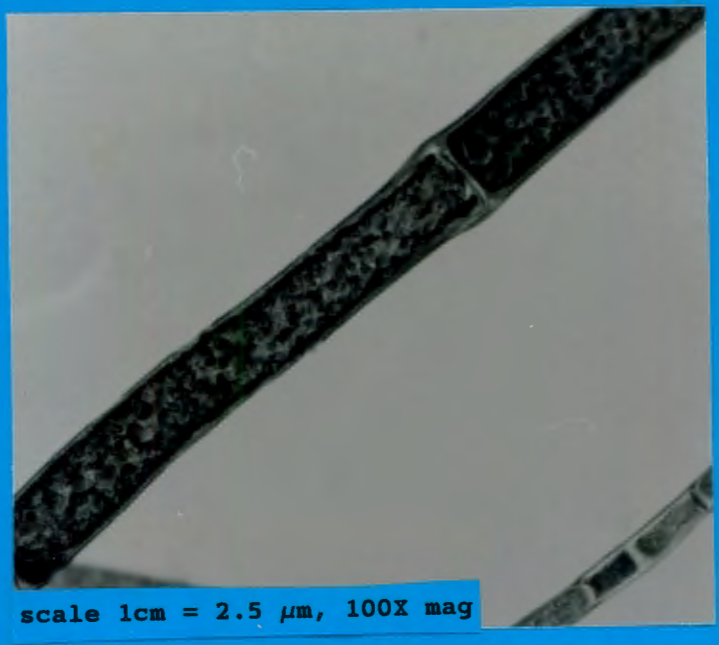
RENEE JOY LE ROUX

BOT 400 W

PHYCOLOGY HONOURS PROJECT 1994

FACTORS INFLUENCING THE GROWTH AND CONTROL OF *CLADOPHORA GLOMERATA* (L.) KUTZ.



ABSTRACT

The growth response of the freshwater macroalga, *C.glomerata*, to different light levels, pH levels and copper concentrations were investigated in the laboratory. Two light experiments were conducted which included a wide range of light intensities from 20-500 μmol photons m^{-2} s^{-1} and 20-200 μmol photons m^{-2} s^{-1}. The maximum growth rate in both light experiments was at a light intensity of 200 μmol photons m^{-2} s^{-1} and the lowest growth rate recorded at 20 μmol photons m^{-2} s^{-1}.

The effect on the growth rates of *C.glomerata* in different pH levels of 6, 7 and 8 with a copper concentration range of 0-2mg.l^{-1} over a 5 day period was investigated. The results showed that the growth rates were significantly reduced at a copper concentration of 0, 0.3 and 0.8 mg.l^{-1}, at pH levels of 6, 7 and 8 respectively and that the pH level of 6 demonstrated the lowest growth rates at all copper concentrations. Furthermore, the effect of copper on cell damage at concentrations of 0, 2, 6 and 10 mg.l^{-1} were examined in pH levels of 6 and 7 over a 4 hour period. It was found that an increasing concentration of copper, increased the extent of cell damage and that cell damage was more profound at a pH level of 6.

It was concluded that the growth rate of C.glomerata could be effectively controlled at light intensities below 20 μmol photons m^{-2} s^{-1} and that the minimum copper concentration required to obtain optimum control depends on the pH of the water, time of residence as well as the algal biomass.

INTRODUCTION

In many countries, including South Africa industrial, agricultural and domestic demands of water are increasing due to an increase in population growth as well as urbanisation. In addition to the pressure exerted by human demands, schemes for the maintenance of a regular water supply in South Africa are subject to environmental problems which include erratic rainfall which may lead to drought conditions and the loss of source supply due to the degradation of the freshwater environments (Joska and Bolton, in press).

The degradation of freshwater systems has received much attention since the quality of water affects the lives of many humans. Ironically, the same activities which require a good water quality pollute the systems with industrial effluent, run-off from agricultural lands and the emittance of sewage. The excessive loading of nutrients and chemical wastes leads to the eutrophication of water systems which may enhance the selective growth of weedy, filamentous algae. Large blooms of weedy algae can be regarded as being detrimental since they block water work systems, lower the O_2 concentration in the water upon decay and reduce the species diversity of the area. Moreover, dense growths of filamentous algae are aesthetically unacceptable, as they hinder recreational activities and often the decomposition of the algae lead to undesirable odours (Palmer, 1980). The excessive pollution of surface waters and the consequent algal blooms have

therefore received considerable attention and control methods have been investigated. However, algae are the primary producers of freshwater systems and therefore play an integral part in the ecosystem. The conditions for excessive growth of the problem algae require careful monitoring in order to determine what control methods or changes should be implemented for the benefit of humans as well for the conservation of aquatic life.

The research on environmental conditions regulating macrophytic algal growth has emphasized those species with the most significant impacts on human utilisation of water (Lembi, *et al* 1988). Such a filamentous nuisance alga, *Cladophora glomerata* (L.) Kütz, has been extensively studied because of its global distribution and its infestation in many eutrophic water bodies (Lembi *et al*, 1988; Dodds, 1991). Dense growths of *C. glomerata* in response to cultural eutrophication have been reported in many areas, including the Great Lakes in northern America (Lorenz *et al*, 1991), rivers in the U.S.A (Leland and Carter, 1984; Dodds, 1991), The Great Stour River in England (Wharfe *et al*, 1984) as well as many dams and rivers in South Africa (Joska and Bolton, in press). Dodds (1991), indeed considered *C. glomerata* to be the most ubiquitous river alga in the temperate northern hemisphere. Comprehensive studies have therefore been undertaken to determine the environmental factors which stimulate the growth of *C. glomerata*. In the Northern Hemisphere, *C. glomerata* has been shown to thrive in high light intensities, strong hydrodynamic

movements and an optimum temperature range of 15-25°C (Whitton, 1970a; Dodds and Gudder, 1992). In addition a high pH level, more than 7 (Whitton, 1970a) and elevated phosphate and nitrogen supplies (Whitton, 1970a; Wharfe *et al*, 1984; Dodds and Gudder, 1992) may enhance and stimulate the growth of *C. glomerata*.

The attempts to control *C. glomerata* growth have focused on long-term plans to lower nutrient levels by improving methods of sewage treatment and industrial effluent (Whitton, 1970a; Wharfe *et al*, 1984). Nutrient reduction would be an ideal solution to reduce the density of *C. glomerata* in South Africa but often not a viable option since many agricultural lands are concentrated around the major water schemes. Since *C. glomerata* apparently thrives in high light intensities, it has been suggested that the shading of canals with trees to reduce light levels may drastically decrease the growth. Shading with bank side trees to control *C. glomerata* growth was shown to be effective in the Avon River, Ontario, Canada (Demal and Fortin, 1987). However, in South Africa, the blocking of light from the canals as a control method has not been considered as an economically viable option. At present in South Africa, the control of excessive algal growth is focused on short-term and temporary methods such as manual removal and the application of copper sulphate (Joska and Bolton, in press). Although manual removal of *C. glomerata* has proven to be successful in some small irrigation schemes, it is often impeded in areas which are inconvenient for access as well as by climatic factors.

Many studies in heavy metal polluted waters have revealed that metal pollution decreases algal productivity and diversity as well as algal species composition (Takamura *et al*, 1989). Although trace amounts of heavy metals are essential for metabolic processes of algae (Steeman-Nielsen and Wium-Andersen, 1970; Hillebrand and De Vries, 1986), higher concentrations inhibit growth or kill the algae. *C.glomerata* has been reported to be sensitive to heavy metals such as copper, lead and zinc (Whitton, 1970b), and can therefore be regarded as a good biomonitor for heavy metal accumulation in freshwater systems (Oertel, 1991). The sensitivity of *C.glomerata* to heavy metals is also exploited by water supply managers to control the excessive growth of *C.glomerata*. Copper sulphate is a commonly used algicide in freshwater environments and is been applied quite extensively to the water systems of South Africa. However, the addition of $CuSO_4$ has not proven to be very effective in alkaline waters, where *C. glomerata* tends to thrive (Whitton, 1970a; Palmer, 1980). In some water schemes in South Africa for example, the Kalkfontein and Hartebeespoort, the addition of $CuSO_4$ after an initial predosing of sulphuric acid to reduce pH is being used as a control to reduce *C. glomerata* growth (Joska and Bolton, in press).

The use of $CuSO_4$ and certainly the addition of sulphuric acid to natural water bodies are environmentally unacceptable and prohibited in many countries. It is therefore essential that

the correct application of $CuSO_4$ is determined whereby maximum growth control is obtained at the minimum $CuSO_4$ concentration and correct pH which is toxic to the nuisance algae.

Although extensive research has been undertaken on *C.glomerata* in other countries, very little research work has been done in South Africa. Populations of a single species in different geographical areas may differ somewhat in their response to environmental conditions. This project has therefore been undertaken to investigate firstly the effect of light as an environmental factor on the growth of *C.glomerata* so as to increase our understanding of its biology. The second component of the project was to determine the appropriate application of $CuSO_4$ as an algicide so as to obtain effective growth control of *C.glomerata*. Investigations were therefore carried out to examine the effect of pH and copper concentration on the growth of *C.glomerata* over a long-term and short-term duration.

MATERIALS AND METHODS

The *C.glomerata* plants used in the experiment were collected from the Kalkfontein Dam Scheme in the Orange Free State by M.A.P. Joska during May 1993. For the experiments clumps of thalli were randomly selected from the plants which had been maintained in the laboratory in aerated tap water under room temperature and dim natural sunlight.

In general all the experiments were carried out in a phytotron unit (growth chamber) under controlled light and temperature conditions. The sole light source was obtained from a flourescent tubing system fitted into the ceiling of the growth chamber and programmed to produce a light-dark cycle. The growth medium used in the experiments was Wood's Hole (Table 1). The nutrient solution was sterilized and in some experiments slightly modified to manipulate the conditions. The pH of the solution was altered by adjusting the pH of the buffer, TRIS, with diluted HCl acid or a 1M NAOH solution and determined using a pH electrode which was connected to an electric pH meter. The glassware was sterilized by soaking in a detergent solution, rinsing, acid washing with a final rinse in distilled water, and then oven dried at 60° C.

Table 1. Components of the synthetic growth medium, Wood's Hole. (after Stein, 1973)

Component nutrients	Stock solution	Dilution ($ml.l^{-1}$)
Macronutrients:		
$CaCl_2.2H_2O$	36.76 $g.l^{-1}$	1
$MgSO_4.7H_2O$	36.97	1
$NaHCO_3$	12.60	1
K_2HPO_4	8.71	1
$NaNO_3$	85.01	1
$Na_2SiO_3.9H_2O$	28.42	1
Micronutrients:		
Na_2.EDTA	4.36 $g.l^{-1}$	1
$FeCl_3.6H_2O$	3.15	1
$CuSO_4.5H_2O$	0.01	1
$ZnSO_4.7H_2O$	0.02	1
$CoCl_2.6H_2O$	0.01	1
$MnCl_2.4H_2O$	0.18	1
$Na_2MoO_4.2H_2O$	0.006	1
Vitamins:		
Thiamine.HCL		0.1 $mg.l^{-1}$
Biotin		0.5 $\mu g.l^{-1}$
Cyanocobalamin		0.5 $\mu g.l^{-1}$
Buffer:		
Tris(hydroxymethyl)-aminomethane	50 g/200ml	2

The light experiments

Tufts of *C.glomerata* plants were weighed after blotting the alga on laboratory tissue paper to remove excess water from the thalli. The initial weights of the plant material used were 250 mg which were placed in 500ml conical glass flasks containing 250ml of Wood's Hole nutrient solution. The pH of the nutrient solution ranged between 8 and 9. Light intensity was measured as photosynthetically active radiation (PAR) in μ mol photons m^{-2} s^{-1} with a portable light meter. A wide range of light intensities between 20-500 μmol photons m^{-2} s^{-1} were obtained by manipulating the position of the conical flasks in relation to the light source as well as placing nylon netting over the flasks for the lower light intensities. A gradient of six light intensities were used which consisted of 20, 50, 125, 200, 350 and 500 μ mol photons $m^{-2}s^{-1}$. In total the experiment comprised 24 conical flasks since each light intensity had 4 replicates which were randomized daily to avoid difference in growth due to variation in local positions. The experiment was conducted over a sixteen day period with the nutrient solution being changed every four days. The time interval of nutrient solution change also coincided with a recording of the weight, measured in a similar manner as the initial weight. For this particular experiment the photoperiod was 14:10h light-dark cycle which is typical of winter photoperiodism. Despite the maintenance of a constant water temperature in the room at bench level,

AERATION?

the water temperature at the six light intensities differed slightly. The water temperatures recorded at the different light intensities were : 17.5°C at 20 μmol photons m^{-2} s^{-1}, 20°C at 50 μmol photons m^{-2} s^{-1}, 21°C at 100, 200 and 350 μmol photons m^{-2} s^{-1} and 25°C at 500 μmol photons m^{-2} s^{-1}.

In addition, to the first light experiment a second experiment was conducted over a shorter time period and using a narrower light intensity range. The light intensity levels were 20, 50, 75, 125, 165 and 200 μ mol photons m^{-2} s^{-1}. Since a narrower range in light intensities were used, the fluctuation in water temperature was not as drastic as the first light experiment. Water temperature was maintained at approximately 15°C ± 1°C. The photoperiod was altered to a 16:8h light-dark cycle simulating summer conditions, that is long day and short nights. The experimental procedure was carried out in the same way as the first experiment, except that the duration was reduced to eight days.

Copper and pH experiments

pH Treatments: The growth of *C.glomerata* was monitored in different pH levels. The Wood's Hole solution was adjusted to yield three pH levels 6, 7 and 8. This experimented was duplicated. Tufts of 40mg of *C.glomerata* plants were weighed and placed into each plastic petri dish which contained 20ml of Wood's Hole solution adjusted to its appropriate pH level.

Although the nutrient solution was buffered the pH tended to fluctuate with photosynthetic activity. For this reason, the pH of the growth medium was monitored and altered daily. The weights of the plants were recorded simultaneously with the change in nutrient solution on the third day as well as on the fifth day when the experiment was terminated. For the duration of the experiment the petri dishes were placed in the growth chamber under a light intensity of 200 μ mol photons m^{-2} s^{-1} and at a water temperature of 21°C ± 1°C.

Copper treatments: The copper treatment experiments were run consecutively at three different pH levels, 6, 7 and 8. At each pH level there were six copper treatments ranging between 0 - 2 mg $Cu.l^{-1}$. The source of Cu was derived from a 1mM $CuSO_4.5\ H_2O$ stock solution which contained 64 mg of $Cu.l^{-1}$. The standard Wood's Hole solution (Table 1), contained a trace amount of $CuSO_4$ which yielded a Cu^{2+} concentration in the order of 2.56 $\mu g.l^{-1}$. Hence to obtain the required Cu concentrations for the experiment the normal $CuSO_4$ component was omitted from the standard Wood's Hole medium and appropriate volumes of the stock solution were added to produce a series containing 0, 0.5, 1, 1.5, 1.75 and 2 mg Cu.l-1.

An initial weight of 50mg ± 1mg of *C.glomerata* plants were placed into each plastic petri dishes containing 20ml of the Wood's Hole Solution adjusted to its required pH. Each copper treatment at the different pH levels had four replicates. The pH of the nutrient solution in each petri dish was monitored and adjusted daily. As with the pH experiment, the weight of the *C.glomerata*

tufts were recorded on day 3 when the nutrient solution was changed as well on day 5, at the end of the experiment. The temperature of the water was maintained at 15°C ± 1°C and light was maintained at a maximum of 200 μmol photons m^{-2} s^{-1} at a 16:8h light-dark cycle.

Supplementing the long-term copper treatment experiments, a short-term assessment of the effect of copper on *C.glomerata* was undertaken. Segments of *C.glomerata* plants were placed into petri dishes with the Wood's Hole growth medium which was modified to contain 0, 2, 6 and 10 mg $Cu.l^{-1}$ at pH levels of 6 and 7. At each copper concentration at the two pH levels, the algal segments were incubated separately for varying time periods, including 10, 30, 60, 120, 180 and 240 minutes, after which they were transferred into separate petri dishes with the standard Wood's Hole Solution. Subsequently, all the algal segments in each petri dish were examined under a compound microscopic in one field of view at a 100X magnification at time intervals of 10, 30, 60, 120, 180 and 240 minutes after incubation to determine the extent of damage incurred by the Cu ions. The effect of the Cu ions was in each case subjectively classified under 7 categories according to the percentage of cells damaged. These were:

0. no cells damaged
1. < 25%
2. ± 25%
3. between 25-50%
4. between 50-75%
5 between 75 -100%
6. ± 100%

The experiment was performed under room temperature and light with no replicates due to the large amount of petri dishes used to include all the relevant cases.

COMPUTATION AND STATISTICAL ANALYSES

The raw data measurements of weight were transformed to specific growth rates which were calculated as doublings per day with the formula:

$$r = \ln (x_2-x_1)/(t_1-t_2)$$

where r = growth rate; x_1 and x_2 represent the weight of the *C. glomerata* tufts at t_1 = day 1 and t_2 = day z, respectively (Hillebrand and De Vries, 1986).

All statistical analyses were executed on a software computer package, Statgraphics v 5.0 using Analysis of Variance or Simple Regressions except for the pH experiments for which a Lord's Range Test (Langley, 1968) was computed. Tests for normality were performed using normal probability plots and homoscedacity was determined by Bartlett's test.

RESULTS

Comparison of the response of the growth rates of C. glomerata to different light conditions.

Laboratory studies were performed under a range of light intensities to elucidate the effect on the growth rate of *C. glomerata*. Significant differences in growth rates with light intensities ranging from 0-500 μmol photons m^{-2} s^{-1}, were found only after days 4 and 8, but not after days 12 and 16, at $p \leq 0.05$ (Table 2).

Table 2. Statistical data from a One-Way ANOVA between growth rate of *C. glomerata* and light intensity over different time intervals.

Days	F-ratio	p
4	2.84	0.04*
8	2.73	0.05*
12	1.83	1.70
16	1.70	0.19

n = 4; df=23;
* denotes a statistical difference

The amount of algal growth over an 8 day period, increased with increasing light intensities from 20 μmol photons $m^{-2}s^{-1}$, the lowest growth rate, to 200 μmol photons $m^{-2}s^{-1}$, the maximum growth level evaluated. At light intensities higher than 200 μmol photons $m^{-2}s^{-1}$, that is at 350 and 500 μmol photons $m^{-2}s^{-1}$, the growth rate of *C. glomerata* decreased (Fig. 1).

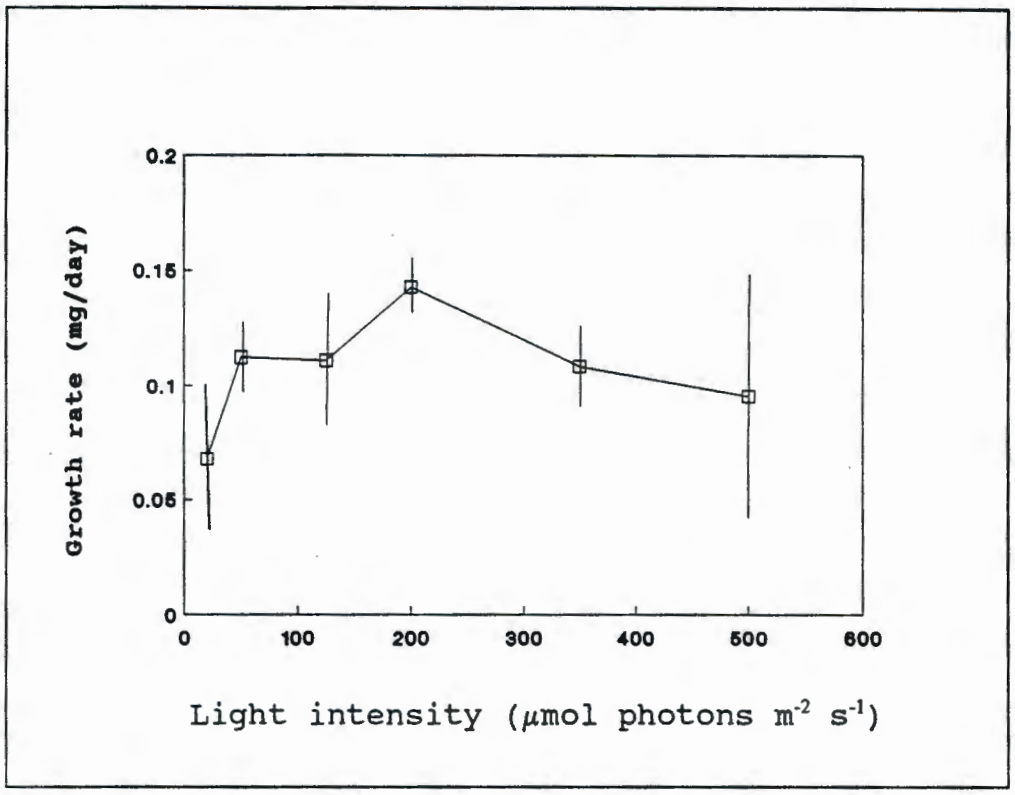


Fig 1. Effect of different light intensities on the growth rates of *C. glomerata* over an 8 day period. Mean ± SD (vertical lines), n = 4

In the second light experiment using a narrower light interval a significant increase in growth rate over 8 days from 20 to 200 μmol photons $m^{-2}s^{-1}$ was interpreted, with a F-ratio of 7.646 and $p \leq 0.05$. In both light experiments, the growth rate curves below 200 μmol photons $m^{-2}s^{-1}$ exhibited a distinct pattern. The growth rates were very similar from 50-165 μmol photons $m^{-2}s^{-1}$, less at 20 μmol photons $m^{-2}s^{-1}$ and higher at 200 μmol photons $m^{-2}s^{-1}$ (Fig 1 and 2). However, in the second light experiment the overall growth rates between 20-200 μmol photons m^{-2} s^{-1} were much lower than the first experiment. The growth rates evaluated in the second

experiment were reduced by 70%, 55%, 49% and 50% to the growth rates in light experiment 1, at 20, 50, 125 and 200 μmol photons $m^{-2}s^{-1}$, respectively (Fig 1 and 2).

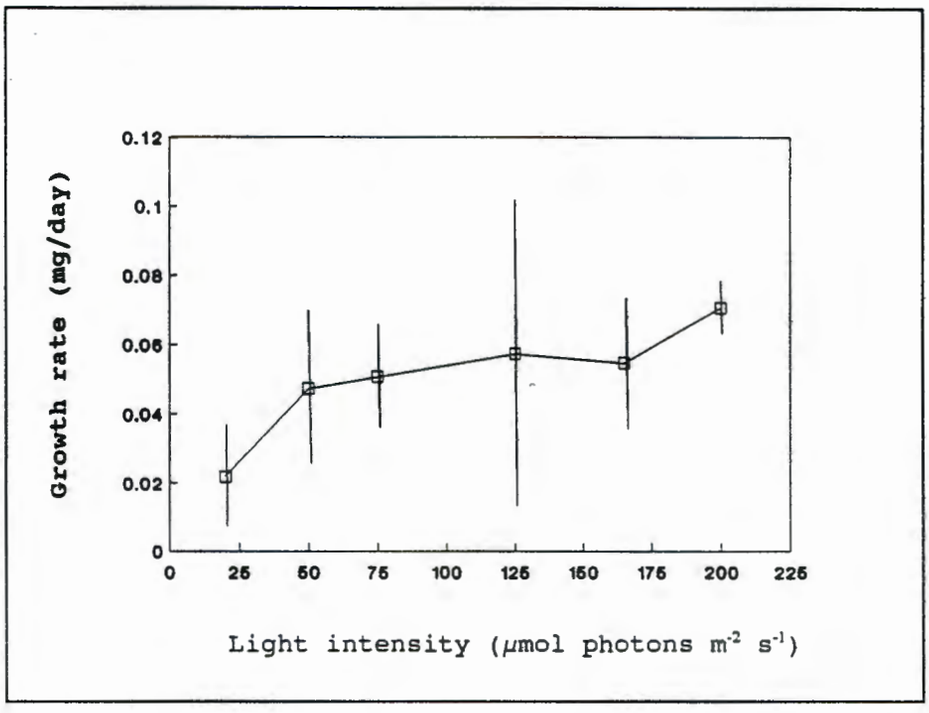


Fig 2. The growth rates of *C. glomerata* at different light intensities over an 8 day period. Mean ± SD (vertical lines), n=4.

The effect of pH and copper on the growth rates of C. glomerata

pH treatments: A significant effect of pH on the growth rate of *C.glomerata* was observed, with a test statistic of 2.042 (Lord's Range Test) and $p \leq 0.05$. A positive relationship between growth rates and pH were obtained after 5 days of incubation. The growth rate at a pH of 8 (alkaline) was 2 and 5 times greater than growth rates in pH levels of 7 (neutral) and 6 (acidic) respectively (Fig 3).

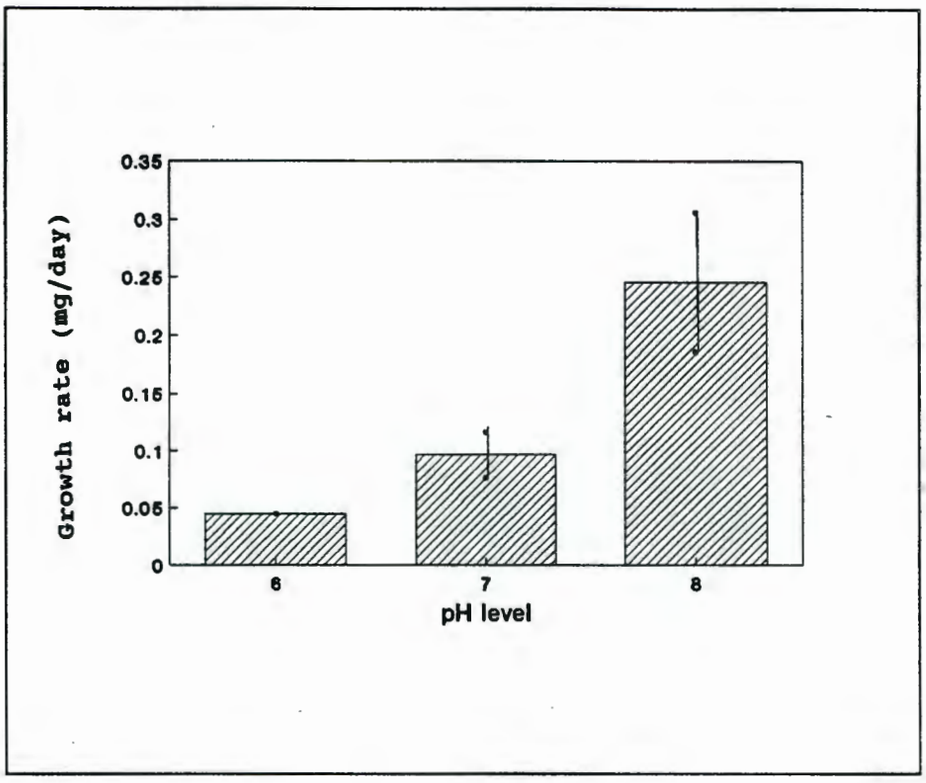


Fig 3. The effect of pH on the mean growth rates of *C.glomerata* after 5 days of treatment. n = 2.

The copper treatments:

Copper combined with pH manipulations had a marked effects on growth rates of *C. glomerata*. A significant ($p \leq 0.05$) negative correlation was observed between growth rates of *C. glomerata* over a five day period and copper concentrations at all three pH levels. (Fig 4 and Table 3).

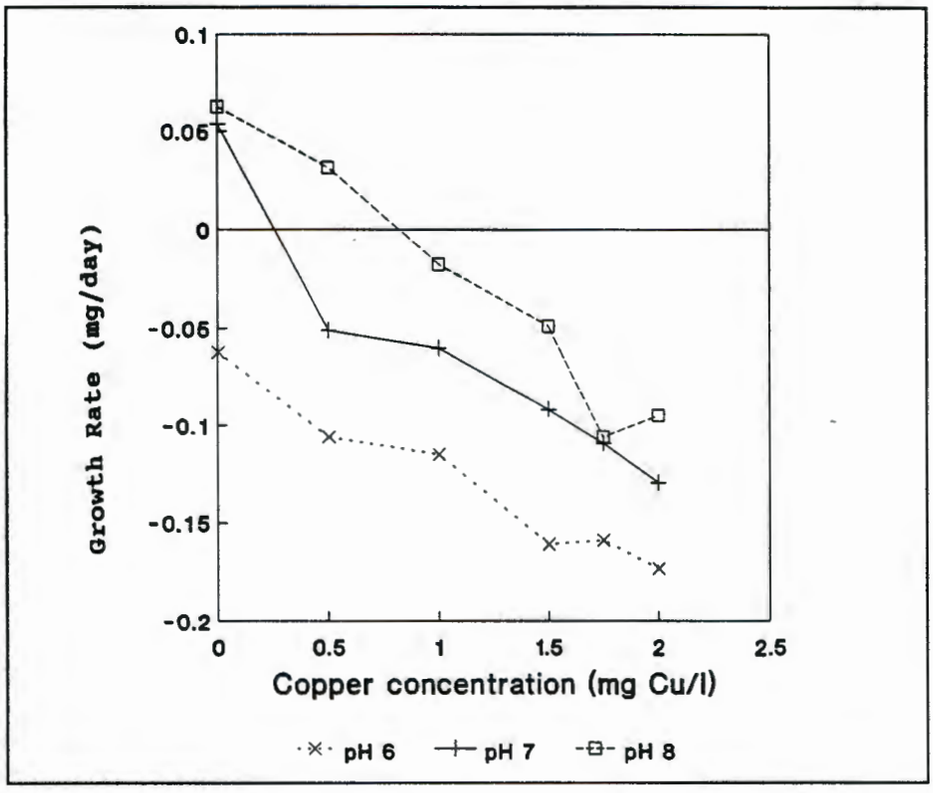


Fig 4. Growth rates of *C. glomerata* in three pH levels at various concentrations of copper. Values are means. n = 4.

Table 3. The correlation between *C. glomerata* growth rates and copper concentrations for 3 pH levels.

pH level	r
6	-0.98 *
7	-0.95 *
8	-0.98 *

n = 4 at each pH
* denotes a correlation significant at $p \leq 0.05$