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Structure and composition of the photochemical apparatus of the Antarctic green alga, *Chlamydomonas subcaudata*

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Abstract

The green alga, *Chlamydomonas subcaudata*, collected from a perennially ice-covered Antarctic lake, was able to grow at temperatures of 16 °C or lower, but not at temperatures of 20 °C or higher, which confirmed its psychrophilic nature. Low temperature (77 K) Chl *a* fluorescence emission spectra of whole cells of the mesophile, *C. reinhardtii*, indicated the presence of major emission bands at 681 and 709 nm associated with PS II and PS I, respectively. In contrast, emission spectra of whole cells of *C. subcaudata* exhibited major emission bands at 681 and 692 nm associated with PS II, but the absence of a major PS I emission band at 709 nm. These results for *C. subcaudata* were consistent with: (1) low ratio of Chl *a/b* (1.80); (2) low levels of PsaA/PsaB heterodimer as well as specific *Lhca* polypeptides as determined by immunoblotting, (3) decreased levels of the Chl-protein complexes CP1 and LHC I associated with PS I; and (4) an increased stability of the oligomeric form of LHC II as assessed by non-denaturing gel electrophoresis in the psychrophile compared to the mesophile. Furthermore, immunoblotting indicated that the stoichiometry of PS II:PS I:CF_1 is significantly altered in *C. subcaudata* compared to the mesophile. Even though the psychrophile is adapted to growth at low irradiance, it retained the capacity to adjust the total xanthophyll cycle pool size as well as the epoxidation state of the xanthophyll cycle. Despite these differences, the psychrophile and mesophile exhibited comparable photosynthetic efficiency for O_2 evolution regardless of growth conditions. P_{max} for both *Chlamydomonas* species was similar only when grown under identical conditions. We suggest that these photosynthetic characteristics of the Antarctic psychrophile reflect the unusual light and low temperature regime to which it is adapted.

Abbreviations: α – photosynthetic efficiency; $\sigma_{PS\,I,PS\,II}$ – absorptive cross section of Photosystem I or II; BBM – Bold's basal medium: CF_1 – coupling factor 1; DOC – deoxycholic acid; $F_{678,681,692,709}$ – 77 K fluorescence emission maxima at the respective wavelengths; LHC I – light-harvesting complex I; LHC II1 – monomeric LHC II; LHC II3 – oligomeric LHC II; μ_{max} – maximum growth rate; PS I – Photosystem I; PS II – Photosystem II; P_{max} – maximum light and CO_2-saturated rate of O_2 evolution; t_{gen} – doubling time; SDS – sodium dodecyl sulfate

Introduction

Phytoplankton which inhabit polar environments are generally adapted to a variety of extreme growth conditions. Algae endemic to Antarctica typically require low temperatures for optimal growth, and are thus, classified as psychrophilic algae (Eddy 1960). Conversely, several Arctic species exhibit a broad range in growth temperatures, and thus are often not obligated to grow under a low temperature regime (Bolton and Lüning 1982). It is thought that Antarctic marine phytoplankton exhibit a requirement for low temperature

due to the persistence of low temperature waters for the last 13 million years in the Southern Hemisphere compared with only 3 million years in the Arctic (Kirst and Wiencke 1995).

Light has been proposed as the most important factor controlling production in polar algal communities (Lizotte and Sullivan 1991; Lizotte and Priscu 1992). Antarctic aquatic systems are exposed to light conditions that can vary greatly as a consequence of seasonal changes in day length as well as changes in the extent of snow and ice-cover. Thus, there are a number of studies regarding the effects of the light regime on algal populations living in Polar aquatic habitats (Buma et al. 1993; Cota 1985; Cota and Sullivan 1990; Kudoh et al. 1997). Wiencke and Fischer (1990) reported that growth rates of three endemic Antarctic species were light saturated between 15 and 20 μmol photons m^{-2} s^{-1}. This characteristic of low light saturation levels for growth was confirmed in three brown and three red Antarctic algal species (Kirst and Wiencke 1995). Thus, it appears that in addition to the low growth temperature requirement, several Antarctic species are obligated to grow at low irradiance to achieve maximal growth rates.

The ability to protect against short-term photoinhibition also has been reported in several polar species (Bidigare et al. 1993; Hanelt et al. 1997). Bidigare and coworkers (1993) observed that the Antarctic snow alga, *Chlamydomonas antarctica*, accumulates the red carotenoid, astaxanthin, to minimize light absorption by the light harvesting apparatus under photoinhibitory conditions. Kirst and Wiencke (1995) observed that the recovery of photosynthesis after photoinhibition in the Antarctic macroalgae *Andenocystis utricularis* and *Palmaria dicipens* was faster than recovery in temperate species. These studies indicate that although Antarctic phytoplankton are very efficient at utilizing low growth irradiance, many possess photoacclimatory mechanisms to survive and quickly recover from short term photoinhibition.

The perennially ice-covered lakes of the McMurdo Dry Valleys near McMurdo Sound, Antarctica, represent some of the most unique habitats in the world. Neale and Priscu (1995, 1998) and Lizotte and Priscu (1998) identified the green alga *Chlamydomonas subcaudata* as the dominant species of the deepest biotic layer (17 to 20 m) in the east lobe of Lake Bonney. A 4 m ice-cover maintains an extremely stable water column year round allowing for stratification of nutrients and phytoplankton assemblages. The absence of turbulence allows the phytoplanton to maintain their position in the water column resulting in their exposure to a relatively stable light regime. The deep layer of the east lobe of Lake Bonney is characterized by temperatures ranging from 0 to $-2\,^{\circ}C$ (Spigel and Priscu 1996), and the perennial ice cover attenuates all but 1 to 3% of the incident of irradiance so that algal populations never receive more than 50 μmol photons m^{-2} s^{-1}. As well, the ice and the shallower algal communities preferentially attenuate wavelengths less than 500 nm and longer wavelengths of more than 600 nm (Lizotte and Priscu 1992). Neale and Priscu (1995) suggested that phytoplankton inhabiting Lake Bonney are always growing under light limiting conditions. Thus, increased efficiency of light absorption and utilization, particularly within the blue-green region of the visible spectrum, would confer an adaptive advantage to this extreme light regime. Adaptation to a particular light quality can involve dynamic adjustments in PS II/PS I stoichiometry as well as modulations in pigment composition of the light harvesting apparatus (Chow et al. 1990). *C. subcaudata* possesses higher levels of the carotenoids lutein, neoxanthin and violaxanthin, and reduced levels of β-carotene in comparison with the mesophilic species, *C. reinhardtii* (Lizotte and Priscu 1998; Neale and Priscu 1995). These authors suggest that *C. subcaudata* exhibits an unusually high efficiency of blue-green light utilization because of the augmentation of the light harvesting apparatus of PS II with light harvesting carotenoids. Furthermore, while some Antarctic phytoplankton may be able to protect against short term photoinhibition, Neale and Priscu (1995) suggest that *C. subcaudata* has lost the ability to adjust because this alga never experiences photoinhibitory light levels. However, a recent study indicated that although this species exhibited a high degree of sensitivity to photoinhibition and UV treatment (Neale et al. 1994), nonphotochemical quenching was detected even at low irradiance levels (Neale and Priscu 1998). Thus, it appears that *C. subcaudata* may have retained the capacity to protect against excess light absorption.

Considering the wide variety of unusual light regimes encountered by polar algae, the majority of Antarctic studies have focussed on the response of photosynthesis to irradiance, particularly with respect to phytoplankton assemblages in their natural environment. Other than the work of Neale and Priscu (1995, 1998), there are few published data regarding the organization and composition of the photosynthetic apparatus of Antarctic lake algae, despite the fact that the light environment is regarded as one of

the most important factors influencing phytoplankton production. As part of a study regarding the mechanism(s) by which psychrophilic green algae adjust to low temperature and irradiance, we have characterized the organization of the light harvesting complexes I and II in *Chlamydomonas subcaudata*, isolated from this unusual, extreme environment. We report specific adjustments in PS I content, the relative abundance of *Lhca* polypeptides and the apparent stability of LHC II organization.

Materials and methods

Growth conditions

Cultures of the green algae *Chlamydomonas reinhardtii* (UTEX 89) and *Chlamydomonas subcaudata* (Neale and Priscu 1995) were grown axenically in 250 ml pyrex tubes and continuously aerated under ambient CO_2 conditions. *C. reinhardtii* was grown in a modified BBM (Nichols and Bold 1965). *C. subcaudata* was grown in BBM supplemented with 0.7M NaCl, to match the salinity content under natural conditions. Cell cultures were grown in thermoregulated aquaria at either 8, 16, or 29 $\pm$ 1 °C. Low (20 μmol photons m^2 s^{-1}) to moderate (150 μmol photons m^2 s^{-1}) growth irradiances were generated by fluorescent tubes (Sylvania CW-40). Irradiance was measured from the center of the growth tubes with a quantum sensor attached to a radiometer (Model LI-189, Licor Inc., Lincoln, NE, USA). Growth kinetics were monitored as the change in optical density at 750 nm. The doubling times (t_{gen}) were calculated as ln $2/\mu$, where μ is the pseudo-first order rate constant for growth (Guillard 1973). Cells were harvested during mid-log phase which represented maximum growth rates.

Thylakoid isolation

Cultures in mid-log phase were harvested by centrifugation at 5000 $\times$ *g* for 10 min and the pellet was resuspended in a Tricine-NaOH (pH 7.8) buffer containing 0.3 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM benzamidine, and 1 mM caproic acid. All isolation buffers contained 1mM phenylmethyl-sulfonyl fluoride which was freshly added at the time of thylakoid isolation. The suspension was passed through a chilled French Pressure Cell twice at 10 000 lb/in^2. The broken cells were centrifuged at 272 $\times$ *g* for 5 min and the supernatant was centrifuged at 23 700 $\times$ *g* for 30 min. The pellet was washed in a buffer containing 50 mM Tricine-NaOH (pH 7.8), 10 mM NaCl, and 5 mM $MgCl_2$ and centrifuged at 13 300 $\times$ *g* for 15 min. Enriched thylakoid membrane preparations were resuspended in the above wash buffer supplemented with 0.1 M sorbitol and 10% glycerol. Samples were stored at -80 °C.

Pigment analysis

Algal cells were harvested by centrifugation and concentrated under vacuum to remove the remaining water. Pigments were extracted with 100% acetone at 4 °C and dim light using 0.1 mm zirconia/silica beads (3 $\times$ 20 s cycle) and a MINI-BEADBEATER^{TM} (Biospec Products, Inc., Bertlesville, OK, USA). Extracts were clarified by centrifugation. The supernatant was filtered through a 0.22 μm syringe filter and samples were stored at -80 °C until analysed.

Pigments were separated and quantified by high-performance liquid chromatography (HPLC) as described previously (Ivanov et al. 1995) with some modifications. The system contained a Beckman System Gold programmable solvent module 126, diode array detector module 168 (Beckman Instruments, San Ramon, CA, USA), CSC-Spherisorb ODS-1 reverse-phase column (5 μm particle size, 25 $\times$ 0.46 cm I.D.) with an Upchurch Perisorb A guard column (both columns from Chromatographic Specialties Inc., Concord, ON, Canada). Samples were injected using a Beckman 210A sample injection valve with a 20 μl sample loop. Pigments were eluted isocratically for 6 min with a solvent system acetonitrile:methanol:0.1 M Tris-HCl (pH 8.0), (72:8:3.5, v/v/v), followed by a 2 min linear gradient to 100% methanol:hexane (75:25, v/v) which continued isocratically for 4 min. Total run time was 12 min. The flow rate was 2 cm^3 min^{-1}. Absorbance was detected at 440 nm and peak areas were integrated by Beckman System Gold software. Retention times and response factors of Chl *a*, Chl *b*, lutein and β-carotene were determined by injection of known amounts of pure standards purchased from Sigma (St. Louis, MO, USA). The retention times of zeaxanthin, antheraxanthin, violaxanthin and neoxanthin were determined by using pigments purified by thin-layer chromatography according to Diaz et al. (1990). Chl content was determined spectrophotometrically using the method of Jeffrey and Humphrey (1975).

Nondenaturing PAGE

Chl-protein complexes were prepared for electrophoretic separation according to Król et al. (1997). Thylakoid samples were washed once in water, once in 1mM EDTA (pH 8.0), and twice in a 50 mM Tricine-NaOH (pH 7.8) resuspension buffer. Samples were resuspended to a DOC:SDS:Chl ratio of 20:10:1 in a 0.3 M Tris-NaOH (pH 8.8) solubilization buffer containing 13% (w/v) glycerol. Nondenaturing electrophoresis was performed on an 8% (w/v) polyacrylamide resolving gel containing a 150 mM Tris (pH 6.35) buffer and a 4% (w/v) stacking gel containing a 40 mM Tris (pH 6.14) buffer. Lanes were loaded with 20 μg Chl and the gel was run at 8 mA for 40 min and 12 mA for 1 h at 5 °C in the dark. The excised lanes were scanned at 671 nm on a Beckman DU 640 spectrophotometer for Chl *a* absorbance and the relative content of each band was determined by the peak area normalized to the total area of the scan.

PsaA/B heterodimer isolation

Thylakoid preparations from *C. reinhardtii* were solubilized in a 60 mM Tris (pH 7.8) buffer containing 1 mM EDTA, 12% (w/v) sucrose and 2% (w/v) SDS to achieve a SDS:Chl of 20:1. Membrane polypeptides were electrophoresed in a 10% (w/v) polyacrylamide resolving gel containing 6 M urea, 0.66 M Tris (pH 8.8), and 5% (w/v) polyacrylamide stacking gel containing 0.125 M Tris (pH 6.8) according to Laemmli (1970). One large well was loaded with 250–350 μg Chl and the gel was electrophoresed for 1 h at 30 mA at room temperature. A Chl-protein band of approximately 120 kD representing the pigment-binding PsaA/B heterodimer was excised. Gel pieces were subsequently loaded on an Elutrap Electro-Separation System (Schleicher & Schuell) and the proteins were electroeluted from the gel at 125 mV at 5 °C for 5 h according to Jacobs and Clad (1986). Protein concentration was measured by the standard bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). Each sample was checked for puritiy which was assessed to be at least 95% pure based on SDS-PAGE as described above.

PsaA/B heterodimer antibody production

New Zealand White male rabbits were inoculated with 300–500 μg protein solubilized in a sodium phosphate buffer (0.02 M $NaPO_4$, pH 7.3; 0.14 M NaCl) and Hunter's Titer Max adjuvant (1:1). Antibodies were precipitated by the addition of 27% (w/v) ammonium sulphate to the serum and incubation at room temperature for 2 h. Antibodies were collected by centrifugation at 10 000 $\times$ *g* for 30 min and the pellet was dissolved in a buffer containing 15 mM potassium phosphate (pH 7) and 50 mM NaCl (Newsted and Huner 1987). The sample was dialysed against the same buffer for 16 h at 5 °C and stored at −80 °C.

SDS-PAGE and immunoblotting

Thylakoid samples were solubilized as described above and loaded on an equal Chl basis (6 μg per lane). Electrophoretic separation was performed as mentioned above with a 15% (w/v) polyacrylamide resolving and 8% (w/v) polyacrylamide stacking gel using a Mini-Protean II apparatus (Bio-Rad) and electrophoresed at 20 °C for 1.5 h at 30 mA. Protein standards were obtained from Sigma.

Thylakoid polypeptides separated by SDS-PAGE were transferred electrophoretically to nitrocellulose membranes (0.2 μm pore size, Bio-Rad) at 5 °C for 1.5 to 3 h at 295 mA. The membranes were pre-blocked with a Tris-buffered salt (20 mM Tris, pH 7.5; 150 mM NaCl) containing 5% (w/v) BSA and 0.01% (w/v) Tween 20. Membranes were probed with the following antibodies: (1) Lhcb2, 1:2000 dilution; (2) PsaA/B heterodimer, 1:2000 dilution; (3) D1, 1:5000 dilution, and α-and β-ATPase were used at a 1:1000 dilution. Antibodies raised against 8 LHC I polypeptides of *C. reinhardtii* (Bassi et al. 1994) were used at the following dilutions: p14.1, p18 and p22.2, 1:1000; p15 and p15.1, 1:2000; p17.2, p18.1 and p22.1, 1:5000. After incubation with the secondary antibody conjugated with horseradish peroxidase (Sigma, 1:20 000 dilution), the antibody complexes were visualized by incubation of the blots in ECL chemiluminescent detection reagents (Amersham) and developed on X-Omat XRP5 film (Kodak).

Low temperature Chl fluorescence

Low temperature (77 K) Chl *a* fluorescence emission spectra of whole cells were collected on a PTI Fluorometer (Model LS-1, Photon Technology International Inc.). Fresh samples collected during mid-log phase were resuspended to a Chl concentration of 5 μg in growth media containing 50% (v/v) glycerol. Samples were dark adapted at 20 °C for 15 min and scanned at 77 K with an excitation wavelength of 435 nm. A slit width of 4 nm for both the actinic light and

the detector was used. All spectra represent an average of 5 corrected scans.

Oxygen evolution

O_2 evolution was measured *in vivo* at the growth temperature with a Clark type aqueous phase O_2 electrode (Hansatech Ltd., King's Lynn, UK). Photosynthetic light-response curves were constructed using a range of irradiance between 20 and 1300 μmol photons m^{-2} s^{-1} according to Falk and Samuelsson (1992). Fresh samples were dark adapted under CO_2-saturated conditions for 15 min. O_2 evolution at each measuring irradiance was recorded on an Omnigraphic 2000 chart recorder (Bauch & Lomb Inc.). Photosynthetic efficiency was determined under light limiting conditions (<50 μmol photons m^{-2} s^{-1}) and estimated as the initial slope of the light response curve and photosynthetic capacity was estimated as the light saturated rate of O_2 evolution and was measured at an irradiance of 1300 μmol photons m^{-2} s^{-1}.

Results

Growth and photosynthetic characteristics

Cultures of *C. subcaudata* exhibited exponential growth (μ_{max} = 0.0198 ± 0.0001) at 8 °C/150 μmol photons m^{-2} s^{-1}, but failed to grow at 29 °C/150 μmol photons m^{-2} s^{-1}, (Figure 1A). The psychrophile did grow at 16 °C (Table 1), but not at 20 °C (data not shown). The mesophilic species, *C. reinhardtii*, exhibited exponential growth at 29 °C/150 μmol photons m^{-2} s^{-1} (μ_{max} = 0.103 ± 0.01) but exhibited minimal growth at 8 °C/150 μmol photons m^{-2} s^{-1} (Figure 1B). These results indicate that *C. subcaudata* is adapted to grow at low temperature, thus confirming its psychrophilic nature (Eddy 1960).

Priscu and Neale (1995) observed that *C. subcaudata* normally grows at an irradiance of less than 50 μmol m^{-2} s^{-1} in its natural environment. Thus, the control growth condition for the psychrophile was designated as 8 °C/20 μmol photons m^{-2} s^{-1} (P-control) and the control growth condition for the mesophile was designated as 29 °C/150 μmol photons m^{-2} s^{-1} (M-control). P-control exhibited about a 12 fold greater doubling time than M-control. The mesophile and the psychrophile were also grown at 16 °C/ 150 μmol photons m^{-2} s^{-1} (16/150) to compare the two species at the same growth regime. The psychrophile grown at 16/150 exhibited about a 2.3 fold greater doubling time than the mesophile (Table 1).

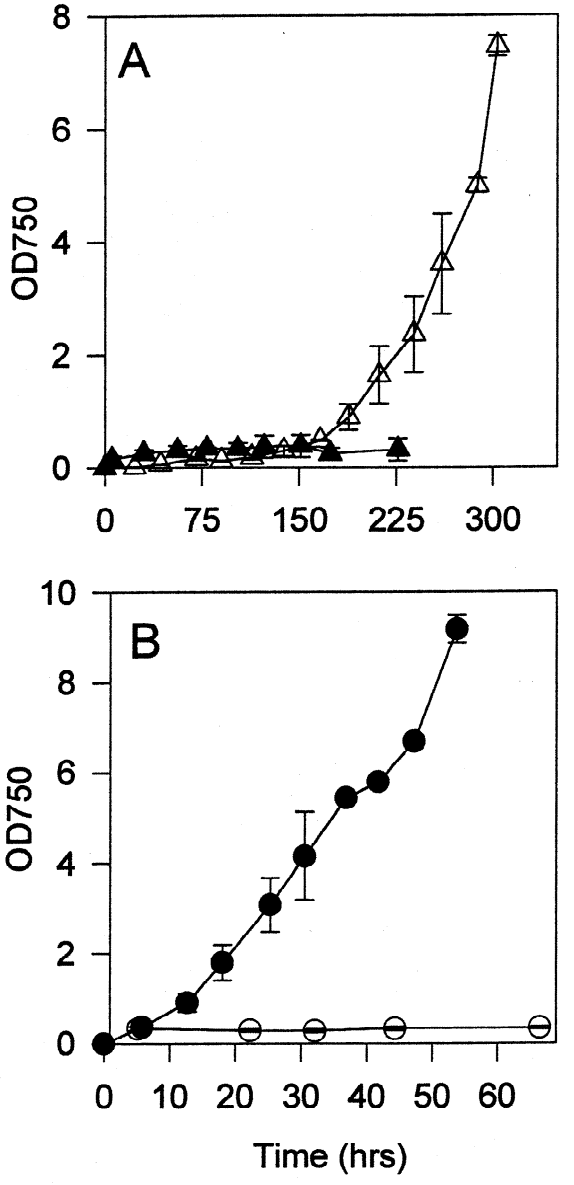

Figure 1. Growth curves, measured as the change in optical density (OD750) as a function of time after inoculation in *C. reinhardtii* (closed) and *C. subcaudata* (open) grown at 8 °C/150 μmol photons m^{-2} s^{-1} (A) and 29 °C/150 μmol photons m^{-2} s^{-1}(B). Bars represent SD; $\geqslant$3.

Although M-control exhibited a P_{max} that was about 6 times higher than that of P-control, both species exhibited similarly high photosynthetic efficiencies (Table 1). However, when grown at 16/150,

Table 1. Growth and photosynthetic characteristics of *C. subcaudata* (P) and *C. reinhardtii* (M) grown under control conditions of 8 °C/20 μmol photons m^{-2} s^{-1} (P-control), and 29 °C/150 μmol photons m^{-2} s^{-1} (M-control), respectively. Both species were also grown under comparable conditions of 16 °C/150 μmol photons m^{-2} s^{-1}. Values represent mean ± SD of at least three experiments. t_{gen}, doubling times (h^{-1}), calculated from exponentially growing cultures; P_{max}, gross photosynthesis (μmol O_2 h^{-1} mg Chl^{-1}), calculated at light-saturated levels of O_2 evolution; α, photosynthetic efficiency (μmol O_2 h^{-1} mg Chl^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$)

Growth regime (°C/μmol m^{-2} s^{-1})	t_{gen}	P_{max}	α	Chl *a/b*
M-control	7.00 ± 0.90	407.18 ± 21.93	0.699 ± 0.078	2.81 ± 0.06
M 16/150	29.2 ± 5.80	177.16 ± 19.13	0.308 ± 0.044	3.11 ± 0.04
P 16/150	67.0 ± 13.2	118.48 ± 37.59	0.443 ± 0.121	2.01 ± 0.20
P-control	85.5 ± 5.30	68.34 ± 11.38	0.770 ± 0.060	1.80 ± 0.05

Table 2. HPLC analysis of photosynthetic pigments extracted from whole cells of *C. subcaudata* (P) and *C. reinhardtii* (M) grown under control conditions of 8 °C/20 μmol photons m^{-2} s^{-1} (P-control), and 29 °C/150 μmol photons m^{-2} s^{-1} (M-control), respectively. Both species were also grown under comparable conditions of 16 °C/150 μmol photons m^{-2} s^{-1}. N, neoxanthin; V, violaxanthin; A, antheraxanthin; Z, zeaxanthin; L, lutein; β-C, β-carotene; EPS, xanthophyll cycle epoxidation state (V + 0.5A/V + A + Z). Values represent mean ± SD (n = 3), and were expressed as mmol pig mol^{-1} Chl *a*

Growth regime (°C/μmol m^{-2} s^{-1})	N	V	A	Z	L	β-C	V + A + Z	EPS
M-control	63 ± 6	93 ± 2	25 ± 2	22 ± 2	266 ± 62	119 ± 10	140 ± 2	0.22 ± 0.18
M 16/150	61 ± 10	75 ± 2	50 ± 4	55 ± 1	747 ± 105	103 ± 11	180 ± 9	0.45 ± 0.09
P 16/150	66 ± 5	69 ± 2	39 ± 5	63 ± 3	447 ± 8	92 ± 21	170 ± 20	0.49 ± 0.04
P-control	63 ± 6	40 ± 1	2 ± 1	7 ± 0.5	302 ± 10	75 ± 1	49 ± 7	0.15 ± 0.09

both species exhibited comparable P_{max} and similarly lower photosynthetic efficiencies (Table 1).

Pigment composition

The psychrophile grown under control conditions exhibited an unusually low Chl *a/b* ratio (1.80) which was about 40% lower than that of the M-control cells (2.81) (Table 1). When both species were grown under the same condition of 16/150, the psychrophile exhibited a Chl *a/b* ratio of 2.01, which was 35% lower than the Chl *a/b* ratio observed in the mesophile. As indicated in Table 2, whole cell extracts of P-control exhibited 40–90% lower levels of the xanthophylls violaxanthin, antheraxanthin and zeaxanthin which reflected a 65% lower level in the total xanthophyll pool size in comparison with the M-control (Table 2). However, both P-control and M-control exhibited similarly low epoxidation states. The total pool size of xanthophyll cycle pigments as well as the xanthophyll epoxidation state (EPS), increased to comparable levels when cultures of the mesophile and the psychrophile were grown at 16/150. In addition, both species exhibited higher levels of lutein when grown at 16/150 in comparison with their control conditions. Thus, the differences in carotenoid composition that were observed between the two species grown under control conditions appeared to be minimized when the mesophile and the psychrophile were both grown at 16/150.

Chl-protein complexes

As expected from previous reports for the separation of Chl-protein complexes from thylakoids of plants and algae (Huner et al. 1987; Król et al. 1997), the nondenaturing gel electrophoresis of the DOC-solubilized membranes from *C. reinhardtii* and *C. subcaudata* resolved several pigment bands. Each pigment band was identified by their characteristic absorption spectra (data not shown) and relative migration in the SDS/DOC non-denaturing gel (Figure 2)

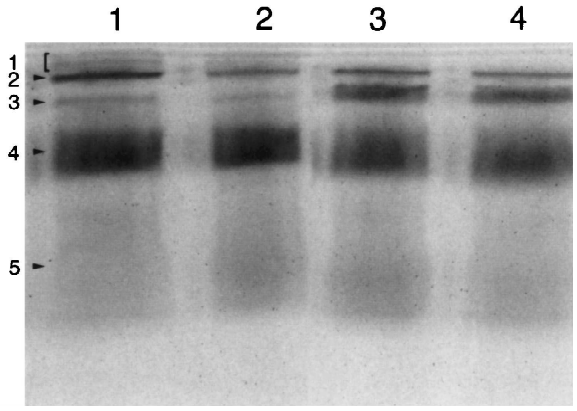

Figure 2. Nondenaturing electrophoretic separation of Chl-binding protein complexes in isolated thylakoids of the mesophile (M) and the psychrophile (P) grown under varying conditions. Lanes were loaded equally with 20 μg Chl. Numbers on the left indicate the separation of the following Chl-protein complexes: 1, LHC I; 2, CP1; 3, oligomeric LHC II; 4, monomeric LHC II; 5, free pigment. Lane 1, M-control (29 °C/150 μmol photons m^{-2} s^{-1}); lane 2, M 16 °C/150 μmol photons m^{-2} s^{-1}; lane 3, P 16 °C/150 μmol photons m^{-2} s^{-1}; lane 4, P-control (8 °C/20 μmol photons m^{-2} s^{-1}).

(Król et al. 1997). Four of these bands were Chl *a/b* binding protein complexes (Figure 2) and were identified as the monomeric (band 4), dimeric, and oligomeric LHC II (band 3), and the PS I light harvesting complex, LHC I (band 1). Two additional protein complexes were Chl *a* binding and were identified as CP1 (band 2) and CPa. The dimeric and CPa bands were not well resolved in Figure 2. The presence of minimal free pigment (band 5) indicates that most of the pigments remained associated with protein during solubilization and separation.

Both P-control and the psychrophile grown at 16/150 exhibited a 5-fold higher ratio of oligomeric: monomeric LHC II (LHC II3:LHC II1) compared to M-control or the mesophile grown at 16/150 (Figure 2; Table 3). The mesophile grown under control conditions exhibited significantly higher levels of LHC I in comparison with P-control which was reflected in more than a 3 fold higher LHC I:LHC II ratio in M-control in comparison with P-control. This trend was also observed when both species were grown at 16/150 (Figure 2, Table 3).

Low temperature Chl fluorescence emission spectra of whole cells

The low temperature (77 K) Chl *a* fluorescence emission of M-control cells exhibited an emission spectrum with maxima at 681 (F_{681}) and 709 nm (F_{709}) (Figure 3A). In contrast, the mesophile grown at 16/150 exhibited a shoulder at around 681 nm and a maximum at 709 nm (Figure 3B). Thus, the mesophile grown at 16/150 exhibited lower levels of F_{681} (F_{681}:F_{709} = 0.18) in comparison to M-control (F_{681}:F_{709} = 0.39). The emission spectrum of P-control cells exhibited an emission maximum at about 681 nm, a maximum at 692 nm, but no major emission maximum at 709 nm (Figure 3A). The psychrophile grown at 16/150 exhibited a blue shift in its emission maximum (678 nm) relative to P-control (681 nm) and a reduction in the contribution of the emission at 692 nm.

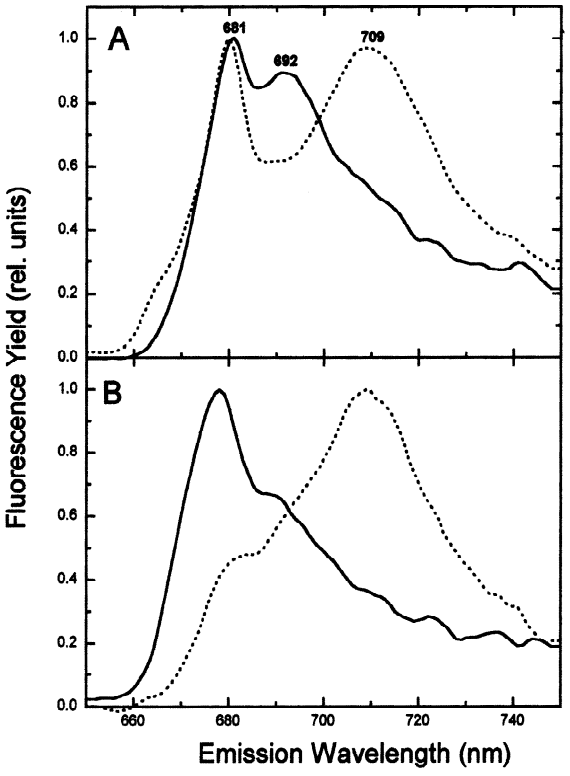

Figure 3. Chl *a* fluorescence emission spectra at 77 K of whole cells of *C. subcaudata* (—) and *C. reinhardtii* (.....) grown at control conditions (A) and 16 °C/150 μmol photons m^{-2} s^{-1}(B). The Chl concentration of all samples was 5 μg/ml and the excitation wavelength was 435 nm. Spectra represent an average of 5 corrected scans. Emission maxima are indicated in nm at the top of the peaks.

SDS-PAGE and Western blotting

The analysis of the SDS-solubilized thylakoid membrane proteins revealed differences in the apparent molecular weight of several polypeptides between the two species (Figure 4). Both species exhibited at least four major polypeptides within the 20–27 kD range.

Table 3. Ratios of Chl-protein complexes of *C. reinhardtii* (M) *and C. subcaudata* (P) grown under control and similar growth conditions. Ratios present relative absorbance values of the Chl-protein bands from lanes excised from nondenaturing gels similar to Figure 2 (n = 2). Lanes were loaded with 20 μg of Chl and scanned at 671 nm. Chl *a* content was expressed as relative peak area as a function of the total area

Growth regime ($^\circ$C/μmol m^{-2} s^{-1})	PS I:PS II	LHC I:LHC II	LHC II3:LHC II1
M-control	1.43	0.13	0.12
M 16/150	0.89	0.18	0.09
P 16/150	0.86	0.03	0.51
P-control	0.71	0.04	0.48

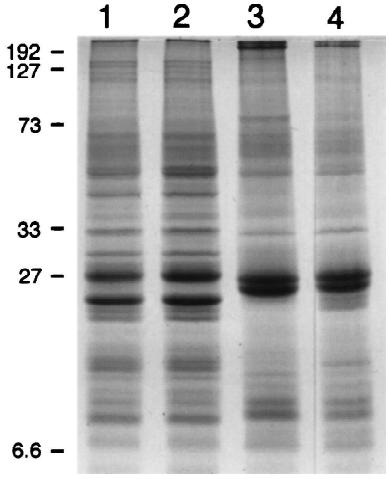

Figure 4. SDS-PAGE of thylakoid membrane polypeptides of the mesophile (M), and the psychrophile (P), grown under control versus the same conditions ($^\circ$C/μmol photons m^{-2} s^{-1}). Each lane was equally loaded with 6 μg Chl. Values left indicate molecular masses (kD) of standards in first lane. Lane 1, M-control; lane 2, M 16/150; lane 3, P 16/150; lane 4, P-control.

The molecular masses of these polypeptides in the mesophile were estimated to be 27, 23, 21 and 20 kD, in contrast to molecular masses of 26, 24, 23 and 22 kD for the psychrophile. Western blot analysis of SDS-PAGE of cells grown under control conditions showed that these polypeptides cross-reacted with an anti-LHC II polyclonal antibody (Figure 5A). Cultures of the psychrophile grown at 16/150 (Figure 4, lane 3) also appeared to lack the 22 kD protein that was observed in P-control (lane 4). Immunoblot analysis confirmed that this polypeptide also cross-reacted with the LHCII antibody (data not shown). While M-control and P-control exhibited comparable levels of PS II reaction centre polypeptide, D1 (Figure 5B), cul-

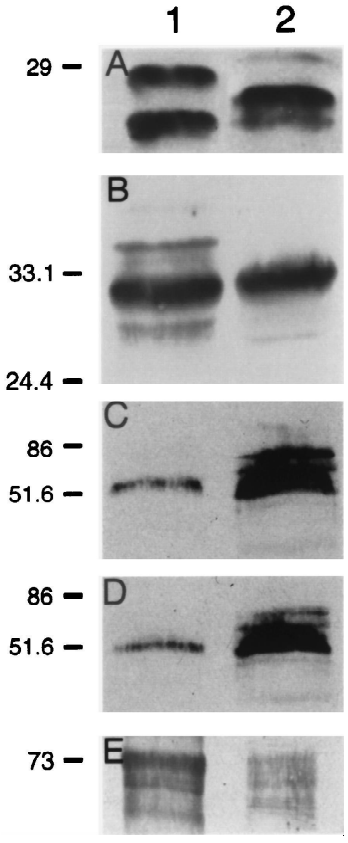

Figure 5. Western blots of SDS-PAGE probed with antibodies raised against αLhcb2 (A), D1 (B), αATPase (C), βATPase (D), and PsaA/B (E) of the thylakoids of the mesophile (M) and the psychrophile (P) grown under control conditions ($^\circ$C/μmol photons m^{-2} s^{-1}). Lanes of SDS-PAGE were equally loaded with 6 μg Chl. Lane 1, M-Control; lane 2, P-Control. Numbers on the left represent molecular (kD) masses of markers.

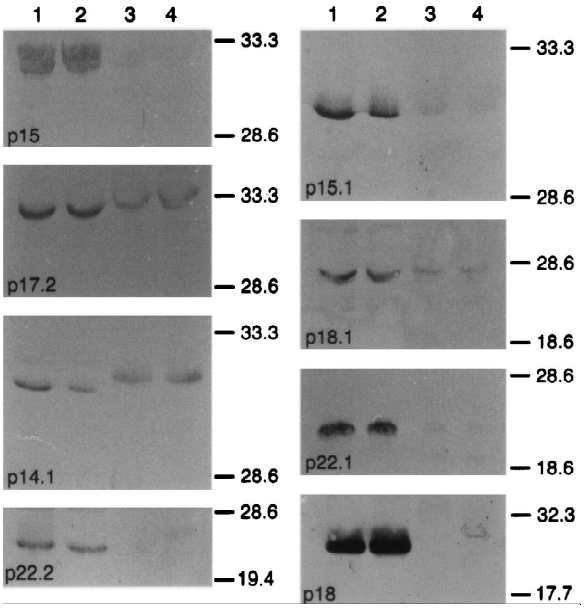

Figure 6. Immunoblot analysis of mesophile (M) and psychrophile (P) thylakoid samples using antibodies raised against 8 *Lhca* polypeptides of *C. reinhardtii*. SDS-PAGE samples were loaded with 6 μg Chl. The identity the polypeptides according to Bassi et al. (1994) is denoted in the lower left corner of each blot. Numbers on the right represent molecular (kD) masses of markers. Lane 1, M-control; lane 2, M 16 °C/150 μmol photons m^{-2} s^{-1}; lane 3, P 16 °C/150 μmol photons m^{-2} s^{-1}; lane 4, P-control.

tures of *C. subcaudata* grown under control conditions appeared to exhibit higher levels of both the α and β subunits of CF_1 on a Chl basis than M-control (Figure 5, C and D). Finally, P-control exhibited reduced levels of the major PS I core polypeptides, PsaA/B, in comparison with M-control (Figure 5E).

LHC I composition

Bassi and coworkers (1994) isolated at least 11 *Lhca* polypeptides in *C. reinhardtii* between the molecular mass of 21 and 31 kD. As expected, isolated thylakoids from *C. reinhardtii* grown at either control conditions or at 16/150, and subsequently probed with antibodies raised against 8 of the LHC I proteins, cross-reacted strongly with all of the antibodies tested (Figure 6, lanes 1 and 2). In contrast, thylakoid samples of P-control cells or of the psychrophile grown at 16/150 did not cross react with anti-p15, p22.2, or p18 antibodies. Furthermore, *C. subcaudata* exhibited greatly reduced levels of p15.1, p18.1, p17.2 and p22.1, but comparable levels of p14.1 relative to *C. reinhardtii* (Figure 6, lanes 3 and 4). Thus, it appears that the psychrophile possesses differentially reduced levels of almost all of the *Lhca* polypeptides compared to the mesophile, regardless of growth regime.

Discussion

The low temperature (77 K) fluorescence emission of M-control cells exhibited two major maxima at 681 (F_{681}) and 709 nm (F_{709}), considered to be associated with LHC II and PS I, respectively (Krause and Weis 1991). In contrast, P-control cells exhibited the F_{681} peak and a second major peak at 692 nm (F_{692}), thought to be associated with fluorescence of the PS II core (Krause and Weis 1991), but the absence of the major emission maximum at 709 nm associated with PS I. Immunoblot analysis indicated reduced levels of the PsaA/B heterodimer (Figure 5E) and either an absence or reduction in 7 of the 8 *Lhca* polypeptides in the psychrophile (Figure 6). Non-denaturing electrophoresis also showed that the psychrophile exhibited a concomitant reduction in LHC I abundance, as indicated by the lower LHC I:LHC II ratios (Table 3). Furthermore, preliminary experiments indicate that the functional absorptive cross section of PS I ($\sigma_{PS\,I}$) of *C. subcaudata* is smaller than the $\sigma_{PS\,I}$ of *C. reinhardtii* (R. Morgan, D. Bruce, N. Huner, unpublished). Thus, the 77 K fluorescence emission indicating a reduction in the PS I emission band at 709 nm can probably be accounted for by the presence of lower levels of the PsaA/B heterodimer as well as the decreased content of specific *Lhca* polypeptides in *C. subcaudata* versus *C. reinhardtii*.

Preliminary experiments indicated that on the basis of 77 K fluorescence, the psychrophile possesses relatively low levels of PS I even when it was grown in blue light, which was similar to the spectral range of the natural conditions found in Lake Bonney. In contrast, when *C. reinhardtii* was grown under growth conditions of identical light quality, cells responded by increasing PS I fluorescence (F_{709}) relative to LHC II fluorescence (F_{681}) (data not shown). Melis et al. (1996) observed that cells of *C. reinhardtii* also adjusted PS II/PS I stoichiometry in response to growth under blue light by increasing amounts of PS I while levels of PS II remained constant. These authors and others (Murakami et al. 1997) suggest that PS I plays a more important role than PS II in stoichiometry adjustments during acclimation to changes in light quality. Thus, the relatively low levels of PS I observed in *C. subcaudata* may reflect a specific adaptation in the

Antarctic species to the extremely stable low light conditions of a narrow spectral distribution. This suggestion is supported by Neale and Priscu (1995) who postulated that *C. subcaudata* may possess a higher PS II/PS I ratio to allow for more efficient use of light within the blue-green region and avoid an imbalance of energy distribution between the two photosystems.

Low temperature fluorescence emission maxima at 685 and 695 nm have been interpreted to be fluorescence associated with the PS II core (Kramer and Amesz 1982; Krause and Weis 1991). The significance of this major F_{692} maximum in the psychrophile is unknown, but may indicate an alteration in the association between LHC II and PS II core complex. Neale and Priscu (1995) observed that *C. subcaudata* exhibited a higher efficiency for utilization of blue-green light (481 nm) than *C. reinhardtii*, measured as turnover rate of PS II photochemistry. These authors and others (Kageyama et al. 1977) suggested that this could reflect a greater efficiency in energy transfer from LHC II to the PS II core complex. On the basis of 77 K fluorescence emission, the mesophile or the psychrophile responded to growth at 16/150 by adjustments at the level of PS II. However, while the mesophile exhibited a reduction in F_{681} relative to F_{709}, the psychrophile exhibited a reduction in F_{692} relative to F_{681}. The psychrophile grown under either condition also possessed higher levels of the oligomeric form of LHC II than the mesophile (Figure 2). Finally, preliminary results suggest that P-control cells possess a larger PS II absorptive cross section ($\sigma_{PS\,II}$) compared to $\sigma_{PS\,II}$ of M-control cells (R. Morgan, D. Bruce, N. Huner, unpublished). Thus, 77 K fluorescence emission spectra, the data from non-denaturing gel electrophoresis (Figure 2) as well as SDS-PAGE (Figure 4) support the suggestion that the *C. subcaudata* also possesses a different LHC II–PS II organization in comparison with *C. reinhardtii* which may reflect differences in energy transfer efficiencies.

Immunoblot analysis in the mesophile and the psychrophile grown under control conditions indicated that the two species exhibited comparable levels of the major PS II core protein, D1. In contrast, P-control appeared to possess higher amounts of both the CF_1 proteins, but lower levels of the PS I core proteins, PsaA/PsaB. Although the data in Figure 5 preclude the determination of absolute stoichiometries, we suggest that the psychrophile appears to exhibit a higher ratio of PS II:PS I than the mesophile. This is consistent with the 77 K fluorescence emission data (Figure 3). Furthermore, *C. subcaudata* appears to have a significantly lower ratio of PS II:CF_1 than *C. reinhardtii*. Thus, the stoichiometry of the thylakoid components appears to have been altered during adaptation to the Antarctic environment.

Neale and Priscu (1995, 1998) observed that *C. subcaudata* possessed relatively high levels of the xanthophylls violaxanthin, neoxanthin, and lutein in comparison to the mesophilic species *C. reinhardtii*. *C. reinhardtii* also exhibited 13% (mol mol^{-1} Chl *a*) β-carotene *versus* 5% in *C. subcaudata* (Neale and Priscu 1995). These researchers suggested that the phytoplankton of Lake Bonney have sacrificed photoprotective mechanisms in favour of an enhanced ability for efficient light harvesting and energy utilization. In contrast, cultures of both species responded to the growth regime of 16/150 not only by increasing the total xanthophyll cycle pigment pool size but also by a 2- to 3-fold increase in the xanthophyll cycle epoxidation state (Table 2). Both antheraxanthin and zeaxanthin are thought to play a major role in dissipating energy as heat under conditions of excess light absorption. This supports the observation made by Neale and Priscu (1998) that samples isolated from Lake Bonney exhibited nonphotochemical quenching, even at low irradiance levels. Thus, we conclude that, under our culturing conditions, *C. subcaudata* has retained the capacity to adjust the xanthophyll cycle upon altered growth conditions, and hence, possesses the capacity for dissipating excess energy as heat (Demmig-Adams 1990).

In summary, the Antarctic psychrophile, *Chlamydomonas subcaudata*, exhibits significant differences in its organization of LHC II–PS II and LHC I–PS I units as well as overall stoichiometry of PS II:PS I:CF_1, with respect to the mesophile, *C. reinhardtii*. However, the psychrophile appears to have retained it is potential to dissipate energy non-radiatively even though it is adapted to growth at low light. Despite these differences, the psychrophile and mesophile exhibit comparable P_{max} and photosynthetic efficiencies when grown under comparable conditions. These characteristics of the Antarctic psychrophile are probably a reflection of the unusual light and low temperature regime to which this organism is adapted.

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References

Bassi R, Soen SY, Frank G, Zuber H and Rochaix J (1994) Characterization of chlorophyll *a/b* proteins of photosystem I from *Chlamydomonas reinhardtii*. J Biol Chem 36: 25714–25721

Bidigare RR, Ondrusek ME, Kennicutt II MC, Iturriaga R, Harvey HR, Hoham RW and MackoSA (1993) Evidence for a photoprotective function for secondary carotenoids of snow algae. J Phycol 29: 427–434

Bolton J and Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Mar Biol 66: 89–94

Buma AGJ, Noordeloos AAM and Larsen J (1993) Strategies and kinetics of photoacclimation in three Antarctic nanophytoflagellates. J Phycol 29: 407–417

Chow WS, Melis A and Anderson JM (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proc Natl Acad Sci USA 87: 7502–7506

Cota GF (1985) Photoadaptation of high Arctic ice algae. Nature 315: 219–222

Cota GF and Sullivan CW (1990) Photoadaptation, growth and production of bottom ice algae in the Antarctic. J Phycol 26: 399–411

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Act 1020: 1–24

Diaz M, Ball E and Lüttge U (1990) Stress-induced accumulation of the xanthophyll photoxanthin in leaves of *Aloe vera*. Plant Physiol Biochem 28: 679–682

Eddy BP (1960) The use and meaning of the term 'psychrophilic'. J Appl Bact 23: 189–190

Falk S and Sammuelsson G (1992) Recovery of photosynthesis and Photosystem II fluorescence in *Chlamydomonas reinhardtii* after exposure to three levels of high light. Physiol Plant 85: 61–68

Guillard RRL (1973) Division rates. In: Stein JE (ed) Handbook of Phycological Methods, pp 289–313. Cambridge University Press, Cambridge, UK

Hanelt D, Melchersmann B, Wiencke C and Nultsch W (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149: 255–266

Huner NPA, Krol M, Williams JP, Maissan E, Low PS, Roberts D and Thompson JE (1987) Low temperature development induces a specific decrease in *trans*Δ^3-hexadecenenoicacid content which influences LHC II organization. Plant Physiol 84: 12–18

Ivanov AG, Król M, Maxwell, D and Huner NPA. (1995) Abscisic acid induced protection against photoinhibition of PS II correlates with enhanced activity of the xanthophyll cycle. FEBS Lett 371: 61–64

Jacobs E and Clad A (1986) Electroelution of fixed and stained membrane proteins from preparative sodium dodecyl sulphate-polyacrylamide gels into a membrane trap. Anal Biochem 154: 583–589

Jeffrey SW and Humphrey GF (1975) New spectrophotometric equations for determining chlorophyll *a*, *b*, *c*1, *c*2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167: 191–194

Kageyama A, Yokohama Y, Shimura S and Ikawa T (1977) An efficient energy transfer from a carotenoid, siphonaxanthin to chlorophyll *a* observed in a deep-water species of chlorophycean seaweed. Plant Cell Physiol 18: 477–480

Kirst GO and Wiencke C (1995) Ecophysiology of polar algae. J Phycol 31: 181–19

Kramer HJM and Amesz J (1982) Anisotropy of the emission and absorption bands of spinach chloroplasts measured by fluorescence polarization and polarized excitation spectra at low temperature. Biochim Biophys Acta 682: 201–207

Krause GH and Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42: 313–49

Król M, Maxwell DP and Huner NPA (1997) Exposure of *Dunaliella salina* to low temperature mimics the high light-induced accumulation of carotenoids and the carotenoid binding protein (Cbr). Plant Cell Physiol 38: 213–216

Kudoh S, Robineau B, Suzuki Y, Fujiyoshi and Takahashi (1997) Photosynthetic acclimation and the estimation of temperate ice algal primary production in Saroma-Ko Lagooon, Japan. J Mar Syst 11: 93–109

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Lizotte MP and Priscu JC (1992) Spectral irradiance and bio-optical properties in perennially ice-covered lakes of the dry valleys (McMurdo Sound, Antarctica). Ant Res Ser 57: 1–14

Lizotte MP and Priscu JC (1998) Distribution, succession and fate of phytoplankton in the dry valley lakes of Antarctica, based on pigment analysis. In: Priscu JC (ed) Ecosystem Dynamics in a Polar Desert, The McMurdo Dry Valley, Antarctica, Vol. 72, pp 229–240. American Geophysical Union, Washington, DC

Lizotte MP and Sullivan CW (1991) Rates of photoadaptation in sea ice diatoms from McMurdoSound, Antarctica. J Phycol 27: 367–373

Melis A, Murakami A, Nemson JA, Aizawa K, Ohki K and Fujita Y (1996) Chromatic regulation in *Chlamydomonas reinhardtii* alters photosystem stoichiometry and improves quantum efficiency of photosynthesis. Photosynth Res 47: 253–265

Murakami A, Fujita Y, Nemson JA and Melis A (1997) Chromatic regulation in *Chlamydomonas renhardtii*: Time course of photosytem stoichiometry adjustment following a shift in growth light quality. Plant Cell Physiol 38: 188–193

Neale PJ, Lesser MP and Cullen JJ (1994) Effects of ultraviolet radiation on the photosynthesis of phytoplankton in the vicinity of McMurdo Station, Antarctica. Ant Res Ser 62: 125–142

Neale PJ and Pricu JC (1995) The photosynthetic apparatus of phytoplankton from a perennially ice-covered Antarctic lake: Acclimation to an extreme shade environment. Plant Cell Physiol 36: 253–263

Neale PJ and Pricu JC (1998) Fluorescence quenching in phytoplankton of the McMurdo dry valleylakes (Antarctica): Implications for the structure and function of the photosynthetic apparatus. In: Priscu JC (ed) Ecosystem Dynamics in a Polar Desert, The McMurdo Dry Valley, Antarctica, Vol. 72, pp 241–254. American Geophysical Union, Washington, DC

Newsted WJ and Huner NPA (1987) Major sclerotial polypeptides of psychrophillic fungi: Temperature regulation of *in vivo* synthesis in vegetative hyphae. Can J Bot 66: 1755–1761

Nichols HW and Bold HC (1965) *Trichosarcina polymorpha*. Gen. Et Sp. Nov. J Phycol 1: 34–38

Spigel RH and Priscu JC (1996) Evolution of temperature and salt structure of Lake Bonney, a chemically stratified Antarctic lake. Hydrobiol 321: 177–190

Wiencke C and Fischer G (1990) Growth and stable carbon isotope composition of cold-water macroalgae in relation to light and temperature. Mar Ecol Prog Ser 65: 283–292