APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1984, p. 234–238
0099-2240/84/020234-05$02.00/0

Vol. 47, No. 2

Nodularia (*Cyanobacteriaceae*) Akinetes in the Sediments of the Peel-Harvey Estuary, Western Australia: Potential Inoculum Source for *Nodularia* Blooms

ANN L. HUBER

Department of Soil Science and Plant Nutrition, University of Western Australia, Nedlands, Western Australia 6009

Received 21 July 1983/Accepted 17 October 1983

The populations of viable *Nodularia* (*Cyanobacteriaceae*) propagules in the sediments of the Peel-Harvey Estuary in Western Australia were assessed over location, time, and depth. The sediments of the Harvey Estuary had greater numbers of *Nodularia* propagules than those of the contiguous Peel Inlet. This was consistent with the distribution of *Nodularia* blooms in the system. The sediment populations of *Nodularia* propagules has increased up to 100-fold at the study sites over a 4-year period during which three blooms have occurred. It is considered that the majority of the propagules are akinetes. The significance of the sediment akinetes in providing the inoculum for rapid onset of *Nodularia* blooms is discussed. The population of akinetes decreased with depth, but viable akinetes were still found at 35 cm, the maximum depth sampled. Bioturbation by polychaete worms is likely to be significant in the distribution of akinetes to these depths.

The Peel-Harvey estuarine system in Western Australia, a shallow estuary with a mean depth of 1 m, is subject to massive planktonic blooms of the cyanobacterium *Nodularia spumigena*. Dense blooms have occurred four times since a study of the estuary began in 1976. A bloom was also reported in 1974. High phosphorus inputs into the estuary, together with the appropriate environmental conditions, have been considered the main causes of these blooms. *N. spumigena* does occur in small numbers in the water column throughout the year. The yearly mean population over the entire estuary, between the bloom periods, is 9.7 CFU/100 ml of water (4). The sediments are likely to play a significant role in the maintenance of this low-density population and in providing an inoculum for the rapid onset of the *Nodularia* blooms. During a bloom, *N. spumigena* forms akinetes which are more resistant to decomposition processes than vegetative cells (1). The akinetes are negatively buoyant, sediment out of the water column with senescing vegetative filaments, become incorporated into the sediment, and have the potential to germinate to form new filaments given the appropriate conditions.

There have been a number of reports of bloom-forming cyanobacteria occurring in sediments between blooms (cf. references 2, 12, 13, 15, and 16). Most have dealt with lake systems. Kappers (6) enumerated the sediment cyanobacterial population in Lake Brielle, but the maximum depth examined was 2 cm. Livingstone and Jaworski (9) showed the presence of akinetes of *Anabaena* sp. to a depth of 60 cm in Rostherne Mere and established the viability of at least a proportion of the akinetes present to a depth of 40 cm.

In the present study, the population of *Nodularia* propagules was enumerated over location, time, and depth. Viable propagules were found to a depth of 35 cm in estuarine sediments. Germinating akinetes from the sediments have been observed, thus confirming their viability and establishing akinetes as a significant fraction of the viable propagules enumerated.

MATERIALS AND METHODS

Nodularia populations in sediments were assessed six times from 1979 to 1982. The sites sampled and the type of sample examined (i.e., water, bulked surface sediment, or sectioned cores) are given in Table 1. The locations of the sampling sites are shown in Fig. 1. A 45-mm inner diameter acrylic corer was used to obtain intact sediment cores. All samples were assessed for viable populations of heterocystous cyanobacteria within 3 days of collection. The material was refrigerated (4°C) until analysis could be done. Storage of up to several months at 4°C did not affect the viability of the *Nodularia* akinetes (unpublished data).

Surface sediments. The overall distribution of viable *Nodularia* propagules in the sediments was determined by using bulked surface samples. These consisted of three cores (4-cm diameter, 5 cm long) which were combined and homogenized. A 10-ml subsample was added to 90 ml of either liquid BG11 medium (Table 2), 5‰ NaCl, or artificial sea salt (Gerrard's Sea Salt) dilution blanks. These were shaken vigorously, and a 1-ml sample was removed and added to 9 ml of the same diluent. The diluting medium did not affect the results. From 0.5 to 5 ml of these final dilutions was filtered through 0.45-μm gridded Millipore filters. The filters were placed onto plates of solidified (1.0% agar) medium made up of filter-sterilized estuary water and supplemented with BG11 nutrients at half strength. The plates were incubated at 22°C under 8 to 13 microeinsteins per m^2/s of light (Grolux). The heterocystous cyanobacterial colonies were enumerated after 4 weeks of incubation. Recovery of *Nodularia* propagules in BG11 medium has been extensively examined and found to be satisfactory. All counts were done in either duplicate or triplicate. Results are reported in terms of viable propagules per milliliter of sediment.

Depth profiles. Sediment samples were obtained by taking a 1-cm thickness of sediment at 5-cm intervals from the core and trimming away all edges to avoid any contamination from the higher layers. Subsamples were diluted and treated as above.

RESULTS

Overall distribution. The numbers of viable *Nodularia* propagules in the top 5 cm of sediment determined from the grid study (17 stations) in March 1981 are shown in Fig. 2. There are two points which should be noted. First, there

TABLE 1. Sampling program

Sampling date	Locations[a]	Sampling procedure
27 July 79	1, 4–8, P46	Water column (surface and bottom), overlying sediment water, bulked surface sediments (5 cm)
11 August 80	1, 4, 7, 31	Water column (surface and bottom), sectioned cores (6 cm)
30 October 80	1, 7, 31	Water column (surface and bottom), sectioned cores (15 cm)
10 April 81	1, 2, 4, 7, 8, 10, 21, 23, 24, 26–32, 36	Bulked surface sediments (5 cm)
6 October 81	1, 4, 7, 31	Water column (surface and bottom), sectioned cores (to 25 cm)
21 April 82	1, 4, 7, 31	Sectioned cores (to 40 cm)

[a] Refer to Fig. 1.

TABLE 2. BG11 medium[a]

Component	Final amt in medium
NaCl	5.0 g/liter
K_2HPO_4	0.039 g/liter
$MgSO_4 \cdot 7H_2O$	0.075 g/liter
$CaCl_2 \cdot 2H_2O$	0.0268 g/liter
$NaHCO_3$	0.02 g/liter
$FeCl_2$ solution[b]	1 ml
Micronutrient mix[c]	1 ml
TAPS buffer[d]	0.05 M[e]

[a] Modified from Hughes et al. (5).
[b] $FeCl_2$ solution, 0.365 g of $FeCl_2$–0.6 g of sodium EDTA in 100 ml of distilled water.
[c] Micronutrient mixture (mg/100 ml): $CoCl_2 \cdot 6H_2O$, 0.08; $MnCl_2 \cdot 4H_2O$, 0.5768; $NaMoO_4 \cdot 2H_2O$, 0.1261; H_3BO_3, 0.05725.
[d] Sigma Chemical Co.
[e] pH adjusted to 8.6 with NaOH.

were high concentrations of viable *Nodularia* propagules in the sediments. The numbers ranged from 1.4×10^2 to 9.5×10^3 propagules per ml of sediment (stations 36 and 31, respectively) on this sampling date. This is equivalent to 7.0×10^6 to 4.8×10^8 viable propagules (CFU) per m^2 of sediment. Second, the Harvey Estuary has higher sediment concentrations of *Nodularia* propagules than does the Peel Inlet. The average concentrations of viable propagules per ml of the Harvey Estuary and Peel Inlet sediments were 3.6×10^5 and 1.0×10^5, respectively. This distribution is consistent with the pattern of *Nodularia* blooms, which usually begin in the Harvey Estuary and later extend into the Peel Inlet and finally enter the ocean by tidal exchange.

Population increase over time. From the first survey in July 1979 to the final one in April 1982, the *Nodularia* population in the sediments has increased about 100-fold in the Harvey Estuary and 20-fold in the Peel Inlet. The mean populations in the top 5 cm of Harvey Estuary and Peel Inlet sediments determined during this study are plotted in Fig. 3. The periods of *Nodularia* blooms are also indicated in Fig. 3. The first bloom in the study period (1977 to current) occurred in 1978. The next began in October 1980. There was no increase in viable propagules from 1979 to September 1980, but a very sharp increase occurred between 11 September 1980 and 30 October 1980, at the beginning of the bloom. It is likely that this increase can be explained in terms of an increase in the number of vegetative propagules from already germinated akinetes or an increase in the number of rapidly germinable akinetes or both. After the 1980–1981 bloom, there was again a large increase in *Nodularia* populations in both the Peel Inlet and Harvey Estuary sediments. No further increase in *Nodularia* populations occurred in the Peel Inlet sediments after the 1981–1982 bloom. However, the population in the Harvey Estuary sediments continued to increase.

Depth profiles. The sediments in the estuary usually consist of black, reduced surface layers with high water and organic matter contents and lower layers of varying clay and

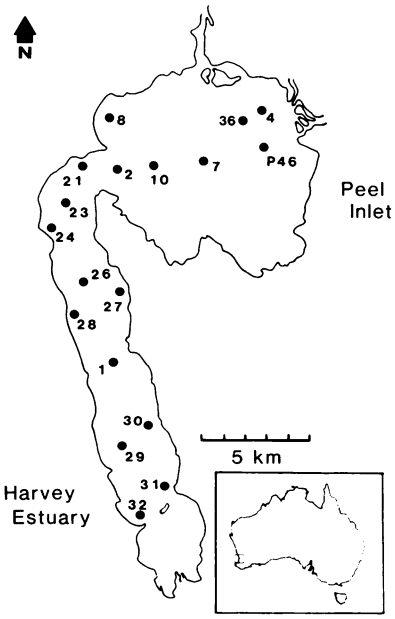

FIG. 1. Sampling stations.

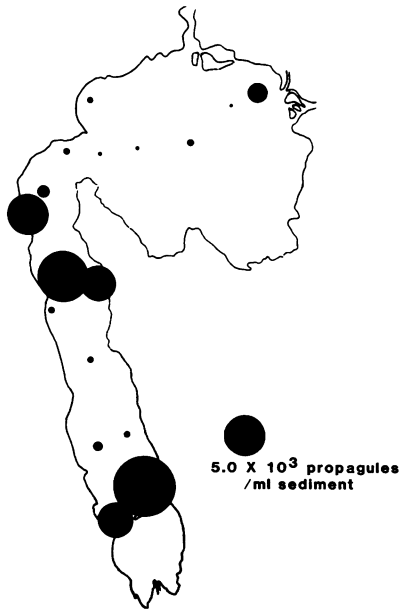

FIG. 2. Distribution of viable *Nodularia* propagules in the top 5 cm of sediments in the Peel-Harvey Estuary, March 1981.

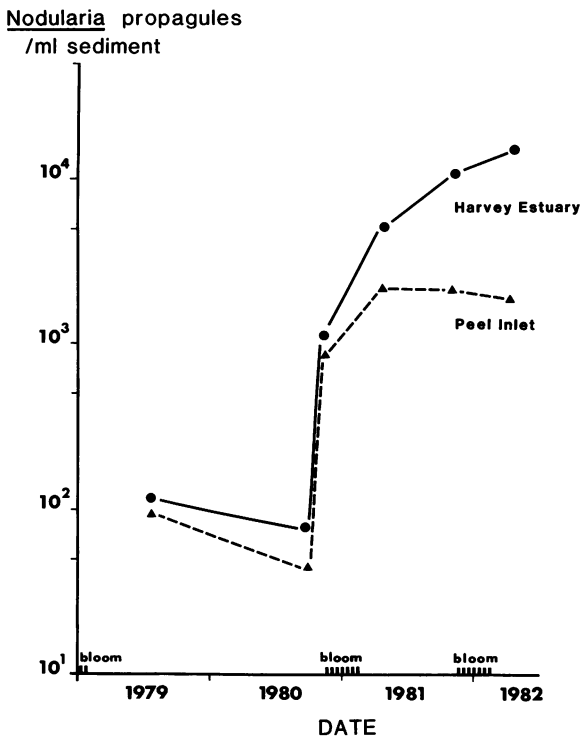

FIG. 3. Change in mean sediment populations (top 5 cm) from July 1979 to March 1982. Note that the means are calculated on the basis of those sites surveyed at each sampling time (see Table 1).

sand content. A typical profile (station 1) is presented in Fig. 4. The transitions between zones are gradual and vary with location. Peel Inlet sediments tend to have lower organic matter contents, especially in the surface layers.

The depth profiles of the population of viable *Nodularia* propagules for stations 1 and 4 in April 1982 (postbloom) are shown in Fig. 5. The lengths of stations 1 and 4 cores were 35 and 30 cm, respectively. The number of viable propagules per ml of sediment decreased with depth. Surface counts (top 1 cm) were very high, 1.1×10^4 and 2.4×10^3 viable propagules per ml of sediment at stations 1 and 4. The numbers declined more rapidly with depth in the station 4 sediment. The pattern of decline was not uniform throughout the profile. In the lower sediments a slight increase was sometimes observed. Overall, the station 4 populations were lower. *Nodularia* propagules were present in station 4 and station 1 sediments to the maximum depths sampled.

DISCUSSION

The literature concerning akinetes has been reviewed recently by Nichols and Adams (11).

Many factors have been implicated in the formation of akinetes, but there appears to be much variation between and even within species. In general, for natural water bodies at least, akinete formation appears to be the result of physiological imbalances (especially changes in cellular carbon to nitrogen ratios) brought about by physical or nutritional changes or both (16, 17).

Lang (8) summarized the literature on the metabolic activities of akinetes of several species of cyanobacteria. These cells have significant respiration rates, lowered photosynthetic activities, and protein synthesis rates similar to those of vegetative cells. Akinetes can have an accumulation of RNA and cyanophycin and a DNA content equal to, or 5 to 30 times greater than, that of vegetative cells. A high DNA content in *Cylindrospermum* akinetes (about 30 times that in vegetative cells) was also shown by Ueda and Sawada (19).

Akinetes have been shown to remain viable for 5 years or more in the dark in a desiccated state. This is to be compared with 15 days for vegetative cells (22). However, the lag time for germination increased with storage time.

Akinete germination appears to be stimulated by (but not necessarily dependent on) high phosphorus concentrations (21), warm temperatures in the range of 20 to 27°C, light, pH 7 to 8, the presence of oxygen, and sodium acetate (23). Lang (8) stated that germination of *Anabaena* akinetes required light, whereas *Nostoc* akinete germination was based on a fixed temporal sequence and not affected by environmental conditions.

RNA synthesis was found to be an initial step in akinete germination, using stored cyanophycin as a nitrogen source (8). Decreases in stored nitrogen and glycogen have also been shown by Wildman et al. (20), along with an increase in lipid bodies and gas vesicles.

From the above reports, the simplest sequence of events involving akinetes of bloom-forming cyanobacteria in sediments would appear to be as follows. Akinetes form when nutrient or physical conditions or both in the water cause physiological imbalances in the vegetative cells. The akinetes then sediment with the moribund vegetative cells but resist decomposition. There are stores of nucleic acids and nitrogen (in the form of cyanophycin) in the akinetes, and an active metabolism is maintained. Under the appropriate physical and nutritional conditions germination occurs, probably initiated by RNA synthesis, using stored nutrients. The sediments, therefore, can potentially act as a source of resistant but metabolically active cells for bloom initiation.

The above scenario is consistent with the pattern of *Nodularia* blooms which occur in the Peel-Harvey Estuary in the current study. It is significant that the sediment populations of viable *Nodularia* propagules have increased up to 100-fold over the course of the three bloom years. Reynolds (13) reported mass germination of spores of *Anabaena* sp. which had overwintered in the sediments of Crose Mere, but this was not always consistent (14). Wildman et al. (20) found akinetes of *Aphanizomenon* sp. in sediment in winter but noted that germination did not take place until

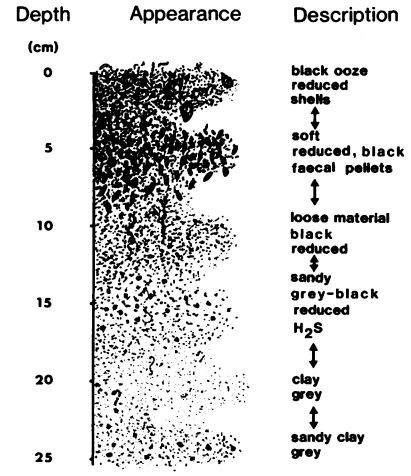

FIG. 4. Physical description of sediment, station 1, 6 October 1981.

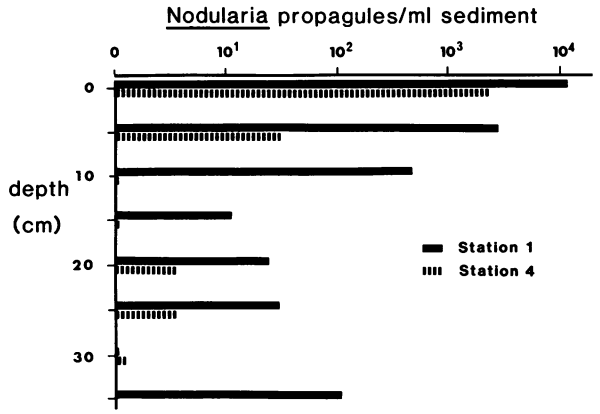

FIG. 5. Distributions of viable *Nodularia* propagules in the top 35 cm of stations 1 and 4 sediments, 21 April 1982.

spring. Conversely, Rother and Fay (16) indicated that germination took place immediately after maturation, and the resultant vegetative filaments formed the overwintering population. In the present study no large population of *Nodularia* filaments was observed in the surface sediments over the winter period. The populations of *Aphanizomenon* sp., *Microcystis* sp., and *Oscillatoria* sp. in the top 2 cm of sediments of Lake Brielle were assessed by Kappers (6). Of these, only *Aphanizomenon* sp. forms akinetes. Over the course of the present study, the concentrations of *Nodularia* propagules in the top 5 cm of sediment in the Peel-Harvey system varied from being similar to the concentrations of cyanobacteria found by Kappers to 100-fold greater.

In the current study, viable *Nodularia* propagules were found in the estuarine sediments to a depth of 35 cm. This was the maximum depth examined. The only reports found dealing with the distribution of viable algae in deep sediments are those of Stockner and Lund (18), who found viable cells of the diatom, *Melosira* sp., to depths of up to 35 cm in sediments of three English Lake District lakes, and Livingstone and Jaworski (9), who established the presence of *Anabaena* akinetes to 60 cm and their viability to 40 cm.

The overall rate of sediment deposition in the Peel-Harvey Estuary has been estimated to be 0.3 mm/year (3). Undisturbed sediments at a 35-cm depth therefore would be over 1,000 years old. It is clearly unrealistic to expect the *Nodularia* propagules found in these sediments to be the same age. Therefore, other mechanisms must occur which result in the presence of viable cells to depths of 35 cm or more. The most obvious of these is bioturbation. Krezoski et al. (7) found high populations of macroinvertebrates in the top 10 cm of lake sediments. They calculated that the sediment homogenization capacity of the worms could exceed the sedimentation rate by four times. McCaffrey et al. (10) found that bioturbation was sufficient to completely mix the top 20 cm of sediment. The report of Stockner and Lund (18) contains a thorough discussion of bioturbation in relation to movement of diatoms through sediments. However, they concluded that bioturbation alone could not explain the depth to which live cells were found, and they estimated the age of the cells to be up to 262 years. The sediments used by Livingstone and Jaworski (9) were permanently deoxygenated and supported no benthic fauna. They estimated the ages of *Aphanizomenon* and *Anabaena* akinetes to be 18 and 64 years, respectively.

In the Peel-Harvey Estuary, polychaete worms (*Boccardia, Ceratoneries*, and *Capitella*) have been found at all depths of the sediment cores examined in this study (A. Huber and P. Dyke, unpublished results). In fact, Treloar (M.Sc. thesis, University of Western Australia, Nedlands, 1978) observed polychaete worms to a depth of 1 m in the Peel Inlet sediments. Their burrows average 2 mm in diameter and are filled with fecal debris. Furthermore, germination of *Nodularia* akinetes from fecal pellets separated from the sediments has been observed (Fig. 6). This would strongly implicate macroinvertebrate-mediated movement of *Nodularia* akinetes through the sediments of the Peel-Harvey estuarine sediments. Although this question needs to be further studied, it is clear that a very large potential inoculum of *Nodularia spumigena* is present in the estuarine sediments, particularly in the surface layers.

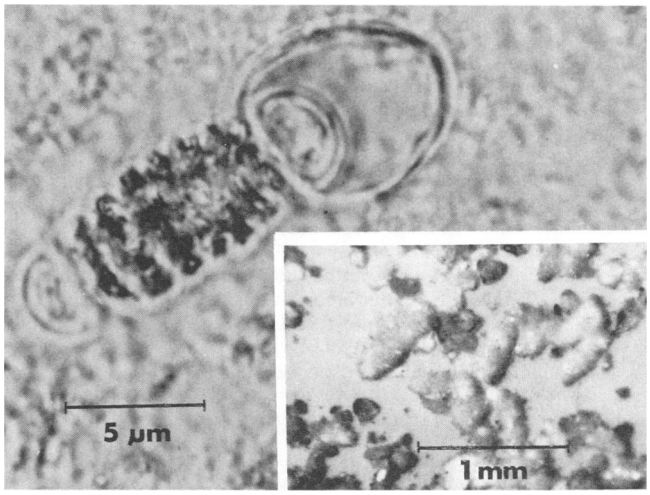

FIG. 6. Germinated *Nodularia* filament from sediment fecal pellet.

ACKNOWLEDGMENTS

This research was supported by a grant from the Department of Conservation and Environment and a University of Western Australia studentship to A.H.

The help of D. K. Kidby, J. O. Gabrielson, R. Lukatelich, and T. Armitage is gratefully acknowledged.

LITERATURE CITED

1. **Fay, P.** 1969. Metabolic activities of isolated spores of *Anabaena cylindrica*. J. Exp. Bot. **20**:100–109.
2. **Ganf, G. G.** 1974. Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda). J. Ecol. **62**:611–630.
3. **Hodgkin, E. P., P. B. Birch, R. E. Black, and R. B. Humphries.** 1980. The Peel-Harvey estuarine system study. Report no. 9, p. 22. Department of Conservation and Environment, Western Australia. 1981.
4. **Huber, A. L.** 1980. Cyanobacteria and nitrogen fixation in the Peel-Harvey estuarine system. Bulletin no. 94. Department of Conservation and Environment, Western Australia.
5. **Hughes, E. O., P. R. Gorham, and A. Zehnder.** 1958. Toxicity of a unialgal culture of *Microcystis aeruginosa*. Can. J. Microbiol. **4**:225–236.
6. **Kappers, F. I.** 1976. Presence of blue green algae in sediments of Lake Brielle, p. 382–386. *In* H. L. Golterman (ed.), Interactions between sediments and freshwater. Dr. W. Junk B. V. Publishers, The Hague, The Netherlands.
7. **Krezoski, J. R., S. C. Mozley, and J. A. Robbins.** 1978. Influence of benthic macroinvertebrates on mixing of profundal sediments in southeastern Lake Huron. Limnol. Oceanogr. **23**:1011–1016.

8. **Lang, N. J.** 1980. Akinetes: resistant spores of advanced cyanobacteria. J. Phycol. **16**(Suppl.):24.
9. **Livingstone, D., and G. M. H. Jaworski.** 1980. The viability of akinetes of blue-green algae recovered from the sediments of Rostherne Mere. Br. Phycol. J. **15**:357–364.
10. **McCaffrey, R. J., A. C. Myers, E. Davey, G. Morrison, M. Bender, N. Luedtke, D. Cullen, P. Froelich, and G. Klinkhammer.** 1980. The relation between pore water chemistry and benthic fluxes of nutrients and manganese in Narragansett Bay, Rhode Island. Limnol. Oceanogr. **25**:31–44.
11. **Nichols, J. M., and D. G. Adams.** 1982. Akinetes, p. 387–412. *In* N. G. Carr and B. A. Whitton (ed.), The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, England.
12. **Preston, T., W. D. P. Stewart, and C. S. Reynolds.** 1980. Bloom-forming cyanobacterium *Microcystis aeruginosa* overwinters on sediment surface. Nature (London) **288**:365–367.
13. **Reynolds, C. S.** 1972. Growth, gas-vacuolation and buoyancy in a natural population of a blue-green alga. Freshwater Biol. **2**:87–106.
14. **Reynolds, C. S.** 1975. Interrelations of photosynthetic behavior and buoyancy regulation in a natural population of a blue-green alga. Freshwater Biol. **5**:323–338.
15. **Roelfs, T. D., and R. T. Oglesby.** 1970. Ecological observations on the planktonic cyanophyte *Gleotrichia echinulata*. Limnol. Oceanogr. **15**:224–229.
16. **Rother, J. A., and P. Fay.** 1977. Sporulation and development of planktonic blue-green algae in two Salopian meres. Proc. R. Soc. Lond. Ser. B. **196**:317–332.
17. **Rother, J. A., and P. Fay.** 1979. Some physiological-biological characteristics of planktonic blue-green algae during formation in three Salopian meres. Freshwater Biol. **9**:369–379.
18. **Stockner, J. C., and J. W. G. Lund.** 1970. Live algae in postglacial lake deposits. Limnol. Oceanogr. **15**:41–58.
19. **Ueda, K., and M. Sawada.** 1972. DNA-content of akinete-cells in *Cylindrospermum*. Cytologia **37**:519–523.
20. **Wildman, R. B., J. H. Loescher, and C. L. Winger.** 1975. Development and germination of akinetes of *Aphanizomenon flos-aquae*. J. Phycol. **11**:96–104.
21. **Wolk, C. P.** 1965. Control of sporulation in a blue green alga. Dev. Biol. **12**:15–35.
22. **Yamamoto, Y.** 1975. Effect of desiccation on the germination of akinetes of *Anabaena cylindrica*. Plant Cell. Physiol. **16**:749–752.
23. **Yamamoto, Y.** 1976. Effect of some physical and chemical factors on the germination of *Anabaena cylindrica*. J. Gen. Appl. Microbiol. **22**:311–323.