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Kenya Journal of Science and Technology Series B (1983) 4: 3-16

Possible factors limiting the growth of an aquatic weed, *Hydrilla verticillata* (L.F.) Royle under tropical conditions

B.N. Kigomo,
Kenya Agricultural Research Institute, Muguga, Kenya

SUMMARY

The aim of this study was to help understand the possible factors limiting the growth of an aquatic weed *Hydrilla verticillata* in the littoral waters of Lake Victoria. The study was confined to investigation of inorganic carbon as source of nutrient and a possible biocontrol of the weed by fresh water snails, *Lymnea natalensis* and *Bulinus* spp found in the lake. A series of experiments were conducted for the purpose of evaluating these factors.

From the studies it was observed that *Hydrilla* is able to photosynthesize in a medium where HCO^-_3 ions are predominant. The weed would thus be at an advantage where carbon dioxide is reduced or lacking as a result of local ecological effects. *Hydrilla* has a maximum growth in a medium of 220 mg per litre (predominantly HCO^-_3 ions) optimum total alkalinity. This would mean that *Hydrilla* can do well in calcareous waters, an observation well supported by the fact that the weed flourishes well in many hard water habitats. Consumption of *Hydrilla* by snails was 34.3% of the total available biomass.

These results show that while fresh water snails may be playing relatively little role in the control of *Hydrilla,* it is most likely that the low inorganic carbon source in Lake Victoria of total alkalinity 55.8 mg per litre (CO^2 + HCO^-_3) may be contributing to the low growth of the aquatic weed.

Further studies on nutrient requirements by *Hydrilla* in relation to the nutrient status in Lake Victoria littoral zone may contribute profitably to the present conclusions.

INTRODUCTION

This study focuses on the markedly reduced growth of the aquatic weed *Hydrilla verticillata* (L.F.) Royle in Lake Victoria and therefore growth controlling factors in tropical waters.

Hydrilla is a perennial, monoecious or dioecious submerged aquatic plant in the family Hydrocharitaceae. The weed has been reported in places such as lakes Tanganyika and Victoria, but the source of introduction in these East African lakes is unknown (Agnew 1974). It may well be an indigenous plant.

Under good growth conditions *Hydrilla* forms a dense mat. The upper portion of the plant towards stem tip is often seen to grow up to the water surface. Individual plants have been found growing in deep waters of 2.5 to 3 metres (Ruttner 1953).

Hydrilla has become one of the most serious aquatic weed problem in many tropical areas (Blanchard 1967). It is such an aggressive weed that it has succeeded in dominating native aquatic vegetation in many places in Florida and is now a more troublesome weed than older weed pests like the African pyle, *Salvinia molesta* Mitchell and water hyacinth *Eichhornia crassipes* (Mart) Solms. Being a substrate submerged plant, its control by herbicides, harvesting or biological means has met mostly with failure and has involved a great deal of financial expenditure (Blanchard 1967). Johnson and Manning (Blanchard 1967) have noted that *Hydrilla* in a reservoir of 10 hectares could spread to over five hectares in just six weeks. The weed is thus a menace to agricultural and recreational uses of water.

In East Africa, *Hydrilla* occurs in the forementioned lakes but some natural factor(s) keep it under control. It is however evident that most aquatic weeds respond favourably to an increase in nutrients. Eutrophication will be a problem in Africa and elsewhere and it may increase growth of the weed.

In the present study, by using laboratory cultural experiments, carbon sources as possible growth limiting factors were investigated. Possible biocontrol of the aquatic weed, by tropical water snails, *Lymnea natalensis* and *Bulinus* spp. was also investigated.

METHODS AND MATERIALS

Natural habitat

Hydrilla plant under study was collected from Jinja, Lake Victoria in shallow waters growing among the littoral macrophytes common to this area. Lake Victoria is a tropical Lake (01° 00'S; 33° 00'E) bordered by Kenya, Uganda and Tanzania. The lake is relatively deep, of immense size (75,000 km^2) and is characterised by a much indented shoreline with many large and shallow swampy bays (figure 1). The shallow bays provide ideal conditions for growth of floating, emergent and submerged macrophytes (appendix 1).

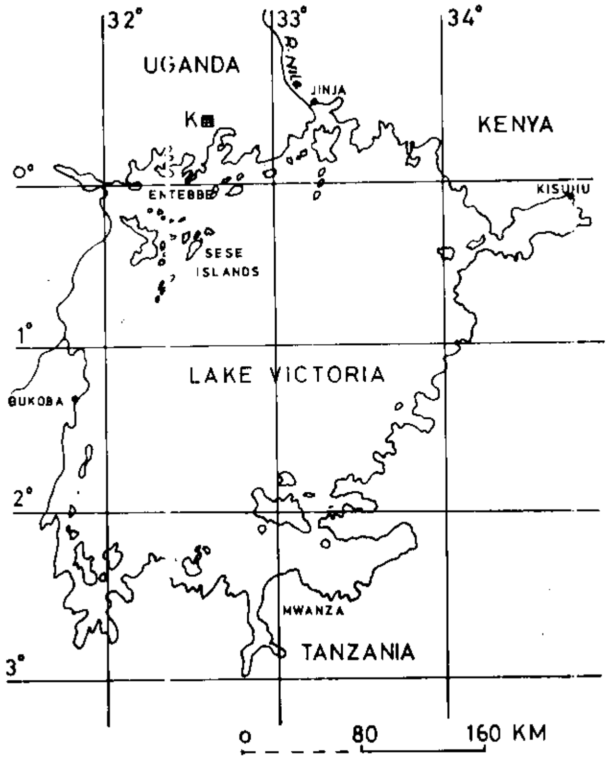

Figure 1. Lake Victoria

Experimental design

Three approaches were followed in the study. The first approach observed the possibility of carbon as a limiting factor in the growth of *Hydrilla,* i.e., could *Hydrilla* utilize other forms of inorganic carbon other than free carbon dioxide? If so, this would possibly give the weed a competitive advantage over other macrophytes within the same habitat.

The second approach involved water quality measurements such as pH, dissolved carbon and oxygen, i.e., environmental conditions for growth.

The third approach involved observation of feeding on *Hydrilla* by some tropical fresh water snails *(Lymnea* and *Bulinus)*; i.e., would these two tropical snails found in Lake Victoria contribute to the supressed growth of *Hydrilla?*

Plant culture

Hydrilla plants originally cultured from Lake Victoria were grown in various tanks approximating 50-70 litre capacity. Cuttings were planted in clay-sand mixture in glass jars. The tanks were then filled with tap water to which 5% strength Hoagland solution and 8% sodium bicarbonate (Na HCO^-_3) were added.

Water in these tanks was changed every two weeks to avoid salt precipitation or depletion of nutrients. These tanks provided the source of the study material. *Hydrilla* plants considered reasonably healthy and long were cut into 6 cm long apical stem sections and six of these sections were planted in one glass jar almost filled with washed sand (pH 6.5 and conductivity 42 micromhos/cm). These glass jars were then placed in experimental aquaria. The latter were almost filled up with respective test solutions containing 5% strength Hoagland solution. Sides of aquaria were covered with two wrappings of aluminium foil to keep the growth of algae to the minimum. The top was covered with glass lid to allow top lighting with ordinary sunlight or artificial light.

Carbon source

Four kilner glass jars planted with *Hydrilla* apical stem sections were placed in each of six aquaria (15-20 litre capacity). Each of the aquaria was supplemented with varying concentrations of an inorganic carbon source (Na HCO_3) in an ascending order of 75, 125, 175 250, 500 and 1000 mg per litre. These aquaria were then placed in a growth room for a period of two to four weeks. Water level in the aquaria was maintained and pH, temperature and total alkalinity monitored after every fourth day in the course of the experiment.

After the second and fourth week of growth, standing crop was determined by recording mean number of leaves, fresh weight, dry weight and shoot length. Harvested crop was washed with distilled water before weighing. Fresh weights were taken after allowing plants to drip dry for two minutes and dry weight was determined after drying the plants in oven at 105°C for 24 hours to constant weight.

In the above experiments carbon source (Na HCO_3) contributed sodium ions to the treatments. It was consequently decided to investigate possible sodium effect to the growth of *Hydrilla.* The procedure used was as above but sodium sulphate (Na_2 SO_4) was used in the place of Na HCO_3.

In all the experiments, growth solutions were changed weekly.

Water quality

Conductivity of water was monitored with a conductivity meter model RC 16 BC, pH was measured by use of pH meter model E350 and temperature by a glass thermometer. Light intensity was measured by use of radiometer model Li 185 while free dissolved carbon dioxide was determined by titrating against sodium hydroxide using phenolphthalein (Golterman 1969).

Dissolved oxygen was determined by the Winkler method. Twenty millilitres of sample was titrated with standard sodium thiosulphate using starch solution. Titrating a 25 ml sample with 0.02N sulphuric acid using a mixed indicator gave total alkalinity

(Taras and Arnold 1971).

Using the above techniques water quality in four different habitats was monitored., The habitats were selected as the most common in which *Hydrilla* would grow. First habitat was an uncovered aquarium simulating normal growth in shallow water while the second habitat was under a mat of floating *Salvinia molesta,* a common weed in shallow tropical water. The third habitat was under a mat of floating weed *Pistia stratiotes* L. another common weed in tropical lakes. The fourth habitat simulated deep water conditions by covering the tank with aluminium foil to create a dark environment.

In the course of growth in uncovered aquaria algal growth problems were experienced. To determine whether this algal growth had any effect on water quality, an experiment was set up. Glass jars with growing *Hydrilla* were introduced into a three litre growth container filled with hard water. Another container was filled with an algal suspension. These containers were tightly covered and placed under a 150 watt shaded light at 30 cm for four days. pH, dissolved CO_2 and oxygen were monitored daily at 0800 and 1800 hours.

Biocontrol

Two aquaria were introduced with assessed *Hydrilla* as regards number of leaves per cutting. The aquaria were filled with stream water. Snails for the biocontrol study were collected from the same stream. *Hydrilla* was allowed to equilibrate for two weeks after which 25 snails (average 16mm length and 8mm maximum width) of a mixed population of *Lymnea* and *Bulinus* were placed in the first aquarium. The second aquarium was kept free of snails.

After 15 days, standing fresh weight was measured and consumption of *Hydrilla* determined as follows:

$$\frac{A - B}{A} \times 100 = C$$

Where A = fresh wt control aquarium,
B = fresh wt snails aquarium, and
C = consumption (%)

Regression analyses of biomass production upon water quality parameters were carried out using the methods of Bailey (1974).

RESULTS

Carbon source

Maximum average increase in number of leaves, fresh weight, dry weight and shoot length was observed at 200 mg per litre (Na HCO_3) total alkalinity (figures 2a, 2b, 2c, 2d, and table 1). Total alkalinity here refers mostly to bicarbonate because pH (8.0 — 8.9) of the cultures will allow existence of HCO^-_3 ions but very little carbonate. There is a positive relationship between fresh weight and total alkalinity with a correlation coefficient of 0.62 (figure 3a) and fresh weight and conductivity with a correlation coefficient of 0.82 (figure 3b), both significant at p = 0.05 and P = 0.01, respectively.

Figures 4a and 4b show biomass production after four weeks of growth in sodium sulphate medium. Growth of *Hydrilla* was slightly depressed at higher sodium concentrations, possibly as a result of increase in conductivity. A low correlation coefficient of 0.11 is observed (figure 4c) and this may be an indication that sodium ions input may not be playing an important role in the overall growth of *Hydrilla* in the present experiments.

Water quality

Figures 5a, 5b, and 5c show difference in growth after three weeks period in different light regimes. Growth under *Salvinia* and *Pistia* (floating weeds) was at a low light intensity while plants grown in open tanks had the highest light intensity. Increase in shoot length and fresh weight was exhibited in the four simulated habitats. Growth in the aluminium covered aquarium showed maximum shoot length, fresh weight and number of branching counts. The uncovered glass aquarium had the lowest biomass output.

Low branching in *Pistia* and *Salvinia* mat habitats may be the result of low light

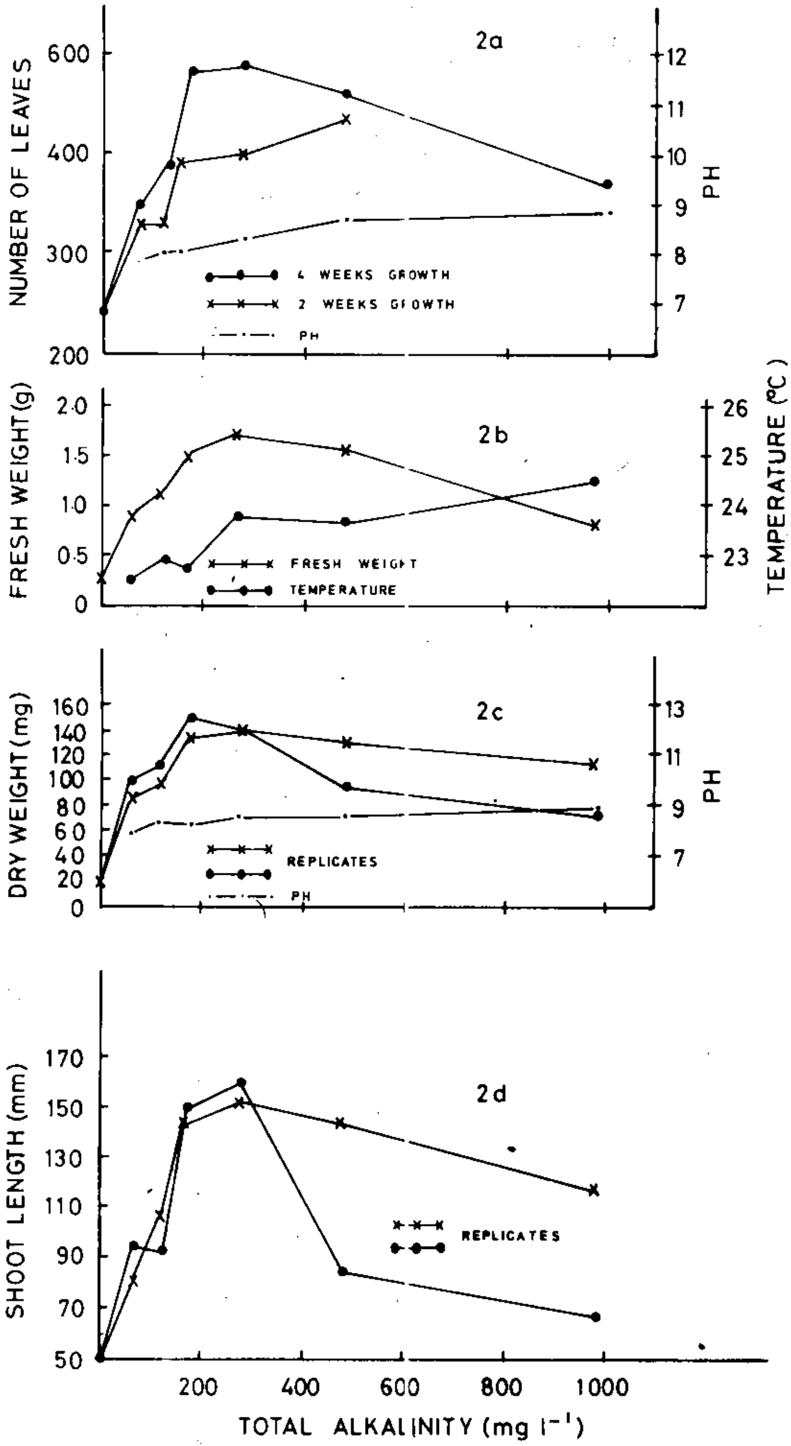

Figure 2. Growth expression by *Hydrilla* in different water qualities and growth periods

Table 1. Mean biomass production and growth expression by *Hydrilla verticillata* in various concentrations of bicarbonate (four weeks growth)

Mean total alkalinity- $CO_2 + HCO^-_3 + CO^{2-}_3$	Fresh weight (g) mg l⁻¹	Dry weight (mg)	Number of leaves	Shoot length (mm)
76	0.9	93	350	87
120	1.1	102	380	98
175	1.5	142	470	148
260	1.6	140	480	156
484	1.5	114	460	114
980	0.8	92	370	91
Overall means	1.25	113.8	418.3	115.7
Coefficient of variation (%)	29	20	14	26

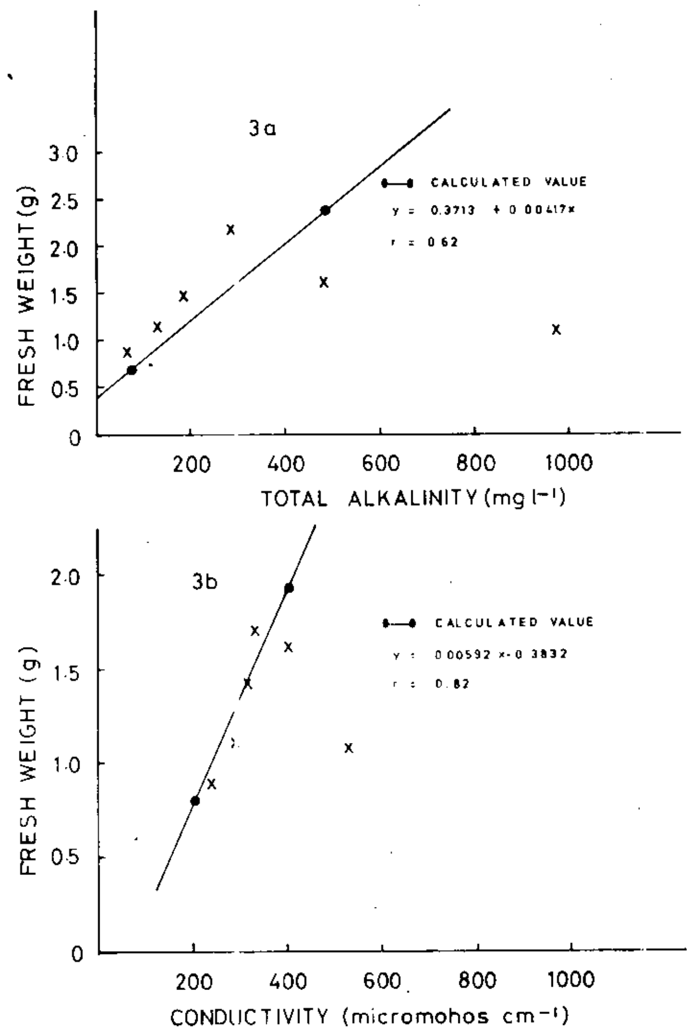

Figure 3. Regression of total alkalinity (3a); and conductivity (3b); upon *Hydrilla* biomass based on four weeks growth

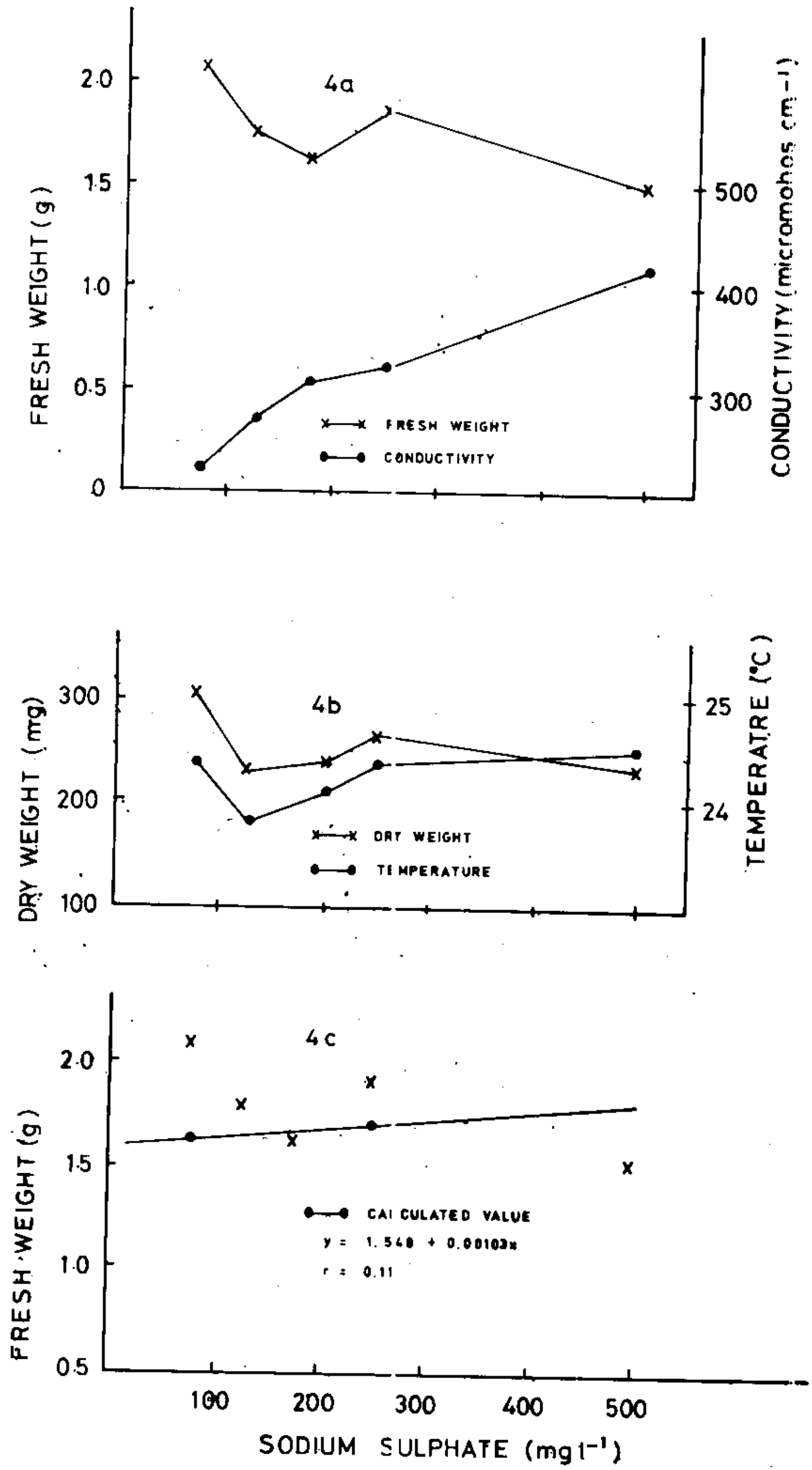

Figure 4. Biomass harvest of *Hydrilla* in Sodium water quality (4a, b) and regression of sodium sulphate upon *Hydrilla* biomass (fresh weight)

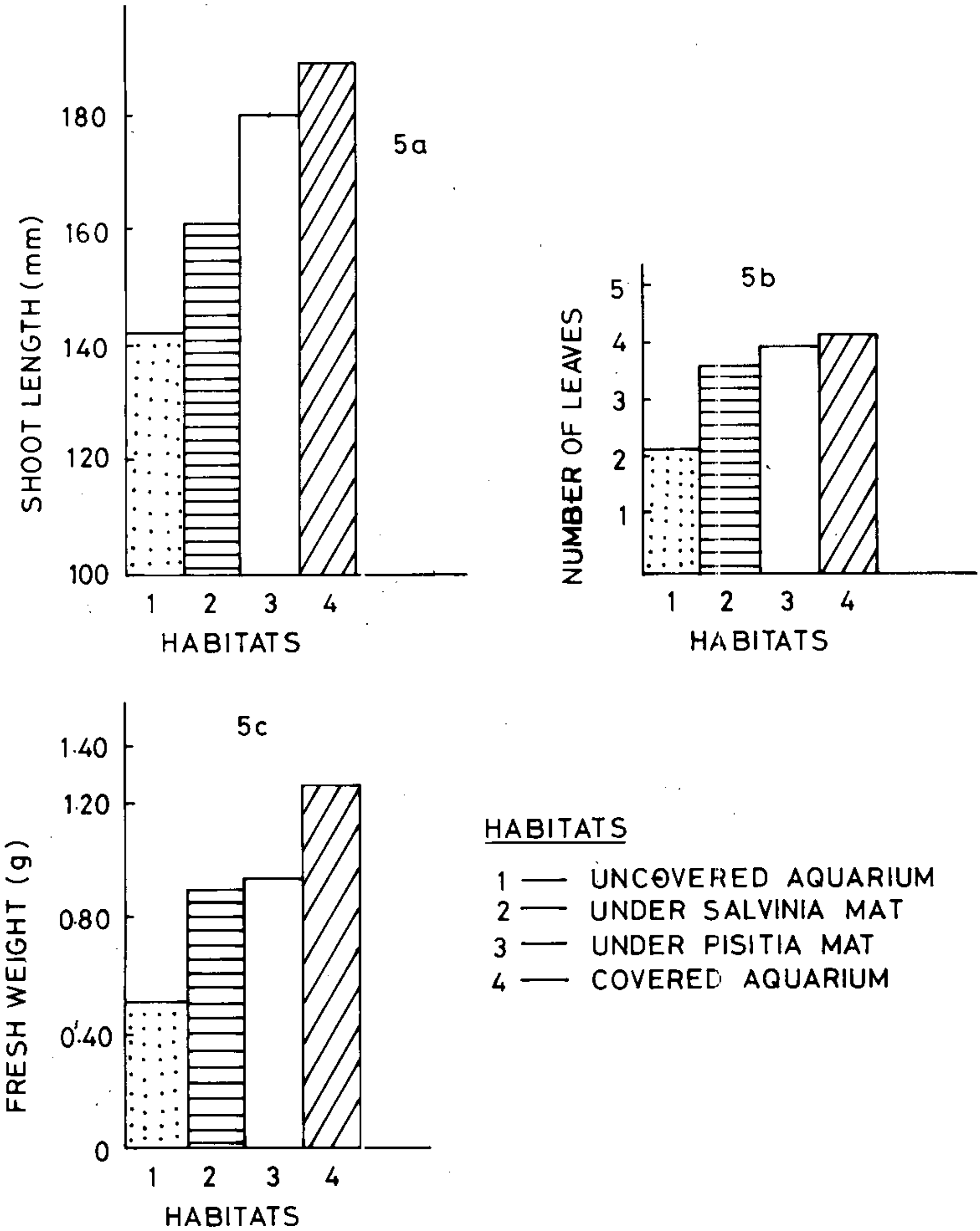

Figure 5. Growth expression by *Hydrilla verticillata* under four different habitats

intensities which promote internode elongation with a limited amount of branching which in effect cause *Hydrilla* to elongate towards water surface where more light is available for photosynthesis. The uncovered aquarium was invaded by algae after six days. It is possible that competition in nutrients between the study plants and algal bloom may have contributed to the suppressed growth in this habitat. The covered habitat with well lighted top had the greatest *Hydrilla* growth.

As pointed out above, changes in water quality status could have been the result of concommitant growth of algae in experimental containers. When similar algal suspension was monitored without *Hydrilla,* it was possible to separate effect of algae on water quality. The algal suspension consisted mostly of *Scenedesmus falcans* Chadat, common in all of the above experiments.

Both the algae and *Hydrilla* show the ability to assimilate both carbon dioxide and bicarbonate ions as source of carbon. In the algal suspension (figures 6a, 6b, 6c) pH rose from 6.3 to a maximum of 11.1 after 58 hours. After this period pH remained almost constant and at the end of experiment was 10.8. In the same container dissolved oxygen rose from 5.5 to 10.8 mg per litre after 24 hours of photosynthesis. After this period oxygen remained almost constant at 7.8 mg per litre. The algal suspension also showed changes in dissolved carbon dioxide from 4.5 mg per litre at start to 0 after 10 hours.

When *Hydrilla* was grown separately the water was characterised by a rise in pH after illumination reaching a maximum of 10. Dissolved oxygen remained high at 9.4 mg per litre. Dissolved carbon dioxide was depleted from growth system after 10 hours (figure 6c).

Biocontrol

Lymnea showed a preference for the top and side leaves of *Hydrilla* while *Bulinus* was often associated with cut off shoot segments. *Lymnea* in several cases was also seen attached to the end of cut off shoot segments. Stems whose leaves were eaten or stem segments cut off by any of these snails did poorly in terms of growth. Cut off leaves fell to the bottom of aquarium where they were likely eaten up by bottom snails or possibly dissappeared through decay.

Damage to *Hydrilla* by snails was assessed through measurement of biomass in control and in the aquaria containing snails. When snails were grazed on *Hydrilla* alone, the mean fresh weight consumed was 34.3%. In the case where other littoral plant species were introduced together with *Hydrilla,* consumption of the latter was 22.2% (table 2).

DISCUSSION

Plants may use CO_2, HCO^-_3 or CO^{2-}_3 (though the latter has not been fully established) to achieve their photosynthetic requirements (Golterman 1969). In the utilization of inorganic carbon by both *Hydrilla* and algal suspension, pH of the system changes. As CO_2 is utilized pH rises and once it is depleted from the system the absolute quantity as well as changes from CO_2 to HCO^-_3 and CO^{2-}_3 will fall. If *Hydrilla* and *Scenedesmus* used only free CO_2, photosynthesis and oxygen production would stop when concentration of CO_2 became negligible (e.g., after 10 hours, figure 6c). At this point rise in pH would also stop. This observation will take place when the pH is a little above 9.0 and if a plant used HCO^-_3 ions then a rise in pH would continue until the pH would be well above 10 (Wetzel 1972; Golterman 1969). Both *Scenedesmus* and *Hydrilla* fulfil the latter requirement in the sense that pH rose to above 10 and oxygen continued to be produced even after carbon dioxide was lacking in the system (figure 6a, 6b, 6c). Unreliability of the method used in this study may be caused by pH changes due to assimilation of NH^+_4 or NO^-_4 ions and possibly by flux of H^+ and OH^- from plants. Hutchinson (1975) and Sculthorpe (1966) have, however, shown that the possible effects of these ions are unlikely to produce major errors.

HCO^-_3 ions may be used directly or in the production of free CO_2 (Golterman 1969).

Table 2. Consumption of *Hydrilla* by snails, *(Lymnea natalensis* and *Bulinus* spp.

	Control aquaria fresh weight (g)	Snail aquaria fresh weight (g)	Difference	Consumption (%)
Hydrilla	7.33	4.20	3.13	41.8
Hydrilla	5.26	3.86	1.40	26.8
Mean	...	...	...	34.3
Mixed macrophyte	5.71	4.44	1.27	22.2

Each treatment value is a mean of four replicates

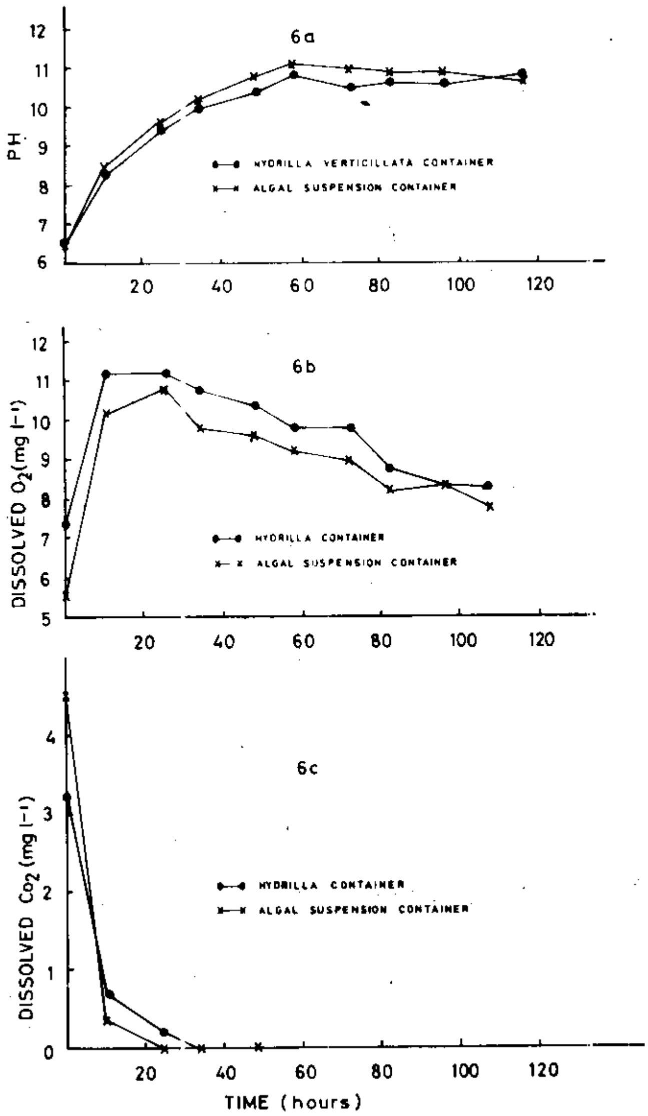

Figure 6. pH (6a), dissolved oxygen (6b) and carbon dioxide (6c) levels during the course of photosynthesis by *Hydrilla verticillata* and some algal species

Most higher water plants make effective use of HCO^-_3 as opposed to bryophytes and submerged land plants. Steeman-Nielsen (Hutchinson 1975) and Gessner (Sculthorpe 1966) have shown that *Elodea canadensis* Michx, *Myriophyllum spicatum* Ponted ex L., *Potamageton lucens* Solms and *Ceratophyllum dermersum* L. could photosynthezise in solutions containing either CO_2 or HCO^-_3. Osterlid (Raven 1970) has shown that *Scenedesmus* and *Hydrodictyon africanum* Miq could utilize HCO^-_3 ions. Wetzel (1972) further states that some algae utilize more HCO^-_3 at a faster rate than higher plants.

The above observations show that *Hydrilla* and *Scenedesmus* are able to photosynthezise at high pH and therefore able to use CO_2 and HCO^-_3 as source of inorganic carbon. Possession of an affinity for HCO^-_3 would be of adaptive significance, particularly for submerged angiosperms.

While potassium plays some role in the growth of macrophytes and algae, sodium has not been found to play any significant physiological role in the growth of macrophytes (Wetzel 1960). Wetzel working with *Najas flexils* L. has shown sodium to cause marked increase in photosynthetic rate when present at about 3-4 mg per litre. Beyond this concentration photosynthetic rate decreased. Brownell (1964) has shown sodium to be a possible microelement for a higher land plant. Allen and Daniel (1955) have shown sodium to be of some importance in the blue green algae group.

Maximum growth of *Hydrilla* occured at an optimum inorganic carbon source of about 220 mg per litre (CO_2 + HCO^-_3). Denny (1972) was able to grow *Hydrilla* in a medium of 160.8 mg per litre total alkalinity. This observation means that *Hydrilla* would likely be limited by lower levels of HCO^-_3.

In Lake Victoria an underlying limestone bottom may help in supplementing inorganic carbon should it be lacking or reduced by high plant populations. Since diffusion of CO_2 from atmosphere into solutions in slow, free CO_2 content of the lake water would rapidly diminish and the situation would have to be rectified by another source. But the littoral region of the lake is well populated (especially bays) with macrophytes and algae and the average total alkalinity (CO_2 + HCO^-_3) in the lake is about 55.8 mg per litre (Haller and Sutton 1975). *Scenedesmus, Chlorella, Hydrodictyon* (all found in Lake Victoria) have been found to be users of CO_2 and have the added advantage of HCO^-_3 utilization (Wetzel 1960). The above situation would contribute in putting *Hydrilla* under inorganic carbon stress.

In most waters where *Hydrilla* growth is pronounced and an important weed, the water is calcareous in nature. These favourable conditions can be noted in Lake Orange, Florida, with a bottom limestone lining and also Silver springs, Florida, where water quality is similar to Lake Victoria except that the total alkalinity is much lower for the latter case and Lousiana lakes where total alkalinity (HCO^-_3) is 140-175 mg per litre and CO^{2-}_3 is 180 — 205 mg per litre (Pirk and Brook 1959). Hutchinson (1975) has shown that several aquatic macrophytes are able to tolerate fairly high alkalinity provided the overall concentration of other electrolytes is not so high.

Alkaline waters are not only nutrient rich but are also sinks of CO_2 and HCO^-_3. CO_2 is supplied by air, by oxidised or reduced subaqueous mud and HCO^-_3 ions originate from the mud (Ruttner 1953). Rich calcareous waters have a constant carbon source that may be lacking in the lake Victoria substrate. This coupled with depletion of HCO^-_3 in the water by phytoplankton production could result in a real shortage of inorganic carbon. Brian (1973) has noted that some species are able to use CO_2 and HCO^-_3 at very low levels for their photosynthesis, but their growth are slow and would probably be at a serious competitive disadvantage compared with species of eutropic lakes or high populations of free Co_2 users. This could be a possible case limiting the growth of *Hydrilla* in Lake Victoria littoral waters.

Results of the above experiments and present study indicate that HCO^-_3 level does influence the distribution of *Hydrilla* in East Africa but it may not be the only determinant. It is therefore appropriate to consider other ions which might be important. Lake Victoria is known for its ionic imbalances (Ruttner 1953).

There may also be some physical factors influencing the natural growth of *Hydrilla*. Lake temperature stratification, lack of a suitable carbon supply as a result of stratification or other factors affecting mineral cycling could be important in the interpretation of the weed's growth status. Phosphorus in the lake has been shown to be reasonably low (Fish 1956). Nitrate increases with depth and is less available at surface waters. All these affect *Hydrilla* growth, especially since the leaves are reported to be the nutrient absorbers in submerged macrophytes (Welzel 1972).

Growth of *Hydrilla* in these experiments was not as pronounced as in nature. There could be some side-effecs from possible ionic quality or phytoplakton invasion of the waters. It is thus of paramount importance to establish the ecological status of Lake Victoria. This should be done before any answer would be forthcoming to some of the questions regarding macrophyte-phytoplankton relationships. Some correlations between macrophyte and phytoplankton distribution in relations to water chemistry of the lake could easily be established.

Effects of local fauna such as snails must be considered. In the present study there was only a low consumption of *Hydrilla* by snails. This could be attributed to the small size of the animals used. Their daily consumption was probably very small. Close observation showed that few of the snails were associated with *Hydrilla* plants at any one time. When other species were introduced, there was reduced consumption of *Hydrilla*. This possibly means that snails are able to feed on other plant species and may as well show a food preference for species other than *Hydrilla*.

Taking into account that there may be a higher population of snails in the littoral zone of lakes, it is possible that these snails could be playing some role in the suppressed growth of *Hydrilla* in Lake Victoria. But water hardness is important in the growth of gastropods and in most of the cases low alkalinity present a limiting factor to normal growth of snails. The low alkalinity of Lake Victoria water may allow development of populations but with reduced size and shell. These factors may contribute to a low consumption of macrophytes by snails and intensive biocontrol in Lake Victoria littoral waters may not be expected.

CONCLUSIONS

Hydrilla verticillata occurs in Lake Victoria littoral zones among other macrophytes. From the present study the weed was observed to have highest biomass output in growth mediums of average 220 mg per litre total alkalinity (CO_2 + HCO^-_3). Elsewhere in calcareous water the weed is so profuse in growth that it outgrows most other weeds associated with it. Growth in Lake Victoria is, however, less profuse. Low average total alkalinity of 55.8 mg per litre (CO_2 + HCO_3^-) in the lake and lack of bottom limestone lining likely to be a constant source of carbon supply to the lake substrate may be limiting the growth of this weed. The presence of rich flora of macrophytes and algae, also users of CO_2 and HCO^-_3 ions as source of inorganic carbon for photosynthesis may be contributing in placing *Hydrilla* under inorganic carbon stress. Further studies in other nutrient requirements by the weed in relation to the nutrient status of the lake littoral zone is suggested. Biocontrol of the weed by snails whose development and life performance is dependent on water hardness was observed to be low.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the supervisory assistance given to me by Dr. J. Gaudet of the Department of Botany, University of Nairobi. His helpful criticisms are highly appreciated.

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B.N. Kigomo,
Kenya Agricultural Research Institute, Muguga
P.O. Box 30148, Nairobi, Kenya

Acceptance date: 30 March 1983

APPENDIX 1

Checklist of the most common macrophyte species in shallow waters of Lake Victoria (Eggeling 1940; Thomas 1941)

Emergent

Cyperus papyrus L.
C. digitatus Lag.
C. rigidifolius L.
C. haspan L.
C. immensus L.

Eleocharis fistulosa Lisk
Hydrocolyle ranuniculoides Lag.
Impatiens procridioides Warb
Limnanthemum zanzibarensis Miq
Ludwigia prostrata Roxb
Nymphaea caerulea L.
Pycreus mundtii Kraenzl
Punicum chlonachne Mez
P. repens L.
Polygonium serrulatum Lag.
Typha latifolia Miq.
Ultricularia exoleta R. Br.
Xyris capensis Thunb.

Submerged

Ceratophyllum demersum L.
Lemna polyrhiza L.
Ottelia ulvifolia Walp.
Potamogeton richardii Solms — Lamb
Sphagnum macrolincarum Dix

Floating

Azolla nilotica Desm.
Lemna Polyrhiza L.
Nymphaea capensis L.
N. zanzibarensis Caspary