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Photosynthetic response of laboratory-cultured *Halophila ovalis* to thermal stress

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ABSTRACT: Chlorophyll *a* fluorescence was able to rapidly detect responses of laboratory-cultured *Halophila ovalis* to acute changes in temperature. Six heating (27.5, 30.0, 32.5, 35.0, 37.5 and 40.0°C) and 6 chilling (10.0, 12.5, 15.0, 17.5, 20.0 and 22.5°C) stress levels were used over a 96 h exposure period, followed by a 5 d recovery period, to provide a comprehensive measure of the overall thermal stress effects and responses. The acute (5 h) response of *H. ovalis* to thermal shock was characterised by a change in photochemical quenching, whilst after 96 h the effective and maximum quantum yields were able to detect temperature changes of ±2.5°C. Maximum fluorescence declined for both heating and chilling, whilst minimum fluorescence was stable for chilling and increased with moderate heating. *H. ovalis* was susceptible to thermal stress outside the optimum photosynthetic range of 25 to 30°C, where extreme temperatures (10.0, 12.5, 37.5 and 40°C) caused a complete collapse of the PSII electron transport system. When thermal stress was applied in darkness, chlorophyll *a* fluorescence was not able to detect the onset of thermal stress (except at 40.0°C). *H. ovalis* tolerated thermal shock from 15 to 30°C for up to 96 h, and was able to completely recover on return to standard growth conditions.

KEY WORDS: Seagrass · Thermal stress · Chlorophyll fluorescence

INTRODUCTION

The wide thermal tolerance of seagrasses enables them to inhabit regions from subarctic coasts, beneath winter ice (Hudson Bay, Canada, 0°C), to rock pools (40°C) in the tropics (Drew 1979, Bulthuis 1983, 1987). High water temperature is frequently linked with shallow water bodies, usually limiting the minimum depth of the seagrass growth (Hillman et al. 1989). Fluctuations in seasonal water temperature of seagrass habitats can be quite large; for example, temperate southeastern Australian waters range from 17 to 23°C (Womersley 1981), whereas in Chesapeake Bay (similar latitude) temperatures range from 8 to 30°C (Evans et al. 1986).

Thermal stress, whether of anthropogenic or natural origin, has a significant impact upon the biogeographical distribution and health of seagrass meadows (Bulthuis 1983, Hillman et al. 1989). Factors capable of modifying the ambient water temperature of seagrass meadows may include: global temperature oscillations (e.g. El Niño), global warming, seasonal and diurnal fluctuations, and heated effluent water from electricity generation and industrial processing plants (Edwards 1995). Seagrass ecology is strongly influenced by thermal conditions; this is well documented where cooling waste-water discharge into estuaries and lakes can increase the ambient water temperature by up to 10°C (Thorhaug et al. 1978, King & Holland 1986, Marshman & Hodgson 1991). Heated effluent water has also been found to alter the local distribution of seagrass species, and to reduce the vigour of the surviving meadows (King & Holland 1986).

As a result of global warming, it has been postulated that sea temperature could rise by 2°C by 2100 (Edwards 1995). Since tropical seagrasses are generally thought to be stenothermal (den Hartog 1970), a minor temperature increase could have a severe impact on the more vulnerable species where they exist at the upper limit of their thermal tolerance.

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Thorhaug et al. (1978) demonstrated that a rise of 4 to 5°C would have dramatic effects on the global distribution of seagrasses. A recent example of mass decline occurred in Florida in 1987, with the loss of 4000 ha of *Thalassia testudinum* that was linked to anthropogenic thermal stress (Edwards 1995).

The effects of thermal stress on the photosynthesis, productivity and morphology of seagrasses (mainly *Zostera marina*) have been widely studied (Drew 1979, McMillan & Phillips 1979, McMillan 1983). Bulthuis (1987) reviewed many investigations into thermal effects on seagrass growth and distribution. Previous investigations have suggested that the effects of thermal modification to seagrass habitats are dependent on the duration of exposure, relative size of temperature change, acclimatisation (thermal history), actinic light regime, and the maturity of the leaf (Thorhaug et al. 1978, Bulthuis 1987, Larcher 1994).

The normal temperature range for seagrasses is thought to be 5 to 35°C, while the optimum temperature range is 28 to 32°C (Hillman et al. 1989). Maintenance of a positive carbon balance may not be achieved during summer periods when increased temperature increases respiration. Since the compensation irradiance increases with temperature, more light is required at higher temperatures to maintain a positive carbon balance under heated conditions (Bulthuis 1987). Hillman (1985) found that laboratory-cultured *Halophila ovalis* had a maximum productivity (2.1 mg dry wt d^{-1}) at 25°C, whilst growth was restricted below 15°C and ceased at 10°C, although the plants were still photosynthetically active. Growth increased from 10 to 25°C, with a 7-fold increase from 15 to 20°C. The wide thermal tolerance of *H. ovalis* is consistent with its wide biogeographical distribution (den Hartog 1970).

Photosystem II (PSII) is the most temperature-sensitive component of the photosynthetic apparatus, compared with the electron transport chain, stromal enzymes, PSI activity and the chloroplasts envelope (Georgieva & Yordanov 1994, Havaux 1994). Long-term adaptations to thermal stress include: thylakoid membrane re-organisation, and heat-shock protein, lipid and *de novo* protein synthesis (Larcher 1994). Short-term (minutes to hours) adaptations to thermal modifications include: accumulation of thermo-protective compounds, alterations to the pH gradient of the thylakoid, and modification of the quenching processes leading to down-regulation (Havaux 1994). The closure of PSII reaction centres can occur within a few minutes, rapidly adapting the plant to the modified thermal conditions. Chlorophyll *a* fluorescence is a sensitive indicator of thermal stress (Georgieva & Yordanov 1994, Larcher 1994).

Under chilling temperatures, the rate of photosynthetic electron transport will be less than normal for any given irradiance, and so the proportion of irradiance in excess due to reduced reaction kinetics will be greater, therefore increasing the plant's susceptibility to photoinhibition (Bilger & Bjorkman 1991, Franklin 1994, Luuk et al. 1994). In this situation, photoinhibition is a regulatory mechanism, whereby the inactivated PSII reaction centres dissipate heat. Photosynthetic down-regulation can occur within a relatively short time frame (minutes to hours) so it is an effective short-term adaptation to thermal modification (Krause 1994). Alternatively, high temperature causes the blockage of the PSII reaction centres by disrupting the PSII water-splitting reaction, reducing the maximum quantum yield and electron flow from the primary PSII electron acceptor (Q_a). Extreme thermal stress causes the complete separation of the PSII reaction centre from the light harvesting complex (LHC).

The objectives of the experiments described in this paper were: to characterise the acute response of laboratory-cultured *Halophila ovalis* (R. Br.) Hook. *f.* to thermal-shock treatments over a wide range of chilling and heating temperatures; to assess the susceptibility of this species to thermal stress; to examine the effect of darkness on the thermal stress responses; and to provide some information on the thermal tolerance and recovery of this plant.

METHODS AND MATERIALS

Plant material. *Halophila ovalis* was collected from Taylor's Bay, Sydney Harbour, Australia (33° 50' S, 151° 15' E) and cultured under laboratory conditions for at least 3 mo prior to experimentation. Growth conditions were: 35 ppt filtered seawater, 120 µmol quanta m^{-2} s^{-1}, photoperiod 16:8 h, grown in terrestrial sandy loam sediment within a recirculating flow-through system (Ralph 1997). Each culture tub (11.5 × 5.5 × 17 cm) contained at least 5 individual plants. From 2 culture tubs, a pair of leaves from the second node behind the meristem were randomly selected and a range of chlorophyll fluorescence parameters were measured.

Experimental treatments. These experiments constituted thermal-shock treatments, rather than acclimation or tolerance trials, as the tissue was directly transferred from growth conditions into the experimental temperature regime, without acclimation. The experimental aquariums (10 l) used for thermal-shock treatments were immersed within a larger aquarium (50 l) containing either an immersion cooler or a heater stirrer unit. Heating treatments were at 27.5, 30.0, 32.5, 35.0, 37.5, 40.0°C and control (25.0°C), maintained by a heater/stirrer unit (Braun Thermomix, 1420). Chilling treatments were at 22.5, 20.0, 17.5, 15.0, 12.5, 10.0°C, and control (25.0°C) maintained by an immersion

cooler unit (Thermoline, TIC). The experiments were run over 2 separate 96 h periods, providing 4 replicate samples. All experimental aquaria were aerated, and the salinity was checked daily. All treatments were maintained under standard growth conditions, as specified above. Temperature in the experimental tanks was stabilised for at least 24 h prior to commencement of experiments.

Recovery. After the completion of the 96 h exposure trial (at each temperature tested), all surviving samples were returned to the standard growth conditions. Effective quantum yield was measured at 09:00 h each day for a further 5 d. Surviving samples were defined as having an effective quantum yield greater than zero at the 96 h measurement. Time zero for the recovery experiment was taken as the 96 h reading of the initial thermal-shock trial.

Dark response. The effects of modified thermal conditions were also assessed in complete darkness. Sample tubs were covered by a small inverted aquarium (10 l), painted black to prevent light penetration. The sample tub and the covering aquarium were immersed in a large controlled temperature aquarium (50 l). These dark samples were exposed to the same thermal treatments as previously described.

Chlorophyll fluorescence. Chlorophyll fluorescence was determined using a PAM-2000 fluorometer (Walz, Germany) and the following parameters measured: minimum fluorescence (F_o), maximum fluorescence (F_m), effective quantum yield ($\Delta F/F_m'$) and the maximum quantum yield (F_v/F_m). For further details on fluorescence determinations, see Schreiber & Bilger (1993). Maximum quantum yield was measured after 15 min exclusion of light using dark-adaptation clips (DLC-8). Effective quantum yield was measured on light-adapted leaves using the same clip to ensure consistent distance between leaf and fibre-optic; however, measurements were taken immediately (without a dark-adaptation period). The fluorescence signal was always sampled at a standard position on the leaf, approximately in the middle of the leaf and on the adaxial surface. All chlorophyll fluorescence measurements were performed underwater. Fluorescence measurements were collected over a discontinuous time-scale, where, after the initial exposure, specimens were measured at hourly intervals for 5 h, then daily (ca 10:00 h) for the following 4 d (Ralph 1997).

Statistical analyses. Two replicate leaf samples from 2 separate tubs (in separate tanks) per treatment were analysed by a 2-factor orthogonal ANOVA to determine whether either a tub or tank effect was significant. If no significant difference between tubs was detected, the tub/tank effect was eliminated, therefore the samples were pooled (n = 4) (Ralph 1997). A 2-factor ANOVA was performed on all chlorophyll fluorescence data to determine the significance of the various treatments, exposure period and interaction between these factors.

RESULTS

F_o and F_m

The percentage of the initial minimum fluorescence (F_o) appeared to be steady during the 5 h period for all heat and chill treatments (Fig 1a). The moderate heat treatments (27.5 to 32.5°C) showed a sustained increase in F_o after 96 h, whilst the 2 extreme heat treatments were completely quenched. After 96 h exposure the 10°C treatment declined to less than 60% of the initial value.

The percentage of the initial maximum fluorescence (F_m) of both heat and chill treatments showed a substantial decline over the 96 h exposure period (Fig. 1b). All heat treatments (except 40.0°C) remained relatively stable over the first 5 h of exposure. The F_m response to chilling temperature was similar to the corresponding heat response, with an overall decline with respect to time, increasing in rate with increased chilling (Fig. 1b). The 37.5 and 40.0°C treatments were completely quenched by 96 h. The 27.5°C treatment showed a 35% decline over the 96 h period, whilst the 32.5 and 35.0°C treatments were about 60 to 70% lower than the original values.

$\Delta F/F_m'$ and F_v/F_m ratio

The effective quantum yield ($\Delta F/F_m'$; Fig. 1c) and maximum quantum yield (F_v/F_m; Fig. 1d) were similar and relatively stable over a wide range of temperatures after the 5 h exposure period. The 40°C treatment after 5 h exposure showed a substantial decrease for both parameters. The thermal response curves after 96 h exposure were significantly more acute than the 5 h curves. F_v/F_m ratio for the control leaves (25.0°C) was found to be approximately 0.7 (Fig. 1d), while a substantial decline occurred for all heat and chill treated specimens over the 96 h exposure period. After 5 h exposure, the F_v/F_m ratio of the 40.0°C treatment was approximately 0.38, about half that of the control. The 40.0 and 37.5°C treatments were both chronically inhibited within 96 h. After 96 h exposure at 27.5°C, F_v/F_m was about 30% below pre-exposure levels; the 30.0°C treatment was reduced by 52%, whilst the 32.5 and 35.0°C treatments were both reduced by about 75%.

The chilled treatments from 10.0 to 17.5°C all declined rapidly in F_v/F_m after the 96 h exposure period. The 10.0 and 12.5°C treatments decreased by

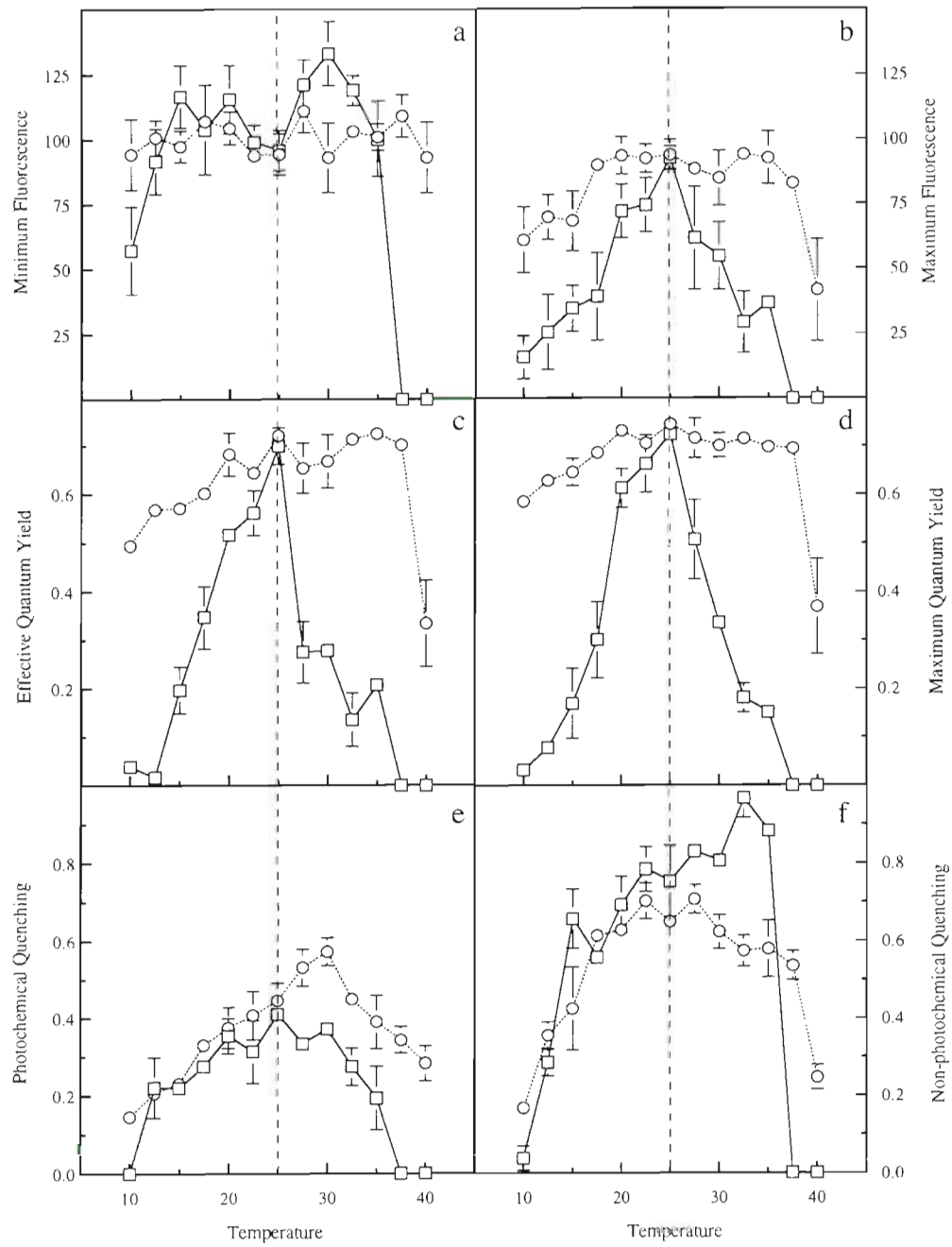

Fig. 1. *Halophila ovalis*. Response of the (a) minimum fluorescence (F_o) (% initial), (b) maximum fluorescence (F_m) (% initial), (c) effective quantum yield ($\Delta F/F_m'$), (d) maximum quantum yield (F_v/F_m ratio), (e) photochemical quenching (qP) and (f) non-photochemical quenching (qN) as a function of temperature over 5 h (○) and 96 h (□). Error bars indicate the 95% confidence interval of the mean (n = 4). The units of F_v/F_m ratio, $\Delta F_v/F_m'$, qP and qN are relative. Vertical dashed line: control temperature

approximately 90% over the 96 h exposure period, whilst the 15.0 and 17.5°C treatments decreased by 74 and 60%, respectively. The 2 moderate chilling treatments (22.5 and 20.0°C) showed a decline in F_v/F_m ratio of 16 and 21%, respectively. The lower the temperature, the lower the F_v/F_m ratio for any period of exposure.

qP and *qN*

The photochemical quenching coefficient (qP) for the control specimens remained steady at about 0.4 over the 96 h period (Fig. 1e). Elevated temperatures (27.5 and 30.0°C) led to a substantial increase in qP during the first 5 h, which was similar to control qP after 96 h. The qP for

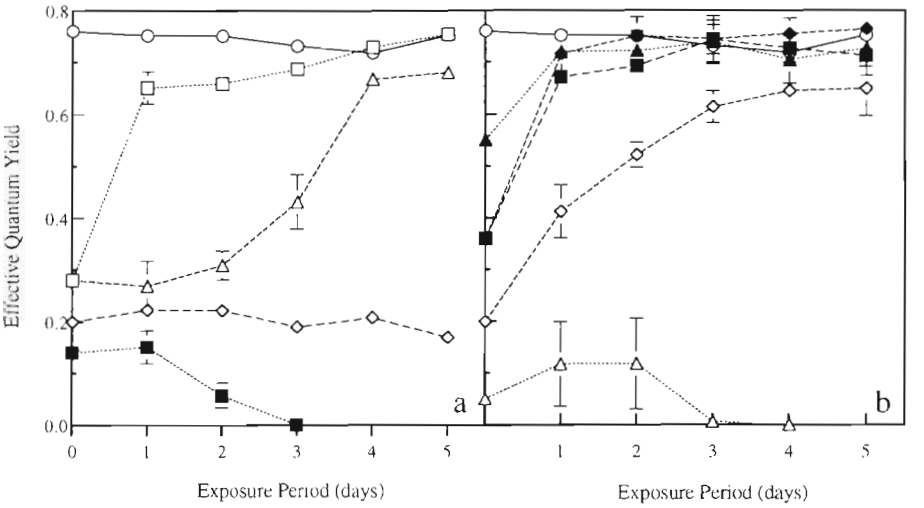

Fig. 2. *Halophila ovalis*. Recovery of the effective quantum yield ($\Delta F/F_m'$) for (a) 4 heating treatments [27.5°C (□), 30.0°C (△), 32.5°C (◇), 35.0°C (■)] and the control (○); and (b) 5 chilling treatments [12.5°C (△), 15.0°C (◇), 17.5°C (■), 20.0°C (▲), 22.5°C (◆)] and the control (○), returned to 25.0°C, as a function of exposure time up to 96 h. Error bars indicate the 95% confidence interval of the mean (n = 4). $\Delta F/F_m'$ was measured in relative units

the higher temperatures (>32.5°C) declined at increasing rates. The *qP* responses for the 10.0, 37.5 and 40°C treatments were completely quenched by 96 h.

The non-photochemical quenching (*qN*) responses for the 2 extreme heat treatments were completely quenched after 96 h (Fig. 1f). The moderate heat treatments (27.5 to 35.0°C) increased the *qN* coefficient up to 5 h relative to the control response. The *qN* of the 32.5°C and 35.0°C treatments increased by more than 20%. The control, 22.5 and 20.0°C treatments showed no obvious change in *qN* up to 96 h exposure (Fig. 1f). The 10.0, 12.5 and 15.0°C treatments declined during the both the 5 and 96 h periods. The 12.5°C treatment declined to 57% of the initial *qN* value by 96 h, and the 10.0°C was almost completely quenched.

Recovery

Recovery from these thermal treatments was dependent upon exposure temperature. The 37.5 and 40.0°C treatments during the 96 h exposure period were irreversibly damaged, and so no recovery was possible (Fig. 2a). The 32.5 and 35.0°C treatments were not irreversibly damaged; however, they were still unable to recover after 96 h at 25°C. The 30.0°C treatment samples were initially less than 30% of the control level; however, after 4 d recovery under standard growth conditions, the samples were within 10% of the control response. Leaves exposed to 27.5°C largely recovered within 1 d and recovered completely after 5 d.

Recovery from 96 h exposure to chilling temperatures was successful for treatments ranging from 15.0 to 22.5°C (Fig. 2b). The 12.5°C treatment failed to recover after 3 d at 25.0°C, as the samples were chronically photoinhibited. The 15.0°C treatment recovered to within 17% of the control level after 5 d. The 17.5, 20.0 and 22.5°C treatments had returned to the level of the controls after 1 d under standard growth temperature.

Dark response

All darkened heat treatments (Fig. 3a) showed a substantially less stressed response than the corresponding samples exposed to normal irradiance (120 µmol quanta m^{-2} s^{-1}) (Fig. 1d). The 22.5°C treatment was consistently lower than all the other treatments over the 5 h exposure period (Fig. 3b). All other darkened treatments showed similar responses to the control. The 2-way ANOVA model found both the heated and chilled treatments to be significantly different ($p < 0.05$) from control responses.

DISCUSSION AND CONCLUSION

Characteristics of acute thermal response

Symptoms of thermal stress were detected in *Halophila ovalis* using chlorophyll *a* fluorescence within 5 h of exposure to heating or chilling conditions. Temperature changes of as little as ±2.5°C led to a fluorescence response. Under heated conditions, *qP* was the most rapid (sensitive) indicator of the thermal-stress symptoms for *H. ovalis*, whilst effective quantum yield detected chilling stress within 5 h. These responses were performed at 120 µmol m^{-2} s^{-1} irradiance, which is slightly below saturation irradiance for this species (Ralph 1997). Increased sensitivity might be found if these experiments were performed at saturating intensity or *in situ*.

After 5 h exposure, *Halophila ovalis* demonstrated a wide tolerance, ranging from 20.0 to 37.5°C, without a

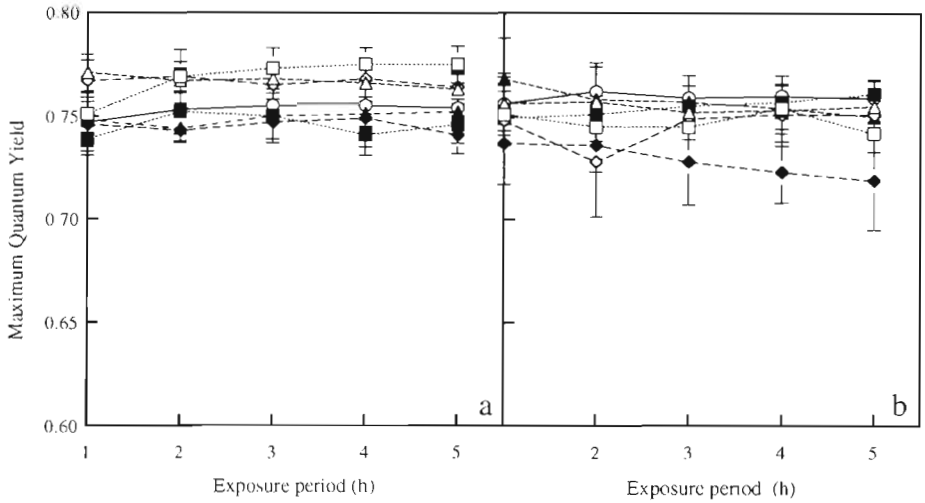

Fig. 3. *Halophila ovalis*. Response of the maximum quantum yield (F_v/F_m ratio) under darkened conditions of (a) 6 heating treatments and control: [27.5°C (□), 30.0°C (△), 32.5°C (◇), 35.0°C (■), 37.5°C (▲), 40.0°C (◆)] and the control (○), and (b) 6 chilling treatments [10.0°C (□), 12.5°C (△), 15.0°C (◇), 17.5°C (■), 20.0°C (▲), 22.5°C (◆)] and the control (○), as a function of exposure time up to 5 h. Error bars indicate the 95% confidence interval of the mean (n = 4). F_v/F_m was measured in relative units

substantial impact upon the F_v/F_m ratio, $\Delta F_v/F_m'$, or qN responses. The optimum photosynthetic range was 25 to 30°C. The extreme treatments (10.0, 12.5, 37.5 and 40.0°C) resulted in chronic photosynthetic stress within 96 h, which could be linked to complete and permanent abolition of either F_v/F_m or $\Delta F_v/F_m'$. These results are consistent with field observations of a decline in *Thalassia testudinum* due to heated effluent from power plants, where an increase of 5°C resulted in denuded seagrass meadows (Thorhaug et al. 1978).

The dramatic decline in F_v/F_m ratio for the extreme treatments (10.0, 12.5, 37.5 and 40.0°C) was linked to chronic inhibition of photosynthesis, as indicated by the F_o and F_m responses being completely quenched during the period of the experiment. The samples were unable to recover from these extreme treatments, suggesting irreparable damage to the chloroplast structure and function. Temperatures further from control (25.0°C) caused greater F_m quenching. The decline in F_m is thought to be heat-induced photoinhibition, which is associated with the closure of PSII reaction centres, due to thermal stress and chloroplast dysfunction (Havaux 1994). Under chilling conditions, F_o was relatively unaffected, whilst F_o increased after 96 h under moderate heating conditions. Luuk et al. (1994) found similar results for tomato seedlings when exposed to chilling temperatures, where the minimum fluorescence remained steady. Steady F_o is thought to be associated with increased thermal deactivation of PSII reaction centres, a regulatory photoprotective mechanism (Havaux 1994, Krause 1994). An increase in F_o (and a decrease in F_m) at elevated temperatures (27.5, 30.0 and 32.5°C) could be associated with reduced trapping efficiency at the PSII antennae and the disassociation of the PSII reaction centre from the LHC. This would prevent captured energy from being transferred, so increasing F_o (Georgieva & Yordanov 1994). This indicates the sensitivity of the photosynthetic tissue to thermal alterations. Anthropogenic changes to the ambient water temperature will therefore severely affect the ecophysiology of impacted seagrasses, and chlorophyll fluorescence provides an accurate monitor of this effect.

Susceptibility to thermal stress

Bilger et al. (1987) suggested that the quenching data reflect functional changes in the photochemistry that are readily reversible, whilst F_o and F_m are indicative of structural alterations that require longer time periods, up to days, for recovery. The present study demonstrates these types of thermal-stress responses. The rapid and complete decline of qP for the 10.0°C treatment suggests membrane damage, eliminating electron transport (Bruggemann et al. 1992). The photosynthetic inactivation and inability of samples to recover at 10.0 and 12.5°C is in contrast to the work of Hillman (1985), who found that laboratory-cultured *Halophila ovalis*, although showing no growth, survived at 10.0°C. During the 5 h exposure, peak photochemical quenching of approximately 0.6 occurred at 30.0°C; therefore, only 40% ($1 - qP = 0.4$) of the Q_a pool was reduced (closed PSII reaction centres), whereas the control specimens were about 60% reduced. This response is in agreement with Hillman et al. (1989); however, over the 96 h exposure the peak qP declined to 25.0°C. This could be an artefact of growing plants at 25°C, or it may be an effect of the below-saturation irradiance.

Quenching coefficients were only sensitive to thermal alterations greater than ±7.5°C. Non-photochemical quenching increased only after 96 h exposure and with increasing temperature. This would be a result of

the simultaneous effects of the increased electron transport and the decline in the Calvin cycle activity, due to the inactivation of temperature-dependent enzymes (Bruggemann et al. 1992). The reduced overall consumption of ATP would lead to an accumulation of stored energy in the thylakoid membrane and an increase in the proton gradient (Bilger & Bjorkman 1991, Koroleva et al. 1994). A change in the pH gradient across the thylakoid membrane results in an increase in *qN* with increased temperature (Koroleva et al. 1994). After 95 h exposure, all short- and long-term adaptations would be functional, and therefore an increase in *qN* would indicate extreme stress associated with thylakoid energisation. The photoprotective role of *qN* was evident in the 15.0°C treatment, where *qN* appeared to recover or acclimate, with a steady increase in *qN* towards the control level. A reduction in *qN* under extreme temperature conditions (10.0, 12.5, 37.5 and 40.0°C) further increased the plants' susceptibility to photoinhibition. Georgieva & Yordanov (1994) found similar results for peas *Pisum sativum*, where non-photochemical quenching decreased under extreme chilling conditions and increased with moderate heating conditions. Seagrasses may be exposed to photoinhibitory conditions during both summer, when increased irradiance is required due to higher water temperatures, as well as during winter, when the photosynthetic tissues have increased susceptibility to low-temperature photoinhibition.

Dark thermal response

One aspect of thermal-induced stress that has received limited attention is the assessment of the effects of temperature under darkened conditions (Gilmore & Bjorkman 1995). The results of the dark exposure experiment support the view that thermal stress mainly affects the photosystems, since over the 5 h exposure period in complete darkness no significant effect was detected over the range 10 to 37.5°C, and at 40.0°C, although slight inhibition occurred. Heating leaves (including *Elodea* sp. and *Potamogeton* sp.) in the presence of low-light resulted in the heat-induced alterations of PSII being considerably alleviated, suggesting that low-light protects the photosynthetic apparatus from heat inactivation (Srivastava & Strasser 1995). Since the photosynthetic apparatus was inactive during the dark exposure period, protein (specifically D-1) and enzyme damage may have occurred, but this would not be apparent until the tissue was exposed to normal light (Gilmore & Bjorkman 1995). Further experiments are required to determine if there were any effects after 5 h, and the capacity for recovery under normal irradiance after dark thermal exposure would indicate the long-term effect of this type of stress. Exclusion of light negated the capacity of chlorophyll *a* fluorescence to monitor the onset of thermal stress.

Tolerance and recovery

Halophila ovalis tolerated thermal-shock from 15 to 30°C for up to 96 h, and completely recovered on return to standard growth conditions. This is consistent with the view that *H. ovalis* is a eurythermal species (den Hartog 1970, Hillman 1985). Recovery from both high and low temperature stress under normal light conditions further indicates the protective role of the down-regulation of PSII reaction centres (Krause 1994). The delay of several days in the recovery of some of the more extreme temperature treatments (Fig. 2a, b) suggests that the photosynthetic enzymes have been inactivated or damaged, and protein synthesis was required to facilitate recovery (Larcher 1994). Given that these investigations were using thermal-shock exposure, not acclimation, it appears that *H. ovalis* is a particularly thermo-tolerant species. Further investigations of the capacity of this species for gradual acclimation would provide valuable insight into its phenotypic plasticity and biogeographical distribution. Acclimation was indicated during the 96 h experiment, with the recovery of *qN* at 15.0°C (Ralph 1997).

These experiments suggest that the photosynthetic condition of *Halophila ovalis* (and possibly other seagrasses) would suffer detrimental effects from short-term or episodic change in temperature. An understanding of the thermal tolerance of seagrasses is essential for appropriate management considerations, such as controlling the thermal effluent damage from cooling water, by allowing recommendations of a maximum temperature change. The ability to identify subtle stress symptoms at the earliest possible time may provide a valuable early warning of disturbances, which if not corrected would ultimately lead to the decline of the meadow.

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