SOME ASPECTS OF THE AUTECOLOGY OF *RHIZOCLONIUM RIPARIUM* (ROTH) HARV. WITH SPECIAL REFERENCE TO ITS GROWTH IN THE MATURATION PONDS OF THE GRAHAMSTOWN SEWAGE WORKS.

by

DEBORAH JANE SNOOK

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Department of Plant Science,
Rhodes University,
Grahamstown,
South Africa.

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Frontispiece. View of the two largest maturation ponds in the Grahamstown Sewage Works with floating mats of Rhizoclonium riparium (Roth) Harv. in the foreground.

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ABSTRACT

During 1982 benthic and floating filamentous algal mats appeared in the maturation ponds of the Grahamstown Sewage Disposal Works. These mats clogged the ponds and reduced the efficiency by which the effluent was purified. As they continued to be a problem despite numerous efforts to remove them, this study was initiated to investigate the alga, establish why it was successful in the pond environment, and how its growth could be controlled.

The physico-chemical environment of a representative maturation pond was characterised while laboratory studies were conducted to investigate the growth, photosynthetic and respiratory characteristics in the alga. The alga was identified as Rhizoclonium riparium (Roth) Harv. although its morphological variability was greater than that reported in the literature. Growth and photosynthetic studies indicated that the alga favoured temperatures between 20 and 30°C and relatively high light intensity (700$\mu E.m^{-2}.s^{-1}$) and that it was highly productive. In addition, the alga exhibited photoadaptive ability, although it seemed to be sensitive to photoinhibition. Its success in the maturation pond was attributed to the favourable physico-chemical environment, particularly the high transparency of the effluent which allowed the penetration of PAR to the pond floor and to the alga's ability to adapt to the change in environment when it

floated from the pond floor to its surface. Although the algal mats contribute to the oxidation of the effuent within the maturation pond, they are generally detrimental to the system because they shade the water column and inhibit wind-induced mixing. Recommendations on methods of controlling of the mats are presented.

1.0 INTRODUCTION

One of the major sources of pollution in the world today is domestic sewage which, when discharged into water courses can threaten health or cause eutrophication and other water quality problems (Khauer, 1975; Nusch, 1975; Solterero, Gasperino and Graham, 1975; Toerien and Steyn, 1975; Grindley, 1983; de Villiers and Malan, 1985). In order to control such problems the organic material, inorganic solutes and bacteria in the sewage must be removed and the effluent re-oxidised before the water is discharged into water courses (Wurtz, 1964; Meiring, Drews, van Eck and Stander, 1968; Golterman, 1975). Whilst the removal of pollutionary materials from domestic sewage is largely achieved by physical processes such as filtration and sedimentation, the final purification of the effluent is usually by means of biological processes (Meiring et al, 1968; McGarry, 1971). In many South African sewage treatment works the final stages involve the use of trickling filters and maturation ponds which reduce the inorganic, organic and bacterial loads of the effluent (van der Post and Toerien, 1974; Pieterse and Shillinglaw, 1976; Shillinglaw and Pieterse, 1977).

The efficiency of the maturation pond system depends on the maintenance of an aerobic effluent since:

1. aerobic bacteria are more efficient in the breakdown of

organic compounds than anaerobic bacteria;

2. the maintenance of an oxidised sediment surface will sequestrate nutrients, and

3. the aerobic environment is suitable for colonisation by higher organisms eg zooplankton and fish.

Oxidation is achieved by several processes including atmospheric diffusion assisted by wind-driven water movement and photosynthetic oxygen production, so it is important that the conditions favouring these processes are enhanced. The optimum design of maturation ponds is such that they should create a self-generating purification system based on the maintenance of a photosynthetically active phytoplankton population (Oswald and Golueke, 1968; Palmer, 1980). This system is illustrated in Figure 1. The ponds are shallow so they are well mixed, the entire depth normally lies within the euphotic zone, and the water is nutrient rich. Bacterial respiration produces carbon dioxide, which stimulates photosynthesis further (Ganipati, 1975). In addition, the algae use and remove inorganic nutrients from the effluent (Palmer, 1980). The aerobic environment encourages colonisation by zooplankton which can increase the productivity of the algae (Porter, 1976) and also feed on bacteria and detritus, further increasing the quality of the discharged effluent.

Maturation ponds are usually colonised by planktonic algae (eg species of Coelastrum, Dunaliella, Spirulina, Anabaena, Chlamydomonas, Micractinium, Scenedesmus, Euglena and

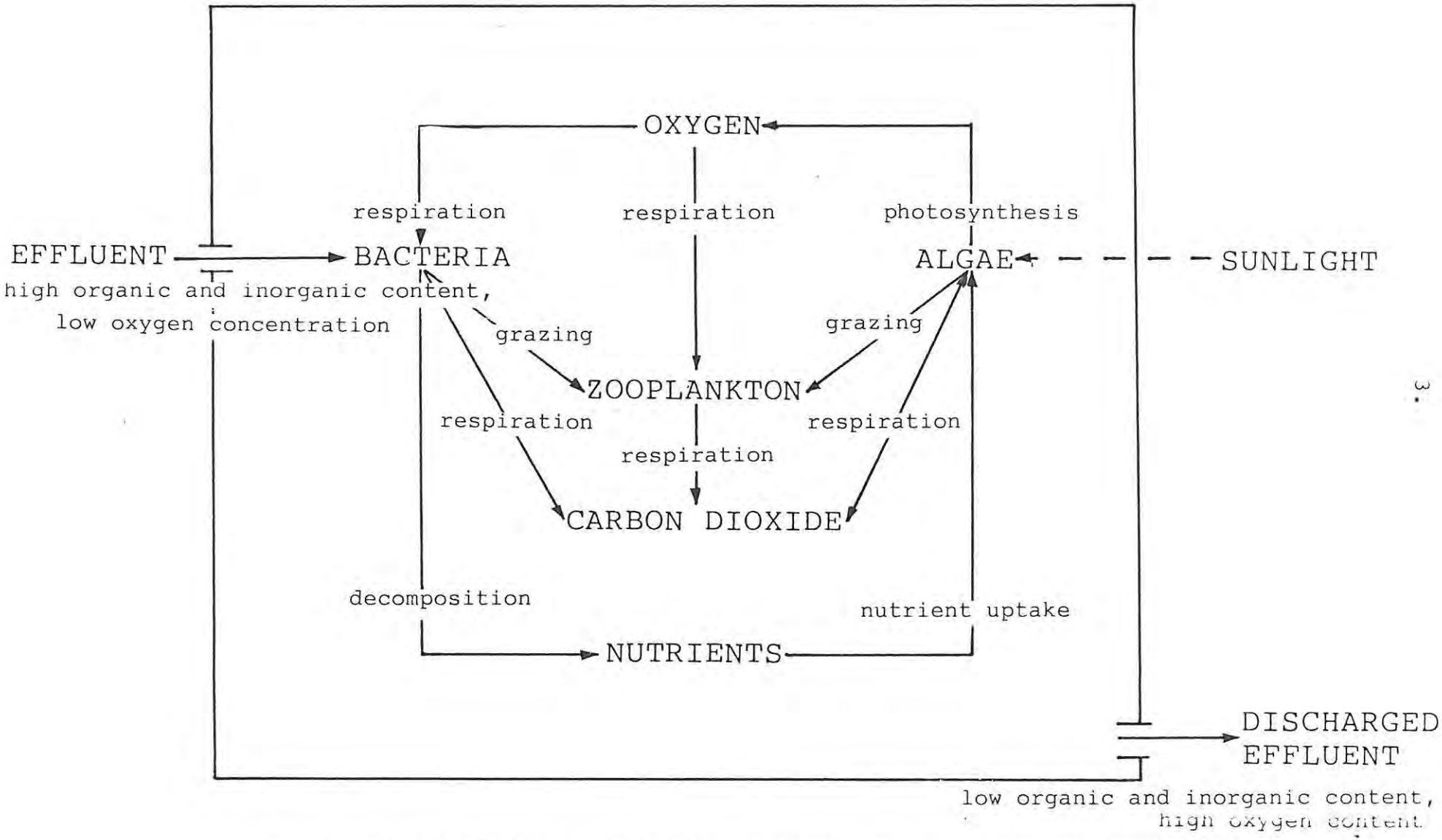

Figure 1. Diagrammatic representation of the self-generating purification system within maturation ponds (modified from Oswald and Golueke, 1968).

Chlorella - Pieterse and Shillinglaw, 1977; ~~van Vuren and Grobbelaar, 1982~~) which, because their cells have a relatively high surface area:volume ratio are efficient converters of light energy (Malone, 1980). By the same token nutrient uptake is more efficient. Large colonial algae are less desirable in such systems, especially when they form thick floating mats which, apart from decreasing the effective area over which light and nutrients can be absorbed, also reduce wind-driven mixing, shade the pond creating light-limited conditions, and release oxygen into the air rather the water (Palmer, 1980). Under these conditions it is unlikely that an aerobic environment could be permanently maintained. In addition, algal mats cause blockages which reduce water flow and this creates problems in the management of maturation ponds as it necessitates the manual removal of mats (Palmer, 1964). Nevertheless, the removal of such material has some benefit in that the nutrients which are incorporated into the biomass are removed from the effluent permanently, rather than being recycled within it (Fekete, Riemer and Motto, 1972).

During 1982 the Grahamstown Sewage Disposal Works experienced a severe infestation of a filamentous green alga which almost completely blocked the Work's final stage maturation ponds. Furthermore, reduced phytoplankton populations were observed in association with this infestation, and officials from the Department of Health reported that the effluent water quality had deteriorated to below the acceptable limits as prescribed

in the Water Act (Reilly, personal communication[1]). The performance of the ponds in the purification system was subsequently investigated by a team of students from the Department of Plant Sciences, Rhodes University.

Roman (1982) and Irwine (1983) examined various aspects of water quality in the sewage works. Both concluded that the quality of the discharged effluent was lower than that of the water in the river into which the effluent was discharged, and was therefore polluting it.

Three studies were initiated to examine the processes occurring in the ponds more closely.

1. Bell (1983) investigated the influence of the avifauna on water quality as it had been suggested that the birds, through defecation, were adding to its organic load. He concluded that the avifauna had no significant effect on water quality since estimates indicated that the total avian organic waste deposited into the pond would only increase COD levels by 0.15%.

2. Snook (1983) examined the role which the phytoplankton played in the oxidation of effluent. She showed that the rate of planktonic respiration usually exceeded the photosynthetic rate, despite the fact that the algae were photosynthetically active. Primary production was limited by the size of the phytoplankton community, which may have been limited by zooplankton grazing.

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1. Mr J. Reilly, Superintendent, Grahamstown Sewage Disposal Works, Grahamstown.

3. This grazing hypothesis was tested by Sugden (1983) using a laboratory model system designed to simulate the physical and chemical conditions within the ponds. She showed that zooplankton grazing could reduce the natural phytoplankton population and thus contribute to the reduction of the ponds' oxidation efficiency.

These results indicated that the maturation pond system was not functioning efficiently as a water treatment system. Furthermore, it was postulated that the inefficiency of the ponds could be attributed to the presence of the algal mats. Correspondence received from the City Engineers of several municipalities (Johannesburg, Pretoria, Cape Town, Port Elizabeth, East London, Port Alfred and Graaf Reinet) indicated that filamentous algal mats occur in many South African sewage works where they are sometimes a problem. Mat removal is costly: in Grahamstown an average of one man day per week was spent during 1983 and 1984 in removing the floating mats manually. Furthermore, at one stage (April, 1983) the problem became so severe that the largest maturation pond had to be emptied, dried and regraded.

Consequently, this autecological investigation was initiated, with the objectives of the study being to:

1. Identify the alga responsible for the problem (Chapter 2);
2. Characterise the physico-chemical environment of the ponds, and the effect of the algal mats on it (Chapter 3);
3. Measure the rates of growth, photosynthesis and respiration (Chapters 4 and 5), and

4. Explain, using the data collected, why the alga was successful in the maturation pond system and to suggest how it might be controlled (Chapter 6).

2.0 MORPHOLOGY, ULTRASTRUCTURE AND TAXONOMY

2.1 INTRODUCTION

At the onset of this investigation little was known about the taxonomic position of the alga concerned. Consequently, its morphology and ultrastructure were examined. In addition, because the alga formed benthic mats which subsequently floated to the pond surface (Plate 1), it was felt that these sub-populations should be compared in order to establish whether there was any significant morphological difference between them.

2.2 MATERIALS AND METHODS

2.2.1 Morphology

Samples of the alga were collected approximately every fortnight over a period of 14 months (May, 1984 to August, 1985). One sample from each population (floating and benthic) was collected from the pond edge (for description of the pond system see Section 3.3), using a long-handled rake.

The taxonomically important morphological features of filamentous green algae, as described by Nienhuis (1975) were routinely measured. These are:

1. filament diameter,
2. length of the cells,
3. thickness of the cell wall,

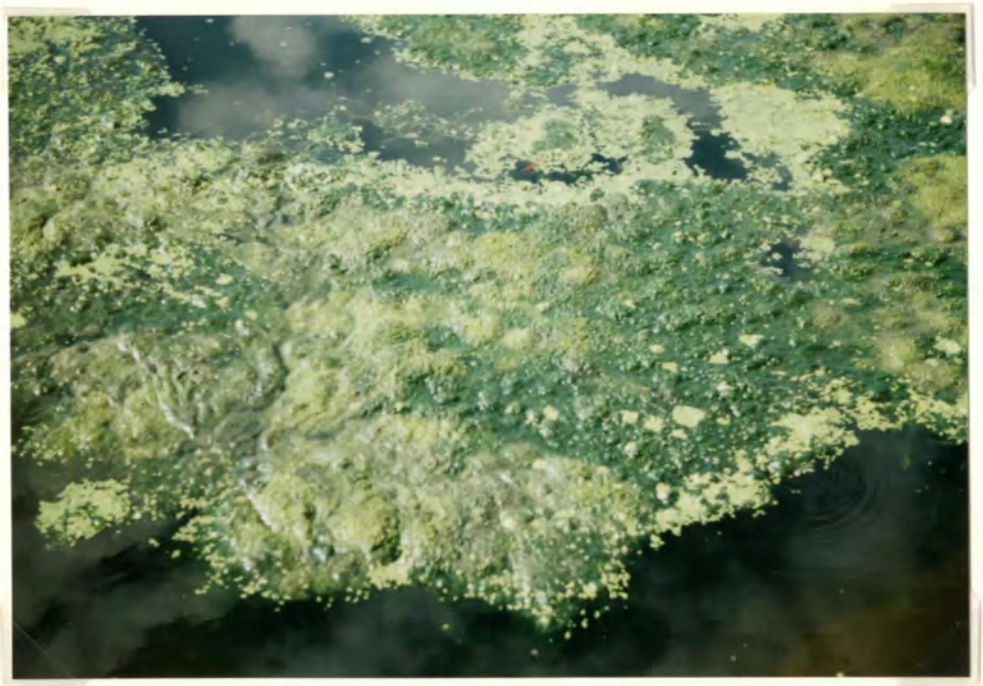

Plate 1. Photograph of a floating mat of the filamentous green alga in the maturation pond of the Grahamstown Sewage Works. Notice the dense filament mesh and the layer of debris and aquatic macrophytes (Lemna sp.) on the mat surface.

4. filament diameter,
5. number of rhizoids,
6. number of lateral branches and
7. number of nuclei per cell.

In addition, after observing that the cells of the benthic mats appeared to be larger than those of the floating mats, the cell volume was calculated using the following formula:

$$\text{Internal Cell Volume } (\mu m^3) = \pi r^2 . l$$

where, $r = \frac{\text{(internal cell width)}}{2}$

$$= \frac{\text{filament diameter} - 2(\text{cell wall width})}{2}$$

l = cell length.

For each sample, sub-samples were mounted in either aceto-orcein or iodine solution and the dimensions of 100 cells measured. Deformed and broken cells were not counted.

The variation in cell dimensions with time and between the floating and the benthic material was tested using two way analysis of variance, using a HP-65 programmable calculator.

2.2.2 Ultrastructure

Material for the examination of cellular ultrastructure was collected on 28 February, 1985. Samples were taken from both benthic and floating mats and washed to remove debris before fixation in 5% glutaraldehyde for 17 hours. Before and after pre-staining in osmium tetroxide for 2 hours the samples were washed in 0.1M phosphate buffer. They were then dehydrated in

an ethanol series (5 minutes in 30, 50, 70, 80, 90 and 100% ethanol) before being infiltrated with a transitional solvent (propylene oxide). Resin infiltration was achieved by passing the filaments through a series (75:25, 50:50 and 75:25%) of propylene oxide:resin (Taab 812/Araldite) mixtures. Samples were left for 12 hours in each mixture and then in 100% resin for 24 hours. Finally, the filaments were concentrated into bundles and placed in resin-filled capsules before the resin was polymerised at 60°C for 22 hours (Cross and Hartley[2], personal communication).

Sections for transmission electron microscopy (TEM) were cut on a LLB 8000 ultramicrotome III using a diamond knife. They were stained using uranyl acetate (20 minutes) and lead citrate (3 minutes) before being examined on a Joel JEM 100CX transmission electron microscope. The results obtained using TEM were supplemented with scanning electron microscopic (SEM) examination using cryotechniques. This was done so that cross-sectional views of the filaments, which were difficult to cut using TEM could be obtained. Fresh material was plunge-frozen and freeze-fractured before examination using a Joel JSM 840 scanning electron microscope.

2.3 RESULTS AND DISCUSSION

2.3.1 Morphology and Ultrastructure

Microscopic examination of R. riparium revealed that it

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2. Messrs R. Cross and A. Hartley, Electron Microscopy Unit, Rhodes University, Grahamstown.

consists of unbranched uniserate filaments made up of cylindrical cells joined end to end (Plate 2) with the concave end of one cell fitting into the convex end of the next (Plate 2). Rhizoids were never observed in the material from the pond but they did develop in laboratory cultured material (Plate 3). The cells had a mean length of 68.3µm, but this varied (CV=21.7%) from 25.9 to 124.7µm (Table 1). Cell diameter varied between 11.8 and 71.8µm, with a mean of 26.2µm (Table 1). The length:width ratio (LWR) of the cells varied from 1.1 to 4.9 (CV=19.6%) with a mean of 2.7. The thickness of the cell wall varied from 0.6 to 4.1µm (mean of 2.1µm) and was related to the diameter of the filaments ($r=0.89$; $n=3600$; $p>0.01$).

Table 1. Morphological characteristics of cells from filaments collected in the field between May, 1984 and August, 1985.

Characteristic	MIN.	MAX.	MEAN	SD*	n
Cell Length (µm)	25.9	124.7	68.3	14.8	3600
Cell Diameter (µm)	11.8	71.8	26.2	1.4	3600
Length:width ratio	1.1	4.9	2.7	0.8	3600
Cell Wall Width (µm)	0.6	4.2	2.1	0.6	3600

*SD- Standard Deviation

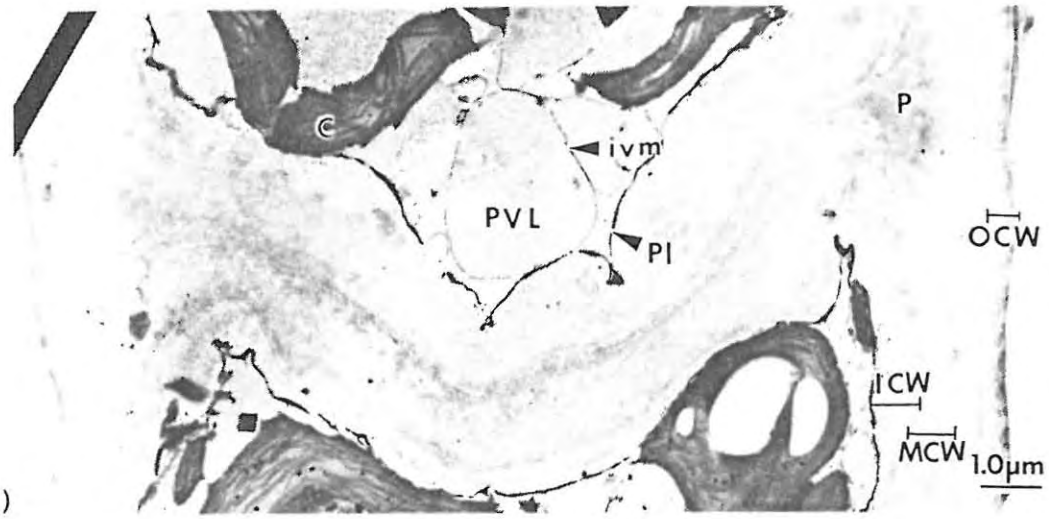
The cells were enclosed by a cell wall and plasmalemma membrane. The parietal nucleus was surrounded by a peripheral vacuolar layer (Plate 5) which was composed primarily of vacuoles, but included ~~organelles such as~~ endoplasmic reticulum and mitochondria. The peripheral vacuolar region of the cell is an unusual feature of algal cells, and appears to be unique to the Cladophoraceae (Chan, Ling-Wong and Wong,

Plate 2. A single filament of the alga showing its undifferentiated filamentous habit and bright green chloroplasts. 0.1mm

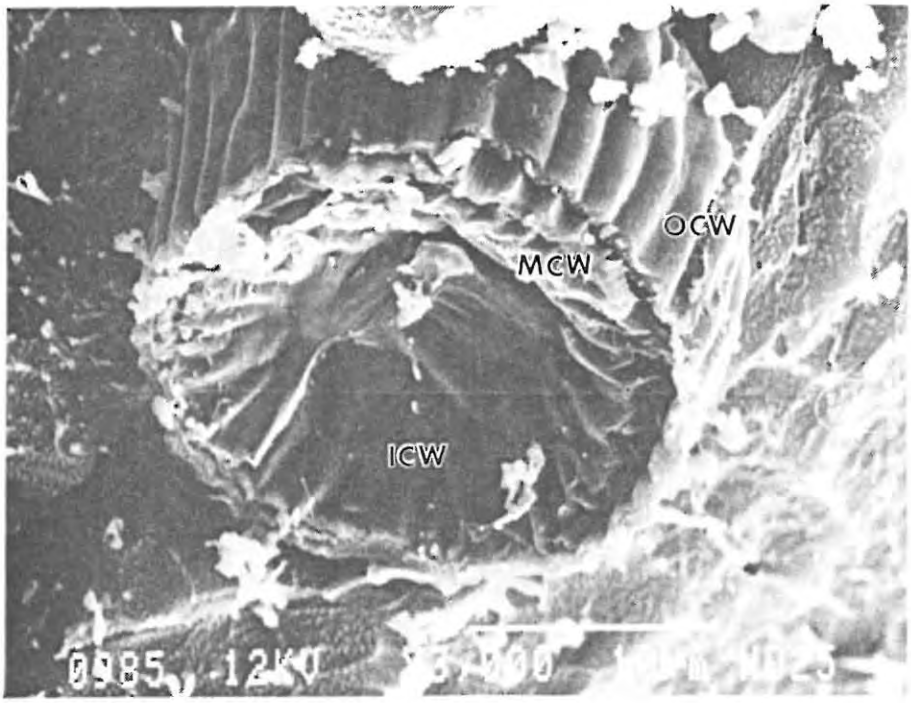
Plate 3. Light micrograph showing a rhizoidal holdfast cell. Rhizoidal cells only developed when the alga was cultured on agar in this study.

0.1mm

Plate 4. (a) Transmission electron micrograph showing the arrangement of the outer cell wall layer (OCW), middle cell wall layer (MCW), inner cell wall layer (ICW) and plasmalemma membrane (Pl) at the cell junction. At the junction the inner cell wall layer peels away from the rest of the cell wall to line the cell ends while the middle cell wall layer widens to form a plug (p). The convex cell-end is filled by the chloroplast (C) and peripheral vacuolar layer (PVL) which is divided by intravacuolar membranes (ivm). (b) and (c) Scanning electron micrographs showing how cells are joined end to end so that the concave end of one cell fits into the convex end of the next.



(a)



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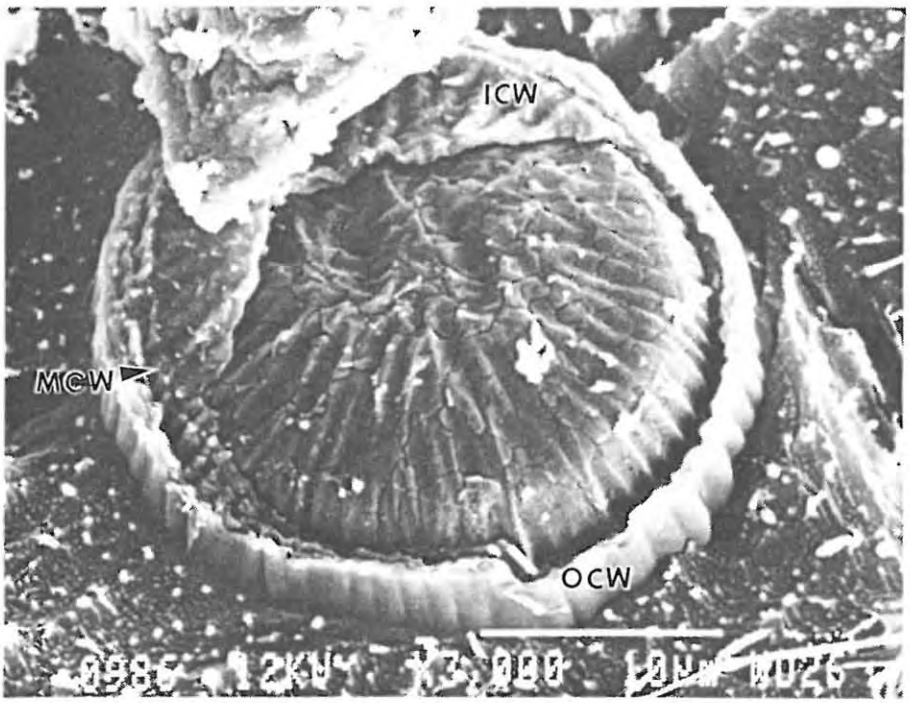

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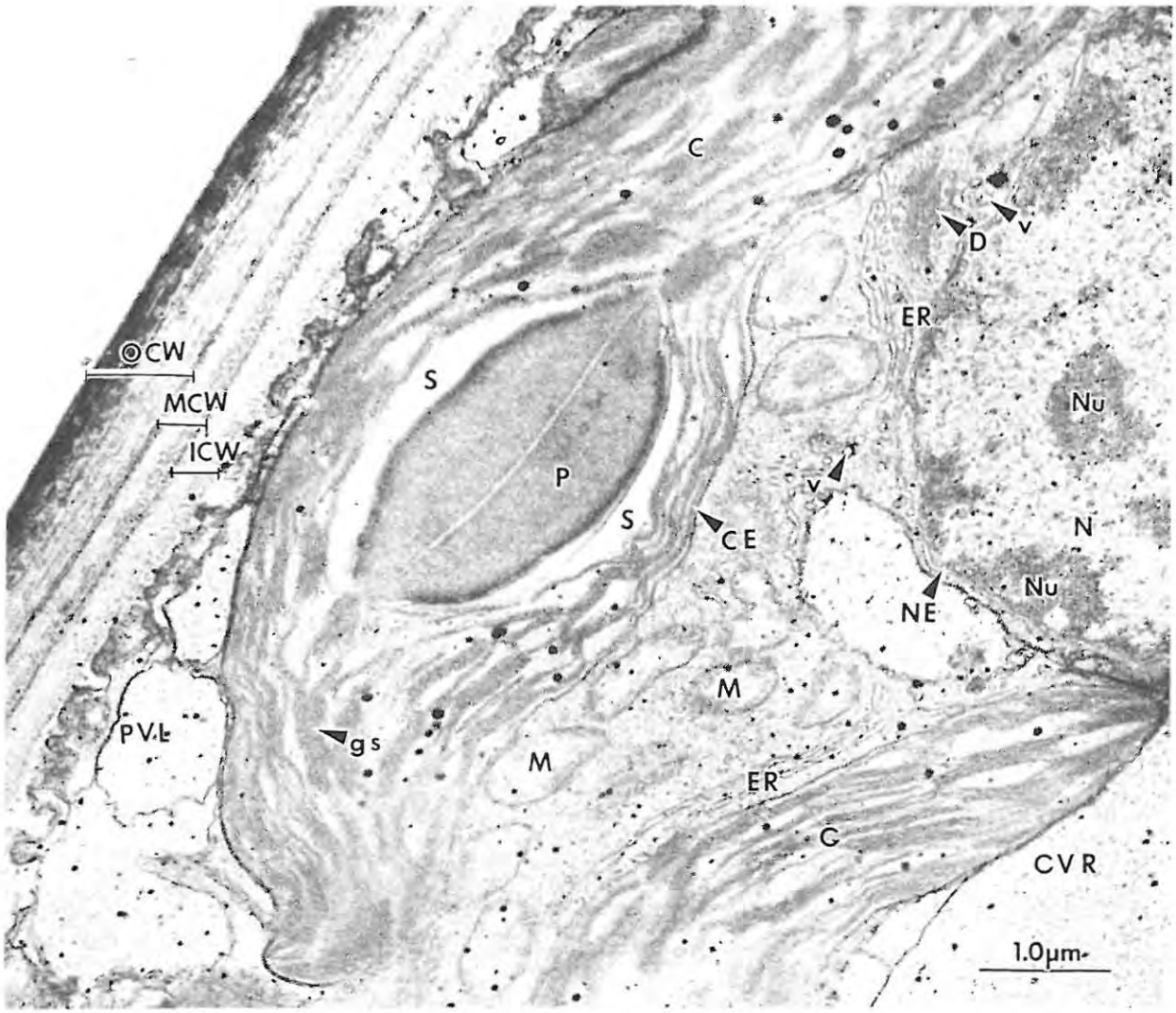


Plate 5. Transmission electron micrograph showing the structure and arrangement of the cell. The cell wall is divided into three layers: an outer densely lamellate cell wall layer (OCW), an amorphous inner cell wall layer (ICW) and an intermediate middle cell wall layer (MCW). The peripheral vacuolar layer (PVL) is comprised primarily of vacuoles which are divided by intravacuolar membranes (ivm). The chloroplast (C) is bounded by a chloroplast envelope (CE). The chloroplast is made up of thylakoid membranes arranged in granal stacks (gs) and pyrenoids (P) which are surrounded by starch grains (S). The nucleus (N) lies within the chloroplast and is bounded by the nuclear envelope (NE) and contains numerous irregularly shaped nucleoli (Nu). Between the chloroplast and the nucleus is the cytoplasmic region of the cell which includes organelles such as mitochondria (M), endoplasmic reticulum (ER), vesicles (v) and a dictyosome (D). The cytoplasmic region of the cell bounds the central vacuolar region (CVR) of the cell.

1978). There is a second vacuolar region in the cell centre, where the large vacuoles are sub-divided by intravacuolar membranes (Plate 6). The vacuolar regions of the cells from floating mats appeared to more highly developed than those from benthic mats (Plate 7). The area between the chloroplast and the central vacuolar region is occupied by the cell nuclei and cytoplasmic organelles such as mitochondria, endoplasmic reticulum and vesicles as well as the nuclei (Plate 6). The nuclei are round and are arranged spirally along the length of the cell (Plate 8). They contain many dark-staining nucleoli of irregular shape, which are differentiated into regions of varying density (Plate 5 and 6).

The cell wall had a lamellate structure composed of alternating microfibrillar and amorphous layers (Plate 5). The wall can be divided into three layers: a dense outer layer where the microfibrils are tightly compacted, particularly in the peripheral region; an intermediate middle layer, and an inner layer which has a greater proportion of amorphous material and so appears less dense (Plate 5). The outer and middle layers enclose the perimeter of the cell while the inner layer divides the adjacent cells (Plate 4). At the point where the cells join, the inner layer peels away to cover the cell ends and the space created between the inner and outer layers is filled by the enlargement of the middle cell wall layer to form a plug (Plate 4). The lamellate structure of the cell wall resembles that of

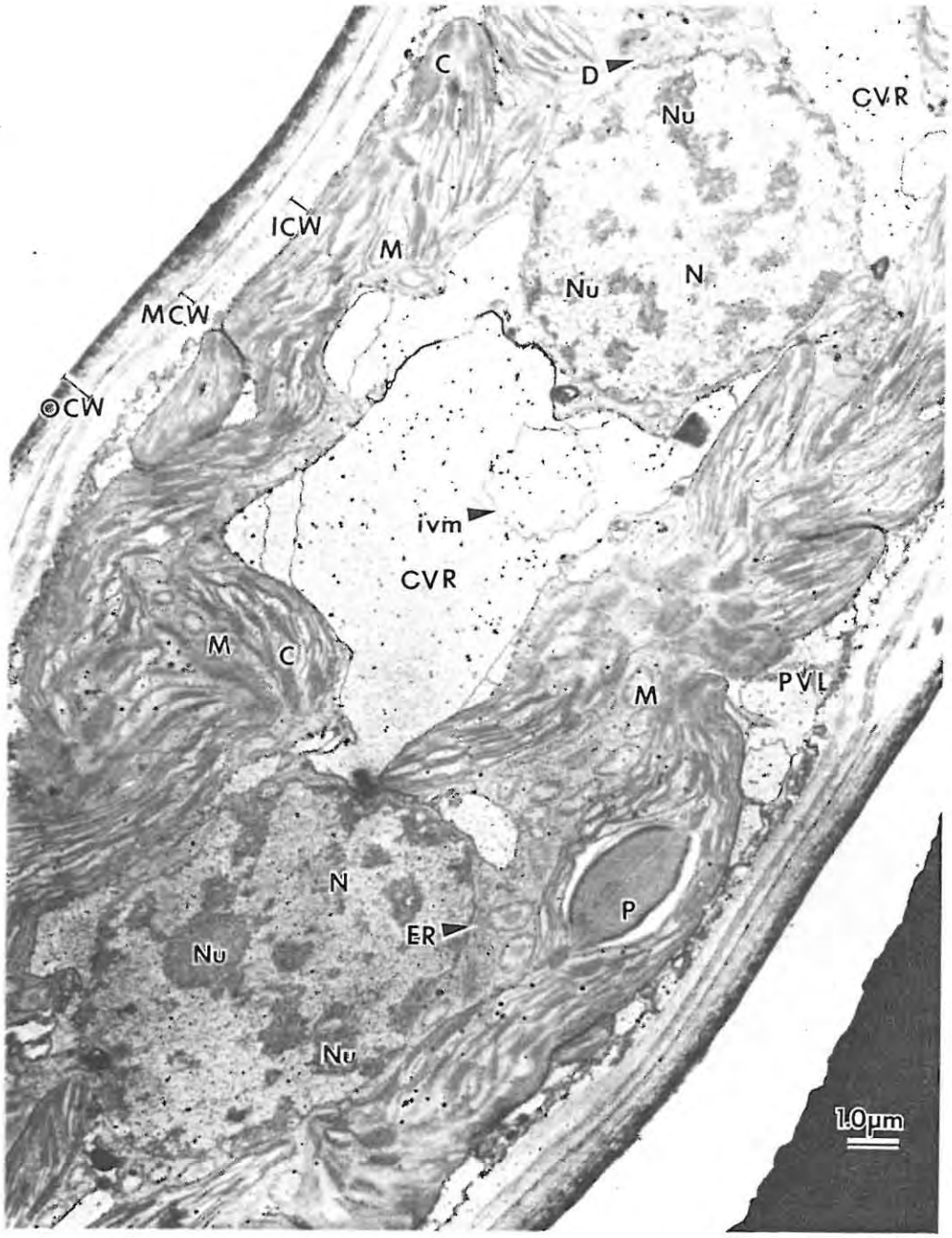
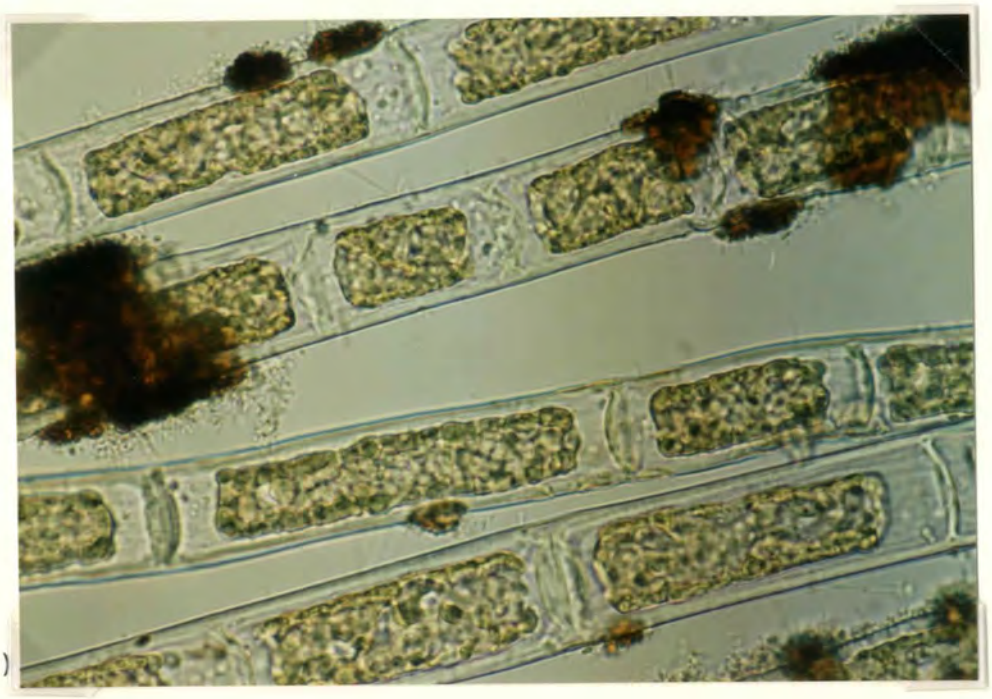


Plate 6. Transmission electron micrograph showing the arrangement of the cell organelles. The cell wall is divided into three regions: the outer cell wall layer (OCW), the middle cell wall layer (MCW) and the inner cell wall layer (ICW). The peripheral vacuolar layer (PVL) lies exterior to the reticulate chloroplast (C) which contains pyrenoids (P). Mitochondria (M), endoplasmic reticulum (ER) and a dictyosome (D) lie within the folds of the chloroplast which surrounds the nuclei (N) and the central vacuolar region (CVR). The nucleus has many irregularly-shaped nucleoli (Nu). The vacuoles are divided by intravacuolar membranes (ivm).

(a)



(b)

Plate 7. Light micrographs showing the difference in the development of the vacuolar regions, and the density of the chloroplast reticulum of cells from (a) floating mats and (b) benthic mats.

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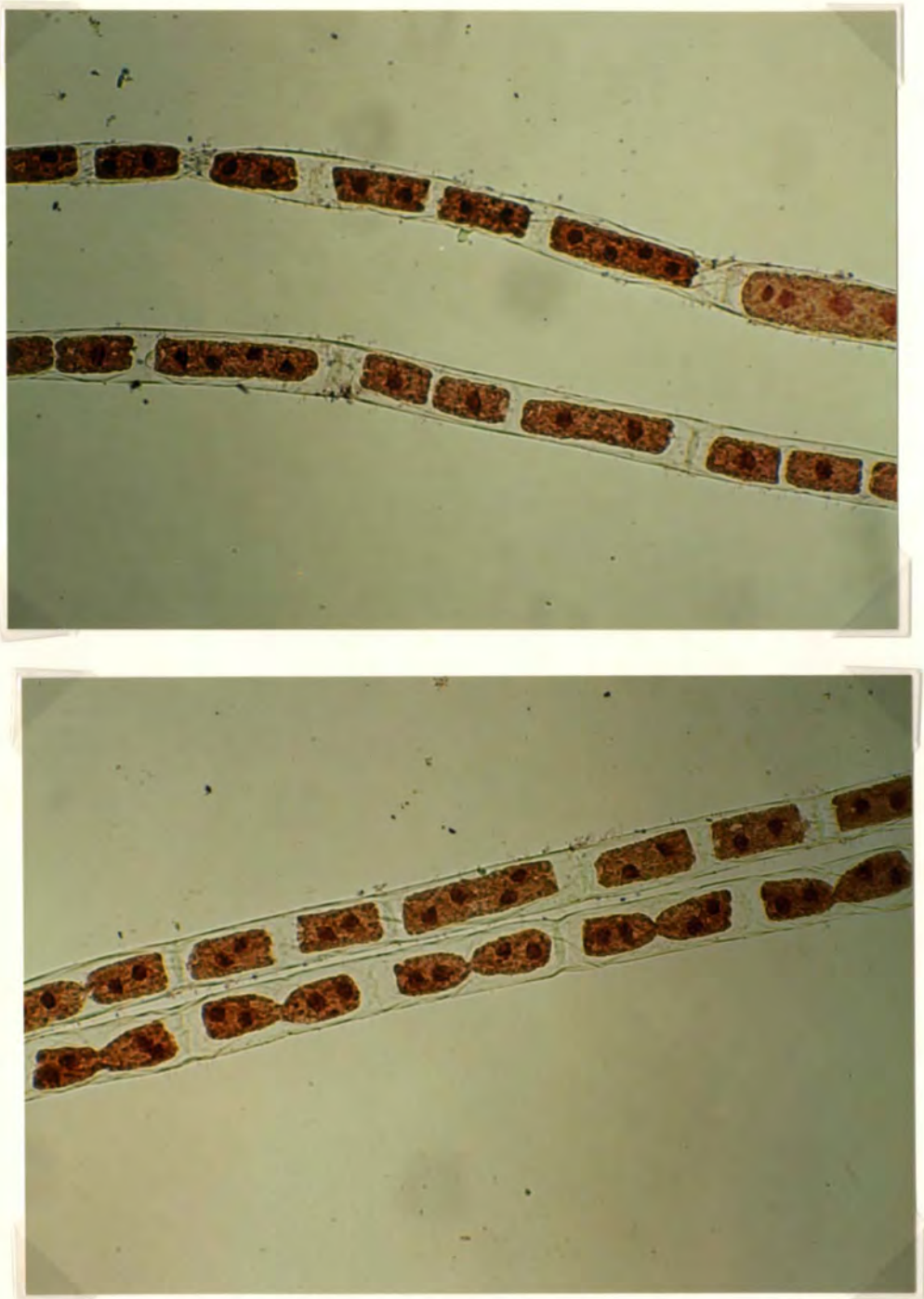


Plate 8. Light micrographs showing the multinucleate nature of the cells. The nuclei are arranged spirally along the length of the cell, (slides were stained with aceto-orcein).

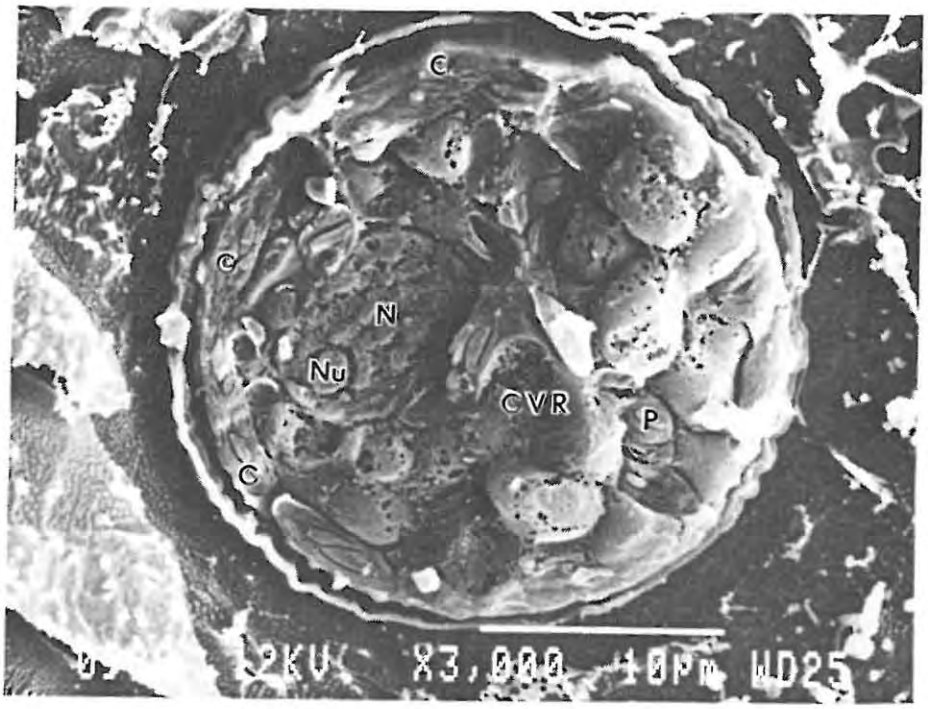
0.1mm

related algae such as Chaetomorpha brachygona (Chan et al, 1978), C. melagonium (Hanic and Craigie, 1969) and Cladophora glomerata (McDonald and Pickett-Heaps, 1976) but the cell wall of these algae have only two, rather than three cell wall layers.

The chloroplast of R. riparium, enclosed by a double membrane which is discontinuous with the endoplasmic reticulum and contains numerous pyrenoids, dominates the cell (Plate 9). The chloroplast reticulum appeared to be more dense in the cells from benthic filaments than from those of the floating mats (Plate 7). The chloroplast lamellae are made up of two thylakoid membranes pressed together and irregularly stacked to form granal and intergranal regions (Plate 5). This arrangement resembles that found in Chara in that the granal regions are more clearly defined than those typical of the green algae (Dodge, 1973). The pyrenoids are simple oval structures, enclosed by two starch grains, lying between the thylakoids and pierced by a single lamella (Plate 5). The number of pyrenoids per cell varied, but appeared to be greatest in cells of ~~floating~~ benthic mats (Plate 10).

Cells were observed to have a variable number of nuclei (1 to 9), with binucleate and quadrinucleate cells being the most abundant (Figure 2). Although the basal nuclei number is one, asynchrony between cytokinesis and karyokinesis during mitosis leads to the occurrence of the multinucleate condition (Bold and Wynne, 1978). The distribution of nuclei number within the cells of alga collected in the maturation

(a)



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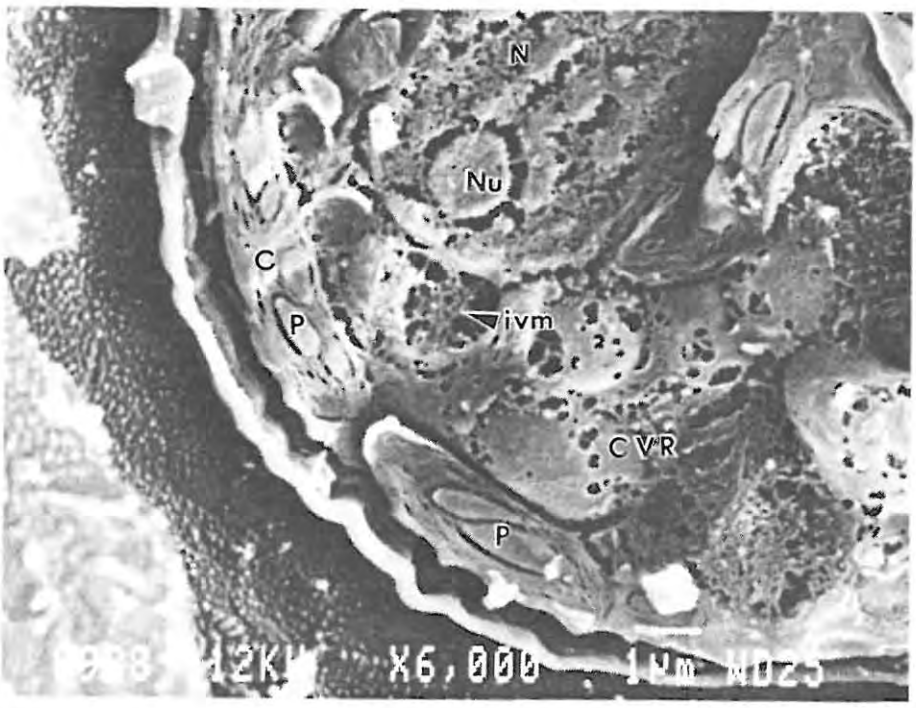
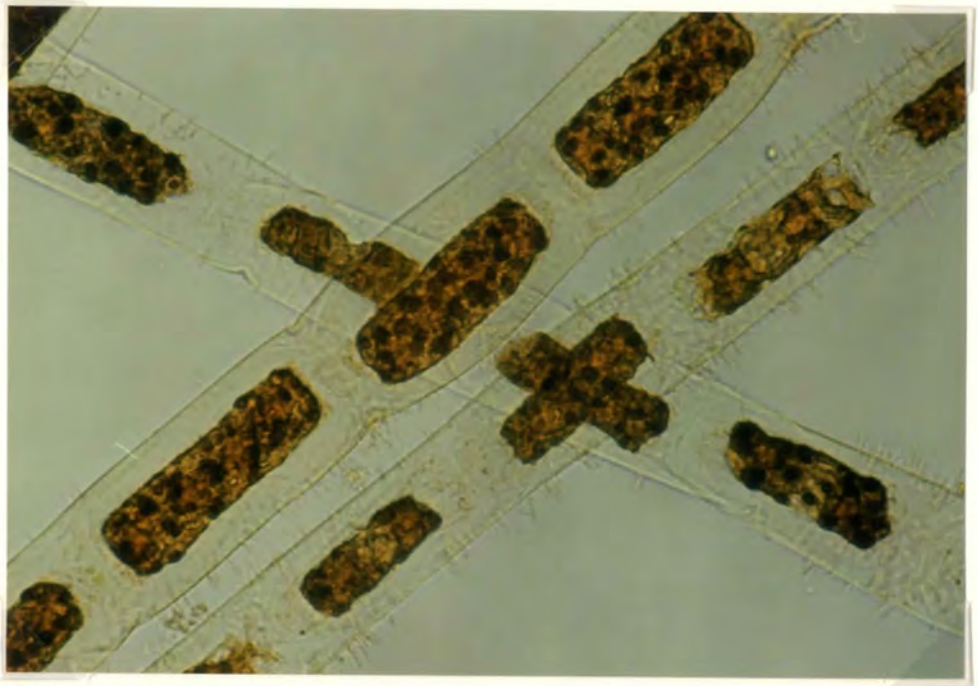


Plate 9. Scanning electron micrograph or a freeze-etched cell showing the arrangement of the reticulate chloroplast (C) around the central vacuolar region (CVR). The chloroplast includes numerous pyrenoids (P). The central vacuolar region is divided by intravacuolar membranes (ivm) and surrounds the nucleus (N) (nucleoli - Nu). (a) Cross-section through a cell. (b) Detailed view of part of the same cell.

(a)



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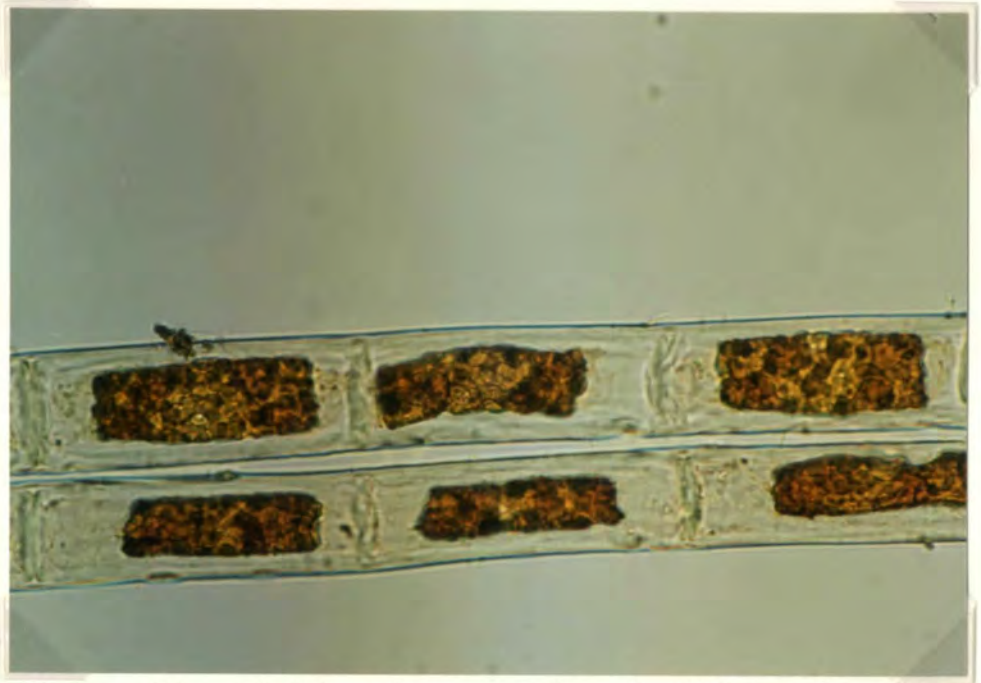

Plate 10. Light micrograph of filaments from benthic and floating mats stained with Lugol's solution to show the starch grains associated with the pyrenoids. The filaments of surface mats (a) have more pyrenoids per cell than those of benthic mats (b). 0.1mm

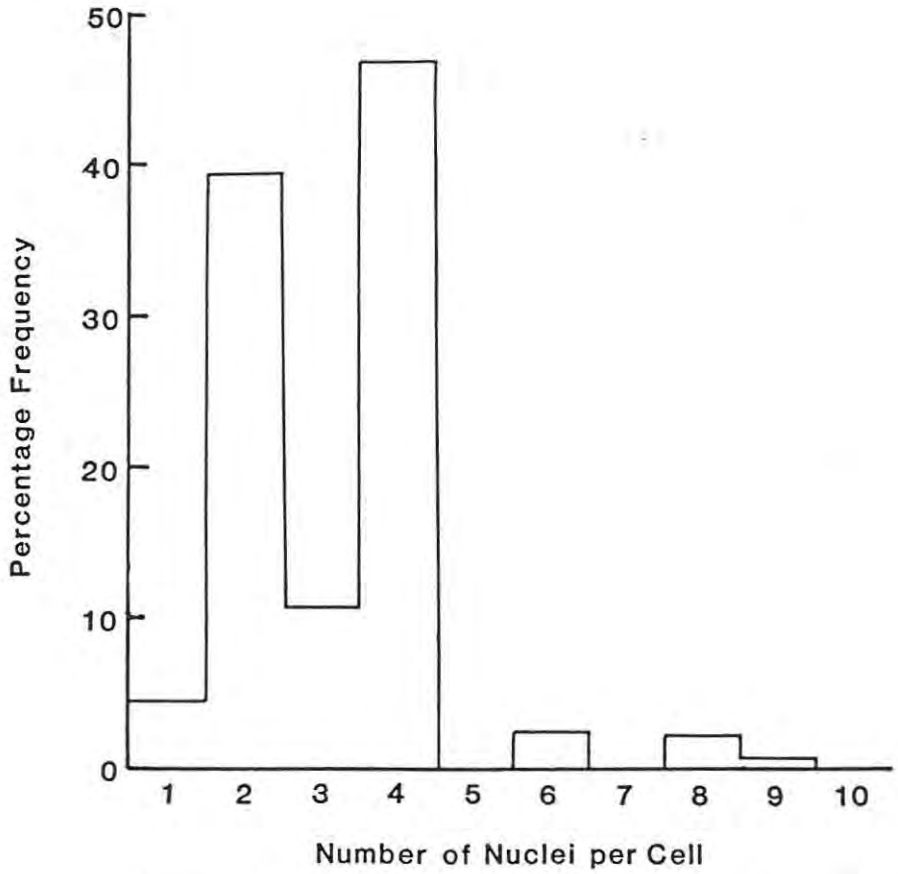

Figure 2. Frequency histogram of the number of nuclei per cell recorded in R. riparium from the maturation ponds.

pond followed a logical relationship. Asynchronous divisions of uninucleate cells give rise to the multinucleate series involving binucleate, quadrinucleate and octonucleate cells (Plate 8). This was the most common series observed in the material studied, accounting for approximately 80% of the cells. A second series, that including cells with three or six nuclei was less common (accounting for 12% of the sample), while a small percentage of the cells were observed to have nine nuclei (approximately 1%). The frequency distribution of nuclei number (Figure 2) is perhaps spurious in that counts included cells which had undergone nuclear division, but had not yet formed the dividing cell plate. Evidence for this postulate is shown in Plate 8. For example, a proportion of the binucleate cells would appear in the quadrinucleate count and some of the quadrinucleate would appear in the octonuclate stage. As cells with 16 or 18 nuclei were not observed, it is likely that eight and nine nuclei per cell is the highest nuclear number for this population, although up to 24 nuclei per cell have been observed in other R. riparium populations (Chapman, 1941).

The number of nuclei per cell appeared to influence the size of the cell. Cell volume increased at the nine nucleate stage, cell length increased to a maximum in the quadrinucleate cells and internal cell wall width remained relatively constant to the quadrinucleate stage and then increased (Figure 3). A similar relationship between cell length and nucleus number has been reported in other algae

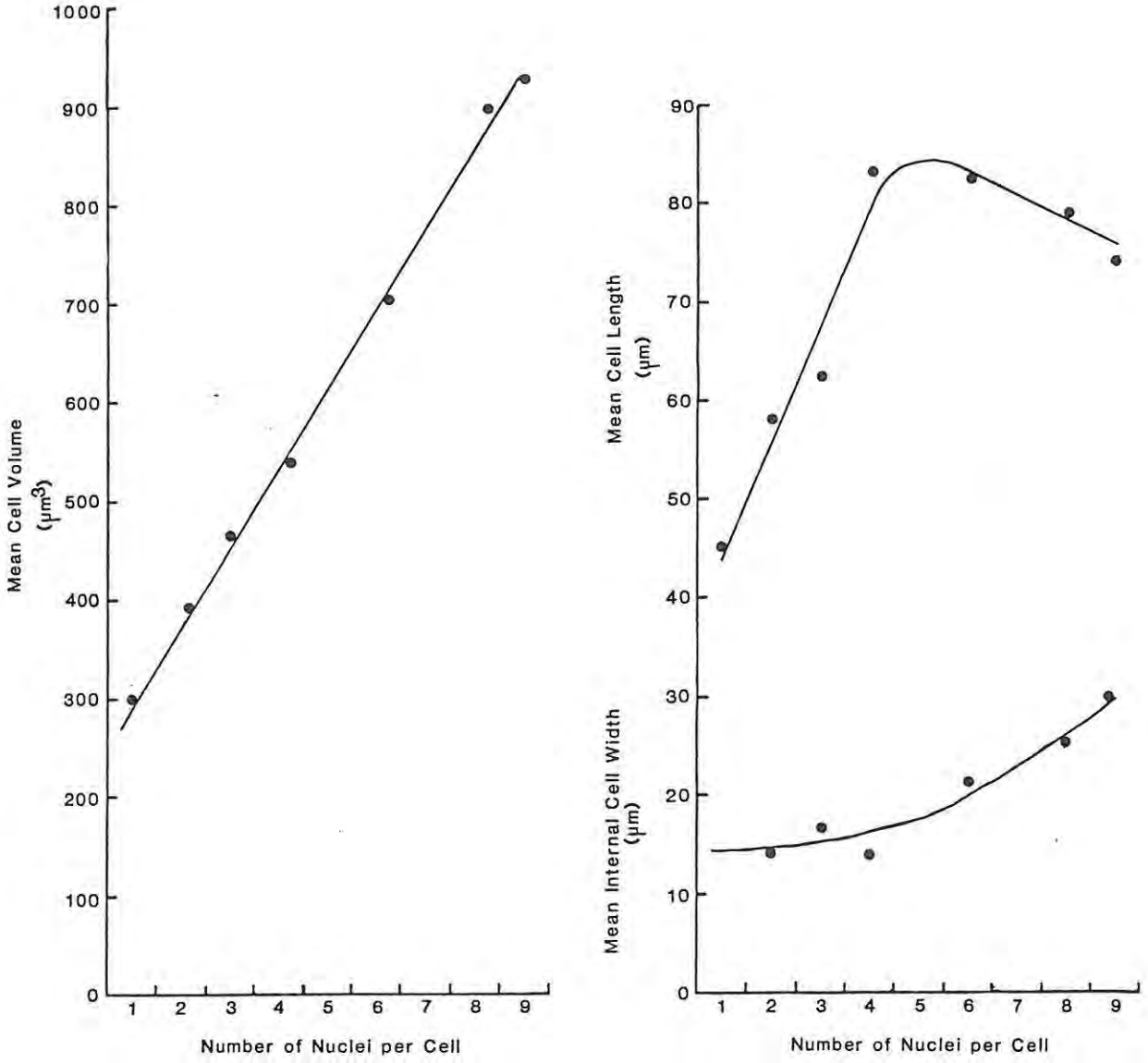

Figure 3. Relationship of cell length, internal cell width and cell volume with the number of nuclei per cell.

(Chapman, 1941; Smith, 1950; Chan et al, 1978), although these authors give no indication that there was a maximum cell length. There appear to be no reports on the relationship between cell volume or internal cell width with nucleus number. The results obtained in this study suggest that the cells increase in length until the maximum cell size is reached, when the nuclei divide. A second nuclear division may take place prior to cytokinesis, giving rise to the alternative nuclei numbers within each series, and accommodated by the increase in cell width. The odd series (cells with three or nine nuclei) arise when one or more of the daughter nuclei are lost during cytokinesis.

The dimensions of the R. riparium cells of the Grahamstown material did not vary significantly during the study period (Figure 4). This suggests that they are not ~~dependent on~~ influenced by the environment (Nienhuis, 1975). The differences in cell length, cell volume and the internal cell width of benthic and floating material were insignificant. However, the cell wall of benthic material was wider than that of floating filaments ($F=4.5179$; $p>005$) and this increased the diameter of these filaments ($F=5.6134$; $p>0.05$). This relationship between cell wall width and filament diameter is typical of filamentous algae, and is usually attributed to differences in the age of the cells (Nienhuis, 1975).

2.3.2 Taxonomy

The presence of grass-green chloroplasts (Plate 2) with

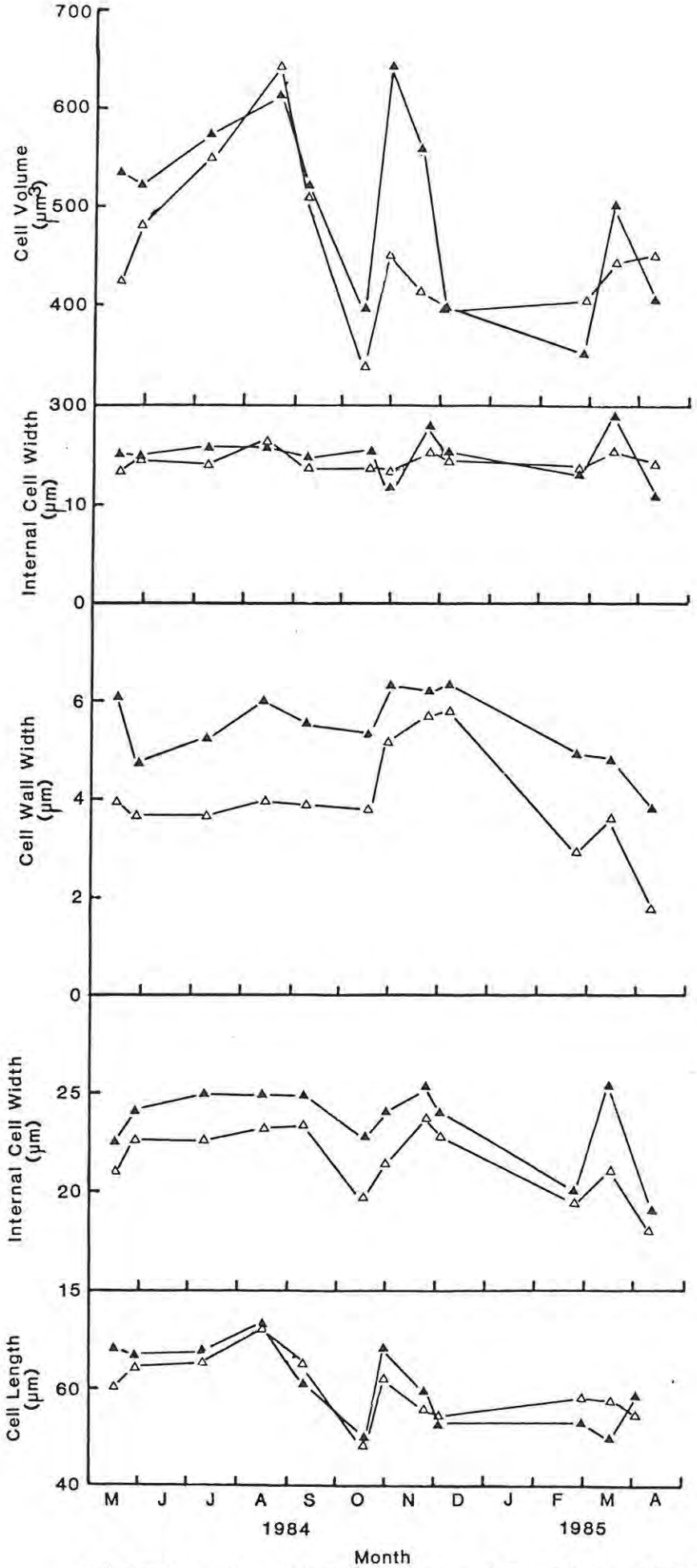

Figure 4. Variation in the cell dimensions of filaments growing in the maturation pond during the study period. (△ - surface material, ▲ - benthic material).

pyrenoids (Plate 10) confirmed that the alga was a member of the Chlorophyta. Its unbranched filaments and relatively narrow cells indicated that it was Rhizoclonium riparium (Roth) Harv. although the range of cell dimensions recorded were greater than those reported in the literature. This identification was later independently confirmed by three algologists (Nienhuis[3], Blair[4] and Compere[5], personal communication).

There is some confusion regarding the separation of the order Cladophorales (which includes Rhizoclonium) from the Siphonales. Fritsch (1935) maintained that the Cladophorales were a well defined group of algae which bore only a superficial resemblance to the Siphonales, but others suggest that the two groups are closely related and should be combined (Oltmanns, 1922, in Fritsch, 1935; Feldmann, 1938, in Chapman, 1964). More recently, Chapman (1964) and van den Hoek (1981) have advocated that the two orders be reduced to family and combined in a new order, the Siphonocladales. This taxonomic position will be adopted here.

The family Cladophoraceae includes the genera Rhizoclonium, Chaetomorpha, Cladophora, Pithophora, Urospora and Spongomorpha (Bold and Wynne, 1978). The genera are separated according to their mode of attachment, branching pattern, the

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3. P.N. Nienhuis, Delta Institute for Hydrobiological Research, 28 Yerseke, The Netherlands.
4. S.M. Blair, Harbor Branch Foundation, RR1, Box 196, Fort Pierce, Florida.
5. P. Compere, Jardin Botanique National, De Belgique, Domaine de Bouchout, B1860 Meise, Belguim.

number and position of nuclei and pyrenoids, cell shape and cell dimensions (Chapman, 1939; Bold and Wynne, 1978; Blair, 1983). Blair (1983) has suggested that the separation of Chaetomorpha and Rhizoclonium is invalid, because recent ecological studies have shown that these characteristics are either dependent on the environment (cell shape, mode of attachment) or vary according to the volume of the cell (number of nuclei and pyrenoids per cell) (Nienhuis, 1975). He also proposed that the similarity between chromosome numbers of the two species (both have a basic number of 6 chromosomes and show polyploidy) reflects a close relationship between them (Blair, 1983). However, a general overview of the family (Table 2) shows that on this basis the relationship between Rhizoclonium and Chaetomorpha is no closer than that between Rhizoclonium and Cladophora or Chaetomorpha and Cladophora (Godward, 1966).

The genus Rhizoclonium contains species with uniserate filaments which are usually unbranched, although some species do have colourless rhizoidal protrusions (Fritsch, 1935; Chapman, 1964). Filaments may be temporarily attached (Chapman, 1964), normally by a basal cell (Blair, 1983) which may have basal lobes (Bold and Wynne, 1978). The cells have a single parietal, reticulate choroplast with a varying number of pyrenoids and are uninucleate or multinucleate (Bold and Wynne, 1978). The LWR* of the cells is less than 6, and usually between 1 and 4 (Blair, 1983). Reproduction is by vegetative fragmentation, and by an isomormorphic alternation

* length : width ratio

Table 2. Chromosome numbers in the Cladophoraceae (Sinha, 1958 and Patel, 1961 in Godward, 1966; Perrot, 1965 in Blair, 1983).

Species	Chromosome Number Mitosis (2n)	Meiosis (n)	Author
Rhizoclonium riparium	36	18	Sinha, 1958
R. implexum	24	--	Sinha, 1958
	24	--	Patel, 1961
R. tortuosum	22	11	Patel, 1961
	24	--	Sinha, 1958
	20	10	Perrot, 1965
R. hieroglyphycum	24, 48	--	Patel, 1961
	24	--	Sinha, 1958
	24	--	Prasad, et al, 1973
Chartomorpha linum	18, 36	--	Sinha, 1958
	36	--	Patel, 1961
Ch. aerea	12	--	Patel, 1961
	18	--	Sinha, 1958
Ch. melagonium	12, 24	12	Patel, 1961
	--	18	Sinha, 1958
Cladophora glomerata	48, 96	--	Sinha, 1958
Cl. crispata	24	--	Sinha, 1958
Cl. fracta	24	--	Sinha, 1958
Cl. flexuosa	24	12	Sinha, 1958
	12, 24	12	Patel, 1961
Cl. sericea	12, 24	12	Patel, 1961
Cl. gracilis	36	--	Patel, 1961
Cl. pellucida	36	--	Patel, 1961
	--	18	Sinha, 1958

of generations with isogamous or anisogamous biflagellate gametes and biflagellate or quadriflagellate zoospores (Nienhuis, 1975; Blair, 1983).

The taxonomy of the genus has created many problems for phycologists, largely because its phenotypic variability was poorly understood when species were being described (Blair, 1983). Recently, phycologists have attempted to define the reliability of the morphological characters used by taxonomists and the extent to which the characters vary with the environment (Nienhuis, 1975; Blair, 1983). The following characters have been used (~~Kutzing, 1843, 1949; Stockmayer, 1890; Setchell and Gardner, 1920; Hamel, 1930; Blinding, 1957; Sinha, 1958~~; all in Nienhuis, 1975; Abbott and Hollenberg, 1976; Blair, 1983):

1. the habit of the plant,
2. filament diameter,
3. LWR of the cells,
4. thickness of the cell wall,
5. presence and shape of the rhizoids and lateral branches,
6. number and shape of the nuclei and their arrangement,
7. number of chromosomes, and
8. mode of attachment.

Nienhuis (1975) has shown that the differences in habit, cell dimensions, cell wall thickness, rhizoid and lateral branch occurrence, and the mode of reproduction varied according to environmental conditions, and could not be used to separate R. implexum and R. riparium. Nevertheless, in the

absence of more reliable, easily examined characters they are still used. For example, Blair (1983) used the habit of the plant, its mode of reproduction and the dimensions of the filaments and the cells to separate R. riparium and R. tortuosum, and Abbott and Hollenberg (1976) separated these species according to their range of cell diameter. As yet no taxonomic significance has been attributed to the data gathered concerning the number of chromosomes in R. riparium, R. implexum, R. tortuosum and R. hierogyphicum (Table 2).

R. riparium was first described, by Roth (1793; in Blair, 1983) as Conferva riparia which had a thin, twisted and bifuculate habit, with cells of width one half the length. Harvey (1845-1851; in Blair, 1983) transferred the species to Rhizoclonium and called it R. riparium. It was described as having filaments 18 to 30μm wide, and cells that were one and a half to two and a half times longer than wide (Koster, 1955). Koster (1955), while revising the Rhizoclonium species of the Netherlands recognised only 3 species: R. riparium (Roth) Harv., R. implexum (Dillw.) Kutz., and R. hieroglyphicum (Kutz.) Stockm.. She sunk the following genera into the single species, R. riparium (Roth) Harv.: Conferva riparia Roth, R. jurgensii (Mert.) Kutz., R. biforme Kutz. f velutinum Kutz., R. interruptum Kutz., R. affine Kutz., R. lacustre Kutz., R. lacustre f velutinum Kutz., R. riparium var implexum (non Dillw.) Rosenv., R. tortuosum (Dillw.) Kutz. and R. riparium f validum Foslie.

R. riparium was described as having filaments, with or without unicellular rhizoidal branches whose cells were 18 to 48µm wide, and had a LWR of 0.5 to 4.5 (mostly 1 to 2). She was uncertain as to the status of R. hieroglyphicum (C. Ag.) Kutz. which, although a freshwater alga, is morphologically similar to the brackish R. riparium in its dried form (Nienhuis, 1975). Nienhuis (1975) used culture experiments to show that there was extreme variability in the filament and rhizoidal morphology of R. riparium populations. As a result of his work he synonymized R. implexum and R. hieroglyphicum with R. riparium (Nienhuis, 1975). He disagreed with Koster (1955) on the sinking of R. tortuosum into R. riparium, preferring to consider it a single species as Chapman (1939) had done. Blair (1983) recognised two species distinguished by their cell diameter (8 to 48µm in R. riparium and 30 to 75µm in R. tortuosum), by their mode of reproduction (isogamous gametes in R. riparium, anisogamous gametes in R. tortuosum) and by their habit (R. riparium occupies the marsh areas and the high littoral zones, R. tortuosum is typically a sub-littoral species).

R. riparium is widely distributed throughout the temperate regions of the world, having been recorded in the Netherlands, Germany, Ireland, Japan, North and South America and in South Africa (Prescott, 1962; Nienhuis, 1975; Abbott and Hollenberg, 1976; Blair, 1983; Seagrief, 1984). It has an extremely diverse habit being holeuryhaline, occupying supralittoral and eulittoral regions on hard or soft

substratum (Nienhuis, 1975). In addition, it may form small mats entwined about other algae, flowering plants or poles; large woolly mats covering terrestrial or semi-terrestrial mud-flats; free-floating mats in quiet pools, or membranous masses in flowing streams (Nienhuis, 1975; Abbott and Hollenberg, 1976). It is probably best known for its place as the dominant green alga in many salt-marshes (Carter, 1933a; Carter, 1933b; Chapman, 1939; Neinhuis, 1975). Although it is not usually associated with eutrophic systems, being described as a typical clean water alga of North American systems (Palmer, 1980), it has been recorded in the maturation ponds of other South African sewage works eg Pretoria, Graaf-Reinet, Macassar township and Cape Receife.

2.4 GENERAL DISCUSSION

A literature search indicated that there has been very little research done on the ultrastructure of R. riparium. Because of the absence of directly comparable ultrastructural reports it was impossible to establish whether the material in the maturation pond was ultrastructurally unusual. However, the difference in cell wall structure to that of other members of the Cladophoraceae suggests that it may be. The general description provided in this study forms a basis for further work into the variability of R. riparium with respect to its benthic or floating position, in addition to differences between this population and those in other areas.

There are a number of reports on the morphological and

cytological variability of Rhizoclonium species, conducted primarily as an attempt to characterise them fully (Koster, 1955; Nienhuis, 1975; Blair, 1983). Like that recorded in the Netherlands (Koster, 1955; Nienhuis, 1975) and North America (Blair, 1983) the morphology of cells in the maturation pond was highly variable. However, the range of morphological characteristics was often wider than previously described. For example, Blair (1983) describes the range of cell diameter in R. riparium as 8 to 48μm, while that of the maturation pond population was from 11.8 to 70.5μm, and the species is usually described as multinucleate (eg Bold and Wynne, 1978) but approximately 10% of the cells examined in this study were uninucleate. As both of these characteristics are used in the taxonomic description of Rhizoclonium species it is important that the full range of morphological variability is known.

Although cell cytology was not investigated in detail there were obvious differences between the cells of the sub-populations with regard to the degree of vacuolisation, the development of the chloroplast reticulum and the number of pyrenoids per cell. The cells of the floating filaments were more vacuolated and had an open chloroplast with many pyrenoids while those of benthic filaments had a dense, dark-green chloroplast with fewer pyrenoids which filled the cell leaving little room for vacuolisation. The same differences in chloroplast structure and colour were recorded between algal samples collected in the supralittoral and sublittoral

regions of a salt-marsh (Nienhuis, 1975). They may be attributed to the differences in the light climate of the two environments (Section 3.3.2). Benthic cells possess elevated chloroplast levels to increase their photosynthetic efficiency by trapping a larger percentage of the attenuated light (Section 5.3). The resultant larger chloroplast is accomodated by the reduction the area of the vacuolar region. The abundance of pyrenoids in floating cells relative to that in benthic cells was due to the difference in the growth rate and the maturity of the cells, as observed in Chaetomorpha brachygona (Chan et al, 1978) and R. riparium (Nienhuis, 1975).

Although the extensive vacuolar system of the Cladophoraceae has been noted by several authors (eg McDonald and Pickett-Heaps, 1976; Scott and Bullock, 1976; Chan et al, 1978) and has been compared to to that of other algae eg Batrachospermum (Brown and Weier, 1970), Euglena gracilis (Osafune, 1973), Penicillus (Turner and Friedmann, 1974), Derbesia tenuissima (Wheeler and Page, 1974) and Coelastrum (Chan, 1978) there is little idea as to their contents. These were not examined in the present study but, because of their staining response to osmium tetroxide it is unlikely that the contents are lipid or gaseous in nature. They would therefore not have reduced the cell density sufficiently to increase mat buoyancy (Hutchinson, 1967). The mats probably became buoyant when oxygen produced during photosynthesis is released into the water, and becomes trapped within the

filament mesh. A similar mechanism has been described for populations of Cladophora (Brand, 1902; Wesenburg-Lund, 1903; both in Norton and Mathieson, 1983), blue-green algae (Phillips, 1963; in Norton and Mathieson, 1983) and Schizonema (Nelson, 1947).

2.5 CONCLUSIONS

1. The filamentous mat-forming alga from the maturation ponds of the Grahamstown sewage works was Rhizoclonium riparium (Roth) Harv. (Chlorophyta, Siphonocladales, Cladophoraceae). It is characterised by unbranched, undifferentiated filaments of uninucleate or multinucleate cells with parietal reticulate chloroplasts, lamellate cell walls and a peripheral vacuolar layer. The material displayed the potential for differentiation in culture, where rhizoidal holdfast cells were formed.

2. The variability of certain taxonomic criteria normally used to identify this species was found to be greater in the material collected in the Grahamstown maturation ponds than those reported in the literature. This applied particularly to cell dimensions and the number of nuclei per cell. This study has demonstrated that caution should be taken before rigidly applying such criteria to identifying this species.

3. Although R. riparium is usually reported to be multinucleate, uninucleate cells were observed in the Grahamstown material. In addition the material exhibited a frequency of nuclear number distribution in which binucleate

and quadrinucleate cells predominated. The maximum number of nuclei per cell observed was 9. The increase in nuclei number was related to cell volume which increased as cells lengthened up to the quadrinucleate stage, after which cell volume was increased by increasing the internal cell width.

4. Benthic and floating populations varied in morphology (filament diameter and cell wall width) and in cytology (chloroplast development, pyrenoid number and size of the vacuolar region). This variability can be ascribed to the difference between the environment at the pond surface and its floor, and to cell age.

3.0 DESCRIPTION OF THE STUDY AREA

3.1 CLIMATE

Grahamstown lies on the border of three climatic zones: the southern steppe, Karroo and south eastern Cape coastal belt regions (Schultze, 1947). The climate is characterised by warm summers and mild winters with occasional frost (Schultze, 1965). Temperatures are similar to those recorded along the coast although diurnal ranges are usually higher (Rhodes University Hydrological Research Unit - unpublished data). Absolute maximum and mimimum temperatures are 45^{o}C and 5^{o}C respectively, and the mean annual temperature is 17^{o}C. February is usually the warmest month with an average temperature of 24.5^{o}C, while July, with an average temperature of 12^{o}C is the coolest (Schultze, 1965). Rain falls throughout the year but there is usually an August/September spring maximum. The mean annual rainfall of the region is 673mm.

3.2 THE GRAHAMSTOWN SEWAGE DISPOSAL WORKS

The Grahamstown Sewage Disposal Works lies in the Beaumont Valley, 5km east of Grahamstown (33^{o}20'S; 26^{o}38'E) at 553m above mean sea level (Figure 5). It was commissioned in 1937 and enlarged in 1978 to cope with the increased sewage load of the expanding city. At the present time approximately 2500m^{3} of raw sewage is treated at the Works daily (Riley,

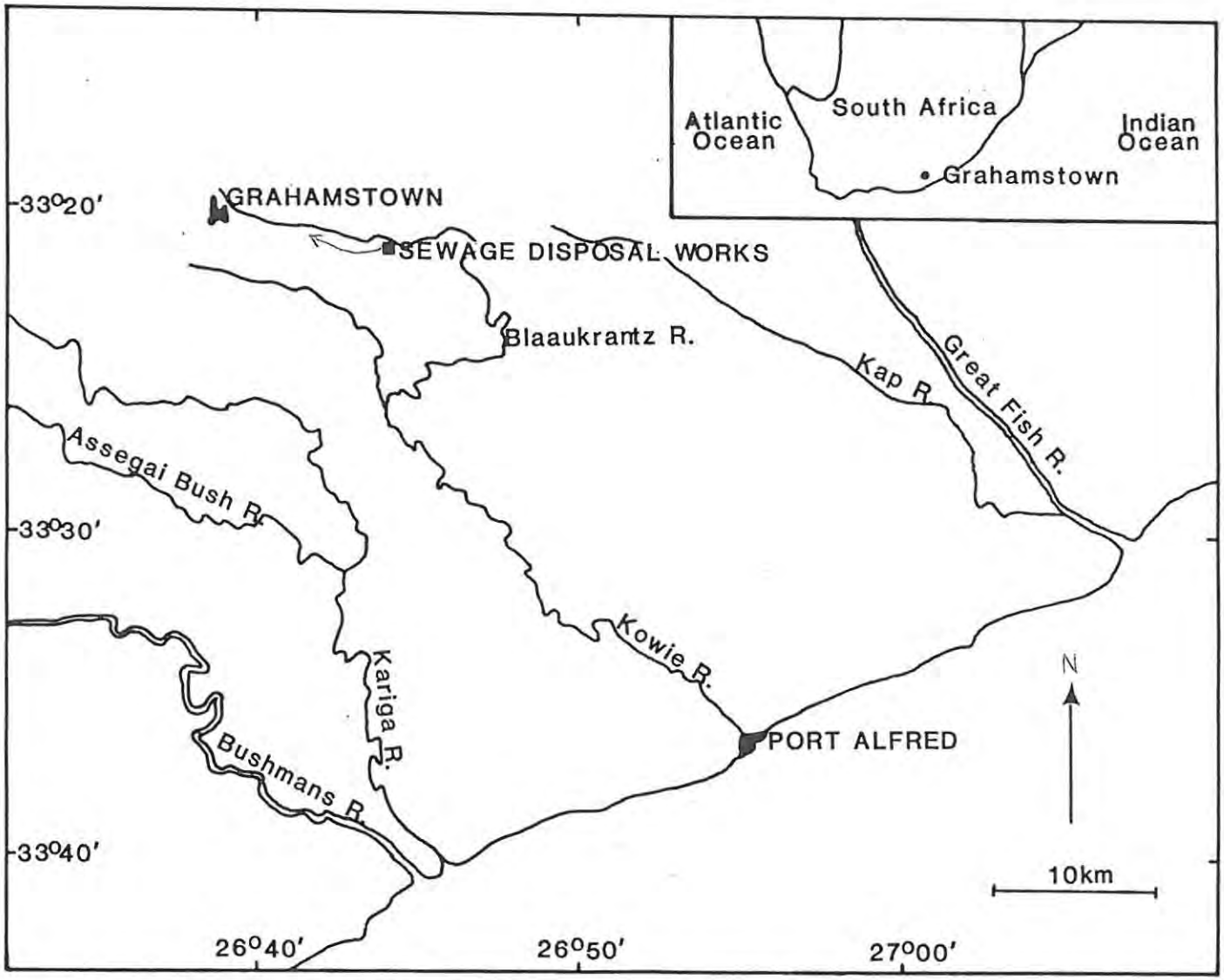


Figure 5. Map of the area between Grahamstown and the coast, showing the position of the Grahamstown Sewage Disposal Works relative to the city and to the drainage system of the area.

personal communication).

The raw sewage enters the Works at point A (Figure 6) where the flow rate is recorded and intractable solids removed by a coarse screen. It then passes through grit channels where the rate of flow is decreased so that the heaviest solids settle out of suspension. The effluent then enters a settling tank where approximately two thirds of the remaining solids and one third of the remaining biochemical load is separated from the effluent by physical sedimentation. The sludge is drawn off to the digesters while the effluent passes through four trickling filters, which are arranged in parallel. These beds of crushed stone support a large heterotrophic community which feeds on the organic matter trickling over them, removing a large proportion of the remaining biochemical load. From these filters the effluent passes through a series of six maturation ponds before being discharged into the Blaaukrantz river (Figure 6).

All six maturation ponds in the Works have earthen floors and cemented walls. Their morphometric characteristics are given in Table 3.

3.3 THE MATURATION POND

Algal mats were most prolific in ponds 4 and 5, although lesser quantities were present in each of the others. This was probably because they were the largest of the maturation ponds and had longer retention times (Table 3). After the largest pond (pond 4) was scoured during April, 1984 it was

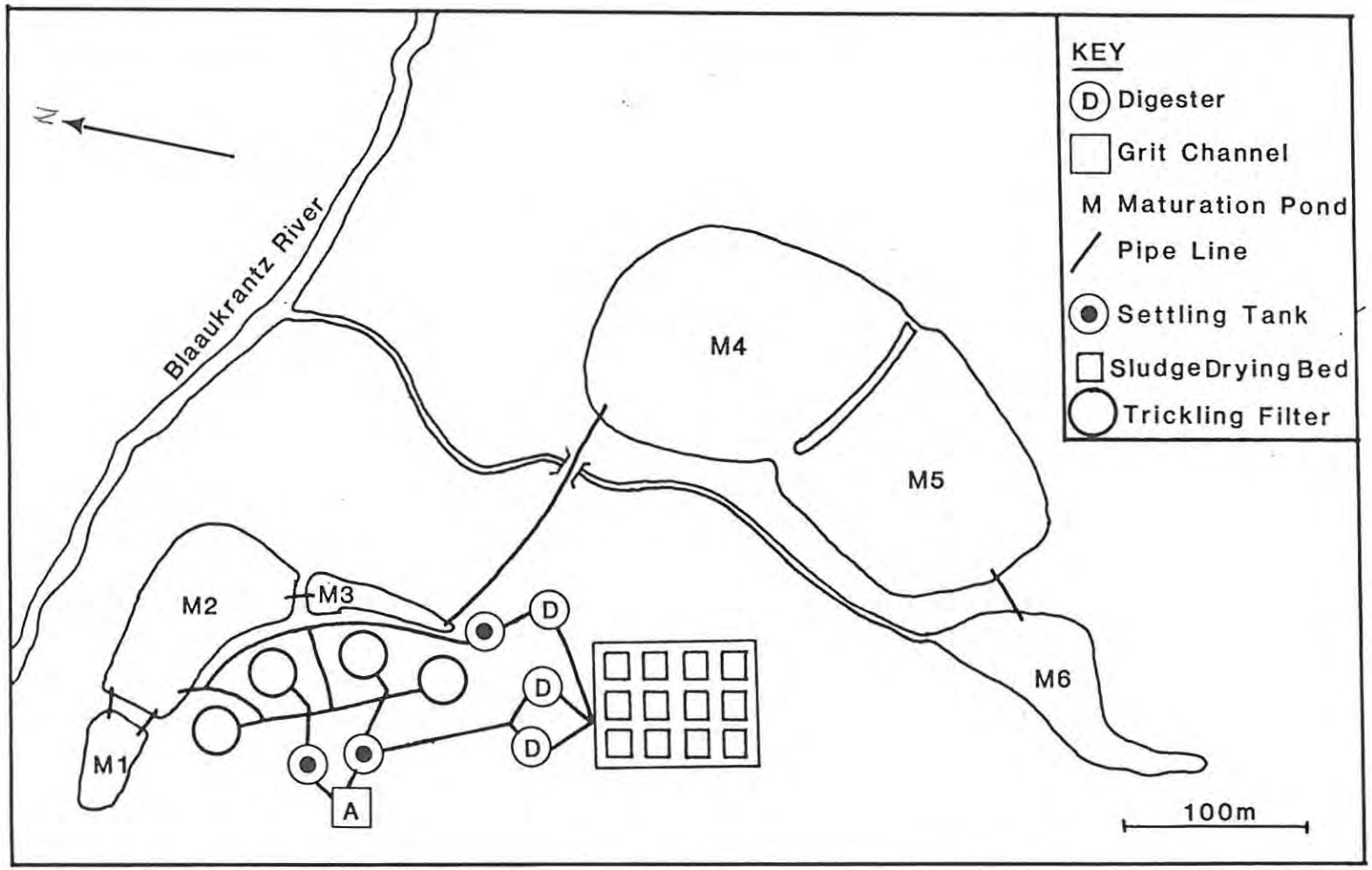


Figure 6. Layout of the Grahamstown Sewage Disposal Works.

Table 3. Morphometric characteristics of the maturation ponds of the Grahamstown Sewage Disposal Works. Pond numbers correspond to those shown in Figure 6.

MATURATION POND NO.	MORPHOMETRIC PARAMETER			
	Surface Area (ha)	Mean Depth (m)	Volume (m^3)	Retention Time (days)
1	0.081	0.8	988.6	0.360
2	0.393	0.8	4897.7	1.959
3	0.049	0.8	602.3	0.241
4	0.911	0.8	11136.4	4.455
5	0.798	1.4	5602.3	2.241
6	0.385	1.1	3636.4	1.455
TOTAL	2.617	-	26863.7	10.746

selected as the study pond since the removal of plant material provided the opportunity to investigate the development of algal mats without complications caused by clogging, shading and other factors.

3.3.1 MATERIALS AND METHODS

Sample Collection and Field Observations

In order to characterise the variation in environmental conditions in which the alga occurred, the pond was examined at approximately fortnightly intervals over a period of 12 months (May, 1984 to April, 1985). As there was no significant spatial variation in water quality within the pond (Roman, 1982; Snook, 1983) sampling was performed at the pond centre. Replicate samples for chemical analysis were collected from a depth of 0.5m using a bilge pump, and were stored in acid-washed polythene bottles.

Profiles of dissolved oxygen (DO) and water temperature were measured between 9.00 and 9.30am on each of the routine sampling occasions and at regular intervals from 6.00am to 6.00pm on the 1 October and 19 November, 1985. On these occasions profiles were also measured under an area in which extensive floating algal material occurred. Measurements were taken at 0.5m (or 0.25m on the special sampling occasions) intervals through the water column. Samples for the determination of DO were collected using a bilge pump and the water transferred to acid-washed glass-stoppered bottles. Samples were fixed in the field whilst oxygen concentrations

were measured on return to the laboratory by Winkler titration (Golterman and Clymo, 1971). Results were converted to percentage saturation corrected for altitude, using the nomogram method of Golterman and Clymo (1971).

Water temperature at each depth was measured (to 0.1^{o}C) by means of a standard mercury thermometer which was inserted into the samples immediately following collection. On 1 October and 19 November, 1985 the temperature profiles within the pond were measured at half-hourly and hourly intervals respectively.

At 12.00 noon on routine sampling visits light profiles were obtained using an integrating-quantum radiometer/photometer (Licor LI-1883) which measured down-welling irradiance in the photosynthetically active range (PAR - 400 to 750nm). Light profiles under algal mats were made at 5cm intervals in covered areas. Light meter readings were used to calculate the mean diffuse attenuation co-efficient of light (k) using the formula from Moss (1980):

$$k = \frac{\ln I_o - \ln I_z}{z}$$

where, I_o is the irradiance at depth zero metres,

I_z is the irradiance at depth z metres, and

z is the distance between depths o and z, in metres.

Preliminary observations of the filamentous algal population within the maturation pond indicated that its size varied seasonally. Quantifying these changes proved to be difficult

because monitoring growth on the pond floor was impossible. Benthic mats could only be examined by diving below the effluent surface which imposed a health risk. Collecting samples from the surface mats was possible, but monitoring their expansion was complicated by the removal of mats by the staff of the Sewage Works in order to keep the system unblocked, and also by wind-driven biomass movement. Attempts made to measure the in situ growth rate by enclosing sample areas with plastic netting were unsuccessful as the alga attached and grew onto the netting rather than rising from the pond floor to the surface thus facilitating its collection for biomass estimates. Furthermore, when the growth on the nets was examined it was clear that the young algal filaments were competing with epiphytic and periphytic communities (mainly diatoms and blue-green algae) for an attachment area, and that filament growth was adversely affected. Isolating the filaments in a diaphanous tube was impossible as cellulose bacteria rapidly degraded the tube and allowed the entry of contaminants. It was finally decided that the extent to which the algal mats covered the pond surface would have to be estimated visually on an aerial basis in order to obtain an indication of the development of algal biomass. It was noted that the floating mats were only a fraction of total biomass, but as their biomass was partially dependent on the benthic mat for their development, benthic expansion was adequately represented.

Algal samples were collected from both benthic and floating

mats. They were washed to remove debris before preserving in 5% formalin. Material was later identified using the keys of Prescott (1978) and Blair (1983).

Laboratory and Chemical Methods

Water samples were returned to the laboratory within 1 hour of collection. Conductivity, pH and alkalinity were measured immediately. Samples were then filtered through Whatman GF/C filters and the filtrate used for the determination of inorganic nitrogen forms (nitrate, nitrite and ammonium) and orthophosphate concentration. The filters were used to measure phytoplanktonic chorophyll a concentrations, corrected for phaeophytin content (Sartory, 1982). All spectrophotometric measurements were made on a Bausch and Lomb Spectronic 20 Spectrophotometer. Calibration standards were prepared with each series of analyses. Samples were diluted so that the nutrient concentration lay within the range measured most accurately by the method used. All analyses were completed within 24 hours of sample collection.

A Zeiss dds (200) meter with pH probe, calibrated against standard buffers of pH 7.00 and 10.00, was used to measure pH. Specific conductivity was measured using a conductivity probe attached to the same instrument.

Total alkalinity was estimated using the titrimetric method recommended by Golterman and Clymo (1971).

Orthophosphate (PO_4-P) concentrations were determined using the molybdate reduction method described by Golterman and

Clymo (1971).

Nitrate (NO_3-N) was estimated using the cadmium reduction method, and nitrite (NO_2-N) using the Griess-Ilosvay method, both described by Mackereth, Heron and Talling (1978).

Ammonium (NH_4-N) was determined using a modification of the phenol-hypochlorite method (Solorzano, 1969).

3.3.2 RESULTS AND DISCUSSION

During the study period the water temperature at the pond's surface varied between 13.1 and 27.0°C (Table 4). On most routine sampling occasions the water temperature at the surface was higher than at the bottom of the pond. The temperature difference between surface and bottom (ΔT) showed wide variation (CV=100%) ranging from 0.0 to 1.5°C, with an average of 0.6°C. Although this data suggests that the pond was usually "stratified", more detailed diurnal observations made on two occasions under different weather conditions indicated that ΔT varied throughout the day (Figure 7). This is typical of shallow water bodies which rarely exhibit stable stratification (Ernst and Reinhardt, 1980).

The differences observed between the temperature profiles of areas covered by mats and the open areas indicate that the mats play a significant role in influencing water temperature. On both special sampling occasions (1 October and 19 November, 1985) surface and bottom water temperature in mat covered areas of the pond were higher than those in

Table 4. Temperature and light conditions recorded in the pond during the study period. The light intensity at 1.2m (the pond bottom) was estimated using the attenuation co-efficient (k) calculated using the formula from Moss (1980). The depth of the photic zone is based on 1% of surface light intensity being the limit of the photic zone (Talling, 1971). ΔT is the difference between the temperature of surface and bottom water.

DATE	Temperature		ΔT	Light Intensity at:		Estimated Depth of the Photic Zone	k area	
	Surface	Bottom		0m	1.2m		covered	uncovered
	°C		°C	$\mu E.m^{-2}.s^{-1}$		m	m^{-1}	
1984								
24 May	16.0	15.3	0.7	918	21	1.46	136.4	3.15
11 July	13.1	12.5	0.6	1383	29	1.55	144.6	2.98
25 July	14.0	14.0	0.0	1242	37	1.57	142.5	2.94
13 August	14.5	14.3	0.2	1181	30	1.51	141.5	3.05
5 September	15.2	15.0	0.0	-	-	1.81	143.9	2.54
21 September	20.8	18.8	2.0	2076	11	1.05	152.8	4.39
5 October	17.2	16.8	0.2	1697	78	2.93	148.7	2.57
18 October	21.0	20.5	0.5	2144	98	1.97	153.4	2.57
1 November	20.5	19.0	1.5	2114	47	1.45	153.1	3.18
20 November	22.0	22.0	0.0	2146	125	1.94	153.4	2.37
4 December	20.9	20.6	0.3	2320	182	2.17	155.0	2.12
1985								
30 January	19.4	18.8	0.6	1989	69	2.02	151.0	2.80
28 February	27.1	26.5	0.6	1781	7	1.01	149.7	4.57
18 March	22.7	22.1	0.6	2022	102	3.08	152.2	2.49
2 April	25.7	24.9	0.8	297	20	2.07	113.9	2.23
Mean	19.3	18.7	0.6	1665	61	1.84	146.4	2.93
S D [+]	4.2	4.1	0.6	584	51	0.59	10.48	2.65
C V [*] (%)	21.8	21.9	100.0	35	84	32.1	7.16	90.49

[+] S D - standard deviation
[*] C V - co-efficient of variance

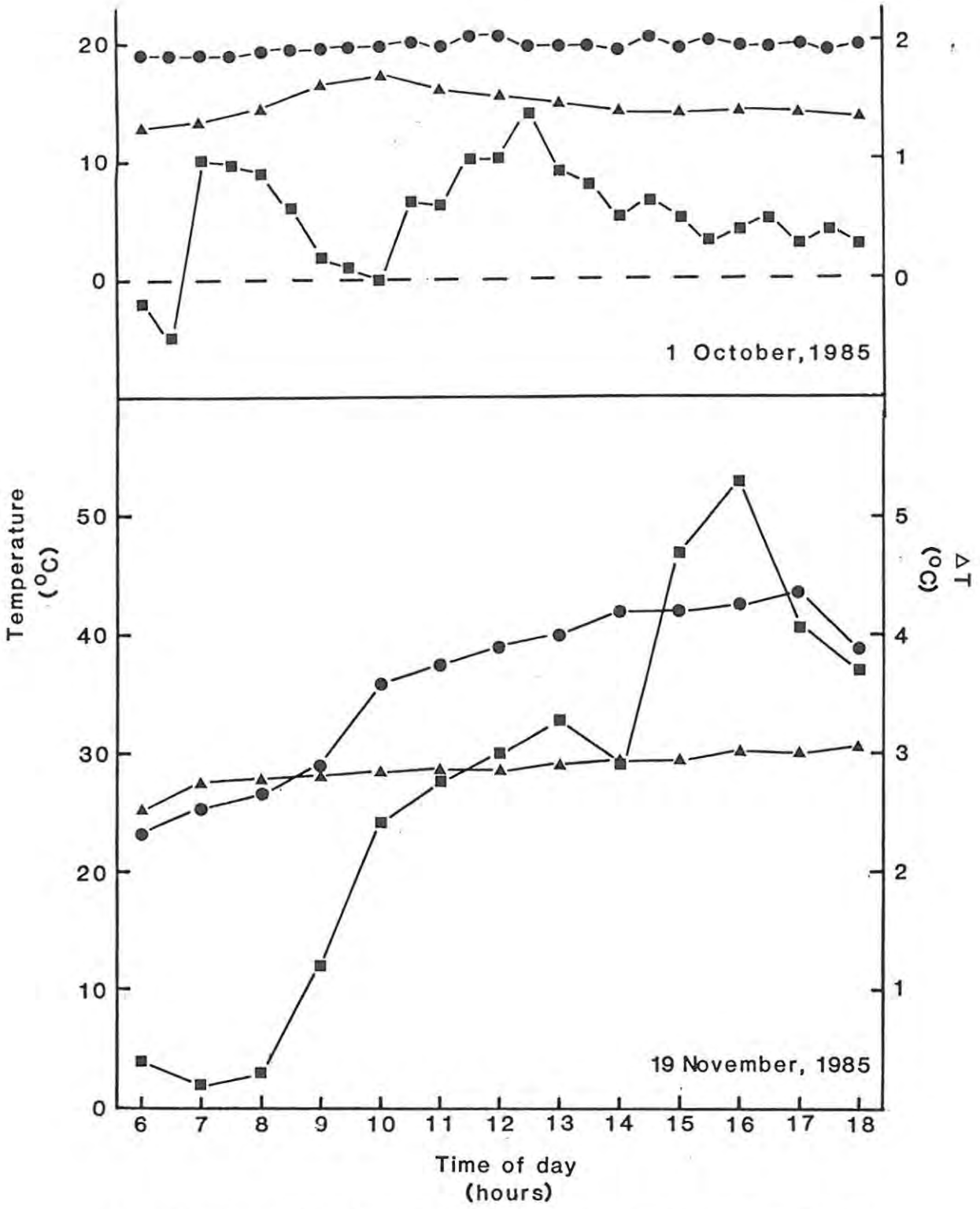


Figure 7. Variation in the air temperature (●), surface water temperature (▲) and ΔT (the difference in water temperature between surface and bottom water - ■) on the 1 October when it was cool, windy and overcast, and on the 19 November when it was clear, calm and exeptionally hot.

the open area (Figure 8). This can be explained on the basis of several influencing factors:

1. Inhibition of evaporative cooling from the water surface;
2. Inhibition of wind mixing so that heat transfer through the water column is limited by the relatively slow process of diffusion;
3. Prevention of the penetration of radiant energy into the water column by increasing the amount of light reflected off the water surface and the absorption of radiant energy. The net result would be a greater than normal proportion of radiant energy being absorbed by the surface waters and an increase in the temperature of the water column.

Light attenuation in natural water is mostly influenced by suspended and dissolved substances (Hutchinson, 1957). Maturation pond water has a high humic content (Plate 11) and with the presence of large phytoplankton populations would be expected to show high k values. Pure distilled water has a k value of 0.05 to $0.10m^{-1}$ for PAR. By comparison, k values for the pond varied between 2.12 and $4.57m^{-1}$ (Table 4). Despite these high values light always penetrated to the bottom of the pond as indicated by light meter readings (Table 4). Using the average k value it is possible to estimate the depth of the euphotic zone (the depth to which 1% of surface light penetrates - Talling, 1971). This depth was usually greater than the maximum depth of the pond, which suggests that the intensity received at the pond floor was capable of supporting positive net photosynthesis.

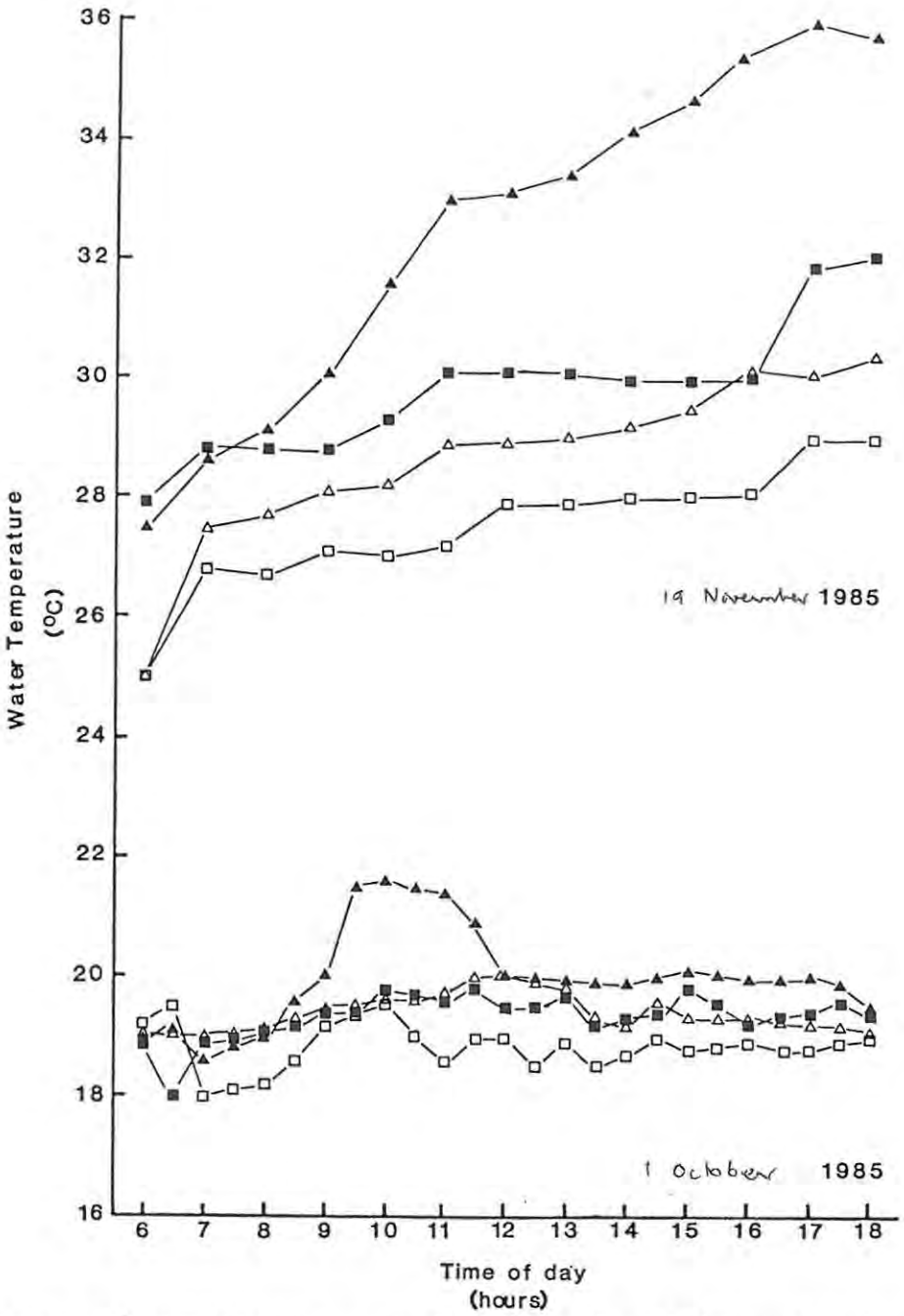


Figure 8. Variation, during the daylight period, in the temperature of surface and benthic waters, in areas of the pond which were covered by floating mats (▲ - surface, ■ - benthic) and those free (△ - surface, □ - benthic) of floating mats. The effect of the mats was compared when weather conditions were overcast and cool (1 October, 1985) and when they were clear and exceptionally hot (19 November, 1985).

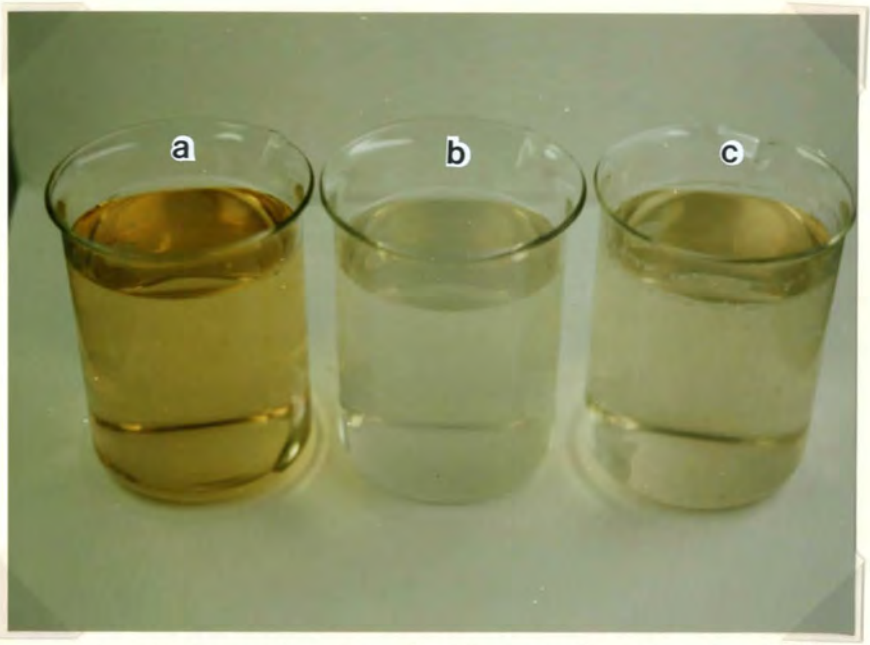


Plate 11. Comparison of the colour of effluent collected at the inflow pipe of pond 4 (a) and the centre of the pond (c) with that of tap water (b). The effluent is stained with dissolved humic substances.

The algal mats have a marked effect on the penetration of light in the pond, in that they increased attenuation (Table 4) such that no radiant penetrated below 5cm. These observations are similar to those reported by Zohary (1985) which showed that no light penetrated 2 to 3cm below the crust of hyperscums on Hartbeespoort Dam. By increasing the amount of light absorbed, as well as that reflected by the debris trapped amid the filaments, the mats considerably diminished the depth of the pond's euphotic zone.

The results of chemical analyses are presented in Table 5. During the study effluent pH varied from 6.7 to 7.7 with a mean of 7.26. Routine monthly measurements of pH (collected by the Leather Industry Research Institute, Grahamstown) indicate that the effluent pH varied more widely than the values in this study suggest. Their records show that during the study period pH varied between 5.7 and 7.6 with a mean of 6.9.

The mean total alkalinity of the effluent was 1.77meq.l^{-1} which indicates that it was generally well buffered despite a wide variation (1.01 to 2.92meq.l^{-1}). As pH was always below 8.4, alkalinity was principally due to the presence of bicarbonate ions (Golterman and Clymo, 1971).

Effluent conductivity varied between 14.99 and 18.85mS.m^{-1}, which is below the quality criteria range for drinking water (30 to 200 mS.m^{-1} - Kempster, Hattingh and van Vleit, 1980). Conductivity was also lower than in effluent from other

Table 5. Chemical characteristics of effluent in pond 4 during the study period.

Date	pH	Total Alkalinity	Conductivity	Dissolved Oxygen		Inorganic Nitrogen			Orthophosphate
		meq.l^{-1}	mS.m^{-1}	% Saturation		NO_3-N	NO_2-N	NH_4-N	PO_4-P
				Surface	Bottom	mgN.l^{-1}			mgP.l^{-1}
1984									
24 May	7.25	2.92	18.85	29.6	24.6	6.84	0.42	17.55	13.46
11 July	7.35	2.10	16.46	63.9	61.1	12.30	0.61	3.16	21.30
25 July	7.27	1.68	15.42	50.9	49.1	21.50	0.65	2.71	21.25
13 August	7.22	1.96	16.20	103.7	54.6	8.10	0.70	1.95	19.12
5 September	7.40	2.08	16.68	92.6	76.9	7.06	0.65	16.03	13.32
21 September	7.13	2.20	16.83	55.6	11.0	4.75	0.61	18.16	15.41
5 October	7.38	2.10	14.99	39.8	25.9	12.58	0.35	14.04	9.09
18 October	7.56	1.16	16.04	116.7	62.0	13.70	0.54	15.08	13.14
1 November	7.70	1.20	16.06	55.6	70.4	15.04	0.27	12.43	14.62
20 November	7.29	1.02	14.99	24.5	30.1	19.90	0.56	13.07	14.62
4 December	7.62	1.01	14.99	31.5	32.4	12.87	0.34	10.71	16.40
1985									
30 January	7.17	1.98	14.13	71.0	53.5	12.60	0.42	7.02	15.29
28 February	6.70	2.10	15.72	53.4	28.1	13.53	0.21	6.98	16.75
18 March	6.92	1.86	16.13	36.1	42.6	12.34	0.27	5.86	16.34
2 April	7.01	1.20	15.98	23.2	23.2	11.60	0.03	9.01	9.05
Mean	7.26	1.77	15.96	56.54	43.0	12.31	0.44	10.25	15.28
S D [+]	0.26	0.57	1.09	28.83	19.7	4.52	0.20	5.46	3.59
C V [*]	3.58	32.26	6.83	50.99	45.8	36.72	45.5	53.32	23.49

[+] S D - standard deviation
[*] C V - co-efficient of variation

sewage works (Table 6). However, the chemical characteristics of the pond, namely the generally high concentrations of all constituents, is possibly a reflection of the nature of sewage which is domestic rather than industrial.

The amount of DO* in a water body at any time is the product of a complex set of interactions between factors which increase the DO concentration (eg temperature, photosynthesis and atmospheric diffusion) and those which reduce it (eg temperature and respiration) (Hutchinson, 1957; Eberly, 1963). In the maturation pond, where there is a high biological and chemical oxygen demand (Irwine, 1983) there would be a tendency toward anaerobic conditions which must be counter-balanced by wind action and photosynthesis if purification is to be successful. Although the DO of surface and bottom waters was generally low, anaerobic conditions were never recorded (Table 5), indicating that there was net input of oxygen into the pond. The difference in the DO concentration of the surface and the bottom waters (ΔDO) varied from -14.8 to 54.7%. On several occasions DO was higher in the bottom waters (eg 1 November, 20 November, 4 December, 18 March), suggesting a certain amount of benthic photosynthesis.

Under cloudy conditions, as experienced on 1 October 1985, DO of both surface and bottom waters increased in the early morning (6am to 8am) but thereafter remained relatively constant (Figure 9a). In the early hours of the morning there was a difference between the DO of surface and bottom

* dissolved oxygen

Table 6. Conductivity and nutrient concentrations in sewage effluent and polluted water bodies.

Source	Conductivity	Nitrogen			Ortho-phosphate	Reference
		NO_3-N	NO_2-N	NH_4-N		
	$mS.m^{-1}$		$mgN.l^{-1}$		$mgP.l^{-1}$	
SEWAGE WORKS						
Cydna	-	16	-	6.7-15.5	-	Allanson, 1961
Delta	-	8	1.9	18.3	-	Allanson, 1961
Bruma	-	16-20	0.4-1.8	7.5-10.2	-	Allanson, 1961
Spokane	-	-	-	-	0.01	Solterero et al, 1975
Daspoort	-	0.2-10.0	-	0.2-7.0	1.0-10.0	Shillinglaw and Pieterse, 1977
Baviaanspoort	79-90	2.0-2.5	1.5-1.7	24-25	5.0-7.5	Walmsley and Toerien, 1978
Kempton Park	86.5-91.4	1.9-2.1	0.2-0.5	4.5-5.4	7.4-7.5	Toerien and Walmsley, 1979
Cape Flats	0.33	-	-	6.0-40.0	-	Bickerton, 1983
SEWAGE OUTFALLS:						
Cape Recife	-	0.55	0.2	3.0	8-4	Emmerson et al, 1983
Fishwater Flats	-	0.07	0.004	0.002	1.0	Emmerson et al 1983
POLLUTED RIVERS:						
Gcuwa River	76.02	-	2.33	0.9	1.15	Du Preez, (1985)
Komani River	81.19	-	19.73	2.18	8.41	Du Preez, (1985)

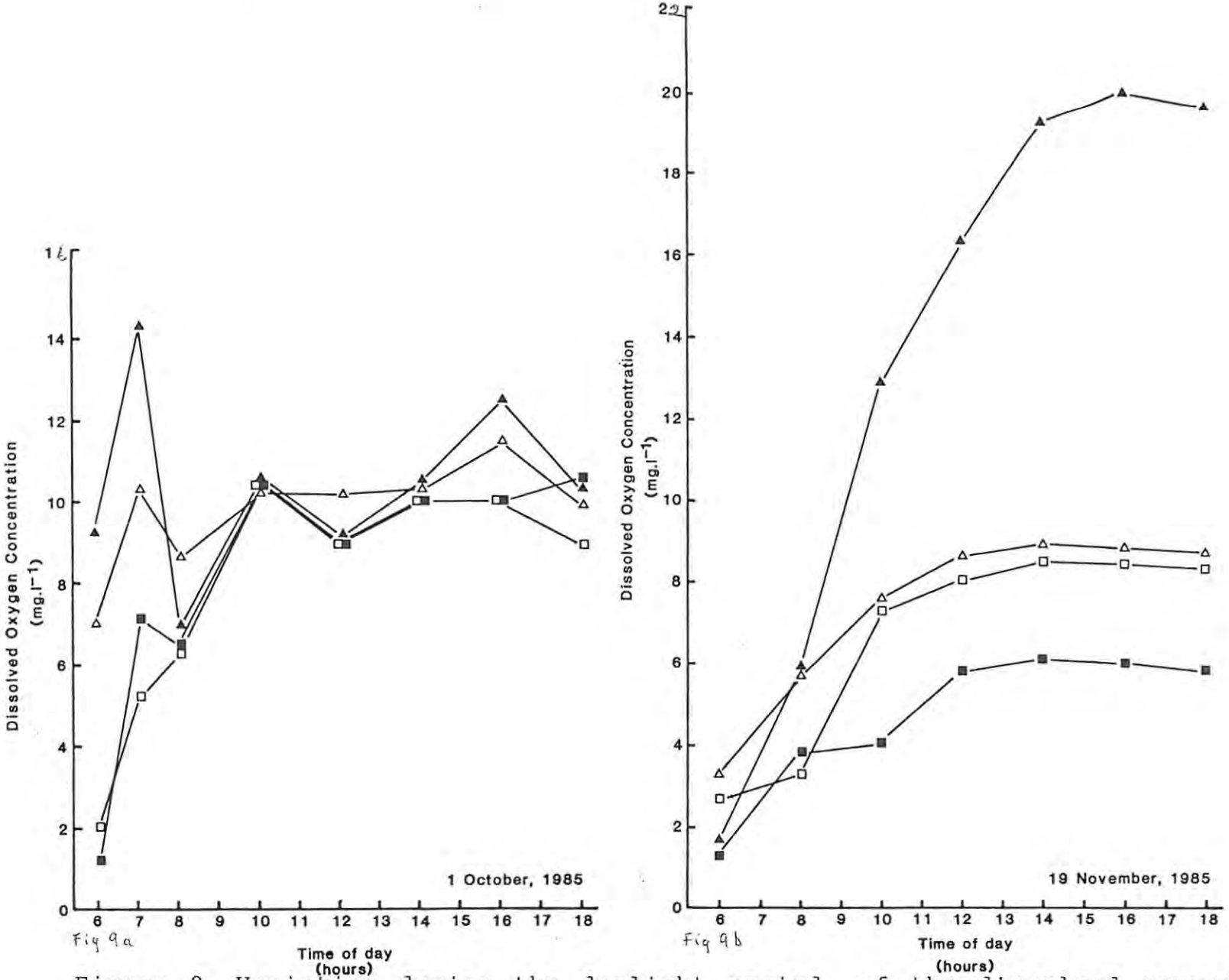


Figure 9. Variation during the daylight period, of the dissolved oxygen concentration of surface and benthic water, in areas of the pond covered (▲ - surface, ■ - benthic) and free (△ - surface, □- benthic) from floating algal mats. The effect of the mats was compared when weather conditions were overcast and cool (1 October, 1985) and when they were clear and exceptionally hot (19 November, 1985).

waters, but from 8am this difference was very small. There were no obvious differences between covered and uncovered areas.

When it was hot, clear and calm (eg 19 November, 1985), the DO concentration of surface waters was higher than that of the benthic layer throughout the day (Figure 9b). In the open areas the DO concentration of surface and benthic waters increased during the morning, reaching an early afternoon maximum which was maintained for the rest of the daylight period. Although ΔDO was observed, it did not change much during the day. In contrast, the surface DO of the areas covered by mats increased dramatically to a late afternoon peak (ΔDO was as high as 14mg.l^{-1}) while that of the benthic water increased only slightly. On this occasion the algal mats showed a marked influence on the DO concentration of the mat covered areas of the pond. These results suggest that:

1. The surface algal mats increase the DO of the pond when environmental conditions favour photosynthesis;
2. There was little benthic oxygen production under surface mats;
3. In open areas the DO concentration increased through the entire water column, possibly as a result of atmospheric oxygen diffusion and benthic photosynthesis, assisted by wind-driven mixing.
4. Vertical mixing within the water column was reduced by the surface algal mats, but horizontal water movement did not appear to be affected.

Dissolved inorganic nitrogen concentration (as NO_3-N + NO_2-N + NH_3-N) in the effluent varied from 10.76 to 33.53mgN.l^{-1} (Table 5). Nitrate was the dominant form of inorganic nitrogen during most of the study period, with a mean concentration of 12.31mgN.l^{-1}. The mean concentration of NO_2-N recorded in the pond was 0.44mgN.l^{-1}, and it varied between 0.03 and 0.70mgN.l^{-1}. Ammonium was present in concentrations ranging from 1.95 to 18.16mgN.l^{-1} and was the dominant inorganic nitrogen form between August and October, 1984 (Table 5). The dominance of NO_3-N for most of the study period suggests that the pond environment was oxidising as such conditions promote microbial nitrification. It could also be attributed to the preferential use of NH_4-N as an inorganic nitrogen source (McCarty and Eppley, 1972; Conway, 1977; McCarthy, 1980).

The average NO_3-N, NO_2-N and NH_4-N concentrations recorded in the Grahamstown effluent exceed those recorded in other sewage works and sewage polluted systems (Table 6). Such high concentrations exceed the limits approved for water entering rivers or dams (Kempster et al, 1980).

The PO_4-P concentration varied between 9.05 and 21.30mgP.l^{-1}, with an average concentration of 15.28mgP.l^{-1} (Table 5). This form of phosphate was more abundant than previously recorded in the same pond (3.47 to 3.84mgP.l^{-1} - Roman, 1982; 10.63 to 13.93mgP.l^{-1} - Snook, 1983) and were higher than levels recorded in many other South African sewage works and sewage polluted systems (Table 6). The situation recorded during

this study are possibly a reflection of the prevailing drought conditions and the water restrictions which led to a more concentrated effluent.

The size of the phytoplanktonic algal population, measured as the concentration of chorophyll a in the pelagic region of the pond, fluctuated widely (CV=190%) (Figure 10). The maximum of 323.0µg.l^{-1} was observed during a spring population pulse dominated by a Eudorina species. This pulse lasted approximately three weeks (mid-July to August, 1984) and was followed by a smaller pulse, in which chlorophyll a concentrations reached 78.89µg.l^{-1} in late October, 1984. During the rest of the study period the phytoplanktonic algal population was small, with the chlorophyll a concentrations remaining below 25µg.l^{-1}. This is unusual because the population pulses recorded in maturation ponds are usually more frequent and of greater amplitude. For example, in Daspoort maturation ponds 12 major pulses were recorded within a period of 12 months, and chlorophyll a concentrations reached 1500µg.l^{-1} (Shillinglaw and Pieterse, 1977). However, the results obtained during the current study are similar to those recorded previously in the pond when the chorophyll a concentrations varied between 0.83 and 23.21µg.l^{-1} (Snook, 1983).

Surface mats were present throughout the study period, but benthic mats were observed to be sparse during August and early September, 1984, when raking the pond floor yielded no benthic material. Surface mats re-appeared within two weeks

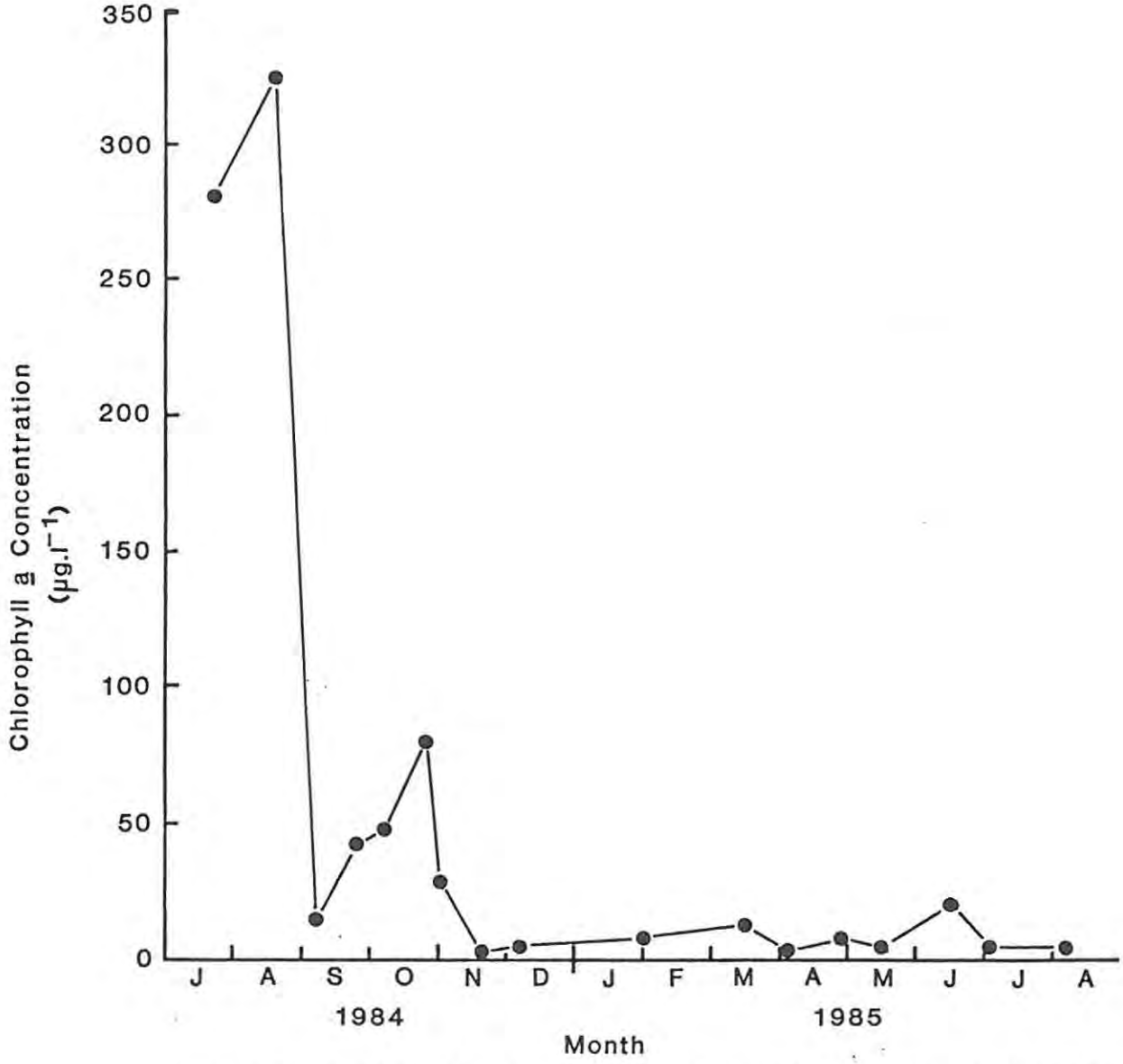


Figure 10. Variation in the size of the phytoplankton population of the maturation pond during the study period, measured as the chlorophyll a concentration in the pelagic region.

of the pond being graded and refilled in April, 1984, covering an area of 5% by May, 1984 (Figure 11). These mats began to proliferate in early summer (September, 1984) reaching a peak biomass which covered 68% of the pond's surface in February, 1985. Between March and August, 1985 there was a progressive decline in the area covered.

The mats were always dominated by R. riparium whose filaments were colonised by epiphytic bacteria, diatoms and blue-green algae (Plate 12). Small quantities of a species of filamentous desmid, Desmidium was found amid the green alga during May and July, 1984, but this alga was never a major component of the algal mats.

3.3.3 GENERAL DISCUSSION

The factors which commonly regulate the size of algal populations are light, temperature, nutrient concentrations, turbulence, dilution and sinking rates, bacterial and fungal infection, and grazing (Pieczynsha, 1971; Ulhmann, 1971; Fogg, 1975; Abeliovich and Azov, 1976; Pieterse and Shillinglaw, 1976; Shillinglaw and Pieterse, 1977; Abeliovich, 1981; Grobbelaar, 1982). The relative importance of these factors vary according to the environment and there may be considerable interaction between them in their effect on algal growth (Abeliovich, 1981).

Although light is important in relation to growth and photosynthesis (Moss, 1980) it also plays a role in the development of algal form (eg colony size in Nostoc),in

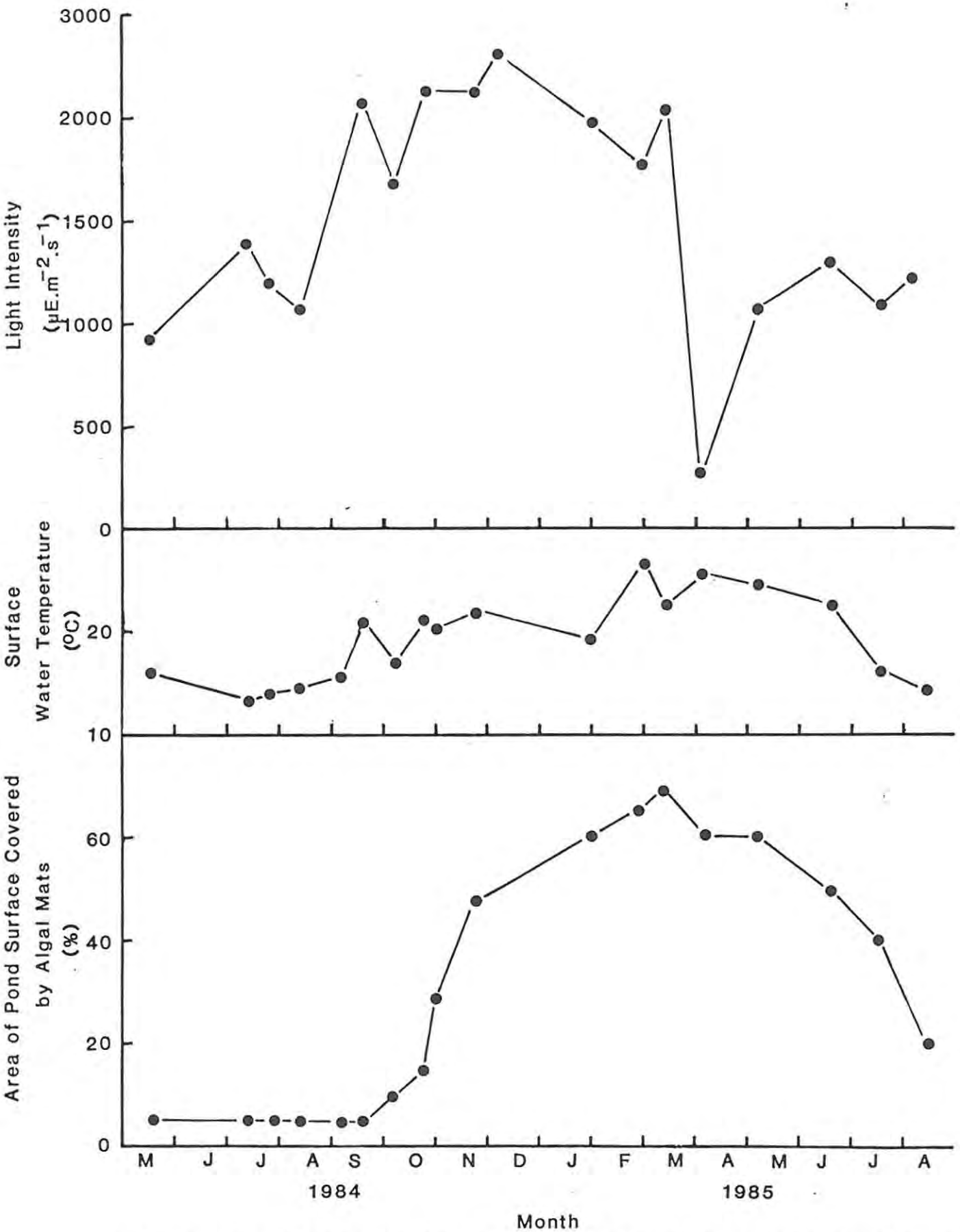


Figure 11. Change in the surface area of the pond covered by floating algal mats during the study period. The variation in surface water temperature (measured at 9.00am) and incident PAR (at 12.00 noon) are also shown.

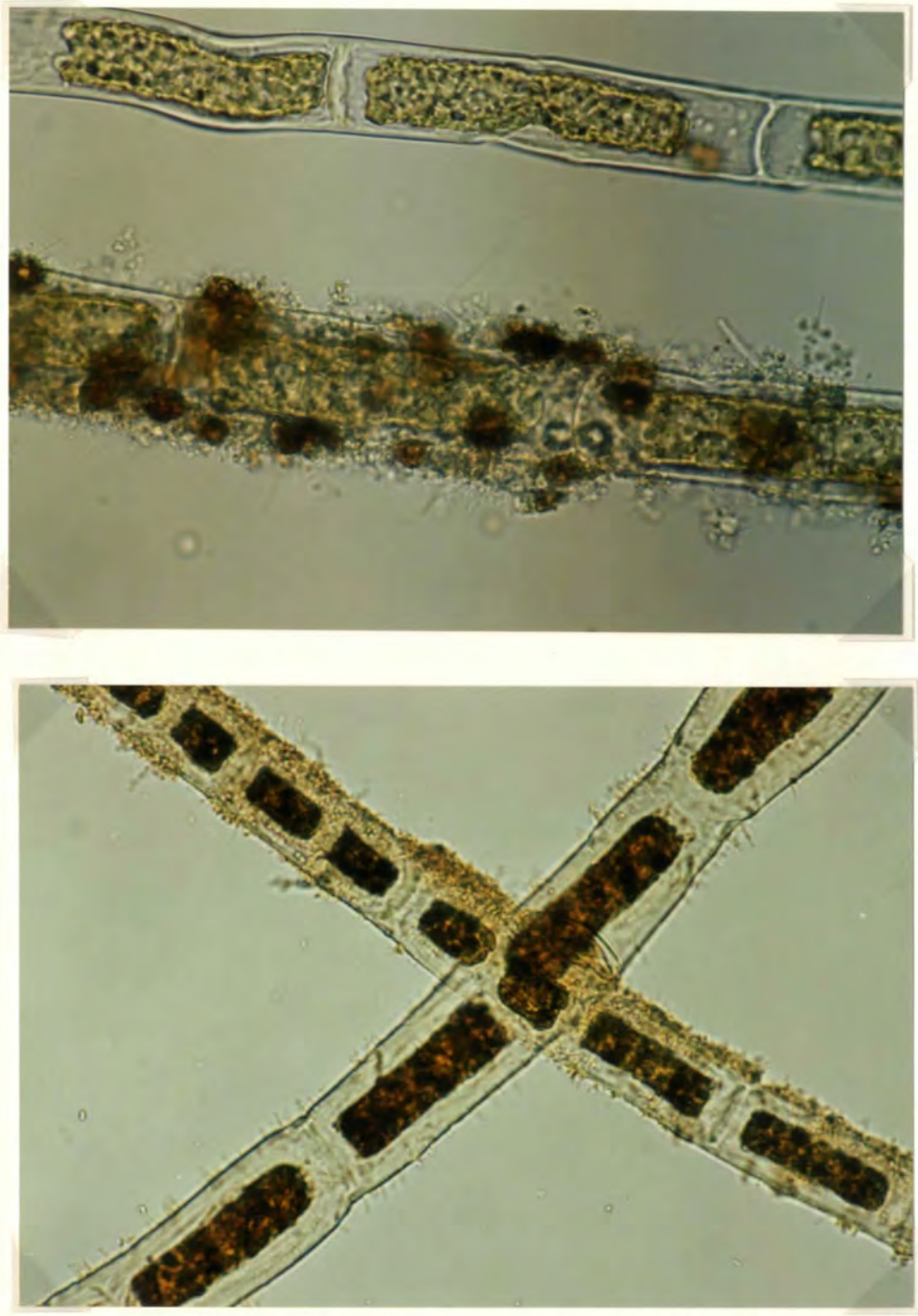


Plate 12. The filaments, particularly those from the surface mats, were colonised by epiphytic bacteria, blue-green algae and diatoms.

ultrastructural variation (eg gas vacuolation in the blue-green algae), as well as changes in the physiological status of the cells (eg changes in the chlorophyll content of cells) (Taylor, 1980). With regard to photosynthesis it is generally accepted that appreciable growth and photosynthesis can only occur in the euphotic zone of a water body (Talling, 1971). More specifically, photosynthetic rates become light-limited when the light intensity falls below a value at which photosynthesis is light saturated (I_k) (Harris, 1978). The value of I_k is species specific, and often lies between 50 and 120$\mu E.m^{-2}.s^{-1}$ (Talling, 1957a; Talling, 1957b). As the euphotic zone in the maturation pond usually included the pond floor, the benthic mats in the pond probably received sufficient light to photosynthesise and maintain growth.

With respect to light climate the situation becomes further complicated when portions of the benthic population float to the surface where they are subjected to higher light intensities and also contribute to an autoshading effect. Photoinhibition, a phenomenon which reduces the rate of photosynthesis, has been observed to affect phytoplankton at intensities as low as 300$\mu E.m^{-2}.s^{-1}$ and can reach 50% at intensities of 1600$\mu E.m^{-2}.s^{-1}$ (Harris, 1980). The effects of photoinhibition are normally manifest as a lowering of photosynthetic activity due to photo-oxidation and bleaching of the chloroplast pigments (Harris, 1978). Since surface mats were observed to exhibit a fading of the green pigment (Plate 1) which was similar to that observed in the photo-

oxidised crust of hyperscums (Zohary, 1985), photoinhibition is possibly a factor which affects the growth and development of surface mats.

The optimum temperature of most green algae lies between 20°C and 30°C (Marre, 1962) and growth patterns usually follow an Arrhenius-type of model (Harris, 1978). Pond temperatures were between 20 and 30°C for most of the summer period and below 20°C from April to September, 1984. However, laboratory experiments indicated that the optimum temperature for the alga was 15°C (Section 4.3.2) and that growth was only slightly depressed at 20°C. This information suggests that temperature conditions in the pond were optimal, or nearly optimal for most of the study period.

The effects of the surface mats on the water body were similar to those recorded for mats of Cladophora (Barbehenn, 1952; Tweed, 1967; Whitton, 1970; all in Whitton, 1970). Mats increase light attenuation so that conditions directly below them become unsuitable for benthic or phytoplankton growth. Water temperatures in areas covered by mats were observed to increase. Thus, if the area covered by algal mats is large, this heating effect could increase metabolic activity within the pond. Finally, the mats decrease vertical mixing in the pond, although they do not appear to interfere with horizontal water movement. This means that the vertical movement of oxygen produced during mat photosynthesis will be limited by the rate of diffusion, and this may inhibit effluent oxidation and thus reduce the efficiency of the

pond.

The effluent within the maturation pond appeared to be a substrate suitable for algal growth, particularly because the elements that most often limit growth (N and P) (O'Kelley, 1968) were always present in concentrations far in excess of algal demand. Ammonium is the form of nitrogen most readily assimilated by algae, becoming growth-limiting at concentrations below 0.5µg atoms $N.l^{-1}$ ($7\mu gN.^{-1}$) (McCarthy, 1980) but inhibiting algal growth at concentrations exceeding 1mM ($14mgN.l^{-1}$) particularly when the water is poorly buffered (Chu, 1942). Although the NH_4-N concentration did exceed $14mgN.l^{-1}$ between September and December, 1984 the algal population was not reduced, suggesting that it was not adversely affected. The uptake of NO_3-N and NO_2-N is usually suppressed in the presence of NH_4-N (McCarthy and Eppley, 1972; Conway, 1977; McCarthy, Taylor and Taft, 1977; Serra, Llama and Cadenas, 1978; MacIsaac, Dugdale, Huntsman and Conway, 1979), which was so abundant in the pond that this source of inorganic nitrogen was probably capable of fulfilling the inorganic nitrogen requirements within the system.

Similarly, the supply of phosphate, as PO_4-P, was present in concentrations in excess of levels which might limit algal growth. Rodhe (1948; in Kulh, 1974) found that the uptake saturation constant for PO_4-P was approximately $20\mu gP.l^{-1}$, while Chu (1943) showed the PO_4-P concentration most suited to algal growth was between 0.018 and $17.800\mu gP.l^{-1}$.

In enriched systems there have been numerous reports of algal growth becoming limited by the inorganic carbon supply (King, 1972; Talling, 1976). However, it seems unlikely that algal growth in the pond would be thus affected since the effluent was well buffered, and pH never exceeded 8.3. Under these conditions algae would have a supply of carbon in the form of carbon-dioxide (CO_2) and bicarbonate ions (HCO_3^+), the inorganic carbon forms most readily assimilated by algae (Talling, 1976).

An unusual feature of the Grahamstown maturation ponds was the virtual absence of phytoplankton. There are many reasons which may explain this phenomenon, the most important being grazing, retention time, inhibition effects of other algae, and sedimentation (Uhlmann, 1971).

Grazing by phytoplankton-consuming zooplankton is often cited as the most important factor regulating phytoplankton growth in maturation ponds (De Noyelles, 1967; Ulhmann, 1971; Pieterse and Shillinglaw, 1976; Shillinglaw and Pieterse, 1977) although some researchers suggest that this is not the case (eg van der Post and Toerien, 1974). No data relating to zooplankton grazing was collected during this study, but it was considered a major factor during earlier studies (Snook, 1983; Sugden, 1983). Large Daphnia populations were observed in the pond during the study period and it seems reasonable to assume that grazing was significant in maintaining low phytoplankton populations. The influence of zooplankton grazing on the benthic material can possibly be considered

negligible in view of the physical dimensions of the filaments (Section 2.3.1)

Retention time is a factor which can reduce phytoplankton populations when effluent flow removes biomass from the system at a rate faster than the natural growth rate (Uhlmann, 1971). In high rate oxidation ponds a steady-state with respect to algal growth can be maintained at a retention time of 5 days (Abeliovich and Azov, 1976). The Grahamstown maturation pond studied had a retention period of approximately 4.5 days (Table 3), a value which approaches the critical level when considering the net removal of biomass by washout.

The only phytoplankton populations of any significance were recorded during the period prior to the re-establishment of the surface algal mats which suggests that the surface mats inhibited phytoplankton growth. This could be achieved either by the production of some kind of growth-inhibiting substance (Shillinglaw and Pieterse, 1977; Palmer, 1980) or by shading. However, as growth was only inhibited once floating mats had developed it seems that the latter might be more important.

The removal of phytoplankton biomass from the water column by sedimentation can be a significant factor in stratified water bodies (Uhlmann, 1971). In maturation ponds the limitations associated with thermal stratification and sinking are largely alleviated because the ponds are shallow and generally well mixed. However, the effect of sedimentation

cannot be assessed from the observations made during the study.

In considering each of the factors and their relative contribution to reducing phytoplankton populations it would seem that the inhibiting effect of shading of light of the algal mats was the most important. Grazing and retention time were probably also important as the development of benthic mats in the maturation pond was only possible because of the suitable light climate, largely brought about by the virtual absence of suspended phytoplankton cells. Factors such as grazing, retention time and sedimentation, which may have reduced phytoplankton biomass obviously do not have any significant effect on the development of the benthic material. Under the present operating conditions the Grahamstown maturation ponds are therefore an ideal ecosystem in which algal mats can develop.

3.3.4 CONCLUSIONS.

1. The maturation pond exhibited ~~unstable~~ no thermal statification. ~~which~~ The temperature fluctuated on a diurnal basis, modified by variable climatic conditions.

2. Light attenuation was relatively low with PAR penetrating to the pond floor on most sampling occasions during the study period. Dissolved humic substances appeared to be the major light attenuating factor.

3. Water chemistry indicated that the pond was a well

buffered system in which inorganic nutrients (NO_2-N, NO_3-N, NH_4-N, PO_4-P and CO_2) were present in abundance. Concentration ranges were above average for sewage, a fact which was possibly a reflection of the prevailing drought conditions.

4. Phytoplankton populations were generally low, a factor which was ascribed to a combination of high zooplankton grazing rates, low pond retention times and shading by floating algal mats.

5. Surface mat development was initially slow, but at the beginning of summer increased so that mats occupied almost 68% of the pond surface. Observations indicate that the mats influence water temperature, DO concentrations and light penetration and as a result play a significant role in the pond ecosystem.

6. R. riparium is able to proliferate from the benthic habit due to favorable light, temperature and nutrient conditions.

4.0 GERMINATION AND GROWTH

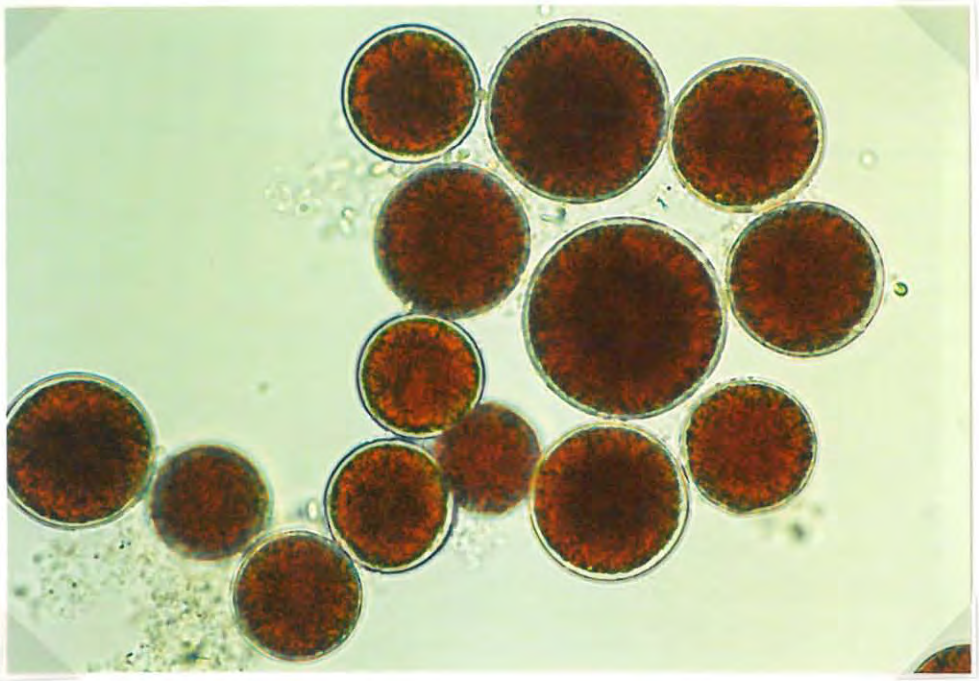
4.1 INTRODUCTION

In order to explain the success of R. riparium in the maturation pond it was necessary to know which conditions favoured its growth. Although many environmental factors affect the growth and development of algae (eg photoperiod, available nutrients and turbulence), water temperature and light intensity are the most important (Bellis and McLarty, 1967; Bellis, 1968; Lorenz and Herdendorf, 1982; Neil and Jackson, 1982). Consequently, the effect of these parameters on growth was investigated. Laboratory cultures were used as it is difficult to control the environment in situ (Bellis, 1968).

During culture in the laboratory it was observed that filaments disintegrated after a period of time, leaving red or orange cells in the bottom of the flasks. Microscopic examination of these cells suggested that they were akinete and zoospores respectively (Plates 13 and 14). These cells are the reproductive initials of R. riparium, and thus constitute part of its life-cycle, which is described as a diplohaplontic alternation of isomorphic generations (Figure 12) (Bold and Wynne, 1978).

Reproduction is primarily by vegetative fragmentation, where filament elongation is by the division of undifferentiated

(a)



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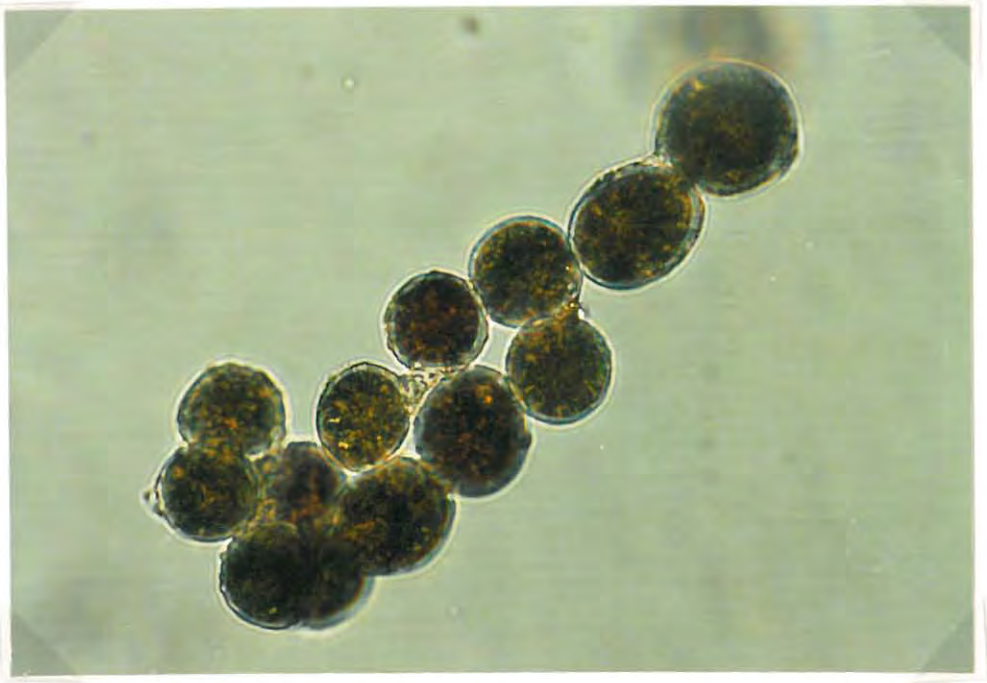
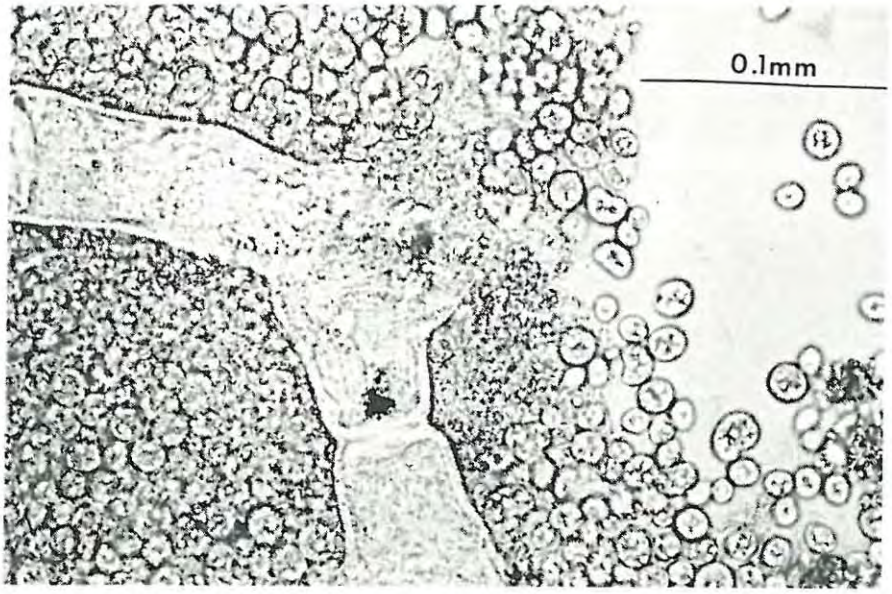


Plate 13. R. riparium akinete cells. (a) Unstained. (b) Stained with Lugol's solution. Notice the bright red color of the cells, the thick cell walls and the abundance of starch in the cells. 0.1mm

(a)



(b)

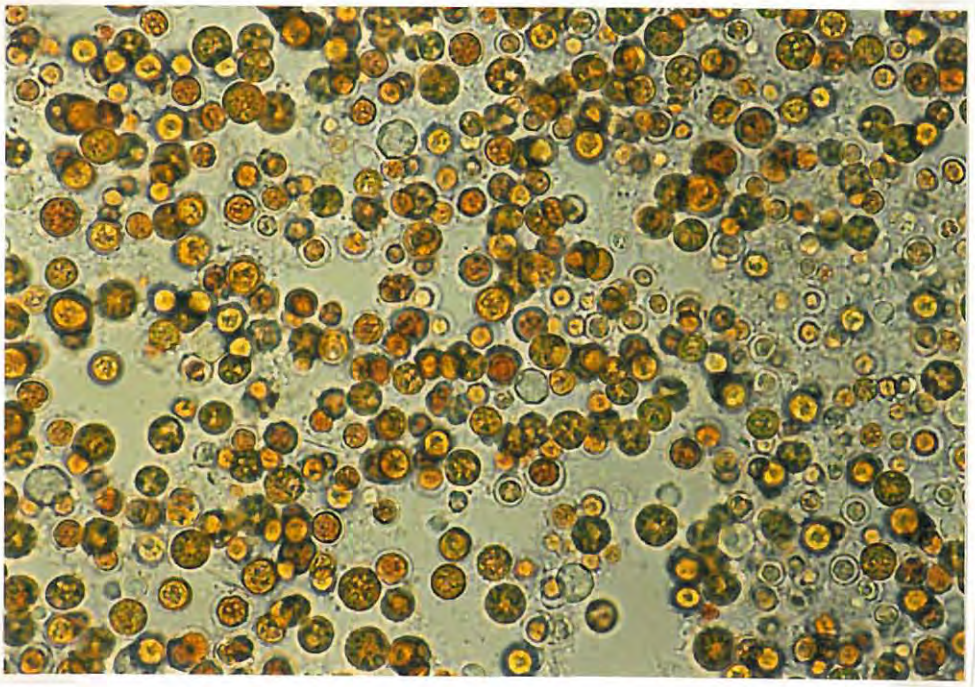


Plate 14. Biflagellate zoospores of R. riparium. Zoospores are released by the rupture of a cell wall (a) and immediately settle, shedding their flagella in the process (b). 0.1mm

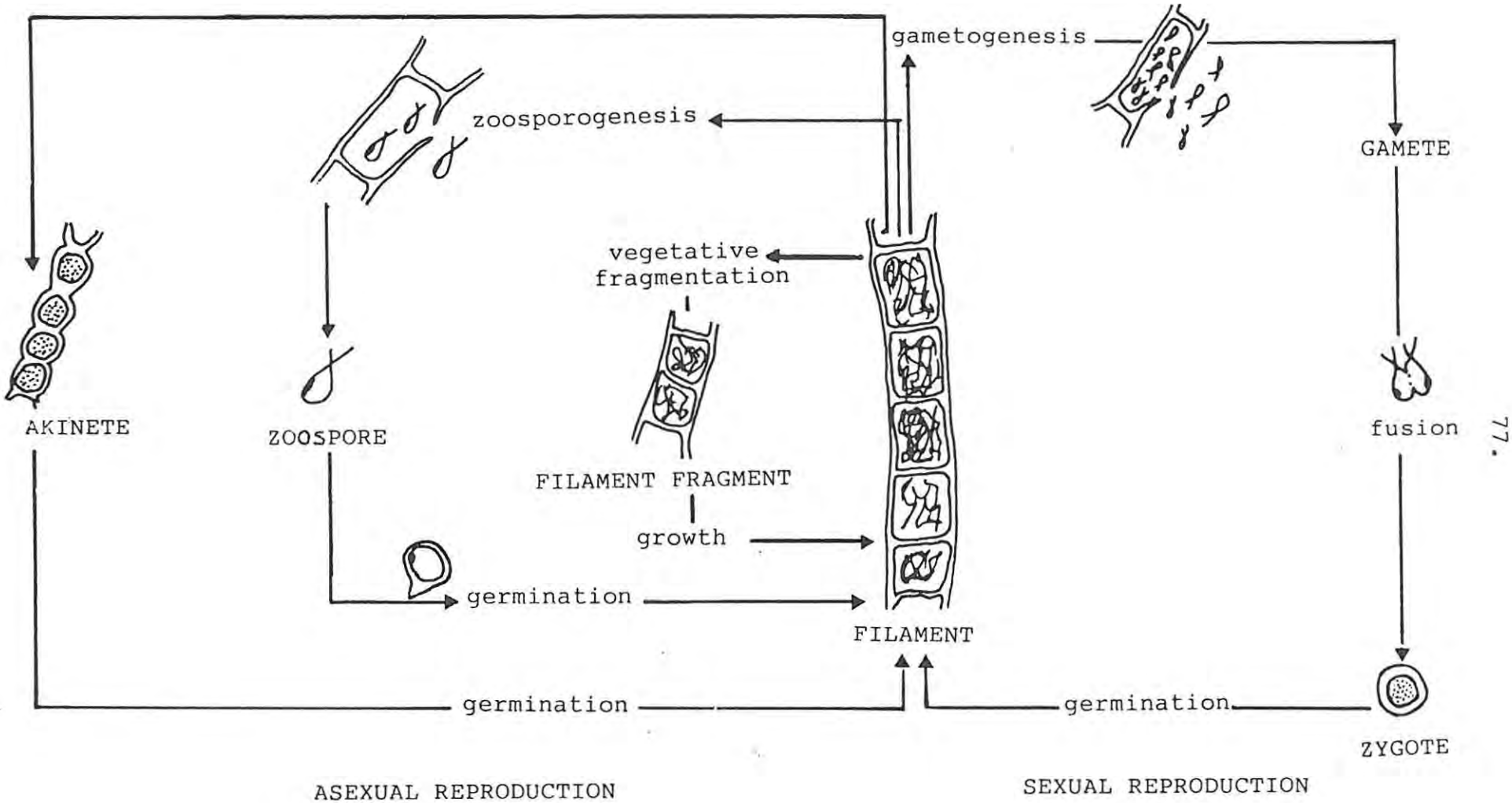


Figure 12. Diagram of the life-cycle of R. riparium.

cells. When stressed, particularly by nutrient limitation or extreme temperature conditions (Bellis, 1968; Whitton, 1970), akinetes form. These cells, usually greater than 40µm in diameter, have a large starch content and thick cell walls which are typical of cells which serve as a resting stage in the life-cycle of algae (Bold and Wynne, 1978). They are usually produced as a row of cells along the filament, only being released when the filaments disintegrate under very extreme conditions (Nienhuis, 1975). More rarely, reproductive initials are produced when the contents of a nonspecific cell divide to form zoospores (between 20 and 60 per cell). These are released through a pore in the cell wall, settle, discard their flagella and germinate once environmental conditions are favourable. Zoospores, which are either biflagellate or quadriflagellate are usually 10µm in diameter (Nienhuis, 1975).

Sexual reproduction is by the production of up to 50 isogamous gametes within a cell. These are released when the cell wall ruptures, and the biflagellate cells resemble zoospores although they are much smaller (diameter is approximately 3.5µm). Zygotes are formed by the fusion of gametes and these will germinate when conditions are favorable (Nienhuis, 1975).

As akinete and zoospores were available for culture experiments, the effect of temperature on their germination was investigated.

4.2 MATERIAL AND METHODS

4.2.1 Culture Methods

Unialgal cultures of R. riparium were isolated from pond material using methods described in Stein (1973). Material was separated into individual filaments which were then washed in tap water, followed by approximately twenty consecutive rinses in sterile water. The filaments were then placed on agar plates (1.5% agar in autoclaved and filtered sewage effluent) using aseptic techniques, and were left in a constant environment room at 15°C, 150μE.m^{-2}.s^{-1}, and a 12 hour light period. Plates were examined daily using a Zeiss binocular dissecting microscope. Any contaminated filaments were re-washed, and attempts to remove the epiphytes made using the agar drag method (Graham, Kranzfelder and Auer, 1985). This treatment, however did not eliminate the epiphytic diatom population and the filaments had to be transferred to a modified medium (autoclaved effluent + 0.1g.l^{-1} germanium dioxide - Markham and Hagmeiter, 1982). Once filaments were unialgal they were transferred to 150ml conical flasks containing 100ml of the modified medium. This was renewed approximately once a month to maintain stock cultures, and was shaken daily. No attempt was made to produce axenic cultures as previous research has shown that R. riparium requires organic compounds produced by bacteria for growth (Thomas, 1963, in Whitton, 1970)

Attempts to grow the alga on a defined inorganic medium (eg

Chu 10, Basal Bold - Nichols, 1973) were unsuccessful. In the past R. riparium has been cultured in media including soil extracts and higher than normal (brackish) inorganic salt concentrations (eg Nienhuis, 1975). However, rather than use one of these media, which were inappropriate, the alga was cultured in sewage effluent. Some of the constituents of sewage effuent are described in Table 7.

4.2.2 Germination Tests

Akinetes and zoospores were stirred into a homogenous suspension from which sub-samples (50ml) were transferred to 100ml conical flasks. These were incubated at 5, 15, 20, 25, 30 and 35^{o}C in an incubation chamber at a light intensity of 150μE.m^{-2}.s^{-1} under a 12 hour light regime. Every alternate day the cells were re-suspended and slides prepared using 1 drop of sample. This was examined and the percentage of germinating cells in 5 light fields (magnification 40 x 10) counted. Germination was considered complete after the first cell had been cut off, i.e. at the two-cell stage.

4.2.3. Growth Tests

Using the unialgal cultures isolated previously, the growth response of R. riparium to various conditions of light intensity (80, 150 and 275μE.m^{-2}.s^{-1}) and temperature (5, 15, 20, 25, 30 and 25^{o}C) was measured as change in algal biomass (dry weight) with time. A 12 hour photoperiod was adopted as the alternating light-dark period represents a more natural condition than the constant irradiance usually used in batch

Table 7: Chemical analysis of autoclaved and filtered sewage effuent.

Chemical	Concentration ($mg.l^{-1}$)
Calcium	45
Chlorine	352
Copper	0.03
Iron	<0.01
Magnesium	19
Manganese	<0.01
Potassium	24
Sodium	264
Zinc	0.1

cultures (Steemann Nielson, 1978).

Sample inoculum was prepared by chopping 0.5g of algal filaments (previously grown under experimental conditions for 5 days) into short sections. These fragments were suspended in 100ml of medium (autoclaved effluent containing germanium dioxide) using a magnetic stirrer to create a homogeneous mixture. Sub-samples (5ml) were drawn off using an autopipette and were tranferred to 100ml medium before rinsing the pipette tip with an additional 50ml medium to remove any remaining material. After inoculating all samples a further five sub-samples were filtered though tared filters to determine the dry weight of the inoculum.

For each experiment 21 inoculated flasks were prepared. The average dry weight of 3 flasks was measured every 3 days over a period of 21 days, and the growth rate was estimated as the specific growth rate using the formula from Toerien, Huang, Radimsky, Pearson and Scherfig (1971):

$$\mu = \frac{\ln X_1 - \ln X_0}{t}$$

where, μ is the specific growth rate (day^{-1}),

X_0 is the biomass at time zero,

X_1 is the biomass at time t, and

t is the time period, in days.

As the sample inoculum had been grown under the experimental conditions prior to the start of the experiment no lag period was observed. Growth from the onset of the experiment was

therefore in the logarithmic phase where a constant growth rate represents the maximum productive capacity of the alga under the test conditions (Steemann Nielson, 1978). At low temperatures (5 and 15°C) growth continued in the logarithmic phase throughout the 21 day study period, but at higher temperatures (>15°C) had entered the stationary phase by the twenty-first day. In the latter flasks, growth rates were calculated during the period over which the logarithmic phase was observed.

4.3 RESULTS AND DISCUSSION

4.3.1 Germination.

When akinete cells were transferred to fresh medium they turned green within 24 hours and then germinated to the two cell stage. Germination was observed at all the temperatures tested and increased with time, although the rate at which germination occurred was highest during the first day (Figure 13a). The germination response was greatest at between 15 and 20°C, although even at these optimum temperatures the response was poor (a maximum of 59.2% of the akinetes had germinated after one week) (Figure 13b). As water temperature increased above 20°C germination was increasingly inhibited, and at 35°C only 7.5% of the akinetes had germinated after 7 days.

Similarly, zoospore germination increased with time, with a maximum rate being recorded during the first two days (Figure 14a). Maximum germination was recorded at 20°C, and 35°C was

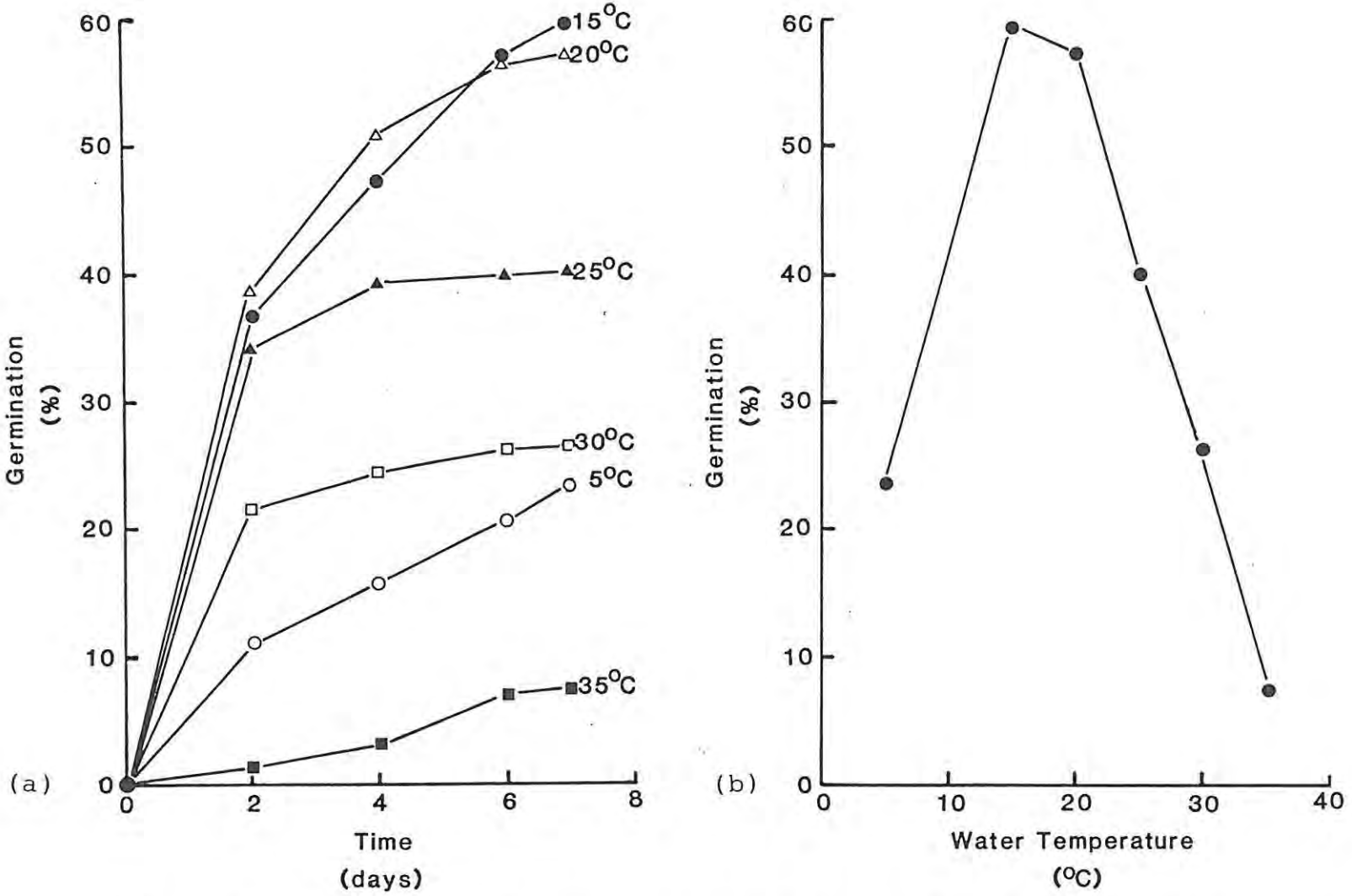


Figure 13. The effect of water temperature on the germination of R. riparium akinetes. (a) The variation in the rate of germination at different water temperatures. (b) Percentage germination after one week.

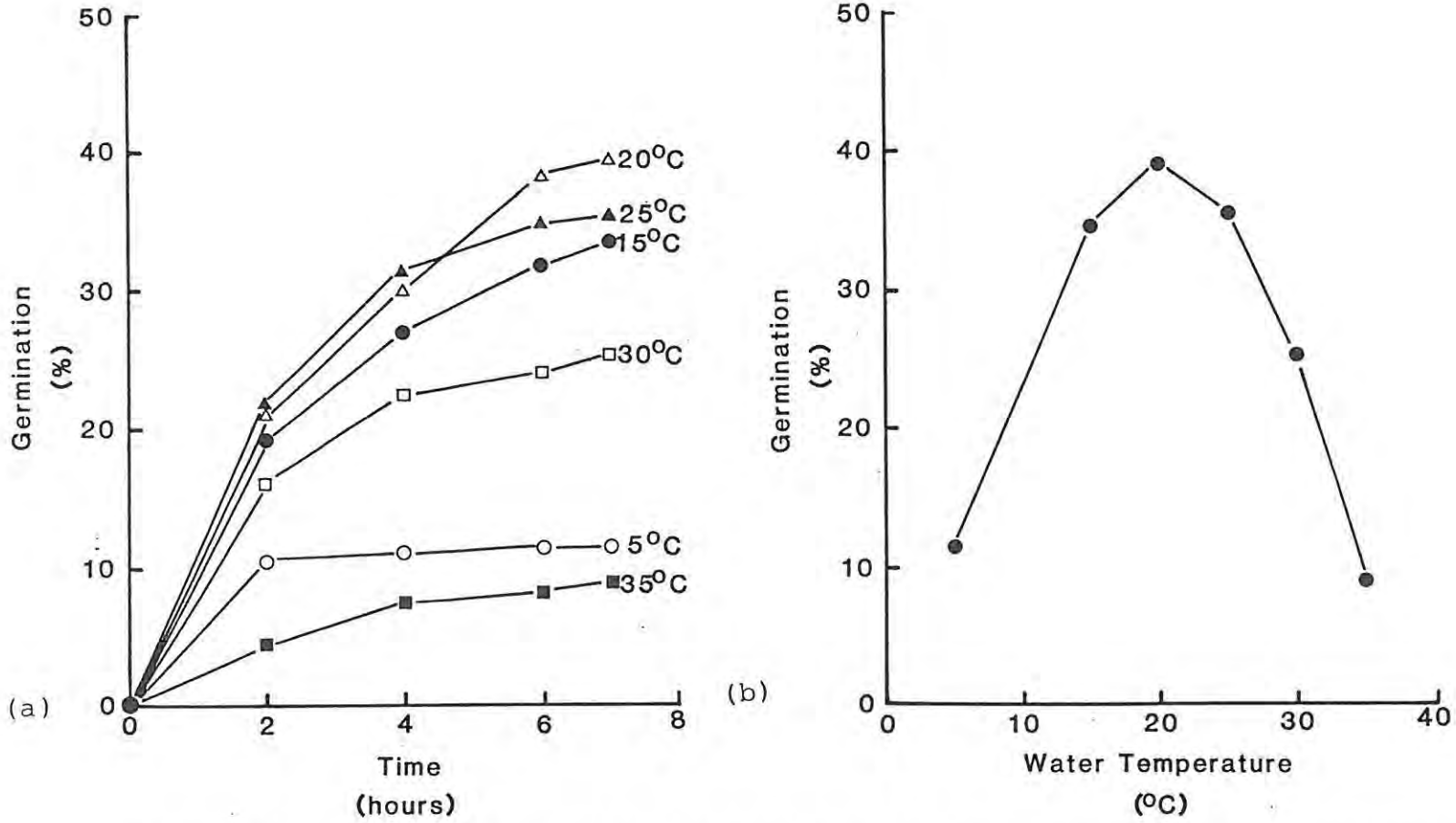


Figure 14. The effect of water temperature on the germination of R. riparium zoospores. (a) The variation in rate of germination at different water temperatures. (b) Percentage germination after one week.

the most unfavourable temperature tested (Figure 14b).

4.3.2 Growth.

The maximum growth response of R. riparium was recorded at 15°C, and the response to temperature did not followed a bell-shaped curve (Figure 15). At the range of light intensities tested (80 to 275μE.m^{-2}.s^{-1}) the growth rate increased with increasing light intensity, except at 35°C where the maximum response was recorded at 80μE.m^{-2}.s^{-1}. At this range of light intensities growth was not light-saturated at 15 and 20°C, although growth appeared to be saturated or approaching saturation at 5, 25 and 30°C. Analysis of variance showed that the effects of both light and temperature were significant (Table 8).

Table 8. ANOVA of specific growth rate as a function of light intensity and temperature.

Source	df	SS	F ratio	Significance
Light	2	0.1246	5.82242	>0.05
Temperature	5	0.4654	8.69094	>0.001
Error	10	0.1071		
Total	17	0.6971		

The growth of R. riparium was highly dependent on water temperature, especially below 15°C where there was a three- to four-fold increase in the growth rate between 5 and 15°C. This corresponds to a Q_{10} value of between 2.6 and 8.7 which reveals a remarkable sensitivity of the growth of this alga to temperature (Healey, 1983 a. Synura). The upper limit of Q_{10} is higher than is commonly reported over the same temperature range (Eppley, 1972; Foy, Gibson and Smith, 1976; Hitchcock,

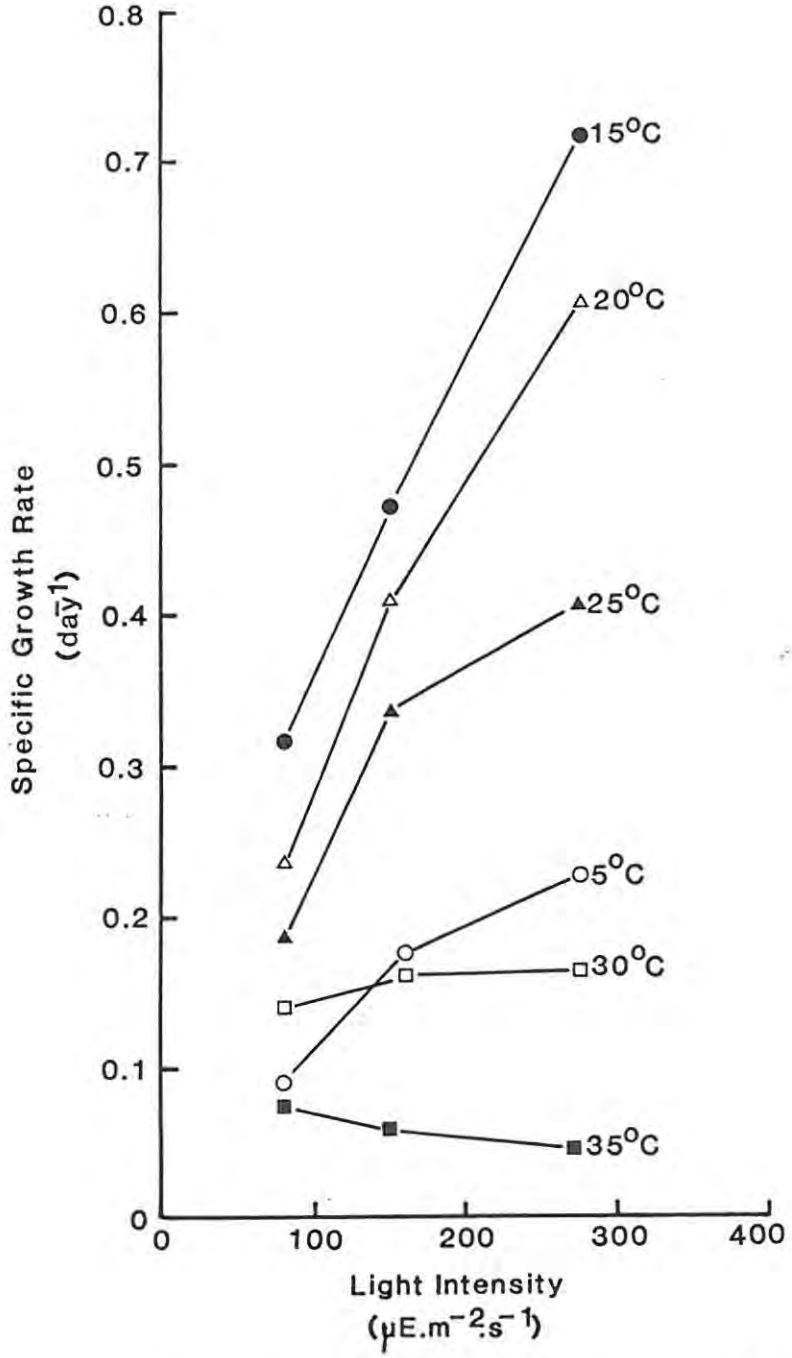


Figure 15. The effect of light intensity and water temperature on the specific growth rate of R. riparium.

1980) although similarly high values have been reported (Curl and McLeod, 1961; Cloern, 1977; Meeson and Sweeney, 1982; Healey, 1983).

The net specific growth rate varied from 0.04 day^{-1} at 80$\mu E.m^{-2}.s^{-1}$ and 35°C to 0.716 day^{-1} at 275$\mu E.m^{-2}.s^{-1}$ and 15°C (Figure 15). The maximum growth rate recorded corresponds to a doubling period of 35.5 hours. These rates are similar to those recorded by Auer and Canale, (1982) in Cladophora glomerata (0.77 day^{-1}). It was, however lower than the growth rate recorded in other studies of Cladophora (eg 1.2 day^{-1} - Zuraw, 1969; 1.4 day^{-1} - Whitton, 1967, in Whitton, 1970) and other filamentous algae (eg Stigeoclonium tenue: 2.0 day^{-1} - Rosemarin, 1982; Oscillatoria redekei: 2.05 day^{-1} - Nicklisch, Conrad and Hohl, 1981).

4.4 GENERAL DISCUSSION

There are few studies concerned with the growth of filamentous algae and many of these have estimated growth from the results of photosynthetic investigations (eg Lester et al, 1974 in Graham, Auer, Canale and Hoffman, 1982; Graham et al, 1982; Graham, et al, 1985). An attempt to measure growth under a variety of environmental conditions was made here with the view to extrapolating the results so as to explain the growth patterns observed in the pond.

The data collected during this study indicated that the optimum environmental conditions for germination and growth varied. In general, R. riparium favours temperatures between

15 and 25°C at which growth was unsaturated at the highest light intensity tested (275μE.m^{-2}.s^{-1}). Temperatures within this range were recorded in the pond on 61% of the sampling occasions (Section 3.3.2) and therefore temperature conditions were favourable for the growth of R. riparium over most of the study.

More specifically, akinetes would have germinated throughout the study period although germination would have have been retarded for a short period in July when temperatures were below 15°C. However, as described earlier, akinetes are usually produced in response to stress (Bellis, 1968; Whitton, 1970), and as the R. riparium population was not stressed (Section 3.3.2) it was unlikely that akinetes would have been produced at all. This is supported by the fact that akinetes were never observed in the samples collected for examination (Section 2.2.1).

Zoospore germination would also have been favoured throughout the study period. The conditions under which algal zoospores are produced vary widely . For example, in Ulothrix zonata they are produced in response to conditions of high temperature and light intensity (Graham, et al, 1985) whilst those of Cladophora glomerata are produced when temperatures are moderate and the light intensity is low (Hoffman and Graham, 1984). As R. riparium produced zoospores under culture conditions (150μE.m^{-2}.s^{-1} and 15°C) it may be assumed that zoosporogenesis occurs at low light and moderate temperature. Under these conditions the zoospores would have

germinated immediately.

Growth, which was greatest at between 15 and 20°C, would have been favoured between September, 1984 and January, 1985 and also in June, 1985. This correlates well with the increase in biomass observed from September to November, 1984 but does not account for the marked decline in biomass after April, 1985 (Figure 11, Section 3.3.2).

Because the growth rate was only measured at relatively low light intensities it is difficult to use these results to explain the growth in the pond environment where light intensities of up to 2320$\mu E.m^{-2}.s^{-1}$ where recorded (Table 4, Section 3.3.2). It was clear that at 30 and 35°C the growth rate was saturated at 275$\mu E.m^{-2}.s^{-1}$, but when temperatures were lower than this, growth was light-limited. In similar algae growth is limited at intensities below between 300 and 1100$\mu E.m^{-2}.s^{-1}$ (Cladophora glomerata - Graham et al, 1982; and Ulothrix zonata - Graham et al, 1985). Light may also inhibit growth, particularly at high temperatures, when there is an increase in the rate of photorespiration (Lorenz and Herdendorf, 1982). Light inhibition was recorded at light intensities of above 1100$\mu E.m^{-2}.s^{-1}$ at 5°C but at only 125$uE.m^{-2}.s^{-1}$ at 30°C in Ulothrix zonata (Graham, et al, 1985) and at above 1200$\mu E.m^{-2}.s^{-1}$ at temperatures between 5 and 25°, but above 600$\mu E.m^{-2}.s^{-1}$ at 30°C in Cladophora glomerata (Graham et al, 1982). Thus, it seems that when water temperature is low algae can utilize light of relatively high intensity, but when water temperature is high

the same light intensities are inhibitory. In R.riparium the same pattern was observed: at 35°C growth was inhibited at 150μE.m^{-2}.s^{-1} but at 15°C ~~and a light intensity of~~ 275μE.m^{-2}.s^{-1} was ~~limiting.~~ most probably light limited.

It seems then, that under the conditions recorded in the maturation pond the growth of at least part of the R. riparium population was favoured during most of the study period. With such a long growth period one would expect that algal biomass would continue to accumulate indefinitely. However, this is not the case in most ecosystems. For example, in the Great Lakes of America Cladophora glomerata and Ulothrix zonata show seasonal succession with the former dominant during the warmest period whilst the latter grows during the cooler months. Both die back in winter when the lake shores are ice-bound and when their biomass is reduced by a process known as sloughing (Bellis and McLarty, 1967; Auer, Canale, Grundler and Matsuoka, 1982; Canale and Auer, 1982; Graham et al, 1985). On the other hand, in oligotrophic environments succession is principally due to the ablility of different species to utilize different concentrations of the limiting nutrient (Fogg, 1975).

As the alga did not appear to be nutrient-limited, growth was probably limited by changes in environmental conditions, especially the decline in radiant energy available for benthic mat growth when shading by floating mats becomes significant. During the first half of the study period, when the area covered by surface mats was relatively small,

benthic growth obviously continued in the unshaded areas, but as the surface mats expanded the unshaded area was reduced and mat growth hindered. Because only a small percentage of the surface mats were photosynthetically active (Section 5.3) the rate at which biomass could be generated was limited so, despite the favourable temperature conditions the area covered by mats declined.

4.5 CONCLUSIONS

1. Aged laboratory cultures of R. riparium produced either akinete cells or zoospores. The germination response of these reproductive initials followed a bell-shaped curve. Germination of akinetes was greatest at 15°C, while that of zoospores was at 20°C.

2. The relationship of growth and water temperature also followed a bell-shaped curve, with the optimum at 15°C.

3. At the light intensities tested growth rate increased with increasing light intensity, except at 35°C where intensities of 150 and 275$\mu E.m^{-2}.s^{-1}$ were growth inhibiting. Because the range of light intensities tested was small (80, 150 and 275$\mu E.m^{-2}.s^{-1}$) it was not possible to measure the maximum specific growth rate of R. riparium.

4. Mat expansion in the pond appeared to be controlled primarily by water temperature, but could be limited by light supply when surface mats shaded a significant area of the pond.

5.0 PHOTOSYNTHESIS AND RESPIRATION

5.1 INTRODUCTION

Photosynthesis and respiration are the basic processes concerned with algal growth and thus estimates of their rates provide considerable insight into the role of certain factors on algal growth and development. Furthermore, in the maturation pond environment photosynthesis and respiration are the dominant processes ensuring the continued functioning of the ecosystem. Since the R. riparium mats were the major component of the primary production community of the pond, its photosynthetic and respiratory characteristics were of considerable importance.

The objectives of this chapter were to:

1. measure photosynthesis and respiration in situ;
2. establish the effects of light and temperature on photosynthesis and respiration;
3. measure the photosynthetic constants such as P_{max} (the light saturated rate of photosynthesis), α (the slope of the light-limited portion of the photosynthetic:irradiance (P:I) curve), I_k (the light intensity at which photosynthesis becomes light saturated) and I_c (the light intensity at which the rate of net photosynthesis is zero), as well as R_D (the dark, or basal respiration rate).
4. establish whether the photosynthetic constants of

material from benthic and floating mats were significantly different.

5.2 MATERIALS AND METHODS

The long-term and short-term effects of environmental conditions on the rates of photosynthesis and respiration were measured using the oxygen light and dark bottle method (OLDB method) and the oxygen electrode method (OE method).

5.2.1 Oxygen Light and Dark Bottle Method.

Algal photosynthesis and an estimate of respiration in surface and benthic material were measured in situ on twelve occasions, using the method described by Vollenweider (1969). Although the OLDB method is not as sensitive as the ^{14}C method (Vollenweider, 1969; Harris, 1978; Harris, 1980), it is simpler to use and gives an estimate of net photosynthesis (Vollenweider, 1969). The problem of measuring the changes in DO accurately can be avoided by using modifications of the Winkler titration method which have increased its precision approximately five times, eg Bryan, Riley and Williams (1976) and Tschumi, Zbaren and Zbaren (1978). Furthermore, when using filamentous algae the magnitude of change in the DO concentration can be controlled by changing the amount of algae enclosed. Regulation meant that the changes could be controlled so that they remained within the range measured most accurately, and also meant that incubation periods could be reduced so that bottle effects (Vollenweider, 1969; Harris, 1978; Moss, 1980) could be minimised. However, it was

important that the amount of alga enclosed be kept to a minimum to avoid self-shading (Wood, 1968).

The procedure followed is illustrated in Figure 16.

Preparation of the incubation medium. The alga was incubated in the sewage effluent to avoid any shock response to change in the growth medium. The effuent was filtered through glass-fibre (GF/C) filters to remove phytoplankton cells and debris which would have contributed to changes in the DO concentration. The incubation vessels and bottles for the determination of initial DO concentration, all acid-washed and pre-rinsed with filtered effluent, were filled using a siphon tube to prevent the inclusion of air bubbles.

Preparation of the algal material. Two algal samples, one from the surface mat (taken from beneath the surface layer filaments of floating mats to ensure that cells were not photo-oxidised) and one from a benthic mat were collected, rinsed under running water and then combed to remove as much of the debris as possible. Microscopic examination indicated that this process also removed much of the epiphyton. Experimental trials had indicated that at low light intensities (less than $50\mu E.m^{-2}.s^{-1}$) changes in DO concentrations were achieved within 3 hours using 90mg dry weight of algae. Consequently, approximately this mass was added to all the dark bottles and to those bottles to be incubated in the bottom half of the water column. To prevent supersaturation less material was added to the surface and sub-surface light bottles. These sub-samples were added to

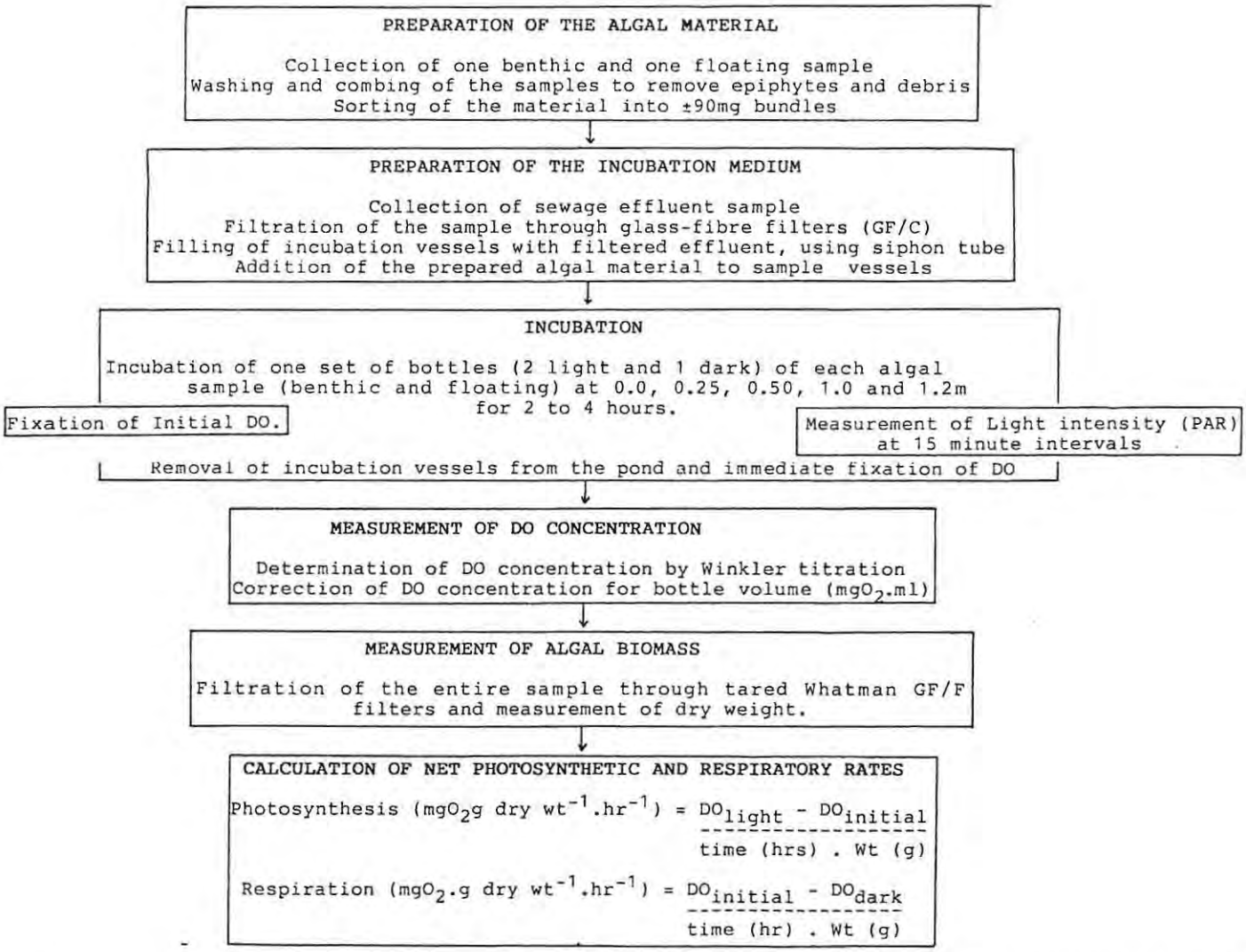


Figure 16. Protocol of the procedure followed for the determination of algal net photosynthesis and respiration in situ, using the oxygen light and dark bottle method.

both the incubation vessels and the bottles used for the determination of initial DO concentration. Care was taken to ensure the even distribution of filaments and to avoid the inclusion of air bubbles.

Incubation. Bottles were fitted into the incubation stands and lowered into the water in an open area for between 2 and 4 hours depending on light intensity. Simultaneously, the DO was fixed for the determination of initial DO concentration. The addition of algal samples to these vessels ensured that any changes caused by their inclusion were corrected for.

During the incubation period PAR was measured every 15 minutes in order to estimate the quantity of radiant energy available to the alga. The average intensity received at each depth was calculated using the attenuation coefficient (Section 3.3.1).

Determination of DO concentration. After incubation the DO of the samples was fixed before they were returned to the laboratory where oxygen concentrations were measured titrimetrically (Section 3.3.1). As the bottles were not all the same size the oxygen concentration was corrected for bottle volume.

Biomass determination. Sample biomass was measured by filtering the contents of the entire bottle after titration through tared glass-fibre (Whatman GF/C) filters. Dry biomass was measured after drying to constant weight at 70^{o}C.

Determination of the photosynthetic and respiratory rates. The rates of photosynthesis and respiration were calculated using the formulae given by Moss (1980). As the mass of alga incubated was not constant all changes in DO were corrected for biomass before the rates were calculated.

5.2.2 The Oxygen Electrode Method.

An estimate of productivity can be obtained by measuring photosynthesis and respiration in situ, but the response to changes in environmental variables is difficult to assess as they cannot be fully controlled (Bellis, 1968). In order to investigate the effects of growth temperature and short-term light intensity changes on photosynthesis and respiration, the OE method, as described by Harris (1978), was used. This has previously been used by Harris (1973), Harris and Lott (1973) and Harris and Piccinin (1977) for phytoplankton, and by Mantai (1974) for filamentous algae. It has the advantage of a rapid response time while the conditions of light and temperature are carefully controlled (Harris, 1980).

A Rank Brothers oxygen electrode was used to measure net photosynthetic and respiratory rates. The light source, focussed on the reaction vessel by means of a convex lens, was an Alpha projector connected to a rheostat to vary the light intensity. Light intensity (PAR) was measured using a Li-Cor-188B photometer. The reaction vessel and its surrounding water jacket reduce the incident light intensity (Harris and Piccinin, 1977) so the mean of readings taken

immediately in front of and behind the vessel was assumed to be the light intensity received by the sample. Water temperature was kept to within 0.10°C of the experimental temperature using a Lauda Thermostat. The oxygen concentration was kept between 30 and 50% saturation by bubbling with nitrogen gas and pH was checked regularly to ensure that it did not fluctuate more than 1.0 unit. These crude control methods were found to give repeatable photosynthetic rates during short-term experiments. Changes in oxygen concentrations were recorded on a Linear 1200 chart recorder, and were calibrated against a standard of known oxygen concentration.

Comparison of the OLDB and OE methods

Although there is some evidence that the results obtained using the OE and ^{14}C methods are comparable (Harris and Piccinin, 1977), there appear to be no direct comparison of the OE and OLDB methods. However, Mantai (1974) found that the photosynthetic rates which he recorded for Cladophora glomerata using the OE method were three to four times higher than those recorded by Wood (1968) and Adams and Stone (1973) who used the OLDB method. He attributed these differences to the variation caused by long-term (OLDB method) and short-term (OE method) exposure, the former being more affected by photoinhibition (Harris and Piccinin, 1977). As photoinhibition occurs after long-term exposure to high light intensities (Harris and Piccinin, 1977), rates measured in situ (OLDB method) over a three hour could only be compared

with those measured over a ten minute period in the laboratory (OE method) when the responses to low light intensities were investigated.

The methods were compared on four occasions when, while samples were being incubated _in situ_, pond material was brought back to the laboratory where the photosynthetic response to light intensities of 20, 50, 100, 150, 200 and 500$\mu E.m^{-2}.s^{-1}$ was measured on the OE. The samples were incubated in freshly collected filtered effluent at the water temperature measured in the pond at collection. After measuring the dark respiration rate the photosynthetic rates at increasing light intensities were estimated over a ten minute interval. To avoid hysteresis, photosynthesis was only measured over a range of increasing light intensities (Falkowski and Owens, 1978) in which the alga was left in darkness for five minutes between each light intensity exposure. Only one sample was tested and its dry weight determined, as described earlier, in order to express photosynthetic rate in terms of biomass. By using alga from the same sample, incubating it at the same temperature, in the same medium, it was hoped that experimental error would be minimised.

Measurement of photosynthetic and respiratory constants

The OE method was also used to establish some of the physiological effects of light and temperature on net photosynthesis and respiration using unialgal laboratory cultures. Although algae are known to adapt to changes in

their environment, the response time varies from between a few seconds (eg fluorescence phenomena and the photochemical reactions following changes in irradiance - Marra, 1980) to a few days (eg the change in photosynthetic constants such as P_{max} and I_k and enzymatic changes - Harris, 1978; Li, 1980). In an attempt to investigate both short-term and long-term photosynthetic responses of R. riparium to changes in light intensity and long-term responses to temperature, samples of R. riparium were cultured in the laboratory under different conditions of light (20 and 300$\mu E.m^{-2}.s^{-1}$) and temperature (5, 15, 20, 25, 30 and 35^{o}C) for one week. The photosynthetic response to rapidly changing light intensities (between 50 to 3000$\mu E.m^{-2}.s^{-1}$) was then measured at constant temperature using the OE. The dark respiratory rate was estimated before the photosynthetic measurements were made.

The rate of change in oxygen concentration was expressed in terms of dry biomass and also chlorophyll a concentrations. The latter was calculated using a mean algal wet weight:chlorophyll a ratio, which varied between benthic and floating material and with growth temperature (Table 9), and the mean algal dry weight:wet weight ratio (0.763). Chlorophyll a concentrations were measured using the method of Sartory (1982) after grinding the filaments with acid-washed sand to break the cell walls and so increase extraction.

Table 9. Variation in the mean chlorophyll a:wet weight ratio recorded in light-adapted (grown at 300μE.m^{-2}.s^{-1}) and shade-adapted (grown at 20μE.m^{-2}.s^{-1}) R.riparium material. (n=40)

Temperature (°C)	5	15	20	25	30	35
Light-adapted material	0.97	2.86	2.86	0.34	0.27	0.24
Shaded-adapted material	1.41	3.39	3.43	1.30	1.24	0.42

5.2.3 Statistical analysis and data processing.

The differences in photosynthetic and respiratory rates recorded for benthic and surface material and for light-adapted (grown at 300μE.m^{-2}.s^{-1}) and shade-adapted (grown at 20μE.m^{-2}.s^{-1}) material were tested using the t-statistic for two means and two way analysis of variance (Bishop, 1980). All analyses were performed on a HP-65 programmable calculator. The data collected using the OE method were fitted to the hyperbolic tangent function of Jassby and Platt (1976). As the model only treats the portion of the photosynthesis:irradiance (P:I) curve up to and including light saturation, the data collected at light intensities exceeding I_k were fitted free-hand.

5.3 RESULTS AND DISCUSSION

5.3.1 Oxygen Light and Dark Bottle Method

Curves of net photosynthetic rates are presented in Figure 17. A number of trends and differences between the surface and the bottom material are evident from these results. For surface material there was a general decrease in the net photosynthetic rate with depth on most of the sampling occasions. However, on a few occasions peak activity was recorded at the sub-surface incubation depth (eg 21 September, 20 November, 18 March). In contrast, the maximum photosynthetic rate for benthic material was usually recorded at 0.25m, and on the majority of sampling dates the rate of net photosynthesis on the pond floor was higher than that

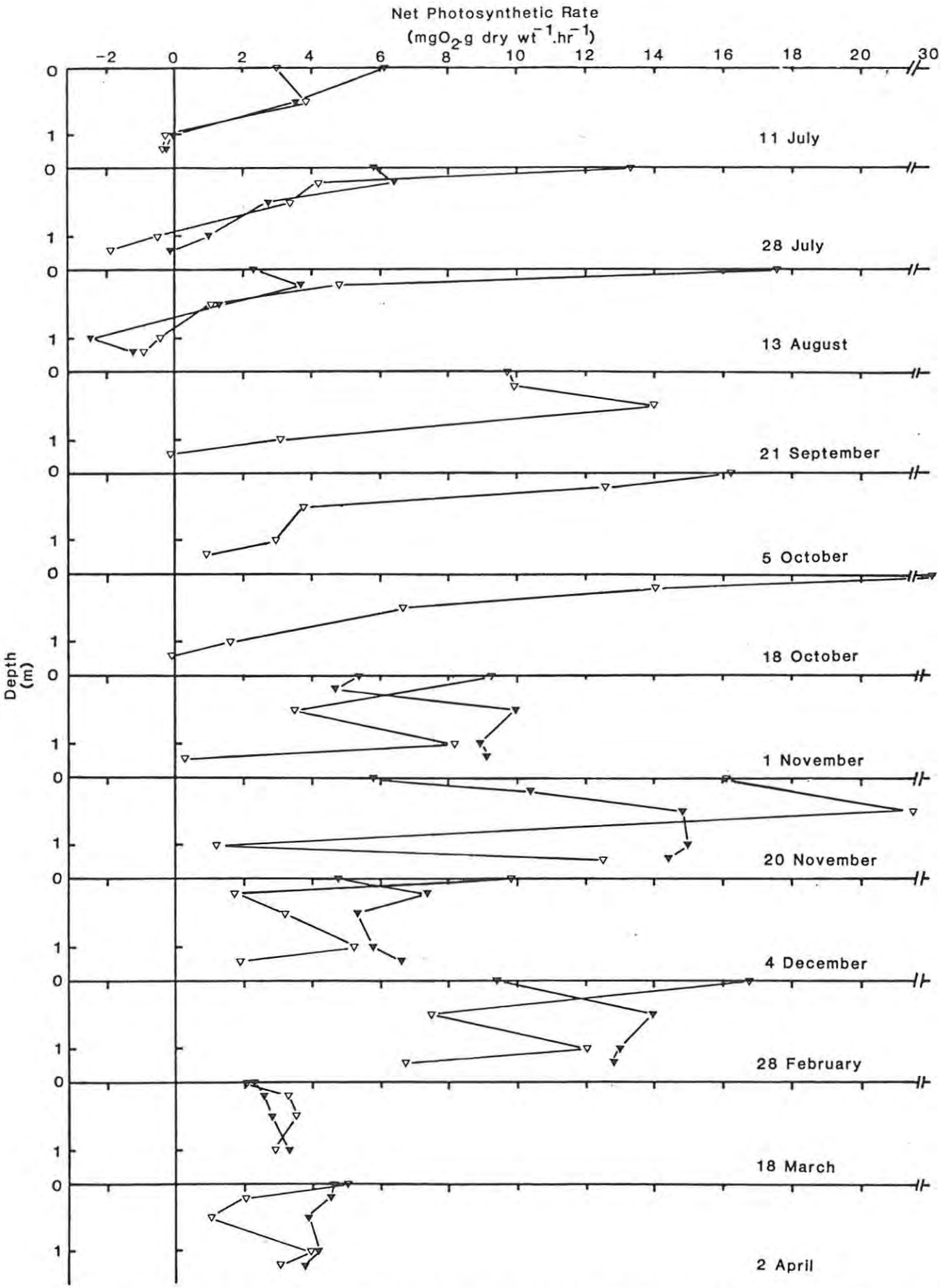


Figure 17. Net photosynthesis:depth curves for R. riparium measured, using the oxygen light and dark bottle method between 11 July, 1984 and 18 March, 1985 (▵ - surface material, ▲ - benthic material).

measured at the water surface (eg 1 November).

The results indicate that net photosynthesis of both benthic and surface material occurred throughout the water column on most occasions. Negative net photosynthetics rates were recorded only occasionally (11 July, 28 July, 13 August).

The maximum rate of net photosynthesis recorded during the study was 30.9mgO_2.g dry $wt^{-1}.hr^{-1}$ (18 October) while the lowest rate was -2.50mgO_2.g dry $wt^{-1}.hr^{-1}$ (13 August). These are comparable to rates reported for other filamentous green algae eg Ulothrix zonata - 16.8mgO_2.g dry $wt^{-1}.hr^{-1}$ (Graham et al, 1985), Cladophora glomerata - 52.4mgO_2.g dry $wt^{-1}.hr^{-1}$ (Mantai, 1974), 13.51mgO_2.g dry $wt^{-1}.hr^{-1}$ (Adams and Stone, 1973), 1.3mgO_2. g dry $wt^{-1}.hr^{-1}$ (Graham et al, 1982), 0.14mgO_2.g dry $wt^{-1}.hr^{-1}$ (Howard-Williams and Allanson, 1981) and algal mats 6.6mgO_2.g dry $wt^{-1}.hr^{-1}$ (Howard-Williams, 1978).

These results present evidence that the net photosynthetic rates of R. riparium mats were sensitive to light intensity and to water temperature, a feature which is to be expected in photosynthetic material. In addition, there were indications that photoinhibition may limit production at high surface light intensities. This seemed to be more apparent in material from the pond floor. In general, there was a difference in the photosynthetic response of benthic and surface material to different light regimes, with the benthic material being most active at light intensities below

those apparently favoured by surface material. The fact that net photosynthesis was almost always above zero supports the earlier conclusion that the light supply to the pond floor was capable of maintaining the growth and development of benthic algal mats (Section 3.3.3).

The mean rate of respiration recorded for benthic and floating material is presented in Table 10. The respiratory rate of surface material was higher than that of benthic material on all occasions. The rate of respiration varied from 1.168 to 6.766mgO_2.g dry $wt^{-1}.hr^{-1}$ in floating material and from 0.971 to 2.316mgO_2.g dry $wt^{-1}.hr^{-1}$ in benthic material. These rates are similar to those recorded for Ulothrix zonata - 1.74 to 6.9mgO_2.g dry $wt^{-1}.hr^{-1}$ (Graham et al, 1985) but are lower than those recorded in other filamentous algae eg Oscillatoria redekei - 5 to 18mgO_2.g dry $wt^{-1}.hr^{-1}$ (Foy and Gibson, 1982) and Cladophora glomerata - 16.66mgO_2.g dry $wt^{-1}.hr^{-1}$ (Graham et al, 1982).

Despite the fact that these trends were observed, they were interpretable on the basis of current knowledge of the behavior of algal material, and were comparable to results obtained in similar research programmes, it is felt that caution should be taken with the acceptance and interpretation of the absolute values recorded. This opinion is expressed because there were marked differences in the rates of net photosynthesis recorded between replicate light bottles incubated at the same depth, and because the respiration rates estimated from changes in the DO of dark

Table 10. Respiratory rates of *R. riparium* measured *in situ* using the OLDB method.

Date	Respiration Rate $mgO_2.g\ dry\ wt^{-1}.hr^{-1}$	
	Benthic Material	Surface Material
1984		
11 July	1.17	2.17
28 July	1.36	3.46
13 August	2.04	2.84
21 September	-	1.16
5 October	-	2.09
18 October	-	1.487
1 November	1.53	3.15
20 November	3.51	6.67
4 December	2.32	4.72
1985		
28 February	0.99	2.29
18 March	1.11	2.33
2 April	0.97	1.35

bottles containing the same material, varied widely (mean CV=180%). It is proposed that the main source of error was the measurement of algal biomass within each bottle because of the epiphytes on and the debris trapped in the filament mesh. This view is supported by evidence that shows that there was more error incurred when measuring the biomass of material collected in the field than measuring that of algae grown in laboratory culture. Assuming that the error in measuring chlorophyll *a* concentration is similar in both, there was twice as much variation recorded using material from the maturation pond when estimating the wet weight:chlorophyll *a* ratio (CV=35% in material from the pond, 17% in material grown in laboratory culture).

It would have been impractical to improve the method by increasing the number of replicate bottles. Previous studies which had investigated the rates of photosynthesis and respiration of filamentous algae could not be used as guides to measuring biomass as they measured dry weight (Howard-Williams and Allanson, 1981; Mantai, 1974; Oates and Murray, 1983; Taylor, 1983), freeze-dried weight (Adams and Stone, 1973; Graham *et al*, 1982), fresh weight (Muller, 1978) or ash-free dry weight (Wood, 1968), all of which would have included the debris causing the error. Neither could biomass be measured as cellular chemical components such as chlorophyll *a* as the sample was acidified during DO determination, and prior removal made DO determination inaccurate. Considering the size and number of samples cell

counts were also impractical.

Considering the error recorded in *in situ* measurements, there was no point in estimating the photosynthetic constants from these data, and the results could not be used to compare the OLDB and the OE method.

5.3.2 The Oxygen Electrode Method

The relationship between light intensity and photosynthesis is a sensitive indicator of photosynthetic efficiency, photosynthetic capacity and the light history of the algal material (Porter, Muscatine, Dubinsky and Falkowski, 1984). Using the OE data collected it was possible to investigate the photosynthetic differences between light-adapted (grown at 300$\mu E.m^{-2}.s^{-1}$) and dark-adapted (grown at 20$\mu E.m^{-2}.s^{-2}$) material, to postulate on the means by which photoadaption is achieved and then to extrapolate these results to discuss the variation between the sub-populations of *R. riparium* in the maturation pond. The results are presented as P:I curves normalised to dry weight (Figure 18), whilst Table 11 gives a summary of some photosynthetic constants of *R. riparium* normalised either to dry weight or to chlorophyll *a* concentration.

In general the P:I curves (Figure 18) followed a typical pattern (Harris and Piccinin, 1977). The rate of net photosynthesis increased with light intensity until photosynthesis was light saturated, where P_{max} was highest in light-adapted material. At light intensities exceeding I_k the

Table 11. Photosynthetic characteristics of light-adapted and shade-adapted R.riparium material.

Characteristic	Light-adapted Temperature (°C)						Shade-adapted Temperature (°C)					
	5	15	20	25	30	35	5	15	20	25	30	35
1. α	0.25	0.26	0.20	0.20	0.32	0.27	0.05	0.09	0.09	0.10	0.13	0.07
2. α	0.19	0.20	0.13	0.15	0.25	0.21	0.04	0.07	0.07	0.04	0.08	0.05
3. I_c	42	71	116	94	67	62	18	24	35	71	44	3.1
4. I_k	500	400	600	551	504	510	572	780	650	616	514	729
5. P_{max}	36.1	56.7	61.8	67.1	51.3	31.7	15.5	17.9	28.0	36.4	32.3	21.1
6. P_{max}	8.4	12.1	15.3	14.2	10.4	3.8	1.3	11.2	12.3	13.0	12.1	4.8
7. R_D	10.56	13.76	19.17	22.89	25.34	27.11	2.60	6.57	8.62	11.51	13.51	19.61
8. R_D	2.25	3.48	4.76	5.94	6.41	8.47	0.63	1.37	1.80	2.88	3.47	4.89

1. α - The slope of the light-limited portion of the P:I curve ($mgO_2.g\ dry\ wt^{-1}.hr^{-1}\ (\mu E.m^{-2}.s^{-1})^{-1}$)
2. α - The slope of the light-limited portion of the P:I curve ($mgO_2.g\ chl\underline{a}^{-1}.hr^{-1}\ (\mu E.m^{-2}.s^{-1})^{-1}$)
3. I_c - The compensation light intensity, at which the rate of net photosynthesis is zero ($\mu E.m^{-2}.s^{-1}$)
4. I_k - The light intensity at which the rate of photosynthesis becomes light saturated ($\mu E.m^{-2}.s^{-1}$)
5. P_{max} - The light saturated rate of net photosynthesis ($mgO_2.g\ dry\ wt^{-1}.hr^{-1}$)
6. P_{max} - The light saturated rate of net photosynthesis ($mgO_2.g\ chl\underline{a}^{-1}.hr^{-1}$)
7. R_D - The dark (basal) respiratory rate, measured after 12 hours of darkness ($mgO_2.g\ dry\ wt^{-1}.hr^{-1}$)
8. R_D - The dark (basal) respiration rate, measured after 12 hours in darkness ($mgO_2.g\ chl\underline{a}^{-1}.hr^{-1}$)

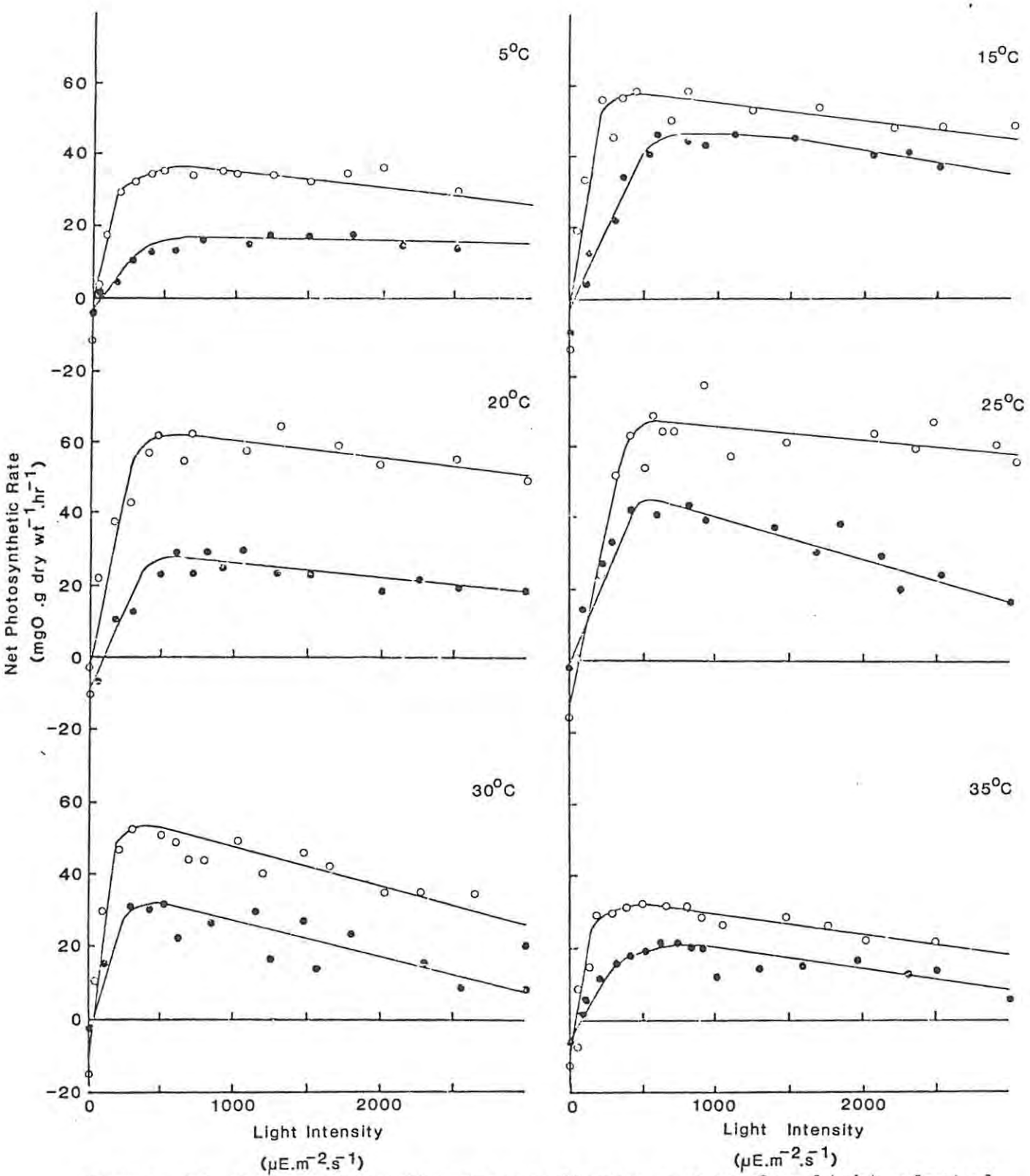


Figure 18. Net photosynthesis:irradiance curves for light-adapted (o) and shade-adapted (●) R. riparium material grown at different temperatures.

rate of net photosynthesis decreased, and this slope increased with water temperature. This may have been an indication of photoinhibtion. A second difference in the P:I curves of light- and shade-adapted material was the slope of the light-limited portion which was greatest in light-adapted material.

The magnitude of α is a reflection of the light utilization efficiency of algae at sub-saturating light intensities (Porter et al, 1981). It is similar to the quantum yield of photosynthesis (ϕ) in all respects except that the latter is independent of chlorophyll a concentration (Bannister, 1974), and is useful as a photosynthetic parameter because it is independent of water temperature (Welschmeyer and Lorenzen, 1981). In R. riparium α was highest in light-adapted material (Tables 11 and 12). Values for shade-adapted material were similar to those recorded in other temperate algae (0.050 to 0.099mgO_2.g dry $wt^{-1}.hr^{-1}.\ \mu E\ m^{-2}\ s^{-1})^{-1}$, but that of the light-adapted alga is relatively high (Foy and Gibson, 1982; Porter et al, 1984).

The I_c values of light-adapted material were significantly higher than those of material which was shade-adapted (Tables 11 and 12; Figure 19). I_c showed a bell-shaped curve with temperature, with a maximum at 20°C in light-adapted material and 25°C in shade-adapted material (Figure 19). These I_c values are relatively high (Kirst, 1981; Beer and Levy, 1983; Carpenter, 1985) but are typical of the green algae which require high light intensities before net photosynthesis

Table 12. A summary of the changes in the photosynthetic characteristics of R. riparium due to the photoadaption applied in this study.

Parameter	Change	Significance
α (dry wt)	decreased 2.8 times	t=7.47 df=10 p>0.001
(chl a)	decreased 3.2 times	t=6.85 df=10 p>0.001
I_c	decreased 1.3-3.0 times	F=16.53 df=1 p>0.01
I_k	increased 1.0-2.0 times	not significant
P_{max} (dry wt)	decreased 1.5-10.3 times	F=40.65 df=1 p>0.01
(chl a)	increased/decreased	not significant
R_D (dry wt)	decreased 1.4-4.0 times	F=120.0 df=1 p>0.001
(chl a)	decreased 1.7-3.6 times	F=86.75 df=1 p>0.001

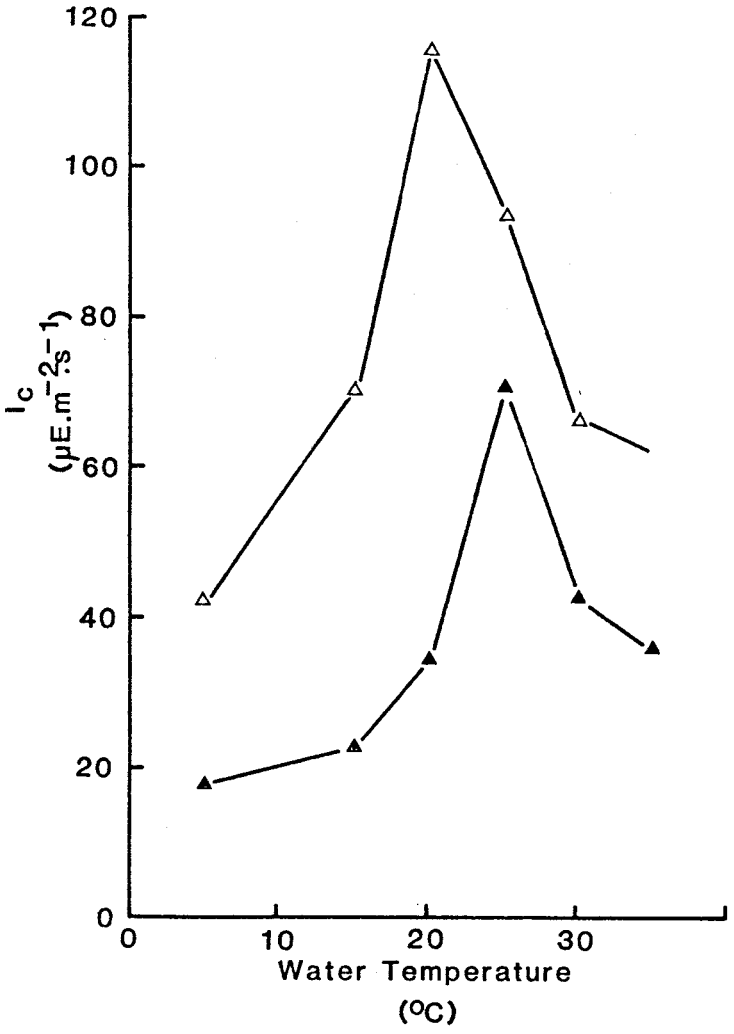


Figure 19. Variation in the I_c value of light-adapted (△) and shade-adapted (▲) R. riparium material, with water temperature.

occurs (Richardson, Beardall and Raven, 1983).

In R. riparium I_k varied between 400 and 729$\mu E.m^{-2}.s^{-1}$ (Tables 11 and 12). These values are higher than those recorded in blue-green algae and temperate phytoplankton populations, but are not as high as those recorded in reef algae (Carpenter, 1985).

When expressed in terms of dry weight P_{max} was highest in light-adapted material (Table 12) as shade-adapted algae are unable to utilise saturating light intensities (Beardall and Morris, 1976). P_{max} was also a function of temperature, reaching a maximum of 67.1mgO_2.g dry $wt^{-1}.hr^{-1}$ and of 36.4mgO_2.g dry $wt^{-1}.\ hr^{-1}$ at 25°C in light- and shade-adapted algae respectively (Figure 20). When normalised to chlorophyll a concentration P_{max} of light- and shade-adapted alga was not significantly different (Table 12), indicating that the light-trapping efficiency of the chlorophyll a was similar despite photoadaption. The photosynthetic pattern observed between light- and shade-adapted R. riparium was similar to that recorded in surface and benthic pond material respectively in that the maximum rate of photosynthesis recorded for the benthic population in situ was lower than that recorded for the surface (light-adapted) population. However, P_{max} values were approximately half those recorded using the OE method, which is similar to the results reported by Mantai (1974).

The rate of dark respiration was related to temperature in

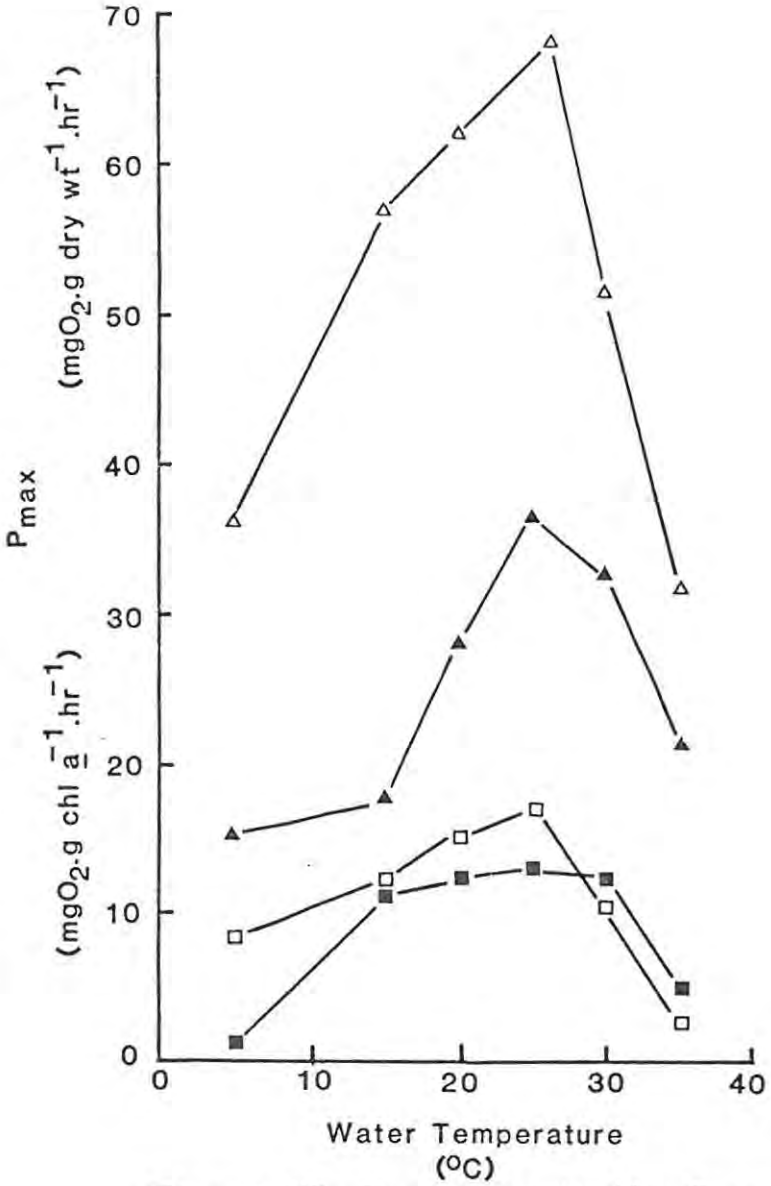


Figure 20. Variation in the P_{max} value of light-adapted (△ - dry weight; □ - chl a) and shade-adapted (▲ - dry weight; ■ - chl a) R. riparium material, with water temperature.

R. riparium (Figure 21) with the R_D increasing with temperature in both light-adapted and shade-adapted material (Table 11). This is the case in most algae (eg net plankton - Harris, 1973; Cladophora glomerata - Graham et al, 1982; reef algae - Porter et al, 1981; Microcystis aeruginosa - Robarts, 1984), and is attributed to the increase in the metabolic activity associated with increasing water temperature (Falkowski, 1981). The Q_{10} values of shade-adapted and light-adapted material were similar (Figure 21). This suggests that that the difference in R_D was due to higher metabolic activity in light-adapted material, rather than to differences in their response to temperature.

5.4 GENERAL DISCUSSION

Photoadaption has been observed in many algae (Jorgenson, 1969; Harris and Lott, 1973; Beardall and Morris, 1976; Prezelin, 1976; Prezelin and Sweeney, 1977; Raven, Smith and Glidewell, 1979; Falkowski and Owens, 1980; Porter, 1980; Harding, Meeson, Prezelin and Sweeney, 1981; Kirst, 1981; Foy and Gibson, 1982; Rivkin, Seliger, Swift and Biggley, 1982; Beer and Levy, 1983; Heine, 1983; Porter et al, 1981; Carpenter, 1985) where it is achieved in one of two ways (Falkowski, 1980; Ramus and Rosenburg, 1980; Ramus, 1981):

1. The Chlorella-type adaption where there is an increase in the photosynthetic pigment concentration of the cell so that there is an increase in the rate of the light reactions.
2. The Cyclotella-type adaption where the enzyme levels are changed so that the rate of the dark reactions are increased.

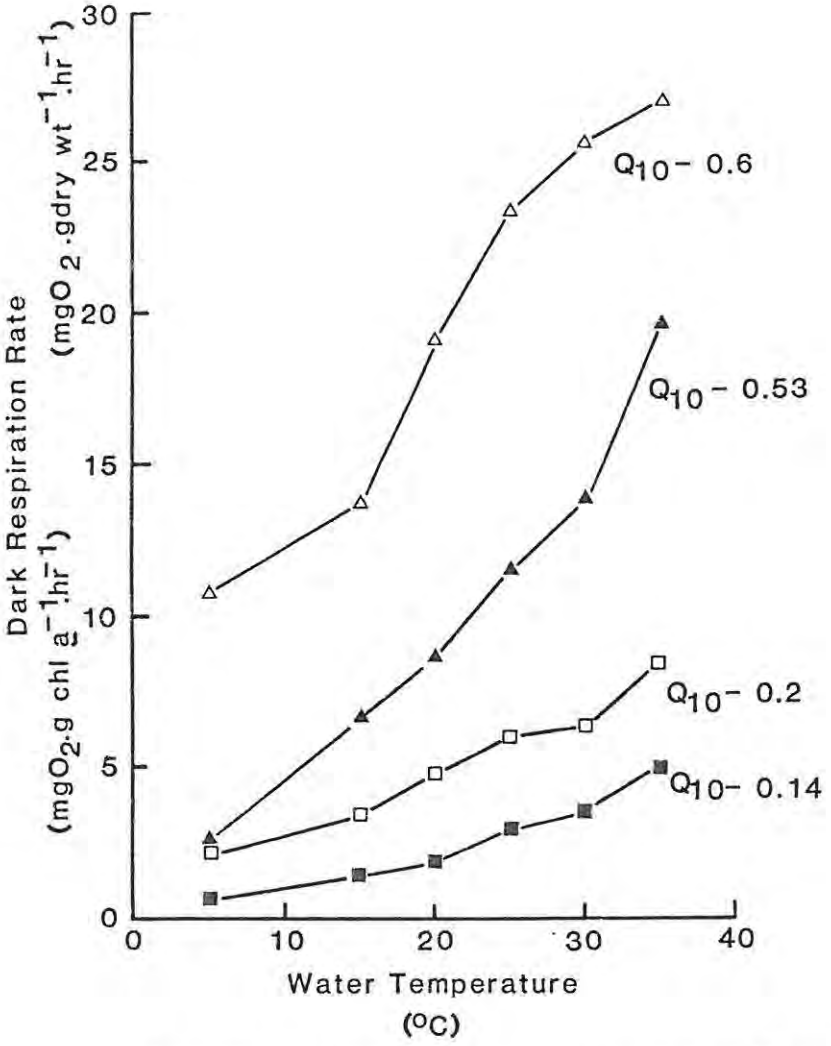


Figure 21 Variation in R_D value (△ - dry weight; □ - chl a) and shade-adapted (▲ - dry weight; ■-chl a) R. riparium material, with water temperature.

The differences in the chlorophyll a:wet weight ratio of light- and shade-adapted algae (Table 9) suggests that photoadaption in R. riparium is achieved by Chlorella-type adaption, which is typical of green algae. By changing the relative proportion of pigments in photosystems I and II (PSI and PSII) the algae can increase their light utilization efficiency. This is achieved by either increasing the size of P_{700} units,which increases the area over which light can be absorbed or, increasing the number of P_{700} units per photosynthetic unit, which increases the rate of electron flow (Prezelin and Sweeney, 1977; Falkowski, 1980). However, the observed differences in the R_D values (Figure 21) suggests that there is a change in metabolic activity during photoadaption. As this is probably achieved by changes in the enzyme levels of the cells, adaption could also follow that typical of Cyclotella. The data presented is insufficient to explain the photoadaption strategy fully.

Several authors (eg Yentsch and Lee, 1966; Beardall and Morris, 1976) have questioned whether the energy used to adapt to changing light environment is worth the gain in photosynthetic efficiency. In relation to algae in a well mixed environment it would seem a debatable point (Falkowski, 1980) but in this situation, where the algal mats are virtually fixed either at the pond surface or its floor, adaption would certainly warrant the physiological changes. During initial mat development the alga would be confined to the pond floor where light intensity would be low throughout

the day and adaption to shade conditions necessary if growth was to occur. Once the mat had become buoyant and floated to the pond surface cells would adapt to the high light environment thereby exploiting the conditions.

The data gathered during this study indicate that R. riparium is a highly productive green alga, able to photosynthesise at a rate of up to 67.1mgO_2.g dry $wt^{-1}.hr^{-1}$ under optimum conditions of light and temperature. Photosynthesis is dependent on water temperature, nutrients, light intensity and the adaptive state of the alga. When light-adapted the alga was able to maintain a high rate of photosynthesis under even extremely high light intensities eg 59mgO_2.mg dry $wt^{-1}.hr^{-1}$ at 25°C and 3000$\mu E.m^{-2}.s^{-1}$ (Figure 18). The relatively high I_k values indicate that unless the light intensity was high photosynthesis would be light-limited. In the maturation pond, where only the surface layer of algal filaments was not shaded it would seem that production is limited by the light supply, even though the shade-adapted material was able to use light more efficiently. However, the I_c value of shade-adapted material was lower than that of material adapted to high light intensities, and net photosynthesis would have been positive above intensities of between 17 and 71$\mu E.m^{-2}.s^{-1}$. In the maturation pond the intensity of light reaching the pond floor at noon was higher than this on all but two sampling occasions (Table 4), so it would seem that although photosynthesis in the benthic mats was limited, photosynthesis occurred.

The optimum temperature for photosynthesis was between 20 and 25°C which was higher than that for growth (Section 4.3.2). This is the case in most algae and is explained in terms of changes in rates of different reactions with temperature so that some reactions are enhanced while others are depressed (Li, 1980). Thus, in the maturation pond where water temperature favoured growth during early summer and the beginning of winter, photosynthesis was favored during mid-summer.

In the maturation pond R_D would have varied between 14 and 23mgO_2. g dry $wt^{-1}.hr^{-1}$ in light-adapted material and from 2 to 20mgO_2. g dry $wt^{-1}.hr^{-1}$ in shaded mats. These rates are relatively high (Graham et al, 1985) and would have reduced gross production during the warm summer months.

The high rate of net photosynthesis, and thus the rapid production of oxygen observed in R. riparium suggests that it would make a valuable contribution to the oxidation of sewage effluent in th maturation pond. This was observed previously when the DO concentration of effluent around the mats was significantly higher than that in open areas (Section 3.3.2). However, their respiratory demand was also high, especially as the filaments supported a large heterotrophic community. Thus, under conditions unsuitable for photosynthesis they would have increased the systems oxygen demand significantly. For example, if water temperature was 25°C and photosynthesis was unsaturated for most of the day the algal mats would have produced approximately 686.8mgO_2.g dry $wt^{-1}.day^{-1}$, and would

have consumed about 251.79mgO_2.g dry $wt^{-1}.day^{-1}$ (a net increase of 435.01mgO_2.g dry $wt^{-1}.day^{-1}$). However, when it was cooler (15°C), and the light conditions were such that the light period was reduced and photosynthesis was light-limited, the balance would be a net increase of only 60.45mgO_2.g dry $wt^{-1}.day^{-1}$. These calculation only include the algal mats which were able to photosynthesise during the daylight period, and so the total population oxygen demand would be greater than this. Thus, when conditions were suitable for photosynthesis by the entire algal population they would have been able to oxidise the ~~the~~ effluent, but when there was significant shading or environmental conditions did not favour photosynthesis they would have been unable to do so. Nevertheless, in the absence of an active phytoplankton population their activity must be considered significant in the purification of effluent.

5.5 CONCLUSIONS

1. R. riparium has a high rate of photosynthesis under optimum conditions. It favours water temperatures between 20 and 30°C and relatively high light intensities (above approximately 600μE.$m^{-2}.s^{-1}$).

2. The alga exhibited photoadaption when grown under conditions of high and low light intensity. Photoadaption appeared to be achieved by changing the chlorophyll a concentration of the cell, and by increasing the enzyme levels.

3. R. riparium was sensitive to photoinhibition. The effect of photoinhibtion was enhanced by increasing growth temperature.

4. Under optimum environmental conditions the algal mats would have made a significant contribution to effluent oxidation, but when photosynthesis was not favoured they would have increased the oxygen demand of the pond.

6.0 GENERAL DISCUSSION

The results of this study indicate that the success of R. riparium in the maturation pond environment can be attributed to:

1. The water temperature of the environment which favours this alga's growth for a large proportion of the year;
2. Low light attenuation by the effluent which enables the penetration of PAR to the pond floor, thus allowing the growth of benthic mats. This is largely the result of the virtual absence of a phytoplankton population;
3. The high concentrations of plant nutrients in the effluent, which laboratory studies showed was a substrate suitable for R. riparium growth;
4. The absence of natural predators in the pond;
5. The alga's ablility to adapt to a changing light regime which allows its continued growth in high and low light intensity environments as it develops on the pond floor and then floats to the surface; and
6. The growth habit of the alga which means that it will not be flushed out of the system by water flow through the ponds.

These factors combine to create an environment in which this alga is able to successfully compete with other species, and can therefore dominate the system.

As the maturation pond system was operating during the study period, the R. riparium mats were performing an essential function as they were the only photosynthetically active

algal population. However, the mats were autoinhibitory and prevented wind-driven mixing so that in the long term they were actually detrimental to the system. The shading of the water column by the surface mats was one of the factors limiting the growth and subsequent development of a phytoplankton population. Such a population could fulfil the positive role played by the mats at present, while not interfering with the physical environment in the same detrimental manner. It is clear that the efficiency of the maturation pond system could be improved by removing the surface mats permanently. Because of the habit of the alga this would require that the benthic mats be removed as well.

The control of aquatic weeds has been effected using a wide variety of methods including harvesting, the use of herbicides and algicides, the introduction of herbivorous predators such as snails, insects and fish and by biological methods which change the environment so that plant growth becomes limited, usually by light or by nutrient supply (Bennett, 1972; Perkins, 1974; Spencer, 1974; von Zon, 1974; N.A.S., 1976). The choice of methods is largely determined by the cost of control in relation to the benefits gained (Mitchell, 1979), and must take into account factors such as the permanence and the specificity of the solution.

Algicides could be used to control mat growth but, this is an expensive method, and their non-specific action makes them unsuitable for this particular system.

Up until this time the control of algal mat growth in the maturation pond has been attempted using harvesting methods. However, as this study indicated, removing the surface mats will actually enhance mat growth by maintaining the benthic light supply. Thus, at best harvesting can only achieve a temporary decline in the size of surface mats. More permanent relief could only be gained by preventing the growth of the benthic ones.

The most efficient means of doing so would be to reduce the pond floor's light supply. However, the light supply to the rest of the pond must not be interferred with if phytoplankton growth is to be maintained. Thus, instead of shading the pond, for example with trees and shrubs (von Zon, 1973) some means of cutting off the light supply at the sediments is required. This may be achieved either directly, by laying black plastic sheets over the floor (eg Weed-Barrier - Walmsley, personal communication[6]) or indirectly by stirring up the pond sediments. The former method is highly efficient, but is expensive and will only ensure that mat growth is inhibited. The indirect method has the advantage that it can be achieved using a bottom-feeding fish, many of which will graze the algal mats and thus remove the alga physically as well.

The ideal fish for this task is the Chinese grass carp (Ctenopharyngodon idella Val.) which has been used widely,

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6. Dr. R.D. Walmsley, Co-operative Scientific Programmes, C.S.I.R., PO Box 395, Pretoria, 0001.

and successfully to control the growth of submerged plant and algal material (von Zon, 1974; N.A.S., 1976; Mitchell, 1977). It has the advantages that it feeds voraciously, consuming several times its body weight as a fingerling, and at least its body weight as an adult and its preferred food is soft plants and algae. In addition, it does not breed easily outside its natural range, and monosex populations can be obtained so there is little danger of the fish interfering with the indigenous fish population should it escape from the ponds. It is suited to the pond environment where the water temperatures are relatively high and stable although it is intolerant of low DO concentrations (N.A.S., 1976). Furthermore, it will not feed on the phytoplankton so grazing will be specific. Alternatively, one of the following fish could be grown in the pond: Tilapia zillii, T. rendalli, T. guineesis, Sarotherodon mossambicus (the Mozambique tilapia), S. niloticus and Hypophthalmichthys molitrix (the silver carp) (N.A.S., 1976), but they would not be as well suited either because they also feed on phytoplankton or because they are not bottom-feeders.

The removal of the algal mats will not ensure the development of an active phytoplankton population, as it seems that phytoplankton growth was also inhibited by zooplankton grazing and by wash-out. Thus, to gain the maximum benefit of removing algal mats the size of the zooplankton population should be reduced, and the retention time of the pond increased to above 5 days. The former could be achieved by

the addition of zooplanktonivorous fish while the pond could be enlarged to increase its retention time.

In conclusion, the algal mats inhibit the development of an active phytoplankton population in the maturation pond, either by reducing the light supply or by producing some kind of growth inhibiting substance. As the phytoplankton are an essentual component in the purification system of maturation ponds, it is vital that the mats be removed. It is recommended that this be done by the introduction of a bottom-feeding herbivorous fish, such as the Chinese grass carp, which would remove algal biomass while preventing the growth of benthic mats by reducing the light supply to the pond floor. As the fish could become a permanent part of the maturation pond system algal regrowth from reproductive initials present in the pond sediments would be prevented. Once the phytoplankton do not have to compete with more successful R. riparium, and grazing and washout were reduced, a population could be established. It would take over the role in effluent oxidation performed by the algal mats at present and, because it would not interfere with the mixing induced by wind action would increase the systems purification efficiency.

REFERENCES

ABBOTT, I.A. and HOLLENBERG, G.J. (1976) Marine Algae of California. Stanford University Press. Stanford, California.

ABELIOVICH, A. (1981) Factors affecting algal growth in high rate oxidation ponds. In: Shelef, G.H. and Soeder, C.J. (Eds), Algal Biomass, Production and Use. North Holland Biomedical Press.

ABELIOVICH, A. and AZOV, V. (1976) Toxicity of ammonia to algae in sewage oxidation ponds. Applied Environmental Microbiology, 31: 801-816.

ADAMS, M.S. and STONE, W. (1973) Field studies on photosynthesis of Cladophora glomerata (Chlorophyta) in Green Bay, Lake Michigan. Ecology, 54: 853-862.

ALLANSON, B.R. (1961) Investigations into the ecology of polluted inland waters in the Transvaal. I. Hydrobiologia, XVIII, 5-76.

AUER, M.T. and CANALE, R.P. (1982) Ecological studies and mathematical modeling of Cladophora in Lake Huron. 2. Phosphorus uptake kinetics. Journal of Great Lakes Research, 8: 84-92.

AUER, M.T., CANALE, R.P., GRUNDLER, H.C. and MAKSUOKA, Y. (1982) Ecological studies and mathematical modeling of Cladophora in Lake Huron. I. Program description and field monitoring of growth dynamics. Journal of Great Lakes Research, 8: 73-83.

BANNISTER, T.T. (1974) Production equations in terms of chlorophyll concentration, quantum yield, and the upper limit to production. Limnology and Oceanography, 19: 1-12.

BEARDALL, J. and MORRIS, I. (1976) The concept of light intensity adaption in marine phytoplankton: Some experiments with Phaeodactylum tricornutum. Marine Biology, 37: 377-387.

BEER, S. and LEVY, I. (1983) Effects of photon ~~fluence~~ rate and light spectrum composition on growth, photosynthesis and pigment relations in Gracilaria sp.. Journal of Phycology, 19: 516-522.

BELL, C.G. (1983) An Investigation into the Effects of the Avifauna on the Ecology of the Maturation Ponds of the Grahamstown Sewage Works. Third Year Project, Plant Science Department, Rhodes University, Grahamstown, South Africa.

BELLIS, V.J. (1968) Unialgal cultures of Cladophora glomerata (L.) Kutz. I. Response to temperature. Journal of Phycology, 4: 19-23.

BELLIS, V.J. and McLARTY, D.A. (1967) Ecology of Cladophora glomerata (L.) Kuzt. in southern Ontario. Journal of Phycology, 3: 57-63.

BENNETT, F.D. (1972) Some aspects of the biological control of aquatic weeds. Proceedings of the Second International Symposium on the Biological Control of Weeds: 63-73.

BICKERTON 1983

BISHOP, O.N. (1980) Statistics for Biology (Third Edition). Longman Group Limited. Essex.

BLAIR, S.M. (1983) Taxonomic treatment of the Chaetomorpha and Rhizoclonium species (Cladophorales: Chlorophyta) in New England. Rhodora, 85: 175-211.

BOLD, H.C. and WYNNE, M.J. (1978) Introduction to the Algae: Structure and Reproduction. Prentice-Hall Inc. Englewood Cliffs.

BROWN, D.L. and WEIER, T.E. (1970) Ultrastructure of the freshwater alga Batrachospermum. I. Thin-section and freeze-etch analysis of juvenile and photosynthetic filament vegetative cells. Phycologia, 9: 217-235.

BRYAN, J.R., RILEY, J.P. and WILLIAMS, P.J.Le B. (1976) A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. Journal of Experimental Marine Biology and Ecology, 21: 191-197.

CANALE, R.P. and AUER, M.T. (1982) Ecological studies and mathematical modeling of Cladophora in Lake Huron. 5. Model development amd calibration. Journal of Great Lakes Research, 8: 112-125.

CARPENTER, R.C. (1985) Relationships between primary productivity and irradiance in coral reef algal communities. Limnology and Oceanography, 30: 784-793.

CARTER, N. (1933a) A comparative study of the algal flora of two salt-marshes. II. Journal of Ecology, 21: 128-208.

CARTER, N. (1933b) A comparative study of the algal flora of two salt-marshes. III. Journal of Ecology, 21: 385-403.

CHAN, A.T. (1978) Comparative physiological study of marine diatoms and dinoflagellates in relation to irradiance and cell size. I. Growth under continuous light. Journal of Phycology, 14: 396-402.

CHAN, K-Y., LING-WONG, S.L. and WONG, M.H. (1978) Observations on Chaetomorpha brachygona Harv. (Chlorophyta, Cladophorales). 1. Ultrastructure of the vegetative cells. Phycologia, 17: 419-429.

CHAPMAN, V.J. (1939) Some algal complexities. Rhodora, 41: 19-28.

CHAPMAN, V.J. (1941) An Introduction to the Study of Algae. Cambridge University Press. Cambridge.

CHAPMAN, V.J. (1964) The Algae. Macmillan and Co. Ltd. London, New York.

CHU, S.P. (1942) The influence of the mineral composition of the medium on the growth of planktonic algae. I. Methods and culture media. Journal of Ecology, 30: 284-325.

CHU, S.P. (1943) The influence of the mineral composition of the medium on the growth of planktonic algae. II. The influence of the concentration of the inorganic nitrogen and phosphate phosphorus. Journal of Ecology, 31: 109-148.

CLOERN, J.E. (1977) Effects of light intensity and temperature on Cryptomonas ovata growth and nutrient uptake rates. Journal of Phycology, 13: 389-395.

CONWAY, H.L. (1977) Interactions of inorganic nitrogen in the uptake and assimilation by marine phytoplankton. Marine Biology, 39: 221-232.

CURL, H. and McLOED, G.C. (1961) The physiological ecology of a marine diatom Skeletonema costatum. Journal of Marine Research, 19: 70-88.

DE NOYELLES, F. (1967) Factors affecting phytoplankton distribution in a double-cell sewage lagoon. Journal of Phycology, 3: 174-181.

DE VILLIERS , G. du T. and MALAN, E. (1985) The water quality of a small urban catchment near Durban, South Africa. Water, S.A., 11: 35-40.

DODGE, J.D. (1973) The Fine Structure of Algal cells. Academic Press. London, New York.

DU PREEZ, A. (1985) The chemical composition of Transkei river water. Water S.A., 11: 41-47.

EBERLY, W.R. (1963) Oxygen production in some northern Indiana lakes. Proceedings of the Seventeenth Industrial Waste Conference Engineering Bulletin of Purdue University. XLVII: 733-747.

EMMERSON, W.D., McLACHLAN, A., WATLING, H.R. and WATLING, R.J. (1983) Some ecological effects of two sewage outfalls in Algoa Bay. Water S.A., 9: 23-30.

EPPLEY, R.W. (1972) Temperature and phytoplankton growth in the sea. Fishery Bulletin, 70: 1063-1085.

ERNST, D. and REINHARDT, D. (1980) Primary productivity measurements and carbon metabolism in Steinhuder-Meer and Lake Dummer. In: Dokulil, M., Metz, H. and Jewson, D. (Eds.) Developments in Hydrobiology. Volume 3. W. Junk Publishers. The Hague, Boston, London.

FALKOWSKI, P.G. (1980) Light-shade adaption in Marine Phytoplankton. In: Falkowski, P.G. (Ed.) Primary Productivity in the Sea. Plenum Press. New York and London. pp 99-117.

FALKOWSKI, P.G. (1981) Light-shade adaption and assimilation numbers. Journal of Plankton Research, 3: 203-216.

FALKOWSKI, P.G. and OWENS, T.G. (1978) Effects of light intensity on photosynthesis and dark respiration in six species of marine phytoplankton. Marine Biology, 45: 289-295.

FALKOWSKI, P.G. and OWENS, T.G. (1980) Light-shade adaption. Two strategies in marine phytoplankton. Plant Physiology, 66: 592-595.

FEKETE, A., RIEMER, D. and MOTTO, H. (1972) Removal of Rhizoclonium from a pond and its relationship to dissolved nutrients. Northeastern Weed Science Society, 26: 193-196.

FOGG, G.E. (1975) Algal Cultures and Phytoplankton Ecology. Athlone Press. London.

FOY, R.H., GIBSON, C.E. and SMITH, R.V. (1976) The influence of day length, light intensity and temperature on the growth rates of planktonic blue-green algae. British Phycological Journal, 11: 151-163.

FOY, R.H. and GIBSON, C.E. (1982) Photosynthetic characteristics of planktonic blue-green algae: Changes in photosynthetic capacity and pigmentation of Oscillatoria redenkei von Door under high and low light. British Phycological Journal, 17: 183-193.

FRITSCH, F.E. (1935) The Structure and Reproduction of the Algae. Volume 1. Cambridge University Press, Cambridge.

GANAPATI, S.V. (1975) Biochemical studies of algal-bacterial symbiosis in high-rate oxidation ponds with varying detention periods and algae. Archiv für Hydrobiolgie, 76: 302-368.

GODWARD, M.B.E. (1966) The Chromosomes of the Algae. Edward Arnold Ltd. London.

GOLTERMAN, H.L. (1975) Developments in Water Science. Elsevier Scientific Publishing Company. Amsterdam, Oxford, New York.

GOLTERMAN, H.L. and CLYMO, R.S. (1971) Methods for the Chemical Analysis of Freshwater. IPB Handbook No. 8, (2nd Edition). Blackwell Scientific Publications. Oxford, Edinburgh.

GRAHAM, J.M., AUER, M.T., CANALES, P. and HOFFMANN, J.P. (1982) Ecological studies and mathematical modeling of Cladophora in Lake Huron. 4. Photosynthesis and respiration as functions of light and temperature. Journal of Great Lakes Research, 8: 100-111.

GRAHAM, J.M., KRANZFELDER, J.A. and AUER, M.T. (1985) Light and temperature as factors regulating seasonal growth and distribution of Ulothrix zonata (Ulvophyceae). Journal of Phycology, 21: 228-234.

GRINDLEY, T.B. (1983) Eeste. In: Heinecken, T.J.E., Bickerton, I.B. and Heydorn, A.E.F. (Eds.) A Summary of Studies of the Pollution Input by Rivers and Estuaries entering False Bay. CSIR Report T/SEA 8301.

GROBBELAAR, J.U. (1982) Potential of algal production. Water S.A., 8: 79-85.

HANIC, L.A. and CRAIGIE, J.S. (1969) Studies on the algal cuticle. Journal of Phycology, 5: 89-102.

HARDING, L.W., MEESON, B.W., PREZELIN, B.B. and SWEENEY, B.M. (1981) Diel periodicity of photosynthesis in marine phytoplankton. Marine Biology, 61: 95-105.

HARRIS, G.P. (1973) Diel and annual cycles of net plankton photosynthesis in Lake Ontario. Journal of the Fisheries Research Board of Canada, 30: 1779-1787.

HARRIS, G.P. (1978) Photosynthesis, productivity and growth: the physiological aspect of phytoplankton. Ergebniesse der Limnologie (Archiv für Hydrobiologie), 10: 1-171.

HARRIS, G.P. (1980) The measurement of photosynthesis in natural populations on phytoplankton. In: Morris, I. (Ed.) The Physiological Ecology of Phytoplankton. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Melbourne. pp 129-187.

HARRIS, G.P. (1984) Physiological responses of phytoplankton to natural light regimes. Journal of Plankton Research, 6: 295-308.

HARRIS, G.P. and LOTT, J.N.A. (1973) Light intensity and photosynthetic rates in phytoplankton. Journal of the Fisheries Board of Canada, 30: 1771-1778.

HARRIS, G.P. and PICCININ, B.P. (1977) Photosynthesis by natural phytoplankton populations. Archiv für Hydrobiologie, 80: 405-457.

HEALEY, F.P. (1983) Effect of temperature and light intensity on the growth rate of Synura sphagnicola. Journal of Plankton Research, 5: 767-774.

HEINE, J.N. (1983) Seasonal productivity of two red algae in a central Californian kelp forest. Journal of Phycology, 19: 146-152.

HITCHCOCK, G.L. (1980) Diel variations in chlorophyll a, carbohydrate and protein content of the marine diatom Skeletonema costatum. Marine Biology, 57: 271-278.

HOFFMANN, J.P. and GRAHAM, L.E. (1984) Effects of selected physiological factors on growth and zoosporogenesis of Cladophora glomerata (Chlorophyta). Journal of Phycology, 20: 1-7.

HOWARD-WILLIAMS, C. (1978) Growth and production of aquatic macrophytes in a south temperate saline lake. International Association of Theoretical and Applied Limnology, 20: 1153-1158.

HOWARD-WILLIAMS, C. and ALLANSON, B.R. (1981) An integrated study on littoral and pelagic primary production in a southern African coastal lake. Archiv für Hydrobiologie, 92: 507-534.

HUTCHINSON, G.E. (1957) A Treatise on Limnology. I. Geography, Physics and Chemistry. John Wiley and Sons, New York.

HUTCHINSON, G.E. (1967) A Treatise on Limnology. II. Introduction to lake biology and their limnoplankton. John Wiley and Sons. New York.

IRWINE, D.W. (1983) Water Quality in the Grahamstown Sewage Works. Third Year Project. Plant Science Department, Rhodes University, Grahamstown.

JASSBY, A.D. and PLATT, T. (1976) Mathematical formulation of the relationshop between photosynthesis and light for phytoplankton. Limnology and Oceanography, 21: 540-547.

JORGENSON, E.G. (1969) The adaption of planktonic algae. IV. Light adaption in different species. Physiologia Plantarum, 22: 1307-1315.

KEMPSTER, P.L., HATTINGH, W.A.J. and VAN VLIET, H.R. (1980) Summarized Water Quality Criteria. Department of Water Affairs, Forestry and Environmental Conservation. Technical Report No. TR 108.

KHAUER, D.R. (1975) The effect of urban run-off on phytoplankton ecology. International Association of Theoretical and Applied Limnology, 19: 893-903.

KING, D.L. (1972) Carbon limitation in sewage lagoons. In Likens, G.E. (Ed.) Nutrients and Eutrophication: The limiting nutrient controversy. Special Symposium of the American Society of Limnology and Oceanography. Volume 2. pp 98-110.

KIRST, G.O. (1981) Photosynthesis and respiration of Griffithsia monilis (Rhodophyceae): effect of light, salinity and oxygen. Planta, 151: 281-288.

KOSTER, J.T. (1955) The genus Rhizoclonium in the Netherlands. Pubblicazioni della Stazione zoologia di Napoli, 27: 335-357.

KUHL, A. (1974) Phosphorus. In: Stewart, W.D.P. (Ed.) Algal Physiology and Biochemistry. Blackwell Scientific Publications. Oxford, London, Melbourne.

LI, W.K.W. (1980) Temperature adaption in phytoplankton: cellular and photosynthetic characteristics. In: Falkowski, P.G. (Ed.) Primary Productivity in the Sea. Plenum Press. New York, London. pp 259-279.

LORENZ, R.C. and HERDENDORF, C.H. (1982) Growth dynamics of Cladophora glomerata in western Lake Erie in relation to some environmental factors. Journal of Great Lakes Research, 8: 42-53.

MACISAAC, J.J., DUGDALE, R.C., HUNTSMAN, S.A. and CONWAY, H.L. (1979) The effects of sewage on uptake of inorganic nitrogen and carbon by natural populations of marine phytoplankton. Journal of Marine Research, 37: 51-65.

MACKERETH, F.J.H , HERON, J. and TALLING, J.F. (1978) Water Analysis: Some Revised Methods for Limnologists. Freshwater Biological Association. Publication No. 360.

MALONE, T.C. (1980) Algal Size. In: Morris, I. (Ed.) The Physiological Ecology of Phytoplankton. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Melbourne. pp 433-464.

MANTAI, K.E. (1974) Some aspects of photosynthesis in Cladophora glomerata. Journal of Phycology, 10: 288-291.

MARKHAM, J.M. and HAGMEITER, R. (1982) Observations on the effects of germanium dioxide on the growth of macro-algae and diatoms. Phycologia, 21: 125-130.

MARRA, J. (1980) Vertical mixing and primary production. In: Falkowski, P.G. (Ed.) Primary Productivity in the Sea. Plenum Press. New York, London. pp 121-138.

MARRE, E. (1962) Temperature. In: Lewin, R.A. (Ed.) Physiology and Biochemistry of Algae. Academic Press. New York, London. pp 541-550.

McCARTHY, J.J. (1980) Nitrogen. In: Morris, I. (Ed.) The Physiological Ecology of Phytoplankton. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Melbourne. pp 191-134.

McCARTHY, J.J and EPPLEY, R.W. (1972) A comparison of chemical, isotopic and enzymatic methods for measuring nitrogen assimilation of marine phytoplankton. Limnology and Oceanography, 17: 371-382.

McCARTHY, J.J., TAYLOR, W.R. and TAFT, J.L. (1977) Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. Limnology and Oceanography, 22: 996-1011.

McDONALD, K.L. and PICKETT-HEAPS, J.D. (1976) Ultrastructure and differentiation in Cladophora glomerata. 1. Cell division. American Journal of Botany, 63: 592-601.

McGARRY, M.G. (1971) Water and protein reclamation from sewage. Processes in Biochemistry: 50-53.

MEESON, B.W. and SWEENEY, B.M. (1982) Adaption of Ceratium furca and Gonyaulax polydra to different temperatures and irradiances: growth rates and cell volumes. Journal of Phycology, 18: 241-245.

MEIRING, P.G., DREWS, R.J.L.C., VAN ECK, H. and STANDER, G.J. (1968) A guide to the use of pond systems in South Africa for the purification of raw and partially treated sewage. CSIR Special Report WAT 34.

MILLER, S.B. and BUHR, H.O. (1981) Mixing characteristics of a high-rate algal pond. Water S.A., 7: 8-15.

MITCHELL, C. (1977) The use of grass carp for submerged weed control. Fish. Res. Publs. N.Z. Min. agric. Fish., 310: 145-148.

MITCHELL, D.S. (1979) Formulating Aquatic Weed Managemant Programmes. Journal of Aquatic Plant Management, 17: 22-24.

MOSS, B. (1980) Ecology of Freshwaters. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston.

MULLER, C. (1978) On the productivity and chemical composition of some benthic algae in hard-water streams. International Association of Theoretical and Applied Limnology, 20: 1457-1462.

N.A.S. (1976) Making Aquatic Weeds Useful: some perspectives for developing countries. National Academy of Science. Washington D.C.

NALEWAJKO, C. and LEAN, D.R.S. (1980) Phosphorus. In: Morris, I. (Ed.) The Physiological Ecology of Phytoplankton. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Melbourne. pp 235-258.

NEIL, J.H. and JACKSON, M.B. (1982) Monitoring Cladophora growth conditions and the effect of phosphorus additions at the shoreline site in northern Lake Erie. Journal of Great Lakes Research, 8: 30-34.

NELSON, T.C. (1947) Some contributions from the land in determining conditions of life in the sea. Ecological Monographs, 17: 337-346.

NICHOLS, H.W. (1973) Growth media - freshwater. In: Stein, J.R. (Ed.) Handbook of Phycological Methods. Culture Methods and Growth Media. Cambridge University Press. Cambridge. pp 7-24.

NICKLISH, A. CONRAD, B. and HOHL, J.G. (1981) Growth kinetics of Oscillatoria redekei Von Goor as a basis to understanding its mass development in eutrophic lakes. International Association of Theoretical and Applied Limnology, 21: 1427-1431.

NIENHUIS, P.H. (1975) Biosystematics and Ecology of RHIZOCLONIUM RIPARIUM (Roth) Harv. (Chlorophyceae: Cladophorales) in the Estuarine Areas of the rivers Rhine, Meuse and Scheldt. PhD Thesis. Rijksuniversiteit te Groningen.

NORTON, T.A. and MATHIESON, A.C. (1983) The Biology of Unattached Seaweeds. In: Round, F.E. and Chapman, D.J. (Eds) Progress in Phycological Research. Volume 2. Elsevier. Amsterdam, New York, Oxford. pp 333-386.

NUSCH, E.A. (1975) Comparative investigations on extent, causes and effects of eutrophication in Western German reservoirs. International Association of Theoretical and Applied Limnology, 19: 1871-1879.

OATES, B.R. and MURRAY, S.N. (1983) Photosynthesis, dark respiration and desiccation resistance of the intertidal seaweeds Hesperophycus harveyanus and Pelvetia fastigiata f. Gracilis. Journal of Phycology, 19: 371-380.

O'KELLEY, J.C. (1968) Mineral nutrition of algae. Annual Review of Plant Physiology, 19: 89-112.

OSAFUNE, T. (1973) Three-dimensional structures of giant mitochondria, dicytosomes and `concentric lamellar bodies' formed during the cell division of Euglena gracilis (Z.) in synchronous cultures. Journal of Electron Microscopy, 22: 51-61.

OSWALD, W.J. and GOLUEKE, G.G. (1968) Harvesting and processing wastegrown microalgae. In: Jackson, D.F. (Ed.) Algae, Man and the Environment. Syracuse University Press. New York. pp 371-390.

PALMER, C.M. (1964) Algae in water supplies of the United States. In: Jackson, D.F. (Ed.) Algae and Man. Plenum Press. New York. pp 239-261.

PALMER, C.M. (1980) Algae and Water Pollution. Castle House Publications. England.

PATEL, R.J. (1971) Growth of members of Cladophorales in experimental culture. Phykos, 10: 40-53.

PERKINS, B.D. (1974) Arthropods that stress waterhyacinth. Proceedings of the Third International Symposium on the Biological Control of Weeds: 49-60.

PIECZYNSHA, E. (1971) Mass appearance of algae in the littoral of several Mazurian lakes. International Association of Theoretical and Applied Limnology, 19: 59-69.

PIETERSE, A.J.H. and SHILLINGLAW, S.N. (1976) Preliminary observations on suppression of algal population density in experimental maturation ponds. S. 122 M/F Conference. First Interdisciplinary Conference on Marine and Freshwater Research in South Africa. Port Elizabeth.

PORTER, J.W., MUSCATINE, L., DUBINSKY, Z. and FALKOWSKI, P.G. (1981) Primary production and photoadaption in light- and shade-adapted colonies of the symbiotic coral Stylophora pistilla. Proceedings of the Royal Society of London, 22: 161-180.

PORTER, K.G. (1976) Enhancement of algal growth and productivity by grazing zooplankton. Science, 192: 1332-1334.

PRASAD, B.N., DUTTA, S. and JAIN, V. (1973) Cytological studies in the genus Rhizoclonium. Phykos, 12: 106-113.

PRESCOTT, G.W. (1962) Algae of the Western Great Lakes Area. Wm.C. Brown Company Publishers. Dubuque, Iowa.

PRESCOTT, G.W. (1978) Freshwater Algae. Third Edition. Wm.C. Brown Company Publishers. Dubuque, Iowa.

PREZELIN, B.B. (1976) The role of peridinin-chlorophyll a-proteins in the photosynthetic light adaption of the marine dinoflagellates, Glenodinium sp.. Planta, 130: 225-233.

PREZELIN, B.B. and SWEENEY, B.M. (1977) Characterization of photosynthetic rythms in marine dinoflagellates. II. Photosynthesis-irradiance curves and *in vivo* chlorophyll fluorescence. *Plant Physiology*, *60*: 388-392.

RAMUS, J. (1981) The capture and transduction of light energy. In: Lobban, C.S. and Wynne, M.J. (Eds) *The Biology of Seaweeds.* Biological Monographs. Volume 17. Blackwell Scientific Publications. Oxford. pp 458-492.

RAMUS, J.S. and ROSENBURG, G. (1980) Diurnal photosynthetic performance of seaweeds measured under natural conditions. *Marine Biology*, *56*: 21-28.

RAVEN, J.A., SMITH, F.A. and GLIDEWELL, S.M. (1979) Photosynthetic capacities and biological strategies of giant-celled and small-celled macro-algae. *New Phytologist*, *83*: 299-309.

RICHARDSON, K., BEARDALL, J. and RAVEN, J.A. (1983) Adaption of unicellular algae to irradiance: an analysis of strategies. *New Phytologist*, *93*: 157-191.

RIVKIN, R.B., SELIGER, H.H., SWIFT, E. and BIGGLEY, W.H. (1982) Light-shade adaption by the oceanic dinoflagellates *Pyrocystis noctiluca* and *P. fusiformis*. *Marine Biologist*, *68*: 181-191.

ROBARTS, R.D. (1984) Factors controlling primary production in a hypertrophic lake (Hartbeespoort Dam, South Africa). *Journal of Plankton Research*, *6*: 91-105.

ROMAN, S.L.E. (1982) An Investigation into the Grahamstown Sewage Works. Third Year Project. Plant Science Department, Rhodes University, Grahamstown.

ROSEMARIN, A.S. (1982) Phosphate nutrition of two potentially competing filamentous algae, Cladophora glomerata (L.) Kutz. and Stigeoclonium tenue (Agardh.) Kutz. from Lake Ontario. Journal of Great Lakes Research, 8: 66-72.

SARTORY, D.P. (1982) Spectrophotometric analysis of chlorophyll a in freshwater phytoplankton. Report No. TR 115, Department of Environmental Affairs, Goverment Printer, Pretoria.

SCHULTZE, B.R. (1947) The climate of South Africa according to the classifications of Koppen, and Thornwaite. South African Geography Journal, 29: 32-42.

SCHULTZE, B.R. (1965) Climate of South Africa. Part 8. General survey. Goverment Printer, Pretoria.

SCOTT, J.L. and BULLOCK, K.W. (1976) Ultrastructure of cell division in Cladophora. Pregametangial cell division in the haploid generation of Cladophora flexuosa. Canadian Journal of Botany, 54: 1546-1560.

SEAGRIEF, S.C (1984) A catalogue of South African green, brown and red marine algae. (Ed. Leistner, O.A.) Memoirs of the Botanical Survey of South Africa. No. 47. Department of Agriculture. South Africa.

SERRA, J.L., LLAMA, M.J. and CADENAS, E. (1978) Nitrate utilization by the diatom Skeletonema costatum. II. Regulation of nitrate uptake. Plant Physiology, 62: 991-994.

SHILLINGLAW, S.N. and PIETERSE, A.J.H (1977) Observations on the algal populations in an experimental maturation pond system. Water S.A., 3: 183-191.

SMITH, G.M. (1950) The Freshwater Algae of the United States. McGraw-Hill Book Company. New York, Toronto, London.

SNOOK, D.J. (1983) Observations on Primary Production and Respiraton in the Maturation Ponds of the Grahamstown Sewage Works. Honours Project. Plant Science Department, Rhodes University, Grahamstown.

SOLORZANO, L. (1969) Determination of ammonium in natural waters by the phenolhypochlorite method. Limnology and Oceanography, 14: 799-801.

SOLTERERO, R.A., GASPERINO, A.F. and GRAHAM, W.G. (1975) Cultural eutrophication of Long Lake, Washington. International Association of Theoretical and Applied Limnology, 19: 1778-1789.

SPENCER, N.R. (1974) Insect enemies of aquatic weeds. Proceedings of the Third International Symposium of the Biological Control of Weeds: 37-47.

STEEMANN NIELSON, E. (1978) Principal aspects concerning the batch techniques in algae assays. International Association of Theoretical and Applied Limnology, 21: 81-87.

STEIN, J.R. (1973) Handbook of Phycological Methods. Culture Methods and Growth Media. Cambridge University Press. Cambridge.

SUGDEN, J. (1983) The Effects of Zooplankton Grazing on Algal Populations in a Shallow, Enriched Pond. Third Year Project. Plant Science Department, Rhodes University.

TALLING,, J.F. (1957a) Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. New Phytologist, 56: 29-50.

TALLING, J.F. (1957b) The phytoplankton population as a compound photosynthetic system. New Phytologist, 56: 133-149.

TALLING, J.F. (1962) Freshwater algae. In: Lewin, R.A. (Ed.) Physiology and Biochemistry of Algae. Academic Press. London, New York. pp 743-758.

TALLING, J.F. (1971) The underwater light climate as a controlling factor in the production ecology of freshwater phytoplankton. International Association of Theoretical and Applied Limnology, 19: 214-243.

TALLING, J.F. (1976) The depletion of carbon dioxide from lake water by phytoplankton. Journal of Ecology, 64: 79-121.

TAYLOR, D.I. (1983) The effects of a major macrophyte regression upon primary production in the littoral of Swartvlei. Archiv für Hydrobiologie, 96: 345-353.

TAYLOR, F.J.R. (1980) Basic biological features of phytoplankton cells. In: Morris, I. (Ed.) The Physiological Ecology of Phytoplankton. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Melbourne. pp 3-56.

TOERIEN D.F., HUANG, C.H., RADIMSKY, J. PEARSON, E.A. and SCHERFIG, J.(1971) Provisional Algal Assay Procedures. SERL Report No. 71-6.

TOERIEN, D.F. and STEYN, D.J. (1975) The eutrophication levels of four South African Impoundments. International Association of Theoretical and Applied Limnology, 19: 1947-1956.

TOERIEN, D.F. and WALMSLEY, R.D. (1979) The chemical composition of the upper Hennops river and its implications on the water quality of Rietvlei Dam. Water S.A., 5: 77-89.

TSCHUMI, P.A., LBAREN, D. and ZBAREN, J. (1978) An improved oxygen method for measuring primary production in lakes. International Association of Theoretical and Applied Limnology, 20: 43-48.

TURNER, J.B. and FRIEDMANN, E.I. (1974) Fine structure of capitular filaments in the coenocytic green alga Penicillus. Journal of Phycology, 10: 125-134.

UHLMANN, D. (1971) Influence of dilution, sinking and grazing rate on the phytoplankton populations of hyperfertilized ponds and microecosystems. International Association of Theoretical and Applied Limnology, 19: 100-124.

VAN DEN HOEK, C. (1981) Chlorophyta: Morphology and Classification. In: Lobban, C.S. and Wynne, M.J. (Eds.) The Biology of Seaweeds. Botanical Monographs, Volume 17. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Melbourne. pp 86-132.

VAN DER POST, D.C. and TOERIEN, D.F. (1974) The retardment of algal growth in maturation ponds. Water Research, 8: 593-600.

VAN VUREN, M.M.J. and GROBBELAAR, J.U. (1982) Selection of algal species for use in open outdoor mass culture. Water S.A., 8: 86-91.

VOLLENWEIDER, R.A. (1969) A Manual for Measuring Primary Production in Aquatic Environments. IBP Handbook No. 12. Blackwell Scientific Publications. Oxford, Edinburgh.

VON ZON, J.C.J. (1974) Studies on the biological control of aquatic weeds in the Netherlands. Third Symposium on the Biological Control of Weeds: 31-38.

WALMSLEY, R.D. and TOERIEN, D.F. (1978) The chemical composition of the waters flowing into Rhoodeplaat Dam. Water S.A., 4: 192-202.

WELSCHMEYER, N.A. and LORENZEN, C.J. (1981) Chlorophyll-specific photosynthesis and quantum yield efficiency at subsaturating light intensities. Journal of Phycology, 17: 283-293.

WHEELER, A.E. and PAGE, J.Z. (1974) The ultrastructure of Derbesia tenuissima (De Notaris) Crouan. I. Organization of the gametophyte protoplast, gametangium, and gametangial pore. Journal of Phycology, 10: 336-353.

WOOD, K.G. (1968) Photosynthesis of Cladophora under unnatural conditions. In: Jackson, D.F. (Ed.) Algae, Man and the Environment. Syracuse University Press. pp 121-133.

WURTZ, A.G. (1964) Some problems remaining in algae culturing. In: Jackson, D.F. (Ed.) Algae and Man. Plenum Press. New York. pp 120-137.

WHITTON, B.A. (1970) Biology of Cladophora in freshwaters. Water Research, 4: 457-476.

YENTSCH, C.S. and LEE, R.W. (1966) Questions on the mechanism of temperature adaption in marine phytoplankton. Marine Biology, 24, 147-156.

ZOHARY, T. (1985) Hyperscums of the cyanobacterium Microcystis aeruginosa in a hypertrophic lake (Hartbeespoort Dam, South Africa). Journal of Plankton Research, 7: 399-409.

ZURAW, E.A. (1969) Culture and physiological requirements of ~~a bacterize~~ Cladophora glomerata (L.) Kutz. from Lake Michigan. Journal of Phycology, 5: 83-85.