

ORIGINAL ARTICLE

A comparative study of the photosynthetic activity among three temperate seagrass species in Northern Japan

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Keywords

Chlorophyll fluorescence; Japan; pulse amplitude modulated; photosynthetic activity; *Phyllospadix iwatensis*; *Zostera asiatica*; *Zostera marina*.

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Abstract

The photosynthetic activity of *Zostera marina*, *Zostera asiatica* and *Phyllospadix iwatensis* shoots from populations of Hokkaido (Northern Japan) was determined using the pulse amplitude modulated (PAM) fluorometer. Several fluorescence parameters were measured as a function of irradiance and leaf age: electron transport rate (ETR), quantum yield, photochemical quenching (qP) and non-photochemical quenching (NPQ). The leaf age determined by the leaf position in the shoot bundle strongly influenced the photosynthetic activity of *Z. marina*, *Z. asiatica* and *P. iwatensis*. Young leaves had the maximum electron transport rate (Zm: Leaf 1 = 15.7, Leaf 2 = 16.3; Za: Leaf 1 = 13.0, Leaf 2 = 12.2; and Pi: Leaf 1 = 12.5, Leaf 2 = 11.7) and showed higher photoprotection (NPQ) than old leaves. Among the studied seagrass species, *Z. marina* had the highest photosynthetic activity (ETR_{max} = 15.3), in accordance with the highest production in the field in comparison with the other two seagrass species. The PAM fluorometry technique showed to be effective in determining intraspecific (among-leaves) and interspecific (among-species) variation in seagrass photosynthetic activity.

Problem

Seagrasses contribute significantly to the production of coastal areas in both temperate and tropical waters (Phillips & McRoy 1980; Stapel & Hemminga 1997) and several examples are reported in literature. They serve as primary producers and provide habitats for various species including commercial invertebrates and fishes (reviewed by Zieman 1982).

In the shallow, oligotrophic coastal waters of the Caribbean Sea, the turtle grass, *Thalassia testudinum* Banks ex König, a dominant seagrass, forms a highly productive meadow (Den Hartog 1970) and *Thalassia hemprichii* (Ehrenberg) Ascherson are widely distributed in shallow coastal areas in the tropics and subtropics of the Western Atlantic and Indo-Pacific, respectively (Den Hartog 1970; Phillips & Meñez 1998; Spalding *et al.* 2003). *Posidonia oceanica* plays an important role in the primary production of oligotrophic marine ecosystems in the Mediterranean sea (Dalla Via *et al.* 1998). *Zostera capricorni*

Aschers is the dominant seagrass species in Moreton Bay, Queensland, Australia, showing approximately 25,000 ha of seagrass meadows (Hyland *et al.* 1989). *Zostera marina* is the dominant seagrass found in the coastal and estuarine areas of the western North Atlantic (Short & Short 2003). In the eastern North Atlantic and Mediterranean, *Z. marina* co-occurs with *Zostera noltii*, which similarly dominates in intertidal and shallow subtidal areas (Marba *et al.* 1996; Harrison 1999).

Such major role of seagrass beds in the coastal marine ecosystems is attributed to their extremely high production. The productivities of tropical and temperate seagrass beds are either similar or higher than tropical rain and temperate deciduous forests (Zieman 1975). Their high production is related to high activity and capacity for photosynthesis.

The photosynthetic rates of seagrasses are typically determined by enclosing the plants or their parts in chambers, and measuring either O₂ evolution or ¹⁴CO₂/H¹⁴CO₃⁻ uptake. This method is extremely intrusive as it

generally involves plant removal from the natural environment (Beer *et al.* 1998). The chlorophyll excitation energy dissipated by fluorescence has an inverse relationship with photosynthetic carbon assimilation (Walker 1988). Because of this complex relationship, several selective measuring techniques which are meant to differentiate the several aspects of fluorescence emissions were developed. One of these techniques is the PAM fluorometry, which, through a special emitter-detector unit allows fluorescence measures to be performed *in vivo* on intact shoots (Schreiber *et al.* 1988). A direct relationship between the photosynthetic electron transport rate (ETR) derived from the fluorescence measurements and the photosynthetic O₂ evolution was demonstrated for seagrass species such as *Cymodocea nodosa*, *Z. noltii* and *Halophila ovalis* (Beer *et al.* 1998; Beer & Björk 2000; Silva & Santos 2004). These results further demonstrated that PAM fluorometry can be used to analyse seagrass photosynthetic performances. Thus, this technique has been successfully used in several studies on seagrasses (Beer *et al.* 1998; Ralph *et al.* 1998; Björk *et al.* 1999; Beer & Björk 2000; Schwarz *et al.* 2000; Seddon & Cheshire 2001; Durako & Kunzelman 2002; Enriquez *et al.* 2002; Major & Dunton 2002; Campbell *et al.* 2003; Silva & Santos 2003, 2004; Ralph & Gademann 2005; Ralph *et al.* 2005).

In Akkeshi Bay, Hokkaido, Japan, four species of temperate seagrasses are found dominating the area: *Z. marina* Linnaeus, *Z. asiatica* Miki, *Z. japonica* Ascherson and Graebner and *Phyllospadix iwantensis* Miki (Nakaoka & Aioi 2001; Aioi & Nakaoka 2003). Considering the massive population, species richness and importance of seagrasses in Akkeshi Bay, it is therefore important to determine their photosynthetic performances in the area, and how the photosynthetic activity in shoots varies with many influential factors such as light condition, competitive inhibition by epiphytes, epifaunal effects, habitat condition and others. Leaf age was found to be an important factor affecting the photosynthetic activity in shoots (Mazzella & Alberte 1986; Alcoverro *et al.* 1998; Ralph *et al.* 2005), and variation in the photosynthetic activity within the shoot would also indicate high production as well as survival of the species. The aim of this study was to clarify the variability in the photosynthetic activity within different leaves in individual shoots of the seagrass species, *Z. marina*, *Z. asiatica* and *P. iwantensis*, by means of the non-intrusive PAM fluorometry method.

Material and Methods

Collection of seagrass samples

The study was conducted in July 2005, corresponding to the period of maximum production for *Z. marina*,

Z. asiatica and *P. iwatensis* meadows at Aininkap (43 °00'N, 144 °51'E), Akkeshi Bay, Hokkaido, Northern Japan (Hasegawa *et al.* 2005; Watanabe *et al.* 2005). Records on shoot density revealed mean values of 560 shoots m⁻² for *Z. marina*, 126 shoots m⁻² for *Z. asiatica* and 3180 shoots m⁻² for *P. iwatensis* (Hasegawa *et al.* 2005; Watanabe *et al.* 2005). Also, it was during this time that epiphytes exhibited their maximum growth and production (Hasegawa 2006) with *P. iwatensis* having the highest amount of epiphytes per leaf surface area (Sasil-Orbita 2006). *Zostera asiatica* is the dominant species occurring from the lowest intertidal to the deepest edge of the subtidal parts of the seagrass bed (ca. 5 m deep), *Z. marina* is restricted to the shallower edge of the bed (intertidal zone to <2 m deep), while *P. iwatensis* is found in the shallow rocky shore of the surf zone (Hamamoto & Mukai 1999). Previous data on relative light intensity (% of the surface irradiance measured in gaps on the sea floor among seagrass stands) were 0.4–16.1% greater at the *Z. marina* and *P. iwatensis* area (1.5 m depth) than at the *Z. asiatica* area 2 m deep (Watanabe *et al.* 2005). All seagrass species were collected in the upper subtidal zone (0–1 m) during low tide. For each seagrass species, 10 shoots were randomly collected. Shoots were brought to the laboratory immediately after collection and sorted. For each shoot, leaves were numbered from the youngest to the oldest, as Leaf 1, Leaf 2 and Leaf 3, respectively. Only three leaves for each shoot were considered in this study as the oldest leaf (Leaf 4) was already non-chlorotic and sometimes dead. Thus, Leaf 3 was considered as the oldest one. Although some leaves were free of epiphytes, all leaves were carefully cleaned using a soft brush to ensure consistency in the treatments.

Measurement of absorption factor

The absorption factor (AF), which represents the fraction of incident PAR that is absorbed by the leaves, was determined in all leaves of *Z. marina*, *Z. asiatica* and *P. iwatensis* according to the method of Beer & Björk (2000). To determine the within-shoot variability in AF, measurements were conducted with respect to different leaf ages based on leaf position in the shoot. AF was measured by placing each leaf (according to the age) on the top of the fluorometer PAR quantum sensor and irradiating perpendicularly at a fixed distance with the instrument optic fibre. Three measurements were taken for each leaf, at the base, the middle and the top, respectively. Then, the mean value was calculated. 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 AF instead of the instrument predefined and commonly used value of 0.84 allowed for the

calculation of absolute ($\mu\text{mol electron m}^{-2}\cdot\text{s}^{-1}$) instead of relative ETRs.

Fluorescence measurements

The chlorophyll *a* fluorescence measurements were carried out for Leaf 1, Leaf 2 and Leaf 3, respectively, of *Z. marina*, *Z. asiatica* and *P. iwatensis*. Time lag between shoot collection and fluorescence analysis was about 20–30 min as the collection area is very close to the laboratory. The rapid light curves (RLCs) were produced by the Diving-PAM (Walz GmbH, Effeltrich, Germany) using an internal program and artificial PPFD (PAR photon flux density). Nine discrete irradiance steps were used (10 s); 10, 43, 78, 121, 205, 263, 343, 466 and 562 $\mu\text{mol quanta m}^{-2}\cdot\text{s}^{-1}$. The tip of the instrument optical fibre was placed at 10 mm from, and perpendicular to the adaxial surface of leaves, using dark leaf clips. Each light increment was followed by the measurement of F_t and by a saturating pulse for the measurement of F_m . The absolute ETR was calculated according to the formula:

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

where Φ_{PSII} is the effective quantum yield $[F_m' - F_t]/F_m'$ (Genty *et al.* 1989), PPFD is the intensity of the photosynthetically active radiation (400–700 nm), 0.5 is a correction factor based on the assumption that the incident photons are absorbed equally by the two photosystems, and AF is the absorption factor which describes the proportion of incident photons absorbed by the photosynthetic pigments. ETR values were plotted to derive the RLCs and the experimental data were fitted to a double exponential decay function described by Platt *et al.* (1980). Several descriptive parameters were used to quantitatively compare RLCs: maximum electron transport rate (ETR_{max}), minimum saturating irradiance (E_k) and light-limited photosynthetic efficiency (α). Photosynthetic recovery was measured in the dark at 10 s, 30 s, 1 min, 2 min and 5 min after the last actinic light that was utilized for the RLCs derivation. Photochemical (qP) and non-photochemical (NPQ) quenching parameters were calculated based on a built-in function of the Diving-PAM (L-CURVE + REC) according to the following equations: $\text{qP} = (F_m' - F_t)/(F_m' - F_0)$ and $\text{NPQ} = [F_m - F_m']/F_m'$.

Measurements of chlorophyll pigments

The extraction of chlorophyll *a* and *b* was performed using *N,N*-dimethylformamide (DMF) according to the methods of Arar & Collins (1992), and concentrations were determined using the extinction coefficient equations of Jeffrey & Humphrey (1975) and Dennison (1990). More in detail, the sampled leaves were cut into three sec-

tions (corresponding to the base, the middle and the top), and each section was further cut into fine pieces and placed in 5 ml DMF tubes. The tubes were kept in the refrigerator for 24–48 h before spectrophotometric analysis (Shimadzu UV-VIS Spectrophotometer, UV-260). Absorbance was read at 647, 664 and 750 nm, respectively.

Statistical analyses

The values of AF, RLCs descriptive parameters and chlorophyll content for leaves of different age were statistically compared in each seagrass species using one-way analysis of variance (ANOVA), with significance level (*P*) of 0.05. The same statistical tool was used to determine the variation in the AF, RLCs descriptive parameters and pigment content among seagrass species. The Tukey's multiple comparison test was performed to identify which treatments were significantly different. All data sets were found to meet the assumptions of normality and equal variance. These statistical analyses were performed using SPSS, Inc. (v8.0) software.

Results

Absorption factor

The mean AF varied among leaves for each seagrass species, but the variation was not significant (Table 1). In all the three analysed species, the oldest leaves (Leaf 3) had the highest AF, while the youngest leaves (Leaf 1) showed the lowest AF value. The variation in AF among the seagrass species was significant ($P < 0.05$). The highest AF values were found in *P. iwatensis* and *Z. asiatica*, while the lowest value was in *Z. marina* (Table 2).

Fluorescence parameters

Rapid light curves

Rapid light curves for *Z. marina*, *Z. asiatica* and *P. iwatensis* are reported in Fig. 1. The ETR of all the seagrass species increased with increasing irradiance until its maximum value and decreased at the highest experimented light intensities. In all the species, the ETR curve for Leaf 1 and 2 presented higher values than Leaf 3. In particular, ETR_{max} and E_k decreased from the youngest to the oldest leaves in all the species, while the light-limited photosynthetic efficiency (α) increased from the youngest to the oldest leaves (Table 3). In *Z. marina* and *P. iwatensis*, only the ETR_{max} showed a significant difference among leaves. However, in *Z. asiatica*, a significant variation in both ETR_{max} and light-limited photosynthetic efficiency (α) was detected among leaves. Additionally, a significant variation was detected in the ETR_{max} , E_k and α among seagrass species (Table 2, Fig. 2). *Zostera marina* had

Table 1. Leaf absorption factor (AF) and chlorophyll content of leaves of different ages in *Z. marina*, *Z. asiatica* and *P. iwatensis* (mean \pm SE, $n = 45$).

species	leaf number	AF	total chlorophyll	chlorophyll a:b
<i>Z. marina</i>	1	0.66 \pm 0.05	4.2 \pm 0.9	2.2 \pm 0.4
	2	0.75 \pm 0.03	6.5 \pm 1.2	2.4 \pm 0.3
	3	0.76 \pm 0.09 NS	7.1 \pm 1.1 NS	2.7 \pm 0.2 NS
<i>Z. asiatica</i>	1	0.79 \pm 0.04	6.5 \pm 1.4 ^a	2.8 \pm 0.9
	2	0.81 \pm 0.04	10.3 \pm 1.1 ^b	3.2 \pm 0.2
	3	0.82 \pm 0.05 NS	11.8 \pm 0.8 ^b ≤ 0.05	3.7 \pm 0.1 NS
<i>P. iwatensis</i>	1	0.81 \pm 0.07	11.4 \pm 1.7	2.9 \pm 0.9
	2	0.83 \pm 0.04	12.4 \pm 1.8	3.4 \pm 0.5
	3	0.85 \pm 0.04 NS	12.8 \pm 2.0 NS	3.8 \pm 0.4 NS

Units of chlorophyll are $\mu\text{g}\cdot\text{cm}^{-2}$. Different apex letters indicate differences in mean values as determined by Tukey's multiple comparison test. NS, non-significant.

higher ETR_{max} , minimum saturating irradiance (E_k) and less acute α . In contrast, both *P. iwatensis* and *Z. asiatica* had lower ETR_{max} , E_k and more acute α .

Photochemical and non-photochemical quenching

The time-dependent recovery of NPQ after the RLC derivation is reported in Fig. 3. All leaves of *Z. marina*, *Z. asiatica* and *P. iwatensis* completely recovered after 5 min in the dark, implying that the plants did not suffer photodamages. However, NPQ of the youngest leaves experienced quick relaxation (30–60 s), while the oldest leaves completely relaxed after 5 min in all species.

Chlorophyll pigments

In all the species, chlorophyll pigments showed an increasing trend with leaf age similarly to what was observed for the leaf AF (Table 1). In all species, in fact, the highest values for both the total chlorophyll content and chlorophyll *a/b* ratio were observed in the oldest leaves (Leaf 3), while the lowest values were recorded in the youngest leaves (Leaf 1). Anyway, only *Z. asiatica*

showed a statistically significant variation in the amount of total chlorophyll, while no significant variations were detected for both *Z. marina* and *P. iwatensis*. Among the seagrass species, *P. iwatensis* and *Z. asiatica* showed a significantly higher total chlorophyll amount and chlorophyll *a/b* ratio, while *Z. marina* had the lowest chlorophyll content (Table 2).

Discussion

In order to calculate the absolute photosynthetic ETR, the AF of leaves needs to be determined (Beer *et al.* 1998; Beer & Björk 2000). The youngest leaves showed the lowest AF value in all the analysed seagrass species; a similar result was reported by Durako & Kunzelman (2002) who first investigated the intraspecific variation in PAR absorption for seagrasses. In addition, a significant variation in PAR absorption was highlighted in the present work among *Z. marina*, *Z. asiatica* and *P. iwatensis* in accordance with literature data even if the AF values that we measured for these seagrasses result higher than those previously reported (0.44–0.72, Beer *et al.* 1998; Beer & Björk 2000; Schwarz *et al.* 2000). PAR absorption and chlorophyll content among leaves in each species were similar in pattern. A more elevated chlorophyll *a/b* ratio in the oldest leaves suggested that these leaves are adapted to higher light conditions, as consistent with their external location in the shoot. The existence of a positive correlation between AF and chlorophyll content is demonstrated also comparing *Z. asiatica*, *P. iwatensis* and *Z. marina*, the first species showing both the highest amount of chlorophyll pigments and the highest PAR absorption. The pattern in AF and chlorophyll content can also be related to leaf thickness, epiphyte density and reciprocal leaf shading. It was observed that the leaves of *Z. marina*, *Z. asiatica* and *P. iwatensis* were increasingly thicker with increasing age of the leaf, *i.e.*, Leaf 1 to 3 (Sasil-Orbita, personal observation). Thicker leaves have more chloroplasts, hence more chlorophyll content per leaf area, with an increased PAR absorbance (Zimmerman 2006). Similarly, *Z. asiatica* and *P. iwatensis* had both thicker leaves which showed higher chlorophyll content and PAR absorption compared with

Table 2. Comparison of leaf absorbance (AF), chlorophyll contents and photosynthetic parameters among the three studied seagrass species.

species	AF	total chlorophyll	chlorophyll a:b	ETR_{max}	E_k	α	P
<i>Z. marina</i>	0.73 \pm 0.02 ^a	5.8 \pm 0.6 ^a	2.3 \pm 0.2 ^a	15.3 \pm 0.4 ^a	29.3 \pm 1.3 ^a	0.22 \pm 0.01 ^a	<0.01
<i>Z. asiatica</i>	0.81 \pm 0.01 ^b	9.5 \pm 0.7 ^b	3.0 \pm 0.1 ^b	12.1 \pm 0.2 ^b	19.0 \pm 0.6 ^b	0.32 \pm 0.02 ^b	<0.01
<i>P. iwatensis</i>	0.83 \pm 0.01 ^b	12.1 \pm 1.0 ^b	3.2 \pm 0.3 ^b	11.6 \pm 0.2 ^b	20.6 \pm 0.8 ^b	0.33 \pm 0.01 ^b	<0.01

Mean values \pm SE; values with different apex letters are significantly different as determined by Tukey's multiple comparison test ($P < 0.05$). NS, non-significant.

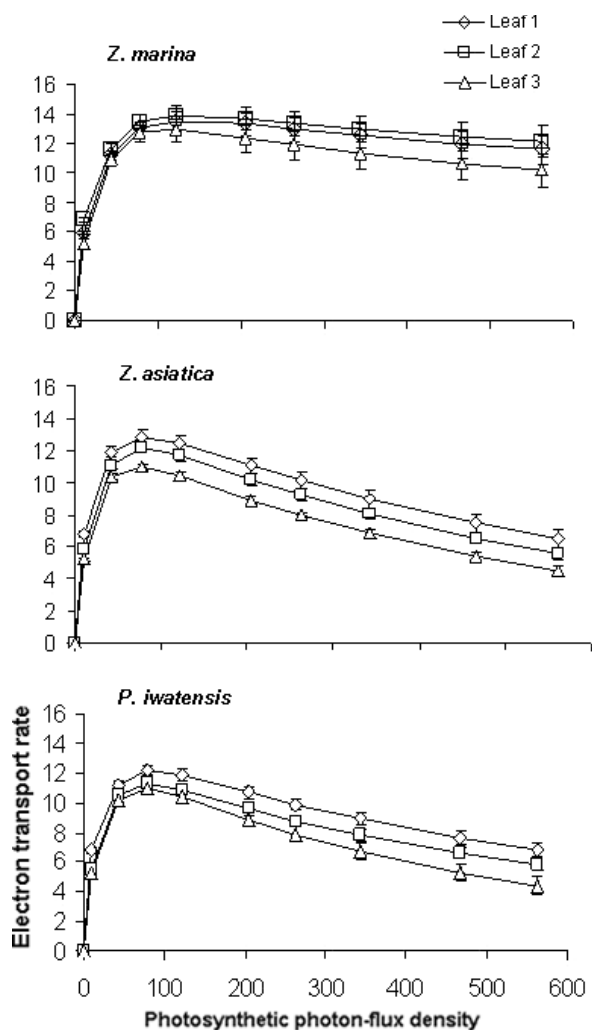


Fig. 1. Rapid light curves of leaves of different ages in *Z. marina*, *Z. asiatica* and *P. iwatensis* (mean ± SE, n = 90).

Z. marina. The presence of epiphytes which were observed to be abundant in the oldest leaves of *Z. marina*, *Z. asiatica* and *P. iwatensis* (Sasil-Orbita 2006) may also contribute to the higher PAR absorption and chlorophyll content of the oldest leaves in the shoot. Additionally, the oldest leaves have greater potential for light absorption compared with the younger leaves which are shaded by the oldest leaves.

The effective quantum yield (Φ_{PSII}) of *Z. marina*, *Z. asiatica* and *P. iwatensis* leaves showed a similar declining pattern at increasing irradiance and Φ_{PSII} and NPQ were correlated. The decline in Φ_{PSII} is, generally, attributed to the increase of energy dissipation via non-photochemical quenching (NPQ), usually associated with the xanthophyll cycle (Ralph *et al.* 2005). The photoprotective component of NPQ (qE and qT), in fact, may allow for the dissipation of incoming photon energy, preventing damages to

Table 3. Photosynthetic parameters derived from rapid light curves of *Z. marina*, *Z. asiatica* and *P. iwatensis* (mean ± SE, n = 90).

species	leaf number	ETR _{max}	E _k	α
<i>Z. marina</i>	1	15.7 ± 0.8 ^a	28.3 ± 2.1	0.20 ± 0.02
	2	16.3 ± 0.8 ^a	33.1 ± 2.4	0.22 ± 0.02
	3	13.8 ± 0.6 ^b	26.6 ± 2.0	0.24 ± 0.02
<i>Z. asiatica</i>	1	≤0.05	NS	NS
	2	13.0 ± 0.4 ^a	19.5 ± 1.3	0.30 ± 0.02 ^a
	3	12.2 ± 0.3 ^a	19.0 ± 1.0	0.32 ± 0.02 ^a
<i>P. iwatensis</i>	1	11.0 ± 0.2 ^b	18.5 ± 0.7	0.34 ± 0.02 ^b
	2	≤0.05	NS	≤0.05
	3	12.5 ± 0.4 ^a	25.5 ± 1.3	0.21 ± 0.02
	1	11.7 ± 0.4 ^a	24.6 ± 1.4	0.24 ± 0.03
	2	10.0 ± 0.3 ^b	24.0 ± 1.5	0.25 ± 0.03
	3	≤0.05	NS	NS

Different apex letters indicate differences in mean values as determined by Tukey's multiple comparison test. NS, non-significant.

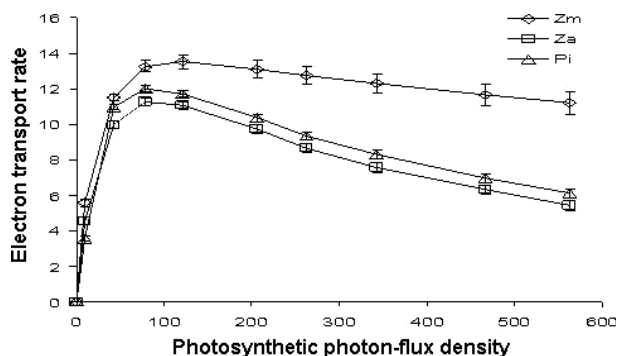


Fig. 2. Rapid light curves of the three seagrass species (mean ± SE, n = 90). Zm = *Zostera marina*, Za = *Zostera asiatica*, Pi = *Phyllospadix iwatensis*.

the photochemical pathway, before the energy is accumulated as reactive intermediate substances in the photosynthetic chain (Ralph *et al.* 2002). The ETR-irradiance curves showed a slight down-turn at elevated irradiances. With traditional P-E curves, this kind of decline was usually associated with photodamages (Henley 1993), but in the RLCs this decline may be better linked to dynamic-down regulation of Photosystem II (the reaction centre contained within the chloroplast and reactive at wavelength of 680 nm with quinone as terminal electron acceptor) as suggested by White & Critchley (1999), because time is probably insufficient in this experimental conditions to induce photodamages. Besides, a total recovery within 5 min was pointed out for all the leaves in the shoot of all the analysed species, demonstrating the absence of photodamages. Moreover, the youngest leaf showed a higher increase of NPQ with increasing

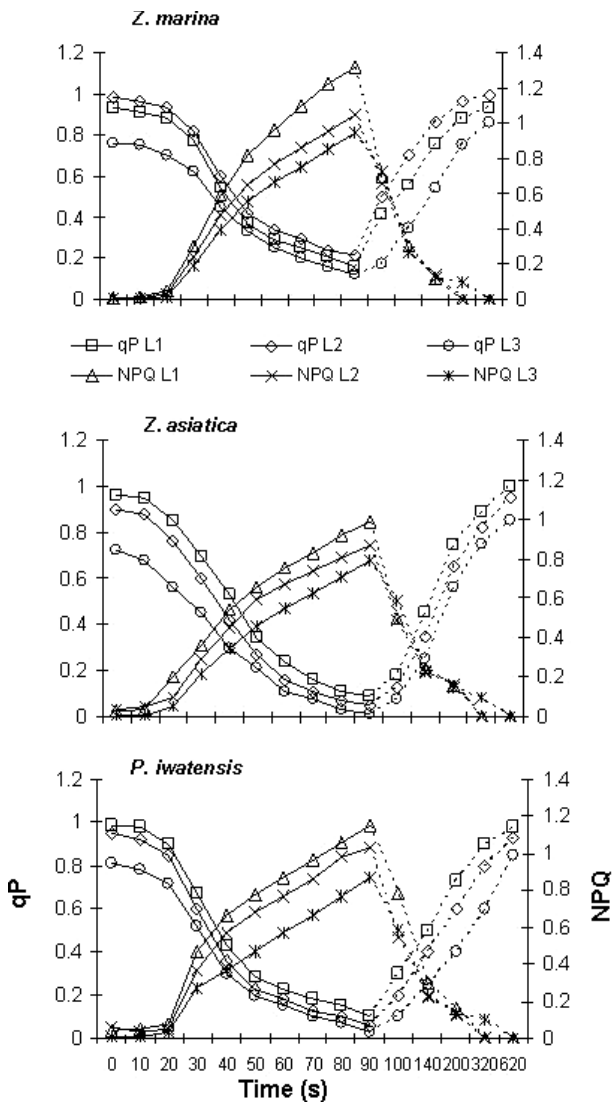


Fig. 3. Pattern of NPQ recovery for leaves of different ages of *Z. marina*, *Z. asiatica* and *P. iwatensis*.

irradiance and a faster recovery of the initial qP value in the dark with respect to the oldest leaf; it may suggest a more efficacious photoprotection in the former than in the latter. The NPQ components with quick relaxation within seconds to minutes are associated with removal of energy-dependent non-photochemical quenching (qE) and state-transition non-photochemical quenching (qT), both linked to physiological mechanisms of photoprotections; whereas, a slower relaxation (>10 min up to hours) is thought to be associated with photodamages (qI), as well as changes in energy distribution in favour of PSII (Horton & Hague 1998; White & Critchley 1999).

As all the three seagrass species were collected in the same area and depth (0–1 m), it was assumed that their

light climate would be similar. Despite this assumption, a significant variation was detected in the RLC data. Several investigations have documented that photosynthesis is not constant along a leaf (Mazzella & Alberte 1986; Ralph & Gademann 1999; Durako & Kunzelman 2002; Enriquez *et al.* 2002; Ralph *et al.* 2005). The results of the present study also suggest that photosynthesis varies among leaves in a shoot. Variation in P_{\max} among leaf blades has been described in *Z. marina* (Mazzella & Alberte 1986), and showed that the maximum photosynthetic rates were higher in young leaves than in old leaves. Very importantly, results on the ontogenic change in the photosynthetic activity of *Z. marina*, *Z. asiatica* and *P. iwatensis* cultured seedlings revealed higher photosynthetic activity in young leaves than in old leaves (Sasil-Orbita & Mukai, unpublished data). In addition, Alcoverro *et al.* (1998) observed that younger age classes (A1: 0–25; A2: 26–50 days) of *Posidonia oceanica* had the maximum photosynthetic rates compared with the older age classes.

Light gradients created by leaf canopy structure of *Z. marina*, *Z. asiatica* and *P. iwatensis* and additional shading by the epiphytes (maximum in July–August) could influence the photosynthetic performance of the different leaves in the shoot. As a consequence of this light availability, gradients in leaf chlorophyll pigment contents also exist among leaves which are dependent upon leaf age. The observed differences in photosynthetic activity among leaves can be attributed in part to differences in leaf chlorophyll content, but leaf age is also a contributing factor. Differences in the minimum saturating irradiance (E_k) and light-limited photosynthetic efficiency (α) observed among leaves can also be ascribed to age differences. These features explain the increased value of α and the decreased E_k values among leaves. The same pattern was observed in the different amounts of chlorophyll content among leaves with differing ages.

Differences in the photosynthetic activity did not only exist among leaves within the shoot (intraspecific) but also among species (interspecific). In spite of low amount of chlorophyll content, *Z. marina* had the maximum electron transport rate and highest minimum saturating irradiance among the three seagrass species. The high photosynthetic activity may contribute to ensure a greater shoot density and branching frequency to this species compared with the co-generic *Z. asiatica* (Watanabe *et al.* 2005). More importantly, this species was found to be the most abundant sublittoral seagrass species in Japan (Aioi 1980). In contrast, the higher shoot density of *P. iwatensis* could not be related to its photosynthetic activity, where it showed a very low value. Nevertheless, its higher shoot density is better related to high nutrient accumulation (Hasegawa *et al.* 2005) and other related factors.

Both *Z. asiatica* and *P. iwatensis* had lower minimum saturating irradiance (E_k) and larger light-limited photosynthetic efficiency (α) compared to *Z. marina*. This result suggests that these species are better adapted to low light conditions, while *Z. marina* with higher E_k and smaller α is adapted to high light conditions. A difference of being adapted to low light conditions exists between *Z. asiatica* and *P. iwatensis*. The low light adaptation of *Z. asiatica* is due to low light level, mostly confined in the deepest area, while the low light adaptation of *P. iwatensis* is not due to low light level, as it is confined in the shallowest area of the seagrass bed, but rather as an adaptive mechanism against epiphytic load (Sasil-Orbita and Mukai, unpublished data).

Conclusions

The variation in the photosynthetic activity among leaves in the shoot was influenced by several factors in which the leaf age had a significant contribution. As a consequence, young leaves had the maximum electron transport rate and showed higher photoprotection than old leaves. Whereas *Z. marina* had the lowest mean amount of chlorophyll pigments, and the highest photosynthetic activity in relation to the highest production in the field compared with *Z. asiatica* and *P. iwatensis*. Accordingly, the photosynthetic characteristics that are shown by each seagrass species are inherent and species-specific, with *Z. marina* as high light-adapted species, and both *Z. asiatica* and *P. iwatensis* low-light adapted species. The PAM fluorometry constitutes an alternative and non-intrusive method of measuring photosynthetic activity, and shows to be effective in determining intraspecific (among-leaves) and interspecific (among-species) variation in seagrasses.

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