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High-temperature tolerance of photosynthesis in the red alga *Chondrus crispus*

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Abstract. *Chondrus crispus* (Stackhouse) is a perennial red seaweed, common in intertidal and shallow sublittoral communities throughout the North Atlantic Ocean. In the intertidal zone, *C. crispus* may experience rapid temperature changes of 10 to 20 C° during a single immersion–emersion cycle, and may be exposed to temperatures that exceed the thermal limits for long-term survival. *C. crispus* collected year-round at Long Cove Point, Chamberlain, Maine, USA, during 1989 and 1990, underwent phenotypic acclimation to growth temperature in the laboratory. This phenotypic acclimation enhanced its ability to withstand brief exposure to extreme temperature. Plants grown at summer seawater temperature (20 °C) were able to maintain constant rates of light-saturated photosynthesis at 30 °C for 9 h. In contrast, light-saturated photosynthetic rates of plants grown at winter seawater temperature (5 °C) declined rapidly following exposure to 30 °C, reached 20 to 25% of initial values within 10 min, and then remained constant at this level for 9 h. The degree of inhibition of photosynthesis at 30 °C was also dependent upon light intensity. Inhibition was greatest in plants exposed to 30 °C in darkness or high light (600 µmol photons $m^{-2}s^{-1}$) than in plants maintained under moderate light levels (70 to 100 µmol photons $m^{-2}s^{-1}$). Photosynthesis of 20 °C-acclimated plants was inhibited by exposure to 30 °C in darkness or high light, but the degree of inhibition was less than that exhibited by 5 °C-grown plants. Not only was light-saturated photosynthesis of 20 °C plants less severely inhibited by exposure to 30 °C than that of 5 °C plants, but the former also recovered faster when they were returned to growth conditions. The mechanistic basis of this acclimation to growth temperature is not clear. Our results indicate that there were no differences between 5 and 20 °C-grown plants in the thermal stability of respiration, electron transport associated with Photosystems I or II, Rubisco or energy transfer between the phycobilisomes and Photosystem II. Overall, our results suggest that phenotypic acclimation to seawater temperature allows plants to tolerate higher temperatures, and may play an important role in the success of *C. crispus* in the intertidal environment.

Introduction

Temperature is one of the most important environmental factors limiting the growth and distribution of marine plants (Lüning 1990). Intertidal macroalgae in boreal and temperate oceans are exposed to considerable seasonal changes in water temperature, and also experience rapid variations in temperature associated with tidal emersion. For example, temperature changes of 10 to 20 C° have been recorded in temperate intertidal habitats during tidal emersion (Dudgeon et al. 1989, Brawley and Johnson 1991). In the Gulf of Maine, coastal surface seawater temperatures vary from −2 °C in winter to 18 °C in summer (Apollonio 1979), while air temperatures can vary from ≤ −20 °C in winter to ≥ 30 °C in summer. In contrast to many terrestrial plants that survive temperature extremes (e.g. freezing) by becoming physiologically inactive, intertidal macroalgae remain physiologically active year-round.

Previous studies have focused on the response of seaweed metabolism to seasonal changes in water temperature (Davison 1987, Davison et al. 1991, Kübler and Davison 1993). Much less is known about the response to sudden changes in temperature associated with tidal emersion, although recent studies have examined the response of intertidal seaweeds to sudden exposures to freezing temperatures (Davison et al. 1989, Dudgeon et al. 1989, Pearson and Davison 1993). In this study, we investigate the effects of sudden increases in temperature which occur during summer low tides (e.g. Brawley and Johnson 1991) on the primary productivity of *Chondrus crispus*, an ecologically dominant and economically important red macroalga in the North Atlantic Ocean. To study the effects of high temperature exposure we measured changes in respiration and photosynthesis in *C. crispus* over a time-course following abrupt transfer between growth temperatures and 30 °C, which is above the upper thermal limit for long-term survival of this species (Lüning 1984). These changes model those occurring during summer tidal immersion–emersion. Desiccation constitutes an important stress factor for intertidal seaweeds (Smith and Berry 1986), and is frequently associated with high temperatures (Brawley and Johnson 1991). Howev-

er, during our experiments, plants were kept immersed to allow us to separate effects of temperature from those of desiccation. This experimental design emulates the situation in intertidal pools and in the dense turfs of *C. crispus* where plants would experience high temperatures but remain wet during summer low tides. Because *C. crispus* is known to undergo phenotypic acclimation of photosynthesis to temperature (Dudgeon et al. 1990, Kübler and Davison 1993), plants from a single population were grown at cold (5 °C) and warm (20 °C) temperatures and were used to study the possibility that high-temperature tolerance was subject to thermal acclimation.

We present evidence that *Chondrus crispus* acclimates to growth temperature; plants acclimated to summer seawater temperature (20 °C) can tolerate brief exposure to extreme high temperatures better than those acclimated to winter seawater temperature (5 °C). Acclimation affected respiratory and photosynthetic metabolism as well as the ability to recover from thermal stress. This ability to tolerate thermal variations may be a key factor in the success of this species in the extremely variable intertidal environment.

Materials and methods

Collection and culture conditions

Thalli of *Chondrus crispus* (Stackhouse) were collected from Long Cove Point, Chamberlain, Maine, USA (43°54'N; 69°28'W) monthly in 1989 and 1990 and transported in seawater to the laboratory at Orono, Maine, within 2 to 4 h. Gametophytes and sporophytes were separated by the resorcinol test (Garbary and DeWreede 1988); gametophytes made up >80% of the population at this site. *C. crispus* gametophytes were grown for 3 wk in laboratory culture at 5, 10, 15, 20 or 25 °C, at a growth-saturating photosynthetic photon-flux density (PPFD) of 100 μmol photons $m^{-2} s^{-1}$ (Fortes and Lüning 1980) in a 16 h light:8 h dark cycle. Ten to twelve small (~5 cm length) gametophytes were acclimated in 3.5 litres of aerated, Provasoli-enriched seawater (PES, Provasoli 1968) in Plexiglas jars. Media were changed twice weekly, at which time plants were cleaned of epibionts by wiping with paper towels.

Growth rates were calculated using the logarithmic growth equation of Evans (1972); tagged plants were blotted with paper towels and weighed at the beginning and end of the growth experiment, which lasted 3 wk. *Chondrus crispus* has an apical meristem, and growth rates were sufficient to allow us to use only apical tissue produced in the laboratory for the photosynthetic experiments. Each experiment included a comparison of responses of plants grown at 5 and 20 °C (cold- and warm-temperature acclimated plants, respectively). These temperatures represent typical winter and summer water temperatures and have been previously shown to produce acclimation of photosynthetic light-harvesting in this species (Kübler and Davison 1993).

Instantaneous effects of temperature on net photosynthesis

Light-saturated photosynthesis of 2 cm-long apical sections of *Chondrus crispus* was measured in a Clark-type oxygen electrode system (Rank Bros. Botisham, England) with 5 ml of 0.45 μm Millipore-filtered seawater (Kuebler et al. 1991). Apices were cut 24 h in advance and held in growth-temperature seawater to allow wound-healing to occur (Bidwell and MaLachlan, 1985). Temperature was controlled by a recirculating water bath connected to the water jacket of the electrode chamber. The PPFD was 500 μmol photons $m^{-2} s^{-1}$, which is sufficient to saturate photosynthesis (Kübler and Davison 1993). Photosynthetic rates were measured at 5C° increments between 0 and 40 °C for five apices of cold- and warm-temperature acclimated plants. Each apical segment was only used for one measurement, and individual measurements took 3 to 5 min.

Effects of prolonged exposure to 30 °C

The effect of several hours exposure to 30 °C was studied on plants grown at 5 and 20 °C. Apices were transferred from growth conditions to the electrode chamber and light-saturated photosynthesis was measured at 30 °C as described in the previous subsection. Apices were then transferred to 100 ml beakers containing 50 ml of PES. The PES was maintained at 30 °C by placing the beakers in a shaking water bath that was illuminated at a PPFD of at ~100 μmol photons $m^{-2} s^{-1}$ by a bank of cool-white fluorescent tubes. Apices were removed from the water bath at 30 min intervals and returned to the electrode chamber to remeasure photosynthesis. Measurements were repeated over a 12 h time-course. The PES in the beakers was changed between measurements (i.e., every 30 min).

The effects of high temperature on respiration were measured over a similar time-course after transfer from growth conditions. Plants were maintained at 30 °C and 100 μmol photons $m^{-2} s^{-1}$ as described above, but oxygen uptake was determined in the dark.

Effect of light level on photosynthesis at 30 °C

In order to determine if light level affects the ability of *Chondrus crispus* to tolerate prolonged exposure to high temperature, apices from cold- and warm-temperature acclimated plants were exposed to 30 °C in three PPFD's: 0,70 and 600 μmol photons $m^{-2} s^{-1}$. These light levels were previously found to be below (70 μmol photons $m^{-2} s^{-1}$) and above (600 μmoles photons $m^{-2} s^{-1}$) the PPFD which saturates photosynthesis for both 5 and 20 °C-grown plants when measured at 30 °C (Kübler 1992, Kübler and Davison 1993). The PPFD in the shaking water bath was adjusted by changing the number of fluorescent tubes illuminating the bath and/or by layering neutral-density screening or black cloth between the lights and the water bath. Rates of light-saturated photosynthesis were determined over a 6 h time-course as described in the previous subsection.

Recovery of photosynthesis after exposure to high temperature

To determine if *Chondrus crispus* could recover from the inhibition of photosynthesis that occurred during prolonged exposure to 30 °C, photosynthetic rates of plants acclimated to 5 and 20 °C were measured at 15 °C before and after exposure to 30 °C at a PPFD of 100 μmol photons $m^{-2} s^{-1}$ for 0.5, 1, 3 or 6 h. Apices were then returned to growth conditions and photosynthetic rates were remeasured at 15 °C over a recovery time-course of 24 h.

Photosynthetic electron-transport rates

The effects of temperatures on photosynthetic electron-transport rates were measured in two ways. Short-term in vitro responses were measured by studying electron transport in isolated thylakoids at a range of temperatures from 0 to 40 °C. Longer term exposure to high temperatures was studied by exposing plants to 30 °C for 1 h, prior to isolating thylakoids and assaying electron transport at 15 °C. The assays and extraction procedures were the same in both experiments. Measurements were made using a suspension of isolated thylakoid membranes prepared by a modification of the methods

of Nordhorn et al. (1976) and Popovic et al. (1983). Approximately 1 g (fresh weight) of apical tissue was ground in a pH 6.1 extraction buffer containing: 50 m*M*, MES, 4% PVP-40, 0.2% BSA, 5 m*M* NaEDTA, 3m*M* ascorbic acid, 3.4 m*M* ascorbic acid, 3.4 m*M* L-cysteine, 1 μ*M* $MnCl_2$, 1 m*M* $MgCl_2$, 1 m*M* $NaNO_3$, 0.5 m*M* KCl and 1 *M* sorbitol, in a mortar and pestle precooled with liquid nitrogen. The resulting slurry was further disrupted in a glass tissue-homogenizer, and suction-filtered through several layers of 100 μm nylon mesh to remove large particles and cell-wall material. The resulting suspension was pelleted by centrifugation of 17 000 rpm for 1 min in a Sorvall SS-2B refrigerated centrifuge set at 5 °C. The pellet was resuspended in a pH 7.6 buffer containing 50 m*M* HEPES, 5 m*M* NaEDTA, 1 m*M* $MnCl_2$, 1 m*M* $MgCl_2$, 1 m*M* $NaNO_3$, and 0.5 m*M* KCl, to give a suspension of ruptured thylakoid fragments which was used in the following assays.

Rates of PSI and PSII electron transport were determined by monitoring oxygen consumption (PSI) or production (PSII) in a Clark-type oxygen electrode (Hansatech Instruments, King's Lynn, England). PSI activity was measured as the transfer of electrons from $DCPIPH_2$ to methyl viologen as described by Mitchell and Barber (1986). PSII activity was measured as the transfer of electrons from water to phenyl benzyl quinone (PBQ) as described by Lein (1978) All measurements were carried out in a reaction volume of 1 ml at saturating PPFD of 500 μmol photons m^{-2} s^{-1}. The short-term response to temperature was measured over a range of temperatures from 0 to 40 °C. The long-term effects of temperature were determined at a standard measurement temperature of 15 °C. Rates of photosynthetic electron transport were quantified on the basis of chlorophyll-*a* concentration in the thylakoid suspension.

Temperature dependence of phycoerythrin fluorescence

The thermal stability of energetic transfer from phycobilisome light-harvesting complexes to photosynthetic reaction centers was measured by studying the temperature dependence of phycoerythrin fluorescence (Kuebler et al. 1991). Phycoerythrin fluorescence increases rapidly (= fluorescence jump) when light energy harvested by the phycobilisomes can not be transferred to PSII. Phycoerythrin fluorescence was produced by excitation at 380±10 nm, at a photon flux density of 20 μmol photons m^{-2} s^{-1}, and measured at 580±10 nm in a Perkin-Elmer Model 650–105 spectrofluorometer. Temperature was increased from 20 to 50 °C at a rate of 1 °C every 5 min. Fluorescence intensity and temperature were recorded continuously on a two-channel chart recorder. A regression of fluorescence yield versus temperature over the linear range of fluorescence increase was used to calculate the temperature at which phycoerythrin fluorescence increased by 100% over the initial value.

Results

Growth responses to temperature

Chondrus crispus grew at temperatures from 5 to 20 °C (Fig. 1), with growth rates increasing from 0.30±0.11 g g^{-1} whole fresh wt mo^{-1} (mean ±SE, $n=10$) at 5 °C to 0.69±0.07 g g^{-1} mo^{-1} (mean ± SE, $n=10$) at 15 °C. Growth rates were similar at 15 and 20 °C; plants did not grow at 25 °C.

Instantaneous effects of temperature on photosynthesis

Fig. 2 shows the effect of temperatures from 0 to 40 °C on rates of light-saturated photosynthesis of plants grown at 5 or 20 °C for 3 wk. Apices were only exposed to incubation temperatures long enough to enable measurements

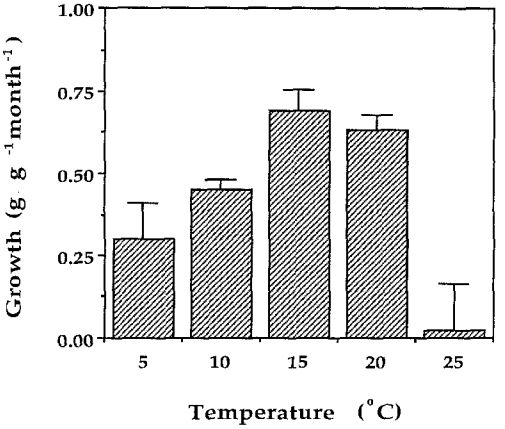

Fig. 1. *Chondrus crispus*. Effect of temperature from 5 to 25 °C on relative growth rates (measured as fresh weight increase per unit whole fresh weight) over 3 wk period. Error bars indicate ±1 SE from mean ($n=10$)

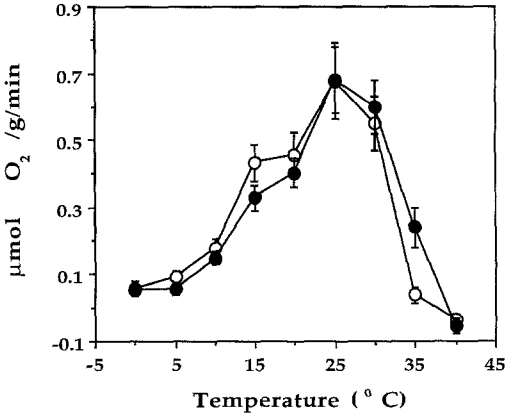

Fig. 2. *Chondrus crispus*. Effect of short-term (3 to 5 min) exposure to temperatures from 0 to 40 °C on rates of light-saturated net photosynthesis for plants grown at 5 °C (o) and 20 °C (•). Error bars indicate ±1 SE from mean ($n=5$)

(3 to 5 min). Both groups of plants had maximal photosynthetic rates (~0.7 μmol O_2mol O_2 g^{-1} min^{-1}) at 25 °C. Photosynthetic rates increased with increasing temperature from 0 to 25 °C (from 0.06 μmol O_2 g^{-1} min^{-1}), were similar from 25 to 30 °C, and then declined sharply above 30 °C. This pattern occurred for both cold- and warm-temperature grown plants, and there were no significant differences between these groups except at 35 °C, where only plants grown at 20 °C achieved net photosynthesis. The temperature range over which photosynthesis occurred is broader than the thermal limits for growth (Fig. 2 cf. Fig. 1).

Effects of extended exposure to 30 °C on photosynthesis and respiration

The effect on photosynthesis of prolonged exposure to 30 °C in shown in Fig. 3. Initial photosynthetic rates were high (~0.75 μmol O_2 $g^{-1}min^{-1}$) in both cold- and warm-temperature grown plants, and were not significantly different. However, photosynthetic rates of cold-temperature grown plants declined rapidly to 20–25% of

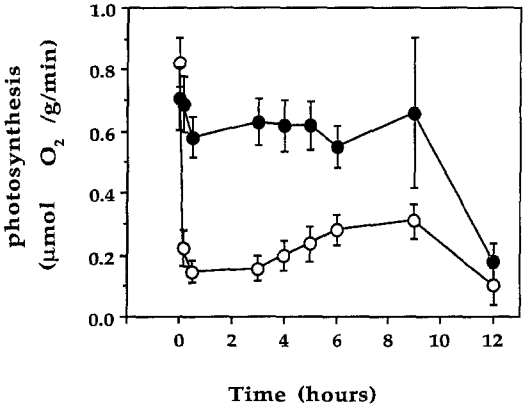

Fig. 3. *Chondrus crispus.* Effect of continuous exposure to 30 °C on rates of light-saturated net photosynthesis for plants grown at 5 °C (○) or 20 °C (●). Error bars indicate ±1 SE from the mean ($n=10$)

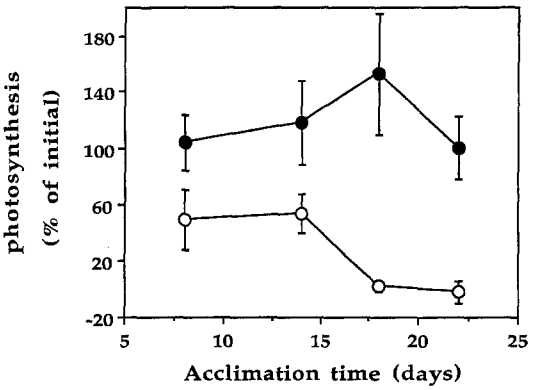

Fig. 4. *Chondrus crispus.* Effect of 30 min incubation at 30 °C on rates of light-saturated net photosynthesis (measured as a % of initial values) for plants acclimated at 5 °C (○) of 20 °C (●) for different periods of time. Error bars indicate ±1 SE from mean ($n=10$)

the initial rate within 10 min, whereas rates of warm-temperature grown plants remained constant. After the initial changes, photosynthetic rates of both 5 and 20 °C-grown plants remained constant for the next 9 h, after which time rates of photosynthesis declined sharply in both groups.

The development of the different responses to prolonged exposure to 30 °C in cold- and warm-temperature grown plants was studied in plants which were preacclimated to 15 °C for 2 wk (other culture conditions described in "Materials and methods – Collection and culture conditons") and then transferred to growth temperatures of either 5 or 20 °C. These data are presented in Fig. 4 and indicate that differences in high-temperature tolerance were well established after 8 d (the earliest time measured). After 8 d of culture, 5 °C-grown plants displayed a 50.9% (SE = 19.9) decrease in photosynthetic rates during a 30 min exposure to 30 °C, whereas 20 °C-grown plants maintained 106.4% (SE = 21.2) of their initial photosynthetic rates in the same treatment.

Effect of light level on photosynthesis at 30 °C

The foregoing results on the effect of exposure to 30 °C on photosynthesis were obtained at a PPFD of 100 μmol photons m^{-2} s^{-1}. Exposure to 30 °C at high PPFD (600 μmol photons m^{-2} s^{-1}) or in the dark, resulted in more severe inhibition of photosynthesis in both cold- and warm-temperature acclimated plants than occurred at a moderate PPFD of 70 μmoles photons m^{-2} s^{-1} (Fig. 5). Although photosynthetic rates of high-temperature acclimated plants were affected by high-temperature treatment in the dark and at high light levels, the inhibition of photosynthesis was more severe for low-temperature acclimated plants under all conditions.

Recovery of photosynthetic rates

Photosynthetic rates recovered upon return to growth conditions after a short (30 min) exposure to 30 °C (Fig. 6). Photosynthesis of cold-temperature acclimated

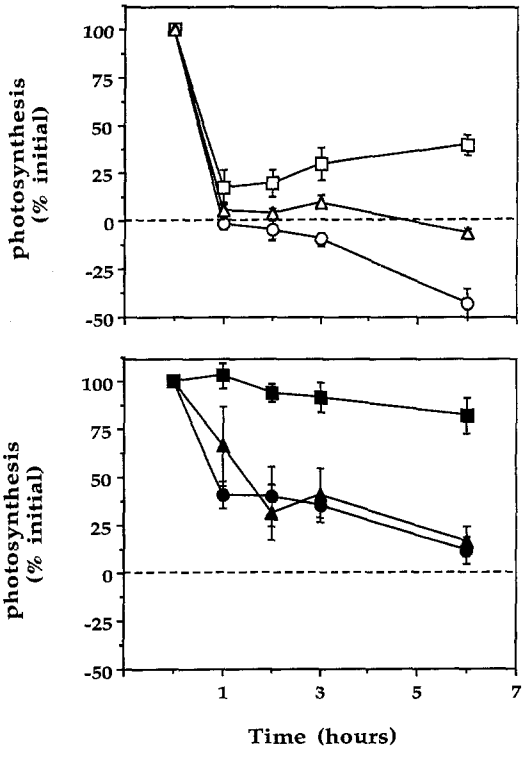

Fig. 5. *Chondrus crispus.* Time-courses of light-saturated net photosynthesis measured following exposure to 30 °C in the dark (○), 70 μmoles photons m^{-2} s^{-1} (□, ■), 600 μmoles photons m^{-2} s^{-1} (△, ▲) for plants grown at 5 °C (open symbols) or 20 °C (filled symbols). Error bars indicate ±1 SE from mean ($n=5$)

plants was more severely inhibited than that of warm-temperature grown plants immediately after exposure to 30 °C. Following transfer to growth conditions, photosynthesis in 5 °C-acclimated plants exhibited an initial rapid recovery which then slowed, with plants taking approximately 9 h to reach initial (pre-exposure) rates. Photosynthesis in warm-temperature acclimated plants was stimulated by 30 min exposure to 30 °C and remained higher than initial photosynthetic rates for at least 8 h. The time required for recovery of 100% of initial photosynthetic rates depended on the duration of exposure to

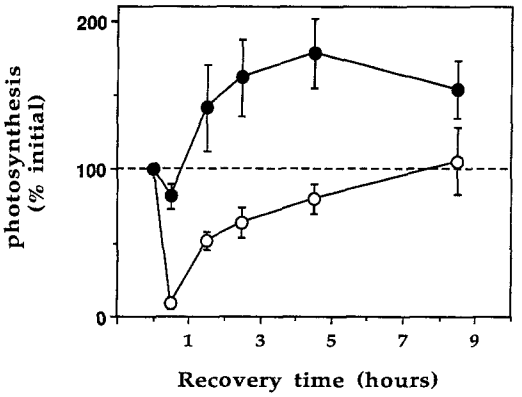

Fig. 6. *Chondrus crispus.* Time-courses of recovery for net light-saturated photosynthesis measured at 15 °C after 30 min exposure to 30 °C. Plants were returned to their respective growth conditions at 5 °C (o) or 20 °C (•) after high temperature exposure and between measurements of photosynthesis at 15 °C. Data are expressed as percentages of pre-exposure photosynthetic rates at 15 °C. Error bars indicate ±1 SE from mean (n = 5)

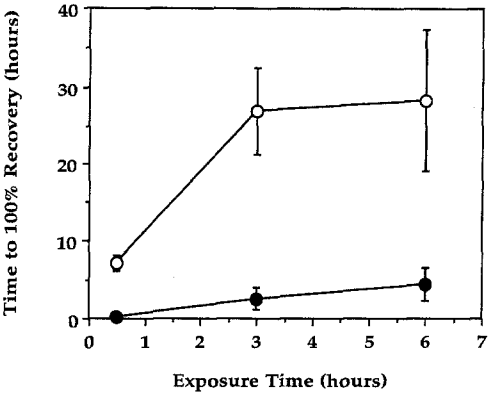

Fig. 7. *Chondrus crispus.* Time required for plants grown at 5 °C (o) or 20 °C (•) to recover 100% of initial net photosynthetic rates after exposure to 30 °C for 0.5, 3, or 6 h. Error bars indicated ±1 SE from mean ($n = 5$)

30 °C (Fig. 7). Longer exposures required longer recovery times. Complete recovery occurred significantly sooner in warm-temperature acclimated plants for all exposure times examined. For example, warm-temperature acclimated plants required 2.6±1.5 h to recover completely from a 3 h exposure to 30 °C, while cold-temperature acclimated plants required >24 h.

Respiratory effects of extended exposure to 30 °C

The response of respiration to extended exposure to 30 °C was similar for cold- and warm-temperature grown plants, although respiration rates were higher in 5 °C-grown plants (Fig. 8). Respiration rates were not significantly different from initial values (0.103±0.018 and 0.072±0.016 μmol O_2 g^{-1} min^{-1} for 5 and 20 °C-grown plants, respectively) during the first 6 h. However, after 6 h at 30 °C, respiration rates increased dramatically in both warm- and cold-temperature grown plants, reaching 0.2 and 0.28 μmol O_2 g^{-1} min^{-1} (5 and 20 °C-grown plants, respectively) by 12 h.

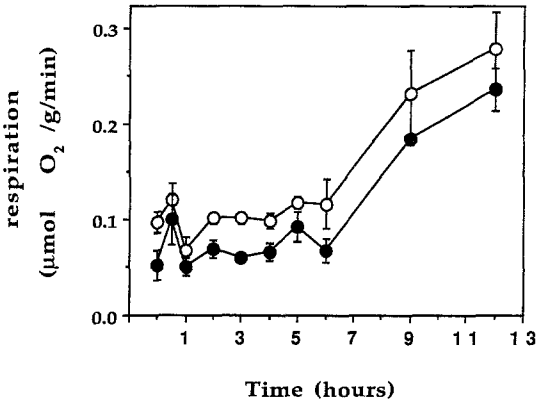

Fig. 8. *Chondrus crispus.* Respiration rates measured over 12 h time-course at 30 °C for plants grown at 5 °C (o) or 20 °C (•). Error bars indicate ±1 SE from mean ($n = 10$)

Photosynthetic electron-transport rates

Photosynthetic electron-transport rates were sensitive to both instantaneous and prolonged changes in temperature. The short-term thermal response of PSI electron-transport rates (Fig. 9) closely paralleled the thermal response of photosynthesis (Fig. 2) in both groups of plants, and did not differ for cold- and warm-temperature acclimated plants except at low temperatures (0 and 5 °C), where the rate of PSI electron transport was significantly higher in cold-temperature acclimated plants. No significant differences in PSII electron-transport rates were found between the two groups of plants (data not shown). Rates of PSI electron transport measured in plants exposed to 30 °C for 1 h (Table 1) were 10% higher than unexposed controls in both cold- and warm-temperature grown plants. PSII activity was not affected by 30 °C exposure in either group of plants.

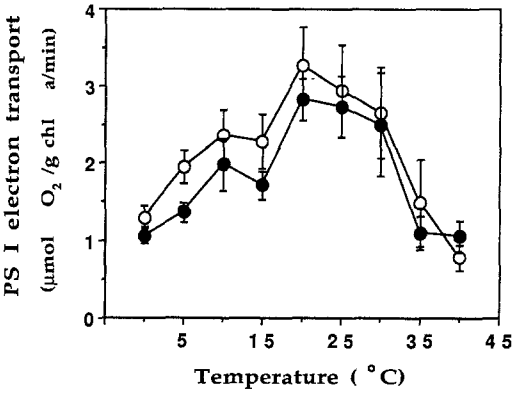

Fig. 9. *Chondrus crispus.* PSI photosynthetic electron-transport rates for plants grown at 5 °C (o) or 20 °C (•) measured over range of temperatures from 0 to 40 °C. Error bars indicate ±1 SE from mean ($n = 5$)

Temperature dependence of phycoerythrin fluorescence

The transfer of light-energy transfer from the phycobilisomes to the photosynthetic reaction centers was not affected by temperatures which inhibited photosynthesis (Fig. 10). Phycoerythrin fluorescence increased abruptly

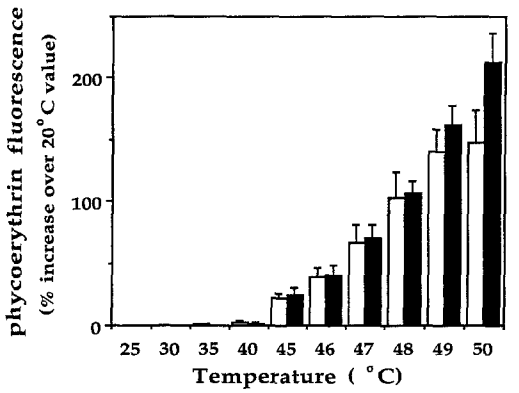

Fig. 10. *Chondrus crispus*. Phycoerythrin fluorescence measured between 30 and 50 °C. Data are expressed as percent increase over 20 °C values for plants grown at 5 °C (open bars) or 20 °C (filled bars). Error bars indicate ±1 SE from mean ($n=5$)

Table 1. *Chondrus crispus*. Photosynthetic electron-transport rates in extracts prepared from plants grown at 5 or 20 °C and exposed to 30 °C for 1 h. Values are expressed as μmol O_2 mg^{-1} chl *a* min^{-1} consumed for Photosystem I (PSI) electron-transport activity or as μmol O_2 mg^{-1} chl *a* min^{-1} produced for Photosystem II (PSII) electron-transport activity. Values in parentheses are standard errors of means ($n=12$). Differences were tested using sign test for paired data, with level of significance set at $p=0.05$

	PSI		PSII	
	5 °C	20 °C	5 °C	20 °C
Control mean (SE)	1.79 (0.25)	2.44 (0.26)	0.084 (0.031)	0.150 (0.036)
Exposed mean (SE)	2.16 (0.28)	2.70 (0.38)	0.133 (0.046)	0.130 (0.031)
Differences: mean (SE)	0.29 (0.13)	0.38 (0.28)	0.004 (0.025)	-0.009 (0.025)
p-value	0.033	0.033	NS	NS

at high temperatures due to the breakdown in the ability to transfer light energy harvested by this pigment to PSII. However, this disfunction occurred at similar temperatures in both 5 and 20 °C-grown plants. Phycoerythrin fluorescence was twice the initial fluorescence intensity at 48.1 °C (SE=0.51) and 47.6 °C (SE=0.27) for 5 and 20 °C-grown plants, respectively. These temperatures are higher than the upper thermal limit for photosynthesis and are not significantly different for the two groups of plants.

Discussion

Growth and photosynthesis

Our data indicate that *Chondrus crispus* was able to grow over the temperature range from 5 to 20 °C (Fig. 1). This is consistent with previously published data for *C. crispus* in the Eastern North Atlantic (Fortes and Lüning 1980). Overall, these data indicate that *C. crispus* is able to grow over most of the temperature range (−2 to 20 °C; Apollonio 1979) encountered in the Gulf of Maine.

Intertidal algae also experience periodic exposure to extreme temperatures above and below seawater temperatures during tidal emersion. Temperatures during tidal emersion may be 10 to 20 C° higher or lower than seawater temperatures (Dudgeon et al. 1989, Brawley and Johnson 1991, Pearson and Davison 1993). Thus, intertidal seaweeds are potentially exposed to rapid extreme fluctuations in temperature twice daily because of semidiurnal tidal emersion. Prolonged exposure to the thermal extremes associated with tidal emersion would be lethal to *Chondrus crispus*. However, it is able to survive extreme temperatures for limited periods of time. For example, *C. crispus* cannot grow or survive prolonged exposure at 30 °C (Fig. 1 of Lüning 1984). Our data indicate that *C. crispus* not only survives short exposures to 30 °C, but maintains high rates of photosynthesis at this temperature for several hours (Fig. 3). Warm-temperature grown plants can photosynthesize at 30 °C for up to 9 h, considerably longer than *C. crispus* would be exposed to air in nature (<6 h; Dudgeon personal communication). Similarly, respiration rates were unaffected by 30 °C for up to 6 h (Fig. 8). Unlike many terrestrial higher plants, that become dormant during periods of environmental stress (e.g. winter), seaweeds remain physiologically active. This strategy is necessary because intertidal seaweeds do not experience prolonged stress-free periods, but instead are potentially exposed to stress twice daily, year-round. In order to survive in the intertidal, seaweeds have evolved mechanisms that confer tolerance to stress or allow rapid recovery from stress on reimmersion. The ability to photosynthesize at high temperatures is one such adaptation, and it is important because it allows primary productivity to continue undisrupted in a variable thermal environment, thereby minimizing the impact of extreme temperatures on growth and competitive ability.

The uncoupling of temperature tolerances of photosynthesis and growth is a common phenomenon that has been observed in higher plants, seagrasses, microalgae and macroalgae (Davison 1987, Geider 1987, Osmond et al. 1987, Raven and Geider 1988, Zimmermann et al. 1989, Kuebler et al. 1991). It has been suggested that growth is hormonally inhibited under stress conditions, although plants may continue to photosynthesize (Chapin 1991). The roles of growth-regulating hormones in macroalgae are not yet well understood, but hormones such as abscisic acid may be important in growth regulation under stress conditions (Bradley 1991). There is some indirect evidence to support this hypothesis; some unicellular algae continue to accumulate carbohydrates after ceasing to grow at extreme temperatures (Geider 1987) Alternatively, the observed differences in thermal tolerances of photosynthesis and growth may be an artifact of the time required to detect changes in biomass. That is, plants may continue to grow for several hours at 30 °C but do not survive for the several days required to measure growth. There is some evidence, based on continuous video-digitizing of projected surface areas, that macroalgal growth rates do change on the timescale of hours (Lüning 1992). This is consistent with the data of Strömgren (1982), who reported changes in elongation rates of fucoid algae during the first 5 to 10 min after a change in temperature. Further development of such sen-

sitive methods to measure growth would indicate if the uncoupling of photosynthesis and growth rates reported here is real or apparent.

Acclimation of high-temperature tolerance

When *Chondrus crispus* gametophytes are grown in laboratory culture at typical winter and summer seawater temperatures (5 and 20 °C, respectively), they develop an increased tolerance to the extreme air temperatures encountered during these seasons. For example, Dudgeon et al. (1990) found that plants grown at 5 °C were less affected by emersion at −20 °C than were 20 °C-grown plants. Our data indicate that plants grown at 5° and 20 °C also have different tolerances of high-temperature (30 °C) inhibition of photosynthesis, but in this case, 20 °C-grown plants are more tolerant. The acclimation response of photosynthesis to growth temperature occurs rapidly and is well established within 8 d (Fig. 4), and potentially may occur sooner. The rapid response insures that *C. crispus* is able to adjust its metabolism to maximize photosynthesis and, potentially, growth in a variable environment.

Growth temperature also affected the ability of *Chondrus crispus* to recover from high-temperature inhibition of photosynthesis. When photosynthetic rates were inhibited by extreme temperatures (Figs. 6, 7), plants acclimated at 20 °C recovered more quickly and more completely than plants acclimated at 5 °C. Photosynthesis of 20 °C-grown plants was stimulated upon return to growth conditions after a high-temperature exposure (Fig. 6). This response suggests that productivity may be stimulated by exposure to high air temperatures during the summer. Stimulation of photosynthesis has been described for low-temperature acclimated *C. crispus* plants upon recovery from brief exposures to freezing temperatures (Dudgeon et al. 1990), and appears to be a common response in many seaweeds following moderate desiccation stress (Brinkhuis et al. 1976, Smith and Berry 1986).

Interactions between high temperature and light

In nature, intertidal seaweeds are exposed to seasonal and tidal changes in environmental factors that occur concurrently with those of temperature (e.g. light and nutrients). These factors may influence the thermal responses described above and must be considered in evaluating the potential ecological effects of high temperatures. For example, individual *Chondrus crispus* plants may experience very different light levels during emersion depending on the time of low water and microhabitat. These range from full sunlight (2000 μmol photons m^{-2} s^{-1} during the summer) to very low levels during nighttime low tides or for plants covered by a dense canopy of larger seaweeds. Because *C. crispus* forms a dense turf, most plants probably experience light levels well below those measured at the surface of the canopy. Our data indicate that light has a profound effect on the response of *C. crispus* to high temperature. Although photosynthesis of 20 °C-acclimated plants was not inhibited by exposure to 30 °C at moderate light levels (70 to 100 μmol photons m^{-2} s^{-1}), inhibition did occur when plants were exposed to darkness or high light (600 μmol photons m^{-2} s^{-1}) (Fig. 3). The combined stresses of high light level, and extreme temperature (either high or low) often result in lower photosynthetic rates, than does the temperature stress alone. At high light levels, plants may absorb more light than can be safely dissipated through biochemical processes if, for example, photosynthesis was partially inhibited by thermal stress (Kyle et al. 1987). The enhanced temperature tolerance at moderate light levels could be due to the fact that these plants are able to maintain chloroplast pH gradients and activated states of the photosynthetic apparatus which require only low light levels (Heldt et al. 1983). The increased inhibition exhibited by plants exposed to 30 °C in the dark may also be related to the thermal stability of light-activation when the plants are returned to the light to measure photosynthesis (Weis 1982).

Mechanisms of high-temperature tolerance

We investigated several mechanisms which potentially could produce the enhanced high-temperature tolerance of photosynthesis in warm-water acclimated plants. We found that the response of net photosynthesis was neither correlated with, nor dependent on, the response of respiration to high temperature. Respiration rates of 5 and 20 °C-grown plants were not differentially affected by prolonged exposure to 30 °C (Fig. 7) and there was no immediate increase in respiration at 30 °C which might explain the decrease in net photosynthesis observed in low-temperature acclimated plants. Both cold- and warm-temperature acclimated plants showed the same pattern of stable respiration rates at 30 °C for up to 6 h. These results indicate that the thermal tolerance of net photosynthesis in warm-temperature grown plants involves stability of some aspect or aspects of photosynthetic metabolism.

Thermal stability of energy transfer from the phycobilisomes to PSII was implicated in high-temperature tolerance of photosynthesis and growth in the red algae *Lomentaria baileyana* and *L. orcadensis* (Kuebler et al. 1991) and breakdown of energy transfer from phycobilisomes to PSII occurs during extreme low-temperature exposure in *Chondrus crispus* (Dudgeon et al. 1990). Although energy transfer broke down at high temperatures in *C. crispus*, the breakdown occurred at temperatures far above those at which photosynthesis is inhibited, and there were no differences in the thermal stability of this process between 5 and 20 °C-grown plants that could explain their responses to 30 °C.

Carboxylase activity of ribulose-1,5-bisphosphate-carboxylase-oxygenase (RuBisCO) were similarly stable to temperatures more than 10 C° higher than those at which photosynthesis was inhibited, and no differences occurred between high- and low-temperature acclimated plants (Kübler and Davison 1993). This indicates that the high temperature tolerance of photosynthesis is not the

result of stabilization of the coupling of light harvesting to photochemical processes or of Rubisco activity.

The observation that high temperature along with high PPFD resulted in more severe inhibition of photosynthesis suggested that the acclimation-dependent tolerance of extreme high temperature may have been related to the ability to withstand photoinhibition, or to recover from photoinhibition. Photosynthetic electron transport is frequently cited as the most thermally labile aspect of the photosynthetic apparatus and the point at which photoinhibitory damage is most evident (Powles 1984, Chetti and Nobel 1987). In this case, there were no differences between 5 and 20 °C-grown plants with respect to high temperature inhibition of photosynthetic electron transport. PSI activity in thylakoids isolated from heat-treated plants was stimulated by 10% over controls (Table 1). This slight stimulation is in agreement with the results of Havaux et al. (1991), who found that PSII was inhibited and PSI stimulated within minutes after transfer to high temperature, in peas. It has been suggested that increased cyclic electron transport around PSI at high temperatures may allow for the production of ATP and presumably dissipation of light energy during otherwise photoinhibitory conditions (Canaani et al. 1989). It is possible that this occurs in *C. crispus*, although in vivo measurements of photosynthetic electron transport are required to test this hypothesis.

Other mechanisms of high-temperature tolerance could explain our observations. For example, a 10 C° increase in the growth temperature of desert succulents results in both the accumulation of heat-shock proteins and an increase in the upper lethal temperature (Kee and Nobel 1986). Increased concentration of heat-shock proteins is associated with thermal acclimation of a variety of organisms (Veirling 1991), and may also occur in *Chondrus crispus*. Alternatively, acclimation to growth temperature may induce changes in membrane composition which affect the stability of membrane-associated proteins, electron-transport chains and transmembrane gradients (Sommerville and Browse 1991). The biphasic nature of the response of 5 °C-grown plants to prolonged exposure to 30 °C, wherein photosynthetic rates are initially high, decline rapidly over the first 10 min and then remain low but stable for several hours, suggests that the difference between plants grown at low and high temperature is not a change in the acute thermally lability of any part of the photosynthetic apparatus. Among the alternative hypotheses are: high-temperature inhibition of the synthesis of component(s) of the photosynthetic apparatus which are themselves thermally stable, or, the thermal instability of some active transport process which would lead to the eventual depletion of a metabolite pool. These hypotheses are both consistent with the pattern of photosynthesis being thermally stable for a limited period, reflecting the turnover time of the components in question.

Overall, our data indicate that the thermal response of net photosynthesis and growth are uncoupled at high temperatures in the red macroalga *Chondrus crispus*. This species also undergoes a thermal acclimation to growth at 20 °C relative to 5 °C, which allows it to photosynthesize during, and completely recover from, temporary exposures to temperatures above its upper thermal limit for growth. Thermally acclimated *C. crispus* is able to completely recover from daily brief exposures to extreme temperature, such as would occur during summer low tides. The physiological basis of the acclimation response is not clear. This acclimation of short-term high-temperature tolerance has important implications for the ecological success of species, such as this one, which experience rapid, large fluctuations in temperature in the intertidal.

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