Effects of daylength and temperature on the growth and photosynthesis of an Arctic cyanobacterium, *Schizothrix calcicola* (Oscillatoriaceae)

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Cyanobacteria are often the dominant phototrophs in high-latitude lakes and streams where they must experience continuously low temperature and extreme variations in daylength. The present study examined the interaction between these two variables for the growth and physiology of an Arctic isolate of the mat-forming species *Schizothrix calcicola* (Agardh) Gomont. Growth rates (μ), photosynthesis (P), respiration (R), pigment composition and *in vivo* absorption characteristics were measured under 15 combinations of daylengths (8:16, 12:12, 16:8, 20:4 and 24:0 L/D) and temperatures (5, 15 and 25 °C). μ increased with increasing temperature (μ_{max} at 5 °C = 0·12 d^{-1}, μ_{max} at 25 °C = 0·28 d^{-1}) and a similar trend was observed in photosynthetic capacity (average P^B_{max} at 5 °C = 4·42 mg C (mg Chl *a*)$^{-1}$ h^{-1}, average P^B_{max} at 25 °C = 5·74 mg C (mg Chl *a*)$^{-1}$ h^{-1}), pigment content and absorbance. Daylength had a positive effect on μ and pigment absorption, but not pigment content. The shape of the μ–daylength curve varied with temperature: μ was a linear function of daylength at 5 °C, but at 15 and 25 °C the relationship resembled a rectangular hyperbola and μ saturated at 16:8 and 12:12 L/D, respectively. The non-linear relationship between μ and daylength at high temperature was related to a reduction in net photosynthesis under extended daylength; at 25 °C, net P under 24:0 L/D was 0·82 ± 0·37 mg O_2 (mg Chl *a*)$^{-1}$ h^{-1}, while under 8:16 L/D, it was 6·54 ± 0·69 mg O_2 (mg Chl *a*)$^{-1}$ h^{-1}. The constant increase in growth with increasing daylength at low temperature may reflect an adaptive tolerance to the combination of cold temperature and continuous daylight during the Arctic summer.

Key words: Arctic, Antarctic, cyanobacteria, growth, photoperiod, photosynthesis, pigments, polar, respiration, temperature

Introduction

Algae in aquatic ecosystems outside the tropics must contend with seasonal variations in daylength and temperature. Although algal growth generally increases with increasing daylength (Castenholz, 1966; Paasche, 1968; Foy *et al.*, 1976; Foy & Gibson, 1993), continuous light can be detrimental to some algal species (Brand & Guillard, 1981; Sicko-Goad & Andresen, 1991; Nielsen, 1992). Among the species that are not harmed by continuous illumination, there are large variations in sensitivity to changes in photoperiod. For example, the growth rate (μ) of a clone of the diatom *Skeletonema costatum* was twice as high under continuous light (24:0 L/D) compared with 12:12 L/D (Gilstad *et al.*, 1993), whereas *Pycnococcus provasolii* grown under continuous light (24:0 L/D) showed only a 1·3-fold enhancement in μ relative to cultures under a 12:12 photoperiod (Iriarte & Purdie, 1993). By normalizing μ to daylength, an estimate of the daily light utilization efficiency of the cells, specifically the growth per unit light dose, is obtained. Application of this analysis shows that *S. costatum* uses light equally efficiently under both a continuous light and light/dark (L/D) cycle whereas *P. provasolii* uses light more efficiently under a L/D cycle. For the latter species, the reduction in growth per unit light dose was associated with decreased photosynthetic capacity and efficiency while respiration remained unchanged (Iriarte & Purdie, 1993). For *S. costatum*, respiration increased concomitantly with daylength while photosynthetic parameters showed little change (Gilstad *et al.*, 1993). Therefore, the optimal daylength and growth per unit light dose may be highly species-specific, suggesting genetic and physiological differences, in part reflecting when and where the algae have been collected (Gallagher *et al.*, 1984).

The effect of daylength on algal growth may be temperature-dependent. For example, in marine diatoms the effect of daylength on growth diminishes at low temperatures (Durbin, 1974; Yoder, 1979; Verity, 1982). Experiments with some of these diatom species have shown that as temperature approaches the optimal temperature for growth, the positive effect of long daylengths on growth becomes more pronounced (Verity, 1982).

Algae in polar regions experience an extreme range of photoperiods, from 24:0 to 0:24 L/D, and continuously low temperatures. The polar algae may be either more

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tolerant or well adapted to these conditions. Gilstad & Sakshaug (1990) showed that the growth of Arctic diatoms isolated from the Barents Sea increased with increasing daylength at −0·5 °C such that μ was a rectangular hyperbolic function of daylength. No similar work has been conducted on mat-forming cyanobacteria from the polar zones. These benthic cyanobacteria are ubiquitous in ice-melt ponds and streams in the Arctic (Vézina & Vincent, 1997) and Antarctic (James *et al*., 1995; Ellis-Evans *et al*., 1998) and they are often the biomass dominants in these aquatic ecosystems (Hamilton & Edlund, 1994; Vincent & Quesada, 1994). Given their prokaryotic cell physiology, these cyanobacteria may differ greatly from the marine Arctic diatoms in their response to the combination of cold temperature and L/D cycles.

The primary aim of the present study was to evaluate the combined effect of temperature and photoperiod on the growth of a cyanobacterium isolated from the high Arctic. Our secondary objective was to identify the physiological mechanisms causing the observed changes in growth. We approached the latter by measurements of photosynthetic parameters, pigment concentration and *in vivo* absorbance, and photosynthetic and respiratory oxygen exchange.

Materials and methods

Isolation and maintenance of stock cultures

The mat-forming cyanobacterium, *Schizothrix calcicola* (Agardh) Gomont, was isolated from an algal mat collected from the nearshore of Meretta Lake at Resolute in the Canadian high Arctic (74° N, 94° W) in August 1995. The algal mat was placed on agar plates containing BG-11 culture medium (Rippka *et al*., 1979) upon collection. The algal material was further streaked onto fresh agar plates. Individual filaments were picked out and transferred into fresh BG-11 liquid medium to start a clonal unialgal culture. The culture was non-axenic but epifluorescence analysis of exponential cultures showed that bacteria constituted $< 2\%$ of the total microbial biomass. The stock culture was maintained under low irradiance (45 μmol m^{-2} s^{-1}) at 13 °C.

Growth assays

We estimated the growth rates of the cyanobacterium *S. calcicola* under 15 combinations of temperature (5, 15, 25 °C) and photoperiod (8:16, 12:12, 16:8, 20:4 and 24:0 L/D). The cultures were grown under an irradiance of 225 μmol m^{-2} s^{-1} which has been found to be sufficient to saturate growth in most Arctic cyanobacteria without inducing photoinhibition (Vézina, 1995). We grew the cyanobacteria under saturating irradiance to ensure that the observed μ–daylength relationships at each temperature were not influenced by light limitation.

The cultures were acclimated to their respective combination of photoperiod and temperature 5 days prior to experimentation. Photoacclimation to light quality (Ohki & Fujita, 1992; Sagert & Schubert, 1995) and quantity (Wyman & Fay, 1986) typically occurs within a cell generation time (Falkowski & LaRoche, 1991). Algae also adjust their bio-optical (Sosik & Mitchell, 1994) and enzymatic properties (Li & Morris, 1982; Davison, 1991) in response to temperature changes. Campbell *et al*. (1995) found that physiological acclimation in *Synechococcus* began within hours of transfer to low temperature and μ reached the acclimated state after the population went through one generation of depressed growth. Hence, in the present study, 5 days of growth is likely to have been sufficient for near-complete acclimation in most of the cultures.

The stock cultures were homogeneously dispersed with a Teflon tissue grinder to produce identical aliquots for inoculation. For each growth assay, 16 125 ml Erlenmeyer flasks containing 100 ml of BG-11 liquid medium were inoculated with an aliquot of stock culture to yield a final optical density at 750 nm (OD_{750}) of 0·015. The change in OD_{750} was used as an index of biomass as there is a strong positive correlation between the two variables (Quesada & Vincent, 1993). Two flasks were chosen randomly for OD_{750} measurements every day over 8 consecutive days. For the 5 °C cultures, we monitored OD_{750} every 4 days due to their slow growth at low temperature. We plotted the natural logarithm of OD_{750} over time and estimated growth rates from the straight portion of the curve using linear regression analysis. In all cases except one, OD_{750} values used in the regression analyses varied between 0·015 and 0·10 so that self-shading of the cultures was minimal. Growth rates were also normalized against daylength to obtain a measure of growth per unit light dose.

Acetone-extractable chlorophyll and carotenoid content

After 4 days of growth, we estimated the chlorophyll *a* (Chl *a*) and carotenoid (CAR) content of the cultures that were used for the growth assays. A 50 ml aliquot of the culture was filtered onto a Whatman 934/AH glass fibre filter which was then wrapped and kept frozen until further analyses. We measured the acetone-extractable Chl *a* and CAR content using the methods outlined in Quesada & Vincent (1993). Briefly, the filters were ground with a Teflon pestle and extracted in 8 ml of 90% acetone at 4 °C in the dark for 30 min. The mixture was then centrifuged for 12 min at 10 000 rpm. The supernatant was assayed for Chl *a* and CAR at 663 and 480 nm, respectively, using a Milton-Roy Spectronic 1001 spectrophotometer. The Chl *a* and CAR concentrations were normalized to optical density of the culture to provide an index of biomass-specific Chl *a* and CAR content.

In vivo *absorbance*

The *in vivo* absorbance of the cultures by carotenoids, chlorophyll and phycobilipigments was measured during exponential growth (i.e. after 14 days of growth for the

5 °C culture and 4 days of growth for the 15 and 25 °C cultures). A sample of the culture previously used for the growth assay was concentrated by sedimentation prior to the spectral scans so that reliable measurements could be obtained. The *in vivo* absorption spectrum was determined from 400 nm to 800 nm with a diode-array spectrophotometer (Hewlett Packard model HP-8452A) fitted with an integrating sphere (Labsphere Inc. model RSA-HP-84). The integration time of each scan was 0·1 s, thereby avoiding problems associated with cell sedimentation during the scan. The absorbance values were recorded at 480, 620, 650 and 680 nm, which have previously been identified as the absorbance maxima for CAR, C-phycocyanin (PC), allophycocyanin (APC) and Chl *a*, respectively, using cultures of mat-forming cyanobacteria grown under low irradiance (Vézina, 1995; Vézina & Vincent, 1997). The OD_{750} of the concentrated culture was then measured on the Milton-Roy spectrophotometer and used as an index of biomass. The absorbance peaks were normalized to OD_{750} for biomass-specific comparisons among cultures. Chl *a*-specific absorption (a^*) was also calculated (Kirk, 1994) to assess the extent of self-shading via packaging effect (Osborne & Raven, 1986).

Photosynthesis versus irradiance

All cultures were acclimated to their experimental conditions, after which they were transferred to 4 l fresh BG-11 medium and allowed to grow for 2 (15 and 25 °C cultures) to 6 days (5 °C cultures). The optical density of the experimental culture ranged from 0·012 to 0·030, which was low enough to prevent self-shading during the photosynthesis experiment. Shortly after the onset of the light period, the exponentially growing cultures were homogenized with a Teflon tissue grinder and dispensed into triplicate sets of 12 20 ml borosilicate scintillation vials. Each vial was spiked with 50 μl of 925 kBq [^{14}C]bicarbonate. One vial in each set was wrapped in aluminium foil and used as an estimate of dark ^{14}C uptake while the rest of the vials were transferred into a white Perspex box consisting of 11 slots. Light transmission into each slot varied from 2·6% to 109% (the latter due to internal reflection) and variation in light transmission was achieved by placing different layers of neutral-density screens over each slot. All samples were incubated in a light- and temperature-controlled incubator at their growth temperature and an ambient irradiance of 1240 μmol m^{-2} s^{-1} shortly after the onset of photoperiod. After 2 h of incubation, the content of each vial was filtered onto a Whatman 934/AH glass fibre filter. The filters were immediately acidified with 100 μl of 1 N HCl to eliminate unfixed ^{14}C. After 24 h, 10 ml of Beckman Ready Safe scintillation cocktail was added and the filters were counted for radioactivity in a Beckman 6500 scintillation counter. Acidified blank filters were used for background correction.

Dissolved inorganic carbon concentration in BG-11 medium was estimated from pH and alkalinity determined by Gran titration using a Hanna Instrument 8521 pH and temperature probe. Triplicate 60 ml aliquots of cultures were filtered onto Whatman 934/AH filters for Chl *a* analyses using the method described above.

All ^{14}C uptake rates in the light were corrected for dark uptake and carbon assimilation values were subsequently normalized to Chl *a*. Prior to fitting any curves through the *P* versus *E* data, we inspected the relationships visually. We fitted the *P* versus *E* function derived by Webb *et al*. (1974) to the data sets that did not display evidence of photoinhibition (i.e. the 5 and 15 °C data sets), and that of Platt et *al*. (1980) was used for the data sets in which photoinhibition was apparent (i.e. 25 °C data sets).

Photosynthetic and respiratory O_2 exchange

Photosynthetic and respiratory oxygen exchange rates were compared among the cultures grown under 8:16 and 24:0 L/D at 15 and 25 °C. The cultures were acclimated to the experimental regime for 5 days. They were then transferred to 2 l of fresh BG-11 medium and allowed to grow for 2 days until they reached exponential phase. The cultures were homogenized with a Teflon tissue grinder. Preliminary experiments showed that this procedure did not harm the cells. Instead, photosynthesis and respiration of the mat-forming cyanobacteria were slightly enhanced, possibly due to the increase in surface-to-volume ratio for light absorption and nutrient uptake when the filaments were dispersed. The homogenized cultures were dispensed into two sets of 12 60 ml BOD bottles. Among the first set of 12 bottles, 6 were used for O_2 measurement in the light at time 0 and time 1 and the other 6 were used for simultaneous Chl *a* measurement. The second set of bottles were used for measurements in the dark. The remaining culture was filtered through Whatman 934/AH filters to remove cyanobacterial filaments. The filtrate was distributed among 6 light and 6 dark bottles for estimation of heterotrophic bacterial respiration in the light and in the dark.

The *P* and *R* incubations were performed under irradiance, temperature and daylength conditions that were identical to the growth conditions of the cultures. For the cultures grown under a L/D cycle, we measured the O_2 concentration at the beginning and the end of the light cycle. Hence, photosynthetic O_2 evolution during the entire light period was estimated under growth irradiance. Meanwhile, the set of BOD bottles used for dark respiration estimation were incubated in the light throughout the light period. Then we estimated respiration rate from the difference between the O_2 concentration at the beginning and the end of the dark cycle to establish the dark respiration rate. Incubation in the light prior to O_2 uptake measurements accounts for enhanced post-illumination respiration (Falkowski *et al*., 1985). Algal respiration rates tend to be high shortly after light-to-dark transition and decrease exponentially afterwards (Markager, 1992). For the cultures grown under continuous light, the dark respiration measurements were

performed simultaneously with the photosynthetic measurements. The O_2 concentrations were determined by Winkler titration using a Mettler DL 21 automatic titrator.

The O_2 exchange rates were estimated as differences in the mean O_2 concentration at time 0 and time 1. All cyanobacterial photosynthetic and respiration rates were corrected for heterotrophic respiration by subtracting the O_2 exchange rates of the filtrate from the rates of the non-filtered culture. The cyanobacteria were growing exponentially so that the Chl *a* concentration might change over the course of the O_2 exchange experiment. We normalized all O_2 uptake and release rates to the geometric mean of Chl *a* concentrations at the beginning and at the end of the experiments to correct for any changes in biomass due to growth during the incubation. The individual errors associated with O_2 and Chl *a* concentrations were propagated to give an estimate of overall errors for the O_2 exchange rates using the propagation equations in Bevington (1969).

Statistical analyses

The effects of temperature and daylength and their interactive effects on growth, acetone-extracted pigment concentrations, pigment ratios and *in vivo* absorbance were tested using Forward Stepwise Regression Analyses. Assumption of normality was tested and met in all cases but assumption of constant variance was not met in four cases where the *in vivo* absorbance of PC, APC, Chl *a* and extracted CAR concentration were the dependent variables. Therefore, these variables were log-transformed prior to statistical analyses. In addition, the absorbance of CAR, the concentration of Chl *a* and CAR:Chl *a* were log-transformed as well because the transformation improved their relationship with temperature. The log-transformation validated the statistical analyses without altering their outcome. We used an analysis of covariance (ANCOVA) to determine the effects of temperature and daylength on growth per unit light dose. Dummy variables were used to designate each temperature treatment. For example, D5 is the variable that distinguishes 5 °C treatment (D5 = 1) from 15 and 25 °C treatments (D5 = 0), and D15 separates 15 °C (D15 = 1) treatment from the other two (D15 = 0), and so forth. Interaction terms for temperature and daylength were also included, so the model becomes:

$$\mu/\text{daylength} = a + b\text{L/D} + c\text{D5} + d\text{D5}\cdot\text{L/D} + \ldots + g\text{D25} + h\text{D25}\cdot\text{L/D} \quad (1)$$

where μ/daylength, L/D and D are growth per unit light dose, daylength and temperature treatments, respectively and $a, b, \ldots, h$ are fitted parameters. *P* versus *E* parameters were compared using two-way analysis of variance. Assumptions of normality and equal variance were met in all these tests.

Results

Growth rates

The growth rate of *Schizothrix calcicola* increased with both temperature and daylength (Fig. 1*A*, Table 1). The effect of temperature on μ was much greater between 5 and 15 °C than between 15 and 25 °C. At 5 °C, μ increased linearly with increasing daylength (Fig. 1*A*) and the μ–daylength relationship can be described by the following equation:

$$\mu = -0{\cdot}028 + 0{\cdot}048\text{L/D}, \quad R^2 = 0{\cdot}97,\ p = 0{\cdot}007 \quad (2)$$

However, at 15 and 25 °C, μ became saturated at 16:8 L/D and 12:12 L/D, respectively (Fig. 1*A*). The Monod equation, which was used to describe μ–daylength relationships in *Oscillatoria agardhii* by Post *et al.* (1986), fits the μ–daylength at 15 °C (Eq. 3) and 25 °C (Eq. 4):

$$\mu = 0{\cdot}43\ \{\text{L/D}/(12{\cdot}5 + \text{L/D})\}, \quad R^2 = 0{\cdot}97,\ p < 0{\cdot}001 \quad (3)$$

$$\mu = 0{\cdot}36\ \{\text{L/D}/(3{\cdot}85 + \text{L/D})\}, \quad R^2 = 0{\cdot}98,\ p < 0{\cdot}001 \quad (4)$$

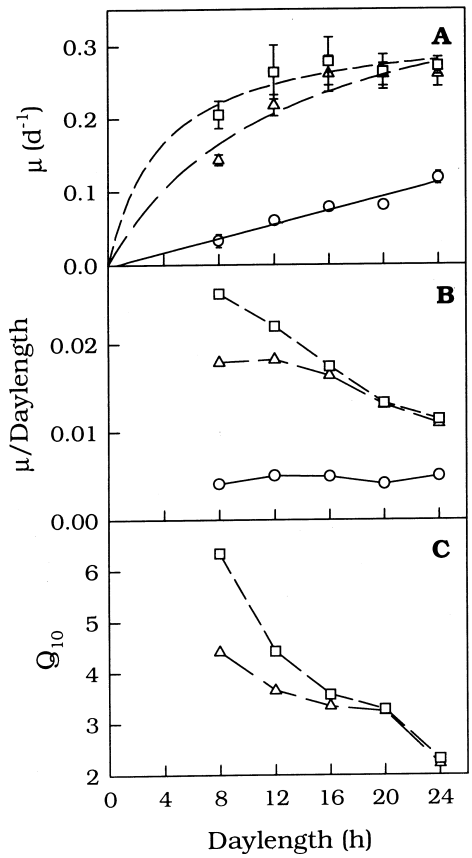

Fig. 1. The effect of daylength on the growth (*A*; μ d^{-1}) and growth per unit light dose (*B*; μ/daylength) at 5 °C (circles), 15 °C (triangles) and 25 °C (squares) and the effect of daylength on Q_{10} values of growth (*C*) between 5 and 15 °C (triangles) and 5 and 25 °C (squares) of *S. calcicola*. Error bars represent standard error of the estimate derived from regression analysis.

Table 1. A regression model predicting growth (μ) from temperature (Temp) and daylength (L/D) and an ANCOVA evaluating the independent and interactive effects of temperature and daylength on growth per unit light dose (μ/daylength) of the cyanobacterium *S. calcicola*

		SE of coeff.	*t*	*p*
μ =	−0·31	0·036	−0·86	0·41
	+0·0050 L/D	0·0018	2·75	0·018
	+0·0092 Temp	0·0013	7·24	< 0·001
	$n = 15$, $R^2 = 0{\cdot}91$, $F = 30{\cdot}0$, $p < 0{\cdot}001$			
μ/daylength =	0·0329	0·0012	27·52	< 0·001
	−0·00048 L/D	0·000071	−6·81	< 0·001
	−0·029 D5	0·0017	−16·9	< 0·001
	−0·010 D15	0·0017	−5·90	< 0·001
	+0·00050 D5·L/D	0·00010	5·004	< 0·001
	−0·00046 D25·L/D	0·00010	−4·58	0·001
	$n = 15$, $R^2 = 0{\cdot}99$, $F = 170{\cdot}3$, $p < 0{\cdot}001$			

D5, D15 and D25 represent dummy variables denoting cultures grown under 5, 15 and 25 °C, respectively.

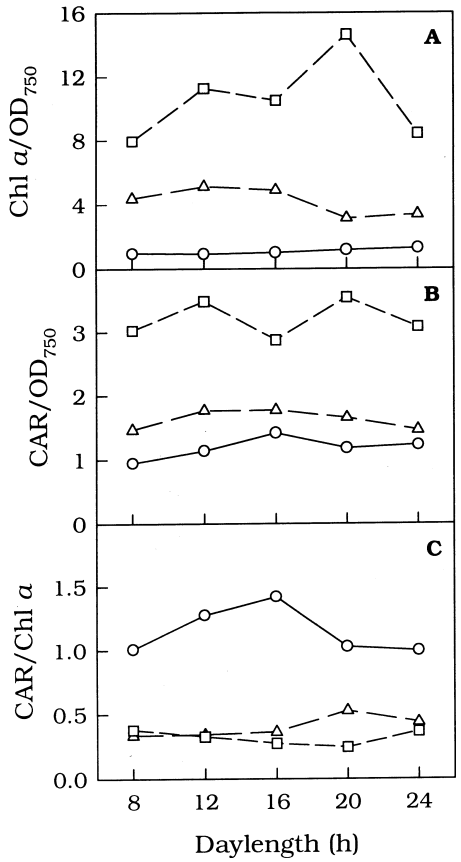

Fig. 2. The change in chlorophyll *a* concentration (*A*; Chl *a* mg l^{-1}), carotenoid concentration (*B*; CAR mg l^{-1}) and carotenoid:chlorophyll ratio (*C*) with increasing daylength and temperature (5 °C, circles; 15 °C, triangles; 25 °C, squares). The Chl *a* and CAR concentrations were normalized to the optical density (OD_{750}) of the cyanobacterial cultures. Values represent means of duplicates.

The interactive effect of temperature and daylength on growth became apparent when μ was normalized to daylength (μ per unit light dose). μ per unit light dose decreased rapidly with increasing daylength at 15 and 25 °C but remained constant at 5 °C (Fig. 1*B*, Table 1). A comparison of Q_{10} values at each daylength also indicated decreasing effect of temperature on μ with increasing daylength (Fig. 1*C*).

Table 2. *t*-statistics from regression analyses for the effects of temperature (Temp) on chlorophyll *a* (Chl *a*) and carotenoid (CAR) content and CAR:Chl *a*

Dependent variable	Independent variable	*t*	*p*
log *Chl a*	Temp	−7·45	< 0·001
log CAR	Temp	11·2	< 0·001
log CAR:Chl *a*	Temp	15·9	< 0·001

Acetone-extractable chlorophyll and carotenoid content

Both CAR and Chl *a* content increased significantly with increasing temperature. However, CAR rose more slowly with temperature than Chl *a* so that CAR:Chl *a* was negatively correlated with temperature (Fig. 2, Table 2). Neither CAR nor Chl *a* were affected by daylength and as a result there was no daylength dependence of CAR:Chl *a* either.

In vivo *absorbance*

The *in vivo* absorbance maxima of PC, APC and Chl *a* were positively correlated with temperature and negatively correlated with daylength, but the absorbance of CAR was only affected by temperature (Fig. 3, Table 3). However, the absorbance of CAR relative to Chl *a* was affected by both temperature and daylength, reflecting the differential effect of temperature on CAR and Chl *a* and the reduced absorption of Chl *a* at longer daylengths (Fig. 4*A*, Table 3). Daylength and temperature had only an interactive effect on PC:Chl *a* ($t = -5{\cdot}91$, $p < 0{\cdot}001$). APC:Chl *a* was negatively related to temperature indicating that Chl *a* absorbance increased at a faster rate with temperature than APC (Fig. 4*C*, Table 3). Chl *a*-specific

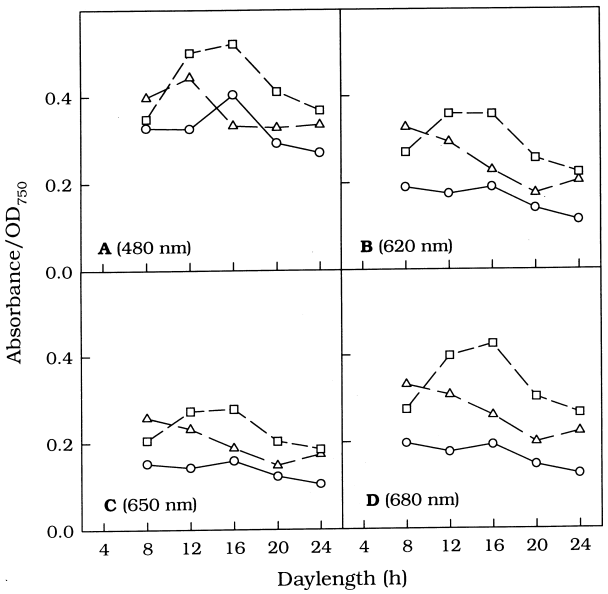

Fig. 3. The *in vivo* absorbance maxima of carotenoid (*A*; 480 nm), C-phycocyanin (*B*; 620 nm), allophycocyanin (*C*; 650 nm) and chlorophyll *a* (*D*; 680 nm) normalized to optical density (OD_{750}) for *S. calcicola* grown under a combination of three temperatures (5 °C, circles; 15 °C, triangles; 25 °C, squares) and five daylengths. Values represent means of duplicates.

Table 3. *t*-statistics from regression analyses for the effects of temperature (Temp) and daylength (L/D) on the *in vivo* absorbance maxima of carotenoid (CAR), phycocyanin (PC), allophycocyanin (APC) and chlorophyll *a* (Chl *a*), the absorbance ratios of CAR:Chl *a*, PC:Chl *a* and APC:Chl *a* and the Chl *a*-specific absorption (*a**)

Dependent variable	Independent variable	*t*	*p*
log CAR	Temp	2·93	0·012
	L/D	NS	
log PC	Temp	6·03	< 0·001
	L/D	−3·97	0·002
log APC	Temp	5·50	< 0·001
	L/D	−3·28	0·007
log Chl *a*	Temp	6·60	< 0·001
	L/D	−2·97	0·012
CAR:Chl *a*	Temp	−6·78	< 0·001
	L/D	2·73	0·018
PC:Chl *a*	Temp	NS	
	L/D	NS	
APC:Chl *a*	Temp	−7·21	< 0·001
	L/D	NS	
*a**	Temp	−6·46	< 0·001
	L/D	NS	

NS, no significant effect.

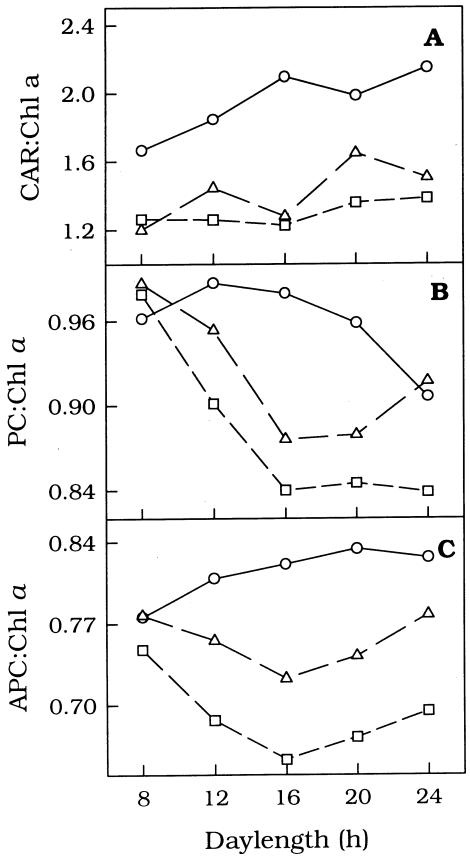

Fig. 4. The absorbance of carotenoid (*A*; CAR), C-phycocyanin (*B*; PC) and allophycocyanin (*C*; APC) relative to chlorophyll *a* (Chl *a*) for *S. calcicola* grown under a combination of three temperatures (5 °C, circles; 15 °C, triangles; 25 °C, squares) and five daylengths. Values represent means of duplicates.

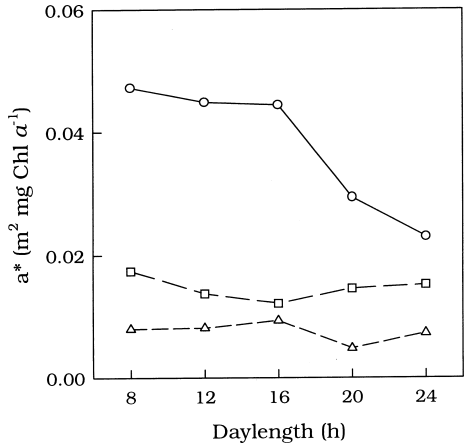

Fig. 5. The Chl *a*-specific absorption (*a**) for *S. calcicola* grown under a combination of three temperatures (5 °C, circles; 15 °C, triangles; 25 °C, squares) and five daylengths. Values represent means of duplicates.

absorption was not significantly affected by daylength but it decreased with increasing temperature (Fig. 5, Table 3).

Photosynthesis versus irradiance

Both temperature ($F = 63{\cdot}4$, $p < 0{\cdot}001$) and daylength ($F = 45{\cdot}3$, $p < 0{\cdot}001$) had significant effects on the maximum photosynthetic capacity (P^{B}_{max}; Table 4). Pairwise comparisons indicated P^{B}_{max} at 5 and 15 °C were not significantly different ($p = 0{\cdot}12$) but they were both significantly lower than P^{B}_{max} at 25 °C ($p < 0{\cdot}001$). The 8:16 L/D cultures did not have significantly different P^{B}_{max} from 12:12 L/D cultures ($p = 0{\cdot}1$) while the P^{B}_{max} of the 16:8 cultures did not differ from that of the 20:4

Table 4. The maximum photosynthetic capacity (P^B_{max} mg C (mg Chl *a*)$^{-1}$ h^{-1}) of *S. calcicola* grown under 15 combinations of temperature and daylength

	Temperature (°C)			
Daylength (h)	5	15	25	Average
8	2·46 ± 0·030	2·89 ± 0·036	6·88 ± 0·14	4·08 ± 0·70
12	3·33 ± 0·050	3·59 ± 0·11	4·31 ± 0·22	3·75 ± 0·16
16	6·84 ± 0·22	4·49 ± 0·18	4·25 ± 0·38	5·19 ± 0·44
20	3·62 ± 0·060	5·53 ± 0·20	5·39 ± 0·41	4·85 ± 0·36
24	5·85 ± 0·17	4·41 ± 0·13	7·87 ± 0·51	6·05 ± 0·52
Average	4·42 ± 0·44	4·18 ± 0·24	5·74 ± 0·41	

Each value is the mean of separate estimates of triplicate *PE* curves (± SE).

cultures ($p = 0·082$). All the other pairwise comparisons between daylength treatments showed significant differences between treatments ($p < 0·001$). Thus, in general, temperature and daylength had positive effects on P^B_{max}. Although the two-way ANOVA indicated significant interactive effects of temperature and daylength on P^B_{max} ($F = 6·67$, $p < 0·001$), no consistent trends were observed (Table 4).

The maximum light utilization coefficient (α^B) of *S. calcicola* was not affected by temperature ($F = 1·46$, $p = 0·25$). Although a significant effect of daylength on α^B was detected ($F = 3·49$, $p = 0·0019$), this stemmed from one outlying data point (Table 5). Cultures exposed to 20:4 L/D had significantly lower α^B compared with all other groups ($p < 0·05$). Interactive effects of temperature and daylength were detected ($F = 3·46$, $p = 0·006$), but apart from the result that 25 °C cultures under continuous irradiance had higher αB than all other treatments (with the exception of the 8:16 L/D at 5 °C treatment), no other trends were observed.

Photosynthetic and respiratory O_2 exchange

Cyanobacteria grown under continuous light had markedly reduced net photosynthetic rates compared with those subjected to a 8:16 L/D regime (Table 6) both at 15 °C ($t = 11·4$, $p < 0·001$) and 25 °C ($t = 7·12$, $p < 0·001$). No significant differences in net photosynthesis were detected between cultures grown at the same daylength but under different temperatures. Respiration was approximately 2-fold higher under continuous illumination compared with 8:16 L/D but the errors associated with the measurements were too large for significant differences to be detected. Gross photosynthesis was only significantly different between the 8:16 L/D at 15 °C and the 24:0 L/D at 25 °C treatments ($t = 3·30$, $p < 0·05$; Table 6).

Table 6. The net and gross photosynthesis (*P*; mg O_2 (mg Chl *a*)$^{-1}$ h^{-1}) and respiration (*R*; mg O_2 (mg Chl *a*)$^{-1}$ h^{-1}) of *S. calcicola* grown under a combination of two temperatures and two daylengths

Temperature (°C)	Daylength (h)	Net *P*	*R*	Gross *P*
15	8	5·92 ± 0·37	−0·49 ± 1·79	6·41 ± 1·82
15	24	2·43 ± 0·38	−0·99 ± 1·13	3·42 ± 1·19
25	8	6·54 ± 0·69	−0·58 ± 13·1	7·12 ± 13·1
25	24	0·82 ± 0·37	−1·38 ± 0·70	2·19 ± 0·79

The *P* and *R* values are means of triplicates (± SE calculated by propagation of errors).

Discussion

Daylength

In contrast to some diatom (Gibson & Fitzsimons, 1991, 1992) and dinoflagellate species (Dixon & Syrett, 1988; Dixon & Holligan, 1989), the cyanobacterium *S. calcicola* does not require a dark period to optimize growth (μ). μ for *S. calcicola* increased with increasing daylength more or less linearly at 5 °C (Fig. 1*A*; Eq. 2). At 15 and 25 °C, the μ–daylength relationship fits the Monod equation (Fig. 1*A*; Eqs. 3 and 4) that describes the μ–daylength relationship of *Oscillatoria agardhii* (Post *et al.*, 1986). The lack of proportionality of μ with daylength at 15 and 25 °C implies that under warm temperature conditions

Table 5. The maximum light utilization coefficient (α^B mg C (mg Chl *a*)$^{-1}$ h^{-1} μmol^{-1} m^2 s) of *S. calcicola* grown under 15 combinations of temperature and daylength

	Temperature (°C)			
Daylength (h)	5	15	25	Average
8	0·095 ± 0·032	0·050 ± 0·0078	0·078 ± 0·015	0·074 ± 0·012
12	0·070 ± 0·0081	0·059 ± 0·0059	0·049 ± 0·0044	0·059 ± 0·0044
16	0·076 ± 0·020	0·057 ± 0·0033	0·046 ± 0·0066	0·060 ± 0·0074
20	0·032 ± 0·0029	0·040 ± 0·0031	0·034 ± 0·0055	0·035 ± 0·0023
24	0·032 ± 0·0050	0·046 ± 0·0061	0·11 ± 0·024	0·063 ± 0·014
Average	0·061	0·050	0·064	

Each value is the mean of separate estimates of triplicate *PE* curves (± SE).

S. calcicola uses light for growth more efficiently at shorter daylengths.

At 15 and 25 °C, the reduction in μ per daily light dose (μ/daylength) was accompanied by reduced net photosynthesis (net *P*) under continuous light (Table 6). These lowered rates could be the result of decreased gross photosynthesis (gross *P*) or increased respiratory losses (*R*), but the variation in our respiration data was too large for any definitive conclusions to be drawn (Table 6). These results based on O_2 exhange contrast with those of the ^{14}C *P* versus *E* experiments which showed that photosynthetic carbon fixation under saturating irradiance (P^B_{max}) increased with increasing daylength (Table 4). The disparity between the ^{14}C uptake and O_2 exchange experiments may stem from differences in the methodology and time-scales used in the two types of *P* determinations.

The *P* versus *E* experiments based on ^{14}C assimilation were conducted within 2 h. Over such short-term incubation, the ^{14}C uptake may reflect net or gross photosynthesis depending on whether cyanobacteria recycle the respired ^{14}C (Williams *et al.*, 1996). If cyanobacteria recycle their respired ^{14}C, ^{14}C uptake over a short period would represent net *P*. Williams & Lefèvre (1996) demonstrated 100% ^{14}C recycling in the diatom *Skeletonema costatum*. However, cyanobacteria have different inorganic C uptake mechanisms from diatoms and there is no evidence that cyanobacteria use respired CO_2 as an inorganic C pool.

The O_2 exchange technique probably underestimated *R* and consequently gross *P* because respiration in the light phase of growth is usually higher than in darkness (Grande *et al.*, 1989; Weger *et al.*, 1989). Weger et *al.* (1989) attributed the enhanced respiration to increased substrate supply from photosynthesis and higher anabolism. Given that we only estimated O_2 consumption in the dark, *R* and gross *P* were likely to be underestimated, and more so in the cultures grown under continuous illumination than in the 8:16 L/D cultures.

The decrease in net and gross *P* under extended daylengths was accompanied by a decrease in light absorption by chlorophyll (Chl *a*) and phycobiliproteins (Fig. 3). Reduced light absorption with increasing daylength has also been observed in the cyanobacteria *Limnothrix redekei* and *Planktothrix agardhii* (Nicklisch, 1998). In contrast, Chl *a* and carotenoid (CAR) content did not change significantly with varying daylength (Fig. 2), suggesting that the cyanobacterial cells reorganized their pigments to control their light-capturing properties; for example, to reduce self-shading via the packaging effect (Osborne & Raven, 1986). However, the absorption per unit Chl *a* (a^*) did not indicate an increase in self-shading with longer daylengths (Fig. 5).

Temperature

μ increased with temperature under all daylengths and the maximum growth rate was observed at 25 °C (Fig. 1*A*, Table 1). The μ values under 24:0 L/D are similar to those reported in Tang *et al.* (1997). The variations in our photosynthetic and respiratory O_2 measurements were too large to calculate the differential effects of temperature on *P* and *R* and their relationship to μ (Table 6).

P^B_{max} derived from the *P* versus *E* curves displayed a positive trend with temperature (Table 4), consistent with the view that carbon fixation at light saturation is enzymatically controlled, and hence a temperature-dependent process (Falkowski & Raven, 1997). The maximum light utilization coefficient (α^B), which is the product of the Chl *a*-specific absorption coefficient (a^*) and maximum quantum yield (Φ_m), was temperature-independent (Table 5). Since a^* increases with increasing temperature (Fig. 5, Table 3), Φ_m probably decreases with temperature such that α^B becomes a temperature-independent parameter. The lack of dependence of α^B on temperature in our case is consistent with many observations elsewhere (Post *et al.*, 1985; Smith *et al.*, 1994; Rae & Vincent, 1998) and is consistent with the view that under light limitation, photosynthesis is primarily limited by light absorption and charge separation, and not electron transport (Falkowski & Raven, 1997).

The decrease in light absorption by CAR and Chl *a* at lower temperatures (Fig. 3*A*, *B*) reflects an overall reduction in pigment content (Fig. 2). The reduced absorbance of phycocyanin (PC) and allophycocyanin (APC) were likely to be related to a decrease in phycobiliproteins at low temperature, but we did not measure these pigments directly (Fig. 3*C*, *D*). The inverse relationship between pigment content and temperature indicates allocation of resources away from light-harvesting components by cyanobacterial cells at low temperature (Raven & Geider, 1988) in response to increased excitation pressure (Maxwell *et al.*, 1994; Król *et al.*, 1997). However, CAR decreased at a slower rate than Chl *a* (Figs. 2*A*, *B*, 3*A*, *B*). Most algae are more prone to photoinhibition at low temperature (Krause, 1993; Rae & Vincent, 1998) and the increased CAR:Chl *a* at low temperature may reflect the increasing need for photoprotection by CAR, for example for the direct quenching of singlet oxygen (Young, 1993; Young & Frank, 1996). Similar effects of low temperature on the CAR:Chl *a* ratio have been observed in the Antarctic mat-forming cyanobacterium *Phormidium murrayi* (Roos & Vincent, 1998).

Interactive effects of daylength and temperature

Daylength and temperature exerted separate effects as well as an interactive effect on the growth of *S. calcicola*. The effect of daylength on μ diminished with increasing temperature, giving rise to the trend of markedly decreasing Q_{10} values with increasing daylength (Fig. 1*A*, *C*). The shape of the μ–daylength curve varies with temperature such that at 15 and 25 °C, it resembles the rectangular hyperbola which typically describes the μ–daylength relationships of many algal species (Gilstad & Sakshaug, 1990; Nicklisch, 1998). At 5 °C, the

μ–daylength relationship is linear (Fig. 1*A*) so that μ per unit light dose (*i.e.* μ/daylength) is constant (Fig. 1*B*). This contrasts with a previous study by Foy *et al.* (1976) on four species of planktonic cyanobacteria. They reported a more dramatic increase in μ with increasing daylength at 20 °C than at 10 °C. The Q_{10} values for four species of cyanobacteria under 6:18 L/D ranged from 1·5 to 2·0, compared with 2·4 to 2·9 under 24:0 L/D. Several studies reported findings similar to Foy *et al.* (1976) on various marine diatoms (Durbin, 1974; Yoder, 1979; Verity 1982). There were no apparent interactive effects of daylength and temperature on pigment content (Table 2) and absorption (Table 3). Thus, the non-linear response of cyanobacterial growth to increasing daylength at high temperature appears to be primarily the result of a reduction of net photosynthesis under extended daylengths (Table 6). The constant daily light utilization efficiency (i.e. growth per unit light dose) of *S. calcicola* under different daylengths at 5 °C may reflect genetic adaptation by this strain to its native Arctic environment where the growing season is characterized by persistent cold temperatures in combination with a highly variable light regime caused by lake ice, snow, cloud and variable daylength.

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