MECHANISM AND ECOLOGY OF EXOGENOUS BICARBONATE USE IN PHOTOSYNTHESIS BY FRESHWATER MACROPHYTES.

by

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CAREER.

I graduated from the University of St Andrews in June 1969 with a First Class Honours Degree in Botany. I was awarded a Natural Environment Research Council Studentship for 2 years research at the University of St Andrews.

In August 1969 I was admitted as a Research Student under Ordinance General No. 12 and under Resolution of the University Court, 1967 No. 1.

DECLARATION.

I hereby declare that the following thesis is based upon work done by me, that the thesis is my own composition, and that it has not been previously presented for a Higher Degree.

The research was carried out at the Department of Botany, University of St Andrews, under the supervision of Professor D.H.N. Spence.

CERTIFICATE

I certify that Myra Ann Black (nee March) spent eight terms of research under my direction, that she has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967, No. 1, and that she is qualified to submit the accompanying Thesis in application for the Degree of Master of Science.

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The author wishes to record her gratitude to Professor D.H.N. Spence of the Department of Botany, University of St Andrews, for supervising the work presented in this Thesis and for his interest and encouragement throughout the whole project. She also wishes to thank Professor J.A. MacDonald of the Department of Botany of the University of St Andrews for providing facilities for research.

CONTENTS

1. INTRODUCTION.

Comparative study of bicarbonate use in photosynthesis.

Many submerged aquatic plants can exchange nutrients between the bathing solution and their leaves (see references in Sculthorpe, 1967: Denny and Weeks, 1968, 1970: Campbell 1972). Most experiments have comprised studies of ion influx and efflux by detached leaves or shoots during photosynthesis particularly in relation to use of bicarbonate which Angelstein (1911) first indicated as a possible exogenous inorganic carbon source in submerged plants.

This thesis deals with detached leaves of Potamogeton species and Fontinalis antipyretica. These experiments may be classed broadly as those concerned with establishing that bicarbonate is used during photosynthesis and by what species, and those, discused presently, concerned with mechanisms. Detached leaves, shoots or thalli of some species of angiosperms and algae photosynthesise more rapidly in a bicarbonate solution

at high pH, with less than 5% unhydrated or free CO_2, than in a solution at lower pH containing less than 10% bicarbonate but an equivalent concentration of unhydrated or free CO_2 (e.g. Steeman-Nielson 1947, Raven 1968). This capacity is taken to imply the use of bicarbonate by the leaf or other green tissue during photosynthesis and is generally attributed to bicarbonate uptake followed by dehydration (Arens, 1930, 1933: Steeman-Nielson, 1947: Raven, 1968) less frequently to dehydration of bicarbonate at the leaf surface free space (Briggs, 1959). Under similar conditions other species (of moss and algae) cannot use bicarbonate (Rutner, 1947: Steeman-Nielson, 1947, 1951, 1952: Österlind, 1951).

A comparative method is employed here; one of its drawbacks is the difficulty of distinguishing between effects on photosynthesis of the external bicarbonate concentration and the external pH so, firstly, results of experiments are described which were designed to discriminate between these factors. Then, by means of the comparative

method, one angiosperm is confirmed and two further angiosperms (all Potamogeton species) are established as bicarbonate users; one moss (Fontinalis antipyretica) is confirmed and an angiosperm (Potamogeton polygonifolius) is established as a non-user. All the species have contrasted distribution in terms of bicarbonate concentrations of their natural waters.

Mechanism of bicarbonate use.

Various workers have demonstrated or deduced uptake into detached leaves of several naturally submerged species, of Ca, K, NH_4, NO_3 HCO_3, HPO_4, SO_4 and Cl ions and export of Ca, Na and OH^- ions: uptake occurring in the dark and at an enhanced rate in light; for PO_4 SO_4 Cl and HCO_3 uptake there is clear evidence of metabolic dependence (Arens, 1930, 1933: Steeman-Nielson, 1946, 1947: Lowenhaupt, 1956). Denny and Weeks, (1970) for detached leaves of Potamogeton schweinfurthii, produced strong evidence for at least partially active transport of bicarbonate coupled to photosynthesis. Raven (1968) showed that photosynthesis with bicarbonate in Hydrodictyon

africanum is more sensitive within defined limits to DCMU (3-(3,4-Dichlorophenyl)-1,1-dimethylurea) than is CO_2 fixation. Ikemori and Nishida (1968) with Ulva pertusa thalli and Raven (1970) with H. africanum found that Diamox (2-acetylamine-1,3,4-thiadiazol-5-sulfonamide) and sulphanilamide respectively, supplied at concentrations that severely inhibited carbonic anhydrase activity had almost no effect on photosynthesis in carbon dioxide solutions but inhibited photosynthesis in bicarbonate solutions. In this present account effects are studied of added DCMU on rates of photosynthesis by an angiosperm bicarbonate user, P. praelongus, and a non-user, P. polygonifolius, in bicarbonate solution and in a solution with an equivalent concentration of free CO_2 only; and the effects are studied of added Diamox on carbonic anhydrase activity and rates of photosynthesis, of P. praelongus, in a bicarbonate solution.

Variations in water chemistry, salinity and alkalinity.

Since one of the striking features of

fresh waters is the wide variations in ionic concentration, the question of bicarbonate use is placed in this general context and in that of the carbon dioxide system in water.

In the lakes of the U.K. alone there is a large range in ionic concentration (total dissolved solids), from about 5mg/l to about 500mg/l. Na and Cl are among the large contributors to this range, but there is a 1000 fold range, from 0.004 to 3.80mM, in concentration of ions comprising total alkalinity (HCO_3^- + CO_3^{--} + OH^-) and from 0.005 to 5.20mM in Ca concentration.

CO_2 in water.

In varying proportions that depend on pH, inorganic carbon in solution comprises carbon dioxide, carbonic acid and bicarbonate and carbonate, see Figure 1. When carbon dioxide dissolves in water, a proportion slowly hydrolyses to give, at equilibrium, 99.6% free CO_2 and 0.4% carbonic acid:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

Subsequently in this account free CO_2 is held to

include and is uncorrected for undissociated carbonic acid. In two reactions that are rapid compared with this hydration reaction, carbonic acid with pK_1 (at 20°C) of 6.39 dissociates:

$$H_2CO_3 \underset{}{\overset{k_1}{\rightleftharpoons}} H^+ + HCO_3^-$$

and with pK_2 (at 20°C) of 10.38, HCO_3^- dissociates in turn:

$$HCO_3^- \overset{k_2}{\rightleftharpoons} H^+ + CO_3^{--}$$

where k_1 and k_2 are, respectively, the first and second dissociation constants of carbonic acid in water. These facts are used in this account to provide, via the derived Henderson-Hasselbach equation and Saruhashi's (1953) Tables, based in turn on that equation, percentage molar fractions of the total CO_2 contributed by free CO_2, bicarbonate and carbonate in solution at various values of pH and temperature.

Bicarbonate concentration and nutrient status and plant distribution.

In fresh waters of high pH only a small proportion of the total CO_2 (2% at pH 8) is free CO_2 (see Figure 1). For the rest of the total

CO_2, contained in the total alkalinity (HCO_3^- + CO_3^{--} + OH^-) we are dealing with situations where bicarbonate is the principal or only contributor and indicates the nutrient status of that water; this is true at least early in the growing season, if not late, of lakes where photosynthetic activity has eventually depleted the carbon pool and increased the OH^- component. It does not apply to calcareous waters, where although bicarbonate alkalinity more or less equals total alkalinity, there is essential nutrient inbalance and limiting levels of for example phosphorous in solution: but it may apply to mud underlying these waters. Accepting these qualifications it follows that some aquatic plants may be typical of nutrient- or bicarbonate-rich sites, others of nutrient-or bicarbonate-poor sites, and others, again, indifferent or ubiquitous in distribution.

Ecology of bicarbonate use.

This raises the general question of the nature and significance of any adaptations by

species to high or low-alkaline sites, in terms of their responses to shortage or excess of certain ions, and the particular question of the role of the bicarbonate ion. The basic argument is that provided disseminules of a species, and space, are available (Spence 1964, 1967) water chemistry determines whether or not a species grows in a particular lake.

This study is concerned solely with supply of bicarbonate or free CO_2 to detached leaves in a bathing solution and in nature a similar pathway is inferred. Steeman-Nielson (1947) established by the comparative method already referred to that Potamogeton lucens and Myriophyllum spicatum were bicarbonate users, and Spence (1964, 1967) found that these species were uncommon and confined in Scottish lochs to waters having alkalinities (about 2.8mM HCO_3^-) similar to those bicarbonate solutions with added Na, K, Cl and SO_4 in which Steeman-Nielson showed that detached leaves or shoots of these species achieved optimum photosynthetic rates.

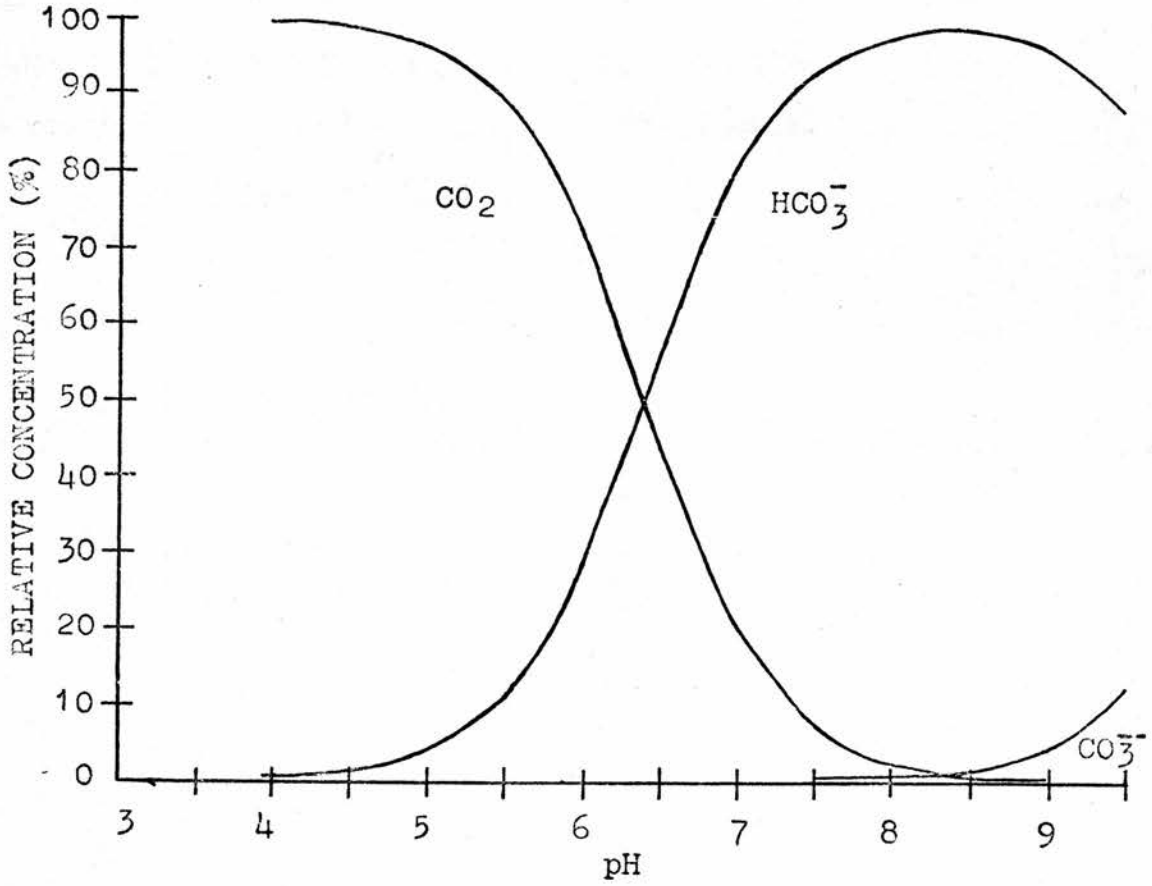
In his review article, Raven (1970)

listed users and non-users, as defined by the comparative method outlined above, among angiosperms, Bryophyta, Charophyta and algae but, because there are no species for which reliable information on both mechanism and ecology are available, it was impossible to state firmly whether possession of the ability to use bicarbonate, or its absence, has any obvious adaptive significance. This study concludes with an examination of this question in relation to the natural distribution of the species studied here.

FIGURE 1

The relative concentrations of carbon dioxide, bicarbonate ion and carbonate ion at different pH values in fresh water at 20°C.

FIGURE 1


100
90
80
70
60
50
40
30
20
10
0
RELATIVE CONCENTRATION (%)
CO_2
HCO_3^-
$CO_3^=$
3
4
5
6
7
8
9
pH

2. MATERIALS.

The plant species chosen for the study were submerged fresh water aquatics and those floating leaved aquatics which possess submerged leaves.

Perennating parts of several species were collected from various Scottish lochs (Table I) and were grown in the greenhouse. A constant supply of fresh plant tissue was thus provided for laboratory experiments.

All the species studied were rooted in a soil-sand mixture and their "aerial" parts were submerged by tap water. Shoots of Fontinalis antipyretica were kept submerged by tap water which was continuously aerated. The water in all containers was changed at regular intervals in an effort to reduce algal infection of the plants.

All plants were grown under mercury vapour lamps and received 16 hours light in every 24 hours.

TABLE I.

Locality and distribution of plant species used.

Species	Locality	Distribution
Fontinalis antipyretica L.	Kirrie Den, Kirriemuir, Angus.	Ubiquitous
Potamogeton lucens L.	White Loch, Castle Kennedy, Wigtonshire.	Rich
Potamogeton perfoliatus L.	Loch Croispol Durness, Sutherland.	Ubiquitous
Potamogeton polygonifolius Pourr.	Loch of Lowes, Dunkeld, Perthshire.	Poor
Potamogeton praelongus Wulf.	Loch Uanagan, Fort Augustus, Inverness-shire.	Ubiquitous

Key to distribution (Spence 1967):

	Total Alkalinity
Rich	$\leq$ 1.2 mM HCO_3^-/l
Poor	< 0.4 mM HCO_3^-/l

3. METHODS.

3.1. Pre-treatment of Plant Tissue.

In all experiments employing Potamogeton leaves, frshly picked leaves were used. This was done to obtain rates of photosynthesis as near as possible to those which would have been attained by an attached leaf. It had been shown, in preliminary experiments, that rates of photosynthesis by leaves detached from the plant and left in distilled wate overnight, were considerably lower than those rates attained by leaves immediately after being detached, (Table II).

TABLE II.

Rates of photosynthesis, by P. praelongus leaves, immediately, and 20 hours, after detachment.

	Rate of Photosynthesis $M\ O_2\ cm^{-2}\ hour^{-1}$
Immediately after detachment	10.5×10^{-7}
20 hours after detachment	7.5×10^{-7}

In the case of F. antipyretica rates of photosynthesis did not decrease with increase in length of time for which the shoot tip had been detached from the parent plant. Freshly picked

shoots of F. antipyretica, however, were used in experiments where possible.

For each species leaves of similar appearance and position on the plant and therefore it is hoped of similar age, were used throughout experimental series. This was done to reduce variability of rates of photosynthesis due to tissue variability.

In the case of P. polygonifolius which possesses floating leaves, only submerged leaves were used in experiments.

3.2. Preparation of Bathing Solution.

Bathing solutions were prepared using distilled water with a low oxygen and carbon dioxide concentration. A low initial CO_2 concentration was achieved in order that it would be insignificant compared with the added carbon. In calculations, initial CO_2 concentration of the distilled water was only considered in relation to solutions with very low concentrations of additional CO_2.

Low O_2 concentrations were used for two reasons. Firstly O_2 has been shown to have an inhibitory effect on photosynthesis particularly when photosynthesis is limited by low CO_2 concentration (Gibbs, Ellyard and Latzko, 1968); secondly, an initial low O_2 concentration would mean a larger percentage increase in O_2, due to photosynthesis, than would be obtained using a solution of high initial O_2 concentration. This latter was particularly important in experiments where rate of O_2 evolution was used as a measure of rate of photosynthesis.

The method chosen to remove O_2 from the water necessarily removed CO_2 as well. For some series of experiments large volumes of bathing solution were required. Boiling such large volumes was hazadous and it was also necessary to cool under Nitrogen gas to prevent re-dissolution of O_2 and CO_2 from the atmosphere. O_2 and CO_2 were, therefore, removed from distilled water by flushing well with N_2 gas for approximtely 30 minutes before the start of an experiment. This method for removing the O_2 and CO_2 is based on Daltons Law of Partial Pressures which states that "the pressure exerted by a mixture of gases is equal to the sum of the separate pressures which each gas would exert if it alone occupied the whole volume". It was found that this method removed sufficient amounts of O_2 and CO_2 from the distilled water to enable it to be used in the preparation of the bathing solutions, (see Table III).

TABLE III.

Concentration of O_2 and CO_2 in distilled water before and after flushing for 30 minutes with N_2 gas.

Gas	Initial Concentration	Final Concentration
O_2	7.4 mg/l	3.4 mg/l
CO_2	0.09 mg/l	0.008 mg/l

Carbon was added to the distilled water in the form of potassium bicarbonate. The pH of the solutions of $KHCO_3$ varied from about 7.8 to 8.3 depending upon the concentrations used. In subsequent text these solutions are referred to as 'bicarbonate' solutions. As can be seen from Figure 1 (page 10) there are appreciable equilibrium concentrations of 'free' CO_2 at these pH values, from 3.7 to 1.2% 'free' CO_2 respectively at 20°C. To enable a calculation of the 'free' CO_2 concentration in these bicarbonate solutions to be made recordings of the pH of the bathing solutions used for each experiment were made.

'Free' CO_2 solutions were prepared by acidifying bicarbonate solutions to pH 4.3 with Normal HCl. At this pH value, at 20°C, there is only 1% HCO_3^- in equilibrium with free CO_2 and H_2CO_3 forms. For example, a 1.0×10^{-3}M HCO_3^- solution, when acidified to pH 4.3, gives a 1.0×10^{-3}M free CO_2 solution. (See Appendix I).

When solutions of intermediate pH were required, $KHCO_3$ was added to distilled water to give the desired free CO_2 concentration at the particular pH required. The percentage of carbon present in the form of free CO_2, at a certain pH value was calculated from Saruhashi's Tables (Appendix II). 1 N HCl was used to acidify solutions, as before.

$CaCl_2$ was added to all solutions unless otherwise stated. The concentration added in all cases was 10^{-4}M. It has been shown by various workers (Steeman-Nielsen 1947, Denny 1967) that the presence of Ca^{++} ion is necessary for the uptake of any appreciable amounts of HCO_3^- ion into

plant cells. Choice of the concentration used was based on the results of Denny (1967).

3.3. Use of an Oxygen Electrode for Measuring the Rate of O_2 Evolution by Photosynthesising Tissue.

A Mackereth oxygen electrode was employed for this purpose. The electrode was first calibrated according to the method of Mackereth (1964). It was then possible to convert readings, in milliamperes, to mg/l, using the calibration chart. Several concentrations of O_2 in solution, determined in this way, were checked by Winkler analysis. (See Appendix III for calibration chart and comparable Winkler readings.)

The apparatus used is shown in Figure 2. The experimental bathing solution was sealed from the atmosphere by means of the perspex box lid. There was thus little probability of equilibration of free CO_2 from the solution taking place with atmospheric CO_2. During an experiment the bathing solution was continuously stirred by means of the magnetic stirrer. Electric current to the stirrer motor ran via a voltage stabiliser, to ensure that any variation in photosynthetic rate was not due to change in stirring rate. The

plastic mesh cage was necessary to prevent the tissue from becoming entangled around the electrode and stirrer. It was so designed to allow free rotation of the stirrer beneath it and freedom of the tissue within it.

Procedure.

The electrode was kept in a solution of Sodium Sulphite, when not in use. Before use, therefore, the electrode was rinsed in tap water and then placed in distilled water at 20°C. This allowed the electrode to equilibrate at the experimental temperature.

In all experiments, unless otherwise stated, the external water bath was maintained at 20°C. The plant tissue was allowed to equilibrate with the bathing solution for at least 20 minutes before readings of O_2 concentration, of the bathing solution were taken. Readings of O_2 concentration were taken at 3 minute intervals, thereafter, throughout the experiment. A

constant check was kept on the temperature of the water bath and of the temperature of the bathing solution, the latter by means of the thermistor incorporated in the electrode. At the end of the experiment the tissue was removed from the perspex box, its area and dry weight determined and the specific leaf area (SLA) of the tissue calculated.

Light Source.

Light was supplied by 5 Osram spotlights of 130 Watts each, which were arranged around the perspex box as shown in Figure 3.

The irradiance incident on the photosynthesising tissue was measured using an ISCO Spectroradiometer (see Appendix X for method). The irradiance (400 - 700 nm) was found to be in the order of 23,000 uW cm^{-2} ($\equiv$ 35,000 lux $\equiv$ 230,000 ergs sec^{-1} cm^{-2}) with all 5 lamps on. This was shown to be a saturating light intensity for photosynthesis for the plants used (page 38)

FIGURE 2

Apparatus employed to follow oxygen evolution from photosynthesising tissue, using an O_2 electrode.

Legend.

1. Mackereth O_2 electrode, lead A connects with an ammeter
2. Membrane around electrode
3. Perspex box, capacity 780ml, containing bathing solution.
4. Box lid
5. Mesh cage containing plant tissue
6. Magnetic stirrer

FIGURE 2

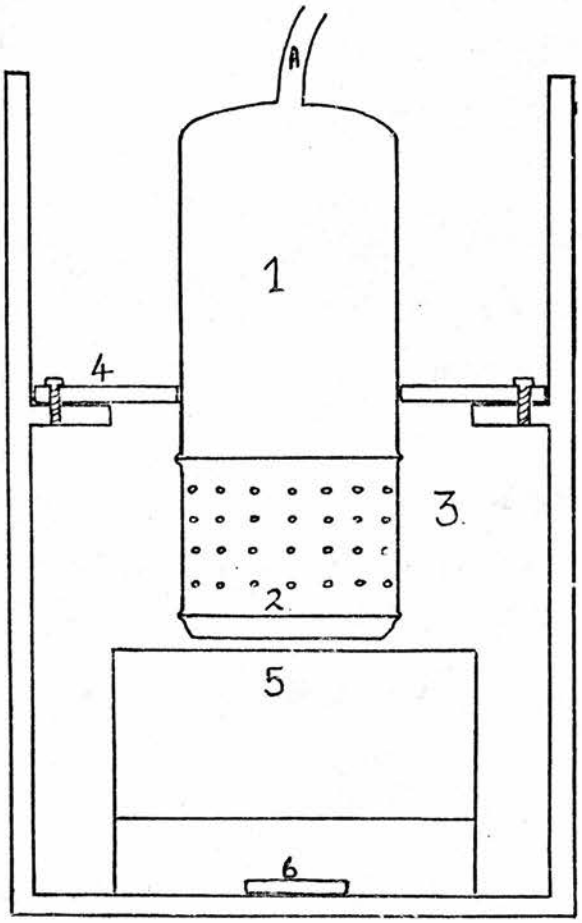


FIGURE 3

Arrangement of spotlights used to irradiate plant tissue

Legend.

1. Temperature control unit
2. Constant temperature water bath
3. Reaction vessel containing plant tissue
4. Cooling tanks
5. Dexian frame
6. Osram spotlights

FIGURE 3

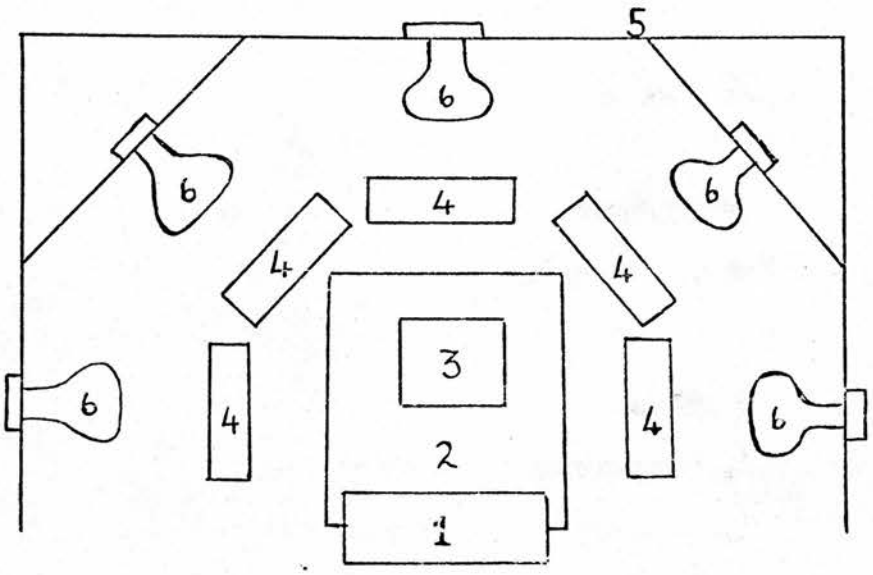


FIGURE 4

Apparatus employed to follow ^{14}C uptake by plant tissue.

Legend.

1. Temperature control unit
2. Mercury thermometer
3. Stirrer
4. Heating coil
5. Constant temperature water bath
6. Reaction vessel
7. Ground glass stopper
8. Magnetic stirrer
9. Stirrer motor
10. Perspex stand
11. Metal weight
12. Cooling coil
13. Cooling tank
14. Heat resistant glass
15. Light source

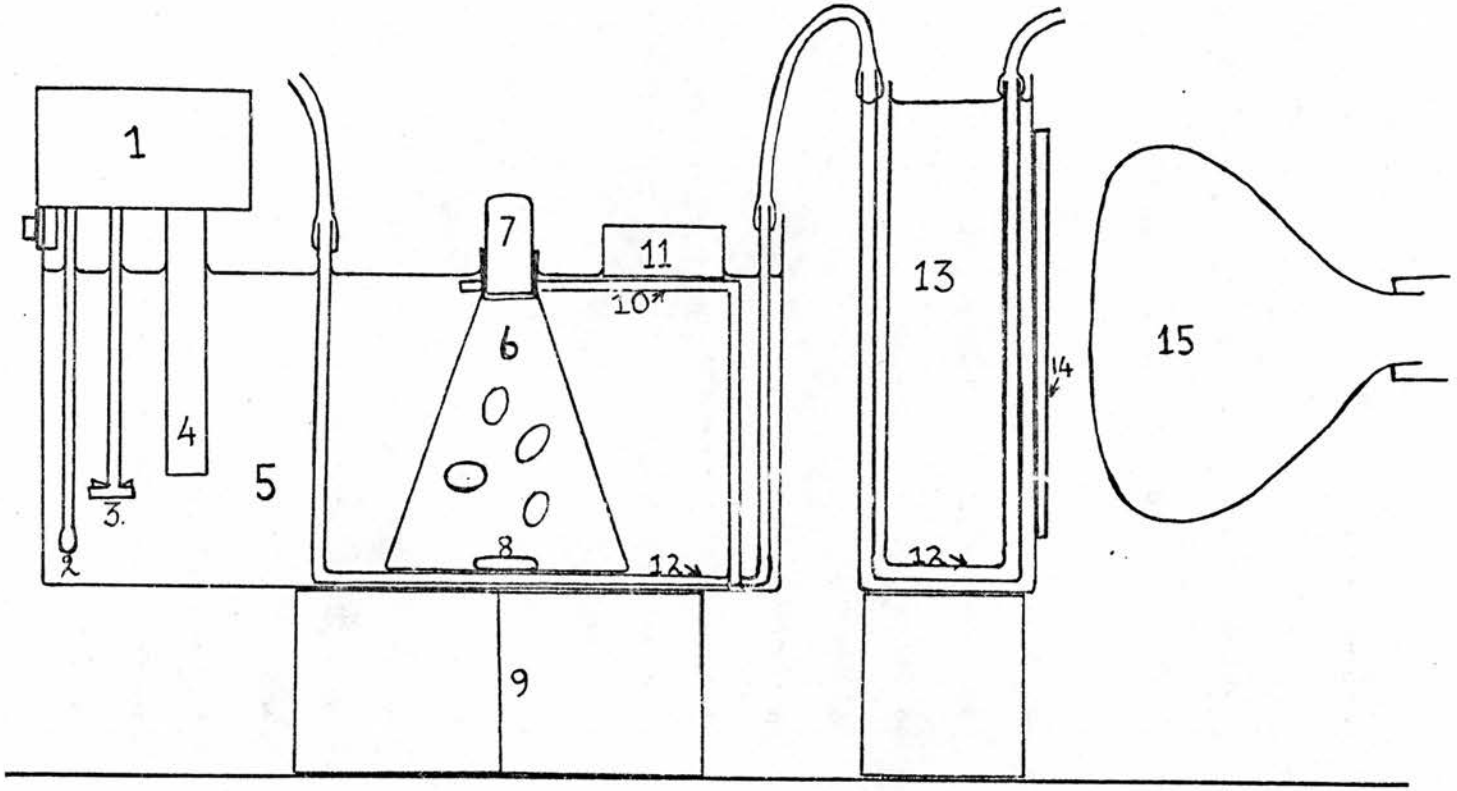


FIGURE 4

Limitations of use of the electrode.

In several initial experiments anomalous results were obtained when using free CO_2 solutions with high CO_2 concentration i.e. 5.0mM CO_2. Results took the form of an initial increase in the meter reading followed by a sharp decrease. These results were found to be caused by the electrode. Approximately half an hour from the start of recordings the meter reading became steady. In experiments using the electrode, therefore, only readings taken after the first half an hour of the experiment were considered in the calculations.

3.4. Determination of Rate of Carbon Uptake by Plant Tissue.

i) In the Laboratory.

This method is based upon determining the rate of uptake of ^{14}C by photosynthesising plant tissue. Plant tissue was allowed to photosynthesise in a bathing solution to which $NaH^{14}CO_3$ had been added. The amount of ^{14}C taken up by the tissue per unit leaf area, was then determined by counting the tissue in a Panax solid scintillation counter. Knowing the specific activity of the bathing solution and the rate of ^{14}C uptake, the rate of Carbon uptake per unit leaf area, could be calculated, (see Appendix IV for specimen calculation). As the rate of uptake of the two isotopes of carbon are not identical (Van Norman and Brown, 1952), the specific activities of the bathing solutions were kept as nearly the same as was feasible, in all experiments, see Table IV.

TABLE IV.

µCi ^{14}C added to bathing solutions of different CO_2 concentrations.

Total CO_2M	µCi 100cm^{-3}
2×10^{-2}	80
1×10^{-2}	40
5×10^{-3}	15
1×10^{-3}	5.0
8×10^{-4}	4.0
5×10^{-4}	2.0
3×10^{-4}	1.5
1×10^{-4}	1.0
5×10^{-5}	0.5

At lower total CO_2 concentrations, the specific activity of the solutions was increased so that it was measurable by the technique employed.

Procedure for determining rate of C uptake, by plant tissue, from solutions of different total CO_2 concentration.

The apparatus was set up as shown in Figure 4. Freshly picked leaves of the species under investigation, were cut into strips or discs (area 0·28 cm²), depending on the size of the leaf, and in the case of F. antipyretica whole leaves were used. 20 tissue pieces were placed in the experimental vessel and allowed to equilibrate in the bathing solution, for 20 minutes, before the radioactive solution was added. A 100 cm^3 Quickfit conical flask was used as a reaction vessel in this series of experiments. By using a close fitting ground glass stopper and having very little air space in the vessel, it is hoped that e uilibration of atmospheric CO_2 with free CO_2 of the bathing solution was kept to a minimum.

In trial experiments tissue pieces were removed from the bathing solution at intervals throughout an experimental period of one hour. Considering the initially limited number of tissue

pieces, however, and the time factor involved in "after treatment" of tissue, only 3 or 4 tissue pieces could be removed at the decided time intervals. It was found that, on occasions, variation in uptake of ^{14}C by the 3 pieces was considerable. An accurate measure of rate of C uptake could not, therefore, be determined by this method.

From these initial experiments, however, an indication was given that over a one hour period, at least, uptake of C was more or less linear with time. It was decided, therefore, to remove all the tissue pieces from the radioactive bathing solution after one hour and to calculate the rate of C uptake from a mean value of counts per minute (CPM) for these pieces, assuming linear rate of C uptake with time. An estimate of Standard Error of the mean value of rate of C uptake could be determined, then. (An average percentage error of 6 was found using 20 samples of tissue.)

Tissue pieces were allowed to photosynthesise in radioactive solution for one hour, being continuously stirred, throughout this period,

by means of the magnetic stirrer. After this time the tissue was removed from the solution, rinsed in distilled water and the areas of the pieces determined. The tissue was then stuck to planchets, using egg albumen, and then completely covered with 10% acetic acid, to remove any unfixed volatile carbon. The tissue was dried and counted.

At the end of the experimental period samples of bathing solution were withdrawn and the carbon present precipitated down with saturated $Ba(OH)_2$, onto weighed planchets. The resulting precipitates of $BaCO_3$ were then dried and counted. CPM were corrected for $BaCO_3$ self absorption.

A correction chart was drawn up to enable a correction for $BaCO_3$ self absorption to be made. It was found necessary to precipitate down onto lens tissue on the planchets, to give a uniform thickness of precipitate. (See Appendix V) A self absorption curve for plant tissue was also produced. It was found, from this, that no appreciable self absorption occurred when tissue

was only of one leaf thickness. No corrections have been made to tissue counts, therefore, (see Appendix VI for curve).

To determine rates of any dark fixation of C, which may have been occurring, a reaction vessel was completely covered with aluminium foil, to exclude light from the plant tissue, throughout an experimental period.

Light Source.

For this series of experiments the light source was as for the series of oxygen electrode experiments, see Figure 3.

Procedure employed for remaining C uptake experiments. e.g. series investigating effects of inhibitors on photosynthesis.

Screw top bottles, each of approximately 25cm^3 capacity, were employed as reaction vessels. These had rubber seals in the lids and a hole punched in the lid. Thus it was possible to inject radioactive solution into the flasks without removing the lids.

Reaction bottles, to which bathing solution and plant tissue had been added, were placed in a metabolic shaker. The shaker was set at minimum shaking rate to ensure the same rate was used in all experiments. The bottles were covered with water which was maintained at 20°C. Plant tissue was allowed to equilibrate with the bathing solution for 20 minutes before radioactive solution was added. Plant tissue was left to photosynthesise in the radioactive solution for one hour. After the experimental period plant tissue and bathing solution were treated and counted as in the previous method.

The areas of plant tissue used in these experiments were determined at the end of each experimental run.

Light Source.

An overhead light source of xenon lights was employed giving a maximum light intensity of 15000 lux. A constant temperature water bath was positioned between the lights and the metabolic shaker to reduce heat reaching the reaction vessels.

ii) In the Field.

Procedure.

Screw top bottles were used as reaction vessels, each with approximately 25cm^3 capacity, as previously described. Bathing solutions used in the field were natural loch water, loch water enriched with HCO_3^- and or Ca^{++}, or 0.1M number 11 Warburg buffer.

One or two leaves taken from plants recently collected from the loch, were placed in a reaction bottle, containing the experimental solution. Radioactive solution was added to the bottle which was then placed in the loch, at about one foot depth, and left for an experimental period of 3 or 4 hours. Replicates were employed and for each solution and plant species tested a dark bottle was also employed.

During the experimental period light incident on the bottles was recorded using an ISCO Spectroradiometer.

Samples of experimental solution and

plant tissue, used in an experiment, were treated and counted as described for laboratory technique. Total Carbon, in the experimental solutions used, was calculated from measurements of total alkalinity and pH, and also by titrating total CO_2 (see Appendix VII for method).

Specific leaf area of representative samples of leaves, for each experimental run, were determined.

4. INVESTIGATION OF DIURNAL RHYTHM, SATURATING LIGHT INTENSITY AND pH EFFECTS ON PHOTOSYNTHESIS.

4.1. Introduction.

Preliminary experiments were carried out, using the oxygen electrode, to establish conditions for the comparative experiments using bicarbonate and free CO_2 solutions.

The first of these preliminary experiments was to ascertain whether or not the aquatic species chosen exhibited a diurnal rhythm of photosynthesis. If such a rhythm did exist then this would obviously necessitate comparable experiments being carried out at the same time of day. Rates of respiration, by the aquatics, were too small to be detected using the oxygen electrode. Any diurnal rhythm in respiration would be unlikely, therefore, to obscure any diurnal rhythm in photosynthesis. An investigation into the existence of a seasonal rhythm of photosynthesis was not carried out.

The effect of increasing light intensity

on the rate of photosynthesis was investigated in order that a light intensity saturating for photosynthesis could be used in comparative experiments.

The effect was studied of varying pH on the rates of photosynthesis in solutions containing a range of free CO_2 concentrations from $1x10^{-5}$ to $1x10^{-3}$N.

4.2. Diurnal Rhythm of Photosynthesis.

F. antipyretica and *P. polygonifolius* were employed for this investigation.

Procedure.

Oxygen evolution by the plant tissue was followed for periods of 7.5 hours, from 10.45 to 18.15 hours. The bathing solution employed had a free CO_2 concentration of 5×10^{-3} M and a pH value of 6.0. The solution was changed every 2 hours to ensure that the CO_2 concentration and pH value remained reasonably constant throughout the experimental period. For each species, two lots of tissue were used alternately throughout the experimental period. This was to avoid deterioration of the tissue caused by such factors as photo-oxidation. When not in use the tissue was left in distilled water, under subdued lighting.

Rates of photosynthesis were calculated for each hourly period of the experiment. A histogram of photosynthesis against time, for each species is shown in Figure 5.

FIGURE 5

Rate of photosynthesis, in terms of oxygen evolved, by *F. antipyretica* and *P. polygonifolius*, over a 9 hour continuous period.

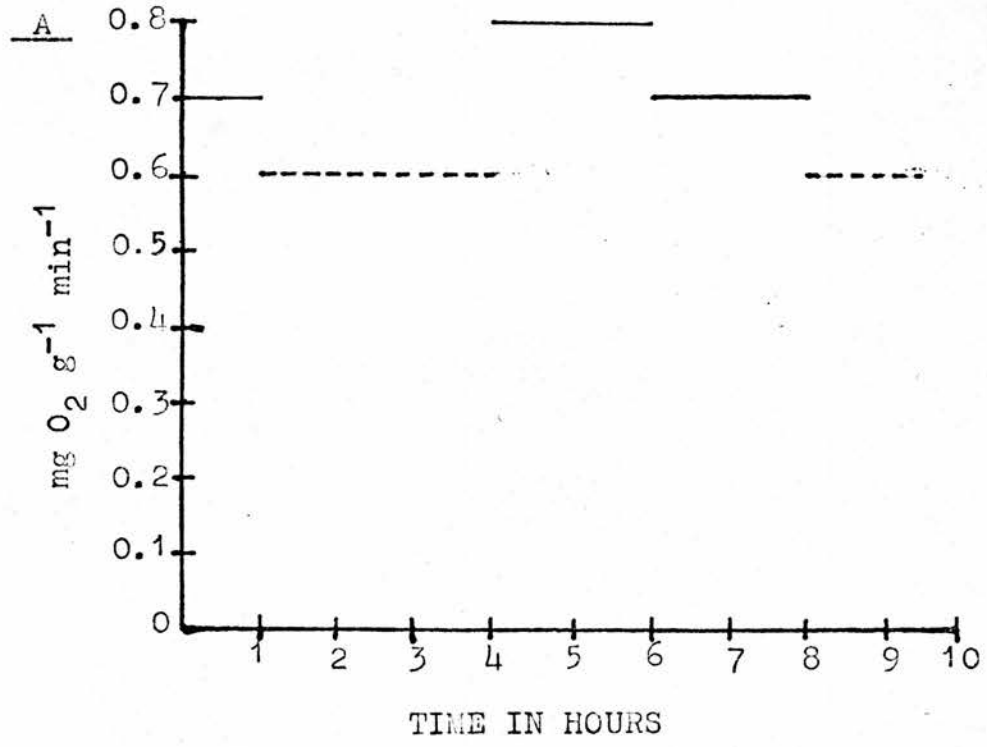
(a) *F. antipyretica*

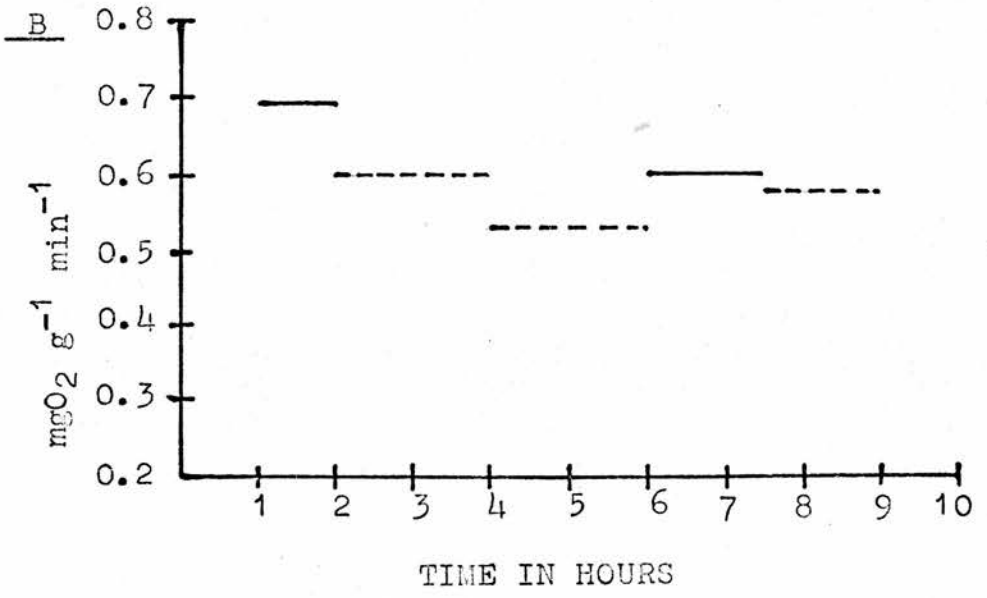
(b) *P. polygonifolius*

(——) lot A tissue

(---) lot B tissue

FIGURE 5


A
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
mg O_2 g^{-1} min^{-1}
1
2
3
4
5
6
7
8
9
10
TIME IN HOURS


B
0.8
0.7
0.6
0.5
0.4
0.3
0.2
mgO_2 g^{-1} min^{-1}
1
2
3
4
5
6
7
8
9
10
TIME IN HOURS

Discussion.

It can be seen from the results, shown in Figure 5, that there is no significant change in the rate of photosynthesis by either species over the experimental period. Although the complete pattern would not be shown, if the tissue did possess a diurnal rythm of photosynthesis, it would at least be evident during an experimental period of more than 7 hours. It has therefore been concluded from these results that neither P. polygonifolius or F. antipyretica exhibit a diurnal rythm of photosynthesis. This fact allows for the comparison of rates of photosynthesis measured at different times of the day.

4.3. Measurement of Respiration.

An attempt was made to determine the rate of any photorespiration which might have been occurring in *P. polygonifolius* and *P. perfoliatus*. A Gilson Respirometer was employed for this purpose. A Warburg 11 0.01M buffer was used as a bathing solution and photosynthesis was inhibited maximally with 10^{-5}M DCMU (see Section 6). To obtain comparable dark respiration rates Warburg buffer was used with no added DCMU and the Warburg flasks were completely covered with foil.

Results.

No dark respiration rates were obtained for either species. A low steady rate of light respiration of 1/10th. and 1/20th. of the photosynthetic rate was obtained for *P. polygonifolius* and *P. perfoliatus*, respectively.

Discussion.

Results of this experiment indicate the

existence of photorespiration in *P. polygonifolius* and in *P. perfoliatus*. The fact that photorespiration is taking place could result in an underestimation of photosynthetic rates in these species. The rates of photorespiration obtained here are, however, very low particularly for *P. perfoliatus*. It must also be remembered that the bathing solution used in these experiments (Warburg buffer) was not "de-oxygenated". If indeed the rates obtained here are true light respiration rates then it is likely that these rates would be depressed in the deoxygenated bathing solutions used for other experiments undertaken in the present study.

If time had been available an attempt would have been made to estimate photorespiration using a ^{14}C efflux method as used by Zelitch (1969). In this method tissue discs are in a $^{14}CO_2$ atmosphere in Warburg vessels. CO_2 free air is then led into the vessels and out through a venting plug into a CO_2 trap. Thus the CO_2 evolved by the tissue may be measured.

4.4. Determination of Saturating Light Intensity.

Procedure.

Rates of oxygen evolution by F. antipyretica P. polygonifolius, P. perfoliatus, P. praelongus and P. lucens were determined under different light regimes, i.e. with 1, 2, 4 and 5 spotlights on. The bathing solution, a 5×10^{-2}M HCO_3^- solution, was the same for each species.

Results.

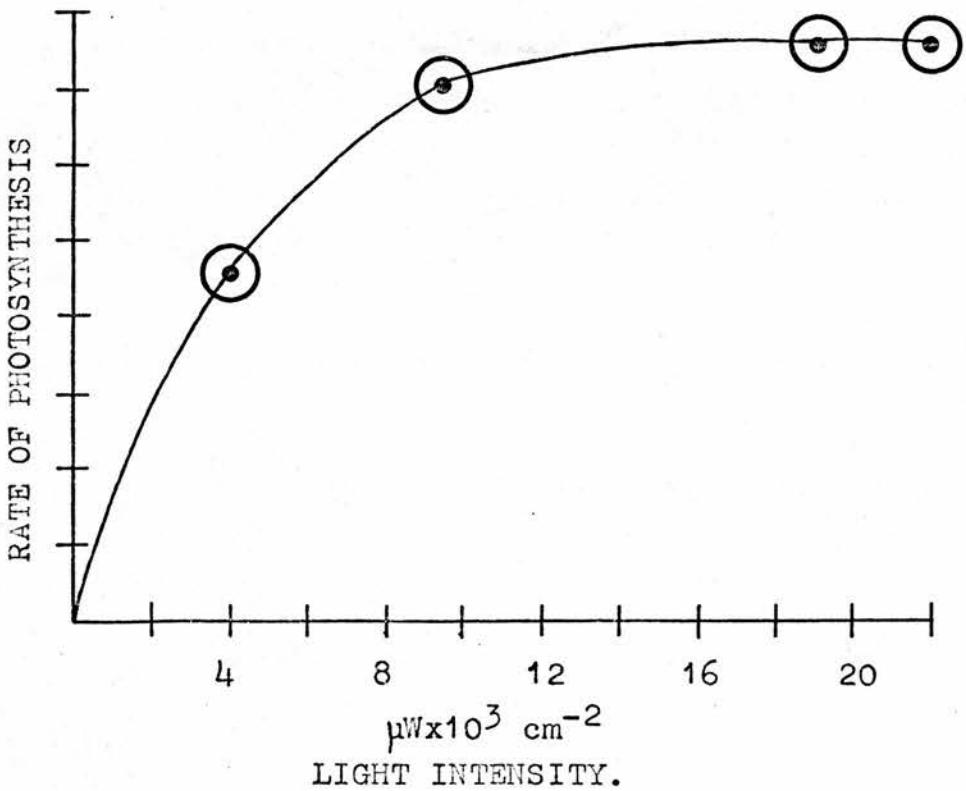
5 lamps were shown to give a saturating light intensity for photosynthesis of all the plant species. Figure 6 shows the typical curve of rate of photosynthesis against light intensity, obtained. Results obtained for P. lucens are exhibited in the figure.

In all subsequent experiments, using the light apparatus shown in Figure 3, 5 lamps were used to ensure a saturating light intensity.

FIGURE 6

Rate of photosynthesis, in terms of oxygen evolved, by *P. lucens*, against increasing light intensity.

FIGURE 6


RATE OF PHOTOSYNTHESIS
4
8
12
16
20
μWx10³ cm⁻²
LIGHT INTENSITY.

4.5. Investigation of pH Effects on Rates of Photosynthesis.

Introduction.

The aim of the following series of experiments was to ascertain whether there was any pH effect on the efficiency of photosynthesis. The classical method for determining whether an aquatic plant is able to use bicarbonate, as an exogenous carbon source for photosynthesis, as well as or instead of free CO_2, involves comparing rates of photosynthesis attained by the species in both free CO_2 and bicarbonate solutions (see Section 1 Introduction, page 2).

However, even though a species may exhibit higher rates of photosynthesis in a bicarbonate solution than it does in a solution of the same free CO_2 concentration, this is not sufficient evidence that the particular species is a "bicarbonate user". It could be argued that the higher pH was the direct cause of the increased rate of photosynthesis, in the bicarbonate solution.

To indicate the validity of this argument for the species used in this study, the following experimental series was carried out.

Procedure.

Using the oxygen electrode, rates of photosynthesis of P. praelongus and P. perfoliatus in bathing solutions of the same free CO_2 concentration but with pH values of 4.3 and 7.3 were followed. At pH 7.3 11% of the total CO_2 is in the free CO_2 form and 89% in the bicarbonate form. Rates of photosynthesis were calculated in terms of mM O_2 evolved per unit leaf area per unit time. The ratio of rates obtained at pH 7.3 to rates obtained at pH 4.3 were then calculated for each concentration of free CO_2 examined. These ratios are shown in Table V, page 42.

In the case of P. polygonifolius rates of photosynthesis in solutions of pH 4.3 and 8.0 over a range of free CO_2 concentrations, were compared. Ratios of rates obtained at pH 8.0 to rates obtained at pH 4.3 are shown in Table VI, page 43.

To obtain further evidence regarding pH effect on rates of photosynthesis, rates of O_2 evolution by P. praelongus were followed in a series of bathing solutions with pH values of

4.3, 5.3, 6.0 and 7.3, each with a free CO_2 concentration of $1x10^{-3}$M. Two sets of tissue were used; rates of photosynthesis of the first set were followed as they were transferred through a series of solutions of increasing pH, from an initial pH 4.3 to a final pH 7.3. Rates of photosynthesis were measured in a second set of tissues which were placed in the opposite sequence of solutions, from pH 7.3 to pH 4.3. This procedure was intended to ensure that any pH effect was indeed an effect on the rates of photosynthesis and not merely an adverse "pre-treatment" effect of extreme pH on the tissue. Results from this experiment are shown in Figure 7.

Oxygen evolution by P. praelongus in solutions of extreme pH values of 4.0 and 9.5 were followed. The pH 4.0 solution used had a free CO_2 concentration of $1x10^{-3}$M and the pH ~~7.3~~ 9.5 solution had a bicarbonate concentration of $5x10^{-2}$M and 0.1N KOH was added to adjust the pH to 9.5. If no depressing effects on the rates of O_2 evolution were observed such solutions could be used in comparative experiments as at pH 4.0 there is less than 0.1% HCO_3^- present and at pH 9.5 there is less than 0.1% free CO_2 present.

TABLE V.

Rate of O_2 evolution, per unit leaf area, at pH 7.3 relative to the rate at pH 4.3, for a series of concentrations of free CO_2.

Concentration of free CO_2 $Mx10^{-4}$	Rate of O_2 evolution at pH 7.3 / Rate of O_2 evolution at pH 4.3	
	P. praelongus	P. perfoliatus
0.1	0.95	-
1.0	0.92	0.79
3.0	1.04	0.94
5.0	0.98	0.97
10.0	0.98	0.97

TABLE VI.

Rate of O_2 evolution, per unit leaf area, by P. polygonifolius, at pH 8.0 relative to the rate at pH 4.3, for a series of concentrations of free CO_2.

Concentration of free CO_2 $Mx10^{-4}$	Rate of O_2 evolution pH 8.0 / Rate of O_2 evolution pH 4.3
0.1	1.00
1.0	0.85
2.0	1.00
3.0	1.07
5.0	1.05

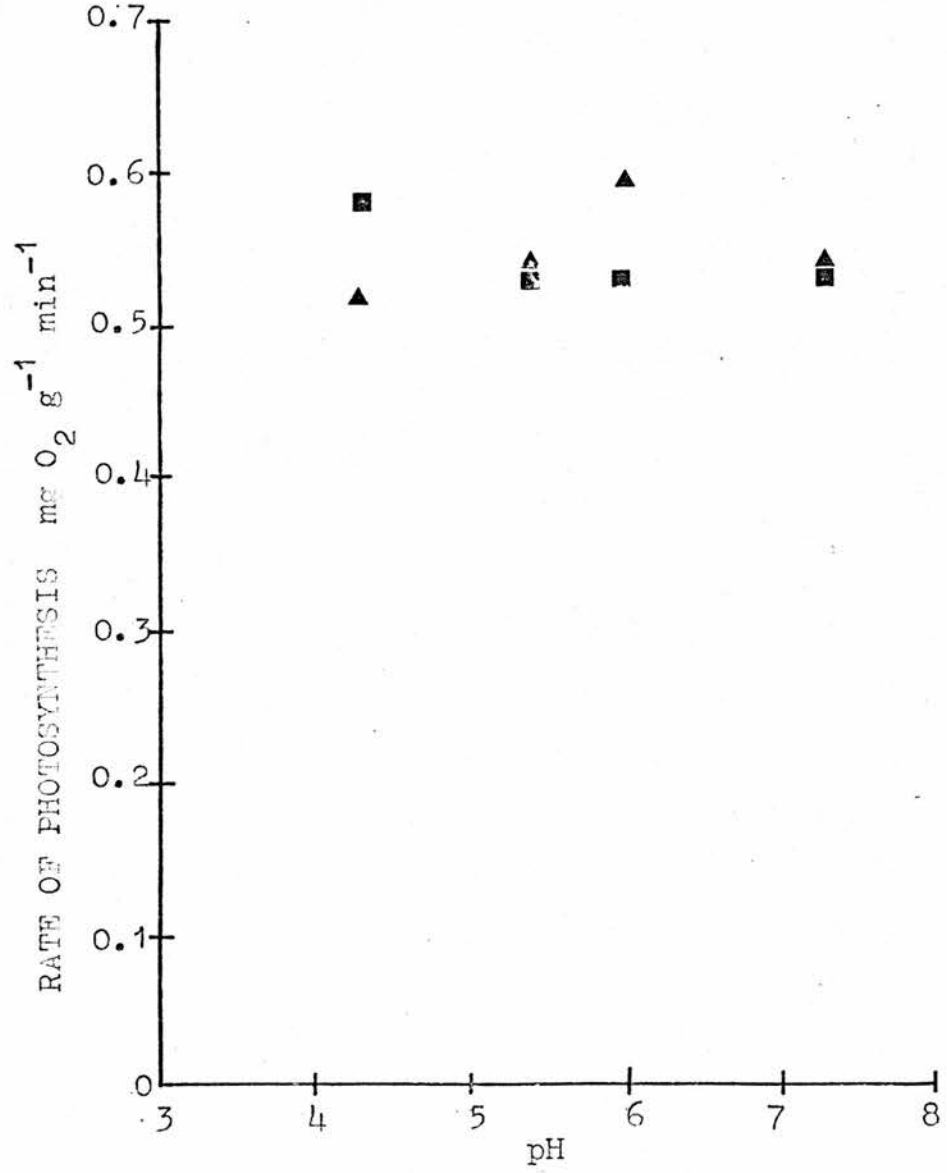
FIGURE 7

Rates of photosynthesis, in terms of oxygen evolved, by P. praelongus in solutions of different pH values, all having free CO_2 concentration of 1×10^{-3} M.

(■) tissue allowed to photosynthesise in pH 4.3 solution first

(▲) tissue allowed to photosynthesise in pH 7.3 solution first

FIGURE 7


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
RATE OF PHOTOSYNTHESIS mc O_2 g^{-1} min^{-1}
3
4
5
6
7
8
pH

Discussion.

From Table V it can be seen that, for P. praelongus and P. perfoliatus, rates of photosynthesis at pH 7.3 do not differ significantly from those at pH 4.3, for the same concentration of free CO_2. It would seem likely that if there were an enhancement of photosynthesis caused by increasing pH of the bathing solution, then this would be apparent over the pH range examined.

At pH 7.3 there is a considerable proportion of HCO_3^- present, indeed 89% of the total CO_2 is in the HCO_3^- form, and 11% in the free CO_2 form. At these concentrations, however, the HCO_3^- ion does not appear to enhance photosynthesis even in the presence of such low free CO_2 concentrations as 0.1 and 1.0×10^{-4}M, which do not approach saturating concentrations for photosynthesis of these species.

In the case of P. polygonifolius rates of photosynthesis in solutions with a pH of 8.0 do not differ significantly from those in solutions with a pH of 4.3. At pH 8.0 97% of the total CO_2

is in the bicarbonate form and 2.5% in the free CO_2 form.

It could be argued, for these three species, that the increase in pH has a depressing effect on the rate of photosynthesis but that this is not apparent as it is compensated for by an enhancement effect of the incresed amount of bicarbonate. It seems improbable, however, that these two factors would operate to cancel each other out exactly, as would be the case for all the species examined in these experiments.

Further evidence that this is not indeed the case, for P. praelongus at least, is shown by an experiment using a bathing solution of pH 6.0, at which pH 71.5% of the total CO_2 is free CO_2 and 28.5% is in the bicarbonate form. Rates of O_2 evolution, by P. praelongus, at this pH, relative to rates at pH 4.3 were 1.00 and 1.05 for free CO_2 concentrations of 5×10^{-4}M and 1×10^{-3}M respectively. Further, rates of O_2 evolution, by P. praelongus , in bathing solutions of four pH values, each with a free CO_2 concentration of

1×10^{-3}M, do not appear to differ significantly from each other, see Figure 7.

Although there appears to be no pH effect on rates of photosynthesis, by P. praelongus, over the pH range 4.3 to 7.3, the extreme pH values of 4.0 and 9.5 do have a depressing effect on the rates. In a 5×10^{-2}M bicarbonate solution, pH 9.5 the rate of O_2 evolution attained by P. praelongus was 6.4×10^{-7}M O_2 hour^{-1} cm^{-2}, which was approximately half the rate observed in a solution with the same concentration of bicarbonate but with a pH of 8.3. The rate of O_2 evolution, by the same tissue, was again followed in the pH 9.5 solution, approximately 2 hours after the first experiment. The rate had decreased to 3.0×10^{-7}M O_2 hour^{-1} cm^{-2}, clearly indicating the adverse effect of this high pH value on the tissue. Similarly the pH 4.0 solution, with a free CO_2 concentration of 1×10^{-3}M, depressed the rate of O_2 evolution to approximately half that attained in a pH 4.3 solution with the same concentration of free CO_2. Such evidence clearly precludes the use of bathing solutions of extreme pH values in

subsequent comparative experiments.

The preceeding experiments have established that a solution pH within the range pH 4.3 to 7.3, for P. perfoliatus and P. praelongus, and within the range pH 4.3 to 8.0 for P. polygonifolius, does not affect the rate of photosynthesis. Steeman-Nielson (1947) made similar observations for P. lucens and F. antipyretica.

All these species are used in subsequent experiments and conclusions of comparative experiments are drawn assuming that there is no pH effect on photosynthesis within the pH range used.

4.6. Experiment on Effects of Solutions of Low or High pH on the Growth of F. antipyretica.

It has been observed that a steady rate of O_2 evolution is maintained, by F. antipyretica, in a solution of pH 4.3 over an experimental period of 3 to 4 hours. When shoots of F. antipyretica were kept in a solution of pH 4.3, however, after 24 hours they began to loose their green colour. After a period of 48 hours in a solution of pH 4.3, all the shoots examined had lost all their chlorophyll. Similar shoots kept in a bicarbonate solution with a pH of 8.3, all other conditions being the same, remained healthy over an experimental period of one week. This would indicate that, in the case of F. antipyretica, a pH as low as 4.3 must be affecting the plant tissue adversely.

5.1. DETERMINATION OF RATES OF PHOTOSYNTHESIS IN BATHING SOLUTIONS OF DIFFERENT CO_2 CONCENTRATIONS AND pH VALUES, USING A ^{14}C TECHNIQUE.

Introduction.

A series of comparative experiments was carried out to ascertain whether the presence of high concentrations of bicarbonate increases the rate of photosynthesis of the plant species under consideration and thus to establish whether or not these species are able to utilise the bicarbonate ion as an exogenous carbon source for photosynthesis.

A ^{14}C technique was employed to determine rates of photosynthesis in this series of comparative experiments. As C uptake by several pieces of tissue, from one solution, could be determined, standard errors of rates of uptake could be calculated. This was considered an advantage of the ^{14}C technique over the oxygen electrode method for measuring rates of photosynthesis. Further a 14C technique was employed to measure rates of photosynthesis in the field. Comparison of field

and laboratory results was made easier and more accurate by using a similar method in the two situations.

To eliminate the necessity of correcting the rates of photosynthesis in bicarbonate solutions for the free CO_2 present in these solutions, bicarbonate solutions of high pH could have been used. At a pH greater than 9.4 there would be no free CO_2 present in solution, all the CO_2 being in the bicarbonate and carbonate forms. If there was a measurable rate of photosynthesis in bicarbonate solutions of such pH then this would clearly be an indication of "bicarbonate use" for photosynthesis (Raven 1968). As has already been shown, however, such high pH values could not be employed for this particular study as they had adverse effects on the plant tissue used.

It has been established that the pH of the bathing solution, within a range of pH 4.3 to 7.3 does not affect the rate of photosynthesis of any of the species used in the following experments. Further a pH value of up to 8.3 does not

appear to adversely affect photosynthesis of the species used. pH 4.3 to 8.3 is, therefore, the maximum pH range used in the following comparative experiments.

Procedure.

A light intensity saturating for photosynthesis of all species used, was employed throughout this series of experiments.

The following bathing solutions were employed: i) bicarbonate solutions with a concentration range of 1×10^{-3}M to 2×10^{-2}M total CO_2; the pH values of these solutions varied from 7.8 to 8.3 depending on the CO_2 concentration, ii) free CO_2 solutions with a concentration range of 5×10^{-5}M to 1×10^{-3}M total CO_2 and a pH of 4.3.

Rates of uptake of carbon from the bathing solutions were then determined, for each plant species, using the method described in the Methods section (page 25). Rates were calculated in terms of Moles of Carbon taken up by the plant tissue per cm^2 leaf area per hour (see Appendix IV for specimen calculation.)

It must be noted that absolute rates of photosynthesis are, in part, a function of specific leaf area (SLA), cm^2 leaf area/ mg leaf dry weight. Although all the rates for the Potamogeton species are only quoted on a leaf area basis sample SLA

determinations were made for each experimental series. While similar leaves were chosen for experimental series (see Method section) this choice was subjective and although SLA of leaves used in each series showed little variation, it is likely that some variation in rates of photosynthesis is due to variation in SLA of the leaves used.

Initial pH of the bicarbonate solutions were determined, using a pH meter, and concentration of free CO_2 present in these solutions were calculated using the values of relative percentages of the CO_2 forms present, as calculated by Saruhashi (1953), for the particular pH value concerned.

Results of these experiments are shown in Table VII. Figures 8 to 12, inclusive, show rates of photosynthesis against free CO_2 concentrations, only, for the two solution types. The standard errors of rates of uptake of carbon, for each CO_2 concentration, are indicated by the vertical lines in the figures.

Rates of carbon uptake in bicarbonate solutions at pH 8 and in solutions at pH 4.3 containing equivalent concentrations of free CO_2, are directly compared in Tables VIII and IX. These rates have been taken from the lines of best fit plotted in Figures 8 to 12 and may, therefore, differ from the rates given, for the same CO_2 concentration, in Table VII, where the actual measured rates are recorded.

TABLE VII

Rates of carbon uptake in bicarbonate solution at pH 8 and in free CO_2 solution at pH 4.3.

Plant species	Bathing solution	Total CO_2 $1x10^{-3}M$	Rate of C uptake $1x10^{-7}M\ cm^{-2}\ hr^{-1}$
P. lucens	Bicarb-onate at pH 8	20	8.44 ± 0.37
		10	4.38 ± 0.23
		5	2.68 ± 0.15
		1	1.25 ± 0.07
		0.5	1.01 ± 0.06
P. lucens	Free CO_2 pH 4.3	1	13.99 ± 0.42
		0.8	13.61 ± 0.65
		0.5	8.96 ± 0.36
		0.3	4.66 ± 0.26
		0.1	1.37 ± 0.10
P. praelongus	Bicarbo-nate pH 8	20	8.68 ± 0.20
		10	7.08 ± 0.39
		5	4.21 ± 0.30
		1	1.98 ± 0.16
		0.5	0.93 ± 0.09

TABLE VII continued.

Plant species	Bathing solution	Total CO_2 $1x10^{-3}M$	Rate of C uptake $1x10^{-7}M\ cm^{-2}\ hr^{-1}$
P. praelongus	Free CO_2 pH 4.3	1	23.58 ± 0.94
		0.8	19.62 ± 1.35
		0.5	6.95 ± 0.66
		0.3	5.80 ± 0.80
		0.1	1.94 ± 0.20
P. perfoliatus	Bicarbonate pH 8	20	4.93 ± 0.49
		10	3.80 ± 0.30
		5	3.71 ± 0.45
		1	1.57 ± 0.21
		0.5	0.88 ± 0.13
P. perfoliatus	Free CO_2 pH 4.3	1	12.14 ± 1.00
		0.8	10.54 ± 0.44
		0.5	5.56 ± 0.44
		0.1	0.63 ± 0.08

TABLE VII continued

Plant species	Bathing solution	Total CO_2 $1x10^{-5}M$	Rate of C uptake $1x10^{-7}M\ cm^{-2}hr^{-1}$
P. polygonifolius	Bicarbonate pH 8	20	6.25 ± 0.29
		10	2.40 ± 0.40
		5	1.17 ± 0.20
		1	1.15 ± 0.09
		0.5	0.51 ± 0.04
P. polygonifolius	Free CO_2 pH 4.3	0.8	12.31 ± 1.50
		0.5	10.40 ± 0.88
		0.4	6.13 ± 0.49
		0.3	5.77 ± 0.47
		0.1	1.37 ± 0.10

TABLE VII continued

Plant species	Bathing solution	Total CO_2 1×10^{-3} M	Rate of C uptake 1×10^{-7} M $mg^{-1} hr^{-1}$
F. anti-pyretica	Bicarb-onate pH 8	20	9.39 ± 0.38
		10	8.40 ± 0.33
		5	6.66 ± 0.28
		1	3.72 ± 0.20
F. anti-pyretica	Free CO_2 pH 4.3	1	19.22 ± 1.86
		0.5	10.40 ± 0.59
		0.3	9.54 ± 0.53
		0.1	9.21 ± 0.52
		0.05	3.01 ± 0.10

TABLE VIII

Rate of Carbon uptake in bicarbonate solution of pH 8 and in solution of pH 4.3 containing equivalent concentration of free CO_2.

		Concentration of free CO_2 M				
Plant species	Bathing solution	$2x10^{-5}$	$3x10^{-5}$	$9x10^{-5}$	$1x10^{-4}$	$2x10^{-4}$
P. lucens	bicarbonate	1.0	1.3	3.2	3.5	6.6
	free CO_2	0.2	0.4	1.4	1.6	3.3
P. perfoliatus	bicarbonate	1.2	1.6	3.0	3.2	4.8
	free CO_2	0.2	0.4	1.0	1.2	2.3
P. praelongus	bicarbonate	1.2	1.8	4.6	5.0	8.2
	free CO_2	0.4	0.6	1.8	2.0	4.0
P. polygonifolius	bicarbonate	0.2	0.4	1.4	1.6	3.5
	free CO_2	0.2	0.4	1.4	1.6	3.5
F. antipyretica	bicarbonate	1.2	2.0	6.0	6.4	8.2
	free CO_2	1.2	2.0	6.0	6.4	8.2

Rates of C uptake in $1x10^{-7}$M C cm^{-2} hr^{-1} for Potamogeton species, $1x10^{-7}$M C mg dry $weight^{-1}$ hr^{-1} for F. antipyretica.

TABLE IX

Ratio of Carbon uptake in bicarbonate solution of pH 8 to Carbon uptake in solution of pH 4.3 containing equivalent concentration of free CO_2.

Plant species	Concentration of free CO_2 M				
	$2x10^{-5}$	$3x10^{-5}$	$9x10^{-5}$	$1x10^{-4}$	$2x10^{-4}$
P. lucens	5.0	3.2	2.3	2.2	2.0
P. perfoliatus	6.0	4.0	3.0	2.7	2.1
P. praelongus	3.0	3.0	2.6	2.5	2.0
P. polygonifolius	1.0	1.0	1.0	1.0	1.0
F. antipyretica	1.0	1.0	1.0	1.0	1.0

FIGURES 8-12

Rates of carbon uptake from bicarbonate solutions pH 8 (□) and from free CO_2 solutions pH 4.3 (o), against free CO_2 concentrations.
Vertical lines represent standard errors of rates of uptake.

FIGURE 8:- P. lucens

FIGURE 9:- P. perfoliatus

FIGURE 10:-P. praelongus

FIGURE 11:-P. polygonifolius

FIGURE 12:-F.antipyretica

FIGURE 8

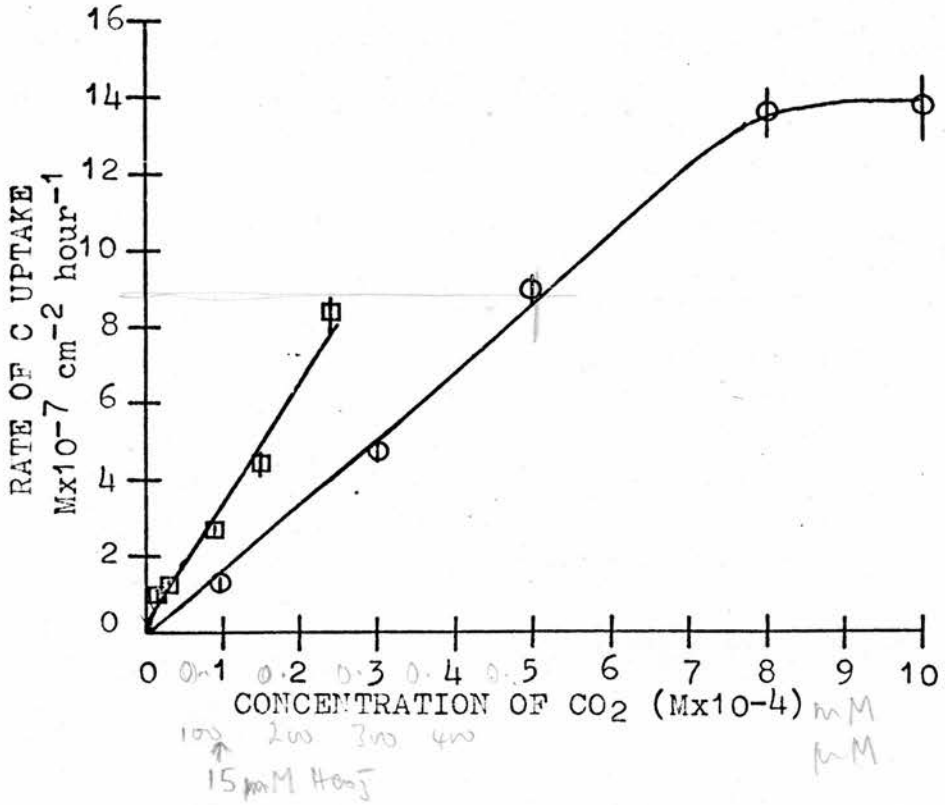

RATE OF C UPTAKE
Mx10-7 cm-2 hour-1
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10
CONCENTRATION OF CO2 (Mx10-4)

FIGURE 9

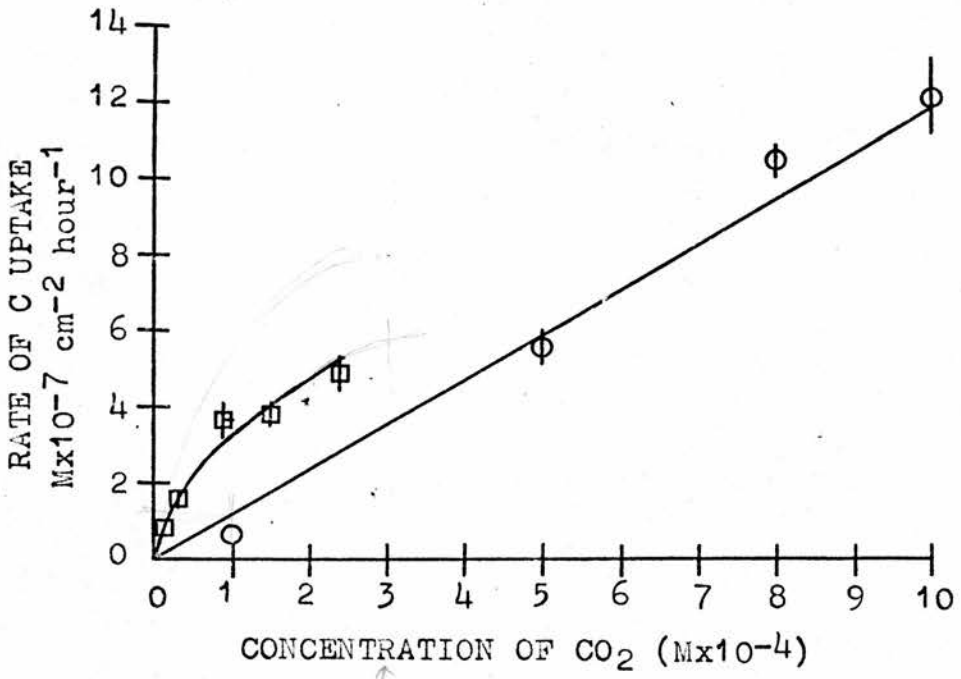
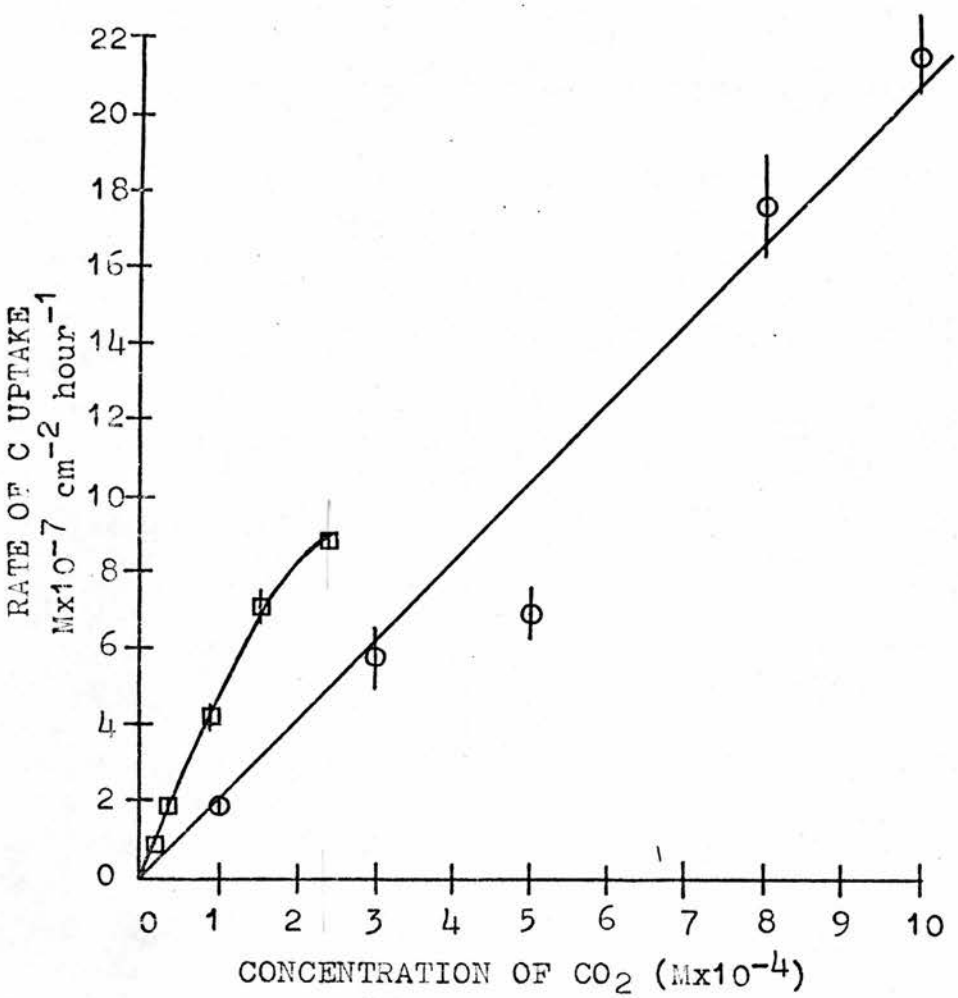

RATE OF C UPTAKE
Mx10-7 cm-2 hour-1
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
7
8
9
10
CONCENTRATION OF CO2 (Mx10-4)

FIGURE 10



15 30 45 mM HCO_3^-

FIGURE 11

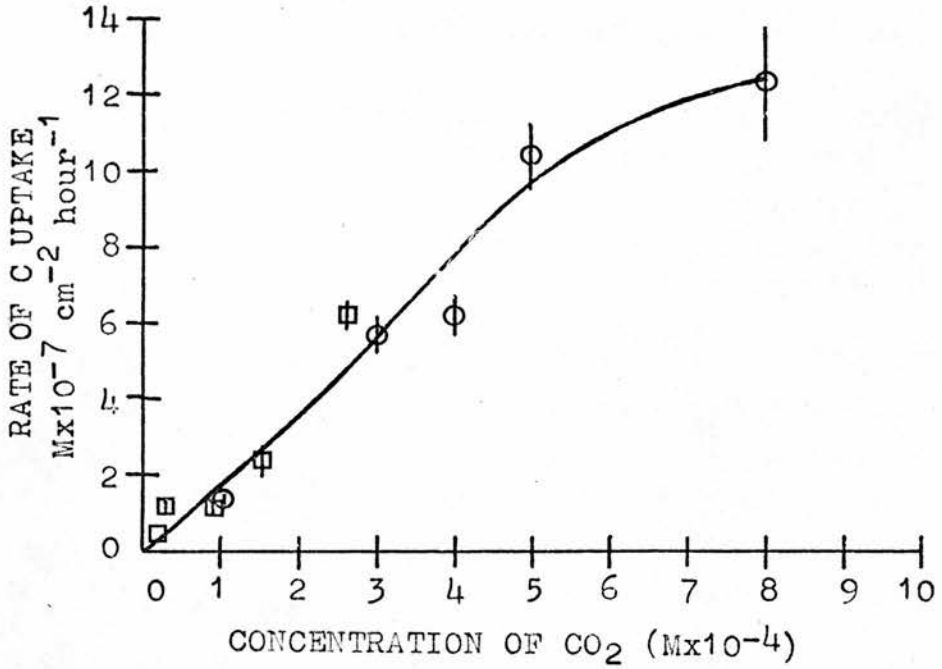
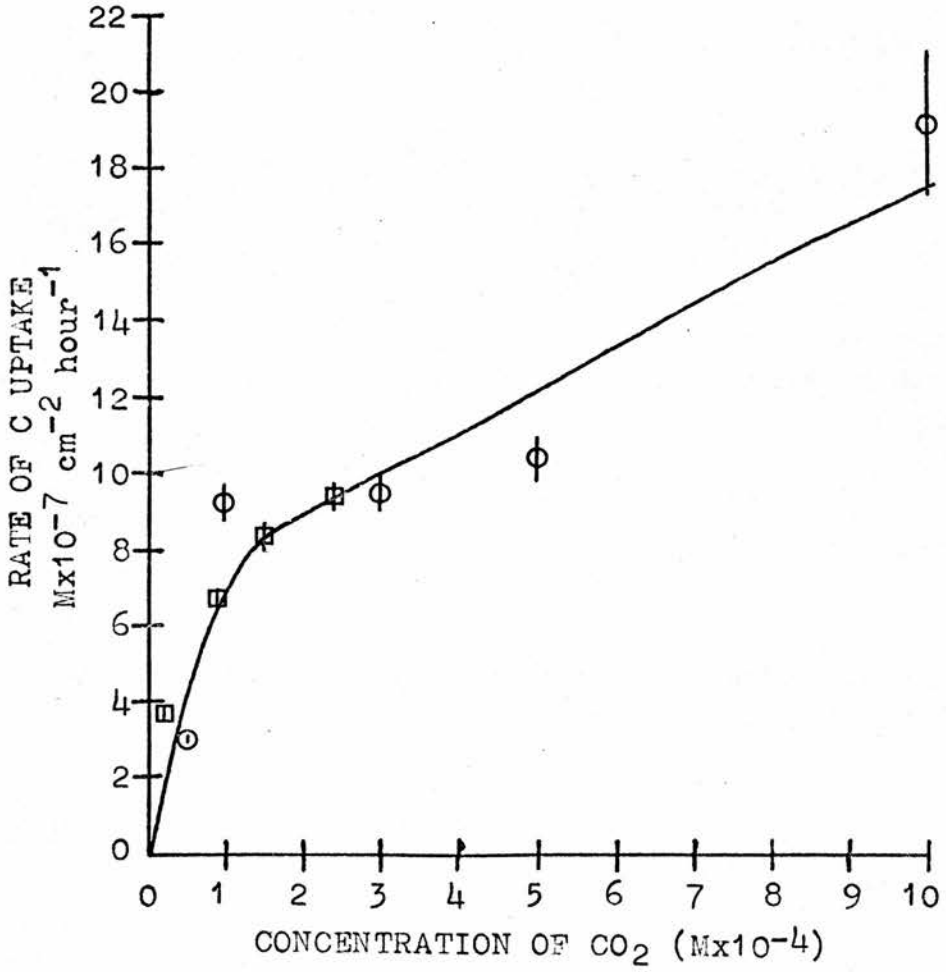

RATE OF C UPTAKE
Mx10⁻⁷ cm⁻² hour⁻¹
0
2
4
6
8
10
12
14
0 1 2 3 4 5 6 7 8 9 10
CONCENTRATION OF CO2 (Mx10⁻⁴)

FIGURE 12


RATE OF C UPTAKE
Mx10^{-7} cm^{-2} hour^{-1}
22
20
18
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10
CONCENTRATION OF CO_2 (Mx10^{-4})

Discussion.

Rates of carbon uptake by the five plant species from the two solution types, were plotted against free CO_2 concentration of the solutions. Considering, first, the plots for P. perfoliatus and P. praelongus, (Figures 9 and 10); in both cases it can be seen that rates of carbon uptake from the bicarbonate solutions are significantly greater than those from the free CO_2, pH 4.3, solutions, for all equivalent concentrations of free CO_2 examined. This significantly higher rate of carbon uptake from the bicarbonate solutions is again exhibited by P. lucens, (Figure 8).

If a plant species is not able to utilise the bicarbonate ion for photosynthesis one would expect a similar plot of rate of carbon uptake against free CO_2 when employing a bicarbonate solution, pH 8, and when employing a free CO_2 solution, pH 4.3. If the plant is able to utilise the bicarbonate ion then one would expect a significantly higher rate of carbon uptake from a bicarbonate solution than from a free CO_2 solution

with equivalent free CO_2 concentrations. This, as we see from Figures 8, 9 and 10, is the case for P. lucens, P. perfoliatus and P. praelongus.

These results, then, are evidence for the utilization of the bicarbonate ion for photosynthesis, by these three plant species, provided that the increase in pH of the bathing solution does not enhance photosynthesis and that the bicarbonate ion is not merely acting as a reservoir of free CO_2. It has been shown, by the experiments of section 4.5, for P. perfoliatus and P. praelongus, and by other workers (Steeman-Nielson, 1947) for P. lucens, that an increase in the pH of the bathing solution does not enhance the rate of photosynthesis.

If the bicarbonate ion was acting as a "reservoir" of free CO_2, as suggested by Briggs (1959), one would expect an increase in the rate of photosynthesis, from that at pH 4.3 to that at pH 7.3 where pH 89% of the total CO_2 is in the bicarbonate form. Experiments of section 4.5, however, show that, for equivalent concentrations of free CO_2, rates of photosynthesis of P. praelongus

and P. perfoliatus are not significantly different in solutions of pH 4.3 and of pH 7.3.

It does not then appear probable that the enhancement of carbon uptake by the bicarbonate solutions at pH 8 is due to the large reservoir of free CO_2, supplied by the bicarbonate ion. As it has also been shown that an increase in pH does not enhance photosynthesis, the results exhibited in Figures 8, 9 and 10 clearly indicate the ability of the three Potamogeton species, P. lucens, P. perfoliatus and P. praelongus, to utilise the bicarbonate ion as an exogenous carbon source for photosynthesis.

Figures 11 and 12 show that, for P. polygonifolius and F. antipyretica respectively, the differences in rates of carbon uptake from the two solution types, for equivalent concentrations of free CO_2, are not significant. This indicates that these two species are not able to utilise the bicarbonate ion directly for photosynthesis. The increase in pH may be depressing photosynthesis and the increase in bicarbonate content of the bathing solution may be enhancing photosynthesis

by an equal, but opposite, amount. As previously discussed, in Section 4.5 it is unlikely that these two factors would operate to cancel each other out exactly.

Conclusions drawn from these results for P. lucens and F. antipyretica, as to their ability to utilise the bicarbonate ion, are in agreement with those of Steeman-Nielson, (1947).

It can be seen from Table VIII that all the Potamogeton species examined achieve similar rates of carbon uptake from the free CO_2 solutions. Ratios of carbon uptake from the bicarbonate solutions to carbon uptake from the free CO_2 solutions, for all the free CO_2 concentrations used, are greater than or equal to 2.0, for P. lucens, P. praelongus and P. perfoliatus, while the ratios for P. polygonifolius are not significantly greater than 1.0. The high ratios for the three former species are accounted for by their improved performance, with respect to photosynthesis, in the bicarbonate solutions. P. polygonifolius shows no improvement of photosynthesis when in the bicarbonate solutions.

Direct comparison of the actual rates of photosynthesis of F. antipyretica with those of the Potamogeton species cannot be made as rates of carbon uptake of the latter are calculated on a leaf area basis, those of the former on a dry weight basis. As with P. polygonifolius, however, the ratios, for F. antipyretica, of rates of carbon uptake from the bicarbonate solutions to uptake from the free CO_2 solutions are not significantly greater than 1.0.

5.2. FIELD EXPERIMENTS

Introduction.

A series of field experiments were carried out with the Potamogeton species used in the laboratory. Rates of carbon uptake, by these species, in the field , were then compared with rates obtained in the laboratory, for equivalent CO_2 concentration and pH values and assuming saturating light intensity for photosynthesis in both situations.

These experiments were carried out to see if similarities occurred between rates of photosynthesis in the laboratory and field. Where such similarities occurred the possibility existed of expanding the conclusions which had been reached from laboratory experiments regarding bicarbonate use, to include explanations of the distribution of the species examined in relation to water chemistry.

The field situation chosen for study was in an area near Durness, Sutherland, Scotland.

Three lochs; Loch Croispol, Loch Lanlish and Loch Glugach and three plant species; Potamogeton perfoliatus, P. praelongus and P. polygonifolius, were under consideration.

In this area of Sutherland runs a limestone belt on which Loch Croispol and Loch Lanlish are found, approximately one mile from each other. These two lochs are typical calcareous lochs with clear water and pH values rising above pH 8, alkalinity values above 3mM HCO_3^-. Locally, P. perfoliatus is confined to Loch Croispol and P. praelongus to Loch Lanlish but neither occur in Loch Glugach which is situated approximately 4 miles inland, in an area of peat bog. This is a typically oligotrophic loch with very brown water and with pH values often lower than pH 6 and alkalinity values less than 0.25 mM HCO_3^-. P. polygonifolius is found growing in this loch but is absent from the other two.

This area then provided a convenient situation for the study of the three Potamogeton species, in relation to their distribution in waters of different chemical composition, as each

species is restricted to one of the three lochs. P. polygonifolius is normally found in poor water, it was, therefore, to be expected that this species should be found only in Loch Glugach. P. perfoliatus and P. praelongus are ubiquitous in their distribution, though are more often found in richer waters.

Rates of carbon uptake by the three species, in each of the three loch waters, were first determined. Rates of carbon uptake from a series of "bicarbonate enriched" loch waters were then determined, all other conditions being comparable.

Procedure.

The method used for all field experiments was as described in Methods section, page

Water from each of the lochs was collected just before each experimental series and alkalinity and total CO_2 content of the loch waters were found by titration (see Appendix VII) and the pH was measured. Glugach loch water was enriched with $KHCO_3$, which was added to give an increase of 5mM in the bicarbonate concentration of the water; the pH value of this bicarbonate enriched water was noted.

Rates of carbon uptake by each species were determined in each of the three natural loch waters and in Glugach water with added $KHCO_3$. All the reaction vessels were placed in approximately one foot depth of water at the edge of Loch Croispol for the experimental period.

From a knowledge of total CO_2 content, pH and "titratable" alkalinity (phenolphthalein alkalinity ($OH^- + CO_3^{--}$) and total alkalinity ($OH^- + CO_3^{--} + HCO_3^-$)), the proportions of free CO_2 and bicarbonate, of each loch water used,

were calculated. The rates of carbon uptake were then compared with rates attained, by each species, in the laboratory for equivalent CO_2 concentrations and pH values (Figure 13).

A control experiment was carried out, using Winkler light and dark bottles (see Appendix VIII for method) in which rates of photosynthesis of P. perfoliatus, were followed in Loch Lanlish water. This was to ascertain whether rates of photosynthesis measured by the ^{14}C method were nearer to a net or gross measurement of photosynthesis. Results from this experiment are shown in Table X.

TABLE X

Rates of C uptake, from loch waters, obtained in the field, compared with rates of C uptake, from experimental bathing solutions with free CO_2 concentrations equivalent to those in loch waters, obtained in the laboratory.

Species	Loch water	Total CO_2 concentration $1x10^{-3}M$	pH	Rate of C uptake $1x10^{-8}Mcm^{-2}hour^{-1}$ Field	Laboratory
P. polygonifolius	Lanlish	1.9	8.3	1.6	2
	Croispol	3.5	8.4	4.2	5
	Glugach	0.3	5.8	7.9	52
	Glugach + HCO_3^-	5.3	8.3	7.8	9
P. praelongus	Lanlish	1.8	8.6	12.5	10
	Croispol	3.4	8.4	14.5	17
	Glugach	0.3	5.8	5.0	56
	Glugach + HCO_3^-	5.3	8.4	15.8	27

TABLE X continued

Species	Loch water	Total CO_2 concentration $1x10^{-3}M$	pH	Rate of C uptake $1x10^{-8}Mcm^{-2}hour^{-1}$ Field	Laboratory
P. perfoliatus	Lanlish	1.9	8.3	12.2	12
	Croispol	3.5	8.2	18.5	21
	Glugach	0.3	5.8	5.2	32
	Glugach + HCO_3^-	5.3	8.3	7.4	22

Rate of photosynthesis of P. perfoliatus, in Lanlish water, measured using Winkler analysis of O_2 content of bathing medium, was found to be $12.3 \times 10^{-8}M\ O_2\ cm^{-2}\ hour^{-1}$

FIGURE 13.

Rates of C uptake, by 3 Potamogeton, species from loch waters (▨), compared with rates of C uptake, by each species, from experimental bathing solutions of equivalent CO_2 concentrations and pH values, (□).

(a) P. polygonifolius

(b) P. praelongus

(c) P. perfoliatus

FIGURE 13(a)

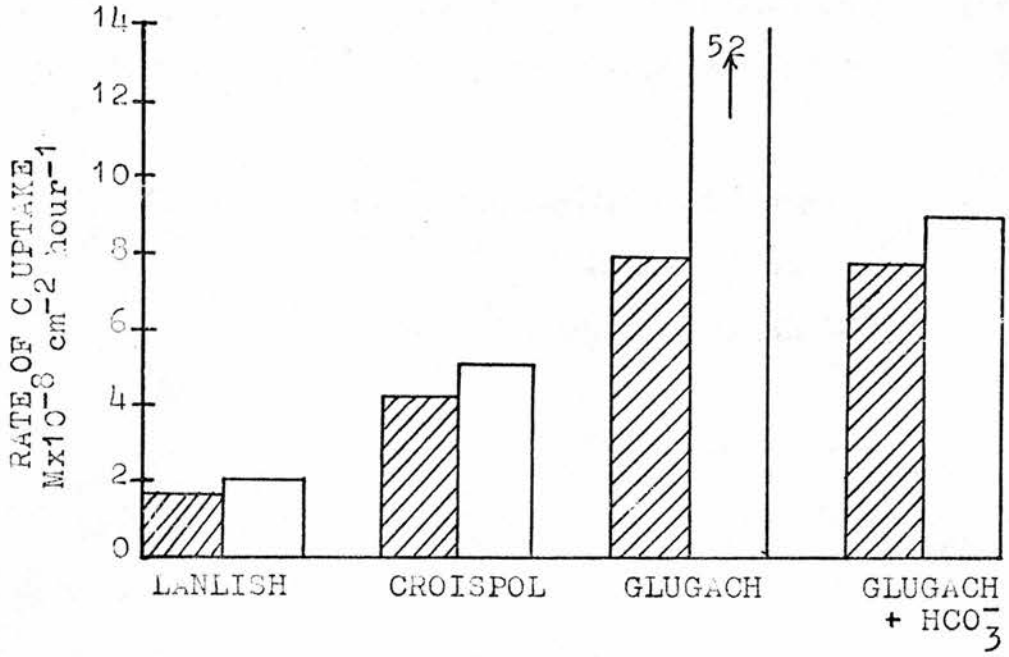

RATE OF C UPTAKE
Mx10⁻⁸ cm⁻² hour⁻¹
0
2
4
6
8
10
12
14
52
LANLISH
CROISPOL
GLUGACH
GLUGACH + HCO⁻₃

FIGURE 13(b)

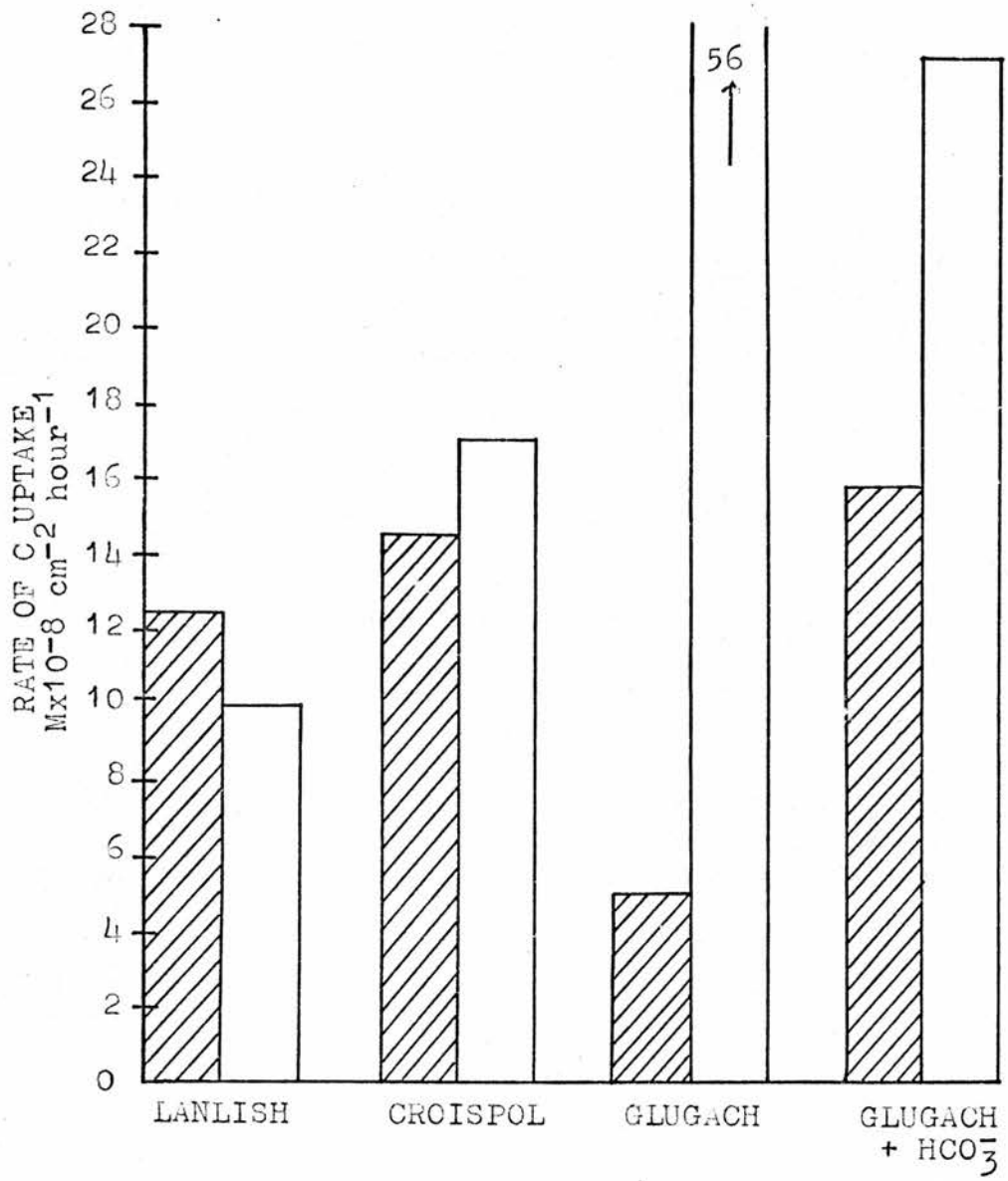
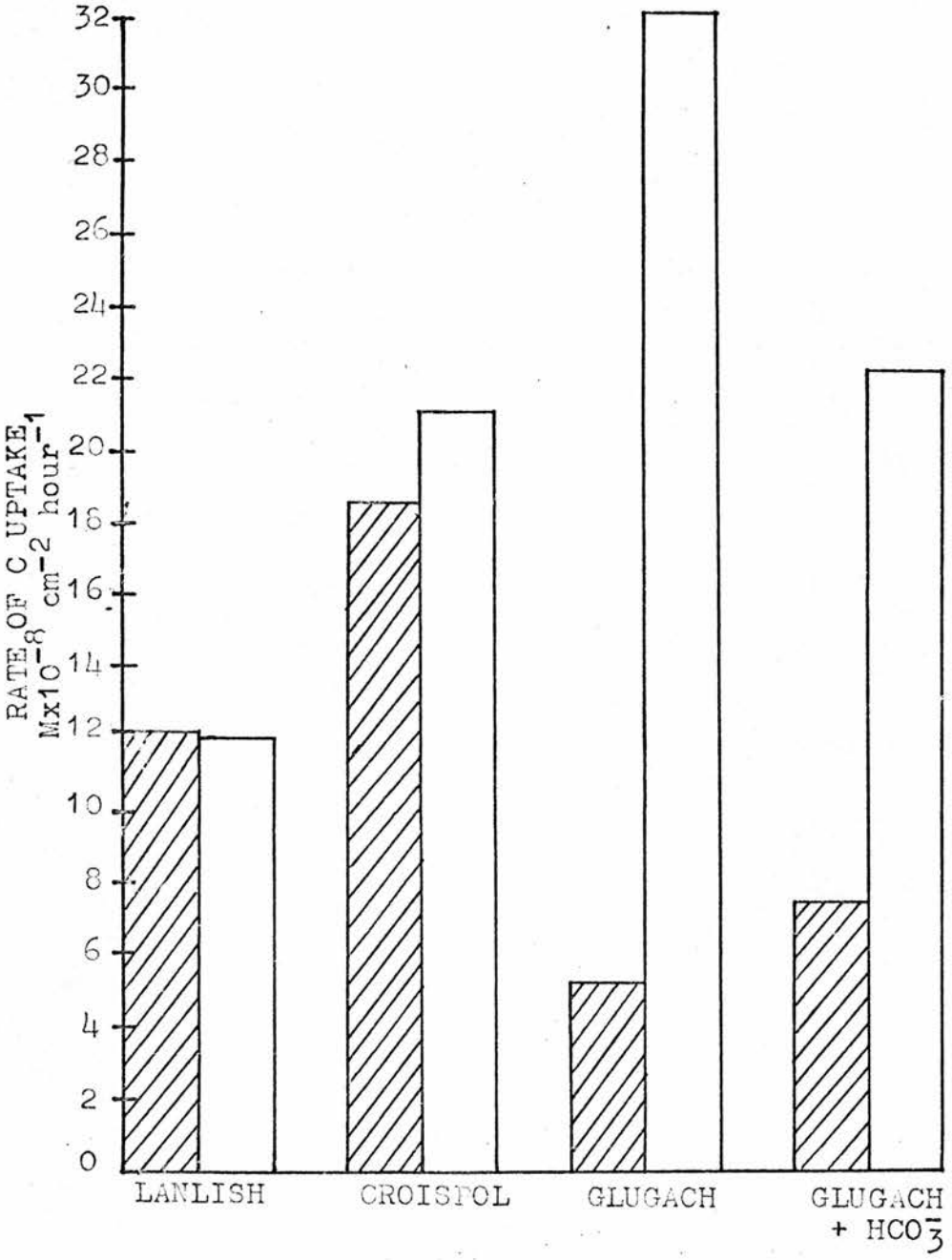

RATE OF C_2 UPTAKE
Mx10-8 cm^{-2} $hour^{-1}$
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
56
LANLISH
CROISPOL
GLUGACH
GLUGACH + HCO_3^-

FIGURE 13(c)


RATE OF C UPTAKE
Mx10^{-8} cm^{-2} hour^{-1}
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
LANLISH
CROISPOL
GLUGACH
GLUGACH
+ HCO_3^-

Discussion.

Rates of photosynthesis, by P. perfoliatus, in loch water, are comparable when determined by two methods, ^{14}C technique and Winkler technique; 12.2×10^{-8}M C cm^{-2} $hour^{-1}$ and 12.3 $\times 10^{-8}$M O_2 cm^{-2} $hour^{-1}$ respectively. This would indicate that rates of photosynthesis, determined in the field by the ^{14}C technique, are near to a net value of photosynthesis at saturating values of light intensity.

Rates of C uptake, by the three Potamogeton species, in the field and laboratory, have been compared directly. Rates attained in the field are the mean values of several separate determinations. The laboratory rates, exhibited in Figure 13, are taken from results of comparative experiments of Section 5.1. Rates, obtained in the laboratory, in solutions of equivalent free CO_2 concentrations to those of the loch waters, have been used for the comparisons. In the case of acid loch water, the field rates have been compared with rates attained in free CO_2, pH 4.3, solutions. For alkaline loch waters, field rates

have been compared with rates attained in bicarbonate solutions, pH 8, with equivalent free CO_2 concentrations.

Errors may arise in making such direct comparisons. The largest of these may be the result of differences in light and temperature regime, and mineral content of bathing solutions, between the two situations. As to the latter, laboratory solutions contained only additional calcium and potassium, from the added $KHCO_3$. Any absolute effects, of minerals, on rates of photosynthesis are not known, although it is known that K^+ and Ca^{++} enhance bicarbonate uptake, by bicarbonate users (Steeman-Nielson, 1947), and that phosphate may enhance rates of photosynthesis. Temperature variation, between the two situations, are on average 5°C; laboratory solutions were always maintained at 20°C while loch waters were approximately 15°C. Differences in light conditions would probably affect rates of photosynthesis more than a 5°C difference in temperature. However, it is possible to compare

rates of photosynthesis, in different light conditions, if we assume light to be saturating for photosynthesis in both situations. This has been shown to be so for laboratory rates, see Section 4.4. In the field, determinations were made during summer months, around mid-day and reaction vessels were placed in only one foot depth of water, so it is likely that light, in the field, was also saturating for photosynthesis.

One error which has arisen in determining field rates of photosynthesis is the result of depletion of C source, in the small reaction vessels, 25ml, over the comparatively long experimental periods of 3 hours. This error has only arisen when using loch water with low total CO_2 concentration, i.e. Glugach water. As seen in Figures 13, a,b and c, for all the Potamogeton species used, rates attained in the field, in Glugach water, are considerably lower than rates attained in the laboratory. If the laboratory rate is considered to be the maximum rate of

photosynthesis, in the particular free CO_2 concentration concerned, then, from the following calculation for P. praelongus, it can be seen that CO_2 available in the reaction vessel in the field would be depleted within an experimental period of approximately one and a half hours.

Laboratory C uptake: 56×10^{-8} M cm^{-2} $hour^{-1}$

Average leaf area = 8 cm^2

Total C uptake in 1 hour = 4.5×10^{-6} Moles

Free CO_2 in Glugach water = 2.8×10^{-4} M

= 7.0×10^{-6} Moles/25ml

Similarly for P. perfoliatus:

Free CO_2 available = 7.0×10^{-6} Moles

Total C uptake in 1 hour = 2.6×10^{-6} Moles

And for P. polygonifolius:

Free CO_2 available = 7.0×10^{-6} Moles

Total C uptake in 1 hour = 3.36×10^{-6} Moles.

The rate attained by P. polygonifolius in Glugach water, although erroneously low, is still higher than that attained in Lanlish and Croispol waters.

With Glugach water, enriched with HCO_3^-, rate of C uptake by P. polygonifolius is similar to that obtained in an equivalent solution in the laboratory. Again, in Lanlish and Croispol waters, both with relatively high CO_2 concentration, rates attained by P. polygonifolius are similar to those obtained in the laboratory. These results confirm the conclusions of the earlier, comparative laboratory experiments that bicarbonate ion is not acting as a carbon source for photosynthesis in this species.

When leaves of P. praelongus are bathed in Glugach water with additional HCO_3^-, rate of C uptake is similar to that obtained in Croispol water. The rate is not as great, however, as the laboratory rate of 27×10^{-8} M C cm^{-2} $hour^{-1}$, which is the rate estimated for a similar bicarbonate solution. When, however, the equivalent laboratory rate is estimated for a free CO_2

solution (i.e. without additional C uptake due to HCO_3^- ion) it becomes 12 x 10^{-8} M C cm^{-2} $hour^{-1}$, which is much nearer the field rate of 15.8 x 10^{-8} M C cm^{-2} $hour^{-1}$.

In the case of P. perfoliatus, rate of C uptake from HCO_3^- enriched Glugach water is considerably lower than rates attained in Croispol and Lanlish waters. This situation for P. perfoliatus is, however, similar to that for P. praelongus, in that laboratory rate estimated for a free CO_2 solution is 8 x 10^{-8} M C cm^{-2} $hour^{-1}$ compared with a field rate of 7.4 x 10^{-8} M C cm^{-2} $hour^{-1}$. Assuming there are no errors of calculation or technique it would thus appear that these species are unable to utilise the HCO_3^- ion when it is supplied in Glugach water.

P. praelongus and P. perfoliatus both attain rates of C uptake in Croispol and Lanlish waters similar to those attained in equivalent laboratory solutions. As already stated rates estimated for Glugach water are erroneously low. It may be pointed out, however, that while rates

in Glugach water, estimated for all three species, are similar, rates of *P. praelongus* and *P. perfoliatus* are approximately 6 times that of *P. polygonifolius* in Lanlish water and 4 times that of *P. polygonifolius* in Croispol water.

Results, using Lanlish and Croispol waters, would indicate that *P. praelongus* and *P. perfoliatus* are able to utilise the HCO_3^- ion available in these loch waters as the laboratory rates are comparable with those obtained in bicarbonate solutions with free CO_2 concentrations equivalent to those in the loch waters.

6. EFFECT OF DCMU* ON RATES OF PHOTOSYNTHESIS.

Introduction.

An investigation of the effect of the herbicide, DCMU, on the rate of carbon uptake, by Potamogeton species, was undertaken.

DCMU has been found to inhibit photosynthesis by specifically inhibiting photosystem 2, (Gingrass and Lemasson, 1965). Results obtained by Raven(1968) using light filters, suggest the existence of a reaction, specific to bicarbonate use requiring photosystem 2. He has used DCMU in order to study the HCO_3^- uptake mechansm in Hydrodictyon africanum, a species which he had established, by comparative experiments, as a HCO_3^- user, (Raven, 1968).

In this present study this approach has been extended to cover both a bicarbonate "user" and a "non-user", P. praelongus and P. polygoni-

* 3-(3,4-Dichlorophenyl)-1,1-dimethylurea

folius respectively, as established by comparative experiments also. The effect of DCMU, on the rate of carbon uptake by these two species, from bicarbonate solutions, pH8, and from free CO_2 solutions, pH 4.3, was investigated.

Procedure.

Problems arise when using DCMU as an inhibitor over a pH range. DCMU will be ionized to varying extents in solutions of different pH values and, therefore, effective concentrations of the inhibitor will differ in solutions of different pH values. Plant tissue can be pre-treated in a neutral DCMU solution and then carbon uptake followed in the desired solutions. As the effect of DCMU is completely reversible, carbon uptake must be followed within an approximately ten minute period after pre-treatment (see Table XI). Apart from this being a very short time for following carbon uptake, this method does not allow for any equilibration of the plant tissue in the experimental bathing solution.

An experiment was carried out to determine percentage inhibition of carbon uptake by 2×10^{-7}M DCMU at three pH values, pH 4.3, 6.0 and 7.3. All the bathing solutions had a concentration of free CO_2 of 1×10^{-3}M.

TABLE XI

Rate of C uptake, by P. praelongus, from a 2×10^{-2}M $KHCO_3$ solution, under different conditions of inhibition.

Conditions.	Rate of C uptake $M \times 10^{-7}$ hour^{-1} cm^{-2}
light	8.55 ± 0.38
light, after 1 hour pre-treatment in neutral DCMU solution (10^{-5}M)	6.74 ± 0.65
light, DCMU (10^{-5}M) added to bathing solution	0.96 ± 0.07

^{14}C uptake was followed for an experimental period of 1 hour.

In preliminary experiments 10^{-5}M DCMU was shown to inhibit photosynthesis maxiamlly.

TABLE XII

Percentage inhibition of photosynthesis, of P. praelongus, by $2x10^{-7}$M DCMU, at three pH values.

pH of solution	Percentage inhibition of photosynthesis
4.3	31.7
6.0	31.9
7.3	29.3

It was decided, because of the results exhibited in Tables Xi and XII, to a dd the DCMU to the experimental bathing solutions directly. By using this method the plant tissue could equilibrate in the appropriate bathing solution and carbon uptake could be followed for periods of at least an hour.

Rates of carbon uptake, by P. polygonifolius and P. praelongus, from the following soluti ns were determined: $1x10^{-3}$M free CO_2 pH 4.3, $2x10^{-2}$M total CO_2 pH 8.3, and $2.4x10^{-4}$M free CO_2 pH 4.3, the latter being the free CO_2 concentration present in a $2x10^{-2}$M bicarbonate

solution at pH 8.3. The effects of four concentrations of DCMU ($5x10^{-8}$M to $1x10^{-6}$M) on rates of carbon uptake were then determined for each species, in each of the three solutions. Rates of carbon uptake in the dark from the three solutions with no added DCMU, were also determined for both species.

This series of experiments were carried out as described in Method section, page 28 The plant tissue was allowed to photosynthesise in the radioactive solutions for periods of one hour. Rates of carbon uptake were then calculated in terms of Moles of carbon fixed per cm^2 leaf area per hour.

Results of these experiments are exhibited in Table XIII and Figures 14 and 15. Table XIII shows percentage inhibition of photosynthesis, in each of the three solution types, by the four concentrations of DCMU. Figures 14 and 15 show rates of photosynthesis against DCMU concentration for the bicarbonate and for the $2x10^{-4}$M free CO_2 pH 4.3 solutions.

TABLE XIII(a)

Rates of carbon uptake, by P. praelongus and P. polygonifolius, from bathing solutions containing different concentrations of DCMU.

P. polygonifolius

Concentration of DCMU (M)	Rates of C uptake, 10^{-7}M C cm^{-2} $hour^{-1}$		
	$1x10^{-3}$M CO_2,pH4.3	$2x10^{-2}$M CO_2,pH8.3	$2.4x10^{-4}$M CO_2,pH4.3
Light Control	11.69 ± 0.74	6.62 ± 0.40	5.90 ± 0.60
5×10^{-8}	7.60 ± 0.53	3.87 ± 0.39	3.98 ± 0.49
1×10^{-7}	3.10 ± 0.22	2.89 ± 0.24	3.36 ± 0.36
3×10^{-7}	1.05 ± 0.07	0.88 ± 0.07	1.11 ± 0.08
1×10^{-6}	0.35 ± 0.02	0.31 ± 0.02	0.29 ± 0.02
Dark Control	0.24 ± 0.02	0.29 ± 0.02	0.09 ± 0.006

TABLE XIII (a) continued.

P. praelongus.

Concentration of DCMU (M)	Rates of C uptake, 10^{-7}M C cm^{-2} $hour^{-1}$		
	$1x10^{-3}$M CO_2,pH 4.3	$2x10^{-2}$M CO_2,pH8.3	$2.4x10^{-4}$M CO_2,pH4.3
Light Control	16.90 ± 1.01	11.8 ± 0.92	5.52 ± 0.41
5×10^{-8}	12.95 ± 0.78	10.18 ± 0.90	4.20 ± 0.36
1×10^{-7}	10.90 ± 0.71	7.73 ± 0.81	2.40 ± 0.34
5×10^{-7}	1.67 ± 0.12	1.02 ± 0.07	0.90 ± 0.10
1×10^{-6}	0.64 ± 0.04	0.55 ± 0.04	0.23 ± 0.06
Dark Control	0.26 ± 0.02	0.24 ± 0.02	0.03 ± 0.002

TABLE XIII(b)

Percentage inhibition, of Carbon uptake, by different concentrations of DCMU.

P. polygonifolius

Concentration of DCMU (M)	Percentage inhibition $1x10^{-3}MCO_2$ pH 4.3	$2x10^{-2}MCO_2$ pH 8.3	$2.4x10^{-4}MCO_2$ pH 4.3
5×10^{-8}	35.0	41.4	30.0
1×10^{-7}	73.5	56.3	40.9
3×10^{-7}	91.0	86.7	79.9
1×10^{-6}	97.0	95.4	94.9

P. praelongus

5×10^{-8}	23.3	13.8	24.0
1×10^{-7}	35.5	34.5	56.5
5×10^{-7}	90.1	91.3	83.7
1×10^{-6}	96.2	95.4	95.8

FIGURES 14 & 15

Rates of carbon uptake from bicarbonate solutions, $2x10^{-2}$M CO_2, (+) and free CO_2 solutions, 2.4 x 10^{-4}M CO_2 (⊕), with different concentrations of added DCMU.

FIGURE 14:- P. polygonifolius

FIGURE 15:- P. praelongus

(Standard errors of the two points only are shown on the figures, these are represented by vertical lines. Standard errors of remaining rates may be found in Table XIIIa.)

FIGURE 14

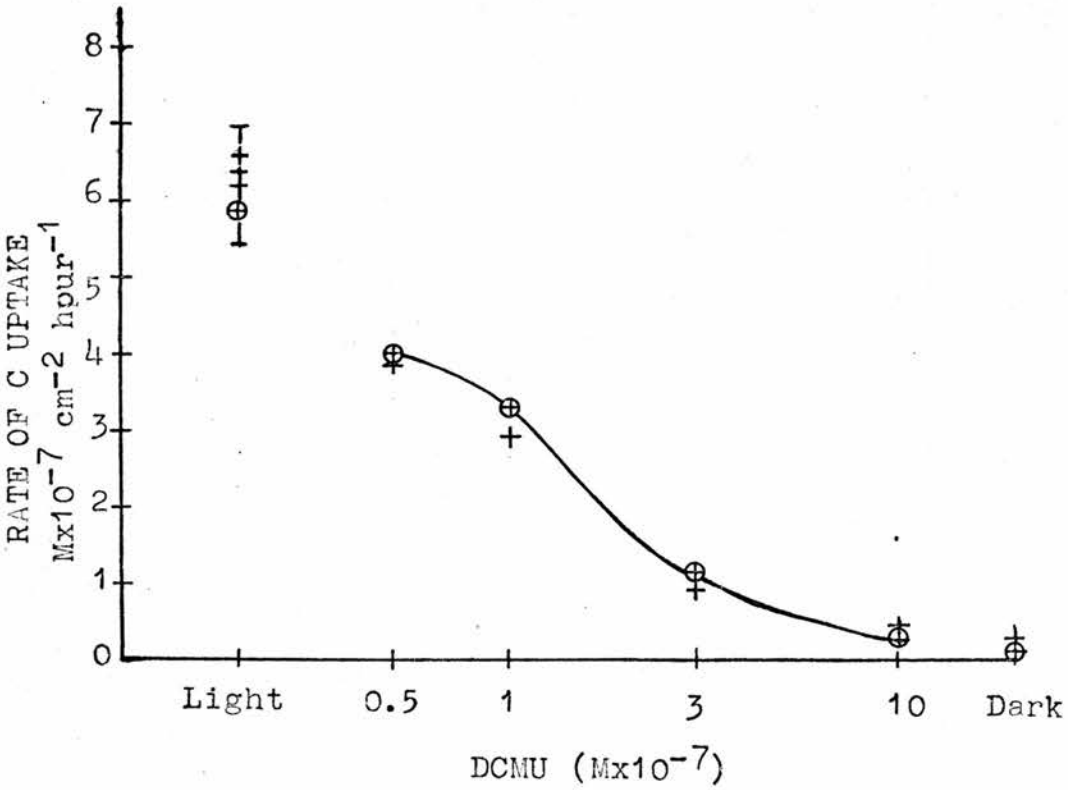
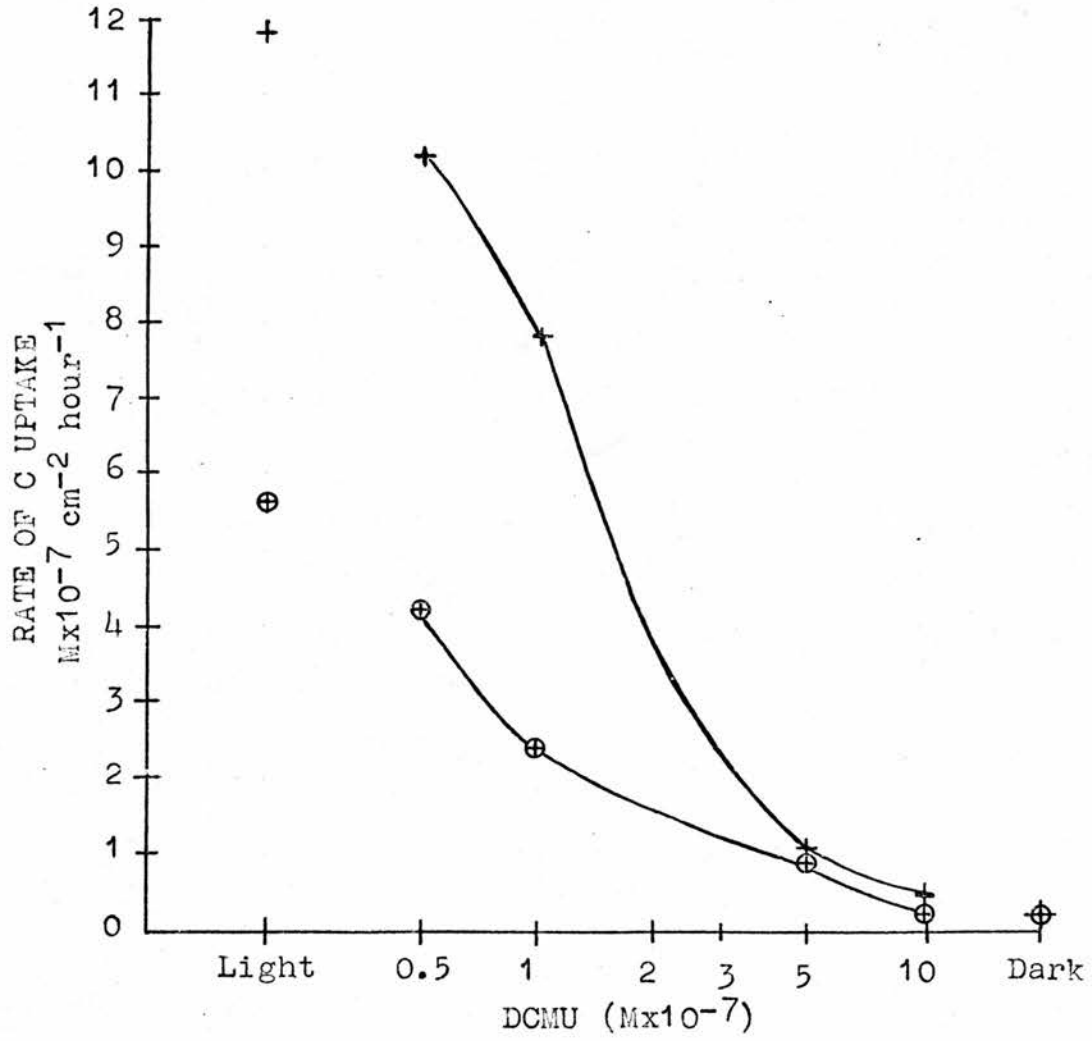

RATE OF C UPTAKE
Mx10^{-7} cm^{-2} hour^{-1}
0
1
2
3
4
5
6
7
8
Light
0.5
1
3
10
Dark
DCMU (Mx10^{-7})

FIGURE 15


RATE OF C UPTAKE
Mx10⁻⁷ cm⁻² hour⁻¹
12
11
10
9
8
7
6
5
4
3
2
1
0
Light
0.5
1
2
3
5
10
Dark
DCMU (Mx10⁻⁷)

Discussion.

Gingras and Lemasson (1965), in their discussion of the mode of DCMU action, propose the existence of a substance E in two oxidation states, the more oxidised of which accepts electrons from photosystem 2. Photosystem 2 acts as reductant, photosystem1 as an oxidant. The action of DCMU is the removal of E^+. Gingras and Lemasson observed that the plateau of a light intensity curve of O_2 evolution by photosynthetic tissue is insensitive to low concentrations of DCMU ($4x10^{-7}M$), i.e. the stedy state concentration of E^+ is smaller but not small enough to affect saturation level. As the concentration of DCMU is increased the net rate of formation of E^+ becomes slower, limiting the reaction.

It can be seen from Figures 14 and 15 that the rates of carbon uptake from the $2.4x10^{-4}M$ free CO_2 solutions are similar, for both species, over the DCMU concentration range tested.

This is then in agreement with results of the comparative experiments, where rates attained by both species, in solutions of low free CO_2 concentrations and low pH, were similar (Table VII). In this 2.4×10^{-4}M free CO_2 solution, at pH 4.3, there is only 0.1% HCO_3^- which, even in the case of a bicarbonate user, is unlikely to act as a significant carbon source for photosynthesis. In this solution then we are only concerned with inhibition of fixation of free CO_2 by both P. polygonifolius and P. praelongus. The absolute levels of this inhibition are the same for both species at all concentrations of DCMU tested.

For illuminated P. polygonifolius leaves without DCMU rates of carbon uptake from the bicarbonate solution and from the 2.4×10^{-4}M free CO_2 solution are not significantly different. The absolute level of inhibition by all concentrations of DCMU used is the same for both the bicarbonate and the 2.4×10^{-4}M free CO_2 solutions, (see Figure 14). This result confirms the conclusions of the earlier comparative experiment, that bicarbonate ion is not acting as a carbon

source for photosynthesis in this species. In both solutions rate of photosynthesis depends on free CO_2 at the same concentration of 2×10^{-4}M. At no concentration of DCMU does the presence of the bicarbonate ion increase the rate of carbon uptake, by P. polygonifolius, above that observed in the 2.4×10^{-4}M free CO_2, pH 4.3, solutions.

It has been assumed that a similar argument to that of Gingras and Lemasson may be applied to the affect of DCMU on a curve of rate of photosynthesis, in terms of carbon uptake, against CO_2 concentration, from the other solutions used in these experiments.

In the case of P. polygonifolius, a 1×10^{-3}M free CO_2 concentration gives a rate of carbon uptake situated on the plateau of a curve of rate of carbon uptake against CO_2 concentration (see Figure 11). This plateau appears to be sensitive to a DCMU concentration of 1×10^{-7}M. With this concentration of DCMU, free CO_2 concentrations of 1×10^{-3}M and 2.4×10^{-4}M give rates of carbon uptake of $3.10 \pm 0.22 \times 10^{-7}$M C cm^{-2}

hour^{-1} and 3.36 $\pm$ 0.36 x 10^{-7}M C cm^{-2} hour^{-1} respectively, (see Table XIII(a), page). It appears then that with 1 x 10^{-7}M added DCMU a concentration of 2.4 x 10^{-4}M free CO_2 gives a rate of carbon uptake situated on the plateau.

In the case of P. praelongus, 1 x 10^{-3}M free CO_2 gives a rate of carbon uptake which may be just before the start of the plateau (see Figure 10). Rate of photosynthesis, at this concentration, is still probably limited by supply of CO_2. A concentration of 1 x 10^{-7}M DCMU does not appear to affect the position of this plateau. With a DCMU concentration of 5 x 10^{-7}M, however, rate of carbon uptake, from 1 x 10^{-3}M free CO_2 solution, is depressed from 16.90 $\pm$ 1.01 x 10^{-7} M C cm^{-2} hour^{-1} to 1.67 $\pm$ 0.12 x 10^{-7}M C cm^{-2} hour^{-1}, (Table XIII(a), page 87). From this table it appears that with 5 x 10^{-7} M added DCMU, a concentration of just greater than 2.4 x 10^{-4}M would give a rate of carbon uptake situated on the plateau. With this concentration of DCMU, rate of photosynthesis, in 2.4 x 10^{-4}M

free CO_2, is no longer limited by CO_2 supply. Only at concentrations of 5×10^{-7}M DCMU, or greater, does uptake of HCO_3^-, by P. praelongus, show apparent greater sensitivity to the inhibitor than does uptake of free CO_2. It must also be remembered that, with this concentration of DCMU, 1×10^{-3}M free CO_2 gives a rate of carbon uptake only 0.5 M C cm^{-2} $hour^{-1}$ greater than that obtained in 2.4×10^{-4} M free CO_2.

The presence of the bicarbonate ion, in solutions of high pH has already been shown to enhance rate of photosynthesis of P. praelongus (see Section 5.1). This is again shown by the results of experiments of this section. With no DCMU, or with added 5×10^{-8}M and 1×10^{-7}M DCMU rates of carbon uptake by P. praelongus are significantly higher in the bicarbonate solution than in the 2.4×10^{-4}M free CO_2 solution. Assuming the difference between rates obtained in the bicarbonate and 2.4×10^{-4} M free CO_2 solution is the result of the bicarbonate ion in the former solution(which has the same free CO_2 concentration of 2.4×10^{-4}M), it is noted that, with 5×10^{-7}M

DCMU any such differences disappear strongly supporting inhibition of bicarbonate uptake.

7. INVESTIGATION OF CARBONIC ANHYDRASE ACTIVITY.

Introduction.

Carbonic anhydrase catalyses the hydration and dehydration of CO_2. The main function of carbonic anhydrase in plants is not known for certain and several possibilities exist. The enzyme may facilitate the diffusion of CO_2 at chloroplast membranes. This facilitated diffusion of CO_2 has been demonstrated, in animals, by Enns, 1967. Graham and Reed, 1971, have postulated the involvement of the enzyme in a buffering action to regulate proton gradient across membranes and HCO_3^- concentration.

Carbonic anhydrase may play a role in increasing the rate of supply of CO_2 from HCO_3^- for carboxylation. Ikemori and Nishida (1967) have shown a greater suppression of photosynthetic activity of Ulva pertusa, a bicarbonate user, at pH 9.0 than at pH 5.6, by Diamox*, a specific

* Diamox = 2-acetylamino-1,3,4-thiadiazol-5-sulfonamide

inhibitor of carbonic anhydrase (Mann and Kielin, 1940).

The presence of carbonic anhydrase in leaves of several aquatic species has been reported; e.g. Ulva pertusa (Ikemori and Nishida, 1968), Helodea canadensis, bicarbonate users, and Fontinalis dalecarlica, a non-user, (Steeman-Nielsen and Kristiansen, 1959). In this study it was hoped to ascertain the presence or absence of the enzyme, in a bicarbonate user and non-user, as identified by the comparative experiments of Section 5.1. If the enzyme were present then the study could be extended and the effect of Diamox on the rate of photosynthesis in bicarbonate and free CO_2 solutions investigated. Time, however, only permitted the start of this study and presence of the enzyme was only demonstrated in P. praelongus. The effect of Diamox on the rate of C uptake, by P. praelongus, from a bicarbonate solution was investigated.

Assay Procedure

The carbonic anhydrase content of P. praelongus was assayed after the method of Ikemori and Nishida (1968). This method is based upon the catalytic activity of the enzyme on the release of CO_2 from $NaHCO_3$, in NaOH, when added to phosphate buffer at pH 6.8.

Leaves of P. praelongus, approximately 1g fresh weight, were homogenised in 5ml 0.01M cysteine and the extract was filtered. The filtrate was made up to 10ml with cysteine solution and then centrifuged at 2500 rpm for 30 minutes and the sediment was resuspended in 5ml cysteine solution. Both the suspension and the supernatant were tested for enzyme activity.

Enzyme activities in leaf extracts were compared with the activity found in blood, which is known to contain carbonic anhydrase.

0.2ml extract was added to 0.5ml phosphate buffer in a Warburg flask; 0.5ml 0.2M

$NaHCO_3$ in 0.02M NaOH was placed in the side arm of the flask. Warburg flasks were covered with foil to prevent gas volume change, due to photosynthesis, occurring. After 10 minutes incubation at 20°C the solution in the side arm was introduced into the main vessel and the resulting evolution of CO_2 followed. 0.02ml 0.01M cysteine was added to the blank flasks instead of extract.

Diamox, a specific inhibitor of carbonic anhydrase, was added to a sample of extract at a concentration of 5mM.

Enzyme activity was observed in the supernatant extracts only. Rates of CO_2 evolution, in the presence of supernatant extract, extract plus Diamox,and blood are shown in Figures 16 and 17.

FIGURE 16.

CO_2 evolved from $NaHCO_3$, in NaOH, when added to phosphate buffer, pH 6.8 alone (■), in the presence of plant extract (▲) and in the presence of blood (●), against time.

FIGURE 16

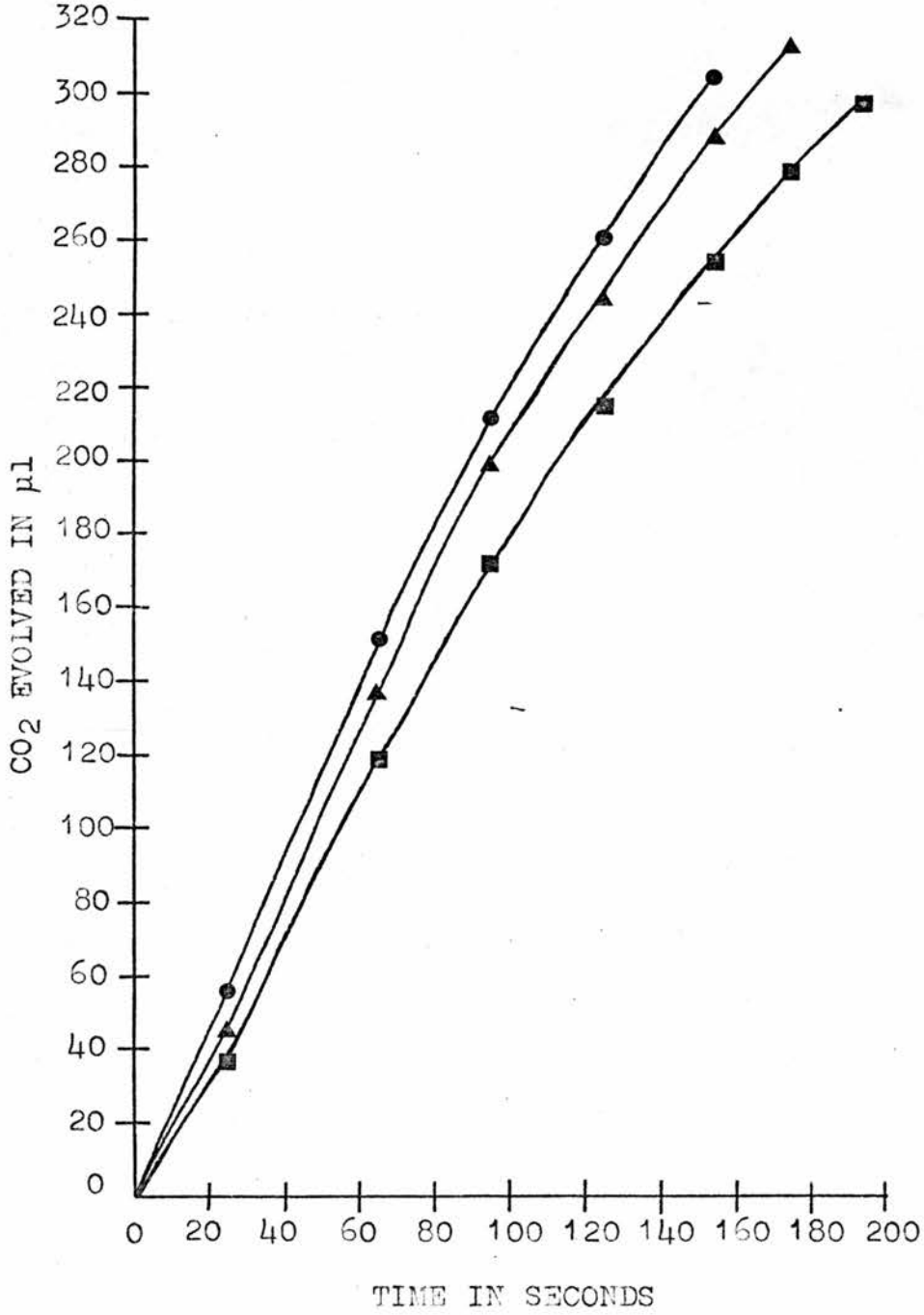
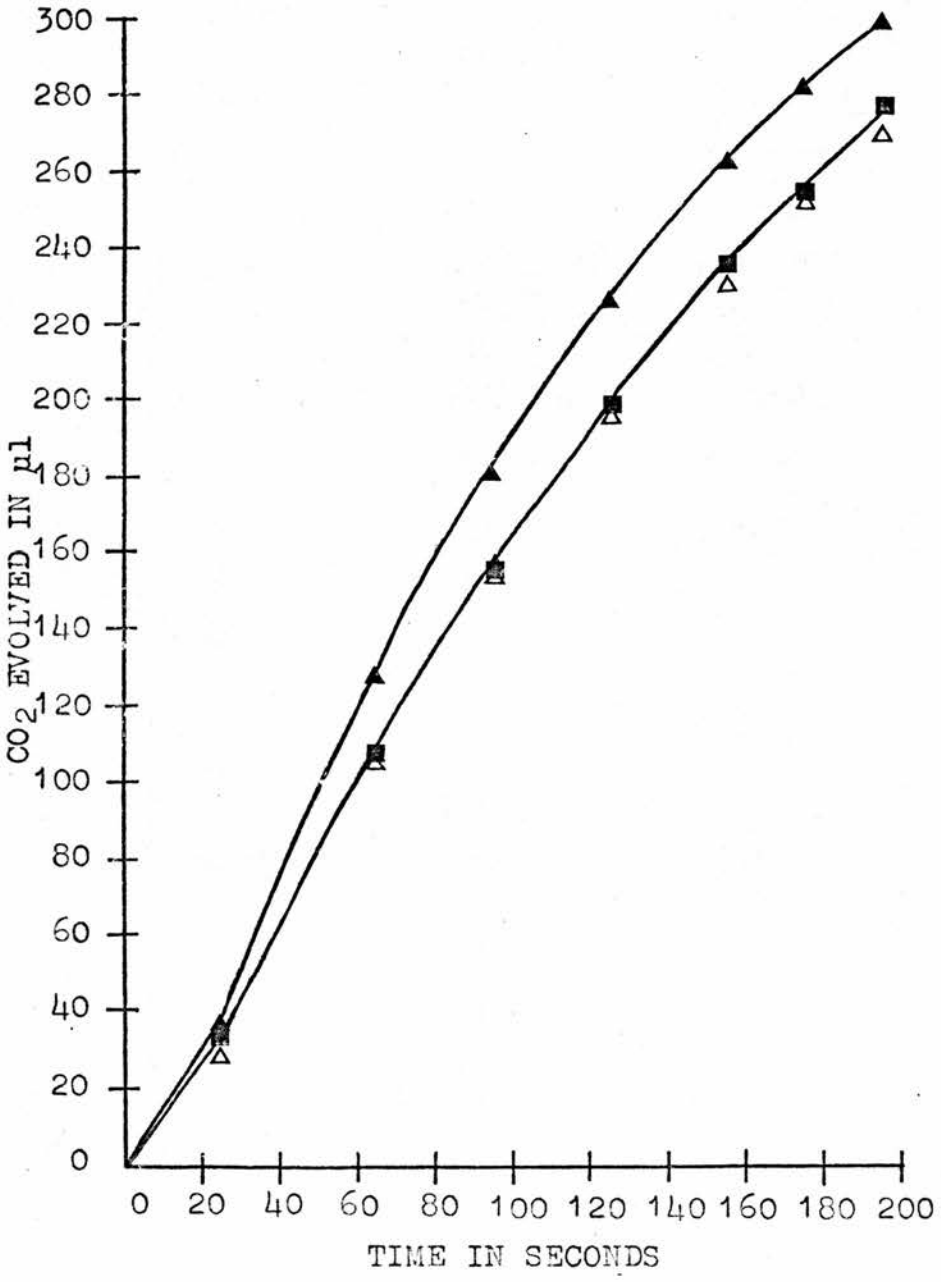

320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
CO_2 EVOLVED IN µl
0
20
40
60
80
100
120
140
160
180
200
TIME IN SECONDS

FIGURE 17

CO_2 evolved from $NaHCO_3$, in NaOH, when added to phosphate buffer 6.8 alone (■), in the presence of plant extract (▲) and in the presence of plant extract with Diamox (△), against time.

FIGURE 17


300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
CO_2 EVOLVED IN µl
0 20 40 60 80 100 120 140 160 180 200
TIME IN SECONDS

Effect of Diamox on Carbon Uptake

The effect of Diamox upon the uptake of carbon, by P. praelongus, was investigated.

Procedure.

A 2×10^{-2}M $KHCO_3$ bathing solution, pH 8.3 was used. The method employed for determining rate of carbon uptake was as described in Method section, page 28 . Diamox was added to the bathing solution to give a concentration of 10^{-4}M. Light and dark controls, without Diamox, were used. The plant tissue was allowed to photosynthesise in the radioactive experimental solutions for one hour.

Results.

Rate of carbon uptake, by P. praelongus, from the solution, without Diamox in the light, was 13.32×10^{-7} M C cm^{-2} $hour^{-1}$. When Diamox was added to the bathing solution this rate was decreased to 6.57×10^{-7} M C cm^{-2} $hour^{-1}$.

Discussion.

Figure 16 shows CO_2 evolved from $NaHCO_3$, in NaOH, when added to phosphate buffer, pH 6.8. It can be seen that rate of evolution is increased in the presence of extract of P. praelongus leaves and in the presence of blood. The latter is known to contain carbonic anhydrase, which would increase the rate of CO_2 evolution as the enzyme catalyses dehydration of HCO_3^-. Results shown in Figure 16, then, indicate that the leaf extract also contains carbonic anhydrase. Further evidence for this is shown in Figure 17. This figure shows that when Diamox, a specific inhibitor of carbonic anhydrase, is added to the leaf extract rate of CO_2 evolution is depressed. From these results it is concluded that P. praelongus leaves do possess the enzyme, carbonic anhydrase.

It has also been shown that Diamox inhibits uptake of carbon, by P. praelongus, from a bicarbonate solution, by 50%.

Ikemori and Nishida (1967) observed that 100mg Diamox suppressed photosynthetic activity of Ulva pertusa fronds 70% at pH 9.0 and by 30% at

pH 5.6. Raven (1970) using sulphanilamide, as an inhibitor of carbonic anhydrase, found a similar differential sensitivity in the bicarbonate user Hydrodictyon africanum .

From their results Ikemori and Nishida proposed that carbonic anhydrase may play an important role in the production of CO_2, from HCO_3^-, for photosynthesis of U. pertusa, a bicarbonate user. The above results would indicate that this may possibly be the case for P. praelongus. Obviously, however, further study, particularly on the effect of Diamox on photosynthetic activity, is needed before conclusions may be drawn. It must also be remembered that carbonic anhydrase has been found in both bicarbonate users and non-users (Steeman-Nielson and Kristiansen, 1949).

8. FURTHER ASPECTS OF THE SIGNIFICANCE OF BICARBONATE USE, FOR PHOTOSYNTHESIS.

Introduction.

In warm still waters pH may rise to values well above 8.5, so free CO_2 content of the water may often be very much lower than 1% of the total CO_2 content. If plants thrive in such conditions and continue to carry out photosynthesis, it may be postulated that these plants are able to utilise the bicarbonate ion as an exogenous carbon source or, if utilising free CO_2 only, that they possess a low CO_2 Compensation Point (C.P.) for photosynthesis and have an efficient use of CO_2 at low CO_2 concentrations. A low CO_2 C.P., approximately 10ppm or less, and an efficient use of CO_2, at low CO_2 concentrations, are characteristics of plants which possess β carboxylation pathway of primary CO_2 fixation.

Plants possessing the Calvin cycle for primary CO_2 fixation have high CO_2 C.P. (50 to

100ppm), and also exhibit low efficiency of CO_2 use at low CO_2 concentrations. This latter may be the result of the phenomenon of photorespiration, the substrate for which may be glycollic acid. The following reaction, catalysed by glycolate oxidase, for photorespiration is proposed by Zelitch (1966):

$CH_2OH\text{-}COOH + O_2 \rightleftharpoons CHO\text{-}COOH + H_2O_2 \rightleftharpoons HCOOH + CO_2 + H_2O$

(glycollate) (glyoxylate)

An investigation of the existence of photorespiration in P. polygonifolius and P. perfoliatus has been carried out (see Section 4.3).

One significant effect of photorespiration could be the maintenance of high intracellular CO_2. Further, in alkaline conditions many plants with high CO_2 C.P. fix ^{14}C via glycollic acid (Gibbs, Ellyard and Latzko, 1968).

The possibility also exists that plants with high CO_2 C.P. may, in solutions having low concentrations of CO_2 and high concentrations of O_2, be able to utilise organic carbon in glycollic acid as they would be equipped with glycollic oxidase. There is evidence that growth of

Chlorella is stimulated by exogenous glycollate, whilst inhibition of glycollate oxidase prevents growth of Chlorella on glycollate.(Lord and Merrett, 1971). Also, at low external CO_2 concentrations, barley leaves have been shown to convert externally supplied glycollate to CO_2, via intermediates of the Calvin cycle, in the chloroplasts (Tamàs and Bidwell, 1971).

In studying aspects of bicarbonate use it was therefore decided to ascertain whether any difference in CO_2 fixation pathway exists between a HCO_3^- user and a non-user. Firstly CO_2 C.P.s for photosynthesis of P. praelongus (user) and P. polygonifolius (non-user) were determined. Secondly gross anatomy of leaves of all the Potamogeton species, considered in this study, was investigated. This investigation was carried out as it is apparent that the presence of chloroplast-containing cells surrounding vascular bundles, i.e. possession of bundle sheaths, is also a characteristic of plants which carry out the B carboxylation pathway of CO_2 fixation (Bjorkman and Gauhl, 1969).

From the transverse sections of the leaves an estimate of the length of diffusive pathway of CO_2 was obtainable. Knowledge of this and of rates of photosynthesis in defined conditions allowed some estimate to be made of the resistances to CO_2 fixation in terms of aqueous diffusive and chemical resistances (in leaves).

8.1. DETERMINATION OF CO_2 COMPENSATION POINTS.

Procedure.

A free CO_2 bathing solution, pH 4.3, was employed. The solution was first saturated with air thus giving a concentration of O_2 of 21%. If there was any difference between the compensation points of the two species then it would be exagerated at high concentrations of O_2. $KHCO_3$ $1x10^{-4}$ M, was added to the solution, which with the initial CO_2 content of the distilled water, gave a solution with a starting concentration of $2x10^{-4}$M free CO_2.

Three leaves of each species were taken and placed in 25ml screw top bottles, one leaf per bottle. It was necessary to have a high initial concentration of ^{14}C so that a steady count obtained from the solution at compensation point was distinguishable from background count. 20uCi ^{14}C were, therefore, added to each reaction bottle. Bottles were placed in the metabolic shaker under the four xenon lamps.

Three 0.1ml samples of bathing solution were removed at regular intervals throughout a six hour

period. These samples were precipitated down and counted as described in Methods section.

Figures 18 and 19 show a semi-logarithmic plot of CPM against time for the bathing solution of each of the six leaves. From the initial and final count rates of each solution and a knowledge of the initial CO_2 concentration, final CO_2 concentrations of the solutions were calculated. These are shown in Table XIV.

TABLE XIV.

Final concentrations of CO_2 in the bathing solutions of P. praelongus and P. polygonifolius, after six hours in the light.

Species	Final CO_2 concentration ($1x10^{-6}M$)	Average CO_2 concentration ($1x10^{-6}M$)
P. polygonifolius	2.37	2.07
	2.03	
	1.75	
P. praelongus	2.07	2.00
	1.92	
	2.00	

FIGURES 18 & 19.

Semi-log. plot of CPM of bathing solution containing photosynthesising tissue, against time.

FIGURE 18:- 3 leaves of P. polygonifolius

FIGURE 19:- 3 leaves of P. praelongus

(□) CPM of bathing solution with no plant tissue.

FIGURE 18

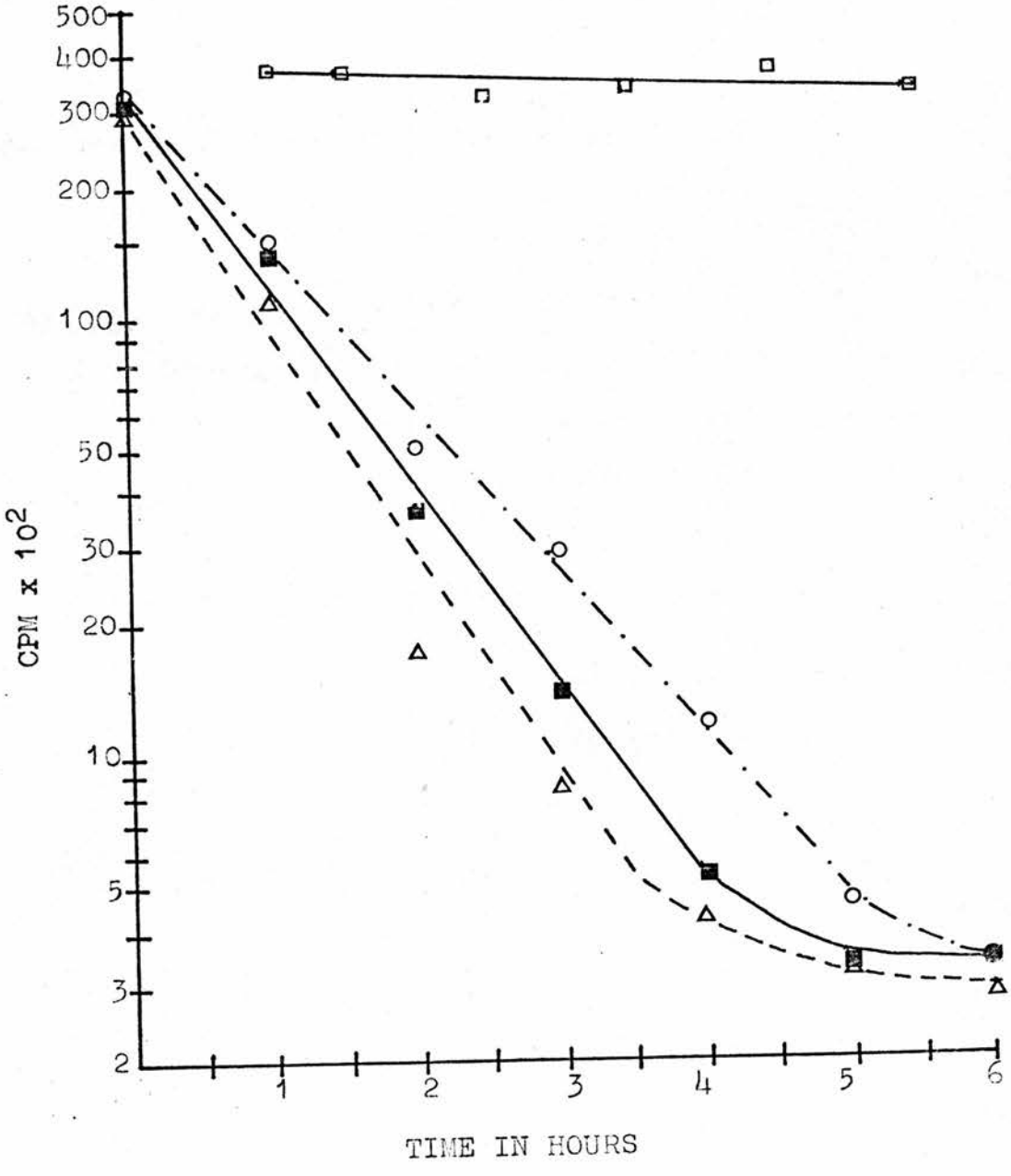
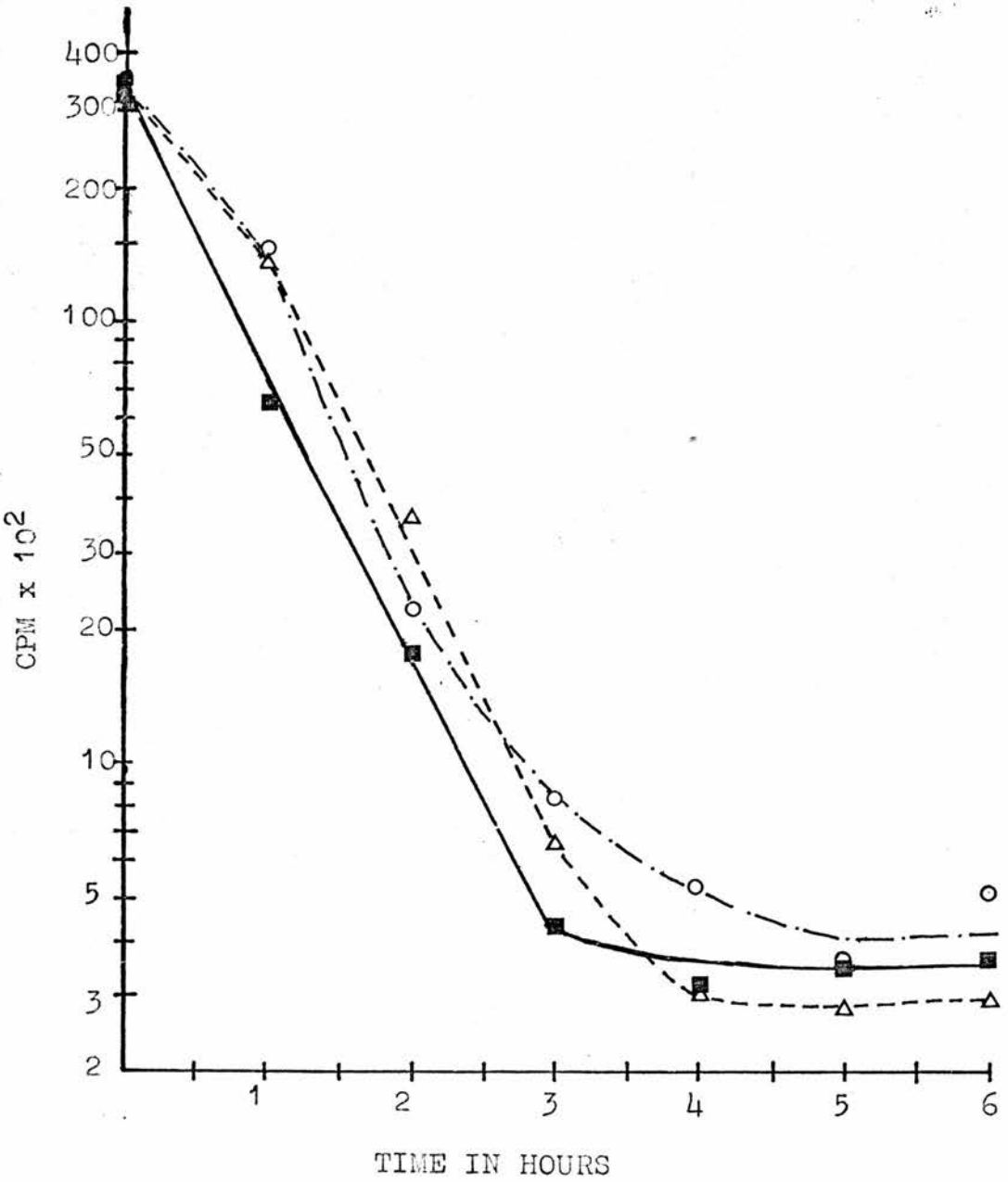

500
400
300
200
100
50
30
20
10
5
3
2
CPM x 10²
1
2
3
4
5
6
TIME IN HOURS

FIGURE 19



8.2(i). LEAF ANATOMY

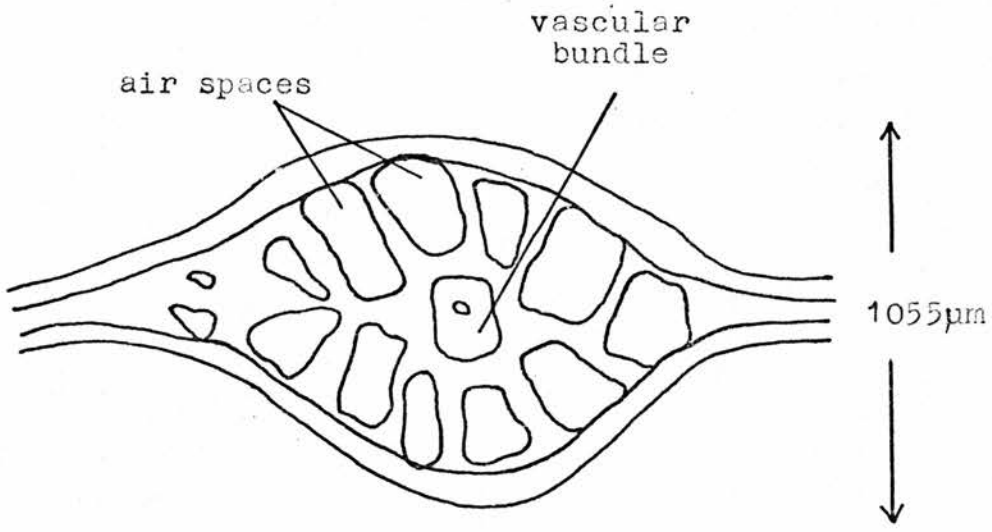
Procedure.

Transverse sections of leaves of P. lucens, P. praelongus, P. perfoliatus and P. polygonifolius (submerged leaves only) were cut. Measurements of leaf thickness and cell dimensions were made and camera lucida drawings of the sections were made.

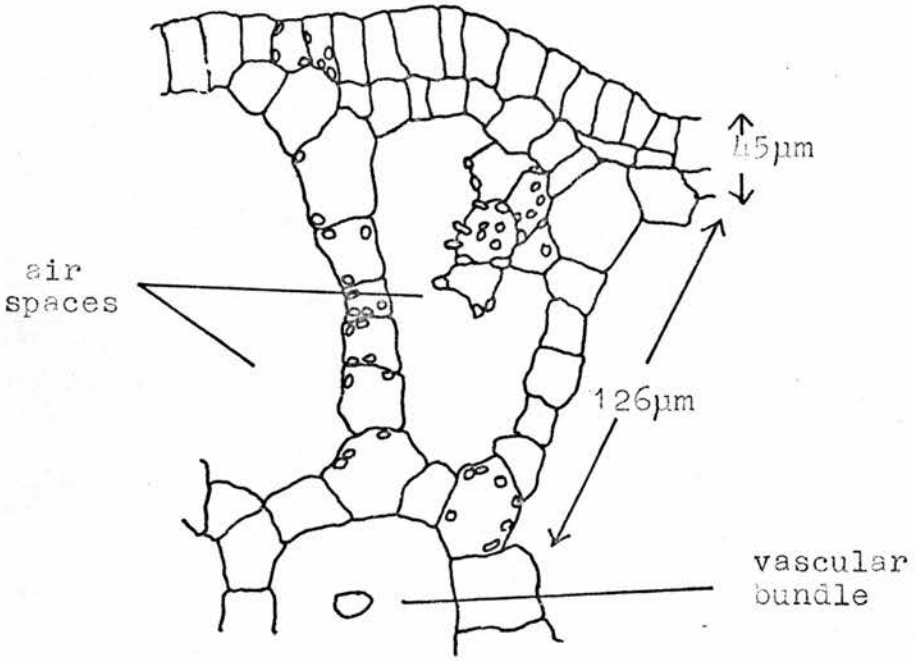
These diagrams are shown in Figures 20 to 23. Dimensions, in um, are noted on the figures.

FIGURE 20

POTAMOGETON LUCENS.



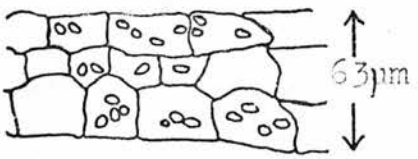
A. T.S. through mid vein region, L.P.



B. T.S. through mid vein region, H.P.

FIGURE 20

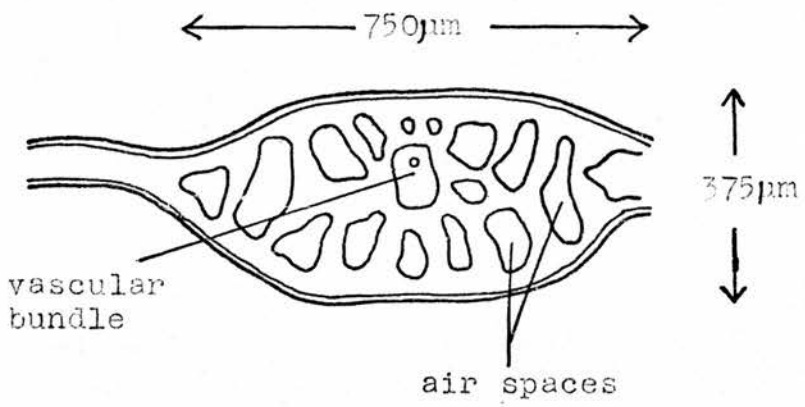
POTAMOGETON LUCENS.



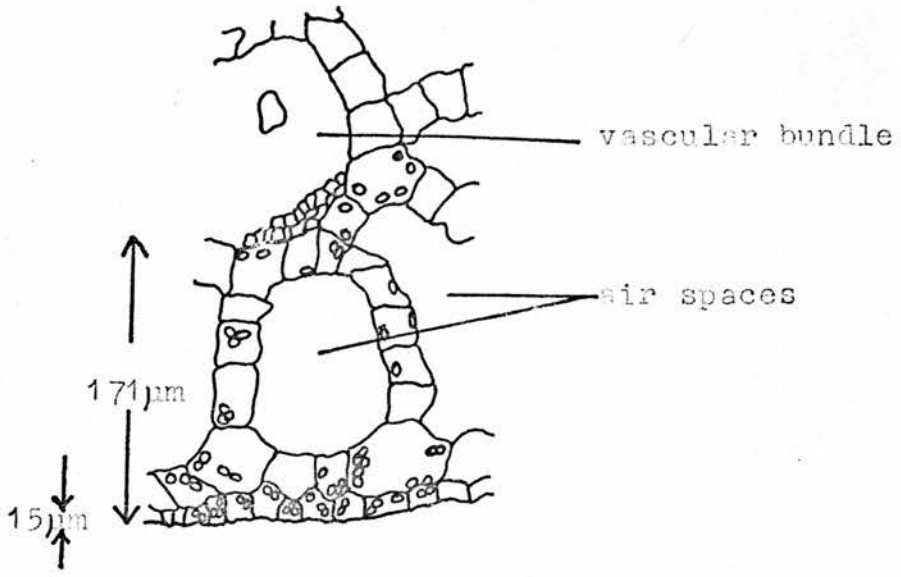
C. T.S. through leaf lamina, H.P.

FIGURE 21

POTAMOGETON PRAELONGUS.



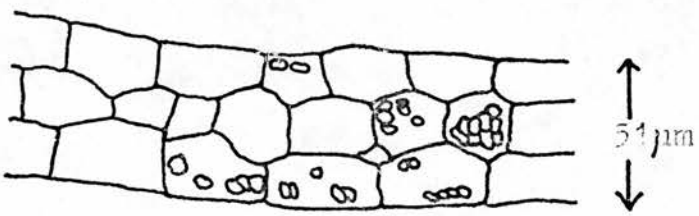
A. T.S. through mid vein region, L.P.



B. T.S. through mid vein region, H.P.

FIGURE 21

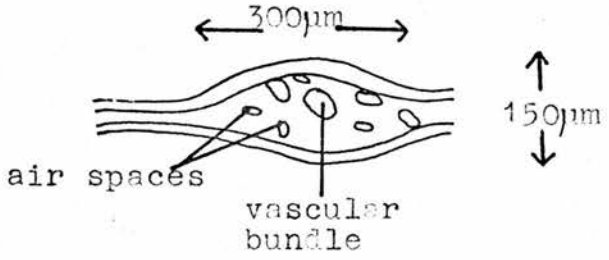
POTAMOGETON PRAELONGUS.



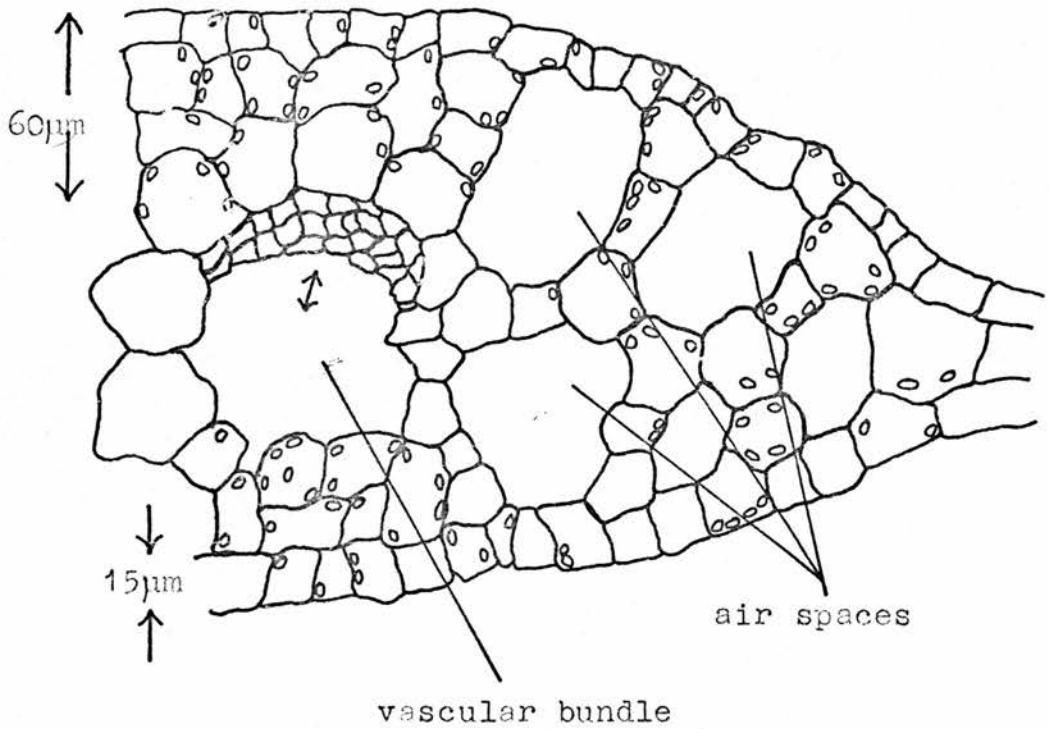
C. T.S. through leaf lamina, H.P.

FIGURE 22

POTAMOGETON PERFOLIATUS



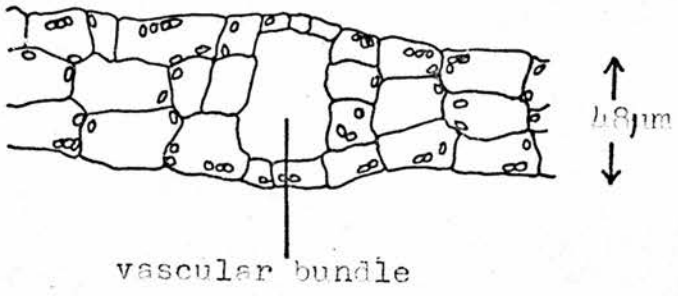
A. T.S. through mid vein region, L.P.



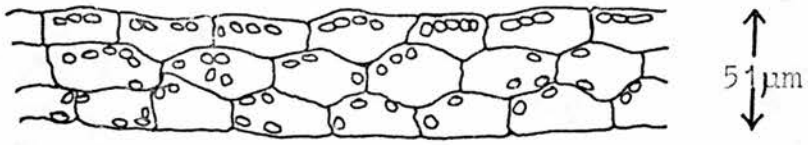
B. T.S. through mid vein region, H.P.

FIGURE 22

POTAMOGETON PERFOLIATUS.



C. T.S. through leaf lamina, H.P.

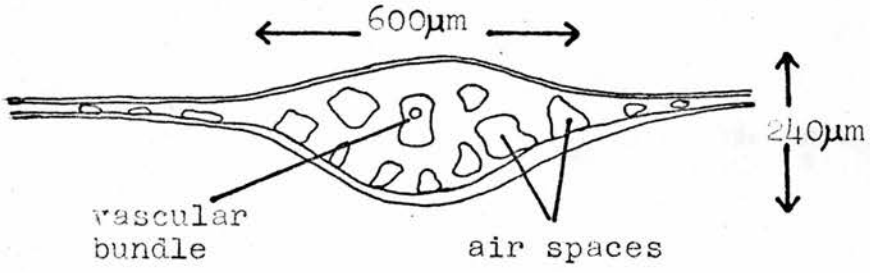


D. T.S. through leaf lamina, H.P.

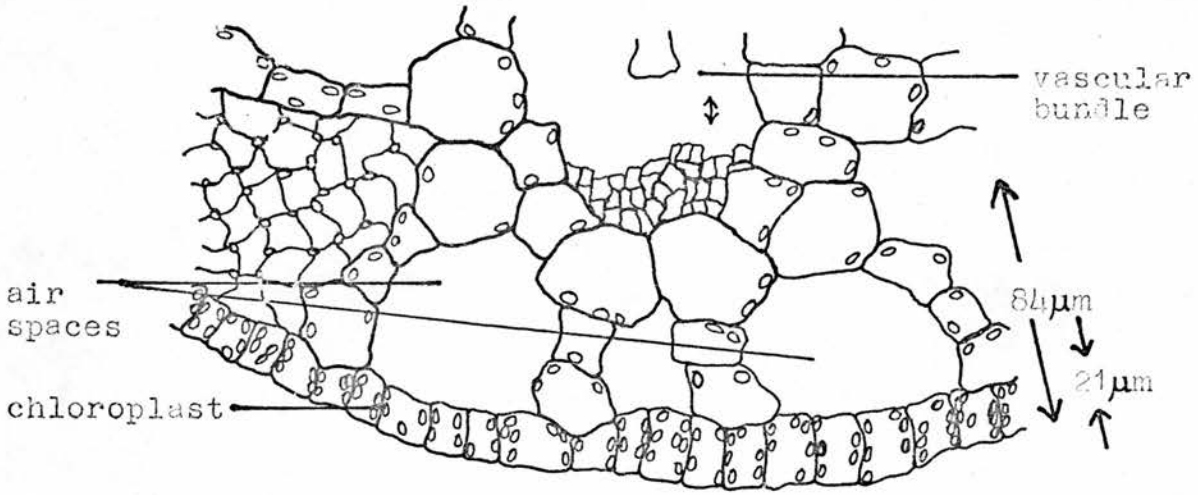
FIGURE 23

POTAMOGETON POLYGONIFOLIUS.

(submerged leaves)



A. T.S. through mid vein region, L.P.

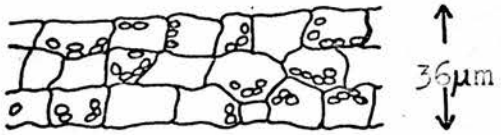


B. T.S. through mid vein region, H.P.

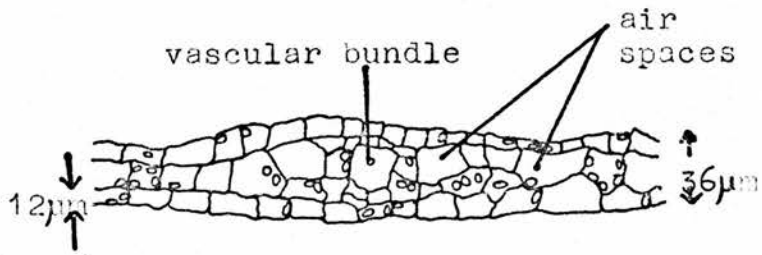
FIGURE 23

POTAMOGETON POLYGONIFOLIUS.

(submerged leaves)



C. T.S. through leaf lamina, H.P.



D. T.S. through leaf lamina, H.P.

8.3 CALCUALTION OF DIFFUSIVE AND CHEMICAL RESISTANCES

Rate of Photosynthesis (PS) = $\frac{\text{Concentration drop}}{\text{aqueous diffusive resistance.}}$

$$= \frac{C_o - C_i}{L/D} \qquad (1)$$

where:

PS is expressed in terms of picomoles C sec^{-1} cm^{-2} cell surface area.

C_o = CO_2 concentration in bulk phase of the bathing medium.

C_i = CO_2 concentration at the site of photosynthesis.

L = length of aqueous diffusion pathway (d, unstirred layer outside leaf and L_i, inside leaf)

D = diffusion coefficient of CO_2 in water = 0.160×10^{-4} cm^{-2} sec^{-1}.

Putting D/L = P the permeability constant, (1) becomes:

$$PS = P(C_o - C_i) \qquad (2)$$

$$PS = kC_i \qquad (3)$$

where:

k = first order rate constant for the primary carboxylation reaction.

Therefore: $P(C_o - C_i) = kC_i$

$$C_i = \frac{PC_o}{k + P} \qquad (4)$$

Substituting for C_i in (3):

$$PS = k \frac{PC_o}{(k+P)}$$

$$PS = \frac{C_o}{1/k + 1/P} \qquad (5)$$

Calculation of Diffusive Resistances.

Errors arise in the calculation of 1/P as estimates only of d can be used. Dainty and Hope(1959) have estimated the thickness of the unstirred layer for Chara cell walls, in a well stirred medium, to be 100 um. Raven (1970) uses a value of 500 um for the thickness of the unstirred layer around large plant organs, in

a poorly stirred medium.

If L_i maximum is taken to be 1/4 thickness of the leaf then for P. lucens, with a leaf thickness of 63 um, (see Figure 20), L_i max. = 16um. Then internal aqueous diffusion resistance = L/D

$$= \frac{16 \times 10^{-4}}{0.160 \times 10^{-4}} \frac{\text{cm}}{\text{cm}^2 \text{ sec}^{-1}}$$

$$= \underline{100 \text{ sec cm}^{-1}}$$

Taking d = 500 µm

$$1/P = \frac{(16 + 500) \times 10^{-4}}{0.160 \times 10^{-4}} \frac{\text{cm}}{\text{cm}^2 \text{ sec}^{-1}}$$

$$= \underline{3225 \text{ sec cm}^{-1}}$$

Taking d = 100 µm

$$1/P = \frac{(16 + 100) \times 10^{-4}}{0.160 \times 10^{-4}} \frac{\text{cm}}{\text{cm}^{-2} \text{ sec}^{-1}}$$

$$= \underline{725 \text{ sec cm}^{-1}}$$

Estimates of aqueous diffusion resistances of leaves of the Potamogeton species, considered in this study, are shown in Table XV.

TABLE XV

Aqueous diffusion resistances of leaves of Potamogeton species.

Species	Leaf thickness	Diffusion resistances sec cm^{-1} Internal resistance	d=100µm	d=500µm
P. lucens	63µm	100	725	3225
P. praelongus	51µm	81	706	3206
P.perfoliatus	51µm	81	706	3206
P.polygonifolius	36µm	56	681	3181

Calculation of Chemical Resistances.

Values of (1/k + 1/P) are calculated using Equation (5) and values of rates of photosynthesis, determined in comparative experiments of Section 5.1.

Rates of photosynthesis determined in Section 5.1 are in terms of Moles carbon cm^{-2} leaf surface area $hour^{-1}$. For substitution in Equation (5) these rates must be converted to picomoles cm^{-2} cell surface area sec^{-1}.

Considering P. praelongus:

Leaf thickness = 51μm

Assuming more or less cuboid cells each cell would have a linear measurement = 17 μm (see Figure 21)

Leaf on average is 3 cells thick; Considering one leaf surface, total number of cells in a volume of leaf 1 cm^2 x 25μm =

$1\ cm^2 = 1 \times 10^8\ \mu m$

$$\frac{10^8 \times 1.5}{17 \times 17} = 52 \times 10^4 \text{ cells}$$

Each cell has a surface area of 17 x 17 x 6 =

1740 μm^2

Total cell surface area in volume of leaf concerned

$$= \frac{1740 \times 52 \times 10^4}{10^8} \text{ cm}^2$$

$$= \underline{9 \text{ cm}^2}$$

In one laboratory experiment, for example, with 1×10^{-4}M free CO_2 rate of photosynthesis of P. praelongus was 2×10^{-7}M C cm^{-2} leaf surface area $hour^{-1}$.

$$PS = \frac{2 \times 10^{-7}}{36 \times 10^2 \times 9} = 6.16 \text{ pM C cm}^{-2} \text{ cell surface area sec}^{-1}$$

Substituting in Equation (5):

$$6.16 \text{ pM cm}^{-2} \text{ sec}^{-1} = \frac{1 \times 10^5}{(1/k + 1/P)} \text{ pM C cm}^{-3} \text{ sec cm}^{-1}$$

$$(1/k + 1/P) = \frac{1 \times 10^5}{6.16}$$

$$= \underline{1.62 \times 10^4 \text{ sec cm}^{-1}}$$

For values of $(1/k + 1/P)$ of other Potamogeton species, with different concentrations of CO_2 see Table XVI.

TABLE XVI

Estimates of $(1/k + 1/P)$ of Potamogeton species in bathing solutions of different CO_2 concentrations.

Species	Total CO_2 concentration pM cm^{-3}	$(1/k + 1/P)$ sec cm^{-1} pH 4.3
P. polygonifolius	5×10^5	1.6×10^4
	1×10^5	1.9×10^4
	1×10^4	1.6×10^4
P. praelongus	5×10^5	1.5×10^4
	1×10^5	1.6×10^4
	1×10^4	1.1×10^4
P. lucens	5×10^5	1.8×10^4
	1×10^5	2.0×10^4
	1×10^4	1.8×10^4
P. perfoliatus	5×10^5	2.7×10^4
	1×10^5	2.7×10^4
	1×10^4	2.5×10^4

Discussion.

From the results in Table XIV, both the bicarbonate user and non-user, P. praelongus and P. polygonifolius, appear to have high CO_2 compensation points, 2.00×10^{-6}M and 2.07×10^{-6}M respectively. These values are equivalent to approximately 50ppm. It has already been shown that P. polygonifolius and P. perfoliatus (the latter being a bicarbonate user) apparently exhibit photorespiration, see Section 4.3.

Figures 20 to 23 show that none of the Potamogeton species investigated have bundle sheaths. It would appear then that these plants carry out photosynthesis via the normal Calvin cycle as opposed to the β-carboxylation pathway.

The dominance of P. lucens, P. perfoliatus and P. praelongus, in waters of high pH and high alkalinity, may then be the result of the ability of the species to utilise the HCO_3^- ion, rather than any efficiency of use of free CO_2 at low external free CO_2 concentrations. It is possible that, in such environmental conditions, a

proportion of the carbon fixed photosynthetically, by these plants, may be via glycollate. The glycollate may then be broken down to maintain high intracellular CO_2 concentrations, in alkaline conditions.

As stated in the introduction to this section, ^{14}C has been shown to be fixed via glycollate, particularly in alkaline conditions and at low external concentrations of CO_2. Pritchard, Griffin and Whittingham (1962) for Chlorella and higher plants, have shown that glycollate is a major labelled product when $^{14}CO_2$ is supplied at low external CO_2 concentrations and high O_2 concentrations. Gibbs, Ellyard and Latzko (1968) have also shown that DCMU decreased the proportion of $^{14}CO_2$ incorporated into glycollate and concluded that some cooperation between the Calvin cycle and photosystem 2 is required for glycollate synthesis. Is it also possible that as DCMU inhibits bicarbonate uptake (Raven 1968) and alkaline conditions enhance ^{14}C fixation via glycollate that bicarbonate uptake is also involved in this co-

operation? Tolbert (1962) has shown that Chlorella excretes glycollate, in conditions which favour HCO_3^- fixation, and he postulates that glycollate excretion and absorption may represent a glycollate-bicarbonate anionic exchange across the cell membrane, without any undesirable loss or absorption of cations.

Considering the non-HCO_3^- user, P. polygonifolius; like the other Potamogeton species investigated, this species has high CO_2 C.P., does not possess bundle sheaths and apparently exhibits photorespiration. It is unlikely, therefore, that P. polygonifolius carries out photosynthesis via β-carboxylation. Rate of photosynthesis, by this species, would be considerably limited by low concentrations of free CO_2 available in alkaline waters and low efficiency of CO_2 use at these low CO_2 concentrations. If, indeed, this species does photorespire it may be postulated that a proportion of the carbon fixed, in conditions of low external CO_2 concentrations, may be via glycollate pathway.

Estimates of diffusive resistances to CO_2 are shown in Table XV. From these results it can be seen that internal diffusive resistance, in P. polygonifolius leaves, is only 56% of that of P. lucens leaves and 70% that of P. praelongus and P. perfoliatus leaves. If one considers, however, the presence of an unstirred layer, around the leaves, through which the CO_2 must diffuse, differences in diffusion resistances of the leaves of the different species, becomes insignificant. This is so even if the width of the unstirred layer ie estimated as low as 100 um. Values in Table XV are for resistance to diffusion of free CO_2 only.

Both chemical and diffusion resistances are considered, together, in estimates of $1/k + 1/P$, Table XVI. These estimates have been made using rates of photosynthesis and CO_2 concentrations in free CO_2 solutions only. Estimates of $1/k + 1/P$ calculated using equation (5), may not be considered as accurate for bicarbonate solutions.

In the case of P. polygonifolius, a non-

bicarbonate user, $1/k + 1/P$, for the species in a bicarbonate solution, may be estimated by considering C_o equal to the equilibrium free CO_2 concentration in the solution. For bicarbonate-users an estimate of $1/k + 1/P$, for the species in a bicarbonate solution, obviously cannot be calculated on this basis. It has been shown that bicarbonate is used as an exogenous carbon source for photosynthesis by these plants, HCO_3^- must then be taken into the cell. If only free CO_2 enters into the primary carboxylation step, bicarbonate must dissociate in the cell. Concentration of free CO_2 available at the site of photosynthesis will therefore depend on the concentration of HCO_3^- which has entered the cell and on the pH of the cell sap.

In estimating values of $1/P$ for the leaves no adjustment has been made for the resistances to diffusion of CO_2, of the cell membranes, i.e. plasmalemma and chloroplast membranes. Water and CO_2 can readily penetrate the plasmalemma (Nobel, 1970). The permeability coefficients for

electrolytes are, however, much smaller than for non-electrolytes (Nobel 1970), hence membranes may hinder entry of HCO_3^- considerably.

Estimates of 1/P, based on diffusion of free CO_2, in leaves, may be lower than those values shown in Table XV. As the unstirred layers are so large compared to the length of diffusion pathway within the leaves, the differences in permeability to CO_2, of the membranes, would make an insignificant difference to 1/P for the leaf and unstirred layer, when considered together. In bicarbonate solutions, however, resistances of the plasmalemma to HCO_3^- diffusion may increase 1/P, of leaves of bicarbonate users, considerably. Further Raven (1968) concluded that some uptake of HCO_3^- into the cells of Hydrodictyon africanum may be active and Denny and Weeks (1971) have indicated active uptake of HCO_3^- into leaves of Potamogeton schweinfurthii. Thus it is difficult to obtain an accurate estimate of 1/P for leaves of bicarbonate users, in bicarbonate solutions. Because of these problems, the remainder of this discussion concerning resistances will be restricted to values

of $1/k + 1/P$ estimated for leaves in free CO_2 solutions only, Table XVI.

Estimates for P. polygonifolius, P. praelongus and P. lucens are similar, approximately 1.7×10^4 sec cm^{-1}, those for P. perfoliatus are somewhat higher, 2.6×10^4 sec cm^{-1}, but all are of the same order of magnitude. In particular estimates for a non bicarbonate user, P. polygonifolius, and for the bicarbonate users, are similar. From estimates of 1/P (Table XV), it can be seen that even in a poorly stirred medium, resulting in an unstirred layer 500 µm thick, 1/k would still be the main factor limiting rate of photosynthesis. These estimates show, however, that stirring should still increase rate of photosynthesis by decreasing d and thus 1/P. For example, in the case of P. praelongus, if d is reduced from 500 µm to 100 µm 1/P is decreased to 706 sec cm^{-1} from 3206 sec cm^{-1} (Table XV). $(1/k + 1/P)$ for P. praelongus, with a rate of photosynthesis of 6.16 pM cm^{-2} sec^{-1} in 1×10^{-4}M free CO_2 solution, has been calculated as 1.62×10^4 sec cm^{-1}.

if, in this situation, d is equal to 500 μm 1/k is equal to 1.30×10^4 sec cm^{-1}. If stirring reduces d to 100 μm in this situation then 1/P becomes 706 sec cm^{-1}, (1/k + 1/P) becomes 1.37×10^4 sec cm^{-1} and rate of photosynthesis 7.3 pM cm^{-2} sec^{-1}. Thus in this theoretical case stirring increases the rate of photosynthesis by almost 2%.

The fact that stirring does affect rate of photosynthesis, i.e. rate of photosynthesis proportional to rate of stirring, has been shown by Westlake et al (reviewed in Sculthorpe, 1967).

It may be added that if d at 500 μm is underestimated and is as large as 1000μm, 1/P for P. praelongus becomes 6300 sec cm^{-1}. If photosynthesis is also underestimated, for example due to unmeasured photorespiration (see Section 4.3), by as much as 1/10th then in the example given above, (1/k + 1/P) becomes 1.47×10^4 sec cm^{-1}. Considering d equal to 1000μm 1/k becomes 8400 sec cm^{-1}. Thus if this situation does exist stirring may alter rate of photosynthesis by P. praelongus, in 1×10^{-4}M free CO_2, by as much as 80%.

9. SUMMARY

The distribution of certain fresh water macrophytes appears to be connected with waters rich in electrolytes. These waters have high alkalinity and high pH values and, therefore little free CO_2 available for photosynthesis. It is proposed then that the distribution of some fresh water plants may be the result of their ability, or inability, to utilise the HCO_3^- ion as a carbon source for photosynthesis.

Four Potamogeton species and Fontinalis antipyretica were studied, by comparative experiments, to determine whether or not they could utilise HCO_3^-. In initial experiments photosynthesis was measured by following rate of O_2 evolution, using an oxygen electrode. Remaining experiments followed rate of C uptake, using a ^{14}C technique.

The Potamogeton species studied in detail were chosen as they differ in their distribution with respect to water chemistry. P. lucens is

restricted to rich waters, P. praelongus and P. perfoliatus are ubiquitous, but are often found in moderately rich to rich waters, and P. polygonifolius is restricted to poor waters.

Preliminary experiments indicated that the species used show no diurnal rhythm of photosynthesis. This allows for the comparison of rates of photosynthesis measured at different times of the day.

It appears that there is no pH effect on rate of photosynthesis, of the species concerned in the study, within the pH range 4.3 to 7.3. It has also been indicated that the HCO_3^- ion is not acting merely as a "reservoir" of free CO_2. The series of comparative experiments show that while all the Potamogeton species investigated attain similar rates of photosynthesis in free CO_2 solutions, P. lucens, P. praelongus and P. perfoliatus utilise the HCO_3^-, and P. polygonifolius and Fontinalis antipyretica do not utilise the HCO_3^- ion, as an exogenous carbon source for photosynthesis.

Since HCO_3^- uptake may be light activated

and, in consequence, sensitive to DCMU (Raven 1968), the effect of DCMU on photosynthetic activity of P. praelongus and P. polygonifolius was tested. For all concentrations of DCMU used rates of photosynthesis of P. polygonifolius are similar in a bicarbonate solution and in a solution with equivalent free CO_2 concentration. If P. polygonifolius was utilising the HCO_3^- ion and if bicarbonate uptake is more sensitive to DCMU than is free CO_2 uptake, then rates attained in the bicarbonate solutions would be depressed below those in the free CO_2 solutions at high concentrations of DCMU.

P. praelongus exhibits higher rates of photosynthesis in bicarbonate solutions than in equivalent free CO_2 solutions, at lower concentrations of DCMU used. With 5×10^{-7} M DCMU, however, carbon uptake due to HCO_3^- appears to be completely inhibited.

These results then further indicate the ability of P. praelongus and the inability of P. polygonifolius to utilise the HCO_3^- ion.

A preliminary investigation of the significance of carbonic anhydrase, to bicarbonate users, was undertaken. Inhibition of action of this enzyme does, in fact, inhibit C uptake, by P. praelongus, from a HCO_3^- solution. The possibility exists, then, that carbonic anhydrase plays a role in the production of CO_2 for the carboxylation process, from HCO_3^-.

Estimates of chemical and diffusive resistances, of Potamogeton leaves, to CO_2, were made, in free CO_2 solutions. From values of 1/P and (1/k + 1/P), it appears that 1/k, the chemical resistance, is the main resistance limiting photosynthesis. Even so, it has been shown that stirring could affect rate of photosynthesis, by decreasing the thickness of the unstirred layer surrounding the leaves and thus decreasing 1/P.

Three of the Potamogeton species were studied in a field situation. In the area chosen for study, P. praelongus and P. perfoliatus are found in rich water lochs and P. polygonifolius is found in a poor water loch.

Rates of photosynthesis attained by

P. praelongus and P. perfoliatus, in their natural loch waters, are similar to those attained in equivalent bicarbonate solutions in the laboratory. This indicates that these species are utilising the HCO_3^- ion, of the loch waters, for photosynthesis. They do not, however, appear to utilise the HCO_3^- when supplied in "HCO_3^- enriched" poor loch water. By comparing laboratory and field results for P. polygonifolius, it appears that this species does not use HCO_3^- ion, from loch water, for photosynthesis.

P. polygonifolius and P. praelongus both have relatively high CO_2 compensation points (50 ppm) for photosynthesis. Anatomical observations shown the absence of bundle sheaths in all the Potamogeton species studied. It is postulated, therefore, that these species do not fix carbon dioxide via the B-carboxylation pathway. It is most likely, then, that none of these species have low CO_2 C.P.s i.e. less than 100ppm. Ability to thrive in waters of high pH and, therefore, low free CO_2 concentration, cannot be the result of an efficient use of CO_2 at low external CO_2 concentrations.

Let us now consider the possible adaptive significance of bicarbonate use or non-use in relation to the natural distribution of the species studied here.

Fontinalis antipyretica has been shown to be unable to utilise the bicarbonate ion. This confirms the results of Rutner (1947) and Steeman-Nielson (1947), but this moss occurrs abundantly even in calcareous waters. It has been suggested, however, that, being a prostrate plant, it might gain from a locally high CO_2 concentration above a decomposing organic mud and that, anyway, CO_2 requirement should be lower at the reduced irradiances of deeper water (Spence, 1967). Raven (1970) adds that diffusion of bicarbonate to cell free space might provide a CO_2 reservoir near the cell membrane.

From the results of experiments conducted in this study, it appears that distribution of the Potamogeton species considered here, could be the result of the ability or inability of the plants to utilise the HCO_3^- ion for photosynthesis.

Non-users, e.g. P. polygonifolius, may be debarred from alkaline waters in a competetive situation. Rate of photosynthesis of such species depend on the concentration of available free CO_2 only. In rich waters, with high pH, this concentration is very low, thus such species could only maintain low rates of photosynthesis in such waters. This is confirmed by the low rate of photosynthesis attained by P. polygonifolius in alkaline Croispol and Lanlish loch waters. In these waters, bicarbonate users, P. praelongus and P. perfoliatus, attain rates of photosynthesis four to six times those of P. polygonifolius.

Both users and non-users perform rather similarly (per cm^2 leaf area) in acid solutions in the laboratory. The absence of P. lucens from low alkaline waters may be fortuitous, or unconnected, at least directly, with shortage of HCO_3^-.

Rates of photosynthesis of P. praelongus and P. perfoliatus, in poor loch water, in the field, were low. These low rates are, however, most likely the result of carbon limitation in 25ml samples over experimental periods of 3 hours.

If this is so rates of photosynthesis must have been similar to laboratory rates in equivalent free CO_2 solutions. These rates would be higher than rates in the alkaline loch waters. As the rates of photosynthesis of these species is dependent only on free CO_2, in solutions with less than approximately 7.7 , then considering the factor of CO_2 supply only, it is expected that these species would be ubiquitous in their distribution, as indeed they are.

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APPENDIX I

CO_2 IN WATER.

Carbon dioxide, in solution, forms carbonic acid which dissociates to form HCO_3^- and H^+; the HCO_3^- may then dissociate to give H^+ and CO_3^{--}:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \overset{k_1}{\rightleftharpoons} HCO_3^- + H^+ \overset{k_2}{\rightleftharpoons} CO_3^{--} + 2H^+ \quad \text{1.1}$$

where: k_1 and k_2 are, respectively, the first and second dissociation constants of carbonic acid in water.

Considering the dissociation of carbonic acid, since the concentration of H_2CO_3 is essentially dependent upon the concentration of CO_2, k_1 may be written as:

$$k_1 = \frac{[H^+]\,[HCO_3^-]}{[CO_2]} \qquad \text{1.2}$$

k_2 may be written as:

$$k_2 = \frac{[H^+]\,[CO_3^{--}]}{[HCO_3^-]} \qquad \text{1.3}$$

These equations apply to CO_2 dissolving in fresh water where the activity coefficients of H^+, CO_2, HCO_3^-, and CO_3^{--} may be considered as unity.

From equation 1.2 the following equation can be derived and is often referred to as the Henderson-Hasselbach equation:

$$pH = pk_1 + \log \frac{[HCO_3^-]}{[CO_2]} \qquad \text{1.4}$$

Similarly equation 1.5 can be derived from 1.3:

$$pH = pk_2 + \log \frac{[CO_3^{--}]}{[HCO_3^-]} \qquad \text{1.5}$$

The dissociation constants, in fresh water, depend only on water temperature. Pressure effects need be considered only when the pH is very small and depth exceeds 1000 meters. Thus, using equations 1.4 and 1.5, the relative proportions of CO_2, HCO_3^-, and CO_3^{--} can be calculated.

Saruhashi (1953) has produced tables of percentage molar fraction of free carbonic acid, bicarbonate ion and carbonate ion in solution, at 2°C intervals of temperature and 0.1 pH intervals of pH, using the above equations. In this study, in calculations involving determination of these percentages, in a solution, these tables have been used.

CALCULATION OF CONCENTRATION OF CO_2 FORMS IN THE BATHING SOLUTIONS.

The following sample calculations use a pk_1 value of 6.392, for 20°C, (Harned and Davis) and a pk_2 value of 10.38, for 20°C (Harned, Samuel and Scholes) (pk values as given in Saruhashi, 1953).

The bathing solutions were prepared as described in Methods section. Carbon was added to the solutions in the form of $KHCO_3$ (Molecular weight 100).

Considering a $1x10^{-3}$M solution of $KHCO_3$, this solution would have a $1x10^{-3}$M concentration of HCO_3^- ion and a $1x10^{-3}$M concentration of carbon. The percentages of the various forms of CO_2 present in solution of a particular pH value may then be calculated using equations 1.4 and 1.5 given above.

Example.

For pH 8.0 and a $1x10^{-3}$M solution of $KHCO_3$:

$$\log \frac{[HCO_3^-]}{[CO2]} = pH-pk1 = 8.0 - 6.392$$

$$= 1.608$$

$$\frac{[HCO_3^-]}{[CO_2]} = 40.5$$

$$\log \frac{[CO_3^{--}]}{[HCO_3^-]} = pH - pK_2$$

$$= 8.0 - 10.38$$

$$= -2.38$$

$$= \bar{3}.62$$

$$\frac{[CO_3^{--}]}{[HCO_3^-]} = 0.0042$$

$$[CO_2] + [HCO_3^-] + [CO_3^{--}] = 1 \times 10^{-3} M$$

solving for $[HCO_3^-]$ $= 0.972 \times 10^{-3} M$ (97.2%)

solving for $[CO_2]$ $= 0.024 \times 10^{-3} M$ (2.4%)

therefore $[CO_3^{--}]$ $= 0.004 \times 10^{-3} M$ (0.4%)

For pH 4.3 and a $1 \times 10^{-3} M$ solution of $KHCO_3$:

$$\log \frac{[HCO_3^-]}{[CO_2]} = 4.3 - 6.392$$

$= -2.092$

$= \bar{3}.908$

$\left[HCO_3^-\right] = 8.1 \times 10^{-3}$

$\left[CO_2\right] = 0.0081$

solving for $\left[CO_2\right] = 0.992 \times 10^{-3}$M (99.2%)

therefore $\left[HCO_3^-\right] = 0.008 \times 10^{-3}$ (0.8%)

The $\left[CO_3^{--}\right]$ is only significant at pH values greater than pH 7.4, at which value $\left[CO_3^{--}\right]$ accounts for 0.1% of the CO_2 forms.

APPENDIX II

$KHCO_3$ TO BE ADDED TO A BATHING SOLUTION TO GIVE REQUIRED FREE CO_2 CONCENTRATION AT pH 7.3.

From Saruhashi's tables (Saruhashi 1953): at 20°C, pH 7.3 there is 11% free CO_2 present in solution.

APPENDIX TABLE I.

Free CO_2 M	Total CO_2 M	$KHCO_3$ g/l
1×10^{-3}	9.1×10^{-3}	0.91
5×10^{-4}	4.5×10^{-3}	0.45
3×10^{-4}	2.7×10^{-3}	0.27
1×10^{-4}	9.1×10^{-4}	0.091
8×10^{-5}	7.3×10^{-4}	0.073
5×10^{-5}	4.5×10^{-4}	0.045
2×10^{-5}	1.8×10^{-4}	0.018

APPENDIX III

CALIBRATION OF THE OXYGEN ELECTRODE.

The electrode was calibrated for a temperature range of 0°C to 25°C.

For the temperature range concerned, figures for the solubility of oxygen in water, in equilibrium with air at 760mm Hg pressure and 100% R.H., were taken from Montgomery, Thom and Cockburn, 1964. These figures were converted to solubility at 751mm Hg, the barometric pressure prevailing when readings were taken, using the formula:

$$Sx = \frac{S\ Px}{760}$$

where: Sx = solubility at pressure Px

S = solubility at 760mm Hg pressure

Px = observed pressure in mm Hg.

The oxygen content of the water was then related to the observed meter reading in μA for each particular temperature. The sensitivity of the electrode was then calculated as μA per mgO_2 per litre of water.

The observed thermistor readings were related to the measured temperature of the water. The sensitivity was then directly related to the thermistor readings. A graph of sensitivity and thermistor reading against temperature was plotted. From this graph a calibration chart was drawn up, see Appendix Table II.

Knowing the thermistor reading and meter reading the concentration of oxygen, in the solution concerned, may be calculated using the figures for sensitivity. This assumes a linear relationship between meter reading and oxygen concentration for each particular temperature. Appendix Figure 1 shows a plot of meter reading against saturated oxygen concentration for a temperature range of 15 to 25°C. A plot of meter reading against oxygen concentration, determined by Winkler analysis, in water at 20°C, shows that this relationship is in fact, linear.

Oxygen concentration of several of the saturated oxygen solutions were found by Winkler analysis. Values of oxygen concentration, estimated in this way, are also plotted against meter reading in Appendix Figure 1.

APPENDIX TABLE II

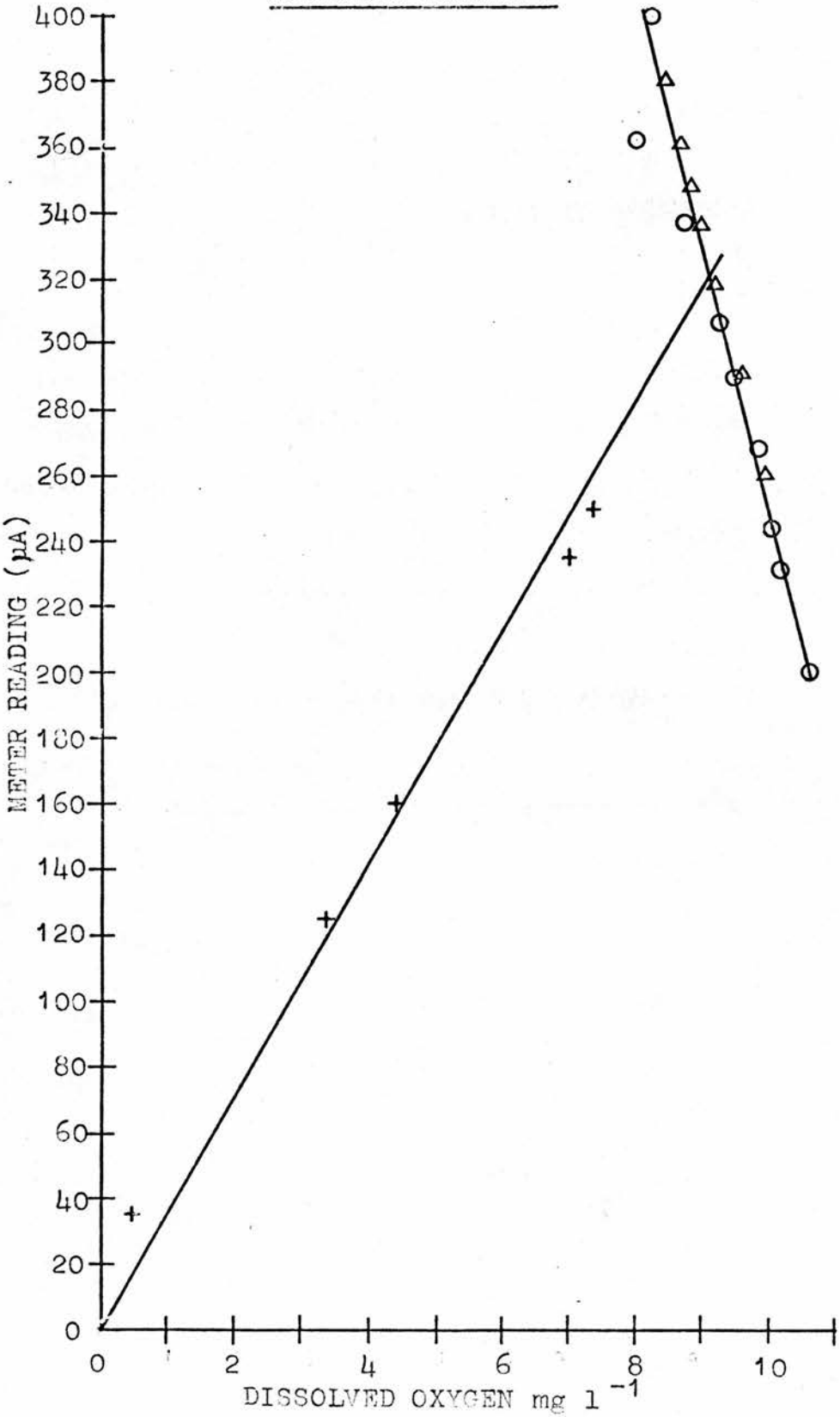
CALIBRATION CHART FOR THE OXYGEN ELECTRODE.

Thermistor reading	Temperature °C	Sensitivity $\mu A/mgO_2/L$
660	15.0	26.3
670	15.25	26.8
680	15.55	27.3
690	15.8	27.8
700	16.05	28.3
710	16.35	28.9
720	16.6	29.4
730	16.9	30.0
740	17.2	30.6
750	17.45	31.2
760	17.75	31.8
770	18.0	32.4
780	18.3	33.1
790	18.6	33.8
800	18.9	34.4
810	19.2	35.1
820	19.5	35.8
830	19.8	36.6
840	20.1	37.3
850	20.4	38.1
860	20.7	38.9
870	21.0	39.7
880	21.3	40.6
890	21.65	41.6
900	21.95	42.6
910	22.3	43.6
920	22.6	44.6
930	23.0	45.7
940	23.3	46.6
950	23.6	47.6
960	24.0	48.7

APPENDIX FIGURE 1

This figure shows a plot of meter reading obtained with the oxygen electrode in saturated O_2 solutions, with temperature of 15 to 25°C;
(o) O_2 concentrations calculated from solubility tables,
(Δ) O_2 concentrations determined by Winkler analysis,
(+) meter reading against O_2 concentratio n of solution at 20°C; concentrations determined by Winkler analysis.

APPENDIX FIGURE 1



APPENDIX IV

SPECIMEN CALCULATION OF RATE OF CARBON UPTAKE BY PHOTOSYNTHESISING TISSUE.

Calculation of rate of carbon uptake by *Potamogeton praelongus* from 10 x 10^{-3}M $KHCO_3$ solution, pH 8.2.

0.1ml samples of bathing solution were precipitated with 0.1ml saturated $Ba(OH)_2$ solution.
(In laboratory experiments three samples of bathing solution were removed before, and three after, the plant tissue had been photosynthesising for one hour. Counts from the last three samples were then corrected for uptake of ^{14}C by the plant tissue. In field experiments three samples were removed after the experimental period only and counts from these samples were corrected for uptake of ^{14}C by the plant tissue.)

Mean solution count = 3990 CPM/0.1ml.
Therefore 1ml of solution would give 39900 CPM

Carbon concentration of bathing solution = 10×10^{-3}M

Carbon concentration of 1ml bathing solution =

1×10^{-5}M

$$\frac{\text{CPM}}{\text{Moles C}} = \frac{39900}{1 \times 10^{-5}} = 3.99 \times 10^{9} \text{ CPM Moles C}^{-1}$$

Mean tissue count after one hour in radioactive solution = 2824 CPM cm^{-2}

$$\frac{\text{Tissue count}}{\text{CPM Moles C}^{-1}} = \frac{2824}{3.99 \times 10^{9}} = 7.08 \times 10^{-7} \text{ M C cm}^{-2} \text{ hour}^{-1}$$

Standard error of tissue count = 5.5%

Rate of Carbon uptake = $7.08 \pm 0.39 \times 10^{-7}$M cm^{-2} hr^{-1}

APPENDIX V

BARIUM CARBONATE SELF ABSORPTION CURVE.

It was found necessary to precipitate the sample solution with $Ba(OH)_2$ onto lens tissue on the planchets. When lens tissue was not used the precipitate dried out covering a more or less circular area, but as the areas were small the errors involved in calculating the areas of these precipitates were considerable. A reliable self absorption curve could not, therefore, be produced in this way. By using lens tissue uniform thickness of precipitates could be reproduced in all experiments.

Procedure.

Planchets were first weighed, together with a single thickness of lens tissue, cut to fit the base of the planchet.

A 10mM $KHCO_3$ solution was employed, with a radioactive concentration of 0.2 μCi/l. Equal volumes of the radioactive solution and saturated $Ba(OH)_2$ solution were delivered directly onto the

planchets. This produced precipitates of $BaCO_3$ with specific activities of 0.004 μCi/mg. Different volumes of solution were used thus producing a range of thickness of precipitates. Two replicates for each of the volumes chosen were prepared. The following volumes of radioactive $KHCO_3$ and $Ba(OH)_2$ solution were used: 1 drop. 0.05ml, 0.075ml, 0.10ml, 0.15ml and 0.20ml.

Planchets were weighed after the precipitates had dried and thickness of the precipitates calculated in terms of $mg/3cm^2$. Counts per minute were recorded for each precipitate.

Appendix Figure 2 shows a plot of per-centage maximum activity against thickness of precipitate. Percentage maximum activity of particular weights of precipitate were calculated from the formular:

$$\% \text{ max. activity} = \frac{\text{observed CPM}}{\text{theoretical CPM}} \times 100$$

From figures of percentage maximum activity correction factors were calculated, for a range of thicknesses of precipitates, by which the counts

obtained from experimental $BaCO_3$ precipitates, were multiplied to give true counts.

APPENDIX TABLE III

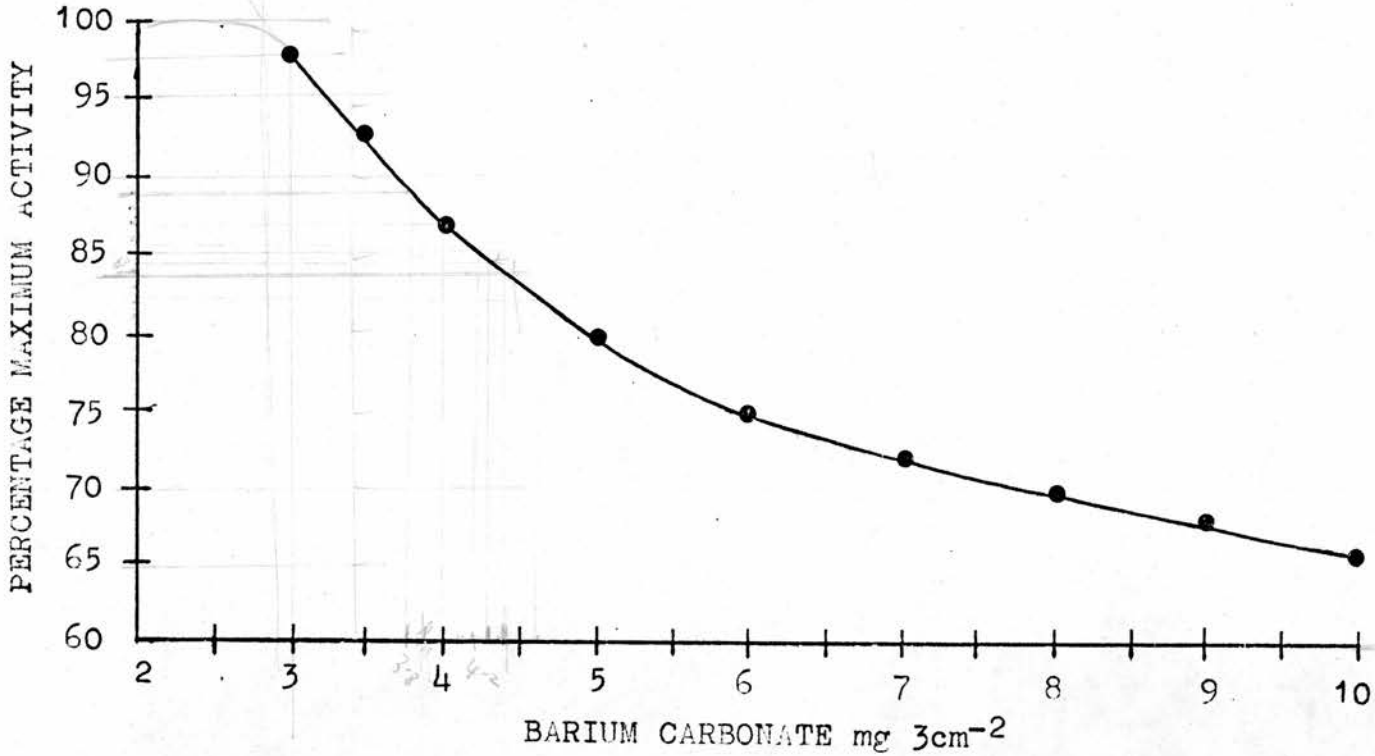
CORRECTION FACTORS FOR $BaCO_3$ SELF ABSORPTION.

Thickness of precipitate mg/3cm^2	% maximum activity	Correction factor
4.5	82.8	1.21
4.6	82.0	1.22
4.7	81.4	1.23
4.8	80.8	1.24
4.9	80.2	1.25
5.0	80.0	1.25
5.1	79.0	1.26
5.2	78.4	1.27
5.3	78.0	1.28
5.4	77.6	1.29
5.5	77.0	1.30
5.6	76.4	1.31
5.7	76.0	1.31
5.8	75.6	1.32
5.9	75.2	1.33
6.0	75.0	1.33

APPENDIX FIGURE 2

Self absorption curve of Barium Carbonate precipitate. The figure shows a plot of percentage maximum activity against thickness of precipitate.

APPENDIX FIGURE 2



APPENDIX VI

PLANT TISSUE SELF ABSORPTION

Self absorption curves for Fontinalis antipyretica and P. praelongus were prepared.

Procedure.

The basic method employed was as described in Methods section. Tissue discs were cut from leaves of P. praelongus and after the experimental period in radioactive solution, the discs were stuck onto planchets. 1,2,3,4, and 5 thicknesses of tissue discs were then counted for radioactivity. Similarly 1,2,3, and 4 thicknesses of leaves of F. antipyretica were counted.

Weights of tissue were estimated by determining dry weights of similar areas of untreated tissue of both species.

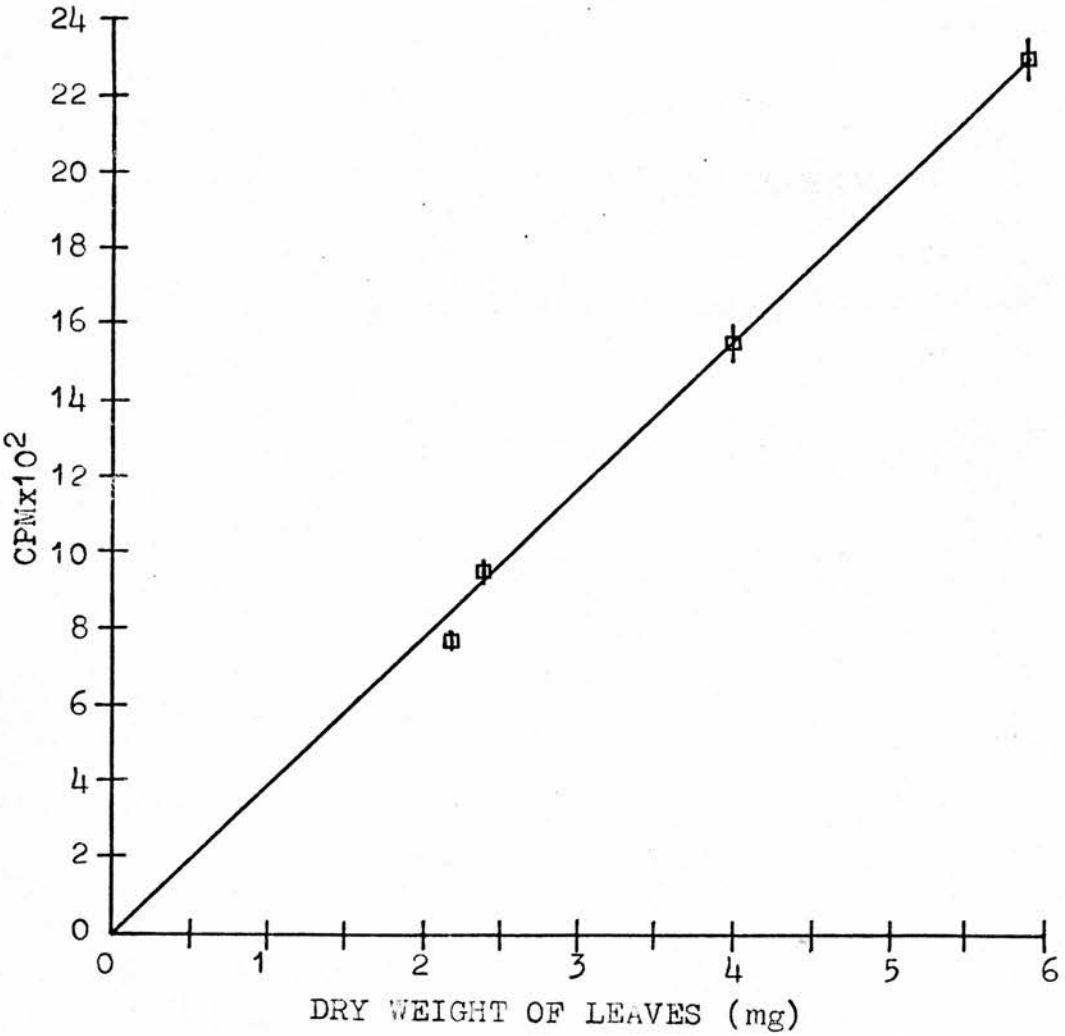
Appendix Figure 3 and 4 show plots of counts per minute against tissue thickness.

From Appendix Figures 3 and 4 it can be seen that when only one thickness of plant tissue is used, of either species, there appears to be no self absorption of β particles by the tissue.

APPENDIX FIGURE 3

Self absorption curve of *F. antipyretica* tissue.

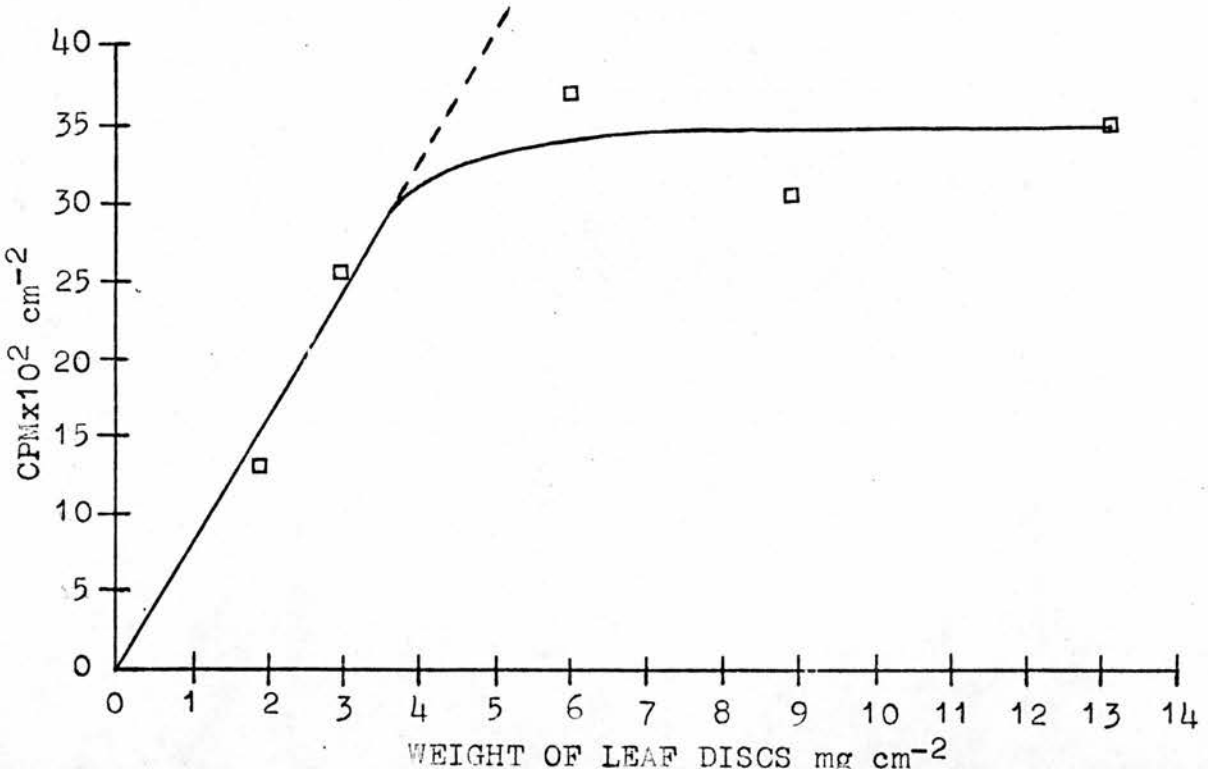
APPENDIX FIGURE 3



APPENDIX FIGURE 4

Self absorption curve for *P. praelongus* tissue.

APPENDIX FIGURE 4



APPENDIX VII

1. DETERMINATION OF TOTAL CO_2 BY TITRATION.

(This method is taken from "Methods for Chemical Analysis of Fresh Waters, IBP Handbook No. 8)

Procedure.

Carbonate-free NaOH solution was prepared by dissolving 50g NaOH in 50ml distilled water, and filtering through sintered glass filters to remove Na_2CO_3. This solution was stored until required when it was diluted to 0.05N, with CO_2-free distilled water, and standardised against standard acid.

100ml samples of bathing solution were taken and phenolphthalein added. If the solution turned pink the sample was titrated with 0.05N HCl until clear, pH 8.3. If the solution was clear on addition of the indicator, the sample was titrated with 0.05N NaOH until the solution had a faint pink colour, pH 8.3.

Mixed indicator was added and 0.05N HCl added until the end point of pH 4.4 was reached.

The following buffers were made up to give standard end point colours: Borax buffer pH 8.3, Acetate buffer pH 4.4.

Calculation.

$$CO_2 \text{ mEq/l} = \frac{\text{ml titrant (between both end points)} \times \text{normality of acid} \times 1000}{\text{ml of sample}}$$

1mEq/l = 44 mg CO_2/l = 12 mg C/l.

2. ALKALINITY

Determination of alkalinity of water samples.

Procedure.

The pH of the water sample was measured using a pH meter.

To 25ml samples of water, phenolphthalein was added. If the solution turned pink, 0.05N HCl was added until the solution was just clear, pH 8.3. Mixed indicator was then added and the solution titrated with 0.05N HCl until the end point of pH 4.4 was reached. If, on addition of phenolphthalein, the solution remained clear, mixed indicator was added directly to the solution which was then titrated to pH 4.4 with the standard acid.

Acetate buffer, pH 4.4, was used to give a standard end point colour with the mixed indicator.

Calculation.

$$\text{Phenolphthalein alkalinity } (OH^- + CO_3^{--}) \text{ mM} = \frac{\text{Vol. titrant ml (to end point pH 8.3) x normality of acid}}{\text{Vol. water sample ml}} \times 10^{-3}$$

$$\text{Total alkalinity mM } (OH^- + CO_3^{--} + HCO_3^-) = \frac{\text{Vol. titrant ml (to end point pH 4.4) x normality of acid}}{\text{Vol. water sample} \times 10^{-3}}$$

Knowing the original pH of the water sample and phenolphthalein and total alkalinity, the proportions of CO_3^{--}, HCO_3^- and free CO_2, in the original sample, may be calculated (see Appendix I)

APPENDIX VIII

WINKLER TECHNIQUE

The following micro-Winkler technique was employed to determine oxygen concentration of bathing solutions and water samples.

Reagents.

50% $MnSO_4$ solution

15% KI & 70% KOH solution

1% Starch solution

Concentrated orthophosphoric acid

N/800 Sodium thiosulphate (standardised by titration with standard potassium iodate.)

Procedure.

Approximately 0.5ml $MnSO_4$ solution and approximately 0.5ml KI & KOH solution were added, by means of a syringe, to the water sample, in a 30ml glass stoppered bottle, (reagents were injected directly into the 25 ml screw top bottles, in field experiments). The bottles were then shaken

when a precipitate of manganous hydroxide formed. The mixture was allowed to stand for about 5 minutes, after which time the precipitate was dissolved, by a few drops of orthophosphoric acid, forming a yellow solution. 5ml samples of this solution were then titrated against standard thiosulphate solution, using a starch solution as an indicator, titrating to a colourless end point.

Calculation

1ml N/800 thiosulphate $\equiv$ 0.01mg O_2

$\equiv$ 0.0075 mls O_2 at $20^{o}C$

Therefore O_2 mg/l water = $\frac{\text{vol. of titrant} \times 0.01 \times 1000}{5}$

APPENDIX IX

FORMULAE USED FOR STATISTICAL ANALYSES OF RESULTS.

1. Standard Deviation (S)

$$S^2 = \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n - 1}$$

where: x = quantity measured

n = number of observations

2. Standard Error. (S.E.)

$$S.E. = \frac{S}{\sqrt{n}}$$

APPENDIX X

DETERMINATION OF IRRADIANCE.

Irradiance incident upon the photosynthesising tissue was determined using an ISCO Spectroradiometer.

In the laboratory experiments using the Osram spotlights, the 6 foot remote probe of the spectroradiometer was placed in the position of the plant tissue facing, in turn, North, South, East and West directions, with respect to the light apparatus. Light energy incident upon the remote probe, when in each direction, was then summed to give total irradiance reaching the plant tissue. (Note: irradiance from each lamp was measured, in turn, and intensities from individual lamps then summed. This gave a total irradiance value similar to that obtained using the former method.) In the laboratory experiments using the overhead xenon light source and in field experiments

the 6 foot probe was placed, with the sensitive face in the horizontal, at the same level as the plant tissue.

Recordings of the spectral intensity, over a spectrum of 380 mu to 750 mu were made. Readings at every 25mu were then taken from the recorded chart, corrected for scale differences and multiplied by correction factors for the 6 foot probe, obtained from a previously prepared calibration chart. Figures thus obtained were then multiplied by the bandwidth and products summed to give a figure equivalent to the area under a curve of spectral intensity against wavelength.

This method gives a measure of irradiance in uW cm^{-2}. This value may be converted to foot candles and thence to lux by multiplying readings at, say, every 25 mu wavelength by a luminosity factor. The sum of these products over the spectrum considered, multiplied by 0.632 gives the light intensity in foot candles. 1 foot candle is equivalent to 10.8 lux.