Impact of temperature acclimation on photosynthesis in the toxic red-tide dinoflagellate *Alexandrium fundyense* (Ca28)

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Abstract. This study assessed the impact of temperature on the photosynthetic activity in the dinoflagellate *Alexandrium fundyense* (Ca28) for cultures grown at 75 μmol photons m^{-2} s^{-1} over a range of temperatures. Increasing light intensity under static temperatures caused a 5-fold decrease in the maximum quantum yield for photosystem II (PSII) (F_v/F_m). Carbon fixation rates mirrored high-light depressions in F_v/F_m. Cells in the presence of streptomycin showed an 83% recovery in F_v/F_m; therefore, only a minor proportion of the decline in F_v/F_m was attributable to PSII damage by bright light. For cells transferred to higher temperatures, F_v/F_m was less sensitive to high light, decreasing only 20–40% compared to the 80–90% decrease observed for cells incubated at their ambient growth temperature. For cells shifted to higher temperatures, the rapid recovery phase of F_v/F_m was not present; therefore, cells did not initiate downregulation of PSII. Higher capacity to maintain electron transport, as indicated by the quantum yields, was confirmed by enhanced carbon fixation. Shifts to lower temperatures significantly increased PSII sensitivity to high light. Overall, these relationships reflect the synergy between photosynthetic light and dark reactions which are differentially impacted by changes in temperature.

Introduction

Temperature is an important environmental variable for understanding the physiological ecology of algae in nature, as it can affect key biological processes, including photosynthesis (Li and Morris, 1982), enzymatic activity (Kristiansen, 1983; Li *et al.*, 1984) and respiration (Ahmed and Kenner, 1977). The impact of temperature on these processes, in turn, influences both maximum (Eppley, 1972) and instantaneous growth rates (Raven and Geider, 1988). Despite this, microalgae acclimate and thrive over a wide range of temperatures. However, acclimation has a metabolic cost resulting in lower growth rates (Raven and Geider, 1988). Photosynthesis provides the chemical currency to absorb the acclimation cost and drive cellular growth; therefore, defining the interaction between light and temperature is essential. These complex synergistic interactions (Geider, 1987; Davison, 1991; Maxwell *et al.*, 1994; Machalek *et al.*, 1996), as well as genotypic and phenotypic responses (cf. Berry and Björkman, 1980), are difficult to define.

The temperature dependence of photosynthesis is most significant during periods of light saturation (Sheridan and Ulik, 1976; Li and Morris, 1982; Zupan and West, 1990) reflecting rate limitation, presumably by carbon metabolism (Li *et al.*, 1984; Stitt, 1986; Sukenik *et al.*, 1987). Despite this, it has been hypothesized

that temperature will also influence light-limited photosynthesis due to the enzymatic nature of phosphorylation and plastoquinone diffusion (Öquist, 1983; Raven and Geider, 1988). In higher plants, acclimation to low temperature exhibits many similarities to high-light acclimation with decreased energy transfer from the light-harvesting complexes to the photosystems (Mawson and Cummins, 1987) and the accumulation of inactive photosystem II reaction centers (PSII) (Krause *et al.*, 1990; Briantais *et al.*, 1992; van Wijk *et al.*, 1993). It has been hypothesized that these temperature-dependent changes compensate for reduced utilization of excitation energy by carbon metabolism (Öquist *et al.*, 1993), reduced rates of D1 protein repair at low temperatures (Hurry *et al.*, 1992; Wünschmann and Brand, 1992), and the inhibition of the xanthophyll energy-quenching mechanisms (Bilger and Björkman, 1991; Demmig-Adams and Adams, 1992).

Defining the impact of temperature on photosynthetic activity has only been conducted on a limited number of algal species (cf. Davison, 1991) and data are particularly sparse for dinoflagellates (Raven and Geider, 1988). This is significant given that temperature is a key environmental parameter impacting the physiological ecology of dinoflagellates. For example, germination of overwintering cysts of *Alexandrium fundyense* is strongly impacted by ambient temperatures (Anderson, 1980). These cysts have been hypothesized to provide the 'seed' populations for blooms which are a recurrent feature in the coastal waters of the northeastern USA (Prakash, 1967; Hurst and Yentsch, 1981; Franks and Anderson, 1992). These coastal blooms are associated with buoyant plumes of low-salinity water, which distribute point-source populations from estuaries and embayments, in the late spring and early summer months, to coastal waters (Anderson and Walls, 1978; Franks and Anderson, 1992). This transport process of seed populations to coastal waters can represent a temperature increase for the populations of up to 5°C during the springtime (Anderson and Stolzenbach, 1985); however, the potential physiological impact of this temperature shift is largely undefined.

This study was initiated to assess the interaction of light and temperature on photosynthetic quantum yields in the toxic dinoflagellate *A.fundyense* (Ca28). We focused on defining long- (month acclimation) and short-term (hour) responses in maximum quantum yield for PSII to temperature. Temperature significantly impacted both the maximum quantum yields of photosynthetic activity and carbon fixation rates. Our results resemble previous findings in higher plants where changes in temperature influence the synergy between electron transport and carbon fixation.

Method

Cultures of *A.fundyense* (Ca28) were grown under 75 μmol m^{-2} s^{-1} fluorescent light (Cool White F40CW) at three temperatures (6, 15 and 24°C ± 0.5°C). The cultures were maintained in exponential growth by diluting them, 50% by volume, with fresh f/2 media on a weekly basis (Guillard and Ryther, 1962). Measurements were made on at least three independently grown cultures.

The maximum (F_m) and minimum (F_0) fluorescence levels were measured using a pulse amplitude modulated fluorometer (PAM) equipped with a high-sensitivity cuvette (ED-101US, Heinz Walz Inc.). *Alexandrium fundyense* is motile and phototactic; therefore, to ensure that cells did not swim out of the region of the cuvette sampled by the PAM fluorescence detector, only 1 ml was placed in the cuvette. Values of F_0 (average of at least three measurements) were measured after a 15 min dark adaptation to alleviate any non-photochemical quenching (Kroon *et al.*, 1993). The maximum fluorescence level was achieved by applying a 600 ms flash of saturating light (>3000 μmol quanta m^{-2} s^{-1}) with a Schott flash lamp (KL-1500). Increasing flash duration and/or intensity resulted in a decrease in the maximum fluorescence level. Values of F_m and F_0 were used to calculate the maximum quantum yield for stable charge separations at PSII [$F_v/F_m = (F_m - F_0)/F_m$)] (see Table I for significant symbols). Values of F_v/F_m were determined as a function of irradiance and temperature.

Light intensity curves

The relationship between F_v/F_m and irradiance was defined using a photosynthetron (Lewis and Smith, 1983). This device allows up to 50 samples to be incubated at constant temperature over a wide range of light intensities (light levels ranged from 0 to 2500 μmol photons m^{-2} s^{-1}). The light was provided by a 75 W tungsten halogen light source. For these measurements, 1 ml aliquots were dispensed into 1 cm cuvettes and then incubated over a range of light intensities. After a 30 min incubation, samples were dark adapted for 15 min prior to the fluorescence measurements (Kroon *et al.*, 1993). Samples were then sequentially removed for F_v/F_m measurements. Each F_v/F_m–irradiance (F_v/F_m–I) curve took <10 min to measure. From the F_v/F_m–I curve, the inhibition slope and inflection point irradiance were derived (Figure 1). The curves were described by linear and log–linear functions. The linear function described the portion of the F_v/F_m–I curve where F_v/F_m did not vary with increasing irradiance (calculated from F_v/F_m values < 50 μmol m^{-2} s^{-1}). At high light levels, the decrease in F_v/F_m with irradiance was described by a log–linear function, from which the slope was derived. The inflection point irradiance represented the irradiance level where the linear and log–linear functions intersected.

Table I. Significant symbols

F_0	Minimum fluorescence level in the absence of non-photochemical quenching (volts)
F_m	Maximum fluorescence level in the absence of non-photochemical quenching (volts)
F_v/F_m	Maximum quantum yield for stable charge separations at photosystem II $F_v/F_m = (F_m - F_0)/F_m$ (dimensionless)
I_k	Minimum irradiance required to saturate carbon fixation rates $I_k = P_{max}/\alpha$ (μmol photons m^{-2} s^{-1})
P_{max}	Maximum carbon fixation rate (mg C m^{-3} h^{-1})
PSII	Photosystem II
α	Light-limited slope of a photosynthesis–irradiance curve [mg C m^{-3} h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$]

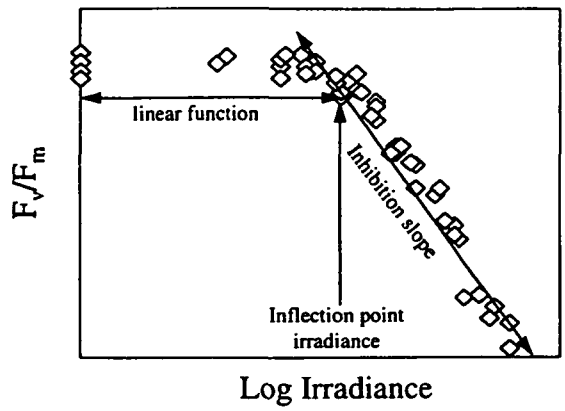

Fig. 1. A F_v/F_m–I curve with the inhibition slope and the inflection irradiance point indicated by arrowed lines.

These data were complemented by photosynthesis–irradiance (P–I) carbon fixation curves which were measured using procedures published by Moline and Prézelin (1996). Samples were incubated over a range of light levels from 0 to 2500 μmol photons m^{-2} s^{-1} (same light intensities used for the F_v/F_m–I curve). Incubation times were for 60 min and incubation temperature was controlled to within 0.2°C. A 60 min incubation was used to allow for significant ^{14}C uptake for these dilute cultures. Samples were acidified and total radioactivity was determined. The radioactive counts for this measurement reflect both the internal cellular label as well as any organic cellular exudates. Non-linear curve fits for P–I data were calculated using the Simplex method of Caceci and Cacheris (1984). Curve fitting provided estimates of the photosynthetic parameters which included the maximum photosynthetic rate [P_{max}; mg C mg^{-1} chlorophyll (Chl) *a* h^{-1}], the minimum irradiance required to saturate photosynthesis (I_k; μmol photons m^{-2} s^{-1}) and the light-limited slope for photosynthesis [α; mg C mg^{-1} Chl *a* h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$]. Estimates of the standard deviations for the P–I parameters were calculated using the procedures described by Zimmerman *et al.* (1987). These productivity values were normalized to Chl *a* concentrations (n = 3). The Chl *a* concentrations were measured fluorometrically using procedures described in Strickland and Parsons (1972). Samples were initially extracted in 100% acetone (diluted back to 90% acetone before reading) to minimize any bias due to chlorophyllase activity (Owens and Falkowski, 1982; Suzuki and Fujita, 1986; Jefferey and Hallengraeff, 1987).

Kinetic responses to high irradiance

Kinetic experiments were conducted to ascertain the time scales of bright light inhibition and recovery in F_v/F_m for cultures grown at 6 and 24°C. For these manipulations, 500 ml culture vessels were submerged in a Neslab circulating waterbath (RTE-211) and incubated under high-intensity (2500 μmol m^{-2} s^{-1})

tungsten light (Cool-Lux FOS-11 100W) for 60 min. During the incubation, subsamples were periodically removed for fluorescence and productivity measurements (see below). The length of this incubation was the same as the carbon fixation measurements. For temperature shift experiments, cultures were pre-incubated (at 75 μmol m^{-2} s^{-1}) at their new temperature for 60 min prior to incubation under high light. This pre-incubation period was sufficiently long to allow the 500 ml culture to come to temperature. After the high-light incubation, the samples were returned to initial light conditions. Subsamples were collected over the next 20–24 h to define the kinetics of recovery from photoinhibitory light. For these kinetic treatments, F_v/F_m, Kautsky induction curves and carbon fixation rates were determined.

Kautsky curves provide information on energy flow from the light-harvesting complexes to the reaction center and the maximum quantum yield. Kautsky induction curves, which are measured in the absence of electron-blocking inhibitors, were determined by recording the rise in fluorescence (sampling rate of 300 μs) first to an intermediate plateau, and then to the maximum fluorescence level (Lavorel, 1959; Munday and Govindjee, 1969; Govindjee, 1995). During the induction measurements, a light-emitting diode (665 nm, 30 μmol photons m^{-2} s^{-1} incident light on cuvette) was triggered in conjunction with a 600 ms flash from a Schott flash lamp (>3000 μmol quanta m^{-2} s^{-1}). The light intensity of the light-emitting diode was measured using a LiCor Model LI190A 2π cosine light meter. During these kinetic treatments, aliquots were incubated with and without the addition of streptomycin sulfate (250 mg l^{-1}; Lesser *et al.*, 1994), which inhibits *de novo* protein synthesis. Comparisons between the Kautsky curves measured in the presence or absence of the streptomycin were used to distinguish what fraction of the high-light F_v/F_m recovery did not require protein synthesis.

To complement the kinetic F_v/F_m treatments (60 min incubation under 2500 μmol m^{-2} s^{-1}), productivity rates were determined for cultures which were incubated in the Neslab circulating waterbath. Here, productivity rates were measured by adding aqueous radioactive sodium bicarbonate ($NaH^{14}CO_3$, final concentration of 185 Bq l^{-1}) to the 500 ml culture 60 min before the high-light incubation. Just prior to the high-light treatment, a 50 ml aliquot was removed to provide an estimate of the productivity rate under the static culture conditions. During the 1 h incubation, 50 ml aliquots were removed to determine productivity rates after 30 and 60 min. Aliquots were filtered onto 0.4 μm Nuclepore filters and carbon uptake for the particulate material was determined on a scintillation counter (Beckman 6400). In contrast to the *P–I* curves, these radioactive counts do not include any radioactively labeled organic cellular exudates.

Results

Long-term acclimation to temperature

Long-term temperature acclimation resulted in significant changes in the carbon fixation *P–I* parameters (Table II). Increasing temperature resulted in a 3- and 2.2-fold increase in Chl-specific P_{max} and α, respectively. Chlorophyll *a* per cell remained constant with temperature (Table II); therefore, higher temperatures

Table II. Photosynthetic characteristics for cultures of *A.fundyense* (Ca28) grown at 75 μmol m^{-2} s^{-1} at different ambient temperatures (6, 15 and 24°C). Production units are mg C mg^{-1} Chl *a* h^{-1}. The irradiance units are μmol photons m^{-2} s^{-1}. The standard error is also presented

Parameter	6°C	15°C	24°C
pg Chl *a* $cell^{-1}$	11.75 ± 2.35	13.50 ± 1.62	10.84 ± 5.42
P versus *I* (Chl specific)			
P_{max}	1.58 ± 0.09	2.04 ± 0.08	4.96 ± 0.10
α	0.012 ± 0.002	0.016 ± 0.002	0.026 ± 0.001
I_k	133 ± 23	128 ± 17	190 ± 10
F_v/F_m versus *I*			
F_v/F_m (at 0 μmol m^{-2} s^{-1})	0.70 ± 0.02	0.66 ± 0.03	0.55 ± 0.03
Inflection point irradiance	105 ± 15	98 ± 22	92 ± 14
Inhibition slope	−0.162 ± 0.003	−0.102 ± 0.004	−0.119 ± 0.003

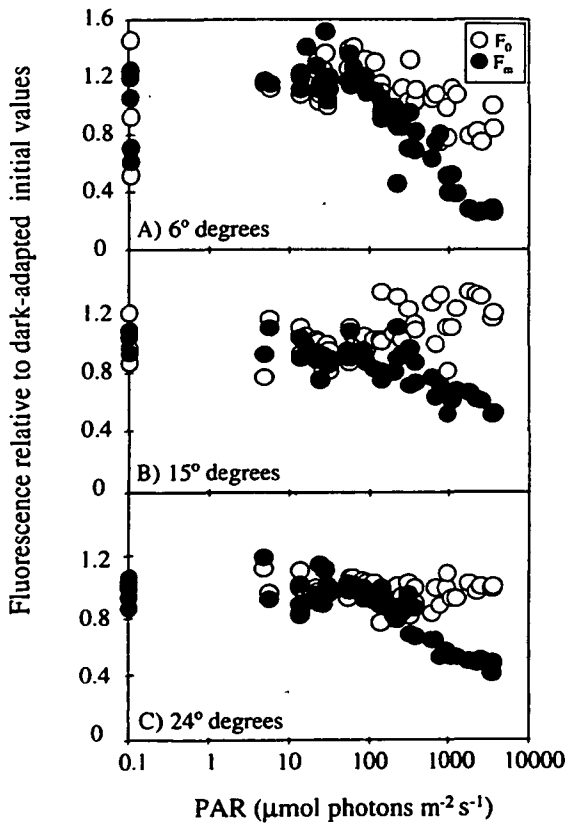

Fig. 2. The irradiance-dependent relationship of the maximum (F_m) and minimum (F_0) fluorescence level for cultures of *A.fundyense* grown at three different growth temperatures. The culture temperature was 6°C (**A**), 15°C (**B**) and 24°C (**C**).

also resulted in increased cell-specific *P–I* parameters. The I_k values were not significantly different for the 6 and 15°C cultures; however, the I_k value was 30% higher for the 24°C culture. Photoinhibition in the *P–I* curves (particulate and exudate carbon) was not detectable.

The temperature-dependent changes in the *P–I* curves were different from those of the F_v/F_m–*I* curves. The dark-adapted values of F_v/F_m were inversely related to temperature (Table II). Increasing light intensity always resulted in decreasing values of F_v/F_m. The declines in the F_v/F_m were largely driven by the light-dependent declines in F_m (Figure 2). The 50–60% decreases in F_m resulted in the majority of the 3-fold decrease in F_v/F_m (Figure 3) despite up to 20% variability in F_0. For the 6°C culture, F_0 decreased with increasing irradiance. In contrast, F_0 increased with irradiance for the 15°C cultures and showed no irradiance dependence for the 24°C culture. There were no significant differences in the inflection point irradiance for the F_v/F_m–*I* curves for cultures grown at different irradiances (Table II). The inhibition slope was greatest for the 6°C culture with no significant differences between the 15 and 24°C cultures (Table II).

The time-dependent declines in F_v/F_m under high irradiances (2500 μmol m^{-2} s^{-1}) were characterized in a series of independent treatments. Light-induced decreases in F_v/F_m occurred rapidly (the majority of the decreases occurred within 30 min). The magnitude and kinetics of the decline in F_v/F_m were similar for cells grown at different temperatures. Values of F_v/F_m decreased by 40% in less than 10 min and, by the end of the 60 min incubations, values were <30% of

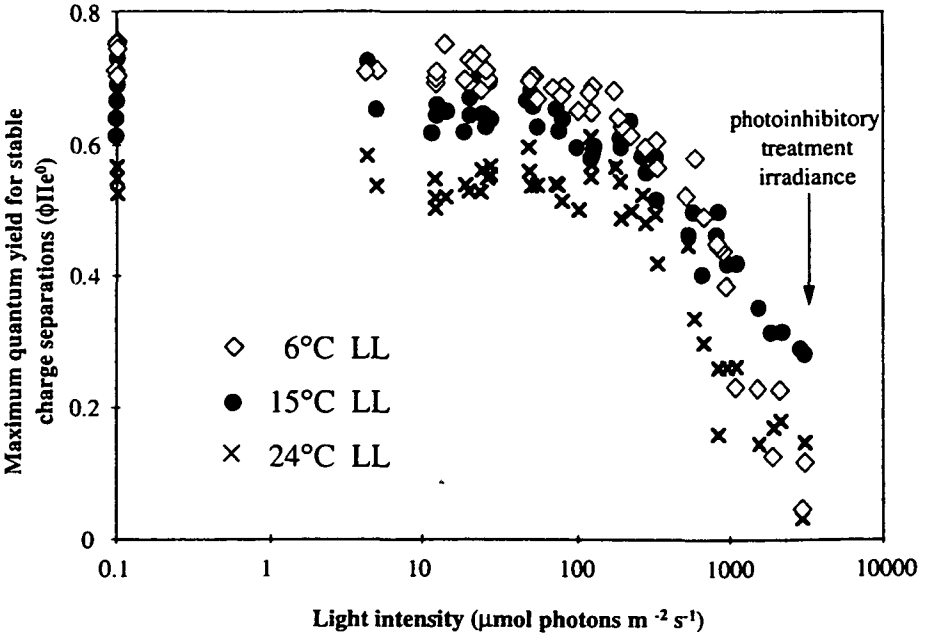

Fig. 3. The irradiance-dependent relationship for the maximum quantum yield for stable charge separations of photosystem II for cultures of *A.fundyense* grown at 6, 15 and 24°C.

the initial F_v/F_m (Figure 4A). The magnitudes of these decreases were equivalent to the independently measured F_v/F_m–I curves (Figure 3). The reduction in F_v/F_m closely mirrored the relative changes in particulate carbon fixation rates measured during the incubation (Figure 4A). Carbon fixation rates declined during the 60 min incubation with no significant differences between the 6 and 24°C cultures. The declines in the carbon fixation are in contrast to the P–I curves where no photoinhibition was detectable. The acid-venting technique used to measure the P–I curves reflects both particulate carbon fixation as well as any organically labeled cellular exudate; therefore, differences between the

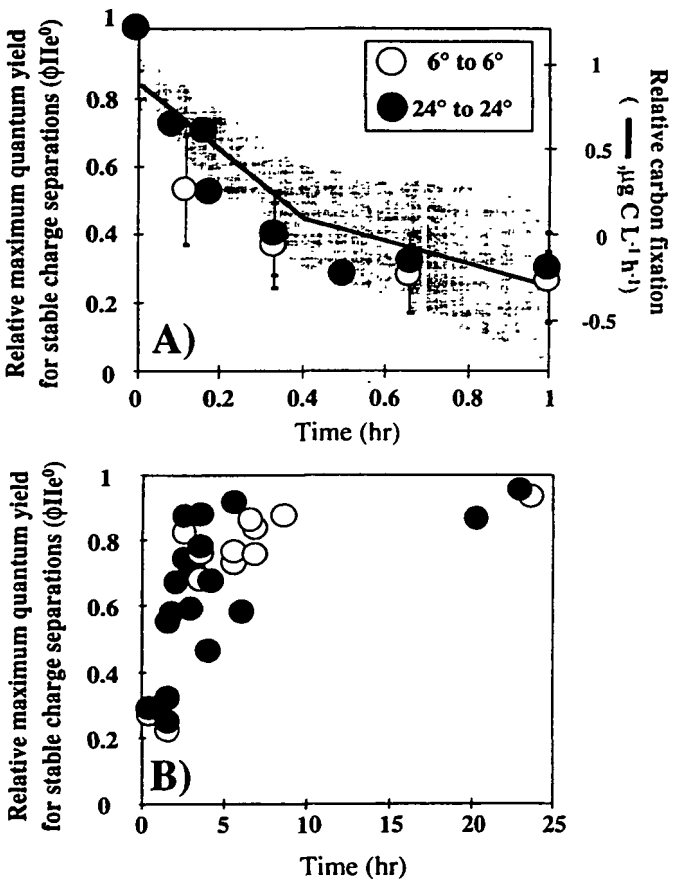

Fig. 4. Kinetic response of the maximum charge separation at photosystem II and volumetric carbon fixation for *A. fundyense* incubated under 2400 µmol m^{-2} s^{-1}. (**A**) The kinetic response of the maximum quantum yield (circles), incubated at the growth temperature (6, 24°C) where the vertical lines indicate the standard deviation around the mean (n = 4). The solid line is the relative carbon fixation rates (24°C) with the gray shaded area representing the standard deviation around the mean (n = 3). (**B**) The time-dependent recovery of the maximum quantum yield after the 1 h photoinhibitory treatment. Cells were maintained at their growth illumination (75 µmol m^{-2} s^{-1}) during the recovery period. The filled circles represent 6°C cultures and open circles represent 24°C cultures.

treatments reflect the amount of cellular exudate. Results here suggest that under high light close to 70% of the carbon fixed was released as a cellular exudate.

The recovery of F_v/F_m reflects the repair of damaged PSII and the relaxation of physiological adjustments which are induced by the high light. Cells incubated at their growth temperatures showed rapid recovery from high light (Figure 4B). Up to 83% of the light-dependent depressions in F_v/F_m recovered within 3 h in the presence and absence of streptomycin (Figure 5). Cells incubated in the presence of streptomycin recovered to within 17% of their initial values. Streptomycin inhibits *de novo* synthesis, therefore 83% of the decline in F_v/F_m reflected physiological adjustments to the high light. The final 17% recovery in F_v/F_m did not occur for cells incubated with streptomycin and reflected repair which

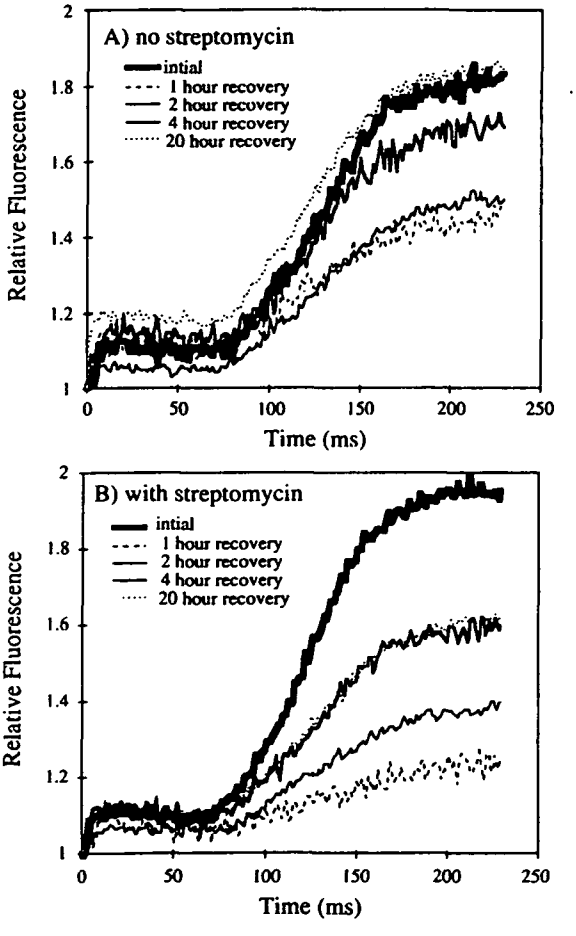

Fig. 5. Bright light inhibition (2400 μmol m^{-2} s^{-1}) and recovery for Kautsky curves of *A.fundyense* cells incubated in the presence of streptomycin. Cells were grown and incubated at 6°C. Fluorescence curves are normalized to a value of one at F_0.

required *de novo* synthesis of damaged PSII components. These repair processes required up to 20 h (Figure 4B).

Responses to temperature shifts

The irradiance dependence in F_v/F_m was sensitive to changes in temperature (Figures 6 and 7, Table III). For cells experiencing short-term increases in ambient temperatures, the slopes of the F_v/F_m–I curves decreased (Figures 6 and 7, Table III). The corresponding changes in the F_v/F_m–I curves were not proportional to the change in temperatures. For example, for the 6°C culture, the inhibition slope for F_v/F_m decreased by 50% for cells transferred to 15°C and only 66% for cells transferred to 24°C (Table III). Results from independent measurements defining the time dependency of high-light response in F_v/F_m were consistent with the F_v/F_m–I curves (Figure 8). For the cells transferred to higher temperatures, after an initial decrease of 20% in the first 10 min, values of F_v/F_m remained relatively constant during the 60 min incubation under high light (Figure 8A). Particulate carbon fixation rates in the same treatment decreased by 50% (Figure 8A, n = 3), compared to the 90% decrease observed in cells incubated at growth temperatures (Figure 4A). These results illustrate the synergistic temperature–light interactions between F_v/F_m and carbon fixation rates. There was no rapid recovery in F_v/F_m for cells shifted to higher temperatures (Figure 8B) and full recovery required close to 20 h.

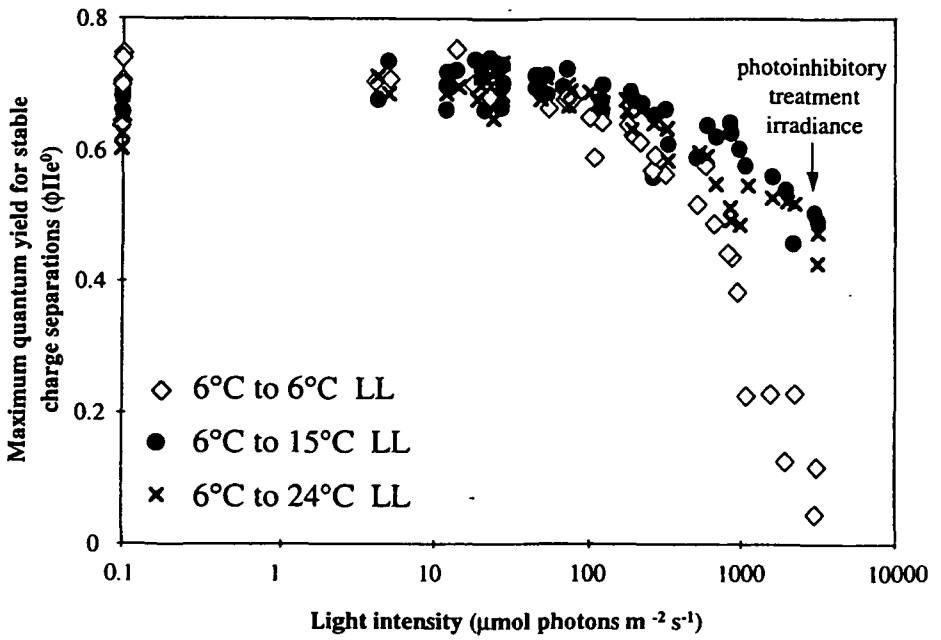

Fig. 6. The irradiance-dependent relationship for the maximum quantum yield for stable charge separations of photosystem II for cultures of *A.fundyense* incubated at 6, 15 and 24°C. The culture growth temperature was 6°C.

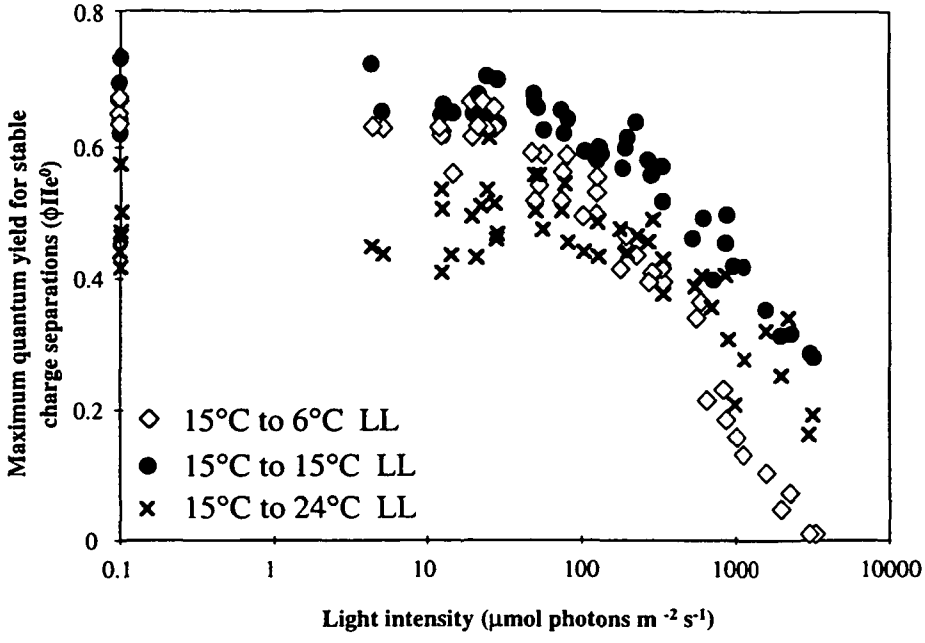

Fig. 7. The irradiance-dependent relationship for the maximum quantum yield for stable charge separations at photosystem II for cultures of *A.fundyense* incubated at 6, 15 and 24°C. The culture growth temperature was 15°C.

Table III. Differences in the inflection point irradiance and the log–linear inhibition slope derived from F_v/F_m versus irradiance curves for cultures under static and shifting temperatures (Figures 2, 5 and 6). Inflection point irradiances and slopes are shown ± 1 SE. Significant differences for temperature shift experiments from the initial growth temperatures are denoted by bold fonts

Treatment	Inflection point irradiance (μmol m^{-2} s^{-1})	Inhibition slope
Static temperature		
6°C	105 ± 15	–0.162 ± 0.003
15°C	98 ± 22	–0.102 ± 0.004
24°C	92 ± 14	–0.120 ± 0.003
Temperature shifts		
6°C to 15°C	99 ± 23	**–0.062 ± 0.001**
6°C to 24°C	140 ± 37	**–0.066 ± 0.003**
15°C to 6°C	**43 ± 11**	**–0.143 ± 0.006**
15°C to 24°C	136 ± 17	**–0.055 ± 0.003**

In contrast, cells which were transferred to lower temperatures experienced an increase in both the inhibition slopes as well as a decrease in the inflection point irradiance in the F_v/F_m–I curves (Figure 7, Table III). For low-temperature treatments, values of F_v/F_m declined rapidly and more precipitously than cells incubated at their culture temperature (Figure 8A versus Figure 4A). Radioisotope

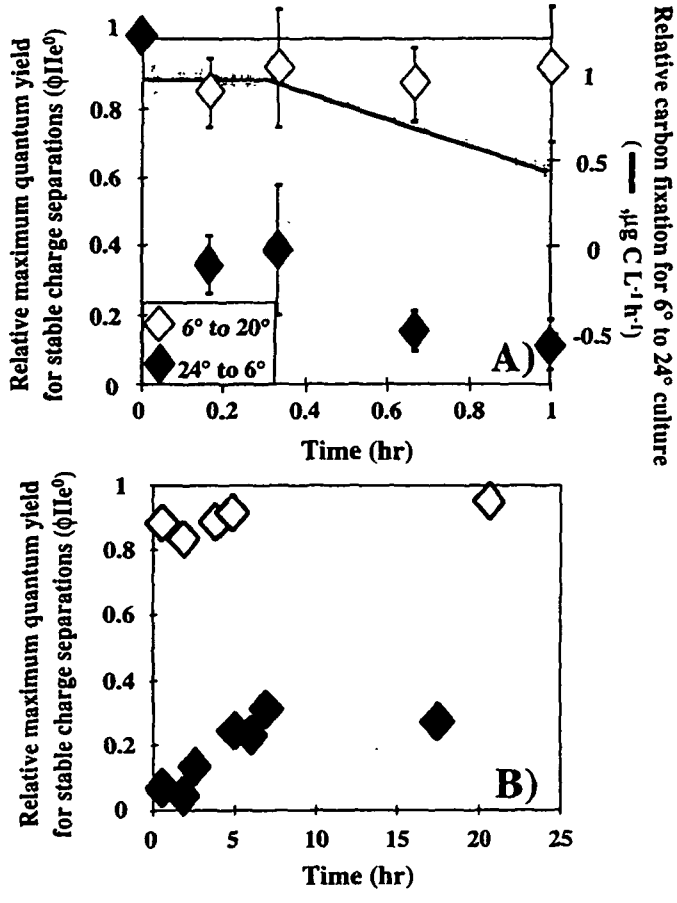

Fig. 8. (A) The kinetic response of the maximum quantum yield (diamonds) and carbon fixation (bold line) to photoinhibitory light (2400 μmol m^{-2} s^{-1}) during temperature shift experiments. The incubation temperature for 6°C cultures was 24°C. The incubation temperature for 24°C cultures was 6°C. Vertical lines indicate the standard deviation around the mean (n = 4). The solid line is the relative carbon fixation rates (24°C) with the gray shaded area representing the standard deviation around the mean (n = 3). (B) The time-dependent recovery of the maximum quantum yield after the 1 h photoinhibitory treatment. Cells were maintained at their growth illumination (75 μmol m^{-2} s^{-1}) and at shifted temperature conditions during the recovery period. The filled diamonds represent 6°C cultures and open diamonds represent 24°C cultures.

measurements were unfortunately not measured during this treatment. Values of F_v/F_m were <10% of the initial values at the end of the 60 min incubation. The recovery rate of F_v/F_m for these cells was low and was not fully recovered after 24 h.

Discussion

Photosynthesis begins with the absorption of radiant energy resulting in stable charge separations at the photosynthetic reaction centers and eventually leads to

the production of oxygen and fixation of carbon. The rate at which electrons are converted into carbon end products is dependent on the rate at which electrons are generated by the light reactions and the rate at which enzymatic dark reactions can utilize those electrons. If the activity of the light reactions exceeds the dark reactions, the resulting build-up of electron pressure will lead to depressed quantum yields and lower photosynthetic activity (for a review, see Baker and Bowyer, 1994). These depressions in the quantum yields for photosynthetic activity can be driven by damage to PSII (Kyle *et al.*, 1984; Powles, 1984; Prasil *et al.*, 1992) and/or a suite of physiological responses induced to minimize damage (Melis and Homann, 1975; Osmond, 1981; Powles, 1984; Demmig *et al.*, 1987; Kroon, 1994). Such 'photoprotective' mechanisms include (i) decreased optical cross-section associated with light-harvesting complexes for the photosynthetic reaction centers (Melis and Homann, 1975; Kroon, 1994), (ii) involvement of the xanthophyll cycle pigment pool in the dissipation of excess energy from the light-harvesting pigment bed (e.g. xanthophyll 'cycling'; Demmig *et al.*, 1987; Demmig-Adams and Adams, 1992; Schubert *et al.*, 1994), (iii) increased synthesis of photoprotective pigments (Falkowski and Laroche, 1991), and (iv) increased photorespiratory capacity of cells (Powles, 1984). Many of these processes are sensitive to changes in environmental conditions and are consistent with results from this study which demonstrated the interaction between temperature and light acclimation on the photosynthetic properties of *A.fundyense*.

Acclimation to ambient temperature

Long-term acclimation to temperature resulted in changes in the photosynthetic properties of *A.fundyense*. Consistent with other algal studies, increasing temperature resulted in higher values of Chl *a*-specific P_{max} (Morris and Glover, 1974; Levasseur *et al.*, 1990; Davison, 1991). These relationships have been related to the temperature-dependent nature of carbon metabolism (Li *et al.*, 1984; Stitt, 1986; Sukenik *et al.*, 1987) which is rate limiting under high light intensities. Under static conditions, the downregulation of PSII under high light was similar for cells grown under high and low temperatures (note, there were minor differences in F_0), with no significant differences in either the high light reduction or subsequent recovery in F_v/F_m. Downregulation of F_v/F_m is related to the redox state of the plastoquinone pool (Krause and Weis, 1991) which is, in turn, a function of coordinated activity between PSI and PSII (Allen *et al.*, 1981). Thus, cellular responses induced to minimize damage of PSII under high light were not sensitive to temperature. This supports the hypothesis that the changes in P_{max} reflected changes in carbon metabolism and not in electron transport.

Trends in Chl-specific α mirrored the temperature dependence in P_{max}. Values of α are a function of cellular absorption and the maximum quantum yield for carbon fixation. Cell size varied by 38% (Jovine, unpublished data) and Chl *a* was relatively constant, suggesting that cellular absorption could not account for the 2-fold changes in Chl-specific α. This suggests that the maximum quantum yield for carbon fixation also increased with temperature. In contrast, values of F_v/F_m showed an inverse relationship to temperature, suggesting that changes in the

maximum quantum yield for carbon fixation did not occur at the level of PSII. The light reactions can be impacted by the temperature dependence of the state transitions (Murata *et al.*, 1975; Chow *et al.*, 1981; Moll and Steinback, 1986; Mawson and Cummins, 1989) and/or plastoquinone diffusion (Raven and Geider, 1988) depending on the proportion of bound and unbound quinone B in PSII (Cao and Govindjee, 1990; Kroon, 1994). The sensitivity of these processes has been hypothesized to reflect changes in the homeoviscous properties of thylakoid membranes (Quinn and Williams, 1985; Mitchell and Barber, 1986). Also, the significant fraction of carbon which was released as exudate could account for the differences between F_v/F_m and the *P–I*-based maximum carbon quantum yield. Our results suggested that under extreme conditions up to 80% of the fixed carbon was released as cellular exudates. These processes would influence photosynthetic activity beyond that measured in dark-adapted quantum yields for stable charge separations at PSII, allowing differences between F_v/F_m and the maximum quantum yield for carbon fixation.

The depressions in the quantum yields and carbon fixation rates can represent physiological responses to the bright light and/or damage to the photosynthetic machinery. Bright light physiological responses can result in depressed dark-adapted PSII quantum yields and are characterized by being rapidly reversible (Demmig *et al.*, 1987; Weis and Berry, 1987; Harbinson *et al.*, 1989), and do not represent damage to the photosynthetic machinery (Foyer *et al.*, 1990). These reversible responses occur on the time scales of minutes to hours and do not require protein synthesis. For cells, incubated at ambient temperatures, high light levels induced significant declines in F_v/F_m. Variability in F_v/F_m can be driven by either the maximum or minimum fluorescence level. Light-induced changes in the maximum fluorescence level have been related to rapidly reversible physiological mechanisms which lead to depressed photosynthetic quantum yields (Powles, 1984). About 80% of the observed decreases in F_v/F_m were driven by decreases in F_m, compared to the 20% impact associated with the irradiance dependence in F_0. This, combined with the rapid recovery in F_v/F_m, suggested that cells induced a downregulation of PSII in response to high light intensities. Given sufficient acclimation time, the cellular responses were similar despite dramatically different temperature regimes.

Increases in ambient temperature

Acclimation to light and temperature are reflected in cellular responses (Berry and Björkman, 1980). Responses of PSII quantum yields were similar for cells grown at different temperatures, suggesting that temperature did not constrain the long-term acclimation to light. Shifts in temperature influenced the responses of PSII quantum yields to bright light. Bright light did not induce large depressions in F_v/F_m in cells shifted to higher temperatures. The detectable declines in F_v/F_m were not rapidly reversible, suggesting photodamage. The amount of this photodamage was similar, regardless of the temperature the cells had been acclimated to. Therefore, increases in temperature eliminated the downregulation of PSII under high light. High quantum efficiencies in PSII under bright light

resulted in enhanced carbon fixation rates (compare Figures 3 and 7); thus, short-term increases in temperature resulted in a higher cellular capacity to maintain productivity rates under high light. This is consistent with temperature acclimation either increasing activity in rate-limiting catalysts and/or increasing catalyst pool size (Hochachka and Somero, 1984). The short-term increases in temperature relieved oxidation pressure in the plastoquinone pool, resulting in no down-regulation of F_v/F_m under high light.

Decreases in ambient temperature

Sudden decreases in ambient temperature can depress photosynthetic activity in photoautotrophs (Berry and Björkman, 1980) and may reflect the impaired activity in either the light and/or dark reactions. In this study, cellular responses normally induced to mitigate photodamage under ambient conditions were always impaired for cells transferred to lower temperatures. The lack of a reversible recovery in F_v/F_m suggested that cells experienced significant photodamage.

In conclusion, temperature acclimation influences the function of PSII in *A.fundyense*. Temperature changes induced significant changes in the irradiance dependence of F_v/F_m which were not evident during static temperature incubations. Taken together, these findings illustrate the difficulty in predicting variability and dynamic behavior in the photosynthetic properties of dinoflagellates in a stochastic environment. Therefore, understanding the synergistic interaction between different environmental variables will be key to describing the variability in the physiology for natural populations.

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