Effects of Sedimentation and Light Intensity on Mat-forming Oscillatoriales with Particular Reference to Microcoleus lyngbyaeus Gomont

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The distribution and abundance of mat-forming Oscillatoriales were investigated at a silt-depositing freshwater site in England over a period of 4 years. Maximum growth occurred during April and May and experiments showed that mat buried by fine sediment could regain the surface by gliding movements. The number of trichomes reaching the surface was light-dependent but chemotactic responses were also apparent. Gliding rates of 1-2 μm s⁻¹ were recorded and the trichomes moved through the sediment as a left-handed screw with a pitch of 60°. A surface illumination of 1 klx (4 W m⁻²) was optimal for mat formation. Light intensities exceeding 2-5 klx produced a photophobic response resulting in lateral trichome migration to regions of lower surface illumination when these were provided. Migration into the sediment was not observed in the surface intensity range 0–10 klx. The onset of light saturation measured by the ¹⁴CO₂ method was 3 klx for Microcoleus lyngbyaeus, close to the optimum for mat development. Experiments employing ¹⁴CO₂ in the presence of sulphide and absence of oxygen suggested that the organisms were adapted to an aerobic environment.

INTRODUCTION

Mat-forming cyanobacteria belonging to the family Oscillatoriales cover extensive areas of shallow water subject to sedimentation throughout the world. In some regions, usually where the water is saline or depositing carbonates, the mats have a considerable stabilizing effect and may bind the sediments sufficiently well to produce a durable substratum (Golubic, 1973; Walter, 1976; Pentecost, 1978) whilst in other sites there is little binding, the development of mats is seasonal and the sediment is shifting continuously. This last environment, although of worldwide occurrence, has received little attention and has not been investigated experimentally. An accreting benthos would appear to hold few advantages for a photoautotrophic organism since survival would depend upon an efficient means of overcoming the obstruction of light caused by the rain of sediment. The ability to perform net photosynthesis under low light regimes associated with turbid water would also be of advantage. Previous studies would suggest that members of the Oscillatoriales have the potential to overcome these disadvantages as many are motile and some planktonic forms are saturated for photosynthesis at low light levels (Zimmermann, 1969). However, data relating to motility in natural habitats are primarily qualitative (Castenholz, 1982) and there are no quantitative estimates of growth of these forms on sediment surfaces. This investigation attempts to describe the cyanobacteria associated with a temperate silt-depositing site and to determine their ability to withstand rapid sedimentation under differing light regimes.

METHODS

Sampling and characterization of cyanobacteria. Samples were collected from a small tributary of the River Medway near Tonbridge, Kent, UK (NGR 51/577465) using a modification of the method described by Round & Heaton (1966). The suspension of silt and cyanobacteria was allowed to settle for 5 h and the total trichome
length measured under a dissection microscope ($\times 50$) under a surface illumination of 2 klx. Trichome length was calculated by the grid method described by Olson (1950). Since the trichomes were initially Poisson distributed on the sediment surface, confidence limits could be calculated for the length estimates. The occurrence of sheath material and polyphosphate bodies was monitored using ruthenium red and Ebel's solution respectively (Fuhs, 1973). Rates of motility were recorded under the microscope at 20°C (illumination 500 lx) with a stopwatch. Trichomes were photographed using Ilford FP4 film and width measurements were made under oil immersion with a Leitz Ortholux microscope. Site temperature, illumination and water chemistry were also monitored (Mackereth et al., 1978).

**Sedimentation studies.** Silt samples from the site were well washed, sieved and autoclaved before use and a slurry was prepared by decanting the supernatant water after standing for 3 h. Mats were allowed to develop in Petri dishes with the sides covered in aluminium foil and a calculated volume of silt suspension was carefully poured over the mat to give depths ranging from 2.5 to 7.5 mm. The surfaces were illuminated in the range 0-1-20 klx and the temperature was maintained at 15°C. The number of trichomes appearing at the sediment surface was measured at regular intervals as described above.

**Incorporation of $^{14}$CO$_2$.** Trichomes of *Microcoleus lyngbyaceus* were harvested free of silt by collecting them from the sides of glass containers and brought into suspension by shaking with Chl 10 medium at pH 7-0 (Chu, 1942). Suspensions were incubated at 15°C under light intensities of 0-1-20 klx with 10 $\mu$Ci Na$^{14}$CO$_3$ of specific activity 3-3 mCi mmol$^{-1}$ (122 MBq mmol$^{-1}$). To study the effect of deoxygenation, two suspensions were prepared, one sparged with nitrogen and the other with air for 2 h prior to incubation. Suspensions were incubated at light saturation in sealed 20 ml glass bottles. Another set of deoxygenated samples was spiked with up to 0.5 mM-$\text{NazS}$ at pH 7.0 and here the Chu 1942). Rates of motility were recorded under the microscope at 20°C (illumination $<0.05$ klx) depending upon the water turbidity. The length of individual trichomes ranged from $<0.05$ to 2.4 mm but usually averaged 0.5-1 mm in both species. Trichome length appeared to be significantly greater early in the year (Fig. 3c, d) during the development of locally dense mats. The frequency distribution of trichome length in *M. lyngbyaceus* during May showed an approximately log-normal form (Fig. 3f) with large numbers of short hormogonia present. A significant correlation ($P<0.05$, Spearman's test) was found between trichome length frequency and the frequency of the distance between successive necridia in intact trichomes (Fig. 3e). This suggests that the sampling procedure caused insignificant damage through trichome breakage. The cells of both species possessed randomly distributed polyphosphate bodies throughout the year and firm, permanent sheaths such as those found in *Lyngbya* were never observed.

Water at the sampling site was 5-40 cm deep and was eutrophic (pH 6.8-7.5, total CO$_2$ 2.4-2.6 mm, Ca 3.5 mm, Mg 0.3 mm, total P 8-10 $\mu$M, NO$_3$ 0.1-0.25 mm, total sulphide $<0.2$ mm,
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Fig. 1. Frequency distributions of trichome widths for samples collected in May 1980–83.

Fig. 2. (a) Trichomes of *M. lyngbyaceus* showing discoid cells, necridia and rounded apices. (b) Trichomes of *O. brevis* (left) with rostrate apices, and two trichomes of *M. lyngbyaceus* (right). Bars 25 μm.
Fig. 3. (a, b) Mean trichome biomass, expressed as trichome length (cm) per cm² of sediment for *O. brevis* (a) and *M. lyngbyaceus* (b). (c, d) Mean trichome length for *O. brevis* (c) and *M. lyngbyaceus* (d) during 1981. (e) Frequency distribution of the distances between adjacent necridia in long trichomes of *M. lyngbyaceus*. (f) Frequency distribution of trichome length in the same sample.

Oxygen-saturated. The sediment consisted of fine silt of mean diameter 13.5 μm and range <0.5–75 μm with about 4% organic matter. *Eh* and pH of the sediment surface ranged from +190 to +430 mV and from 6.8 to 7.5 respectively. There was a black anoxic zone at 2–3 cm depth and the light extinction coefficient for the sediment was 18 mm⁻¹.

**Response of *M. lyngbyaceus* to sedimentation**

Burial beneath 2.5 mm sediment resulted in almost complete recovery of mats within 3 h with a surface light intensity of 2 klx (Fig. 4a). Burial under 5 mm gave a similar initial rate of recovery but this was not sustained. Burial beneath 7.5 mm sediment resulted in the emergence of only 30% of the mat after 5 h (Fig. 4a). The recovery rate in the light was similar to that in the dark under 5 mm sediment. Comparable results were obtained with *O. brevis*.

Trichome emergence was influenced by the light intensity with the greatest recovery occurring at 1 klx when mats were buried beneath 3 mm sediment (Fig. 4b). The same result was obtained when recovery was recorded after 8 h burial using a greater range of surface light intensities (Fig. 4c). In both experiments, recovery in the dark was significant but always lower than that obtained with the optimum surface illumination.
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Fig. 4. (a) Recovery of M. lyngbyaceus after burial beneath sediment of the following depths: 2.5 mm (○); 5 mm (○); 7.5 mm (□). Dark control, 5 mm. Trichome length per unit area was measured at various times after burial, and expressed as a percentage of the value before burial. (b) Recovery after burial beneath 3 mm sediment as a function of surface light intensity: 1 klx (○); 5 klx (■); 10 klx (□); dark (■). (c) Recovery after 8 h burial beneath 3 mm sediment as a function of light intensity (log scale). (d) Percentage of trichomes remaining on the illuminated half of a mat after 24 h. The other half was shaded by foil. Total trichomes on entire mat = 100%. (e) Recovery of mats buried beneath 3 mm sediment with illumination at 1 klx. Light intensity was increased to 10 klx (arrow, △) after 4 h. (f) Relative photosynthetic 14CO2 uptake as a function of light intensity. The vertical bars in (a), (b), (c) (e) and (f) represent 95% confidence limits.

Two further experiments were conducted to test for trichome migration away from the surface. When part of a mat was shaded by foil, trichomes were only found to migrate into light of intensity 1 klx. At intensities of 0.5, 2.5 and 5 klx there was little or no tendency to migrate in either direction but at 10 klx, trichome migration away from the light was significant (Fig. 4d). This migration was lateral: no vertical migration was detected. When a mat was illuminated initially at 1 klx and the illumination then increased to 10 klx, vertical migration of the trichomes was again found to be insignificant over a period of 4 h (Fig. 4e).

The mean gliding rates of M. lyngbyaceus and O. brevis were 2.1 (range 1.95–2.2) and 1.33 (range 1.08–1.58) μm s⁻¹ respectively at 20°C and 0.5 klx. Both species moved through the sediment in the sense of a left-handed screw with a pitch of 59–63°. A significant negative
correlation \((r = -0.78, P < 0.05)\) was found between trichome width and helix pitch in \(O. \text{ brevis}\) but there was no correlation between the gliding rate and trichome length.

The rate of mat recovery was found to be partially dependent upon the nature of the sediment, since mats covered with 3 mm clean, washed sand of 360 \(\mu\)m average diameter failed to recover after 6 h although the trichomes remained motile.

**Uptake of \(^{14}\)CO\(_2\)**

The relationship between \(^{14}\)CO\(_2\) uptake and light intensity is shown in Fig. 4(f). The onset of light saturation occurred at 3 klx and above 5 klx a decline in uptake was evident. When trichomes were incubated with sulphide adjusted to pH 7, uptake showed a continuous decline as sulphide concentrations increased from 0.02 to 0.5 mM and was almost entirely inhibited at 0.5 mM when compared with a deoxygenated control. When aerated and deoxygenated uptake rates were compared, the former were found to be significantly higher than the latter \((P = 0.05)\).

**DISCUSSION**

The sedimentation rates calculated for the site investigated, and the depth of sediment likely to cover the trichomes, were less than the maximum employed in the experiments. This suggests that mats are capable of re-forming at the surface even in the absence of light. This would be important to the organisms since light intensities at the sediment surface were extremely low when sedimentation was in process.

The inhibition of \(^{14}\)CO\(_2\) uptake under anoxic conditions and in the presence of sulphide suggests that gradients of oxygen and sulphide within the sediment might be the stimulus causing the trichomes to migrate in the dark. The occurrence of chemotactic behaviour is also supported by the observation that trichomes moved away from the sediment surface in the dark when an oxygen gradient was present. Castenholz (1982) noted a chemotactic response to sulphide by *Oscillatoria terebriformis*, but there are problems in identifying the response since high sulphide levels are usually associated with an absence of oxygen and elevated levels of ferrous iron and carbon dioxide, any one of which might be the causative factor. The rate of recovery of trichomes in the dark might be expected to fall as the depth of sediment above is increased. This is apparent from the results (Figs. 4a, b) but the effect is not so great as might be expected. The cause of this is unclear but may be due to differences in concentration of dissolved substances in different batches of sediment or variation in the respiratory activity of the buried trichomes, which must affect the immediate environment.

With an average gliding rate of 2.1 \(\mu\)m s\(^{-1}\), *M. lyngbyaceus* should be capable of surfacing within 2 h when buried beneath 7.5 mm of silt, but the rate of recovery was much lower than this. One possible explanation is a reduction in motility caused by the increased sediment compaction with depth, but it is more likely to be a result of the longer path length the trichomes took to reach the surface. The trichomes were unable to orientate themselves perpendicular to the surface, and arrived at low angles, usually less than 10°, so that the distance travelled was much greater. This also helps to explain the incomplete recovery of deeply buried mats. The trichomes were found to move along straight paths through the sediment since the particles were small and easily pushed aside. The inability of trichomes to move through sand appears to result from the fact that movement in straight lines was excluded and the efficient screwing-motion made ineffective.

Light-induced trichome motility has been investigated in detail in the laboratory. Optimum photokinesis in *Phormidium ambiguum* occurred at 1 klx (Nultsch & Hellman, 1972) and at 0.2 klx in *P. autumnale* (Nultsch, 1962). The cyanobacteria so far investigated exhibit positive phototaxis in the range 0.05–10 klx (Castenholz, 1982) so it would seem that the group as a whole, with the *Oscillatoriaceae* in particular, are adapted well to low light regimes. Light intensity is clearly the main factor controlling mat formation in *M. lyngbyaceus* and the response of trichomes to different light regimes is of considerable interest. It would appear from these experiments that trichomes, once at the sediment surface, prefer to glide laterally rather than back into the sediment when the light regime becomes unsuitable. This behaviour may be due to
the presence of substances dissolved in the interstitial water which produce a negative chemotactic response, but this conflicts with the results where the trichomes remained buried yet apparently viable and motile. The trichomes are apparently incapable of migrating into the sediment and can only alter their light regime by lateral migration, by moving over each other and causing self-shading or by awaiting further sedimentation. Migration back into the sediment is unlikely to provide a particularly suitable light climate for the trichomes unless fine adjustments of position could be made. For example, with a surface illumination of 15 klx, the trichomes would need to be positioned beneath 0.11–0.18 mm of sediment to achieve the optimum light intensity of around 0.5–2 klx. Self-shading would occur when the trichomes clump together to form dense mats although the cells at the surface would not be protected. This clumping behaviour occurs in most motile Oscillatoria species but it was insignificant in the experiments performed here.

Regular migrations into sediments by O. terebriformis have been reported by Castenholz (1982). The causes were unknown although they did not appear to be entirely light-dependent. The only possible means by which the species investigated here could undergo vertical migration would depend upon the deposition of an extremely fine and loose sediment. Such sediments do occur in the Medway system but the development of mats upon these has not been investigated. Clearly, the nature of the sediment is important to mat formation and will determine which kinds of organisms will be present. The helical path described by the trichome surfaces results in a screwing effect which would facilitate movement around small obstacles but the negative correlation between helix pitch and trichome width has no clear explanation.

The Oscillatoriaceae have been classified into a confusing number of forms (Geitler, 1932; Desikachary, 1968) although several attempts to rectify this situation have been made (Drouet, 1968; Rippka et al., 1979; Polderman, 1980). However, there is still no satisfactory division of the family for ecological work, which necessitates a compromise between the classical system of Geitler and the system proposed by Rippka et al. (1979) for the strains in pure culture. The value of filament diameter in the classification of algae has been questioned by Miller & Hoshaw (1974) but the results obtained here and those of Golubic & Focke (1979) suggest that these measurements have considerable use in distinguishing between populations of filamentous cyanobacteria.

The regular seasonal development of M. lyngbyaceus, which occurred throughout the Medway system, cannot be attributed to any single factor but was probably due to a combination of the following: increased competition from eukaryotic algae, especially Nitzschia sigma and Spirogyra nitida; increasing grazing pressure from gastropods during summer, and decreased sediment disturbance during summer caused by lower rainfall and greater soil cover. Nutrient limitation was ruled out since the water was continuously eutrophic. The flotation and downstream loss of large areas of dense mat also occurred and this must reduce the likelihood of any long-term establishment of mats in these sites.

REFERENCES


