Adjustment of photoprotection to tidal conditions in intertidal seagrasses

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Light adaptive strategies were studied in a comparative analysis of the congeneric seagrass species Zostera muelleri *and* Zostera marina *at two case study areas in New Zealand and Germany. Surveys in intertidal seagrass meadows were conducted from pre-dawn until sunset on days when either low or high tide coincided with noon. The results show marked fluctuations of photophysiology (optimum and effective quantum yield, non-photochemical quenching, cycling of xanthophyll cycle (XC) pigments) over daily and tidal cycles. At both locations, we observed a near complete conversion (de-epoxidation) of violaxanthin to zeaxanthin at times with high irradiance and a rapid and complete re-epoxidation under subsequent lower light conditions. At the New Zealand site we also observed significantly larger XC-pigment pool sizes in seagrass leaves sampled in a week when low tide coincided with noon (larger daily fluence and higher maximum irradiance), compared with leaves sampled in a week when high tide was at noon. This dynamic adjustment of xanthophyll pool size has not been previously reported for intertidal seagrasses. It adds to our understanding of an important adaptive feature in a highly dynamic light environment and to the general ecology and adaptability of seagrasses.*

INTRODUCTION

Seagrass beds are among the most productive marine ecosystems, forming habitats for many species and playing significant roles in coastal protection and climate stability (Hughes *et al.*, 2009; Fourqurean *et al.*, 2012; Ondiviela *et al.*, 2014). In recent decades there has been a worldwide loss of seagrass (Waycott *et al.*, 2009). The overarching reasons for this are habitat destruction and pollution, and the loss of water clarity through sediment loading and eutrophication (Short *et al.*, 2011). About one third of the global area of seagrass meadows has been lost since the 19th century and the current rate of loss is about 7% year^{-1}, which is accelerating (Waycott *et al.*, 2009). The importance and vulnerability of seagrass beds underline the need for a thorough understanding of their ecology and adaptability to be able to prevent further losses and help improve restoration activities.

Intertidal seagrasses populate a very variable habitat, characterized by strong fluctuations in temperature, desiccation and irradiance (Björk *et al.*, 1999; Silva & Santos, 2003). In particular, seasonal and diurnal fluctuations of the light conditions in the intertidal can be extreme, and at low tide seagrasses can be exposed to irradiances >2000 μmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR). With the incoming tide, *in situ* irradiance decreases rapidly, particularly in turbid water (Serodio & Catarino, 1999). In clear water the decrease in irradiance upon submersion is less pronounced, but short-term light flashes of PAR >5000 μmol photons m^{-2} s^{-1} can occur due to the wave-focusing effect (Wing & Patterson, 1993; Schubert *et al.*, 2001). However, the highest *in situ* irradiance normally coincides with mid-day low water. In contrast, irradiance at a mid-day high tide is considerably less, but stronger variations in light climate during the day occur (increase of irradiance in the morning, decrease with incoming tide, increase again with outgoing tide, decrease with decreasing light in the evening). Changing light conditions in the intertidal suggests a requirement for rapid adjustment by intertidal photosynthetic organisms, which need to optimize light harvesting when irradiance is low and to protect their photosynthetic apparatus against photodamage when irradiance is high. Mechanisms of photoprotection comprise processes in the antenna complexes of PSII that decrease the transfer of excitation energy to the reaction centre (Franklin *et al.*, 2003). These are dissipative reactions, resulting in a transient reduction in photosynthetic efficiency, which is rapidly reversible. If the decrease in photosynthetic efficiency is due to photoinactivation (also referred to as photodamage) there is a loss of PSII reaction centre function due to a loss of D1 protein and the reversion is much slower or even impossible (Franklin *et al.*, 2003). Both terrestrial and aquatic plants have evolved adaptive physiological traits to cope with high and fluctuating irradiances (Demmig-Adams & Adams, 1992; Schwarz *et al.*, 2000; Ensminger *et al.*, 2001; Silva & Santos, 2003; Kono & Terashima, 2014). While photosynthetic activity usually follows the increase and decrease of diurnal irradiance, midday depressions of photosynthetic activity have been observed (Hanelt *et al.*, 1994). This is particularly common in macrophytes growing in the intertidal (Huppertz *et al.*, 1990; Silva & Santos, 2003).

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One widely found photoprotective mechanism is the xanthophyll cycle (XC), which includes the rapid and reversible de-epoxidation of violaxanthin (Vx) to antheraxanthin (Ax) and zeaxanthin (Zx) under high light (Demmig-Adams, 1990; Jahns & Holzwarth, 2012). Under low light or overnight re-epoxidation from Zx over Ax to Vx takes place (Demmig-Adams, 1990). The XC is found in higher terrestrial plants (Yamamoto et al., 1962; Bilger & Björkman, 1990; Demmig-Adams, 1990), diatoms (Lavaud et al., 2002), dinoflagellates (Ambarsari et al., 1997; Brown et al., 1999), brown algae (Uhrmacher et al., 1995) and green algae (Bischof et al., 2002). Although there have been few studies documenting the XC in seagrasses, most have been conducted under laboratory conditions or in outdoor tanks (Dawson & Dennison, 1996; Flanigan & Critchley, 1996; Ralph et al., 2002; Marín-Guirao et al., 2013). To date, only three studies have been carried out in situ (Collier et al., 2008; García-Sánchez et al., 2012; Silva et al., 2013). However, these focused on subtidal rather than intertidal seagrasses where radiation levels are much more variable. In our study areas (New Zealand and Germany), seagrass meadows occur predominantly in the intertidal (Hily et al., 2003; Inglis, 2003). There is clearly a lack of field studies characterizing the essential processes of photoacclimation and photoprotection in seagrass from intertidal habitats, which is the focus of this study. We hypothesize that intertidal seagrasses dynamically adjust the xanthophyll cycle as an efficient photoprotection mechanism in response to highly fluctuating light conditions.

The seagrass genus Zostera comprises nine species and inhabits the intertidal and subtidal along the coast of all continents, except Antarctica (Moore & Short, 2006), facilitating comparative studies in different geographic regions. Here we wanted to separate general vs specific responses to varying radiation, temperature, and local and regional environmental effects as well as genetic/intrinsic differences between congeneric species. To achieve this we chose two distant study areas – one located in the northern hemisphere in a cold temperate climate (off the island of Sylt in the Northern Frisian Wadden Sea, North Sea) and the other in the southern hemisphere in a warm-temperate climate (Tauranga Harbour, North Island, New Zealand). To take into account different tidal settings we conducted two whole day surveys during summer from pre-dawn until sunset, one when low tide occurred at noon and one when high tide occurred at noon. We used these settings for an in-depth characterization of light-protective mechanisms of seagrass in highly variable light environments.

MATERIALS AND METHODS

Study sites and species

Tauranga Harbour is a meso-tidal estuarine lagoon at the east coast (Bay of Plenty) of the North Island of New Zealand with an average tidal range of ~1.5 m. The study site was located in the northern reaches of the harbour on the outer part of a small sand spit enclosing a sub-estuary (for coordinates see Table 1). The site can be characterized as moderately wave exposed. Median grain sizes

Table 1. Abiotic conditions and coordinates of the study sites in Germany (GER) and New Zealand (NZ).

Site	Coordinates		Noon tide state	Atmospheric light (on sampling day)		In situ light	In situ temperature (°C)			Water coverage (h:min)			
				max. irradiance (μmol photons m⁻² s⁻¹)	daily fluence (mol photons d⁻¹)	max. irradiance (μmol photons m⁻² s⁻¹)	max	min	mean	max	min	mean	
Sylt/Königshafen, GER	Lat	55.02665	HT	1678	56	1520	30	14	21	05:20	04:05	04:41	submersed
	Long	8.43165	LT	1816	40	1289	29	14	20	07:17	05:59	06:27	exposed
Tauranga Harbour, NZ	Lat	−37.48603	HT	2219	61	1377	36	15	22	06:40	05:46	04:45	submersed
	Long	175.95352	LT	2252	62	2866	38	14	22	09:55	07:35	08:50	exposed

Noon tide state: HT, high tide at noon; LT, low tide at noon.

of the sediment at this site are around 250 μm and the mud content (<63 μm) is low (<5%) (Kohlmeier *et al.*, 2014).

The German study area is called Königshafen and is located in the Wadden Sea off the island of Sylt. The area/embayment is well studied and a detailed description can be found in Reise (1985), and a review on the current status in Schumacher *et al.* (2014). The average tidal range is 1.8 m. The study site is called Tonnenlegerbucht and is located in the south-east of the Königshafen (for coordinates see Table 1). The small bay is enclosed by a sand spit in the east and by a dyke to the south and the west. The site can be described as sheltered and is characterized by a mean grain size of 140 μm (Lefebvre and Laurens, unpublished data).

Zostera muelleri Irmisch ex Ascherson (1867) is the genus' representative in the South-west Pacific and its range covers the coasts of Australia, Papua New Guinea and New Zealand (Moore & Short, 2006; Matheson *et al.*, 2011). *Zostera marina* Linnaeus (1753) (eelgrass) is a very widespread species in the northern hemisphere (Moore & Short, 2006) and in the German Wadden Sea it grows in vast mixed meadows with the dwarf eelgrass *Zostera noltei* Hornemann (1832) as well as in smaller monospecific meadows. The meadow studied here was dominated by *Z. marina*, with some small patches of *Z. noltei*.

Set-up and sampling scheme

Sampling was conducted under summer conditions in January/February 2011 in New Zealand and in July 2011 in Germany. Whole day surveys, one on a day where low tide was at noon (hereafter LT) and one with high tide at noon (hereafter HT) were carried out at both study sites. One survey included continuous recording of incident atmospheric light (as PAR), regular sampling of photosynthetic parameters (via PAM-fluorometry), pigments and *in situ* light from pre-dawn until sunset. Nine plots, three at the upper, middle and lower levels of the respective seagrass meadow, were marked at each site on the day before the survey. Pilot studies had shown that there were no strong differences in physical, chemical or biological parameters among levels. The positioning of the nine replicate plots at the three levels was done to take natural variability due to vertical position (and thus potential differences in, for example, submersion times and desiccation stress) into account, if necessary. The distance between plots at each level was 10 m and distance between levels was ~50 m. Sampling for PAM-fluorometry, pigment content and *in situ* light was conducted at ~3 h intervals. For each sampling, one seagrass shoot per plot was chosen. PAM-measurements and sampling for pigment concentrations were conducted on the second or third youngest leaf per shoot. When the seagrass was submerged samples were taken by snorkelling, taking care to prevent sediment resuspension and changes in water transparency.

PAM-Fluorometry

For determination of the effective and maximum quantum yields of PSII, measurements of chlorophyll a fluorescence were conducted with a submersible pulse amplitude modulated fluorometer (Diving-PAM, Walz, Effeltrich, Germany). The effective quantum yield ($\Delta F/Fm'$) was determined using a sample holder (Universal sample holder, Walz, Effeltrich, Germany), which also ensured simultaneous measurements

of incident light next to the leaf. After determination of $\Delta F/Fm'$ the leaf was darkened using a leaf clip (Diving LC, Walz, Effeltrich, Germany). After 10 min of darkening the maximum quantum yield (Fv/Fm) was measured. Non-photochemical quenching (NPQ) was calculated: NPQ = (Fm-Fm')/Fm'. After the PAM-measurements leaf material was sampled for pigment analysis.

Light and temperature

Atmospheric light was measured using a 2π cosine-corrected sensor (LI-COR Li-192, Lincoln, USA) and logged using a data logger (Li-1500, Lincoln, USA). The sensor was located near the study site and measurements were taken every minute, and the mean over 5 min was logged. *In situ* light was measured next to the seagrass leaves using the mini-quantum sensor of the PAM-fluorometer simultaneously with PAM-measurements. The sensor was calibrated against a 2π cosine-corrected sensor (LI-COR Li-192, Lincoln, USA). Temperature was logged *in situ* during the whole survey periods at 5 min intervals with TidBit® temperature loggers (Onset Computer Corporation, Bourne, USA).

Pigments

The leaf material previously used for *in situ* measurements of PAM-fluorescence was cleaned of sediment and visible epiphytes, placed in vials, kept dark, brought back to shore within 15 and 25 min and frozen in liquid nitrogen. Samples were stored until analysis at −80°C. Pigment analysis was conducted following the protocol described in Kohlmeier *et al.* (2014). In short, the lyophilized samples were ground using an homogenizer (Fast Prep, Lysing Matrix D, MP Biomedical, Germany) and extracted in 90% acetone for 24 h at 4°C. Analyses were performed by reversed phase HPLC, applying a gradient according to Wright *et al.* (1991). Peaks were detected at 440 nm, and identified and quantified by co-chromatography with pigment standards for chlorophyll a, chlorophyll b, lutein, antheraxanthin (Ax), zeaxanthin (Zx), violaxanthin (Vx), neoxanthin and β-carotene (DHI Lab Products, Hørsholm, Denmark). All extraction procedures were conducted in a low-light environment.

The de-epoxidation state was calculated according to the formula

$$DPS = \frac{Zx + 0.5Ax}{Vx + Ax + Zx}$$

(Thayer & Björkman, 1990).

Although we endeavoured to minimize the time between sampling and freezing of the pigment samples, some epoxidation may have taken place before freezing and thus DPS *in situ* may have been higher.

Statistics

Statistical analyses were performed using R (Version 2.14.0) and R Commander (1.8–4). One-way analysis of variance (ANOVA) followed by Tukey post-hoc tests were performed on pooled pigment data and on selected data from the daily cycles (see below). Where data did not meet the assumptions of normality and variance homogeneity, data transformations

were conducted prior to analysis. In cases where data transformations did not lead to normal distribution and/or homogeneity of data, a non-parametric test (Kruskal–Wallis) was performed on un-transformed data, followed by non-parametric post-hoc tests (Wilcoxon with Bonferroni correction).

For NZ_HT there were two sampling times around mid-day and these results were pooled for subsequent analyses. In order to facilitate direct comparisons between the two sites and the two contrasting tidal settings, selected time points within the daily cycles were analysed. To compare the noon LT and HT settings we analysed the New Zealand (NZ) data. To compare the two study areas, the differences between German (GER) HT and NZ_HT were analysed. We could not include the GER_LT data, due to unfavourable weather conditions on the sampling day.

RESULTS

Daily fluence rates during the sampling period were comparable at both sites (~10% lower at the GER site, see Table 1), despite the different geographic latitudes, with longer days at the GER site, but higher maximum irradiance at the NZ location. Maximum incident irradiance at the GER site was 1680 and 1820 μmol photons $m^{-2} s^{-1}$ for HT and LT, respectively. At the NZ site the maximum incident irradiance was 2220 and 2250 μmol photons $m^{-2} s^{-1}$ for HT and LT, respectively. Maximum *in situ* irradiance was comparable (between 1300 and 1500 μmol photons $m^{-2} s^{-1}$) for both GER surveys and the NZ_HT survey, but was nearly twice as high for NZ_LT (~2900 μmol photons $m^{-2} s^{-1}$). On the day of the GER_LT survey the light/weather conditions were unfavourable, with rapidly changing incident light (due to clouds), markedly lower daily fluence (40 mol photons $m^{-2} d^{-1}$) and low maximum *in situ* irradiance of PAR (Table 1, Figure 1).

Mean *in situ* temperatures were between 20 and 22°C at both sites and for all four surveys, with comparable minimum temperatures and slightly higher maximum temperatures at the NZ site (Table 1). Submersion times at the study sites ranged between 4 and 6 h, depending on the monthly tidal status and the location within the seagrass meadow (see Table 1).

In all surveys the same general patterns were observed. The effective quantum yield (ΔF/Fm') decreased with increasing light and it increased again when the light was decreasing (Figures 1C, D and 2C, D). The optimum quantum yield of PSII, Fv/Fm (as a measure of the intrinsic photochemical quantum yield of PS II) decreased slightly in some cases, but it increased again at the last measurement (in the evening) (Figures 1C, D and 2C, D). Both the NPQ and the DPS were increasing and decreasing in parallel with incident light conditions (Figures 1E, F and 2E, F). The pigments of the xanthophyll cycle also showed similar responses in all four surveys: Vx decreased with increasing light and increased again in the evening (Figures 1G, and 2G, H). Ax had intermediate levels and fluctuated only slightly. Zx increased strongly with increasing light conditions, but also decreased again in the evening. A near complete conversion indicated by high levels of Zx, intermediate levels of Ax and low levels of Vx occurred when the irradiance was high (Figures 1A, B, G, H and 2A, B, G, H). Following this, there was a high

DPS with levels of up to 0.8 with high irradiance conditions (Figures 1E, F and 2E, F).

The unfavourable weather conditions during the GER_LT meant that a restricted number of tidal conditions could be analysed statistically. For the comparison of NZ_HT and NZ_LT the data showed one clear peak or one minimum in the case of the LT study (a clear peak of irradiance, DPS and NPQ and Zx and a minimum for ΔF/Fm', Fv/Fm and also for Vx), while the data of the HT study showed a bimodal pattern. There were statistically significant differences for PAR (P<0.001), ΔF/Fm' ($P < 0.001$), DPS ($P < 0.0001$) and for Vx ($P < 0.0001$), Ax ($P = 0.002$) and Zx ($P < 0.001$) (and lutein, $P = 0.014$; data not shown). There were no statistically significant differences for Fv/Fm or NPQ. In a second analysis (for comparison of study areas), NZ_HT and GER_HT were compared and here we found statistically significant differences for PAR ($P < 0.001$), Fv/Fm ($P = 0.002$) and lutein ($P = 0.001$; data not shown). NPQ, ΔF/Fm' and pigment concentrations were not statistically different.

Data from each survey were pooled to examine differences in mean pigment content. Absolute pigment concentrations and total pigments (Table 2) were significantly higher at the GER site than at the NZ site ($P < 0.01$). No statistically significant differences were observed between the two tidal settings within the GER data set. In the comparison of the NZ data set, differences were found for the absolute values (μg pigment per mg dry weight): the XC pool (Vx + Ax + Zx) was significantly higher for LT ($P < 0.001$). Again there were no differences within the GER data set for the relative values (pigments per Chl a). The NZ data showed that β-carotene and lutein were significantly higher when LT was at noon ($P < 0.001$). And, as was also seen for the absolute values, higher levels of the XC pool (Vx + Ax + Zx) per Chl a were found ($P < 0.001$). Also the ratio of chlorophylls to carotenoids was lower when LT was at noon ($P < 0.001$).

In summary, there was a high activity of the XC with a near complete conversion of Vx to Zx (Figures 1G, H and 2G, H). We have seen similar patterns in both species with rapidly changing pigment compositions following the daily fluctuations in incident PAR (Figures 1A, B and 2A, B). For the NZ seagrass, differences were also observed in pigment concentrations (Table 2). Especially the XC pool sizes changed with tidal settings: when LT occurred at noon, there was a larger XC pool compared with when HT occurred at noon.

DISCUSSION

The results of our study are an illustrative example of the significance of the XC in a highly dynamic light environment. Under most conditions only low or intermediate levels of Ax were observed, indicating a complete de-epoxidation to Zx when levels of PAR were high. This is different to other studies on the XC in seagrasses (Ralph *et al.*, 2002; Collier *et al.*, 2008; García-Sánchez *et al.*, 2012) that found only an incomplete conversion, related to an accumulation of Ax and only very low levels of Zx. This may have been due to the fact that our study was conducted in an intertidal habitat using *in situ* light conditions (in contrast to subtidal habitat or artificial light in other studies).

The dynamic regulation of the XC indicates its significant role in photoprotection. Values in the literature for the light saturation point (Ek) for *Zostera* are very variable; they range
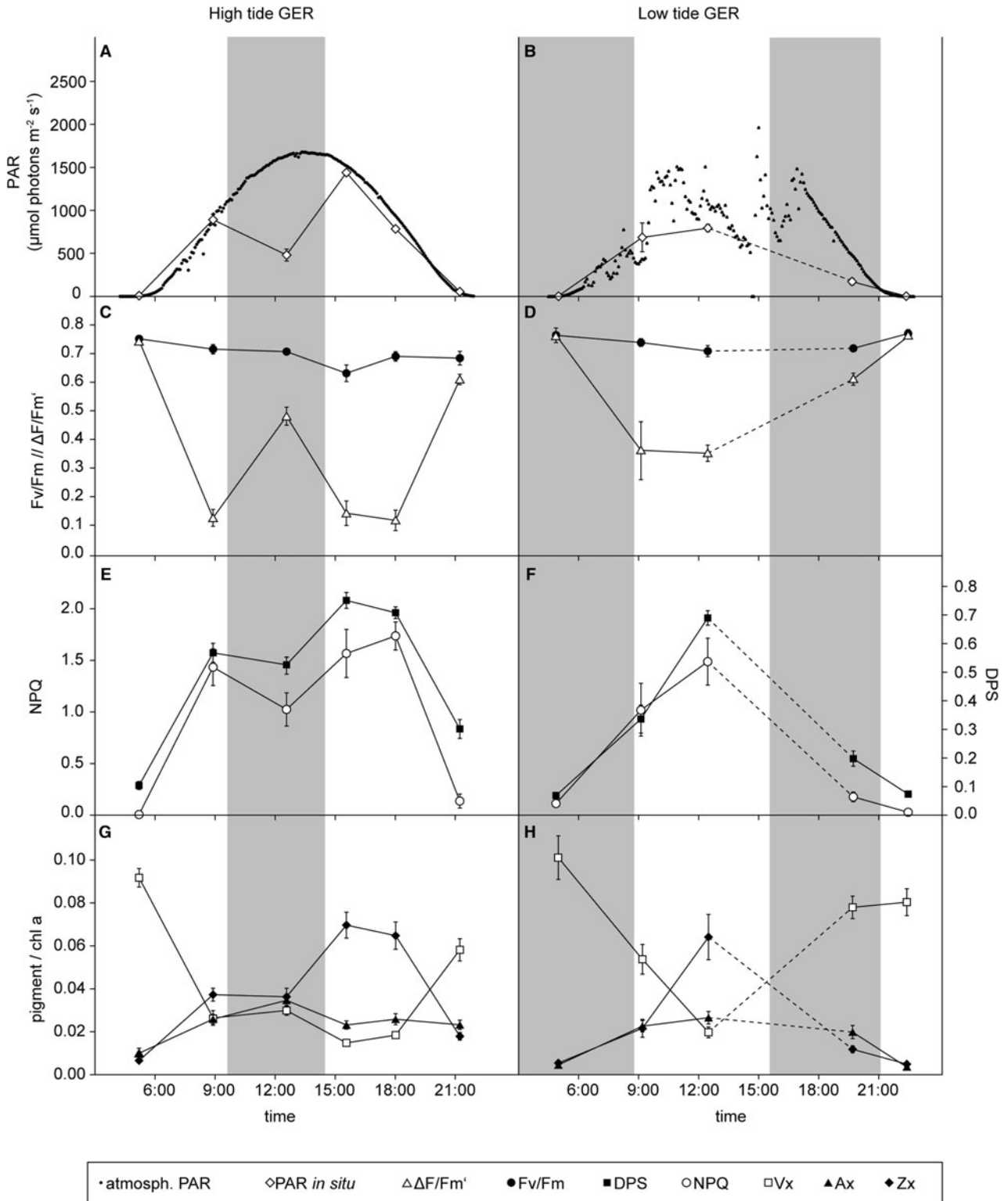
Fig. 1. German site – atmospheric and *in situ* irradiance (as photosynthetically active radiation – PAR) at noon HT (A) and at noon LT (B), effective quantum yield ($\Delta F/F_m'$) and optimum quantum yield (F_v/F_m) of PSII at noon HT (C) and noon LT (D); non-photochemical quenching (NPQ) and de-epoxidation state (DPS) at noon HT (E) and at noon LT (F); xanthophyll pigment (violaxanthin Vx, antheraxanthin Ax, zeaxanthin Zx) concentrations (as ratio per Chl a) at noon HT (G) and at noon LT (H); N = 3 at pre-dawn and post-sunset measurements of the LT survey, all others N = 9, except atmospheric PAR: $n = 1$; error bars represent $\pm$ 1 SE. The grey area indicates times when the sites were submerged.

from 80–900 μmol photons m^{-2} s^{-1} (Leuschner & Rees, 1993; Vermaat, 1997; Ralph *et al.*, 2002; Schwarz, 2004). Although, we did not conduct photosynthesis *vs* irradiance curves, our results confirm high light adaptation for both *Zostera* species,

as full operation of the XC was only observed under PAR irradiances above ∼900 μmol photons m^{-2} s^{-1}. Maximum quantum yield (F_v/F_m) dropped only to values of just below 0.5 and at sunset the F_v/F_m had recovered completely, giving
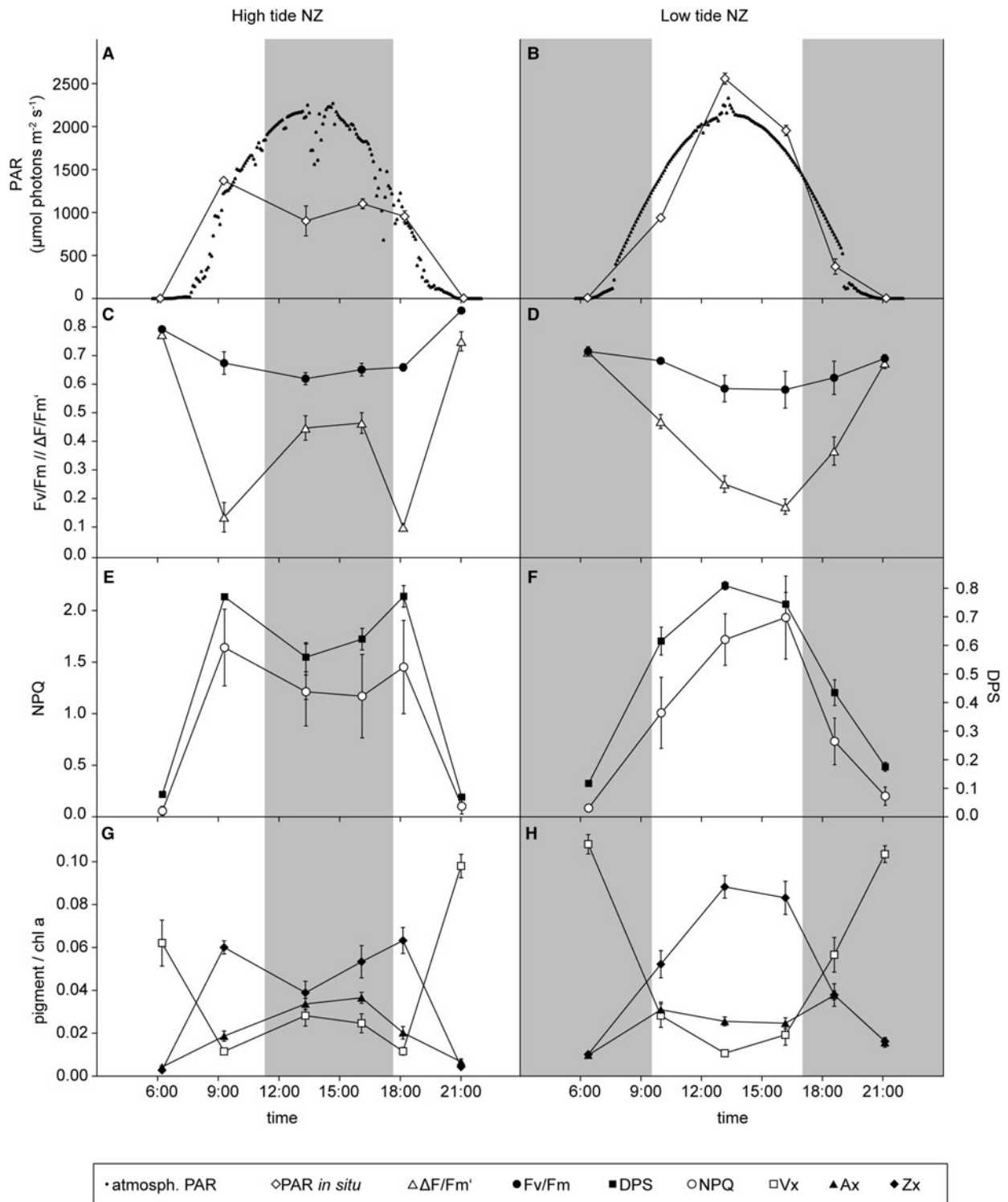
Fig. 2. New Zealand site – atmospheric and *in situ* irradiance (as photosynthetically active radiation – PAR) at noon HT (A) and at noon LT (B), effective quantum yield ($\Delta F/Fm'$) and optimum quantum yield (Fv/Fm) of PSII at noon HT (C) and at noon LT (D); non-photochemical quenching (NPQ) and de-epoxidation state (DPS) at noon HT (E) and at noon LT (F); xanthophyll pigment (violaxanthin Vx, antheraxanthin Ax, zeaxanthin Zx) concentrations (as ratio per Chl a) at noon HT (G) and at noon LT (H); N = 9 except atmospheric PAR: N = 1; error bars represent ± 1 SE. The grey area indicates times when the sites were submerged.

no indication of prolonged photoinactivation or photodamage for either species, even at very high PAR. This is further supported by very high NPQ values and an active XC, indicating effective photoprotection mechanisms (Demmig-Adams, 1998; Goss & Jakob, 2010).

A dynamic adjustment of the XC pool sizes was also observed. When LT coincided with noon, seagrass leaves at the New Zealand site contained significantly higher concentrations of XC pigments. This is in accordance with studies that have reported higher pool sizes in terrestrial sun plants

Table 2. Results of pigment analysis, with the data representing mean values $\pm$ standard errors (GER_HT: N = 54; GER_LT: N = 33; NZ_HT: N = 49; NZ_LT: N = 54). For each row of data different letters (a, b, c) indicate significant differences ($P < 0.05$) among the four surveys.

	P	GER_HT	GER_LT	NZ_HT	NZ_LT
Absolute (in $\mu g\ mg^{-1}$ DW)					
Chlorophyll a	<0.001	4.42 $\pm$ 0.180 a	4.29 $\pm$ 0.247 a	3.09 $\pm$ 0.117 b	3.11 $\pm$ 0.131 b
Chlorophyll b	<0.001	1.801 $\pm$ 0.076 a	1.736 $\pm$ 0.102 a	1.025 $\pm$ 0.041 b	1.019 $\pm$ 0.043 b
β-Carotene	<0.001	0.204 $\pm$ 0.009 a	0.204 $\pm$ 0.011 a	0.142 $\pm$ 0.006 b	0.167 $\pm$ 0.007 b
Lutein	<0.001	0.510 $\pm$ 0.019 a	0.477 $\pm$ 0.029 a	0.286 $\pm$ 0.014 b	0.320 $\pm$ 0.014 b
Neoxanthin	<0.001	0.170 $\pm$ 0.008 a	0.168 $\pm$ 0.009 a	0.117 $\pm$ 0.007 b	0.115 $\pm$ 0.005 b
Vx + Ax + Zx	<0.001	0.446 $\pm$ 0.018 a	0.440 $\pm$ 0.025 a	0.301 $\pm$ 0.014 b	0.395 $\pm$ 0.019 a
Total pigments	<0.001	7.549 $\pm$ 0.303 a	7.310 $\pm$ 0.407 a	4.964 $\pm$ 0.187 b	5.121 $\pm$ 0.216 b
Relative					
Chl b/Chl a	<0.001	0.406 $\pm$ 0.003 a	0.405 $\pm$ 0.003 a	0.331 $\pm$ 0.002 b	0.328 $\pm$ 0.002 b
β-Carotene/Chl a	<0.001	0.047 $\pm$ 0.001 a	0.048 $\pm$ 0.001 a	0.046 $\pm$ 0.001 a	0.054 $\pm$ 0.001 b
Lutein/ Chl a	<0.001	0.117 $\pm$ 0.002 a	0.114 $\pm$ 0.004 a	0.093 $\pm$ 0.003 b	0.103 $\pm$ 0.001 c
Neoxanthin/Chl a	0.0014	0.039 $\pm$ 0.001 a	0.040 $\pm$ 0.001 a	0.038 $\pm$ 0.002 b	0.037 $\pm$ 0.000 b
Vx + Ax + Zx/Chl a	<0.001	0.102 $\pm$ 0.002 a	0.105 $\pm$ 0.004 a	0.099 $\pm$ 0.004 a	0.127 $\pm$ 0.003 b
Chl/Carot	<0.001	4.686 $\pm$ 0.080 a	4.643 $\pm$ 0.097 a	5.052 $\pm$ 0.186 a	4.179 $\pm$ 0.058 b

Chl = Chlorophylls (chlorophyll a and chlorophyll b); Carot = carotenoids (β-carotene, lutein, neoxanthin, violaxanthin (Vx), antheraxanthin (Ax), zeaxanthin (Zx)).

than in shade plants (Thayer & Björkman, 1990; Königer et al., 1995; Demmig-Adams, 1998) or even sun and shade leaves (Björkman & Demmig-Adams, 1994). Other studies have shown that among closely related species there can be strong differences in the type and size of the XC (Matsubara et al., 2009; Stamenković et al., 2014). Within one species or even one specimen, the XC pool size can increase significantly within one day (Björkman & Demmig-Adams, 1994) to 10 days (Eskling & Åkerlund, 1998). In the case of Zostera, our results clearly show that XC pool sizes depended on the tidal setting (LT or HT at noon). For subtidal seagrass in tank experiments, Ralph et al. (2002) found that the XC pool sizes may increase within one day. In our study we have seen an increase of the XC pool size within one week in the New Zealand data set, correlating with increasing fluence in accordance with the timing of the tides.

Pigments other than those of the XC did not show any clear cycling (data not shown), indicating that alternative energy dissipation pathways, such as the lutein-epoxide cycle that operates in some plants (García-Plazaola et al., 2002; Matsubara et al., 2009), was probably not active in the two species examined. However, the levels of lutein were significantly higher in the NZ_LT plants, indicating an important role for lutein in photoprotection (Jahns & Holzwarth, 2012) as also found for the seagrass Cymodocea nodosa (García-Sánchez et al., 2012).

For the first time in intertidal seagrasses, our study has demonstrated the high activity of the XC and a near complete conversion from Vx to Zx. We observed similar patterns in both species with rapidly changing pigment compositions following the daily fluctuations in incident PAR. Moreover, at the NZ site, differences were seen in pigment concentrations especially those of the XC between the different tidal settings. Our results therefore corroborate our hypothesis, that intertidal seagrasses dynamically adjust the XC as an efficient photoprotective mechanism in response to highly fluctuating light conditions. Both the high activity of the XC and the dynamic adjustment of XC pool size to tidal conditions appear to be important adaptive features of intertidal seagrass, enabling it to thrive in an extremely variable light environment. Our results underline the known high-light adaptation

of seagrasses and help explain the success of the genus Zostera in intertidal habitats. In the context of the global decline of seagrass meadows and increased sedimentation and eutrophication, further work is needed on the impact of light reduction on seagrasses.

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