Physiological characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability

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ABSTRACT: Physiological plasticity has the capacity to prolong seagrass survival under reduced light conditions. However, when light is attenuated across a depth gradient, the relative importance of this over other mechanisms for tolerating long-term light reduction (such as morphological adjustments and shoot loss) has been questioned. This study aimed to describe a number of the physiological characteristics of *Posidonia sinuosa* Cambridge et Kuo along a depth-related gradient of light availability (1.6 to 9.0 m depth) and infer how these characteristics are important for the long-term maintenance of the meadow. Rapid light curve-derived parameters, light harvesting pigments, photoprotective pigments and nutrient and carbohydrate concentrations exhibited few differences among depth strata, but showed some (albeit limited) adjustment between the seasons. It was inferred that some physiological plasticity is possible in *P. sinuosa* but that differences in the depth-related gradient of long-term light availability were not sufficient to induce physiological differences, even at the depth limit. Shoot density reductions, which reduce the effects of self-shading, possibly offset depth-related light reductions. Because the physiological characteristics we examined did not explain the adaptations by *P. sinuosa* to the long-term gradient of light availability, they may not be useful indicators of long-term light reduction.

KEY WORDS: Light reduction · Depth gradient · Photosynthetic responses · Pigments · Carbohydrates · Self-shading

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INTRODUCTION

Seagrasses are exposed to a highly variable light environment owing to the range of depths they colonise (Duarte 1991), seasonal cycles of irradiance (Alcoverro et al. 2001a) and fluctuating water quality (Longstaff & Dennison 1999). Maximisation of light utilisation through a number of physiological changes can lower the compensation irradiance, or the minimum light required to sustain plants over annual cycles, and can prolong survival under conditions of reduced light availability (Dennison & Alberte 1985). If these changes are insufficient to maintain a positive

carbon balance, utilisation of carbohydrate reserves provides an additional source of carbon with which to prolong tolerance to reduced light (Burke et al. 1996, Lee & Dunton 1997).

During periods of light reduction, photosynthetic adjustments can improve light capture and conversion to chemical energy. For example, the ratio of chlorophyll *a* to chlorophyll *b* (chl *a*:*b* ratio; Longstaff & Dennison 1999) can be reduced, as can maximum photosynthetic rate and saturating irradiance (Dennison & Alberte 1985, Ruiz & Romero 2001, Ralph & Gademann 2005), while chlorophyll concentration can be increased (Dennison & Alberte 1985, Abal et al. 1994,

Lee & Dunton 1997, Longstaff & Dennison 1999), as can photosynthetic efficiency (α; Durako et al. 2003). Responses of the photoprotective xanthophyll pigments in seagrasses to changes in light availability are not as well known (with the exception of Flanigan & Critchley 1996, Ralph et al. 2002). In response to increased light, photosynthetic down-regulation diverts energy to the xanthophyll cycle as a mechanism to avoid photosystem damage (Demmig-Adams et al. 1996). De-epoxidation of the xanthophyll violaxanthin to antheraxanthin and then zeaxanthin removes excess irradiance from the photosystem as heat (Demmig-Adams et al. 1996). The diversion of energy away from photosynthesis leads to a reduction in the maximum quantum yield of Photosystem II (PSII; variable fluorescence:maximum fluorescence ratio, F_v:F_m) and, as such, increases in xanthophylls cycle products are associated with reductions in the F_v:F_m ratio and increases in non-photochemical quenching (Demmig-Adams et al. 1996, Ralph et al. 2002). Given their role in the quenching of excess irradiance, seagrass plants growing in low-light environments should maintain lower concentrations of xanthophyll pigments than plants in high-light environments.

Other physiological responses to light reduction include a reduced $\delta^{13}C$ signature of leaves owing to higher selectivity against ^{13}C when growth is reduced, and increased tissue nutrient and amino acid concentration (Abal et al. 1994, Longstaff & Dennison 1999). Reductions in carbohydrate concentrations in the leaves and rhizome are often detected during shading because carbohydrates are remobilised to sustain respiratory and growth requirements (Burke et al. 1996, Lee & Dunton 1997, Kraemer & Hanisak 2000).

Physiological responses precede morphological adjustments and shoot loss (Longstaff & Dennison 1999) and are seen as short-term responses to light availability. As such, physiological characteristics may be useful early warning monitoring tools of a light-related impact on seagrass meadows. A depth-related gradient of long-term light availability represents a steady and persistent light reduction with depth, and therefore provides an opportunity to identify how these physiological characteristics enable seagrasses to persist under long-term sub-optimal light conditions.

The ability to acclimate to depth-related light reduction should confer an important advantage to all species that occupy such gradients, and a number of species have demonstrated physiological adaptation with depth (Dennison & Alberte 1985, Olesen et al. 2002, Schwarz & Hellblom 2002). However, the importance of physiological responses of *Posidonia sinuosa* and the similarly slow-growing, meadow-forming *Posidonia oceanica* have been questioned (Masini et al. 1995, Alcoverro et al. 1998, Olesen et al. 2002). Mechanisms

that optimise light capture and carbon fixation/utilisation operate at a number of levels, including meadow-scale (shoot density), shoot-scale (morphology) and leaf-scale (physiological changes). It has been proposed that in such meadow-forming species the physiological adaptations are not utilised along depth gradients, and that meadow-scale changes are instead more important (Olesen et al. 2002). These physiological responses can, however, operate in these species in response to shading (Ruiz & Romero 2001). In an earlier study (Collier et al. 2007) we described the morphology and growth of the meadow-forming seagrass *P. sinuosa* along a depth-related gradient of light extending from 1.6 m to the depth limit at 9.0 m. As with *P. oceania* (Olesen et al. 2002), shoot-density differences dominated, which should have reduced respiratory load and self-shading at deeper sites. Shoot density reduction enables greater light penetration through the canopy and was therefore considered a meadow-scale adjustment. Leaf growth was the same among all depth strata in summer, even at the depth limit, suggesting that sufficient physiological and morphological adjustments had been made to enable optimal leaf growth at the shoot level. While meadow-scale shoot loss appeared to dominate, physiological adaptation to the depth-related light gradient should have also optimised shoot performance. However, the meadow-scale responses may have been sufficient so that physiological responses were not necessary over the long-term.

Before using these physiological characteristics as monitoring tools, it is crucial that their response under conditions of long-term light reduction be identified in order to provide sufficient background for their interpretation and use in management. The aim of this research was to characterise a number of physiological characteristics of the meadow-forming seagrass *Posidonia sinuosa* that have previously been reported as responsive to light availability, with a particular emphasis given near the depth limit where light availability is sub-optimal.

MATERIALS AND METHODS

Study locations. The study was conducted in Cockburn Sound (CS) and Warnbro Sound (WS) in southwestern Australia, where monospecific stands of the locally dominant species *Posidonia sinuosa* grow on steep, sub-tidal depth gradients ranging from approx. 1 to 9 m depth. CS was located on north-east Garden Island, which is protected from the dominant southwesterly wind and swell. Sampling at CS was carried out in winter (June 2002) and summer (January to February 2003) at 6 depths: 1.6, 4.0, 5.7, 6.5, 8.3 and 9.0 m (lowest astronomical tide), which are herein referred to

as sites CS1, CS2, CS3, CS4, CS5 and CS6 respectively. Sampling effort was concentrated nearer to the depth limit, where the greatest differences were expected owing to the approach of compensating light levels. The deepest 5 sites were within close proximity to each other on a steep slope leading to a basin (32° 09′ 37″ S, 115° 40′ 47″ E), while the shallowest site was located approx. 800 m away, closer to Garden Island (32°09′36″S, 115°40′16.″E). The location of sampling in WS was Safety Bay, located in the north-east of the sound, which is more exposed to south-westerly winds. Sampling at WS was carried out in summer only (January to February 2003) at the same 6 depths, and sites are herein referred to as WS1, WS2, WS3, WS4, WS5 and WS6 respectively. Again, the deepest 5 sites were located within close proximity (32° 18′ 57″ S, 115° 42′ 52″ E), while the shallowest site was located approx. 150 m away, closer to the mainland shore (32° 18′ 54″ S, 115° 42′ 50″ E). The sites were sampled for a variety of physiological parameters.

Sampling and analysis. *Carbohydrates (soluble sugars and starch):* Six replicate rhizomes and shoots were randomly collected from each depth, placed in plastic bags and immediately stored on ice prior to storage at –18°C. Samples were later analysed for soluble sugar concentration according to the method of Dubois et al. (1956). For rhizome material, 0.1 to 0.2 g from a segment nearer to the terminal shoot (Internode 4) and an older segment (Internode 10) were analysed separately (3-way ANOVA, Season × Depth × Segment). Starch levels showed no difference between these segments and so data were pooled for final statistical analysis, whereas sugar data for both segments were presented separately (Segment × Location: p < 0.01). All analyses on leaves were carried out on the youngest fully mature leaf (usually Leaf 1 or 2). Rhizome material was finely ground with acid-washed sand, and leaf material was ground in liquid nitrogen because grinding in sand did not effectively macerate leaves. Sugars were twice extracted in ethanol at 60°C for 20 min. A trial determined that this extraction regime was as effective as 3 shorter extractions. The extract was analysed for sugar content using the phenol-sulphuric acid colorimetric method. Samples were gelatinized at 100°C for 15 min and then solubilised in 70% perchloric acid. Starch content was then analysed using the phenol-sulphuric acid colorimetric method (Quarmby & Allen 1989).

Nutrient content and $\delta^{13}C$: Leaf samples for $\delta^{13}C$ and nutrient content (%N and %C) were randomly selected from the pre-scraped above-ground biomass samples. Only the youngest fully mature leaf was selected. Dried samples were finely ground in a mixer mill (Retsch MM 200) and analysed for $\delta^{13}C$, %N and %C carbon using a mass spectrometer (ANCA-NT Europa Scientific, Crewe) interfaced with a 20-20 isotope

ratio mass spectrometer (Europa Scientific, Crewe). Isotope signatures were determined by comparison with a working laboratory reference material, which had been previously calibrated against various International Atomic Energy Agency (IAEA) or National Institute of Standards and Technology (NIST) standard reference materials with a precision of <0.1‰. All $\delta^{13}C$ are traceable to the internationally accepted Vienna PeeDee Belemnite (VPDB; for ^{13}C) scales.

Pigments: At each depth, 6 replicate shoots were randomly collected and placed immediately on ice in the dark, prior to storage at –18°C. In a darkened room, the youngest fully mature leaf was scraped free of epiphytes, finely chopped and extracted in N,N-dimethylformamide at 4°C for 72 h in darkness. Spectrophotometric determination of chlorophyll concentrations were performed on the extracts according to the equations of Wellburn (1994).

Additional shoots were collected for analysis of accessory pigments. Whole shoots were collected, wrapped in foil whilst under water and placed in liquid nitrogen upon return to the surface. Samples were stored at –86°C. In a darkened room, a whole mature leaf, including all material emerging from the leaf sheath to the leaf tip, was scraped free of epiphytes. Leaf material was ground in a cold, glass mortar with acid-washed sand and cold (–4°C), HPLC-grade 90% acetone. The sample was sonicated in an ice bath for 20 min, allowed to extract for 12 h at 4°C and sonicated for a further 20 min. The extract was then analysed for pigment concentration on a high performance liquid chromatograph (Waters) comprising a 600 controller, 717 plus refrigerated autosampler and a 996 photodiode array detector. Concentrations of pigments were determined from a combination of standards (Sigma) and from purified pigments isolated from algal cultures.

PAM fluorometry. Photosynthetic characteristics were measured in 6 replicate leaves using a Diving-pulse amplitude modulated (PAM) fluorometer (Walz). The sites were measured in a randomised order between 10:00 and 14:00 h on cloudless days to capture the midday period of maximum electron transport (Ralph & Gademann 1999, Campbell et al. 2003). No data were collected for WS5. All measurements were made on the youngest mature leaf, 15 cm from the top of the leaf sheath (lower-mid section), which is the most mature section that was consistently epiphyte free and where the highest effective quantum yield is expected (Durako & Kunzelman 2002). The leaf was held 5 mm from the tip of the fibre-optic cable in a dark-adaptation clip. Rapid light curves (RLCs) were initiated immediately after the clip was fitted using the internal step-wise program, which measures effective quantum yield [$\phi_{PSII} = (F_m' - F)/F_m'$], where F is background fluorescence and F_m' is maximum fluorescence

yield after providing a 0.8 s saturating pulse at each irradiance step. Preliminary data indicated that the appropriate range of irradiance steps to cover saturating and non-saturating irradiance throughout the depth range was 1 to 900 µmol m^{-2} s^{-1}.

Electron transport rate (ETR) was calculated as the product of the effective quantum yield and the irradiance provided by the internal halogen lamp and a standard absorptance factor (AF) (ϕ_{PSII} × photosynthetic photon flux density [PPFD] × 0.5 × 0.84). A standard absorptance factor was used here because the best means to directly measure absorptance continues to be debated among the scientific community. As such, all values are considered relative ETR (rETR) because leaf absorptance was not directly measured (Durako et al. 2003). rETR was plotted against irradiance, and the photosynthetic characteristics (photosynthetic efficiency in light-limiting conditions, α; maximum relative electron transport rate, rETR$_{max}$; and saturating irradiance, E_k) were calculated by fitting the hyperbolic tangent model of Jassby & Platt (1976) using Sigmaplot 2001 (version 7.0, SPSS). For CS only, where sufficient light data were available (Collier et al. 2007), hours of photosynthetically saturating irradiance (H_{sat}) were calculated from E_k and the measured light data at each depth.

Following measurement of the RLCs, leaves were dark-adapted for 10 min as recommended by Beer et al. (2001) in order to measure 'maximum' or 'potential' quantum yield of PSII $(F_m - F_0)/F_m = F_v/F_m$). Maximum quantum yield was then determined by measurement of background fluorescence (F_0) and maximum fluorescence (F_m) of dark-adapted leaves after providing a 0.8 s saturating pulse. Quenching coefficients were calculated according to the equations $(F_m - F_m')/F_m'$ for non-photochemical quenching (NPQ), and $(F_m' - F)/(F_m' - F_0)$ for photochemical quenching (qP).

Statistical analysis. All data were tested for normality and homogeneity of variances (Levene's median test). If either assumption failed, data were log or square-root transformed to achieve the highest Levene's score. If transformation still did not satisfy assumptions of ANOVA, the p value was set to 0.01 to minimise the risk of a Type I error (Underwood 1997). For conforming data, significance was determined at p < 0.05. Significant effects of the season and site (depth) were tested using a 2-way ANOVA with site and season as random factors. Location and site differences were tested using a 2-way ANOVA (Location × Site) for all data collected in summer at CS and WS. Season and location were analysed as fixed factors, while site was a random factor. Tukey's post-hoc analysis was used to further determine differences between the sites sampled, and a *t*-test was used to determine differences at the same depth between season and location.

RESULTS

Carbohydrates

Total soluble sugars in the leaves ranged from 4 to 12 mg g^{-1} fresh wt. There was no clear trend in sugar concentrations in leaves with increasing depth (Fig. 1A,B), though there were significant Site × Season and Site × Location interactions (p < 0.05; Table 1). Sugar concentrations did not differ among sites at CS in winter, while the lowest concentration was recorded from the 4.0 m site in summer. At WS, which was only sampled in summer, the deepest 2 sites had a lower leaf sugar concentration than the shallowest. This was not the case at CS.

Starch concentrations in the leaves were about 2 to 3 times greater than those of soluble sugars (17 to 32 mg g^{-1} fresh wt) (Fig. 1D,E). Like sugars, starch in leaves showed no trend with depth, but at CS there was a Site × Season interaction (p < 0.01; Table 1). In winter, the highest starch concentration occurred at CS3 and the lowest at CS1. In summer, CS5 and CS6 exhibited the lowest starch concentrations. At all sites except CS5, starch concentrations were higher in summer than in winter.

In the rhizomes, total soluble sugar concentrations ranged from 18 to 93 mg g^{-1} fresh wt, and at CS these concentrations were significantly lower in winter than in summer (p < 0.001; Table 1); however, there was no significant effect of site (Fig. 1A). When analysed according to location, a significant Segment × Location interaction was observed (p < 0.01; Table 1). The data are therefore separately presented for the younger (Internode 4) and older rhizome segment (Internode 10) (Fig. 1B,C). At both locations, sugar concentration was significantly higher in the older segment (Internode 10) than in the younger segment (Internode 4) (CS: p < 0.05; WS: p < 0.001). A significant effect of location on sugar concentration in the rhizomes was observed, but the difference was dependent on the site sampled (Site × Location: p < 0.05). At Sites 1 (p < 0.01), 3 and 4 (p < 0.05), sugar concentration was significantly higher at WS than CS for both segments. Starch concentration in the rhizome ranged from 4 to 12 mg g^{-1} fresh wt and was significantly higher at WS than at CS (p < 0.05), but was unaffected by the site sampled.

Nutrient content and δ^{13}C

At CS, and owing predominantly to a significant (p < 0.001) increase in %N in winter (Fig. 2A), the carbon to nitrogen ratio (C:N ratio) was significantly lower (p < 0.001; Table 1) in winter (Fig. 2B). In summer, there was a significant effect of depth on the C:N ratio and
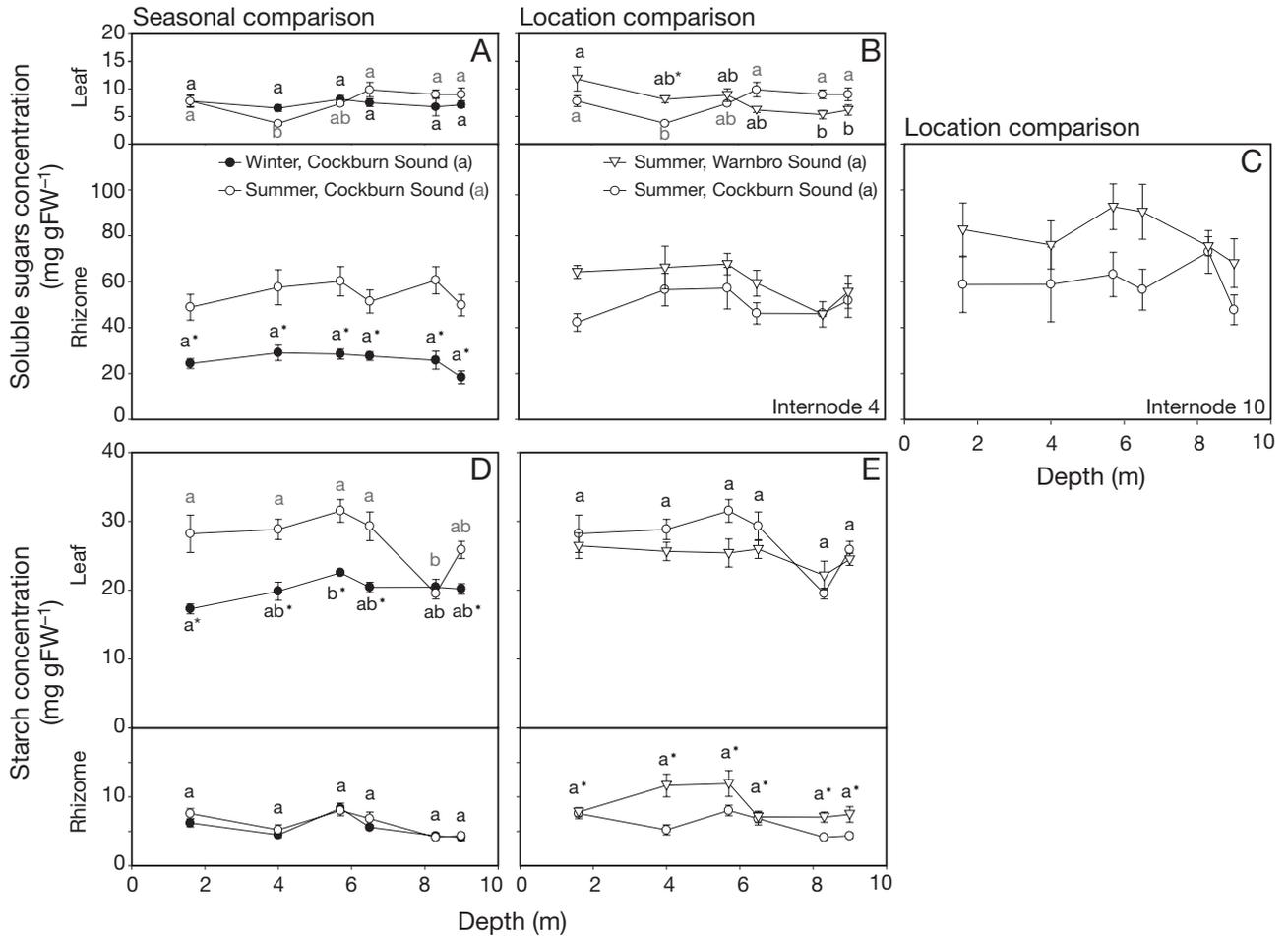
Fig. 1. *Posidonia sinuosa*. Carbohydrate concentration in leaves and rhizome at 1.6, 4.0, 5.7, 6.5, 8.3 and 9.0 m (Sites 1 to 6, respectively). Total soluble sugar concentration in (A) leaves (above axis) and rhizome (below axis) at Cockburn Sound (CS) in winter 2002 and summer 2003, (B) leaves and rhizome Internode 4 at CS and Warnbro Sound (WS) in summer 2003, and (C) rhizome Internode 10 at CS and WS in summer 2003. Starch concentration in leaves (above axis) and rhizome (below axis) at (D) CS in winter 2002 and summer 2003 and (E) CS and WS in summer 2003. Values are mean ± SE (n = 6). Sites with the same lower-case letters did not differ significantly within season or location. Differently shaded letters are independent of each other.
*p < 0.05

%N; however, this was affected by the location sampled (Site × Location: p < 0.05). At CS there was no effect of site, while at WS the deepest 3 sites had a significantly (p < 0.01) lower C:N ratio than the shallowest site. With regard to %N at WS, the only significant difference (p < 0.05) observed was that between WS1 and WS5.

The $\delta^{13}C$ content significantly increased with depth at both locations in summer (p < 0.01; Table 1). The 2 shallowest sites were significantly more depleted in the heavier carbon isotope than were the deepest 3 sites (Fig. 2F). At CS, the effect of site was influenced by the season sampled (Site × Season: p < 0.05) because there was no significant differences among the sites sampled in winter. In summer, $\delta^{13}C$ was significantly less negative than in winter at all depths.

Chlorophyll

Total chlorophyll (chl *a* + *b*) concentration was significantly affected by site (Table 2), but the site at which a difference was observed was dependent on both Season (Site × Season: p < 0.01; Table 3) and Location (Site × Location: p < 0.01). At CS in summer, total chlorophyll concentration was lower at CS1 than at CS3, while at WS the observed differences did not vary consistently with increasing depth.

The chl *a*:*b* ratio was not affected by season but was affected by Site in summer (Table 2); however, the site at which a difference was observed depended on the location sampled (Site × Location: p < 0.01). At CS the chl *a*:*b* ratio was lowest at CS1, while at WS the ratio was lowest at WS3 and highest at WS4.

Table 1. *Posidonia sinuosa*. Results of 2-way ANOVA (top) examining the effects of site and season or site and location on carbohydrate concentration, soluble sugars and starch and $\delta^{13}C$ and C:N ratios. Also shown are results of 3-way ANOVA (bottom half) examining the effects of site, rhizome internode number (4 or 10) and location or season. Data were transformed where necessary to meet the assumptions of ANOVA. ns: not significant

Seasonal test / Location test

Independent variable	df	Sugars in leaves (mg g⁻¹ fresh wt) MS	F	p	Starch in leaves (mg g⁻¹ fresh wt) MS	F	p	%N MS	F	p	C:N ratio MS	F	p	δ¹³C MS	F	p
Seasonal test																
Site	5	19.706	1.797	ns	0.12	1.25	ns	0.074	2.076	ns	31.292	1.800	ns	5.999	2.182	ns
Season	1	1.867	0.170	ns	1.50	15.80	ns	2.654	74.767	<0.001	926.762	53.318	<0.001	156.554	56.942	<0.001
Site × Season	5	10.969	2.394	<0.05	9.48	5.16	<0.001	0.036	0.993	ns	17.382	1.266	ns	2.749	2.444	<0.05
Location test																
Site	5	0.367	0.424	ns	0.96	4.07	ns	0.087	0.972	ns	63.383	1.194	ns	11.692	13.695	<0.01
Location	1	0.005	0.006	ns	0.74	3.13	ns	0.0001	0.001	ns	11.140	0.210	ns	0.035	0.041	ns
Site × Location	5	0.866	9.545	<0.05	0.24	1.40	ns	0.090	2.672	<0.05	53.098	3.302	<0.05	0.854	0.754	ns

Season, site and internode test

Independent variable	df	Seasonal test — Sugars in rhizome (mg g⁻¹ fresh wt) MS	F	p	Starch in rhizome (mg g⁻¹ fresh wt) MS	F	p	Location test — Sugars in rhizome (mg g⁻¹ fresh wt) MS	F	p	Starch in rhizome (mg g⁻¹ fresh wt) MS	F	p
Site	5	0.273	1.330	ns	58.547	8.428	ns	486.281	0.623	ns	1.133	3.485	ns
Season (or Location)	1	19.476	158.290	<0.001	8.623	2.483	ns	7553.968	16.490	<0.01	4.716	10.929	<0.05
Internode	5	1.312	5.694	ns	1.881	0.197	ns	7415.283	18.187	<0.01	0.151	3.957	ns
Site × Season (or Location)	5	0.123	0.826	ns	3.471	0.570	ns	458.979	5.320	<0.05	0.434	2.970	ns
Site × Internode	5	0.231	1.549	ns	9.546	1.571	ns	408.136	4.731	ns	0.037	0.254	ns
Internode × Season (or Location)	1	0.148	0.997	ns	9.097	1.497	ns	1119.822	12.601	<0.01	0.016	0.109	ns
Site × Season (or Location) × Internode	5	0.149	1.246	ns	6.089	1.655	ns	86.269	0.237	ns	0.146	0.992	ns

Carotenoids

Of the xanthophyll cycle pigments, there was no zeaxanthin detected. Of the others, relative violaxanthin, antheraxanthin and total xanthophyll cycle pigment concentrations were not significantly affected by site (Fig. 3). There was significantly less relative antheraxanthin in winter than in summer at CS ($p < 0.01$; Table 3). For the accessory pigments there was a significant effect of season and site on all pigments; however, no clear trend with increasing depth was apparent (Table 2). Neoxanthin and lutein concentrations were significantly lower at CS4 than CS1 in summer and winter, while β,β-carotene concentrations were lowest at CS2 and CS4.

Rapid light curves

While RLC-derived parameters demonstrated significant differences among sites, these differences did not follow a clear trend with increasing depth, varying according to location for α and both location and season for $rETR_{max}$ and E_k (Fig. 4). The most distinctive trend was at CS, where α was significantly higher in winter than in summer but did not vary among sites ($p < 0.01$; Table 3). In contrast, there was a significant effect of site on α at WS, whereby α was significantly lower at WS1 than at WS3, WS4 and WS6. For $rETR_{max}$, CS3 and CS5 had the highest rate compared with other sites in winter, while CS4 had a higher $rETR_{max}$ than did CS1 in summer. At WS, $rETR_{max}$ increased with depth and was significantly higher at WS4 and WS6 than at WS1. At CS, E_k was highest at CS3 and CS5 in winter, and at CS4 and CS5 in summer. Only CS4 differed significantly between the 2 sampling occassions. At WS, E_k was higher at WS6 than at all other depths. E_k was higher at WS6 than at CS6, but higher at CS4 than at WS4. The number of light-saturating hours of photosynthesis (H_{sat}) ranged from 11.5 to 8.6 h from the shallowest
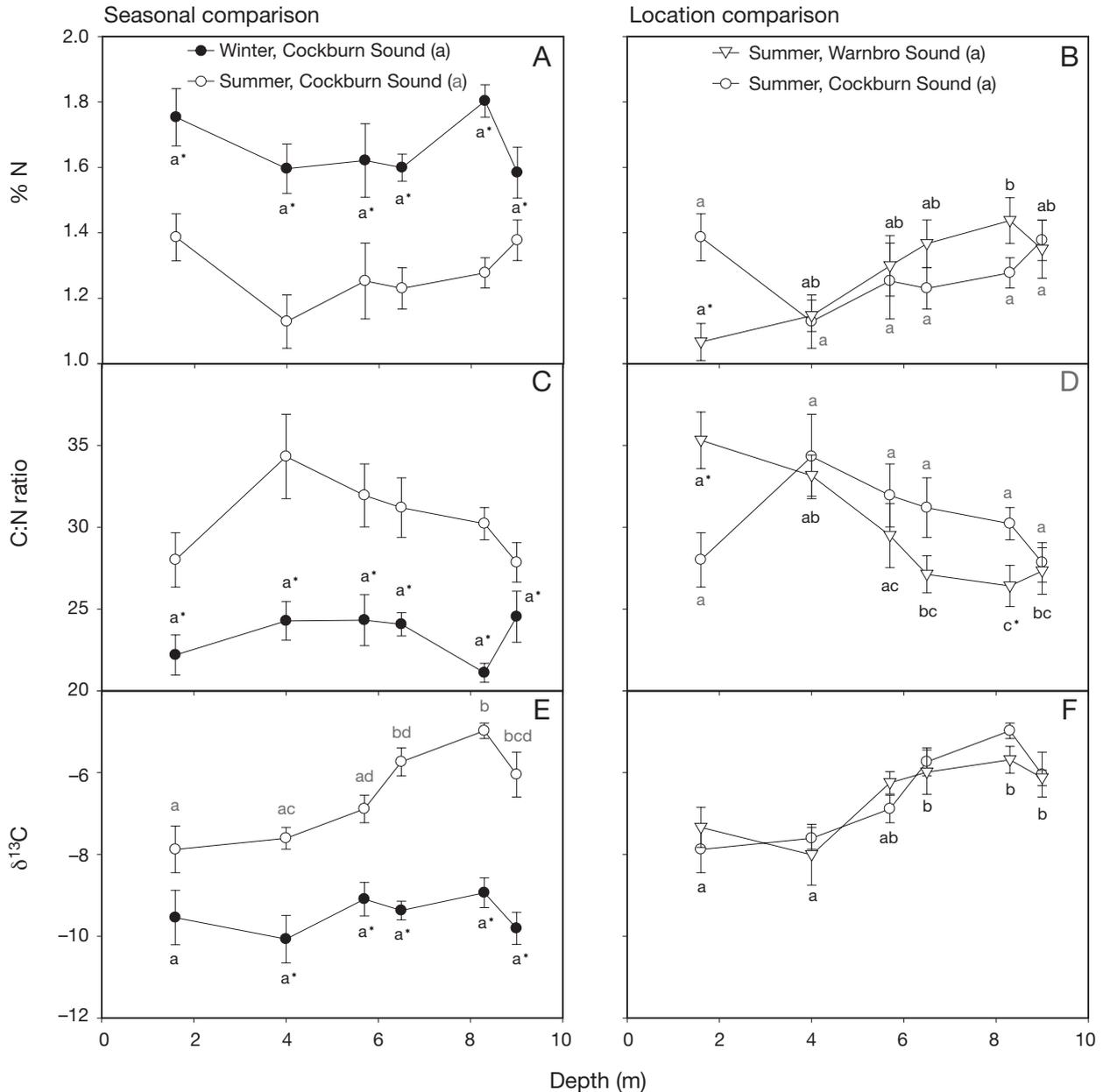
Fig. 2. *Posidonia sinuosa.* Nutrient content at Cockburn Sound (CS) in winter 2002 and summer 2003 (left), and CS and Warnbro Sound in summer 2003 (right) at 1.6, 4.0, 5.7, 6.5, 8.3 and 9.0 m (Sites 1 to 6, respectively): (A,B) %N, (C,D) C:N ratio, and (E,F) δ^{13}C. Values are mean ± SE (n = 6). Sites with the same lower-case letters did not differ significantly within season or location. Differently shaded letters are independent of each other. *p < 0.05

to the deepest sites in summer, and from 8.0 to 1.6 h from the shallowest site to CS5 in winter; E_k was higher at CS5 than at CS6 in winter (Table 4).

Maximum quantum yield and quenching analysis

There was no significant difference observed in maximum quantum yield (F_v:F_m ratio) among sites

sampled at either location (p > 0.05; Tables 3 & 5). However, the F_v:F_m ratio was significantly lower in summer (ranging from 0.73 to 0.75) than in winter (ranging from 0.82 to 0.83) (p < 0.001; Table 3).

Season did not have a significant effect on qP or NPQ. NPQ was affected by site, but the nature of the effect depended on the location sampled (Site × Location: p < 0.05). At WS there was no effect of Site, while at CS the NPQ was higher at CS3 than at CS6.

Table 2. *Posidonia sinuosa.* Pigment concentration at Cockburn Sound (CS) in winter (June 2002) and summer (January 2003) and Warnbro Sound (WS) in summer (January 2003) at 1.6, 4.0, 5.7, 6.5, 8.3 and 9.0 m (Sites 1 to 6, respectively): chl *a* + *b*, chl *a*:*b* ratio, and accessory pigments neoxanthin, lutein and β,β-carotene (data not shown for accessory pigments at WS). Parentheses: SE (n = 6). Sites with the same lower case letters did not differ significantly within season or location. *p < 0.05

Location	Season	Site					
		1	2	3	4	5	6
Chl *a*+*b* (mg g^{-1} fresh wt)							
CS	Winter	4.48 (0.18)^{a}*	4.71 (0.16)^{a}*	4.10 (0.57)^{a}	3.10 (0.23)^{a}	2.91 (0.45)^{a}	2.80 (0.34)^{a}*
	Summer^{A}	0.98 (0.8)^{a}	1.60 (0.20)^{ab}	2.48 (0.31)^{b}	1.87 (0.49)^{ab}	1.65 (0.35)^{ab}	1.39 (0.16)^{ab}
WS	Summer	2.46 (1.02)^{ac}*	0.85 (0.29)^{bc}*	2.84 (1.31)^{a}	1.27 (0.78)^{c}	1.98 (0.55)^{abc}	1.66 (0.57)^{abc}
Chl *a*:*b* ratio							
CS	Winter	1.8 (0.4)^{a}	2.0 (0.3)^{a}	2.0 (0.6)^{a}	2.4 (0.3)^{a}	2.5 (0.2)^{a}	2.6 (0.2)^{a}
	Summer^{A}	1.9 (0.1)^{a}	2.4 (0.1)^{b}	2.3 (0.3)^{b}	2.2 (0.2)^{b}	2.4 (0.1)^{b}	2.4 (0.1)^{b}
WS	Summer	2.1 (0.2)^{ab}*	2.2 (0.1)^{ab}*	2.0 (0.1)^{a}	2.5 (0.3)^{b}	2.4 (0.2)^{ab}	2.2 (0.2)^{ab}
Neoxanthin (μg g^{-1} fresh wt)							
CS	Winter	18.3 (3.3)^{a}*	14.3 (3.5)^{ab}*	25.0 (2.4)^{ab}*	11.0 (1.9)^{b}*	16.9 (3.9)^{ab}*	14.6 (3.2)^{ab}*
	Summer	17.8 (2.0)^{a}	12.5 (2.2)^{a}	12.4 (3.1)^{ab}	7.4 (2.8)^{b}	11.0 (2.4)^{ab}	11.6 (3.9)^{ab}
Lutein (μg g^{-1} fresh wt)							
CS	Winter	135.8 (10.4)^{a}*	105.3 (10.5)^{ab}*	146.6 (14.6)^{ab}*	86.5 (10.0)^{b}*	134.4 (8.1)^{a}*	119.9 (6.8)^{ab}*
	Summer	122.1 (8.6)^{a}	83.3 (13.4)^{ab}	88.7 (18.0)^{ab}	64.2 (20.4)^{b}	104.8 (7.4)^{a}	87.5 (21.4)^{ab}
β,β-carotene (μg g^{-1} fresh wt)							
CS	Winter	63.0 (4.9)^{ab}*	52.8 (4.8)^{a}*	69.6 (5.1)^{b}*	47.3 (2.1)^{a}*	66.7 (3.6)^{bc}*	57.0 (3.7)^{ac}*
	Summer	52.6 (4.2)^{ab}	49.3 (2.2)^{a}	68.8 (4.4)^{b}	47.8 (5.1)^{a}	51.3 (2.5)^{bc}	50.8 (6.3)^{ac}

^{A}Only different when analysed according to location

DISCUSSION

Photosynthetic responses to the light gradient

The photosynthetic parameters measured here indicate limited physiological differences among sites along the depth-related light gradient. Studies on other species demonstrate that chl *a* fluorescence can describe the photokinetics of seagrasses growing in different light environments (Ralph & Gademann 2005). These include increased photosynthetic efficiency (α) to maximise photosynthetic performance in the light-limiting region of the curve, a reduced maximum electron transport rate (rETR$_{max}$), a reduction in the saturating irradiance (E_k) (Schwarz & Hellblom 2002, Campbell et al. 2003, Silva & Santos 2003) and changes to maximum quantum yield (F_v:F_m ratio) (Major & Dunton 2002, Campbell et al. 2003). However, these trends are inconsistent: due to a number of field-based environmental scale-related considerations, there are as many reports of no response or a response that does not follow the light gradient as there are of an observed response (e.g. Pirc 1986, Dawes 1998, Olesen et al. 2002, Durako et al. 2003). With the exception of a lower α at WS1, the current study and previous research using oxygen evolution (Masini et al. 1995) revealed few differences in any photosynthetic parameter in *Posidonia sinuosa* among depth strata, including RLC-derived parameters, maximum quantum yield and quenching. While seasonal variation of α and photoadaptation under severe light reduction (Collier 2007) indicate that some plasticity is possible in this species, the adaptive process of the *P. sinuosa* plants to the depth-related light gradient did not include a change in these photosynthetic characteristics.

The attenuation of light with increasing water depth was just one process acting to influence the light environment at the epidermis of seagrass leaves. Additional attenuation of light occurs within dense seagrass canopies, which places the mid-lower section of leaves in an even lower light environment (Masini et al. 1995, Enríquez et al. 2002). In response, the lower sections of *Thalassia testudinum* leaves in dense canopies are shade-adapted and photosynthetic quantum yield may be considerably elevated (Durako & Kunzelman 2002, Enríquez et al. 2002), and in *Posidonia australis* the ETR$_{max}$ was lower near the leaf base (Ralph & Gademann 1999). Also, in a dense *Posidonia oceanica* meadow, leaves demonstrated shade adaptation in the lower section (10 to 15 cm from the sheath), with increased α and reduced rETR$_{max}$ and E_k compared with the mid and upper sections (Collier 2007). Measurements were made on the lower-mid region of leaves in the current study in order to minimise the influence of epiphytes (Ralph & Gademann 1999) and regions of leaf necrosis (Durako & Kunzelman 2002) near the upper leaf sections. As such, in the dense

Table 3. *Posidonia sinuosa*. Results of 2-way ANOVA examining the effects of site and season or site and location on the $F_v:F_m$ ratio and the rapid light curve parameters (photosynthetic efficiency, α; relative maximum electron transport rate, rETR$_{max}$; saturation irradiance, E_k), the quenching co-efficients (non-photochemical quenching, NPQ; photochemical quenching, qP), total chlorophyll and the chl $a:b$ ratio. Results of 2-way ANOVA examining the effects of depth and season only for the accessory pigments lutein, β,β-carotene and neoxanthin and the xanthophylls (violaxanthin, antheraxanthin and total xanthophyll concentration). Data were transformed where necessary to meet the assumptions of ANOVA. ns: not significant

Independent variable	df	$F_v:F_m$ ratio MS	F	p	α (μmol electrons m⁻² s⁻¹ [μmol photons m⁻² s⁻¹]⁻¹) MS	F	p	rETR$_{max}$ MS	F	p	E_k (μmol photons m⁻² s⁻¹) MS	F	p	Chl (mg chl $a+b$ g⁻¹ fresh wt) MS	F	p
Seasonal test																
Site	5	0.0004	1.453	ns	0.007	2.270	ns	2.264	1.584	ns	0.670	2.190	ns	0.035	0.902	ns
Season	1	0.1160	442.021	<0.001	0.084	28.854	<0.01	0.059	0.041	ns	0.254	0.833	ns	1.103	28.550	<0.01
Site × Season	5	0.0003	1.029	ns	0.003	2.323	ns	1.429	5.186	<0.001	0.306	5.186	<0.001	0.038	3.714	<0.01
Location test																
Site	5	0.0005	0.542	ns	0.009	0.450	ns	427.855	2.855	ns	0.1040	2.330	ns	0.155	1.121	ns
Location	1	0.0032	3.749	ns	0.061	3.252	ns	42.481	0.286	ns	0.0223	2.330	ns	0.015	0.106	ns
Site × Location	5	0.0009	2.292	ns	0.019	8.101	<0.001	149.061	4.486	<0.01	0.0045	0.502	<0.05	0.139	3.718	<0.01

Independent variable	df	Chl $a:b$ ratio MS	F	p	NPQ MS	F	p	qP MS	F	p	Lutein (μg g⁻¹ fresh wt) MS	F	p	β,β-carotene (μg g⁻¹ fresh wt) MS	F	p
Seasonal test																
Site	5	0.683	3.372	ns	0.097	0.532	ns	0.001	0.329	ns	4477.790	12.331	<0.01	576.776	8.733	<0.05
Season	1	0.078	0.387	ns	0.871	4.782	<0.01	0.003	1.025	ns	11094.734	29.795	<0.01	527.661	7.928	<0.05
Site × Season	5	0.203	2.747	ns	0.182	2.471	ns	0.003	1.995	ns	363.122	0.402	ns	66.046	0.688	ns
Location test																
Site	5	0.044	1.379	ns	0.078	0.247	ns	0.003	4.252	ns						
Location	1	0.006	0.199	ns	0.008	0.025	ns	0.024	35.363	<0.01						
Site × Location	5	0.032	5.193	<0.01	0.316	2.991	<0.05	0.001	0.124	ns						

Independent variable	df	Neoxanthin (μg g⁻¹ fresh wt) MS	F	p	Violaxanthin (mmol mol[chl $a+b$]⁻¹) MS	F	p	Antheraxanthin (mmol mol[chl $a+b$]⁻¹) MS	F	p	Total xanthophyll (mmol mol[chl $a+b$]⁻¹) MS	F	p
Location test													
Site	5	0.921	5.329	<0.05	21.152	3.848	ns	0.04	0.75	ns	20.611	2.671	ns
Season	1	1.267	7.247	<0.05	48.243	8.778	<0.05	0.99	20.12	<0.01	14.938	1.946	ns
Site × Season	5	0.173	0.579	ns	5.497	1.016	ns	0.05	1.06	ns	7.716	1.416	ns

meadows at shallower locations, photosynthetic parameters are probably responding to the attenuated light environment within the dense canopy instead of to the shallow, high-light environment.

Earlier we described the strong reduction of shoot density with increasing depth and the probable implications in terms of reduced self-shading (Collier et al. 2007). When shoot density declines, considerably more light penetrates through the canopy so that the lower sections of leaves growing in deeper water may receive comparable light levels to plants at shallower depths (Dalla Via et al. 1998). Like *Posidonia oceanica* (studied by Dalla Via et al. 1998), *Posidonia sinuosa* forms dense meadows in shallow water with high light, but becomes more sparse at deeper, low-light sites (up to 88-fold reduction in shoot density between 1.6 and 9.0 m depth; Collier et al. 2007). This is likely to reduce self-shading and increase light penetration to leaves. Light availability at the leaf surface was not measured as a part of the current study; however, the similar photosynthetic characteristics among the depth strata suggest that it could be comparable. In contrast, when seagrasses are shaded, changes in photosynthetic characteristics occur (e.g. Dennison & Alberte 1985). The current study suggests that these differences may not necessarily continue in the long term because the whole meadow and the morphology of the plant adapt to increase light availability at the leaf surface. Further investigation into the short-term vs. long-term responses of this species is warranted.

Some photosynthetic parameters suggest that light availability in this region of the canopy
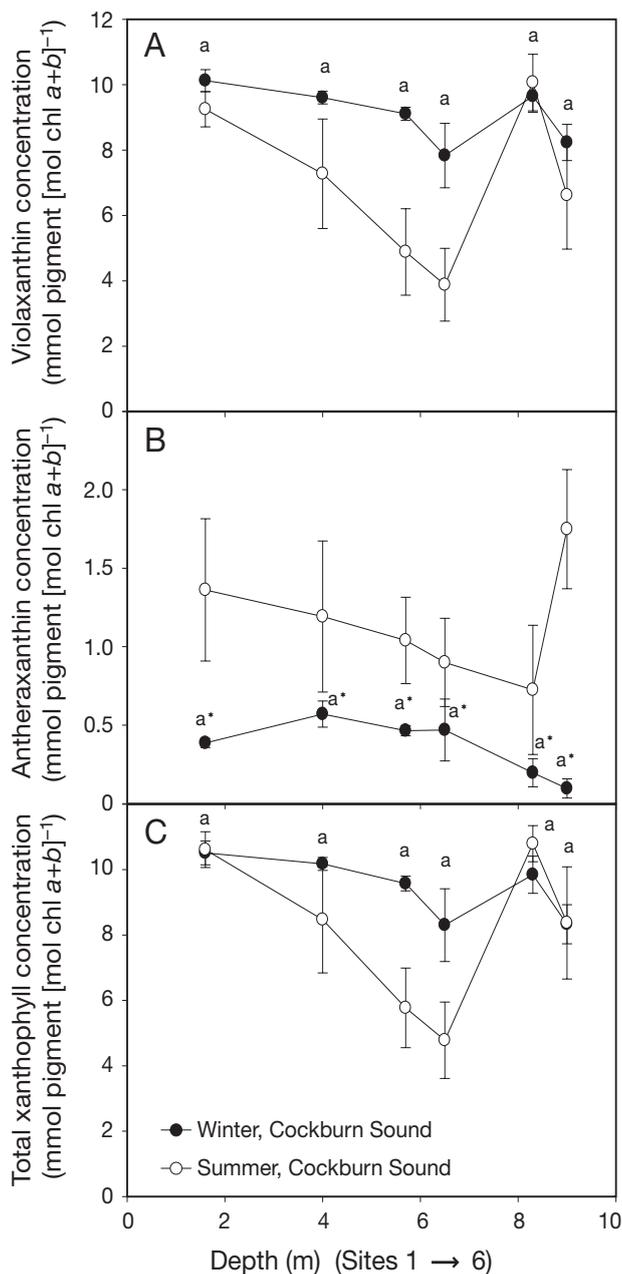
Fig. 3. *Posidonia sinuosa*. Xanthophyll cycle pigment concentrations relative to chlorophyll concentration at Cockburn Sound (CS) in winter 2002 and summer 2003 at 1.6, 4.0, 5.7, 6.5, 8.3 and 9.0 m (Sites 1 to 6, respectively): (A) violaxanthin, (B) antheraxanthin, and (C) total xanthophyll concentration. Values are mean ± SE (n = 6). Sites with the same lower-case letters did not differ significantly within season or location. $^{*}p < 0.05$

even increases with depth. At both CS and WS in summer, rETR$_{max}$ and E_k were elevated at deeper sites when measured at the lower-mid level of the canopy. The high photosynthetic capacity at depth may support the leaf growth rates that are known to be equal among all depths in summer, including the depth limit

(Collier et al. 2007). Photosynthetic responses at the lower-mid level of the canopy may therefore not reflect the overall light conditions of the meadow. Any future consideration of the use of these parameters as indicators of the long-term light environment should include self-shading effects on light pre-history. Measurements made nearer to the top of the canopy may more appropriately represent ambient light availability, but the practical application of measuring leaf tips depends on epiphyte density and leaf integrity.

Total xanthophyll concentrations in *Posidonia sinuosa* (6 to 12 mmol pigment [mol chl $a+b$]$^{-1}$) were similar to those reported for *Zostera capricorni* (4 to 5 mmol pigment [mol chl $a+b$]$^{-1}$) by Flanigan & Critchley (1996), but substantially lower than those of *Z. marina* (approx. 300 mmol pigment [mol chl $a+b$]$^{-1}$) (Ralph et al. 2002). The values recorded for *Z. marina* are typical of terrestrial sun-adapted plants. Plants adjust their total xanthophyll cycle pigment pool in response to total irradiance, such that shade plants maintain significantly less xanthophyll than sun plants (Thayer & Björkman 1990, Adams et al. 1996). Concentration of antheraxnathin was not significantly elevated at shallow sites compared with deeper sites, even though the whole leaf (from leaf base to tip) was analysed. Self-shading by the canopy and epiphyte cover on the leaf tips may ameliorate the effects of high light exposure at the shallow depths. Even in January, when peak daily light availabilities were reached, only partial conversion of xanthophyll products occurred (whereby violaxanthin was converted to antherxanthin); no zeaxanthin was detected.

Other depth-related influences

Changes observed along a depth-gradient may also result from environmental factors other than light. For example, sediment reduction-oxidation (redox) potential may be affected by light availability. We measured redox potential but did not observe a significant difference among depths at this site (Collier et al. 2007). Other sediment characteristics such as nutrient availability were not measured and therefore cannot be dismissed. Photosynthetic characteristics of *Posidonia oceanica* that did not follow the depth-related light gradient were also previously attributed to the influence of thermoclines (Pirc 1986). Temperature was not measured in the current study, but vertical temperature stratification is unlikely to be significant in the generally well-mixed shallow waters of Cockburn and Warnbro Sounds.

The lack of strong light-related trends in the physiological attributes of *Posidonia sinuosa* may also reflect limitation by other major resources. For example, %N
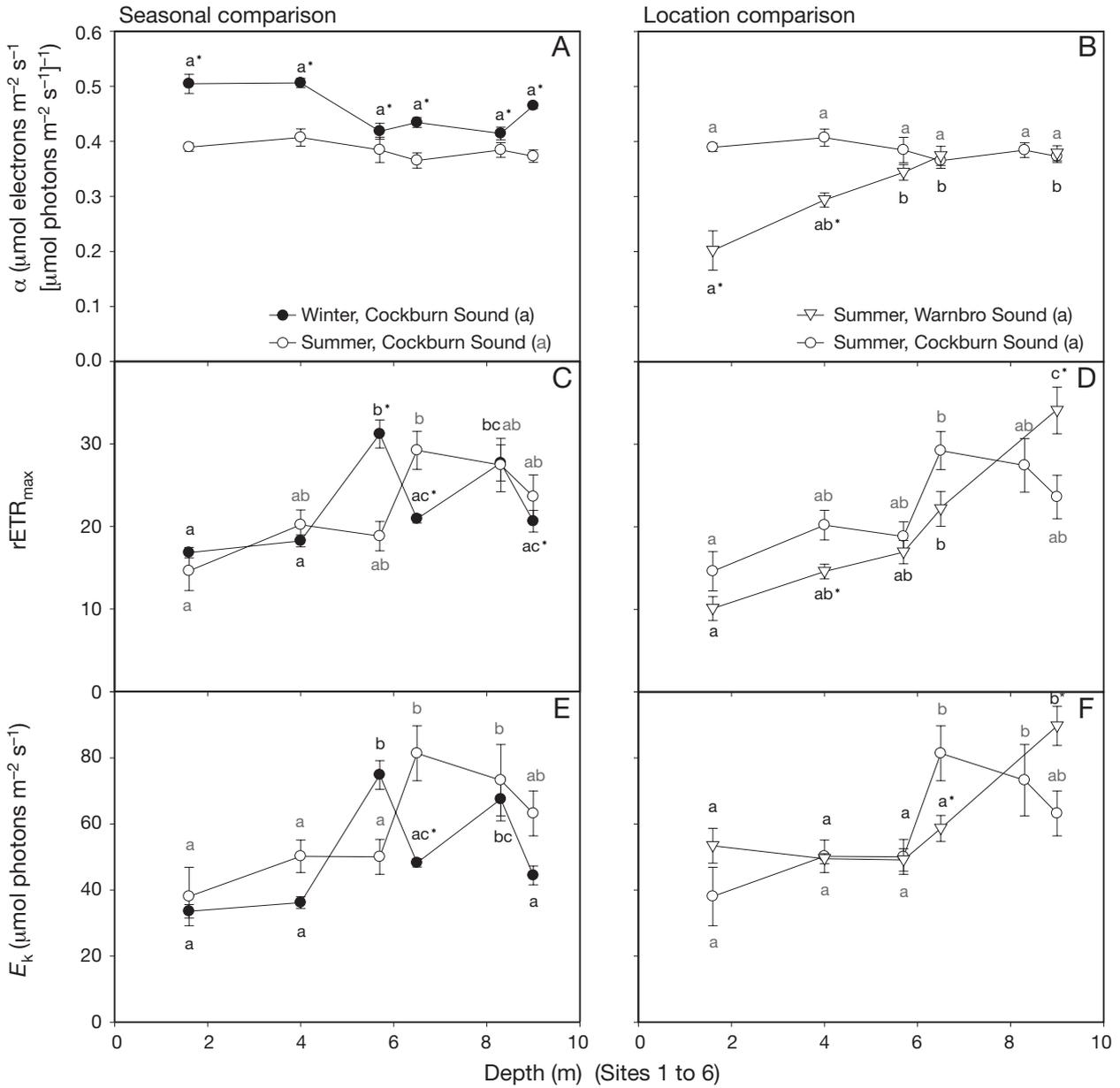
Fig. 4. *Posidonia sinuosa.* Rapid light curve parameters at Cockburn Sound (CS) in winter 2002 and summer 2003 (left), and CS and Warnbro Sound in summer 2003 (right) at 1.6, 4.0, 5.7, 6.5, 8.3 and 9.0 m (Sites 1 to 6, respectively): (A,B) α, (C,D) rETR$_{max}$, and (E,F) E_k (see Table 3 for definitions of parameters). Values are mean ± SE (n = 6). Sites with the same lower-case letters did not differ significantly within season or location. Differently shaded letters are independent of each other. *p < 0.05

Table 4. Hours of light-saturated photosynthesis (H_{sat}) at 6 sites (depths) at Cockburn Sound (CS) in winter 2002 and summer 2003, calculated from saturating irradiance (E_k) and measured light availability recorded every 15 min (Collier et al. 2007)

Season	Site					
	CS1	CS2	CS3	CS4	CS5	CS6
Winter	8.0	7.3	3.3	4.6	1.6	2.8
Summer	11.5	11.2	10.8	10.6	10.1	8.6

of leaves ranged from only 1.1 to 1.4 % at both locations in summer, well below the 1.8 % level considered indicative of nutrient limitation for seagrasses (Duarte 1990). Generally, there were few significant differences among depths (with the exception of the shallowest site at WS), but N limitation at all depths may have offset the significance of light availability. In oligotrophic zones, the response of photosynthetic characteristics and pigment pools to light can be restricted by

Table 5. *Posidonia sinuosa.* Maximum quantum yield (F_v:F_m ratio), non-photochemical quenching (NPQ) and photochemical quenching (qP) at Cockburn Sound (CS) in winter (June 2002) and summer (January 2003) and Warnbro Sound (WS) in summer (January 2003). Parentheses: SE (n = 6). Sites with the same lower-case letters did not differ significantly within season or location. *p < 0.05

Site	Season	Site					
		1	2	3	4	5	6
F_v:F_m							
CS	Winter	0.824 (0.004)[a]*	0.833 (0.004)[a]*	0.816 (0.011)[a]*	0.821 (0.006)[a]*	0.831 (0.008)[a]*	0.823 (0.016)[a]*
	Summer	0.735 (0.006)[a]	0.746 (0.003)[a]	0.749 (0.004)[a]	0.730 (0.011)[a]	0.749 (0.006)[a]	0.749 (0.004)[a]
WS	Summer	0.771 (0.005)[a]	0.750 (0.012)[a]	0.747 (0.009)[a]	0.749 (0.008)[a]	nd	0.752 (0.015)[a]
NPQ							
CS	Winter	0.018 (0.016)[a]	0.014 (0.014)[a]	0.011 (0.011)[a]	0.000 (0.000)[a]	0.003 (0.003)[a]	0.016 (0.013)[a]
	Summer[A]	0.048 (0.048)[ab]	0.114 (0.091)[ab]	0.292 (0.060)[a]	0.170 (0.077)[ab]	0.176 (0.158)[ab]	0.002 (0.002)[b]
WS	Summer	0.144 (0.086)[a]*	0.145 (0.101)[a]	0.024 (0.022)[a]	0.078 (0.045)[a]	nd	0.176 (0.082)[a]*
qP							
CS	Winter	0.980 (0.009)[a]	0.976 (0.008)[a]	0.954 (0.021)[a]	0.972 (0.014)[a]	0.937 (0.015)[a]	0.959 (0.016)[a]
	Summer	0.940 (0.034)[a]	0.989 (0.007)[a]	0.987 (0.012)[a]	0.986 (0.007)[a]	0.989 (0.004)[a]	0.966 (0.010)[a]
WS	Summer	0.908 (0.022)[a]	0.918 (0.036)[a]	0.908 (0.038)[a]	0.973 (0.015)[a]	nd	0.940 (0.024)[a]

[A]Depth differences were significant only when analyzed according to location

N availability (Alcoverro et al. 2001b). However, δ^{13}C signatures indicated that C limitation at shallower depths was unlikely because the leaves were depleted in ^{13}C and not enriched, the latter of which would be expected under conditions of C limitation. When light is reduced, the δ^{13}C signature of seagrass reduces owing to a reduced photosynthetic and productivity demand for carbon, allowing more ^{13}C discrimination (Cooper & DeNiro 1989, Abal et al. 1994, Hemminga & Mateo 1996). The less negative values at the deeper site would instead suggest C limitation at depth. A reversal of this trend here may reflect the hydrodynamic nature of the meadow rather than the light status. The shallow sites are generally highly dynamic, allowing conditions of reduced boundary layers to develop at the leaf surface (Fonesca & Kenworthy 1987). In the current study, the deeper sites may have experienced less water movement around the leaves in summer when the swell was reduced, which may have created thicker diffusion boundary layers around the leaves and lead to elevated δ^{13}C signatures.

Seasonal photosynthetic responses

Over seasonal cycles, seagrasses are exposed to variation in at least 2 key environmental variables: light intensity and temperature. These seasonal fluctuations are often associated with photosynthetic adaptation (e.g. Dennison 1987, Perez & Romero 1992). Generally, temperature effects are the most pronounced near the upper and particularly the lower boundaries of the optimum temperature range (e.g. Masini & Manning 1997). Temperature extremes at the study site of the present study ranged from approximately 16 to 23°C, the mid and optimum temperature range for *Posidonia sinuosa* photosynthesis. As such, temperature effects on photosynthetic parameters were probably less significant than light-related effects. The consistent and substantial seasonal response of α at all depths indicates the capacity for photosynthetic adaptation to seasonal light availability in *P. sinuosa*. An elevated α is frequently reported in low-light adapted plants (Ralph & Gademann 2005), which can be linked to the seasonal variability of this parameter (e.g. Agawin et al. 2001, Campbell et al. 2003). The elevated α in winter would improve the capacity of the photosystems to utilise reduced light in winter. During winter, leaves were operating in this light-limiting region (below saturating irradiance) for most of the day, particularly at the 4 deeper sites. Adaptation of the other photosynthetic parameters (rETR$_{max}$ and E_k) is possible in *Posidonia* spp. under conditions of extreme light deprivation (Ruiz & Romero 2001, 2003, Collier 2007), but was not observed in the current study in relation to seasonal differences in light availability.

The increase in the concentration of the xanthophyll cycle product antheraxanthin and the decrease in the F_v:F_m ratio at all depths indicate that the photoprotective cycle was more active during summer than in winter in *Posidonia sinuosa*. Xanthophyll cycle pigments undergo rapid conversion in response to high light levels (Demmig-Adams et al. 1996), and as such the elevated antheraxanthin were indicative of higher light levels at all depths during summer. Although highly variable and not significantly different, NPQ was generally also higher in summer. However, the total xanthophyll pigment pool did not significantly change between summer and winter.

The difference in H_{sat} between shallow and deep locations was only 25% in summer but 65 to 80% in winter, suggesting that physiological differences among depths were greatest during winter. However, in general, the photosynthetic parameters responded to seasonal light variation equally among depths; the only depth-dependent response of the photosynthetic apparatus was chlorophyll concentration at the 2 shallowest and the deepest site. Elevated %N during winter probably enabled the increases in chlorophyll (and other accessory carotenoid pigments). Higher chlorophyll concentration may enhance light capture — albeit at reduced efficiency as concentration increases (Enríquez 2005) — when plants are exposed to reduced irradiance.

Carbohydrate reserves

The capacity to store considerable quantities of carbohydrates is an important and distinctive feature of the structurally large seagrasses, and their responses should be considered in terms of an annual cycle, because growth can be de-coupled from photosynthesis (Alcoverro et al. 2001a). The limited seasonal photosynthetic responses of *Posidonia sinuosa* to changes in irradiance may be compensated by the ability to access stored carbohydrates. The formation of carbohydrate reserves requires light to be near to saturation levels for considerable periods of the day (Alcoverro et al. 2001a), so peak concentration tends to occur towards the end of summer or in autumn (Pirc 1985, Alcoverro et al. 2001a, Collier 2007). This would have occurred just after the timing of sampling in the current study. The elevated soluble sugar concentrations in the rhizome and starch in the leaves of *P. sinuosa* at all depths during summer indicate that light availability and photosynthetic C fixation had been sufficient for the production of carbohydrate reserves. The use of carbohydrate reserves for over-wintering (Pirc 1985, Burke et al. 1996) is probably important for *P. sinuosa*, because reserves were already substantially depleted by early winter (June). Soluble sugars in the rhizome showed the largest seasonal difference, though starch in the leaves was also significantly reduced in winter.

Carbohydrate concentrations were the same among depth strata (with few exceptions) in both seasons, suggesting that the pattern of utilization and storage were similar at all depths. While this study examined only 2 seasons, more frequent carbohydrate analysis of *Posidonia sinuosa* over 1 year produced similar results (Collier 2007). It was surprising that, in winter, carbohydrate depletion at deeper sites was not greater than at shallow sites: it was expected that the plants growing in deeper water would rely more heavily on carbohydrate reserves during winter. Furthermore, the ratio of above- to below-ground biomass increases with depth (Collier et al. 2007), which is expected to increase the demand on carbohydrate reserves at deeper sites. Similar trends in carbohydrate concentrations over a 30 m depth gradient were also observed in *Posidonia oceanica* over an annual cycle (Pirc 1985). Meadow-scale adjustments (shoot reduction) with depth may be sufficient to reduce C demand and the requirement to draw-down on carbohydrate reserves during winter, yet enable accumulation of reserves during summer.

In conclusion, the gradient of light corresponding to depth resulted in reduced *Posidonia sinuosa* shoot density but did not correlate with changes in physiological characteristics. While laboratory and *in situ* manipulative experiments indicate that changes in photosynthetic parameters may be an important mechanism enabling seagrasses to endure short-term light reduction (Dennison & Alberte 1985, Ruiz & Romero 2001), they probably exaggerate their importance when compared with field situations with long-term light reduction (Olesen et al. 2002). Where meadow-scale shoot density reductions and concomitant (but minor) shoot-scale morphological changes (Collier et al. 2007) effectively open the canopy to greater light availability, adaptation is dominated by meadow- and shoot-scale differences rather than by leaf-scale physiological changes. During seasonally induced light reduction in winter, carbohydrate reserves are used at all depths to support growth and respiration. The application of these physiological characteristics as monitoring tools to identify long-term light reduction is probably not effective for *P. sinuosa* and other similar meadow-forming species.

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