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ORIGINAL ARTICLE

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Interactive effects of salinity and irradiance on photoprotection in acclimated seedlings of two sympatric mangroves

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Abstract The synergistic effects of irradiance and salinity on leaf angle, the photochemical efficiency of photosystem II and photosynthetic pigment composition of mangroves were studied in a factorial experiment. Seedlings of *Aegiceras corniculatum* (L.) Blanco (Myrsinaceae) and *Avicennia marina* (Forstk.) Vierh var. *australasica* (Walp.) Moldenke (Avicenniaceae) were grown under salinity treatments (0, 5, 25, 50, 75, and 100% artificial seawater), in full sunlight or under shade cloth (transmitting 30 or 70% sunlight), during summer and autumn. Significant species' differences and effects of salinity and growth irradiance were found for key measures. Depressions in F_v/F_m due to salinity and growth irradiance were chronic, they were least in 25% seawater and in 30% sunlight, and greater in low and high salinity, and higher irradiance. A diurnal depression of F_v/F_m was superimposed on the chronic depression, and was greater for *Ae. corniculatum* than *Av. marina*. Increases in leaf angle; and increases in the size, and de-epoxidation state of the xanthophyll cycle pigment pool afforded protection from adverse effects of excess excitation energy. Adverse effects of the highest salinities on β,β-carotene and β,ε-carotene biosynthetic pathways were suggested, particularly in *Ae. corniculatum*. The ecological significance of differences in species' extent and temporal patterns of response are discussed.

Keywords Carotenoids · Leaf angle · Light · Photoinhibition · Xanthophyll cycle

Introduction

Variation in mangrove community structure along physicochemical gradients may depend on species' differences in physiological tolerances, including responses of photosynthesis to irradiance and salinity (Snedaker 1982; Ball and Critchley 1982; Ball 1988; McKee 1995). It has been hypothesised that species-specific strategies for photoprotection may determine canopy dominance (Lovelock and Clough 1992), and differences in mangroves' capacities for photosynthesis and protective dissipation of excess excitation energy along salinity gradients may impact on plant performance and the distribution of species (Ball 1996).

Exposure to excess irradiance can lead to photoinhibition, which is characterised by a light-dependent reduction in the intrinsic quantum yield of photosynthesis and a loss of photosystem II (PSII) activity (Osmond 1994). Photosynthesis of mangrove leaves becomes light-saturated at incident photon flux densities of 40% sunlight or less (Ball and Critchley 1982; Andrews et al. 1984; Carter et al. 1990; Cheeseman et al. 1991; Cheeseman 1994; Youssef and Saenger 1998). Hence, irradiance may often be excessive. In mangroves, photoinhibition occurs under high irradiance in canopies in the field, but the degree to which photoinhibition is sustained varies (Attiwill and Clough 1980; Ball and Critchley 1982; Björkman et al. 1988; Cheeseman et al. 1991, 1997; Kitao et al. 2003). There have been no laboratory-based studies of photoinhibition under varying growth irradiance.

Photoprotection allows downregulation of the photosynthetic apparatus to balance light energy receipt and use (Osmond 1994). In mangroves, inhibition of zeaxanthin (Z) formation has been associated with more sustained depression of F_v/F_m (the potential maximal photochemical efficiency of photosystem II) in seedlings (Demmig-Adams et al. 1989). Consistent with a key role of the xanthophyll cycle in photoprotection, in mature canopies pool sizes were lower in the shade compared with sunlit leaves and in leaves with steeper angles, and large pool size was accompanied by higher midday de-epoxidation state (Lovelock and Clough 1992). Steep angles of display in exposed leaves also help maintain photosynthetic efficiency (Björkman et al. 1988).

In mangroves, effects of increasing salinity on photosynthetic carbon assimilation may be detrimental (Ball and

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Farquhar 1984; Sobrado 1999b), beneficial (Naidoo and von Willert 1995; Werner and Stelzer 1990) or negligible (Pezeshki et al. 1990). Salinity-dependent limitations to photosynthetic carbon metabolism may increase the potential for photoinhibition (Powles 1984). However, responses reported for mangroves vary. In field studies of mature canopies, greater depressions of F_v/F_m in habitats with hyposaline compared with seawater salinities (Naidoo et al. 2002), contrast with no differences in F_v/F_m in habitats with hypersaline compared with seawater salinities (Sobrado and Ball 1999). Although glasshouse-grown seedlings showed lower F_v/F_m when grown in 100% seawater compared with 10% seawater (Björkman et al. 1988), another study found no significant effects on F_v/F_m of an increase in the salinity of the sodium chloride solutions in which seedlings were grown (Sobrado 1999a). There have been no laboratory-based studies of photoprotection under varying salinity in mangroves. However, a study of mature canopies found no differences in xanthophyll cycle pool size or de-epoxidation state in habitats with hypersaline compared with seawater salinities (Sobrado and Ball 1999).

My study (Christian 1999) tested the hypotheses of Lovelock and Clough (1992) and Ball (1996), as stated above. The study species *Ae. corniculatum* and *Av. marina* are sympatric in southeastern Australia (West et al. 1985; Busby and Bridgewater 1986), where they tend to dominate mangrove communities in the upper and lower reaches of estuaries, respectively (Clarke and Hannon 1970; Owen 1978). Usually, *Av. marina* occurs as a tree or shrub in the overstorey, and *Ae. corniculatum* shrubs occur in monospecific stands or as an understorey beneath *Av. marina* (Christian 1999).

A factorial experiment was used to test three hypotheses: (1) interactions between high light and adverse salinity exacerbate photoinhibition, (2) this effect is greater in *Ae. corniculatum* than *Av. marina* and (3) differences in leaf display and chlorophyll (chl) and carotenoid composition contribute to species-specific strategies of photoprotection along salinity and light gradients.

Materials and methods

Plant material

Propagules of *Ae. corniculatum* and *Av. marina* were collected in August and October 1993, respectively, at Cullendulla Creek, Batemans Bay, New South Wales (35°42′S, 150°12′E). Propagules were grown in sand culture in 50% (v/v) seawater, made by diluting artificial seawater with tap water, in a glasshouse in Canberra (35°18′S, 149°12′E). Day and night air temperatures were approximately 25°C and 15°C, respectively, and seedlings received approximately 70% sunlight. When the first pair of leaves was fully expanded and shedding of cotyledons commenced in *Av. marina*, cotyledons remaining on *Av. marina* were removed. Fresh weights of all seedlings were measured. Seedlings were replanted in sand in perforated pots (140 mm diameter, approximately 1 L volume). Each pot was placed in a 2-L tub and immersed in 50% seawater with added nutrient solution (10% Hoagland's; Hewitt 1966).

Experimental procedure

A split-plot experiment was conducted outdoors from 20 January to 1 May 1994. Restricted randomisation was used. Three shade shelters were used in each of three north-south oriented blocks. Shade treatments were achieved using no shading (100% sunlight) or shade-cloth transmitting either 30 or 70% sunlight. Species (*Av. marina* and *Ae. corniculatum*) and salinity treatment (0, 5, 25, 50, 75 or 100% seawater) were assigned to one of 12 positions in each shelter. Three pots containing seedlings of similar fresh weight were assigned to each position. Pots were assigned to one of three harvests (H1: 0 days; H2: 52 days; and H3: 101 days), as part of a growth analysis study (Christian 1999).

Once plants were positioned, salinities were brought to treatment levels in increments of 10% seawater or less by replacement of bathing solutions twice daily. Concentrations of Hoagland's solution were increased concurrently to 30%. Thereafter, pots were aerated through a 16 mm microspray jet inserted in the sand. Solutions were topped-up with tap water daily and were replaced weekly.

Conditions

Daily global solar radiation data for Canberra were obtained from the Bureau of Meteorology (Radiation Network Exposure Data). Photosynthetic photon flux densities incident on a horizontal surface (PPFD) were measured with a quantum sensor (model 190s, LI-COR, Lincoln, Nebraska) above the leading shoot apex of each plant at intervals through the day on 9 February, 23 March, and 21 and 27 April. Daily maximum and minimum air temperatures in each shelter were recorded from 11 February 1994.

Leaf selection

For each H3 plant a fully expanded, north to west facing, exposed leaf, which had developed under the experimental treatment was tagged for all measurements. New leaves were tagged when growth led to shading. Not all 108 plants were tagged because under adverse treatments plants did not grow enough new large leaves.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured in situ on sunny days. On three days (2 February, 23 March, and 21 April) I made consecutive one- to two-hourly measurements throughout the day from predawn until dusk. Predawn and midday measurements were also made on 28 April, concurrent with sampling for pigment analyses.

The dark-adapted minimum (F_o) and maximum (F_m) chlorophyll fluorescence were measured with a field-portable, time-resolving fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). Leaves were first dark-adapted and then exposed for 5 s to saturating red light. The ratio of variable to maximal fluorescence F_v/F_m, was calculated as $(F_m-F_o)/F_m$. Measurements made predawn represent the true dark-adapted state. During daylight, dark-adaptation was for 5 min, allowing relaxation of fluorescence quenching associated with thylakoid membrane energisation (Krause et al. 1983; Demmig et al. 1987).

Leaf display and photosynthetic pigments

The midrib angle of each tagged leaf was measured with a protractor and shot-line. Predawn and at midday, one to four 0.95 cm^2 discs were punched from each leaf, frozen in liquid nitrogen and stored at -80°C. Discs were powdered in liquid nitrogen and extracted for 5 min with 100% AR grade acetone in the presence of $NaHCO_3$. The solvent was then diluted with water to 80% acetone and extracted for 10 min more. The sample was centrifuged (5 min, 5,000 rpm, 2°C) and the supernatant collected and stored under $N_{2(g)}$ at -80°C.

Chlorophyll concentrations were determined by absorption spectroscopy (Ultraspec II spectrophotometer, Pharmacia LKB, Uppsala, Sweden) using wavelengths and extinction coefficients as in Porra et al. (1989). The filtered (0.45 μm) supernatant was analysed for carotenoid pigments using the high-performance liquid chromatography (HPLC) method of Gilmore and Yamamoto (1991). Solvent A1 (acetonitrile–methanol–Tris–HCl buffer 0.1 M pH 8.0, 72:8:3) was used. Absorption was measured at 440 nm using a chromatograph with a variable wavelength detector (Waters model 490). Spherisorb ODS-1 nonend-capped columns (5 μm particle size, 250 mm × 4.6 mm, Alltech Associates Aust. Pty. Ltd.) and guard columns were used. Elution of pigments was as described in Roden and Ball (1996). All sample injections were 40 μl. Pigment concentrations were calculated using calibrations based on standards (as described in Robinson et al. 1993).

The de-epoxidation state (DPS) of xanthophyll cycle pigments, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) was calculated as (Z+0.5A)/(V+A+Z). The xanthophyll cycle pool size (VAZ/chl) was calculated as (V+A+Z)/(chl a+b).

Statistical analyses

Data were analysed using GenStat (5th edition, releases 3.1 through to 7.2, Rothamsted Experimental Station 1994 and VSN International). The description of factorial models is as in Wilkinson and Rogers (1973). Fixed effects were Shade, Species, Salinity and Hour (where repeated measures are compared for two occasions). Random effects were block, shelter, position and time.

Where the variance was heterogeneous the data scales were transformed to equalise variances. In the case of F_v/F_m, the constant 0.84 was taken as an upper limit and when necessary, the following transformation was used:

$$f\,(F_v/F_m) = \ln\,(0.84 - F_v/F_m) \qquad (1)$$

The data scale is important for determining whether synergistic effects of treatment factors occur. For example, when two factors show multiplicative effects (i.e., an interaction), on a linear scale:

$$a = b \times c \qquad (2)$$

transformation to a logarithm scale:

$$\ln a = \ln b + \ln c \qquad (3)$$

makes the relationship additive.

For balanced data, analyses of variance were used. For unbalanced data, linear mixed models were fitted using restricted maximum likelihood estimation (REML; Patterson and Thompson 1971). The full model was fitted and terms were then successively dropped from nested submodels where changes in deviance tests (χ^2 test) were not significant ($p<0.05$), to arrive at the final, most parsimonious sub-model. The analysis of deviance for the final model is summarised (in the figure and table captions) as: the residual deviance (RD) and degrees of freedom (df) for the final model; and the change in RD and df of the submodel when each term was dropped, and its χ^2 significance level.

Repeated measures made on different days were pooled as there was no evidence for autocorrelation between dates within a subject. Repeated measures made on more than two occasions through the day (e.g., PPFD) were explored by plotting the response through time. Where all subjects showed consistent patterns, responses of each were modelled by fitting nonlinear curves to the data (Payne et al. 1994). A summary measure was then derived for each fitted curve, which was analysed as above.

Models of significant ($p<0.05$) treatment effects are presented graphically for the highest order factors and interactions that were significant. For data that did not require transformation, figures show the means and average standard errors. Where the variate was transformed, results are presented as backtransformed means and 95% confidence intervals [i.e., mean (lower CI, upper CI)]. Where all effects were not significant the null model was fitted using REML to obtain the correct error term, and the grand mean and 95% CI are given in the text.

Equations describing the relationships between F_v/F_m and several measures of photoprotection were examined by fitting linear mixed models to the data collected on 28 April. Fixed effects of treatment factors (Shade, Species, Salinity and Hour), three covariates (leaf angle, VAZ/chl and DPS), and all possible two-way interactions were modelled (random effects: block/shelter/position/time). The variates were transformed where necessary. Marginal predicted values of the response variate were estimated for given values of the explanatory variates as in Welham et al. (2004).

Results

Experimental conditions

The mean daily global radiation over the experimental period was 19.5 MJ m^{-2} day^{-1} (6.9, 32.1). The 30, 70 and 100% sunlight treatments, received PPFDs at midday of 349 (283, 421), 927 (818, 1,043) and 1,263 (1,135, 1,398) μmol m^{-2} s^{-1}; and total daily photon receipt of 11.3 (7.4, 16), 26.7 (20.5, 33.6) and 39.4 (31.8, 47.8) mol m^{-2}, respectively. There were no significant effects of Shade on the maximum [26.9°C (26.7, 27.1)] or minimum [8.65°C (8.55, 8.75)] air temperatures within each shelter.

Chlorophyll fluorescence

Repeated measurements throughout the day indicated that F_v/F_m was reduced through the morning, and recovered in the afternoon (data not presented). The minima for F_v/F_m occurred at midday [1206 hours EST (1140, 1234)], and the time of occurrence was not significantly affected by any factor. Variation in F_o was large and differences with time of day were not significant (data not presented).

Predawn, on average, *Av. marina* showed a 2% lower F_v/F_m than *Ae. corniculatum* (Fig. 1a). However, at midday on average, *Ae. corniculatum* showed an 11% lower F_v/F_m than *Av. marina*. On average, F_v/F_m was 14% lower in plants grown in full sunlight compared with 30% sunlight (Fig. 1b). On average, compared with 25% seawater, F_v/F_m was reduced by 7% in freshwater and 13% in 100% seawater (Fig. 1c). Additive effects of shading and salinity on log-transformed F_v/F_m data indicate multiplicative effects on the original scale, and a greater adverse effect of high growth irradiance under adverse salinity. The frequent need for transformation of the F_v/F_m data indicates that variation in this ratio was greatest when photoinhibition was greatest. This may be because of variation between plants in the extent of resistance to photoinhibition, and/or because of the low signal-to-noise ratio which arises when chlorophyll fluorescence is most quenched.

Leaf display and photosynthetic pigments

Leaf angles were increasingly vertical with increasing growth irradiance (Fig. 2a) and increasing salinity (Fig. 2b), but did not differ significantly with species. The salinity level did not affect the response to growth irradiance. The total chl concentration per unit leaf area was maximal in 25% seawater and decreased in high and low salinity (Fig. 2c), but was not significantly affected by shading, species or time of day. The molar ratio of chl *a* to

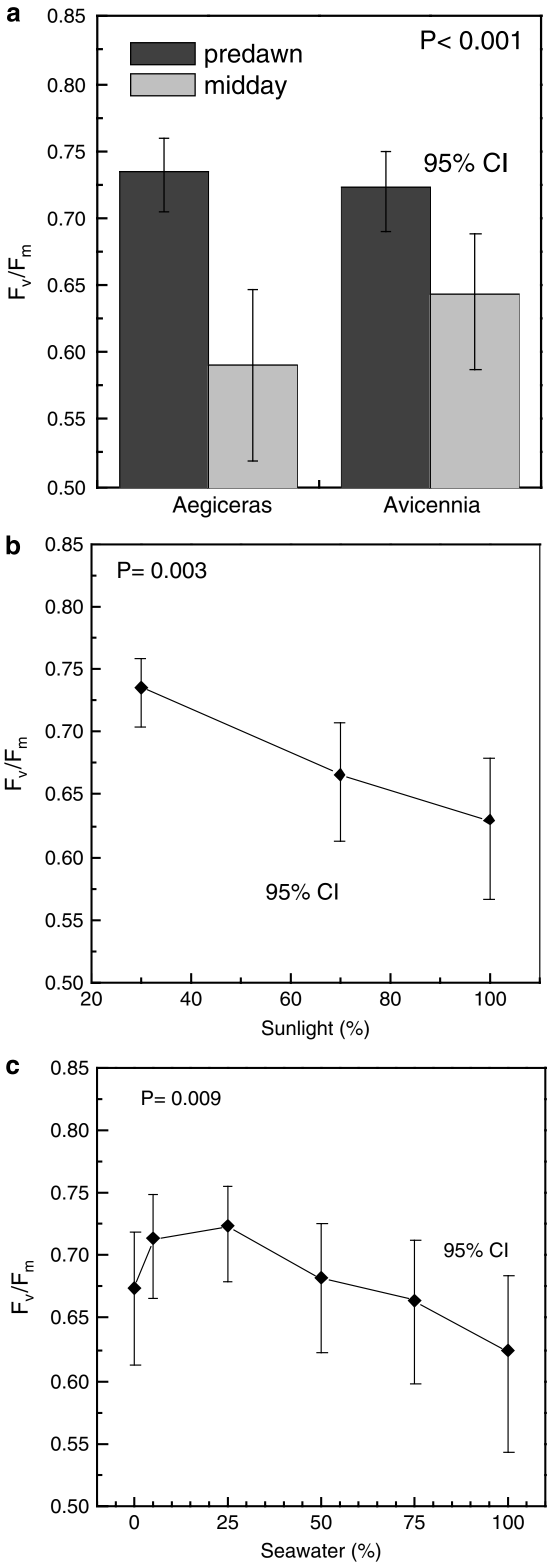

Fig. 1 Effects of **a** Species.Hour (ΔRD=14.4, df=1), **b** Shade (ΔRD=11.4, df=2), and **c** Salinity (ΔRD=15.4, Δdf=5) on F_v/F_m in *Ae. corniculatum* and *Av. marina* seedlings grown in a three-way factorial pot trial under three shading and six salinity treatments. Backtransformed means and 95% CIs for the final linear mixed model (residual deviance = 116.58, df=315) fitted to $\ln(0.84 - F_v/F_m)$ using REML. Data for three dates (23 March, 21 and 28 April 1994) were pooled

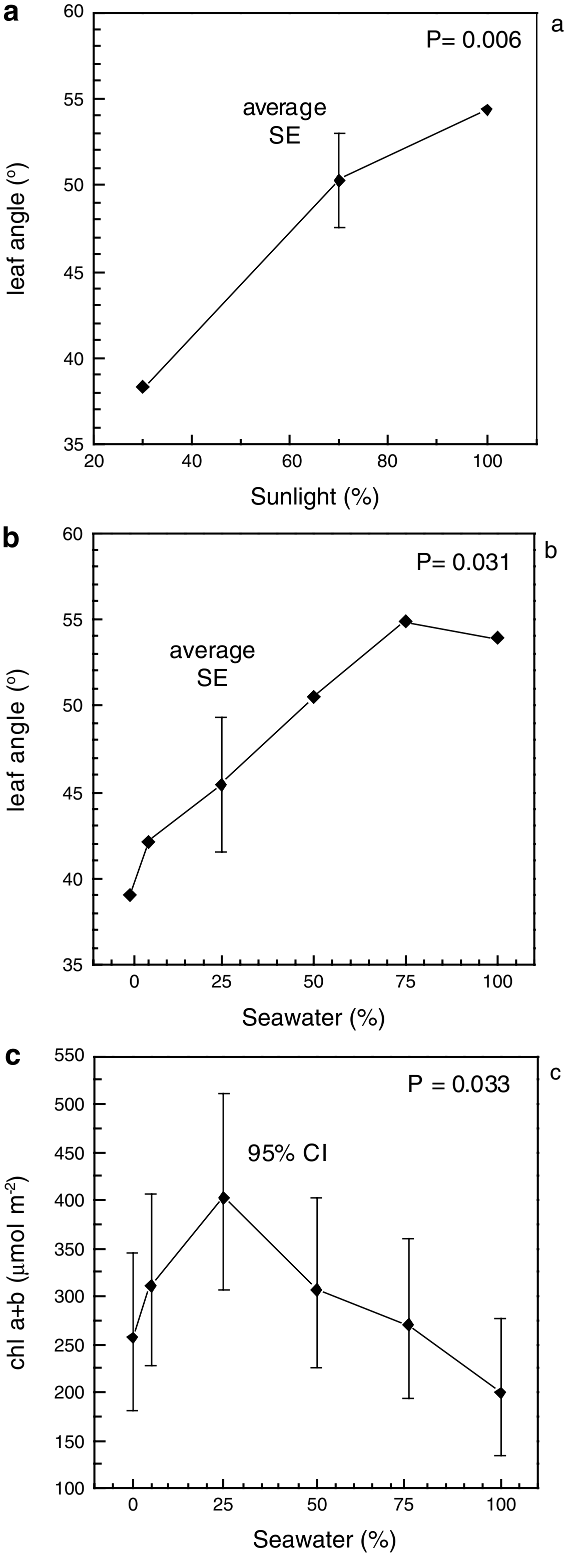

Fig. 2 Effects of **a** Shade (ΔRD=10.31, Δdf=2) and **b** Salinity (ΔRD=12.30, Δdf=5) on leaf angles (from the horizontal). Significantly different means for the final linear mixed model (residual deviance = 539.23, df=78) fitted using REML. **c** Effect of Salinity (ΔRD=12.08, Δdf=5) on the total leaf chlorophyll per unit leaf area (28 April 1994). Backtransformed means and 95% CIs for the final linear mixed model (residual deviance = 434.05, df=106) fitted to (chl a+b (μmol m^{-2}))$^{1/2}$ using REML

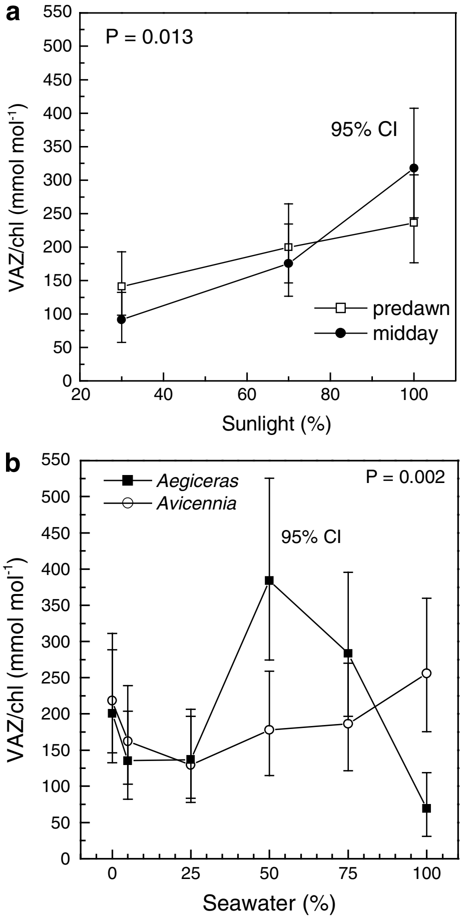

Fig. 3 Effects of **a** Shade.Hour (ΔRD=8.693, Δdf=2), and **b** Species.Salinity (ΔRD=18.37, Δdf=5) on xanthophyll cycle pool size relative to total chlorophyll (*VAZ*/chl), . Backtransformed means and 95% CIs for the final linear mixed model (residual deviance = 83.46, df=92) fitted to ln(100 + [*VAZ*/chl {mmol mol^{-1}}]) using REML

b [3.07 (2.71, 3.43)] was not significantly affected by any of the treatments.

Xanthophyll cycle pool sizes relative to chl increased with increasing growth irradiance (Fig. 3a), due to an increase in xanthophylls per unit leaf area (not presented). Pool sizes in *Av. marina* were lowest in 25% seawater and increased in lower and higher salinities (Fig. 3b). The two species had similar pool sizes in 0–25% seawater; however, for *Ae. corniculatum* pool sizes increased and then decreased with further increases in salinity. The salinity response was partly driven by changes in chl per unit leaf area (Fig. 2c); and *Av. marina* had greater concentrations of xanthophylls per unit leaf area than *Ae. corniculatum* (data not presented). The DPS increased at midday compared with predawn (Fig. 4a and b). *Aegiceras corniculatum* had lower DPS predawn and higher DPS at midday than *Av. marina* (Fig. 4a). At midday, DPS increased with increasing growth irradiance (Fig. 4b). The DPS was higher in 100% seawater than at other salinities (Fig. 4c).

The ratio of β-carotene (βC) to chl varied significantly with salinity and time (Fig. 5a), but not species

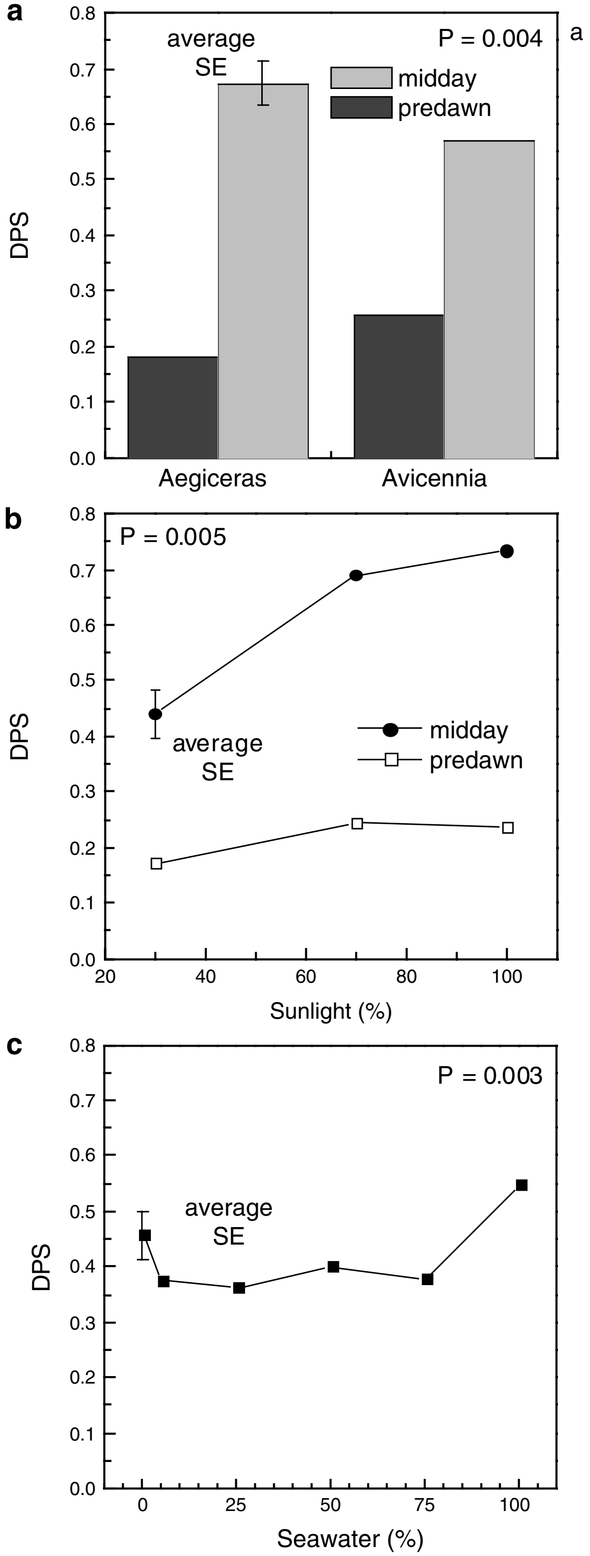

Fig. 4 Effects of **a** Species.Hour (ΔRD=8.127, Δdf=1), **b** Shade.Hour (ΔRD=10.57, df=2), and **c** Salinity (ΔRD=17.62, Δdf=5) on the de-epoxidation state of the xanthophyll cycle pigments ($DPS=[Z+0.5A]/[V+A+Z]$). Significantly different means from the linear mixed model (residual deviance = 255.08, df=102) fitted using REML

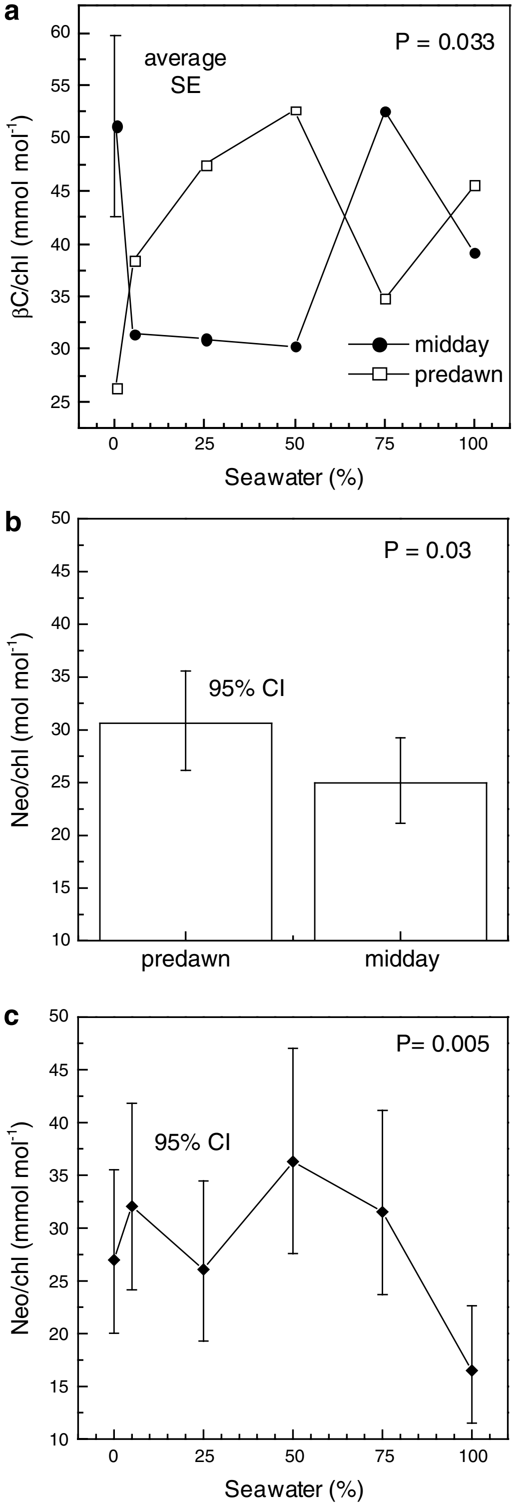

Fig. 5 Effects of **a** Salinity.Hour (ΔRD = 12.16, df = 5) on the ratio of β-carotene to total chlorophyll (βC/chl). Significantly different means from linear mixed model (residual deviance = 795.10, df=99) fitted using REML. Effects of **b** Hour (ΔRD=4.701, Δdf=1), and **c** Salinity (ΔRD=16.59, Δdf=5) on the ratio of neoxanthin to total chlorophyll (Neo/chl). Backtransformed means and 95% CIs for the linear mixed model (residual deviance = 74.32, df=104) fitted to $\ln(10+(\text{Neo/chl [mmol mol}^{-1}]))$ using REML

or shade treatment. Concentrations of βC per unit area [9.76 μmol m^{-2} (7.73, 12.04)] showed no significant effects of any factors (for square-root transformed data). The ratio of neoxanthin (Neo) to chl decreased at midday compared with predawn (Fig. 5b). Ratios were highest in moderate salinities and decreased in 100% seawater (Fig. 5c), but did not differ significantly with species. These patterns were associated with lower Neo concentrations per unit leaf area (data not presented) at midday compared with predawn, and in 100% seawater.

The ratio of lutein (L) to chl increased with increasing growth irradiance and in shaded treatments ratios decreased at midday compared with predawn (Fig. 6a). In *Av. marina,* ratios were lowest in 25% seawater and increased in lower and higher salinities (Fig. 6b). The two species had similar ratios in 0–25% seawater. In *Ae. corniculatum,* ratios increased and then decreased with further increases in salinity. These patterns were associated with lower L concentrations per unit leaf area (data not presented) at midday compared with predawn, in *Ae. corniculatum* compared with *Av. marina,* and with increasing salinity.

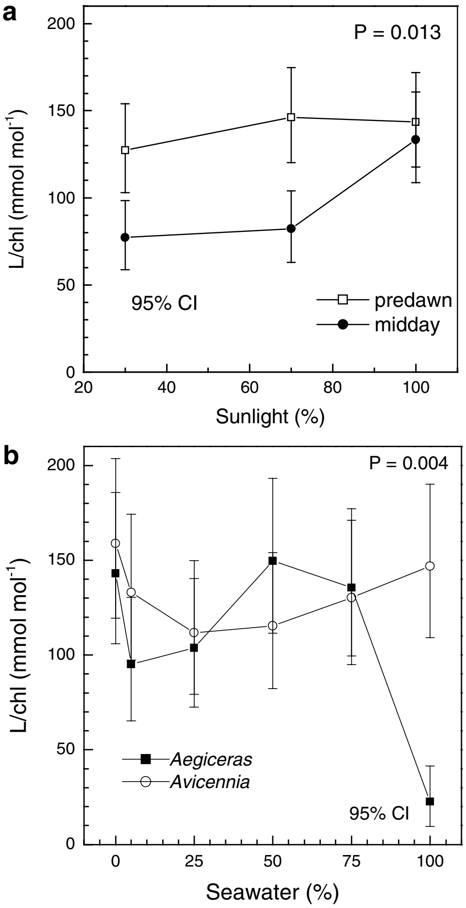

Fig. 6 Effects of **a** Shade.Hour (ΔRD=7.094, Δdf=2), and **b** Species.Salinity (ΔRD=17.26, Δdf=5) on the ratio of lutein to total chlorophyll (L/chl). Backtransformed means and 95% CIs for linear mixed model (residual deviance = 281.89, df=92) fitted to (L/chl [mmol mol^{-1}])$^{1/2}$ using REML

Relationships between measures of photoinhibition and photoprotection

Decreases in leaf angle, increases in xanthophyll cycle pool size under high growth irradiance, and increases in DPS, particularly in *Ae. corniculatum,* were associated with decreases in F_v/F_m (Table 1). Hour affected only the intercept and not the slope of these relationships and Salinity had no significant effects. The models indicated that for a given *Ae. corniculatum* seedling growing under high irradiance, individuals with lower leaf angle, larger xanthophyll cycle pool size and higher DPS had lower F_v/F_m (Fig. 7). If the seedling was grown under low irradiance the effect of pool size was less and if the mangrove was an *Av. marina* seedling, the effect of DPS was less.

Discussion

Photoinhibition in leaves developed under different salinity and irradiance treatments

High growth irradiance led to sustained depressions in F_v/F_m, consistent with depressions of F_v/F_m in sunlit leaves compared with shaded leaves in mature canopies of *Ae. corniculatum* and *Av. marina* (Björkman et al. 1988). Salinity extremes also led to persistent depressions of F_v/F_m, consistent with at least 10% lower F_v/F_m for seedlings of *Av. marina* grown in 100% seawater compared with 10% seawater, under high irradiance (Björkman et al. 1988). Consistent with Hypothesis 1, these effects of high growth irradiance and salinity were multiplicative. Such ‘chronic’ photoinhibition (sensu Osmond 1994) due to high light and salinity in mangroves has been attributed to a regulatory, protective increase in the rate constant for radiationless energy dissipation in the light-harvesting antennae (Björkman et al. 1988).

Sustained depressions in the photochemical efficiency of PSII were greater in *Av. marina* than *Ae. corniculatum,* contrary to Hypothesis 2. However, transient diurnal reductions were superimposed on the sustained depressions in F_v/F_m and were greater in *Ae. corniculatum* than *Av. marina*. This transient component is consistent with responses observed in leaves of *Av. marina* (Attiwill and Clough 1980) and *Rhizophora stylosa* (Cheeseman et al. 1997) canopies in the field, and for glasshouse-grown *Avicennia germinans* (Sobrado 1999b). Midday reductions in the efficiency of PSII in high light were recovered shortly after sunset (Cheeseman et al. 1997) or predawn (Sobrado 1999b) and were thought to result from protective downregulation rather than damage. The responses of the two species, therefore, differ most in their temporal pattern and lie on a continuum with *Ae. corniculatum* showing responses more similar to the pattern of diurnal photoinhibition observed by Cheeseman et al. (1997) and *Av. marina* showing responses more similar to the chronic photoinhibition observed by Björkman et al. (1988).

Table 1 Coefficients of the linear mixed models describing the relationships between the extent of photoinhibition and measures of photoprotection under the imposed treatments on 28 April 1994. Models were fitted using restricted maximum likelihood estimation (REML). Coefficients are given for the final model (residual deviance = 105.73, df=95). Variation in ln(0.84 – F_v/F_m) was explained by Hour. ΔRD=33.05, Δdf=1, $p<0.001$), leaf angle (ΔRD=6.162, Δdf=1, $p=0.013$), shading-dependent effects of the xanthophyll cycle pool size (Shade.[ln(100+VAZ/chl (mmol mol^{-1}))]; ΔRD=11.64, Δdf=2, $p=0.003$) and species-dependent effects of the de-epoxidation state (Species.DPS; ΔRD=22.48, Δdf=1, $p<0.001$)

Shade	Species	Hour	Intercept	Slope.[leaf angle (°)]	Slope.[ln(100+VAZ/chl (mmol mol^{-1}))]	Slope.[DPS]
30	*Aegiceras*	Predawn	−2.90	−0.006	−0.089	1.606
70	*Aegiceras*	Predawn	−2.52	−0.006	0.557	1.606
100	*Aegiceras*	Predawn	−2.68	−0.006	0.578	1.606
30	*Avicennia*	Predawn	−2.55	−0.006	−0.089	0.590
70	*Avicennia*	Predawn	−2.17	−0.006	0.557	0.590
100	*Avicennia*	Predawn	−2.33	−0.006	0.578	0.590
30	*Aegiceras*	Midday	−2.29	−0.006	−0.089	1.606
70	*Aegiceras*	Midday	−1.91	−0.006	0.557	1.606
100	*Aegiceras*	Midday	−2.07	−0.006	0.578	1.606
30	*Avicennia*	Midday	−1.94	−0.006	−0.089	0.590
70	*Avicennia*	Midday	−1.56	−0.006	0.557	0.590
100	*Avicennia*	Midday	−1.73	−0.006	0.578	0.590

Photoprotection mechanisms

Chlorophyll *a*/*b* ratios were typical of sun plants (Anderson et al. 1988). Lower leaf chl content in salinity extremes may represent a protective strategy in which the capture of photons is reduced to match the capacity for energy transduction and use, as has been suggested for sunlit leaves of *Xylocarpus granatum* in the field (Kitao et al. 2003). Lower chlorophyll may also reduce leaf absorptance and enable avoidance of high leaf temperatures when stomatal conductance is low (Havaux and Tardy 1999).

Steeper leaf angles under high growth irradiance and in more saline treatments may be adaptive in avoiding exposure to excess photons (Björkman et al. 1988) and leaf temperatures (Andrews et al. 1984) when the sun is near the zenith. Additive effects of shading and salinity on leaf angle, and a small slope of the relationship between log-transformed F_v/F_m and leaf angle suggest that temperature was the more important causal driver.

My models (Table 1, Fig. 7) reflected the role of steep leaf angles in avoiding photoinhibition, and a role for the xanthophyll cycle in protective downregulation, and were consistent with Hypothesis 3. The species- and shading-dependent nature of the relationships presumably reflects the importance of lumen pH and other factors in the development of nonradiative dissipation and depression of F_v/F_m (Gilmore and Yamamoto 1992, 1993; Gilmore and Govindjee 1999; Müller et al. 2001). Nonlinear relationships between reductions in F_v/F_m and the extent of de-epoxidation of the xanthophyll cycle pool are in accordance with other reports (Adams et al. 1994; Cheng 2003).

Xanthophyll cycle pool sizes and DPS reflected historic exposures to excess irradiance, and excess irradiance at the time of sampling, respectively, as in other studies (e.g., Laing et al. 1995). In general, depression of F_v/F_m was associated with increases in the pool size and increases in the midday DPS of the xanthophyll cycle pigments, suggesting a key role for *Z* (and A) in the protective dissipation of excess excitation energy. The pattern of response of xanthophyll cycle pool size to salinity suggested an increase in the capacity for *Z*-associated protective dissipation of excess excitation energy in salinity extremes, except at the highest salinity in *Ae. corniculatum.*

Aegiceras corniculatum and *Av. marina* showed similar responses of the xanthophyll cycle pool size to growth irradiance and had similar leaf angles. Species differences in DPS, and its effect on F_v/F_m were key factors. This is in contrast to mature canopies of Rhizophoraceae, for which species' differences in strategies for photoprotection were manifest as differences in pool size or leaf angle (Lovelock and Clough 1992), suggesting phylogeny or ontogeny may influence strategies of photoprotection. The lesser diurnal depression and relaxation of depression of F_v/F_m, and lesser diurnal de-epoxidation and re-epoxidation of xanthophylls in *Av. marina* than *Ae. corniculatum*, suggests a greater protective, longer-term down-regulation in *Av. marina* and more extreme but transient protective response under excess excitation in *Ae. corniculatum* (Adams et al. 1994).

Predawn depression of F_v/F_m in *Av. marina* and high salinity may be related to the presence of de-epoxidised xanthophylls (as observed in another study of *Av. marina,* Sobrado and Ball 1999), and sustained engagement of xanthophyll cycle-dependent thermal dissipation (Adams et al. 1994; Verhoeven et al. 1997). Such sustained engagement in the dark could occur if the trans-thylakoid pH gradient was maintained by ATP hydrolysis (Gilmore and Yamamoto 1992; Gilmore and Björkman 1995). In leaves of *Av. marina* and seedlings grown under salinity extremes, a sustained higher DPS should result in more rapid induction of nonradiative dissipation of excess excitation energy. In leaves of *Ae. corniculatum* and seedlings grown under moderate salinity, lower predawn DPS may result in slower development of nonphotochemical quenching under conditions of excess excitation, requiring minutes to hours for de-epoxidation (Bilger et al. 1989; Demmig-Adams 1990; Thayer and Björkman 1990; Adams et al. 1994).

An adverse effect of high salinity on the β,β-carotene pathway was suggested by lowered levels of VAZ in *Ae. corniculatum* and lowered levels of Neo in both species in 100% seawater. Greater Z-dependent quenching might therefore be expected in *Av. marina* than in *Ae. corniculatum* in 100% seawater. Reductions in Neo at midday and in high salinity have implications for the synthesis of abscisic acid (Lee and Milborrow 1997; Niyogi 1997; Schwartz et al. 1997).

An adverse effect of high salinity on the β,ε-carotene pathway in *Ae. corniculatum* was suggested by lowered levels of L in this species in 100% seawater, which may have adverse effects on the capacity for nonradiative dissipation of excess excitation energy (Pogson et al. 1998). Increasing growth irradiance resulted in an accumulation of L relative to chl, as in previous studies (Thayer and Björkman 1990; Niyogi 1997), which may enhance nonphotochemical quenching (Niyogi 1997; Pogson et al. 1998). Diurnal midday declines in L under low growth irradiance suggest the possible operation of a lutein-epoxide cycle (Bungard et al. 1999).

Possible ecological significance of species' differences

The two sympatric species showed different extents of chronic and diurnal photoinhibition, diurnal de-epoxidation of the xanthophyll cycle pigments, and effects of high salinity on L and xanthophyll cycle pool sizes. Species' differences may be of ecological significance, and be indicative of their relative performance along salinity and irradiance gradients in habitats where they co-occur.

The greater long-term down-regulation and lesser diurnal depression of the photochemical efficiency of PSII in leaves of *Av. marina* suggest this species will be favoured under conditions of persistent excess photon dose, for example, during periods of consecutive sunny days, in exposed environments, and at high salinities. However, this strategy may result in lower net carbon gain under low excitation pressure, such as in the understorey or in habitats with moderate salinities. Greater diurnal depressions in the photochemical efficiency of PSII, but lesser long-term downregulation in leaves of *Ae. corniculatum* suggest this species will be favoured under conditions where excess excitation is less frequent, for example during cloudy weather, in shaded environments, and at low salinities. However, this strategy may result in lower net carbon gain under high excitation pressures, such as in exposed sites or in habitats with high salinities.

The chlorophyll fluorescence characteristics of leaves provide a rapid assay of the activity of PSII, and a useful noninvasive tool for monitoring the responses of plants to stress in numbers appropriate to addressing ecological questions (Ball et al. 1994). Confidence in upscaling of the leaf level patterns observed here to whole plants and communities requires additional measurements of

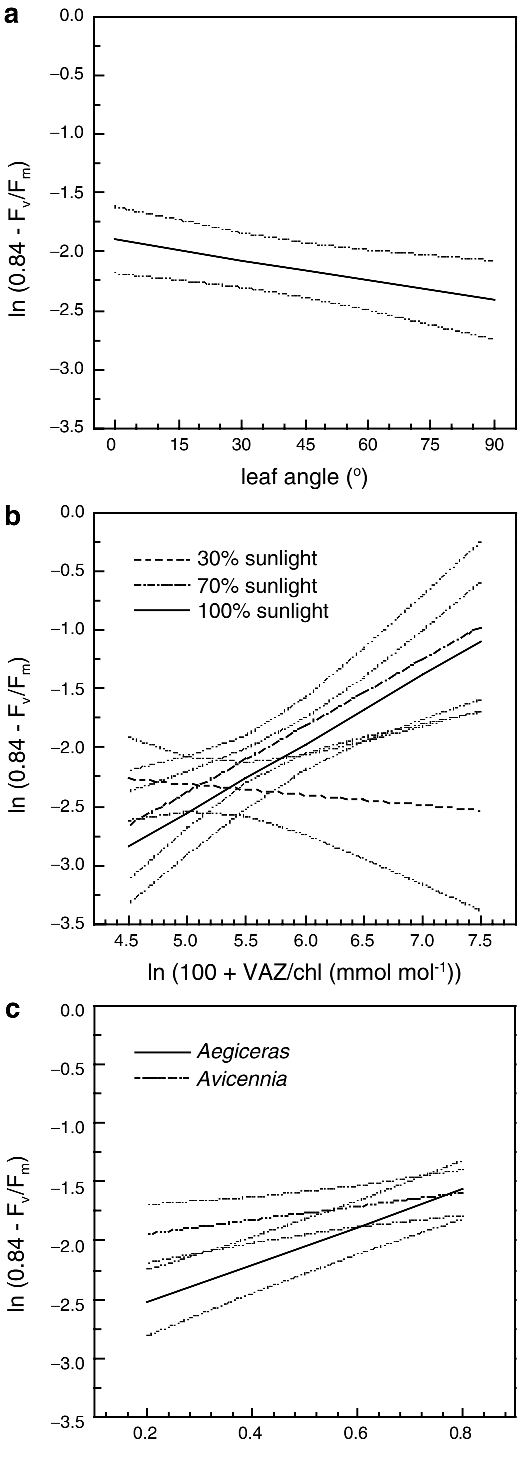

Fig. 7 Predicted values of transformed F_v/F_m for given values of a range of measures of photoprotection, for the models given in Table 1. *Dotted lines* show the 95% confidence intervals in all cases. **a** Effect of leaf angle at midday for leaves of *Ae. corniculatum* in 100% sunlight with the mean xanthophyll cycle pool size and mean de-epoxidation state. **b** Effect of xanthophyll cycle pool size under three shading treatments at midday for leaves of *Ae. corniculatum* with an angle of 45° and the mean de-epoxidation state. **c** Effect of de-epoxidation state in *Ae. corniculatum* and *Av. marina* at midday for leaves with an angle of 45° and the mean xanthophyll cycle pool size

processes at intermediate scales (Jarvis 1995; Körner 1995). I have therefore explored the relationship between the responses reported here and species-specific interactive effects of light and salinity on the net assimilation rate for the whole canopy, and the growth, survivorship and distribution of the two species in the field (Christian 1999).

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