KW 75

NATIONAL INSTITUTE FOR WATER RESEARCH

AND

INSTITUTE OF WATER POLLUTION CONTROL
(SOUTHERN AFRICAN BRANCH)

OBSERVATIONS ON THE ECOLOGY GROWTH AND PHYSIOLOGY OF *MICROCYSTIS AERUGINOSA* IN THE LABORATORY AND IN THE FIELD

by

W E Scott
National Institute for Water Research
Council for Scientific and Industrial Research
P O Box 395, Pretoria 0001

Paper to be presented at Symposium on
"HEALTH ASPECTS OF WATER SUPPLIES"
CSIR Conference Centre, Pretoria
15 November 1979

OBSERVATIONS ON THE ECOLOGY, GROWTH AND PHYSIOLOGY OF *MICROCYSTIS AERUGINOSA* IN THE LABORATORY AND IN THE FIELD

W.E. Scott
National institute for Water Research,
P.O. Box 395, PRETORIA, 0001

ABSTRACT

As a result of potential toxin production the blue-green alga *Microcystis aeruginosa* is a serious problem in eutrophic impoundments in South Africa. This study reports on growth characteristics of *M. aeruginosa* in the laboratory and is supplemented with field observations. It was found that *M. aeruginosa* can grow at a fast rate over a range of light intensities, giving it a competitive advantage over other algae. The alga adapts physiologically to different light intensities by changing its gas vacuole content and pigment composition. At high light intensities there is an increase in gas vacuole and a decrease in chlorophyll content, while the reverse is true at low light intensities. The pigment and gas vacuole changes enable *M. aeruginosa* to grow fast under a variety of conditions and partly explain the success of this alga in nature.

INTRODUCTION

Toxic blooms of blue-green algae, especially strains of *Microcystis aeruginosa*, are a potential health hazard. It is therefore important to have a better understanding of factors that will promote growth and toxin production by these algae. Schwimmer and Schwimmer (1964, 1968) have documented the occurrence of algal toxicity throughout the world. From the numerous records it appears that the degree of toxicity in waterblooms is extremely variable. Our observations on a number of dams have tended to confirm this variability in toxicity. The occurrence of toxicity was sporadic in regular samples collected at Hartbeespoort, Rietvlei and Roodeplaat dams over a period of a year. In these three dams toxicity was associated with the presence of *M. aeruginosa* forma *aeruginosa*. Blooms showing a predominance of *M. aeruginosa* forma *flos-aquae*, *M. wesenbergii*, *Anabaena circinalis* or *A. flos-aquae* were generally not toxic (Scott and Hauman, unpublished).

As a result of the sporadic occurrence of toxicity an assumption can be made that toxicity is genetically controlled and that the trigger for toxin production could possibly be the presence of a virus (Hauman, Barlow and Scott, 1979) or a plasmid which may operate under certain specific environmental conditions. Preliminary experiments to test these assumptions are still in progress and the results are not yet ready to be reported. This lecture will rather focus on the results obtained on the growth of *Microcystis* at different light intensities in the laboratory. The laboratory measurements were supplemented by field observations. The data show that *Microcystis* is able to adapt physiologically to different light intensities and make it possible to partly explain the reason for the successful growth of *Microcystis* in nature.

MATERIALS AND METHODS

Culture Conditions

The *Microcystis aeruginosa* culture used in laboratory experiments originated from a toxic bloom which occurred in Hartbeespoort Dam during 1974 (Toerien,

Scott and Pitout, 1976) and was isolated according to the procedure described by Scott (1974). During cultivation the alga changed from its colonial form to a unicellular form in a similar fashion to that described for *Microcystis aeruginosa* NRC-1 (Zehnder and Gorham, 1960). The culture has been given the number WR88 in the NIWR culture collection and was the source of the culture UV-006 in the culture collections of the University of the Orange Free State (Krüger and Eloff, 1977).

Cultivation of the alga was in 250 mℓ Erlenmeyer flasks equipped with a side arm allowing direct daily reading of the optical density (OD) with an EEL colorimeter (EEL Instruments, Ilford, U.K.). Each flask contained 150 mℓ of Volk and Phinney's medium (1968) with the following modifications: EDDHA-Fe was replaced by EDTA-Fe to give a concentration of 1,2 mg/dm³ and Hoagland's trace element solution was replaced with the A5 (Stainer, Kunizawa, Mandel and Cohen-Bazire, 1971) trace element mixture.

The cultures were maintained at 25 °C in a 16 hours light:8 hours dark cycle at 60 - 70 % relative humidity in a CONVIRON controlled environment chamber (Controlled Environments, Winnipeg, Canada). The light source in the CONVIRON consists of a mixture of fluorescent and incandescent lamps both manufactured by Sylvania Inc. Light intensity received by the cultures was manipulated either by changing the distance of the cultures from the light source or by covering the culture flasks with one or two layers of black silk netting. The light intensities received by the cultures were measured with a Lamoda Li-Cor Li-185 photometer in units of lux (lx). Light intensity was not uniform on the different shelves in the CONVIRON, showing a variation of about 20 %. To ensure that replicate cultures received approximately the same light intensity while the experiments were in progress, the flasks were randomly placed on the shelves after the daily OD measurements.

Three light intensities (3 600, 5 700 and 18 000 lx) were initially used. After 12 days of growth three replicates from 5 700 lx were transferred to 720 lx and three to 230 lx, whilst three flasks grown at 18 000 lx were transferred to 720 lx. The experiment was continued for 28 days.

Growth Measurements

Optical density values were obtained daily immediately after the 6 hour dark period in the 24 hour light:dark cycle and growth rates (k) were calculated from

$$k = OD\ day\ (x + 1) - OD\ day\ (x)$$

Gas Vacuole Determinations

An estimate of the amount of gas vacuoles present in a *M. aeruginosa* suspension was obtained by comparing the OD before and after gas vacuole collapse by a 6 atmosphere pressure application (Walsby, 1969). The amount of gas vacuoles was expressed as a percentage using the following formula:

$$\%\ \text{Gas vacuoles} = \frac{(\text{OD before collapse}) - (\text{OD after collapse})}{(\text{OD before collapse})} \times 100$$

Gas vacuole content was determined on selected days during the course of experiment after the aseptic removal of 10 mℓ subsamples from each flask.

Pigment Determinations

Pigment composition of the algal cultures was analysed by comparison of absorption peaks at 440 nm for carotenoids and 662 nm for chlorophyll after extraction in acetone.

In field material (see below) pigments were determined after extraction of chlorophyll and carotenoids in 80 % acetone. The exact amount of pigments present in the extracts were calculated from the extinction coefficients given by Allen (1968). The amount of pigment is presented per unit dry weight. It was not possible to express the pigment analyses of laboratory experiments on a dry weight basis because the relatively small amounts of cultured material available precluded accurate dry weight determinations.

Field Incubation of *Microcysts*

A natural population of *Microcystis aeruginosa* was sampled from the surface of Hartbeespoort Dam in the late afternoon (17h30; 7 March 1979). Approximately 5 ℓ of this population, which appeared to be unialgal, was placed in a beaker stirred continuously and 100 cm^3 aliquots were pipetted into dialysis tubing. The tubes were sealed and suspended overnight in dam water. At dawn (06h00; 8 March 1979) the tubes were suspended at selected depths from a float in the deepest part of the dam. To ensure that the tubes remained at the selected depths (0, 3 and 28 metres) sinkers were attached to them. Samples were collected 24 and 48 hours after the start of incubation for dry weight and pigment analyses.

Dry Weight Determinations

The dry weight of algae present in a dialysis bag was measured by filtering the total contents (100 cm^3) through a preweighed Nucleopore filter with a pore size of 8 µm . The filters were dried at room temperature for 2 days in a dessicator containing phosphorus pentoxide. Previous experiments have established that 2 days were sufficient to give a constant dry weight.

Other Field Measurements

Temperature, oxygen and pH values in Hartbeespoort Dam were measured with a Martek Instrument (Martek Inc. California, USA). Light penetration into the water was measured with a Lambda Li-Cor Li-185 photometer fitted with an underwater sensor which recorded photosynthetic active radiation in µEinsteins/m^2/s . The surface value was taken as 100 % light penetration and the depths of 1 % and 0,01 % light penetration were calculated from the original measurements.

RESULTS

Growth of *Microcystis* at Different Light Intensities

Identical sigmoid growth curves were observed when *Microcystis aeruginosa* was grown at 3 600, 5 700 and 18 000 lx (curves (a), (b) and (c) in Figure 1). The initial lag phase decreased as the light intensity increased. This is clearly illustrated if the first 8 days of the three curves are compared. It appears therefore that once *Microcystis* has adapted to a particular light intensity growth can continue for several days at a rate independent of the actual amount of light received by the algae. The optimum growth rates recorded at the three different light intensities are given in Table 1.

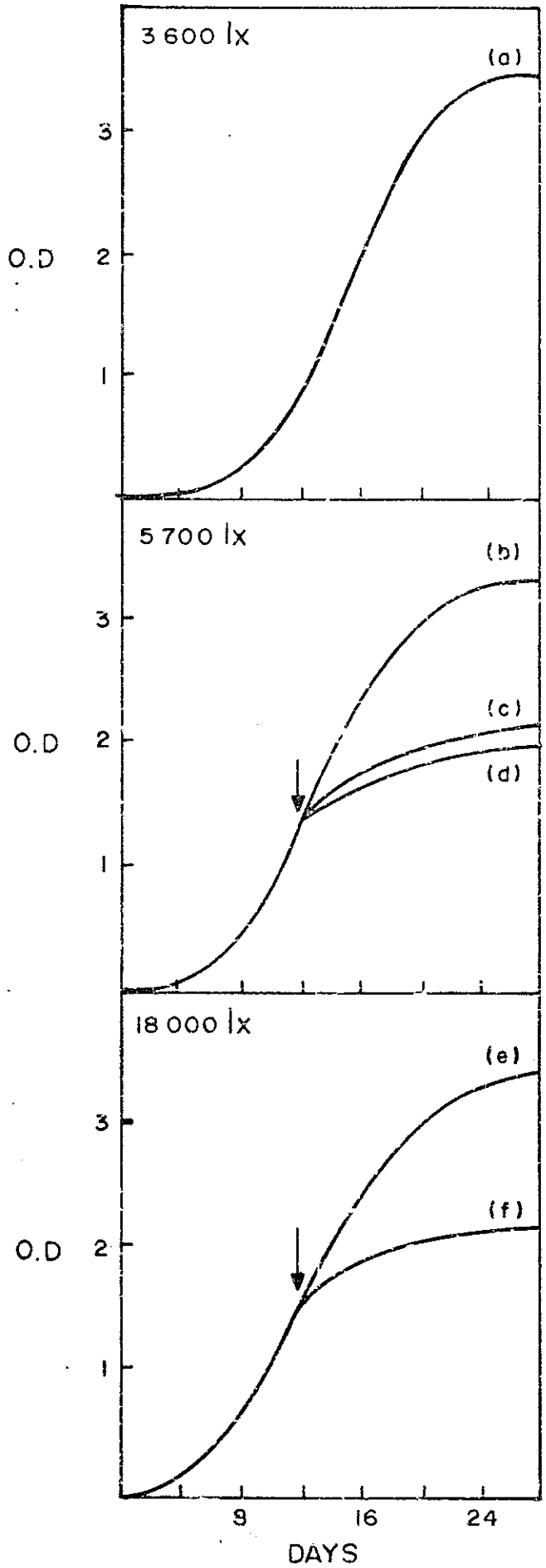

FIGURE 1. Growth curves of *M. aeruginosa* at different light intensities, (a) 3 600 lx, (b) 5 700 lx, (c) 720 lx, (d) 5 700 → 230 lx, (e) 1 800 lx (f) 1 800 lx → 720 lx. Arrows indicate transfer to lower light intensities on the 12th day.

TABLE 1
OPTIMUM GROWTH RATES (k) OF *MICROCYSTIS AERUGINOSA* AT DIFFERENT LIGHT INTENSITIES

Light intensity	k
3 600 lx	0,37
5 700 lx	0,395
18 000 lx	0,385

Although the growth rates were very similar at the different light intensities (Table 1), the physiological state of the algae changed markedly at the different light intensities. The cultures grown at 3 600 lx had a normal green appearance while the cultures at 18 000 lx turned to a yellow colour after a few days. The cultures at 5 700 lx had a yellow-green pigmentation between the two other treatments. On the 12th day of the experiment flasks grown at 5 700 and 18 000 lx were transferred to lower light intensities of 720 and 230 lx respectively (Figure 1). These cultures turned green in a few days (Table 2) but were unable to resume growth at high rates. It appears therefore that light intensities lower than 720 lx are not able to support vigorous growth of *Microcystis*.

TABLE 2
CHLOROPHYLL TO CAROTENOID RATIOS ESTIMATED FROM ABSORPTION PEAKS FOR *MICROCYSTIS* GROWN AT DIFFERENT LIGHT INTENSITIES. ARROWS IN THE FIRST COLUMN INDICATE TRANSFER FROM A HIGHER TO A LOWER LIGHT INTENSITY ON THE 12th DAY

Light intensity (lx)	Chlorophyll/Carotenoid ratio on day 12	19	28
3 600	1,11	0,9	0,68
5 700	0,4	0,35	0,33
5 700 → 720	0,4	0,81	1,19
5 700 → 230	0,4	0,63	0,91
18 000	0,19	0,30	0,19
18 000 → 720	0,19	0,56	1,01

In addition to the changes in pigmentation there were also changes in the gas vacuolation of the algae (Table 3).

TABLE 3
GAS VACUOLE CONTENT OF *MICROCYSTIS* GROWN AT DIFFERENT LIGHT INTENSITIES. ARROWS IN THE FIRST COLUMN INDICATE TRANSFER FROM A HIGHER TO A LOWER LIGHT INTENSITY ON THE 12th DAY

Light intensity (lx)	Gas vacuole content on day 12	19	28
3 600	41,7	44,5	54,8
5 700	58,6	62,0	64,8
5 700 → 720	58,6	48,0	46,7
5 700 → 230	58,6	50,0	47,0
18 000	61,5	64,2	62,9
18 000 → 720	61,5	48,6	42,7

The following conclusions can be made from the data in Table 3:

(a) Gas vacuole content increases with increasing light intensities and the converse is true when cultures are transferred from higher to lower light intensities and

(b) gas vacuole content increases as the cultures become senescent.

The laboratory observations on pigmentation of *Microcystis aeruginosa* was supplemented with field observations in Hartbeespoort Dam, where the *Microcystis* colonies were trapped in dialysis tubes kept at a fixed depths for periods up to 48 hours. The experiments were performed at a time when there was a thick *Microcystis* bloom in the dam reaching surface chlorophyll *a* concentrations in excess of 500 mg/m^3. Typical mid-day conditions observed in the dam while the experiment was in progress, are illustrated in Figure 2. The dam showed high surface pH and oxygen values. The oxygen concentration at 1 metre depth was less than half of the surface concentration and at 12 metres depth the water was anaerobic. The thick *Microcystis* scum reduced the light penetration to virtually zero at 2 metres depth.

The results of pigment analyses of *Microcystis* cells maintained at different depths are illustrated in Figure 3. Cells maintained at the surface of the dam had a linear drop in the chlorophyll/carotenoid ratio which was a direct result of a large decrease in the total chlorophyll content. Chlorophyll content of cells maintained at 3 m depth and at the bottom (28 metres) both increased over the incubation period. The carotenoid content of the cells did not change as much as the chlorophyll content of the cells. At the surface total carotenoid content decreased over the incubation period as did the chlorophyll content of these samples. At 3 metres depth and at the bottom the carotenoid content of the cells increased with the nett result that the chlorophyll/carotenoid ratios were slightly lower than at the beginning of the experiment.

DISCUSSION

The similar optimum growth rates of *Microcystis aeruginosa* at light intensities ranging from 3 600 lx to 18 000 lx indicate that light saturation for *Microcystis* growth is reached at relatively low light intensities. Provided that there is no extensive shading by a surface bloom, the intensity of 3 600 lx will correpsond to the light intensity normally received at a depth of approximately 4 to 5 metres in a typical non-silty Transvaal impoundment such as Hartbeespoort or Rietvlei Dam. The ability of *Microcystis aeruginosa* to grow fast at relatively low light intensities will give it a competitive advantage over other algae in these impoundments and partly explains why such large numbers of this alga frequently develop in these impoundments.

A further competitive advantage is the presence of gas vacuoles in the cells of *Microcystis aeruginosa*. The presence of gas vacuoles make the alga more buoyant with the result that the alga will not sink to depths where the light intensity may be limiting. Waaland, Waaland and Branton (1971) presented evidence that gas vacuoles reduced the amount of light absorbed by photosynthetic pigments in *Nostoc muscorum* and concluded that these structures acted as light shields. Walsby (1972) and Shear and Walsby (1975) questioned the validity of these results and suggested that the main function of gas vacuoles was buoyancy regulation. Dinsdale and Walsby (1972) showed that transfer of *Arabaena flos-aquae* from low (50 lx) to

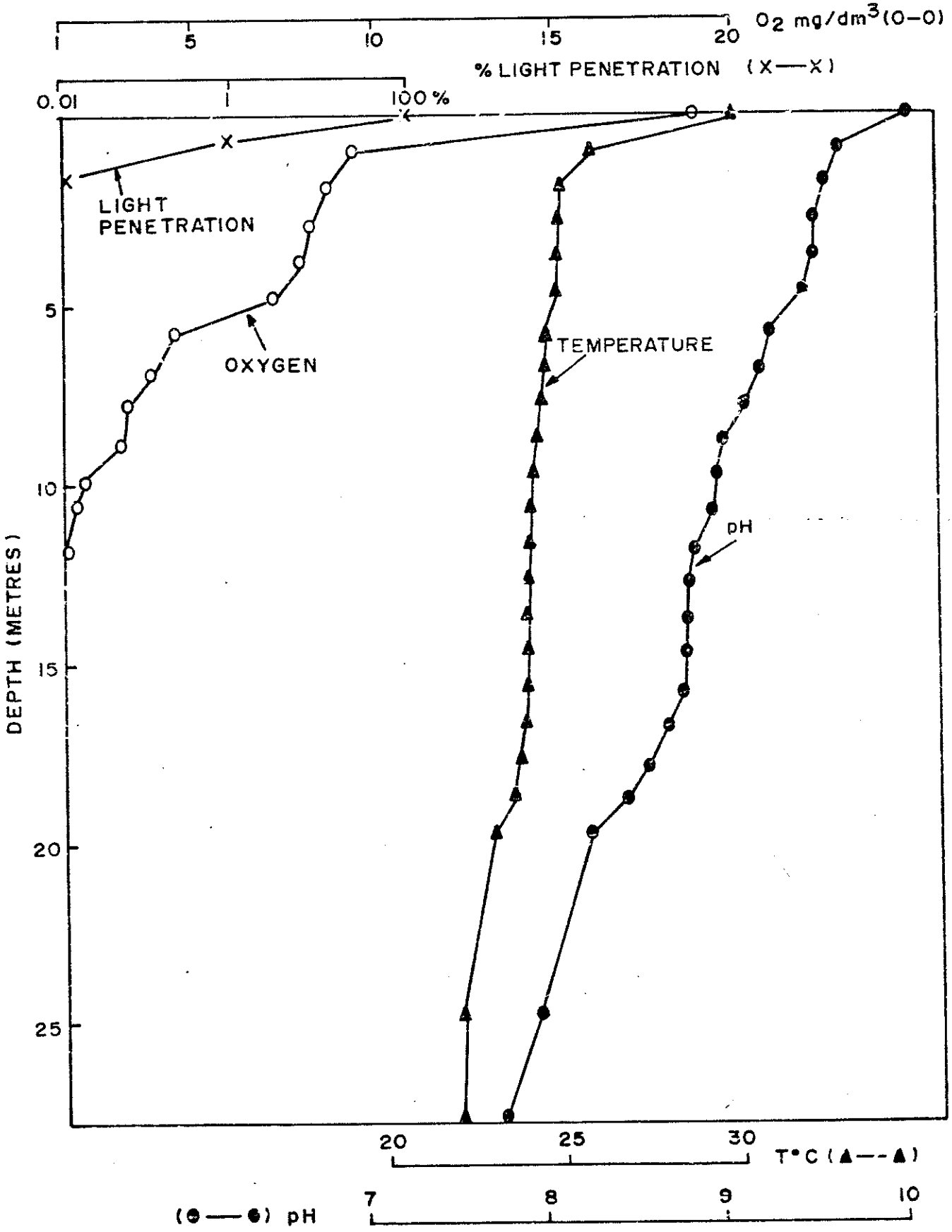

FIGURE 2. Profiles of percentage light penetration, oxygen, temperature and pH in Hartbeespoort Dam (12h00; 7th March 1979).

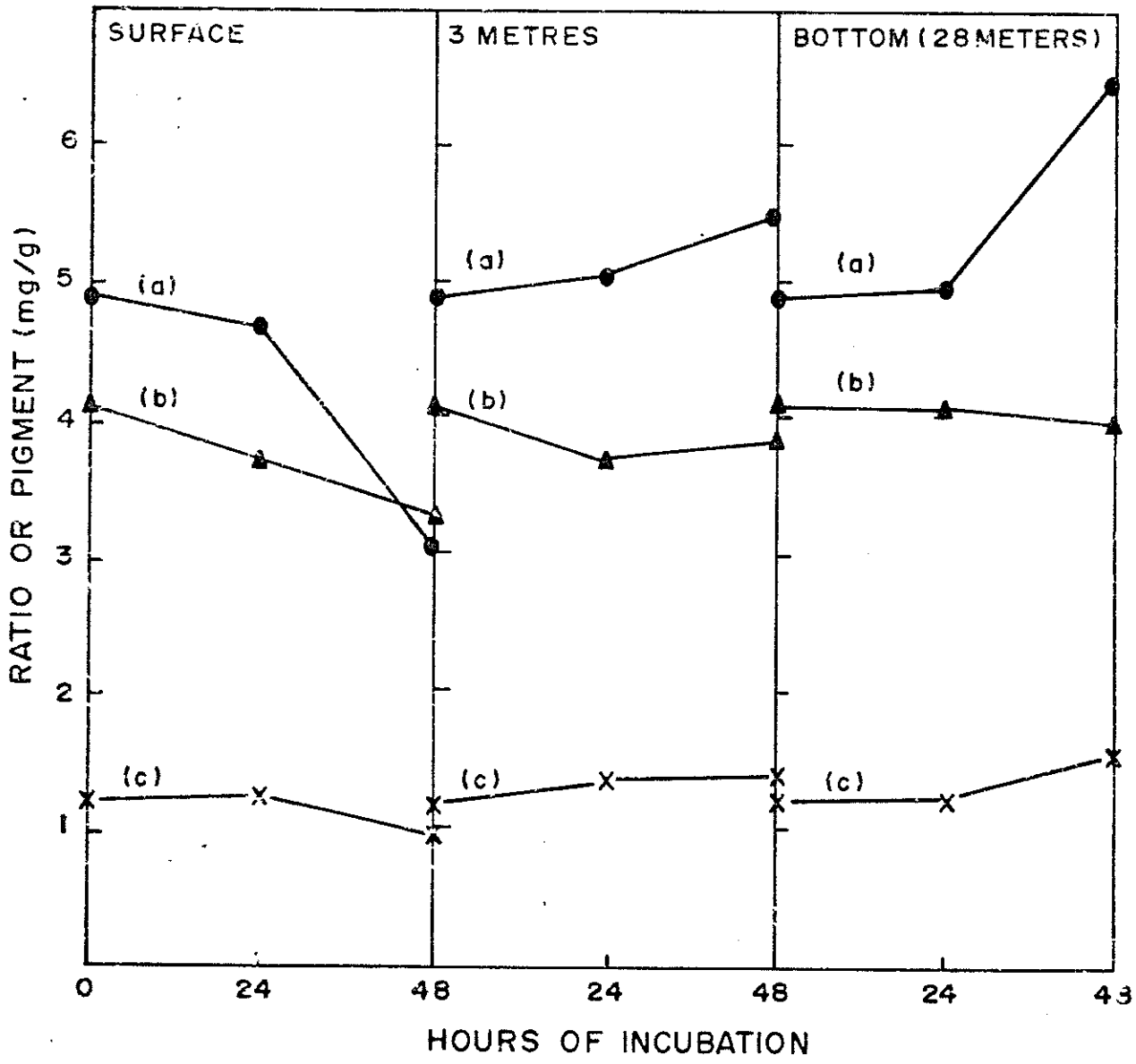

FIGURE 3. Changes in the pigment content and chlorophyll/carotenoid ratio of *Mycrocystis* cells incubated at fixed depths in Hartbeespoort Dam (a) ·——· chlorophyll *a* content (b) ▲——▲ chlorophyll/carotenoid ratio and (c) x——x carotenoid content.

high (10 000 lx) light intensities resulted in a decrease in gas vacuole content. We were unable to confirm these observations with *Microcystis aeruginosa* (Table 3) and the present results support the idea that gas vacuoles may act as light shields. In addition senescent cultures were found to have a higher gas vacuole content.

When *Microcystis aeruginosa* is continuously exposed to high light intensities the alga adapts by changing its pigment composition. This is achieved by reducing the chlorophyll content of the cells. At lower light intensities and in complete darkness more chlorophyll is synthesized (see Figure 3). *Microcystis aeruginosa* is therefore able to adapt to changes in the light climate by changing its pigment composition and gas vacuole content, so that it can maintain high growth rates. Suggestions to control unwanted blue-green algal blooms, and by implication potential toxicity, in eutrophic systems by managing conditions in such a way to stimulate the growth of more desirable green algae, appear unlikely to succeed at this stage because of the adaptability of the blue-green algae.

ACKNOWLEDGEMENTS

The author wishes to thank his colleagues D.J. Barlow, J.H. Hauman and S.I. Funke for technical assistance and help in the preparation of this lecture. A more extensive report on these observations, supplemented by an ultrastructural investigation by D.J. Barlow is in preparation. The Director of the NIWR is thanked for permission to present this paper.

REFERENCES

ALLEN, M.M. (1968) Photosynthetic membrane system in *Anacystis nidulans*. *J. Bact.* 96, 736-841.

DINSDALE, M.T. and WALSBY, A.E. (1972) The interrelations of cell turgor pressure, gas-vacuolation and buoyancy in a blue-green alga. *J. exp. Bot.* 23, 561-570.

HAUMAN, J.H., BARLOW, D.J. and SCOTT, W.E. (1979) Virus-like inclusions in *Microcystis aeruginosa*, isolation and preliminary characterisation. Submitted to *S. Afr. J. Sci.*

KRÜGER, G.H.J. and ELOFF, J.N. (1977) The influence of light intensity on the growth of different *Microcystis* isolates. *J. Limnol. Soc. sth. Afr.* 3, 21-25.

SCHWIMMER, D. and SCHWIMMER, M. (1964) *Algae and Medicine.* In "Algae and Man" (D.F. Jackson ed.) pp. 368-412, Plenum Press New York.

SCHWIMMER, D. and SCHWIMMER, M. (1968) *Medical aspects of phycology* In: "Algae, Man and Environment" (D.F. Jackson ed.) pp. 279-358, Syracuse University Publ. Syracuse, N.Y.

SCOTT, W.E. (1974) The isolation of *Microcystis*. *S. Afr. J. Sci.* 70, 179.

SHEAR, H. and WALSBY, A.E. (1975) An investigation into the possible light - shielding role of gas vacuoles in a planktonic blue-green alga. *Brit. phyc. J.* 10, 241-251.

STANIER, R.Y., KUNISAWA, R., MANDEL, M. and COHEN-BAZIRE, G. (1971) Purification and properties of unicellular blue-green algae (order Chroococcales), *Bact. Rev.* 35, 171-205.

TOERIEN, D.F., SCOTT, W.E. and PITOUT, M.J. (1976) *Microcystis* toxins: Isolation, identification, implications. *Water S.A.* 2, 160-162.

VOLK, S.L. and PHINNEY, H.K. (1968) Mineral requirements for the growth of *Anabaena spiroides* in vitro. *Can. J. Bot.*, 46, 619-630.

WAALAND, J.R., WAALAND, S.D. and BRANTON, D. (1971) Gas vacuoles. Light shielding in blue-green algae. *J. Cell Biol.* 48, 212-215.

WALSBY, A.E. (1969) The permeability of blue-green algal gas-vacuole membranes to gas. *Proc. R. Soc.* B 173, 235-255.

WALSBY, A.E. (1972) Structure and function of gas vacuoles. *Bact. Rev.* 36, 1-32.

ZEHNDER, A. and GORHAM, P.R. (1960) Factors influencing the growth of *Microcystis aeruginosa* Kütz. emend. Elenkin. *Can. J. Microbiol.* 6, 645-660.