

Photosynthetic performance of temperate seagrasses in Northern Japan

¹Maria L. S. Orbita, ²Hiroshi Mukai

¹ Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, Iligan City, Philippines; ² Field Science Education and Research Center, Kyoto University, Kyoto, Japan. Corresponding author: M. L. S. Orbita, mlwsasil@yahoo.com

Abstract. The photosynthetic activity of temperate seagrass species in Akkeshi Bay was assessed using the newly developed submersible, pulse-amplitude modulated (PAM) fluorometer: Diving-PAM. The rapid light curve (RLC) was used to compare the photosynthetic activity of cultured seedlings and mature *in situ* *Zostera marina*, *Zostera asiatica*, *Zostera japonica* and *Phyllospadix iwatensis*. Several fluorescence parameters were measured as a function of irradiance generated by the Diving PAM; electron transport rate, quantum yield, photochemical (qP), non-photochemical quenching (NPQ) and recovery period. Seedling and mature species of *Z. japonica* with less acute alpha had the highest maximum ETR and minimum saturating irradiance. A similar pattern in the photosynthetic activity between cultured seedlings and mature seagrasses was found. A high variation occurred for rapid light curve parameters among species, providing validity to this new PAM fluorometry method in determining species-specific mechanisms and adaptive responses of seagrasses.

Keywords: PAM fluorometer, photosynthetic activity, temperate seagrasses.

Introduction. The assessment of photosynthetic activity in seagrasses can now be made *in situ* without removing or enclosing plants in chambers (Masini et al 1995). The use of chlorophyll-fluorescence techniques and the recent development of an underwater PAM fluorometer have made such measurements possible under ambient light conditions (Schreiber & Bilger 1993; Schreiber 2004). Such technique has been used not only in measuring mature plants present in natural population, but also in young seed-generated species of seagrasses (Orbita & Mukai 2009).

In PAM fluorometry, some important dependent parameters can be generated and used to assess the photosynthetic performance of a plant. For example, the effective quantum yield (Φ_{PSII}) has been used to assess photosynthetic activity. When effective quantum yield is measured, as well as the irradiance, then it is possible to estimate the amount of electrons moving between the photosystems, i.e. electron transport rate or ETR. The electron transport rate (ETR) was found to be closely related to the photosynthetic activity when measured by oxygen evolution or CO₂ uptake (Beer et al 1998; Silva & Santos 2004). Such measurements (Φ_{PSII} & ETR) will assess the leaf's kinetics or ability to cope with different light intensities (Beer et al 2001). Moreover, the result of such a set of measurements is called a rapid light curve. It has been known that rapid light curves (RLC's) were used to measure the actual photosynthetic rate (as irradiance-dependent PSII electron transport) of the *in situ* seagrass leaves. RLC's plot the electron transport rate (ETR) versus irradiance. From the RLC, it is possible to determine the maximum ETR (E_{max}), minimum saturating irradiance (E_k) and light-limited photosynthetic efficiency (α). Ralph et al (1998) demonstrated the utility of the rapid light curves in determining species differences, diurnal cycle and quenching response related to species-specificity in various species of seagrasses, such as *Posidonia australis*, *Posidonia sinuosa*, *Amphibolis antarctica*, *Amphibolis griffithii* and *Halophila ovalis*. Results showed that among the five species of seagrasses, *H. ovalis* had the highest maximum ETR with a high photosynthetic activity. Likewise, it was further emphasized in

the results that by means of chlorophyll *a* fluorescence measurements, a wide variety of seagrass species with several different growth forms revealed a diversity of responses with a wide range of photosynthetic activity. On the other hand, additional information about the mechanisms controlling the overall photosynthetic activity can be gained from quenching analysis. The photon energy captured by a chlorophyll *a* molecule can either drive photosynthesis (photochemical quenching, qP), be emitted as fluorescence, or be converted as heat (non-photochemical quenching, qN or NPQ). Heat dissipation is linked to the xanthophyll cycle, which protects the photosynthetic apparatus from high-light damage. Quenching analysis monitors the development of qP and qN (or NPQ) which are both measures of the energy flow into heat (Schreiber 2004). Also, non-photochemical quenching and Φ_{PSII} are correlated, where Φ_{PSII} decreases with increasing irradiance, as more electrons accumulate at the PSII acceptor side and there is a relative increase in non-photochemical quenching or heat energy dissipation (Schreiber 2004). A comparison of photochemical (qP) to non-photochemical (NPQ) quenching indicated that seagrasses in shallow water receiving high light have a high capacity for non-photochemical quenching (e.g. light protection) compared to seagrasses in deep water (Ralph et al 1998). In addition, non-photochemical quenching (NPQ) also monitors the recovery period of plant photosystems after the rapid-light curve (RLC) and allows its various components to be distinguished. Furthermore, all of those mentioned fluorescence parameters can be used to determine species differences as the photosynthetic activity and adaptive responses in seagrasses are said to be species-specific (Ralph et al 1998).

In Akkeshi Bay, Hokkaido, Japan, four species of temperate seagrasses are found dominating the area, namely; *Zostera marina* L., *Zostera asiatica* Miki, *Zostera japonica* - Ascherson & Graebner and *Phyllospadix iwantensis* Miki. Besides, *Z. marina* and *Z. japonica* are found in an adjacent Akkeshi-ko Estuary where *Z. japonica* dominates the intertidal area. Additionally, *P. iwantensis* is observed to be dominant in the intertidal area of Akkeshi Bay. Although *Z. asiatica* is observed to be restricted in the deeper area, however, it was found co-occurring with *Z. marina* in the intertidal and subtidal area (Watanabe et al 2005).

Consequently, seagrasses are considered as one of the major primary producers in Akkeshi Bay aside from phytoplankton and seaweeds. For instance, *Z. marina* and *Z. asiatica* were estimated to have a total production of 163.3 t DW y⁻¹ (Watanabe et al 2005), while *P. iwantensis* had an average production of 3,789 µg DW/shoot/day (Hasegawa et al 2005), hence, contribute greatly to the enhanced productivity of Akkeshi Bay. Considering the massive population, high production and species richness of seagrasses in Akkeshi Bay, it is therefore important to determine species differences in terms of photosynthetic activity in the area. In this way, one could understand the distinct characteristics of each species especially their light adaptation. Very importantly, the adaptive mechanisms of seagrasses which are found to be species-specific could be related to their distribution pattern as it reveals their light preference while understanding the role of light in the maintenance of seagrass meadows.

In order to establish an accurate determination of species-specificity, the photosynthetic activity and light adaptive responses of mature *in situ* and cultured seedling species of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwantensis* were compared.

The aim of this study was to compare the pattern in photosynthetic activity between mature *in situ* and cultured seedling species of seagrasses namely, *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwantensis*. The second objective was to demonstrate the utility of the rapid light curves and several chlorophyll *a* fluorescence parameters to determine species differences and quenching response related to species specificity.

Material and Method. Seed-bearing shoots of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwantensis* were collected during the months of July and August 2004. The shoots of *Z. marina*, *Z. asiatica* and *P. iwantensis* were collected at Aininkap, Akkeshi Bay, Hokkaido, Japan (43°00' N, 144°51' E), while the shoots of *Z. japonica* were collected at Akkeshi-ko Estuary (43°01' N, 144°53.4' E). After collection, the seed-bearing shoots were brought to Akkeshi Marine Station and were placed inside the outdoor running seawater tanks. The seagrasses were allowed to release their seeds in outdoor running tanks. The

seeds were then separated from the detrital remains of the spathe through several steps of screening and winnowing, and held in ambient flowing seawater for several months prior to actual culture (Granger et al 2000).

Seeds of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* were planted in the third week of April 2005. The sandy sediments that were collected from Aininkap, Akkeshi Bay were used for planting *Z. marina* and *Z. asiatica* seeds, while the muddy sediments that were collected from Akkeshi-ko Estuary were used for planting *Z. japonica* seeds. Except for *P. iwatensis*, about 24 seeds of each seagrass were buried at 7 cm depth in individual plastic planter (26.5 cm x 26.5 cm). On the other hand, seeds of *P. iwatensis* were properly attached and anchored on rocks covered with plastic nets, for this plant will not grow if buried under the sediment. There were six replicates made for each seagrass species. The seeds were then allowed to germinate in outdoor tanks with running seawater, under conditions of ambient light and temperature. Every week, the tank walls were scrubbed to remove the periphyton, and the epiphytes were gently removed from the leaves by hand. The water column remained clear throughout the experiment, as water would continually flow through the tanks.

During the month of August 2005, 10 mature shoots of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* were collected in *Zostera* and *Phyllospadix* meadows at Aininkap (43°00' N, 144°51' E), Akkeshi Bay, Hokkaido, northern Japan, while *Z. japonica* shoots were collected in an adjacent Akkeshi-ko Estuary (43°01' N, 144°53.4' E). The seagrass samples were then brought to Akkeshi Marine Station for chlorophyll *a* fluorescence measurements.

The absorption factor (AF), which represents the fraction of incident PAR that is absorbed by the leaves, was determined simultaneously in all leaves of mature *in situ* shoots and cultured seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* according to the method of Beer et al (2001). AF was measured by placing each leaf on top of the fluorometer PAR quantum sensor and irradiating each leaf perpendicularly at a fixed distance with the instrument's own optic fiber. Irradiance reaching the sensor with and without leaf was recorded, and AF was calculated as the percentage of light absorbed by the leaf. The use of this specific absorption factor instead of the instrument's pre-defined and commonly used value of 0.84 allowed the calculation of absolute ($\mu\text{mol electron m}^{-2} \text{s}^{-1}$) instead of relative electron transport rates.

All chlorophyll *a* fluorescence measurements for mature shoots and seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* were carried out simultaneously in August 2005. During this month, all seedling species were healthy and fully grown. Maximum leaf length of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* were \approx 25.0, 21.0, 28.0 and 20.0 cm, respectively. Measurements were taken at the base, middle and tip of all leaves of the shoots and mean values were calculated. The rapid light curve (RLC) was produced by the Diving-PAM (Walz GmbH, Effeltrich, Germany) under the control of an internal program using artificial PPFD (PAR photon flux density). Nine discrete irradiance steps were used (10 s); 10, 43, 78, 121, 205, 263, 343, 466 and 562 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Each light increment was followed by a saturating pulse and these data were plotted to create the RLC. The fluorescence parameters were measured using an automatic run and Φ_{PSII} , ETR, photochemical (qP) and non-photochemical (NPQ) quenching parameters were calculated at each step. The absolute electron transport rate (ETR) was calculated according to the formula:

$$\text{ETR} = \Phi_{\text{PSII}} \times \text{PPFD} \times 0.5 \times \text{AF}$$

Where: Φ_{PSII} - the quantum yield or $[Fm' - F] / Fm'$ (Genty et al 1989),
PPFD - the intensity of the photosynthetically active radiation (400 to 700 nm),
0.5 - a correction factor based on the assumption that the incident photons are absorbed equally by the pigments of the 2 photosystems,
AF - the absorption factor which describes the proportion of incident photons absorbed by the photosynthetic pigments.

The units of ETR are $\mu\text{mol electron m}^{-2} \text{s}^{-1}$ and PPFD are $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Photosynthetic recovery was measured in darkness at 10 s, 30 s, 1 min, 2 min and 5 min after the last actinic light period of a RLC. Photochemical (qP) and non-photochemical (NPQ) quenching parameters were determined according to the following equations:

$$qP = (Fm' - F) / (Fm' - F_0) \text{ and NPQ} = [Fm - Fm'] / Fm'$$

Photosynthetic parameters, i.e. maximum electron transport rate (ETR_{max}), minimum saturating irradiance (E_k) and light-limited photosynthetic efficiency (α) were estimated from the rapid-light curves using the model described by Platt et al (1980).

The variation in descriptive parameters (ETR_{max} , E_k and α) of RLC's among all species of mature *in situ* and seed-generated (seedlings) seagrasses was determined using the one-way analysis of variance model (ANOVA, significance level, P of 0.05). All datasets were found to meet assumptions of normality and equal variance. The Tukey's multiple comparison tests was performed to identify which treatments were different. The analyses were performed using the SPSS, Inc. (v8.0).

Results and Discussion. The mean absorption factor (AF) of mature *in situ* shoots and cultured seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* is shown in Table 1.

The absorption factor was higher in mature shoots compared to the cultured seedlings but both values were lower than the 0.84 set as default for the Diving-PAM, as they were subsequently used when calculating absolute ETR's. *Z. japonica* had the highest mean AF (0.82 ± 0.05 ; 0.68 ± 0.03) for both mature and seedlings followed by *Z. marina* (0.73 ± 0.10 ; 0.59 ± 0.02), *P. iwatensis* (0.77 ± 0.14 ; 0.58 ± 0.03) and *Z. asiatica* (0.70 ± 0.10 ; 0.56 ± 0.02), respectively.

Table 1
Leaf absorbance (AF) of mature *in situ* shoots and cultured seedlings of *Zostera marina*, *Zostera asiatica*, *Zostera japonica* and *Phyllospadix iwatensis* (mean \pm S.E., n = 5)

Species	Mature	Seedlings
<i>Zostera marina</i>	0.73 ± 0.10	0.59 ± 0.02
<i>Zostera asiatica</i>	0.70 ± 0.10	0.56 ± 0.02
<i>Zostera japonica</i>	0.82 ± 0.05	0.68 ± 0.03
<i>Phyllospadix iwatensis</i>	0.77 ± 0.14	0.58 ± 0.03

The fluorescence yield (Φ_{PSII}) displayed as a function of actinic irradiance for mature shoots and cultured seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* is shown in Figure 1a & b. All mature and seedling species of seagrasses followed a similar pattern in the effective quantum yield (Φ_{PSII}), where Φ_{PSII} declined with increasing actinic irradiance due to the inherent release of heat or energy via non-photochemical quenching or NPQ (drop of Fm'). NPQ dissipates the incoming photon energy, preventing damage to the photochemical pathway, before the energy is accumulated as reactive intermediate substances in the photosynthetic chain (Ralph et al 2002). The mean effective quantum yield curve of mature and cultured seedling shoots of *Z. japonica* was more elevated compared to the other species which implies a higher photosynthetic activity under a series of irradiance. This was followed by *Z. marina*, *P. iwatensis* and *Z. asiatica*. In addition, *Z. asiatica* had the lowest Φ_{PSII} curve for both mature and seedlings (Figure 1b).

Previous study revealed that *Z. japonica* had considerably high photosynthetic activity while *Z. asiatica* had very low activity (Orbita & Mukai 2009). Possible reasons were attributed to their morphological differences as well as to their habitat condition.

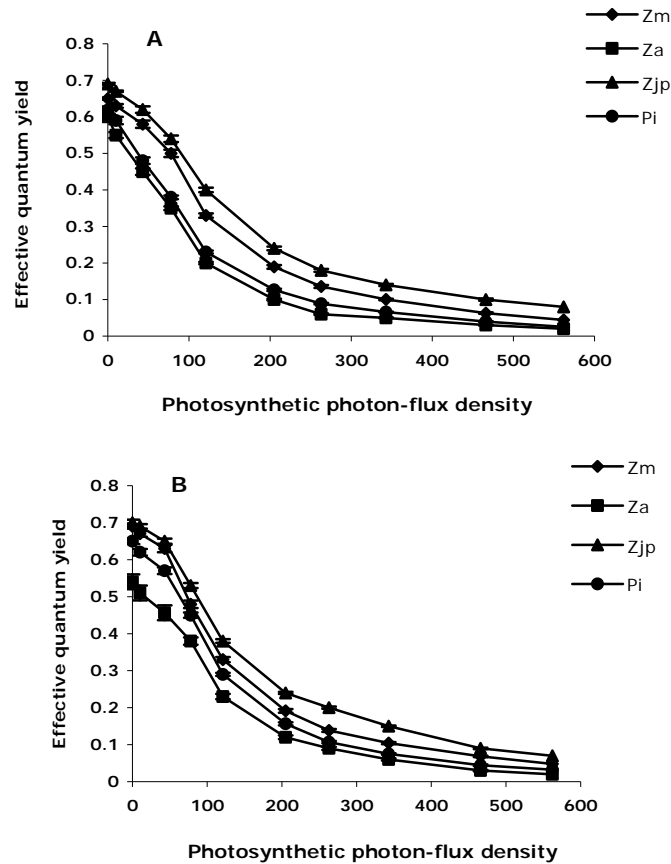


Figure 1. The effective quantum yield curve of mature in situ shoots (A) and cultured seedlings (B) of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* as a function of PPFD (mean \pm S.E., n = 10).

Figure 2a & b show the RLC for mature *in situ* shoots and cultured seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis*. As depicted in Figure 2a & b, the ETR of all mature shoots and seedlings showed a similar pattern where ETR declined with increasing irradiance. This decline in ETR curve could be linked to dynamic down-regulation of PSII, not photoinhibition as there is insufficient time for photodamage to occur (White & Critchley 1999). *Z. japonica* showed a more elevated ETR followed by *Z. marina*, *P. iwatensis* and *Z. asiatica* (lowest), respectively. A significant variation in the maximum ETR, minimum saturating irradiance and light-limited photosynthetic efficiency was found among species for both mature and seedlings (Table 2, $P < 0.005$). *Z. japonica* mature shoots had the highest maximum ETR (18.1 ± 0.46), minimum saturating irradiance or E_k (32.5 ± 1.78), and less acute α (0.21 ± 0.02) followed by *Z. marina* (15.8 ± 0.39 , 28.5 ± 0.84 , 0.24 ± 0.02) and *P. iwatensis* (13.6 ± 0.84 , 24.6 ± 0.82 , 0.27 ± 0.02), respectively. On the other hand, *Z. asiatica* had the lowest ETR_{max} (9.8 ± 0.23), E_k (18.7 ± 0.51) and larger α (0.31 ± 0.05). Subsequently, the RLC measured from cultured seedlings showed a similar pattern with that in mature shoots (Figure 2b) with *Z. japonica* having the highest ETR_{max} (14.2 ± 0.23) and E_k (35.6 ± 1.19) and less acute α (0.20 ± 0.02). This was followed by *Z. marina* (12.0 ± 0.33 , 30.8 ± 1.36 , 0.25 ± 0.01) and *P. iwatensis* (10.1 ± 0.20 , 29.3 ± 1.23 , 0.28 ± 0.01). In contrast, *Z. asiatica* had the lowest maximum ETR (8.7 ± 0.33), minimum saturating irradiance (20.8 ± 2.9) and larger α (0.30 ± 0.02). The low ETR_{max}, E_k and larger α of *Z. asiatica* would show that its response to light was lower compared to the other species. In addition, the maximal rates of photosynthesis in *Z. asiatica* could be achieved at low irradiance as revealed by low E_k , hence, producing low maximum photosynthetic rate.

Table 2

Photosynthetic parameters derived from rapid light curve or RLC in mature in situ shoots and cultured seedlings of *Zostera marina*, *Zostera asiatica*, *Zostera japonica* and *Phyllospadix iwatensis*. ETR_{max} = maximum electron transport rate; E_k = minimum saturated irradiance; a = light-limited photosynthetic efficiency (mean \pm S.E., n = 10)

Species	Mature			Seedlings		
	ETR_{max}	E_k	a	ETR_{max}	E_k	a
<i>Zostera japonica</i>	18.1 \pm 0.46 ^a	32.5 \pm 1.78 ^a	0.21 \pm 0.01 ^a	14.2 \pm 0.23 ^a	35.6 \pm 1.19 ^a	0.20 \pm 0.02 ^a
<i>Zostera marina</i>	15.8 \pm 0.39 ^b	28.5 \pm 0.84 ^b	0.24 \pm 0.01 ^b	12.0 \pm 0.33 ^b	30.8 \pm 1.36 ^b	0.25 \pm 0.01 ^b
<i>Phyllospadix iwatensis</i>	13.6 \pm 0.84 ^c	24.6 \pm 0.82 ^c	0.27 \pm 0.02 ^b	10.1 \pm 0.20 ^c	29.3 \pm 1.23 ^c	0.28 \pm 0.01 ^b
<i>Zostera asiatica</i>	9.8 \pm 0.51 ^d	18.7 \pm 0.51 ^d	0.30 \pm 0.05 ^c	8.7 \pm 0.33 ^d	20.8 \pm 2.9 ^d	0.31 \pm 0.02 ^c
	0.00 [*]	0.00 [*]	0.00 [*]	0.00 [*]	0.00 [*]	0.00 [*]

Letters represent differences in group means according to Tukey's multiple comparison tests.

*Significant difference ($P < 0.05$).

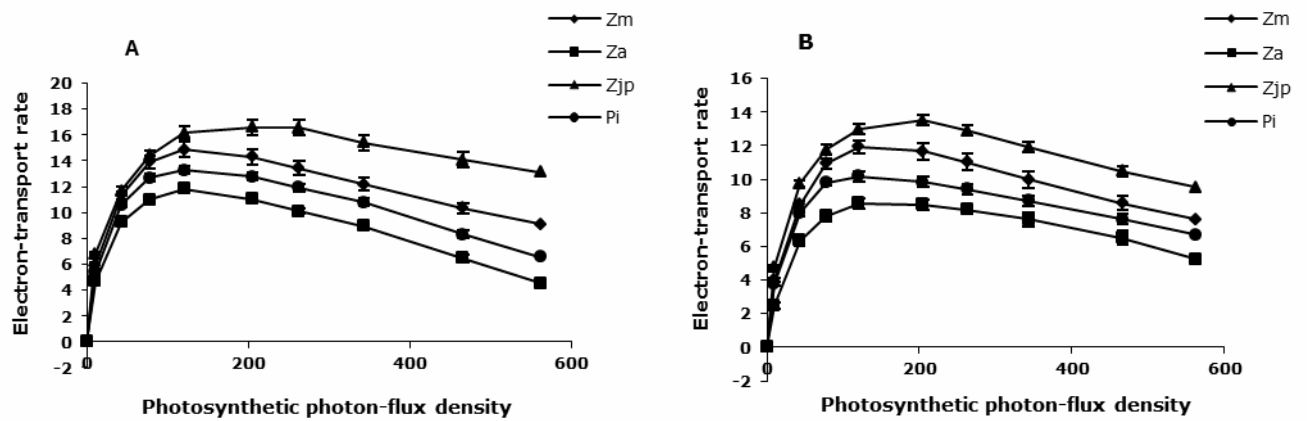


Figure 2. The rapid light curves of mature *in situ* shoots (A) and cultured seedlings (B) of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* (mean \pm S.E., $n = 10$).

The quenching parameters (qP and NPO) for mature *in situ* shoots and cultured seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* are shown in Figure 3a & b.

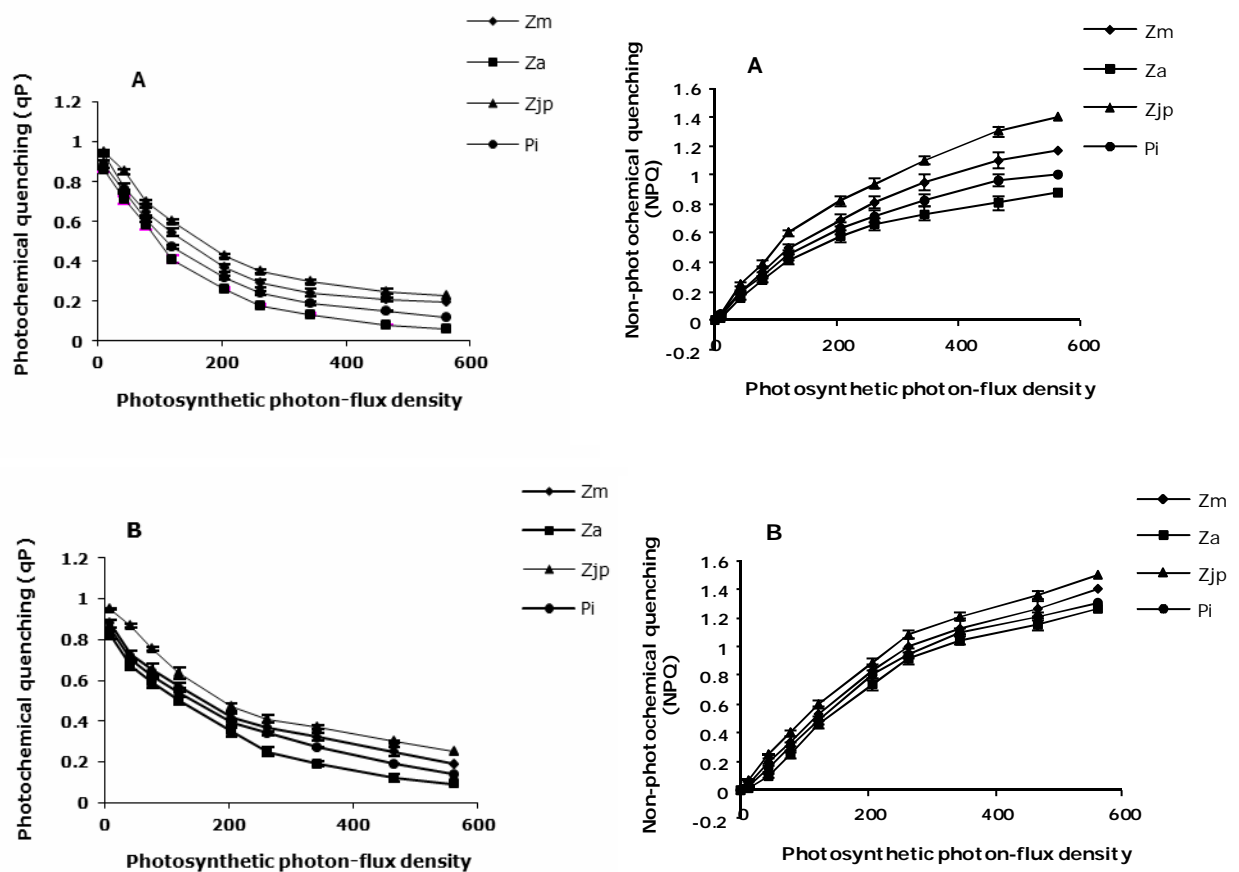


Figure 3. Photochemical (qP) and Stern-Volmer non-photochemical quenching (NPO) of mature *in situ* shoots (A) and cultured seedlings (B) of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* as a function of PPFD (mean \pm S.E., $n = 10$).

Quenching coefficients plotted as a function of PAR showed a clear increase in NPQ with increasing irradiance, and a steady decline in qP. Again, *Z. japonica*, *Z. marina* and *P. iwatensis* had greater increase in NPQ, while *Z. asiatica* had lower increase. In addition, the decline in qP was lesser in *Z. japonica*, *Z. marina* and *P. iwatensis* while greater in *Z. asiatica*. This further implies that *Z. japonica*, *Z. marina* and *P. iwatensis* were not limited by photosynthesis and showed a greater capacity to dissipate heat as a form of light protection compared to *Z. asiatica*. Moreover, the pattern in qP and NPQ among mature and cultured seedling species was similar.

Figure 4 & 5 illustrate the time-dependent response of ETR, Φ_{PSII} and NPQ during the course of a RLC and the subsequent 5 min recovery period. All mature and cultured seedling species of seagrasses completely recovered after 5 min darkness, implying that the plants did not undergo photoinhibition. However, the NPQ component of *Z. japonica*, *Z. marina* and *P. iwatensis* experienced quick relaxation (30–60 s), while *Z. asiatica* completely relaxed after 5 min. The result was not too critical as recovery period was still fast, even with *Z. asiatica* which was expected to have slow relaxation period. The component of NPQ which relaxes quickly (30–60 s) is thought to be associated with removal of energy dependent non-photochemical quenching (qE) and is linked to the relaxation of the ΔpH across the thylakoid membrane. Whereas, a slower relaxation (>10 min up to hours) is thought to be associated with photoinhibition (qI), as well as changes in energy distribution in favour of PSII (Horton & Hague 1998; White & Critchley 1999).

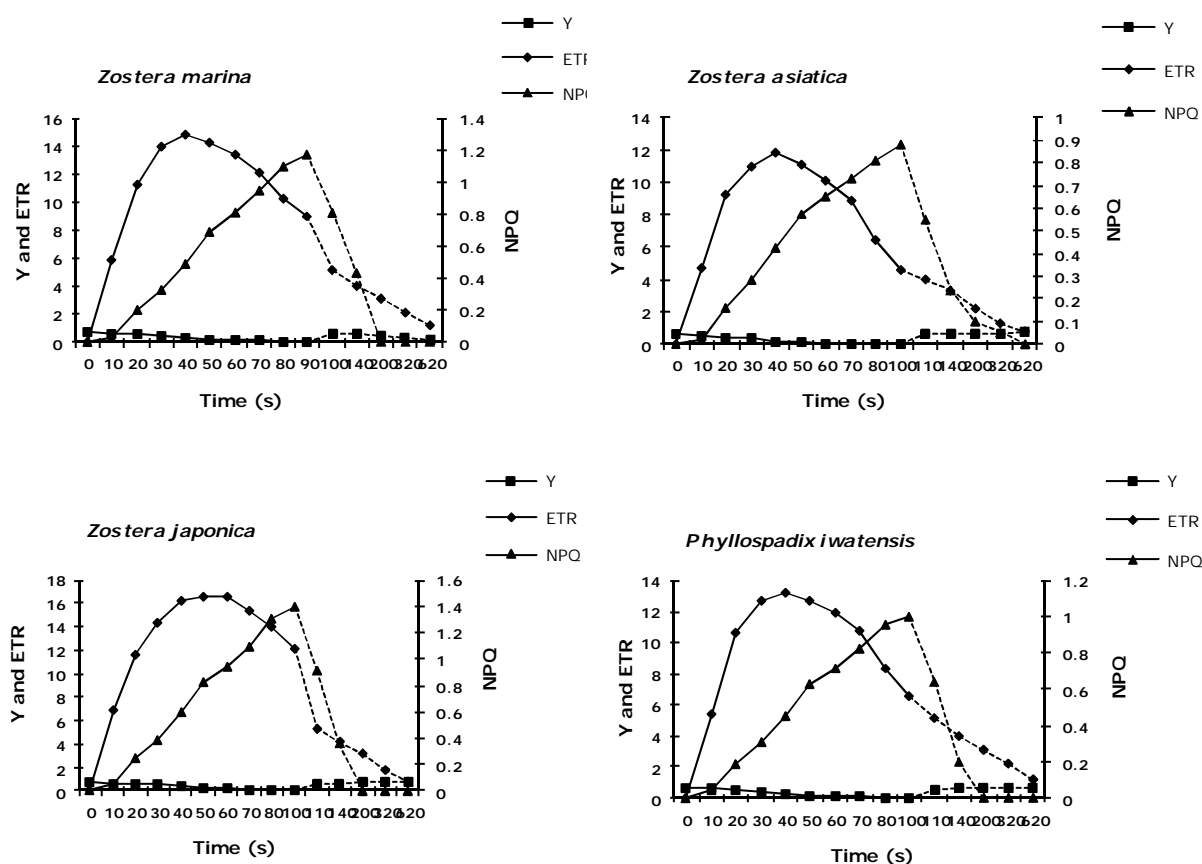


Figure 4. The rapid light curve and recovery of mature *in situ* shoots of *Zostera marina*, *Zostera asiatica*, *Zostera japonica* and *Phyllospadix iwatensis* as a function of time. ETR, Φ_{PSII} and NPQ are plotted against time. RLC data are connected by solid lines while recovery data are connected by dashed lines (mean \pm S.E., n = 10).

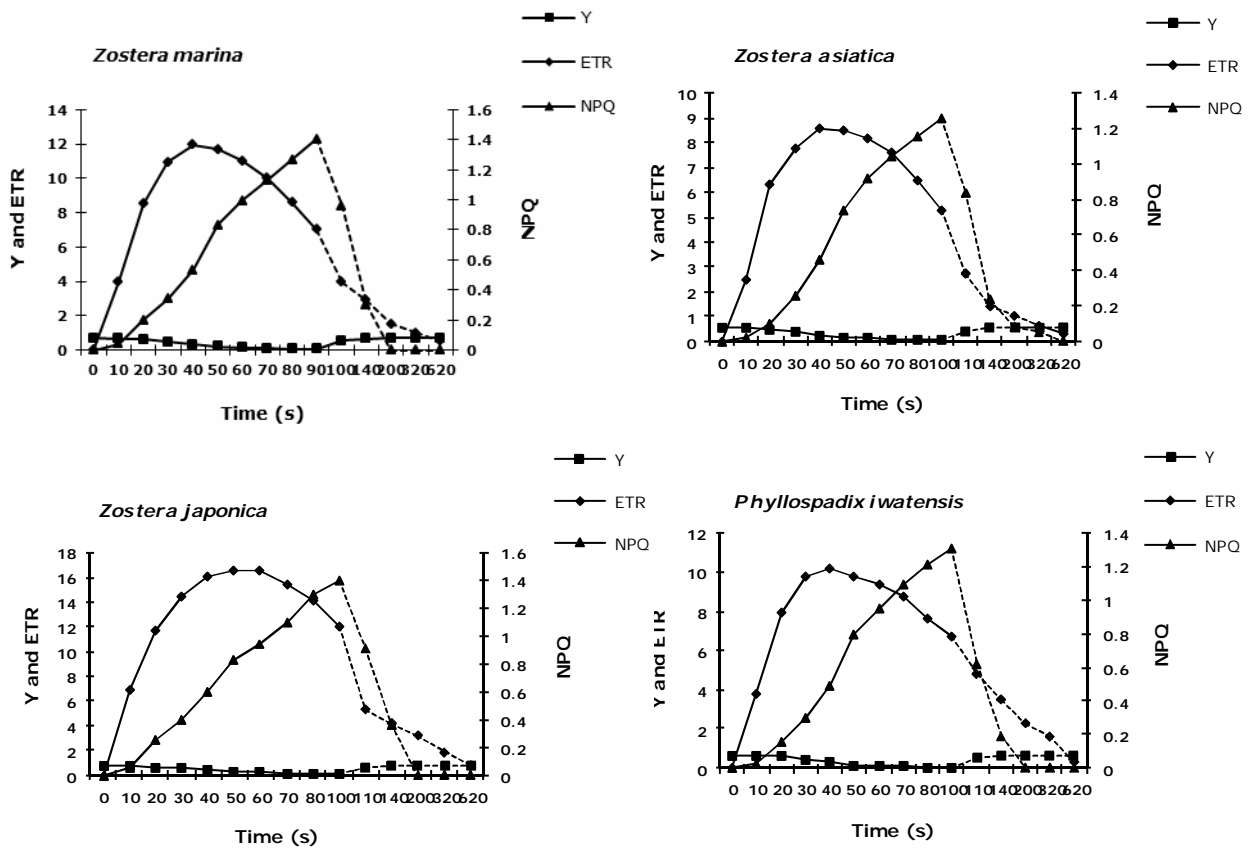


Figure 5. The rapid light curve and recovery of cultured seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* as a function of time. ETR, Φ_{PSII} and NPQ are plotted against time. RLC data are connected by solid lines while recovery data are connected by dashed lines (mean \pm S.E., n = 10).

The result in RLC among species (both mature shoots and seedlings) could be related to their distribution pattern. *Z. japonica* having the highest ETR_{max} and E_k is found dominating the intertidal area of Akkeshi-ko Estuary. Likewise, *P. iwatensis* which is observed to be restricted in the intertidal and *Z. marina* which is found in the shallower area of Akkeshi Bay have both higher ETR_{max} and E_k . In contrast, *Z. asiatica* which is observed to be confined in the deeper area of Akkeshi Bay had the lowest ETR_{max} and E_k . Similarly, the capacity for light protection was high in *Z. japonica*, *Z. marina* and *P. iwatensis* while it was low in *Z. asiatica*. Moreover, in Akkeshi Bay, *Z. asiatica* was found to have higher shoot production compared to *Z. marina*, and that this large shoot size was found to be an adaptive response in capturing photons under low light conditions (Watanabe et al 2005).

Conclusions. The pattern in the descriptive parameters (Φ_{PSII} , ETR_{max} , E_k , α) and quenching responses generated from the RLC for mature *in situ* shoots and cultured seedlings of *Z. marina*, *Z. asiatica*, *Z. japonica* and *P. iwatensis* was similar. Hence, it can be inferred that the photosynthetic activity and light adaptation of seagrasses are distinct and species specific. *Z. japonica* had the highest photosynthetic rate followed by *Z. marina*, *P. iwatensis* and *Z. asiatica*. The pulse-amplitude (PAM) fluorometry constitutes an alternative and non-intrusive method of determining photosynthetic rates in seagrasses. The similarity in the pattern of photosynthetic activity between mature *in situ* seagrasses and cultured seedlings can be considered as one of the key aspects in assessing the utility of rapid light curve to determine species specificity. The light

adaptations among species were sufficient as it reveals their light preference and distribution pattern in the natural environment. In this context, the rapid-light curve or RLC was indeed an effective technique in evaluating species differences in either types of shoots such as seedlings and mature seagrasses.

Acknowledgements. We would like to thank Dr. Ronaldo R. Orbita, and staff of Akkeshi Marine Station, Hokkaido University, for their invariable assistance to our research. We are indebted to MONBUKAGAKUSHO scholarship grant for M. L. S.Orbita supporting our research.

References

- Beer S., Vilenkin B., Weil A., Veste M., Susel L., Eshel A., 1998 Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Mar Ecol Prog Ser* 174:293-300.
- Beer S., Björkmann M., Gademann R., Ralph P., 2001 Measurement of photosynthetic rates in seagrass. In: *Global seagrass research methods*. Short F., Coles R. (eds), pp. 183-197, Elsevier Science, B.V.
- Genty B., Briantais, J., Baker N., 1989 The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990:87-92.
- Granger S., Traber M., Nixon S., 2000 Propagation of *Zostera marina* L., from seed. In: *Seas at the millennium: an environmental evaluation*. Sheppard C. (ed), pp. 55-58, Elsevier Science, Oxford.
- Hasegawa N., Iizumi H., Mukai H., 2005 Nitrogen dynamics of the surfgrass *Phyllospadix iwatensis*. *Mar Ecol Prog Ser* 293:59-68.
- Horton P., Hague A., 1998 Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non- photochemical quenching. *Biochim Biophys Acta* 932:107-115.
- Masini R., Cary J., Simpson C., McComb A., 1995 Effects of light and temperature on the photosynthesis of temperate meadow-forming seagrasses in WA. *Aquat Bot* 49:239-254.
- Orbita M. L., Mukai H., 2009 Ontogenic change in the seedling photosynthetic activity of four temperate seagrass species in Northern Japan. *Asia Life Sci* 18:99-110.
- Platt T., Gallegos C., Harrison W., 1980 Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J Mar Res* 38:687-701.
- Ralph P., Polk S., Moore K., Orth R., Smith W., 2002 Operation of the xanthophyll cycle in the seagrass *Zostera marina* in response to variable light. *J Exp Mar Biol Ecol* 271:189-207.
- Ralph P., Gademann R., Dennison W., 1998 In situ seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. *Mar Biol* 132:367-373.
- Schreiber U., 2004 Pulse-amplitude (PAM) fluorometry and saturation pulse method. In: *Chlorophyll fluorescence: A signature of photosynthesis*. Advances in Photosynthesis and Respiration Series. Papageorgiou G., Govindjee (eds), Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Schreiber U., Bilger W., 1993 Progress in chlorophyll fluorescence research. In: *Progress in botany*. Dietmar B., Heidelberg U., Darmstadt K., Kadereit J. (eds), pp. 151-173, Springer-Verlag, Berlin.
- Silva J., Santos R., 2004 Can chlorophyll fluorescence be used to estimate photosynthetic production in the seagrass *Zostera noltii*? *J Exp Mar Biol Ecol* 307:207-216.
- Watanabe M., Nakaoka M., Mukai H., 2005 Seasonal variation in vegetative growth and production of the endemic Japanese seagrass *Zostera asiatica*: a comparison with sympatric *Zostera marina*. *Botanica Marina* 48(4):266-273.
- White A., Critchley C., 1999 Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. *Photosynth Res* 59:63-72.

Received: 19 October 2013. Accepted: 19 November 2013. Published online: 29 November 2013.

Authors:

Maria Luisa Sasil Orbita, Mindanao State University-Iligan Institute of Technology, College of Science and Mathematics, Department of Biological Sciences, Philippines, Iligan City 9200, Bonifacio Avenue, e-mail: mlwsasil@yahoo.com

Hiroshi Mukai, Field Science Education and Research Center, Kyoto University, Kyoto, Japan, e-mail:

Mukai@kais.kyoto-u.ac.jp.

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Orbita M. L. S., Mukai H., 2013 Photosynthetic performance of temperate seagrasses in Northern Japan. AAB Bioflux 5(3): 152-162.