species. *J Exp Bot.* 56:777–786.
- Puijalón S, Lena JP, Bornette G. 2007. Interactive effects of nutrient and mechanical stresses on plant morphology. *Ann Bot.* 100:1297–1305.
- Ralph P, Durako M, Enriquez S, Collier C, Doblin M. 2007. Impact of light limitation on seagrasses. *J Exp Mar Biol Ecol.* 350:176–193.
- Ralph PJ, Gademann R. 2005. Rapid light curves: a powerful tool for the assessment of photosynthetic activity. *Aquat Bot.* 82:222–237.
- Reckendorfer W, Funk A, Gschöpf C, Hein T, Schiemer F. 2013. Aquatic ecosystem functions of an isolated floodplain and their implications for flood retention and management. *J Appl Ecol.* 50:119–128.
- Renger G. 2010. The light reactions of photosynthesis. *Curr Sci.* 98:1305–1319.
- Rooney RC, Carli C, Bayley SE. 2013. River connectivity affects submerged and floating aquatic vegetation in floodplain wetlands. *Wetlands.* 33:1165–1177.
- Schreiber U, Bilger W, Neubauer C. 1995. Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of in vivo photosynthesis. In: Schulze E, Caldwell MM, editors. *Ecophysiology of photosynthesis*. Berlin: Springer Berlin Heidelberg; p. 49–70.
- Schreiber U, Quayle P, Schmidt S, Escher BI, Mueller JF. 2007. Methodology and evaluation of a highly sensitive algae toxicity test based on multiwall chlorophyll a imaging. *Biosens Bioelectr.* 22:2554–2563.
- Schutten J, Dainty J, Davy AJ. 2005. Root anchorage and its significance for submerged plants in shallow lakes. *J Ecol.* 93:556–571.

- Seródio J, Vieira S, Cruz S, Coelho H. 2006. Rapid light-response curves of chlorophyll fluorescence in microalgae: relationship to steady-state light curves and non-photochemical quenching in benthic diatom-dominated assemblages. *Photosynth Res.* 90:29–43.
- Shi K, Zhang Y, Liu X, Wang M, Qin B. 2014. Remote sensing of diffuse attenuation coefficient of photosynthetically active radiation in Lake Taihu using MERIS data. *Remote Sens Environ.* 140:365–377.
- Smith FA, Walker NA. 1980. Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO₂ and HCO₃⁻ and to carbon isotopic discrimination. *New Phytol.* 86:245–259.
- Stevens CL, Hurd CL. 1997. Boundary-layers around bladed aquatic macrophytes. *Hydrobiologia.* 346:119–128.
- Stewart HL, Carpenter RC. 2003. The effects of morphology and water flow on photosynthesis of marine macroalgae. *Ecology.* 84:2999–3012.
- Strand JA, Weisner SEB. 2001. Morphological plastic responses to water depth and wave exposure in an aquatic plant (*Myriophyllum spicatum*). *J Ecol.* 89:166–175.
- Thomas FIM, Cornelisen CD, Zande JM. 2000. Effects of water velocity and canopy morphology on ammonium uptake by seagrass communities. *Ecology.* 81:2704–2713.
- Vretare V, Weisner SEB, Strand JA, Granéli W. 2001. Phenotypic plasticity in *Phragmites australis* as a functional response to water depth. *Aquat Bot.* 69:127–45.
- Wang P, Zhang Q, Xu YS, Yu FH. 2016. Effects of water level fluctuation on the growth of submerged macrophyte communities. *Flora.* 223:83–89.
- Woolhouse HW. 1974. Longevity and senescence in plant. *Sci Prog.* 6(1):123–147.
- Wu G, de Leeuw J, Skidmore AK, Prins HHT, Best EPH, Liu YL. 2009. Will the three gorges dam affect the underwater light climate of *Vallisneria spiralis* L. and food habitat of Siberian crane in Poyang Lake? *Hydrobiologia.* 623:213–222.
- Xie YH, Deng W, Wang JD. 2007. Growth and root distribution of *Vallisneria natans* in heterogeneous sediment environments. *Aquat Bot.* 86:9–13.
- Xu WW, Hu WP, Deng JC, Zhu JG, Li QQ. 2016. How do water depth and harvest intensity affect the growth and reproduction of *Elodea nuttallii* (Planch.) St. John? *J Plant Ecol.* 9:212–223.
- Xu WW, Hu WP, Deng JC, Zhu J, Zhou NN, Liu X. 2016. Impacts of water depth and substrate type on *Vallisneria natans* at wave-exposed and sheltered sites in a eutrophic large lake. *Ecol Eng.* 97:344–354.
- Xu, NN, Tong X, Tsang Po-Kueug E, Deng H, Chen XY. 2011. Effects of water depth on clonal characteristics and biomass allocation of *Halophila ovalis* (Hydrocharitaceae). *J Plant Ecol.* 4(4):283–291.
- Yang YQ, Yu D, Li YK, Xie YH, Geng XH. 2004. Phenotypic plasticity of two submersed plants in response to flooding. *J Freshw Ecol.* 19:69–76.
- Zhang M, Kong F, Xing P, Tan X. 2007. Effects of interspecific interactions between *Microcystis aeruginosa* and *Chlorella pyrenoidosa* on their growth and physiology. *Int Rev Hydrobiol.* 92(3):281–290.
- Zhang Y, Liu X, Yin Y, Wang M, Qin B. 2012. Predicting the light attenuation coefficient through Secchi disk depth and beam attenuation coefficient in a large, shallow, freshwater lake. *Hydrobiologia.* 693:29–37.
- Zhu GR, Li W, Zhang M, Ni LY, Wang SR. 2012. Adaptation of submerged macrophytes to both water depth and flood intensity as revealed by their mechanical resistance. *Hydrobiologia.* 696:77–93.