PROBLEM BLOOMS OF *CLADOPHORA GLOMERATA* AND *OEDOGONIUM CAPILLARE* IN SOUTH AFRICAN IRRIGATION CANALS

by

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I. EXECUTIVE SUMMARY

The biology and environmental tolerances of the problem algal weeds *Cladophora glomerata* and *Oedogonium capillare* have been studied, both *in situ* in canal systems, and in culture in the laboratory.

Specifically, the aims of this project as submitted in the Research Proposal to the Water Research Commission, were as follows –

1. To continue algae-centered research on problem blooms of macroalgae in rivers and canals in South Africa.

2. To study the life history and ecology of two major problem species (*Cladophora glomerata* and *Oedogonium capillare*) *in situ* to gain information on seasonal growth and recruitment.

3. To investigate on site, what specific conditions (water quality, seasonality, substratum, water depth, flow rate, temperature etc.) are associated with recruitment and rapid growth of the main problem species.

4. To document the life history patterns of two major problem species (*C. glomerata* and *Oedogonium capillare*) in the laboratory, in a variety of environmental conditions for comparison with *in situ* investigations.

5. To test different life history stages of the two main problem species in the laboratory with selected current and potential chemical control methods in a variety of environmental conditions, in a search for optimum conditions and possible sensitive life history stages to provide information for management strategies.

6. To visit sites of blooms of macroalgae which may arise during the course of the project to document the contribution of different algae to, and the extent of these problems (subject to time and financial contraints).

7. To provide a consultancy service during the course of the project for interested parties on the identification and aspects of the management of blooms of freshwater macroalgae.

8. To summarise and disseminate available information on the control of these problems, and other interested parties, through personal contact and the local literature.

- *C. glomerata* growths in sections of the Oranje-Riet and Kalkfontein Irrigation Systems in the south-western Free State and *O. capillare* growths in two canal systems off the Breede River in the south-western Cape of the Republic of South Africa were used for *in situ* study.
- The Oranje-Riet and Kalkfontein Irrigation schemes differ in many ways from the Breede River Irrigation scheme. The first two schemes are supplied by large dams and the latter scheme is a river-fed system with a limited seasonal dam input.
- Water in the Oranje-Riet and Kalkfontein schemes generally has pH levels above neutral and high levels of the cations Ca^{2+} and Mg^{2+}. The Breede River, in the upper sections, has pH levels below neutral and low cation levels.
- Differences between the Oranje-Riet and Kalkfontein systems were electroconductivity levels, with an average difference of 300 $\mu S.m^{-1}$, and turbidity with an average difference of 40 NTU. Canal maintenance was also markedly better in the Oranje-Riet canal system and *Cladophora glomerata* growth was far less.
- Two systems of physical monitoring were used. The initial method, which used sections of actual canal wall cut into squares, proved to be too problematic. There were difficulties in transport and microscopic examination of the surface. However, the second method, using plastic petri dishes which could easily be removed, transported and the surface microscopically observed, was found to be reliable and simple to use.
- Despite our requests for information on copper sulphate dosaging it was found that undisclosed dosing was carried out by the Kalkfontein Irrigation Board and also, illicit dosing by individual farmers/persons took place.
- Dosing with either copper sulphate or other chemicals can be seen on careful examination of the plastic petri dishes. Placing a drop of H_2SO_4 solution on the surface a blue tinge appears and this indicates $CuSO_4$ presence. This is an effective test even after a week in canal water. If copper dosage had taken place there were commonly many empty diatom frustules still adhering to the petri dish surface.

- Importantly, it was seen from our petri dish monitoring systems that *Cladophora glomerata* can establish itself via recruitment throughout the year and, in fact, is not a strictly seasonal alga in these canals. This was also confirmed with the common presence of *Cladophora* growths floating down the canal.
- The growth of *Oedogonium capillare* in the Breede River system was found to be sparse during the project period. Its seasonal massive growths are apparently confined to the La Chasseur system and these are probably light and temperature controlled.
- Control of *O. capillare* growth is easily controlled with a simple copper sulphate dosage system, prior to its recorded and expected appearance. pH is commonly below 7 and therefore little chelation of the copper sulphate takes place.
- The Sanddrift Canal in the Breede River system has relatively high conductivity levels and some growth of a *Cladophora* species, probably *C. fracta*, was found in late summer and autumn.
- In the Breede River systems the algal growths are closely monitored by the bailiffs and early dosing with copper sulphate and careful canal maintenance effectively controlled algal growth during the period of this project. The bailiffs consider that algal growths become noxious in the years after droughts.
- It became apparent during this period of study that a close but simple monitoring system would enable early and effective chemical dosage to prevent the massive growths of *C. glomerata* in the Oranje-Riet and Kalkfontein Canal systems. Also, that the use of a draw down system in the canals, as a method to control *C. glomerata* growth, should be considered.
- Molecular systematic studies performed by U.S. researchers on material sent to them from project personnel demonstrated that the main South African problem alga is indeed *Cladophora glomerata*, and is genetically closely related to samples of the species from different parts of the world.
- During the period of this contract we identified a number of different algal species which were found to occur in different irrigation systems in South Africa. No major algal blooms were reported to us during this period.
- Possible control methods which can be used for the two problem species are given in this report and the Appendix explains some of the causative factors and control options for algal blooms.
- *Cladophora glomerata* is an difficult organism to grow in culture, but reasonable growth was eventually obtained using Cape Town tap water supplemented by nutrients.

- Maximum growth rates of *Cladophora glomerata* were obtained at a temperature of 25°C, with slightly reduced growth at 15, 20 and 30°C. The alga died at 35°C, and remained healthy without growing at 10°C.
- Light saturation points shifted with temperature, reaching their maximum at 25°C at both long day (126µmol m^{-2} s^{-1}) and short day (148µmol m^{-2} s^{-1}) photoperiods.
- In order to reduce the growth rate of *Cladophora* in canals by shading, the shading would have to reduce the incident light considerably. At 15 °C (the temperature range when there are generally *Cladophora* problems in the canals) growth rates at 55µmol m^{-2} s^{-1} were light saturated, while at 20 °C they were at ca. 50% of maximum. Bearing in mind that full sunlight at midday is up to ca. 2000µmol m^{-2} s^{-1}, only relatively low light levels are required for good growth.
- The culture studies suggest that seasonal growth of *Cladophora glomerata* in South African freshwater systems should occur from mid-spring to mid-autumn, when water temperatures are at or above 15°C. Optimum growth should occur in summer, when temperatures are above 20°C.
- Accumulative Cu uptake, Cu uptake rates and photosynthesis at different levels of Cu were determined by exposing *Cladophora* tufts to a Cu concentration range from 0-10mg Cu l^{-1} at a solution pH of either 8, 7 or 6 for experimental periods ranging from 30 to 240 minutes.
- Accumulative Cu uptake by the alga and uptake rates increased with increasing medium Cu concentrations and decreasing solution pH's at all algal incubation time periods. Accumulative uptake increased with time until it reached a saturation point at all medium concentrations after one hour at pH 8 and two hours at pH 6 and 7. Cu uptake rates were highest within the first 30 minutes of algal incubation at all conditions tested.
- Growth rates over 5 days of pieces of *Cladophora* filament in culture were measured, following treatment for 30 minutes in a range of copper concentrations. These growth rates were only affected by Cu concentrations above 20mg l^{-1} at pH 7 and above 6mg l^{-1} at pH 6, with no reduction in growth occurring at pH 8 at all Cu concentrations tested.
- The experiments suggest that in order to control *Cladophora* a minimum of 30 minutes exposure to 6 mg l^{-1} Cu at pH 6 is necessary. Higher pH would need much higher copper concentrations, and large, tightly-packed growths of *Cladophora* may also need more prolonged dosages.

- Culture experiments suggested that the South African *Cladophora* tested has somewhat higher temperature optima, and high Cu tolerances, compared to literature investigations on *Cladophora* from some other world regions. It is likely that the populations of *C. glomerata* in South African irrigation canals have become genetically more tolerant to copper than is the case in populations which have not been exposed to the metal.
- The minimum copper concentration required to effectively reduce algal blooms will depend on a number of water chemistry parameters, algal density and water flow parameters. The toxicity of copper is related not only to the pH, but also to a number of other aspects of the water chemistry, such as the presence of organic chelating compounds and alkalinity. It is predicted that different levels of copper treatment will be required to produce the same toxic effect in different water systems.
- A preliminary experiment on the effects of fermented barley straw on growth of *Cladophora* in culture suggested that the barley straw had no effect. In any case, the use of fermented barley straw seems only to be applicable as an algal control measure in still or very slow-flowing waters.
- A rapid assay technique was tested to assess the relative tolerance of filamentous algae to copper. Using this method it was shown that *Oedogonium capillare* is extremely sensitive to Cu, in fact having a similar sensitivity to *C. glomerata*.

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III. LIST OF FIGURES

IV. LIST OF TABLES

TABLE OF CONTENTS

2.3 *In situ* investigations – Oranje-Riet & Kalkfontein Irrigation Systems

3. Breede River Canals - *Oedogonium capillare*

3.3 *In situ* Investigations

4. General Discussion

1. General Introduction

Scientific investigations related to the presence of nuisance algae in rivers, lakes and dams have been and are still being undertaken, worldwide. However, there are few reports of research in respect of algae in irrigation canals. Most commonly, it is the alga, *Cladophora glomerata*, although others such as *Oedogonium* spp., *Rhizoclonium* spp., *Chara* spp., *Nitella* spp., *Enteromorpha* spp., *Hydrodictyon* spp. and *Stigeoclonium* spp. are recorded as forming problem growths in rivers, dams and reservoirs. The cosmopolitan occurrence of *C. glomerata* has made it the subject of much research. This research project was mainly concerned with those problem algae which occur in irrigation canals, and it is with reference to man-made waterways such as irrigation canals to which most attention and comments are made in this report. Du Plessis & Davidson (1996) reported that *C. glomerata* was found to cause 80% of algal problems in irrigation canals in South Africa. They further state that the nuisance growths of this alga cause a 25 – 50% loss of water in irrigation distribution systems. *O. capillare* occurs as a nuisance alga in the Breede River irrigation system in the Western Province. Water conditions in this southern area are significantly different to those occurring in the Free State and northern regions of South Africa. pH and calcium carbonate levels are generally lower and there is a strong flushing of river systems in a regular winter rainfall pattern (Dallas & Day, 1993). The control of these two algal species has largely been achieved by the regular application of chemicals often combined with manual/mechanical clearance. However, growing public awareness of the ecological damage associated with chemical treatment has directed recent attention to biological methods of control and the limitation of nutrient input to rivers and waterways.

Specifically, this project aimed at studying the life history, ecology of *C. glomerata* and, in less detail, *Oedogonium capillare*, their *in situ* recruitment and growth as related to the pertaining conditions of water quality, seasonality, substratum, water flow rate, temperature, salt content (measured as electroconductivity) and light irradiance in South African conditions, and to ascertain the similarity or not between these findings and those of published research in other countries. Further, *in vivo* investigations in the laboratory were undertaken to establish growth and developmental parameters in different light and temperature conditions, photosynthetic rates, and mortality rates as a result of the addition of different concentrations of copper sulphate.

At present the largest single users of water in South Africa are irrigation schemes. Mining and power stations are the second largest users of water, but, very importantly, the economic return of water usage by this latter sector is 70 times greater than that of irrigation products (Rawhani, 1991). Irrigation schemes of a very basic nature were constructed in ancient Egypt (Ellis *et al.*1991). Since some irrigation schemes such as the Olifants River in the then Cape Province were started in 1797 (Bruwer & van Heerden, 1991) it could be suggested that South African problems with aquatic weeds started soon after this time since it has been shown that alteration to the "normal" aquatic system will affect the environmental balance. Current irrigation methods have been directly linked to increasing salinity in South African rivers and soils (Flugel, 1993). Irrigation development and control was not nationally co-ordinated until the 1960's and the first National Irrigation Symposium was held in 1967 (van Zyl & Bredell, 1991). We were unable to find early (pre 1970) references to problems related to algae in irrigation canals in South Africa. In fact, it was disconcerting to find that few, if any, scientific records had been kept of the occurrence of algal blooms in irrigation systems in South Africa. Thus, we are unable to make any sort of meaningful comparison with our findings.

2. Oranje-Riet and Kalkfontein Canals – *Cladophora glomerata*

2.2. Introduction

Unfortunately, no scientific documentation relating to the original presence of *C. glomerata* in these canals was found. The presence of *C. glomerata* in the original rivers from which these irrigation canal systems originate, the Orange and Riet, was recorded long before the dams, the Vanderkloof and Kalkfontein were constructed, *viz.* by Fritsch (1918) and Pocock (1966), respectively. However, the increasing cost of removal of the huge growths of this alga in irrigation canals, by both manual, mechanical and chemical methods, brought this problem to the attention of the Department of Water Affairs (DWAF) and subsequently, the Water Research Commission. Similar problems in the Hartbeespoort canals fed by the Hartbeespoort Dam on the Crocodile River provoked investigations and reports by Bruwer (1980), De Wet (1980), Prinsloo (1985), Du Plessis & van der Merwe (1991) and Bruwer (1993). Currently, twelve irrigation schemes ranging from the winter rainfall areas in the Cape to the Summer rainfall areas in the Northern Cape, Free State, Gauteng, Mpumalanga and Northern Provinces have serious management problems related to the growth of nuisance algae (Du Plessis & Davidson, 1991).

One section of this project undertook the investigation into the *in situ* growth of *C. glomerata* in the Sarel Hayward and Kalkfontein canals in order to establish its growth habit and occurrence related to various physical parameters which were found over a period of two and a half years. The results of these investigations are reported in Section 2.3 of this report. *In vitro* experiments which took place in our laboratories in the Botany Department at the University of Cape Town are reported in Section 2.4 of this report and formed the major section of the MSc Thesis by Ms. R. le Roux of this Department. In this latter section the results of the laboratory investigations will be presented. We will comment on and compare these findings with similar research results in the literature.

As has been stated, a great deal of research has been undertaken on the alga *C. glomerata* and as part of this project report we obtained as much of this literature as was available. It is this published information that allows us to compare the results of our findings and ascertain whether the *C. glomerata* as found in South Africa “behaves” in a similar way to those growths of *C. glomerata* in other situations, worldwide.

2.2. Literature survey of *Cladophora* spp. – with special reference to *Cladophora glomerata*

2.2.1. Taxonomy

The genus *Cladophora* was erected by Kützing (1843) who transferred many species from the Linnean genus *Confervae*. Kützing (1843) also erected the genus *Aegagropila* whose species are now considered as conspecific with those of *Cladophora* (Hoek, 1976). The genus *Cladophora* contains both marine and freshwater representatives, Kützings original 85 marine species and varieties from Germany and Austria being reduced to 27 marine species and varieties in Europe by Hoek (1976). The number of freshwater *Cladophora* are considerably less, ten according to Hoek (1976). Of these freshwater species only three have been reported to occur outside of Europe - *C. aegagropila, C. fracta* and *C. glomerata*. Despite this paucity in number of freshwater species (as compared to the marine) *C. glomerata*, through its noxious presence in rivers, lakes and irrigation canals, has become one of the most widely investigated freshwater algae. Since we are here concerned with the freshwater *Cladophora*, only these species will be mentioned as examples.

In recent years the systematic position of the Chlorophyta has been intensively investigated with special focus on ultrastructure. The genus *Cladophora* along with *Rhizoclonium* and *Spongiomorpha,* have been placed in their own Class, the Cladophoraceae (Hoek *et al*. 1997). The Cladophorales are distinguished by having: multinucleate cells, numerous chloroplasts in each cell, bilenticular pyrenoids, and cell walls which are composed of a specialised crystalline cellulose with characteristic microfibrillar lamellae which are visible with a light microscope (Hoek, 1976).

The major feature which distinguishes the genus *Cladophora* from the other two genera is its habit of branching. However, the genus is also notable for the range of this habit Hoek (1976). For example, in the freshwater group, *C. basiramosa* and *C. rivularis* plants, may be found which exhibit no branching whatsoever. Conversely, *C. glomerata* and *C. aegagropila*, are markedly branched with specific features which make them readily identifiable. This weak branching pattern in *C. basiramosa* and *C. rivularis* make them easily confused with species in the genus *Rhizoclonium* (Hoek, 1976) or *Pithophora* (Spencer & Lembi, 1981). These latter two genera were reported in as occurring in South Africa (Pocock, 1966) and *Rhizoclonium* spp. were commonly found as occurring in dams and rivers during the course of our study.

The genus *Cladophora* is defined as being filamentous with multinucleate cells which are filled with numerous, disc-like, chloroplasts. Growth occurs by apical/intercalary cell division. Branches may be laterally or apically inserted, the latter leading to pseudodichotomy (apparent division of main filament cell) as in *C. glomerata* and *C. fracta*. However, the initial type of branch insertion may be masked in older sections of the main filament where further cell growth has taken place. Plants are usually attached to the substrate either by a disciform holdfast or rhizoidal growths from the lower portion of the cells in the basal sections of the thallus. Most importantly, most species in the genus show a range of morphology. Branching patterns particularly, may vary within species. During the course of this study we found this to be especially true for *C. glomerata*. This “plastic” morphology which appears be controlled by physical and chemical conditions pertaining at the site of growth leads to some difficulty in absolute species definition. Some freshwater species e.g. *C. aegagropila*, *C. fracta* and *C. glomerata* have been reported as occuring in salinities as high as 18‰. This is of interest since it is being increasingly reported that South African rivers are becoming salinised due to poor agricultural practices (Ghassemi *et al*. 1995). During the course of this study we were asked to supply research workers in California in the USA with samples of *C. glomerata* and *C. fracta* which we found during our field work. As a result of their DNA investigations Marks &

Cummings (1996) showed that South African *C. glomerata* is clearly the same species as samples designated *C. glomerata* from other parts of the world. They also suggested that freshwater *Cladophora* may comprise very few or possibly only one genetic species which is ecologically and morphologically variable.

In South Africa, up to 1993, *C. glomerata* was the only species reported as occurring in freshwater. However, *C. fracta* was found in irrigation canals in the Breede River in 1992 (Joska & Bolton, 1994). Considering the difficulties in accurately identifying species in *Cladophora* it would not be surprising if further freshwater members of this genus were, in fact, found to occur in this country. *C. fracta* has been recorded from Lake Nyassa and forms of *C. glomerata* have been reported from Lake Albert and Lake Victoria in central East Africa (Evans, 1962).

2.2.2. Life history

Reproduction may be either asexual or sexual, or both; by means of biflagellate and/or quadriflagellate zoospores. Hoek *et al.* (1997) consider that *C. glomerata* lacks sexual reproduction and that it has only been found to produce asexual biflagellate zoospores. Vegetative reproduction is frequently found and fragments of the filament which break off produce rhizoids at the proximal end of the filament and will readily attach to suitable substrates. Most species can also produce akinetes (specialised, thick-walled cells) in unfavourable conditions, such as when nutrient availability is reduced (Hoek *et al*. 1997). Careful monitoring of the life history has usually only been done by laboratory culture studies. Not all species of *Cladophora* have been seen to undergo all types of reproduction and in some only one type of reproduction may be known (Whitton, 1970). In many species the method of reproduction is “poorly understood” (Dodds & Gudder, 1992)

Zoospores are released from the cell by means of a pore/s which is/are formed in a specific region of the cell, dependant upon the species. In *C. glomerata* the settled zoospore develops rhizoidal outgrowths shortly after initial cell development. These attach the plant firmly to the substrate. *C. fracta* develops rhizoids from the initial cell, but seldom becomes attached and usually remains free-floating. *C. aegagropila* is only known to reproduce vegetatively and is also seldom attached to the substrate (Hoek, 1976).

2.2.3. Growth *in situ*

The presence of *C. glomerata* as a dominant algal species has commonly been linked to moderately polluted or mildly polluted water conditions (Whitton, 1970; Auer & Canale, 1982a,b; Biggs, 1987; Entwisle, 1989). However, in severely polluted waters e.g. below sewage outlets, it is often not present (Blum, 1957). The question as to why *C. glomerata* should grow to form the major part of the algal biomass in these semi-polluted conditions and not, for instance, some other nuisance algal species has not been convincingly answered. There does however seem to be a generalised concensus amongst researchers that *C. glomerata* has two annual growth peaks, spring and late summer (Whitton, 1970; Auer & Canale, 1982a). These growth peaks may vary according to the geographical location, the peaks occurring later in warmer areas (Bellis & McLarty, 1967). Whitton (1970) reported that after the spring growth peak there was an increasing length of time before the second (and occasionally greater) growth peak which was related to distance from the equator. The controlling influence of these growth peaks has been considered as temperature (Thurman & Kuehne, 1952) but some researchers feel that other overlying factors (singly or in combination), such as nutrient availability, grazing, ecotype variance and irradiance reaction could cause the double growth peak (Dodds & Gudder, 1992). C. *glomerata* has been reported as growing *in situ* in a wide range of temperatures (Whitton, 1967;1970; Chudbya, 1965; 1968). This wide range may occur annually in a single geographic location e.g. 3° - 20° C in Poland (Chudbya, 1965) or in a range of geographic locations e.g. North American boreal forest to desert-chapparal (Sheath & Cole, 1992). Importantly it has also been found growing throughout the year albeit in not such obvious masses (Blum, 1957; Chudbya, 1965; Entwisle, 1989)

There is general consensus that increased flow enhances *C. glomerata* growth and different flow regimes affect branching patterns (Whitton, 1970; Parodi & Caceres, 1991; Dodds & Gudder, 1992; Bergey *et al*, 1995). Chudbya (1965), working in the Skawa river in Poland, collected C. *glomerata* throughout the year and reported that the branching patterns changed according to physical conditions relevant at each season. Chudbya (1968) found that the *C. glomerata* growing on hard substratum in the Skawa River in Poland formed “turf-like” rhizoidal growths.

Growth enhancement in strong water flow is due to the fact that the plant is exposed to a greater turnover of nutrients, and the number of branches gives an increased surface area for absorption of these nutrients. Most reports of the presence of *C. glomerata* are made from flowing rivers. In these situations the large growths are very obvious. Lentic water populations (such as dams and lakes) of *C. glomerata* have also caused troublesome growths, as in the Great Lakes in North America, but the majority of the growths in these lakes occurred in areas where there was some flow or wave action (Rosemarin, 1982). We have observed that *C. glomerata* is the dominant alga in the Kalkfontein Dam, above the irrigation canals, but its length seldom exceeds 5 cm probably due to the still water condition.

2.2.4. Environmental tolerances

2.2.4.1. Light

Plants use radiation energy in a biochemical reaction i.e. photosynthesis which takes place in the chloroplasts, to produce potential energy. Photosynthetically active radiation is generally only available to plants in the 400-700 nm wavelengths (Wetzel, 1983). In the case of algae light is measured as photon flux densities measured as micromoles of light quanta per square metre per second (µmol $m^{-2}\cdot s^{-1}$). This quantum measure is additive, which means that it is possible to multiply up to how many moles of light quanta a particular area receives per day. Light energy is attenuated (absorbed by conversion to heat) as it travels through a body of water and the degree of attenuation is also affected negatively by the amount of dissolved organic matter and inorganic particles. Where there is a large/deep volume of water passing rapidly, as in irrigation canals, the larger number of particles often further decreases light (Stevenson, 1996).

There are conflicting reports on the amount of lowest irradiance required by *C. glomerata* for growth. Napolitano (1994) found that *C. glomerata* had significantly increased concentrations of chlorophylls *a* and *b* and phospholipids when light intensities were reduced. He further found that the concentrations of chlorophyll a and phospholipids were correlated to the ash-free dry mass, thus showing that a reduction in light intensity within the bounds of their system did not negatively affect the growth of this alga. Similarly, Adams & Stone (1973) found that the growth of *C. glomerata* was not limited by low light conditions in Lake Michigan. Whitton (1970) concluded that although he had found in experiments that growth rate increased when light intensity was increased, other researchers had found that light was not the single factor contributing to growth. Light levels lower than 35µ mol m^{-2} s^{-1} were reported to limit *C. glomerata* growth by Graham *et al.* (1982) and 29µ mol m^{-2} s^{-1} by Lorenz *et al*. (1991), both from the Great Lakes in North America. Higher minimum light requirements for *C. glomerata* growth in Lake Erie were observed by Lorenz and Herdendorf (1982). The maximum depth of *C. glomerata* colonization in their study coincided with light intensities above 50μ mol m^{-2} s^{-1} which indicated that values of less than 50 μ mol m^{-2} s^{-1} were limiting to growth at water temperatures of 15° C.

Hoek (1976) reported that *C. glomerata* cell diameters decreased on exposure to increased sunlight and Whitton (1967) reported that branching increased with increasing light intensity. Conflicting reports are given as to the relationship between *C. glomerata* growth and turbidity. Turbidity is measured as the amount of vertical light extinction through a body of water. Turbidity may be caused by organic or inorganic particles and microscopic algae and invertebrates in the water. It may be simply measured by a Secchi disc or, more scientifically, by spectrophotometry (NTU meter) (Wetzel, 1983). Steynberg & Pieterse (unpublished) working at Vereeniging, South Africa on water from the Vaal River, found that *C. glomerata* grew to its greatest volumes at low turbidities (< 40 NTU). There is not always an obligate relationship between high conductivity and turbidity, thus clear water may have high conductivity levels (pers.observ.). The importance of a minimal amount of light necessary for *Cladophora* growth was shown by Demal & Fortin (1987) who found that shading by bankside trees was responsible for a 60% decrease in growth. Towns (1981) working in New Zealand, used artificial shading to exclude all but 6% of normal light and this caused the disappearance of all visible algae.

Growth of plants increases with increase in light, until a light saturation level is reached, and with very high light levels there is also a reduction in photosynthesis and growth (called photoinhibition). The amount of light which causes the maximum growth of *C. glomerata* has also been studied on a number of occasions. Hoffman and Graham (1984) reported that isolates of *C. glomerata* from Lakes Mendota, Madison and Wisconsin yielded maximum dry weight production at 125 μ mol m^{-2} s^{-1} at 25° C. Similarly growth of *C. glomerata* collected from streams in the Spessart Mountains, Germany, increased with increasing light up to 100 μ mol m^{-2} s^{-1} with no saturation evident at 15° C (Leukart and Hanelt, 1995). Lorenz & Herdendorf. (1991) reported that *C. glomerata* collected from Lake Erie and subsequently cultured at 15° C at a 14 hour day length, demonstrated a higher saturation light level for growth of 168 μ mol m^{-2} s^{-1} for growth. Robinson and Hawkes (1986) and Whitton (1967) described maximum light intensities, of 6000 lux (ca. 120 μ mol m^{-2} s^{-1}) and 150 μ mol m^{-2} s^{-1} respectively, for *C. glomerata* growth when exposed to continuous illumination at 20° C.

2.2.4.2. Temperature

As with the conflicting published reports on *C. glomerata* growth in different light conditions the same can be said for published reports on growth at differing temperatures. *C. glomerata* has been found growing in a wide range of temperatures, between 6 and 37ºC (Whitton, 1970). However, temperatures at which growth peaks occur vary considerably. These growth peaks have been reported at 9ºC by Chudbya (1965) and 25ºC by Dodds & Gudder (1992). In laboratory cultures, optimal growth rates between 15ºC and 20ºC were reported by Robinson & Hawkes (1986) but between 15ºC and 30ºC by Bellis (1968). Bellis (1968) concluded that the periodic growth peaks of *C. glomerata* were influenced by the seasonal variation in water temperature. In a set of experiments linking a range of temperatures (1ºC to 35ºC) to a range of light intensities (12μ mol m^{-2} s^{-1} to 1235 μ mol m^{-2} s^{-1}), Graham *et al* (1982) found that maximum photosynthesis occurred between 13 and 17ºC and from 300 to 600μ mol m^{-2} s^{-1} in *C. glomerata* collected from Lake Huron in North America. Storr & Sweeney (1971) conducted experiments linking the growth of *C. glomerata* from the Great Lakes to a range of temperatures at two different light periods, 12 and 14 day light lengths. They found that the greatest growth occurred at 18°C in a 14 hour day which corresponded with the two growth peaks in the Great Lakes in May/June and again in August. The slight decrease in growth rate at higher water temperatures *in situ* in July also corresponded with the *in vitro* increased temperature (26°C). Schölm (1968) who collected *C. glomerata* (and a number of other algal genera) from rivers in Austria, found that *C. glomerata* was frost resistant in winter and heat resistant in summer. Heat and frost resistance decreased according to length of exposure in water with temperature extremes of 40°C and 2°C . He concluded that certain algae have exogenous and endogenous control factors which enable them to withstand these extremes, albeit often for only 4 hours. These temperature resistance factors were also shown to be linked to day lengths.

2.2.4.3. pH

pH is the measurement of hydrogen ion activity and is a logarithmic value (Day, 1990). A pH value of 7 is considered as neutral, lesser values are acidic and greater values are alkaline, and the pH of natural water may range between <2 to 12. (Wetzel, 1983).

There is consensus on reports of the preferred pH growth limits of *C. glomerata*, but few conclusions as to why this is the preferred pH range. *C. glomerata* growth is usually always associated with water which has a pH of 7 or greater (Whitton, 1970; Holmes & Whitton, 1998). However, Bellis (1968a) found that growth was inhibited at pH's > 10. Studying filamentous algal communities in streams with low pH (< 6.67) in The Trossachs in Scotland, Kinross *et al.* (1993) found that algae with a cell diameter > 35 µm did not occur in these streams [*C. glomerata* has recorded cell diameters of ranges from 21 to 250 µm (Hoek, 1976)]. In higher plants, those which have cells with a high calcium requirement (as is found in *C. glomerata*) nutrient uptake is increased at higher pH's (Marschner, 1986). Thus, it seems that in *C. glomerata*, the minimal level of calcium requirement is possibly linked to better growth at higher pH's. However, increased pH can negatively affect nitrogen (as NH_4-N) assimulation (Wetzel, 1983).

2.2.4.4. Conductivity

Conductivity, in the case of freshwater measurement as electrical conductivity, is a measure of all dissolved ions at 25°C. A unit of conductivity is given as microSiemens per centimetre - $\mu S\ cm^{-1}$, or also sometimes as $mS\ m^{-1}$ (milli Siemens per metre) where electrical conductivity levels are exceptionally high (Day, 1990). Commonly also, measurements are given for Total Dissolved Solids (TDS) in freshwaters. TDS measures the major ions such as Na^+, $K^{+,}$ Ca^{2+}, Mg^{2+}, HCO3-, CO_3^{2-}, Cl^-, SO_4^{2-} and the nutrients NO_3^- and PO_4^{3-}. The relationship between TDS and conductivity has been estimated as – TDS (mg l^{-1}) = conductivity (mS m^{-1}) x 6.6 (Dallas & Day, 1993). Biggs & Price (1987) and Biggs (1988) reported that *C. glomerata* was the dominant alga in New Zealand rivers where conductivity levels were high (> 300 mS m^{-1}) and also that growth of *C. glomerata* increased relevant to the increase in conductivity. The dominant presence of *C. glomerata* in water with high conductivity has also been reported by Blum (1957) in Michigan, Sheath & Cole (1992) at many sites across the USA, Chudbya (1968) in Poland, and Whitton (1970) in England.

2.2.4.5. Nutrients

The role of the nutrients, nitrogen and phosphorus, in the growth of *C. glomerata* has been the subject of much experimentation and discussion. Many researchers (see Whitton, 1970; Auer & Canale, 1982b; Freeman, 1986; Winterbourn, 1990; Planas *et al.* 1996) consider that phosphorus, as available PO_4-P (orthophosphate), is the major limiting nutrient for *C. glomerata* growth. Nitrogen, as available NO_3-N (nitrate) and NH_3-N (ammonia), although necessary for *C. glomerata* growth was found to be limiting only at very low levels whereas water containing less than 1.0 mg/ℓ total inorganic phosphorus was limiting for growth (Pitcairn & Hawkes, 1973). However, high concentrations of ammonia were found to be toxic, and nitrate concentrations over 7 mg/ℓ NO_3-N produced no increase in growth (Robinson & Hawkes, 1986). Lohman & Priscu (1992) found that nitrogen was a limiting factor for growth of *C. glomerata* collected from the Columbia River in Montana, USA. They proposed that the variation in their findings to those of other researchers who collected *C. glomerata* from other sites was due to "differences in watershed geology". This controversy in the literature was noted by Wharfe *et al.* (1984) who investigated the effects of a sewage works effluent in the River Great Stour in England. Using available information for a 30 year period they concluded that although phosphorus levels were often high (1 mg/ℓ) *C. glomerata* was not always the dominant alga and that factors other than the high nutrient levels contributed to the nuisance growths which occurred. Hoek (1976) considered that freshwater *Cladophora* species appear to be "bound" to eutrophic or semi-eutrophic conditions. Nitrogen and phosphorus are not the only nutrients critical for *C. glomerata* growth. Minimum requirements of 1.2/ℓ mg calcium and 0.7 mg/ℓ magnesium is known to be necessary (Whitton, 1970). *C. glomerata* also requires silicon, boron, thiamine, zinc and vitamins B_1 and B_{12} for growth (Dodds & Gudder, 1992).

A most interesting finding was made by Bellis (1968b) which was not seemingly investigated by later researchers. He found that growth of *C. glomerata* was inhibited not by pH levels but by calcium, where levels below 5 mgℓ^{-1} caused "disruption of individual vegetative cells". High concentrations (70 mg ℓ^{-1}) were not inhibitory. This is possibly due to the fact that *C. glomerata*, as well as all other members of the class Cladophorophyceae, have a specific type of layering of the microfibrils in the cell walls which is composed of cellulose I, this type of cellulose is the same as that found in higher plants and not common in other algae (Hoek *et al*., 1997). Research in higher plants has shown that low levels of calcium cause a breakdown in cellulose cell walls, inhibition of nutrient uptake, and that new cell wall structural formation is inhibited (Marschner, 1986). South African rivers flowing in the areas north of the South-western Cape have all either Ca and Mg or Ca, Mg and Na as dominant or co-dominant cations (Dallas *et al*. 1994).

2.2.4.7. Flow

It has been shown that water flow is both advantageous for *C. glomerata* growth and also, by temporary cessation, a means of control. The variation in algal morphology and boundary layer thickness is related to the benefits of nutrient uptake in situations of flow stress was reviewed by Raven (1992) *Cladophora* growing in water with high velocities is apparently able to assimulate nutrients more efficiently than in still water or water with low velocities (Whitford & Schumacher, 1961). Steinman *et al*. (1992) showed that branched filamentous (*Draparnaldia glomerata*) and unbranched filamentous (*Rhizoclonium* sp.) green algae have the highest surface area to volume ratio (SA:V). They also showed that these algae were the most efficient in phosphorus uptake and carbon fixation. *Cladophora* in fast flowing streams and rivers was found to grow long tufts which showed morphological variation and these variations in branching altered the flow pattern within the tufts (Dodds, 1991a,b). However, it was found that these long tufts growing in the Yarra River in Australian were prone to breakage (Entwisle, 1989). Biggs (1988) found that *Cladophora/Rhizoclonium* dominated communities were found in New Zealand rivers with low flow power (kW m^{-1} between 0.01 and 0.10). *C. glomerata* growths in in Eel River in California were studied over a period from 1987 to 1989 by Power (1990). She found that the overwintering *Cladophora* turfs growing on the river bed developed long tufts (up to 6 m) in late spring and early summer. Many of these long growths became detached and formed floating mats which were then caught against large boulders or collected on the river edges in slower flowing areas. Bergey *et al*. (1995) also working in the Eel River investigated *C. glomerata in situ* at differing flow velocities on cobbles. It was found that the morphology of the plant did not change initially when water velocity regimes were interchanged, but that in time the plants on cobbles transferred to higher velocity regimes showed a fragmentation of cells at the tips of the plants. Conversely, both Hoek (1976) and Parodi and Caceres (1991) found that *C. glomerata* branched more frequently in high but stable water velocities.

2.2.4.8. Emergence/submergence effects

A marked decrease in biomass due to exposure of *C. glomerata* growths from the Colorado River in Arizona was investigated by Usher & Blinn (1990). Growths which were exposed for either a single 12- and 24-hour period, or for cycles of exposure and submergence, showed an approximate 45% decrease in biomass. Outer branches in the single period exposure plants were weakened and broke off after re-submergence. Repeated exposure/submergence over a two week period gradually weakened the entire plant. Further *in situ* flow and *in vitro* temperature manipulation regimes on *C. glomerata* growths were conducted in the same river by Blinn *et al*.(1995) in 1992 and 1993 when river discharge rates varied between 142 and 566 m^3/s. Growths subjected to a twelve hour exposure for two and five days were reduced in biomass by 38% and 70%, respectively. *Cladophora* growths exposed to a temperature of -2ºC for a period of 3 hours were reduced in biomass by 50% within one day. Blinn *et al*. concluded that in managed water systems the biomass of *C. glomerata* could be substantially reduced by either a two day exposure regime or, especially in winter, a short term exposure to freezing night temperatures. Dodds (1991) also noted the disappearance of *C. glomerata* growths in an irrigation ditch off the Madison River in the USA. Re-appearance, after resumption of water flow, took place only 5 months later. He concluded that the exposure of the basal rhizoidal system had been negatively affected by this exposure.

2.2.5. Community ecology

The relationship between *Cladophora* and other algae and animals (including man) has a number of facets: as a competitor for space and nutrients, as a source of food, as a refuge from predators, as a "sink" organism for heavy metals, as an indicator species for water quality, and as a nuisance species incurring cost and labour effort to remove (see Whitton, 1970; Dudley *et al.*,1986; Steinman & Macintire, 1991; Power, 1990; Feminella & Resh, 1991; Du Plessis & Davidson, 1996). Power (1992) found that winter flooding in northern California rivers promoted *Cladophora* blooms due to the reduction in grazer densities. The most commonly occurring epiphytes on *Cladophora* are diatoms (Whitton, 1970) especially the genera, *Cocconeis, Diatoma, Fragilaria, Gomphonema, Melosira, Rhoicosphenia* and *Synedra* in British and North European rivers. Bergey *et al*. (1995), working in the Eel River in California, USA, found the commonest diatom genera associated with *C. glomerata* were *Acnanthes, Cocconeis, Epithemia* and *Gomphonema.* Dodds (1991) found that the diatom genus, *Nitzschia,* was the commonest epiphyte on *C. glomerata* collected from rivers in Montana, USA. Separating the *Nitzschia* from the *C. glomerata* filaments, Dodds established that there was no competition for nitrogen or phosphorus and the presence of the diatoms reduced the water flow drag effect on the alga. It was suggested that this decrease in the drag effect would probably reduce the likelihood of the alga being detached from the substrate. However, it was found that when water velocity rates decreased the photosynthetic rate of *C. glomerata* was reduced due to the epiphytic diatom "shading" effect. The shape of the diatom, *Cocconeis,* is well suited as an epiphyte on algal filaments as it is concave on one side of its valve system (Round *et al.*, 1996). *C. glomerata* must compete with other algae and invertebrates for substrates, and Power (1992) found that *C. glomerata* was a late succesional dominant species in the Eel River and its tributaries in northern California. November floods removed all macroscopically visible algae from substrates, but in March to May diatoms, blue-green algae and the filamentous algal genus *Zygnema* dominated the substrate. *C. glomerata* filaments became visible in late May and by June covered 80% of the substrate in some sites.

In a series of variant substrate and exclusion *in situ* experiments in Rattlesnake Creek in California, inter-relationships between *C. glomerata* and a filamentous blue-green alga, *Nostoc*, and the invertebrate grazers, *Agepetus celatus*, *Micrasema* spp (caddisflies), *Baetis* spp.(mayfly) were studied by Dudley and D'Antonio (1991). The results showed that *C. glomerata* was significantly affected by grazing but not restricted by substrate variance. Dodds (1991) also found that the mayflies, *Baetis tricaudatus* and *Trycorythodes minutus*, removed 75% of the diatom epiphytes from *C. glomerata* collected in the Madison River in Montana, USA. He suggests that this removal of the diatom epiphytes allows for more efficient photosynthesis in low light conditions. Also, Dodds (1990) showed that an extract of *C. glomerata* inhibited photosynthesis in the epiphytic diatom *Nitzschia fonticola*. Decomposing *C. glomerata* has been shown to produce a substance that inhibits the growth of *Phragmites* (Dodds & Gudder, 1992). The negative effects on the growth of the aquatic angiosperms, *Potomogeton pectinatus* and *Elodea canadensis* in the presence of *C. glomerata* was also shown by Ozimek *et al*. (1991) . Although *C. glomerata* is a comparatively high source of nitrogen for invertebrates (Newman, 1991) it has not been found as a preferred food source for grazers (Dodds & Gudder, 1992). Rather, its complex branching habit and dense growths provide relatively protective environments for epiphytes and epifauna (Moore, 1976).

Competitive interactions between grazers and *C. glomerata* were shown by Brönmark *et al*. (1991). Dense growth of this alga was inhibited by the grazing snails, *Lymnaea stagnalis* and *L. peregra*. This reduced algal growth in turn allowed for an increased presence of phytoplankton which was consumed by the tadpole of the frog, *Rana temporaria*. Densities of *C. glomerata* growth are also markedly reduced by the sedentary aquatic caterpillar, *Petrophila confusalis* (Bergey *et al*. 1995). In an experiment using artificial streams, Sarnelle *et al*. (1993) showed that grazing by the snail, *Physella* sp., significantly reduced the overstorey growth of *C. glomerata* and that the downstream growth gradient of this alga was also reduced. Further, the grazing of the overstorey promoted the growth of understorey microalgae previously inhibited by shading and reduced nutrient supply. Dudley (1992) demonstrated the complex relationships of macroalgae and herbivores by showing that although some herbivores consume epiphytic diatoms to the benefit of *C. glomerata* growth, the young algal sporelings are grazed by other herbivores. He found that there was a critical two-week growth period which was sufficient for *C. glomerata* to attain a size refuge. Whereas young/newly formed cells were readily grazed, older cells had developed a thicker cell wall. McShaffrey & McCafferty (1991) showed that the mayfly, *Ephemerella needhami*, had an intimate association with *C. glomerata* for "life cycle synchronization, spination, coloration and feeding behaviour", where *C. glomerata* was used as a refuge, and for food and food-gathering. There are conflicting reports as to the susceptibility of *C. glomerata* to grazing (Dodds, 1991). Whitton (1970) reported that most of the invertebrates associated with *C. glomerata* growths either fed on its epiphytes or were animals which used the alga as a refuge.

2.2.6. Control and management

C. glomerata was noted as a dominant alga in Lake Erie in North America in the early 19th century (Taft & Kishler, 1973) but its occurrence as a problem alga was more commonly reported in America (Blum, 1957) and England (Butcher, 1933) in the 1930's when its appearance was linked to polluted water conditions. The cost of removal of *C. glomerata* growths can impact strongly on operation costs in water supply systems. In 1982, the annual cost of control of noxious water weeds (mainly *C. glomerata*) in the Salt River Project Canal in Arizona was US$1.5 million (Lembi *et al*. 1988).

Algicides, especially copper sulphate, have been recommended for the control of algal growths since 1904 (Sladeckova & Sladecek, 1967). However, the longterm negative effects of copper sulphate dosing has been recorded (Hanson & Stefan, 1984) and environmentalist groups have increasingly lobbied for the reduction in use of herbicides and pesticides in general (Gallagher & Haller, 1990). In an official British governmental publication, Chancellor (1958), lists the methods of control of algae. His comment that "no generally satisfactory method of controlling algae has yet been discovered" probably pertains now, as then. Although copper sulphate was listed as the main controlling chemical he also mentions chlorine, sodium arsenite and rosin amine compounds. Potassium permanganate as an algicide was tested by Fitzgerald (1966) and Kemp *et al*. (1966). These researchers considered it to be less hazardous and more effective than copper sulphate. However, it is more corrosive than copper sulphate and can form harmful precipitates. In 1967, Sladeckova & Sladecek reviewed the literature then available on algicides, both organic and natural products. Andrews *et al*. (1986) published a comprehensive review on algal control methods. He reported on biological, mechanical and chemical methods of control including the economic costs and benefits, environmental impact and health hazards. All these investigations and recommendations were for the Madison Lakes in North America which are, for the greater part, closed lentic water systems. In summary, he did not recommend one particular type of control method, but rather consideration of specific requirements, economic restraints and long term hazards pertaining in each situation. Two

recently investigated methods of algal control are of interest. Firstly, the biological control method using barley straw (Gibson *et al*. 1990; Barratt & Newman, 1993; Pillinger *et al*. 1994) and secondly, a nitrogen enrichment method for irrigation systems (Merezhko *et al.*, 1992). Published results with the barley straw method indicate that it is successful in closed (lentic) systems and only with some algae, Further experiments and the analysis of the substances that are produced by the barley straw to cause this effect on algae are being undertaken in the United Kingdom (Dr. Dave John, British Museum of Natural History, *pers.comm.*)
Experiments undertaken in the Tatarbunar irrigation canals near Kiev in Russia showed that the additon of nitrogen fertilisers to irrigation water so that the nitrogen concentration was ≥ 100 mg/ℓ, destroyed the *Cladophora* filaments within three days, was safe and cheap, and that the nitrogen enriched water acts as a fertiliser when used in irrigation (Merezhko *et al.*, 1991).
In South Africa, where the *C. glomerata* growths have become a serious problem in irrigation canals in many areas, copper sulphate, in combination with a predose of H_2SO_4 as a means of reducing pH, is used extensively (Du Plessis & Davidson, 1996).

2.3. *In situ* investigations – Oranje-Riet & Kalkfontein irrigation systems

2.3.1. Brief history of schemes

Our sites Nos. 1 & 2 were on the Oranje-Riet Canal System (formerly the Sarel Hayward Canal) below the Scheiding Pumping Station. The Oranje-Riet Canal System was completed in 1987 and is fed by the Vanderkloof Dam (formerly the P K le Roux Dam) (Davies & Day, 1998). The Vanderkloof Dam was completed in 1977 and is situated 140km downstream of the Gariep Dam (formerly the Hendrik Verwoerd Dam) which was completed in 1971. Both these dams lie on the Orange River in the south-eastern Free State, between latitudes 25°30´ and 26°00´, and longitudes 30°00´ and 31°00´. The Orange River flows through this area, which is a semi-arid plateau where the winters are cold to mild and dry, and summers warm to hot with occasional rain. The average rainfall is poor (500 mm) and unreliable, and prior to the provision of irrigation water for this area, regular crop production was hampered by cyclic droughts (Reader's Digest Illustrated Atlas of Southern Africa, 1994; Orange River Project Map, 1994). The Scheiding Pumping Station raises the water level from the Vanderkloof Dam Canal by 45 m to deliver water at a rate of 15.6 m^3/sec to the Oranje-Riet Canal which is approximately 112.6 km long. This canal supplies continuous annual water for 3787 hectares

of irrigated land. This canal system is under the control of the Department of Water Affairs and Forestry offices near Jacobsdal (*pers.comm*. Ms.E. Dorfling). See Figure 2.1.
Our sites, Nos. 3, 4 and 5 were on the Kalkfontein Canal System which is supplied by the Kalkfontein Dam. The Kalkfontein Dam lies on the Riet River and was completed in 1938. Close to the town of Koffiefontein in the south eastern Free State, this dam is considerably smaller than the Vanderkloof Dam (storage capacities 321 million m^3 and 3172.6 million m^3, respectively) and supplies only 7.4 m^3 of water per second at the head of the canal system. Despite having a 50% less supply capability than the Oranje-Riet System, the main canal delivers water to 3046.3 hectares of irrigated land annually (*pers.comm*. Ms.E. Dorfling). The Riet River is markedly smaller than the Orange. Its headwaters lie south of Bloemfontein and it eventually enters the Modder River close to Jacobsdal. Many of the small rivers which join it flow only temporarily (Reader's Digest Illustrated Atlas of Southern Africa, 1994). The main canal system is 104.6 km long and, since the end of 1995, control of its water supply and maintenance was given over to the Kalkfontein Irrigation Board independent of the jurisdiction of the Department of Water Affairs and Forestry. All the end and drainage waters from both the Oranje-Riet and Kalkfontein Canal Systems flow into the Vaal River before its confluence with the Orange River at Douglas, in the Northern Province (formerley the Cape Province). See Figure 2.2.

2.3.2 Site descriptions

All the sites for our experiments were chosen by officials from the Department of Water Affairs and Forestry office at Jacobsdal. We designed the grids which were metal frame inserts in the canal wall to hold the original sample concrete squares and requested that they be placed in the canal walls, the top levels of the grids to be at the average flow top water line. It should be explained that daily flow rates, and thus water levels, vary. Flow rates are related to supply request and thus in times of summer rainfall when water demand decreases, there will be a drop in water level in the one week. The canals in both systems are constructed of pre-stressed concrete slabs with a thickness of never less than 20 cm. The wall texture is rough and the joins of the canal sections are sealed with bitumen. Canal widths at our sites varied from 10 to 2.5 m and depths from 4 to 1 m.

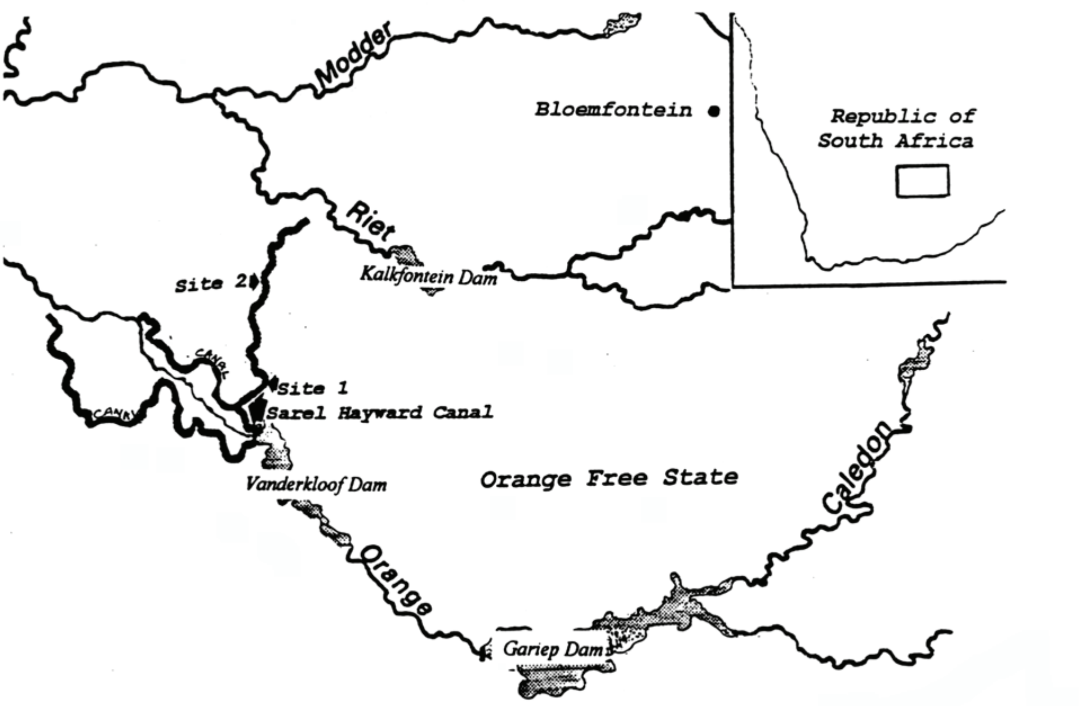


Fig. 2.1. The Gariep and Vanderkloof Dams on the Orange River and, arrowed, the Sarel Hayward Canal and Sites 1 and 2.

The concrete canal walls are cleaned both manually and mechanically. The silt, plants and algae which are removed may be simply dumped on the land at the sides of the canal or removed by trucks. Maintenance and cleaning of the canals is usually carried out once annually and at this time no water flows in large sections of the canals for approximately one week. Dosage with copper sulphate is carried out according to a prescribed method (Du Plessis, 1992), most often with the pre-dosing of H_2SO_4 in order to reduce the pH of the water. In canal systems controlled by the Department of Water Affairs and Forestry (DWAF), permission for dosage must be applied for and their methods and procedures strictly adhered to. In canal systems not controlled by DWAF, dosage with copper sulphate should also be applied for, but this is not always done. Also, since copper sulphate is freely available to farmers, “private dosing” *viz* without permission from DWAF, is often carried out, usually without the pre-dosage of H_2SO_4. The results of this “private dosing” will be commented on in further sections of this report.

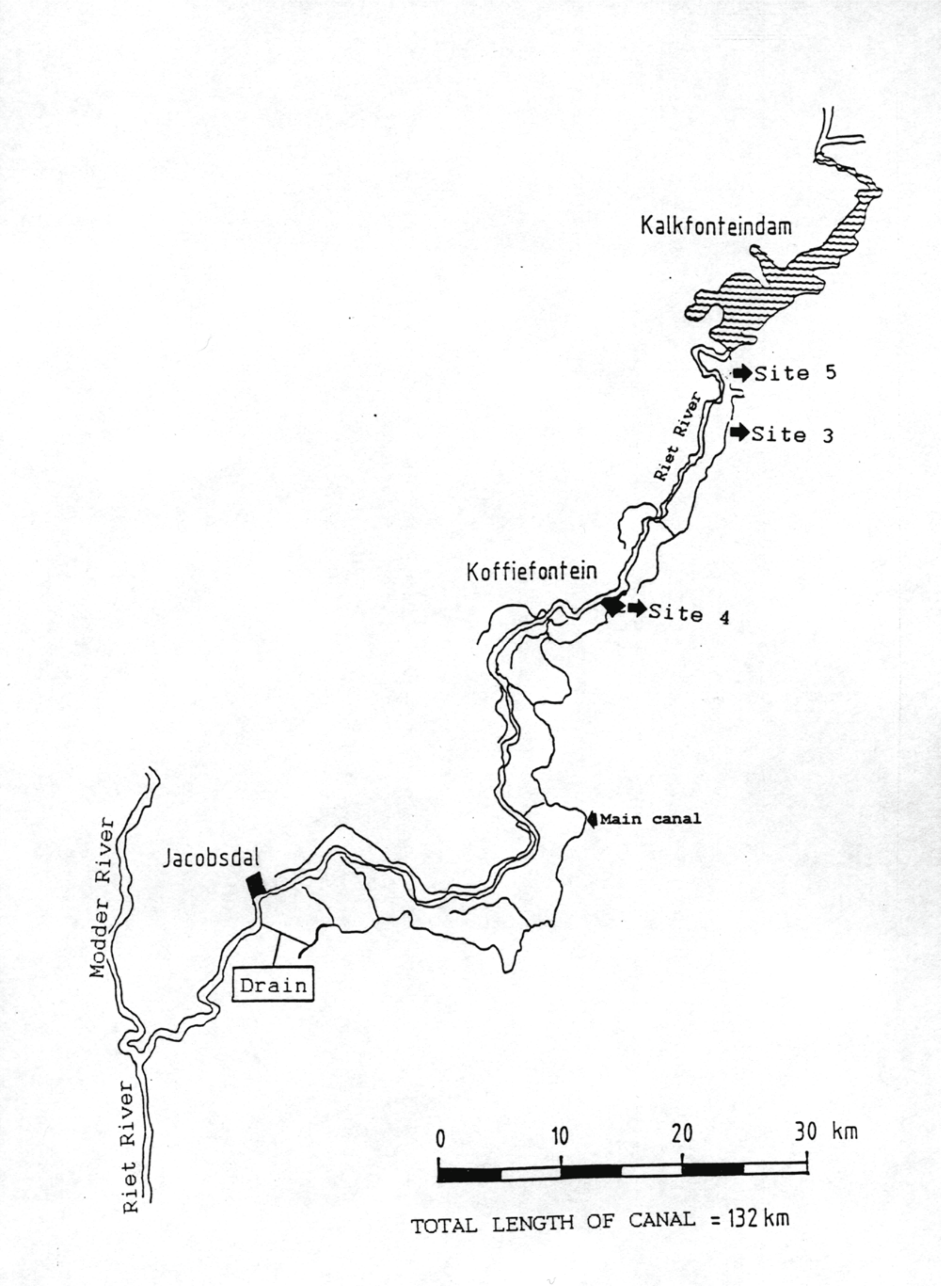


Fig. 2.2. Showing the positions of Sites, 3,4 and 5 on the canal system off the Kalkfontein Dam on the Riet River. (Map redrawn after Du Plessis, 1992)

Site 1

This site was situated on the Oranje-Riet Canal some 70 kilometers from the Scheiding Pumping Station and 39 km by road from Jacobsdal. This was the widest and deepest section of all five sample sites. It will be seen from the monthly sampling results that , on a number of occasions, the water flow was such that we could not get down to the sample grid. A rope ladder was used to access the grid as it had been placed fairly low on the canal side.

Site 2

This was 102 km by road from the first site and also on the Oranje-Riet Canal system. At this lower end of the canal system the canal was only 2 m wide and approximately 1.5 m deep. Water flow was considerably slower and access to the grid obtained directly from within the canal itself. Due to "control" site problems in our sampling of the Kalkfontein Canal System we were unable to continue sampling at this site after September, 1996. Time factors, due to the long distances which had to be covered, made the inclusion of this site in the one day sampling of the systems an impossibility after this date.

Site 3

This was 103 km by road from Site 2, 20 km from the town of Koffiefontein on the Kalkfontein Canal System. The canal is approximately 5 m wide and 2.5 deep with a strong water flow. Access to the grid was obtained by using a rope ladder. On two occasions the dirt road to the site was impassable due to muddy conditions.

Site 4

This site was next to the town of Koffiefontein, alongside the Finch Diamond Mine. The canal was the same depth and width as Site 3 and the grid was accessed by means of a rope ladder.

Site 5

It became apparent in early 1996 that despite our request that we be informed when copper sulphate dosages were made, copper sulphate was being used without our being told. We therefore spoke to the Kalkfontein Irrigation Board and told them of our findings and that we would require a Control Site above which no copper sulphate was placed in the canal. We therefore elected to place a monitoring board (these will be described later) just below the inflow from the Kalkfontein Dam into the canal system. This site was 10 km from Site 3 and 20 km from Site 4. This board was suspended by means of a chain into the canal. Access for light, conductivity and temperature measurement was gained by rope ladder into the canal. Water flow was usually fast and in full volume at this site.

Unfortunately, despite our request that no copper sulphate be applied above this site we found evidence that it had, in fact, been used on at least two occasions.

2.3.3. Material and methods for fieldwork

Sites 1, 3 and 4 were sampled every month from January, 1995 to June, 1997. Site 2 was sampled monthly from January, 1995 to September, 1996 and Site 5 was sampled monthly from October, 1996 to June, 1997. However, no sites were sampled in August, 1996 due to attendance at a Congress in Dublin, Ireland. Travel to and from the canal sampling sites in the Free State was by air and each sampling occasion to all the sites was completed in one day. This enabled us to examine the sample blocks and perspex dishes within 24 hours after collection.

At each sampling occasion the conductivity, pH, temperature and light incidence at three depths (surface, 12 cm and 25 cm) were measured. Two water samples were also collected to be taken back to the laboratory for turbidity measurement. Conductivity was measured with a Y.S.I. Electroconductivity Meter in $\mu S.cm^{-1}$, this meter also measures temperature. The temperature was always recorded at a depth of approximately 20 cm below the water surface, at a level equal to the middle of the sampling block grid. pH was measured using Merck Spezialindikator papers of two levels, pH 4.0 – 7.0 and 6.5 – 10.0. Light incidence was measured with a SKYE Light meter and recorded as $\mu mol\ m^{-1}s^{-1}$. Two water samples, one from the surface water and one from water at a depth of 20 cm were collected in plastic bottles which had been pre-washed in the laboratory with distilled water. Water speed was calculated (with three replicates) measuring the time taken for an orange to cover the distance of 10m on the water surface.

The grids constructed by the DWAF officials were made of metal, approximately 50 cm square and divided into 9 compartments on three levels. These had been set into the canal wall so that the surface was flush with the existing canal wall. Two restraining metal bars were attached by hinges on one side of the grid. These were opened and then fastened after the sample blocks were removed and replaced on each sampling occasion. This closure, in most cases, prevented loss of the sample blocks due to the strong flow conditions which occur in the canals. Two types of sample substrate were used during the sampling period. Initially, in order that the sample substrate be of the same surface as the canal we took broken pieces of canal wall and cut these to blocks, 15 x 15 cm. The original surface remained on the block face and in order that there was no undue movement of the block in each grid section we used cut squares of sponge to "cushion" each block. We placed 3 blocks in each of the top and bottom sections of the grid and on each sampling occasion the blocks were removed, marked and placed in a bucket for transportation to the laboratory where they were examined for algal growth. Prior to each sampling occasion the blocks were thoroughly washed and scrubbed with a brush, rinsed with distilled water and then dried in an oven at 70ºC overnight.

Due to the difficulties in the microscopic examination of the blocks, and lack of *Cladophora* recruitment on the blocks of wall material, we changed the algal sampling method in June, 1996. The second type of sampling method used Perspex® sheets. The sheets measured 45 x 45 cm and two rows of three aluminium bolts, 25 cm apart were fixed on the sheets. One half of a perspex laboratory Petri dish (8cm Ø), with a hole in the base was fixed onto each of the six bolts and secured with a wing nut. These six perspex dishes, three replicates for each of the two levels, were removed and replaced on each sampling occasion. The three dishes for each level were placed in clean, marked, plastic bags at each sampling site and transported back to the laboratory for examination. Each dish was lightly sanded on one half in order to establish whether there was preferred settlement on a roughened surface. During the sampling period the canals were cleaned on a number of occasions, but no official dosaging of copper sulphate according to the methods as described by Du Plessis (1992a,b) was undertaken by DWAF officials as far as we were aware. The presence, or not, of *C. glomerata* growth on the canal walls or floating past in the water was noted on each sampling occasion and when this alga did occur large samples were collected for the *in vitro* experiments in the laboratory. Sample blocks and perspex petri dishes were maintained at a low temperature in the buckets with bags of ice. In the laboratory the three sample blocks from the upper and lower levels from each site were examined macroscopically and microscopically (dissecting microscope) for the presence of algae. Three samples were tested from each of the two bottles of canal water collected at each site with a Hach Turbidity Meter. Results were recorded as NTU's and the mean taken for each site.

We did not measure nutrients in the water of the canals, as it was thought that one sample per month would not add significantly to the data already available from regular sampling of water quality by Water Affairs. We include here our own summary of data selected from that available from Water Affairs, to add information particularly on nitrate and phosphate conditions in the canal systems. This provides a clear idea of the nutrient conditions in the two source dams for the canal systems over a period when *Cladophora* was a problem.

2.3.4. Results

The results of the observations of algal growth on the sample blocks and petri dishes for each site will be given at the end of the results section. Algal growth as seen on the canal walls during the sampling period have been included within the results for each site.
Sites 1 and 2 in the Oranje-Riet Canal System differed in water flow rate and presence of algae. Site 2 was the smallest (width and depth) section of canal sampled.

Site 1

Figure 2.3, shows the temperature, conductivity and pH measurements made at Site 1. There is wide annual temperature range. The lowest temperature, 6.5ºC, was recorded in July, 1996 and the highest temperature, 23ºC, in January 1996. Conductivity variation was small, ranging from 92 to 165 $\mu S/m^2$; slightly reduced conductivity readings occurred in the summer months but in general the conductivity recorded was low. pH readings ranged between 6 and 7.8 during the entire sampling period, being mostly slightly below 7. pH peaks were not related to increased water temperatures nor showed any seasonal relationships. This site showed the greatest variation in water level. On four occasions the water level was well below the grid and on 5 occasions the volume and speed of the water in the canal was so great that we could not get to the grid as the rope ladder would not remain stable. Light attenuation measurements were calculated as percentage decrease from surface to 25 cm depth. Figure 2.4 shows the water speed, light attenuation and turbidity measurements for Site 1. Greatest water speed was measured in November, 1996 when it was 1.4 $m\ s^{-1}$ and the lowest in June, 1997 when it was 0.2 $m\ s^{-1}$, although it was generally greater than 0.8$m\ s^{-1}$. Turbidity levels were low in 1995 (decreasing from 50 to 10 NTU’s), but increased markedly in May, 1996 and remained over 60 NTU’s until the end of the sampling period, with occasional values over 80 NTU’s. This increase in turbidity level from April, 1996 through to July 1997 compared to the stability of the conductivity levels underlines the fact that these two physical conditions are not necessarily related. Light attenuation loss fluctuated between 50 and 80% for the whole of 1995 and the first three months of 1996, but this 30% difference in loss dropped to 25% for the

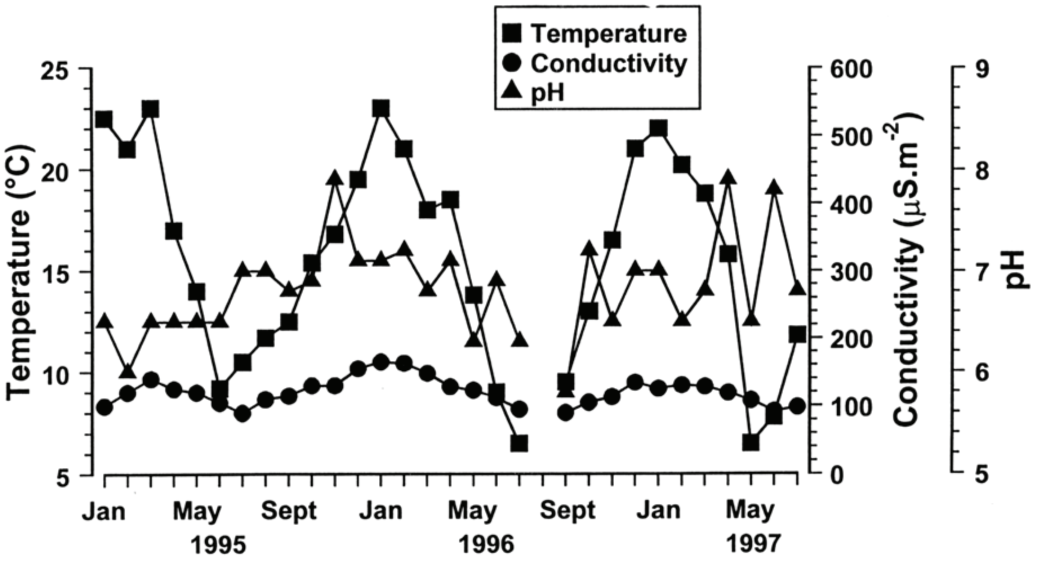


Fig. 2.3. Temperature, conductivity and pH measured during the sampling period at Site 1 on the Sarel Hayward Canal System.

rest of the sampling period. No *Cladophora glomerata* was seen to be growing on the canal walls during the sampling period. However, a 30-50% cover of the blue-green alga *Oscillatoria* sp. was seen on the walls in May, August, September and November 1995, in May and June, 1996 and in May, 1997.

Site 2

As previously mentioned this site was sampled from January, 1995 to September, 1996 only and not for the entire sampling period until July, 1997.

Figure 2.5 shows the temperature, conductivity and pH measurements for Site 2. These values are extremely similar to those for Site 1. Highest temperature, 25ºC, was recorded in January 1996, and the lowest, 5.8ºC, was recorded in July 1996. There is a difference of, 2ºC (highest), and 0.7ºC (lowest) between Site 1 and Site 2. pH measurements varied between 6.4 and 7.8, almost the same as for Site 1. The conductivity measurements mirrored those found at Site 1. Figure 2.6, shows the water speed, turbidity and light attenuation percentage loss for this site. Water speed varied little during the sampling period and ranged only from 0.1 to 0.3 $m.s^{-1}$. Turbidity ranged between 10 and 90 NTU's with a similar peak in April, 1996, as was found in Site 1. Light attenuation fluctuated, showing a marked difference to Site 1, between December, 1995 and March, 1996. A 10 to 20% cover of *C. glomerata* was seen growing on the canal walls in October, 1995, and in March, 1996.

In the Sarel Hayward canal, Sites 1 and 2, small "ropes" of *C. glomerata* were occasionally seen floating past in the canal water during the summer months but this was uncommon and this canal was cleaned at least once annually during the sampling period. Results for Sites 3, 4 and 5, which were on the Kalkfontein Canal System, are recorded as they were visited, which is also the order in their distance from the Kalkfontein Dam outlet into the canal.

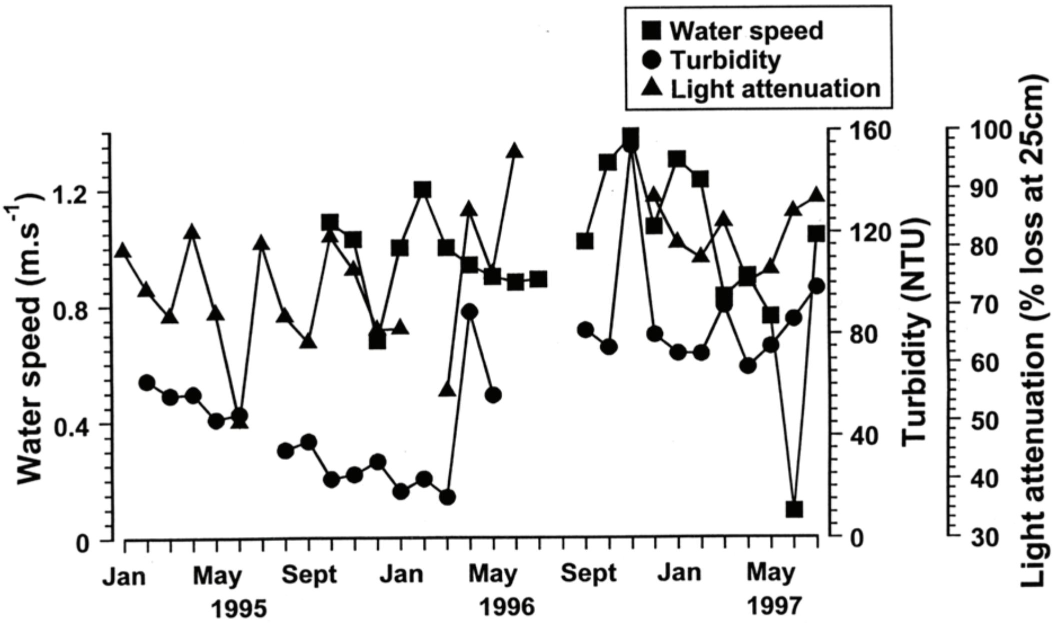


Fig. 2.4. Water speed, light attenuation and turbidity measured during the sampling period at Site 1 on the Sarel Hayward Canal System

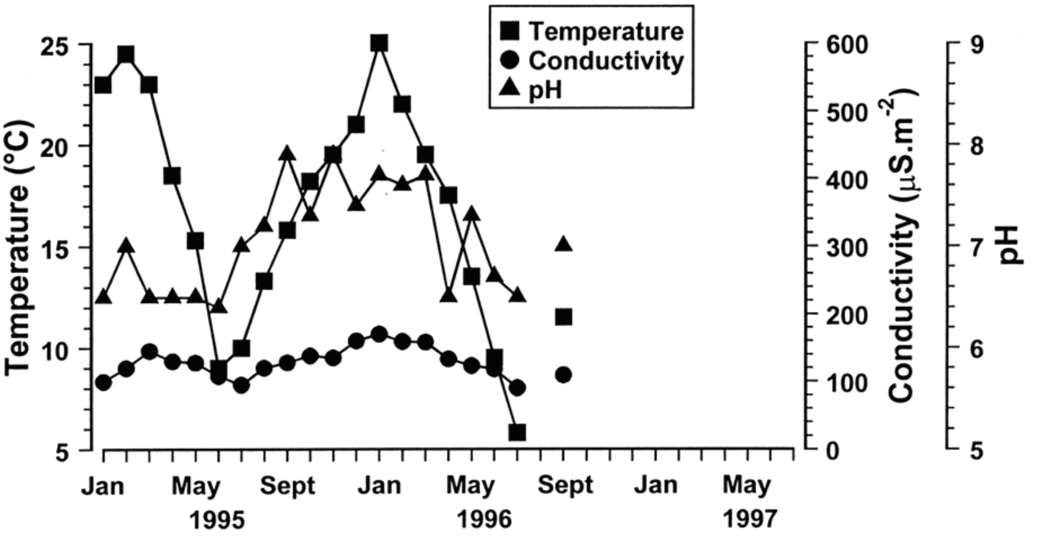


Fig. 2.5. Temperature, conductivity and pH measured during the sampling period at Site 2 on the Sarel Hayward Canal System.

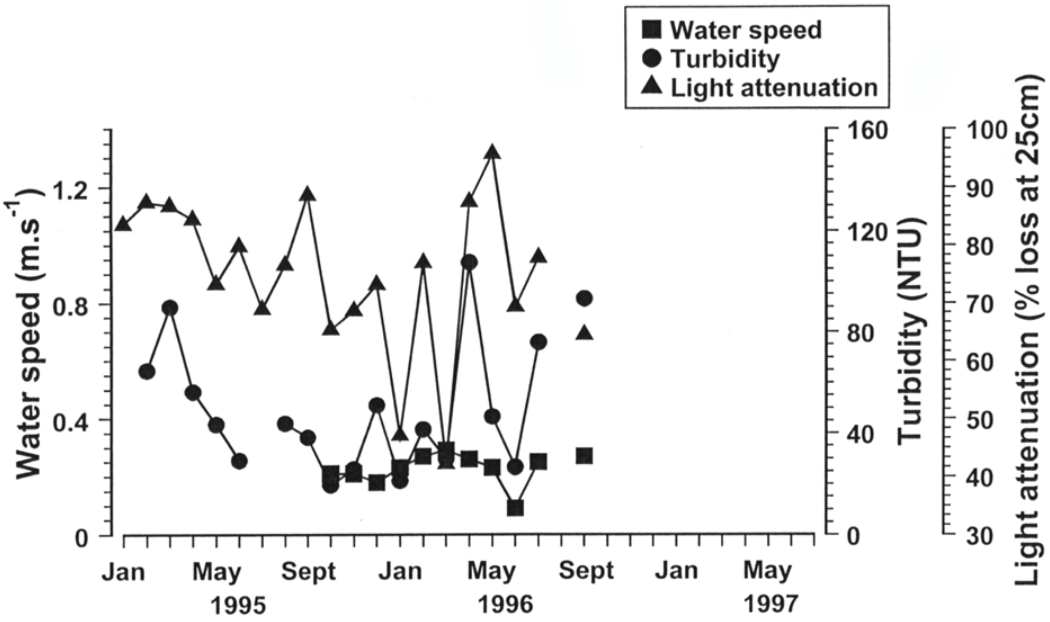


Fig. 2.6. Temperature, conductivity and pH measured during the sampling period at Site 5 on the Kalkfontein Canal System.

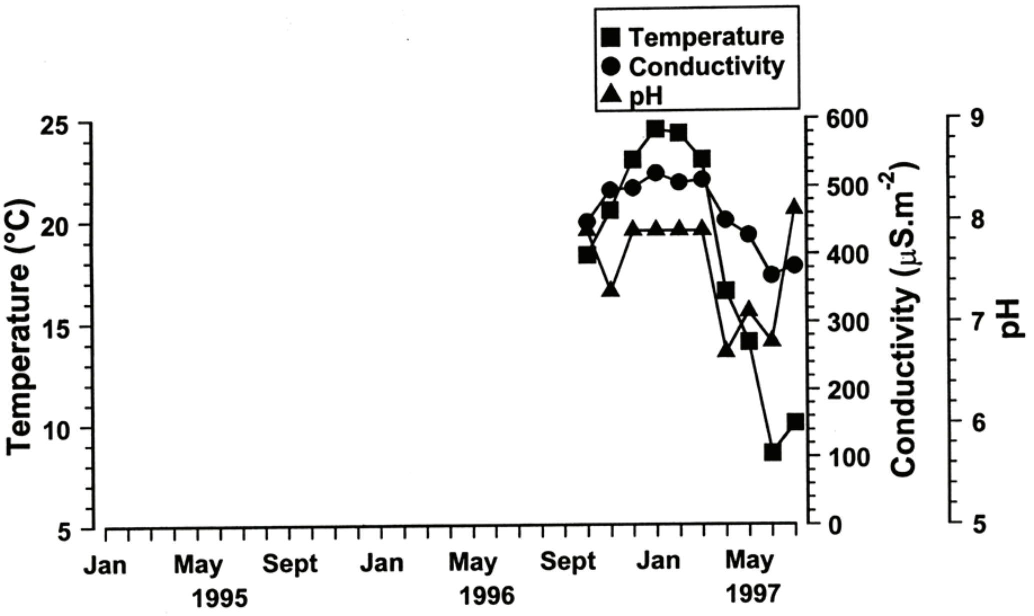


Fig. 2.7. Temperature, conductivity and pH measured during the sampling period at Site 5 on the Kalkfontein Canal System.

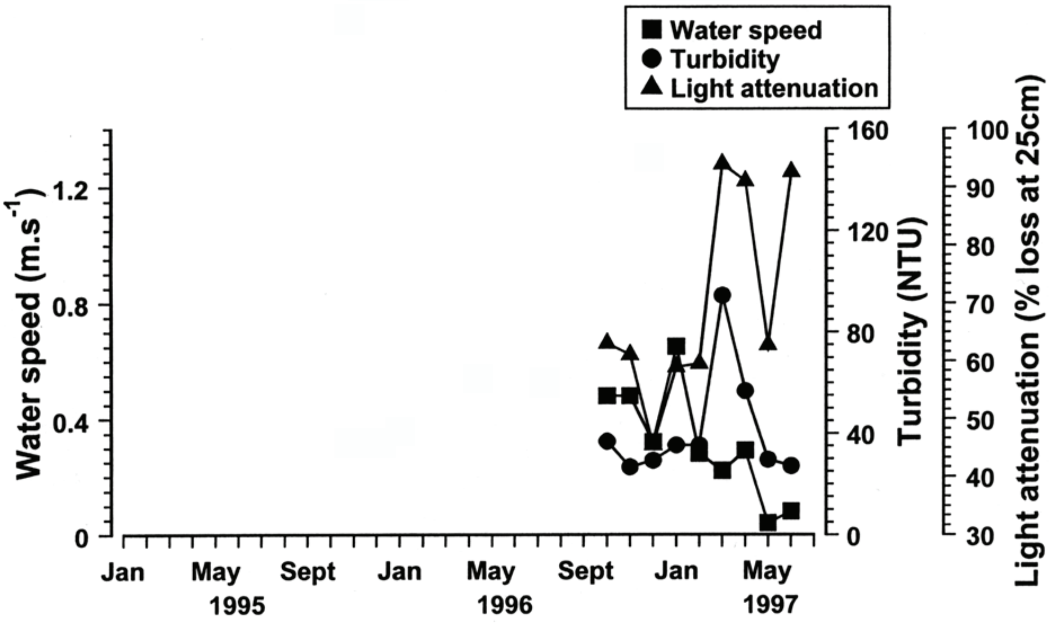


Fig. 2.8. Water speed, light attenuation and turbidity measured during the sampling period at Site 5 on the Kalkfontein Canal System.

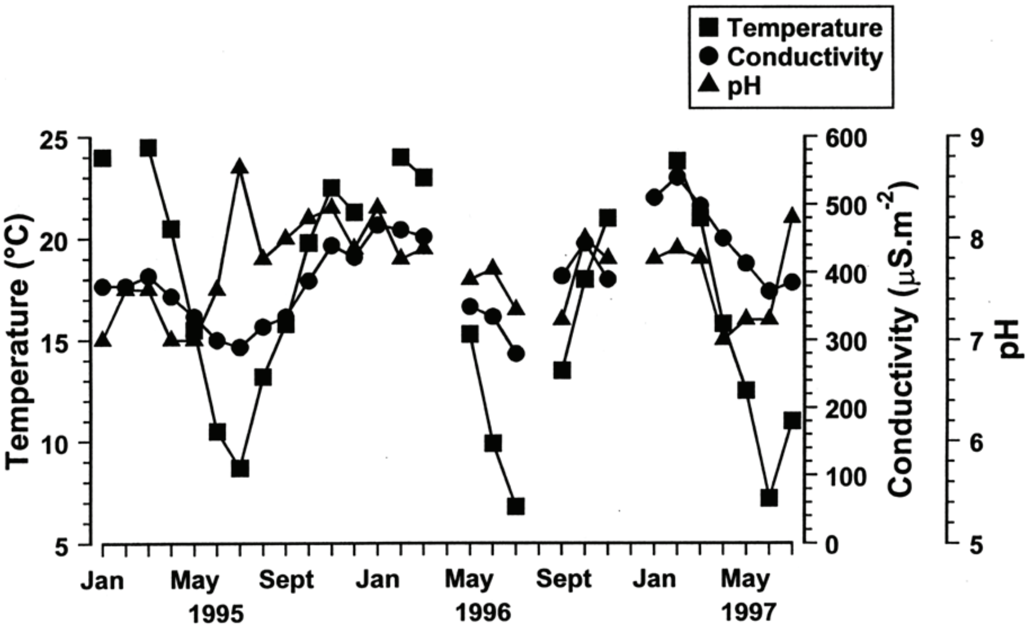


Fig. 2.9. Temperature, conductivity and pH measured during the sampling period at Site 3 on the Kalkfontein Canal System.

Site 5

This site was visited from September, 1996 to July, 1997 and was originally intended as a Control site for this canal system. Figure 2.7 shows the temperature, conductivity and pH measurements as recorded for this site. There was an expected seasonal range in water temperatures with the highest temperature, 24.5ºC, in January 1997, and the lowest temperature, 8.5ºC, in July, 1997.

Conductivity measurements ranged from 368 to 520 $\mu S/m^2$, increasing in the summer months and decreasing in winter. pH range was between 6.7 and 8.2. Figure 2.8 shows the water speed, turbidity and light attenuation percentage loss for this site. In general water speed decreased at this site over the sampling period although a peak increase occurred in January, 1997. Turbidity ranged from 30 to 94 NTU's, with a notable increase in late summer, March, 1997. Light attenuation percentage loss was linked to turbidity, increasing to 94% in March, 1997. There was a marked growth of *Cladophora glomerata* on the canal walls from January to June, 1997, when the canal walls were cleaned. This growth had reached lengths of 5 m by May, 1997. The alga grew from the top water level (which remained almost constant) to a depth of approximately 0.75 m down the sides of the canal.

Site 3

Figure 2.9 shows the temperature, conductivity and pH measurements recorded over the sampling period. Temperatures followed seasonal conditions, the highest temperature recorded was 27.5ºC in January, 1996 and the lowest, 7.2ºC in July, 1996. Conductivity values ranged from 280 to 540 $\mu S/m^2$ during the sampling period with peaks occurring in the summer months. pH values also peaked in the summer months but these did not always coincide with the temperature increases and decreases. Highest pH was 8.7 in July, 1995, and the lowest 7.0 in January, 1995. Figure 2.10 shows the water speed, turbidity and light attenuation percentage loss figures recorded during the sampling period. Water speeds generally decreased durimg the sampling period with the lowest speed recorded in June, 1997. Light attenuation shows six monthly peaks and a marked increase to 94% in April, 1997 from a 36% low in July, 1996. *C. glomerata* was seen growing on the canal walls in October, 1995, July and October, 1996 and January, 1997. However, clumps of *Cladophora glomerata* were seen floating past in the canal water in August and October, 1995, March, May, June, July and October, 1996 and in April, 1997.

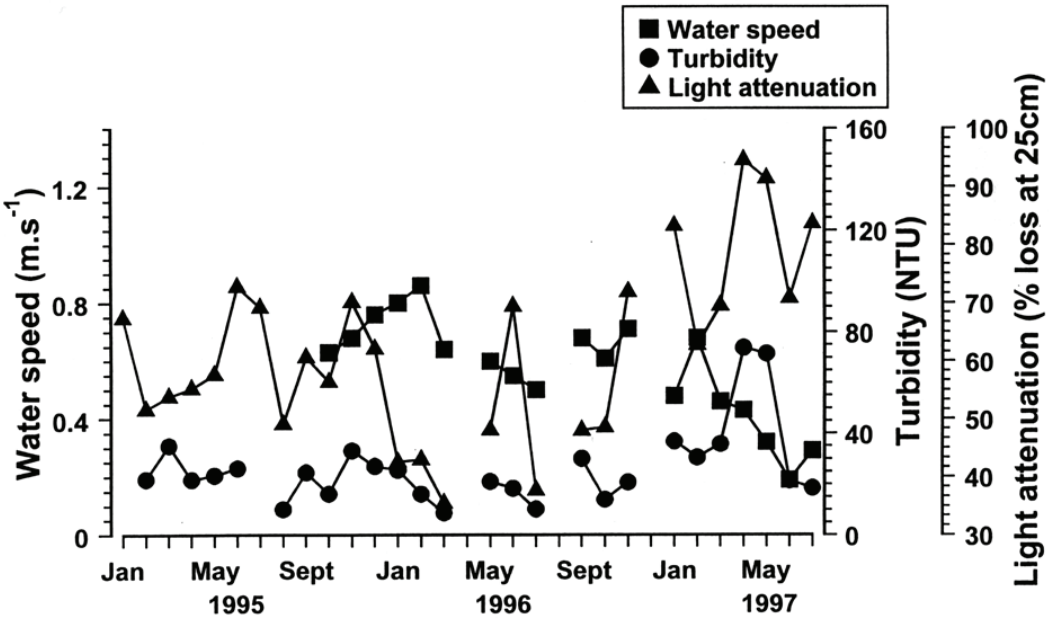


Fig. 2.10. Water speed, light attenuation and turbidity measured during the sampling period at Site 3 on the Kalkfontein Canal System.

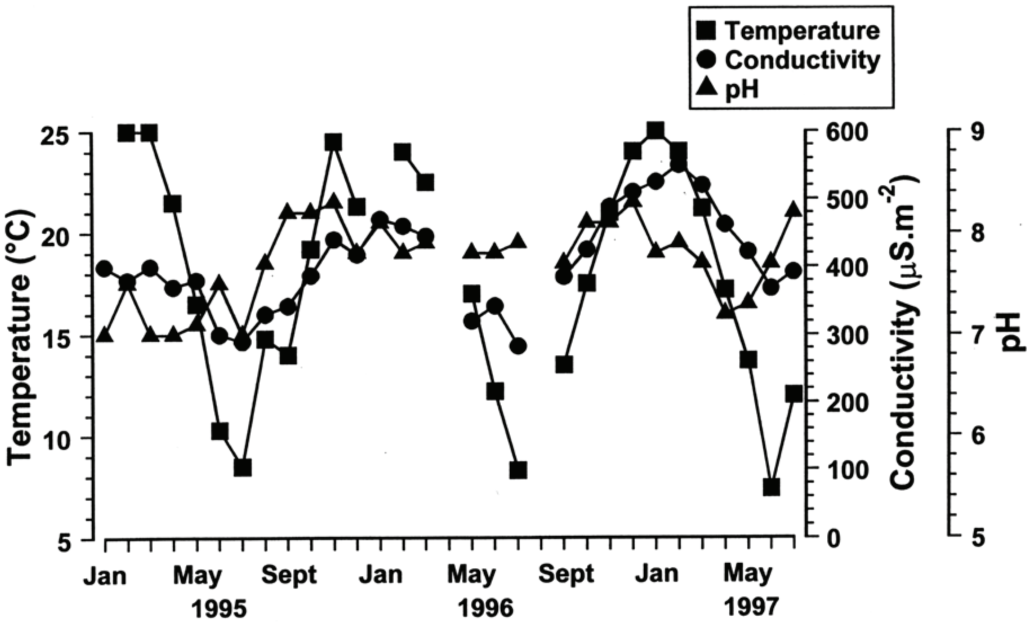


Fig. 2.11. Temperature, conductivity and pH measured during the sampling period at Site 4 on the Kalkfontein Canal System.

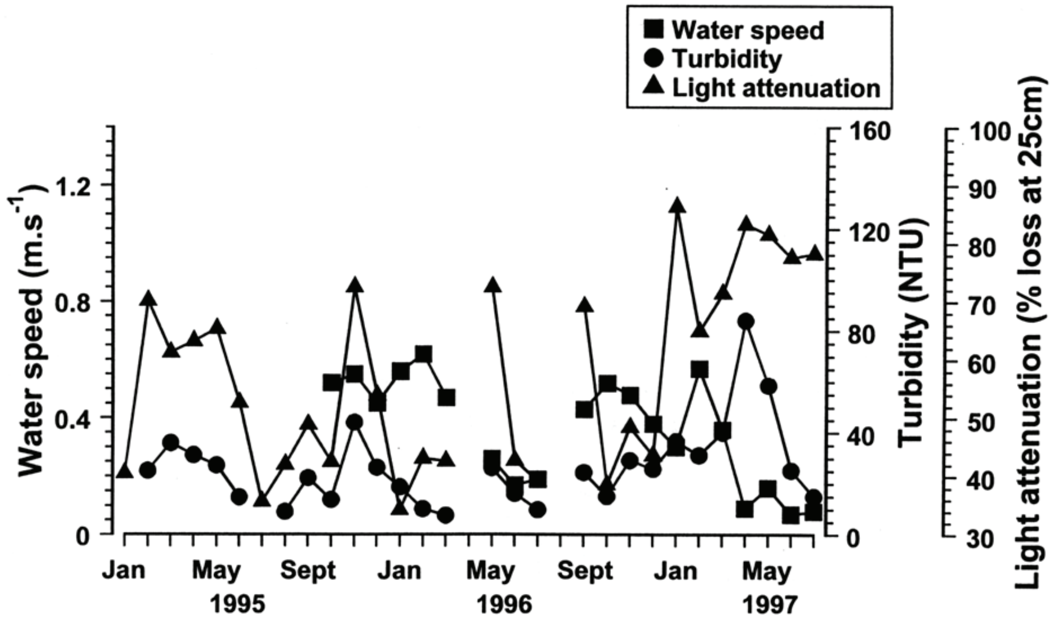
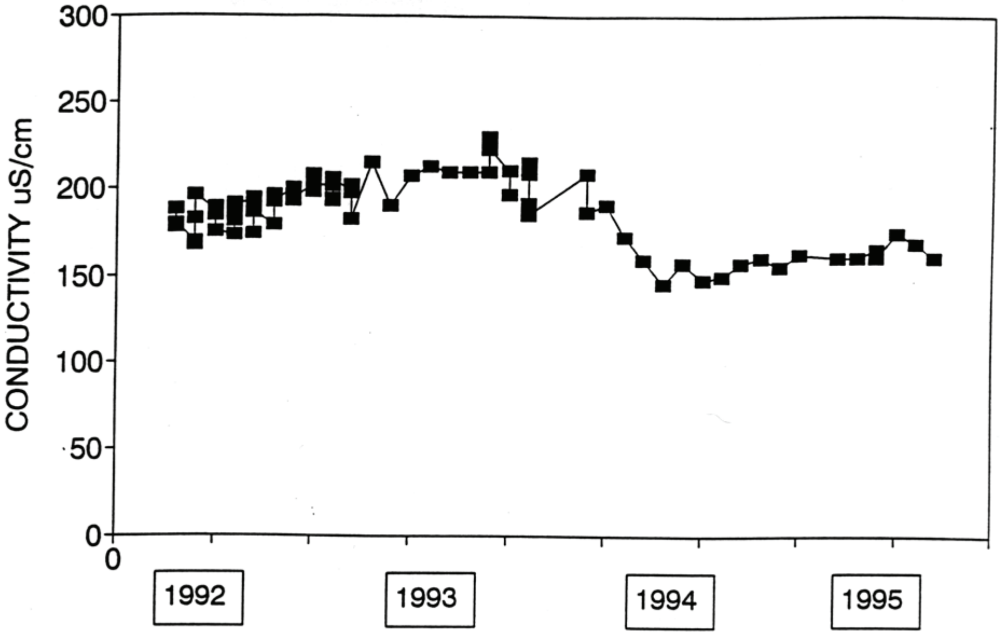


Fig. 2.12. Water speed, light attenuation and turbidity measured during the sampling period at Site 4 on the Kalkfontein Canal System.



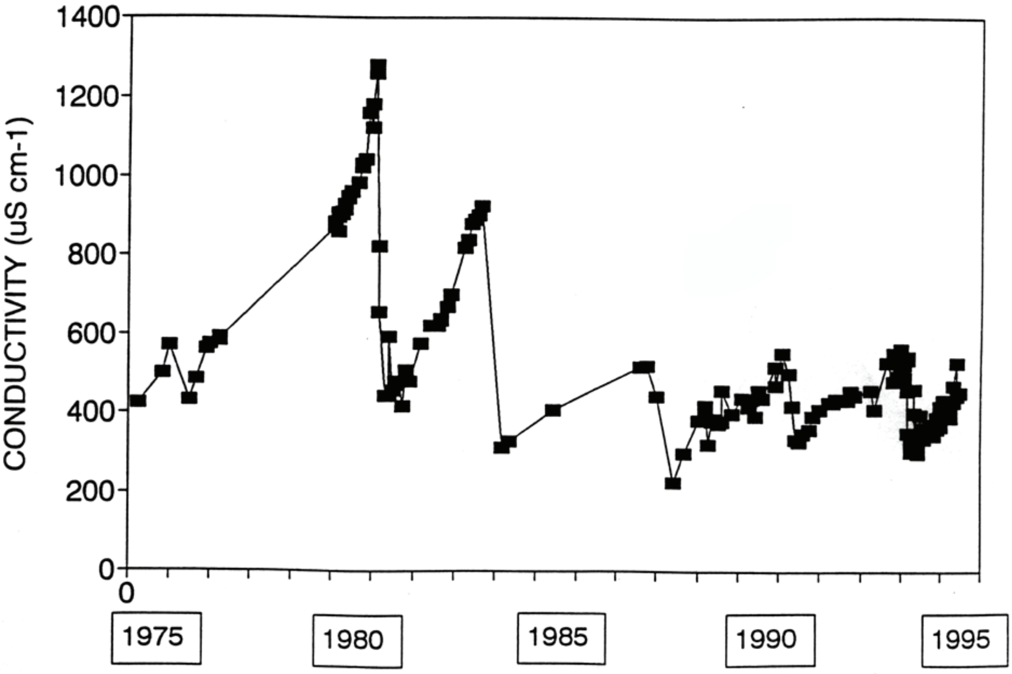
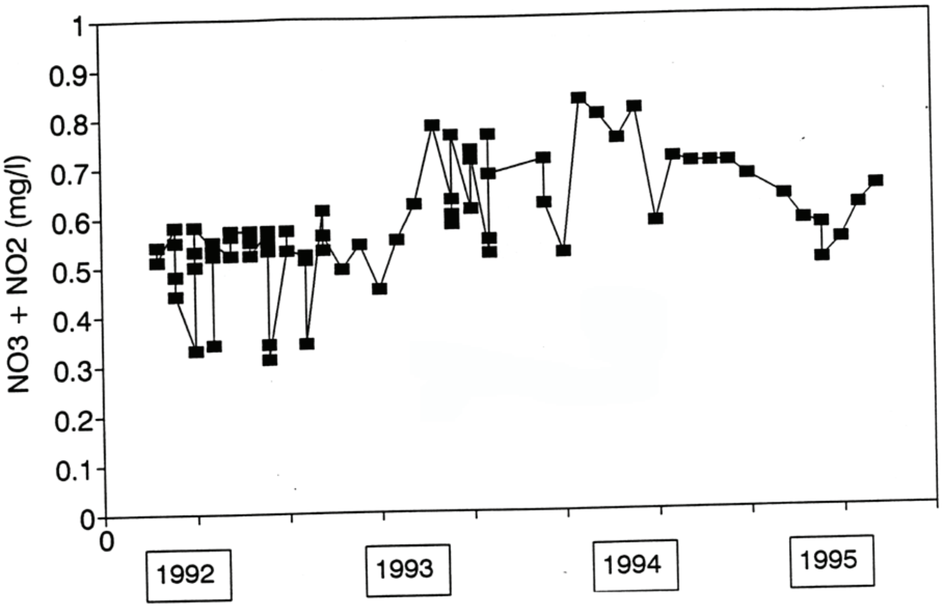


Fig. 2.13 (top) Conductivity levels in the Vanderkloof Dam from 1992 to 1995.

Fig. 2.14 (bottom) Conductivity levels in the Kalkfontein Dam from 1975 to 1995.



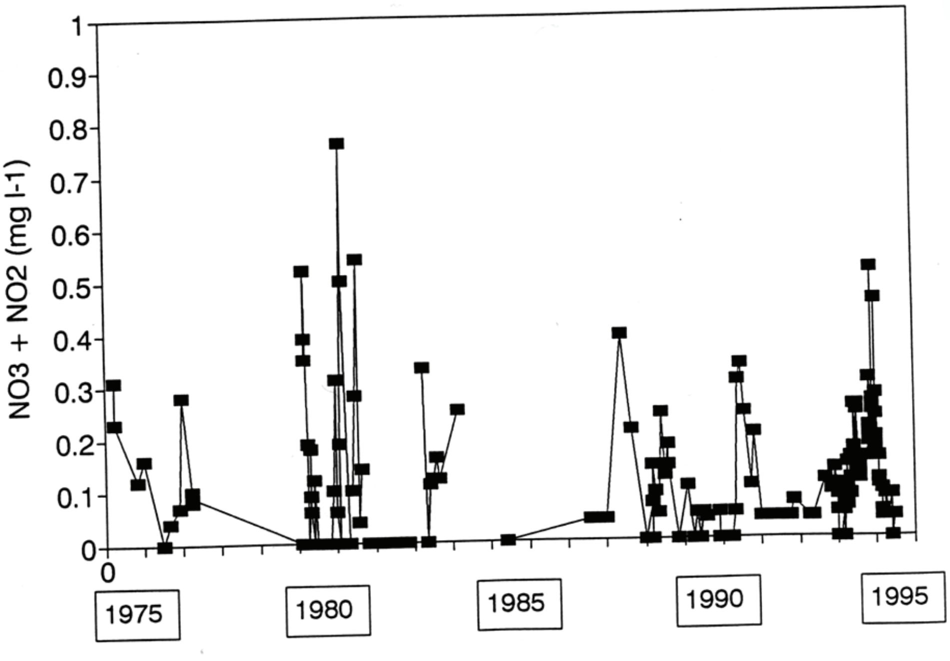


Fig. 2.15 (top). Nitrogen levels in the Vanderkloof Dam from 1992 to 1995.

Fig. 2.16 (bottom). Nitrogen levels in the Kalkfontein Dam from 1975 to 1995.

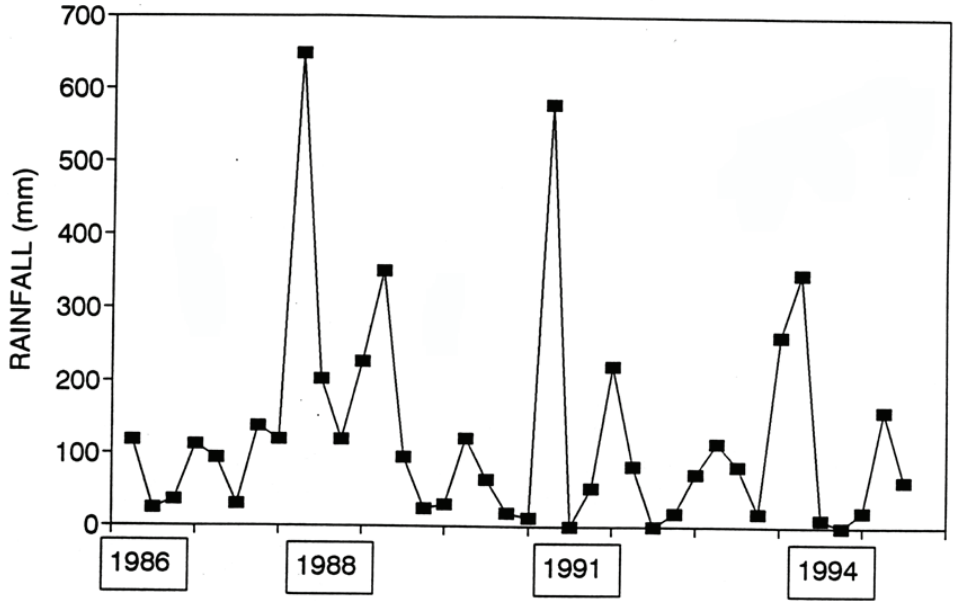
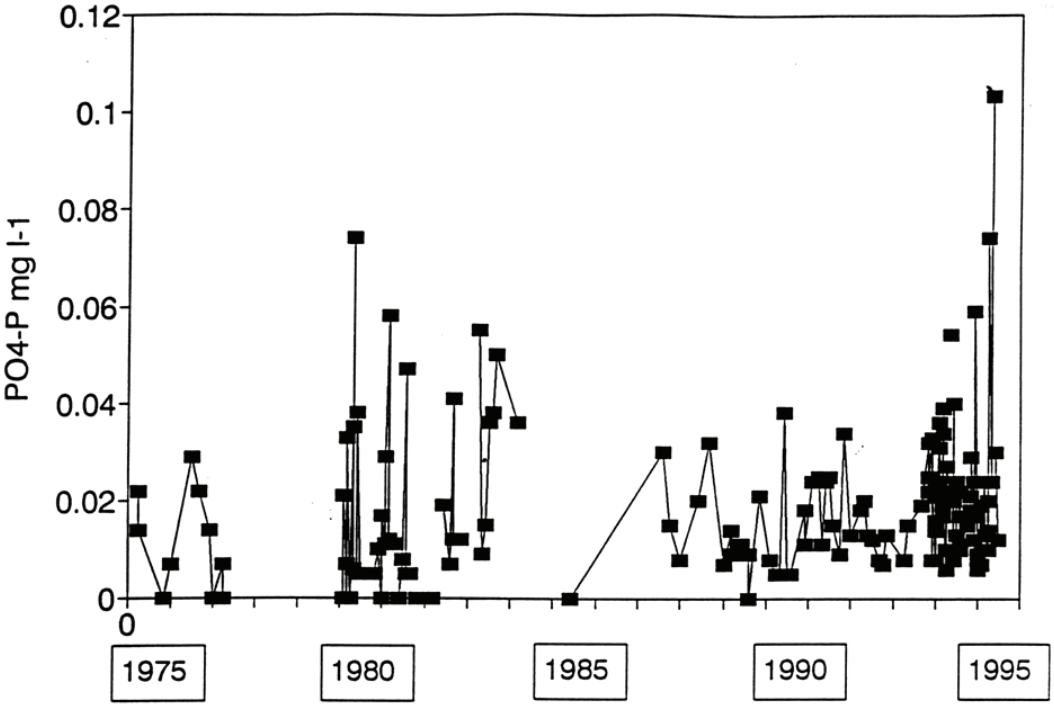


Fig. 2.17. Rainfall figures, as measured at Jagersfontein in the Riet River catchment from 1986 to 1994



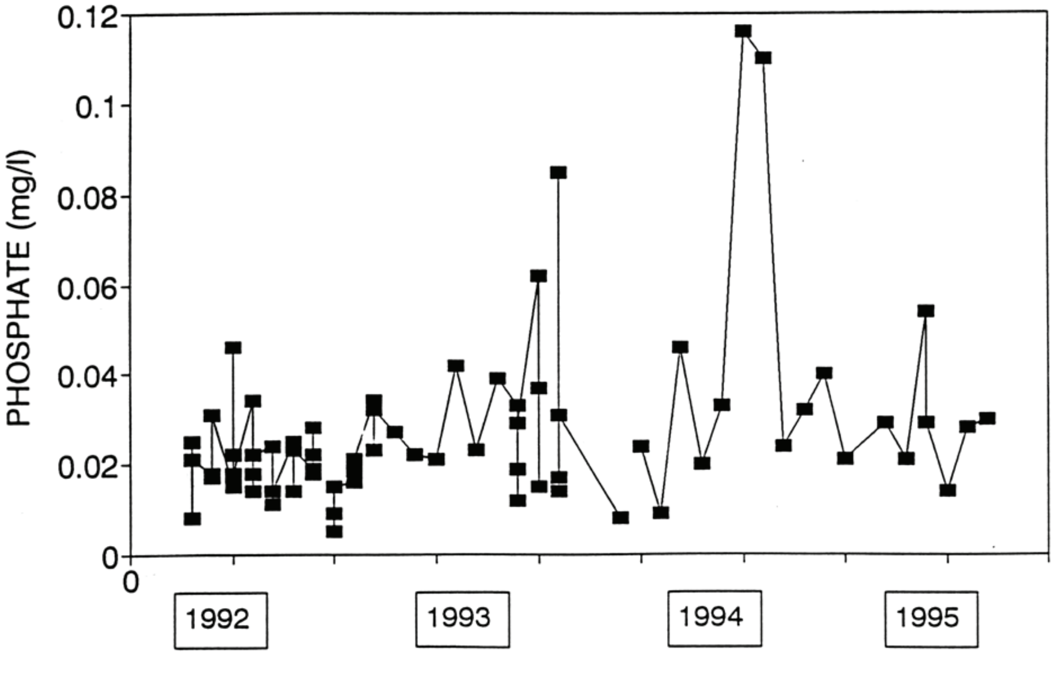


Fig. 2.18 (top). Phosphate (as P) measured in the Kalkfontein Dam from 1975 to 1995

Fig. 2.19 (bottom) Phosphate (as P) measured in the Vanderkloof Dam from 1992 to 1995

Site 4

Figure 2.11 shows the temperature, conductivity and pH measurements recorded at this site over the sampling period. Temperatures peaked during the summer months and this period of increased temperature in summer was more prolonged in 1995. The highest temperature of 28ºC was recorded twice, in January, 1995 and 1997 and the lowest, 7.4ºC in June, 1997. Conductivity values peaked in the summer months, with a gradual increase in peak values occurring in 1997. Conductivity values ranged from 283 to 550 µS.m^{-1} . pH values ranged from 7 to 8.9 and show a slight increase during the summer months but do not always follow the seasonal temperature ranges. Figure 2.12 shows the water speed, turbidity and light attenuation percentage loss figures recorded during the sampling period. Water speed decreased over the sampling period and in June, 1997 the lowest water speed of 0.05 m s^{-1} was recorded compared to the highest speed of 0.65m s^{-1} in February 1996. Light attenuation loss peaked every four to six months except for the last six months of the sampling period when high attenuation losses were recorded. The greatest percentage loss, 86%, occurred in January, 1997. *Cladophorsa glomerata* was observed growing on the canal walls in January, February, April, 1995, October, 1996, and January, 1997. *C. glomerata* was also seen floating past in the canal water in January, February, April, August, 1995 and in April, 1997.

The conductivity in both systems fluctuates with water level, rising as the dam level falls. It is clear from DWAF data that, as with our own data, the Kalkfontein system (Fig 2.13) generally has a much higher conductivity (indicating the level of dissolved salts) than the Orange/Riet system (Vanderkloof Dam, Fig 2.14). With very low dam levels around 1980 there was a salinization problem in the Kalkfontein dam (conductivity briefly over 1200 µS cm^{-1}), whereas from 1990 the levels fluctuate in the range 300-550 µS cm^{-1}. In contrast, since 1990 the levels in the Vanderkloof dam have been in the range 150-200 µS cm^{-1}. Dissolved nitrogen levels in the two systems are always below 0.8 mg l^{-1}, with levels being generally higher in the Vanderkloof dam (usually 0.5-0.7 mg l^{-1} ; Fig. 2.15) than the Kalkfontein dam (usually below 0.4mg l^{-1}; Fig. 2.16). The peaks of dissolved nitrogen level in the Kalkfontein system are produced as 'spikes'which correlate with rainfall events in the Riet River catchment (see data for Jagersfontein in Fig. 2.17), but even these peaks are lower than the general levels in the Vanderkloof system. Levels of dissolved phosphorus in the two systems were variable, with little clear pattern. In the both systems, levels were generally between 0.01 to 0.04 mg PO_4-P l^{-1}, with occasional spikes above 0.05 mg PO_4-P l^{-1} (Figs 2.18, 2.19)

Algal growth on sample blocks and petri dishes.

During the first period of sampling, from 1995 to June, 1996 the sample blocks, cut from pieces of the canal wall and placed in two rows of three replicates, were brought back to the laboratory for examination. These were found to be unsuitable as it was found that initially no algae were observed to grow on the blocks, despite algal growth on the actual canal walls. When algae were found growing on the blocks during the last period of using this method it was found that accurate examination was impractical and unsatisfactory. From September 1996 to July, 1997, when the plastic petri dishes were use d at Sites 1, 3, 4 and 5, satisfactory results were obtained. These are shown in Table Nos. 1 a & b, 3 a & b, 4 a & b and 5 a & b. Numeric values represent percentage cover on petri dish surface and ~ sign indicates that algae appeared dead.

Table 1a. Showing algal growth during the sampling period on the upper row plates at site 1 on the Sarel Hayward Canal System. ~ = dead

Site 1 (top)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophoraglomerata									E	E	
Stigeoclonium tenue									X	X	
Protoderma sp.									P	P	
Oedogonium sp.									O	O	
Cocconeis sp.							15~	5	S	S	5
Other diatoms								1	E	E	2
Oscillatoria sp.								1	D	D	

Table 1b. Showing algal growth during the sampling period on the lower row plates at site 1 on the Sarel Hayward Canal System. ~ = dead

Site 1 (lower)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophora glomerata									E	E	
Stigeoclonium tenue									X	X	
Protoderma sp.									P	P	
Oedogonium sp.									O	O	
Cocconeis sp.							2~	5	S	S	5
Other diatoms								<1	E	E	1
Oscillatoria sp.									D	D	

Table 2a. Showing algal growth during the sampling period on the upper row plates at site 3 on the Kalkfontein Canal System. ~ = dead

Site 3 (top)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophoraglomerata											
Stigeoclonium tenue	<1	1			1			<1			
Protoderma sp.											
Oedogonium sp.											
Cocconeis sp.	10	1~	3~		1~		1~	<1			3
Other diatoms	<1							<1			1
Single cell blue-greens		5			<1	<1					<1

Table 2b. Showing algal growth during the sampling period on the lower row plates at site 3 on the Kalkfontein Canal System. ~ = dead

Site 3 (lower)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophora glomerata											
Stigeoclonium tenue	<1	30~			1						
Protoderma sp.											
Oedogonium sp.											
Cocconeis sp.	10	30~	3~		1~		15~	<1			
Other diatoms	<1				<1						
Single cell blue-greens		4			<1	<1					<1

Table 3a. Showing algal growth during the sampling period on the upper row plates at site 4 on the Kalkfontein Canal System. ~ = dead

Site 4 (top)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophora glomerata	<1				30						
Stigeoclonium tenue	10	30~		3						10	10
Protoderma sp.				1	5		4	4		5	5
Oedogonium sp.				3	1						
Cocconeis sp.	20	30~	5		2		10~	5	7	10	10
Other diatoms	<1				<1						
Single cell blue-greens			2	1	1	5		4			

Table 3b. Showing algal growth during the sampling period on the lower row plates at site 4 on the Kalkfontein Canal System. ~ = dead

Site 4 (lower)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophora glomerata	<1				20						
Stigeoclonium tenue		5~	<1	2					<1	8	10
Protoderma sp.					5		5		<1	4	5
Oedogonium sp.	<1				1						
Cocconeis sp.	8	5~	5~		1		5	5	5	8	10
Other diatoms	2				<1						
Single cell blue-greens		5		3	1	5	<1	2	1		1

Table 4a. Showing algal growth during the sampling period on the upper row plates at site 5 on the Kalkfontein Canal System. ~ = dead

Site 5 (top)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophora glomerata		50	2	1				<1			
Stigeoclonium tenue	90	40	25	3	10	5		<1	<1	40	40
Protoderma sp.						3	5				
Oedogonium sp.											
Cocconeis sp.	30	<1	25	3	10	5~	45	20~	1		30
Other diatoms		<1							<1		
Single cell blue-greens				3	1	1					

Table 4b. Showing algal growth during the sampling period on the lower row plates at site 5 on the Kalkfontein Canal System. ~ = dead

Site 5 (lower)	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
Cladophora glomerata		45		1			<1		40		
Stigeoclonium tenue	60	40	10~	3	3			<1	5		
Protoderma sp.				1	3	2					
Oedogonium sp.											
Cocconeis sp.	20	<1	20~	3	10	30~	50	20~	2	35	30
Other diatoms		<1									
Single cell blue-greens		<1		2		1	7				

2.3.5. Discussion

It became apparent early in the sampling program that there was a notable difference in the physical and biological character in the two canal systems. Water temperatures were the only exception to this. There is a marked difference in the size and storage capacity of the two supply dams. The Vanderkloof Dam has a capacity of 3 172.667 million m^3 and a capacity surface area of 13 340.2 hectares, the Kalkfontein Dam has a capacity of 321 m^3 and a capacity surface area of 4 598.7 hectares. It would be expected that the water from the Van Der Kloof Dam would have a lower temperature than that of the Kalkfontein Dam not only due to the dam size, but also because the water speed is so much greater in the Oranje-Riet Canal at Site 1. Neither dam has offtake variation selection. This similarity in water temperatures is possibly due to the fact that readings were made at a depth not greater than 25 cm where ambient air temperature effects are a controlling factor. The conductivity measurements for all five sites are shown in Figure 2.20.

Conductivity measurements are, on average, four times greater in the Kalkfontein Canal System than in the Oranje-Riet System. All five sites in both systems follow the two main trends in conductivity measurements over the distance between the sites. These differences between the two systems are probably due to the fact that not only is there a difference in dam size and therefore sediment precipitation, but more importantly, that the dams are on different rivers, the Orange and the Riet. Also during the sampling period, the level of the Kalkfontein Dam fell by nearly 50% and was, at the end of the sampling period, only 17% full. Very little rain had fallen in the drainage area of the Kalkfontein Dam. The level of the Vanderkloof Dam remained seasonally constant which was due to the fact that it forms part of the water control system of the Orange River (Davies *et al*, 1993) and is supplied with water from the Gariep Dam upstream and that rainfall in its drainage area had been within normal limits. The Orange River has water with the dominant cations, Ca^{2+} and Mg^{2+} while the Riet River has water with the co-dominant cations, Ca^{2+}, Mg^{2+} and Na^{+} (Dallas *et al*, 1994). Due to the fact that there was little or no precipitation in the drainage area of the Riet River, the higher conductivity levels in the Kalkfontein Canal System could be explained by water offtake with high levels of accumulated sediment in the lower levels of the dam. The conductivity peaks in the Kalkfontein System are also linked to the seasonal temperature increases whereas the Oranje-Riet System conductivity levels show only a minimal increase with temperature. It would appear that the presence of Na^{+} contributes to the increase in conductivity levels in the Kalkfontein System. Water quality data measurements supplied by the Department of Water Affairs indicate higher levels of Na^{+} in the Riet River and its tributaries.

pH levels at all five sites are shown in Figure 2.21. Higher pH levels were measured in the Kalkfontein Canal System sites (3, 4 & 5) than in the Oranje-Riet System sites (1 & 2) throughout the entire sampling period.

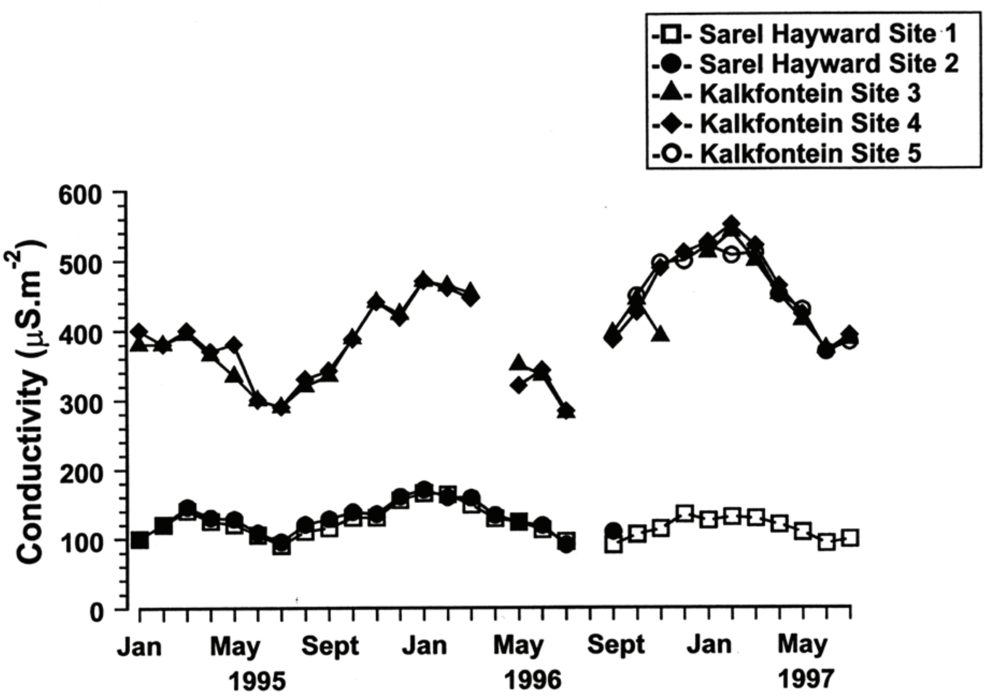


Fig. 2.20. Conductivity as measured at all five sites during the sampling period.

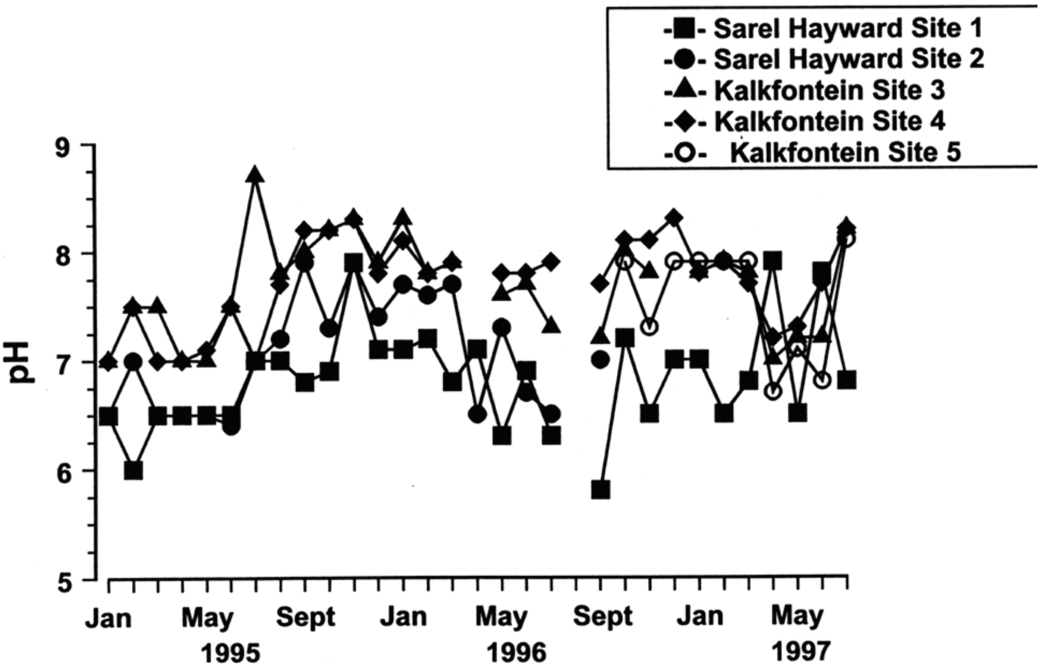


Fig. 2.21. pH as measured at all five sites during the sampling period.

Water speed measurements are shown in Figure 2.22. Water speed and volume was higher during the time of greatest irrigation demand in summer in all the sites, except for Site 2 on the Oranje-Riet System. Site 2, being the smallest section of canal sampled is at the lower end of the system where strong water volumes are required to a far lesser extent. The greatest variation in water speed is seen in Site 1 which was the largest section of canal of all the sites. Water speeds peaked in summer in the Kalkfontein System, but showed a gradual decrease at the end of the sampling period. This was possibly due to the marked decline in the water level of the Kalkfontein Dam which would impose restrictions on the amount of water supplied. Water speeds measured in both systems reflect the irrigation water demand for a 24 hr period only. Farmers request water requirements daily from the relevant supply sources, local Department of Water Affairs office or relevant Irrigation Board. Daily requirements may vary according to crop requirement, available water and financial constraint thus the water volumes in the canals may vary on a daily basis. As the the commonest crops (maize, wheat and sunflowers) are annual and often biseasonal (winter and summer plantings) overhead irrigation is used in this area.

Turbidity levels for all sites are shown in Figure 2.23. Turbidity levels are notably higher in sites 1 & 2 in the Oranje-Riet Canal System than in sites 3, 4 & 5 in the Kalkfontein Canal System. The DWAF data on dissolved nutrients shows that nutrients, while being sufficient in both systems for *Cladophora* growth if other conditions are favourable, are not an important controlling factor of the presence of large growths of *Cladophora* in these two systems. The general levels of dissolved nitrogen and phosphorus, although obviously high enough to cause a *Cladophora* problem in the Kalkfontein system, are not very high by international standards (B. Whitton and J. Eaton, *pers. comm.*). The canal system studied which did not have a *Cladophora* problem during the period of the investigation has water from the Vanderkloof dam, which has higher general nutrient levels than the system with a great problem. It is clear from this data that there are sufficient nutrients in the Orange/Riet canal system for large growths of *Cladophora*, and it thus must be another factor or combination of factors which prevents such growth.

The higher water turbidity levels, greater water speed and marked changes in water levels as found in the Oranje-Riet System can be related to the reduced amount of algae in these canals. It was also observed that there was a more regular canal cleaning in the latter system due possibly to better control measures. Major algal growth, mainly *C. glomerata*, was observed in the Kalkfontein Canal System. Two large growth phases were observed for *Cladophora* *glomerata* on the petri dish monitoring plates from the Control site. Firstly, in October (Spring) and secondly in May (Autumn). Some *C. glomerata* filaments were found in November and December, 1996 and again in March and April, 1997. No similar growth phases of *C. glomerata* sporelings were found on the petri dishes at Sites 3 and 4, below the Control site and thus below the copper dosage sites. However, there was one exception to this, in January, 1997, when there was a growth of *C. glomerata* filaments on the petri dishes at Site 4. No similar growth occurred at any of the other sites. This apparently unusual growth may possibly have occurred because there was a localised release of spores from *C. glomerata* in a side weir upstream or, a more likely reason being that copper sulphate had been applied in a small dosage above the Control site (there was no filament growth on the Control site dishes) and this dosage had been effective in control of *C. glomerata* growth at Site 3 as well. It was noted throughout the sampling period that the effect of the copper sulphate dosage diminished from the usual point of application (just below the Control site) to Site 4, approximately 20 km downstream.

There was no difference in *C. glomerata* sporelings attachment on the smooth or roughened surfaces. In fact, during the spring growth phase there was a massive growth of sporelings on the very smooth surface of the Perspex® base plates. It can also be seen from the results of algal growth on the petri dishes that dead algal filaments and cells *viz*. *Stigeoclonium tenue* and *Cocconeis* sp. were found in November, 1996, and February and April, 1997 at Site 5 and in October and November, 1996 and in March, 1997. This was probably due to the fact that copper sulphate had been used by the Kalkfontein Irrigation Board to control the algal growth. We also found individual bags of copper sulphate suspended in the canal at various places. The common occurrence of the diatom *Cocconeis* sp. and the filamentous alga, *Stigeoclonium tenue*, was a useful indicator of the application times and effect of copper sulphate dosage. *Cocconeis* is a cosmopolitan genus which has some species noted for their tendency to be primary colonisers (Winterbourn, 1990). Because of their shape, oval and concave on the underside, they adhere firmly to most surfaces by means of secreted mucilage (Round, *et al*, 1996). *Stigeoclonium tenue* is a cosmopolitan filamentous algal species which establishes itself initially with a basal growth of filaments which adhere firmly to the substrate. Upright filaments grow from this basal attachment. This algal species is noted for its ability to grow in water with high levels of organic pollution and resistance to metallic poisons (John & Johnson, 1991).

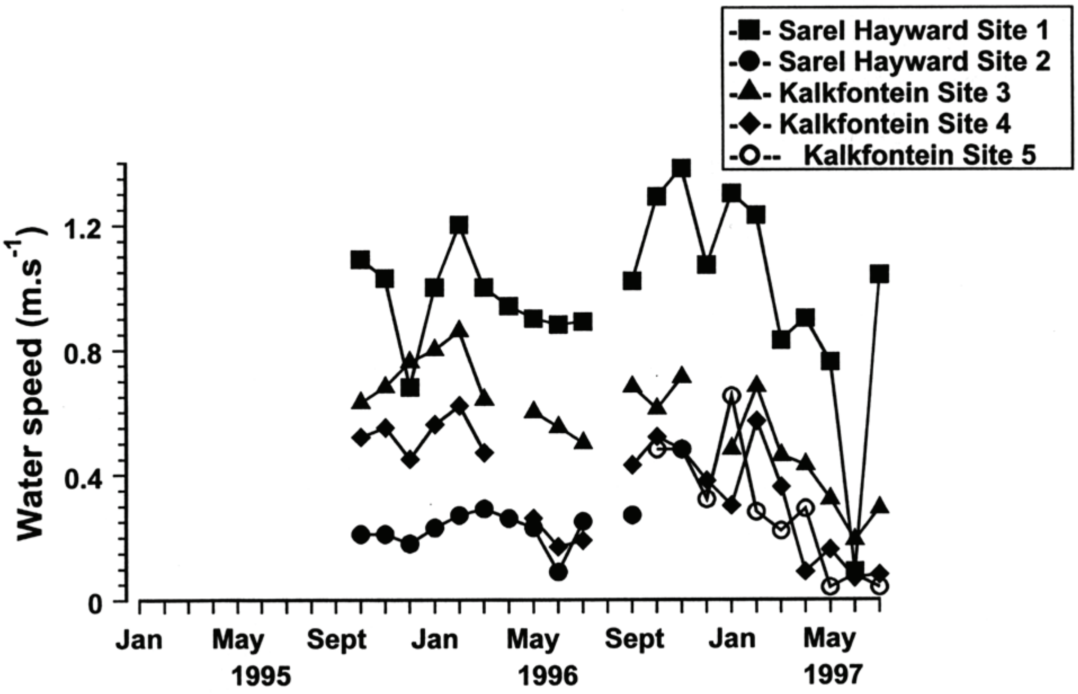


Fig. 2.22. Water speed as measured at all five sites during the sampling period.

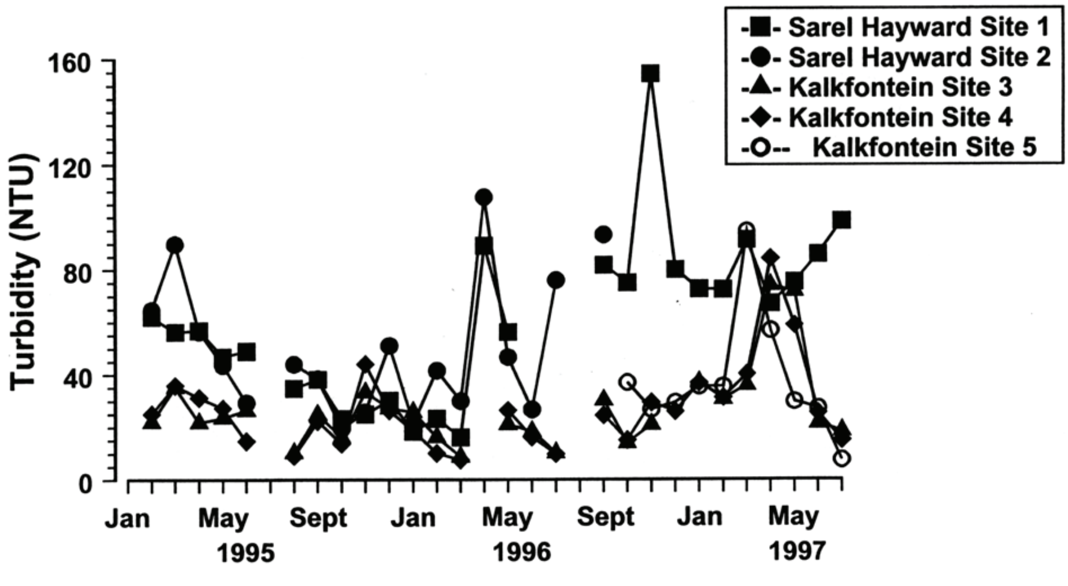


Fig. 2.23. Turbidity as measured at all five sites during the sampling period.

Stigeoclonium tenue and *Cocconeis* sp. formed the dominant cover on the petri dishes from the Control site in the Kalkfontein Canal System in September, 1996 and were present on the dishes from this site throughout the sampling period. The growth of *S. tenue* was less on the lower petri dishes and this was possibly due to the fact that it prefers higher light intensities for growth (Steinman *et al*, 1989).

Protoderma sp., an alga with a completely prostrate growth habit appeared on the Control site and Site 4 petri dishes in summer 1996 and maintained its presence until the end of the sampling period in July, 1997. It did not appear on the dishes at Site 3. This is probably due to the fact that it was negatively affected by the copper sulphate dosing which took place just above this site. A single-celled blue-green alga occurred on the petri dishes at all the sites throughout the year, but in small groups with widely dispersed patchy distribution. This alga was not affected by the copper sulphate dosing and was seen to have chlorophyll when other algae were present only as empty filaments and cells.

Cladophora glomerata was not seen growing on the canal walls at either of the Oranje-Riet Canal System sites (1 & 2) during the monitoring period. It was however seen floating past in the water in September and October, 1995. A noticeable growth of *C. glomerata* filaments was seen on the sponges behind the sample tiles at Site 2 in these months. The lack of an appreciable growth of *C. glomerata* at Site 1 is probably due to the fact that when water levels were above the sample grid the speed and volume of water passing over the canal walls inhibited the attachment of *C. glomerata* filaments or spores. Site 1 was also subject to a constant change in water level. The general lack of noxious growths of *C. glomerata* at both sites (1 & 2) may also be related to the fact that this canal system is carefully monitored and cleaned on a regular basis.

C. glomerata grew on the canal walls in the Kalkfontein Canal System at sites 3 and 4 in March (autumn), 1995, and on the sponges behind the sample tiles in October and November (spring), 1995, on the canal walls in June and July (winter) 1996. All the sites in the Kalkfontein System had *C. glomerata* growing on the walls from January to March (mid-summer) 1997. It would appear from these observations that *C. glomerata* is able to establish itself at all times of the year, but is prevented from doing so by the dosing of copper sulphate on an *ad hoc* basis. The Kalkfontein Dam has a *C. glomerata* “turf” present throughout the year. It is therefore surmised that the canal system is “inoculated” with *C. glomerata* throughout the year, either *via* broken off filaments or by spores. *C. glomerata* collected in the Kalkfontein System was brought back to the laboratory on a regular basis and maintained in ordinary tap water with airstone aeration on the laboratory windowsill. This laboratory maintained culture was commonly seen to have broken off filaments which were attached to the glass of the container by means of rhizoids. These cultures remained healthy for some months in these conditions. . No *Cladophora glomerata* collected from the Kalkfontein System was seen to produce spores during the monitoring period, although it cannot be absolutely certain that spores were not in fact produced (as it was only monitored monthly). Importantly, it appears that *C. glomerata* can grow on the canal walls throughout the year in the Kalkfontein Canal System as is suggested by its presence in most months. Its growth is rapid, and it was observed to have reached lengths of approximately 5 m in two months. The control of its growth by the use of copper sulphate is effective in the short term, but long term control is problematic. No algae was seen at the Control site in June and July (mid-winter), 1997, but any suggestion that *C. glomerata* does not grow in the cold water of the winter months is negated by the observation that it was seen growing on the canal walls in June and July in 1996. These contradictory observations suggest that our request (of the Kalkfontein Irrigation Board), that no copper sulphate dosages be applied above the Control site during our monitoring period, was ignored.

Another effect of these continued dosages of copper sulphate over a period of many years is that invertebrates, which would possibly graze on the *C. glomerata* and reduce the massive growths, have been eradicated (Andrews *et al*.1986)

2.4 *In vitro* experiments

In vitro experiments enable researchers to compare reactions of experimental plants or animals in controlled physical and chemical conditions. These controlled conditions allow comparisons of plant and/or animal reaction within certain parameters. Experimental conditions can test maximum and minimum levels which are above or below those recorded in natural (*in situ*) situations. The results of *in vitro* experiments are thus often used as a method of prediction where natural conditions may change or as a method of comparison of reaction in same or similar species which occur in other climatic regions. This latter possibility of change in reaction according to climatic region could possibly be of importance in the control of the cosmopolitan nuisance algal species, *Cladophora glomerata*.

2.4.1. Introduction to experiments and rationale

A major section of research undertaken in this project was the *in vitro* study of growth at differing light and temperatures, and reaction to differing copper sulphate concentrations measured as both photosynthesis and growth. A preliminary experiment was also carried out on the effect of barley straw liquor as a biological control option. This set of experiments, using *C. glomerata* collected from the Kalkfontein Canal System, was conducted by a Masters student, Ms. R. le Roux, and the published results formed the basis of her Master of Science thesis in the Botany Department at the University of Cape Town (graduated December 1998). Further detail can thus be obtained in le Roux (1998).

2.4.1.1. Literature review - the relationship of *Cladophora glomerata* and heavy metals

Trace amounts of heavy metals are essential for algal metabolic processes and growth, however high concentrations have often been reported to have toxic effects on cells, mainly as a result of their ability to denature protein molecules (Gadd and Griffiths, 1978). The sensitivity of *Cladophora glomerata* to heavy metals has been exploited in the control of nuisance algal blooms in freshwater ecosystems since the beginning of the 20th century (Cedeno-Maldonado and Swader, 1972). Whitton (1970b) illustrated that of 37 species of Chlorophyta studied from flowing waters, *C. glomerata* was 36th in order of resistance, that is extremely sensitive, to Zn; 32nd to Cu^{2+} and 37th to Pb. In a laboratory investigation by McHardy and George (1990), toxic effects in *C. glomerata* cells, grown in a Chu #10 solution with a Zn concentration range from 0-4 mg l^{-1} and at a pH of 7.2 for one week, were first noted when the alga was exposed to 0.4 mg Zn l^{-1}. In these cells abnormalities occurred in the cytoplasm which were not evident in the cells of the control treatment. The *Cladophora* cells which were exposed to Zn concentration of 4.0 mg l^{-1} were observed to be completely dead. Thomas (1962 cited in Whitton, 1970a) reported slightly lower toxic levels of heavy metals in the regions of ± 0.1 mg Zn l^{-1} and ± 0.05 mg Cu l^{-1} for *Cladophora*. Leland and Carter (1984) indicated still lower Cu concentrations of 5 g l^{-1} which essentially inhibited the colonization of *Spirogyra* spp. and *Cladophora* spp. for a limited time period in the Convict Creek, California. Algal densities comparable to the control did not appear until 12 weeks after dosing.

Although many case studies explicitly demonstrate the sensitivity of *Cladophora* to heavy metals thus indicating an effective control measure, Eipper (1959) maintained that *Cladophora* is not exceptionally sensitive to heavy metals, since adequate control was not effectively attained at a concentration of 1 mg Cu l^{-1}. Observations by Bellis (1968) similarly showed good growth of *C. glomerata* despite it being exposed to a culture medium containing 2 mg Zn l^{-1}. In the study by Betzer and Kott (1969) filaments of a *Cladophora* sp. collected from the Saline channel (Israel) and cultured in various concentrations of Cu^{2+} were only adversely affected at concentrations as high as 2-4 mg Cu l^{-1} when exposed to the Cu for 4 days. These findings could be an indication that some *C. glomerata* strains have developed tolerance mechanisms against heavy metals as demonstrated by Hillebrand and De Vries (1986). In the latter study it was demonstrated that different strains of *C. glomerata* varied in their tolerance level to copper. Within a copper concentration range of 0.002-0.58 mg l^{-1} the three strains reacted differently, with one being extremely sensitive whilst one was very tolerant. Furthermore, it was detected that strains collected in the same place exhibited different tolerance levels. The growth rate of the most tolerant strain was decreased by 10% from 0.002 to 0.58 mg Cu l^{-1}, whereas the growth rate declined by 50% in the sensitive strain of *C. glomerata* when exposed to the same Cu concentration range. The study clearly indicated that tolerant and sensitive strains of *C. glomerata* exist in nature. Whitton (1970a), however, observed growth sensitivity of 20 different populations of *C. glomerata* exposed to Zn, Cu and Pb with no significant detectable differences between any of them,despite the fact that one of the algal populations was extracted from a Cu polluted stream.

Although algae may have evolved to mitigate the effects of heavy metals, many researchers have ascertained that environmental conditions have an underlying influence on the biological toxicity of heavy metals. Gadd and Griffiths (1978) have maintained that there are three basic abiotic factors which need to be assessed before the biological toxicity of the metal can be established.

These factors include firstly, the prospect of environmental constituents binding the heavy metal ions. Stokes (1983) maintained that the biological toxicity of heavy metals is attributed mainly to the concentration of free ionic metals rather than to the total copper concentration. In aquatic habitats, heavy metals can be bound and removed from the water by organic sediments, which effectively reduce the total metal ion concentration in solution and subsequently biological toxicity. In the study by Walker and Colwell (1974), bacteria, mainly *Pseudomonas* spp., collected from Colgate Creek in Baltimore Harbour, were capable of degrading petroleum oil despite it being enriched with a high Hg content. The high Hg- resistance of *Pseudomonas* had been partially credited to the binding capacity of mercury with specific organic complexes in the oil thereby reducing the free ionic Hg concentration. Likewise other organic compounds for example citrate, cysteine, glutamate and EDTA (Ethylene-diamine-tetraacetate) can have profound effects on biological responses when included in growth media (Gadd and Griffiths, 1978). These compounds act as chelators, that is, the complex formed by a ligand (a Lewis base attached to a metal atom in a complex) that can bond with two or more atoms to a metal atom (Ebbing and Wrighton, 1990). Many laboratory studies have demonstrated the amelioratory effect of chelators on copper toxicity. MacLeod et al. (1967) showed that metabolic injury to the bacterial cells of *Aerobacter aerogens* was evident when exposed to a diluent of distilled water and $Cu^{2+.}$ However, the toxic effects of Cu^{2+} on *Aerobacter aerogens* were reduced or prevented by the addition of yeast extract or cysteine to the diluent. Similarly, in a study by Zimmerman (1966) toxic effects of copper to the bacteria, *Serratia marcescens*, were relieved by the addition of copper-chelating agents which sequestered the free Cu^{2+} ions. In the study by Stokes and Hutchinson (1975) a relationship between the toxicity of copper to laboratory cultures of *Scenedesmus* (a green alga) and the complexation of copper by the synthetic chelator, EDTA was revealed. The toxic effect of copper was masked in the presence of EDTA up to a point where the copper concentration exceeded the binding capacity of the EDTA. At this point, copper uptake into algal cells increased dramatically and cell division was inhibited. McKnight et al. (1983) therefore proposed that when investigating the minimum toxic concentration of a metal, the optimal strategy would be to first add enough copper to titrate the ligand. The concentration of copper required would vary with ligands since synthetic chelators such as EDTA and triethanolamine appear to bind copper more strongly than natural ligands such as humic and fulvic acids. Accordingly the cupric ion activity for synthetic ligands will be lower than natural ligands for similar copper and ligand concentrations.

The capacity for ligands to bind Cu^{2+} ions could explain the results obtained by numerous laboratory researchers e.g. Eipper (1959), Bellis (1968) and Kott and Betzer (1969) who found good *C. glomerata* growth despite relatively high heavy metal concentrations. In comparison, in media without complexing agents, toxicity may be pronounced. Steeman Nielsen and Wium-Andersen (1970) revealed that a copper concentration as low as $5\mu g\ l^{-1}$ was toxic to cells of the unicellular green alga, *Chlorella pyrenoidosa*, when EDTA and citric acid was omitted from the growth medium.

Despite the substantial body of evidence supporting the proposal that free cupric ions are the main determinants of biological toxicity, there are also indications that biological activity is not directly related to the concentration of ionic Cu. Florence et al. (1983) indicated that total growth inhibition of the marine diatom, *Nitzschia closterium* Ehr., was obtained by the addition of lipid-soluble Cu complexes with a 5×10^{-8} M 8-quinolinol synthetic ligand. They attributed the toxicity of the lipid-soluble Cu complexes to their ability to catalyse the intercellular formation of highly destructive hydroxyl free radicals obtained from molecular O_2 . Tubbing et al. (1993) demonstrated that minor additions of total Cu in the range of 0.05-0.16 M already affected functional and structural aspects of bacterial and phytoplankton communities in spite of rather high concentrations of EDTA, suspended matter and dissolved organic carbon. Likewise Tubbing et al. (1994) showed that chelex-bound Cu induced biological toxicity in the diatom *Selenastrum capricornutum* (Reinsch.) which was cultured in Cu and EDTA enriched synthetic medium or River Rhine water. These studies indicate that complexed Cu contributes to Cu toxicity which may have significant consequences, since in freshwater systems the percentage of organically bound Cu may be very high and the fraction of free Cu is usually less than 10% (Tubbing et al., 1994).

Secondly, the toxicity of heavy metal ions to algae can be remarkably masked by the presence of other ions. Anions such as PO_4^{2-}, SO_4^{2-} and CO_3^{2-} and cations such as Mg^{2+}, Ca^{2+} and Fe^{2+} or Fe^{3+} are able to reduce heavy metal toxicity by precipitation, depending on their concentrations and the pH of the solution (Gadd and Griffiths, 1978). In the study by Abelson and Aldous (1950) the toxic effects of Ni, Co, Cd, Zn and Mn to many bacterial species including *Escherichia coli*, were reduced in media with a high Mg^{2+} content compared to media containing the same metal concentrations but a low Mg^{2+} content. Steeman-Nielsen et al.(1969) demonstrated that the presence of high Fe in a medium had a detoxifying effect on copper to the alga *Chlorella pyrenoidosa*. This has been attributed to the prospect that the Cu ions were adsorbed to the negatively charged sides of ferric hydroxide. Similarly, Cu toxicity to *Nitzchia closterium* was reduced by adsorption of Cu^{2+} on Mn^{3+} and Fe^{2+} hydroxide coatings around the cell, with Mn^{3+} being more effective than Fe^{2+}, possibly due to its ability to scavenge the toxic superoxide free-radical (Stauber and Florence, 1985). The masking effect of the Ca^{2+} ion was illustrated by Rai and Raizada (1985), who found that the uptake of Cu^{2+} and Pb^{3+} by *Nostoc muscorum* (cyanobacterium) was reduced by 10^{-3}M Ca^{2+} , with Ca^{2+} diminishing the effects of the heavy metals studied. A more recent investigation by Issa et al. (1995) likewise showed that when the green alga, *Kirchneriella lunaris* (Kirchner) Mobius, was incubated in the presence and absence of Ca, the growth rate was inhibited by all heavy metals applied including Cd^{2+} , Co^{2+}, Mn^{2+} and Ni^{2+}. However, the effect of heavy metal inhibition on the growth rate was more pronounced in cultures containing heavy metals alone with higher growth rates observed in Ca-containing cultures.

Thirdly, the availability of heavy metals to biological organisms is considerably affected by the pH in an aquatic environment (Gadd and Griffiths, 1978) assuming that free ionic metals ions are the main determinants of biological toxicity. In the model by Lin and Benjamin (1992) the relationship between solution pH and the concentration of metal ion particles has been conceptualized. The fate of metal ions, e.g. Cu^{2+} is governed by adsorption/desorption processes which occur at the interface between the aqueous solution and cationic oxides and hydroxides. The O_2 donor group (either OH or O^-) on the surface can react with cations in solution as expressed by the equation:

$$SOH + M^{z} \quad SOM^{(z-1)} + H^{+}$$

where SOH donates a singly protonated surface oxide site, and M represents a cationic adsorbate.

The pH of a solution is determined by the -[logarithm of the H^+ concentration], therefore by increasing the concentration of H^+ the solution pH will decrease (Ebbing and Wrighton, 1990). In the above model, when an increase in the solution pH (low H^+ concentration) occurs the forward reaction commences which results in an increase in the sorption of the cationic metals, e.g. Cu^{2+}. Eventually, a point will be reached with time when the concentration of H+ increases sufficiently to initiate the reverse reaction   The reverse reaction would accordingly be favoured with decreasing solution pH resulting in an increase in free cationic metals. Lin and Benjamin (1992), acknowledge that the presence of complexing ligands may alter the adsorption characteristics of metals substantially. Organic complexing ligands are often strong adsorbates themselves, which complicates the interpretation of results when they are added to systems containing metals and oxide sorbents.

Winner (1986) investigated the interactive effects of water hardness on the chronic toxicity of Cd to *Daphnia pulex* (a freshwater animal). Water hardness is indirectly related to pH (Ca^{2+} or Mg^{2+} concentrations affect the H^+ concentration in the solution through compound complexation (Ebbing and Wrighton, 1990), with an increase in water hardness resulting in an increase in pH and vice versa. The survivorship of the daphnids, exposed to 5 ug Cd l^{-1} for 20 days, increased with an increase in water hardness from 58-115 mg l^{-1}. Very few studies (Macfie, 1994) have focused on the relationship between pH and Cu^{2+} availability and its effects on algae with no investigations found pertaining to *C. glomerata*. However in general, McKnight et al. (1983), suggested that for Cu-sensitive algae, low dosages of $CuSO_4$ (0.01ppm) should be effective in controlling algal growth regardless of water pH and organic complexing content. In the case of a copper tolerant alga, excluding alkaline water, control should be possible with dosages of ± 1 ppm. In alkaline water, precipitation of $CuCO_3$ and hydroxides prevents cupric ion activities (that is, toxicity) being sufficiently high to limit the growth of a Cu-tolerant nuisance alga. The effective dosage of an alga with intermediate Cu sensitivity exhibits the most dependence on water chemistry. In acidic waters, low dosages of Cu (0.10 ppm) should be effective. In water with a relatively high organic content, enough Cu must be added to titrate the humic complexing agents (± 0.10 ppm Cu) before a toxic effect will be obtained. Likewise dosages greater than 0.10 ppm are required in alkaline waters because of Cu precipitation.

2.4.2 Materials and methods

2.4.2.1 Collection and stock cultures

C. glomerata was collected from the Kalkfontein irrigation canal scheme (Free State) in December 1996, January and February 1997. *C. glomerata* tufts squeezed of excess water were transported in plastic bags to the Botany Department (University of Cape Town) within 24 hours. Stock culture materials cleaned of macroscopic debris were placed into 1litre glass jars containing aerated tap water at 20° C at a light intensity of 60µ mol photons m^{-2} s^{-1} supplied by cool white fluorescent tubes (Osram L30W/20) set on a 16:8 light-dark cycle. Stock cultures were maintained for at least 2 weeks prior to experimental procedures but never exceeding laboratory age of 1 month.

Cladophora glomerata proved to be an extremely difficult organism to cultivate, and this has been shown by a number of previous authors in the literature. It proved impossible to grow the alga successfully in a defined medium (that is one which is based on distilled water with additions, so that the composition is completely known). After many months of attempts to find a suitable medium (detailed in le Roux 1998), reasonable growth was obtained in a medium based on Cape Town tap water, with nutrient additions.

For all the experiments, unless otherwise stated, the basic culture medium used was aged tap water (2 weeks) supplemented with 14 mg N l^{-1} and 2 mg P l^{-1} added as $NaNO_3$ and K_2PO_4 respectively, which will be referred to as the **enriched tap water medium** in subsequent text. Culture media were pre-heated for 2 hours at 90° C in a Memmert stainless steel water bath and cooled to room temperature overnight before each experiment was conducted. All solution pH levels were maintained by adding 1ml of a 2 M Tris (hydroxymethyl)-aminomethane buffer per litre of the enriched tap water medium. The buffer solution was manipulated to yield required pH levels by adding drops of either dilute HCL acid or a 1 M NaOH solution. All solution pH measurements were processed using a calibrated Beckman 40 pH meter. The source of Cu, excluding the negligible natural supply of Cu in the tap water, was derived from a 0.03 mM $CuSO_4$ stock solution. Aliquots were added to the enriched tap water medium prior to each experimental run to yield the final required Cu concentrations in mg l^{-1}. All glassware utilised was soaked in detergent, acid washed with dilute hydrochloric acid, rinsed three times with distilled water and oven dried at 60° C.

2.4.3. Growth at differing light and temperature regimes

2.4.3.1 Introduction

Conflicting reports have been published regarding the light requirements of *C. glomerata* (Neel, 1968; Whitton, 1970; Bolus & Lund, 1974; SandJensen, 1989; Dodds & Gudder, 1992). Similarly conflicting reports have been published regarding the temperature requirements for growth of *C. glomerata* (Waern, 1952; Chudbya, 1965; Bellis & McLarty, 1967; Graham, 1982). The growth *in vitro* of *C. glomerata* collected from the canal system at Kalkfontein was studied in order to ascertain whether its growth was similar to *C. glomerata* from other parts of the world as reported by various researchers. The experiments are summarised here, but are presented in more detail in le Roux (1998).

2.4.3.2 Methods

2.4.3.2.1 Experimental setup

All the growth experiments were conducted in a growth chamber with an air temperature maintained at 10° C. An overhead light bank fitted with three fluorescent tubes (GEC Alsthom 65W) generating cool white light was used as the sole source of irradiance. Light intensity was measured in μ mol m^{-2} s^{-1} as photosynthetically active radiation (PAR) using a Skye SKP 200 light meter. Light intensities of 0, 25, 45, 75, 100 and 150 μ mol m^{-2} s^{-1} were obtained by manipulating the distance of the flasks away from the light source as well as shielding the experimental flasks with nylon shade cloth.

Temperatures examined were 10° C, 15° C, 20° C, 25° C, 30° C and 35° C. Water temperature was manipulated by placing the flasks into perspex water baths (47cm x 25cm x 13 cm) which were fitted with Tempette Junior TE-8J (Techne) thermostats. The experimental flasks rested on white material sheets which were elevated to approximately ½ the height within the water baths. The water levels in the water baths were always maintained at the 100-ml mark on the flasks to ensure that the algal filaments were always exposed to the selected experimental temperature. The required temperature for each experiment was monitored for at least three days prior to the experimental run in order to eliminate any fluctuations. Photoperiods namely a long-day, 16:8 light-dark cycle and a short-day, 8:16 light-dark cycle were simulated by connecting an automatic time switch to the light source. The entire set-up was surrounded by two layers of shade cloth and black plastic bags to prevent any outside light from penetrating the systems.

2.4.3.2.2 Experimental procedures

C. glomerata segments of 10 mm each were cut under water in a glass culture dish lid, under which a grid block constituting of squares with dimensions of 1 mm by 1 mm each was taped, using a Kyowa Optical Stereomicroscope Model SD-2PL. Segments were cut from unbranched filaments in order to obtain uniform filament lengths and morphology. Since the apices were normally branched the segments were cut from the intercalary regions. The dissected segments were placed into 100-ml Erlenmeyer flasks, with each one containing 50ml of aged tap water supplemented with 14 mg N l^{-1} and 2 mg P l^{-1} added as $NaNO_3$ and K_2HPO_4 respectively. These N and P concentrations which were added far exceeded the minimum limiting values for *C. glomerata* growth as suggested by Pitcairn and Hawkes (1973). For all experiments, the tap water was collected in May 1996 and stored in 20-L plastic containers maintained at 20°C in the dark. By allowing the tap water to stand for at least two weeks before utilisation, any chlorine gas dissipates. The N and P enriched tap water medium was sterilised by steaming in a Memmert stainless steel water bath for 2 hours at 90°C and cooled to room temperature overnight. The pH, measured with a Crison pH/mV meter (Model 506), ranged between 8-8.5 for all the experiments.

Twelve experiments were conducted over a 5 day period each, operated at one of the 6 specified temperatures at either a long-day or short-day photoperiod at all the 6 manipulated light intensities. Individual experiments comprised of 4 replicate flasks, containing twenty *C. glomerata* filaments each, exposed to the 6 light intensities thereby yielding a total of 24 experimental flasks. No aeration was supplied since bubbling itself may have affected *C. glomerata* growth and it was virtually impossible to obtain a constant rate in all the flasks. However the mouths of the flasks were loosely covered with polythene, to allow spontaneous diffusion of ambient air into the water medium. At the termination of the experiment the algal filament lengths were remeasured by applying the same grid-technique used as when dissected.

2.4.3.2.3 Computation and statistical analyses

The raw data measurements of the filament lengths were transformed to specific growth rates using the formula

$$\mathbf{r = \ln (L_2\text{-}L_2)/(t_1\text{-}t_2)}$$

where r = growth rate, L_1 and L_2 represent the average filament lengths of the 20 segments in each test tube, at t_1 = day 1 and t_2 = day z, respectively (Hillebrand and De Vries, 1986)

Growth rate data were fitted to a Hyberbolic Tangent Function after Jassby and Platt (1976), which models the Photosynthetic-Irradiance curve. The original formula:

$$\mathbf{P = P_{max} \tanh (I/I_k)}$$

was manipulated and photosynthetic parameters represented by P were replaced by growth parameters, G, yielding the formula:

$$\mathbf{G = G_{max} \tanh (I/I_k)}$$

where G = estimated Growth rate, G_{max} = light saturated Growth rate, tanh = hyberbolic tangent function, I = measured Irradiance (light intensity) and I_k = half-saturation light point.

A user-specified regression was computed by applying a modified version of the equation above.

$$G = (Gmax *(tanh**(I/Ik)-(1/tanh) **(I/Ik))/ (tanh) **(I/Ik) + (1/tanh) **(I/Ik)))$$

where tanh = 2.718281828

The Rosenbrock and Quasi-Newton estimation method was employed and the regression was fitted with a 2-D function and observed values. Light saturation points at Gmax were calculated by multiplying the half-saturation light point (I_k) by 2.

All statistical analyses were executed on a software computer package, Statistica v 5.0 using STA-NLV (Non-linear estimations) or Anova/Manova bases. Computed r-values of the non-linear regression lines were generated by the STA-NLV package. Statistical significance were obtained by applying either an Analysis of Variance (F-ratios) or Kruskall-Wallis test (H). Tests for normality were performed using parametric probability plots and homoscedacity was determined by the Cochran's (Chi2) or Bartlett's tests (B). Statistical significances were denoted where α was < 0.05.

2.4.3.3 Results

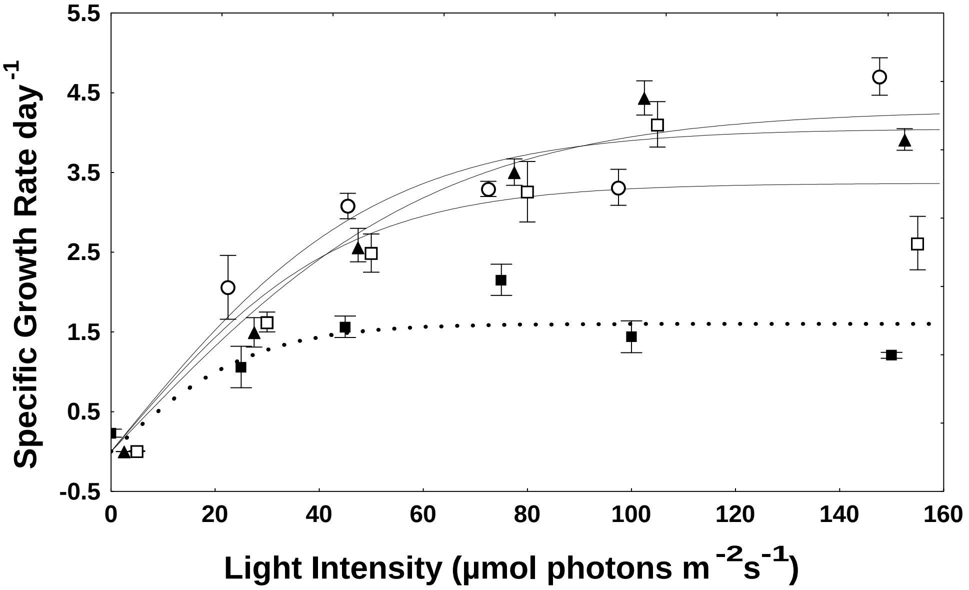
Algal growth rates were primarily affected by temperature (Fig. 2.24a, b). At 10° C, although the algal filaments remained a healthy green colour, no growth occurred irrespective of light intensity and photoperiod. At 35° C, the maximum temperature investigated, all the algal filaments turned white and died within 5 days. Growth rates were similar for temperatures between 20° C and 30° C at most light intensities, and at both photoperiods (Fig. 2.24a, b), however they were significantly reduced at 15° C, long-day (Fig. 2.24a). Growth rates at 15° C, long-day photoperiod, were decreased by approximately 40% at light intensities of 25, 45 and 75 μ mol m^{-2} s^{-1} and by 65% at the two higher irradiance levels of 100 and 150 μ mol m^{-2} s^{-1} compared to the growth rates at the optimal temperature. At the short-day photoperiod, growth rates at 15° C were similar to the growth rates at 20° C, 25° C and 30° C at light intensities from 0-75 μ mol m^{-2} s^{-1} but at higher light intensities growth rates were reduced by ca. 30-35%.

Estimated maximum growth rates (G_{max}) at light saturation point were extrapolated from the non-linear regression curves. Gmax increased with an increase in temperature from 10° C (no growth observed) to 25° C where the highest values, 4.29 m day^{-1} (long-day) and 1.36 m day^{-1} (short-day) were recorded and reduced by 20% and 15% at 30° C at long-day and short-day photoperiods respectively. At the long-day photoperiod, G_{max} was significantly lower at 15° C, being 40% of G_{max} at 25° C. At a short-day photoperiod, Gmax at 15° C, was significantly reduced to 68% of G_{max} at 25° C.

In general, maximum growth rates (G_{max}) of the algal filaments exposed to long-day and short-day photoperiods exhibited a tendency to shift their light saturation points with temperature. At a long-day photoperiod, light saturation points increased proportionately from 55 μ mol m^{-2} s^{-1} at 15° C to 125μ mol m^{-2} s^{-1} at 25° C where the highest estimation was predicted, with a decline to 88 μ mol m^{-2} s^{-1} at 30° C . At the short-day photoperiod, the light saturation points increased from 55 μ mol m^{-2} s^{-1} at 15° C to 147 μ mol m^{-2} s^{-1} at 25° C with a reduction to 112μ mol m^{-2} s^{-1} at 30° C. The light saturation point of 36 μ mol m^{-2} s^{-1} at 20° C (short-day) however was much lower than at all the other temperatures. Light saturation points were similar at both photoperiods at 15° C, however the light saturation point at 20° C, short-day, was 64% lower compared to the long-day light saturation point at 20° C. At 25° C and 30°C, the light saturation points at the long-day photoperiods were both ca. 20 μ mol m^{-2} s^{-1} less than the light saturation points at the short-day light cycle.

Photoperiod also exhibited a significant effect on the growth rates of *C. glomerata*. The algal growth rates were noticeably higher at a long-day photoperiod (16:8 light-dark cycle) compared to the short-day photoperiod (8:16 light-dark cycle) at all the temperatures and light intensities which sustained growth (Fig. 2.25). G_{max} estimates were higher at a long-day at temperatures of 15° C, 20°C, 25° C and 30° C, compared to the short-day light cycle. The magnitude of the ratios was greatest at 20° C and 30° C, with G_{max} being *ca*. 3 times more at the long-day relative to G_{max} at the short-day photoperiod.

a. Long day photoperiod



b. Short day photoperiod

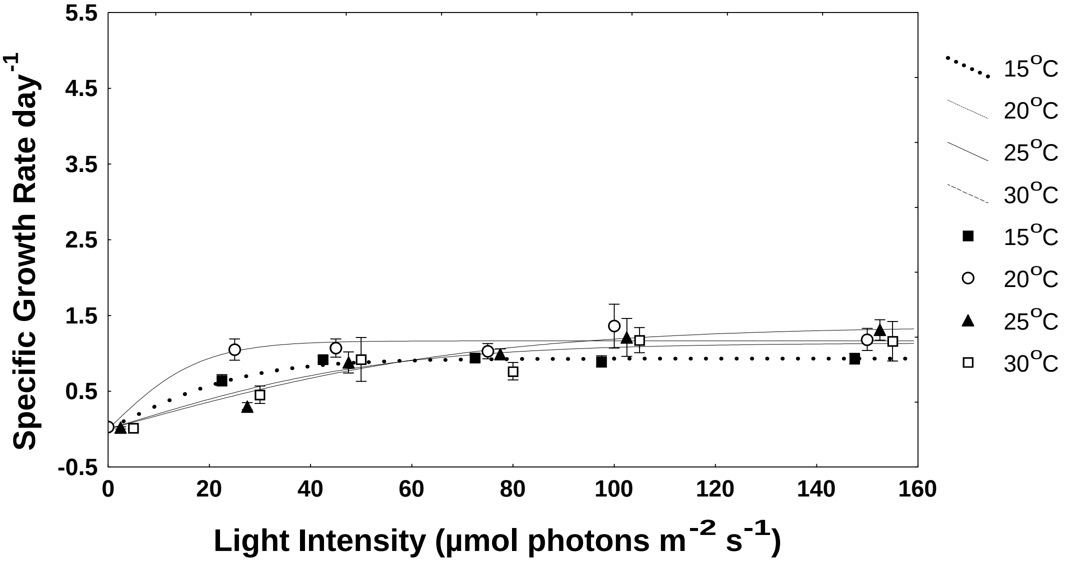


Fig. 2.24. The effects of light intensity and temperature on the specific growth rate of *C. glomerata* at two photoperiods a. Long-day (16:8 light dark cycle) and b. Short-day (8:16 light-dark cycle). Means (n=4) are plotted with ± S.E. (vertical lines) and fitted with non-linear regression lines (line graphs)

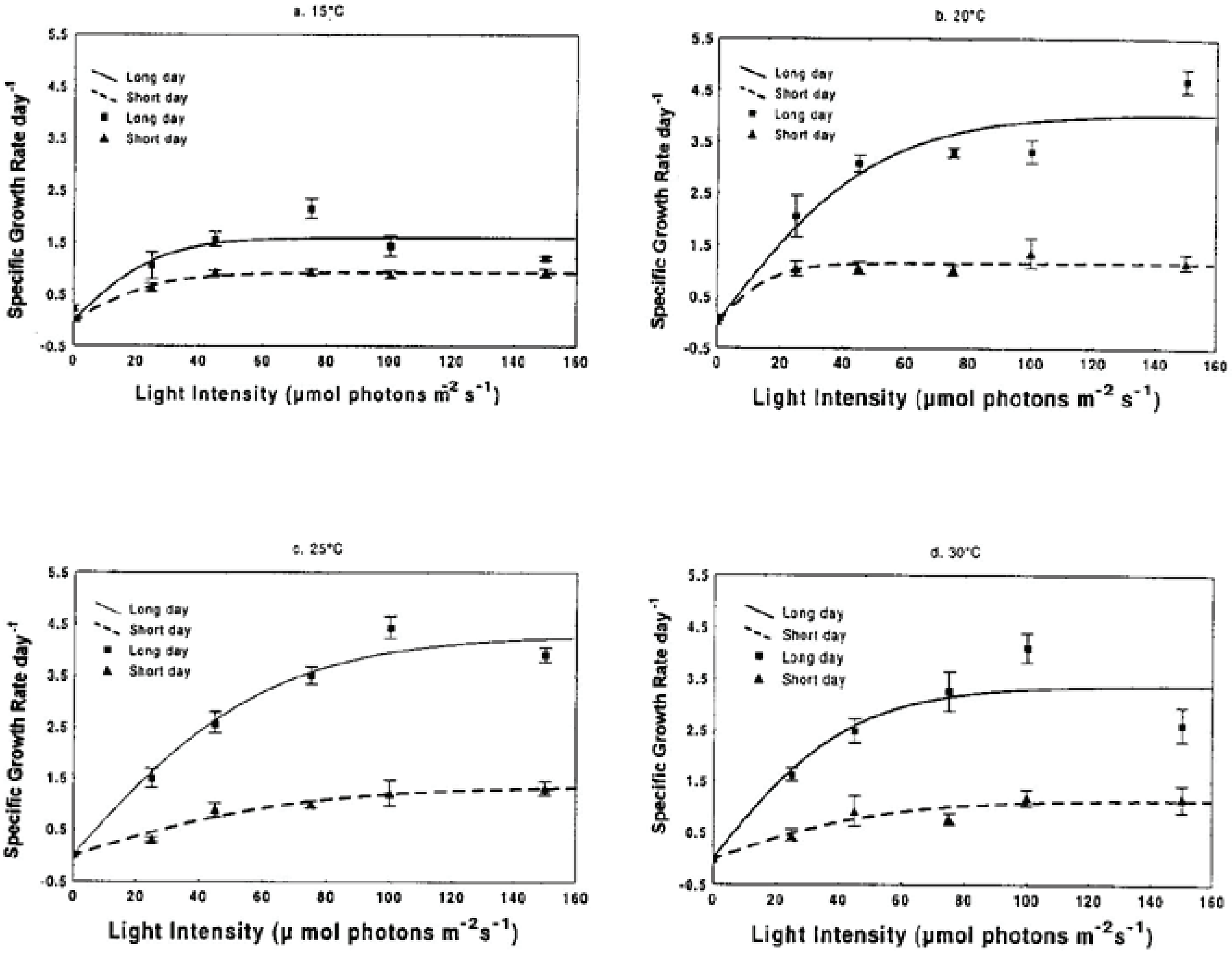


Figure 2.25. The effects of light intensity and photoperiod on the specific growth rates of *C. glomerata* at different temperatures. a. 15°C, b. 20°C, c. 25°C, d. 30°C. Symbols represent the data means (n=4) plotted with ± S.E. (vertical lines). The line graphs are the non-linear regression curves.

2.4.3.4 Discussion

Wong *et al*. (1978) state that temperature plays an important role in *C. glomerata* dominance especially when nutrients are readily available. In this research study, at both the long- and short-day photoperiods (16:8 or 8:16 light/dark cycles) the maximum growth rate of *C. glomerata* at light saturation point was optimal between 20° C and 25° C, slightly suppressed at 15° C and 30° C, with 35° C being lethal and 10° C allowing survival, but not growth (Figs. 2.24a, b). Similar temperature growth effects were reported by Whitton (1967) who collected *C. glomerata* from the River Wear in England, Bellis (1968 b) who cultured *C. glomerata* derived from vegetative plants growing attached to stones in Medway Creek, Lake Ontario, Hoffman and Graham (1984) who harvested *C. glomerata* from Lakes Mendota, Madison and Wisconsin (United States of America) and Robinson and Hawkes (1986) who worked on unialgal cultures of *C. glomerata*. Optimum growth for *C. glomerata* was reported to be between 20° C and 25° C whilst temperatures below 10° C and above 30°C were limiting boundaries of detectable growth.

Slightly lower optimum and maximum limiting temperatures for growth were described by Storr and Sweeney (1971) who cultured *C. glomerata* at a temperature range of 14.5° C to 26° C at a light intensity of 800 foot candles for 30 days, collected from the Oswego region of Lake Ontario and Wong *et al*. (1978) when correlating the effects of water temperature and seasonal pattern of *C. glomerata* in seven river systems in southwestern Ontario. In both studies optimum temperature for growth was reported to be 18° C although Wong *et al*. (1978) noticed a lower upper temperature tolerance of 23.5° C compared to Storr and Sweeney (1971) who observed reduced growth at 26° C.

Likewise, different temperature responses of *C. glomerata* photosynthesis have been reported. In a study by Graham *et al*. (1982) photosynthesis measured as O_2 evolution was optimum between 13° C and 17°C for *C. glomerata* which was collected during spring time from Lake Huron, Harbor Beach, Michigan. *Cladophora glomerata* collected from the upper layers of the water in Leeds and Liverpool canals, Merseyside, and subsequently cultured in the laboratory displayed optimum photosynthesis at a slightly higher temperature of ca. 20° C (Simpson and Eaton, 1986). Adams and Stone (1973) reported yet a higher water temperature range, between 19° C and 24° C, for optimal net photosynthetic activity of *C. glomerata* collected from Green Bay, Lake Michigan. More recent laboratory studies by Lester *et al*. (1988) using *C. glomerata* plant materials collected from the same region (Green Bay, Lake Michigan) indicated still higher optimal temperatures for net photosynthesis, from 28° C to 31° C, which varied with season.

These diverging findings of *C. glomerata's* response to temperature by various researchers could be attributed to many factors. Inconsistent culture conditions for example, algal age, photoperiod, light intensity and nutrient medium used may inherently affect the alga's growth response to temperature. Moreover, the different temperature regimes in the habitats of *C. glomerata* reported may likely be a factor in selecting for ecotypic, sub-specific variation.

The influence of light intensity in controlling *C. glomerata's s*easonal dominance has received secondary importance compared to temperature (Graham *et al*., 1982). Maximum growth of *Cladophora glomerata*, collected from the Kalkfontein irrigation canals in this study, occurred with a light saturation point of 125μ mol m^{-2} s^{-1} at a temperature of 25° C with a long day length of 16 hours. Similarly Hoffman and Graham (1984) reported that isolates of *C. glomerata* from Lakes Mendota, Madison and Wisconsin yielded maximum dry weight production at 125 μ mol m^{-2} s^{-1} at 25° C. However higher light intensities required to support optimum growth at water temperatures of 15° and 20° C compared to this study were reported by other researchers. At 15° C, irrespective of day length, *C. glomerata* growth in this study was saturated at a low light intensity of 55μ mol m^{-2} s^{-1} (Table 2.4.1) compared to *C. glomerata* collected from streams in the Spessart Mountains, Germany, where growth increased up too 100 μ mol m^{-2} s^{-1} with no saturation evident at 15° C (Leukart and Hanelt, 1995). Lorenz *et al*. (1991) reported that *C. glomerata* collected from Lake Erie and subsequently cultured at 15° C at a 14 hour day length, demonstrated yet a higher optimum light intensity of 168 μ mol m^{-2} s^{-1} for growth. Compared to the current study where maximum growth for *C. glomerata* at 20° C at a 16 hour day length was at 100 μ mol m^{-2} s^{-1} , Robinson and Hawkes (1986) and Whitton (1967) both described somewhat higher maximum light intensities, of 6000 lx (ca. 120 μ mol m^{-2} s^{-1}) and 150μ mol m^{-2} s^{-1} respectively, for *C. glomerata* growth when exposed to continuous illumination at 20° C.

Minimum light levels which restrict *C. glomerata* growth were not temperature dependent in this study. Growth rates were only significantly impeded in total darkness, 0 μ mol m^{-2} s^{-1} with 25μ mol m^{-2} s^{-1} , the next incremental light level, supporting some growth from 15° C to 30°C at both photoperiods examined (Fig. 2.24a, b). Higher minimum light requirements for *C.*

glomerata growth in Lake Erie were observed by Lorenz and Herdendorf (1982). The maximum depth of *C. glomerata* colonization in their study coincided with light intensities above 50 μ mol m^{-2} s^{-1} which indicated that values of less than 50 μ mol m^{-2} s^{-1} were limiting to growth at water temperatures of 15° C. Indeed it is predicted from the present results from the Jassby and Platt (1976) equation that maximum growth rates at 15° C for both short- and long-day photoperiods occurred at light saturation points around 55 μ mol m^{-2} s^{-1} . Although in the studies by Graham *et al*. (1982) and Lorenz *et al*. (1991) slightly lower minimum light levels of less than 30 μ mol m^{-2} s^{-1} were reported to be limiting to growth at 15° C.

The different maximum and minimum light levels reported for *C. glomerata* growth and photosynthesis in various studies at similar temperature ranges could be attributed to many factors. Firstly, different environmental conditions under which stock cultures were maintained could affect light requirements especially when algal growth is not monitored over long time periods. Algal stock cultures in this study were maintained at a light intensity of 60μ mol m^{-2} s^{-1} set on a 16 hour day length. The optimum light intensity for growth at 15°C however was much lower in this study, 55 μ mol m^{-2} s^{-1}, compared to the optimum light intensity for growth, of 100μ mol m^{-2} s^{-1} , reported by Leukart and Hanelt (1995) despite that their stock cultures were maintained under a much lower light intensity of 10-20 μ mol m^{-2} s^{-1}.

The second factor which may likely influence optimum light requirements for growth or photosynthesis is photoperiod. In this study the light saturation points at maximum growth were higher at a short day length (8 hours) compared to the long-day length (16 hours) at all temperatures except at 20° C. Likewise Hoffman and Grahams' (1984) study showed that dry weight production at a 8:16 light-dark cycle was greatest at 250 μ mol m^{-2} s^{-1} whereas under long-days (16:8 light-dark cycle) greatest production occurred at 125μ mol m^{-2} s^{-1} . However, the optimum light intensity for *C. glomerata* growth of 150μ mol m^{-2} s^{-1} reported by Whitton (1967) was somewhat higher than the the optimum light intensity reported by Robinson and Hawkes (1986), of 120 μ mol m^{-2} s^{-1} , despite consistent photoperiod conditions of continuous irradiance at 20° C.

Thirdly, the higher light requirements reported by other researchers compared to this study may be an artefact of whether algal clumps were used rather than algal filaments which were dissected in this study. The use of algal clumps may lead to self-shading thereby shifting the light requirements to higher levels. However in the study by Leukart and Hanelt (1995) where algal filament tips of *C. glomerata* were cultured at 15° C, a higher optimum light intensity for growth (100μ mol m^{-2} s^{-1}) was measured compared to this study (55 μ mol m^{-2} s^{-1}) at 15° C. The last factor which is likely to explain the different light requirements for *C. glomerata* growth despite similar culture conditions is ecotypic, sub-specific variation.

The optimum irradiance level where maximum growth occurred, expressed by the light saturation points in this research study, shifted with changes in temperature. At sub-optimal temperatures for *C. glomerata* growth, illustrated to be 15° C, 20° C and 30° C, at a 16:8 hour light-dark cycle the light saturation points were lower than at the optimum temperature of 25° C. The short-day photoperiod (8:16 light-dark cycle) showed a similar pattern except at 20° C where the light saturation point was drastically reduced to 36μ mol m^{-2} s^{-1} . The low light saturation point of 36μ mol m^{-2} s^{-1} predicted could be a function of the possible onset of reproductive activity. In a study by Hoffman and Graham (1984) zoosporogenesis in *C. glomerata* was significantly induced by a short-day photoperiod of 8 hours at a temperature range between 15° C and 20° C with light intensity of less than 60μ mol m^{-2} s^{-1} playing a less important role. In the current study, reproductive activity by means of either zoospores, zoosporangia or sporelings was not observed. Furthermore, it was difficult to ascertain whether empty cells were a function of zoospore release or merely injury from cutting. The dubious low light saturation point at 20° C at a short-day photoperiod may however be a statistical artefact. Since growth rates were similarly low at all light intensities (Fig. 2.25b) examined, a shift in one data point may generate a slightly different non-linear regression curve which would completely change the computed light saturation point.

Shifts in the light saturation points for *C. glomerata* photosynthesis were likewise recorded as water temperatures changed from sub-optimum to optimum. Leukart and Hanelt (1995) reported that photosynthesis of laboratory-grown *C. glomerata* at 15° C at various light intensities set on a light cycle of 14 hours was saturated at 60-67μ mol m^{-2} s^{-1}. A higher photosynthetic saturation point of 380 μ mol m^{-2} s^{-1} for *C. glomerata* collected from the Leeds-Liverpool canal system, Merseyside, was recorded when optimum water temperatures for photosynthesis was 20°C (Simpson and Eaton, 1986). The study by Lester *et al.* (1988) reported that optimum net photosynthesis in *C. glomerata* collected from Green Bay, Lake Michigan, occurred at water temperatures between 28° C and 30° C which coincided with a much higher light saturation range from 345-1125μ mol m^{-2} s^{-1}. However *C. glomerata* collected from Lake Huron, Harbor Beach Michigan, was shown by Graham *et al.* (1982) to photosynthesize optimally between light intensities as high as 300-600μ mol m^{-2} s^{-1} despite lower water temperatures, ranging between 13° C and 17° C.

The general shift in light saturation points with temperature can be construed as a function of resource allocation between photosynthesis and downstream reactions (Raven and Geider, 1988). At sub-optimal low or high temperatures compared to at optimal temperatures, temperature-sensitive processes, for example, respiration would require a greater allocation of resources in order to maintain the same level of cellular activity. This means that the allocation of resources to pigments and polypeptides related to light-harvesting systems is reduced at sub-optimal temperatures which indirectly results in lower light saturation points exhibited by the alga at maximum growth rates. Conversely, Leukart and Hanelt (1995) maintain that at optimum temperatures for growth or photosynthesis the content and activity of Rubisco (the first carboxylating enzyme in the C3 photosynthetic pathway) are high thereby increasing the photosynthetic capacity. Thus an increase in the density of the photosynthetic units would shift the saturation point to higher irradiance levels.

Kelly *et al*. (1983) assert that photosynthetic efficiency is a function of light availability (that is, duration for which the light is available) and algae often respond to changes in light conditions by adjusting the concentrations of photosynthetic pigments, nucleic acids and other cellular components which ultimately affect specific growth rates (Schlesinger and Shuter, 1981). At short day lengths the alga will have higher concentrations of photosynthetic pigments in order to optimise the use of available light compared to long- day photoperiods. Subsequently the light saturation points for algae exposed to short day lengths will be higher than for algae grown at longer day lengths. This pattern was clearly demonstrated in this study, where light saturation points at G_{max} were higher at a short-day photoperiod compared to the longer day photoperiod between 15° C and 30° C, except at 20° C (for reasons explained in earlier text). The increase in resource allocation to photosynthetic apparatus under short daylengths accompanies a negative growth effect which is explicitly shown in Figs. 2.25 (a- d). where the growth rates of *C. glomerata* were lower at a 8:16 light-dark cycle compared to the longer day length of 16 hours at all temperatures which sustained growth. The growth of *C. glomerata*, collected from the Oswego region of Lake Ontario, comparably improved from a 12 hour day to a 14 hour day when exposed to a temperature range of 14.5° C to 26° C at a light intensity of 800 foot candles for 30 days (Storr and Sweeney, 1971). Robinson and Hawkes (1986) likewise demonstrated that the growth rate of unialgal cultures of *C. glomerata* increased progressively as the duration was increased up to a maximum of 24 h light d^{-1}. Hoffman and Graham (1984) claim that the observed pattern of photoperiod on vegetative growth is an indirect consequence of reproductive activity. In their study it was noticed that zoosporogenesis in *C. glomerata* was predominantly promoted by short-day photoperiods (8:16 light-dark cycles), at a temperature range between 15° C and 20° C. It was further observed that algal filament growth was drastically reduced in cultures which underwent sporulation. However, reduced filament length at 20° C, short-day photoperiod was not observed in this study, indicating that reproductive activity was not being initiated.

2.4.4. Effects of copper and pH

2.4.4.1 Introduction

The main objective of this experimental section was to determine the correct application of $CuSO_4$ as an algicide whereby maximum growth control is obtained at the minimum Cu dosage and correct pH which is toxic to *C. glomerata*. Subsequently, pertinent questions needed to be asked which include:

1. How do the solution pH, medium Cu concentration and time of algal incubation in the Cu affect the uptake of Cu by *C. glomerata*?
2. How is the biological activity, measured either as photosynthesis or growth, of *C. glomerata* affected by Cu uptake?
3. Are the effects of Cu on growth and photosythesis permanent or temporary?

2.4.4.2 Methods

2.4.4.2.1 Photosynthetic experimental procedures

C. glomerata was acclimatized for 3 days prior to each experiment. All experiments were conducted in a perspex water bath, dimensions 60 cm x 35 x by 20 x, fitted with a B.Braun Melsungen AG thermomix 1460 connected to a Fryka-Kaltetechnik (DLK 300) cooling unit. Water temperature was maintained at 25 ± 1°C (within the optimum temperature range for *C. glomerata* photosynthesis reported by Adams and Stone 1973; Lester et al., 1988) with continuous illumination supplied by Osram Vialox 66 NAV-E 400W mercury vapour bulbs at 300µ mol m^{-2} s^{-1}, the estimated light saturation level for photosynthesis reported by Graham et al., 1982; Simpson and Eaton, 1986; Lester et al., 1988.

In total twelve photosynthetic experiments were conducted with a matrix of three medium pH levels of either 6, 7 or 8 and four exposure times to the Cu of 30, 60, 120 or 240 minutes. For the individual experiments Cu concentrations of 0, 2, 6 and 10 mg Cu l^{-1} were employed. Algal tufts of ca. 0.5 g wet weight each were placed into 100-ml Erlenmeyer flasks filled with 100ml of the sterilised enriched tap water medium supplemented with Cu, with each Cu concentration having three replicate flasks. After algal exposure to Cu for the various time periods and at each pH level, *C. glomerata* tufts were directly transferred, after shaking off excess Cu supplemented medium, to the enriched tap water medium without Cu addition at a pH of 8.

2.4.4.2.2. Photosynthetic measurements

Photosynthesis was measured using a YSI Model 50B, dissolved oxygen meter calibrated to 100% air saturation. The O_2 probe covered with a thin membrane permeable to gases determines the amount of O_2 in the solution in mg l^{-1}. For each photosynthetic measurement a total of twenty-seven 310-ml Pyrex glass bottles fitted with airtight screw lids were filled with the enriched tap water medium (0 mg Cu l^{-1} and pH 8) bubbled for 5 minutes with liquid N in order to reduce the initial O_2 content. Bottles were gently lowered at a 45° angle into the bucket containing the medium until completely filled and subsequently closed whilst submersed. Care was taken so that air bubbles in the bottles were limited. Three replicate bottles at each Cu concentration with *C. glomerata* tufts were exposed to light in order to determine net photosynthesis. Respiration was determined by inserting the algal tufts into three replicate bottles, at each Cu concentration, darkened by covering with two layers of heavy duty aluminium foil. Pyrex glass bottles filled with the enriched tap water medium without alga were included with each experiment to measure the initial O_2 concentration of the medium prior to incubation and another measurement, serving as a control, taken 1 hour after incubation in order to eliminate microbe activity and external parameters. The photosynthetic measurements were taken 1 hour after algal transfer to the Pyrex glass bottles (the minimum time required to observe a detectable change in the O_2 probe reading) which were incubated in the water bath. Each measurement was taken after the probe was stabilized (± 30 seconds) with continuous manual agitation of the probe. The photosynthetic measurements for each experiment were taken 1 hour and 24 hours after algal incubation in the Cu. Between measurements the algal materials were placed into 100-ml Erlenmeyer flasks containing 100 ml of the enriched tap water medium (pH 8).

2.4.4.2.3 Copper uptake experiments

Atomic absorption spectrophotometry analysis was performed by Cameron Chemical Consultants in order to determine the uptake of Cu by *C. glomerata*. Samples of the enriched tap water medium containing the different Cu concentrations after exposure to *C. glomerata* tufts for 30, 60, 120 and 240 minutes at pH levels of 6, 7 and 8 were collected. Samples of the enriched tap water containing the different Cu concentrations without alga were also collected which served as controls in order to eliminate the possible uptake of Cu by bacteria, although the media were sterilised prior to experimental run. Tap water was collected to determine the natural supply of Cu in tap water thereby serving as a blank. All samples were frozen at -18°C until analyzed. Samples were defrosted by allowing them to thaw at room temperatures for a 24 hour period and subsequently analyzed for total copper using a Varian AA 375 atomic absorption spectrophotometer at a λ of 324,8 nm. A standard curve was used to extrapolate sample Cu concentrations in mg l^{-1}.

2.4.4.2.4 Growth experiments

Intercalary segments of *C. glomerata*, 10 mm each, were cut under water in a glass culture dish lid under which a grid block constituting of squares with dimensions of 1 mm by 1 mm each was taped. A Kyowa Optical Stereomicroscope Model SD-2PL was used. Ten segments of *C. glomerata* were placed into 100-ml Ehrlenmeyer flasks each containing 100 ml of enriched tap water. Cu was added as $CuSO_4$ yielding final medium concentrations of 0, 2, 6, 10 and 20 mg Cu l^{-1} with each Cu concentration adjusted to pH levels of 6, 7 and 8. In total there were 15 combinations with 3 replicates each giving a total of 45 experimental flasks. Experimental flasks containing the *C. glomerata* segments were exposed to the Cu at the 3 pH levels for a period of 30 minutes at ca. 60 mol photons m^{-2} s^{-1} at ± 25° C after which they were transferred to 100ml Ehrlenmeyer flasks each containing 100 ml of enriched tap water without added Cu adjusted to a pH level of 8. Growth was monitored 1, 2, 3 and 5 days after Cu exposure using the same grid technique applied at the start of the experiment. The growth medium in each flask was replaced daily.

2.4.4.2.5 Data transformation and statistical analysis

2.4.4.2.5.1 Photosynthetic experiments

Net Photosynthesis = O_2 concentration in Light bottle - Initial O_2 concentration of the medium

Respiration = Initial O_2 concentration **-** O_2 concentration in Dark bottle

All concentration values (mg O l^{-1}) were converted to mg O_2 g algal dry weight^{-1} hr^{-1} using the formula:

O_2 concentration (mg O_2 l^{-1}) / volume (L) * incubation time (h) * algal dry weight (g)
(with volume = 0.31 litre and incubation time = 1 hour)

Gross Photosynthesis (mg O_2 g algal dry weight^{-1} hr^{-1}) = (Net Photosynthesis + Respiration)- control

For comparison, the measured photosynthetic values obtained were converted to percentages, with the control treatments 0 mg Cu l^{-1}, at each pH level and incubation time in the Cu, representing the maximum photosynthesis exhibited (100%).

2.4.4.2.5.2 Copper uptake experiments

The Cu absorbed by the alga was determined indirectly by subtracting the remaining Cu concentration in the medium which was exposed to the alga from the Cu concentration determined in the solution without algal exposure. The resulting Cu concentrations values were converted to mg Cu g algal dry weight^{-1} using the formula:

Cu concentration (mg l^{-1})* volume / dry weight (g)
(where volume = 0.31 l).

Copper uptake rates by *C. glomerata* expressed in mg Cu g algal dry weight^{-1} h^{-1} were calculated by dividing the result from the above by the incubation time of the alga in Cu.

2.4.4.2.5.3 Growth experiments

Raw measurement data were transformed to growth rates using the following equation (Hillebrand and De Vries, 1986)

$$\mu = \ln (L_x-L_1)/ \Delta t$$

where μ = growth rate, L_x = average filament length in each flask at day x (with x being 1, 2, 3 or 5 days) and L_1 = initial average filament length in each flask and Δ t = the change in time

All data analyses were computed on a software computer package, Statistica v 5.0 using Anova/Manova bases. Statistical significances were obtained by applying One-way or Two-way Analysis of Variances tests (F-ratios). Tests for normality were performed using parametric probability plots and homoscedacity was determined by the Cochran's (Chi^2 test) or Bartlett's tests (B). Statistical significances were denoted where α was < 0.05.

2.4.4.3 Results

2.4.4.3.1 Photosynthesis

In general algal photosynthesis at 2, 6 and 10 mg Cu l^{-1} was observed to be lower than the control treatments (0 mg Cu l^{-1}) at all pH levels and algal incubation times in Cu and therefore was expressed as a percentage of the control. Photosynthesis measured 1 hour and 24 hours after algal exposure to Cu (which will be referred to as P1 and P2 respectively in subsequent text) for 30, 60, 120 and 240 minutes at media pH levels of 8, 7 and 6 are represented in Figures 2.26 to 2.29. The short-term effect of Cu on photosynthesis is indicated by P1 and the extent of photosynthetic recovery is represented by P2.

P1 was significantly affected by Cu concentration after algal incubation in Cu for 30 minutes (Fig. 2.26 a-c) at pH 7and pH 6 but not at pH 8. P1 and P2 were not significantly different from each other at all Cu concentrations at pH 8, pH 7 and pH 6 after 30 minutes of algal incubation in the Cu, indicating no photosynthetic recovery. However, P1 and P2 were notably reduced at all Cu concentrations at pH 6 compared to pH levels of 8 and 7.

After 60 minutes of algal exposure to Cu (Fig. 2.27a-c), P1 was significantly affected by Cu concentration at all pH’s. There was no significant change between P1 and P2 at all Cu concentrations at pH 8, but P1 and P2were statistically different at pH 7and pH 6. P1 at pH 7 increased slightly from ca. 70% at 2 mg Cu l^{-1} and 6 mg Cu l^{-1} and from 58% at 10 mg Cu l^{-1} to more than 90% as indicated by P2 in Fig. 2.27 b. At solution pH 6, P2 at Cu concentrations of 6 mg Cu l^{-1} and below increased from ca. 40% to ca. 80% indicating some degree of recovery, although at 10 mg Cu l^{-1} photosynthesis remained depressed (Fig. 2.27 c). At solution pH 6, P1 was dramatically reduced, with Cu concentrations above 2 mg Cu l^{-1} being less than 40% of the control compared to the reductions at pH 7 and 8 which ranged between 60% and 80% (Fig. 2.27 a-c).

After 120 minutes of algal incubation in Cu (Fig. 2.28 a-c), P1 was significantly different with an increase in Cu concentration at pH 7 and pH 6, but not at pH 8. No significant variation occurred between P2 and P1 at pH 8 (and pH 7 but P2 was observed to be distinctly higher than P1 at pH 6at all Cu concentrations. At 2, 6 and 10 mg Cu l^{-1}, P2 increased by approximately 30% compared to P1(Fig. 2.28 c). No significant relationship was demonstrated between pH and photosynthetic inhibition at all Cu concentration at P1 and P2 with algal exposure to Cu for 120 minutes.

After 240 minutes of algal incubation in Cu (Fig. 2.29 a-c), P1 decreased significantly with an increase in Cu concentration at all pH's. P2 was higher than P1 at all Cu concentrations (Figs. 2.29 a and c). There was no significant effect of pH on P2 at all Cu concentrations, however P1 was significantly reduced with a decrease in pH at 10 mg Cu l^{-1}. P1, when *C. glomerata* was incubated in 10 mg Cu l^{-1}, was reduced to less than 50% at pH 8, 40% at pH 7 and below 30% at pH 6 compared to the control treatments.

The time of algal incubation between 30 and 240 minutes had relatively little effect on the resulting photosynthesis.

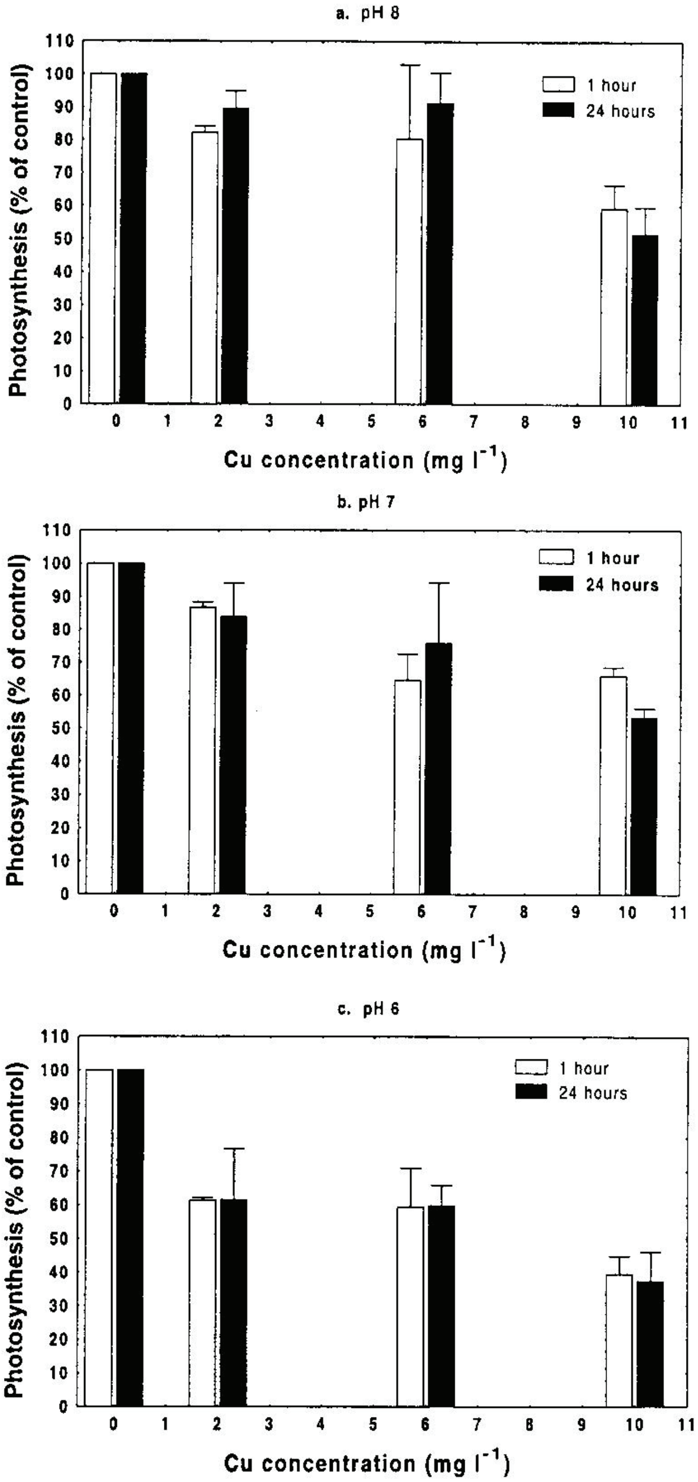


Fig. 2.26. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 30 minutes at three pH levels. a. pH 8, b. pH 7, c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments which were assumed to photosynthesize maximally at each pH level.

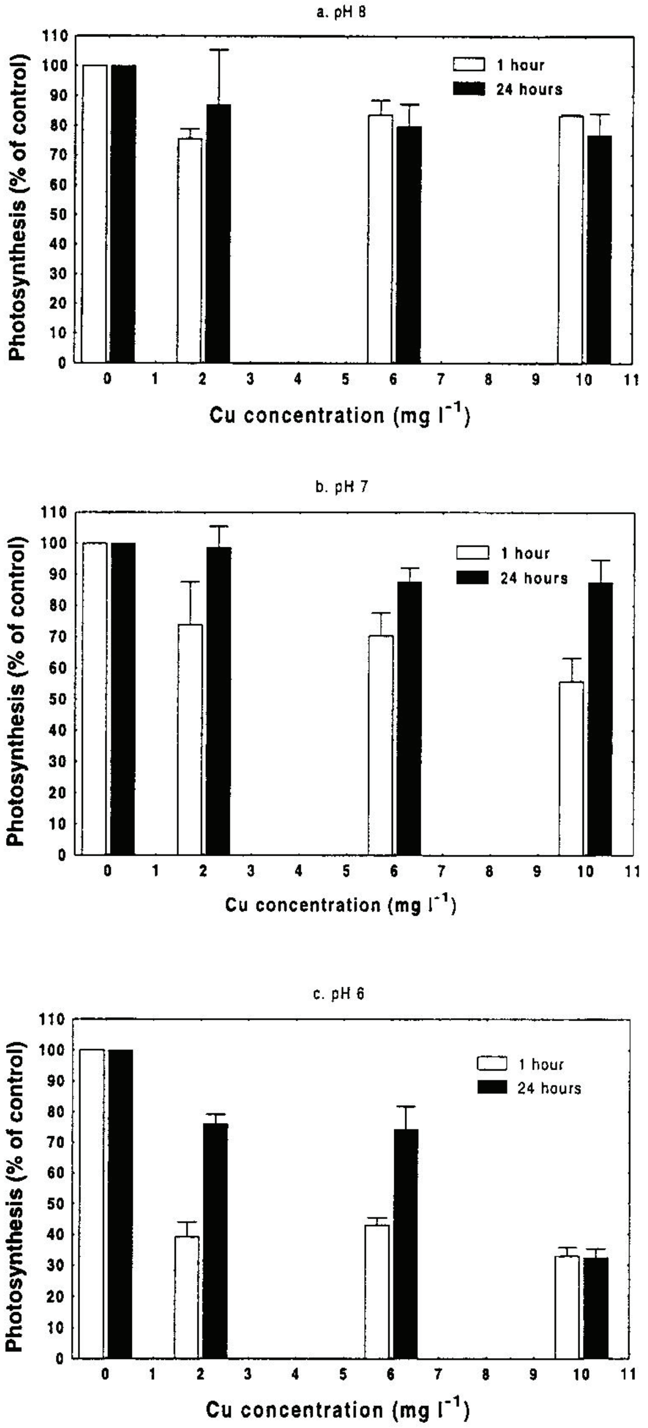


Fig. 2.27. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 60 minutes at three pH levels. . a. pH 8, b. pH 7, c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments which were assumed to photosynthesize maximally at each pH level.

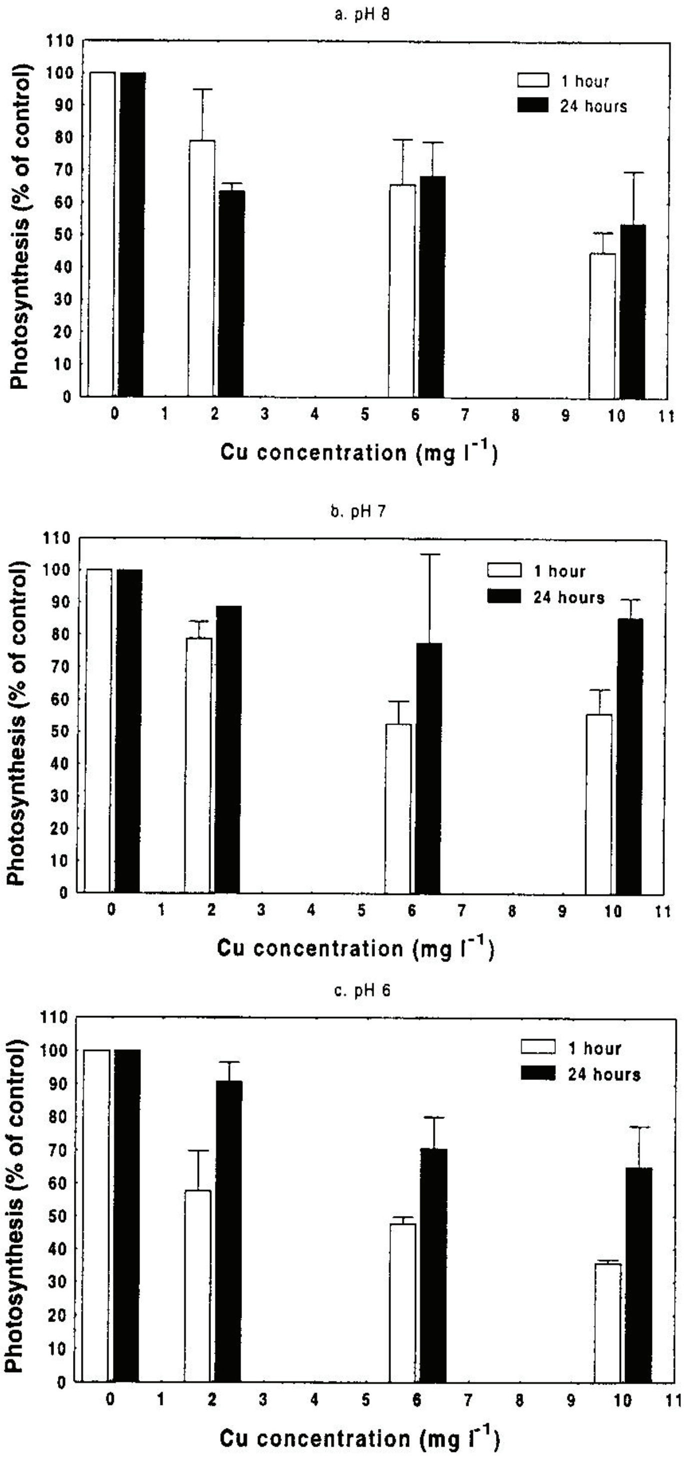


Fig. 2.28. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 120 minutes at three pH levels. . a. pH 8, b. pH 7, c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments which were assumed to photosynthesize maximally at each pH level.

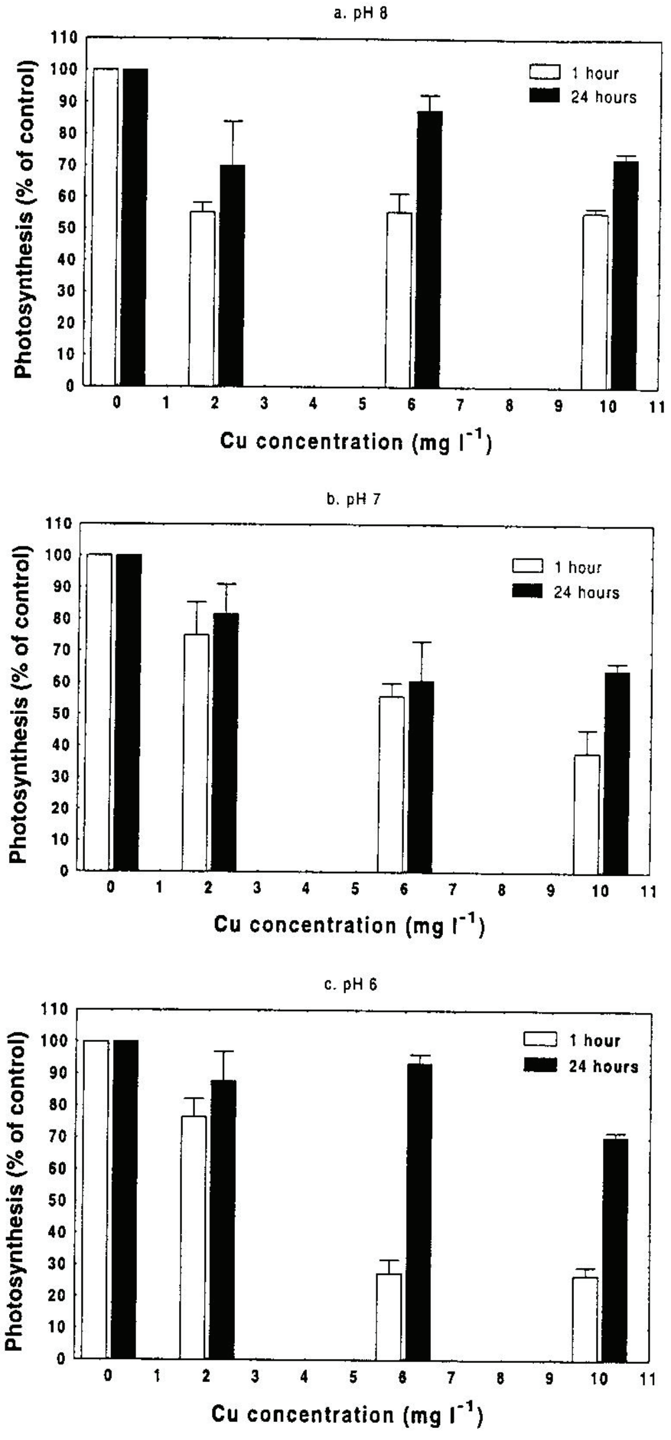


Fig. 2.29. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 240 minutes at three pH levels. . a. pH 8, b. pH 7, c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments which were assumed to photosynthesize maximally at each pH level.

2.4.4.3.2 Copper uptake

2.4.4.3.2.1 The effects of pH on accumulative Cu uptake and uptake rates by *C. glomerata*

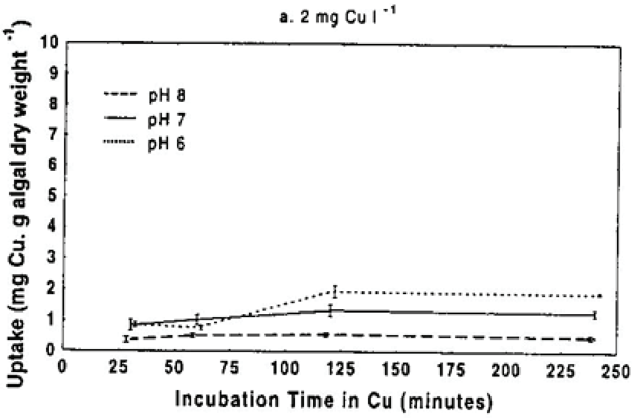
The accumulative uptake of Cu by the algal cells increased significantly with a decrease in pH, from 8 to 6, at all algal incubation times in the Cu as shown in Fig. 2.30 a-c. Uptake rates were not significantly different between the pH levels at the various algal incubation times at Cu concentrations of 2 mg l^{-1} (Fig. 2.31a) and 6 mg l-1 (Fig. 2.31b), however they were significantly different at a Cu concentration of 10 mg l^{-1} (Fig. 2.31c). Uptake rates by *C. glomerata* incubated in 10 mg Cu l^{-1} (Fig. 2.31c.) at a medium pH of 6 were higher than at both pH levels of 7 and 8 with a medium pH of 8 yielding the lowest uptake rates at all incubation times. Uptake rates were approximately 3, 2, 5 and 6 times higher at a solution pH 6 at incubation times of 30, 60, 120 and 240 minutes respectively compared to uptake rates at a solution pH of 8.

2.4.4.3.2.2 The effects of *C. glomerata* incubation time in Cu on accumulative Cu uptake and uptake rates

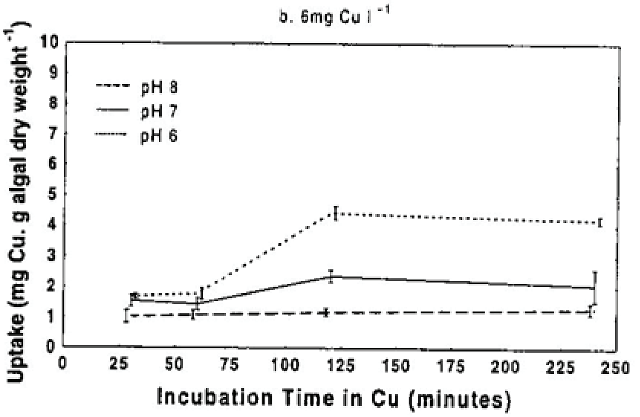
Accumulative Cu uptake was not significantly different with an increase in algal incubation time in the Cu at all Cu concentrations at a pH of 8 (Fig. 2.32 a). However, at the two lower pH levels of 7 (Fig. 2.32b) and 6 (Fig. 2.32c), accumulative Cu uptake increased dramatically within the first 2 hours after which uptake saturation was reached at Cu concentrations of 2, 6 and 10 mg Cu l^{-1}. As illustrated in Figure 2.33a-c the uptake rates of Cu in *C. glomerata* were significantly decreased with an increase in incubation time at all Cu concentrations at pH 8, pH 7 and pH 6. Uptake rates were highest after algal incubation for 30 minutes in the Cu and declined sharply after 1 hour with gradual uptake gradients with further increases in incubation times.

2.4.4.3.2.3 The effects of Cu concentration on the accumulative Cu uptake and uptake rates by *C. glomerata*

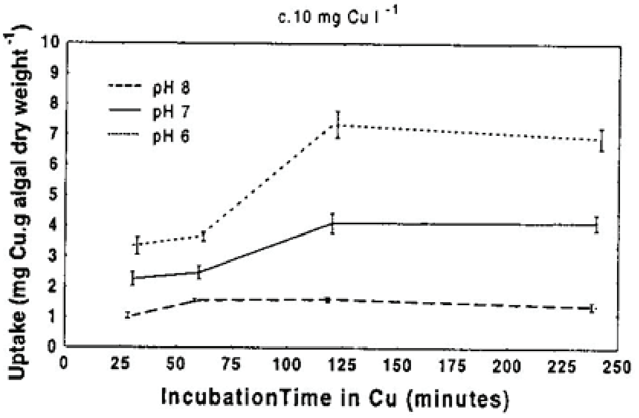
Accumulative Cu uptake and uptake rates increased significantly with Cu concentrations from 0-10 mg l^{-1} after algal incubation in Cu for 30, 60, 120 and 240 minutes at all pH levels (Fig. 2.32 a-c and Fig. 2.33 a-c). At solution pH 8, the maximum Cu uptake rates by *C. glomerata* increased with medium concentration from 0-2 mg Cu l^{-1} afterwhich the rates stabilized at 2 mg Cu g algal dry weight^{-1} h^{-1} when exposed to 6 and 10 mg Cu l^{-1}. Maximum uptake rates at solution pH 6 and 7, however continued to increase linearly with an increase in medium Cu concentration to maxima of 4.5 and 6.7 mg Cu g algal dry weight^{-1} h^{-1} respectively at 10 mg Cu l^{-1}.



a. 2 mg Cu l $^{-1}$



b. 6mg Cu l $^{-1}$



c.10 mg Cu l $^{-1}$

Fig. 2.30. Accumulative Cu uptake by *C. glomerata* with an increase in incubation time at different solution pH levels. *C. glomerata* was exposed to different medium Cu concentrations of a. 2mg Cu l $^{-1}$, b. 6mg Cu l $^{-1}$, c. 10mg Cu l $^{-1}$. Data are means (n=6) with ± S.E. (vertical lines).

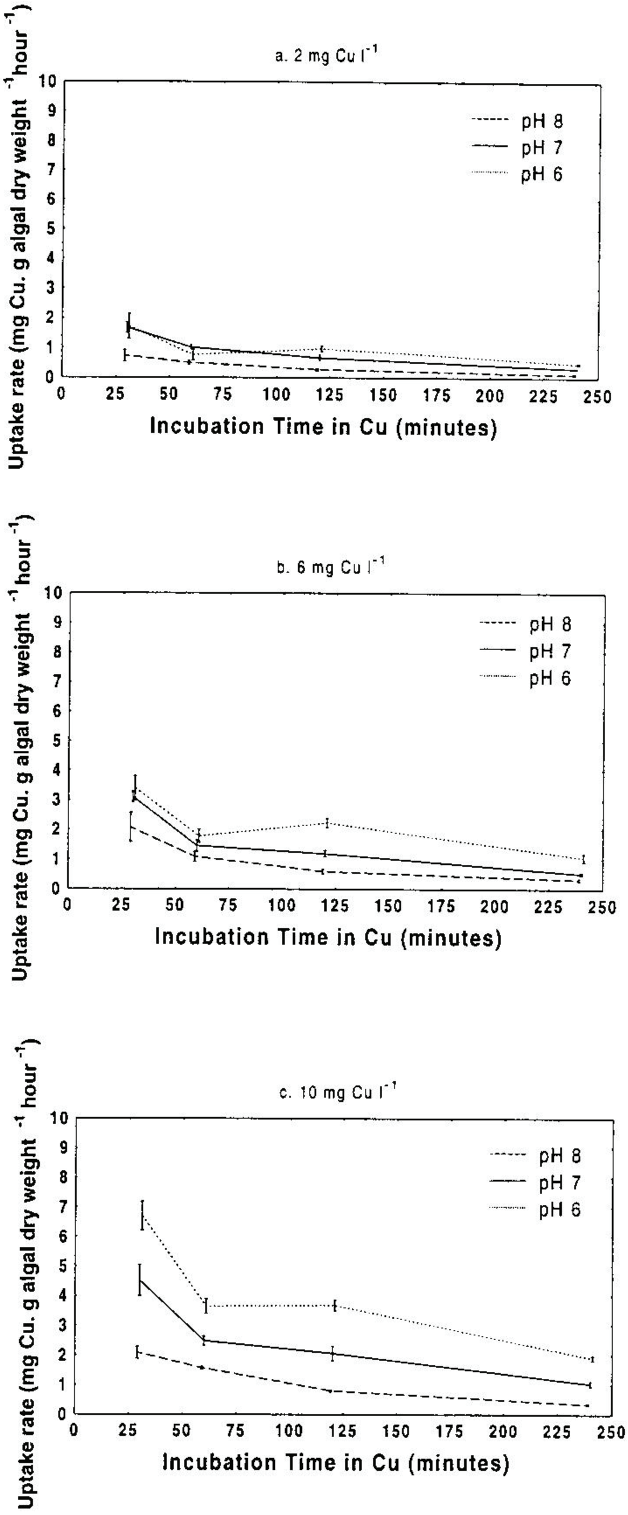
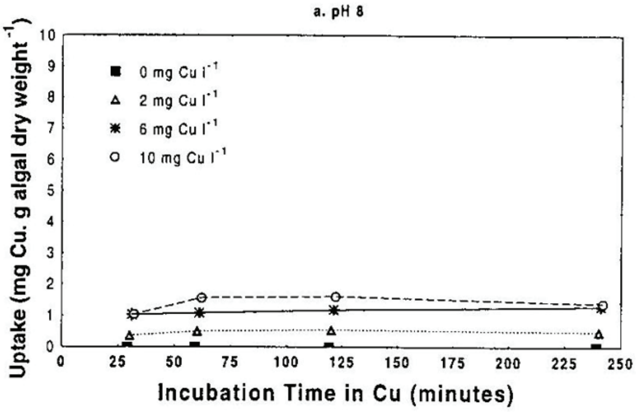
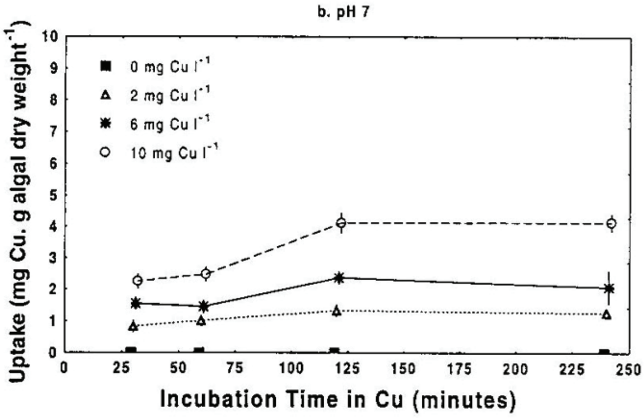


Fig. 2.31. Cu uptake rates by *C. glomerata* with an increase in incubation time at different solution pH levels. *C. glomerata* was exposed to different medium Cu concentrations of a. 2mg Cu l^{-1}, b. 6mg Cu l^{-1}, c. 10mg Cu l^{-1}. Data are means (n=6) with ± S.E. (vertical lines).

a. pH 8



b. pH 7



c. pH 6

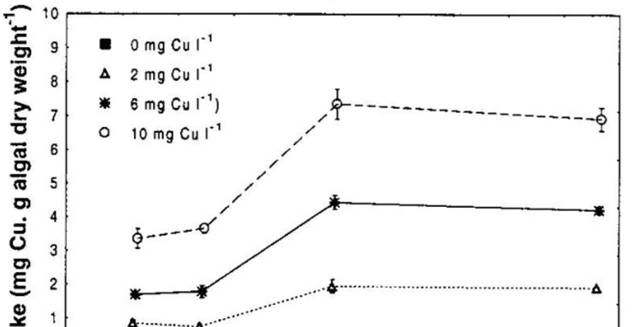


Fig. 2.32. Accumulative Cu uptake by *C. glomerata* with an increase in incubation time at different medium Cu concentrations. *C. glomerata* was exposed to solution pH levels of a. pH 8, b. pH 7, c. pH 6. Data are means (n=6) with ± S.E. (vertical lines).

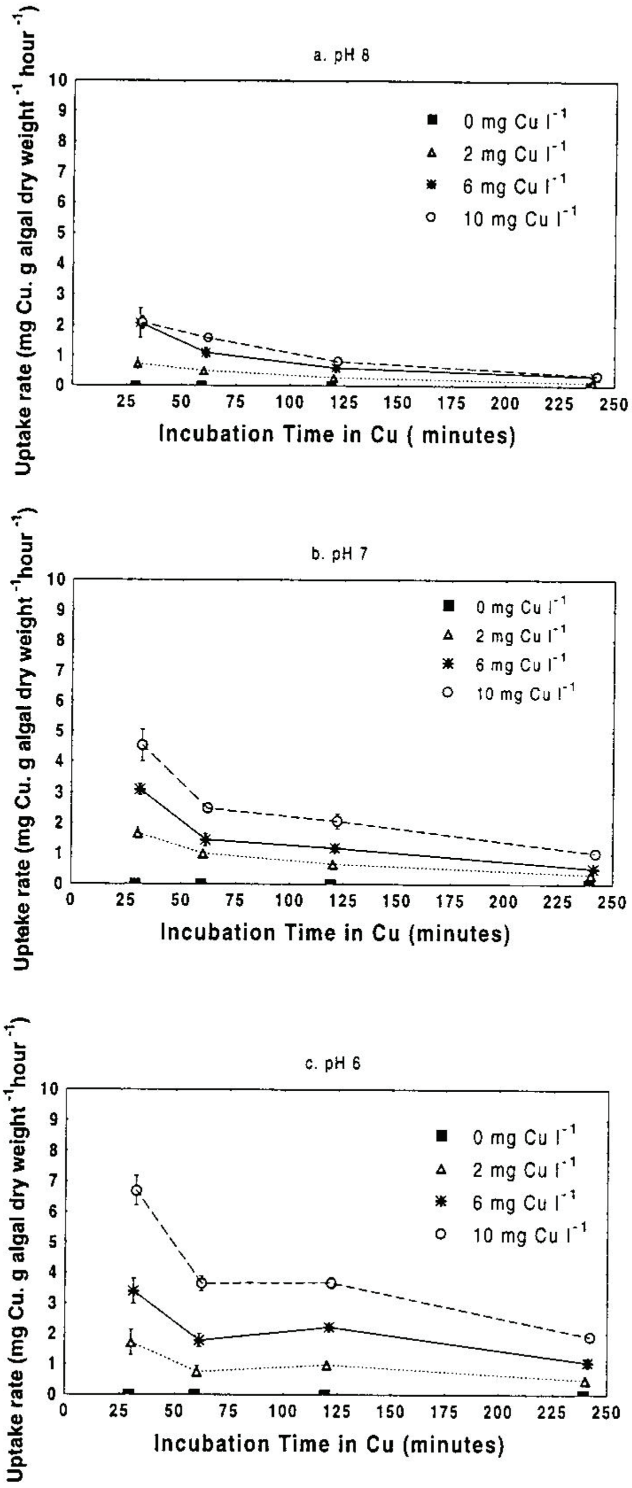


Fig. 2.33. Cu uptake rates by *C. glomerata* with an increase in incubation time at different medium Cu concentrations. *C. glomerata* was exposed to solution pH levels of a. pH 8, b. pH 7, c. pH 6. Data are means (n=6) with ± S.E. (vertical lines).

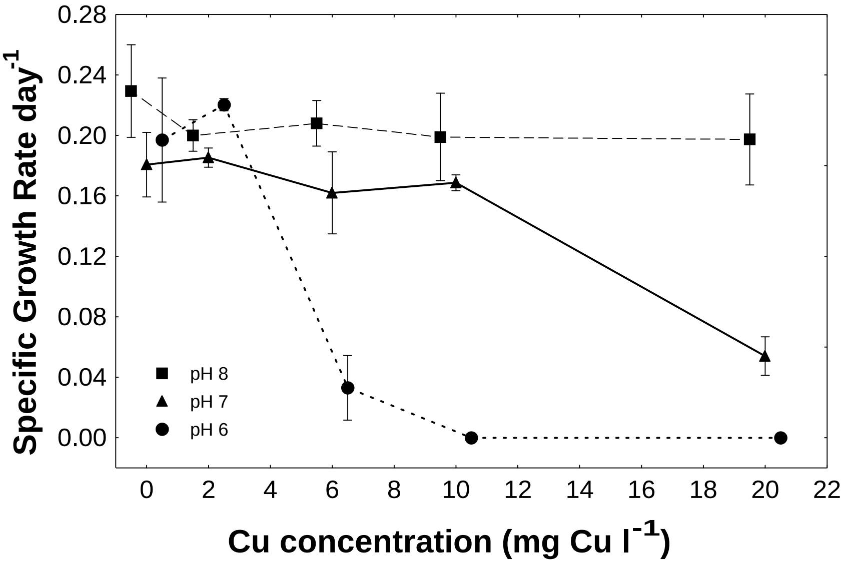


Fig. 2.34. The specific growth rate of *C. glomerata* after 5 days, when incubated in different medium Cu concentrations at three pH levels for 30 minutes. Data plotted are means (n=3) with ± S.E. (vertical lines).

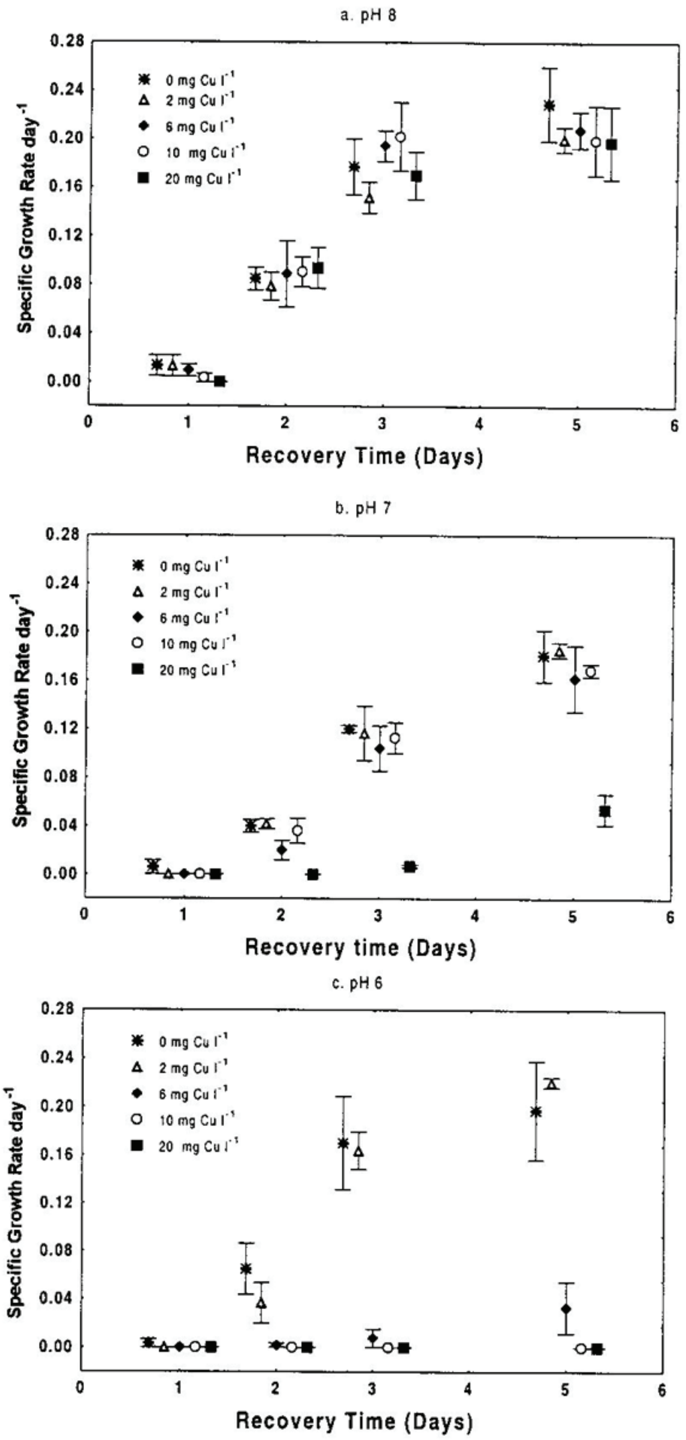


Fig. 2.35. The specific growth rate of *C. glomerata* with time, after incubation in different medium Cu concentrations at three solution pH levels for 30 minutes. a. pH 8, b. pH 7, c. pH 6. Data are means (n=3) with ± S.E. (vertical lines).

2.4.4.4 Growth rates

Growth rate of *C. glomerata* after exposure to Cu for 30 minutes at concentrations of 0, 2, 6, 10 and 20 mg Cu l^{-1} at pH levels of 6, 7 and 8 was observed to decrease significantly with a decrease in pH levels at Cu concentrations above 2 mg Cu l^{-1} after a 5 day observational period (Fig. 2.34). At Cu concentrations of 0 mg Cu l^{-1} (the control treatment) and 2 mg Cu l^{-1}, it is evident that pH has no significant effect on the growth rates (Fig. 2.34). When comparing the recovery of the filaments with time, the growth rates of *C. glomerata* after exposure to medium Cu concentrations of 0-20 mg l^{-1} for 30 minutes at pH 8 (Fig. 2.35a) were not significantly different at each time period observed irrespective of medium Cu concentration. At pH 7 (Fig. 2.35b), *C. glomerata* growth rates were similar at Cu concentrations between 0-10 mg Cu l^{-1} at each time period. However they were significantly reduced at 20 mg Cu l^{-1} for the first three days with a slight recovery after day 5, but not to the same extent as the lower concentrations. A significant effect of pH and Cu was observed on the growth rate of *C. glomerata* with time at a pH 6 after exposure to Cu for 30 minutes. Similar positive growth rates at 0 and 2 mg Cu l^{-1} were observed, although no growth of the filaments occurred within the first 3 days after Cu exposure to 6 mg Cu l^{-1}. A slight recovery was although observed after day 3 with the growth rate being 15% of the growth rates displayed by the filaments exposed to 0 and 2 mg Cu l^{-1}. The filaments exposed to 10 and 20 mg Cu l^{-1} lost their chlorophyll after day 1 and died.

2.4.4 5 Discussion

Accumulative Cu uptake is a measure of the quantity of Cu taken up by the algal cells and the uptake rate indicates the speed at which Cu is taken up by the algal cells. Both these parameters and hence heavy metal toxicity to *C. glomerata* may be dependent on the Cu^{2+} concentration in the medium which is indirectly dependent on medium pH (Gadd and Griffiths, 1978), algal incubation time in the Cu, the nutritional status of the alga (Reuter et al., 1981) and the alga's ability to mitigate heavy metal toxicity (Macfie, 1994).

Stiff (1971), reported that inorganic alkaline substances bind Cu^{2+} in neutral and high pH ranges and decreasing the pH liberates cupric ion from these complexes. Thus, it was postulated that the observed differences in copper toxicity in test waters of different hardness is due to the degree of Cu^{2+} binding with inorganic anions (Miller and MacKay, 1980). Accordingly, at a pH below neutral, metals exist as free ionic cations, but at a pH above neutral, the ionic cations bind to inorganic substances and precipitate as insoluble hydroxides or oxides (Gadd and Griffiths, 1978 and McKnight et al., 1983). Since many heavy metal hydroxides are insoluble, it could be inferred that the toxicity of heavy metals to algae is greatest at low pH and declines as pH increases, assuming that free ionic Cu is the main determinant of algal toxicity as suggested by Stokes (1983).

By applying the above rationale it may be assumed that Cu uptake would increase with decreasing solution pH. In this study accumulative Cu uptake increased with a decrease in pH at all Cu concentrations and at all incubation times in Cu (Fig. 2.30 a-c). In accordance biological activity in the form of algal photosynthesis, measured 1 hour after algal exposure to Cu, was observed to be increasingly impeded by lowering the pH level from 8 to 6 as revealed in the experiments where *C. glomerata* was exposed to Cu for 30, 60 and 240 minutes (Figs. 2.26-2.28). Likewise, it was shown that growth rates of *C. glomerata* were reduced to a greater extent with decreasing pH levels over the 5 day observational period (Figs. 2.35 a- c).
The effect of pH on Cu^{2+} uptake may however be masked by low copper concentrations. In the study by Macfie (1994) cultures of *Chlamydomonas reinhardtii* (Ehr.) exposed to 0-20M Cu at pH levels of 5 and 7 for 5 days demonstrated that the algal cell wall provided some protection from potentially toxic concentrations of Cu. It was however noticed that the cell wall only provided protection at low Cu concentrations with algal cell densities reduced to similar amounts at higher Cu concentrations. Macfie (1994) suggested that at high cationic concentrations the metal binding sites on the cell wall may become saturated with a further lack of protection of the cell membrane. The notable increase in uptake rates by *C. glomerata* with decreasing pH levels at a Cu concentration of 10 mg Cu l^{-1} (Figs. 2.31 c) and not at 2 and 6 mg Cu l^{-1} (Figs. 2.31 a and b) in this study may be partially attributed to the above phenomenon. At solution pH 8, the uptake rates are expected to be lower than at pH 7 and 6 at all Cu concentrations due to the binding capacity of Cu^{2+} to inorganic substances (Figs. 2.32 a-c). At 2 and 6 mg Cu l^{-1} at pH 6 and 7 the concentration of Cu^{2+} ions could probably be sufficient to bind to the cell wall in order to reduce the uptake rate however not high enough, as 10 mg Cu l^{-1} would be, to saturate all the metal binding sites. The effect of pH at low Cu concentrations may also be masked in the presence of ligands (Gadd and Griffiths, 1978). Although no synthetic ligands were added to the culture medium in this study, naturally occurring chelators may have been present in the tap water which may explain why the uptake rates obtained were only affected by pH at Cu concentrations of 10 mg Cu l^{-1} (Fig. 2.31c). This relationship is illustrated by the growth rates of *C. glomerata* after 5 days when exposed to Cu for 30 minutes at pH 6, 7 and 8. Growth rates were only affected by pH levels at Cu concentrations above 2 mg Cu l^{-1} (Fig. 2.34).

The concentration of Cu in the medium from 0-10 mg l^{-1} had significant effects on the uptake of Cu into the algal cells at all three pH levels (Figs. 2.32 and 2.33 a-c). A solution Cu concentration of 6 mg Cu l^{-1} was needed before maximum uptake rates were stabilized at a pH of 8, and at a solution pH of 7 and 6 the maximum uptake rates progressively increased with an increase in medium Cu concentration. Similarly, in a study by McHardy and George (1990), the uptake of Zn by *C. glomerata* increased with increasing concentrations in the test solutions when exposed to solution Zn concentrations from 0.15 - 4.0 mg l-1 with solution pH ranging between 7.2 and 8.4. Uptake was proportional to concentration of test media up to a Zn concentration of 0.4 mg l^{-1}. At concentrations between 1.0 and 4.0 mg.l-1 Zn, the uptake rate stabilized.

Whitton et al. (1981) state that many aquatic macroalgal species have the ability to take up heavy metals from water, producing an internal concentration greater than the surroundings. The maximum amount of Cu accumulated by *C. glomerata* examined in this study was 7 mg Cu g algal dry weight^{-1} when exposed to a medium Cu concentration of 10 mg Cu l^{-1}, pH 6 and for an incubation time in the Cu of 2 hours (Fig. 2.4.9c). In a field study by McHardy and George (1985) the uptake of heavy metals by *C. glomerata*, isolated from an urban river which received a supply of polluted urban runoff, was examined. Heavy metal concentrations were present in higher concentrations in the algal tissue, in the range of 8.08 -16.6 µg g^{-1}, than in the water itself. Trollope and Evans (1976) recorded concentrations of copper, 50-60 µg g^{-1}, in *Cladophora* algal tissue collected from freshwater sites in the Swansea Valley. The diverse levels of Cu accumulated in the tissue of *C. glomerata* may indicate a sensitive response to change in ambient levels (Oertel, 1991).

The increase in medium Cu concentrations from 0-10 mg Cu l^{-1} affected the biological activity of *C. glomerata* through photosynthesis and growth. Photosynthesis, measured 1 hour after algal exposure to Cu, was reduced with an increase in Cu concentration from 0-10 mg Cu l^{-1} when *C. glomerata* was exposed to Cu for 30 to 240 minutes as shown in Figs. 2.26-2.28 (b and c). Similarly, the growth rates of *C. glomerata* were reduced significantly with an increase in concentration above 2 mg Cu l^{-1} at pH 6 and 7 with a Cu concentration of more than 6 mg l^{-1} completely killing the alga at a solution pH of 6 (Fig. 2.34). Previous studies investigating the minimum Cu concentration required to reduce growth in *C. glomerata* reported much lower values, usually less than 1 mg Cu l^{-1} [Leland and Carter (1984), McHardy and George (1990) and Thomas (1962)] however Betzer and Kott (1969) indicated a much higher concentration of 2-4 mg Cu l-1 before effective control was obtained.

The different minimum Cu concentrations obtained by various researchers in determining effective growth control of *C. glomerata* may partially be attributed to the chemical status of the medium as discussed in the Introduction. For this reason McKnight et al. (1983) suggested that a general procedure in determining the minimum effective $CuSO_4$ dosages would be to calculate the cupric ion activity as a function of total copper added from the relevant physico-chemical characteristics of the aquatic body and compare that with the cupric ion activity that is toxic to the resident nuisance algal species.

The minimum Cu concentration required to obtain effective algal growth control may be affected by the duration for which the alga is exposed to Cu. The lower minimum Cu concentrations reported above by other researchers is usually indicative of algal exposure to the Cu for long time periods (ca. 1 week), however in the current growth investigation and photosynthetic experiments, *C. glomerata* filaments were only incubated in the Cu for 30 minutes or 30-240 minutes respectively. The shorter incubation periods are especially relevant to studies focusing on the use of copper to control algal infestations in lotic systems.

Although Cu concentration and pH affected photosynthesis in some of the individual experiments, there was no consistent decrease in photosynthesis (1 and 24 hours after incubation in Cu), when *C. glomerata* was exposed to decreasing solution pH and increasing Cu concentrations, with an increase in algal incubation time in the Cu. An obvious explanation is unclear since experimental procedures were constant. However, an unavoidable possible factor affecting the results may be algal density. The uptake distribution and amount of Cu into the algal cells may not have been constant throughout the algal tuft. This may have resulted in certain cells being affected by Cu whilst others were not, resulting in inconsistent photosynthetic activity with an increase in algal incubation time in Cu, with decreasing pH levels and increasing Cu concentrations. Oertel (1991) showed that differences between the layers of periphyton heavy-metal concentration and also in the uptake rate of metals existed. A more rapid and changeable uptake and release of metals occurred in the lower strata compared to the upper strata resulting in relatively higher concentrations of heavy metals in the periphyton in the lower strata. The enhanced uptake rates of heavy metals in the lower strata were promoted by higher water temperatures, dissolved oxygen and water turbulence.

The accumulative uptake of Cu by *C. glomerata* was affected by incubation time until saturation was reached. At pH 8 accumulative uptake was saturated at some point between 30 and 60 minutes (Fig. 2.32 a) of algal incubation which coincides with the incubation time where the maximum uptake rate occurred (Fig. 2.33). The effect of algal incubation time in Cu subsequently had no effect on accumulative uptake at pH 8. Despite the fact that maximum uptake rate was after 30 minutes of algal incubation in the Cu at pH 7 and 6, accumulative uptake saturation was reached after 2 hours of algal incubation in Cu. The question of "Why does accumulative Cu uptake saturate?" may be ascribed to Cu^{2+} depletion in the solution. The lower level of available of Cu^{2+} ions could explain why saturation occurs faster at pH 8 than at pH 6 and 7. However, uptake saturation cannot be attributed to total Cu^{2+} depletion since uptake rates had not equalled zero even after 240 minutes of algal incubation in the Cu at all Cu concentrations and pH levels (Figs. 2.33 a-c). Uptake saturation may however be a factor of the alga's inability to absorb any more Cu^{2+} ions due to a saturation of the internal cell concentration. Although the internal cell concentration may have been saturated, Cu was still taken up but at much reduced rates (Figs. 2.33 a-c) This was probably a consequence of the replacement of the Cu^{2+} which was utilised by the alga in order to maintain algal physiology (Hillebrand and De Vries, 1986).

The maximum uptake rates of Cu by *C. glomerata* were attained within the first 30 minutes of algal incubation in the Cu at all Cu concentrations and pH levels (Figs. 2.31 a-c). Vymazal (1984), similarly demonstrated that the periphytic filamentous algae, *C. glomerata* and *Oedogonium rivulare* (Le Clerc.) Link, exhibited rapid uptake of Cu. Maximum Cu uptake by *C. glomerata* appeared during the first hour of exposure with approximately 89.9% of total copper (36 $\mu g\ l^{-1}$) removal within this period. McHardy and George (1990) however reported that the uptake of Zn by *C. glomerata* was dramatic in the first 10 minutes. The deviating uptake rates of heavy metals reported above may not only be consequences of different chemical compositions of the test medium but also of the algal's vigour. For example, Oertel (1991), illustrated that young filaments of *C. glomerata* collected from the littoral zone of the River Danube played a more dominant role in metal uptake than old ones. The nutritional status of the alga likewise could affect Cu uptake. Reuter et al. (1981), reported that copper uptake is dependent on silicon nutrition, with more copper being assimilated by silicon limited cells. Indirectly, this observation could be related to the finding by Macfie (1994) who suggested that the cell wall plays a role in ameliorating the effect of possibly toxic concentrations of heavy metal ions. Since Moore and Traquair (1976) claimed that Si nutrition is important for cell wall formation in *C. glomerata*, it would follow that the less developed the cell wall when the algal cells are Si limited the higher the uptake of heavy metal ions. From the above discussion, it is apparent that Cu uptake is governed by solution pH, media Cu concentration and time of algal incubation in the Cu. It is also evident that an increase in Cu uptake causes an increase in growth reduction and photosynthetic inhibition. We should now consider whether the effects of Cu on algal growth and photosynthesis permanent or not.

Although recovery of photosynthesis, that is 24 hours after algal exposure to Cu, was observed in some cases, no consistent patterns were observed with pH, Cu concentration and algal incubation time in Cu (Figs. 2.26-2.28). As explained previously this could be an artefact of some algal cells being affected by the Cu whilst those which were not were still capable of photosynthesising. However, the growth rates of *C. glomerata* after exposure to Cu concentrations above 10 mg Cu l-1 at pH 6 indicated that the effects of Cu on the algal cells were irreversible since complete death occurred within the first day, indicating complete toxicity (Fig. 2.35a). The growth rates of the algal cells exposed to 6 mg Cu l^{-1} at a pH 6 (Fig. 2.35c) and 20 mg Cu l^{-1} at pH 7 (Fig. 2.35b), showed some signs of recovery after day 3. The recovery of the growth rates could indicate that the concentration of Cu in the cell is not sufficient to cause extensive and permanent damage. For example, growth rates of the algal cells, exposed to a medium pH 8, were similar irrespective of medium Cu concentration over the 5 days (Fig. 2.35a). This observation probably indicates that the low Cu uptake amount of less than or equal to 1 mg Cu. g algal dry weight^{-1}, at solution pH 8 and algal incubation time in the Cu for 30 minutes (Fig. 2.32 a), was insufficient to cause irreparable damage. In this study the amount of Cu in the algal tissue increased with an increase in the algal incubation time in the Cu at a solution pH of 6 from 3.5 mg Cu. g algal dry weight^{-1} after 30 minutes of algal incubation in the Cu to ca. 7 mg Cu. g algal dry weight^{-1} after a 2 hour algal incubation time in the Cu (Fig. 2.32 c).

2.4.5. Barley straw experiment

2.4.5.1 Introduction

The use of barley straw to control algal growth is a new and possibly promising method of biological control. It is not known whether it can be used to control the growth of *C. glomerata* or at what strengths or conditions.

2.4.5.2 Methods

Barley straw liquor was prepared from decomposed barley straw according to the method as used by Pillenger *et al*. (1994), involving 15g of barley straw per 20 litres of aged water).
50 ml of autoclaved and non-autoclaved barley straw medium was used in flasks in each of

which 10 segments of *C. glomerata* of 10 mm length had been placed. These were compared with growth in control flasks containing enriched tap water medium. These flasks were placed in a long day (16:8) light cycle at 80 µmol m^{-2} s^{-1} at 25ºC for 5 days. After 5 days the algal segments were re-measured in order to ascertain growth.

2.4.5.3 Results and discussion

In this preliminary experiment there was no significant difference between growth of segments in the barley straw extract compared to the control. It thus could not be demonstrated that the barley straw is capable of controlling *C. glomerata* growth, even in still conditions in laboratory flasks. It seems extremely unlikely that the use of barley straw could be of any benefit to attempts to control *Cladophora* growths in fast-flowing canal systems.

2.4.6. General discussion of *in vitro* findings

The use of $CuSO_4$ as an effective algicide and the predosing of sulphuric acid in alkaline water in order to enhance the algicidal effects have been scientifically confirmed in these culture studies. The minimum Cu concentration required to obtain effective control will depend greatly on the water chemistry in the canals, algal mat density and the application method. In many of the irrigation canal systems $CuSO_4$ is either discarded into the canals as a once-off instant event or by filtration using perforated bags. The residence time of the Cu in the water and its availability to the alga is usually for short time periods depending on the water flow rate in the canals. Consequently, when water flow is fast, the use of perforated bags would be more effective and when water flow is slow or static, an instantaneous application of $CuSO_4$ would suffice. Since maximum uptake rates occurred within the first 30 minutes after algal exposure to Cu (Figs. 2.4.8 and 2.4.10) it may be assumed that these application methods should be successful. As a basic guideline, it is recommended that an application of a minimum of 6 mg Cu l-1 at a solution pH 6 for an incubation period of 30 minutes will effectively control *C. glomerata* growth at low alga densities. However increased water pH would require higher minimum Cu concentration and dense algal mats would probably require repeated dosages of $CuSO_4$ to achieve effective growth control. Minimum Cu concentrations required to obtain effective algal growth control, which are suggested by this study, greatly exceed the Cu concentration, 0.8 mg l-1, applied to irrigation canals when the water pH is reduced to 6 (Du Plessis, 1992 a, b).

The growth responses of *C. glomerata* to various environmental factors, including temperature, light intensity and photoperiod, may not be true reflections of field responses, but do provide some indication of optimum conditions required to support growth. On the other hand laboratory studies could also yield an indication of unfavourable growth conditions which could be simulated in the field to reduce prolific nuisance growths of *C. glomerata*. It would be implausible to manipulate temperature and photoperiod conditions *in vivo*, however the amount of light reaching water surfaces could be controlled. In an experiment by Demal and Fortin (1987), luxuriant growth of *C. glomerata* in the Avon River, southwestern Ontario was shown to be impeded by shading certain areas with cloth. *Cladophora glomerata* growth, measured as dry weight, was reduced by 70% compared to algal growth in a control unshaded site, which received approximately 73% (i.e. 1300-3600μmol m^{-2} s^{-1}) more light than the shaded areas which received between 480 and 1332 μmol m^{-2} s^{-1}. In a detailed study by Dawson and Kern-Hansen (1979) in the area of mid-Jutland, Denmark, the biomass of the submerged aquatic macrophytes, *Ranunculus peltatus* (Schank), *Elodea canadensis* (Rich.) and *Potamogeton crispus* (L.) were vastly reduced by artificially shading the streams. The biomass of these macrophytes were also reduced in naturally shaded areas compared to open areas however the differences varied with seasonal and annual light fluctuations.

The response of *C. glomerata* growth to light intensity may be exploited by water system managers to mediate deleterious effects of growths. If placements of water work systems are placed at a depth below that which minimizes *C. glomerata* colonization, intake fouling may be minimized (Lorenz *et al*., 1991). The concept of blocking light from water bodies has a few shortcomings. Firstly, light levels fluctuate seasonally as well as with water turbidity. In field observations by Lester *et al*. (1988) and Lorenz and Herdendorf (1982) *C. glomerata* colonization depth and vertical distribution in Lake Erie varied with location depending on turbidity, sediment load in the water and the local degree of light attenuation. In the Zuikerbosch canal at the Rand Water Supply Station, South Africa, huge growths of *Cladophora* sp. were observed in 1983 when water turbidity levels were low. Subsequently, the biomass of *Cladophora* sp. seemed to fluctuate according to water turbidity levels, being depressed at high turbidity and enhanced at low turbidity (Steynberg, unpublished). Secondly, in South Africa, the use of shade cloth does not seem to be readily accepted because of the high costs involved (Joska and Bolton, 1994 a). Thirdly, manipulating the light availability in canals and rivers may have detrimental effects on other aquatic organisms (Dawson and Kern-Hansen, 1979). Furthermore, in the current study, *C. glomerata* growth was only reduced at low light intensities, below 25 μmol m^{-2} s^{-1}, and negligible growth was only obtained at 0 μmol m^{-2} s^{-1} irrespective of temperature and photoperiod conditions examined. These low light intensities required to impede *C. glomerata* growth suggest that virtually all incoming light would need to be obstructed from the canals and that water work systems should be placed at water depths below that which obtain light intensities sufficient to support growth. Since the light intensity penetrating the water column at and above 25 cms below the water surface exceeded maximum light requirements to support optimum growth throughout the year, it would advisable if water work systems are placed at water depths more than 25cm below the water surface.

Water extracts containing decomposed barley straw residues have recently been shown to have antialgal activities towards green algae and cyanophytes. Welch *et al*. (1990) demonstrated that the presence of rotting barley straw in a section of the Chesterfield canal (United Kingdom) significantly reduced the amount of *C. glomerata* during the second season after the initial application. In a laboratory study by Gibson *et al*. (1990) the presence of rotting barley straw in water inhibited the growth of planktonic and filamentous algae. The inhibitory effect was most effective after a six month period at 20° C. When the straw was autoclaved all the inhibitory effects were lost which suggested that bacterial populations within the straw were the main determinants of the inhibitory action. Likewise Newman and Barrett (1993) reported that growth of the blue-green alga, *Microcystis aeruginosa* Kütz, was inhibited by the presence of decomposing barley straw in the water. Regrowth of the cells exposed to inhibitory concentrations were however similar to the control, when transferred to a barley straw-free medium.

The application of decomposed barley straw in South African water systems has only been occasional over the past two years. In our preliminary culture study the growth rates of *C. glomerata* filaments were similar irrespective whether they were exposed to autoclaved and unautoclaved media of either decomposed barley straw water extract or nutrient enriched tap water. Although more scientific experimental work needs to be conducted in South Africa to determine which environmental conditions, *in vitro* and *in vivo*, will be required to effectively decay the barley straw and subsequently inhibit algal growth, it is very unlikely that the barley straw method would be useful in fast-flowing canal systems.

2.5 Discussion

2.5.1. Relationships between *in situ* and *in vitro* results

The seasonal distribution of *C. glomerata* in the Kalkfontein irrigation canal scheme, from where the materials were collected for this laboratory research study, coincides with the optimum and limiting temperature ranges for growth as reported earlier. In the Kalkfontein system the water temperatures in winter drop below 10° C which coincided with reduced or negligible biomass of *C. glomerata* occurring in the irrigation canals. In summer, the water temperatures generally rose above 20° C but seldom above 25° C. Thus the temperature conditions throughout the summer in this system seem to be optimal for growth of *C. glomerata*, as demonstrated in the culture experiments.

According to findings in this laboratory study and field observations, light intensity appeared to play a less signifcant role in the seasonal distribution patterns of *C. glomerata* in the Kalkfontein canal system compared to temperature. *In vivo* measurements of light intensity in water fluctuate depending on time of the day, water turbidity, weather conditions and water depth. Light intensities at the water surface of the Kalkfontein irrigation canals generally ranged between 1000 and 1500 μ mol m^{-2} s^{-1} in the summer, spring and autumn months and between 600 and 800μ mol m^{-2} s^{-1} during winter. The percentage of total light loss at 25 cms below the water surface for the period of January 1995 to July 1997 was usually less than 90%. These measurements indicate that light intensities below the water surface where *C. glomerata* is found, were generally higher than the maximum light requirements to support optimum growth at water temperatures between 15° C and 30° C which were reported in the laboratory experiments (Table 2.4.1.).

The seasonal distribution pattern of *C. glomerata* in the Kalkfontein canal may also be affected by photoperiod. Day lengths (data received from the South African Weather Bureau in Pretoria) during the winter season in the Kalkfontein canal region are much shorter (10 ±1 hours of daylight) than during the summer season (15 ±1 hours of daylight). As reported in this laboratory study the maximum growth rates of *C. glomerata* at light saturation point (Table 2.4.1.) were reduced at short-day photoperiods compared to long- day photoperiods at all temperatures examined

3. Breede River Canals – *Oedogonium capillare (*Linnaeus) Kützing 1843

3.1 Introduction

The Breede River Canal System is different in many ways from the Oranje-Riet and Kalkfontein Canal Systems. This canal system is in the winter rainfall area of the Breede River valley in the Western Province of South Africa. The winter rains are dependable and flash flooding of the rivers in this area commonly occur on an annual basis. The Breede River has a permanent flow throughout the year albeit considerably lower in the summer months. The canal system is much older than the Orange River systems and the canals themselves are smaller (water depth rarely greater than 90 cm) and throughout most of their length can be safely and easily accessed by researchers. This latter fact has a negative aspect as will be commented on later.

In the literature, the names Breede and Breë are both given to this river. Both names mean the same (broad river) and are deemed correct, but for the sake of uniformity we will use the name Breede in this report. Most literature investigates the irrigation and water resources development of the Breede (see Schreüder *et al*, 1988 and Murray *et al*. 1989) however recent environmental awareness has influenced increasing research into water quality and environmental impact studies (see Dallas *et al*, 1994) and biological research (see Coetzer, 1986 and Joska and Bolton, 1994)

The seasonal problematic growths of the alga *Oedogonium capillare* were first reported on by Joska & Bolton (1994). A fuller investigation of the occurrence and various aspects of growth potential of this alga, both *in situ* and *in vitro*, were considered necessary. This was accordingly undertaken as part of this whole project together with the research on the alga *Cladophora glomerata*.

3.2. Literature survey

3.2.1. Taxonomy

The algal species, *O. capillare*, is one of many species which occur in the genus *Oedogonium*. The genus was originally named by Link (1820) but the family in which it occurs, the Oedogoniaceae, was only named by De Bary in 1854. The family has three genera, *Oedogonium* (with unbranched filaments), *Bulbochaete* (with branched filaments and setae) and *Oedocladium* (branched filaments but no setae).

The first major taxonomic publication on the family was published by Hirn (1900). Although most of the species which he described occurred in Europe and North America some were sent to him from Australia and Africa. He described 191 recognised species (not including sub-species) and 8 which "required further investigation". Tiffany (1930), working in North America, but examining species sent to him from all over the world, described 195 species (not including sub-species) and 41 species "imperfectly known". In his publication, Oedogoniacees Africaines, Gauthier-Lièvre (1963;1965) described 193 species, all of which he had found in North Africa and 2 species "imperfectly known". This latter publication confirms the fact that most species of *Oedogonium* are cosmopolitan. *O. capillare* was found by Hodgetts (1925) in Stellenbosch in the Western Cape Province. These relatively similar species numbers were not the same as the finding of Mrozinska (1981), who described 465 species (not including sub-species) and a further 61 species "requiring further investigation". Her descriptions include species not personally investigated and it is likely that some species "overlapping" has occurred.

Most importantly, except for three species *viz. O. undulatum*, *O. nodulosum* and *O. reinschii*, *Oedogonium* specimens may only be identified to species level if in a reproductive state. Further, the reproductive state is not often found in field collections and the plant is usually coerced into a reproductive state with *in vitro* manipulation. This latter fact is the reason why most freshwater phycologists are not able (by reason of time and facilities available) to identify *Oedogonium* to species level. This phenomenon is not peculiar to *Oedogonium* and occurs in many other genera. This is unfortunate, as it is becoming evident that certain algal species occur in certain water quality conditions and are thus reliable indicators of problem conditions.

3.2.2. Life history

The very distinctive vegetative cells of this genus make it easily recognised. The apical “caps” which appear after cell division indicate the number of cell divisions which have occurred (Tiffany, 1930). Results of detailed research into the cell and cell division in *Oedogonium* was published by Pickett-Heaps & Fowke (1969; 1970a,b). Mrozinska (1981) states that vegetative reproduction, in the form of akinetes on the basal rhizoids, occurs quite widely in the genus *Oedogonium*. Asexual reproduction occurs in vegetative cells in the filament. The entire contents of the vegetative cell form an oval shaped cell which escapes from the top of the via a break in the cell wall. After release from the cell it can be seen that one end of this oval zoospore has a small colourless region surmounted by a ring of cilia (Tiffany, 1930). *O. cardiacum* was found to have 120 of these cilia (Mrozinska, 1981) which serve to propel the zoospore in a circular motion. After a short time the zoospores settle on the substrate and the colourless end forms the basal disc which attaches the plant. Zoosporogenesis was thoroughly investigated in two species of *Oedogonium*, “large” and “small” by Pickett-Heaps (1971).

Sexual reproduction is more complicated. Male antheridia and female oogonia are present, either on one filament (monoecious) or on separate filaments (dioecious). However, Tiffany (1930) reported that at least two species were both monoecious and dioecious. Where the plants are dioecious, the male filaments may be the same size as the female filaments – macrandrous; or, few celled filaments called dwarf males which are described in three different ways. If the dwarf male is epiphytic on the female filament it is termed as nannandrous. A dwarf male formed from an antheridium on a monoecious plant is termed gynandrosporous, but a dwarf male which has developed from an antheridium on a dioecious plant is termed as idioandrosporous. More complicated developments may occur and these are described in Hirn (1900), Tiffany (1930) and Mrozinska (1981,1991). Gusseva (1931) found that *O. capillare* could develop parthenogenetically. An oospore results from a fertilised oogonium. Oospores are variously ornamented, e.g. ribbed, echinate, dentate, and this ornamentation is characteristic of the species. Oospores can occur singly, or in consecutive cells in the filament. The outer wall of the oospore is able to withstand drought and records of long lived spores are not uncommon (Hirn, 1900; Buzer *et al*, 1985). The oospores have a three layered wall, inner, middle and outer. The outer wall is commonly coloured brown or reddish-brown. Although most oospores are released from the split oogonium some have been recorded as remaining on the parent filament Mrozinska (1981) until germination. Released oospores usually fall to the substrate where they can wait for favourable conditions for germination.

In *Oedogonium* the oospore divides just after fertilisation, into four haploid zoospores (Mrozinska, 1985). On germination of the oospore the released zoospores settle after a short time of swimming and in the first cell division the lower cell becomes the basal cell which may produce either short rhizoids or a disc-like holdfast for attachment to the solid substrate or plant (Tiffany, 1930). It should be noted that most species of *Oedogonium* occur in freshwater only. One of those species which can occur in brackish water is *O. capillare* (Hirn, 1900; Mrozinska, 1981).

Tiffany (1930) reported that there was a recognisable periodicity in the "fruiting records" of *Oedogonium*. He found that the vegetative zoospore production could take place at most times of the year, but that oospore formation was restricted to early summer and autumn. Rawitscher-Kunkel & Machlis (1962) reported that the female filament of *Oedogonium* species produced a sperm attractant hormone.

3.2.3. Growth

Despite the fact that there are so many species of *Oedogonium* the literature available is restricted to those few which have been identified. This may be compared to *Cladophora glomerata*, where one species is the subject of so much research and regularly published literature. Most often in ecological descriptions the number of different algae are listed as genera, with recognisably different species of *Oedogonium* defined by means of the different vegetative cell diameter and, rarely, the different appearance of the vegetative cell contents.

A description of *O. capillare* is given in this section although some of the information is taxonomic in nature. This species is dioecious and maccandrous (male filaments approximately the same size as female filaments). There is usually a single oogonium very seldom exceeding the width of the normal vegetative cell. Vegetative cells 35 – 60 µm; oogonia 35 – 65 µm. The three recognised forms of this species – f. *madagascariense*, f. *stagnale* and f. *capillare* may have filaments 5µm lesser or greater than those recorded for *O. capillare*. This species, and its forms, have been recorded worldwide. As previously noted *O. capillare* is exceptional in that it occurs in brackish and slightly saline conditions as well as in freshwaters. (Hirn, 1900; Tiffany, 1930; Gauthier-Lièvre, 1964; Mrozinska, 1981). Mrozinska (1981) also found that it was often associated with *Enteromorpha* sp. in slightly saline water conditions and grew well in both still and flowing water. This was confirmed in our research and *O. capillare* was found in Theewaterskloof Dam, near Cape Town, the Breede River and its associated irrigation canals and also the irrigation canals off the Gamtoos River in the Eastern Cape Province. In 1902, Fritsch published the results of a study into the growth of five species of *Oedogonium*, including, *O. capillare*. He found that the end cell of the filament had a distinctly pointed tip and also that the attachment cell produced two different types of rhizoid holdfasts. The first type of plant which had grown from a firm substrate produced a disc-like rhizoidal attachment. The second type of rhizoid, very elongated, often in a group of three, developed from plants which were not attached.

Different growth peaks for *Oedogonium* species in four continents are shown by Mrozinska (1981) and those for Africa are April and November (although it must be noted that most work on these algae in Africa has been carried out in tropical regions).

3.2.4. Environmental tolerances

3.2.4.1. Light

In a study of algal distribution in lakes, Stevenson *et al*. (1996) found certain *Oedogonium* spp. (not identified) preferred the upper eulittoral zone with high light, turbulence with a marked temperature fluctuation.

3.2.4.2. Temperature

De Nicola and McIntyre (1990) reported that zoospore production in some *Oedogonium* spp. was temperature limited. Also that in heated sites, such as those where thermal discharge took place, *Oedogonium* spp. were one of those groups of filamentous algae (with *Spirogyra* sp. and *Cladophora* sp which became dominant over a previously diatom dominated site. However, in a listing of those algal genera which occur in the New Zealand thermal areas, *Oedogonium* is not mentioned (Cassie, 1989). Biggs (1990) sampling a wide range of New Zealand rivers found that *Oedogonium* communities occurred mainly at temperatures between 9 and 21ºC.

3.2.4.3. pH

Oedogonium species are considered to prefer a pH range of 4.4 – 7.5. They also appear to replace acid-loving algae when conditions become somewhat more alkaline (Planas *et al*, 1996). Using simulated sulphuric acid and also ammonium sulphate rain conditions Schuurkes *et al* (1987) found that water conditions changed dramatically with pH levels sometimes dropping to 3.5. The previously dominant plant species were replaced by the algae

Oedogonium and *Mougeotia* species. Mrozinska (1991) found that certain species were either acid or alkaline loving.

3.2.4.4. Conductivity

Biggs (1990), working in New Zealand rivers found that conductivity was one of the most important environmental factors for algal community association. In the seven algal community groups studied he found that the *Oedogonium/Melosira* group dominated in water with low to medium conductivities (0 – 200 µS.cm^{-1}). In Finnish rivers, Eloranta & Kwandrans (in Stevenson *et al.*1996), found *Oedogonium* species in water with conductivities of 350-420 µS.cm^{-1}

3.2.4.5. Nutrients

In a study of shallow freshwater habitats in The Netherlands, Simons (1994) found that *Oedogonium*, with a range of 20 species mixed, formed a major part of the filamentous algal community in moderately eutrohpic and mesotrophic water. Biggs (1987) made a similar finding when studying streams and slow running waters in New Zealand.

However, in his study in 1990, in New Zealand, Biggs found that the *Oedogonium/Melosira* communities were predominant in water where there were medium to high amounts of dissolved reactive phosphorus and silica.

3.2.4.6. Flow

Most species of *Oedogonium* collected from a wide range of sites in what is now the Eastern Province of South Africa, and examined by Fritsch & Stephens (1921) occurred in still or slow running water conditions. However, specimens of *Oedogonium* were collected from fast flowing streams as well as slow water sites in Natal by Fritsch & Rich (1924). *Oedogonium*/*Melosira* communities in a range of New Zealand rivers occurred mainly in low flow sites and this factor could be one related to strength of attachment to substrate (Biggs, 1990). Tiffany (1930) found that 54% of *Oedogonium* species were found in permanent ponds compared to 6% in streams in the North Central States of the United States. Mrozinska (1981) found that seven species of *Oedogonium* appeared to prefer fast-flowing water conditions, amongst these was *O. capillare*. In Finnland, Eloranta & Kwandrans (in Stevenson *et al.*1995), found *Oedogonium* spp. in current velocities from 70 – 160 cm s^{-1}.

3.2.5. Community ecology

In a study of diatoms epiphytic on filamentous algae in freshwater streams in Chile, Parra *et al* (1984) found that *Oedogonium* spp. were the second most preferred algae for diatom settlement (*Cladophora glomerata* was the most preferred alga). *Oedogonium* species have been found to be subject to parasitism by amoebae, such as *Vampyrella sp*. and also as preferred substrate algae by dinoflagellates (Sheath et al., 1996). Some *Oedogonium* spp. are commonly grazed and also used for case building by chironomid larvae (Bott, 1996). Conversely, Steinman *et al* (1992), found that certain species of *Oedogonium* which had high phosphorus uptakes were grazer resistant.

3.2.6. Control and management.

The occurrence of *Oedogonium* as a nuisance species with massive growths is apparently only found in South Africa and occasionally New Zealand (Biggs, 1990) Also, although when it occurs it is seasonal and usually in spring and late summer it does not occur in massive growths every year (Joska & Bolton, 1994). In India, Thailand and other rice growing countries in the East, *Oedogonium* spp. may be the dominant algae growing as epiphytes on the deepwater rice plants (Aziz & Ahmed, 1992). In the Netherlands, Simons (1994), found that the percentage cover of *Oedogonium* could vary from site to site and from year to year. The dominant growths of this genus were, in some cases, only due to two species.

3.3. *In situ* investigations

3.3.1. Brief history of scheme

The Breede River valley lies in the southwestern part of the Western Province. It rises in the Ceres area and gains its major water from the Molenaars, Holsloot, Smalblaar and Jan du Toits Rivers. Some other original tributaries e.g. the Hex River have been seasonally lost due to water abstraction in their upper reaches. The Breede River, in its entirety, is divided into five flow regions – Upper Breede River, Hex River, Middle Breede River, Riviersonderend and Lower Breede River. It flows into the Indian Ocean at San Sebastian Bay on Cape Infanta. The major irrigation development is in the middle Breede River area which has four major canal systems which feed off the river from (1) near Worcester – the La Chasseur Canal, (2) above Robertson – the Robertson Canal, (3) 10 km below Robertson – The Angora Canal, and (4) the confluence of the Kogmanskloof and Breede Rivers above Bonnievale. The first recorded development of irrigation canals off the Breede River was in 1864. Originally the canals were privately developed by various farmers but the first irrigation board was proclaimed in 1898 for the Robertson Canal System. The Sanddrift and La Chasseur Canal irrigation boards were proclaimed in 1910 and the Angora Canal Board in 1917. They are still privately run boards maintaining their autonomy from the Department of Water Affairs and Forestry as far as is legally possible (Schreuder *et al*, 1988). Increasing problems of salinity have led to intensified research into assessment of the effects of the canal systems and their influence on salinisation (Murray, Biesenbach & Badenhorst, 1989) and benthic invertebrate communities in the Breede River (Coetzer, 1986). Increasing algal problems were noted by Joska & Bolton (1994).

In this project it was decided to sample and compare the results from two sites from the top (La Chasseur) and lower (Sanddrift) canal systems.

3.3.2. Site descriptions

Figure 3.1 shows the position of the two canal systems on the Breede River and the four sample sites.

Site 1 at the top of the La Chasseur canal was below the Villiersdorp road and approximately 4 km from the original canal offtake point. The canal here is 3 m wide and 1 m deep. Here the canal walls are quite rough with many small stones embedded in the concrete. The natural vegetation has been removed for at least 20 m on either side of the canal and this condition appears to be regularly maintained.

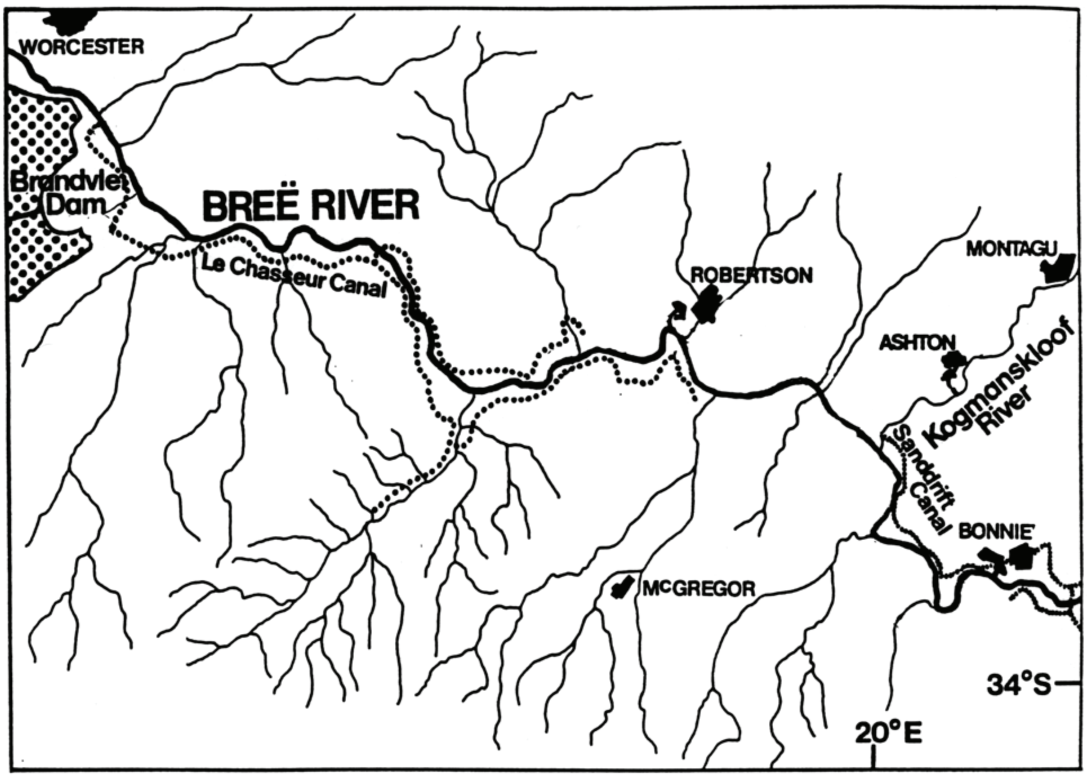


Fig. 3.1. Map of the Breede River (Breë River) showing the La Chasseur and Sanddrift Systems, with Sites 1, 2, 3 and 4 arrowed.

Site 2 at the lower end of the La Chasseur canal on the McGregor road approximately 2 km outside Robertson. The canal had narrowed to 0.5 m wide and 40 cm deep. The walls are only slightly rough. Natural vegetation ("fynbos") grows thickly to within 30 cm of the canal sides.

Site 3, the top end of the Sanddrift Canal, just below its offtake at the confluence of the Kogmanskloof and Breede Rivers. The canal is approximately 4 m wide and 1.5 m deep. The walls are slightly rough and most of the encroaching vegetation (aliens) has been cleared for 5 m on either side of the canal. This site proved to be somewhat problematic as the monitoring board was vandalised/stolen on three occasions. On the advice of the local Water Bailiff we moved the monitoring board to a site about 3 km towards Bonnievale. This second **Site 3** was on the Goudmyn road just before the Rooibrug. The canal has already narrowed considerably, to approximately 2 m wide and 1 m deep. Unfortunately, this site too proved to be susceptible to vandals and although physical measurements continued to be taken, algal monitoring with the petri dishes became impossible as the monitoring board was again stolen.

Site 4, the lower end of the Sanddrift canal was approximately 5 km west of Bonnievale on a privately owned farm. The canal here is approximately 1 m wide and 50 cm deep with slightly roughened concrete surfaces.

3.3.2. Materials & methods for fieldwork

All the sites were sampled for a full year, from March 1996 to May 1997. It was possible to travel to all four sites and return to the laboratory with the collected samples within one day. At each sampling occasion the conductivity, pH, temperature and light incidence at three depths (surface, 12 cm and 25 cm) were measured.

Conductivity was measured with a Y.S.I. Electroconductivity Meter in µS cm$^{-1,}$ this meter also measures temperature. The temperature was always recorded at a depth of approximately 20 cm below the water surface, at a level equal to the middle of the monitoring board. pH was measured using Merck Spezialindikator papers of two levels, pH 4.0 – 7.0 and 6.5 – 10.0. Light incidence was measured with a SKYE Light meter in µmol m^{-2} s^{-1}. Water speed was calculated (with three replicates) measuring the time taken for an orange to cover the distance of 10m on the water surface. Turbidity was not measured at any of these sites as water clarity enabled us to see a Secchi Disc at the bottom of all the canals.

Due to sampling experiences at the Oranje-Riet and Kalkfontein Canal systems we used only the Perspex® monitoring boards for algal sample collection at the Breede River sites.
These sheets measured 45 x 45 cm and two rows of three aluminium bolts, 25 cm apart were fixed on the sheets.. Each sheet had a loop of chain fixed at either side on the top this was used to suspend the monitoring board in the water. One half of a laboratory plastic petri dish, with a hole in the base was fixed onto each of the six bolts and secured with a butterfly screw. These six dishes, three replicates for each of the two levels, were removed and replaced on each sampling occasion. The three dishes for each level were placed in clean, marked, plastic bags at each sampling site and transported back to the laboratory for examination. Each dish was lightly sanded on one half in order to establish whether there was preferred settlement on a roughened surface. Sample petri dishes were maintained at a low temperature in the buckets

with bags of ice. In the laboratory the petri dishes from the upper and lower levels from each site were examined microscopically (compound microscope) for the presence of algae. During the sampling period the Sanddrift canal was emptied on two occasions.

3.3.4. Results

The results of the observations of algal growth on the petri dishes for each site will be given at the end of the results section.

Site 1 – La Chasseur top.

This site close to the intake of water off the Breede River probably reflects the physical conditions pertaining in the river water. Figure 3.2 shows the temperature, conductivity and pH measurements for the sampling period. The temperature reflects the normal seasonal increase and decrease in temperature. The lowest temperature, 10.5ºC was recorded in August (late winter) and the highest, 23.5ºC , in December (mid summer). Conductivity ranged between 28 $\mu S.cm^{-1}$, in September, 1996 and 108 $\mu S.cm^{-1}$ in July, 1996. It can be seen in Figure 3.2 that there was increased conductivity in the winter months, dropping to a low in spring and then a slight increase and steady maintenance from November 1996 to May, 1997. pH values remained below 6 for the entire sampling period. The highest pH value recorded was 5.7 in October, 1996.

Water speed and light attenuation measurements are shown in Figure 3.3. Water speed remained fairly constant although there was a slight decrease in January, February and March 1997 when irrigation demands are increasing. Light attenuation percentages show three marked peaks, in April and October, 1996 and March 1997. Overall the range in light attenuation was 40%, but as previously stated the water in these canals is generally relatively clear.

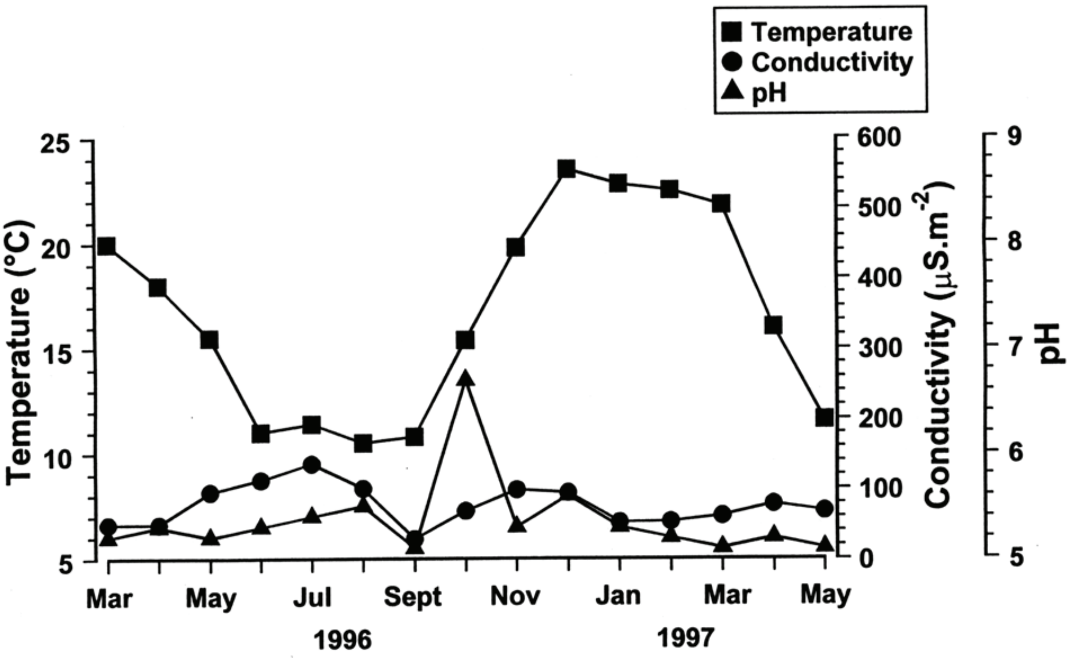


Fig. 3.2. Temperature, conductivity and pH measured at Site 1, Breede River.

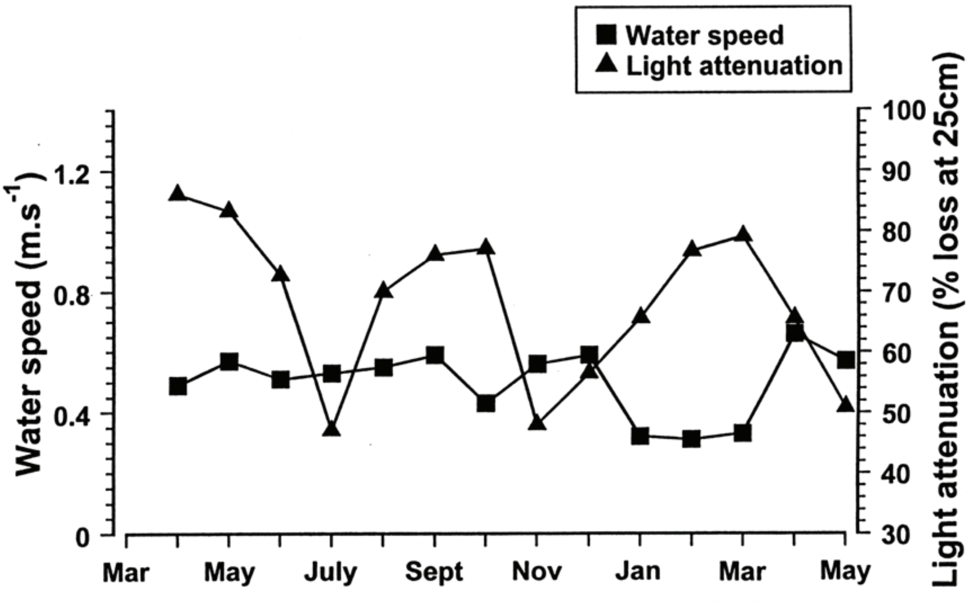


Fig. 3.3. Water speed and light attenuation measured at Site 1, Breede River.

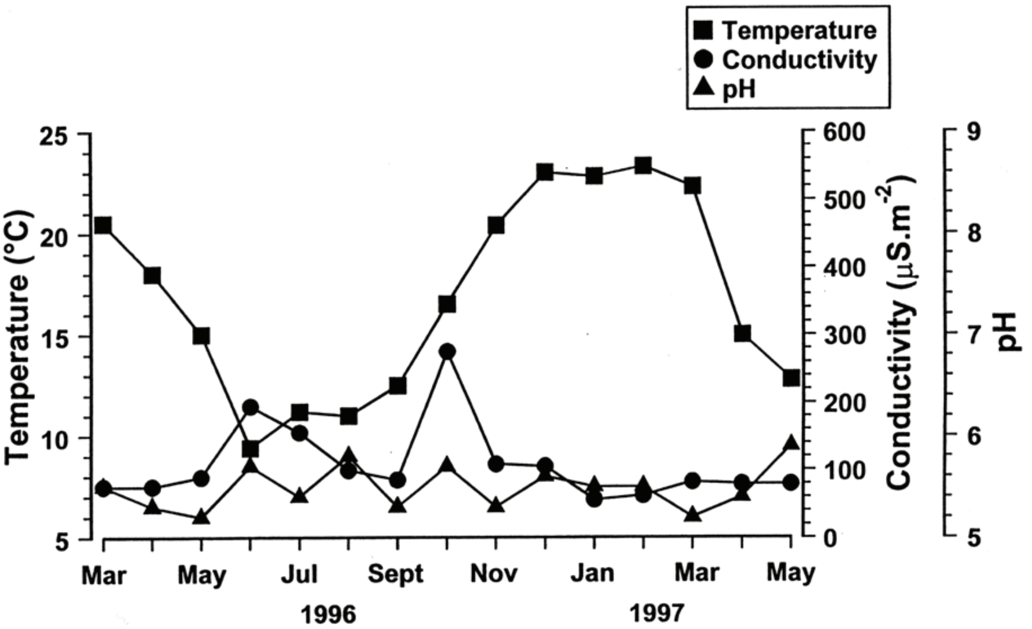


Fig. 3.4. Temperature, conductivity and pH measured at Site 2, Breede River.

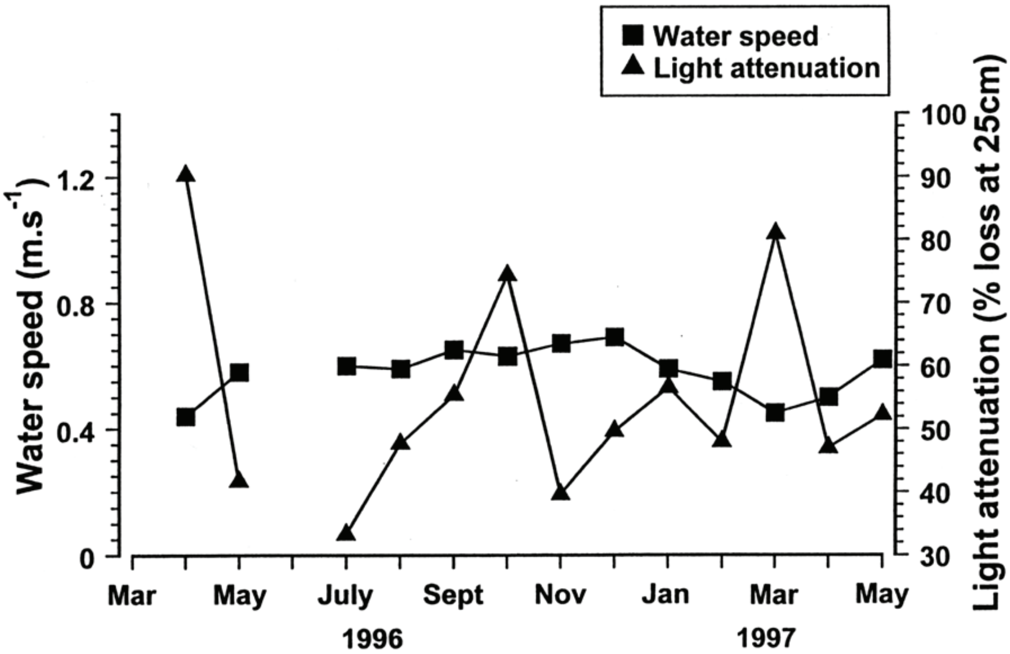


Fig. 3.5. Water speed and light attenuation measured at Site 2, Breede River

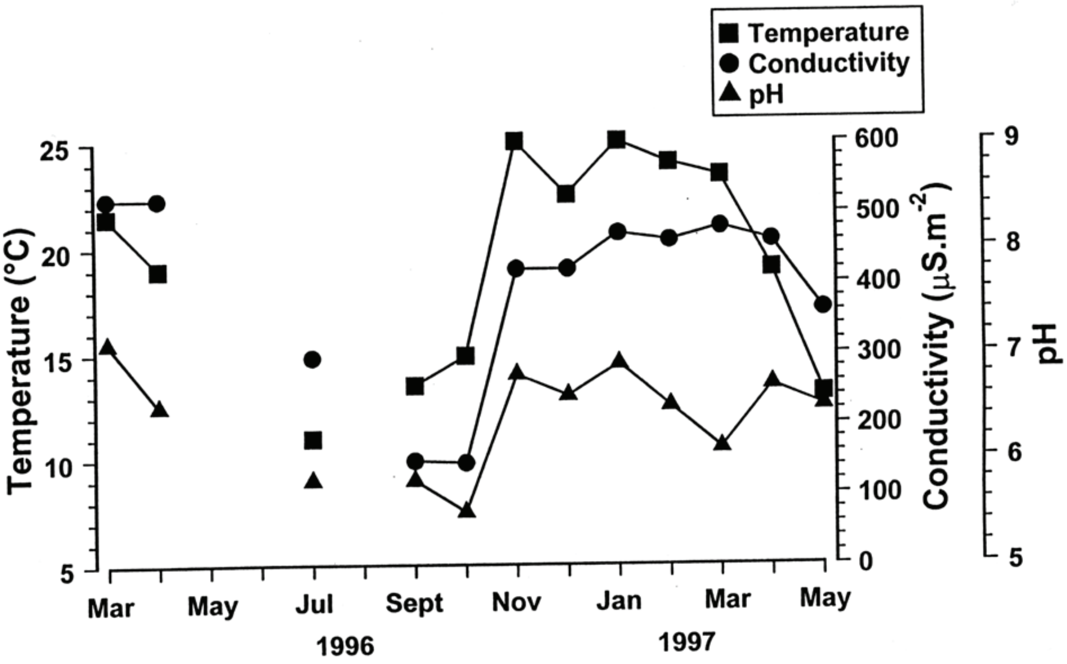


Fig. 3.6. Temperature, conductivity and pH measured at Site 3, Breede River

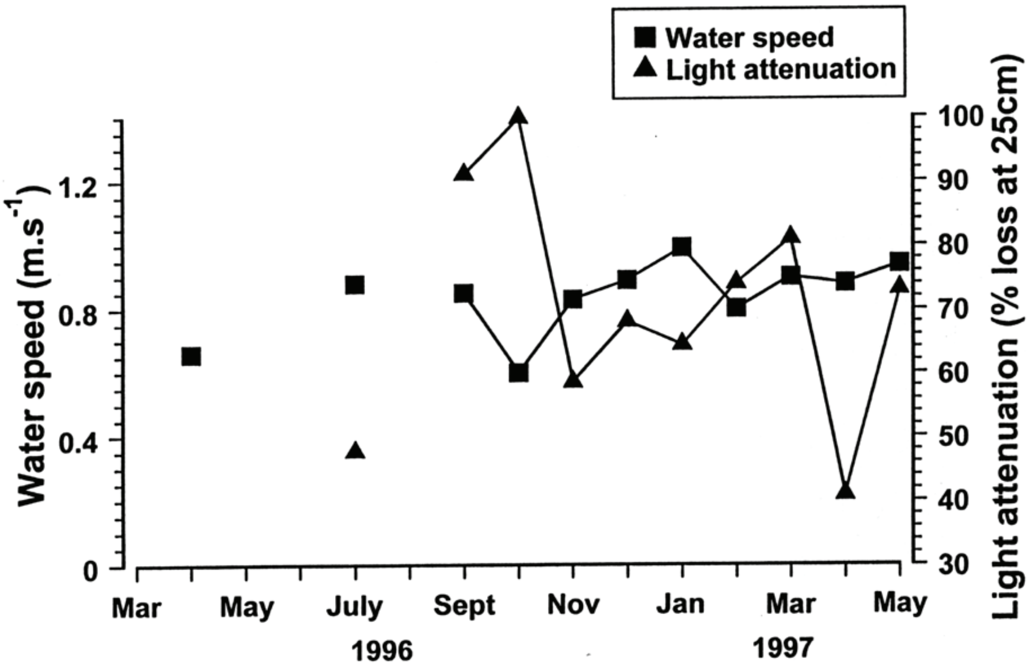


Fig. 3.7. Water speed and light attenuation measured at Site 3, Breede River.

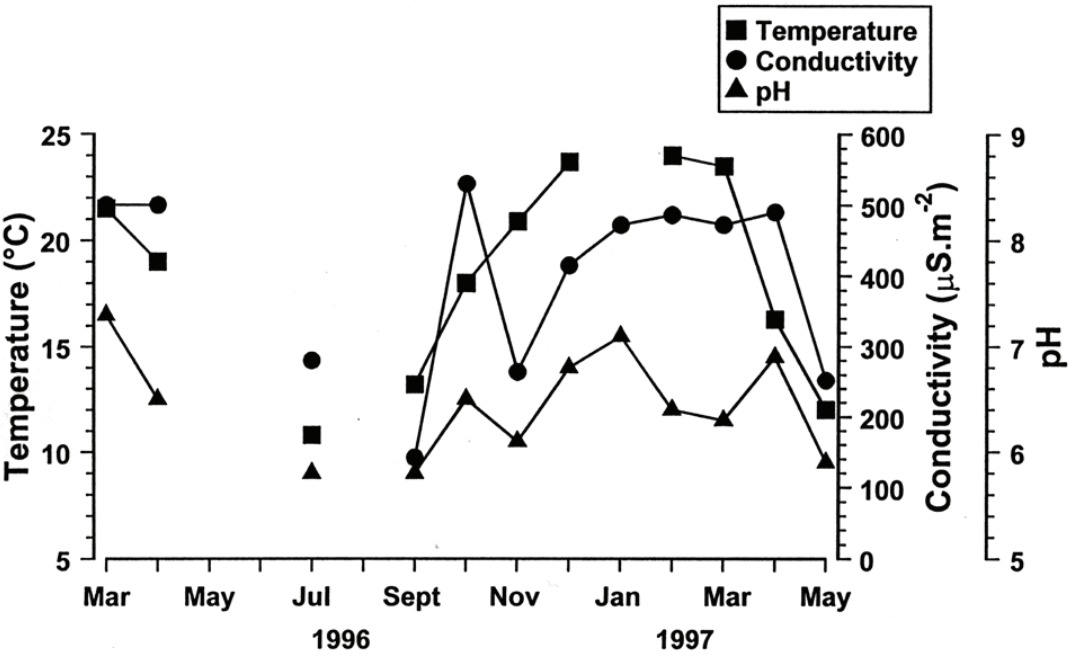


Fig. 3.8. Temperature, conductivity and pH measured at Site 4, Breede River

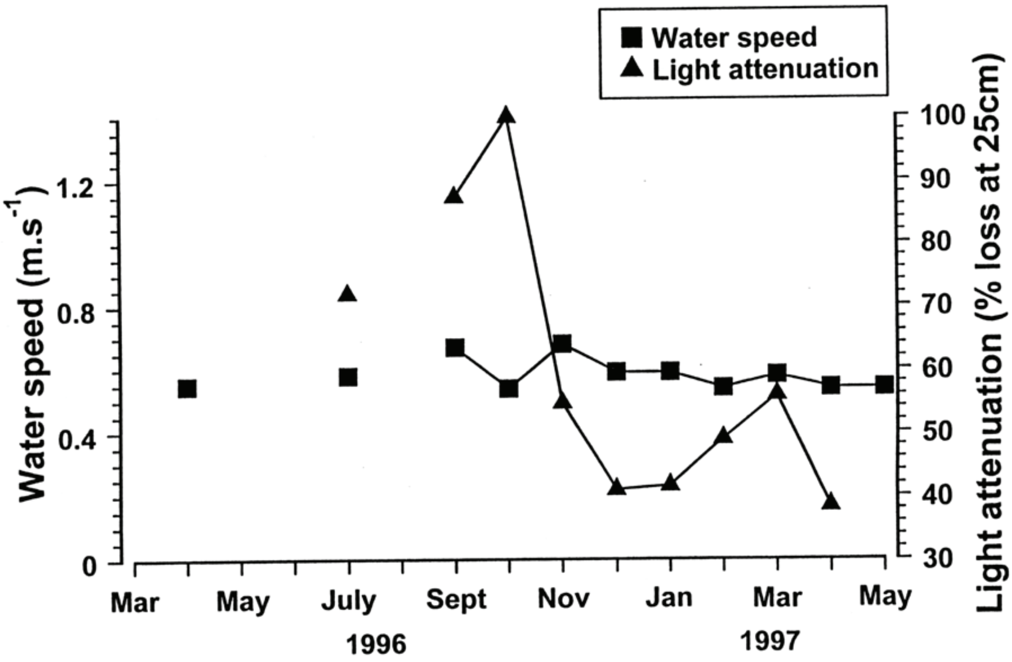


Fig. 3.9. Water speed and light attenuation measured at Site 4, Breede River.

Site 2 – La Chasseur lower

Figure 3.4 shows the temperature, conductivity and pH measurements as recorded during the sampling period. The temperatures mirror the readings as obtained for the top of this canal with summer seasonal increases. The conductivity measurements also mirror those of the top of the canal except for that of October, 1996 when it was 275 µS.cm^{-1} compared to 68 µS.cm^{-1} for the top of the canal. pH measurements remained constant and within a narrow range during the sampling period.

Figure 3.5 shows the water speed and light attenuation figures. These measurements were not taken in June, 1996 as the water was only 3 cm deep in the canal. Water speed remained fairly constant with a slight decrease (0.5 m/sec) from December, 1996 to March 1997 as was seen in the measurements from the top of the canal. Light attenuation figures reflected the same three peaks as seen in the top of the canal, but the peaks with steeper increases than those for the top of the canal, approximately 50 to 80% compared to 65 to 80% over a two month period.

Site 3 – Sanddrift top

Figure 3.6 shows the temperature, conductivity and pH measurements as were able to be taken at this site during the sampling period. In May, June and August the canal was dry, probably for cleaning purposes. The measurements from September, 1996 to May, 1997 indicate a summer increase and winter decrease as would be expected. Conductivity and pH measurements which were higher than those at the La Chasseur sites, showed a seasonal increase and decrease with temperatures.

Water speed and light attenuation percentages are shown in Figure 3.7. Water speed remained fairly constant with a range difference of 0.4 m/sec during the sampling period. Light attenuation showed a far greater range of difference with a 100% measurement in October, 1996, dropping to 40% in April, 1997.

Site 4 – Sanddrift lower

Figure 3.8 shows the temperature, conductivity and pH measurements for this site during the sampling period. As with the top site on this canal, May, June and August no measurements were taken as the canal was dry. The temperature measurements mirror the seasonal increases and decreases as found in the top site. Conductivity showed a marked increase in October, 1996 which then dropped again in November, but a steady increase and then final decrease in April and May 1997, related to seasonal temperatures is seen. pH measurements were similar to those of Site 3 with increases linked to temperature and conductivity measurements. Conductivity ranged from 110 to 520 µS.cm^{-1} over the sampling period with a marked increase from September to October in 1996.

Water speed and light attenuation percentages are shown in Figure 3.9. As would be expected water speeds are similar to those in Site 3, but the light attenuation figures, whilst showing the same peak in October, 1996 show a more marked and continued decrease from this month until the spring of 1997.

The algae found on the upper and lower rows of petri dishes, on the monitoring boards, are shown in the groups below. This sampling period reflects the algae found from March to December, 1996 and January to February, 1997. The algae listed are the main species observed using a compound microscope. Other aquatic plants found e.g. the moss, *Fontinalis antipyretica*, will be commented on in the discussion section. The percentage cover of the respective algae are shown as numbers for each month.

3.3.5. Discussion

The two different canals in this system show marked differences in most of the physical measurements and in the composition of the algal species found on the plastic monitoring plates. Biological monitoring methods are a major point of contention amongst research workers (Whitton, 1991), but the use of plastic petri dishes for algae has been used, apparently successfully, by John & Moore (1985) and John & Johnson (1991) in algal research in the United Kingdom. A factor which can complicate a sampling method is time. If sampling is done over too short a period the sample may not represent the range of possible species, but too long a sampling period may lead to competitive loss of species. Some algal sampling may be destructive in that the samples collected are not analyzed according to species range, but for chlorophyll *a* content. This latter method was not applicable to our project as the aim was to ascertain whether specific genera dominated (by percentage settlement) during a given period of time over a full years' monitoring. The settlement of the algae on the two different types of substrate surface showed no convincing consistency, but rather a general tendency for filamentous algae to settle on the roughened surface and the most dominant diatom, *Cocconeis* sp. to settle on the smooth substrate surfaces. Some overlap of surface settlement preference was always present and non-sedentary diatom species e.g. *Navicula* sp. were found randomly on both surfaces. Although the rim of the petri dish must afford some slight protection from an abrasive flow effect it is possible that the filamentous algae having less well developed rhizoidal bases may be swept off the smooth surfaces.

As may be seen from Figure 3.10, temperatures remained consistently similar over the full sampling period in both the canal systems at all four sites. Winter water temperatures did not drop below 9ºC and summer temperatures did not rise above 25ºC. Figure 3.11 shows the conductivities measured in the two canals over the sampling period. The Sanddrift canals have raised conductivities from the onset of the increase in water temperatures in September (spring) through the summer until May. In the La Chasseur canal system the conductivity does not increase above 200 µS.cm^{-1} whilst the conductivity in the Sanddrift canal systems rose to 500 µS.cm^{-1}.

In a previous project (Joska & Bolton, 1994) it was found that at the point just above the canal intake from the Breede River the very high conductivity of the confluent Kogmanskloof River influences the Breede water conductivity considerably. This Kogmanskloof River water reflects the poor quality of the irrigation return flow water (Coetzer, 1986). Figure 3.12 shows the pH values as recorded over the sampling period for all four sites. Apart from a sudden and unsustained increase in pH at Site 1 when the pH values were raised in the Sanddrift sites from September until March, at the end of summer.

Figure 3.13 shows the water speeds as measured at the four sites. All the canals had a fairly regular water speed except for a slight decrease at Site 1 (La Chasseur – top) from December until March. While to lower sites on both systems had a similar water speed throughout the sampling period, La Chasseur top site had the lowest water speed and Sanddrift top site had the highest water speeds. As mentioned previously the water is not turbid and a Secchi Disc is clearly visible at all depths. Since the temperature, light and water speed measurements do not show a large range difference in either canal system it is concluded that the dissimilar conductivity and pH values are the factors which cause the marked differences in algal species type and coverage in the canals. *Oedogonium capillare* is very rare in the Sanddrift canal. In April, a few filaments of this alga were found in the petri dishes at Sites 3 (lower), Site 4 (upper and lower). Although the total percentage cover of the petri dishes was similar in both canals at all four sites the major algae which formed this cover in the Sanddrift canal system were diatoms, mainly in the genera, *Cocconeis, Navicula, Fragilaria, Synedra* and *Gomphonema*.

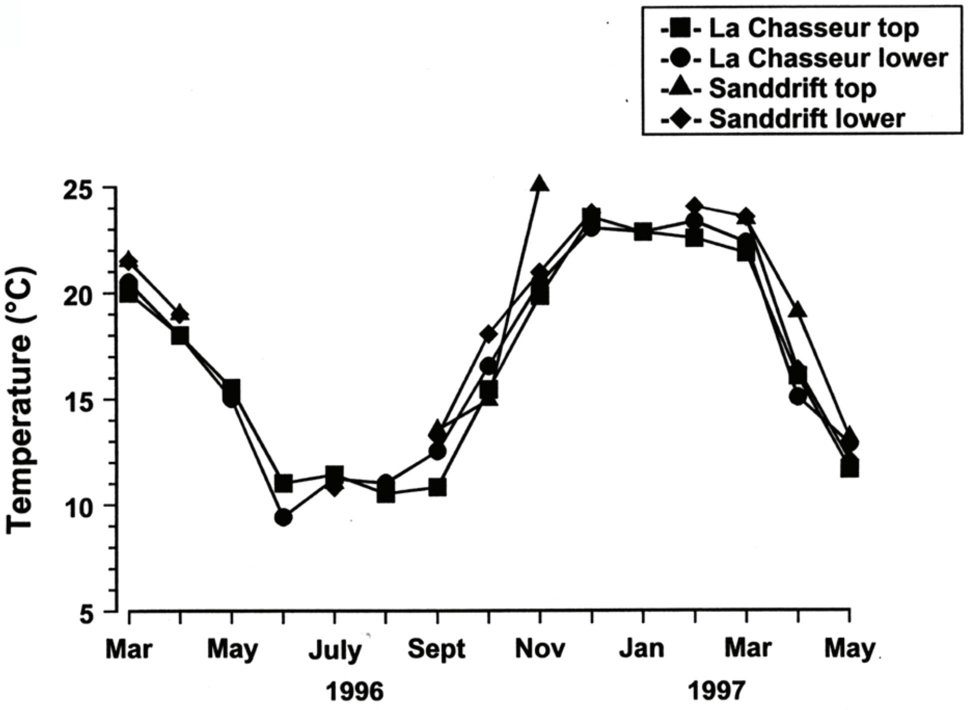


Fig. 3.10. Temperatures measured during the sampling period at all four sites, Breede River.

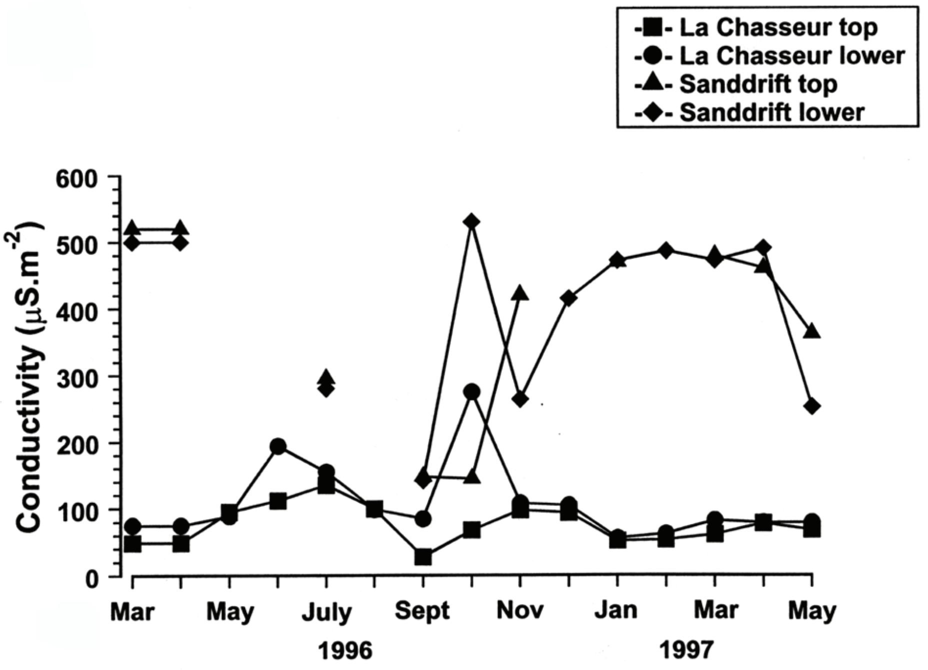


Fig. 3.11. Conductivities measured during the sampling period at all four sites, Breede River.

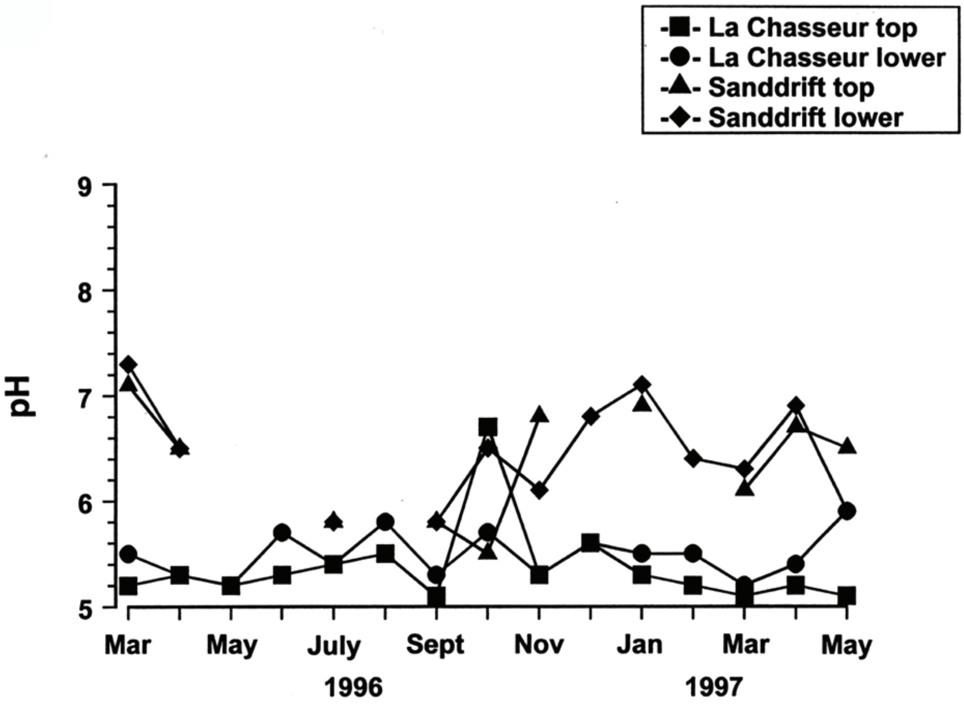


Fig. 3.12. pH measured during the sampling period at all four sites, Breede River.

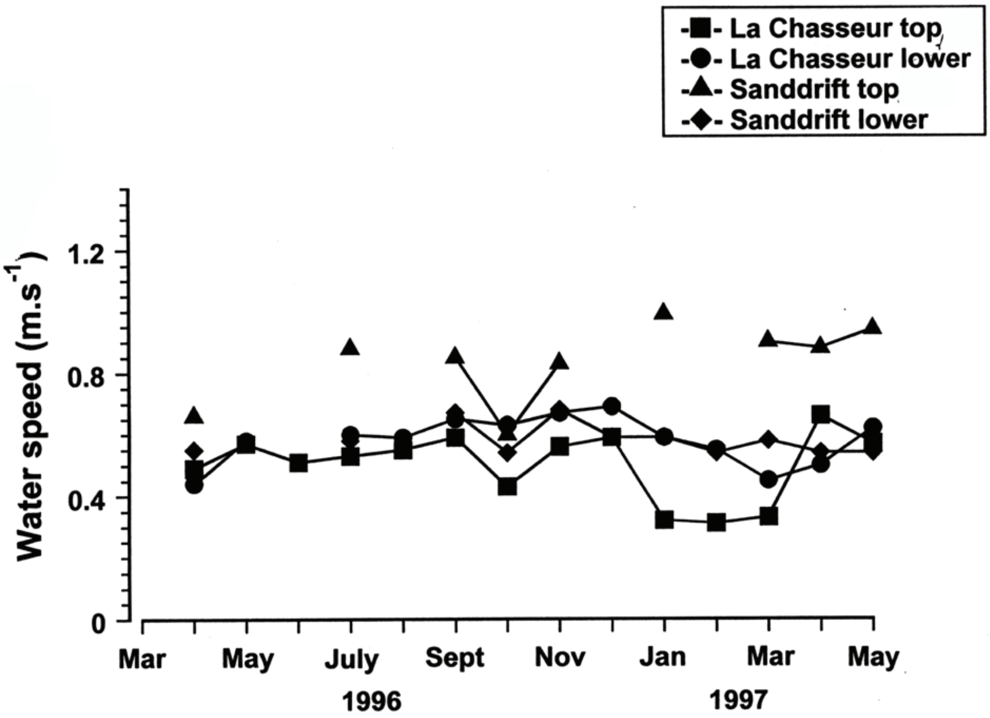


Fig. 3.13. Water speed measured during the sampling period at all four sites, Breede River.

At Site 4, some *Stigeclonium* sp. and *Protoderma* sp. were found in March and April in autumn. As this canal system was emptied in May, June and August it is difficult to make any assumptions on the presence of these algae.

Both canal systems were “dosed” with copper sulphate during the sampling period. Exact times and quantities of $CuSO_4$ used are not certain, but the bailiff on the La Chasseur system applies a “preventative dose” in spring, September/October. The bailiff at the Sanddrift canals informed us that he applied 25 kg of $CuSO_4$ every 3 km of the canal using the suspended bag method during September. At Site 1 the canal walls were covered, for most of the sampling period, with the aquatic moss, *Fontanilis antipyretica*. *O. capillare* was often found as an epiphyte on this moss. During the sampling period *O. capillare* was rarely found growing on the canal walls in the La Chasseur canal and in very small amounts, but *Stigeoclonium* sp. was found on the walls of Site 2 in May, July, August and September, 1996 and in January and February, 1997. During a previous project Joska & Bolton (1994) *O. capillare* was the alga which produced noxious growths in November in the La Chasseur canal system. Conductivity and pH measurements taken at that time were slightly higher than those measured at these sites during this project. There is little doubt that the regular and probably timeous dosages of $CuSO_4$, using the slow release bag method, control the growth of filamentous algae. The continued occurrence of diatoms, especially *Cocconeis* sp. appear to confirm their ability to colonise stable substrates repeatedly despite regular dosage with $CuSO_{4.}$ The occurrence of *Cocconeis placentula* to become the dominant species in mildly eutrophic water has been recorded in England (Kelly *et al*, 1996).

Table 5a. Showing algal growth during the sampling period on the upper plates at site 1 on the La Chasseur Canal on the Breede River.

Site 1 (upper)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare	*1*	*E*		*2*	*4*	*2*	*5*		*2*			
Stigeoclonium sp.	*2*	*X*	*5*	*1*	*2*				*10*			
Protoderma sp.		P		3	2	3						4
Desmids		O		2	3							4
Diatoms	15	S	18	24	25	14	10	6	40	15	20	20
Blue-green algae		E										
Total % cover	**18**	D	**23**	**32**	**36**	**19**	**15**	**6**	**52**	**15**	**20**	**28**

Table 5b. Showing algal growth during the sampling period on the lower plates at site 1 on the La Chasseur Canal on the Breede River.

Site 1 (lower)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare	1	2		3	3	1	10		2	2	3	1
Stigeoclonium sp.	6	2		2		2	1	1	2			
Protoderma sp.							1					
Desmids				3		10	12					
Diatoms	27	20	8	11	17	25	21	15	18	40	30	14
Blue-green algae		5										
Total % cover	**34**	**28**	**8**	**19**	**20**	**38**	**45**	**16**	**22**	**42**	**33**	**15**

Table 5c. Showing algal growth during the sampling period on the upper plates at site 2 on the La Chasseur Canal on the Breede River.

Site 2 (upper)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare	45	E	6	4			5	E	1	6	10	15
Stigeoclonium sp.	5	X		2		1	2	X	1		2	5
Protoderma sp.		P						P				2
Desmids	3	O						O				
Diatoms	15	S	22	15	15	25	40	S	35	20	30	5
Blue-green algae		E					1	E				1
Total % cover	**68**	D	**28**	**21**	**15**	**26**	**48**	D	**48**	**26**	**42**	**28**

Table 5d. Showing algal growth during the sampling period on the lower plates at site 2 on the La Chasseur Canal on the Breede River.

Site 2 (lower)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare	35	5	5	2			2	8	5	20	20	10
Stigeoclonium sp.	1		2	3			1			2		
Protoderma sp.												
Desmids									3	6	3	5
Diatoms	18	60	40	25	15	15	20	70	40	25	15	10
Blue-green algae												
Total % cover	**54**	**65**	**47**	**30**	**15**	**15**	**23**	**78**	**48**	**53**	**38**	**25**

Table 6a. Showing algal growth during the sampling period on the upper plates at site 3 on the Sanddrift Canal on the Breede River.

Site 3 (upper)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare			E	E		E						S
Stigeoclonium sp.			M	M		M	1					T
Protoderma sp.			P	P		P						O
Desmids			T	T		T						L
Diatoms	15	11	Y	Y	30	Y	40	50	15	5	2	E
Blue-green algae							20	30			2	N
Total % cover	**15**	**11**			**30**		**61**	**80**	**15**	**5**	**4**	

Table 6b. Showing algal growth during the sampling period on the lower plates at site 3 on the Sanddrift Canal on the Breede River.

Site 3 (lower)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare		1	E	E		E						S
Stigeoclonium sp.			M	M		M						T
Protoderma sp.			P	P		P						O
Desmids			T	T		T						L
Diatoms	35	40	Y	Y	30	Y	35	50	5	8	5	E
Blue-green algae	2											N
Total % cover	**37**	**41**			**30**		**35**	**50**	**5**	**8**	**6**	

Table 6c. Showing algal growth during the sampling period on the upper plates at site 4 on the Sanddrift Canal on the Breede River.

Site 4 (upper)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare		1	E	E		E	E				E	
Stigeoclonium sp.		10	M	M		M	X			1	X	3
Protoderma sp.	10		P	P		P	P				P	
Desmids			T	T		T	O				O	
Diatoms	50	60	Y	Y	30	Y	S	25	40	50	S	20
Blue-green algae							E				E	
Total % cover	**60**	**71**			**30**		D	**25**	**40**	**51**	**D**	**23**

Table 6d. Showing algal growth during the sampling period on the lower plates at site 4 on the Sanddrift Canal on the Breede River.

Site 4 (lower)	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Oedogonium capillare		1	E	E		E						
Stigeoclonium sp.			M	M		M	5					
Protoderma sp.	5		P	P		P						
Desmids	2		T	T		T						
Diatoms	40	60	Y	Y	22	Y	70	65	45	22	15	20
Blue-green algae	2											
Total % cover	**49**	**61**			**22**		**75**	**65**	**45**	**22**	**15**	**20**

In February, 1997, at Site 4, *Cladophora glomerata* was found in sparse clumps growing at the bottom of the canal. We also found an *Oedogonium* species with a filament diameter of 10 µm and *Spirogyra* sp. The lowering of the quality of the water in the Breede River with the irrigation return flow and poor rainfall for the last three years will possibly lead to noxious growths of other algae which are already known to be present in the Breede River system. These algae are *C. fracta*, *Enteromorpha* sp. and *Compsopogon coeruleus.* Also the Simulid larvae, which were found to form massive coverage on the sample dishes and the canal walls in the Sanddrift system in January and February. This larva develops into pest swarms of black fly which are a source of great irritation to stock and man alike.

3.4. Culture experiments on *Oedogonium*

3.4.1 Introduction to experimental methods and rationale

The species of *Oedogonium* causing a problem in the Breede River canal systems (the most common species having been identified as *O. capillare* from occasionally fertile specimens) proved very difficult to grow in culture. We had difficulty keeping it alive in culture for more than a few days. It was decided to carry out short term experiments on the *Oedogonium*, while concentrating our efforts on *Cladophora glomerata*, as the latter is by far the most serious problem in South African canal systems. *Oedogonium* is only a temporary problem in spring in the Breede River system, and has never been mentioned as a problem elsewhere in South Africa.

In preliminary experiments carried out over short time periods (a few days), the *Oedogonium* survived and grew best at 10 and 15°C, but dying rapidly at 20°C and higher.
A rapid assay technique for copper tolerance was thus developed, and used to compare the tolerances to elevated copper concentrations of various green filamentous algae, including *O. capillare* and *C. glomerata*.

The rapid bioassay method (described in more detail in Privett 1994) was derived from a method originally developed for assay of the copper tolerance of marine filamentous ship-fouling algae (Goodman *et al*. 1976). The technique is based on the idea that copper will kill some or all of the cells of the alga. This relative cell death can be measured by demonstrating which cells in the algal filament have damaged cell membranes. The method of Goodman *et al*. (1976) is based on the premise that living plant cells plasmolyse in hypertonic solutions (osmotic potential differences cause water to leave the cell, and the cell membrance to visibly shrink away from the cell wall), whereas dead cells do not. The method involved treating the algae with a range of copper concentrations for a brief period, followed by immersion in a strong sugar solution, the latter to identify the relative numbers of live and dead cells in the filament.

3.4.2 Methods

The method for the rapid assay was as follows:

1. Roughly equal sized samples (ca. 250mg) of the algae were separated with forceps and washed in distilled water.
2. These were transferred to one of seven 20 ml petri-dishes (one filled with distilled water as a control, the others filled Woods Hole medium at pH 6.5 (without EDTA) containing copper concentrations of: 0.1, 1, 2.5, 5, 7.5, 10mg l^{-1}). The experiment was run in triplicate.
3. After one hour the filaments were removed from the copper solutions and place in 50 ml of fresh Woods Hole medium in glass flasks gently shaken. (Note: the optimum period of one hour in the copper solution was the result of preliminary experiments; see Privett 1994).
4. After one hour on the shaker, the filaments were removed from the Cu, rinsed with distilled water, damp dried on filter paper, and transferred to petri-dishes containing 33% w/v sucrose solution.
5. After 10 minutes, the material was mounted on a microscope slide in the sucrose solution, and the number of plasmolysed and unplasmolysed cells counted under a compound microscope. 50 cells were scored in 5 random fields of view.

The results were statistically analysed using a Type 1, two-factor Analysis of Variance, and the Tukey test for determining significant differences between populations means.

Four species of green algae were tested with the rapid assay for comparison of their copper tolerances:

- 3 species from the Breede River canal system: *O. capillare*, *Stigeoclonium tenue*, *Spirogyra* sp.
- *Cladophora glomerata* from Kalkfontein.

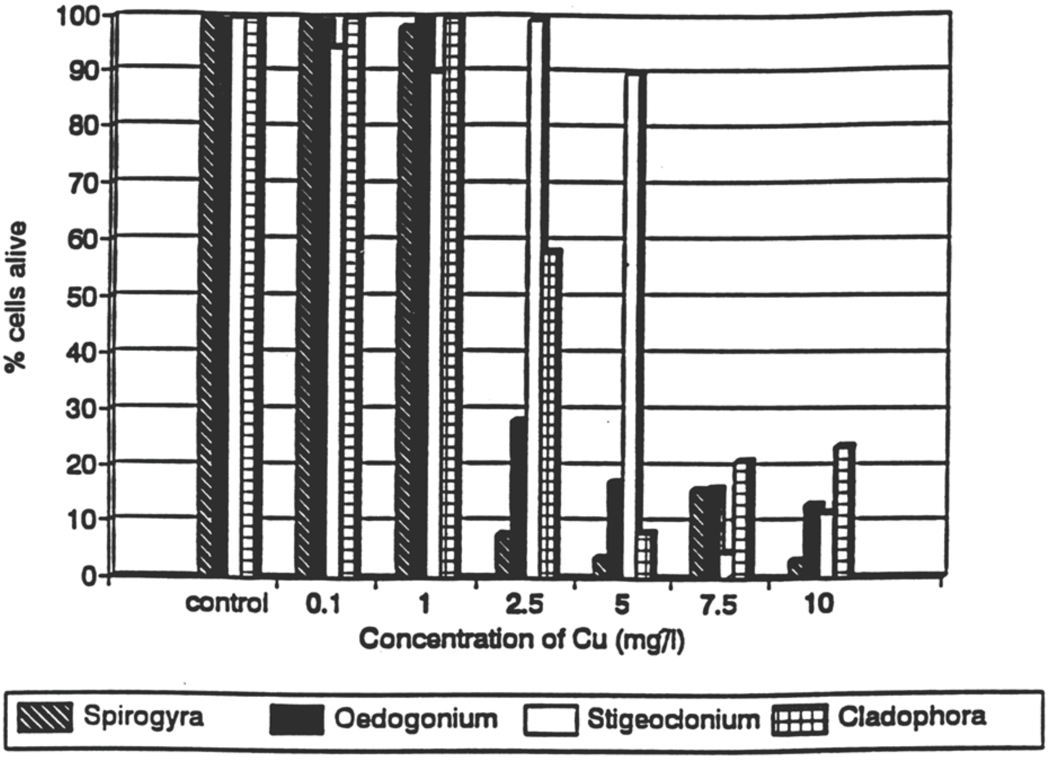


Fig. 3.14. Damage caused to four filamentous algal species exposed to copper for one hour at the various concentrations.

3.4.3 Results

A copper concentration of up to 1µg l^{-1} had little effect on any of the four species, with just about all cells surviving (Fig. 3.14). A concentration of 2µg l^{-1} killed most cells of *Oedogonium* and *Spirogyra*, and 40% of the *Cladophora* cells, but had little effect on the *Stigeoclonium*. 5µg l^{-1} Cu again had little effect on *Stigeoclonium*, but killed almost all cells of the other three species. 7.5µg l^{-1} Cu and above killed more than 75% of the cells of all four species. The results for *Stigeoclonium* were significantly different from those of the other 3 species (Tukey's test), although data for the other species was not significantly different.

3.4.4 Discussion of *in vitro* findings

Although it did not prove possible to grow *Oedogonium capillare* for any length of time in culture, preliminary experiments suggest that it has a rather low temperature tolerance. Relative growth rates of over 0.05 per day were obtained at 10 and 15°C over a few days using cut segments. The growth ability over this range ties in with the habitat of the species, generally producing large growths in the canal system in winter and spring (usually July-September) when the water temperatures are relatively low, and is different from the higher temperature tolerances of *Cladophora glomerata*. It is very likely that blooms of *O. capillare* in the Breede River system are inhibited by high summer temperatures, and thus possible that copper treatment need not be continued throughout the summer in this system.

The rapid essay test reveals that this species of *Oedogonium* is very sensitive to copper treatment. Indeed by this technique *Oedogonium* is at least as sensitive as *Cladophora glomerata*, backing up the empirical evidence of the success of Cu treatment of *Oedogonium* blooms in the Breede River canal system.

4. General Discussion

4.1 Noxious algal growths in South Africa and other countries

Although noxious algal growths must have occurred in rivers, dams and controlled water flow systems in South Africa since the growth of the larger towns and cities, few published reports on their appearance exist. Real concern over the problems caused by these algal growths, especially *Cladophora glomerata*, provoked minor research in the 1980's when the irrigation schemes fed by the large dams built in the 1970's were negatively affected by these growths. Early reports by Bruwer (1980), Prinsloo (1985) highlighted the problems caused by *C. glomerata* but were not pursued with any marked results. Research into the problems caused by *Microcystis aeruginosa* in the Haartbeesport Dam were published by Zohary & Breen (1989) and Zohary & Madeira (1990). Lack of accurate/regular record keeping has undoubtedly been the cause of some confusion as to when and where these algal growths occur. The report by du Plessis and Davidson (1996) lists the major water schemes/irrigation systems and the extent of the algal problems affecting them. One of the concluding recommendations made in this report stresses the importance of monitoring programs. The noxious growths of *Oedogonium capillare* which occurred in the Breede River canals have not been reported elsewhere and, it would seem, are more readily controlled not only by chemical but physical conditions. Some noxious growths of *Chara* sp. were found to occur in the irrigation canals near Vredendal in the Western Province, but their appearance is not regular.

C. glomerata has been the source of major problems in freshwaters worldwide as indicated in the reviews by Whitton (1970) and Dodds and Gudder (1992). Especially well researched was the major noxious growths of *C. glomerata* in the Great Lakes in North America. A large portion of the research results from the Great Lakes research was published in Journal of Great Lakes Research, Vol. 8, Number 1 in 1982. However, Herbst predicted the problem in 1968. Whereas most noxious algal species occur in either still or running waters, *C. glomerata* is one of those algae which can occur in fast flowing and very slow flowing water conditions. The alga, *Pithophora oedogonia* has produced noxious growths in some lakes in the United States (Lembi *et al*,1997) but its growth is restricted to lakes and dams. Although *P. oedogonia* has not been recorded in South Africa a species from the same family, *Rhizoclonium* sp, is commonly found in large growths in dams in the Western Cape.

4.2 **Comparison of control methods – suitability, cost, effect and result**

Basically there are three methods of control – biological, mechanical and chemical. All three methods are used worldwide according to opinions, labour availability and cost.

4.2.1 Biological control methods may make use of –

4.2.1.1 Competitive plant growth where the competing plants utilise the same nutrients which are available, but are able to grow faster and use the available nutrients faster.

4.2.1.2 Competitive plant growth where physical parameters of the water (e.g. pH) or chemical parameters of the water via allelopathic effects (McCracken *et al* 1980) are altered, thus affecting the growth of the original plants.

4.2.1.3 Competitive plant decomposition where the enzymes, or other substances, released during the decomposition process may inhibit the growth of the original plants. This is the effect of barley straw decomposition (Pillinger *et al.* 1994) where the lignin which is released by the barley straw produces a substance (possibly a peroxidase) which affects the growth of the algal cells.

4.2.1.4 Grazing by herbivores such as invertebrates (McAuliffe, 1998) or fish (Gallagher& Haller, 1990).

4.2.1.5 Infection and resultant algal cell destruction by fungi. Such as the effect of the fungus *Acremonium kiliense* on the alga *Cladophora glomerata* (Patrick *et al*, 1983).

Suitability, cost, effect and result of biological methods

Environmentalist groups lobby vociferously for the singular use of this method of algal control. However, many methods can only be used in certain situations and are not effective on all algae. Competitive plant growth effects are a new field of research and have not been used purposely as a control method to any large extent that we are aware of. The commercial production of the allelopathic substances produced by some aquatic plants would seem a worthwhile avenue of research. Research of these substances would be costly however. Manipulation of the physical/chemical parameters of the water has been used with moderate success. When huge noxious growths of *Microcystis aeruginosa* occurred near Kommetjie on the Cape Peninsula, large amounts of salt were used in the water to raise salinity levels and discourage further growth of this plant. Costs involved were moderate, but the long term effects of altering the natural water salinity have not been assessed.

Some care must be taken with the preparation of barley straw where the straw must be loosely packed in net bags to prevent it floating away and blocking outflow vents etc. It takes approximately six weeks for the barley straw to decompose before the enzymes are produced and some algae are not negatively affected by the enzymes in the decomposing barley straw (Dr.D.John *pers.comm.*, and this study). Barley straw has only been found as effective in still or very slow moving waters and thus it would be of little use in fast flowing irrigation canals as was found when bags were placed at the water outflow point below the Kalkfontein Dam. Barley straw is relatively inexpensive and has not been shown to have any detrimental effects. The evaluation of barley straw for the control of algal growth in controlled water areas in South African was investigated by Du Plessis & Du Toit (1996).

Grazing by invertebrates and vertebrates (especially the Chinese Grass Carp, *Ctenopharyngod*on *idella* or other carp species) only control the plant growth to a certain level. Also, Grass Carp are thought to feed on aquatic macrophytes only, not algae. In irrigation canals no visible algae are acceptable as the smallest filaments may block fine jets at the spray outlets. It is likely that the long term usage of copper sulphate has eliminated any invertebrates which may have inhabited the original algal growths. The introduction of exotic fish species to control algal growths is problematic and expensive. Weather and physical conditions of the water may differ negatively from their original habitat and cause seasonal death. More importantly, introducing an exotic species often produces very negative effects on indigenous animal species (Gallagher & Haller, 1990).

Fungal pathogens have been found to negatively affect the growth of some algae, but in order to have a usefully large effect the pathogen would have to occur in widespread growths. This has not been seen in natural conditions and investigation into the culture and effective innoculation of the pathogen into the "target" plant should be carefully researched. The introduction of exotic pathogens is dangerous and could infect plants other than the original noxious alga (Bott & Rogenmuser, 1980).

The introduction of supposedly "safe" biological control agents could upset the ecological balance and, as with any control methods these too should all be critically evaluated.

4.2.2 Mechanical methods may be those in which –

4.2.2.1 The large growths of noxious algae are harvested either by manual or mechanical means as described by Bruwer (1980) where *C. glomerata* was originally controlled by this method in the Hartbeespoort and other irrigation canals.

4.2.2.2 Water drawdown is used (Blinn *et al*. 1995). The water level, in controlled flow situations such as irrigation canals is dropped so as to expose the algae for a period of time, thus causing a reduction or even cessation in growth. Natural drawdown effects, with the resultant negative effects on algal growth, have been reported on by Power (1992).

4.2.2.3 Mechanical shading effects by using shadecloth or other methods of reducing light (Andrews `. 1986) have only been partially successful as was found in South African irrigation canals (Bruwer *pers.comm*.).

Suitability, cost, effect and result of mechanical methods

These methods are often very suitable and, in the case of manual methods, can be especially effective in small irrigation canals. However, during the time that the canals are cleaned the water flow is suspended and this may create some problems especially if it is during a crop development period. Mechanical cleaning of the Hartbeespoort canals is currently estimated at

R250 000 per annum (Du Plessis and Davidson,1996). The length of the canals and the change in width and depth also require a number of different types of machines to be used. Labour is used where available, but is also expensive.

Water draw down is a promising and fairly new method of algal control. Water flow rates are decreased but not stopped altogether, therefore there is no great inconvenience to irrigation routines. Another aspect of this drawdown effect is, that if done at suitable times, the algal growths are not only left without water cover, but are also exposed to a marked drop in temperature at night.

Ideally, irrigation canals would not have algal growth (or other plant growth) if they were covered. Unfortunately, the permanent covering of the present canal system would be totally inhibitive in cost. The planting of shade trees alongside the canals would also markedly reduce the light, but the trees would have to have passive root systems so as not to interfere with the concrete canal walls and would require attention in the early stages of growth. Covering the canals with shadecloth would only be practical where canal widths were fairly narrow and there is always the problem of illegal removal.

4.2.3 Chemical controls of various types are far more widely used –

4.2.3.1 Chemical applications give a rapid and seemingly effective result and require less labour for dosage.

4.2.3.2 Although trademark chemicals such as Diquat®, Paraquat®, Endothall®, Roundup® and Touchdown® are registered for use in freshwater, by far the most common and comparatively inexpensive chemical used is copper sulphate.

4.2.3.3 Some other chemicals have been proposed and used for algal control, such as sodium arsenite (Chancellor, 1958), potassium permanganate (Fitzgerald, 1966), the swimming pool chemical HTH (Steynberg & Pieterse, 1994) and, as proposed by some Russian scientists in Kiev (Merechko *et al*, 1991), excessively heavy application of nitrogen fertilisers to irrigation water.

Suitability, cost, effect and result of chemical methods

Chemicals can be readily obtained and their application can be effected without immediate obvious evidence. This occurred during the time that we were doing field work on the Orange and Riet River Irrigation Systems. Copper sulphate is not expensive and can be applied without public knowledge. This surreptitious dosing with chemicals could cause harm to animals and people who may use the water which has been "dosed". It must be borne in mind that most all chemicals will have only a short term effect on the growth of algae. Algal growth will reoccur without regular redosing. Unfortunately, all irrigation system water in South Africa has a return flow into natural rivers. This then ensures that most of the chemicals applied to kill algae in a water managed situation will appear in unmanaged water situations.

Trademark chemicals are relatively expensive compared to the cost of copper sulphate, but some have been especially developed with more environmentally sensitive "target" species and a short effective life. Unfortunately, all chemical dosing has a very negative effect on any animals in the water. The continued application of the chemicals has "killed" certain rivers as has been found in Europe. Whereas animals are killed by the application of chemicals it has been shown that certain algal species become resistant to chemicals and develop strains which persist in growth (Whitton, 1970).

Now, in many countries in Europe and the United Kingdom, there is an extremely vigilant control on the use of all herbicides. The development of environmentally acceptable herbicides is very expensive and lacks impetus whilst the relatively inexpensive dosage with seemingly harmless chemicals continues (Gallagher & Haller, 1990). The application of large amounts of nitrogenous fertilisers to irrigation water which appears to limit algal growth is interesting (Merezhko *et al*. 1991). It is suggested that farmers would not need to fertilise crops as the suitable fertilisers were already present in the irrigation water. However, not all crops have similar fertiliser requirements and, according to the irrigation method, there would be an increased number of salts in the soils, and of course increased nutrients in natural water courses into which the unusued canal water would flow. Salinisation is already a serious problem in certain areas in South Africa (Coetzer, 1986).

Copper sulphate should be applied to water which has a pH of approximately 6. However, most South African waters tend to be alkaline with pH's of >7. Thus the successful application of copper sulphate is carried out with a pre-dosage of sulphuric acid which reduces the pH of the water. The procedures used in this type of copper sulphate dosaging is described in detail by Du Plessis (1992a; b). Various control options for algal and aquatic plant control in the Madison Lakes in North America, but which apply to most countries are described by Andrews *et al*. (1986).

4.3 Recommendations for future research

4.3.1. The major thrust of future research in South Africa should be applied to the reduction and if at all possible to the elimination of the original sources of nutrient pollution *viz*. nitrogen and phosphorus. This is a very difficult problem to address as there are commercial, industrial and agricultural sources of pollution.

4.3.2. A regular and simple form of monitoring, not only of rivers, but also of irrigation canals would be of great advantage in algal control. If monitoring programs could be set up and algal development monitored then the massive growths which presently cost so much to remove would not develop. Experimental drawdown could be used when algal growths were a few centimeters long, but this would only be possible if accurate monitoring was carried out. The cost benefit of this would be well worth the effort. In 1992 in Australia only the massive blue-green blooms which occurred in the Murray-Darling River system, and threatened the drinking water supplies, caused enough concern to provoke the founding of a national Algal Management Working Group (Creagh, 1992).

4.3.3. It was noticed that the joining sections of the concrete canal walls were sealed with a bitumen product. No algae grew on this bitumen. Research into the reason for this and a possible practical application thereof should be investigated.

4.3.4 Some canals were very irregularly cleaned whereas others received a regular biannual cleaning. These latter canals had notably fewer algal growths than the former. It is possible that the drawdown effect was also taking place in these latter canals. Investigation into the advisability or not of regular cleaning should be undertaken.

4.3.5 Natural rivers, natural and man-made water impoundments and irrigation canals require, in some aspects, different methods of control. Research into where these differences occur and how control methods should differ should be undertaken and become part of a training program for those people involved in water work.

5. Conclusions

Our knowledge of freshwater algae in South Africa is relatively limited and early workers such as Miss Edith Stevens and Miss M A Pocock did not pursue their initial research into these plants. Research has only become a priority since the massive growths of *Cladophora glomerata* caused problems in the Hartbeespoort Irrigation System. The findings of this project seem to indicate that closer attention by way of on site monitoring and the taking of accurate records of occurrence will go some way to enhance our knowledge of the noxious growths of *C. glomerata*. In time, the continued use of chemicals will affect natural systems into which most irrigation water flows. The demand for potable water will certainly increase and this will become prohibitively expensive to produce if purification methods are to be extended to exclude introduced chemicals. Biological control methods are far more difficult to pursue in fast flowing water conditions, such as irrigation canals. Canal cleanliness and maintenance should be carefully monitored as are the “first line of defence” against algal infestations. No algae can be permanently removed, but their occurrence can be curbed to easily manageable levels. During the course of this project it has been noted that the relevant authorities in the Department of Water Affairs and Forestry are making an active and impressive effort to combat noxious algal growths in water bodies under their jurisdiction. Hopefully, this enlightened approach will spread to other relevant authorities and those members of the public sector who can effect eradication of one of the original problems, eutrophication.

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Appendix I: Recommendations for algal control in managed water supply systems

1. Introduction
2. Algal growths – why, when, where?
3. Control options.
   a. Monitoring – first line of defence.
   b. Manual and mechanical
   c. Chemical
   d. Biological
4. Conclusions.

1. Introduction

Algae are best described as plants which do not have flowers and which generally grow in water. Some plants which do have flowers may also grow in water, either attached to the bottom of the river or dam, or free floating. These plants are called aquatic angiosperms and can, as do some algae, cause major problems in managed water supply systems. Algae may be obvious growths, visible to the naked eye, or so small that a microscope with a powerful magnification is needed to see them. Algae, as all the other plants and animals, are grouped into like types and a brief description of the most important ones that cause problems in water systems is given here so that their position in the plant system levels becomes clear. Algae were one of the earliest forms of life on earth and the first so called “primitive” algae were closely related to what are today commonly called, blue-green algae. The term “blue-green” comes from their usual colour as seen under the microscope.

Blue-green algae have fossils which are three billion years old and they are often considered as the main link between bacteria and plants. Blue-green algae have no obvious chloroplast within their cells, but do photosynthesise and produce oxygen and even more importantly they can fix nitrogen from the atmosphere. They can occur as single cells or colonies of single cells or filaments of single cells. Although very small they occur in such large numbers that their group appearance is often noticeable to the naked eye. They may colour the water green, red or black and sometimes produce a characteristic musty odour. Unfortunately, they may also produce toxins which can kill livestock and cause medical problems for man as a result of direct contact. In South Africa the blue-green alga, *Microcystis aeruginosa*, has occurred in massive noxious blooms and the water in dams may look like pea soup.

Single-celled algae in the group Chlorophyta (green algae) can form large growths which may colour the water a strong dark green. These are usually phytoplankton (free floating individual plants) and their noticeable appearance often indicates a nutrient enrichment of the water by a number of means. These points of enrichment will be discussed later. A few filamentous algae, also in the Chlorophyta, can form noxious growths and the commonest of these in South Africa is probably *Cladophora glomerata*. There are also the algae, *Chara* and *Nitella* which have "roots" and some of these are also known to cause problems in irrigation canals in South Africa.

Algae,like any other plants, require nutrients for growth. These major nutrients are composed from carbon, hydrogen, nitrogen, oxygen and phosphorus. When these nutrients are supplied in super abundance as from sewage outflows, agricultural seepage and domestic effluent the algae, and in actuality certain noted species, grow at an above normal rate, inhibit the growth of other species and form what are termed as "blooms" which may choke an entire river or dam and cause immense problems in managed water systems. Managed water supply systems differ from normal water systems in a number of ways. Normal water systems are here considered as natural rivers and streams. In actual fact all rivers, after the point at which they are first dammed, become "managed" as natural flow patterns are altered. Thus, there are very few parts of rivers in South Africa which can be considered as natural.

In managed water supply systems the water is directed from its normal path into a man-made channel. This channel may be directly in the earth or further modified and lined with concrete. Water may be driven along this channel by means of gravitational flow or by physical force e.g. pump. Compared to a natural system, these channels, especially those which are made of concrete, force the water along an unchanging regular path at a regular pace with no normal bankside vegetation. Thus, managed water supply systems substantially change, by rigid regulation, the physical effects on the water but not the chemical effects. This imbalance in effects leads to a disturbance in the normal ecological controls of the organisms which occur in the water. Managed water supply systems have been an essential part of effective organised agriculture for centuries in many countries. Although the negative effects of these managed water systems are now recognised, ways in which these effects can be reduced have only been the subject of research for the last 10 to 15 years.

2. Algal growths – why, when, where?

Algae, in their different forms, are one of the most important groups of organisms whose presence is essential in all water systems. Algae are the primary food source for other organisms in the food chain. Algae, in the process of photosynthesis, produce oxygen which is vital for life for most other organisms. Algae, apart from being a preferred food source for some animals, form growths in which animals can hide to avoid predators and in which the harsh effects of water flow are moderated. In natural systems there is a balance between algal growth, grazers and chemical and physical effect. In modern terms this is called “ecological balance”. In managed water systems these checks on algal growth are often lacking and, there is the further increasingly common problem of nutrient enrichment leading to what is called, eutrophication. This nutrient enrichment is usually the major reason why algal growths become such a problem, but it has been said that “nutrient supply determines how much can grow, but other factors determine the if, the what and how fast” (Pickup, 1992).

When do algal growths occur? This is often determined by increases in temperature and light *viz.* in the spring, or when the high temperatures of summer are decreasing *viz.* in autumn. In natural river or lake conditions there are often changes in water levels and velocities during the seasons and these changes will often negatively affect the growth of algae. In managed water systems there is usually a regular level and flow of water thus allowing algae to grow in the same position throughout the seasons. If water temperatures are not extreme and light levels never drop below a necessary minimum then growth of certain algae will continue throughout the year. Most of the “problem” algal species can reproduce sexually, asexually or vegetatively thus giving them a wide range of opportunity for constant establishment on a suitable substrate.

Where algae establish themselves and grow is often very varied in the constantly changing natural situation. However, in managed water systems, especially where there are concrete or other solid substrate canal walls, there is a constant availability of same settlement space. There are often limited changes in physical and chemical water conditions and there are usually few if any changes in species which will establish themselves. A change in conditions could lead to the growth of a competitor, but nuisance algal species are often able to grow in a wide range of conditions not suitable for a potential competitor. They can outgrow competitors and so limit available nutrients and cause light inhibition by shading. *Cladophora glomerata,* one of the commonest algal nuisance species in the world has been found to grow in a wide range of light, nutrient, temperature and water flow conditions especially in managed water systems and especially where increased amounts of phosphate have been added to natural waters i.e. in the Great Lakes in North America (Lembi, 1988).

3. Control options

In past years the control of nuisance growths of algae was commonly maintained with the use of chemicals, most commonly copper sulphate and a few registered herbicides such as Diquat® and Midstream™**.** However, growing concern over the long term effects of copper sulphate on riverine and lake ecosystems systems has led to its use being severely restricted in Europe and the United Kingdom. One of the main problems in the control of algae has been the fact that the initial establishing growths are microscopic and it is only when the growths become obvious that control is obtained only by using large amounts of copper sulphate or herbicide.

a. Control options - Monitoring – first line of defence

In 1991 the Murray-Darling River in Australia turned green as a result of a massive blue-green algal bloom and a national outcry forced various state water authorities to assess the cause and prevention of such blooms (Creagh, 1992). A number of states have instituted monitoring programmes which will give early warning of possible reocurrence of such blooms. A similar programme is proposed in South Africa by the Department of Water Affairs & Forestry. In the United States many hundreds of school groups have instituted monitoring programmes in many state river systems. These many programmes may be viewed on the Internet by using the search words “water monitoring”. Simple monitoring systems are easily set up with very basic materials. A simple list of equipment would be –

1. **Algal monitoring board**

Algal spores are readily attracted to white plastic and the green growths which appear are seen when small. White plastic lids from jars can be used, and may be fixed to a wooden or metal board with a removable screw. The wooden board should be suspended in the water but prevented from moving in the current.

2. **Thermometer to measure water temperature**

As most thermometers are easily broken, a metal housing for the thermometer with a ring on top for an attaching cord is useful.

3. **Secchi disc**

This is a very serious sounding name for an extremely simple and commonly used scientific measurement. A hole is made through the lid of a large plastic bucket. A strong nylon rope, about 5 meters in length, is threaded through the hole in the lid and attached to a metal weight/s on the underside of the lid. A knot is made next to the hole on the top side of the lid. This lid is lowered into the water and when the white lid can no longer be seen a measure is taken of the length of rope from the top of the lid to the water surface. This measurement, given in centimeters, indicates the amount of water turbidity. Water turbidity indicates how much light is able to travel through the water and is available to the algae for photosynthesis.

4. **To measure the water speed**, one uses - 3 oranges (for each sampling occasion), a stop watch and a 10 meter length of rope. The time it takes for an orange to travel on the water surface along a measured distance is used as an indication of water speed. Three replicate measurements are made and the mean of the three measurements recorded.

To establish a monitoring programme the following basic steps should be taken.

- **i.** **A permanent site/s should be selected** at different points along the canal/river. The same sites should be used throughout the sampling period.
- **ii.** **To record algal growths**, the sites should be visited at least once a month, preferably weekly or fortnightly, throughout the year. White lids on the boards should be removed, placed in a clean plastic bag, and replaced with a new lid. Lids should be cleaned and soaked in a 10% bleach solution overnight before use. Lids from the monitoring boards should be assessed for percentage of algal cover. A magnifying glass can be used, but a dissecting microscope is preferable.

iii. **On each sampling occasion**, the time, date, water temperature, water speed and turbidity measurement should be recorded. An effort should be made to identify the types of algae and this could be done using Prescott (1978).

Although this suggested monitoring programme looks very simplistic it can provide most useful information. The monitoring programme can be carried out after a simple and short training period. Most importantly, an advance warning of noxious algal growths can be obtained and control action can be taken which would be less costly and ecologically harmful for the water system. A very simple subjective measurement of assessment of algal cover was suggested by Quinn *et al*. (1992) and is as follows –

Periphyton (Algal) Abundance Classification

Class	*Observation*
0	Not visible on handheld stones
1	Visible on handheld stones
2	Visible on bed (walls) covering few surfaces (<20% cover)
3	Visible on bed (walls) covering many surfaces (20 – 50% cover)
4	Visible on bed (walls) covering most surfaces (50 - 80% cover)
5	Visible as a complete cover on bed (walls) (80 –100%)

In addition a record of local rainfall figures should be kept.

b. Control options - Manual and Mechanical

These methods of control usually entail the emptying of the canals for at least two days. This is often during a period when there is an increased demand for irrigation water. In irrigation canals which are many kilometers long the cost of manual cleaning can be inhibitive. Mechanical cleaning, with large specialised equipment is even more costly, but is the only option for the removal of thick sediments which collect at the base of the canals. In South Africa, the canal width and depths in one system vary considerably and therefore the large heavy mechanical cleaners do not have access to the narrower sections of the canals. Here the cleaning must be done by well organised groups of manual cleaners using shovels and buckets. In some canal systems, farmers whose land bounds on the canals are asked to provide the groups of workers at least once a year for this cleaning process. This works well in the systems where it has been in practice for a number of years. However, its introduction as a new method in other irrigation schemes may not be as effective. The growth of algae is controlled by the minimum amount of light necessary for the process of photosynthesis. In canal systems algal growth usually only occurs to a depth where sufficient light can penetrate. Using a 24 to 48 hr period of water level decrease in canals the growth of algae can be inhibited. This method has the drawback of decreased water supply to the farmers during the 24 to 46 hr period.

c. Control options - Chemical

Although chemical methods of control appear to be the most effective and efficient, in terms of costs, there are problems associated with their continued use. Most importantly, the algae are only controlled (eliminated) for a limited period of time. Algal spores and pieces of filament can be carried by many means to re-infect a previously cleaned site. Algae and spores are also carried from the river/dam into the managed water supply system. Many of the managed water supply systems have had repeated dosages of copper sulphate or other herbicides for many years. In these systems the natural algal grazers have been eliminated and are unlikely to re-establish themselves. In water which has a pH of 7 or greater the use of ordinary copper sulphate is not successful. At increased pH’s the copper chelates (forms a tight and difficult to break group) with other organic molecules and sinks into the sediment. In South Africa, especially in the hard waters of the northern rivers, pH has been decreased using a pre-dosage of sulphuric acid. This is a hazardous procedure for the dosage operators and there is the further possiblity that the sulphuric acid combines with substances in the sediment to produce unpleasant smelling hydrogen sulphide. Copper sulphate is a comparatively inexpensive option for the chemical control of algae, but it too may produce long-term problems. Potassium permanganate, chlorine, hypochlorites, silver nitrate and sodium aresenite are inorganic compounds which have been proposed for biological control of algae by various researchers and are discussed by Sladeckova & Sladecek (1967), but none of these compounds have been used to any great extent because of problems associated with their use.

A novel method of chemical control has been proposed from researchers in Russia. They used super dosages of nitrogenous fertiliser in the irrigation water and the resulting very high levels of nitrogen in the water eliminated the growth of algae. They further suggested that the farmers could be asked to contribute financially to this chemical control as they would not need to fertilise their crops if this method of algal control was used.

d. Control options - Biological

Environmental groups have long proposed that the continued use of copper sulphate and other herbicides would lead to the "death" of a river. The use of barley straw to control algal growth was made as a chance discovery by a farmer in England. Since the first reports appeared suggesting that the effect of the rotting barley straw was to control the growth of the algae research has indicated that the use of barley straw has limitations. However, its use is simple – 2 g. of barley straw/m^3 of water. The effects will only be noticed after approximately 6 weeks when the straw has begun to rot (Newman & Barrett, 1993). The following information should be considered –

1. The substance produced by the rotting barley straw has not been completely identified.
2. The effect of the substance produced by the rotting straw is lost in medium to fast running water.
3. Although the growth of algae is inhibited by the rotting straw, it has no effect on aquatic flowering plants and removal of the algae may encourage an increase in growth of the flowering plants (Pillinger *et al*, 1994) .
4. Recent experiments have shown that the substance in the rotting barley straw only inhibits the growth of some algae, not all algae (Dr David M. John, pers comm.).

Although the rotting barley straw has little effect in running water it has proved most successful in dams which had nuisance growths of *Rhizoclonium* sp. Barley straw is comparatively inexpensive and can be used in all water types. The controlling effect of the rotting barley straw is not permanent and re-applications must be made every year. The straw must be lightly packed into relatively fine mesh nylon/plastic bags (e.g. orange bags) which should be firmly anchored.

A project to investigate the use of grass carp for controlling unwanted plant growth (both algae and flowering plants) in South Africa rivers and dams is currently underway in Gauteng province. These fish consume large amounts of plant matter daily, but cause loss of other vegetation as they disturb the bottom sediments and clouding of the water. Despite the assurances of the breeders who produce the triploid variants of these carp which are supposed to be incapable of breeding and therefore spreading as an exotic species in natural rivers and wetlands some problems have occurred. In some situations these carp became preferential feeders *i.e* would only consume certain types of aquatic vegetation, and also "escaped" into rivers and were found to be able to breed and infest waterways as happened in the United States (Andrews, 1986; Lembi *et al.*, 1988).

5. Conclusions

Ideally, if canals and waterways were covered to prevent light from getting to the water surface then it is unlikely that the problems caused by algae and other aquatic plants would occur. However, the cost and practicality of covering all the existing canals makes this proposal unfeasible. It should be noted however that at Upington, in the Northern Cape province, the newly constructed drainage canals are all covered. The use of shade cloth was proposed and tried out in some canals, but also found to be impractical. The planting of trees which would shade the water could also be tried, but may not be practically possible in many South African conditions.

It has become apparent during this study that the lack of accurate data as to the times of occurrence and duration of algal growths has been a major handicap in the control of growths. Using simple monitoring methods the initial growth cycles of algae may be observed and, far more easily controlled. Some of the more basic control methods are also inconvenient for the farmers. The use of drawdown over a 48 hour period at the time of initial algal growth would be an extremely cost effective method of control. Co-ordinated manual canal cleaning by the farmers has been found to work well in the Clanwilliam and Citrusdal canals in the Western Province. It must be pointed out however that these canals are generally considerably smaller than many of those in the rest of the country. More stringent governmental/municipal control of sewage effluent phosphate and nitrogen levels would lessen the nutrients available for algal and other plant growth. In a study in the United Kingdom it was found that the sources of phosphate in water were – detergents 17%, animal excreta 25%, sewage 30% and fertiliser run-off 20%. These percentages are, within small limits, the same for most countries in the world. The reduction of phosphate levels in detergents was proposed in the United Kingdom but it was found the cost increase of the detergent was inhibitive and would lead to a large decrease in sales for the major manufacturers and was therefore not pursued! (Pickup & Meddle, 1992)

It is important that we accept that the algae have a very important role in freshwater systems and that their appearance in our man-made systems are to be expected. Early detection with early treatment will lead to more efficient control and control cost reduction.