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Photosynthetic Characteristics of Marestail Using PAM Fluorometry

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Abstract. In this work, we used PAM fluorometry to examine photosynthetic characteristics of marestail (*Hippuris vulgaris*) across a water temperature gradient in three lakes. Three lakes were studied across a gradient in water temperature, with low water temperature conditions in Grass Lake and Arrow Bamboo Lake, high water temperature conditions in Five Colored Lake. Chlorophyll *a* concentrations of marestail differed strongly among the three lakes, with the highest value in Grass Lake. In three lakes, F_v/F_m of marestail declined as irradiance increased during the day, and recovered as light level decreased from noon until sunset. Photochemical quenching and non-photochemical quenching of marestail in Five Colored Lake were significantly higher than the other lakes. Our understanding of higher photosynthetic activity in Five Colored, as it relates to main environmental factors such as temperature.

Introduction

The pulse-amplitude modulated (PAM) fluorometry technique for measuring chlorophyll (Chl) *a* fluorescence associated with photosystem II is a widely used non-invasive and rapid method to monitor in real-time the functional state of photosynthetic organisms. In particular, the application of PAM fluorometry to marine macrophytes has been a powerful tool for assessing physiological stress under different environmental conditions.

Jiuzhaigou (JZG) is located at the transition of the Tibetan Plateau and Sichuan Basin in the northern region of Sichuan Province, China. The Park is characterized by mountainous and karst topography and a series of crystal clear, blue-green lakes, pools, and waterfalls resulting from carbonate calcified dikes. It is the only park in China to have the combined certifications of UNESCO World Heritage Site, World Biosphere Reserve, and Green Globe 21, representing a model for ecological protection and sustainability in China (IUCN 2006). It has been a forerunner for promotion of ecotourism in China and even in Asia. Since early 1980s tourism industry in Jiuzhaigou has been developing rapidly and number of tourists has increased exponentially after 1990s when highways linking outside were renovated. Statistics shows that the number of tourists in Jiuzhaigou had jumped to 2.187 million in 2006 from 27.529 thousands in 1984. However, environmental changes have affected the lakes in JZG in the last 20 years, the increasing sedimentation in lakes is a major problem requiring implementation of large-scale control measures in Jiuzhaigou. It has been shown that macrophytes grow well and promote the sedimentation, in order to minimise the environmental and develop lake sedimentation management strategies. Here, we examine photosynthetic characteristics for marestail occurring in three lakes using Junior-PAM fluorometry in order to understand how variable these estimates are among lakes.

Materials and Methods

JZG is located in a mountainous region in the eastern rim of Qinghai–Tibetan Plateau (32.88 °–33.33 °N, 103.77 °–104.08 °E), encompassing an area of 650 km^2 and spanning from about 2000 to 4880 m above sea level. JZG is a headwater watershed, with precipitation as the sole water source. Annual precipitation is 539–771 mm, and 90% of precipitation falls during the rainy season (April to October). Approximately 1000 residents live in six villages within JZG and three of these villages are located in the Rize, Zechawa, and Shuzhang Valleys. Marestail was measured in Grass Lake (GL), Arrow Bamboo Lake (ABL) and Five Colored Lake (FCL).

Following chlorophyll fluorescence measurement, six plants of study species were harvested. Plants were kept in a dark cooler with ice and transported to the laboratory for processing. Approximately 1.6 g of the mature leaves (wet weight) was collected from each plant. Leaves were ground with a mortar and pestle in 6.4 ml of 80 % cold acetone, then placed in a culture tube and centrifuged at 3000 rpm for 10 min, and supernatant was collected. Using a spectrophotometer (UV-1240-PC, Shimadzu), absorbances was recorded at 663 and 646 nm. We used following equations to calculate chlorophyll *a* and *b* (Arnon 1949) where OD is optical density:

$$\text{Chlorophyll } a = 1.27(\text{OD663}) - 0.269(\text{OD645}) \quad (1)$$

$$\text{Chlorophyll } b = 2.29(\text{OD645}) - 0.468(\text{OD663}) \quad (2)$$

PAM chlorophyll fluorescence was measured with a Junior-PAM (Walz, Germany). Measurements were obtained between 7: 00 and 17: 00 at both sites. Measures of maximal quantum yield (Fv/Fm) of Photosystem II (PSII) were made on 10 replicate leaves from separate plants. The leaves were dark acclimated for 20 min. Values of Fv/Fm were determined from the equation:

$$F_v = F_m - \text{Fo} \quad (3)$$

with F_o being the initial chlorophyll fluorescence after dark adaptation when all reaction centers are open, and F_m being the maximal chlorophyll fluorescence after dark adaptation when all reaction centers were closed following a saturating flash of light.

As the induction kinetics curves of low light and high light could be measured in the laboratory, the plants were carefully collected and transported. The rhizome-systems must be intact and maintained in shaded conditions by water from the sampling site. Fluorescence induction kinetics curves were performed on leaves dark acclimated for 20 min with application of constant actinic irradiance (low light, 190 $\mu mol \cdot m^{-2} s^{-1}$ and high light, 1150 $\mu mol \cdot m^{-2} s^{-1}$) using the LED light source of the Junior-PAM, separated by fourteen saturating light pulses at 20 s intervals, initiated 42 s after the first saturation pulse. Non-photochemical quenching (NPQ) was calculated according to the equation:

$$\text{NPQ} = (F_m - F_m') / F_m' \quad (4)$$

Meantime, photochemical quenching (qP) was also calculated as:

$$\text{qP} = (F_m' - F) / (F_m' - F_o') \quad (5)$$

where F is the fluorescence yield in the light acclimated state, F_m' is the maximum fluorescence and F_o' is the minimum fluorescence in the light acclimated state. NPQ and qP were made on 3 replicate leaves of each species.

Photosynthetic Active Radiation (PAR) and water temperature were measured at a 2 h interval from 7:00 to 17: 00. Water temperature was measured with glass thermometer. In situ ambient PAR was measured using AccuPAR Lp-80 Ceptometer.

Data Analysis

All results are presented as means ± SE. We used 1 way ANOVA to test for the significant differences among variables. Tukey's HSD test was used to establish significant differences between individual means.

Results

Leaf chlorophyll *a* concentrations ($P < 0.01$) of marestail differed strongly among the three lakes, with the highest value in GL. No significant differences were found in chlorophyll *b* concentrations and chlorophyll *a/b* ratios of marestail among the three lakes (Table 1).

A typical diurnal response underwater PAR occurred over the day from 7: 00 to 17: 00 with minimum PAR occurring at 07: 00 and peak PAR occurring at 13: 00 for the three lakes (Figure. 1). The underwater PAR was less than 73 $\mu mol \cdot m^{-2}\ s^{-1}$ at 07: 00, and exceeded 1800 $\mu mol \cdot m^{-2}\ s^{-1}$ during the midday in full sun (12: 00 – 14: 00) in three lakes. No significant differences were found in c PAR of marestail among the three lakes Hourly water temperature was significantly higher in FCL than GL and ABL, with daily maximum of 20.6 ℃, 11.2 ℃ and 11.2 ℃ in the above three lakes, respectively. Water temperature of GL was not significantly different from that of ABL.

Table 1 Chlorophyll *a*, chlorophyll *b* concentrations (mg Chl g fw $^{-1}$) and chlorophyll *a*/*b* of marestail collected from three lakes.

	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a*/*b*
GL	0.688 ± 0.051[a]	0.273 ± 0.037[a]	2.766 ± 0.363[a]
ABL	0569 ± 0.039[a]	0.260 ± 0.032[a]	2.263 ± 0.139[a]
FCL	0.426 ± 0.009[b]	0.234 ± 0.025[a]	1.931 ± 0.176[a]

Note: Values represent means (± SE), n = 6. For each parameter values with different superscript letters are significantly different (P < 0.05) (Tukey's test).

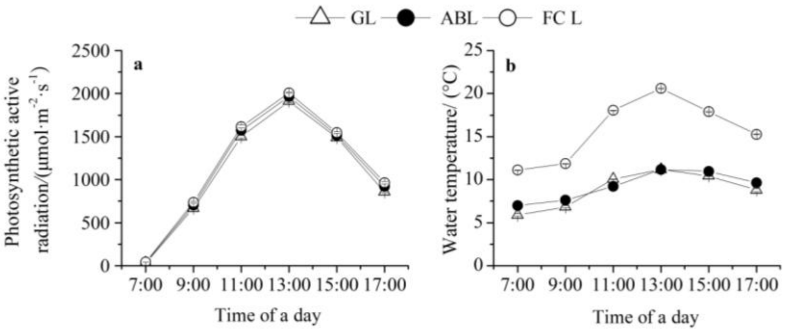

Figure. 1 Diurnal variation in Photosynthetic active radiation and water temperature in three lakes.

F_v/F_m of marestail in three lakes showed a similar pattern over the diurnal cycle with the highest values recorded at low PAR at 7:00 and 17:00. In three lakes, F_v/F_m of marestail declined as irradiance increased during the day, and recovered as light level decreased from noon until sunset (Figure. 2). However, at the end of the day the F_v/F_m values in both GL and ABL were significantly lower than those measured at the beginning of the day.

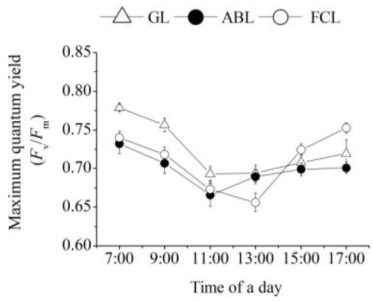

Figure. 2 Diurnal variation in F_v/F_m of marestail in three lakes.

Under low light and high light, qP and NPQ of marestail in FCL were significantly higher than that in ABL and GL, but there were no significant differences between ABL and GL (Figure. 3).

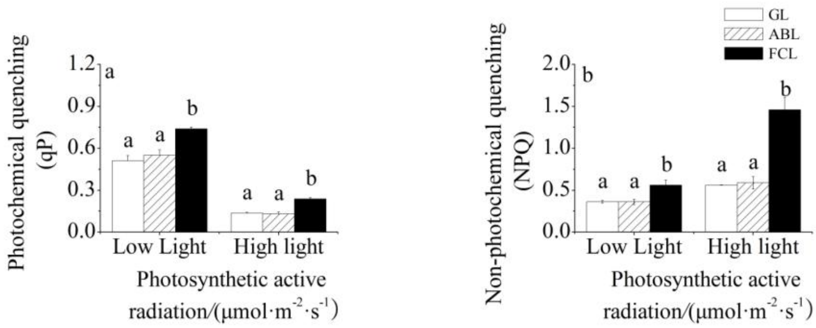

Figure. 3 Photochemical quenching and non-photochemical quenching of marestail under low and high light in three lakes.

Discussion

Marestail had higher chlorophyll *a* concentration at the low water temperatures in both GL and ABL, but lower chlorophyll *a* concentration at high water temperature in FCL. This is similar to the results of Madsen and Brix (1997) who found that *Ranunculus aquatilis* had high chlorophyll *a* concentration at low temperature [1]. However, Santamaría et al. (1998) reported that chlorophyll *a* concentration increased with increasing temperature in *Ruppia drepanensis* [2]. Differences in acclimation strategy of the different species, particularly whether they rely on morphological (e.g., shoot elongation) or photosynthetic (e.g., leaf chlorophyll and protein content) plasticity, might explain these conflicting results regarding the effect of temperature on chlorophyll concentration [3].

A post midday depression in dark-adapted maximum quantum yield (F_v/F_m) was found for marestail in three lakes. This is similar to F_v/F_m of *Halophila ovalis* and *Amphibolis antarctica*, which were influenced by irradiance during the diurnal cycle with a midday depression [4]. Such photoinhibitory mechanisms may serve to protect plants by down regulating the yield of PSII chemistry without causing photodamage and serve to protect photosynthetic apparatus during summer when photon flux is high. Moreover, F_v/F_m of FCL showed full recovery at sunset. The total recovery indicates that no significant photodamage occurs under the highest irradiance levels and that the observed decline of this parameter value is fundamentally due to the activation of photoprotective mechanisms [5]. The recovery of F_v/F_m in FCL was faster than that in GL and ABL. This is similar to the results of Franklin (1994) who found that recovery of F_v/F_m for *Ulva rotundata* was at least twice as fast at 25 ℃ than at 10 ℃ [6]. But, F_v/F_m of GL and ABL did not show full recovery at 17:00. These results suggest that low temperature may exacerbate the adverse effects of

UV-B on important physiological variables such as F_v/F_m [7]. Furthermore, many plant species display slow recovery from photoinhibition at low temperature, possibly because of slow rate of repair of the D-1 protein in PSII [8].

In this study, the relationship between qP and NPQ showed two distinct states: high-light conditions, where qP was low and NPQ was high; and low-light state, where qP was high and NPQ was low. The qP and NPQ response of most of the species indicates a switch between two light climates, and this could be associated with the fact that under elevated light the NPQ processes were dominant. These are in agreement with investigations on *Codium adherens*, *Enteromorpha muscoides*, *Ulva gigantea*, *Ulva rigida* [9]. In general, marestail in GL and ABL showed lower qP than that in FCL as a result of the low water temperature induced reduction in ETR_{max}. However, the higher NPQ observed in FCL in response to higher temperature may be associated with big pool size of xanthophyll cycle pigments in the phyllodes [10], so F_v/F_m of marestail in FCL displayed full recovery at 17:00. Thus marestail in FCL may be more tolerant to high PAR than that in the other two lakes due to higher water temperature.

Conclusions

We found differences in the photosynthetic of marestail. Water temperature is an important resource for marestail in photosynthesis.

Acknowledgements

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