Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature

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Received 8 December 2004; received in revised form 24 April 2005; accepted 15 September 2005

Abstract

This study uses chlorophyll a fluorescence to examine the effect of environmentally relevant (1–4 h) exposures of thermal stress (35–45 °C) on seagrass photosynthetic yield in seven tropical species of seagrasses. Acute response of each tropical seagrass species to thermal stress was characterised, and the capacity of each species to tolerate and recover from thermal stress was assessed. Two fundamental characteristics of heat stress were observed. The first effect was a decrease in photosynthetic yield (Fv/Fm) characterised by reductions in F and Fm. The dramatic decline in Fv/Fm ratio, due to chronic inhibition of photosynthesis, indicates an intolerance of Halophila ovalis, Zostera capricorni and Syringodium isoetifolium to ecologically relevant exposures of thermal stress and structural alterations to the PhotoSystem II (PSII) reaction centres. The decline in Fm represents heat-induced photoinhibition related to closure of PSII reaction centres and chloroplast dysfunction. The key finding was that Cymodocea rotundata, Cymodocea serrulata, Halodule uninervis and Thalassia hemprichii were more tolerant to thermal stress than H. ovalis, Z. capricorni and S. isoetifolium. After 3 days of 4 h temperature treatments ranging from 25 to 40 °C, C. rotundata, C. serrulata and H. uninervis demonstrated a wide tolerance to temperature with no detrimental effect on Fv/Fm, qN or qP responses. These three species are restricted to subtropical and tropical waters and their tolerance to seawater temperatures up to 40 °C is likely to be an adaptive response to high temperatures commonly occurring at low tides and peak solar irradiance. The results of temperature experiments suggest that the photosynthetic condition of all seagrass species tested are likely to suffer irreparable effects from short-term or episodic changes in seawater temperatures as high as 40–45 °C. Acute stress responses of seagrasses to elevated seawater temperatures are consistent with observed reductions in above-ground biomass during a recent El Niño event.

Keywords: Seagrass; Elevated temperature; Global warming; El Niño; Tropical; Australia

1. Introduction

Shallow water tropical seagrasses are exposed to a range of environmental stresses, including high solar radiation, ultra-violet radiation, desiccation and temperature fluctuations. Fluctuations in seasonal seawater temperatures in tropical habitats range from 19.8 to 41 °C (McKenzie, 1994; McKenzie and Campbell, 2004). Critical thermal stress has been reported in temperate seagrasses at temperatures exceeding 35 °C (Bulthuis, 1983; Ralph, 1998), but few studies have examined temperature responses of tropical seagrass species (Fong and Harwell, 1994; Terrados and Ros, 1995). Tropical species of seagrasses generally increase their photosynthesis at elevated temperatures (Perez and...
Romero, 1992; Terrados and Ros, 1995). However, temperatures rising above the normal upper limit of 35 °C can inhibit carbon production in plants because high temperatures bring about increased respiration (Bulthuis, 1983; Ralph, 1998) and photosynthetic enzyme breakdown (Bruggeman et al., 1992; Ralph, 1998). Light requirements for carbon production are also greater at higher temperatures because of increased compensation irradiance (Bulthuis, 1987).

Thermal stress has a significant impact on the biogeographical distribution and condition of seagrass meadows (Bulthuis, 1983; Hillman et al., 1989; McMillan, 1984; Ralph, 1998). Climatic factors influencing the ambient water temperature of seagrass meadows include global temperature oscillations (e.g. El Niño), global warming, seasonal and diurnal fluctuations (Short and Neckles, 1999). Anthropogenic causes of seawater temperature increases include the release of heated effluents into shallow embayments where seagrasses proliferate (Thorhaug et al., 1978). Seawater and air temperatures are factors that limit the upper distributional limits of seagrass meadows. Seagrasses in shallow pools of water are not only exposed to elevated temperatures during midday sun exposure, but they are also subject to desiccation during low tidal periods and high PAR and UV radiation (Dawson and Dennison, 1996; Durako and Kunzelman, 2002). Mean sea temperature increases of up to 2 °C may have a severe impact on species of seagrass that survive at the upper limit of their thermal tolerance (Ralph, 1998). Increases in seawater temperatures of up to 10 °C above the seasonal average can occur in tropical shallow water pools especially during spring low tides and during midday solar exposure (McKenzie and Campbell, 2004; www.seagrasswatch.org). During these events seagrasses may be exposed to elevated seawater temperatures for periods of 3 to 4 h. High seawater temperatures and desiccation have negatively affected seagrass meadows in a number of areas worldwide (Seddon and Cheshire, 2000; Walker and Cambridge, 1995; Erftemeijer and Herman, 1994) with one episode of seagrass loss linked to an El Niño climatic pattern (Seddon and Cheshire, 2000).

The effects of thermal stress on photosynthesis, productivity and morphology of seagrasses have been examined mostly in temperate habitats (Drew, 1979; McMillan and Phillips, 1979; McMillan, 1983; Bulthuis, 1987). These studies suggest that changes in seawater temperature on seagrass habitats are dependent on a number of interacting factors including: duration of exposure, acclimatisation (thermal history), light regime and leaf maturity (Bulthuis, 1987; Seddon and Cheshire, 2000). Fong and Harwell (1994) suggest that the productivity of tropical seagrass species starts to decline above 30 °C but few studies have examined the thermal tolerance of tropical seagrass species to changes in seawater temperature. Thorhaug et al. (1978) reported that at temperatures elevated 3–4 °C above ambient, Thalassia testudinum showed evidence of reduced standing crop and productivity, and that tropical plants were more tolerant than subtropical plants to elevated temperature. However, some species (e.g. Halophila ovalis) with a wide geographical tolerance have a broad temperature tolerance (Ralph, 1998) but tolerance of tropical seagrass species to periods of high temperature exposure is less studied.

We used measures of effective photosynthetic quantum yield to examine the effect of 1–4 h exposures of thermal stress (35–45 °C) on seagrass photosynthetic yield over a 3-day period. A pulse amplitude modulated (PAM) fluorometer was used to measure effects of thermal stress on chlorophyll a fluorescence within PhotoSystem II (PSII), the most temperature sensitive component of the photosynthetic apparatus (Ralph, 1998). The objectives of the present study were to: characterise the acute response of 7 species of tropical seagrass to thermal stress; assess each species’ capacity to tolerate thermal stress and to assess the capacity of tropical seagrasses to recover from thermal stress.

2. Methods

Six species of seagrass (Cymodocea rotundata, Cymodocea serrulata, Halodule uninervis, Halophila ovalis, Syringodium isoetifolium, Thalassia hemprichii) were collected at Green Island in northern Queensland, Australia (38°15′ S 145°21′ E). Zostera capricorni was collected from Cairns Harbour, Queensland, Australia (16°53′ S, 145°46′ E) (Fig. 1). All plants were collected in the afternoon prior to the start of the experiment and acclimated overnight in oxygenated seawater maintained at 26 °C.

Three temperature treatments were conducted over three 5-day periods in May 2003. Each experiment consisted of a control temperature treatment at 26 °C (n = 8), representing the ambient temperature of seawater at Green Island in May, and three elevated temperature treatments (each n = 8) consisting of plants exposed to 35, 40 or 45 °C. Plants were held in separate 2 l containers with oxygenated re-circulating seawater. All containers were immersed within a larger aquarium (60 l), where they were acclimated overnight (26 °C).

On each experimental day, the 2 l containers of plants were directly transferred to the re-circulating...
treatment seawater system at 1100 h, which was then elevated to the treatment temperature over a period of 15–30 min. Plants were exposed to treatment temperatures (35, 40 or 45 °C) for 4 h (from 1100 to 1500 h).

The photochemical efficiency of PhotoSystem II (PSII) and the effective quantum yield of photochemistry ($\Delta F/F_{m}$) were evaluated by subjecting light adapted leaves to saturating pulses of light using a pulse ampli-
tude modulated (PAM) fluorometer (Diving-PAM) (Walz, Germany). For each seagrass plant the basic parameters of chlorophyll fluorescence \( (F, F_{m'}) \) were measured on the leaf shoot adjacent to the meristem, on 8 replicate leaves of each seagrass species, every hour from 1100 to 1500 h. The mid-section of each leaf (3 cm from meristem) was held in a leaf clip (Walz, DIVING LC) and fluorescence measurements were made underwater with the light probe joined to the leaf clip. A weak pulsed red light (<1 μmol quanta m\(^{-2}\) s\(^{-1}\)) was applied to determine \( F \) in an illuminated state. A saturating pulse (800 ms of 8000 μmol quanta m\(^{-2}\) s\(^{-1}\) PAR) was then applied to \( F_{m'} \) in an illuminated state. The change in fluorescence \( (F_m - F_v) \) caused by the saturating pulse \( (F_{m'} - F) \) in relation to the maximal fluorescence \( (F_{m'}) \) is a measure of quantum yield \( (\text{Eq. (1)}) \). The effective quantum yield is given by \( \Delta F/F_m \) (Eq. (1)). The separation of photochemical quenching (qP) and non-photochemical quenching (qN) of chlorophyll fluorescence provides insight to the regulatory processes occurring within the photosynthetic apparatus (Bolhär-Nordenkampf et al., 1989). The quenching characteristics at each time interval were calculated from \( F_v, F, \) and \( F_{m'} \) (Bolhär-Nordenkampf et al., 1989).

\[
Y = \frac{(F_{m'} - F')}{F_m} = \frac{F_v}{F_{m'}} \quad (1)
\]

where

\( Y \) = effective quantum yield

\( F_{m'} \) = maximal fluorescence in light adapted leaves

\( F' \) = initial fluorescence in light adapted leaves

\( F_v = (F_{m'} - F') \)

At 1500 h all replicate plants were returned to acclimation conditions at 26 °C overnight. On days 2 and 3, the experiment was repeated. On days 4 and 5 all plants were retained at 26 °C to determine recovery rates following thermal stress. Parameters of chlorophyll fluorescence \( (F, F_{m'}) \), photochemical quenching (qP) and non-photochemical quenching (qN) were made on 8 replicate leaves of each species at 1100 h on each day for control and treatment plants.

The proportion of incident irradiance absorbed by leaves was determined for 5 replicate leaves for each species. Each leaf was placed over the light sensor of the Diving-PAM and incident saturating PAR recorded with and without leaf according to the method described by Schwarz and Hellblom (2002). The light path distance was 3 mm, as that gave consistent optimal readings of quantum yield of 0.70–0.80 for dark adapted leaves healthy leaves. The measures were made to determine if there were differences in leaf absorbance that may explain responses to seawater temperature exposure.

Mean quantum yield values at 50 h (end of temperature exposure period) and 96 h (end of recovery period) were analysed relative to mean control values to provide a summary of plant responses at the 3 temperature exposure treatments. A three way ANOVA (SYSTAT vers. 10.2) was used to test for the effect of species, experiment (35, 40 and 45 °C) and treatment (control, temperature exposed). Data were arcsin square-root transformed prior to analysis.

3. Results

3.1. Absorbance factors

The proportion of incident irradiance absorbed by leaves ranged from 0.70 to 0.79 for \( C. \) serrulata, \( C. \) rotundata, \( H. \) uninervis, \( T. \) hemprichii, \( S. \) isoetifolium and less than 0.70 for \( H. \) ovalis and \( Z. \) capricorni (Table 1). Habitat characteristics of all species are also presented (Table 1).

3.2. Quantum yield relative to controls

The percentage of effective quantum yield of plants exposed to temperature treatments relative to control

<table>
<thead>
<tr>
<th>Species</th>
<th>Absorbance factor (± 95% confidence interval)</th>
<th>Ecological niche</th>
<th>Substrate</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymodocea rotundata</td>
<td>0.72 ± 0.02</td>
<td>Mid-intertidal–shallow subtidal</td>
<td>Sand</td>
<td>Green Island reef-flat</td>
</tr>
<tr>
<td>Cymodocea serrulata</td>
<td>0.79 ± 0.02</td>
<td>Shallow subtidal</td>
<td>Sand</td>
<td>Green Island lagoon</td>
</tr>
<tr>
<td>Halodule uninervis</td>
<td>0.70 ± 0.03</td>
<td>Mid-intertidal–shallow subtidal</td>
<td>Sand</td>
<td>Green Island reef-flat</td>
</tr>
<tr>
<td>Halophila ovalis</td>
<td>0.56 ± 0.04</td>
<td>Upper intertidal–deep subtidal</td>
<td>Sand</td>
<td>Green Island reef-flat</td>
</tr>
<tr>
<td>Thalassia hemprichii</td>
<td>0.78 ± 0.01</td>
<td>Mid-intertidal–shallow subtidal</td>
<td>Sand</td>
<td>Green Island reef-flat</td>
</tr>
<tr>
<td>Zostera capricorni</td>
<td>0.48 ± 0.03</td>
<td>Upper intertidal–shallow subtidal</td>
<td>Mud</td>
<td>Cairns Harbour mud-bank</td>
</tr>
<tr>
<td>Syringodium isoetifolium</td>
<td>0.73 ± 0.04</td>
<td>Shallow-deep subtidal</td>
<td>Sand</td>
<td>Green Island lagoon</td>
</tr>
</tbody>
</table>
treatments was calculated at 50 and 96 h. At 50 and 96 h the exposure/control percentage of effective quantum yield of *C. rotundata*, *C. serrulata*, *H. uninervis* and *T. hemprichi*, at 35 and 40 °C, ranged from 86% to 105%; for *H. ovalis* and *S. isoetifolium* these values ranged from 43% to 69% and 61% to 81%, respectively (Fig. 2). At 45 °C (50 and 96 h) all species had effective quantum yield values 5–37% of controls (Fig. 2). Response was significantly different between control and exposed for each treatment and for all species at both 50 (Table 2) and 96 h (Table 3) treatments.

3.3. Fluorescence values (*F* and *F*ₘₐₓ)

For light adapted *C. rotundata*, *C. serrulata* and *H. uninervis* the initial minimum fluorescence (*F*) was relatively steady when exposed to 35 °C, 40 °C and respective control treatments during the 3-day 4 h exposures (Fig. 3). A few times during the 5-day experiment lower *F* values were evident for *S. isoetifolium* and *T. hemprichi* at 35 °C compared with controls, and for *H. ovalis*, *S. isoetifolium* and *T. hemprichi* at 40 °C compared to controls (Fig. 3). At 45 °C the decline in *F*, compared with control treatments, occurred after 24 h in all species and persisted during the 2-day recovery (25 °C) period.

No difference in maximum fluorescence (*F*ₘₐₓ) could be detected between control (26 °C) and thermal treatments (35, 40 °C) for *C. rotundata*, *C. serrulata* and *H. uninervis* during the 3-day exposure and 2-day recovery (25 °C) period (Fig. 4). Similarly no difference between control and 35 °C treatments was found for *Z. capricorni* and *H. ovalis*. In contrast lower *F*ₘₐₓ values relative to controls were found for *T. hemprichi* and *S. isoetifolium* at 35 °C and for *T. hemprichi*, *S. isoetifolium* and *H. ovalis* at 40 °C during the 3-day thermal exposure and 2-day recovery period. For *Z. capricorni* lower *F*ₘₐₓ values at 40 °C were found relative to controls during the final day of thermal treatment and day 1 of recovery (Fig. 4). For all species *F*ₘₐₓ declined by greater than 50% in the first 2 h of 45 °C exposure, and during day 2 of 45 °C exposure *F*ₘₐₓ declined to less than 80% of the initial value in all species (Fig. 4).

3.4. Effective quantum yield (*ΔF*/*F*ₘₐₓ)

For *C. rotundata*, *C. serrulata*, *H. uninervis* and *Z. capricorni* the effective quantum yield was relatively steady when exposed to 35 °C, 40 °C and respective control (26 °C) treatments during the 3-day 4 h exposures (Fig. 5). At 40 °C, *ΔF*/*F*ₘₐₓ values for *S. isoetifolium*, *Z. capricorni* and *H. ovalis* were 10–35% lower than control plants (Fig. 5). A decline in *H. ovalis* *ΔF*/*F*ₘₐₓ to 50% of initial values occurred during the 2-day post-thermal stress recovery period (Fig. 5). At 45 °C a rapid decline in *ΔF*/*F*ₘₐₓ after 1 h was observed for all species and persisted during the 3-day exposure and 2-day recovery period.

A significant three way interaction between species, experiment and treatment at 50 h was explained by similar yield values of all species at 35 °C relative to controls, lower yield values for *H. ovalis* and *S. isoetifolium* at 40 °C compared to all other species and controls and lower values for all species at 45 °C.
compared to controls. A similar pattern was evident at 96 h, except that *S. isotetifolium* at 40°C was not lower than controls suggesting recovery of this species after exposure to 25°C.

3.5. Quenching coefficients (qP and qN)

There was no apparent difference in qP between control plants and those exposed to 35°C. Similarly for plants of *C. rotundata*, *C. serrulata*, *H. uninervis*, *S. isotetifolium*, *T. hemprichii* and *Z. capricorni* exposed to 40°C, photochemical quenching (qP) values did not differ from respective control (26°C) treatments during the 3-day 4 h exposures (Fig. 6). For *H. ovalis* and qP values at 40°C were up to 20% lower compared with controls. A decline in *H. ovalis* qP to 75% of initial values occurred during the 2-day post-thermal stress recovery period (Fig. 6). At 45°C a rapid decline in qP after 1 h was observed for all species and persisted during the 3-day exposure and 2-day recovery period. Non-photochemical quenching remained near 0 for most species at 35°C, 40°C and respective control (26°C) treatments (Fig. 7). At 40°C non-photochemical quenching (qN) ranged between 0.2 and 0.6 on days 1 and 3 and increased to 0.8 during the recovery period. By day 2 at 45°C non-photochemical quenching (qN) was 10–12 fold higher than respective control (25°C) treatments.

4. Discussion

The key finding of this study was that *C. rotundata*, *C. serrulata*, *H. uninervis* and *T. hemprichii* were more tolerant to thermal stress than *S. isotetifolium*, *Z. capricorni* and *H. ovalis*. After 3 days of 4 h temperature treatments ranging from 26 to 40°C, *C. rotundata*, *C. serrulata* and *H. uninervis* demonstrated a wide tolerance to temperature with no effect on $\Delta F/F_m$ responses. These three species are restricted to subtropical and tropical waters and their tolerance to seawater temperatures of 40°C is likely to be an adaptive response to high temperatures commonly occurring at low tides and peak solar irradiance. Both *C. rotundata* and *C. serrulata* were also more tolerant for the initial 4 h treatment of 45°C compared with all other species. Experimental responses in the present study occurred at less than 100 A mol m$^{-2}$ s$^{-1}$, below saturation for these species (unpubl data), and the sensitivity of seagrass species is likely to be greater at high in situ irradiances during peak solar intensities. Indeed Ralph (1999) found that elevated light and osmotic stress increased the sensitivity of *H. ovalis* to thermal

### Table 2

Three way ANOVA of the effects of temperature (control vs exposed), treatment (35, 40, 45°C) and species on effective quantum yield in 8 seagrass species at 50 h after exposure to temperature treatments for 4 h over a 3-day period (n = 328)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-squares</th>
<th>df</th>
<th>Mean-square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>7.862</td>
<td>2</td>
<td>3.931</td>
<td>302.238</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment</td>
<td>5.064</td>
<td>1</td>
<td>5.064</td>
<td>389.344</td>
<td>0.000</td>
</tr>
<tr>
<td>Species</td>
<td>0.203</td>
<td>6</td>
<td>0.034</td>
<td>2.601</td>
<td>0.018</td>
</tr>
<tr>
<td>Temperature × treatment</td>
<td>7.089</td>
<td>2</td>
<td>3.544</td>
<td>272.531</td>
<td>0.000</td>
</tr>
<tr>
<td>Temperature × species</td>
<td>0.550</td>
<td>12</td>
<td>0.046</td>
<td>5.327</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment × species</td>
<td>0.137</td>
<td>6</td>
<td>0.023</td>
<td>1.758</td>
<td>0.108</td>
</tr>
<tr>
<td>Temperature × treatment × species</td>
<td>0.286</td>
<td>12</td>
<td>0.024</td>
<td>1.830</td>
<td>0.043</td>
</tr>
<tr>
<td>Error</td>
<td>3.720</td>
<td>286</td>
<td>0.013</td>
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<td></td>
</tr>
</tbody>
</table>

Data were arcsin square-root transformed prior to analysis.

### Table 3

Three way ANOVA of the effects of temperature (control vs exposed), treatment (35, 40, 45°C) and species on effective quantum yield in 8 seagrass species at 96 h after exposure to temperature treatments for 4 h over a 3-day period (n = 328)

<table>
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<th>Source</th>
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<th>Mean-square</th>
<th>F-ratio</th>
<th>P</th>
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<tbody>
<tr>
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<td>4.410</td>
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<tr>
<td>Treatment</td>
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<td>6.968</td>
<td>581.836</td>
<td>0.000</td>
</tr>
<tr>
<td>Species</td>
<td>0.671</td>
<td>6</td>
<td>0.112</td>
<td>9.342</td>
<td>0.000</td>
</tr>
<tr>
<td>Temperature × treatment</td>
<td>7.089</td>
<td>2</td>
<td>3.544</td>
<td>272.531</td>
<td>0.000</td>
</tr>
<tr>
<td>Temperature × species</td>
<td>0.550</td>
<td>12</td>
<td>0.046</td>
<td>5.327</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment × species</td>
<td>0.137</td>
<td>6</td>
<td>0.023</td>
<td>1.758</td>
<td>0.108</td>
</tr>
<tr>
<td>Temperature × treatment × species</td>
<td>0.286</td>
<td>12</td>
<td>0.024</td>
<td>1.830</td>
<td>0.043</td>
</tr>
<tr>
<td>Error</td>
<td>3.533</td>
<td>295</td>
<td>0.012</td>
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</table>

Data were arcsin square-root transformed prior to analysis.
Fig. 3. Minimum fluorescence (F) for each seagrass species exposed to control (○) (26 °C) and treatment (■) (4 h at 35, 40 and 45 °C) seawater temperatures, for each treatment day and subsequent recovery period. The break in the data-line represents the end of each exposure period.
Fig. 4. Maximum fluorescence (Fm') for each seagrass species exposed to control (○) (26 °C) and treatment (■) (4 h at 35, 40 and 45 °C) seawater temperatures, for each treatment day and subsequent recovery period. The break in the data-line represents the end of each exposure period.
Fig. 5. Effective quantum yield ($Y$) for each seagrass species exposed to control (○) (26°C) and treatment (■) (4 h at 35, 40 and 45°C) seawater temperatures, for each treatment day and subsequent recovery period. The break in the data-line represents the end of each exposure period.
Fig. 6. Photochemical quenching (qP) for each seagrass species exposed to control (○) (26 °C) and treatment (■) (4 h at 35, 40 and 45 °C) seawater temperatures, for each treatment day and subsequent recovery period. The break in the data-line represents the end of each exposure period.
Fig. 7. Non-photochemical quenching (qN) for each seagrass species exposed to control (○) (26 °C) and treatment (■) (4 h at 35, 40 and 45 °C) seawater temperatures, for each treatment day and subsequent recovery period. The break in the data-line represents the end of each exposure period.
stress, suggesting that seagrass species may be even less tolerant to high seawater temperatures during midday saturating light exposure.

Symptoms of thermal stress found for *H. ovalis*, *S. isoetifolium* and *Z. capricorni* at 40 °C suggest a lower thermal tolerance and a susceptibility to ecophysiological damage when climatic conditions result in atypically high seawater temperatures. All are eurythermal species with a broad thermal tolerance (10–35 °C) found growing in both tropical (McKenzie, 1994) and temperate waters (Ralph, 1998; Campbell et al., 2003a,b). Their low tolerance to high temperatures (>35 °C) suggests that their photosynthetic condition would suffer detrimental effects from episodic increases in water temperatures greater than 35 °C. Ralph (1998) also found that *H. ovalis* was intolerant to temperatures above 37 °C over a 5 h period. The quantity of light absorbed by leaves of *H. ovalis* is lower than other species examined (Table 1) suggesting it has lower leaf thickness and chlorophyll pigmentation. These characteristics may in part explain its susceptibility to temperature relative to other species. McMillan (1983) also found that *H. ovalis*–minor complex was susceptible to temperature with no survival of small leaved cultures after 18 h exposure to 39 °C and similarly no survival of larger leaf cultures after 48 h exposure to 39 °C.

The susceptibility of *S. isoetifolium* to temperature may be associated with its cylindrical morphology (high surface area to volume ratio), the presence of a thin leaf cuticle and thin vascular-bundle cell walls (Kuo, 1993). Such leaf anatomy and ultrastructure is advantageous for nutrient uptake and gaseous exchange and this species has been found to have a relatively high production rate (0.92 g DW m⁻² d⁻¹) compared with other species used in this experiment (0.01–0.50 g DW m⁻² d⁻¹) (Duarte and Chiscano, 1999). The morphology, however, would also expose a proportionally high area of leaf tissue per volume to stress from increased seawater temperature. It is a species that previously has been restricted to subtidal waters because of an intolerance to high temperatures (i.e. 40 °C) in intertidal reef habitats (McMillan, 1983; Bridges and McMillan, 1986). McMillan (1983) reported that both *S. isoetifolium* and *S. filiforme* cultures were least tolerant to temperatures of 39 °C relative to 7 other tropical genera. This susceptibility was attributed to its lack of distribution in shallow waters that are exposed to high air and seawater temperatures. At elevated temperatures damage to photosynthetic tissue is likely to arise from a loss of specific enzyme activity and changes in transport of molecules and to membrane function (Ralph, 1998). Damaged cells are characterised by a brownish-black discoloration of cell wall and contents (Biebl and McRoy, 1981; Walker and Cambridge, 1995).

We observed two fundamental characteristics of heat stress to seagrasses using saturation PAM fluorometry. The first effect was a decrease in photosynthetic yield characterised by reductions in *F* and *F₉/₁₅*'. The dramatic decline in *F₉/₁₅*' ratio, due to chronic inhibition of photosynthesis, indicates an intolerance of *H. ovalis*, *S. isoetifolium* and *Z. capricorni* to ecologically relevant exposures of thermal stress. The decline in *F* and *F₉/₁₅*' is indicative of structural alterations to the PSI reaction centres that can take several days to recover (Ralph, 1998; Macinnis-Ng and Ralph, 2004). The decline in *F₉/₁₅*' represents heat-induced photoinhibition related to closure of PSI reaction centres and chloroplast dysfunction (Havaux, 1994). Such a response is likely due to a combination of factors including increased respiration, photoinhibition and enzyme breakdown. For both *Z. capricorni* and *H. ovalis* the inability to recover following 3 days of elevated temperature exposure (40 and 45 °C) suggests irreparable damage to chloroplast structure and PSI function. The resulting burden to the carbon balance of the plant would limit the survival potential of seagrass leaves at temperatures greater than 40 °C.

The second effect was an increase in non-photochemical quenching, coupled with a decrease in photochemical quenching. Non-photochemical quenching is considered to reflect a mechanism for photo-protection which is designed to protect the over-reduction of the photosynthetic electron transport chain by dissipation of excess absorbed light energy in the PSI antenna system as heat (Demmig-Adams, 1990). Increases in non-photochemical quenching in *H. ovalis* after 96 h exposure to temperatures greater than 30 °C have been reported (Ralph, 1998), and the current study shows that non-photochemical quenching rapidly increased at temperatures of 40 °C and above. Rapid and complete decline of qP in three species at 40 °C and in all 7 species at 45 °C suggests chloroplast membrane damage eliminating electron transport and/or temperature-dependent enzyme inactivation (Bruggeman et al., 1992). As thermal stress was observed in all 7 seagrass species after 1 h at 45 °C, heat induced photoinhibition and closure of PSI reaction centres occurred rapidly and was irreparable. Damage to PSI function in heat stress has been reported for 1 species of seagrass (Ralph, 1999) and some corals (Jones et al., 1998). The lack of recovery by all species
at 45 °C suggests that photosynthetic enzymes have been inactivated or damaged (Bruggeman et al., 1992) and acclimation to these temperatures for short (4 h) exposure periods is unlikely. The resultant reduced consumption of ATP can lead to an accumulation of stored energy in the thylakoid membrane and an increase in the proton gradient within PSI (Ralph, 1998). Excess absorbed light energy unable to be used in the photosynthetic process is dissipated with the resulting increase in non-photochemical quenching (qN) and decrease in photochemical quenching (qP), as demonstrated in this study. The recovery of Z. capricorni photosynthetic yield after exposure to high temperatures indicates a protective role of PSII down-regulation consistent with that found previously in H. ovalis (Ralph, 1998).

Results from the present study show that relatively short exposures (<4 h) of elevated seawater temperatures can damage seagrass physiology. Such conditions are most likely to occur from November to May when high solar irradiance and high seawater temperatures combine to inhibit photosynthesis. Such findings are consistent with declines in seagrass abundance during the recent climatic anomalies in 2001/2002 in northern Queensland (Roelfs et al., 2003; Campbell et al., 2003a,b). Average seawater temperatures during the austral summer of 2001/2002 increased by 1–2 °C (Berkelmans et al., 2004; Jones et al., 2004) and elevated seawater temperatures (>35 °C) were recorded in shallow pools at low tide (McKenzie and Campbell, 2004). Meteorological data suggests that negative tides were infrequent during solar noon periods in the austral summer of 2001/2002, suggesting that exposure and desiccation of seagrass leaves at low tide could not fully explain seagrass dieback.

The results of temperature experiments here suggest that the photosynthetic condition of all seagrass species tested is likely to suffer irreparable effects from short-term or episodic changes in seawater temperatures as high as 40–45 °C. Species differ in their thermal tolerance making some (e.g. H. ovalis, S. isoetifolium, Z. capricorni) more susceptible to thermal stress than others. Tolerance to temperatures up to 40 °C in C. rotundata, C. serrulata, H. uninervis and T. hemprichii demonstrates an adaptive tolerance of PSII reaction centres and chloroplasts to temperature increases. H. ovalis, S. isoetifolium and Z. capricorni would appear least thermo-tolerant of all species examined and that may explain in part their biogeographical distribution and recent loss in some tropical habitats in north Queensland (unpubl data).

It may at first appear unusual that both H. ovalis and Z. capricorni were the species least tolerant to temperatures of ≥40 °C, as species appear well adapted to grow in upper intertidal areas where shallow pools of water can exceed 40 °C. The results of temperature experiments here suggest that they cannot tolerate extended periods (>4 h) of temperatures ≥40 °C. Z. capricorni is near the northern limit of its distribution in north Queensland, suggesting it may be intolerant of seawater temperatures at latitudes above 10° S. In contrast H. ovalis and S. isoetifolium are widespread throughout tropical latitudes and morphological characteristics would appear to limit their tolerance to high seawater temperatures. The ability of H. ovalis leaves to grow and turnover rapidly may in part compensate for shoot loss during excessive seawater temperature rises. The small leaves of H. ovalis also flatten on the sediment surface during low tide, a feature that keeps them moist and protects them from desiccation (Björk et al., 1999). On the other hand S. isoetifolium is restricted to shallow subtidal waters and its ecological niche suggests a genetic intolerance to excessive temperatures, although other ecological factors are likely to determine the niche of seagrasses within habitats experiencing extreme changes in environmental conditions.

An understanding of the thermal tolerance of tropical species of seagrass is important to understand the stress symptoms of seagrass ecosystems to climatic changes that may lead to changes in seagrass species composition and seagrass decline. Implications of the loss of species and change in species dominance have ramifications for herbivorous marine animals that target seagrass for food and animals that use seagrass areas for habitat.

Acknowledgments

We thank Rudi Yoshida and Kate Pritchard for their assistance in conducting laboratory experiments and Rob Coles for providing review comments. This work was supported by the Australian Cooperative Research Centre Program through the CRC Reef Research Centre, and the Department of Primary Industries and Fisheries, Queensland. [SS]

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