

PHOTOSYNTHESIS OF TWO MORPHOLOGIES OF *NOSTOC PARMELIOIDES*
(CYANOBACTERIA) AS RELATED TO CURRENT VELOCITIES AND
DIFFUSION PATTERNS¹

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ABSTRACT

Colonies of the stream-inhabiting cyanobacterium *Nostoc parmelioides* Kützing often contain a single endosymbiotic dipteran larva *Cricotopus nostocicola* (Wirth), which induces a morphological change from small, spherical colonies to larger, ear-shaped colonies. At a current velocity of $0 \text{ cm} \cdot \text{s}^{-1}$, whole colonies containing the midge showed overall rates of $^{14}\text{CO}_2$ uptake and nitrogenase activity that were higher than those when the midge was absent (sphere-shaped colonies). Spherical colonies incubated at current velocities of $5\text{--}10 \text{ cm} \cdot \text{s}^{-1}$ did not show higher rates of $^{14}\text{CO}_2$ or $^{15}\text{N}_2$ incorporation than those with the larvae (ear-shaped colonies). Ear-shaped colonies extended well into regions of higher current velocity, whereas spherical colonies did not. Photosynthesis of ear-shaped colonies was stimulated by increased current velocity, increased inorganic C and decreased O_2 concentrations. Moreover, levels of O_2 at the surface of midge-inhabited colonies decreased with increased current velocity. The morphological change induced by the larva is detrimental (lowers photosynthesis and N_2 fixation) in quiescent water but not at current velocities above $10 \text{ cm} \cdot \text{s}^{-1}$. This is probably a result of higher diffusion of O_2 and CO_2 associated with the midge-induced morphology.

Key index words: current; inorganic carbon; mutualism; nitrogen fixation; *Nostoc*; photosynthesis

Nostoc parmelioides is attached to rocks in streams and is inhabited by the chironomid midge larvae

Cricotopus nostocicola or *C. fuscatus* (Wirth). *N. parmelioides* with endosymbiotic *C. nostocicola* is found throughout the United States (Wirth 1957, Boesel 1983, Ward et al. 1985). Each colony is inhabited by only one 3rd or 4th instar larva which eats material inside the colony, receives protection from predators (Brock 1960), increases attachment strength of *N. parmelioides* (Dodds and Marra 1989) and may increase *N. parmelioides* photosynthesis (Ward et al. 1985). The larva causes a change in *N. parmelioides* morphology from a small sphere (<5 mm diameter), which is tightly appressed to stream bottom rocks, to a larger (>10 mm diameter) ear-shaped colony which protrudes from the rock's surface (Brock 1960).

This relationship is unusual because other well documented examples of a directed mutualism in a stream between a primary consumer and a primary producer are rare (Gregory 1983, except see Resh and Houpp 1986, Dodds and Castenholz 1988a). No mechanism for how the midge larva affects *N. parmelioides* photosynthesis has been established. The midge may also affect N_2 fixation by *N. parmelioides*. These interactions were examined taking into account the micro-environmental variation experienced by spherical and ear-shaped colonies.

MATERIALS AND METHODS

Algal material was collected from McRae Creek, Lane County, Oregon ($44^{\circ}15' \text{ N}$, $122^{\circ}14' \text{ W}$) or from Hyalite Creek, Gallatin County, Montana ($45^{\circ}32' \text{ N}$, $111^{\circ}0' \text{ W}$). Colonies contained late

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TABLE 1. ^{14}C uptake and nitrogen fixation of ear-shaped and spherical *Nostoc* colonies as measured in test tubes with no flow and on rocks in a re-circulating chamber. Numbers in parentheses give ± 1 SD. *P* and *t* values are the results of the pooled *t* test between means with and without the midge larvae.

Treatment	Ear-shaped	Spherical	n	df	<i>t</i>	<i>P</i>
$^{14}\text{CO}_2$ uptake ^a						
In test tube	0.107 (0.050)	0.241 (0.174)	6	10	1.80	0.06 > <i>P</i> > 0.05
On rock	0.113 (0.133)	0.119 (0.152)	3	4	0.65	<i>P</i> > 0.30
Acetylene reduction ^b						
In test tube	0.288 (0.111)	1.45 (1.06)	3	4	3.67	0.025 > <i>P</i> > 0.01
$^{15}\text{N}_2$ uptake in flow ^c						
On rock	0.188 (0.008)	0.153 (0.015)	4	6	1.14	0.10 > <i>P</i> > 0.05

^a $\mu\text{mole C}\cdot\text{h}^{-1}\cdot\text{dry g}^{-1}$.

^b $\mu\text{mole acetylene}\cdot\text{h}^{-1}\cdot\text{dry g}^{-1}$.

^c Atom-% ^{15}N .

3rd or 4th instar larvae as determined by head-capsule width and larval coloring (Brock 1960) or no midge larvae. Colonies were transported to the laboratory, kept at 4°C and used within two days of collection.

Rate of O_2 production and O_2 concentration were measured in the laboratory using either Clark-type or cathode oxygen microelectrodes (Revsbech and Jørgensen 1986). All measurements of O_2 production and O_2 concentration were made with the microelectrode pushed against the top surface of *N. parmelioides*. Spatial resolution of the O_2 electrodes was 100 μm (Revsbech and Jørgensen 1983). Measurements on the localized effect of larvae on photosynthetic O_2 production and on effects of nutrient enrichment on O_2 production were made on colonies collected from the field and returned to the laboratory where they were removed from rocks and placed on an agar surface in a controlled temperature water bath. Measurements were done at ambient stream temperature (ca 10°C). Samples were illuminated with a "cool" fiber-optic source (Dolan Jenner Industries Inc., Fiberlite, 190) at a photon fluence rate of 1500 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ as measured with a LI-COR Li-190SB quantum sensor. NaHCO_3 was added to stream water, and pH was adjusted with HCl or NaOH to test for NaHCO_3 and pH effects on photosynthesis after a 12 h incubation in the altered stream water solutions.

Photosynthesis of whole colonies was estimated using $^{14}\text{CO}_2$ uptake. Test tube experiments were done in 6, 20-mL screw cap test tubes each containing 10 mL stream water, about 1 g (wet weight) of *N. parmelioides*, and 0.02 $\mu\text{Ci}\cdot\text{mL}^{-1}$ ^{14}C - NaHCO_3 . Three colonies with and three without midge larvae were tested. Samples were incubated at streamside under natural light for 2 h with shaking every 30 min. Individual colonies were then removed, washed thoroughly with stream water and dried. Colonies were treated with dimethyl sulfoxide to extract fixed carbon (Filben and Hough 1984) and counted for 20 min in Scintiverse E cocktail (Fisher) on a Beckman LS 100C liquid scintillation spectrometer. The alkalinity of the water was estimated titrimetrically (Standard methods 1975).

Photosynthesis of spherical and ear-shaped colonies which were still attached to rocks was measured in rectangular 2.5 L plexiglass recirculating chambers (Ward et al. 1985) which had ^{14}C - NaHCO_3 added to a final concentration of 0.02 $\mu\text{Ci}\cdot\text{mL}^{-1}$ and were incubated in the stream. These chambers had regions of maximum current velocity of 100 $\text{cm}\cdot\text{s}^{-1}$ with a mean velocity of 5–10 $\text{cm}\cdot\text{s}^{-1}$ as measured with a thermistor flow meter (La-Barbera and Vogel 1976). After 3 h the reaction was stopped by removing the rocks and rinsing them well with stream water. Three colonies with no midge and three colonies with a midge were dried and analyzed for $^{14}\text{CO}_2$ incorporation as described above.

Acetylene reduction of individual colonies was determined in 15 mL screw cap tubes with butyl rubber stoppers. Approximately 1 g wet weight of *N. parmelioides* was added to each tube with 9

mL stream water and 1 mL acetylene, leaving a total headspace of 6 mL. Tubes were incubated for 4 h with hourly shaking under cool white fluorescent lights at 100 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (measured with Biospherical Instruments QSL-100 light meter). One mL of the headspace gas was sampled after 4 h and analyzed for ethylene content on a Shimadzu GC-mini2 gas chromatograph with a flame ionization detector. The 1 m column was packed with Poropak N and conditions of operation were as follows: injection temperature, 80°C; column temperature, 60°C; and N_2 carrier gas at 40 $\text{mL}\cdot\text{min}^{-1}$.

$^{15}\text{N}_2$ uptake was used to assay nitrogenase activity of whole colonies still attached to rocks since acetylene reduction would not give a per-colony estimate of N_2 fixation. Rocks with attached colonies were incubated in the rectangular recirculating chambers described above. The chamber was closed, and stream water was bubbled (while circulating) with argon for 15 min to remove $^{15}\text{N}_2$. A headspace of 150 mL containing 79% $^{15}\text{N}_2$, 20% O_2 , and 1% CO_2 was added, followed by a 4 h incubation in the stream. Colonies were removed and dried. Dry samples were weighed and analyzed for ^{15}N atom % enrichment by emission spectroscopy after Dumas combustion (Timperley and Priscu 1986).

Experiments with controlled current velocity ($^{14}\text{CO}_2$ uptake and O_2 concentration as a function of velocity) were conducted in an oval recirculating chamber in which current velocity was controlled by regulating voltage supplied to the re-circulation pump. Current velocities were measured 2 mm upstream from the point of *N. parmelioides* attachment (the thermistor flow meter had a 2 mm spatial resolution). To ascertain the effect of flow on $^{14}\text{CO}_2$ uptake, baffles which caused localized regions of widely varied flow were added to the oval recirculating chamber. The velocity at the point of attachment of 45 ear-shaped *N. parmelioides* colonies was determined. $^{14}\text{CO}_2$ was added to the chamber as described above, and the chamber was incubated under sunlight (1960 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 4 h, after which colonies were analyzed for $^{14}\text{CO}_2$ incorporation as described above.

To measure the effect of reduced O_2 tension on photosynthesis, five test tubes with *N. parmelioides* were bubbled with N_2 for 15 min. Five control tubes were not bubbled. $^{14}\text{CO}_2$ was added (as above) to all tubes which were then incubated under sunlight for 2 h at 1960 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Colonies were analyzed for $^{14}\text{CO}_2$ incorporation as described above.

The relationship between current velocity 2 mm above the colony and O_2 concentration within 100 μm of the surface of ear-shaped colonies was measured with O_2 microelectrodes as described above. In these laboratory experiments, the colonies remained attached to rocks, and current velocity was varied over individual colonies. O_2 measurements were made on five colonies at five different velocities each.

Flow profiles over rocks were measured in the laboratory where current velocity was established in the same direction across the rocks as in the stream. Current velocity of water 5 cm from the

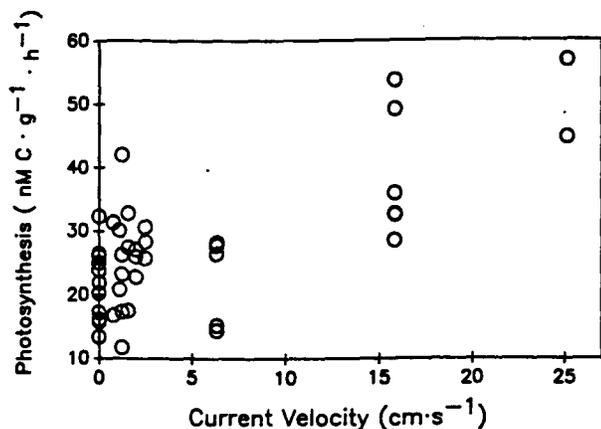


FIG. 1. Whole colony $^{14}\text{CO}_2$ incorporation of 45 ear-shaped *N. parmelioides* colonies attached to rocks as measured in a recirculating chamber at various current velocities.

rocks was matched to the current velocity 5 cm above the rocks when they were still in the stream. The vertical position of the thermistor flow meter was controlled using a micro-manipulator and vertical profiles of current velocity were measured every 2 cm along the rock with a vertical resolution of 0.33 cm.

To measure larval respiration rates, four midge larvae were removed from their colonies and each was placed in a 1.2 mL incubation chamber at 16° C for 1 h. Changes in dissolved O_2 were monitored continuously for 20 min with oxygen microelectrodes.

Statistical comparisons were done with pooled *t* tests when two means were compared because sample sizes were small. One way ANOVA was used to compare multiple means.

RESULTS AND DISCUSSION

Photosynthesis (measured by $^{14}\text{CO}_2$ uptake) and nitrogen fixation (measured by acetylene reduction) of whole colonies in test tubes (quiescent water) were higher in spherical than ear-shaped colonies (Table 1). However, photosynthetic rates of spherical and ear-shaped colonies were not statistically different when colonies remained attached to rocks and measurements were made at an average current velocity of 5–10 $\text{cm} \cdot \text{s}^{-1}$ (Table 1). $^{15}\text{N}_2$ incorporation by spherical colonies was nearly equal to incorporation by ear-shaped colonies in flowing water. The data indicate a relationship between flow and photosynthesis; flow affected nitrogen fixation in a similar manner to photosynthetic results.

Photosynthetic rates of ear-shaped colonies were

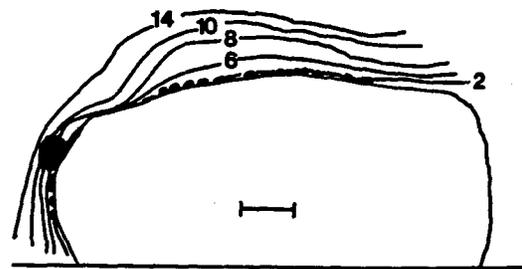


FIG. 2. Current velocity contours over one rock with attached *N. parmelioides* colonies (dark spots). The small spots were spherical colonies and were uninhabited by midge larvae. The large colony protruding from the rock surface was ear-shaped and contained 1 larva. The bar is 2 cm, current velocity is in $\text{cm} \cdot \text{s}^{-1}$. The open channel current was from right to left.

higher at higher current velocity (Fig. 1). Linear regression of the data in Figure 1 showed a significant positive correlation between current velocity and photosynthesis ($P < 0.005$). Current velocity also can increase algal respiration and ^{32}P uptake (Schumacher and Whitford 1965), growth of *Nostoc pruniforme* (Dodds and Castenholz 1988b), and photosynthesis by a phytoplankton (Lehman 1978) and marine and freshwater macrophytes (Westlake 1967, Wheeler 1980).

A typical current velocity profile (Fig. 2) shows a rapid drop in current velocity approaching the rock surface. The profile also shows an ear-shaped colony protruding farther into a region of higher flow than spherical colonies. The higher current velocities experienced by ear-shaped colonies attached to rocks may be related to the fact that the spherical colonies have higher rates of photosynthesis than ear-shaped colonies in quiescent water but equal rates when measured under flow while still attached to rocks.

Increased current velocity can increase diffusion. Diffusion of a required nutrient can limit algal productivity (Whitford 1960, Schumacher and Whitford 1965, Pasciak and Gavis 1974, Lehman 1978). Therefore, increased photosynthesis of *N. parmelioides* with increased current velocity may be related to higher rates of diffusion.

Increased supply of a limiting nutrient can cause an increase in photosynthesis. Inorganic carbon stimulated photosynthesis by 530% at the *N. parmelioides* surface (Table 2). This was probably not caused by bicarbonate buffering since HEPES buffer

TABLE 2. Effect of pH, CO_2 , and NH_4^+ on photosynthesis of ear-shaped *Nostoc* colonies in quiescent stream water. Photosynthesis was measured at the surface of the *Nostoc* colony with an oxygen microelectrode. *P* and *t* values represent pooled *t* tests between the control (unamended stream water) and all other treatments. One way ANOVA of the treatments showed significant variance among treatments ($F = 3.53$, $P < 0.05$).

Treatment	mmole $\text{O}_2 \cdot \text{min}^{-1}$	SD	n	df	<i>t</i>	<i>P</i>
Control (pH 7.81)	2.38	0.46	10			
+10 μM NH_4^+	1.92	0.41	10	18	-0.128	$P > 0.35$
+ NaH_2CO_3 (pH 8.07)	4.15	2.28	10	18	2.40	$0.025 > P > 0.01$
+ NaH_2CO_3 (pH 7.81)	15.1	3.68	9	17	6.61	$0.0005 > P$
pH 8.05	1.92	0.64	8	16	-1.781	$0.05 > P > 0.025$
0.001 M HEPES (pH 7.75)	3.37	1.18	10	18	2.477	$0.025 > P > 0.01$

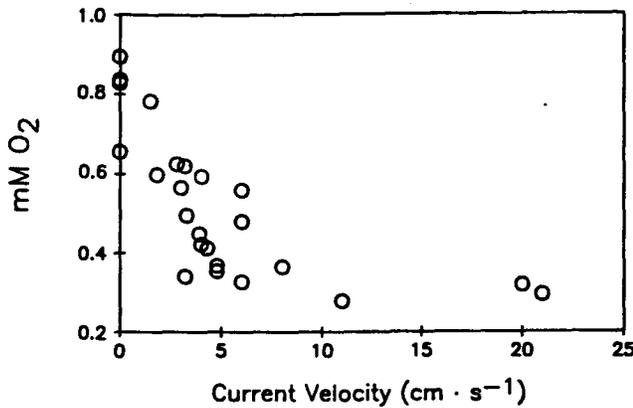


FIG. 3. Oxygen at the surface of five ear-shaped *N. parmelioides* colonies related to current velocity.

at ambient stream pH only increased photosynthesis by 30%. Bicarbonate increased photosynthesis 1.7 fold at pH 8.07 and 6.3 fold at pH 7.81. The latter was probably a result of higher CO_2 availability at the lower pH. Therefore, increased photosynthesis with higher current velocity may have been associated with increased inward diffusion of inorganic C. Wheeler (1980) described how increasing current velocities from stasis to $1 \text{ cm} \cdot \text{s}^{-1}$ could increase diffusion of inorganic C and photosynthesis rates in a marine macrophyte.

Increased export of O_2 from *N. parmelioides* colonies extending into regions of higher current velocity may also be related to increased photosynthesis rates measured at higher current velocities. High levels of oxygen can be toxic to photosynthetic algae (Raven and Beardall 1981). O_2 concentration at the surface of the ear-shaped colonies decreased with increasing current velocity (Fig. 3). At about $10 \text{ cm} \cdot \text{s}^{-1}$ and above, O_2 levels at the surface of the colony were at saturation ($0.27 \mu\text{M O}_2$). In addition, O_2 was inhibitory to photosynthesis. Ear-shaped *N. parmelioides* colonies which were bubbled with N_2 gas for 15 min to lower O_2 tension showed higher $^{14}\text{CO}_2$ uptake rates ($3.02 \pm 1.45 \times 10^6 \text{ DPM} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$, $n = 5$) than colonies that were not bubbled ($1.99 \pm 0.69 \times 10^6 \text{ DPM} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$, $n = 5$, $0.10 > P > 0.05$). Therefore, alleviation of O_2 toxicity also may have been a factor in increased photosynthesis with higher current velocities. O_2 inhibition did not appear to be as important as increased availability of inorganic C. It is possible that the combination of increased CO_2 availability and lower O_2 at higher current velocities resulted in lowered photorespiration and increased photosynthetic efficiency.

Photosynthesis rates (O_2 production) were higher and O_2 levels lower at the colony surface immediately above a midge larva than in areas uninhabited by a midge (Fig. 4). Brock (1960) suggested that the midge might cause a morphological change from spherical to ear-shaped colonies by selectively eating the interior of the colony. However, the measured localized stimulation of photosynthesis by the midge

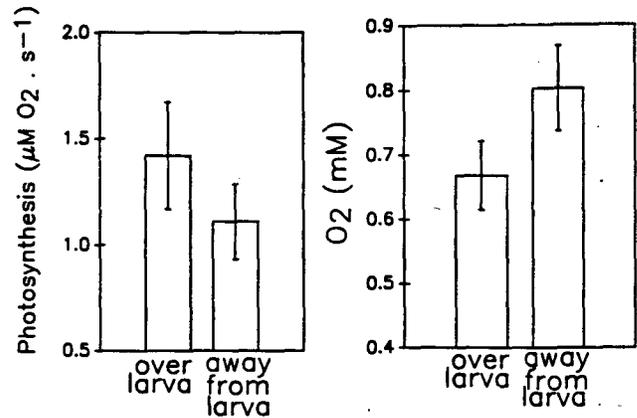


FIG. 4. O_2 concentration and photosynthesis rates at the surface of an ear-shaped *N. parmelioides* colony directly above or away from the midge larva as measured by an O_2 microelectrode. Current velocity = 0. Error bars represent 95% confidence interval for all measurements, $n = 8$. There was a significant difference ($P < 0.05$) between the areas immediately above the midge and the areas away from the midge in both O_2 concentration and photosynthesis.

larva may mean that areas inhabited by the larva grow more quickly, perhaps contributing to formation of the ear-shaped morphology of inhabited colonies.

Mean larval respiration was $0.06 \mu\text{mole O}_2 \cdot \text{L}^{-1} \cdot \text{h}^{-1} \cdot \text{midge}^{-1}$. This respiration rate should be viewed with caution since measurements were made on unfed larva which had been removed from their colony. Assuming a respiratory quotient of 1, and given the carbon uptake rates measured using $^{14}\text{CO}_2$, the midge larvae supplied about 5% of the C requirement of an average (12 mg dry wt) colony. The midge larva probably does not cause a large increase in whole colony photosynthesis by increasing the supply of CO_2 , but increased CO_2 supply may partially explain the localized stimulation of photosynthesis rates by the midge (Fig. 4). NH_4^+ addition had no effect on photosynthesis (Table 2). Therefore, it is unlikely that N remineralization by the larva accounts for higher rates of photosynthesis in regions inhabited by the larva.

Ward et al. (1985) reported distinctly higher rates of photosynthesis in colonies containing midge larvae (ear-shaped) than in spherical colonies. Data here show roughly equal rates of photosynthesis and nitrogen fixation of ear-shaped and spherical colonies experiencing current velocities of $5\text{--}10 \text{ cm} \cdot \text{s}^{-1}$. In addition, nitrogen fixation and photosynthesis appear to depend on current velocity (diffusion). The differences between my results and those reported by Ward et al. (1985) may be due to different flow regimes. However, since Ward et al. (1985) did not specify current velocities, this explanation is speculative.

A larva can alter micro-environmental conditions within a *N. parmelioides* colony. More importantly, the midge larva alters the morphology in such a way

that the current velocities surrounding the colony are higher, and diffusion of materials into and away from the colony may be more rapid. Increased diffusion can enhance photosynthesis. Therefore, the effect of the midge larva on *N. parmelioides* is probably a result of varied flow and diffusion effects on the ear-shaped morphology.

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