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Strategies of thermal adaptation by high-latitude cyanobacteria

EVONNE P. Y. TANG* AND WARWICK F. VINCENT

Département de biologie & Centre d'études Nordiques, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4

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SUMMARY

Although mat-forming cyanobacteria dominate many freshwater ecosystems in the Arctic and Antarctic, their optimal temperature for growth (T_{opt}) is usually much higher than the temperature range of their native habitat. The present study compared the temperature dependence of growth, pigment composition and absorbance, photosynthesis and photosynthate partitioning for two strains of cyanobacteria with contrasting T_{opt} values; *Phormidium subfuscum*, isolated from McMurdo Ice Shelf, Antarctica, and *Phormidium tenue*, collected from the Kuparuk River in the tundra region of northern Alaska. *Phormidium subfuscum* grew between 5 and 20°C with a T_{opt} of 15°C whereas *P. tenue* showed detectable growth from 10 to 40°C and a T_{opt} of 30°C. Light utilization efficiency, photosynthetic capacity and the irradiance at the onset of light saturation increased with increasing temperature up to T_{opt} in both strains. The cellular concentrations of chlorophyll a (Chl a) and carotenoid (CAR) and the *in vivo* absorbance maxima for Chl a, CAR, C-phycocyanin and allophycocyanin changed little for *P. subfuscum* but all these variables increased across the temperature range up to T_{opt} for *P. tenue*. Neither *P. subfuscum* nor *P. tenue* showed changes in relative carbon allocation with varying temperature, suggesting that gross biochemical alterations are not a characteristic of temperature acclimation in these cyanobacteria. We conclude that the eurythermal cyanobacterium *P. tenue* optimizes growth over a wide range of temperatures by adjusting its light-capturing as well as carbon fixation characteristics, whereas stenothermal *P. subfuscum* relies on changes in carbon fixation without concomitant shifts in pigment content.

Key words: Antarctic, Arctic, cyanobacteria, carbon partitioning, photosynthesis, pigment.

INTRODUCTION

There are relatively few studies on the relationship between cyanobacterial growth and temperature perhaps because, until recently, it was generally assumed that cyanobacteria prefer high temperatures ($\geqslant$20°C; Reynolds, 1984; Robarts & Zohary, 1987). However, mat-forming cyanobacteria constitute a major component of many freshwater algal communities in perennially cold Arctic (Vézina & Vincent, 1997) and Antarctic (James *et al.*, 1995) lakes, ponds and streams. Tang *et al.* (1997) found that, among 27 isolates of polar cyanobacteria, the temperature optimum for growth was highly variable. In the two most extreme cases, *Phormidium subfuscum* grew over the temperature range 5–25°C with an optimal temperature for growth of 15°C whereas for *Phormidium tenue*, μ increased with temperature over the range 5–35°C (Tang *et al.*, 1997). Like all polar cyanobacteria examined to date, both isolates are tolerant rather than adapted to the cold water environment (Castenholz & Schneider, 1993; Tang *et al.*, 1997; Roos & Vincent, 1998).

Polar cyanobacterial isolates may have developed adaptive strategies so that they can withstand and grow under the sub-optimal temperature regime of their native habitats. For example, cyanobacteria may adjust their photosynthetic apparatus in order to acclimate to the prevailing temperature. They tend to have decreased photosynthetic capacity (P_{max}) at low temperatures due to depressed activity of ribulose-1,5-bisphosphate carboxylase (Rubisco) (Li *et al.*, 1984; Raven & Geider, 1988). Therefore, a potential adaptive strategy for polar cyanobacteria would be to decrease their concentrations of chlorophyll a and light harvesting pigments (Geider, 1987; Davison, 1991) while increasing the activity of Rubisco (Li & Morris, 1982).

Although the concentration of chlorophyll a may decrease with decreasing temperature, carotenoid

*Author for correspondence (present address) Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, MD 21037, USA (tel +1410 798 4424 ext. 353; fax +1301 261-7954; e-mail *tang@serc.si.edu*).

concentrations often remain high (Young, 1993) because low temperature induces photoinhibition (Falk *et al.*, 1990; Davison, 1991; Krause, 1993). Carotenoids may act as a screening pigment that blocks excess light, as well as scavenging reactive oxygen species, thereby decreasing the damaging effects of intense illumination at low temperatures (Krause, 1993). Cyanobacteria exposed to different temperature regimes may therefore differ in their relative pigment composition.

Polar cyanobacteria may also alter their resource allocation in order to acclimate to the cold. For instance, they may direct more assimilated carbon (C) towards protein synthesis (Somero, 1978). All enzymically-based physiological processes such as nutrient uptake and carbon assimilation are retarded at low temperatures (Falkowski & Raven, 1997). Thus, cyanobacteria may increase their C allocation towards protein in order to increase enzyme production that may offset lowered enzyme activity (Somero, 1978). Increased lipid content has also been suggested as a possible strategy for temperature adaptation (Smith *et al.*, 1994), and elevated percentages (31–80°C) of C incorporation into lipid have been reported in Arctic ice algae (Smith & Morris, 1980; Smith *et al.*, 1989). The range of these physiological acclimations to changing temperature is likely to differ between stenothermal and eurythermal phototrophs.

The purpose of the present study was to compare the temperature dependence of growth and photosynthesis of two strains of high-latitude mat-forming cyanobacteria with radically different temperature optima and range for growth, *P. subfuscum* and *P. tenue*. We also compared their C allocation, pigment composition and *in vivo* absorbance maxima of carotenoid (CAR), chlorophyll a (Chl a), C-phycocyanin (PC) and allophycocyanin (APC). We hypothesized that eurythermal *P. tenue* has a greater flexibility in resource allocation and pigment composition than stenothermal *P. subfuscum*, thereby allowing the former to adjust to a wider range of temperatures.

MATERIALS AND METHODS

Cultures and their maintenance

Phormidium subfuscum Kütz. was isolated from a microbial mat community growing in a lake in the McMurdo Ice Shelf, Antarctica (78° S, 166° E). The Ice Shelf area is a polar desert environment with summer temperatures that range from −1.1 to −6.1°C with a mean of −3.2°C (Vincent, 1988). The freshwater lakes in this area are characterized by stable, low water temperatures in the range 0–8°C (Vincent, 1988; Castenholz & Schneider, 1993). *Phormidium tenue* (Menegh.) Gom. was isolated from a periphyton community (rock scraping) in the Kuparuk River. This river is located in the Long Term Ecological Research (LTER) site of the Toolik Lake area, Alaska (68° N, 149° W), which lies in a lowland tundra zone and experiences a mean summer air temperature of 10°C (Milner *et al.*, 1995). The average summer water temperature of the river is 8–10°C (Hershey *et al.*, 1995).

The cyanobacterial samples were placed immediately after collection onto agar plates containing BG-11 medium (Rippka *et al.*, 1979). The field material was transferred onto fresh agar plates where individual filaments were picked off and transferred to BG-11 liquid medium in order to obtain a clonal culture. Stock cultures were maintained at 13°C under continuous photosynthetic active radiation (PAR) of 45 μmol m^{-2} s^{-1}, which was provided by cool white fluorescent tubes and measured by a QSL photometer (Biospherical Instruments Inc., San Diego, CA, USA) equipped with a 4π sensor.

Pre-acclimation and growth assays

All cultures were pre-incubated under experimental conditions for 5 d before experimentation in order to allow the cyanobacterial cultures to photoacclimate and to respond to temperature changes by adjusting their pigment composition (Sosik & Mitchell, 1994) and enzymic properties (Li & Morris, 1982; Davison 1991). Most cyanobacteria acclimate to the prevailing spectral quality and quantity (Falkowski & LaRoche, 1991) and to temperature (Campbell *et al.*, 1995) after one cell generation. In the present study, all cultures would have undergone one doubling, with the exception of *P. tenue* at 5°C.

Growth rates for *P. subfuscum* (between 5–25°C at 5°C intervals) and *P. tenue* (between 5–40°C at 5°C intervals) were measured under a photosynthetic photon fluence rate (PPFR) of 225 μmol m^{-2} s^{-1} over the waveband 400–700 nm. Growth curves were established by monitoring changes in optical density (OD_{750}) over time. At each temperature, 16 Erlenmeyer flasks containing 100 ml of BG-11 medium were inoculated with a small quantity of pre-acclimated culture to achieve a final turbidity of 0.015. The filaments were not in suspension, but rather were allowed to form benthic aggregates, as in their natural habitat. At each sampling time, two flasks were chosen randomly and the cultures were homogenized with a Teflon tissue-grinder (Type R2R1, Caframo Inc., Wiarton, ON, Canada), after which their optical densities at 750 nm (OD_{750}) were measured. The OD_{750} of *P. subfuscum* was monitored every day for 8 d whereas the OD_{750} of *P. tenue* was measured every 12 h due to their rapid growth. Measurements were made every 4 d over a 28 d period at 5°C for both strains in order to allow sufficient response time for detectable changes. The relationships between log OD_{750} and time were plotted and regression lines were fitted to the

apparently linear portion of each curve for estimation of exponential growth rates. At least five points were used for curve-fitting.

Pigment content

During the mid-exponential growth phase, an aliquot of culture was filtered onto MFS® borosilicate microfibre filters (equivalent to Whatman GF/F filters; nominal porosity of 0.7 μm). The filters were wrapped in aluminium foil and kept frozen for analysis of CAR and Chl a. CAR and Chl a were extracted by grinding in 90% acetone using a Teflon tissue-grinder and quantified using the method outlined in Quesada & Vincent (1993). This extraction method gives a high efficiency for microbial mats relative to other solvents and extracts even though it is less efficient than sonication (Downes *et al*., 1993).

In vivo *absorbance*

The *in vivo* absorbance spectra were measured for each culture during mid-exponential growth phase using a HP-8425A diode array spectrophotometer (Hewlett Packard (Canada) Ltd, Kirkland, QC, Canada) connected to a RSA-HP-84 integrating sphere (Labsphere Inc., North Sutton, NH, USA). A 50 ml aliquot of culture was first concentrated by sedimentation, and its *in vivo* absorption spectrum was measured from 400 to 800 nm with an integration time of 0.1 s, in order to circumvent cell sinking effects. The culture was then diluted and the OD_{750} was measured with a Milton-Roy (Rochester, NY, USA) Spectronic 1001 spectrophotometer. The *in vivo* absorbance value at 750 nm was subtracted from all absorbance values for background correction. The corrected absorbance was then normalized to biomass (i.e. OD_{750}). The absorbance peaks at 480, 620, 650 and 680 nm correspond to CAR, PC, APC and Chl a, respectively.

Photosynthesis versus irradiance curves

The relationships between photosynthesis (P) and irradiance (E) were established using [^{14}C]bicarbonate uptake. *Phormidium subfuscum* and *P. tenue* were pre-acclimated at each temperature and PPFR for 5 d before experimentation, after which they were transferred to 4 l of fresh BG-11 medium. Cultures were then allowed to grow to mid-exponential phase and an optical density of 0.010–0.040 which was low enough to prevent self-shading during the incubation. The sample was dispersed with a Teflon tissue-grinder and then mixed by a magnetic stirrer to ensure homogeneity while it was dispensed into a triplicate series of 13 60-ml culture bottles. Each bottle was spiked with 0.1 ml of 0.5 μCi ml^{-1} of [^{14}C]bicarbonate to yield a final activity of 8.3×10^{-4} μCi ml^{-1}. Twelve bottles were incubated in the light while the last one was wrapped in aluminium foil and incubated in the dark. The triplicate series of bottles were incubated in a temperature-regulated linear incubator similar to those described in Jassby & Platt (1976) and Frénette *et al*. (1993). Briefly, the incubator was a box with a glass front, consisting of nine slots perpendicular to the glass. Temperature control was achieved by continuous water circulation through the incubator. Illumination was provided by a series of nine 12V tungsten halogen lamps, and two blue (Philips TLD18), one green (Philips TLD17) and one cool-white (Philips TLD33) fluorescent tubes. This combination of light sources resulted in a visible spectrum similar to natural sunlight (Markager *et al*., 1998). The PAR received by the 12 bottles ranged from 55 to 1062 μmol m^{-2} s^{-1}. The three sets of 12 culture bottles were stacked together and incubated in three of the nine slots for 2 h. Uptake of C was terminated by filtration on to MFS borosilicate microfibre filters. The filters were then transferred into 20 ml scintillation vials where 0.1 ml of 1N HCl was added to each filter. Unbound $NaH^{14}CO_3$ was converted to $^{14}CO_2$ by this treatment and was allowed to dissipate overnight, after which 10 ml of Beckman Ready Safe scintillation cocktail was added. The vials were kept in the dark for 24 h to decrease chemiluminescence and then counted in a Beckman (Mississauga, ON, Canada) LS 6500 scintillation counter equipped with an internal quench curve. Uptake of ^{14}C in the dark was subtracted from that measured in the light, before calculation of C uptake. The dissolved inorganic C content in BG-11 medium was estimated from pH and alkalinity by Gran-titration (Wetzel & Likens, 1990) using a Hanna Instrument 8521 pH meter equipped with a temperature probe. For the alkalinity measurement, 60 ml of culture was titrated with 0.1 N HCl until the first equivalence point was reached. Alkalinity was then estimated from normality of the acid, the volume of acid used to reach first equivalence point and the sample volume, using the equation in Wetzel & Likens (1990). Neither *P. subfuscum* nor *P. tenue* displayed any sign of photoinhibition at the highest experimental irradiance. Therefore, the equation of Webb *et al*. (1974) along with a re-iterative fitting procedure (Sigmaplot version 3.02, Jandel Scientific, Corte Madera, CA, USA) was used in order to describe the relationship of P versus E for the 13 sets of data.

Endproducts of photosynthesis

To study the relative partitioning of recently fixed C into low molecular weight metabolites, lipid, protein and polysaccharides fractions, the [^{14}C]bicarbonate experiments were repeated and filtered samples of cyanobacteria were frozen (−20°C) in the dark until analysis. These samples were subsequently extracted and assayed for the four classes of photosynthate

(low molecular weight metabolites, lipid, protein and polysaccharides) following the method of Li *et al*. (1980).

RESULTS

Growth rates

Phormidium subfuscum grew over the temperature range 5–20°C with maximum growth rates at 15°C while *P. tenue* grew over the temperature range 10–40°C and showed most rapid growth at 30°C (Table 1). The Q_{10} value was 2.4 over the range 5–15°C for *P. subfuscum*, and 2.5 over the range 10–30°C for *P. tenue*. Thus the growth rates of both isolates vary with temperature at about the same rate even though the latter grew over a wider temperature range. A pairwise comparison of growth rates between 5 and 20°C showed that *P. subfuscum* had significantly higher rates of μ than did *P. tenue* at 5°C ($t = 10.1$, $P<0.001$) and 10°C ($t = 11.8$, $P<0.001$). However, *P. tenue* had superior growth rates compared with *P. subfuscum* at 15°C ($t = 5.6$, $P<0.001$) and 20°C ($t = 24.0$, $P<0.001$).

The growth rates obtained from this study were compared with published growth data on the same two isolates by Tang *et al*. (1997) who used cultures that were not acclimated to the growth conditions. In all but three cases (*P. subfuscum* at 25°C and *P. tenue* at 5°C, 15°C), the pre-acclimated cultures had significantly higher growth rates than did the non-acclimated cultures (Table 1).

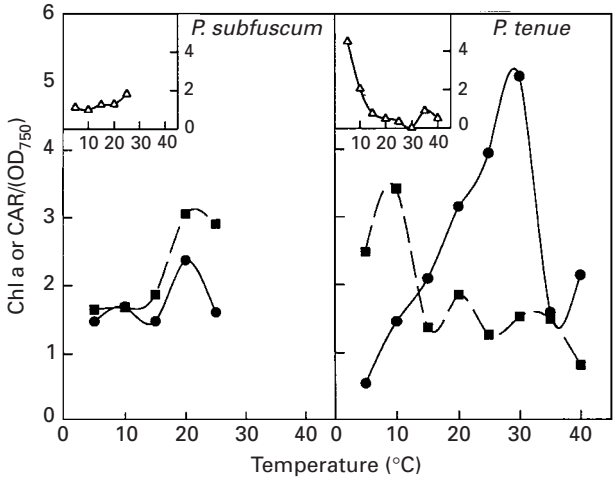

Fig. 1. The relationship between temperature and the concentrations of chlorophyll a (Chl a, circle) and carotenoid (CAR, square) for *Phormidium subfuscum* and *P. tenue*. The relationship between the ratio of carotenoid to chlorophyll a (CAR:Chl a, triangle) and temperature is also shown in the top left hand corner.

Pigment content

The Chl a content of *P. tenue* increased with increasing temperature up to 30°C above which it declined rapidly. However, the cellular carotenoid content varied little with temperature resulting in a decline of the CAR:Chl a ratio with increasing temperature (Fig. 1). For *P. subfuscum*, both CAR and Chl a did not show any trend with changing

Table 1. *The effect of temperature on the growth* (μ; ±*SE of the estimate derived from regression analysis*) *of the cyanobacterial isolates* Phormidium subfuscum *and* P. tenue

Temperature (°C)	Pre-acclimated culture μ (d^{-1})	Non-acclimated culture μ (d^{-1})	*t*	*P*
P. subfuscum				
5	0.11±0.0111	0.013±0.005	22.9	<0.001
10	0.21±0.014	0.16±0.013	8.77	<0.001
15	0.26±0.025	0.21±0.030	3.65	<0.01
20	0.21±0.021	0.13±0.006	11.6	<0.001
25	0	0.11±0.028	3.92	<0.02
P. tenue				
5	0	0.13±0.018	6.96	<0.01
10	0.14±0.020	0.19±0.030	4.73	<0.001
15	0.34±0.042	0.18±0.058	7.61	<0.001
20	0.49±0.030	0.16±0.005	32.7	<0.001
25	0.68±0.019	0.11±0.028	51.2	<0.001
30	0.84±0.12	0.23±0.017	14.5	<0.001
35	0.74±0.12	0.41±0.097	6.74	<0.001
40	0.36±0.020	NA		

The cyanobacteria were acclimated to the respective experimental conditions 5 d before the growth assays were performed. The growth rates of the two isolates (listed as O-025 for *P. subfuscum* and O-120 for *P. tenue*) published in Tang *et al*. (1997) are listed for comparison. Note, however, that Tang *et al*. (1997) did not pre-acclimate the cultures before experimentation.
NA denotes that no measurement was taken.
The *t*-statistic (*t* and *P*) for each comparison are displayed.

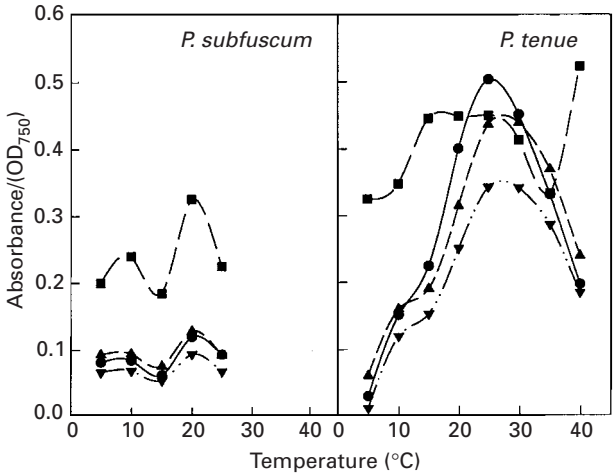

Fig. 2. The effect of temperature on the *in vivo* absorbance maxima normalized to optical density (OD_{750}) of chlorophyll a (Chl a, circle), carotenoid (CAR, square), C-phycocyanin (PC, triangle apex uppermost) and allophycocyanin (APC, triangle base uppermost) for *Phormidium subfuscum* and *P. tenue*.

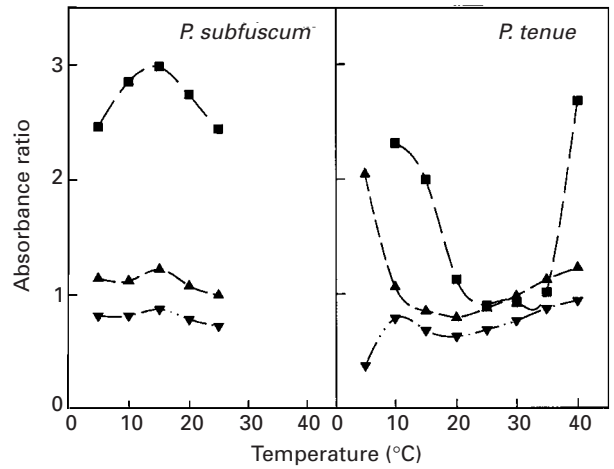

Fig. 3. The effect of temperature on the *in vivo* absorbance of carotenoid (CAR, square), phycocyanin (PC, triangle apex uppermost) and allophycocyanin (APC, triangle base uppermost) relative to chlorophyll a (Chl a) for *Phormidium subfuscum* and *P. tenue*.

temperature. There were minimal variations in CAR:Chl a with changing temperature over the range 5 and 20°C (Fig. 1).

In vivo *absorbance*

The *in vivo* absorbance maxima for CAR, PC, APC and Chl a of *P. subfuscum* did not show any apparent trend with temperature (Fig. 2). As a consequence, there was little variation in the PC:Chl a and APC:Chl a ratios (Fig. 3). However, the CAR:Chl a ratio increased with temperature up to 15°C, above which it decreased again (Fig. 3). In marked contrast, the *in vivo* absorbance of CAR, PC, APC and Chl a of *P. tenue* all increased with temperature up to 25°C, after which the absorbance reached a plateau and then declined (Fig. 2). Since the rate of increase of Chl a absorbance with temperature was higher than that of other pigments, the relationships between CAR:Chl a, PC:Chl a, APC:Chl a and temperature all followed a U-shaped pattern (Fig. 3).

Photosynthesis versus irradiance curves

Both the light utilization efficiency (α) and photosynthetic capacity (P_{max}) of *P. subfuscum* increased with increasing temperature up to T_{opt} for growth, with Q_{10} values of 2.6 and 3.5, respectively (Table 2). At super-optimal temperatures, α dropped substantially whereas P_{max} displayed a more gradual decrease with increasing temperature. The light utilization efficiency of *P. tenue* showed slight variations with temperatures between 5 and 25°C. However, α increased considerably between 25 and 30°C, resulting in an overall Q_{10} value of 2.4 for the range 5–30°C. Photosynthetic capacity, on the other hand, increased steadily with temperatures up to 35°C and the Q_{10} between 5 and 30°C was 4.3 (Table 2). Light saturation (E_k) increased with increasing temperatures for both isolates.

We performed pairwise comparisons of α, P_{max} and E_k between *P. subfuscum* and *P. tenue* at each temperature. The values of α was generally higher in *P. subfuscum* than in *P. tenue* between 5 and 15°C and the trend was reversed at 20°C or greater (Table 2). A comparison of P_{max} between the two strains displayed the same trend and statistically significant differences were detected at all temperatures. The E_k of *P. subfuscum* was higher than that of *P. tenue* at 5°C but no significant differences were detected at other temperatures (Table 2).

The photosynthetic rate of the cyanobacteria at their growth irradiance (225 μmol m^{-2} s^{-1}) was estimated from the P versus E curves. The Q_{10} values of *P. subfuscum* and *P. tenue* were 3.2 and 3.6, respectively. Between 5 and 15°C, P at the growth irradiance was significantly higher in *P. subfuscum* than in *P. tenue*. At 20°C and above, *P. tenue* showed significantly higher values of photosynthesis than *P. subfuscum* (Table 2). In both isolates, photosynthetic rates increased with increasing rate of growth at sub-optimal temperatures. Photosynthesis was generally higher for a given rate of growth at super-optimal temperatures than at sub-optimal temperatures (Table 2).

Photosynthetic endproducts

In a separate set of P versus E experiments, the ^{14}C assimilated by the cyanobacteria was partitioned into four components; low molecular weight compounds, lipid, protein and polysaccharides. To study the effect of temperature on C partitioning, we averaged the percent allocation into each fraction at 12 PPFR for each temperature. There was no apparent relationship between relative C allocation and temperature for either cyanobacterium (Table 3).

Table 2. *Photosynthetic parameters for* Phormidium subfuscum *and* P. tenue *at different temperatures*

Temperature (°C)	α (mg C (mg Chl a)$^{-1}$ h^{-1} µmol m^{-2} s^{-1} × 10^{-2})	P_{max} (mg C (mg Chl a)$^{-1}$ h^{-1})	E_k (µmol m^{-2} s^{-1})	P at 225 µmol m^{-2} s^{-1} (mg C (mg Chl a)$^{-1}$ h^{-1})
P. subfuscum				
5	1.4 ± 0.064**	1.20 ± 0.13**	84.5 ± 5.93**	1.11 ± 0.11*
10	1.7 ± 0.028	1.48 ± 0.032**	94.7 ± 15.0***	1.34 ± 0.025***
15	3.6 ± 0.043**	4.14 ± 0.23**	119 ± 18.4***	3.49 ± 0.055***
20	1.7 ± 0.011	3.34 ± 0.22*	200 ± 25.8*	2.26 ± 0.032*
25	0.92 ± 0.009**	3.27 ± 0.21**	364 ± 43.0***	1.52 ± 0.099***
P. tenue				
5	0.64 ± 0.081**	0.30 ± 0.028**	49.6 ± 11.7**	0.29 ± 0.024*
10	1.4 ± 0.33	1.09 ± 0.058**	86.2 ± 23.9***	0.99 ± 0.026***
15	1.5 ± 0.27**	2.70 ± 0.16**	185 ± 35.9***	1.89 ± 0.15***
20	1.7 ± 0.20	4.84 ± 0.40*	295 ± 49.0*	2.60 ± 0.13*
25	1.5 ± 0.10**	6.82 ± 0.36**	451 ± 55.9***	2.70 ± 0.11***
30	5.4 ± 0.66	11.1 ± 0.32	214 ± 36.2	7.31 ± 0.46
35	4.0 ± 0.49	12.2 ± 1.15	314 ± 37.2	6.30 ± 0.66
40	8.3 ± 2.6	8.01 ± 0.35	120 ± 39.6	6.86 ± 0.96

α, initial slope of the *P* vs E curve; P_{max}, light saturated photosynthetic rate; E_k, light saturation. Photosynthetic rate (P) at the growth PPFR (225 µmol m^{-2} s^{-1}) was estimated by the equation of Webb *et al*. (1974). Means of triplicates (± SE) are displayed.
Significant difference between *P. subfuscum* and *P. tenue* at: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Table 3. *The effect of temperature on the percent allocation of assimilated* ^{14}C *into low molecular weight metabolites, lipid, protein and polysaccharide fractions of the cyanobacterial isolates* Phormidium subfuscum *and* P. tenue

Temperature (°C)	Low molecular weight metabolites	Lipid	Protein	Polysaccharides
P. subfuscum				
5	36.90 ± 3.1	14.39 ± 1.8	21.13 ± 2.8	27.58 ± 0.9
10	25.32 ± 1.4	6.50 ± 0.3	42.91 ± 1.6	23.89 ± 0.5
15	23.69 ± 1.7	13.05 ± 0.6	43.96 ± 2.1	19.31 ± 1.0
20	30.05 ± 1.9	9.29 ± 0.7	38.41 ± 2.3	22.26 ± 0.6
25	20.52 ± 1.6	9.13 ± 0.5	51.24 ± 1.4	19.10 ± 0.6
P. tenue				
5	38.47 ± 1.9	16.59 ± 1.2	25.81 ± 1.2	19.13 ± 0.9
10	30.33 ± 1.9	10.14 ± 0.5	30.76 ± 1.4	28.77 ± 1.9
15	29.23 ± 1.7	8.70 ± 0.4	40.01 ± 1.4	24.88 ± 1.3
20	35.65 ± 1.3	10.00 ± 0.4	35.21 ± 1.1	18.14 ± 0.4
25	27.00 ± 1.3	14.39 ± 1.4	42.25 ± 1.3	18.78 ± 0.7
30	33.67 ± 1.3	9.84 ± 0.5	35.35 ± 1.1	21.15 ± 0.4
35	30.85 ± 1.1	12.07 ± 0.5	39.77 ± 1.2	17.80 ± 0.4
40	45.80 ± 1.5	7.26 ± 0.3	33.29 ± 1.4	13.66 ± 0.3

The values represent averages (± SE) of percent allocation for triplicate samples at 12 different PPFDs ranging from 55 to 1062 µmol m^{-2} s^{-1}.

Therefore, we averaged the percent C allocated into each fraction over the entire temperature range for each PPFR and examined the relationship between C partitioning and PPFR. Both isolates incorporated a larger proportion of their photosynthate into protein at low irradiances than at high irradiances (Fig. 4). The opposite trend was observed for low molecular weight metabolites. ^{14}C incorporated into lipid and polysaccharides showed little variation with PPFR (Fig. 4).

DISCUSSION

The two mat-forming cyanobacteria examined here had strikingly different optimum temperatures for growth (Table 1), despite the fact that both isolates were collected from the polar regions. The differences in T_{opt} may reflect differences in thermal regime of the native habitats of each species. *Phormidium subfuscum* lives within the perennially cold McMurdo Ice Shelf where meltwater temp-

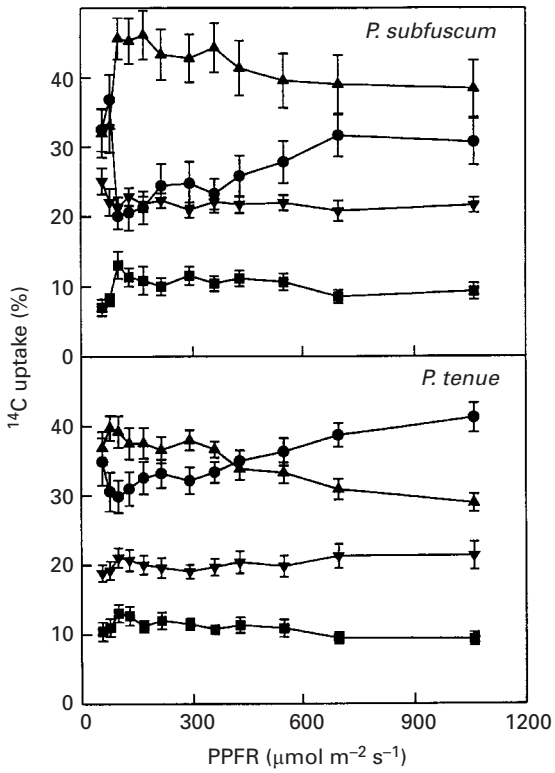

Fig. 4. The percent allocation of assimilated ^{14}C into low molecular weight metabolites (circles), lipid (square), protein (triangle apex uppermost) and polysaccharide fractions (triangle base uppermost) as a function of photosynthetic photon fluence rate (PPFR).

eratures rarely rise above 5°C, whereas *P. tenue* was isolated from the warmer and thermally more variable environment of an Arctic tundra stream. However, both strains were highly responsive to warming as indicated by their high Q_{10} values for growth (μ) suggesting that their growth and accumulation of biomass occur mostly during the period of maximum temperatures within the short Arctic or Antarctic summer.

The comparison of growth data obtained from this study and those of Tang *et al.* (1997) demonstrated the positive effects of acclimation. The growth rates of pre-acclimated cultures were one to nine times higher than those of non-acclimated cultures. Campbell *et al.* (1995) found that even though physiological acclimation in *Synechococcus* began within hours after transfer to low temperature, the population still underwent one generation of severely depressed growth before μ reached its new acclimated value. The measurements in Tang *et al.* (1997) are therefore likely to have reflected a similar effect. The enhanced μ after acclimation is probably the result of physiological changes, such as pigment content (Fig. 1) and changes in activity of Rubisco (Li & Morris, 1982) that help to optimize growth under various temperature regimes. The non-acclimated growth rates in Tang *et al.* (1997) may be more representative of cyanobacterial growth in thermally fluctuating habitats, such as the Kuparuk River, where there are frequent temperature changes (Hershey *et al.*, 1995) with insufficient time for the cells to acclimate fully. The acclimated growth rates presented in this study reflect the growth of cyanobacteria in stable environments such as the ice-covered lakes of Antarctica, and perennially cold environments such as ice shelf meltpools (Vincent, 1988).

The magnitude of difference in acclimated versus non-acclimated cultures differed between *P. subfuscum* and *P. tenue*. Acclimated growth rates were approx. 1.5-fold higher than non-acclimated values of μ in *P. subfuscum*, except for the 5°C culture where there was an 8.5-fold increase after acclimation. Acclimated μ in *P. tenue* was at least 1.8-fold higher than non-acclimated μ with the exception of the 5 and 10°C cultures (Table 1). The greater shift during acclimation in *P. tenue* suggests that this species was better able to adjust to varying temperature conditions.

The analysis of pigment content also showed a greater flexibility of *P. tenue* relative to that of *P. subfuscum*. *Phormidium tenue* decreased its pigment content with decreasing temperature (Fig. 1), possibly in response to increased PSII excitation pressure (Maxwell *et al.*, 1994; Huner *et al.*, 1996; Król *et al.*, 1997). However, carotenoid content (CAR) decreased at a slower rate with temperature than Chl a resulting in elevated CAR : Chl a at low temperature (Fig. 1). The differential decrease in pigment content can be explained by the photoprotective role of CAR. At low temperatures, cyanobacteria are more susceptible to photoinhibition because of the decreased use of photochemically generated reductant and, hence an increased proportion of excess light available to PSII (Huner *et al.*, 1998). Low temperatures are likely to amplify the effects of photoinhibition further by depressing all enzymic processes, including the rate of repair of damage to PSII (Krause, 1993). CAR reduces the damaging effect of high light by screening excess light and quenching triplet Chl and singlet oxygen excitation (Young, 1993; Young & Frank, 1996). The variations in *in vivo* absorbance of CAR and Chl a for *P. tenue* were similar to the variations in pigment content (Fig. 2). The *in vivo* absorbance of PC and APC also decreased with decreasing temperature (Fig. 2), indicating that the cyanobacterium decreased synthesis of the light harvesting complex (Raven & Geider, 1988). The PC : Chl a and APC : Chl a ratios displayed a UCR-shaped pattern with increasing temperature (Fig. 3). This has been observed previously in many strains of polar cyanobacteria (Tang *et al.*, 1997). In contrast to *P. tenue*, the pigment content and absorbance of *P. subfuscum* did not display any particular trend with temperature (Figs 1, 2).

For both *P. subfuscum* and *P. tenue* P_{max} increased with increasing temperature (Table 2), which is consistent with the view that light-saturated photo-

synthesis is limited by C fixation and thus sensitive to temperature changes (Sukenik *et al.*, 1987; Wilhelm, 1990). The dependence of P_{max} and light utilization efficiency (Table 2) on temperature is likely to reflect the re-organization of the light-harvesting complex during thermal acclimation. However, in both isolates, P_{max} rose more rapidly than α with increasing temperature so that E_k (light saturation) was positively correlated with temperature (Table 2). In four out of 13 cases, E_k was higher than the growth irradiance. Photoinhibition was not detected in either *P. subfuscum* and *P. tenue* even at 1062 $\mu mol\ m^{-2}\ s^{-2}$, which is consistent with previous laboratory studies on filamentous cyanobacteria (Vézina & Vincent, 1997). These cyanobacteria may experience photoinhibition in their natural habitat because of concomitant exposure to UV radiation, although such effects can be substantially offset by various acclimation processes (Vincent & Quesada, 1994; Roos & Vincent, 1998).

The relationship between photosynthesis at growth irradiance and temperature correspond well with the relationship between μ and temperature (Tables 1, 2). The values of μ per unit photosynthesis tend to be higher at super-optimal temperatures than at sub-optimal temperatures, reflecting the differential effects of temperature on gross P and μ. Measurement of photosynthesis was based on ^{14}C and performed over 2 h, so that only a small proportion of fixed C would have entered the respiratory pathway (Li & Goldman, 1981). Therefore, it was most likely gross P that was measured, rather than net P, which corresponds more closely with μ. Since respiration tends to increase more rapidly than gross P at high temperatures, the difference between gross P and net P becomes more pronounced with increasing temperature.

Both *P. subfuscum* and *P. tenue* allocated most of their photosynthate to protein (Table 3, Fig. 4), as expected for actively growing cyanobacteria under nutrient-replete conditions (Morris, 1981). The percentage allocation into lipid ranged from 6.5 to 16.6 (Table 3) which is within the range reported in the literature (Wainmann & Lean, 1992). Incorporation of ^{14}C into polysaccharides in this study varied from 13.7 to 28.8% but most values were similar to the 18–20% range (Table 3) reported for cultures of pico-cyanobacteria (Cuhel & Waterbury, 1984; Howard & Joint, 1989). Allocation of C into protein decreased with increasing PPFR (Fig. 4), in accordance with previous findings. However, the decrease in relative allocation towards polysaccharides that usually accompanies the reduced protein photosynthesis (Li & Morris, 1982; Rivkin, 1989) was not observed. Instead, an increase in low molecular weight metabolites was found, consistent with Gleitz and Kirst's observations (1991).

The partitioning of photosynthate did not vary with temperature (Table 3), which corresponds to the observations of Smith *et al.* (1994) but contrasts to those of Hawes (1990), who reported inhibition of protein synthesis at low temperatures in Antarctic freshwater phytoplankton. The disparity between the findings of Hawes and that of the present study may result from a lack of temperature acclimation in the earlier work. Depressed protein synthesis was an instantaneous response to temperature shift. Our results support the view that temperature acclimation is usually not characterized by alterations in biochemical composition (Li & Morris, 1982; Raven & Geider, 1988).

In summary, the cyanobacterium *P. tenue* (10–40°C) can grow over a temperature range twice that of *P. subfuscum* (5–20°C). The eurythermal nature of *P. tenue* may be attributed to its flexibility in pigment content and photosynthetic capacity which can be adjusted to optimize light harvesting, C fixation and hence growth over a broad temperature range. By contrast, stenothermal *P. subfuscum* lacks this adaptive flexibility, and may be restricted to colder and thermally less variable habitats.

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