# Photosynthetic characteristics of the terrestrial blue-green alga, *Nostoc flagelliforme*

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# Photosynthetic characteristics of the terrestrial blue-green alga, *Nostoc flagelliforme*

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Photosynthetic responses of rewetted *Nostoc flagelliforme* to $CO_2$, desiccation, light and temperature were investigated under emersed conditions in order to characterize its ecophysiological behaviour in nature. Net photosynthesis increased to a maximum rate at about 30% water loss, then decreased, while dark respiration always decreased with the progress of desiccation. Light-saturated photosynthesis and dark respiration were significantly reduced at 8 °C, but remained little affected by changes of temperature within the range of 15–35 °C. Photosynthetic efficiency ($\alpha$) was maximal at the beginning of desiccation and then reduced with increased water loss. Saturating irradiance for photosynthesis was about 194–439 $\mu$mol quanta $m^{-2}$ $s^{-1}$, being maximal at about 30% water loss. No photoinhibition was observed at irradiances up to 1140 $\mu$mol $m^{-2}$ $s^{-1}$. Light compensation points were about 41–93 $\mu$mol $m^{-2}$ $s^{-1}$. Photosynthesis of *N. flagelliforme* was $CO_2$-limited at the present atmospheric $CO_2$ level. The $CO_2$-saturated photosynthesis increased with increase of irradiance (190–1140 $\mu$mol $m^{-2}$ $s^{-1}$) and temperature (8–25 °C) and decreased significantly with water loss (0–75%). Photosynthetic affinity for $CO_2$ was sensitive to temperature and irradiance. The $CO_2$ compensation point ($\Gamma$) increased significantly with increased temperature and was insensitive to irradiance. Desiccation did not affect $\Gamma$ values before water loss exceeded 70%. Photorespiratory $CO_2$ release did not occur in *N. flagelliforme* at the current atmospheric $CO_2$ level.

**Key words**: blue-green alga, $CO_2$, cyanobacterium, desiccation, light, *Nostoc flagelliforme*, photorespiration, photosynthesis, temperature

## Introduction

*Nostoc flagelliforme* is a terrestrial blue-green alga, distributed in arid or semiarid areas in Algeria, China, Czechoslovakia, France, Mexico, Mongolia, Morocco, Russia, Somalia and USA (Li, 1991). It has been used by the Chinese as a food and herbal ingredient for hundreds of years. Market demands for this alga are increasing with economic growth in China, while its supply is heavily reduced due to over-exploitation. A number of studies have been carried out in China on the ecology, physiology, morphology, culture and resources of *N. flagelliforme*, which have been reviewed recently (Gao, 1998). Nevertheless, more physiological information is needed to understand its growth in nature and culture.

*N. flagelliforme* experiences extreme environmental changes in its natural habitat where the yearly precipitation is usually less than 300 mm, and daily and yearly temperature differences as large as 16 °C and 95 °C, respectively (Qian *et al.*, 1989). The surface temperature on the arid bare lands is as

high as 78 °C in summer and as low as $-40$ °C in winter (Li, 1991; Dai, 1992). *N. flagelliforme* showed great heat resistance while dry, but was damaged by heat treatment when wet or immersed (Mei & Cheng, 1990). When the alga was naturally dry, both net photosynthetic and dark respiratory activities showed strong resistance to heat treatment. However, pretreatment of wet *Nostoc* at 65 °C led to death and temperatures above 45 °C resulted in zero photosynthetic activity (Mei & Cheng, 1990).

*N. flagelliforme*, like other terrestrial *Nostoc* species, can remain desiccated for months or years and recover metabolic activity fully within hours to days after rehydration (Dodds *et al.*, 1995). Rehydration of the species has been the subject of several studies (Scherer *et al.*, 1984, 1986; Gao *et al.*, 1998*a, b*; Qiu & Gao, 1999). The time required for maximal photosynthetic and respiratory activities to recover after rehydration was dependent on the time of storage in dryness. Longer storage required more time for recovery (Scherer *et al.*, 1984; Potts, 1996, 1999; Qiu & Gao, 1999). It has been demonstrated recently that the photosynthetic recovery

of *N. flagelliforme* is a light-dependent (Gao *et al.*, 1998*a*) and a potassium-requiring process (Qiu & Gao, 1999). Shi *et al.* (1992) investigated the relationship of photosynthesis in *N. flagelliforme* to light and temperature while immersed. This study provides information on the photosynthetic characteristics of the species in ideal conditions, but little about the photosynthesis found in nature, where it is exposed to air for most of its life history. *N. flagelliforme* grows on the surface of bare lands, arid or semiarid areas where water flows and evaporates very rapidly. Photosynthetic responses to light and temperature in air can be significantly different from those in water, as has been shown in *Fucus spiralis* (Madsen & Maberly, 1990), *Halosaccion americanum* (Oates, 1986), *Hesperophycus harveyanus* (Oates & Murray, 1983) and *Pelvetia fastigiata* (Oates & Murray, 1983). While in air, photosynthetic activities may be sensitively related to water status or desiccation of the thallus; however, little is known on this aspect for *N. flagelliforme*.

Blue-green algae generally utilize $CO_2$ as their primary carbon source. The supply of $CO_2$ can limit their growth, and this limitation derives from both physical and chemical factors (Pierce & Omata, 1988). Atmospheric $CO_2$ concentration is increasing due to industrial combustion of fossil fuels, and its subsequent ecological impacts on photosynthesis and growth of plants are of general concern (Bowes, 1993). However, little is known about the utilization of inorganic carbon ($C_i$) in this terrestrial blue-green alga and its photosynthetic responses to elevated $CO_2$. The aim of this study was to investigate the photosynthetic responses of *N. flagelliforme* to environmental factors ($CO_2$, desiccation, light and temperature) and acquire insight into the photosynthetic patterns of *N. flagelliforme* in nature.

### Materials and methods

*Nostoc flagelliforme* (Berk. & Curtis) Bornet & Flah. was collected at Siziwangqi, Inner Mongolia, and stored dry for 2–3 years until used for experiments. Samples were rewetted in $BG_{11}$ medium (Stanier *et al.*, 1971) in an incubator at 40 $\mu$mol m$^{-2}$ s$^{-1}$ and four temperatures (8, 15, 25 and 35 °C) for 9–11 hours, which was long enough for the photosynthetic activity to recover fully (Scherer *et al.*, 1984; Gao *et al.*, 1998*a*; Qiu & Gao, 1999).

Each rewetted sample was spread on a plastic net as a mat of less than 8·6 cm in diameter, similar to the size of mats of *N. flagelliforme* that have been reported in nature (Gao, 1998). Water drops on the mat were removed with filter paper before the initial wet weight was determined. The algal mats were exposed to air under the same temperature and light conditions as for rewetting, and were weighed at intervals to assess water loss. Dry weight was measured after the samples had been dried at 80 °C
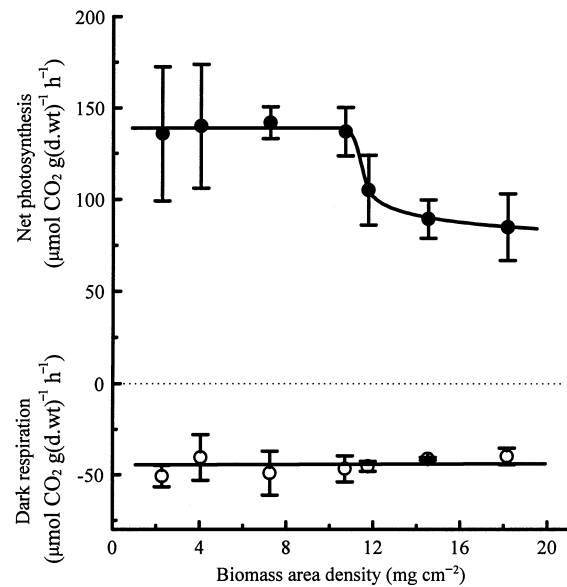


**Fig. 1.** Responses of net photosynthesis and dark respiration of *Nostoc flagelliforme* to the biomass area density of algal mat. Algal samples were rehydrated at 25 °C and 40 $\mu$mol m$^{-2}$ s$^{-1}$ and measured at 25 °C and 770 $\mu$mol m$^{-2}$ s$^{-1}$ in the open system. Data points are means of 3 mats $\pm$ SD.

for 20–24 hours and cooled in a desiccator. Water loss (WL, %) was calculated as follows:

$$WL = (W_w - W_t)/(W_w - W_d) \cdot 100 \qquad (1)$$

where $W_d$ is dry weight, $W_t$ is the instantaneous weight of samples measured at certain intervals, and $W_w$ is the initial wet weight.

The photosynthetic $CO_2$ uptake rate in air was measured by infrared gas analysis (CGT-7000, Shimadzu). A micro-incubator (Radnoti Glass Technology Inc.) with a water jacket for temperature control was used as the assimilation chamber, and the algal mat was placed in the chamber on the net. The sample size for each measurement was about 0·6 g (d.wt) or less (large samples exhibited self-shading and reduced photosynthesis, Fig. 1). Illumination was provided by a halogen lamp and irradiance was measured with a quantum sensor (SKP 200, ELE International). The photosynthetic irradiance response (P–I) curves were investigated at 8, 15, 25 and 35 °C. Irradiance was controlled by changing the distance of the lamp from the assimilation chamber, and always increased from zero to the maximum. The algal mat was adapted to each irradiance for 3–4 min. Photosynthetic responses to $CO_2$ were measured under various irradiances (190, 430, 770 and 1140 $\mu$mol m$^{-2}$ s$^{-1}$) and temperatures (8, 15, 25 and 35 °C) with 21% $O_2$. Different levels of $CO_2$ were obtained by adding pure $CO_2$ to air or by absorbing some $CO_2$ from it with 1 mol l$^{-1}$ NaOH solution. Atmospheres with various $CO_2$ concentrations were stored in different gasbags. The photosynthetic response to $CO_2$ was determined from 1500 $\mu$l l$^{-1}$ $CO_2$ to $\Gamma$. The $CO_2$ compensation point ($\Gamma$) was recorded as the $CO_2$ concentration in the assimilation chamber constant for 8–12 min when measured with low concentrations of $CO_2$ (20–40 $\mu$l l$^{-1}$). Low $O_2$ air (2%) was obtained by mixing air with nitrogen (1:9 v/v) and adding a known amount of $CO_2$. Photosynthesis was measured either in an open or in a closed system. In the open system, net

photosynthesis ($P_n$) and dark respiration ($R_d$) [$\mu$mol $CO_2$ g (d.wt)$^{-1}$ h$^{-1}$] were determined as follows:

$$P_n \text{ or } R_d = (A - B) \cdot F \cdot 60 \cdot 273 / [(273 + T) \cdot 22.4 \cdot W_d] \qquad (2)$$

where $A$ and $B$ are the $CO_2$ concentrations in the inlet and outlet air from the chamber, respectively, $F$ is the air flow rate (l min$^{-1}$), and $T$ is the temperature in the assimilation chamber (°C). In the closed system, $P_n$ and $R_d$ were determined as follows:

$$P_n \text{ or } R_d = C/t \cdot V \cdot 273/(273 + T) \\ \cdot 1/22.4 \cdot 1/W_d \cdot 60 \qquad (3)$$

where $C/t$ is the negative slope of $CO_2$ concentrations in the closed system over a time interval of about 1·5 min, $V$ is the volume (0·579 l) of the closed system.

Parameters for P–I curves were analyzed according to Jassby & Platt (1976) and Henley (1993):

$$P = P_m \cdot \tanh(\alpha \cdot I/P_m) + R_d, \\ I_k = P_m/\alpha, \ I_c = -R_d/\alpha \qquad (4)$$

where $I$ is irradiance, $P$ is photosynthetic activity at a given irradiance, $P_m$ is light-saturated photosynthesis, $I_k$ is saturating irradiance for photosynthesis, and $I_c$ is light compensation point. The ascending slope at limiting irradiances, $\alpha$, was calculated to assess the photosynthetic efficiency. Parameters for the photosynthetic response to $CO_2$ were obtained by fitting photosynthetic activities at various $CO_2$ concentrations with the Michaelis-Menten formula.

$$v = V_{max} \cdot [S]/(K_{0.5}(CO_2) + [S]) \qquad (5)$$

where, $K_{0.5}(CO_2)$, i.e. the $CO_2$ concentration required to give half-maximal photosynthetic rate, was used to define the photosynthetic affinity for $CO_2$, and $V_{max}$ was the $CO_2$-saturated rate of photosynthesis.

The responses of various parameters to environmental factors ($CO_2$, desiccation, light and temperature) were analyzed according to multiple ANOVA (analysis of variance) followed by the Tukey multiple comparison test. Canonical correlation analysis was used to describe the roles of environmental factors (desiccation and temperature) on photosynthetic parameters ($P_m$, $R_d$, $\alpha$, $I_k$ and $I_c$) for P–I curves.

## Results

Net photosynthesis and dark respiration of *N. flagelliforme* were investigated as a function of the biomass area density of algal mat (Fig. 1). Dark respiration was not affected by the increased mass of each mat. However, net photosynthesis decreased when the mass exceeded 0·63 g dry weight, equivalent to a biomass area density of 11 mg cm$^{-2}$. The net photosynthesis was reduced by about 40% when the algal mass was in excess of 1 g dry weight or when the biomass area density exceeded 17 mg cm$^{-2}$.

The water status of *N. flagelliforme* also affected photosynthesis (Fig. 2). Net photosynthesis increased from the beginning (fully hydrated), reached a maximum at a water loss of about 30%, and then decreased with further water loss. The maximal net

photosynthetic rate was about 26% higher than the initial value. About half of the maximal net photosynthetic rate was maintained even with a water loss of about 80%. Dark respiration decreased with increased water loss, and dropped to half of the initial value at water loss of about 70%.

P–I curves of *N. flagelliforme* at various levels of temperature and water loss are shown in Fig. 3. Even at the highest experimental irradiance (1140 $\mu$mol m$^{-2}$ s$^{-1}$), there was no apparent photoinhibition. Light-saturated photosynthetic rates ($P_m$) were reduced by 63–75% at 85% water loss and were highest at 15–25 °C (Fig. 4). $P_m$ values at 8 °C were 53–73% of those at 25 °C. However, $P_m$ values at 35 °C were only 2–36% lower than those at 25 °C. As temperature increased from 8 to 15 °C, the $P_m$ values increased by 46%, 64%, 69% and 69% at water loss levels of 0, 26%, 55% and 85%, respectively. The dark respiration ($R_d$) decreased with water loss and increased slightly with temperature. The rates of dark respiration at 8 °C were only 23–72% of those at other temperatures. This difference was significant (Tukey, $p < 0.05$). Photosynthetic efficiency ($\alpha$) decreased significantly (ANOVA, $p < 0.01$) with increased water loss at all temperatures (Fig. 4). Water loss of 85% reduced the photosynthetic efficiency by 59%, 67%, 59%, 76% at 8, 15, 25 and 35 °C, respectively. The values of $\alpha$ at 8 °C were 42–64% of those at 25 °C, and were significantly (Tukey, $p < 0.05$) raised by the elevation of temperature. However, the differences in photosynthetic efficiency among 15, 25 and 35 °C were insignificant (Tukey, $p > 0.05$). Saturating irradiances for photosynthesis ($I_k$) were significantly (ANOVA, $p < 0.05$) affected by both temperature and water loss (Fig. 4). Less light was required for
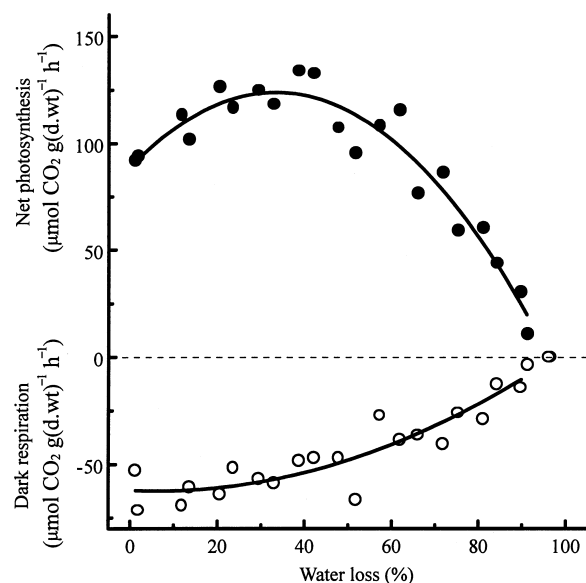


**Fig. 2.** Responses of net photosynthesis and dark respiration of *Nostoc flagelliforme* to water loss. Other details as Fig. 1.
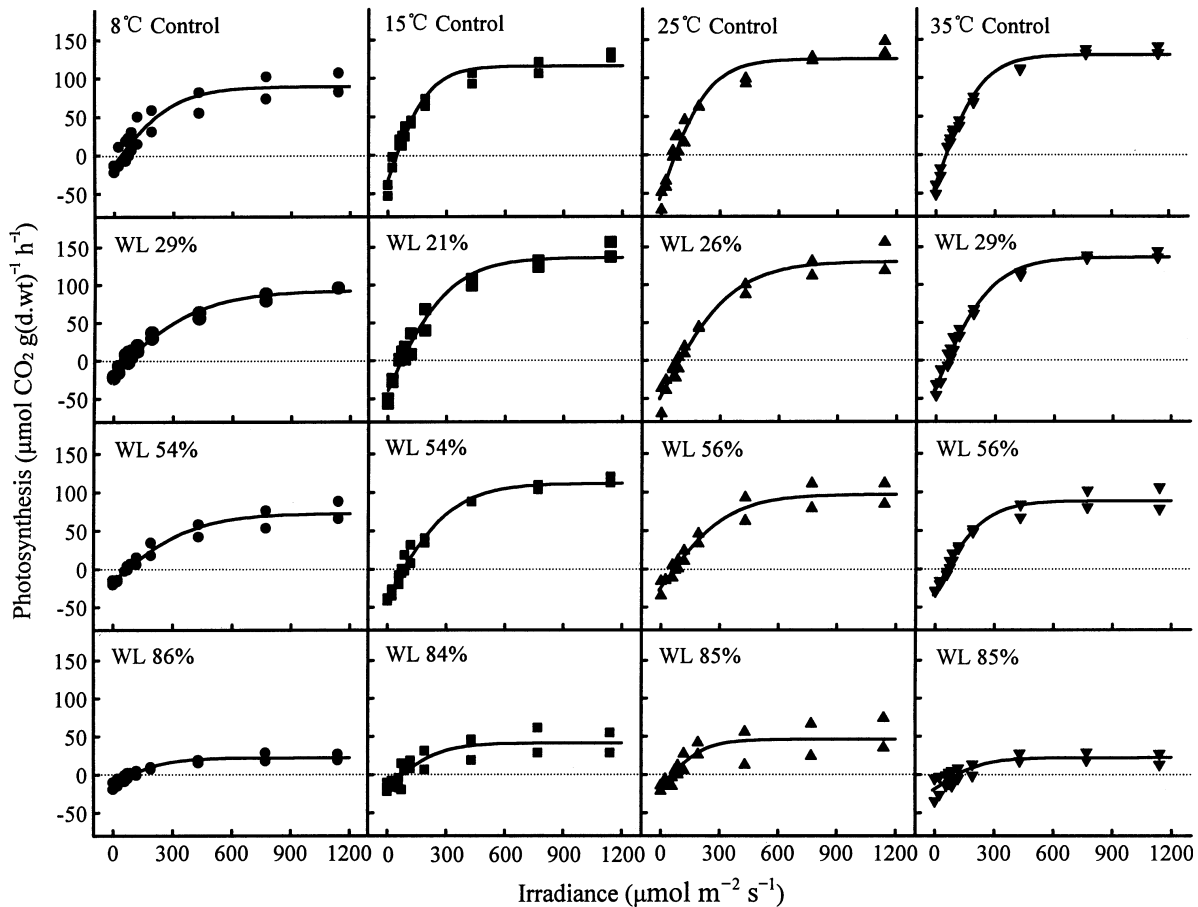
**Fig. 3.** Photosynthesis of *Nostoc flagelliforme* as a function of irradiance at various temperatures and degrees of water loss (WL). Algal samples were rehydrated at 40 $\mu$mol m$^{-2}$ s$^{-1}$ and the experimental temperatures (8, 15, 25 and 35 °C), and measured in the open system. Less than 8 % water was lost during each measurement. Wet samples (0 % water loss) were used as control. Each P–I curve shows the mean from two separate mats.
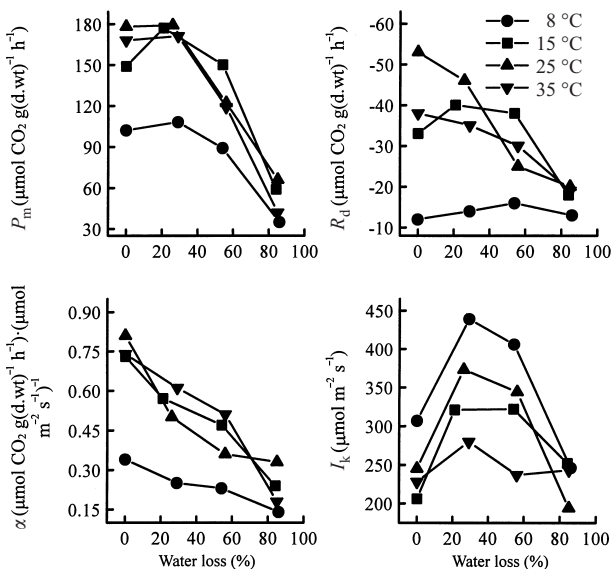


**Fig. 4.** Responses of light-saturated photosynthesis ($P_m$), dark respiration ($R_d$), photosynthetic efficiency ($\alpha$) and saturating irradiance for photosynthesis ($I_k$) of *Nostoc flagelliforme* to water loss at various temperatures. Data points are derived from curves shown in Fig. 3.

photosynthesis to be saturated when the alga was fully wet or highly desiccated than in the range of 30–55 % water loss (Tukey, $p < 0.05$). However, the

differences in $I_k$ between 26 % and 55 % water loss and between 0 and 88 % were insignificant (Tukey, $p > 0.05$). Increased water loss from 26 to 85 % reduced the $I_k$ values by 44 %, 21 %, 48 % and 13 % at 8, 15, 25 and 35 °C, respectively. In all states of desiccation, more light was needed for the photosynthetic saturation at low temperature than at higher temperatures. Responses of light compensation point ($I_c$) to desiccation at various levels of temperature are shown in Table 1. The $I_c$ values were variable, but were not significantly affected by either water loss or temperature (ANOVA, $p > 0.05$).

Canonical correlation analysis between environmental factors (temperature and water loss) and the photosynthetic parameters ($P_m$, $R_d$, $\alpha$, $I_k$ and $I_c$) is shown in Table 2. The first canonical root was significant ($p < 0.01$). The absolute value of the canonical weight for $\alpha$ was the largest, so that $\alpha$ was more sensitive to water loss than $P_m$, $R_d$, $I_k$ and $I_c$. On the other hand, the absolute value of canonical weight for water loss was larger than that for temperature. Therefore, effects of water loss on the photosynthesis of *N. flagelliforme* were more obvious than those of temperature.

**Table 1.** Light compensation points ($I_c$; $\mu$mol m$^{-2}$ s$^{-1}$) of *Nostoc flagelliforme* estimated from the P–I curves (Fig. 3).

| Water loss range (%) | Temperature (°C) | | | |
|---|---|---|---|---|
| | 8 | 15 | 25 | 35 |
| Control (0) | 41 | 45 | 68 | 51 |
| 21–29 | 59 | 72 | 90 | 56 |
| 54–56 | 72 | 82 | 69 | 59 |
| 84–86 | 87 | 78 | 61 | 93 |

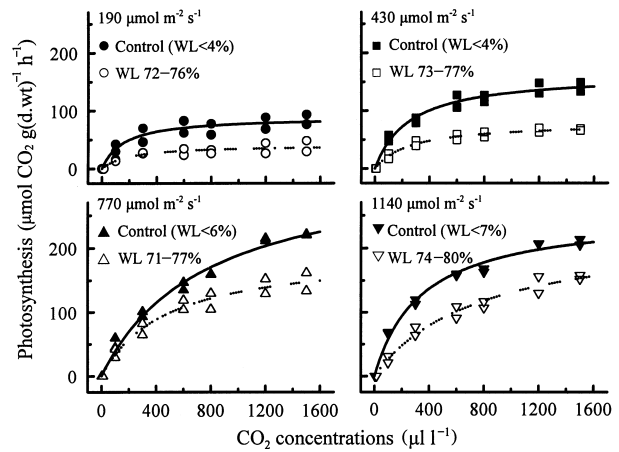Each value is the mean for two replicate curves.



**Fig. 5.** Photosynthetic $CO_2$ assimilation as a function of $CO_2$ concentration for *Nostoc flagelliforme* at four irradiances and two levels of water loss (WL). Algal samples were rehydrated at 40 $\mu$mol m$^{-2}$ s$^{-1}$ and 25 °C and measured at the experimental irradiances (190, 430, 770 and 1140 $\mu$mol m$^{-2}$ s$^{-1}$) and 25 °C in the closed system. Each curve shows the mean from two separate mats.

The photosynthetic responses of *N. flagelliforme* to $CO_2$ concentration at various irradiances are shown in Fig. 5. The $CO_2$-saturated photosynthetic rate ($V_{max}$) increased significantly with increase of irradiance from 190 to 1140 $\mu$mol m$^{-2}$ s$^{-1}$ (ANOVA, $p < 0.01$; Table 3). The photosynthetic affinities for $CO_2$ ($1/K_{0.5}(CO_2)$) at low irradiances (190 and 430 $\mu$mol m$^{-2}$ s$^{-1}$) were significantly (Tukey, $p < 0.05$) higher than those at high irradiances (770 and 1140 $\mu$mol m$^{-2}$ s$^{-1}$; Table 3). The $CO_2$ compensation points ($\Gamma$) were insensitive to irradiance (ANOVA, $p > 0.10$; Table 3).

The photosynthetic responses of *N. flagelliforme* to $CO_2$ concentrations at various temperatures are shown in Fig. 6. The $CO_2$-saturated photosynthetic rate ($V_{max}$) increased with temperature from 8 °C to 25 °C and decreased at 35 °C (ANOVA, $p < 0.05$; Table 4). The photosynthetic affinity for $CO_2$ ($1/K_{0.5}(CO_2)$) decreased significantly (ANOVA, $p < 0.05$) with increase of temperature from 8 °C to 35 °C (Table 4). $CO_2$ compensation points ($\Gamma$) were significantly higher at 35 °C than at 8, 15 and 25 °C (Tukey, $p < 0.05$; Table 4).

The $CO_2$-saturated photosynthetic rate ($V_{max}$) of *N. flagelliforme* was decreased significantly by 75 %

water loss from the alga (Tukey, $p < 0.05$), but water loss had less effect on the photosynthetic affinity for $CO_2$ (Tables 3, 4). $\Gamma$ was minimal at about 36 % water loss ($\Gamma < 2$ $\mu$l l$^{-1}$ $CO_2$), but increased when water loss exceeded about 70 % (Fig. 7).

The effect of $O_2$ concentration in the gas phase on photosynthetic and respiratory rates is shown in Fig. 8. Rates of net photosynthesis and dark respiration were similar at 2 % and 21 % $O_2$, both when the algal mats were fully hydrated and at a water loss of 83 % (Paired $t$-Test, $p > 0.10$).

## Discussion

The biomass density of *N. flagelliforme* mats decreased photosynthetic activity when it exceeded

**Table 2.** Canonical correlation analysis between environmental factors (T and WL) and the photosynthetic parameters ($P_m$, $R_d$, $\alpha$, $I_k$ and $I_c$) from the P–I curves (Fig. 3) for *Nostoc flagelliforme*

| Number | Eigenvalue | Canonical root | Chi–square | d.f. | Probability |
|---|---|---|---|---|---|
| 1 | 0·7578 | 0·8705 | 46·767 | 10 | 0·0000** |
| 2 | 0·2695 | 0·5191 | 8·478 | 4 | 0·0756 |

| Canonical weights | | |
|---|---|---|
| *First set of variables* | Root 1 | Root 2 |
| $P_m$ | −0·4454 | 1·1844 |
| $R_d$ | −0·8395 | −1·0838 |
| $\alpha$ | −1·3644 | −2·1084 |
| $I_k$ | −0·2735 | −1·5047 |
| $I_c$ | −0·2125 | −0·2690 |
| *Second set of variables* | Root 1 | Root 2 |
| T | −0·2309 | 0·9730 |
| WL | 0·9730 | 0·2309 |

T, temperature; WL, water loss.

**Table 3.** $CO_2$-saturated rates of photosynthesis ($V_{max}$), photosynthetic affinity for $CO_2$ ($1/K_{0.5}(CO_2)$) and $CO_2$ compensation point ($\Gamma$) of *Nostoc flagelliforme* at different irradiances

| Irradiance ($\mu$mol m$^{-2}$ s$^{-1}$) | $V_{max}$ ($\mu$mol $CO_2$ g(d.wt)$^{-1}$ h$^{-1}$) | | $K_{0.5}(CO_2)$ ($\mu$l l$^{-1}$ $CO_2$) | | $\Gamma$ ($\mu$l l$^{-1}$ $CO_2$) | |
|---|---|---|---|---|---|---|
| | Control | WL 75% | Control | WL 75% | Control | WL 75% |
| 190 | 88 | 39 | 151 | 163 | 2·5 | 14·0 |
| 430 | 159 | 71 | 207 | 234 | 2·0 | 9·0 |
| 770 | 221 | 153 | 370 | 306 | 3·0 | 10·0 |
| 1140 | 233 | 155 | 259 | 376 | 4·5 | 15·0 |

The parameters were estimated from Fig. 5; each value is the mean from two replicate P–$CO_2$ curves.
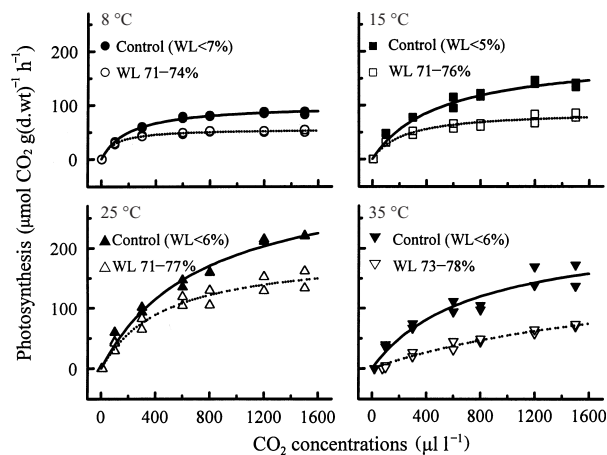WL, water loss.



**Fig. 6.** Photosynthetic $CO_2$ assimilation as a function of $CO_2$ concentration for *Nostoc flagelliforme* at four temperatures and two levels of water loss (WL). Algal samples were rehydrated at 40 $\mu$mol m$^{-2}$ s$^{-1}$ and the experimental temperature (8, 15, 25 and 35 °C) and measured at 770 $\mu$mol m$^{-2}$ s$^{-1}$ in the closed system. Each curve shows the mean from two separate mats.

11 mg cm$^{-2}$ due to self-shading. Increased mass in each mat of the same diameter resulted in increased biomass density, which enhanced the effect of self-shading within the mat. Self-shading thereby lowered the light availability to the alga, and decreased photosynthesis. In non-disturbed areas, mats of *N. flagelliforme* could be as large as 8 cm in
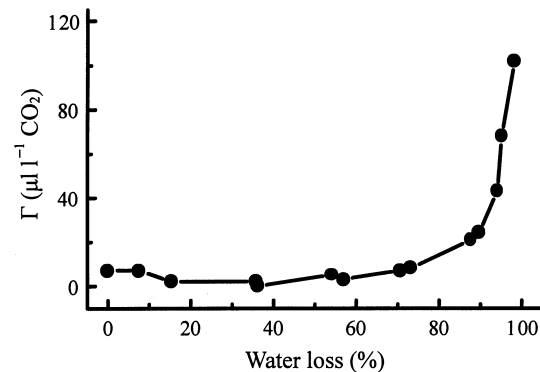


**Fig. 7.** Response of $CO_2$ compensation point ($\Gamma$) of *Nostoc flagelliforme* to water loss. Algal samples were rehydrated at 40 $\mu$mol m$^{-2}$ s$^{-1}$ and 25 °C, and measured at 770 $\mu$mol m$^{-2}$ s$^{-1}$ and 25 °C in the closed system.

diameter, with 18–37 mats m$^{-2}$, each weighing about 0·6–0·9 g d.wt (Gao, 1998). Correspondingly, the biomass density of each mat may be about 12–18 mg cm$^{-2}$. Therefore, self-shading may occur in these areas, especially when the solar radiation is lower than the irradiance employed in the present study (770 $\mu$mol m$^{-2}$ s$^{-1}$).

This study demonstrated that net photosynthesis and dark respiration of *N. flagelliforme* were affected markedly by the levels of water loss. Maximal photosynthetic rates of *N. flagelliforme* occurred at about 30% water loss. Gas exchange

**Table 4.** $CO_2$-saturated rates of photosynthesis ($V_{max}$), photosynthetic affinity for $CO_2$ ($1/K_{0.5}(CO_2)$) and $CO_2$ compensation point ($\Gamma$) of *Nostoc flagelliforme* at different temperatures

| Temperature (°C) | $V_{max}$ ($\mu$mol $CO_2$ g(d.wt)$^{-1}$h$^{-1}$) | | $K_{0.5}(CO_2)$ ($\mu$l l$^{-1}$ $CO_2$) | | $\Gamma$ ($\mu$l l$^{-1}$ $CO_2$) | |
|---|---|---|---|---|---|---|
| | Control | WL 75% | Control | WL 75% | Control | WL 75% |
| 8 | 91 | 67 | 208 | 113 | 2·5 | 5·0 |
| 15 | 143 | 82 | 351 | 203 | 4·5 | 8·0 |
| 25 | 221 | 153 | 370 | 306 | 3·0 | 10·0 |
| 35 | 169 | 70 | 422 | 552 | 18·5 | 77·5 |

The parameters were estimated from Fig. 6; each value is the mean from two replicate P–$CO_2$ curves.
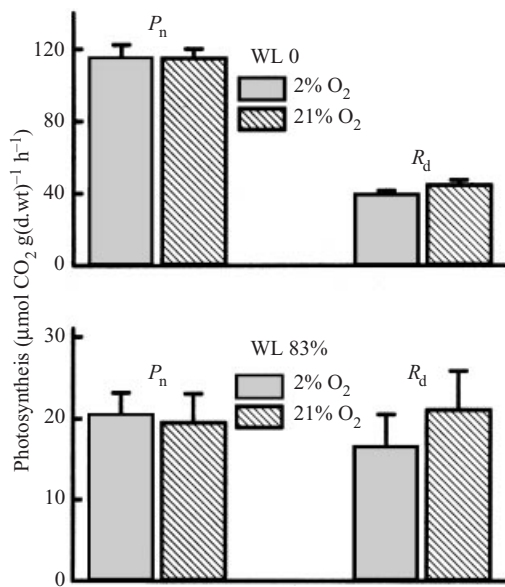WL, water loss.

**Fig. 8.** Net photosynthesis ($P_n$) and dark respiration ($R_d$) of *Nostoc flagelliforme* in air containing ambient (21 %) and reduced (2 %) $O_2$ concentrations. Algal samples were rehydrated at 25 °C and 40 $\mu$mol m$^{-2}$ s$^{-1}$, and measured at 770 $\mu$mol m$^{-2}$ s$^{-1}$ and 25 °C in the closed system. Bars indicate the means of 7 mats $\pm$ SD. WL, water loss.

during photosynthesis can be enhanced when the water film on the thallus is lost due to desiccation. Much of the biomass of *Nostoc* is polysaccharide sheath (Potts, 1996, 1999; Hill *et al.*, 1997; Huang *et al.*, 1998). Thus, the 30 % water loss here is probably in part due to sheath drying, and the sheath in *N. flagelliforme* might buffer the cells from this degree of desiccation. However, we cannot determine how much water loss is due to sheath drying and how much actual cell desiccation occurs with the present experimental design. Further desiccation reduced net photosynthesis, probably due to intracellular water loss. This phenomenon is consistent with those reported in *Ascophyllum nodosum* (Bewley, 1979), *Fucus distichus* (Quadir *et al.*, 1979), *F. spiralis* (Madsen & Maberly, 1990), *F. vesiculosus* (Bewley, 1979), *Halosaccion americanum* (Oates, 1986), *Porphyra linearis* (Lipkin *et al.*, 1993) and *P. yezoensis* (Gao & Aruga, 1987). The photosynthetic activities of these seaweeds were also stimulated by moderate desiccation (compared to the fully hydrated emersed state), presumably because of a reduction in the aqueous diffusion barrier for $CO_2$ (Oates, 1986; Davison & Pearson, 1996).

In the present study, 45–66 % of the photosynthetic activity in *N. flagelliforme* remained at a water loss on a whole thallus basis of 70–80 %. In the semiarid habitats of *N. flagelliforme*, the temperature shows a marked day-night shift and the relative humidity can be 80–90 % at night; *N. flagelliforme* may have 20–30 % of the fully hydrated content of water in the early morning (Dai, 1992; Gao, 1998). About 50 % of the photosynthetic activity was found at 80 % water loss, i.e., about 20 % of the fully hydrated water content. This finding supports the suggestion that *N. flagelliforme* could grow by absorbing dew in nature (Gao, 1998). Gao *et al.* (1998*b*) found that about 40–50 % PSII photochemical efficiency ($F_v/F_m$) was found at such a water content, which agrees well with the present study in terms of photosynthetic rate.

The present study showed that desiccation affected the photosynthetic efficiency ($\alpha$) and light requirement ($I_k$) of *N. flagelliforme*. The values of $\alpha$ decreased with increased desiccation, implying that the efficiency of light-harvesting and energy conversion in photosynthesis were reduced. Saturating irradiances for the photosynthesis of *N. flagelliforme* were in the range 194–439 $\mu$mol m$^{-2}$ s$^{-1}$. The maximal $I_k$ values occurred at about 30 % water loss and the $I_k$ values at water loss less than 30 % or more than 55 % were 52–87 % of the maximal $I_k$. As reported by Beardall & Morris (1976) for *Phaeodactylum tricornutum* and confirmed by Henley (1992) for *Ulva rotundata*, $I_k$ often correlates better with $P_m$ than with $\alpha$, both in comparing individuals acclimated to different irradiances and in the same individual during diurnal P–I changes. During the progress of desiccation at all temperatures used, the maximal $I_k$ values of *N. flagelliforme* were always associated with the maximal light-saturated photosynthetic activity at about 30 % water loss. It has been suggested that a low value of $I_k$ usually indicates inefficient use of high irradiance rather than efficient use of low irradiance (Henley, 1993). The efficiency with which *N. flagelliforme* used high irradiance decreased when the alga was fully rehydrated or getting dry. This can be attributed to an increased diffusion barrier for $CO_2$ when fully hydrated, and to a reduced performance in light-harvesting, energy conversion or carbon assimilation when the alga had lost more than 30 % of the fully hydrated water content. Shi *et al.* (1992) found that saturating irradiance for the photosynthesis of *N. flagelliforme*, when submerged in BG$_{11}$ medium at 23 °C, was about 1200 $\mu$mol m$^{-2}$ s$^{-1}$. This is much higher than the $I_k$ values reported in the present study. *N. flagelliforme* is always exposed to air in nature even immediately after rain. Therefore, only the saturating irradiance for photosynthesis in air reflects the algal light requirement in its habitat.

Our results showed that the maximum photosynthetic rates of *N. flagelliforme* at ambient $CO_2$ occurred over a temperature range of 15–35 °C (Fig. 4) rather than at a single optimum temperature (25 °C) as reported by Shi *et al.* (1992). The phenomenon of broad temperature optima for photosynthesis has also been shown in macroalgae (Oates & Murray, 1983; Madsen & Maberly, 1990). The availability of $C_i$ may affect the shape of photosynthesis-temperature responses, particularly

in those marine macroalgae in which ambient $C_i$ levels are subsaturating (Surif & Raven, 1989; Madsen & Maberly, 1990). An increase in substrate affinity with decreasing temperature can compensate for the reduction in the maximal photosynthetic activity by reducing the temperature dependence of the reaction (Berry & Björkman, 1980; Berry & Raison, 1981). Such responses have been reported for ribulose-1,5-bisphosphate carboxylase (Rubisco) from Antarctic diatoms (Descolas-Gros & de Billy, 1987) and the kelp *Laminaria saccharina* (Davison, 1987). Our measurements were conducted under emersed conditions. Photosynthesis of *N. flagelliforme* was not saturated at ambient $CO_2$ (360 $\mu$l l$^{-1}$). Thus, $C_i$ limitation could account for the occurrence of broad temperature optima for the photosynthesis of *N. flagelliforme* in Fig. 4 (Surif & Raven, 1989; Madsen & Maberly, 1990). Shi *et al.* (1992) measured the photosynthesis of *N. flagelliforme* in $BG_{11}$ medium, the $CO_2$ concentration of which can reach 48 $\mu$mol l$^{-1}$. Only about 15 $\mu$mol l$^{-1}$ $CO_2$ is available in solutions in equilibrium with air. Therefore, the discrepancy between our result (broad temperature optima for photosynthesis under emersed conditions) and that of Shi *et al.* (1992) (a single optimum temperature in $BG_{11}$ medium) can be understood. A single optimum temperature for photosynthesis in *N. flagelliforme* would be expected to appear with the increase of $CO_2$ concentration for the photosynthetic measurements, and this is confirmed by the data for $V_{max}$ at different temperatures (Table 4). Although dark respiration of *N. flagelliforme* was lowered significantly at 8 °C, it showed little change with temperature between 15 °C and 35 °C. In one of the habitats of *N. flagelliforme*, Yongden in Gansu province, the months with mean temperatures above 8 °C are May to September. During this period, the daily temperature difference is 11·0–16·4 °C. In such an environment, *N. flagelliforme* would be expected to maintain a high photosynthetic activity during daytime and low dark respiration at night, which benefits its daily net production.

It has been suggested that $\alpha$ is less sensitive to temperature than $P_m$ and $R_d$ (Davison, 1991; Henley, 1993), but the photosynthetic efficiency ($\alpha$) of *N. flagelliforme* was significantly lower at 8 °C than at the other temperatures examined (15, 25 and 35 °C). Although the initial photochemical reactions of photosynthesis are independent of temperature, many associated aspects of photosynthesis, such as enzymes of photophosphorylation, electron transport and plastoquinone diffusion are temperature dependent (Davison, 1991). Therefore, $\alpha$ may still be significantly affected by temperature, as observed in the present study.

The photosynthesis of *N. flagelliforme* was found to be $CO_2$-limited at the present atmospheric $CO_2$ concentration. $CO_2$ will probably increase from the present level (350 $\mu$l l$^{-1}$) to twice that value (700 $\mu$l l$^{-1}$) within the next century, and such a change could bring about a 13 % to 61 % increase in the rate of photosynthesis of *N. flagelliforme*. This increase was greater at high temperatures (25 or 35 °C) and high irradiances (770 or 1140 $\mu$mol m$^{-2}$ s$^{-1}$), and $CO_2$ enrichment enhanced the photosynthesis of *N. flagelliforme* even when desiccated. Therefore, enrichment of $CO_2$ at high temperature and irradiance would be effective in the cultivation of *N. flagelliforme*, because higher $CO_2$ levels were required to saturate its photosynthesis at higher temperatures and irradiances.

The $CO_2$ compensation point ($\Gamma$) is the concentration of $CO_2$ at which photosynthetic uptake balances photorespiratory/respiratory release in the light. Low values (0–10 $\mu$l l$^{-1}$ $CO_2$) indicate reduced photorespiration, while $O_2$-sensitive values above 50 $\mu$l l$^{-1}$ $CO_2$ indicate an enhanced photorespiratory rate. The $\Gamma$ values reported for most microalgae are very low, and similar to those of $C_4$ plants (Birmingham & Colman, 1979; Birmingham *et al.*, 1982; Beardall, 1989). That of *N. flagelliforme* was lower than 10 $\mu$l l$^{-1}$ $CO_2$ with water loss less than 70 % at 8–25 °C. These values are very much lower than those reported for $C_3$ higher plants.

The photosynthesis of *N. flagelliforme* did not appear to be $O_2$-inhibited at atmospheric levels of $O_2$ and $CO_2$. This contrasts with *N. parmelioides*, for which $O_2$ was inhibitory to photosynthesis (Dodds, 1989). Dark respiration is usually saturated by about 2 % $O_2$ whereas photorespiration increases significantly with $O_2$ concentration in the gas phase. The low $\Gamma$ value and apparent absence of photorespiration suggest that there may be a $CO_2$-concentrating mechanism (CCM) in *N. flagelliforme*, similar to that demonstrated in several aquatic blue-green algae (Kaplan *et al.*, 1980; Badger & Andrews, 1982; Pierce & Omata, 1988; Colman, 1989; Miller *et al.*, 1990; Kaplan & Reinhold, 1999). However, *N. flagelliforme* is terrestrial and may possess a different CCM as an adaptation to its dry habitats. There was clear evidence for the operation of a CCM within some *Nostoc*-lichens (Badger *et al.*, 1993; Palmqvist, 1993). Their photosynthesis showed high efficiency at low $CO_2$ and was not affected by $O_2$. However, the CCM within them appeared to be somewhat less efficient than in other blue-green algae, and it was suggested that they lacked $HCO_3^-$ uptake (Palmqvist, 1993). Further evidence on the nature of $C_i$ utilization in *N. flagelliforme* is needed.

The relationship between photosynthesis and environmental factors reflects the adaptability of *N. flagelliforme* to its terrestrial habitats. Desiccation affected its photosynthetic efficiency ($\alpha$) and light

requirement and the photosynthetic activity was stimulated by moderate desiccation. Our results support the suggestion that *N. flagelliforme* could grow by absorbing dew in nature. The availability of $C_i$ affected the shape of photosynthesis-temperature responses. Although *N. flagelliforme* might possess a less efficient CCM, its photosynthesis was $CO_2$-limited at the current atmospheric $CO_2$ concentration and it would benefit from atmospheric $CO_2$ rise. The enrichment of $CO_2$ at high temperature and irradiance would be effective in the cultivation of *N. flagelliforme*.

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## References

BADGER, M.R. & ANDREWS, T.J. (1982). Photosynthesis and inorganic carbon usage by the marine cyanobacterium, *Synechococcus* sp. *Plant Physiol.*, **70**: 517–523.

BADGER, M.R., PFANZ, H., BÜDEL, B., HEBER, U. & LANGE, O.L. (1993). Evidence for the functioning of photosynthetic $CO_2$-concentrating mechanisms in lichens containing green algal and cyanobacterial photobionts. *Planta*, **191**: 57–70.

BEARDALL, J. (1989). Photosynthesis and photorespiration in marine phytoplankton. *Aquat. Bot.*, **34**: 105–130.

BEARDALL, J. & MORRIS, I. (1976). The concept of light intensity adaptation in marine phytoplankton: some experiments with *Phaeodactylum tricornutum. Mar. Biol.*, **37**: 377–387.

BERRY, J.A. & BJÖRKMAN, O. (1980). Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.*, **31**: 491–543.

BERRY, J.A. & RAISON, J.K. (1981). Responses of macrophytes to temperature. In *Physiological Plant Ecology, I. Response to the Physical Environment. Encyclopedia of Plant Physiology New Series*, Vol. 12A (Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H., editors), 277–338. Springer-Verlag, Berlin.

BEWLEY, J.D. (1979). Physiological aspects of desiccation tolerance. *Annu. Rev. Plant Physiol.*, **30**: 195–238.

BIRMINGHAM, B.C. & COLMAN, B. (1979). Measurement of carbon dioxide compensation points of freshwater algae. *Plant Physiol.*, **64**: 892–895.

BIRMINGHAM, B.C., COLEMAN, J.R. & COLMAN, B. (1982). Measurement of photorespiration in algae. *Plant Physiol.*, **69**: 259–262.

BOWES, G. (1993). Facing the inevitable: plants and increasing atmospheric $CO_2$. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **44**: 309–332.

COLMAN, B. (1989). Photosynthetic carbon assimilation and the suppression of photorespiration in the cyanobacteria. *Aquat. Bot.*, **34**: 211–231.

DAI, Z.J. (1992). Review of *Nostoc flagelliforme* research (in Chinese). *J. Ningxia Univ* (Nat. Sci), **13(1)**: 71–77.

DAVISON, I.R. (1987). Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to changes in growth temperature. *J. Phycol.*, **23**: 273–283.

DAVISON, I.R. (1991). Environmental effects on algal photosynthesis: temperature. *J. Phycol.*, **27**: 2–8.

DAVISON, I.R. & PEARSON, G.A. (1996). Stress tolerance in intertidal seaweeds. *J. Phycol.*, **32**: 197–211.

DESCOLAS-GROS, C. & DE BILLY, G. (1987). Temperature adaptation of RuBP carboxylase: kinetic properties in marine Antarctic diatoms. *J. Exp. Mar. Biol. Ecol.*, **108**: 147–158.

DODDS, W.K. (1989). Photosynthesis of two morphologies of *Nostoc parmelioides* (cyanobacteria) as related to current velocities and diffusion patterns. *J. Phycol.*, **25**: 258–262.

DODDS, W.K., GUDDER, D.A. & MOLLENHAUER, D. (1995). The ecology of *Nostoc. J. Phycol.*, **31**: 2–18.

GAO, K.S. (1998). Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. *J. Appl. Phycol.*, **10**: 37–49.

GAO, K.S. & ARUGA, K. (1987). Preliminary studies on the photosynthesis and respiration of *Porphyra yezoensis* under emersed conditions. *J. Tokyo Univ. Fish.*, **47(1)**: 51–65.

GAO, K.S., QIU, B.S., XIA, J.R. & YU, A.J. (1998*a*). Light dependency of the photosynthetic recovery of *Nostoc flagelliforme. J. Appl. Phycol.*, **10**: 51–53.

GAO, K.S., QIU, B.S., XIA, J.R., YU, A.J. & LI, Y.G. (1998*b*). Effect of wind speed on the water-losing process and photosynthetic efficiency of *Nostoc flagelliforme. J. Appl. Phycol.*, **10**: 55–58.

HENLEY, W.J. (1992). Growth and photosynthesis of *Ulva rotundata* (Chlorophyta) as a function of temperature and square wave irradiance in indoor culture. *J. Phycol.*, **28**: 625–634.

HENLEY, W.J. (1993). Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *J. Phycol.*, **29**: 729–739.

HILL, D.R., KEENAN, T.W., HELM, R.F., POTTS, M., CROWE, L.M. & CROWE, J.H. (1997). Extracellular polysaccharide of *Nostoc commune* (cyanobacteria) inhibits fusion of membrane vesicles during desiccation. *J. Appl. Phycol.*, **9**: 237–248.

HUANG, Z.B., LIU, Y.D., PAULSEN, B.S. & KLAVENESS, D. (1998). Studies on polysaccharides from three edible species of *Nostoc* (Cyanobacteria) with different colony morphologies: Comparison of monosaccharide compositions and viscosities of polysaccharides from field colonies and suspension cultures. *J. Phycol.*, **34**: 962–968.

JASSBY, A.D. & PLATT, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.*, **21**: 540–547.

KAPLAN, A., BADGER, M.R. & BERRY, J.A. (1980). Photosynthesis and the intracellular inorganic carbon pool in the blue-green alga *Anabaena variabilis*: response to external $CO_2$ concentration. *Planta*, **149**: 219–226.

KAPLAN, A. & REINHOLD, L. (1999). $CO_2$ concentrating mechanisms in photosynthetic microorganisms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **50**: 539–570.

LI, S.H. (1991). Ecology of the terrestrial alga *Nostoc flagelliforme* Berk. et Curt. in China. *J. Phycol.*, **27 (Suppl.)**: 45.

LIPKIN, Y., BEER, S. & ESHEL, A. (1993). The ability of *Porphyra linearis* (Rhodophyta) to tolerate prolonged periods of desiccation. *Bot. Mar.*, **36**: 517–523.

MADSEN, T.V. & MABERLY, S.C. (1990). A comparison of air and water as environments for photosynthesis by the intertidal alga *Fucus spiralis* (Phaeophyta). *J. Phycol.*, **26**: 24–30.

MEI, J.X. & CHENG, Z.J. (1990). Effects of temperature on physiological activities of *Nostoc flagelliforme* Born. et Flah (in Chinese with English summary). *J. Western-north Normal Uni.* (Nat. Sci), **1**: 75–85.

MILLER, A.G., ESPIE, G.S. & CANVIN, D.T. (1990). Physiological aspects of $CO_2$ and $HCO_3^-$ transport by cyanobacteria: a review. *Can. J. Bot.*, **68**: 1291–1302.

OATES, B.R. (1986). Components of photosynthesis in the intertidal saccate alga *Halosaccion americanum* (Rhodophyta, Palmariales). *J. Phycol.*, **22**: 217–223.

OATES, B.R. & MURRAY, S.N. (1983). Photosynthesis, dark respiration and desiccation resistance of the intertidal seaweeds *Hesperophycus harveyanus* and *Pelvetia fastigiata* f. *gracilis. J. Phycol.*, **19**: 371–380.

PALMQVIST, K. (1993). Photosynthetic $CO_2$-use efficiency in lichens and their isolated photobionts: the possible role of a $CO_2$-concentrating mechanism. *Planta*, **191**: 48–56.

PIERCE, J. & OMATA, T. (1988). Uptake and utilization of inorganic carbon by cyanobacteria. *Photosynth. Res.*, **16**: 141–154.

POTTS, M. (1996). The anhydrobiotic cyanobacterial cell. *Physiol. Plant*, **97**: 788–794.

POTTS, M. (1999). Mechanisms of desiccation tolerance in cyanobacteria. *Eur. J. Phycol.*, **34**: 319–328.

QIAN, K.X., ZHU, H.R. & CHEN, S.G. (1989). The ecological conditions for *Nostoc flagelliforme* and their analysis (in Chinese with English summary). *Acta Phytoecologica et Geobotanica Sinica.*, **13**: 97–105.

QIU, B.S. & GAO, K.S. (1999). Dried field populations of *Nostoc flagelliforme* (Cyanophyceae) require exogenous nutrients for their photosynthetic recovery. *J. Appl. Phycol.*, **11**: 535–541.

QUADIR, A., HARRISON, P.J. & DEWREEDE, R.E. (1979). The effects of emergence and submergence on the photosynthesis and respiration of marine macrophytes. *Phycologia*, **18**: 83–88.

SCHERER, S., ERNST, A., CHEN, T.W. & BÖGER, P. (1984). Rewetting of drought-resistant blue-green algae: time course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation. *Oecologia*, **62**: 418–423.

SCHERER, S., ERNST, A., CHEN, T.W. & BÖGER, P. (1986). Recovery of adenine-nucleotide pools in terrestrial blue-green algae after prolonged drought periods. *Oecologia*, **68**: 585–588.

SHI, D.J., ZHOU, G.F., FANG, S.X., QIU, Y.Y., ZHONG, Z.P. & CUI, Z.Y. (1992). Studies on photosynthesis, respiration and morphology of *Nostoc flagelliforme* (in Chinese with English summary). *Acta Botanica Sinica*, **34**: 507–514.

STANIER, R.Y., KUNISAWA, M.M. & COHEN-BAZIRE, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev.*, **35**: 171–201.

SURIF, M.B. & RAVEN, J.A. (1989). Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and the Laminariales (Phaeophyta): ecological and taxonomic implications. *Oecologia*, **78**: 97–105.