Studies on the Distribution and Productivity of Submerged Freshwater Macrophytes

by

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A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy

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DECLARATION

I hereby declare that this thesis is of my own composition, that it is based on an accurate record of work carried out by me, and that it has not been previously presented in application for a higher degree.

Robert M. Campbell

St. Andrews, September 1971.

CERTIFICATE

I certify that Robert M. Campbell has spent twelve terms of research under my direction, that he has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967 No. 1, and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

D.H.W. Spence

St. Andrews, September 1971.

CAREER

I graduated Bachelor of Science from the University of St. Andrews in June 1967 with a second class (division one), honours degree in Botany. In October of that year I was awarded a Science Research Council post-graduate studentship and admitted as a research student into the department of Computational Science of the same university, graduating in October 1968 with the degree of Master of Science, awarded on the presentation of a thesis entitled "Conversational Programming Techniques". The same month I was admitted as a candidate for the degree of Doctor of Philosophy of the University of St. Andrews under Ordinance General No. 12, Resolution of the University Court, 1967, No. 1.

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I also wish to express my thanks to the Natural Environment Research Council for the award of a research grant without which my studies could not have been undertaken.

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ABBREVIATIONS

cpm	counts per minute
cps	counts per second
C-14	isotopic form of carbon of atomic weight 14
DOC	dissolved organic carbon
EDTA	ethylenediaminetetra - acetic acid
GLC	gas-liquid chromatography
GM	Geiger-Muller
HMDS	hexamethyldisilazane
IBP	International Biological Programme
LAI	leaf area index (m^2 leaf area/1 m^2 ground area)
NTA	nitrilo triacetic acid
P-32	isotopic form of phosphorus of atomic weight 32
SCUBA	self contained underwater breathing apparatus
SLA	specific leaf area (cm^2 leaf area/mg leaf dry weight)
TCA	trichloracetic acid
TMCS	trimethylchlorosilane
TRIS	tris(hydroxymethyl)methylamine

INTRODUCTION

Between the years 1897 and 1909, and under the direction of Sir John Murray and Lawrence Pullar, a bathymetrical survey of the Scottish freshwater lochs was carried out. In the first volume of results from this survey West (1910) gives an account of his comparative study of the dominant phanerogamic and higher cryptogamic flora of aquatic habit in seven lake areas of Scotland, a work of considerable achievement and only lately surpassed by Spence's (1964) account of the macrophytic vegetation of freshwater lochs, swamps and associated fens and his (1967) hypotheses outlining the factors controlling their distribution. These two workers have provided a sound basis for a number of studies concerned with the freshwater vegetation of Scottish lochs of which the present thesis is one.

Freshwater macrophytes include vascular plants, bryophytes and members of the algal family Characeae. The present studies have centred around the angiosperms and in particular the genus Potamogeton. By combining the schemes adopted by Tansley (1949) and Spence (1964), Sculthorpe (1967) has produced a life-form classification for freshwater macrophytes (Table 1.).

TABLE 1

The life-form classification of aquatic macrophytes (adapted from Sculthorpe, 1967).

A. Macrophytes attached to the substratum.

(1) Emergent e.g. Phragmites spp., Glyceria spp.

(2) Floating leaved e.g. Nymphaea spp., Nuphar spp.

(3) Submerged e.g. Elodea spp., Littorella spp.

B. Free floating macrophytes e.g. Lemna spp., Utricularia spp.

The studies presented in this thesis have been concerned with the submerged macrophytes which are attached to the substratum, and not at all with the emergent or free floating forms.

In so much as this thesis deals with the distribution of submerged freshwater macrophytes, data are presented to illustrate and partly to explain both the distribution of macrophytes between different lochs and the distribution or zonation of macrophytes within the one loch.

According to Yapp (1958) productivity is a word which has been used with some ambiguity. It is often taken as a synonym for production, a word which itself has two meanings, being both the act of producing and that which is produced.

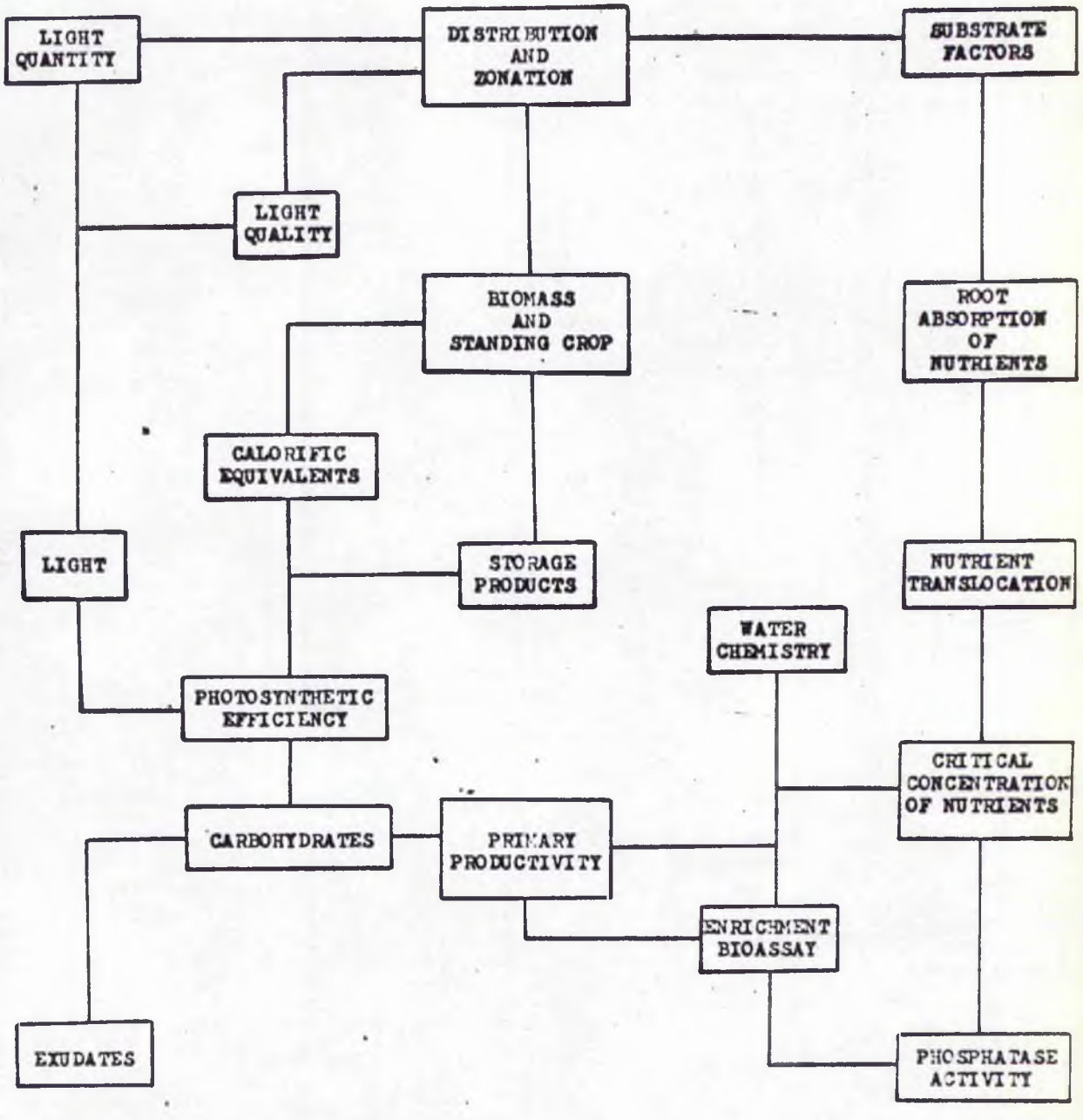
Productivity often suggests capabilities, abundance and usefulness to man but, however, is usually termed something that can be measured as a mathematical rate and it is this latter definition which is adopted in this thesis; primary productivity being the rate of accumulation of organic matter by green plants as created through photosynthesis. Considerable effort has been directed towards prescribing a precise meaning to productivity with reference to submerged macrophytes and in assessing some of the factors which influence it.

The project on which this thesis is based was formulated around several postulates concerning the factors influencing the growth of freshwater macrophytes. The justification for such a project stems not only from any intrinsic merits but also from a recognition, under the growing threat of water pollution, of the uncertainty in predicting the dynamic interactions of environmental influences upon aquatic plants.

The data with which this thesis are concerned come partly from investigations carried out in a series of lochs which, based upon the previous surveys of West (loc.cit.) and Spence (loc.cit.), probably cover the spectrum of this freshwater habitat in Scotland. These field investigations have been supplemented with laboratory studies on various physiological aspects of macrophyte ecology. The scope of

the investigations has been wide (Figure 1). Estimates of macrophyte standing crop and biomass have been determined and calorific equivalents computed. Storage carbohydrates have been identified and photosynthetic efficiencies calculated from measures of incident spectral intensity recorded during in situ primary productivity experiments. The role of the roots of macrophytes has been sought in relation to their ability to absorb nutrients, and discussed with respect to the availability of nutrients for growth, as assessed by tissue analysis and enzymic and enrichment bioassays. The combined results have been interpreted in the light of the current literature with the unifying theme of expanding knowledge of the distribution and productivity of submerged freshwater macrophytes.

FIGURE 1



Schematic representation of the relationships between the various aspects of macrophyte distribution and productivity which have been investigated.

CHAPTER 1

BIOMASS ESTIMATES

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CHAPTER 1

BIOMASS ESTIMATES

INTRODUCTION

The literature on quantitative and semi-quantitative investigations of higher aquatic plants is voluminous (Wetzel, 1965). Much of this has, however, been reviewed by Gessner (1955, 1959 in German), Penfound (1956), Raspopov (1963 in Russian) and Wetzel (1964a).

With reference to the Scottish lochs no estimates of the vegetation biomass appear to be on record, previous investigators, by virtue of the scope of their tasks, being confined to producing either species lists (West, 1910) or *ad hoc* classifications (Spence, 1964). In this present study biomass determinations have been made in several lochs, not only to obtain figures for this parameter, but to simultaneously investigate the quantitative distribution and zonation pattern of submerged macrophytes. In the light of the findings of Pearsall (1920) these studies were also embarked upon to investigate any possible substrate correlations with the distribution of submerged aquatic vegetation. For this reason a limited sediment analysis,

based on the parameters measured by Pearsall (loc.cit.), has also been carried out. This latter investigation has concentrated on the shallow-water, rosette species Littorella uniflora (L) Ashers and Lobelia dortmanna L., in an attempt to determine if the previously reported distribution of these species (Spence, 1964) can be partly explained on the basis of a substrate preference.

Westlake (1965a) emphasises the necessity to distinguish between the terms biomass and standing crop when sampling aquatic macrophytes for production estimates. Standing crop refers generally to the mass of above-ground plant material sampled at one time from a given area while the use of biomass implies that all parts of the plants are sampled. This distinction is recognised to be an important one (Wetzel, 1965) and thus the root systems and storage organs of macrophytes cannot be neglected. In the present study all estimates are of biomass unless otherwise stated.

LOCATIONS

Estimates of the biomass of subaquatic vegetation were made in the following freshwater lochs at various times in 1969 and 1970 (Map 1):-

Loch Achtriochtan (NN 144567) a highland, brown-water loch in Glen Coe, Argyll.[1]

Loch Croispol (NC 390680) a clear-water, limestone loch near Durness, Sutherland.

Dubh Loch (NN 377963) a small, dark, peaty-water loch near Rowardennan, Stirlingshire.

Loch Lanlish (NC 385685) a clear-water, limestone loch similar to, and adjoining, Loch Croispol near Durness, Sutherland.

Loch Leven (NO 145015) a lowland, nutrient enriched loch near Kinross, Kinrosshire.

Loch Lomond (Sheet 53, 7th series) a large, poor-water, lowland loch.[2]

Loch of the Lowes (Loch Lowes) (NO 050440) a poor-water loch near Dunkeld, Perthshire.

Loch Lunagan (NH 370070) a brown-water loch near Fort Augustus, Invernesshire.

1. Terminology follows Spence (1964).

2. Only one small area near Drymen, Stirlingshire was investigated (NN 375955).

MAP 1

Locations of lochs investigated

▲ Durness

△ Loch Uanagan

● Loch Achtriochtan

○ Loch of the Lowes

■ Loch Lomond

□ Loch Leven

METHODS

Collection of Samples

Samples of the vegetation were collected by cropping all the plants, both above and below soil parts, within a square quadrat of side 25 cm. The quadrat frame was constructed of brightly painted wire to ensure easy detection underwater. Sampling was pseudo-random along lines at right angles to the shore ranging from shallow to deep water and generally to the depth limit of colonisation by rooted vegetation. The shallow water samples were gathered by wading using, to examine the quadrat, a perspex viewer, constructed from a motor tyre inner tube, which floated on the water surface. The deeper samples were collected by snorkelling and aqualung diving. Mesh bags were used for holding the sampled material which were kept moist in buckets until return to the laboratory or field station. The quadrat size of 1/16 m^2 also proved convenient for larger samples; these being measured merely by laying the quadrat frame beside a previously cropped area and thus made the use of a large cumbersome frame unnecessary. In most cases a mud core of size 12 x 4 cm was collected by a small hand corer at each sample site and this was retained in a polythene bag until return to the laboratory where they were

placed in a deep freeze until required for analysis. The shallower depths were recorded with a metre rule while the deeper ones were measured using a diver's depth gauge, the calibration of which was periodically checked using a marked line suspended from a buoy. In one case, Loch Leven, where the submerged vegetation was neither obvious nor abundant a number of trawls were carried out by dragging a weighted, pronged grab behind a slowly moving boat for about 200 yd. The composition of the vegetation caught by the grab was recorded as species lists, no samples were retained for biomass estimates and no sediment samples taken.

Treatment of Plant Samples

The constituent species of each quadrat sample were separated and, where possible, individuals of any species counted, washed free of mud and sand and divided into root and shoot portions. Each sample was first air dried and the plant parts of each species wrapped in foil and dried in a force draught oven at 105°C until constant weight. By addition of the weights for constituent species a figure for the total plant biomass of each sample was obtained.

Treatment of Sediment Samples

Mechanical analysis

Mechanical analysis of the sediment into three portions:-

(1) coarse or gravel fraction

(2) sand fraction

(3) silt and clay fraction

was carried out.

After pH and conductivity measurements had been completed on the wet sediment samples the total sample was oven dried in a force draught oven at 105°C. The whole of the oven dried sample was first weighed and then gently ground in a mortar and pestle before being placed in a 2 mm sieve. That portion retained by the sieve was weighed as the coarse or gravel fraction and expressed as a percentage of the total sediment. That portion passing through the sieve was termed fine earth and as such was used to determine the loss on ignition and percentage organic carbon.

Ten grams of fine earth were weighed out into a 600 ml tall beaker and 100 ml of 12 N hydrogen peroxide added. The mixture was heated gently on an electric hot plate until no more frothing occurred and when the addition of hydrogen peroxide produced no reaction it was assumed that

all the organic matter had been oxidised. The suspension was gently washed through a 0.2 mm sieve and that retained weighed as the sand fraction and expressed as the percentage of total sediment sample. That fraction passing through the sieve was termed the silt and clay fraction.

Chemical analysis

The following chemical properties of the sediment samples were measured:-

(1) pH

(2) conductivity

(3) loss on ignition

(4) organic carbon

pH. An aliquot of the wet mud sample was shaken with distilled water (1:3, mud:water) in a conical flask on a mechanical shaker for one hour and the pH measured with a PYE Dynacap pH metre and glass electrode standardised against a buffer solution.

Conductivity. Measurements were made on the same sample as pH using a Mullard conductivity bridge. Results were expressed as μmhos.

Loss on ignition. Approximately one gram of oven dried sediment was accurately weighed into a tared crucible and ignited for three hours in a muffle furnace at 450°C.

The crucible was allowed to cool in a vacuum desiccator and reweighed. The loss on ignition was expressed as % loss on ignition.

Organic carbon. Organic matter in the mud was oxidised with potassium dichromate and the unreduced dichromate back titrated to give a figure for percentage oxidised carbon following the basic method of Walkley and Black as given in Piper (1950). One gram of oven dried sediment was weighed out into a 500 ml conical flask and 10 ml of 1 N potassium dichromate added followed by 20 ml of concentrated sulphuric acid. The flask was heated on an electric hot plate for 20 minutes with occasional shaking to ensure that all the sediment particles were oxidised and had not adhered to the sides of the flask. If, after this time, black organic material still remained further additions of dichromate and acid were made until all the material was oxidised. The oxidised soil sample mixture was made up to 200 ml with distilled water and 10 ml 85% phosphoric acid added along with 1 ml of diphenylamine indicator. Three aliquots (50 ml) were then back titrated against 1 N ferrous sulphate. A blank titration in which the standard ferrous sulphate was used to titrate 50 ml aliquots of a mixture consisting of 10 ml potassium dichromate, 20 ml concentrated sulphuric acid, 170 ml distilled water, 10 ml 85% phosphoric acid and 1 ml diphenylamine indicator served to standardise the

potassium dichromate.

The result was calculated as follows:-

$$\text{oxidised carbon} = \frac{V_1 - V_2}{W} \times 0.3$$

where V_1 is the value of the potassium dichromate added, V_2 the value of ferrous sulphate used in the back titration and W the weight of the oven dried sediment sample and where 1 ml of 1 N potassium dichromate is equivalent to 3 mg of carbon. Doubling the value for the % of carbon in the soils is recognised to be a fair estimate of the organic matter.

RESULTS AND DISCUSSION

Loch Achtriochtan

Table 1.1 presents the biomass data from quadrat samples taken in September, 1969 listed in order of water depth. The relative species composition of this biomass estimate is given in Table 1.2. It will be noted firstly, that the biomass was variable and secondly, that the relative species composition changed with water depth. Subularia aquatica L. and Lobelia dortmanna L. were the major constituents of the very shallow water samples while Littorella uniflora (L) Ashers, Isoetes lacustris L. and finally Juncus bulbosus var. fluitans (Lam.) D.R. predominated in the deeper water. Colonisation by rooted vegetation was deemed to end at 450 cm. Figure 1.1, constructed by taking mean values for biomass estimates made at about the same water depth, illustrates, on the basis of g dry weight plant material per square metre loch floor, the change in biomass with increasing depth of water.

Relatively more samples were taken in the shallow water of up to 100 cm than in the deeper parts of the loch, to obtain data on the two rosette species Littorella uniflora and Lobelia dortmanna which, as can be seen from Table 1.2, made up the bulk of the vegetation in this shallower water.

Table 1.3 lists the data on the numbers of individual rosette plants present in the quadrat samples. It can be seen that there was a considerable degree of variation between samples and that this is especially true of Littorella uniflora where counts varied between zero and 309. Plotting the data on the numbers of Littorella uniflora and Lobelia dortmanna plants per sample against their respective dry weight totals gives distributions (Figures 1.2a and 1.2b) which are sensibly linear, indicating some degree of uniformity in the size of the plants; note, however, that the mean dry weight of individual Lobelia dortmanna plants was twice that of Littorella uniflora plants. Data on the relative proportions of roots and shoots of these two species (Figure 1.3) illustrates not only the important contribution of the roots to the plant biomass but also that there is a certain amount of variation in this root/shoot ratio. It is of note that no Littorella uniflora plants were present in the sample which exhibited the highest shoot/root ratio for Lobelia dortmanna and, generally, there was a trend towards an inverse relationship between the root/shoot ratios of these species.

The samples collected in deeper water recorded the presence of an extensive bed of Juncus bulbosus which ran parallel to the shore and extended down a gentle slope to the depth limit of colonisation where plant growth ended

abruptly. Isoetes lacustris and Littorella uniflora were frequent components of the vegetation along with the J.bulbosus but J.bulbosus alone was recorded asthe deepest coloniser. Though the J.bulbosus was of great bulk and covered a large area of the substratum it constituted comparatively little to the biomass in terms of dry weight per unit area (Table 1.1).

Table 1.4 presents the results from the analyses of the sediment samples. As might be expected the shallow water samples had the largest quantities of gravel and sand while the deep samples were predominantly silt and clay. The values for the percentage carbon and percentage loss on ignition provide a fairly constant figure for the organic matter in the sediment except in the three deepest samples where the figure for loss on ignition was large and correlated with a high estimate for the silt and clay fraction. The variation in organic matter content of the sediment over the first 100 cm of water depth, with its physical composition, is illustrated in Figure 1.4. Both pH and conductivity values varied. The highest pH value was recorded in the sample with the lowest silt and clay fraction and the lowest in the samples with high silt and clay components. Conductivity did not appear to vary directly with pH though the two highest values were recorded in the samples with the two lowest pH values.

Direct correlations of plant distribution with components of the sediment are not obvious though J. bulbosus was confined to the finer sediments of high organic content while Littorella uniflora and Lobelia dortmanna predominated in the shallow water, coarser and less organically rich areas.

TABLE 1.1

Biomass of submerged vegetation sampled in Loch Achtriochtan during September 1969 (sample size 1/16 m^2).

Sample number	Depth of water (cm)	G. dry weight plant material/sample	Biomass, G. dry weight/m^2
3	22	0.24	3.84
4	22	0.49	7.84
1	25	0.15	2.40
5	28	8.57	137.12
2	30	2.65	42.40
6	45	17.68	282.88
7	55	15.27	244.32
8	71	6.30	100.80
9	72	11.02	176.32
10	80	8.51	136.16
11	100	8.98	142.68
12	150	15.92	254.72
14	240	13.96	223.36
13	300	2.09	33.44
15	450	1.32	21.12

TABLE 1.2

Relative percentage species composition of the biomass of submerged vegetation sampled in Loch Achtriochtan during September 1969.

Sample number	Depth of water (cm)	Littorella uniflora	Lobelia dortmanna	Isoetes lacustris	Subularia aquatica	Juncus bulbosus var. fluitans
3	22	-	41.3	-	58.7	-
4	22	-	89.4	-	3.1	7.5
1	25	25.4	66.9	-	7.7	-
5	28	88.2	11.8	-	-	-
2	30	94.1	4.6	1.2	0.1	-
6	45	65.8	12.2	1.0	-	21.0
7	59	64.0	19.5	9.8	-	6.7
8	71	50.4	20.4	7.1	0.7	21.4
9	72	9.0	19.5	2.5	-	69.0
10	80	10.4	22.6	37.5	-	29.5
11	100	52.3	12.4	14.8	0.3	20.2
12	150	14.6	-	7.6	0.2	77.6
14	240	-	-	-	-	100.0
13	300	-	-	-	-	100.0
15	450	-	-	-	-	100.0

TABLE 1.3

Numbers of individual plants present in biomass samples of the submerged vegetation collected in Loch Achtriochtan during September 1969 (sample size 1/16 m^2).

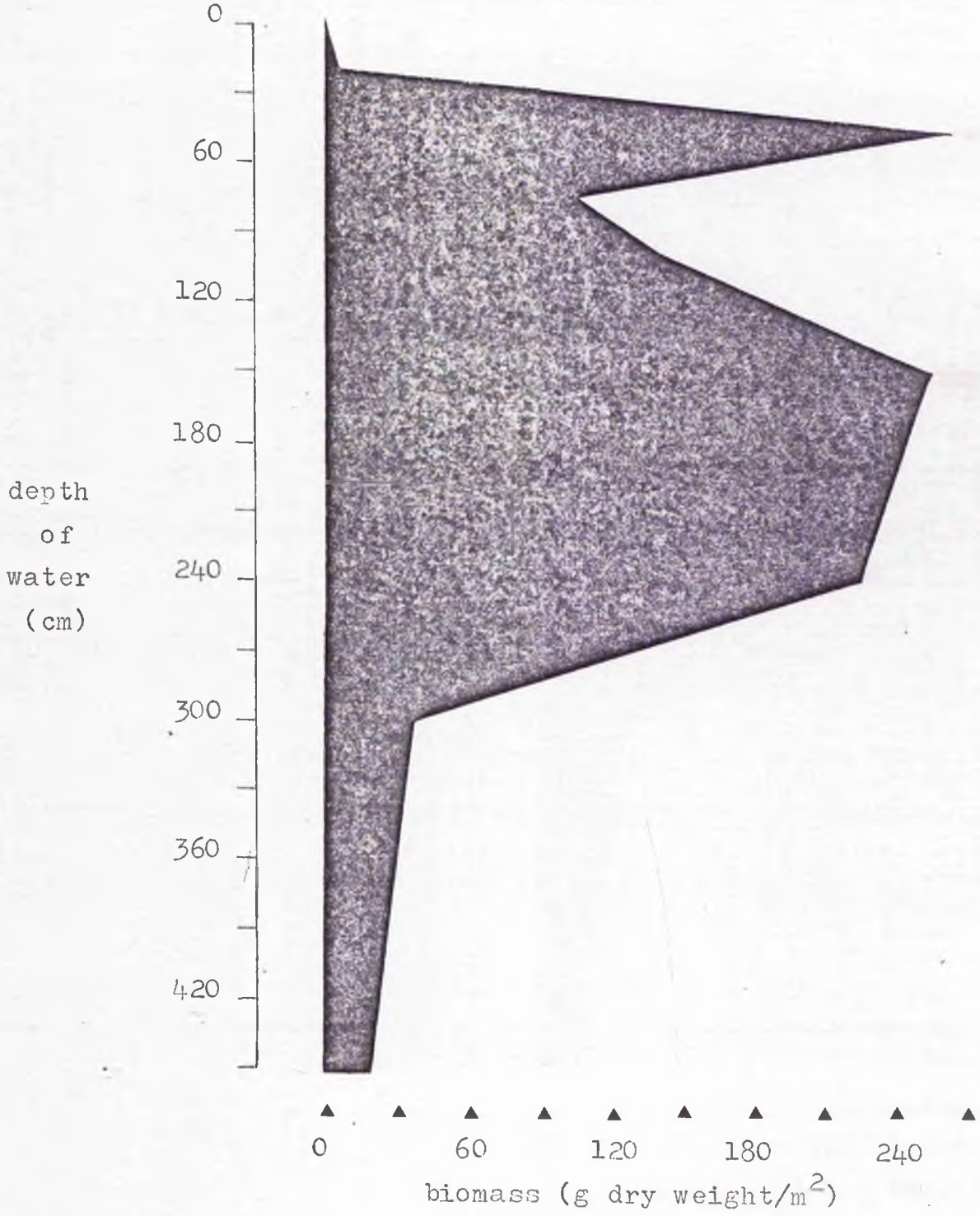
Sample number	Depth of water(cm)	Littorella uniflora	Lobelia dortmanna	Isoetes lacustris	Subularia aquatica
3	22	0	1	0	29
4	22	0	5	0	4
1	25	2	1	0	4
5	28	170	12	0	0
2	30	43	2	0	1
6	45	309	30	6	0
7	55	193	29	8	0
8	71	100	18	5	5
9	72	22	21	3	0
10	80	23	16	29	0
11	100	120	10	17	7
12	150	64	0	16	1
14	240	0	0	0	0
13	300	0	0	0	0
15	450	0	0	2	0

TABLE 1.4

Analyses of sediment samples collected from Loch Achtriochtan during September 1969.

Sample number	Depth of water (cm)	pH	conductivity (umhos)	% loss on ignition	% carbon	% gravel	% sand	silt /clay
4	22	6.2	18	2.5	4.0	30.3	-	-
3	22	6.3	16	3.2	2.1	12.8	47.1	38.0
1	25	7.1	17	3.1	3.0	18.6	47.0	31.4
5	28	6.2	19	4.2	4.4	0.6	40.1	54.9
2	30	6.9	30	3.2	2.9	9.8	48.3	39.0
6	45	6.9	20	4.7	2.4	0.4	34.9	62.3
7	55	6.0	20	7.4	3.4	0.5	20.6	75.5
8	71	5.0	35	7.8	3.6	0	10.4	86.0
9	72	6.0	-	8.1	4.3	0	-	-
10	80	6.2	-	8.2	4.8	0	13.0	82.2
11	100	6.0	19	6.7	3.6	1.8	49.2	45.4
12	150	6.1	-	10.1	1.2	0	3.6	95.2
14	240	5.7	10	20.1	4.7	0	0.6	93.7
13	300	5.6	47	17.5	4.6	0	5.5	89.3

FIGURE 1.1



Biomass (g dry weight/m^2) of submerged vegetation sampled in Loch Achtriochtan during September 1969, plotted against depth of water (cm).

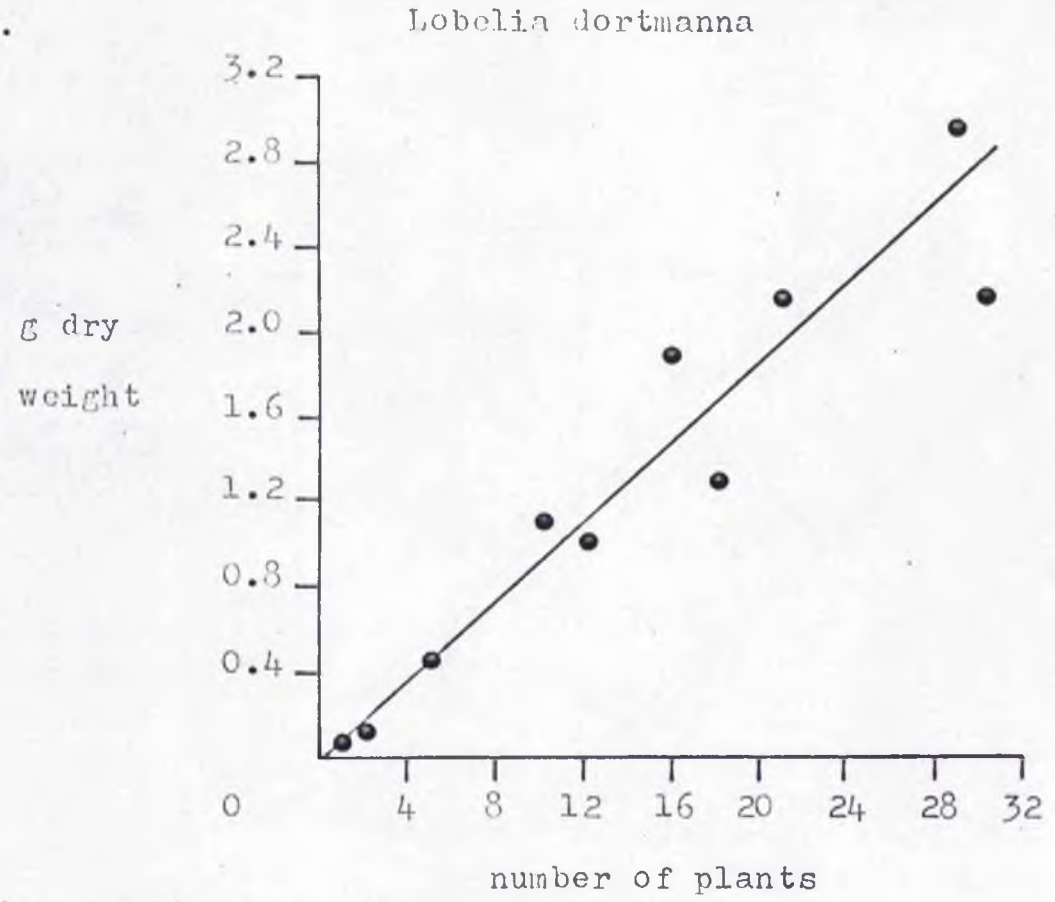
FIGURE 1.2

(opposite)

Numbers of individual plants of: A, Littorella uniflora; B, Lobelia dortmanna; sampled in Loch Achtriochtan during September 1969, plotted against the total dry weight. Sample size was 1/16 m^2.

FIGURE 1.2

B.


Lobelia dortmanna
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
g dry weight
4
8
12
16
20
24
28
32
number of plants

A.

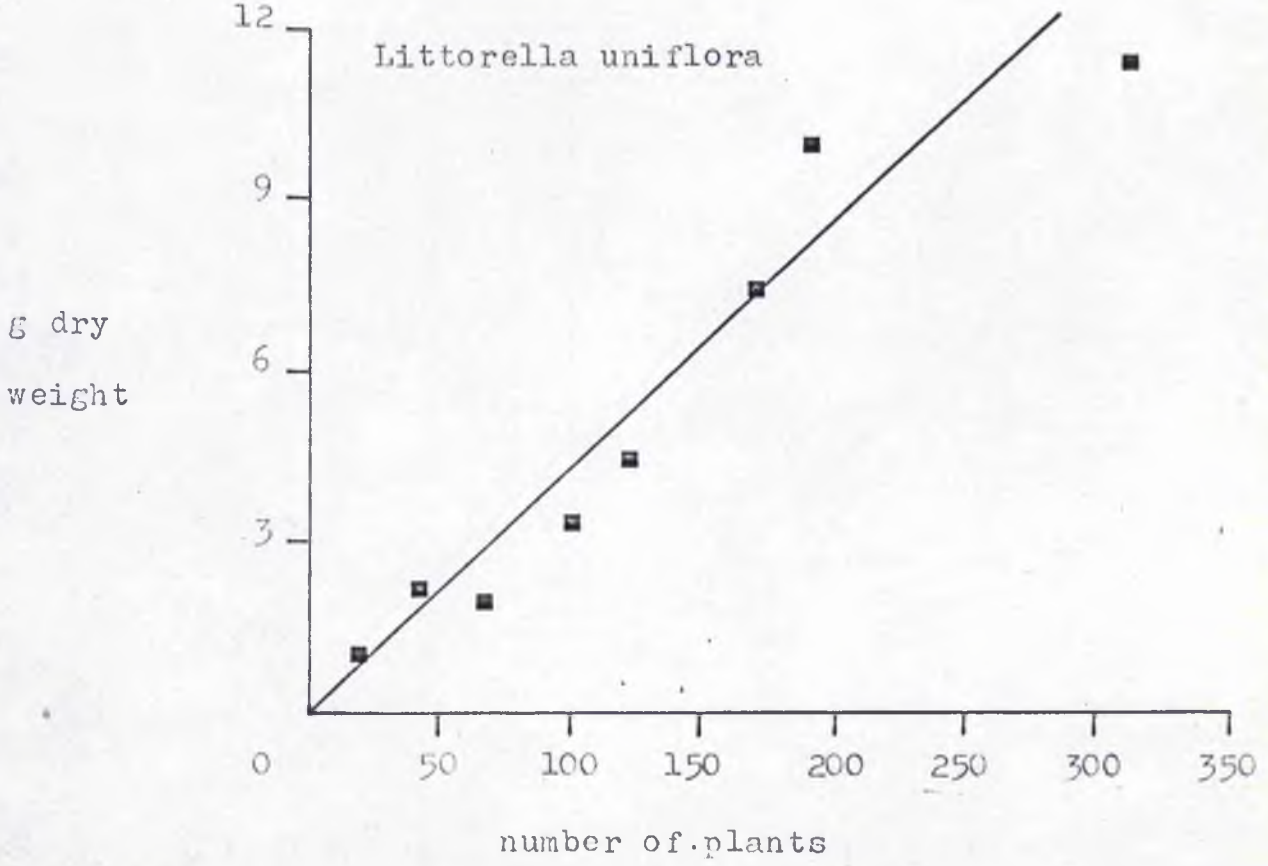
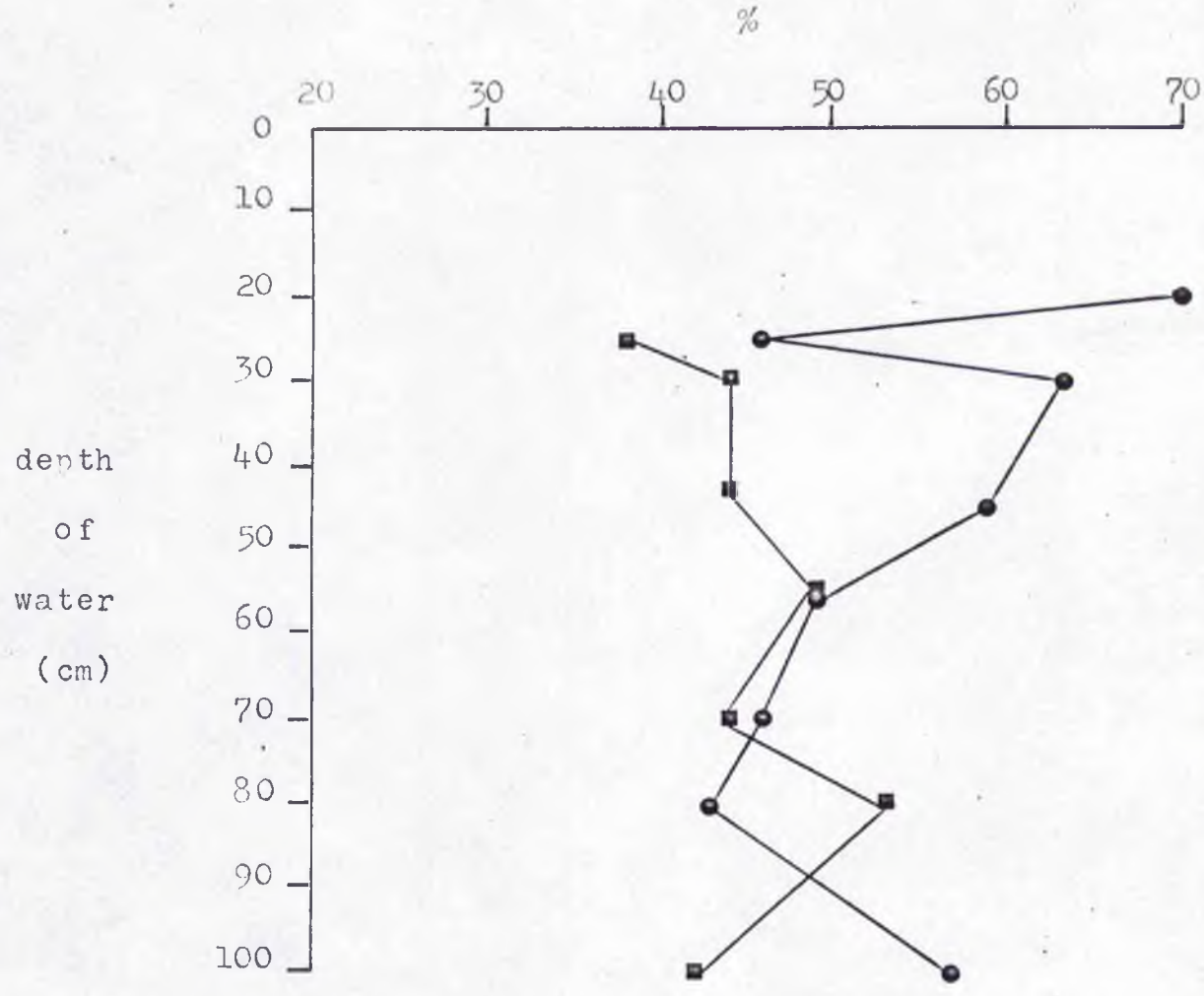
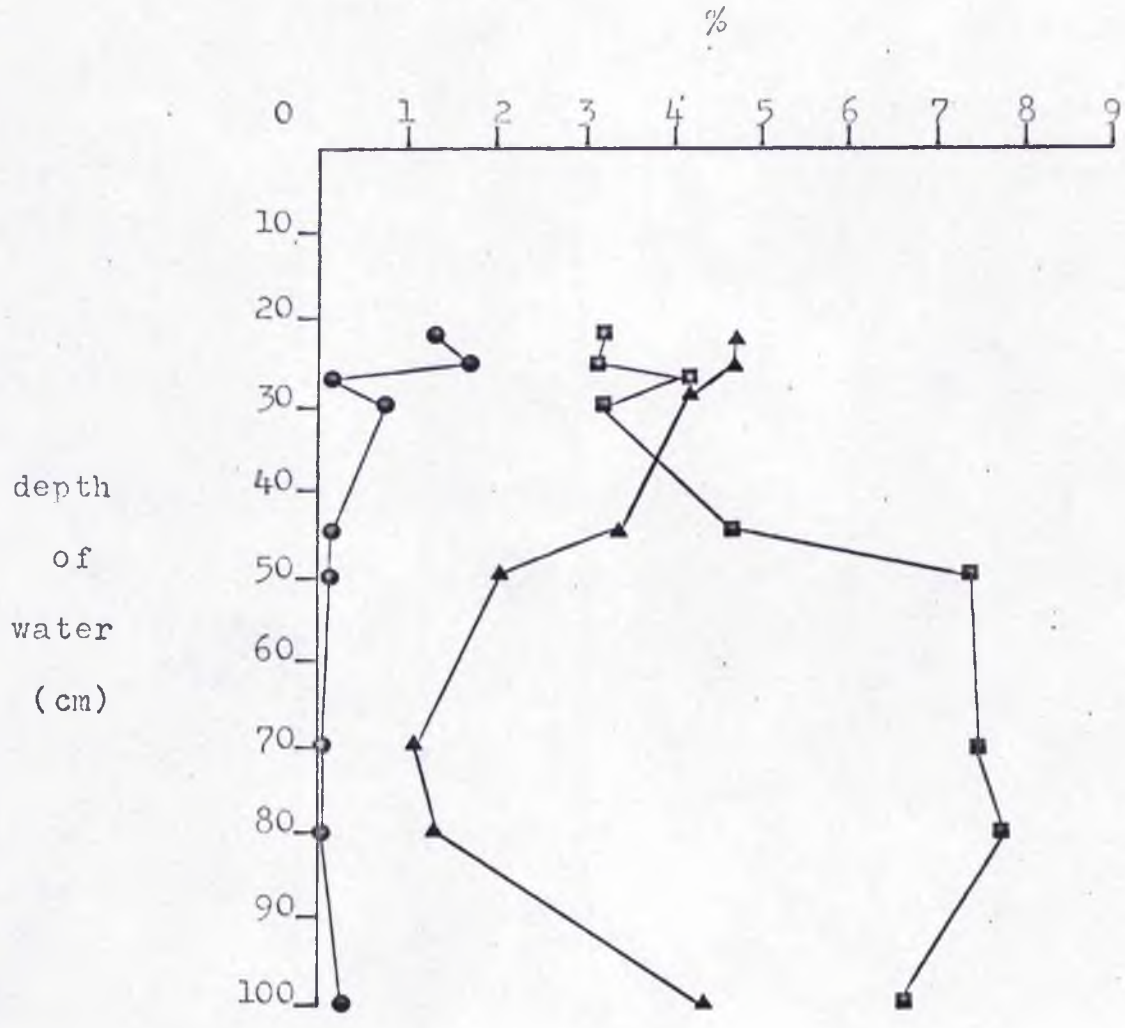

Littorella uniflora
12
9
6
3
0
g dry weight
50
100
150
200
250
300
350
number of plants

FIGURE 1.3



Shoots of Littorella uniflora (■) and Lobelia dortmanna (●) sampled in Loch Achtriochtan during September 1969; plotted as a percentage of plant dry weight against sample water depth.

FIGURE 1.4



Percentage gravel (●), percentage sand (▲) (X axis x 10), and percentage loss on ignition (■) of sediment samples collected in Loch Achtriochtan during September 1969, plotted against sample water depth.

Loch Damacan

Data on the composition and biomass of the vegetation have been previously collected from this loch (Spence *et al.*, unpublished) and are presented here (Figure 1.5) for reference only. The investigation reported here was an attempt to gather information on the shallow water communities. Table 1.5 presents data on the numbers of individual plants, their biomass and relative composition computed from a series of quadrat samples taken between water depths of 30 cm and 150 cm. The two species of this shallow water zone are *Littorella uniflora* and *Lobelia dortmanna*; the two rosette species found in a similar niche in Loch Achtriochtan.

As in Loch Achtriochtan there was considerable variation between samples, in terms of both biomass and species numbers. The relative species composition of the samples is illustrated in Figure 1.6. This shows that *Littorella uniflora* was the dominant plant in terms of numbers in nearly all the samples in which both *Littorella uniflora* and *Lobelia dortmanna* occurred but, due to the larger size of the *Lobelia dortmanna* plants, *Littorella uniflora* plants represented the greater part of the biomass, in terms of dry weight, in only two samples.

Examining the mean dry weights of the plants (Figure 1.7)

it can be seen that the relationship between the mean dry weight of individual Lobelia dortmanna plants and Littorella uniflora plants was not constant but varied with a depth component. This variation was represented in both the roots and the shoots, being more pronounced in the latter where it was emphasised through the high shoot/root ratio of the Lobelia dortmanna plants. The roots of the Lobelia dortmanna plants contributed not more than 31% to the biomass of the plant whereas for Littorella uniflora the mean figure for root biomass was in excess of 50%.

The analytical data from the sediment samples is presented in Table 1.6. All samples had a low silt and clay fraction and high sand and gravel components. The sample with the lowest gravel component had the highest estimate of organic content and came from the area which supported the second highest vegetation biomass; the sample with the highest biomass came from the area with the highest silt/clay component. pH varied little, though the lowest conductivity value was recorded from the sample with the highest organic content. No correlation of any of the sediment properties with depth is apparent and similarly any postulates concerning the relationship of the vegetation and the sediment would be purely speculative.

TABLE 1.5

Data on the biomass of submerged vegetation computed from samples collected in Loch Vanagan during September 1969 (sample size 1/16 m^2)

Sample number	Depth of water(cm)	g.dry weight plant material	Number of plants	% roots
		Littorella uniflora		
20	30	8.96	419	-
21	40	0.42	12	52.8
22	45	0.51	9	56.7
17	60	0.77	40	54.1
18	70	1.08	48	46.3
19	90	2.08	51	56.9
23	150	0.17	9	58.9
		Lobelia dortmanna		
20	30	-	-	-
21	40	0.91	16	28.1
22	45	0.52	5	25.2
17	60	0.68	13	31.6
18	70	0.89	7	25.1
19	90	2.18	10	32.5
23	150	0.55	5	26.1

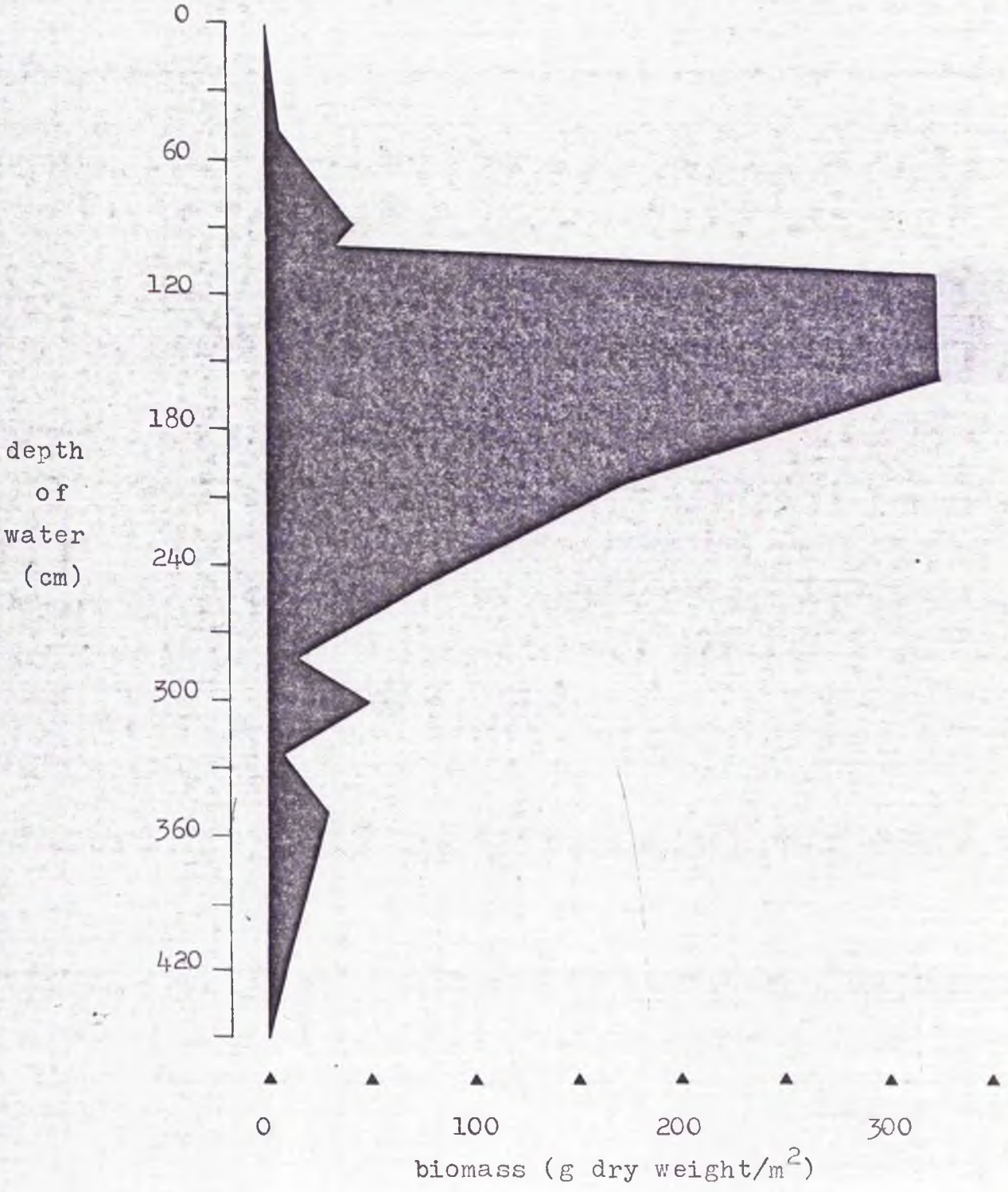
Sample number	Depth of water(cm)	Total g.dry weight plant material/sample	Biomass, g.dry weight/m^2
20	30	8.96	143.24
21	40	1.32	21.24
22	45	1.03	16.48
17	60	1.45	23.23
18	70	1.97	31.44
19	90	4.26	68.24
23	150	0.72	11.67

TABLE 1.6

Analyses of sediment samples collected from Loch Danagan during September 1969.

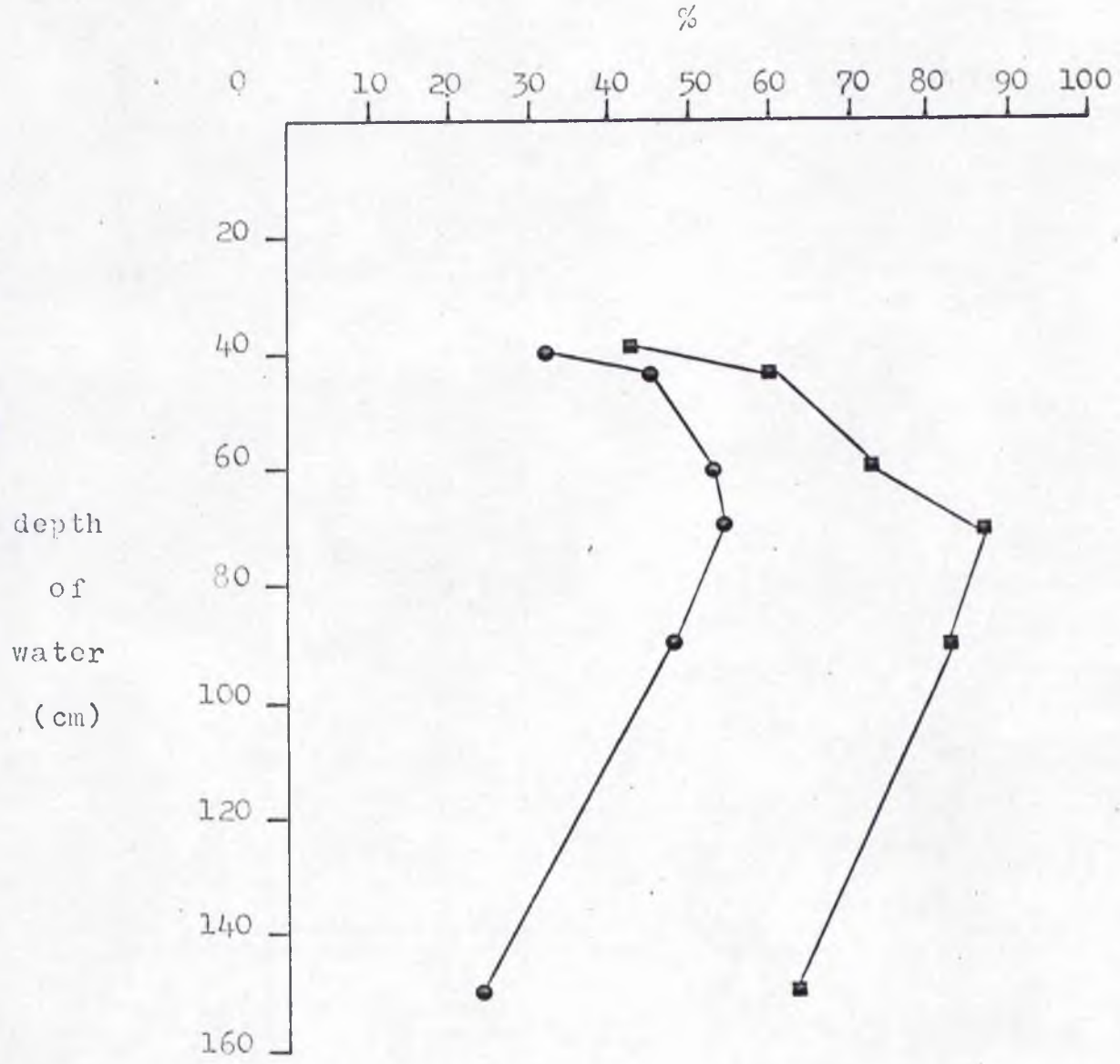
Sample number	Depth of water (cm)	pH	conductivity (umhos)	% loss on ignition	% carbon	% gravel	% sand	% silt /clay
20	30	6.9	22	5.2	2.1	26.0	54.8	17.1
21	40	6.6	21	1.6	1.7	13.7	75.6	9.0
22	45	6.5	29	11.0	1.9	15.3	74.0	8.8
17	60	6.7	25	2.0	1.5	35.0	55.3	8.3
18	70	6.5	20	6.3	2.1	36.8	45.5	14.6
19	90	6.5	13	16.6	12.8	5.8	72.5	9.0
23	150	6.7	17	1.4	4.6	53.8	42.2	2.0

FIGURE 1.5



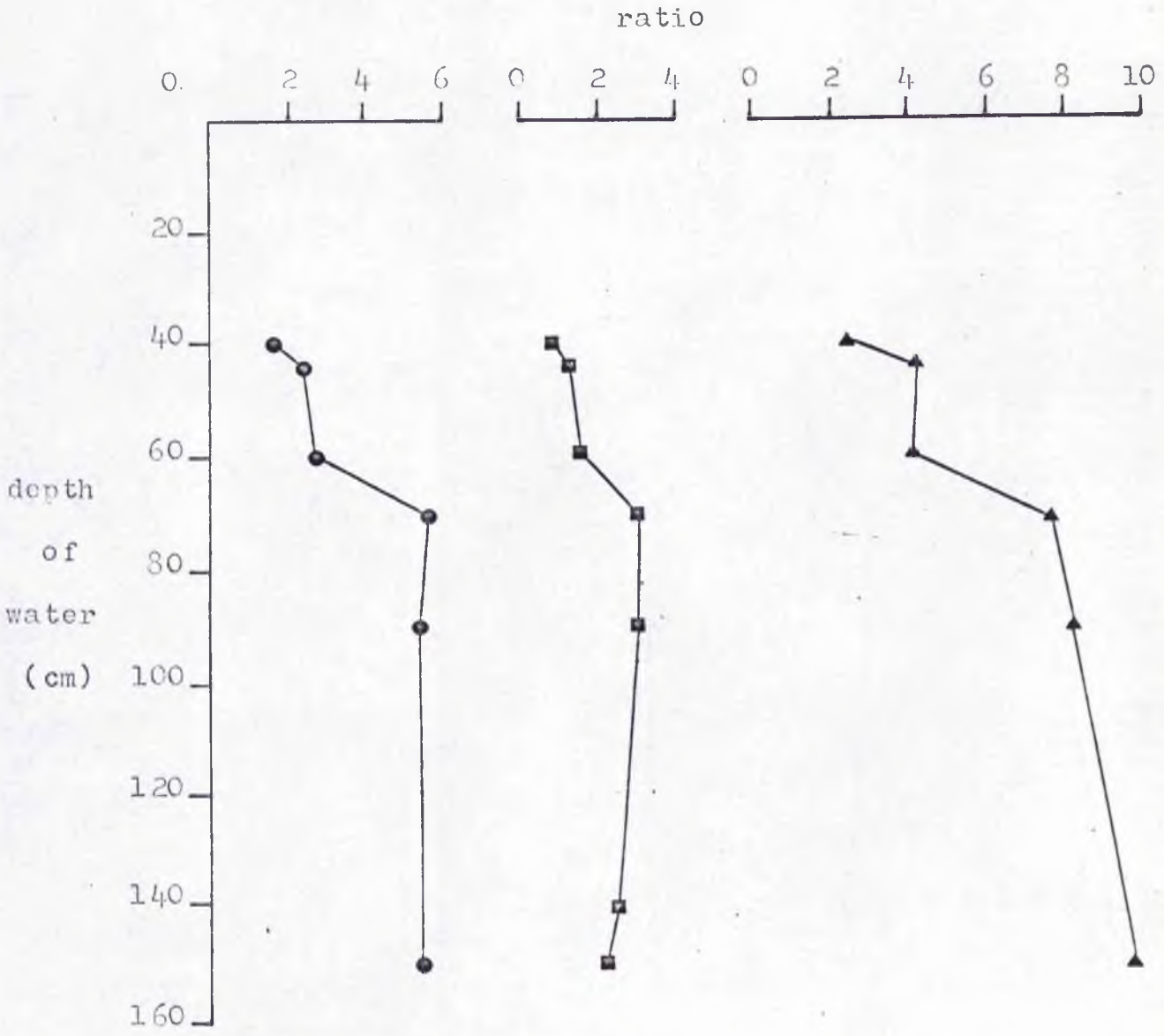
Biomass (g dry weight/m^2) of the submerged vegetation in Loch Uanagan, plotted against depth of water (cm). Redrawn from Spence et al.(unpublished).

FIGURE 1.6



Proportion of Littorella uniflora plants in a mixed sward of Littorella uniflora and Lobelia dortmanna, sampled in Loch Uanagan during September 1969. Represented as a percentage, on the basis of plant numbers (■) and total plant dry weight (●) per sample and plotted against sample water depth. Sample size 1/16 m^2.

FIGURE 1.7



Ratio of mean weights of Lobelia dortmanna : Littorella uniflora in a mixed sward of these species sampled in Loch Uanagan in September 1969. Represented as a ratio of total plant dry weights (●), root dry weights (■) and shoot dry weights (▲) and plotted against sample water depth. Sample size 1/16 m^2.

Loch of the Lowes

In this study a number of transects, running at right angles to the southern shore and extending from shallow water to the depth limit of colonisation by rooted vegetation, were sampled at regular depth intervals. A few randomly selected shallow water samples were collected from the more sheltered northern shore. Table 1.7 lists the biomass data obtained from these samples and Figure 1.8 plots the mean biomass per square metre against depth. The species which contributed to the vegetation were Subularia aquatica, Littorella uniflora, Lobelia dortmanna, Myriophyllum alterniflorum D.C., Potamogeton graminous L., Elodea canadensis Michx., Isoetes lacustris, Nitella opaca Agardh, Potamogeton obtusifolius L. and Potamogeton pusillus L. sec. Dandy and Taylor. Though species weights and numbers varied, as did the biomass as a whole, Littorella uniflora was the predominant member of the shallow water areas (up to 100 cm) both in terms of plant numbers and plant dry weight. A zone of Isoetes lacustris occupied a large area of the loch floor from about 100 cm to 150 cm depth of water where the loch bottom began to shelve off steeply at approximately 25 m from the shore. The deeper vegetation was sparse, Potamogeton perfoliatus and P. obtusifolius dominated while P. pusillus was the deepest coloniser being recorded,

in this study, at a depth of three metres. This zonation is seen in Table 1.8 and in figure 1.9 which gives the numbers of individual plants recorded in the samples taken off the south shore between 20 cm and 150 cm depth of water. The samples collected from the northern shore were of a similar composition to those collected from the southern shore but tended to be of larger biomass; one sample (1/16 m^2 area) recorded a biomass of over 11 g dry weight plant material and was composed of nearly 300 individual plants.

Table 1.9 presents the analyses of the sediment samples. As in the previous analyses, gravel and sand content decreased while the silt and clay component increased with sampling depth, even though at 240 cm only 50% of the sediment consisted of the finer particles and some 1% of gravel still remained; a situation to be contrasted with that in Loch Achtriochtan. Loss on ignition and carbon determinations provided a reasonably consistant estimate of the organic content of the sediment which appeared to vary very little, though the highest estimates coincided with high values for silt and clay. pH varied between 6.1 and 7.9 though with no obvious correlations. Little relationship between the sediment properties measured and the vegetation is apparent, some analyses are, however, incomplete.

TABLE 1.7

Biomass of submerged vegetation sampled in Loch of the Lowes during September, 1969. Samples 49 - 53 collected from the northern shore, the rest from the southern shore (sample size 1/16 m^2).

Sample number	Depth of water(cm)	g.dry weight plant material /sample	biomass, g. dry weight/m^2
42	20	1.40	22.40
36	23	1.17	18.72
38	23	1.73	27.68
41	23	2.49	39.84
43	25	2.21	35.36
34	60	2.12	33.92
35	60	0.72	11.52
37	60	2.68	42.88
39	60	1.59	25.44
40	60	1.63	26.08
44	100	1.03	16.48
45	100	0.98	15.68
46	100	0.92	14.72
47	100	0.67	10.72
48	100	0.99	15.84
29	150	2.87	45.92
30	150	0.74	11.84
31	150	2.09	33.44
32	150	2.66	42.56
33	150	0.94	15.04
24	240	0.04	0.64
25	240	0.02	0.32
26	240	0.64	10.24
27	240	1.07	17.12
28	240	0.55	8.80
49	20	0.33	5.28
50	20	11.25	180.00
51	100	2.94	47.04
52	100	4.66	74.56
53	100	2.30	36.80

TABLE 1.8

Relative percentage species composition of the biomass of submerged vegetation sampled in Loch of the Lowes during September 1969.

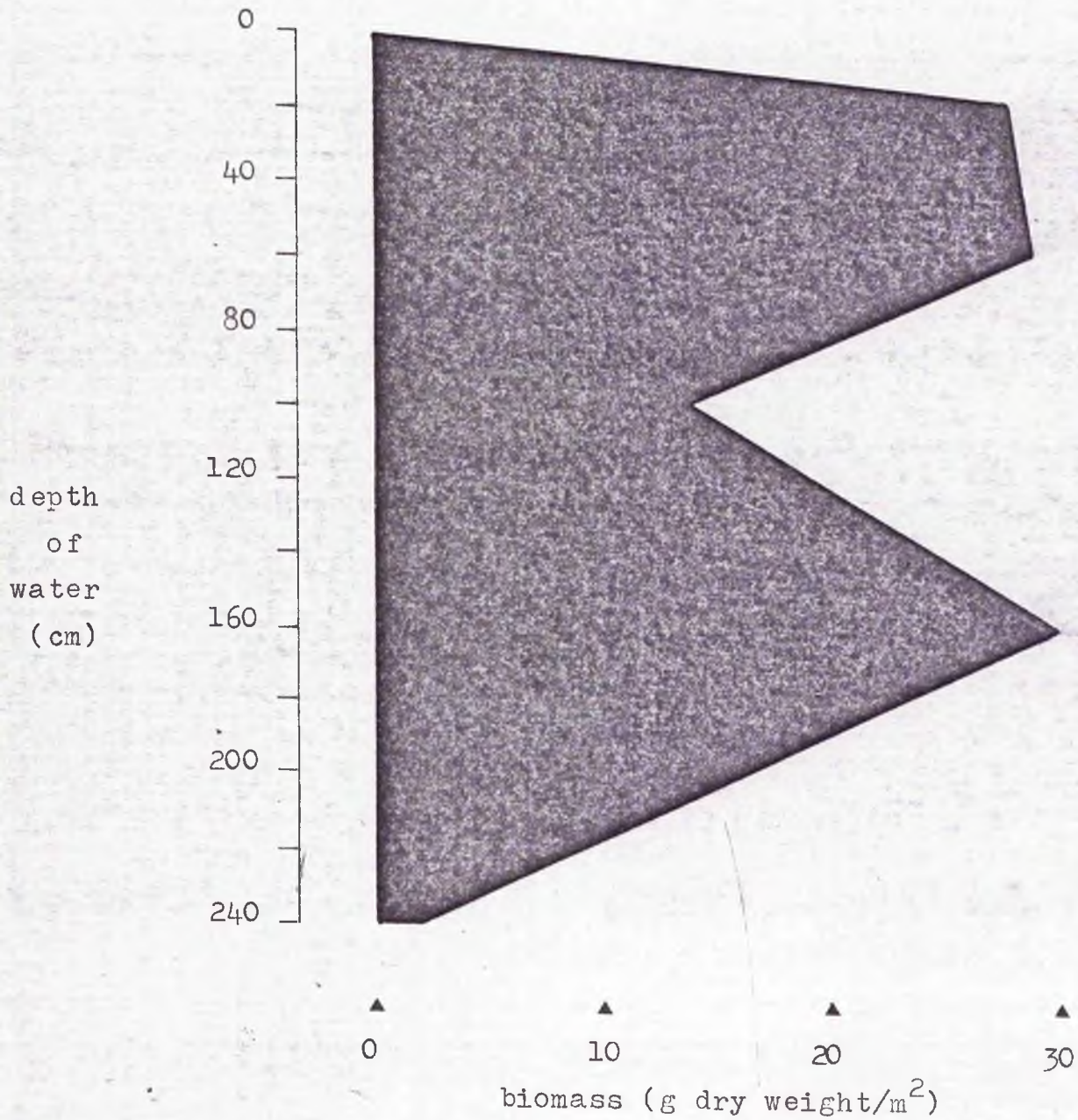
Depth of water(cm)	Littorella uniflora	Lobelia dortmanna	Isoetes lacustris	Potamogeton species	others
25	62	28	2	0	8
60	89	2	6	0	3
100	85	1	11	0	3
150	4	0	94	0	2
240	0	0	0	99	1

TABLE 1.9

Analyses of sediment samples collected in Loch of the Lowes during September 1969.

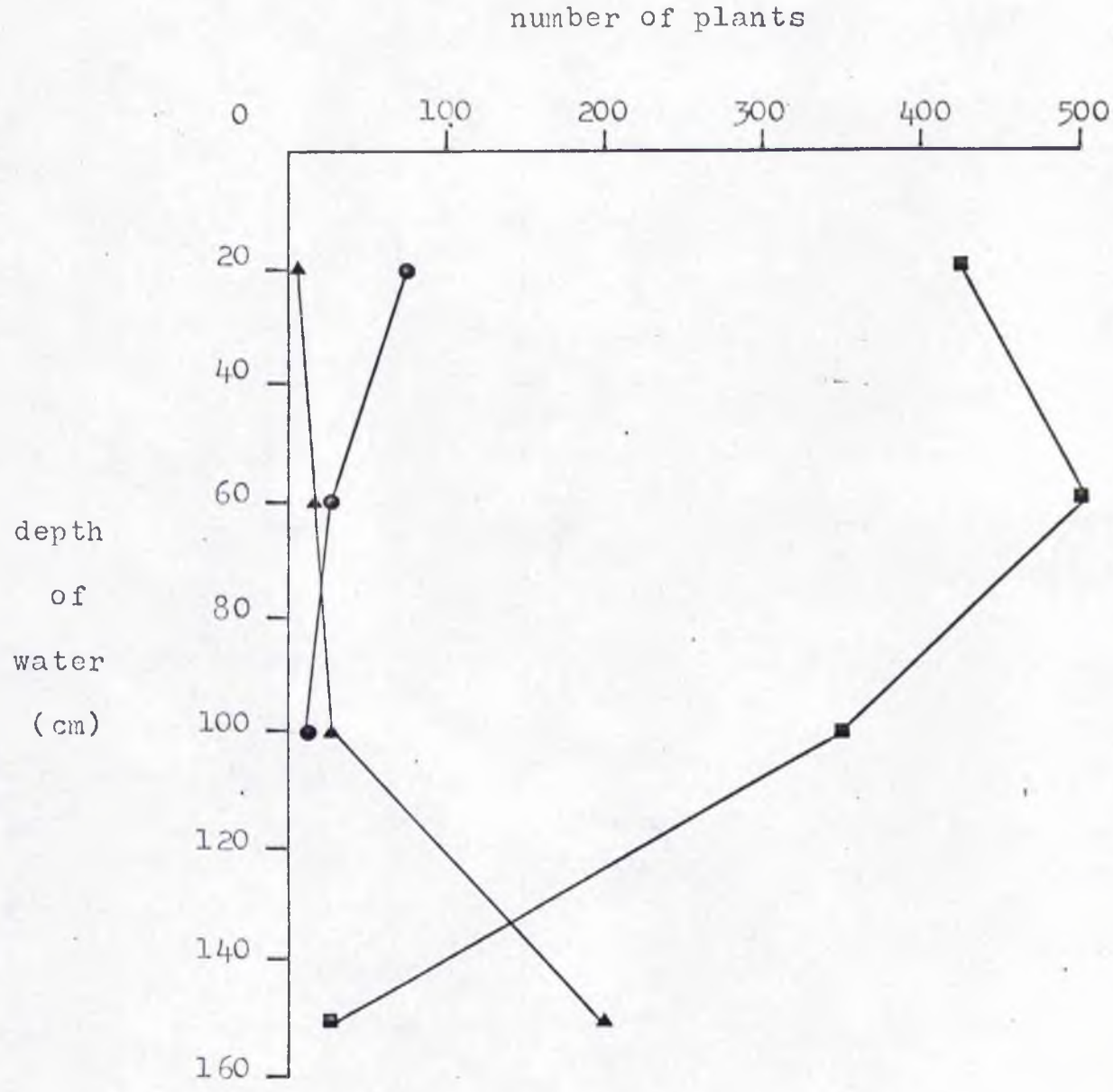
Sample number	Depth of water (cm)	pH	conductivity (umhos)	% loss on ignition	% carbon	% gravel	% sand	% silt /clay
42	20	6.1	15	0.6	1.9	71.2	-	-
36	23	6.7	27	5.0	3.9	46.7	-	-
38	23	7.3	49	0.3	1.1	63.1	32.2	2.5
43	25	6.6	24	1.1	1.8	35.3	-	-
41	23	6.6	91	2.8	-	78.1	-	-
34	60	6.4	110	2.7	2.7	14.9	69.7	10.0
35	60	6.9	20	1.5	4.8	5.4	-	-
37	60	7.0	26	4.0	-	4.8	-	-
39	60	7.4	39	1.0	-	14.7	-	-
40	60	6.8	27	0.9	4.0	7.0	60.9	28.1
44	100	6.9	28	1.3	1.1	1.6	-	-
45	100	7.9	61	1.2	1.2	4.6	61.6	32.6
46	100	7.4	42	1.5	1.2	12.7	42.3	42.8
47	100	7.2	38	1.3	-	0.5	55.1	44.4
48	100	7.9	73	1.4	1.2	3.2	61.6	33.0
29	150	6.6	21	1.2	2.2	0.9	85.8	12.1
30	150	6.1	28	1.3	1.0	0.6	81.0	17.4
31	150	6.3	46	1.5	0.5	0.6	82.3	16.6
32	150	6.3	31	1.9	3.5	1.2	75.3	20.0
33	150	6.3	41	1.0	0.5	2.0	82.3	15.2
24	240	7.1	-	5.2	3.3	0.5	40.5	55.7
25	240	7.0	-	5.2	3.3	0.6	38.1	58.0
26	240	7.3	-	5.5	3.6	1.0	38.4	57.0
27	240	7.3	-	3.8	2.3	0.7	52.3	44.7
28	240	6.7	32	3.1	1.0	3.2	51.3	44.5
28	240	6.2	27	4.4	0.9	1.0	44.1	54.0
49	20	7.0	-	1.4	1.2	0.2	-	-
50	20	6.4	13	2.6	2.1	0.8	-	-
51	100	6.3	21	3.5	1.0	0.1	51.1	47.8
52	100	6.3	14	2.7	2.1	-	-	-
53	100	6.5	19	3.1	0.9	0.4	45.8	53.9

FIGURE 1.8



Biomass (g dry weight/m^2) of submerged vegetation sampled in Loch of the Lowes during September 1969, plotted against depth of water (cm).

FIGURE 1.9



Numbers of Littorella uniflora (■), Lobelia dortmanna (●) and Isoetes lacustris (▲) plants, recorded as the mean of five samples at each depth, plotted against sample depth. Samples collected in Loch of the Lowes in September 1969.

Loch Leven

No quantitative data on the biomass of the submerged vegetation was obtained from this loch which is characterised by its high algal productivity and almost complete absence of rooted macrophytes. One hundred drags covering an extensive area of the loch were made in the summer of 1970 by towing a pronged grab behind a boat. No beds of vegetation were detected and the only obvious vegetation was found growing in the harbour area. The most common species found was Zannichellia palustris L. generally recognised to be an indicator of eutrophic waters. Table 1.10 gives the full list of macrophyte species recorded in the survey.

TABLE 1.10

Submerged species of aquatic macrophytes recorded in a survey of Loch Leven during the summer of 1970.

Callitriche spp.
Chara aspera (Deth)Willd.
Elodea canadensis Michx.
Fontinalis antipyretica Hedw.
Littorella uniflora (L)Aschers.
Polygonus amphibius L.
Potamogeton crispus L.
P.filiformis Pers.
P.obtusifolius Mert & Koch.
P.perfoliatus L.
Ranunculus aquatilis L.
Zannichellia palustris L.

Loch Croispol

A survey of the distribution and biomass of the submerged vegetation in Loch Croispol was carried out in June, 1970. As before, quadrat samples were collected by wading, snorkelling and aqualung diving, and the species composition and oven dry weight determined. Sampling was carried out along two transects at right angles to the shore and in different parts of the loch. In one case samples were collected at fixed intervals along the transect line while in the other, a series of samples were collected from varying depths down to the limit of macrophyte colonisation. The sites for the transects were chosen to represent areas which, from an extensive snorkelling reconnaissance, appeared to differ greatly in species cover and abundance and thus would serve to illustrate the extent of variation among the subaquatic vegetation.

Table 1.11 presents the data collated from the samples collected along the first transect while Table 1.12 gives similar data from the second sampling area. It is immediately noticeable that the two sets of transect samples reflect vegetation which is decidedly different, both in its composition and mass, though it must be remembered that Transect B did extend to a much greater depth than Transect A and thus not only different areas were sampled but also

different depth zones. Transect B extended a greater distance from the shore than A and reached a large "hole" in the loch where the substratum fell away steeply down to a depth of 50 feet (17 m) as measured on a diver's depth gauge. Several samples were taken around the rim of this depression at a water depth of 150 cm and serve to reflect the range in vegetation biomass. The steep slopes of the hole supported a bed of Potamogeton perfoliatus and little else except a few strands of Myriophyllum spicatum L. Colonisation extended down to approximately six metres where it abruptly ended leaving only isolated fragments occurring any deeper and none at more than seven metres.

The high values determined for the biomass of the two Chara species are due to large deposits of calcium carbonate ($CaCO_3$) which cover these algae and which have been determined to account for up to 80% of the biomass of these plants (Wetzel, 1960). To determine the extent of this $CaCO_3$ encrustation in the present investigation, weighed subsamples of the dry plant material were treated with dilute hydrochloric acid. Additions of acid were continued until no more bubbles were evolved, whereupon the plants were gently washed in water, oven dried and reweighed. The loss in weight was expressed as a percentage of the original weight. A mean figure of 75% $\pm$ 0.5 was obtained from ten determinations of the extent of $CaCO_3$

encrustation on the two Chara species. No differences were apparent either between species or within samples of the one species from different areas, as was reported by Wetzel (loc.cit.). Figures 1.10 and 1.11 plot the biomass of the vegetation (corrected for $CaCO_3$) with depth while Figure 1.12 presents data obtained from a previous investigation (Spence et al., unpublished).

It is frequently reported (Sculthorpe, 1967) that the submerged bryophytes and the Charophytes penetrate to the deepest depths in the freshwater environment. This is not the case in Loch Croispol where in both an underwater visual survey and the biomass data presented in Table 1.12, Potamogeton perfoliatus was recorded as the deepest coloniser. A more detailed survey of the extent of this deep growing P.perfoliatus was thus undertaken.

The extent of the pondweed bed was surveyed underwater by two divers using the "leap-frog" technique. This involved a diver swimming with a line of known length until it was taut, when the other diver, who was retaining the other end of the line, swam over the first diver and repeated the process until the area to be surveyed was covered. The number of "leap-frogs" were noted (with a pencil on a piece of formica, which served as an excellent underwater writing board in all these studies) and from a knowledge of the length of the line the area covered was computed.

At each leap-over point the depth was noted and at intervals the depth limit of colonisation by the P.perfoliatus was found by the same "leap-frog" technique. By these methods the extent of the P.perfoliatus vegetation was estimated to stretch for 10 metres across the edge of the deep hole while it extended to six metres depth of water which represented about ten metres of ground, and thus the area of the vegetation was in the region of 1000 square metres. The slope of the substratum, which consisted of unstable marl, fell away at a steep angle approaching 1 in 2.

A further 18 standing crop samples were collected from the P.perfoliatus zone. These were gathered to represent the degree of variation of the stand. The samples were from a ground area of 1/4 m^2, measured by combining four samples cropped using the square 1/16 m^2 quadrat. Before the samples were oven dried to constant weight the number of shoots in each sample was counted and their length measured. Two of the samples were chosen to compute values for the leaf area index of the stand (LAI: area of leaves (m^2) over 1 m^2 of ground). All the leaves of these plants were detached and their areas drawn around on paper. The paper outlines were cut out and weighed and, from a knowledge of the unit area weight of the paper, the area of the leaves computed. This figure gave a value of the leaf area over 1/4 m^2 of ground and by multiplication a figure

for the LAI was obtained. Once the leaves had been outlined they were returned to their samples to be included in the estimate of the standing crop.

Westlake (1965a) recommends that, where possible, figures for the standing crop or biomass of aquatic vegetation should be presented in terms of ash-free dry weight. For this reason ten aliquots of the dried plant material were powdered in a mortar and pestle and the loss on ignition of 0.1 g quantities determined after four hours ignition at 450°C in a muffle furnace. The results of this investigation on the ash content of P.perfoliatus are presented in Table 1.13 along with the estimates of standing crop, LAI and related data.

The sediment of Loch Croispol was soft, unsolidified marl, rich in calcium carbonate and contained many gastropod shells. All but the shallowest areas were rich in organic matter and had high silt and clay contents. The loch was stratified with respect to temperature and oxygen during the summer months and Figure 1.13 presents the results of one such temperature and oxygen profile determined with a Mackereth portable oxygen electrode and temperature probe.

Plates 1A and 1B in Appendix II illustrate some of the submerged vegetation in Loch Croispol.

TABLE 1.11

Biomass of submerged vegetation sampled in Loch Croispol (Transect A) during June 1970 (sample size 1/16 m^2)

Distance from shore(m)	Depth of water (cm)	Species composition	g.dry weight plant material/sample	Total g. dry weight plant material	Biomass g.dry weight/m^2
0	7	Juncus articulatus	10.66	10.66	170.6
3	28	Littorella uniflora	20.58	20.58	329.6
6	40	Myriophyllum alterniflorum	5.39	28.19	451.0
		Littorella uniflora	22.80		
9	50	Littorella uniflora	32.70	35.15	562.4
		Myriophyllum alterniflorum	2.45		
12	68	Chara papillosum	52.25	52.25	836.0
15	80	Chara papillosum	128.25	128.33	2,053.3
		Potamogeton natans	0.08		
18	85	Chara papillosum	132.24	132.24	2,115.8
21	90	Chara papillosum	104.50	104.52	1,672.3
		Potamogeton natans	0.02		
24	95	Chara papillosum	83.60	83.60	1,337.6
27	95	Chara papillosum	209.00	209.00	3,344.0

TABLE 1.12

Biomass of submerged vegetation sampled in Loch Grotaig (Transect E) during June 1970 (sample size 1/16 m^2).

Distance from shore(m)	Depth of water (cm)	Species composition	g.dry weight plant material/sample	Total g. dry weight plant material	Biomass g.dry weight/m^2
4	33	Littorella uniflora	23.40	23.40	373.4
7	56	Littorella uniflora	4.62	4.62	73.9
10	72	Chara aspera	2.16	2.16	34.6
13	89	Chara aspera	1.48	1.48	23.7
16	90	Myriophyllum spicatum	9.90	9.90	158.4
24	155	Chara aspera Potamogeton pectinatus Chara papillosum	14.30 0.15 0.10	14.55	232.8
24	150	Potamogeton perfoliatus Chara papillosum	1.27 30.40	31.67	506.7
28	150	Potamogeton pectinatus Chara aspera	0.05 26.84	26.89	430.4
28	150	Utricularia minor Potamogeton pectinatus Chara aspera Chara papillosum	0.16 0.50 0.31 4.94	5.91	94.6

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TABLE 1.12 continued:-

Distance from shore(m)	Depth of water (cm)	Species composition	g.dry weight plant material/sample	Total g. dry weight plant material	Biomass g.dry weight/m^2
23	150	Chara papillosum	12.35	12.68	202.9
		Potamogeton pectinatus	0.33		
28	150	Chara aspera	12.09		
		Chara papillosum	13.07	29.82	413.1
		Potamogeton pectinatus	0.66		
28	270	Potamogeton perfoliatus	1.30		
		Potamogeton pectinatus	0.66	2.03	32.5
		Chara papillosum	0.07		
28	420	Potamogeton perfoliatus	1.68		
		Myriophyllum spicatum	0.01	1.71	27.4
		Utricularia minor	0.02		
28	480	Potamogeton perfoliatus	0.55	0.55	8.8

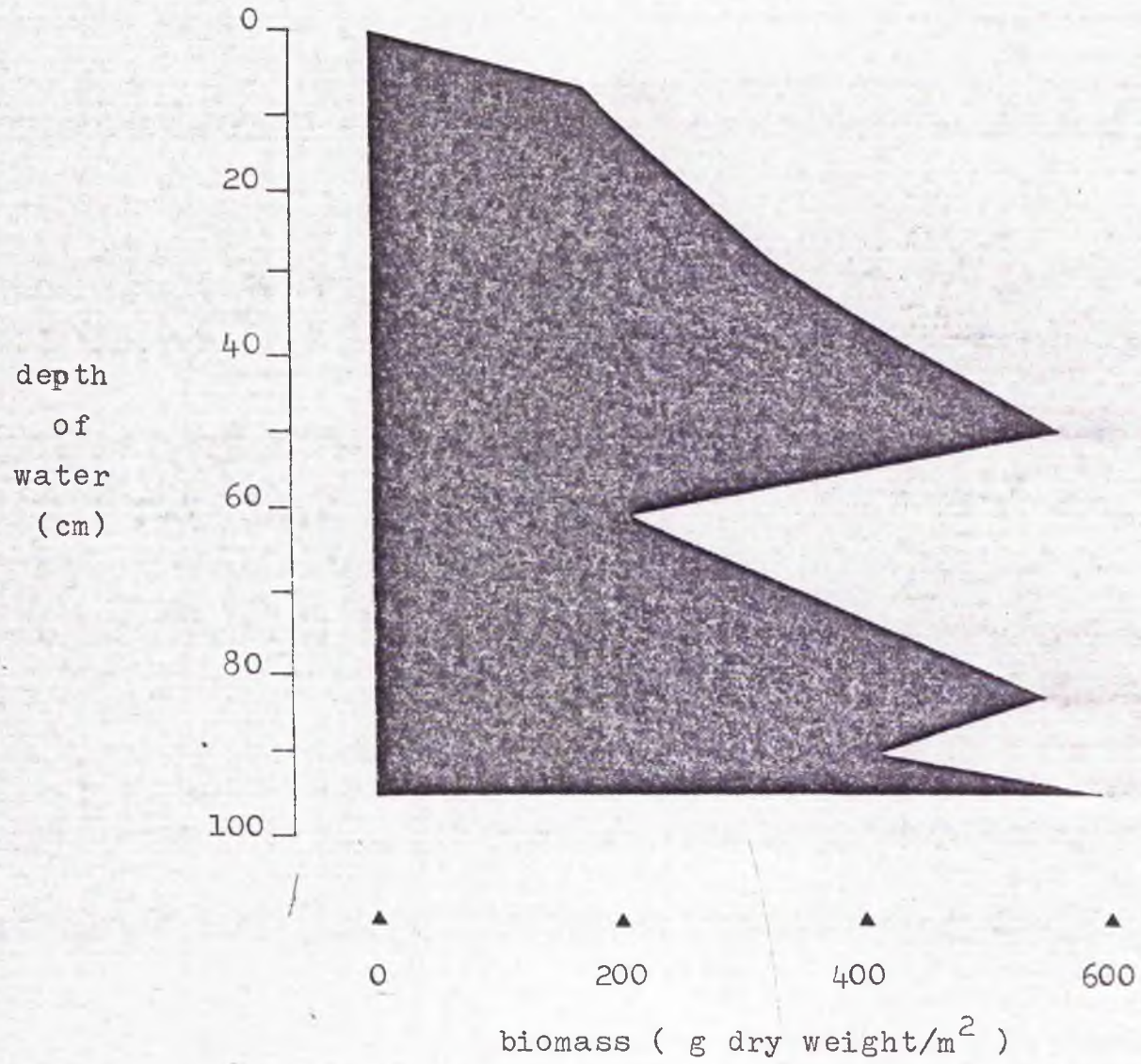
The assistance of the Honours Botany class (1970), in sorting and weighing some of the above samples, is gratefully acknowledged.

TABLE 1.13

Standing crop of Potamogeton perfoliatus in Loch Sroiapol collected during August 1970 (sample size 1/4 m^2).

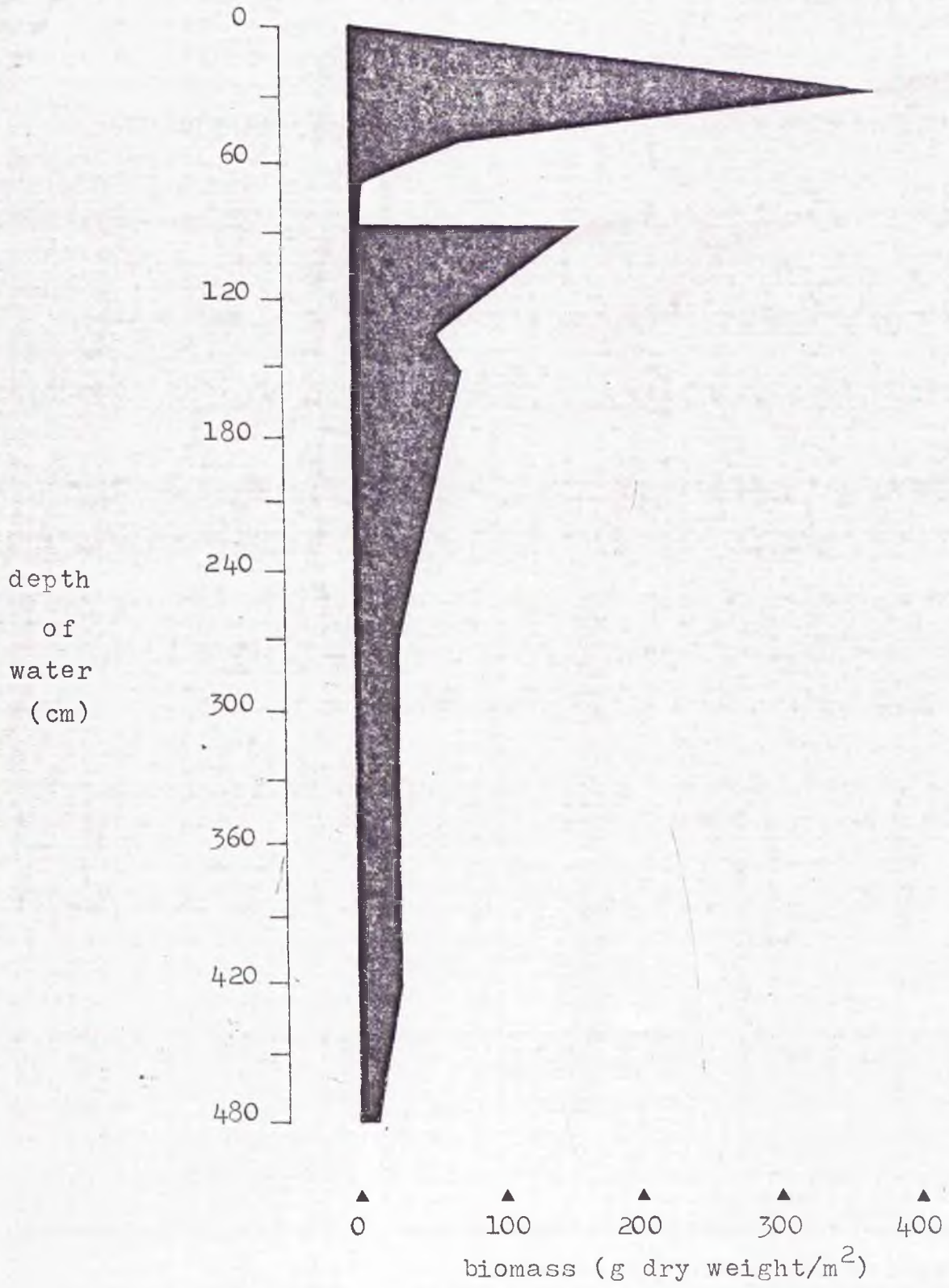
Depth of water(m)	Number of shoots/ sample	Mean shoot length(cm) /sample	Plant dry weight, g./sample	Standing crop of shoots g. dry weight/m^2	Leaf area index
2.5	8	21.10	1.21	4.84	-
2.5	14	29.70	2.43	9.72	-
2.5	17	20.30	2.35	9.40	-
3.0	3	16.60	0.26	1.04	-
3.3	43	33.80	9.17	36.68	4.39
3.3	12	52.60	2.37	9.48	-
3.3	31	32.80	5.15	20.60	-
3.5	21	22.20	3.29	13.16	-
4.0	17	24.00	2.56	10.24	-
4.0	2	17.30	0.21	0.84	-
4.3	4	8.40	0.19	0.76	0.17
4.6	16	24.40	3.00	12.00	-
4.6	25	23.90	5.03	20.12	-
5.0	18	13.10	2.25	9.00	-
5.0	16	32.50	3.00	12.00	-
5.0	8	25.20	1.32	5.28	-
5.0	18	12.30	1.72	6.88	-
5.0	4	15.90	1.30	5.20	-

FIGURE 1.10



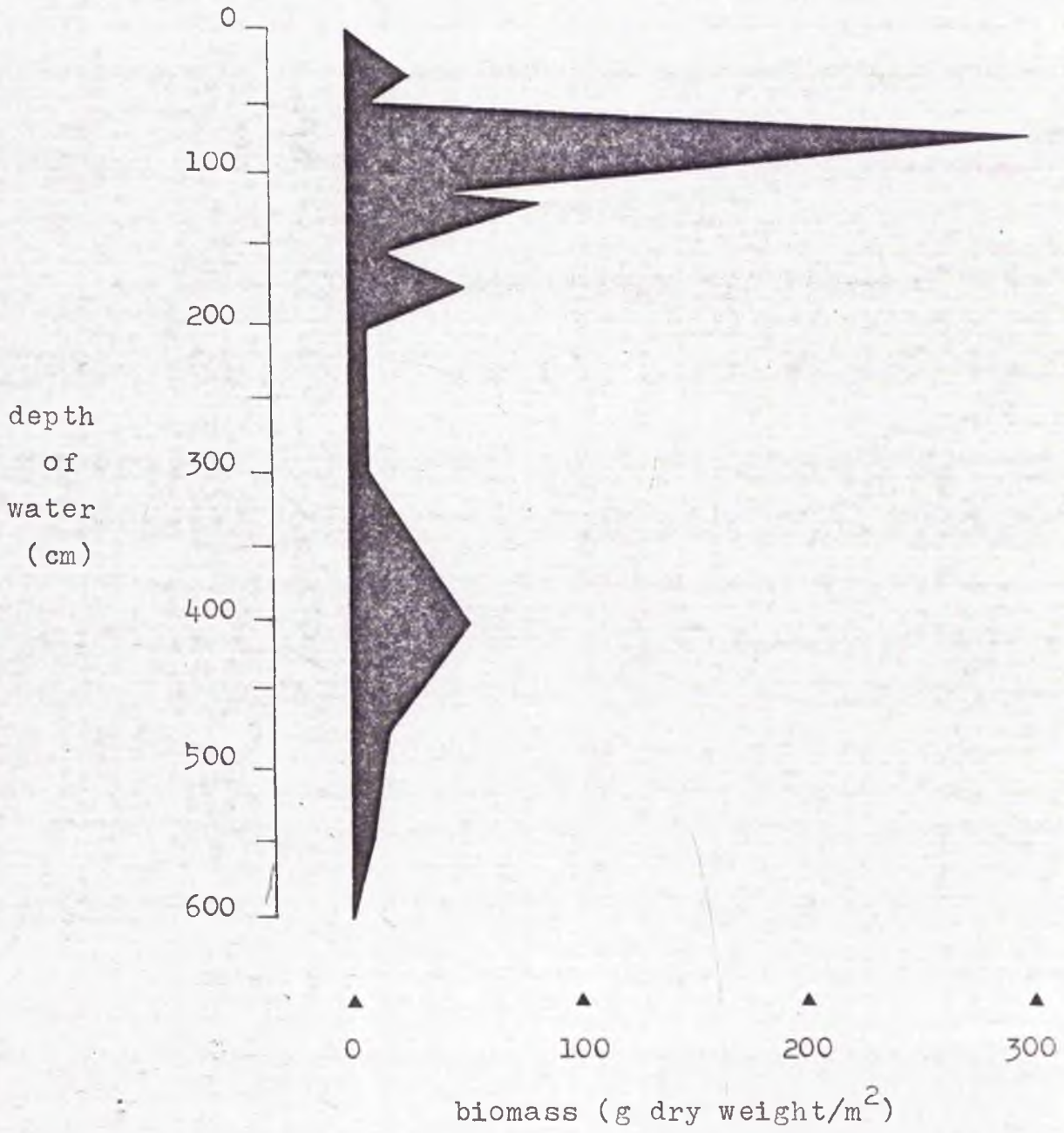
Biomass (g dry weight/m^2) of submerged vegetation sampled in Loch Croispol during June 1970 (Transect A), plotted against depth of water (cm).

FIGURE 1.11



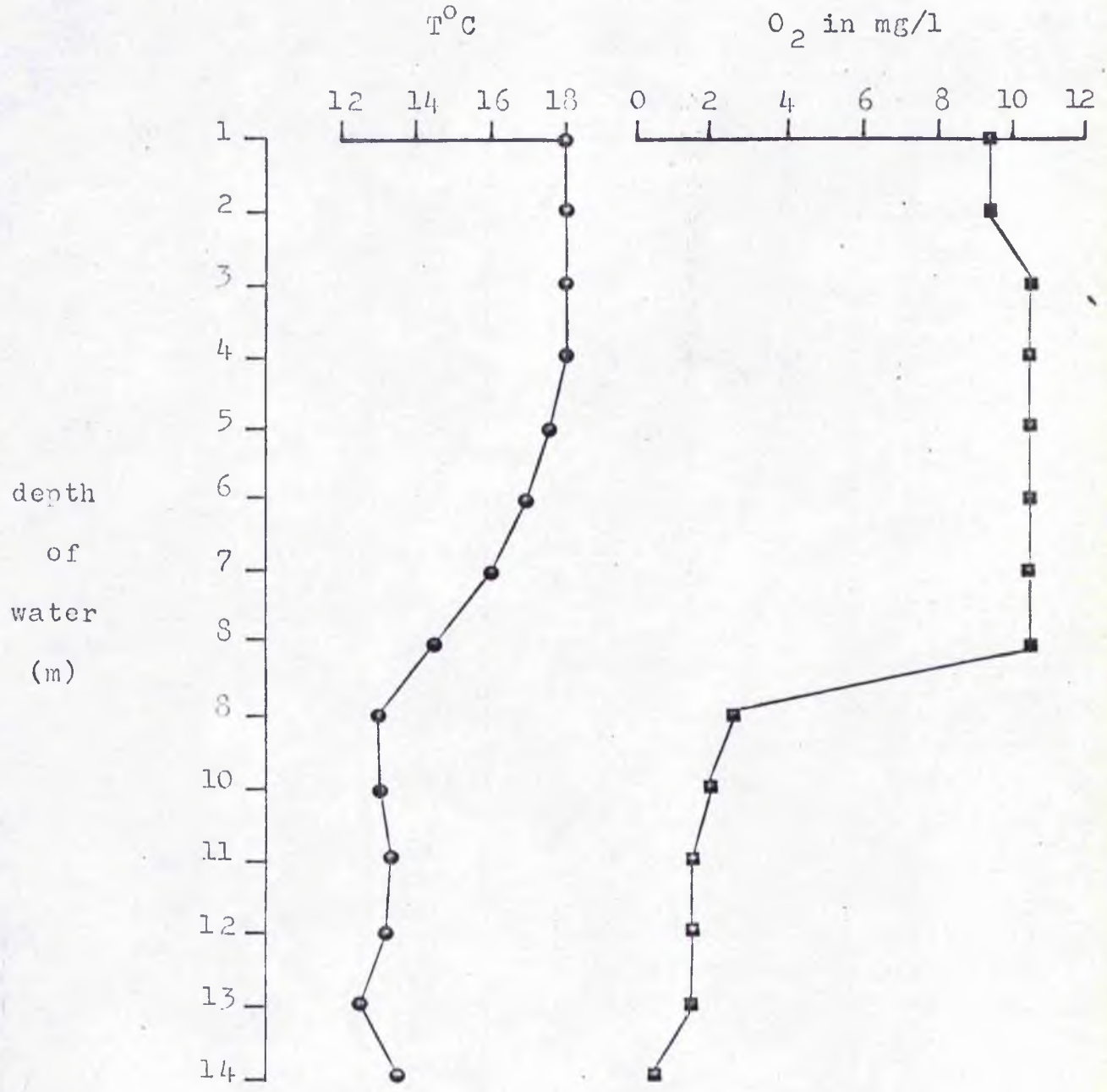
Biomass (g dry weight/m^2) of submerged vegetation sampled in Loch Croispol during June 1970 (Transect B), plotted against depth of water (cm).

FIGURE 1.12



Biomass (g dry weight/m^2) of the submerged vegetation in Loch Croispol, plotted against depth of water (cm). Redrawn from Spence et al.(unpublished).

FIGURE 1.13



Temperature (^{o}C) and oxygen (mg oxygen/l) profile measured, using a Mackereth oxygen probe, in Loch Croispol on 17.7.70.

Loch Lanlish

Only four biomass samples were taken from Loch Lanlish. These were collected as before and were taken to obtain an estimate of the crop size of Potamogeton praelongus which by visual inspection, when swimming underwater, covered a large area of the loch between the depths of two and four metres. Deposits of $CaCO_3$ were obvious on both plants of Chara species and P.praelongus and this was estimated at 75% by weight for the Chara species and 20% by weight for the P.praelongus using the methods previously employed to obtain the degree of $CaCO_3$ encrustation on Chara species from Loch Croispol. Table 1.14 presents the results of the biomass determinations in terms of $CaCO_3$ free oven dry weight.

There was considerable variation among the four biomass samples from Loch Lanlish but, however, the major part of this was made up of variation in the biomass of the macrophytic alga Chara papillosum while P.praelongus showed much less variation, though its biomass pattern is illustrated as being low in the shallower and deeper samples and highest in the one sample taken at 3.3 m, in the depth area where, by visual estimate, growth appeared to be most luxurient.

The contours of the loch in the area where the samples

were taken were measured by the "leap-frog" method, used previously in Loch Croispol, and the P.praelongus found to be growing on a steep slope with a mean gradient of at least 1 in 1.5 which in places was vertical and not unlike that reported from Loch Baille na Ghobhainn, Lismore (Spence et al. unpublished). Colonisation by P.praelongus was aided by downward directed runners and by fallen stems rooting at the nodes. The depth limit of colonisation was determined as 4.5 metres and the deepest colonising species was P.praelongus; no Chara species extending much beyond three metres depth of water.

TABLE 1.14

Biomass of submerged vegetation sampled in Loch Lanlish during June 1970 (sample size 1/16 m^2).

Depth of water (m)	Species composition	g.dry weight plant material/sample	Total g. dry weight plant material	Biomass g.dry weight/m^2
2.2	Potamogeton praelongus	2.23		
	Chara papillosum	1.28	3.55	56.9
	Bryophyte species	0.04		
2.2	Potamogeton praelongus	3.19		
	Chara papillosum	12.87	17.06	272.7
	Bryophyte species	1.00		
3.3	Potamogeton praelongus	4.41		
	Chara papillosum	0.72	5.23	83.6
	Bryophyte species	0.10		
4.0	Potamogeton praelongus	2.21		
	Bryophyte species	0.04	2.25	36.0

Loch Lomond

Six biomass samples were collected from a sheltered bay in this loch. They are by no means thought to represent the production of the loch but were collected to illustrate species relationships within a mixed sward of Littorella uniflora, Isoetes lacustris and Lobelia dortmanna and to investigate the possible substrate preferences of these species. Table 1.15 presents this biomass data along with figures for species numbers, plant mean weights and root/shoot ratios.

There was a certain degree of variation between the samples both in terms of species numbers and dry weights. Lobelia dortmanna was the least abundant of the species and varied least ranging between zero and ten plants per sample while Littorella uniflora plants numbered 236 in one sample and zero in another. Three of the samples contained more than 150 Littorella uniflora plants while the other three had less than 20. Isoetes lacustris was the dominant plant in terms of dry weight in all the samples but not so in numbers, varying between 33 (1.77 g) and 102 (11.3 g). The depth distribution of the species indicated a greater biomass of Littorella uniflora in the three shallower samples than in those taken in deeper water, though a similar distribution did not hold for Isoetes lacustris, the biomass

of which appeared to vary with the organic content of the sediment as measured by loss on ignition (Figure 1.14). Sample 6 (Table 1.15) with the highest biomass of Isoetes lacustris and by far the largest plant mean weight, contained no Littorella uniflora plants and the low biomass in Sample 5 can probably be accounted for by a large (12%) gravel fraction in the sediment. The root/shoot ratios of the plants also varied. For Littorella uniflora and Lobelia dortmanna the roots never contributed more than 30% to the plant biomass while for Isoetes lacustris the roots averaged between 40% and 60%. In all cases the roots contributed greatest to the plant biomass where the sediment analysis indicated a high gravel content.

TABLE 1.15

Data on the biomass of submerged vegetation computed from samples collected in Loch Lomond during October 1969.

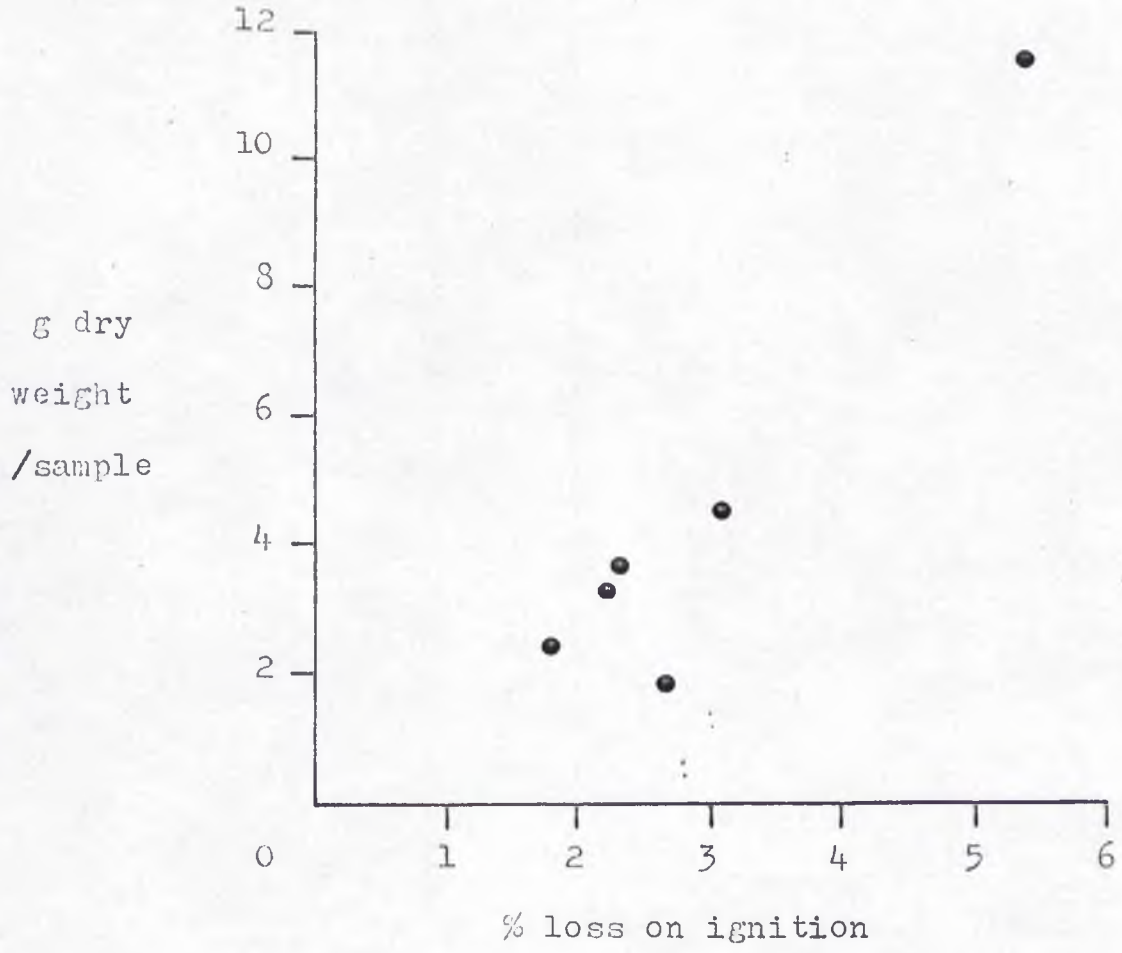
Sample number	Depth of water(m)	g.dry weight plant material/sample	Number of plants/ sample	Plant mean dry weight (mg)	% roots
		Littorella uniflora			
1	2.0	2.36	236	11	44.9
2	2.0	1.88	166	11	49.4
3	3.0	2.89	173	16	49.0
4	3.5	0.33	28	12	35.5
5	3.5	0.30	19	16	47.7
6	4.0	-	-	-	-
		Lobelia dortmanna			
1	2.0	0.21	10	21	36.5
2	2.0	0.07	2	35	29.6
3	3.0	0.13	4	33	29.2
4	3.5	-	-	-	-
5	3.5	0.18	6	30	47.1
6	4.0	0.25	1	25	28.9
		Isoetes lacustris			
1	2.0	3.13	82	38	60.8
2	2.0	2.37	70	34	49.6
3	3.0	3.92	70	56	50.5
4	3.5	4.49	70	64	49.1
5	3.5	1.77	33	36	60.7
6	4.0	11.80	102	116	40.1

cont. over page/

TABLE 1.13 continued:-

Sample number	Total g.dry weight plant material/sample	Biomass, g.dry weight/m^2
1	5.88	94.08
2	4.32	69.12
3	6.94	111.04
4	4.82	77.12
5	2.25	36.00
6	12.05	192.80

FIGURE 1.14



Relationship between the biomass (g dry weight/ sample) of Isoetes lacustris and the percentage loss on ignition of the sediment in which the plants were growing. Samples collected in Loch Lomond in October 1969. Sample size 1/16 m^2.

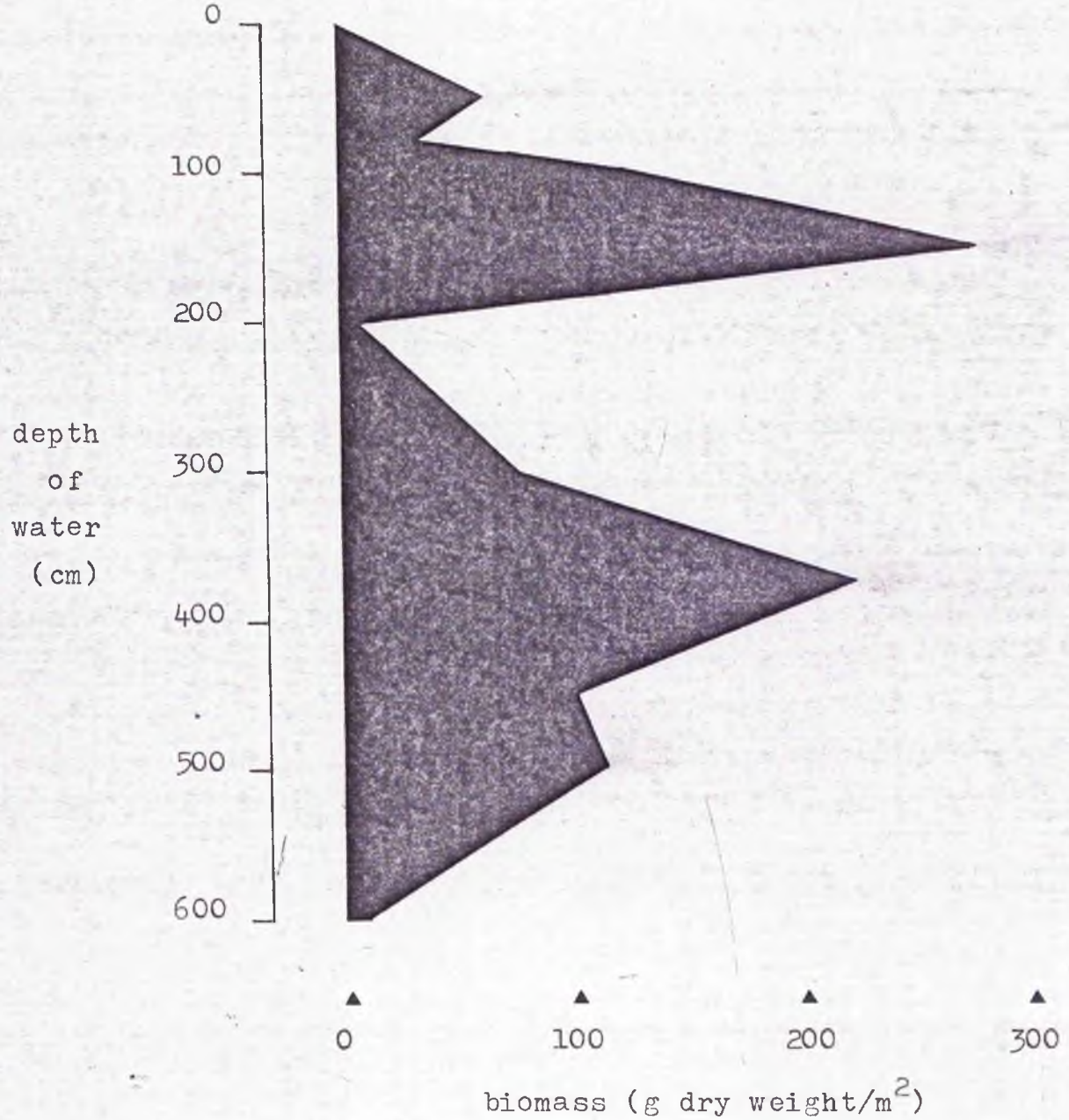
Dubh Loch, Bowardennan.

The biomass of the submerged vegetation in this loch was estimated from twelve samples collected along a transect running through water depths of 45cm to 600cm. Sampling proved difficult not only due to the fact that the loch floor dropped very steeply downwards a few metres from the shore but also because of poor visibility. Beyond a depth of two metres it proved difficult for a diver to read his depth gauge unless brought very close to his face mask, visibility was not improved by the vast clouds of fine sediment which arose when the substrate was disturbed and thus all the deep water samples were collected solely by touch.

Figure 1.15 represents the variation in the biomass with depth. The species which made up this total were Littorella uniflora, Lobelia dortmanna, Isoetes lacustris, Myriophyllum alterniflorum, Sphagnum species and Juncus bulbosus var. fluitans. The peaks in Figure 1.15 correspond, going from shallow to deep, with the dominant zones of Littorella uniflora, Myriophyllum alterniflorum, Juncus bulbosus var. fluitans and Sphagnum species. Sphagnum species were recorded as the deepest coloniser and the limit of colonisation was determined as six metres. No substrate samples were collected in Dubh Loch although

from its handling texture and the ease with which clouds of sediment could be disturbed it would appear that organically rich silt and clay predominated.

FIGURE 1.15



Biomass (g dry weight/m^2) of submerged vegetation sampled in Dubh Loch during October 1969, plotted against depth of water (cm).

GENERAL DISCUSSION

Gravimetric estimates of the biomass of subaquatic vegetation have been made in several north temperate lakes (Wetzel, 1964). These studies vary in thoroughness and in detail depending upon the objectives of the particular investigation. Visual estimates have been used to assess the general abundance and distribution of macrophytes in some studies on the food of waterfowl (Siegler, 1941, 1943). Relative estimates have also been carried out from aerial photography (Edwards and Brown, 1960) and from the changes in the transmission of light through water (Westlake, 1964). Though some workers have employed rakes to collect semi-quantitative samples of the subaquatic vegetation (Swindale and Curtis, 1957) the majority of studies have been carried out using some form of grab or sampler, operated either manually or mechanically from a boat. Many of these sampling devices have been adapted from those routinely used in the collection of benthic fauna and sediment (Petzger and van Engel, 1942) though Grøntved (1957, 1958) used a corer in shallow water while Forsberg (1959, 1960) devised a shearing apparatus for use in soft sediments to cut off plants below ground level.

Wood (1963) reports on a study comparing the merits of an aqualung diver cropping quadrat samples and a boat

operated grab in assessing the biomass of submerged vegetation, concluding that grab sampling can underestimate the extent of the vegetation by up to one third and hence caution must be exercised in the interpretation of grab collected material. In the present studies it has been found that it is also easy to overestimate the depth limit of colonisation when using a boat operated grab, an error only revealed by diving in lochs previously surveyed by grab sampling. Loch Baille na Ghobhainn on the island of Lismore has been reported (West, 1910) as having dense beds of Fontinalis antipyretica growing at depths greater than 12 metres, yet diving has revealed the limit of colonisation to be nearer six metres with only a few isolated plant fragments growing beyond (Spence et al., unpublished). Many lochs and lakes have very steep sides (e.g. Loch Baille na Ghobhainn, Loch Croispol) and it is possible that gravity and currents carry plant fragments downwards and that in many cases it is these which are retrieved by grabs and samplers. Westlake (in Vollenweider, 1969) states that grabs only provide an alternative to the use of divers for sampling subaquatic vegetation in very deep water, but that no grab method is entirely satisfactory. Errors include those inherent in sampling small areas, removing the plant roots inadequately and wrongful inclusion or exclusion of plants at their edges.

Prior to the present studies divers with wet or dry

suits and Self Contained Underwater Breathing Apparatus (SCUBA) have been used in several determinations of the biomass of subaquatic vegetation in both the marine and freshwater environments (Crossett and Larkum, 1965; Wygaard, 1958; Zaspopov, 1963). Wood (1963) and Yager et al.(1966) describe some useful pieces of simple apparatus and give helpful suggestions for underwater working though some of these, especially for writing underwater, seem unduly complicated.

Numerous attempts have been made to convert biomass data into measurements of productivity by integrating biomass dry weight data on a seasonal basis (Ikusima, 1966; Moore and Hartman, 1966). Although agreeing that, with certain reservations, the method is applicable, Wetzel (1965) has found conflicting results in comparison with other methods of assessing productivity. With regard to assessing productivity figures from the data obtained in the present investigation, which was one of the overall aims of the study, it was thought that the considerable variation in the biomass estimates, based on apparently uniform stands of vegetation, in the first set of samples was in excess of the expected changes in monthly biomass as predicted from other studies (e.g. Ikusima, 1966: approximately 20 g/m^2/month) and that to overcome this and obtain estimates of low statistical variation a very large

number of samples would be required. This was not only a practical consideration but a biotic one since a large number of samples could not be regularly taken from the same stands of vegetation without encroaching upon areas disturbed by previous sampling. Thus the biomass and standing crop data in no way reflect the productivity of the lochs investigated but rather their production.

The biomass of higher plants is variable in space and time and hence comparison of crop data is difficult unless careful attention is paid to the programme of sample collection though, on the whole, samples collected near to the time of maximal seasonal biomass, which for aquatic plants is recognised to be at the time of flowering in late summer, are generally comparable (Westlake, 1963a). Spatial variation, as has been found in the present studies, can also be non-random but related to an increasing depth gradient.

With regard to the variation in subaquatic vegetation along this gradient of increasing depth, Spence and Campbell (unpublished) have constructed a simple, computer-based, information-retrieval system, which stores survey data collected from 99 localities in Scotland (Spence, 1964). Using this system of computer data-processing, ecological information on the distribution of aquatic macrophytes can be easily obtained. Table 1.16 gives the depth ranges of

TABLE 1.16

Range in depth of Potamogeton species as processed from Spence (1964) from Spence and Campbell (unpublished).

Species	No. of samples	Range in depth(cm)	Mean depth (cm)	Standard deviation of mean depth
P.alpinus Balb.	6	105-190	135	-
P.berchtoldii Fieb.	12	55-300	114	64
P.crispus L.	7	32-310	161	-
P.filiformis Pers.	18	5-150	54	43
P.gramineus L.	38	24-300	111	62
P.lucens L.	1	-	185	-
P.natans L.	84	-37-200	71	52
P.x nitens Weber	4	60-130	80	-
P.obtusifolius Mert & Koch	24	50-310	128	60
P.pectinatus L.	13	5-205	68	52
P.perfoliatus L.	27	25-285	111	60
P.polygonifolius Pourr.	23	-5-150	17	33
P.praelongus Wulf.	5	125-280	199	-
P.pusillus L.	8	60-300	157	-
P.x zizii Koth.	14	-50-210	116	67

several species of Potamogeton and serves to illustrate the possible variation in species composition with depth of water. Some of the factors which influence such zonation and restriction have been investigated by Spence and Chrystal (1970) and are discussed again later in this thesis. This depth distribution of species has been illustrated by all the biomass data presented, where it has been shown that different species contribute differentially to the vegetation biomass at different depths e.g. in Loch of the Lowes Littorella uniflora is the major constituent of the shallow water vegetation while Potamogeton species dominate in the deeper water. As far as the spatial variation in total biomass of subaquatic vegetation varies with a depth component this is again illustrated in all the biomass data presented, and though no one depth in all lochs can be said to support the highest biomass, this is generally attained within the first two metres of water while, after this peak, the total biomass decreases with depth.

The depth of water to which colonisation by rooted plants extended varied in the lochs investigated, being deepest in the clear, limestone Loch Croispol and shallowest in the brown water lochs. Colonisation in the eutrophic Loch Leven was practically non-existant. Dubh Loch appeared anomolous in having Sphagnum species growing at a depth of six metres and this was the deepest coloniser.

Bryophytes and macrophytic algae are reported as deep water colonisers by Sculthorpe (1967) who gives reports of occurrences of Drepanocladus species and Fontinalis species at depths of 18 and 20 metres (Fassett, 1930; Juday, 1934) while Nitella opaca has been reported to grow even deeper at 27 metres in Lake Towada, Japan (Jinbo et al., 1955). Pearsall and his co-workers have reported depth limits of colonisation from several of the English Lakes and these are given in Table 1.17 along with the figures obtained in the present study and others from the literature. The general restriction of the rooted vegetation to the comparatively shallow regions of lakes is considered to be a function of the penetration of light (Sculthorpe, 1967) and this is discussed specifically in Chapter 9 of this thesis. With reference to some of the lochs in the present investigations it is thought that the restriction in rooting depth might well be, in part, a result of an inability to grow on steep, unstable slopes.

The results of the sediment analysis carried out in the present study add little to Spence's (1964) data, and the findings are in general agreement. No definite causal correlations have been established between any of the sediment properties investigated and the abundance of any particular plant species, though relationships have been illustrated both between the various sediment properties

TABLE 1.17

Depth limit of macrophyte colonisation in some freshwater lakes.

Depth limit(m)	Lake	Reference
2.3	Sweeney Lake	Wilson, 1937
2.75	Bassenthwaite	Pearsall, 1918
3.0	Lake Wanlak	Bernatowicz,1969
3.0	Loch of the Lowes	
4.0	Rathwaite	Pearsall, 1918
4.3	Windermere	Pearsall and Hewitt, 1933
4.5	Loch Achtriochtan	
4.5	Loch Uanagan	
5.0	Lake Mendota	Denniston, 1922
5.0	Weber Lake	Potzger and van Engel, 1942
6.0	Loch Baille na Ghobhainn	
6.0	Dubh Loch	
6.0	Loch Lanlish	
6.0	Lake Sniardwy	Bernatowicz et al., 1968
6.5	Lake Mendota	Hickett, 1921
6.5	Windermere	Pearsall, 1918
7.0	Loch Croispol	
7.0	Lake Muskellunge	In Sculthorpe, 1967
7.7	Wastwater	Pearsall, 1920
10.0	Ennerdale	Pearsall, 1920
10.0	Green Lake	Hickett, 1921
11.0	Lake Grane Langsø	Nygaard, 1958
20.0	Crystal Lake	Juday, 1934
27.0	Lake Towarda	Jimbo et al., 1955

themselves and between some sediment properties and the vegetation. The large biomass of Littorella uniflora found in the shallow water on sandy sediments may well be related to this plant's tolerance of wave action and not to any preference for substrate, and similarly the dominance of the Potamogeton species in the deep water on silty sediment, rich in organic matter, may not necessarily be a causal relationship but is, perhaps, due to a preference for still water or even low light intensity. Spence (1967) criticises the constantly perpetrated ideas of Pearsall (1920) that it is the properties of the sediment which controls the distribution of aquatic macrophytes and concludes, as must be done in the present study, that there is no evidence from any source to support the ideas that there is any precise correlation between one species of aquatic plant and a substratum of a defined composition. The dependence of the growth of aquatic plants upon the nature of the substratum centres around the question of function and role of their roots. This problem has been investigated and results are presented in a later chapter in this thesis.

In his review of plant productivity Westlake (1963) concludes that the biomass of submerged aquatic plants ranges between 0.2 and 10 Kg dry weight/m^2. Table 1.18 lists the maximum biomass recorded in this and other unpublished studies on some Scottish lochs which indicate

TABLE 1.1R

Maximum biomass estimates of submerged macrophytes in some Scottish lochs.

Loch	Biomass, g. dry weight/m^2
Achtriochtan	283
Croispol	310[1]
Croispol	1000
Luba	280
Lanlish	273
Lowes	130
Lomond	193
Vanagan	325[1]

1. Spence et al.(unpublished).

that these are far from being what Westlake (loc.cit.) would term "fertile sites". Data from other sources (Table 1.19) also indicates that a great many other lakes can, as far as submerged freshwater macrophyte production is concerned, also be termed poor and perhaps leads to the conclusion that the natural productivity of these plants has been overestimated by Westlake (1963) and by Penfound (1956). The latter extrapolated daily productivity data from standing crop values and concluded that the productivity of submerged aquatic macrophytes could well be much in excess of that reported from most terrestrial communities.

Blinks (1955) has investigated the biomass of several species of marine algae (Table 1.20). His figures agree with those predicted by Westlake (1963) and the conclusion must be reached that the marine submerged macrophytes (the seaweeds) have a greater biomass than the freshwater macrophytes. This differential production is amply expressed in Figure 1.16 which compares the biomass figures of Loch Ganagan with estimates made of the biomass of Laminaria hyperborea forest.

An important consideration when utilising biomass figures as an index of plant production is that though biomass may be measured at the end of the growing season the results do not necessarily only reflect the products of that seasons growth. Many aquatic macrophytes are

TABLE 1.19

Biomass estimates of submerged freshwater macrophytes

Biomass, g.dry weight plant material/m^2		References
82 - 212	Vallisneria denseserralata	Ikusima, 1966
162	Lake Sniardwy	Bernatowicz et al., 1963
170	Lake Warniak	Bernatowicz, 1969
200 - 500	-	Moore & Hartman, 1966
228	Lake Grane Langeø	Nygaard, 1953
240	Myriophyllum verticillatum L.	Forsberg, 1960
310	Nitella mucronata Miq. Osbysjon	Forsberg, 1960
680	Ceratophyllum demersum L.	Forsberg, 1960
1,130	Chara fragilis Desv.[1]	Forsberg, 1960

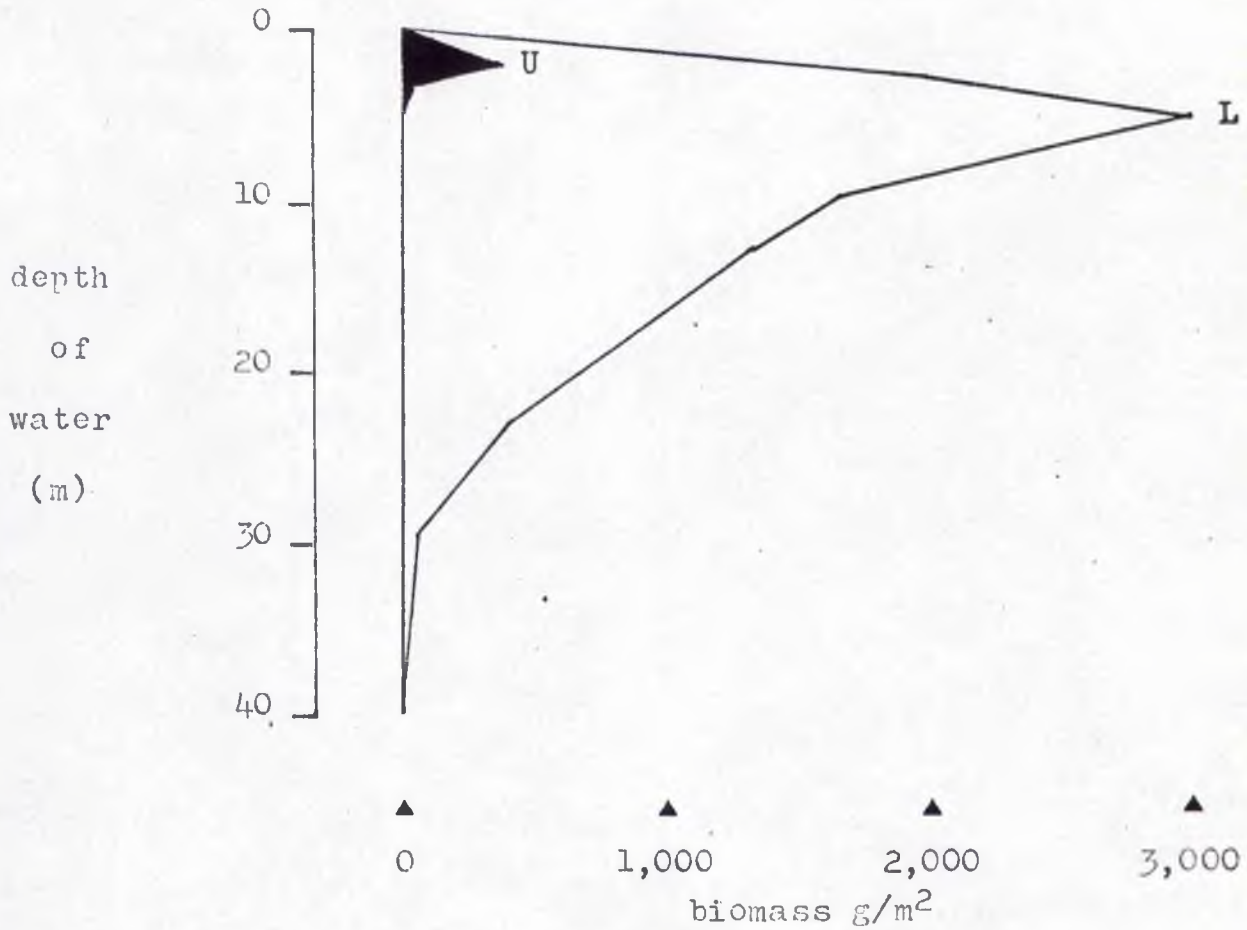
1. Calcium carbonate not removed.

TABL. 1.20

Biomass estimates of marine algae (from Blinks, 1955)

Species	Biomass, g.dry weight/m^2
Ulva spp.	70
Porphyra spp.	300
Fucus spp.	2,130
Laminaria spp.	4,400

FIGURE 1.16



Comparison of the biomass (g dry weight/m^2) of the submerged vegetation in Loch Uanagan (U), (Figure 1.5), with that of a Laminaria hyperborea "forest" (L) in the sea off Cornwall. Laminaria hyperborea data from Bellamy (unpublished).

perennial (e.g. Littorella uniflora, Potamogeton praelongus, Chara species) and hence a biomass figure may measure the accumulated results of many years production. Not only this but the fact that some populations are subject to high rates of mortality and grazing (Mathews and Westlake, 1969) which means that the terminal biomass figures do not necessarily reflect the total production of the vegetation, adds to the difficulties in the interpretation of biomass data. It is worthy of note here that the bulk of the vegetation in Loch Croispol from which was recorded the highest estimate of biomass, is made up of two perennial Chara species though, in the same loch, the August estimate of 10 g/m^2 as the mean standing crop of Potamogeton perfoliatus reflects only one summers growth, as diving in late May revealed a complete absence of shoots of this species.

Bearing in mind the limitations of the data presented, it must still be concluded that the biomass estimates made in Scottish lochs indicate that the freshwater macrophytes of those habitats are poor producers of organic matter when compared to other terrestrial and marine communities.

The amount of dry matter produced by the photosynthesis of plants in unit time depends, among other things, upon the size and efficiency of the photosynthetic system. The total area of leaves or photosynthetic tissue present

per unit area of land (LAI) defines the size of this photosynthetic system while the efficiency can be expressed in terms of the mean rate of organic matter produced per unit leaf area, per unit energy absorbed. It has been shown that for one stand of Potamogeton perfoliatus in Loch Creispol the LAI was variable and ranged between values of less than one to just greater than four. These are low figures when compared to other communities (e.g. Laminaria species forest approached ten, Robertson, pers.com.). LAI figures are, however, not available for any of the other species investigated in this study though Ikusima (1966) recorded figures between 4.8 and 9.3 in his studies with Vallisneria denseserrulata and concluded that the low productivity of aquatic macrophytes was due, in part, to their low photosynthetic efficiency, a subject which is discussed in a later chapter of this thesis.

C H A P T E R 2

CALORIFIC VALUES

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C H A P T E R 2

CALORIFIC VALUES

INTRODUCTION

The calorific equivalent of biological material is frequently used in ecological production studies for ease of comparison of data, yielding information more meaningful than estimates made on the basis of dry matter, ash-free dry matter or carbon. For this reason, in the present study the calorific content of a number of aquatic macrophytes have been estimated by means of a Ballistic Bomb Calorimeter. On the basis of organic carbon content and proximate analysis Westlake (1965b) has predicted figures for the energy content of most aquatic macrophytes to lie between 4.3 and 4.8 Kcal/g organic matter. Boyd (1970) has measured the calorific content of several species of aquatic macrophyte directly by bomb calorimetry finding a mean energy content of 4.1 Kcal/g dry weight. Cummins and Wuycheck (1971) provide extensive tables of calorific values for biological material from many different trophic levels, designed to aid studies on ecological energetics and give average values

for aquatic angiosperms of 4.6 Kcal/g ash-free dry weight (3.9 Kcal/g dry weight).

METHODS

Calorific analysis was carried out using a Gallenkamp CB-370 Ballistic Bomb Calorimeter.

A known weight (usually about 0.5 g) of powdered oven dry (105°C) plant material contained in a standard metal crucible was ignited electrically and burned in an excess of oxygen (25 ats. pressure) in the bomb and the maximum temperature rise of the top of the bomb measured with a thermocouple and recorded from the deflection of a galvanometer needle. The rise in temperature (i.e. scale deflection on the galvanometer) was compared with that produced from a series of combustions carried out with different quantities of thermochemical grade benzoic acid of known calorific content (figures 2.1, 2.2) and a value obtained for the heat released from the bomb and hence the calorific content of the sample. After ignition, the crucible was allowed to cool in a desiccator and then re-weighed to obtain the ash content of the sample. Results were expressed in terms of calories per gram of tissue dry

weight and per gram ash-free dry weight of tissue, where one calorie is defined as the heat required to raise the temperature of 1 g of water 1°C (equivalent to 4.186 joules). Care was taken to ensure that the samples were well compacted before ignition and in the case of the benzoic acid standards this required cautious melting. A standard length (two inches) of gun cotton was used as a fuse between the firing wire and the sample. The calibration graph (Figure 2.2) was adjusted to account for the calorific value of this by burning a series of fuses of increasing number and computing the calorific content of one. Correction was also made for the galvanometer deflection produced when the bomb was fired with no sample.

The plant material analysed was collected from four lochs at several periods during the course of one summer and were chosen to represent the range of submerged broad leaved species of Potamogeton typical of these localities. Samples of Littorella uniflora, Lobelia dortmanna, Isoetes lacustris and Chara species were also analysed. Several specimens of each species were collected and bulked for analysis. Generally only the photosynthetic tissues were analysed though the calorific content of the roots and rhizomes of Potamogeton praelongus was measured on one occasion and of the roots of Littorella uniflora and Lobelia dortmanna on two other occasions.

FIGURE 2.1 and FIGURE 2.2

(opposite)

Calibration graphs for determining the calorific equivalents of plant material.

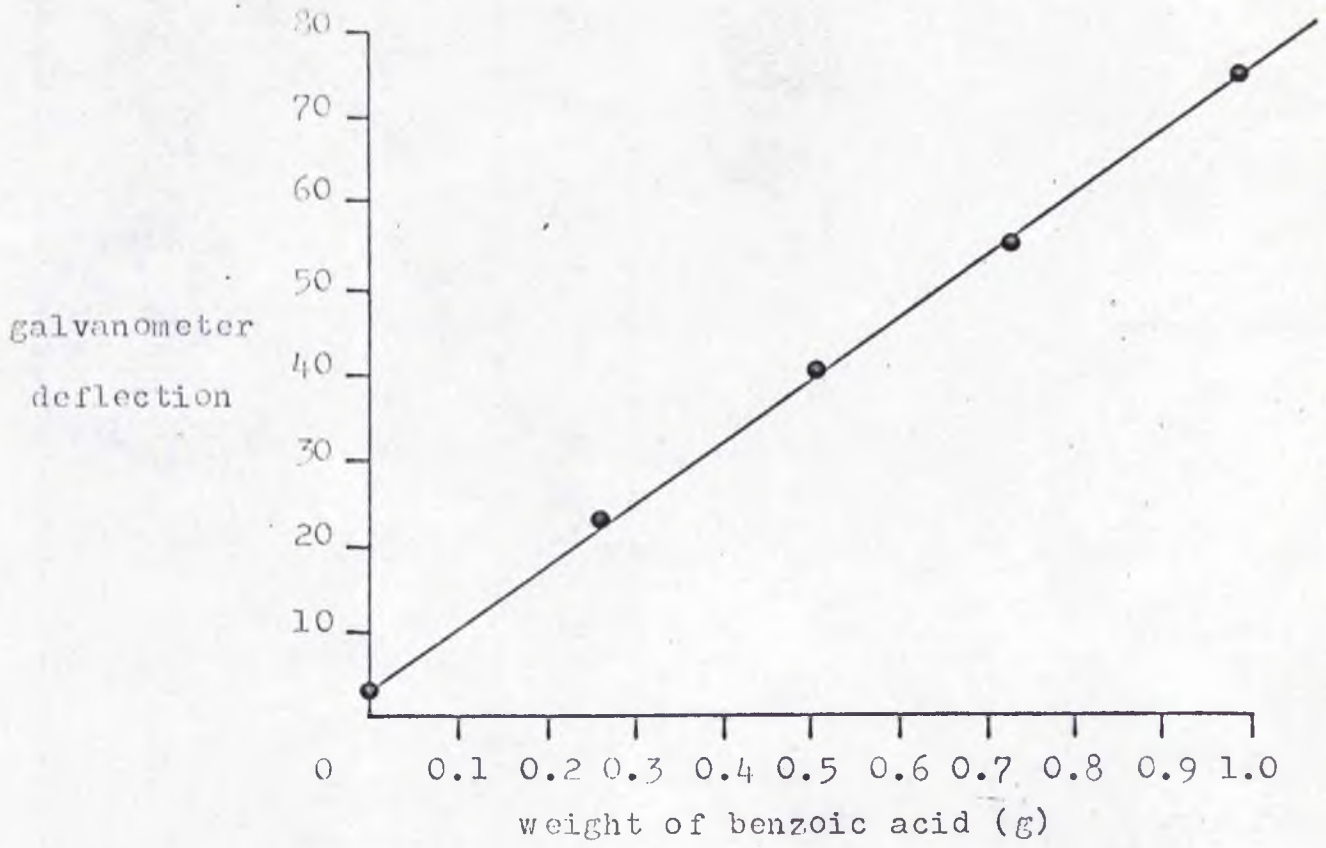
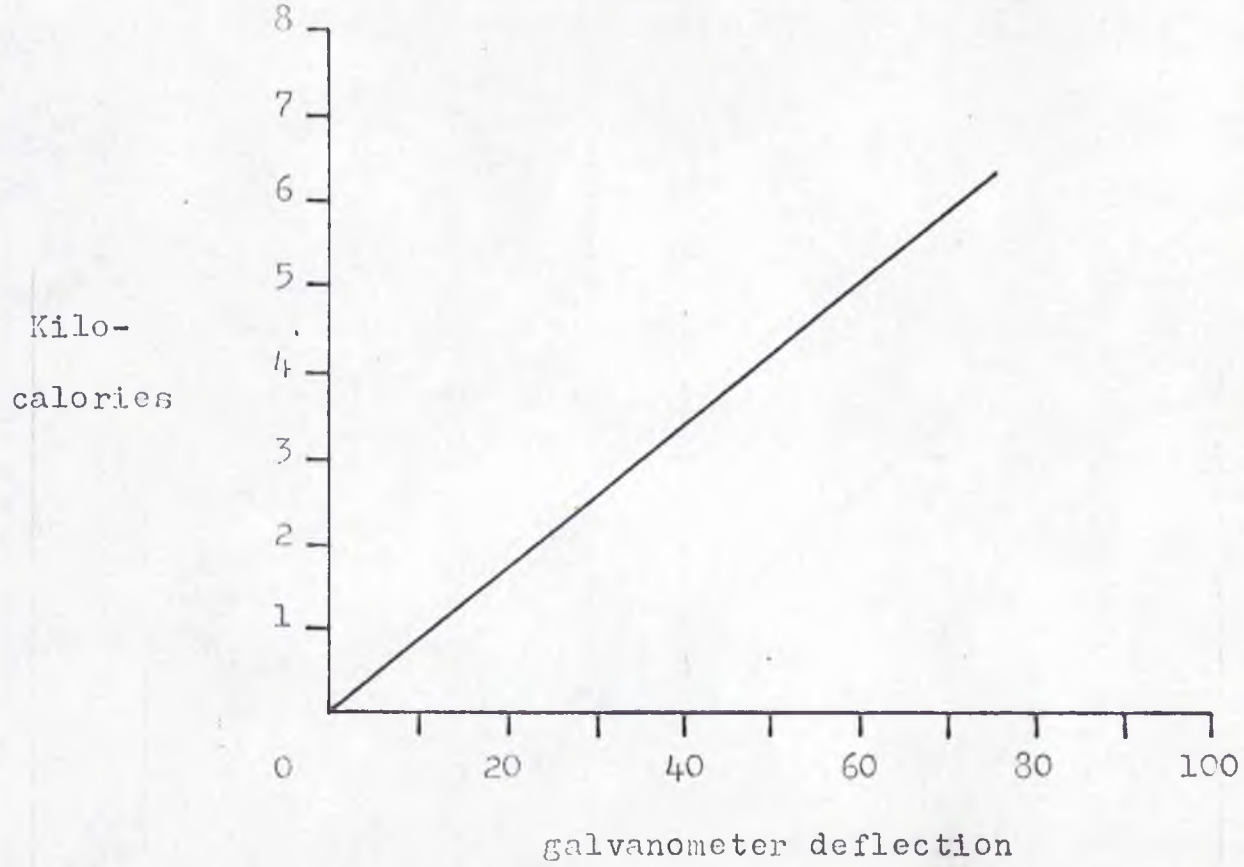

80
70
60
50
40
30
20
10
galvanometer deflection
0
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
weight of benzoic acid (g)

FIGURE 2.2


8
7
6
5
4
3
2
1
Kilo-calories
0
20
40
60
80
100
galvanometer deflection

RESULTS AND DISCUSSION

The theoretical figures for the calorific value of whole organisms fall within a range based upon the value of 5400 cal/g ash-free dry weight for the average pure carbohydrate, and 4100 to 9500 cal/g ash-free dry weight for fats (Cummins and Wuycheck, 1971). Actual organisms can be expected to exhibit only a part of this range with an average closer to that of protein (5100 cal/g ash-free dry weight). Boyd (1970) conducted a study of the amino acid and protein content of vascular aquatic plants in relation to calorific values, to determine if these levels fluctuated with variations in protein concentrations. He found that the amino acid composition of the proteins in aquatic plants is relatively constant. A variation in total protein content existed, and this was much greater than the variation in calorific values.

Based on the assumption that most field programmes do well to operate at a sampling variance of less than 10% Cummins and Wuycheck (loc.cit.) recommend that only differences of 500 - 1000 cal/g should be considered significant differences in most ecological studies. Calorific variations of lesser magnitude are probably accounted for by reliable differences within the population sample. This criterion is sustained in the present investigation in which only

differences in calorific content greater than 500 cal/g ash-free dry weight are held to be significant.

The calorific content of Potamogeton perfoliatus plants is recorded in Table 2.1, P.praelongus in Table 2.2 and P.x zizii in Table 2.3.

Firstly, treating the calorific estimates for the species individually as they appear in the tables and regarding only the values of calories per gram ash-free dry weight as a more meaningful comparison (Westlake, 1965a), the lowest of the measured values for the leaves of P.perfoliatus is 4366 cal/g ash-free dry weight (June, Loch Croispol) and the highest 6811 cal/g ash-free dry weight (September, Loch Vanagan) the overall mean being 5364 cal/g ash-free dry weight. Corresponding figures for the stems of the same species are 4086 cal/g ash-free dry weight (November, Loch Croispol), 4914 cal/g ash-free dry weight (August, Loch Croispol) and 4423 cal/g ash-free dry weight.

Bearing in mind the acceptable degree of variation within samples (500 cal/g ash-free dry weight) there is a significant difference within the overall calorific values recorded for P.perfoliatus leaves and stems and a significant difference between the mean calorific values of the two tissues. Within each loch there is, at least on one sampling occasion, a significant difference between the energy content of the leaves and the stems, being most

TABLE 2.1

Ash content (% dry weight) and calorific values (cal/g dry weight and cal/g ash-free dry weight) of leaves and stems of specimens of Potamogeton perfoliatus from Loch Croispol, Loch of the Lowes and Loch Uanagan.

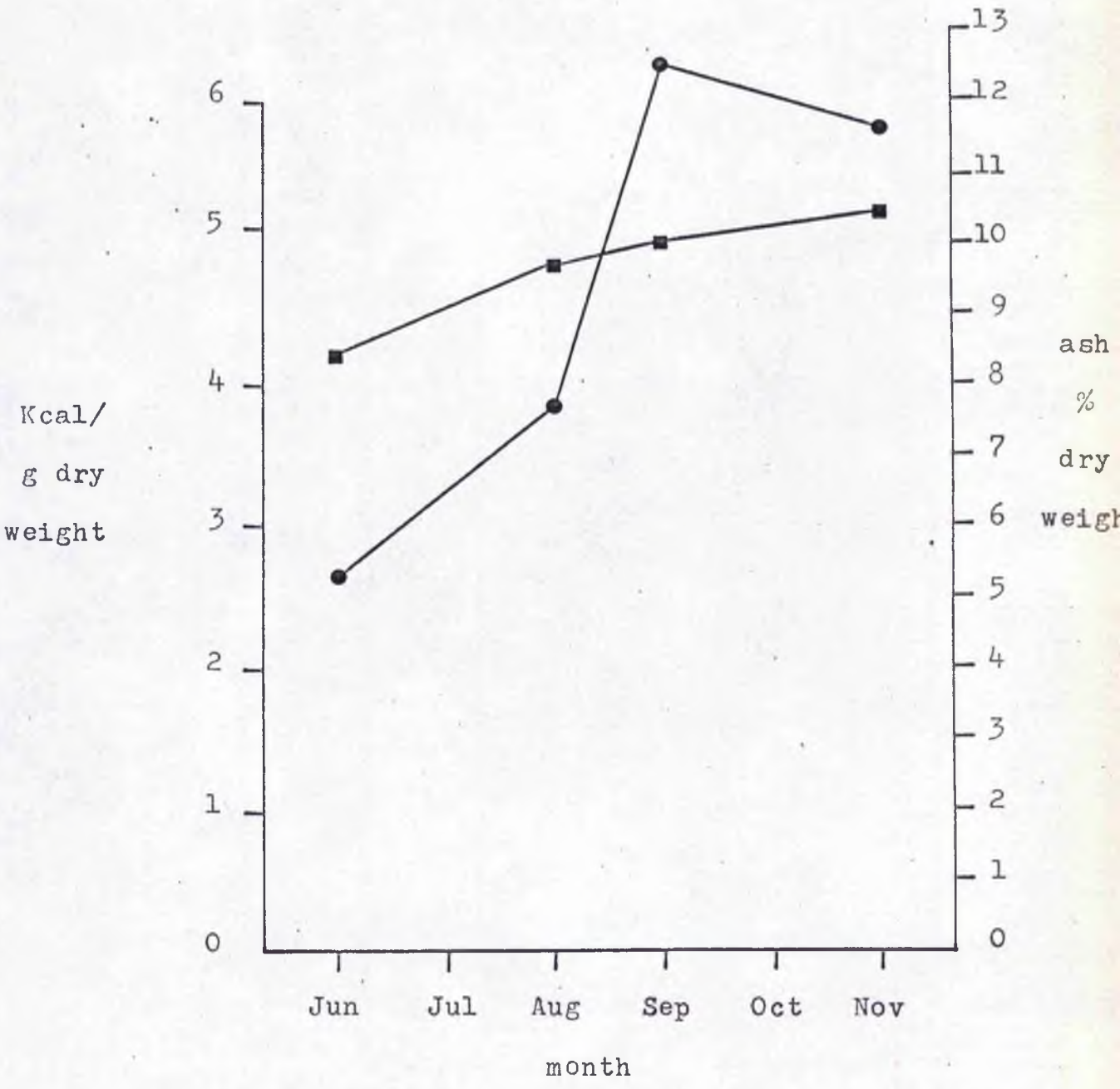
	LEAVES			STEMS		
Month	ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight	ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight
			LOCH CROISPOL			
June	5.3	4133	4366	5.3	3984	4122
Aug	7.7	4364	4730	6.1	4614	4914
Sept	12.5	4409	5036	12.0	3928	4462
Nov	11.6	4525	5177	13.1	3551	4086
			LOCH OF THE LOWES			
June	9.6	5162	5710	2.4	3898	4098
	8.0	5633	6143			
Aug	8.3	5408	5927	7.9	3972	4315
	6.5	4283	4579			
			LOCH UANAGAN			
July	8.6	6010	6583	2.7	4464	4589
Aug	8.4	4690	5129	7.6	4434	4795
Sept	8.7	6222	6811		no value	

pronounced in the July values for Loch Uanagan where there is a difference of 2000 cal/g ash-free dry weight. Similarly, within each loch sampled there is a significant difference in the calorific content of samples collected from different times, this being reflected to a greater extent in the leaf tissue rather than in the stem tissue.

Comparing the three lochs on the basis of the only sampling period common to all three (August), the variation in calorific content of the leaves is within the acceptable range of variation, while an apparently significant difference exists between the calorific content of the stems of specimens collected from Loch Croispol and Loch of the Lowes. On the other hand the mean calorific content of the leaves of all the specimens collected from Loch Uanagan differs greatly (by approximately 1000 cal/g ash-free dry weight) from that of those collected from the other two lochs, which have similar mean values. The mean calorific content of the stems are similar from all localities.

Generally, there are insufficient samples for there to be any clear picture of a seasonal variation in calorific content though (Figure 2.3) the calorific content of the leaves of P.perfoliatus collected from Loch Croispol appears to increase throughout the season. This is not accompanied by a corresponding increase in the calorific content of the stems but is paralleled by a general increase in ash content

FIGURE 2.3



Seasonal variation in the ash (●) and calorific content (■) of leaves of Potamogeton perfoliatus collected from Loch Croispol.

of both leaves and stems. This increase in ash content (though less than the 33.3% previously determined in Chapter 1) is most probably the result of an increase in the amount of calcium carbonate encrustation. Loch Croispol, as noted in Chapter 1, is a limestone loch and it is not uncommon for calciumcarbonate to form exo-skeletons to plants growing in these habitats (Wetzel, 1960). It will also be recalled that the growing season for the shoots of P.perfoliatus in this loch commences in early June which would account for the low values of ash content in this period. In both Loch of the Lowes and Loch Uanagan it is unlikely that calcium carbonate contributed greatly to the ash content of the leaves which remained fairly stable. The ash content of the stems from these two locations, however, more than doubled between the sampling dates and accompanied a slight increase in the calorific value.

Potamogeton praelongus was collected from two sites: Loch Lanlish and Loch Uanagan. The range in calorific content exhibited by P.praelongus leaves is 4492 cal/g ash-free dry weight (August, Loch Lanlish) to 6411 cal/g ash-free dry weight (August, Loch Uanagan) with a mean of 5160 cal/g ash-free dry weight. Corresponding figures for the stems are 3826 cal/g ash-free dry weight (July, Loch Uanagan) 5458 cal/g ash-free dry weight (May, Loch Lanlish) and

TABLE 2.2

Ash content (% dry weight) and calorific values (cal/g dry weight and cal/g ash-free dry weight) of leaves and stems of specimens of Potamogeton praelongus from Loch Lanlish and Loch Uanagan and of roots and rhizomes of the same species from Loch Uanagan

Month	LEAVES			STEMS		
	ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight	ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight
LOCH LANLISH						
May	5.3	4502	4754	4.7	5214	5458
June	3.7	4882	5067	8.3	3951	4320
Aug	7.8	4141	4492	4.0	3953	4115
Sept	6.9	5160	5543	6.2	4445	4741
Nov	6.5	4283	4579	7.9	3972	4315
LOCH UANAGAN						
May	5.0	5055	5321	3.5	5007	5192
July	5.9	5033	5354	2.7	3721	3826
Aug	8.2	5884	6411	2.1	4953	5062
Sept	4.5	4693	4915	2.1	5278	5392
ROOTS AND RHIZOME						
May	2.2	3282	3356			
	3.5	4253	4408			
	2.8	4016	4131			
	2.1	3495	3569			
	2.7	3762	3866			

4713 cal/g ash-free dry weight. There is thus considerable, significant, variation within the recorded calorific equivalents for this species. For all samples collected during the same period (barring one) the calorific content of both leaves and stems collected from Loch Uanagan were significantly greater than the corresponding values of plants from Loch Lanlish, while in just over half the samples the calorific content of the leaves from any one loch was greater than that of the stems of the same specimens. A seasonal fluctuation in calorific content seems apparent. The peak in leaf calorific value corresponds with fruit formation and, as with P.perfoliatus, occurs in the autumn. Variations in the ash content of P.praelongus in the limestone Loch Lanlish were not as large as that found for P.perfoliatus in Loch Croispol and values were smaller than the estimates of calcium carbonate recorded by using the method of acid addition (Chapter 1).

Four samples of the roots and rhizomes of P.praelongus collected from Loch Lanlish in May gave an average ash content of 2.7% and a mean calorific equivalent of 3866 cal/g ash-free dry weight both being lower than the corresponding figures for leaves and stems.

The data presented from the analysis of P.x zizii from Loch Uanagan is different from that of P.perfoliatus

TABLE 2.3

Ash content (% dry weight) and calorific values (cal/g dry weight and cal/g ash-free dry weight) of leaves and stems of specimens of Potamogeton x zizii from Loch Uanagan and of leaves of P.obtusifolius from Loch of the Lowes.

Month	LEAVES			STEMS		
	ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight	ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight
			LOCH UANAGAN			
July	5.6	4692	4971	4.8	5488	5762
Aug	4.0	4711	4508	4.6	4991	5231
Sept	7.3	4278	4615	6.7	3811	4067
			LOCH OF THE LOWES			
Aug	13.6	6132	7093			
Oct	8.8	7218	7915			

and P.praelongus from the same location. On all three sampling dates the calorific content of the stems was significantly different from that of the leaves. No significant difference was found among the calorific values of the leaves collected on different sampling dates and a mean of 4698 cal/g ash-free dry weight was recorded. On the other hand the calorific content of the stems dropped significantly from 4762 cal/g ash-free dry weight in July to 4067 cal/g ash-free dry weight in September. The mean of the three determinations was 5020 cal/g ash-free dry weight.

Two samples of P.obtusifolius leaves collected from Loch of the Lowes in August and October were also analysed and gave the extremely high values of 7093 cal/g ash-free dry weight (August) and 7915 cal/g ash-free dry weight (October). High values for the ash content were also recorded.

Table 2.4 summarises the mean leaf calorific content of the four specimens of Potamogeton investigated. The exceptionally high calorific value for P.obtusifolius is significantly different from all the others as is that of P.perfoliatus from Loch Vanagan: apart from P.obtusifolius which does not grow in Loch Vanagan, material from Loch Vanagan has the highest mean leaf calorific content for all the species investigated, as well as the highest actual

values.

TABLE 2.4

The mean calorific value of leaves of specimens of Potamogeton obtusifolius, P.perfoliatus, P.praelongus and P.x zizii (cal/g ash-free dry weight) collected from Lochs Croispol, Lanlish, Lowes and Vanagan.

Location	P.obtusifolius	P.perfoliatus	P.praelongus	P.x zizii
Croispol	-	4812	-	-
Lanlish	-	-	4887	-
Lowes	7504	5293	-	-
Vanagan	-	6174	5500	4698

Results of the calorific value determinations carried out with the three rosette species Littorella uniflora, Lobelia dortmanna and Isoetes lacustris are reported in Table 2.5. For both Littorella uniflora and Lobelia dortmanna the calorific content of the green photosynthetic shoot was significantly greater than that of the roots while, from both the localities, the values for Lobelia dortmanna (both shoot and root) were significantly greater than those for Littorella uniflora. No significant

TABLE 2.5

Ash content (% dry weight) and calorific values (cal/g dry weight and cal/g ash-free dry weight) of specimens of *Littorella uniflora*, *Lobelia dortmanna* and *Isoetes lacustris* from Loch Achtriochtan and Loch of the Lowes.

Species	Loch		Ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight
Littorella uniflora	Achtriochtan	(shoots)	3.2	3675	3798
	"	(roots)	3.1	3213	3285
"	Lowes	(shoots)	4.1	4163	4341
"	"	(roots)	1.9	3265	3329
Lobelia dortmanna	Achtriochtan	(shoots)	2.7	4896	5032
	"	(roots)	2.0	4308	4394
"	Lowes	(shoots)	8.1	4479	4873
"	"	(roots)	8.5	3986	4020
Isoetes lacustris	Achtriochtan	(whole plants)	3.8	3765	3913

differences in the calorific content of the plants from the two localities are apparent except for the shoots of Littorella uniflora collected in Loch of the Lowes which have a greater calorific content than those from Loch Achtriochtan. The mean combined calorific value for Littorella uniflora from Loch of the Lowes (3835 cal/g ash-free dry weight) agrees well with the mean value for a series of determinations on samples of whole plants from the same loch (3991 cal/g ash-free dry weight). The single determination carried out with whole plants of Isoetes lacustris gave a calorific equivalent of 3913 cal/g ash-free dry weight lying between the values for Littorella uniflora and Lobelia dortmanna.

Three duplicate determinations of the calorific equivalent of two species of the macroalgal genus Chara were carried out and the results are given in Table 2.6. The duplicate determinations agree well and there appears to be no significant differences between the samples. The high ash content is a result of the calcium carbonate encrustation covering these algae; while the low calorific values are in line with those predicted by Westlake (1965b). Note again the discrepancy in the values of the ash content recorded here and the estimates of calcium carbonate recorded in Chapter 1.

TABLE 2.6

Ash content (% dry weight) and calorific values (cal/g dry weight and cal/g ash-free dry weight) of specimens of Chara aspera and Chara papillosum from Lochs Croispol and Lanlish.

Species	Loch		ash content, % dry weight	cal/g dry weight	cal/g ash-free dry weight	mean cal/g ash-free dry weight
Chara aspera	Croispol	i	62.6	1144	3056	2790
		ii	59.6	1020	2524	
Chara papillosum	Croispol	i	56.6	1137	2621	2700
		ii	57.1	1194	2779	
Chara papillosum	Lanlish	i	56.2	1259	2879	2803
		ii	55.4	1217	2726	

Little data are available on the calorific content of submerged aquatic macrophytes to yield direct comparisons with the present investigations. Boyd (loc.cit.) lists calorific equivalents for several species but for none of these involved in the present study. His mean value is much lower than those recorded here for Potamogeton species which are also larger than the "grand mean" value of 4639 cal/g ash-free dry weight for aquatic primary producers quoted by Cummins and Wuycheck (loc.cit.). The present results, especially the values for P.obtusifolius must

therefore, perhaps, be viewed with caution.

Westlake (1965b) finds that there is only tentative evidence of seasonal variation in the calorific contents of freshwater macrophytes, and he deduces that variations in ash content are due to increases in deposition of calcium carbonate as has also been suggested here.

For more meaningful comparative purposes the results of the calorific contents have been expressed in terms of calories per gram ash-free dry weight as recommended by Westlake (1965b). Since most ecologists have been concerned with converting biomass data to calorific equivalents, it is also useful to present the data in the form most frequently employed in the publications of biomass data; dry weight. This allows the conversion and comparison of much of the information present in the literature although it must be stressed that such conversions can often be misleading, especially where species may be covered by marl encrustation (c.f. the 50% ash content of Chara species).

Utilising the biomass figures of Chapter 1 a value can be computed which, bearing in mind the inherent limitations, is an estimate of the quantity of energy bound up by the submerged aquatic plants in a loch. Carrying out these computations the bed of Potamogeton perfoliatus in Loch Croispol of standing crop 10 g dry weight/m^2 (Table 1.13) and mean calorific content 4.2 Kcal/g dry weight (Table 2.1)

can be expected to contain approximately 42 Kcal of energy per square metre. The P.praelongus bed in Loch Lanlish of standing crop 50 g dry weight/m^2 (Table 1.14) and approximate mean calorific content of 5Kcal/g dry weight (Table 2.2) has an energy equivalent close to 250 Kcal per square metre. Loch Banagan, from the figures of Spence et al. (unpublished and Figure 1.5) has a mixed population of Potamogeton species which from the data in Tables 2.1, 2.2 and 2.3 must have an energy value in the region of 200 Kcal/square metre. The Littorella uniflora/Lobelia dortmanna communities in Loch Achtriochtan and Loch of the Lowes, from the data in Tables 1.1 and 1.7, and using mean figures of calorific content from Table 2.5 have energy equivalents near to 600 and 120 Kcal per square metre respectively. The dense beds of the macrophytic algae Chara aspera and C.papillosum found in Loch Croispol (Tables 1.11 and 1.12) have (Table 2.6) energy equivalents of up to 3000 Kcal per square metre of loch floor covered.

These calorific totals calculated above may be more meaningful than biomass data for comparing the production of different lochs. It must be remembered, however, that these figures may be the result of many years of growth. This is the case for the Chara beds in Loch Croispol though, on the other hand, the figure of 42 Kcal/m^2 for P.perfoliatus

in the same loch represents the energy stored by the shoots of this species in only one season of growth. Calorific values also provide a useful measure of the efficiency of primary production and this is discussed in Chapter 5 of this thesis.

CHAPTER 3

PRIMARY PRODUCTIVITY

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CHAPTER 3

PRIMARY PRODUCTIVITY

INTRODUCTION

Compared with numerous studies of the production rate of submerged aquatic macrophytes based upon changes in their biomass and standing crop, there are relatively few reports on actual measurements of the process of photosynthesis under natural conditions.

Thomas (1955) and Talling (1961) have reviewed the literature dealing generally with photosynthesis under natural conditions and Verduin (1952, 1953), Rabinowitch (1951) and Altman and Dittmer (1964) have tabulated data on the photosynthetic rates of many plant species as measured under optimal, near natural conditions. Strickland (1960) has reviewed and evaluated methods useful for measuring the productivity of phytoplankton while Wetzel (1965) has discussed techniques and problems associated with the measurement of the productivity of periphyton and vascular aquatic plants. More recently Vollenweider (1969) has edited an IBP manual on methods of measuring primary productivity in the aquatic environment, concerned largely with

the techniques which have been evolved to measure photosynthesis *in situ*. A portion of this volume is concerned with aquatic macrophytes. A brief review of methods used to measure *in situ* production rates of submerged aquatic macrophytes now follows.

A very rough approximation to the gross photosynthesis of macrophytes in flowing streams can be gained by measuring the diurnal oxygen curves of the water at points along the stream. This method is fully described by Odum (1956) and has been used by Edwards and Owens (1962). These methods provide, however, only approximate values for the productivity of macrophytes and are really a measure of community metabolism. The oxygen technique has also been applied to the still waters of gravel pits in an attempt to measure the diurnal productivity of macrophytes (Goulder, 1969, 1970) but here again only approximate values can be obtained. Diurnal changes in pH and CO_2 have also been applied in a few instances to measure the productivity of macrophytes (Odum and Hoskin, 1958; Verduin, 1952) while Buttner (1960) has used changes in specific conductance of the water as his measure of photosynthetic activity. As for the oxygen studies above, such methods give a measure of the community metabolism rather than of the macrophytes alone.

Several investigators (in Wetzel, 1964a) have adapted a technique similar to that employed with phytoplankton by

enclosing portions of plant tissue in light and dark bottles and estimating the oxygen contents of the water in the bottles before and after incubation in a lake or stream. Several problems associated with this method are discussed by Nygaard (1953) and are raised again by Wetzel (1965). For instance, removing plants from the substratum with a grab, and bringing them to the surface to place them in flasks imposes upon them abnormal conditions, which may influence any productivity estimates. The main problem of such methods of determining an accurate value for the rate of photosynthesis of vascular hydrophytes lies with the basic concept, that changes in the oxygen content of water in which the plants are incubated reflects the rate of photosynthesis of the plant. This may not be so, as much of the oxygen evolved in photosynthesis can be stored in the abundant lacunal system present in the majority of aquatic angiosperms.

Hartman and Brown (1967) have demonstrated by gas chromatography that the lacunal system of several macrophyte species is composed of oxygen, carbon dioxide, nitrogen and methane, and that there is a diurnal accumulation of oxygen in the lacunal tissues and only a slow diffusion out. Results from these studies showed a lag between peak values for dissolved oxygen in the water and in the lacunae and thus the photosynthetic activity of the plant is not immediately reflected in the oxygen content of the water;

the lacunal system apparently functioning as a reservoir for metabolic gases. These observations cast grave doubts upon the validity of utilizing the dissolved oxygen content of water as the sole measure of macrophyte photosynthesis. In the present investigations an *in situ* carbon - 14 technique has been developed to measure the productivity of aquatic macrophytes and to investigate, through enrichment bioassay (Goldman, 1960), if this rate is limited by the supply of available phosphate, a nutrient known to be important for growth, yet only occurring in low concentrations in natural waters (Hem, 1970). See also Table 3.24 p. 140

METHODS

Introduction

The carbon - 14 tracer techniques commonly used for estimating the productivity of aquatic plants are thought by some authors to be simple (in Vollenweider, 1969) but, however, the interpretation of the results obtained by the carbon - 14 method is in the opinion of others (in Strickland, 1960) unfortunately not so simple.

The use of radioactive carbon to measure the uptake of carbon by marine phytoplankton was first developed by Steeman-Nielsen (1951) and countless studies on both marine and freshwater phytoplankton have been reported since, all of which show little variation in the basic technique. The literature on the use of C-14 to measure primary production of aquatic macrophytes is sparce and is dominated by the work of Petzel who was perhaps the first to realise the possibility of extending and adapting Steeman-Nielsen's (loc.cit.) original technique to both periphyton and vascular hydrophytes. The following account summarises what appears to be the most acceptable modern procedures for determining the productivity of aquatic plants using C-14.

In the C-14 technique the rate of incorporation of tracer carbon into organic matter of the plants during

photosynthesis is used as a measure of the rate of primary production. The total available carbon content of the water must be known and a definite quantity of C-14 tracer added. By determining the C-14 content of the plant tissue after the experimental exposure, it is only necessary to multiply the amount of C-14 found, by a factor corresponding to the ratio between the total available carbon in the water and the C-14 added to this at the start of the experiment, to obtain a value for the total amount of carbon fixed in the period of the experiment.

Strickland (1960) estimates that a minimum addition of 10 µCi of C-14 is necessary to gain an accurate idea of phytoplankton production in oligotrophic seas while Vollenweider (1969) advocates the use of 1 to 3 µCi per 125 ml water for phytoplankton studies in lakes of moderate productivity. Wetzel (1964b) used 10 to 20 µCi C-14 per litre of lake water in his studies with freshwater macrophytes. There is clearly no upper limit to the amount of radioactive carbon which can be added except as dictated by economy, coincidence errors in counting and health and contamination hazards. The tracer C-14 is usually added to the experimental bottles in the form of carbonate or bicarbonate and dispensed from an accurately formulated stock solution by means of a long-needled hypodermic syringe. The containers used in phytoplankton studies are generally

glass bottles and these have also been used for macrophytes by Hartman (1967) though Wetzel (1965) prefers and recommends the use of plexiglass chambers which can be placed in the substrate and enclose complete plants.

Various types of isotope detector have been employed to count the radioactivity present in samples of phytoplankton after incubation with C-14 and a Geiger-Muller (G.M.) tube is generally accepted as being adequate (Vollenweider, loc.cit.). Several workers have more recently employed gas phase counting which involves combustion of all carbon to carbon dioxide, measuring this volume and then determining the fraction of $C\text{-}14O_2$ in an ionising detector. This apparatus is 100% efficient in the detection of radioactivity and thus determines the total C-14 fixed. All other types of detector are proportional; but this, however, is no great disadvantage as all samples, whether tissue or bathing solutions, are counted with the same efficiency i.e. the same fraction of the total counts is recorded for all samples. All Wetzel's studies with aquatic macrophytes have involved gas-phase counting of the plant samples after van Slyke chemical combustion to convert organic carbon of the plants to carbon dioxide, while in his work with macrophytic marine algae, Drew (1969) has counted ethanol and acid extracts of the plant tissues using a gas flow G.M. detector.

The total photosynthetically available inorganic carbon in the water is generally calculated by measuring the pH and temperature and determining the total alkalinity by either potentiometric or colour indicator titration. Saunders et al. (1962) have constructed a convenient table of conversion factors. The C-14 in the water is usually measured by precipitating a known volume with excess barium hydroxide, filtering the precipitate and determining its radioactivity count rate.

Methods

Aqualung diving apparatus was used to collect the experimental plant material and carry out underwater manipulations. Plants were collected from carefully measured depths and brought to the surface. Leaves were detached, washed in lake water and placed in foil-wrapped, glass bottles containing 25 ml of lake water. These operations were carried out using a dark cloth to protect the plant material from direct sunlight. When all the experimental bottles had been prepared a known quantity of C-14 labelled sodium carbonate or sodium bicarbonate, usually 2 μCi, was injected with a long needle syringe through a serum cap and the bottles were shaken to ensure mixing of the isotope. The diver then returned to the collection site and clipped

the bottles on a frame anchored in the substratum (Plate 2), removing the foil from all but the dark, equilibration bottles. After a noted exposure time of approximately three hours the diver retrieved the bottles, each one being again wrapped in aluminium foil before being brought to the surface. At the shore station the leaves were removed from each bottle in turn and gently washed in distilled water. The washed leaves were outlined on paper and divided as necessary, then placed on warm aluminium planchets (2.1 cm diameter, type 25 MA) using a little albumin as adhesive. The material was heat-killed and washed with a little dilute acetic acid to remove any possible inorganic carbonate. The radioactivity of the dry planchets was measured using a Panax solid scintillation counter and scaler. All planchets were counted three times to a set count of at least 1,000. Count rates were expressed as cpm/leaf.

Two five ml aliquots of loch water from each bottle were precipitated with excess 0.1 M barium hydroxide solution and allowed to stand for 24 hours in a closed vessel. The precipitates of barium carbonate were filtered under vacuum through a Buchner funnel onto previously weighed 2.1 cm diameter glass-fibre filter papers (Whatman GF/A). These were removed from the funnel, dried, reweighed and their radioactivity measured by placing them on planchets and counting as before. The weight and area of the precipitate

was used to correct the count rate for self absorption of the beta radiation. The weight of the precipitate was also used to calculate the total inorganic carbon content of the loch water, a value which was cross-checked by the determination of the temperature, pH and alkalinity of the water and with reference to the conversion tables of Saunders et al.(1962) mentioned previously. See also Appendix 1A

From a knowledge of the specific activity of the loch water, and the count rates of the tissue and barium carbonate precipitate, a figure representing the total amount of carbon fixed by the leaf during the incubation period, was determined (a fully worked example is given in Appendix I). The area of each leaf was found by planimetry of the previously traced outline and the rates of carbon fixation were finally expressed as µg carbon fixed per square centimetre leaf area per hour.

When the plant material was collected several leaves additional to those actually required for the in situ experiment were retained. These leaves had their areas computed by the same method as the others, but in addition they were dried for 24 hours at 105°C in a force draught oven, cooled in a vacuum desiccator and then weighed. Knowing the leaf area and leaf dry weight the specific leaf area (SLA: cm^2 leaf area/mg leaf dry weight) of the plant material was found. This conversion factor permitted the

results of the productivity experiments to be given in terms of µg carbon fixed per mg leaf dry weight per hour.

Standardisation of C-14 solutions

Radioactive carbon was purchased from the Radiochemical Centre, Amersham as either $Na_2{}^{14}CO_3$ or $NaH^{14}CO_3$ and was delivered in sterile ampoules in concentrations of 1 mCi/ml. Working solutions of 4 µCi/ml were carefully prepared by dilution of the stock solution with sterile distilled water. These working stock solutions were maintained in a refrigerator in sterile, sealed containers until utilised. Standardisation of the initial source was carried out by calculation from the data supplied by the Radiochemical Centre and from the measure of the radioactivity in a barium carbonate precipitate when a known quantity of the isotope was precipitated with barium hydroxide. This method is inferior to gas-phase counting as recommended by Vollenweider (1969) and used by Wetzel (1965) but gave reproducible results with different batches of stock solution.

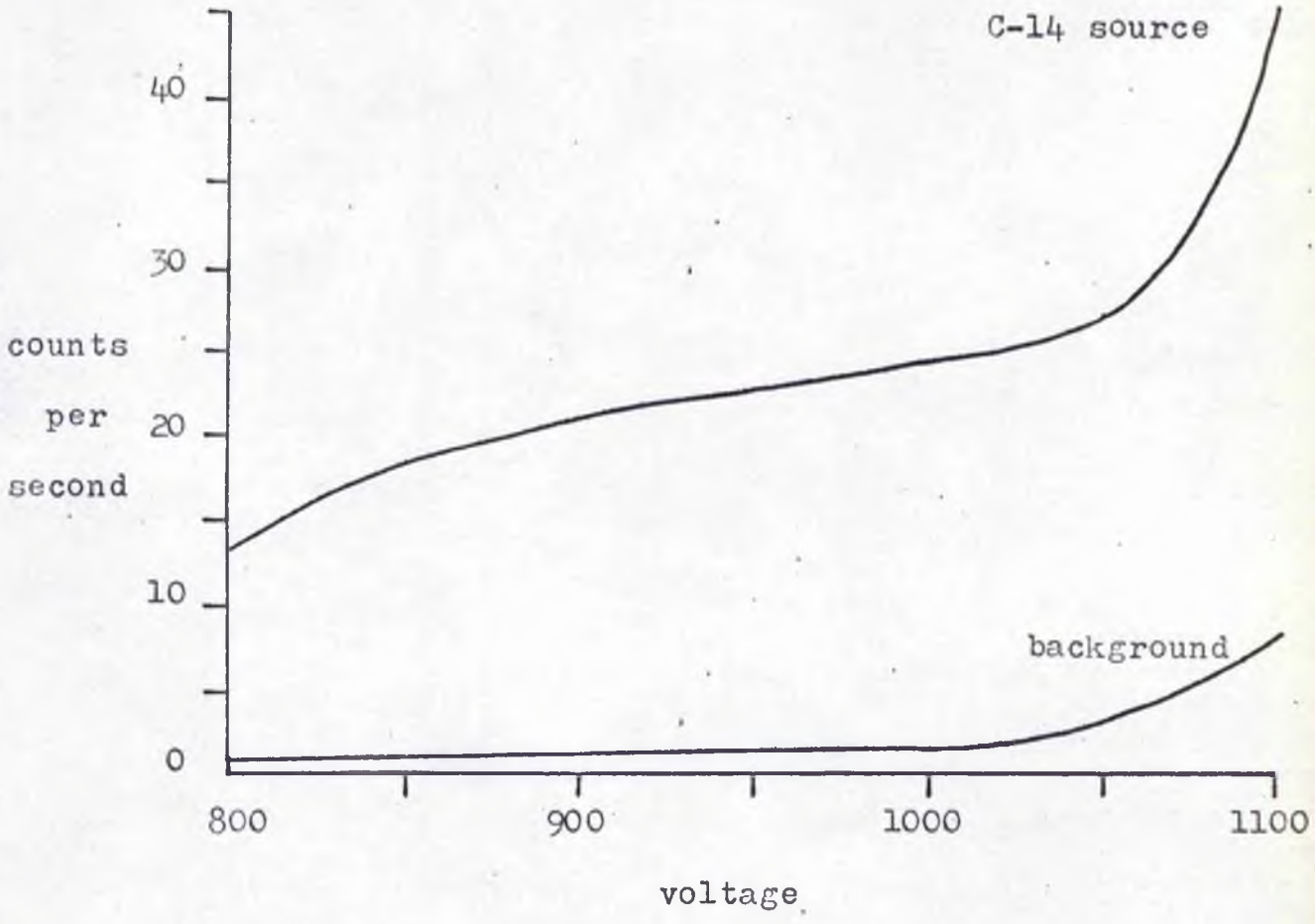
Radiation detection

All estimates of radioactivity were made using a manually operated Panax windowless solid scintillation counter employing a 1" x 0.032" anthracene crystal mounted

in a perspex disc as the fluor. The principle of operation of the apparatus relies on the fact that the ionising radiations produced from a radioactive source induce fluorescence in the anthracene crystal. The fluorescence is channelled through a photomultiplier and after being converted to an electrical pulse, which is proportional to the original magnitude of the ionising particle, is recorded on a digital scaler unit. As for a G.M. detector, the sensitivity of the detector is proportional to the voltage which is passed across it. All counting was therefore performed on the plateau of the detector response as determined by plotting the count rate of a C-14 radioactive source against voltage (Figure 3.1). Due to its electronics the detector is subject to a considerable amount of "noise" which was minimised firstly, by keeping the tube completely light-tight and secondly, by selecting an operating voltage so that the ratio of counts in a test sample to that of background was large. A plot indicating the relationship is given in Figure 3.2.

Anthracene crystals are particularly suited for the detection of the soft beta radiation produced by C-14 (0.155 MeV). The slight negligible dead time (1 μsecond) compared to that of the traditional G.M. tube (400 μseconds) lends considerable advantage at high count rates where no corrections are required to be made for undetected counts.

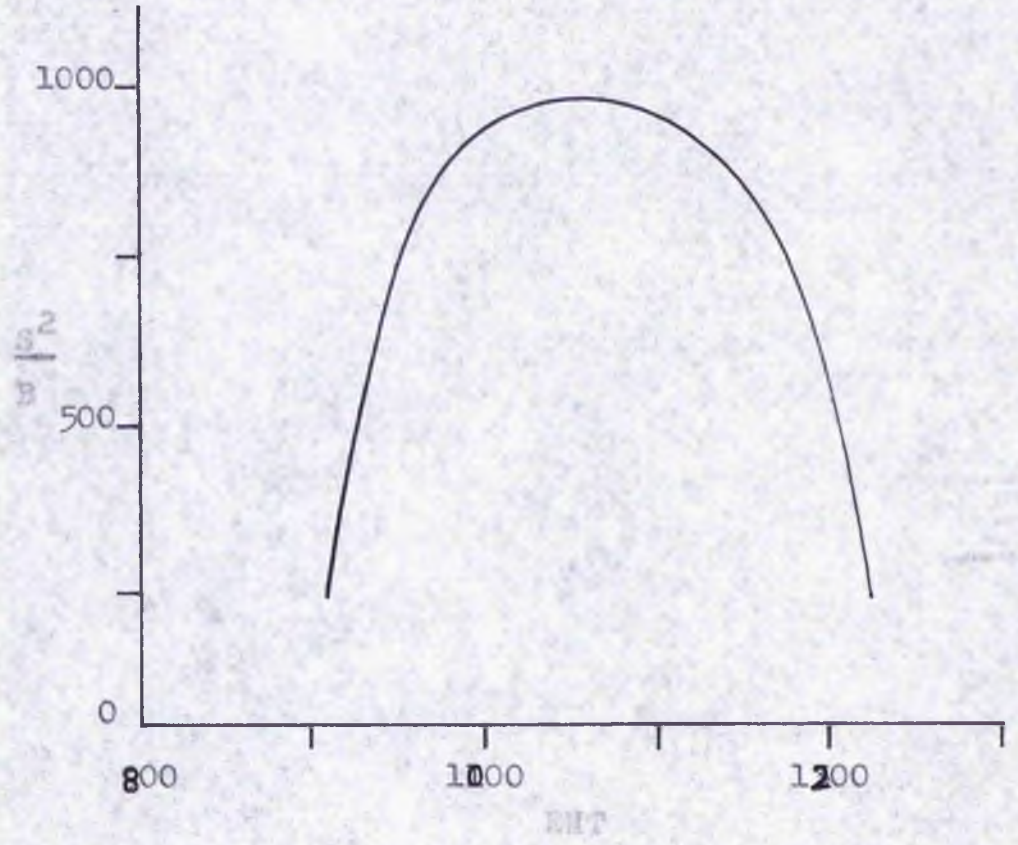
FIGURE 3.1



Voltage plateau of the solid scintillation counter

Anthracene crystals have, however, been little used in the detection of beta emissions, as most of the recently developed gas-flow GM tubes have practically the same efficiency.

FIGURE 3.2



Relationship between voltage across radiation detector (EHT) and background (B) and source (S) radiation (S^2/B plotted against EHT).

Statistics of counting

Radioactive decay is a random process and is thus subject to the normal laws of statistics. The standard deviation of any activity determinations of a radiochemical is limited according to the expression:-

$$\frac{100}{(N \times T)^{\frac{1}{2}}}$$

where N is the number of counts observed per minute from the radioactivity detector (proportional to the concentration of the radioactive substance) and T is the time in minutes during which the counting takes place. The coefficient of variation thus obtained may be used in Gaussian statistics (Strickland, 1960) and, therefore, for a 0.05 probability criterion results can be no better than $\pm$ 2% when 10,000 counts are recorded. Similarly, if less counts are recorded then the level of uncertainty due purely to the "counting error" is raised accordingly. For practical purposes in routine work it is undesirable to have counting times greater than a few minutes (especially as in the present investigation all sample changing was manual) and thus a suitable activity of isotope must be used in an experiment to give a statistically adequate count rate in a short period of time. All radioactive material was counted for a set count of not less than a 1000 counts and usually much higher

(10,000 counts) though for several low-activity samples a fixed counting period of five minutes was employed. The levels of isotope used in the experiment were chosen to produce a level of radioactivity in the plant tissues which was at least ten times that of the background radiation.

Self-absorption and scattering

The radiochemical techniques involved in working with C-14 are somewhat complicated by the fact that this isotope emits only a very soft beta radiation (0.155 MeV). This subject is covered extensively by the treatise of Calvin et al.(1949). The main radiochemical problem which faces users of C-14 for primary productivity estimates is self absorption; that is, the absorption of the weak beta particles by the same substance from which they are being emitted. The beta rays from C-14 are both absorbed and scattered. Absorption is mainly a function of thickness (mass/unit area or mg/cm^2). Because of a near cancellation of several effects the final absorption pattern of the beta particles is approximately exponential with the thickness of the absorber (Strickland, 1960).

If A_g^0 is the activity (cpm) per unit thickness at zero thickness, and Ag is the observed activity per unit thickness when the thickness is g mg/cm^2, then the relationship:-

$$A_g = A_0^0 \times \frac{1 - \exp(-N \times g)}{N \times g}$$

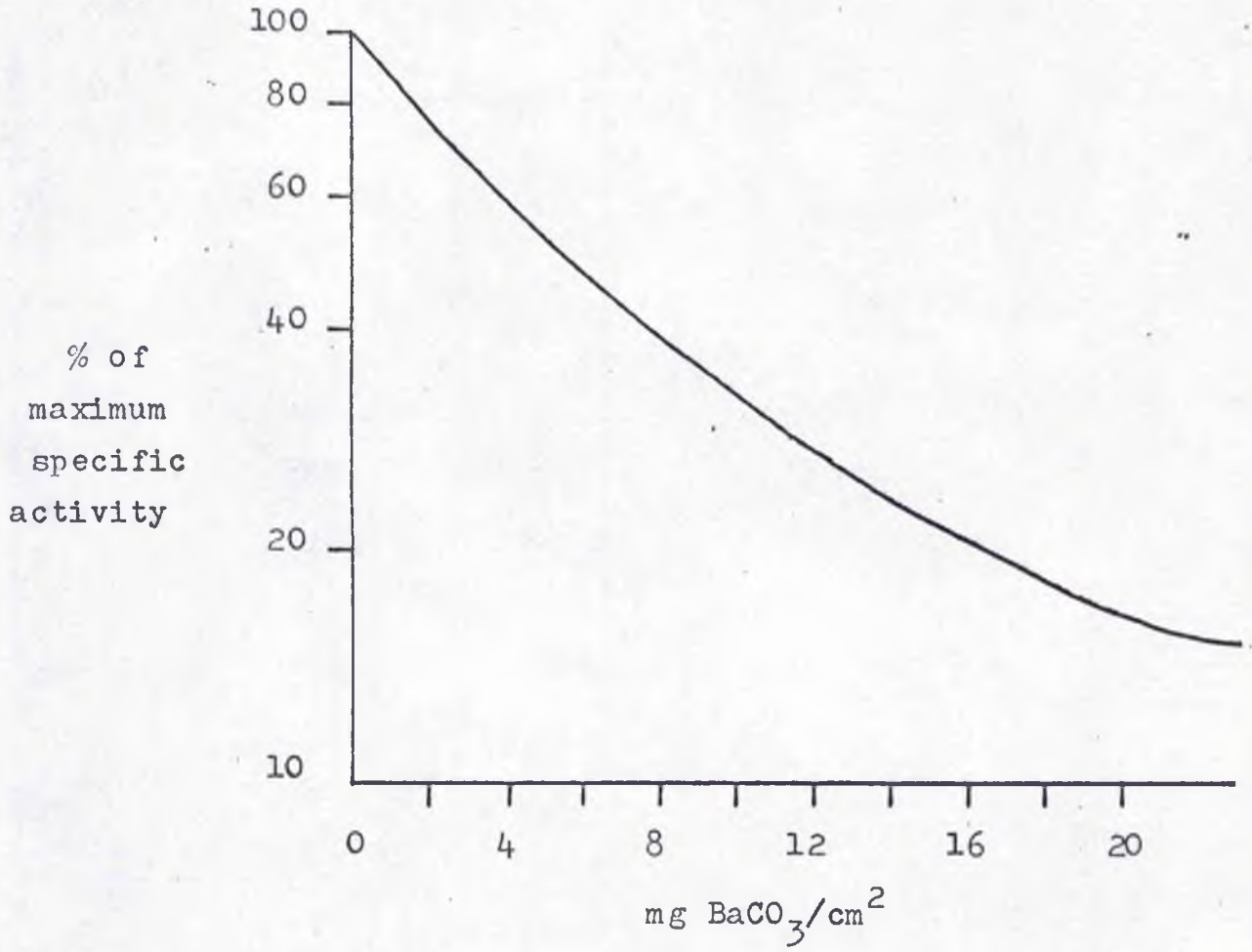
(Strickland, 1960)

holds to a good approximation of g up to 10 mg/cm^2. N is known as the self absorption coefficient and the equation assumes that the specific activity of the isotope is the same for all the samples.

In the present investigation a barium carbonate self-absorption curve was prepared (Figure 3.3) according to the method of Steeman-Nielsen (1952) by which a series of solutions containing increasing amounts of inorganic carbon but the same quantity of C-14 were precipitated with barium hydroxide and the resulting barium carbonate precipitate filtered onto a constant mass pre-weighed filter, dried, reweighed and then counted. Accordingly each filter should have theoretically the same activity (cpm) but the measured activity decreased with increasing thickness. The plot of count rate against weight of precipitate per unit area was extrapolated to zero thickness and the curve presented in Figure 3.3 was constructed and used to estimate the true activity of the barium carbonate precipitates.

The above technique, though common, has been criticised by Hendler (1959), Sorokin (1962) and by Vollenweider (1969) and, while its slight inaccuracy is recognised, it is considered acceptable for the present purposes since modifications advocated by other workers, although often complicated

FIGURE 3.3



Self-absorption correction curve for barium carbonate

experimentally, have resulted in little difference in the shape of the self absorption curve over the range required in the present study. The self absorption curve was used to calculate absolute count rates for the barium carbonate precipitates of the loch water. The plant tissue was counted at zero thickness.

Unlike self absorption the scattering of beta particles depends upon the nature and thickness of the material causing the scatter. The nature of these scattering effects and the geometry of the counting assembly used, make it impossible to make any generalisations (Strickland, 1960). It is, however, usually recognised that scattering and geometric effects are relatively constant unless much extraneous solid matter is present. Both self absorption and scattering can, however, be elegantly overcome by adopting a gas-phase counting technique which is 100% efficient. Such a solution has also been adopted by Wetzel (1965) and Hartman (1967).

Discussion of Methods

If the amount of organically bound C-14 in the plant tissues is to represent the absolute measure of gross photosynthesis the following conditions must be fulfilled:-

(1) The rate of assimilation of C-14 must be the same as C-12.

(2) No C-14 must be incorporated into organic compounds except through photosynthesis.

(3) No C-14 must be lost through respiration.

(4) No organic matter must be lost by excretion.

It is generally accepted that none of these conditions are completely fulfilled. At high light intensity the importance of some of them may be only slight but many workers find it necessary to introduce a correction term in their calculations of carbon fixation to cover all of these inaccuracies.

Some discussion has centred around the problem of isotopic discrimination between C-12 and C-14 isotopes of carbon during photosynthesis and in general, there has been difficulty in deciding what isotope discrimination factor should be applied to allow for the fact that the rate of primary carboxylation by the heavier isotope of carbon will theoretically be slower than the natural process. The subject is viewed from the standpoint of phytoplankton photosynthesis by Steeman-Nielsen (1955) who advocates what

Strickland (1960) terms the "commonsense" figure of 5 to 6% and this has been used by several workers, in phytoplankton studies. As yet no discrimination factors have been specifically determined for macrophytes and the few workers (Wetzel, 1965; Hartman, 1967) who have used C-14 to study their productivity have adopted the 5 to 6% figure of Steeman-Nielsen (loc.cit.). In the present study no isotopic discrimination factor has been used and the estimates of primary productivity may therefore be correspondingly small. It is felt, however, along with Jitts (1957), that in the absence of what is known to be an accurate value for a discrimination factor none should be applied and the results viewed accordingly. This is held to be preferable to applying some composite correction term which supposedly accounts for an accumulation of immeasureable inaccuracies.

While the C-14 method is perhaps among the most sensitive yet developed for the determination of photosynthetic carbon fixation by plants, interpretation of the results from its application presents some difficulties. The greatest concern is over the question of whether the method measures net or gross photosynthesis, or some value between these two. The literature dealing with the subject is large. Basically the problem revolves around the question of whether or not compounds containing newly fixed carbon can be quickly recycled through respiration and in general,

respiratory carbon reutilised in photosynthesis.

In his review of primary productivity methods for marine phytoplankton Strickland (1960) concludes that in suitably designed experiments the C-14 method gives a measure between gross and net photosynthesis but that the position may vary between one species and another. It is possible that C-14 uptake values are nearer to net than to gross photosynthesis but this is not established as a universal rule for all species of marine phytoplankton. It may, however, be assumed from the studies of several workers (in Steeman-Nielsen, 1957) that C-14 labelled carbon atoms taken into algal cells are not normally respired or lost by autolysis to any appreciable extent for at least five to ten hours and therefore any short term C-14 uptake experiments will give a measure of photosynthesis close to net values while, if longer exposure times are employed (Vollenweider and Nauwerck, 1961), the situation is more complicated and difficult to interpret.

Little is known of the respiratory rates of aquatic macrophytes (in Spence and Chrystal, 1970) but under good light conditions they are recognised to be low in comparison to photosynthesis. Few workers have attempted to measure macrophyte respiration <u>in situ</u> (Wetzel, 1965) and the problem as to whether C-14 fixed by photosynthesis is recycled through respiration has not been directly investigated. However

it is considered safe to assume that a similar situation exists for macrophytes as there does for phytoplankton and it is recommended (Vollenweider, 1969) that the results of short term C-14 experiments be treated as corresponding to approximate net rates of photosynthesis. Support for this viewpoint comes from the work of Hartman and Brown (1967) who did not find any C-14 labelled carbon dioxide in the lacunal tissues of Elodea species during short-term C-14 productivity experiments. In the present studies the rates of photosynthesis measured using the C-14 technique are considered to approximate to the net rates.

When plant tissues containing predominantly only one isotopic form of carbon (C-12) are placed in an environment containing a substantial amount of another carbon isotope (C-14) then some physical exchange will occur between the two isotopic forms irrespective of that due to photosynthesis or respiration. By diffusion and exchange, ions of the medium external to the tissue can migrate into regions in cells and tissues without becoming involved in either metabolic processes or transport systems. Cations such as carbonate are subject to these reversible exchanges with the apparent free space (AFS) of the plant which, according to Wetzel (1969), can contribute up to 10% of the plant volume. This diffusion pattern obeys the laws of diffusion kinetics and thus occurs with equal magnitude in light or dark

conditions. Strickland (1960) recommends that such isotopic exchange cannot be neglected and for this reason in all the C-14 productivity experiments carried out in the present study at least one bottle in any experimental treatment has been a dark equilibration, control bottle.

The non-photosynthetic uptake of inorganic carbon without actual fixation into organic compounds is also thought (Wetzel, 1965) to occur to a small degree by an active process, and this is possibly greater in the light than in the dark. In the present studies it has been impossible to differentiate between an actual dark uptake of carbon and a physical exchange of the different isotopic forms and thus the dark bottles give a combined estimate of both of these processes. In the calculation of the C-14 fixed in the productivity experiments the "dark" values are assumed to account for all of the non-photosynthetic uptake. Hartman (1967) has shown that, though "dark fixation" rates of aquatic macrophytes appeared to vary with water temperature, under favourable light conditions "dark fixation" was seldom above 1% of the measured fixation rates in the light. This figure is in agreement with the results obtained from the algal studies of Steeman-Nielsen (1960) and with what has been predicted by Strickland (1960). Subtracting the dark "equilibration" values of the C-14 fixed from the light values renders a figure for carbon fixation which, in the

present investigations, is assumed to have been incorporated into the plant tissues in no other way apart from photosynthesis.

It is known that, under certain conditions, an appreciable quantity of C-14 labelled photosynthesate can be excreted from the cells of aquatic macrophytes (Wetzel, 1965, 1969b) during photosynthesis. This presents a possible source of error in the use of C-14 techniques to measure primary productivity. Little is known of this topic, however, (Chapter 4) and while it is generally recognised that excretion occurs (Wetzel, 1965) and that the magnitude of such losses is dependent upon the environmental conditions (Wetzel, 1969b), no precise in situ measurements have been made. Extracellular losses are not accounted for in the calculations presented in Appendix I and are considered unimportant where only estimates of net photosynthesis are required. The magnitude of extracellular losses are important, however, when estimates of gross photosynthesis are required. These excreted products of photosynthesis must not be confused with the extracellular contamination which results from the precipitation of the tracer radioactive carbon as monocarbonates (Wetzel, 1965). Such contamination on the surface of the plant tissue, which would introduce another error in the C-14 method was, in the present study, removed by washing with dilute acetic acid. Wetzel (1969,

in Vollenweider) believes this a simpler than, but inferior method to, exposure to fumes of hydrochloric acid.

Another serious error in the radiocarbon technique could arise if a substantial fraction of the carbon taken from the water by plants were in the form of carbamino complexes rather than simple inorganic carbon, as may be the case for marine phytoplankton (Neuberg et al.,(1957). The possible magnitude of such errors is unknown but could possibly be high in water of high organic content and in experiments of short duration. In the experiments reported here it is assumed that all the carbon incorporated by photosynthesis originates from simple inorganic forms.

Perhaps the greatest possible error in the C-14 method of estimating productivity is the possible deleterious effects of the presence of a radioactive element. Holm-Hansen et al.(1958) have, however, provided the much-needed evidence that, in fact, the presence of radioactive carbon per se has no short term adverse effects on the metabolism of plants.

RESULTS

Estimates of the primary productivity of submerged macrophytes have been carried out in Loch of the Lowes, Loch Leven, Loch Vanagan and in Lochs Borralie, Croispol and Lanlish, the three limestone lochs near Durness, Sutherland (Map 1). For water chemistry data see Table 3.24 p.140

Loch of the Lowes

Results from the first experiment with Potamogeton perfoliatus are given in Table 3.1.

TABLE 3.1

Loch of the Lowes 29.6.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus when incubated with loch water and loch water + 20 ug phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

Loch water	Loch water + phosphate
0.49 ± 0.13[1]	1.43 ± 0.10
Number of replicates	4
Incubation depth	2.5 m
Water temperature	13°C

1 Standard error of the mean (Bailey, 1959)

The addition of phosphate to the loch water significantly[1] increased the mean rate of carbon fixation of the P.perfoliatus leaves.

The results given in Table 3.2 are from the second experiment which was carried out with both P.obtusifolius and P.perfoliatus.

TABLE 3.2

Loch of the Lowes 12.8.70

Mean rates of carbon fixation by leaves of Potamogeton obtusifolius and P.perfoliatus when incubated with loch water and with loch water + 20 µg phosphate as K_2HPO_4

(µg carbon/cm^2 leaf area/hour)

	Loch water	Loch water + phosphate
P.obtusifolius	6.13 ± 2.51	33.37 ± 0.02
P.perfoliatus	5.66 ± 0.02	30.53 ± 4.80

Number of replicates 4

Incubation depth 3 m

Water temperature 17°C

1 In all instances means are taken to be significantly different when the addition or subtraction of their errors produces no overlap in these values.

These results indicate that, firstly, the basic mean rates of carbon fixation of the two species were not significantly different and that, secondly, these basic rates were increased by the addition of phosphate to the loch water. The degree of enhancement was approximately the same for both species. These results are presented in the form of a histogram in Figure 3.4.

The last of the experiments in Loch of the Lowes was with P.obtusifolius. The mean rate of carbon fixation of leaves of this species is given in Table 3.3.

TABLE 3.3

Loch of the Lowes 19.10.70

Mean rate of carbon fixation by leaves of Potamogeton obtusifolius (µg carbon/cm^2 leaf area/hour).

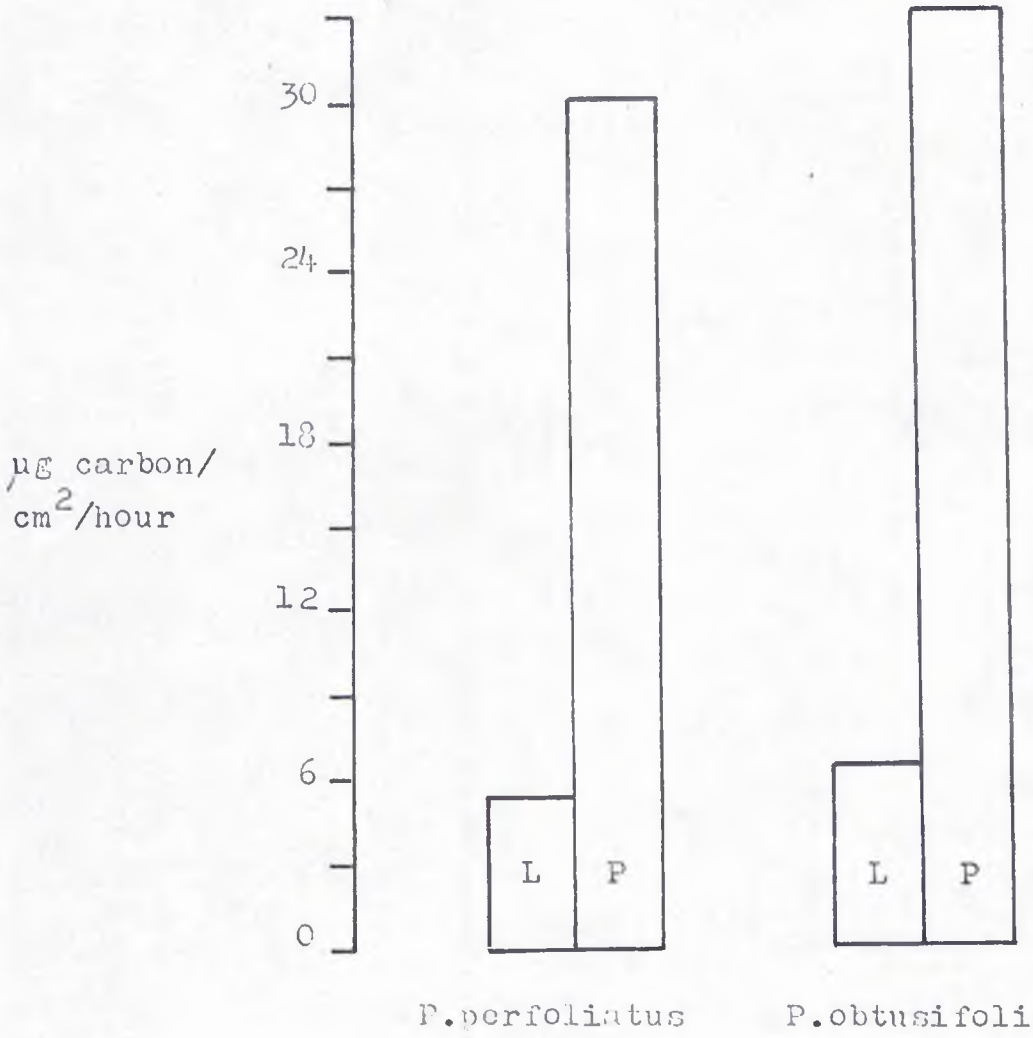
2.89 ± 0.42

Number of replicates 10

Incubation depth 1.5 m

Water temperature 9°C

FIGURE 3.4



Mean rates of carbon fixation by leaves of Potamogeton obtusifolius and P.perfoliatus measured in Loch of the Lowes on 12.8.70, when incubated with loch water (L) and with loch water + 20 µg phosphate (P) as K_2HPO_4 (µg carbon/cm^2 leaf area/hour). Incubation depth was three metres and water temperature 17°C.

Loch Leven

The first of the Loch Leven experiments was carried out with plant material of P.obtusifolius originating from the laboratory and allowed to grow in the loch for one month.

TABLE 3.4

Loch Leven 3.7.70

Mean rate of carbon fixation by leaves of Potamogeton obtusifolius after one month of growth in Loch Leven (μg carbon/cm^2 leaf area/hour).

	9.37 ± 1.90
Number of replicates	8
Incubation depth	2 m
Water temperature	$14^{\circ}C$

Results are given in Table 3.4 which records the fact that an appreciable rate of carbon fixation has been measured from specimens of an aquatic macrophyte after growth in a loch characteristically poor of macrophytic vegetation.

Having established that macrophytes could photosynthesise in Loch Leven two more experiments were carried out to investigate the effect of addition of phosphate on the rate of carbon fixation. The first of these experiments

was carried out with leaves of P.obtusifolius collected on the previous day from Loch of the Lowes. It was hoped to compare specimens from the two lochs but no P.obtusifolius could be found on that occasion in Loch Leven.

TABLE 3.5

Loch Leven 13.3.70

Mean rates of carbon fixation by leaves of Potamogeton obtusifolius from Loch of the Lowes, when incubated with water from Loch Leven and Loch of the Lowes and with these loch waters + 20 µg phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

Loch Leven water	Loch Leven water + phosphate	Loch of the Lowes water	Loch of the Lowes water + phosphate
1.90 ± 0.20	2.03 ± 0.99	2.06 ± 0.55	5.49 ± 1.11

Number of replicates 4

Incubation depth 3 m

Water temperature 17°C

The results presented in Table 3.5 illustrate that the rates of carbon fixation of the leaves in both Lowes and Leven water were practically the same. The addition of 20 µg of phosphate as K_2HPO_4 to the 25 ml of bathing solution in each bottle produced a significantly increased mean rate of

carbon fixation only in the Loch of the Lowes water.

One other *in situ* experiment was carried out in Loch Leven in December 1970 with *P.obtusifolius* leaves collected from plants growing in the harbour, where specimens of this species were abundant at this time. Results are expressed in Table 3.6.

TABLE 3.6

Loch Leven 17.12.70

Mean rates of carbon fixation by leaves of *Potamogeton obtusifolius* when incubated with loch water and with loch water + 20 µg phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

Loch water	Loch water + phosphate
1.15 ± 0.16	16.03 ± 1.51

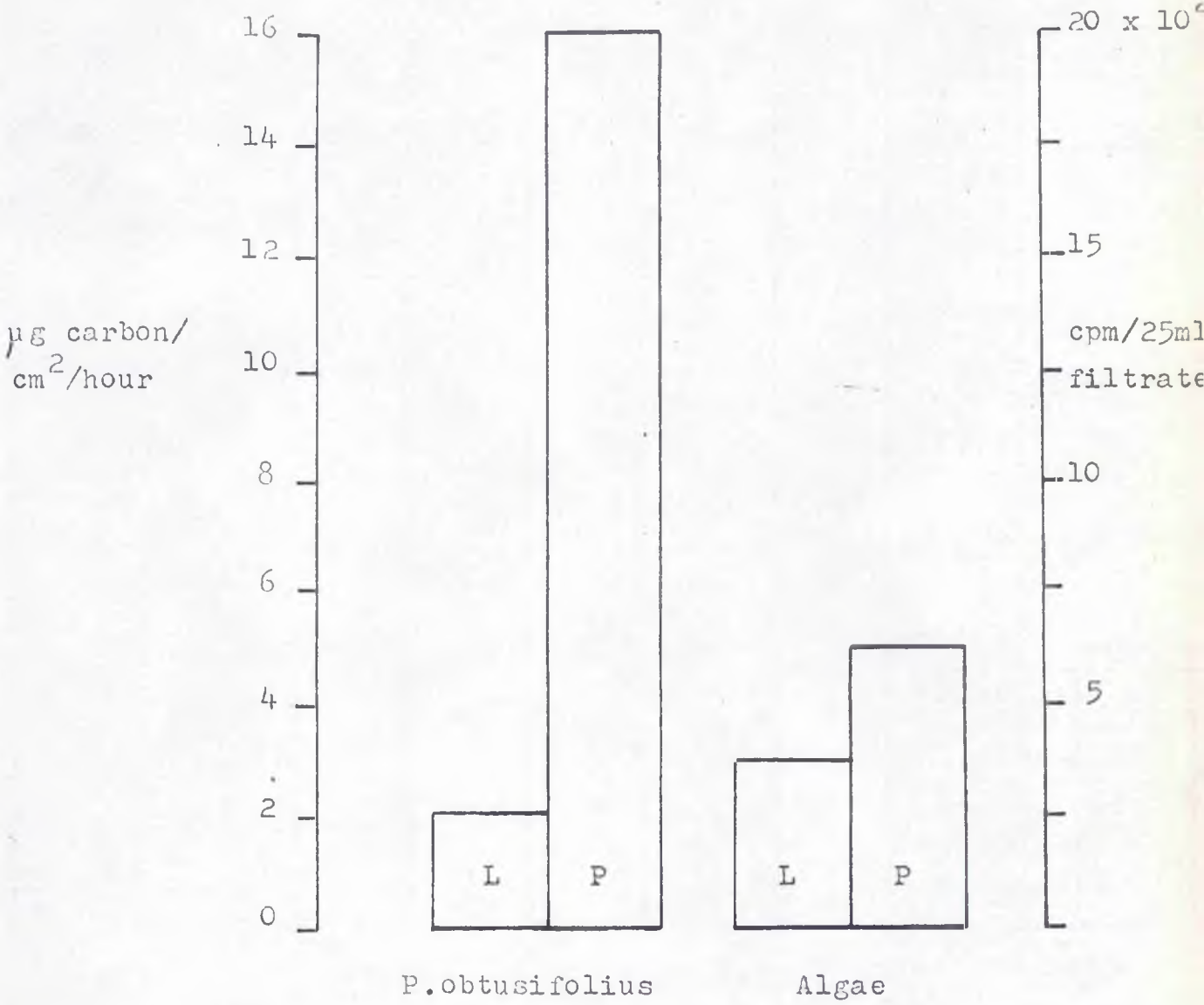
Number of replicates 5

Incubation depth 0.5 m

Water temperature 4°C

The addition of phosphate to the loch water produced a ten fold increase in the rate of carbon fixation of the leaves. It would appear that the addition of phosphate to the loch water somehow provided a more favourable climate for photosynthesis. It is of interest here, that, on the particular date of the experiment, there was no prominant algal bloom

FIGURE 3.5



Mean rates of carbon fixation by leaves of Potamogeton obtusifolius measured in Loch Leven on 17.12.70, when incubated with loch water (L) and loch water + 20 µg phosphate (P) as K_2HPO_4 (µg carbon/cm^2 leaf area/hour). Algal carbon fixation rate in the same incubation bottles expressed as cpm/25 ml filtrate. Incubation depth was 0.5 metres and water temperature 4°C.

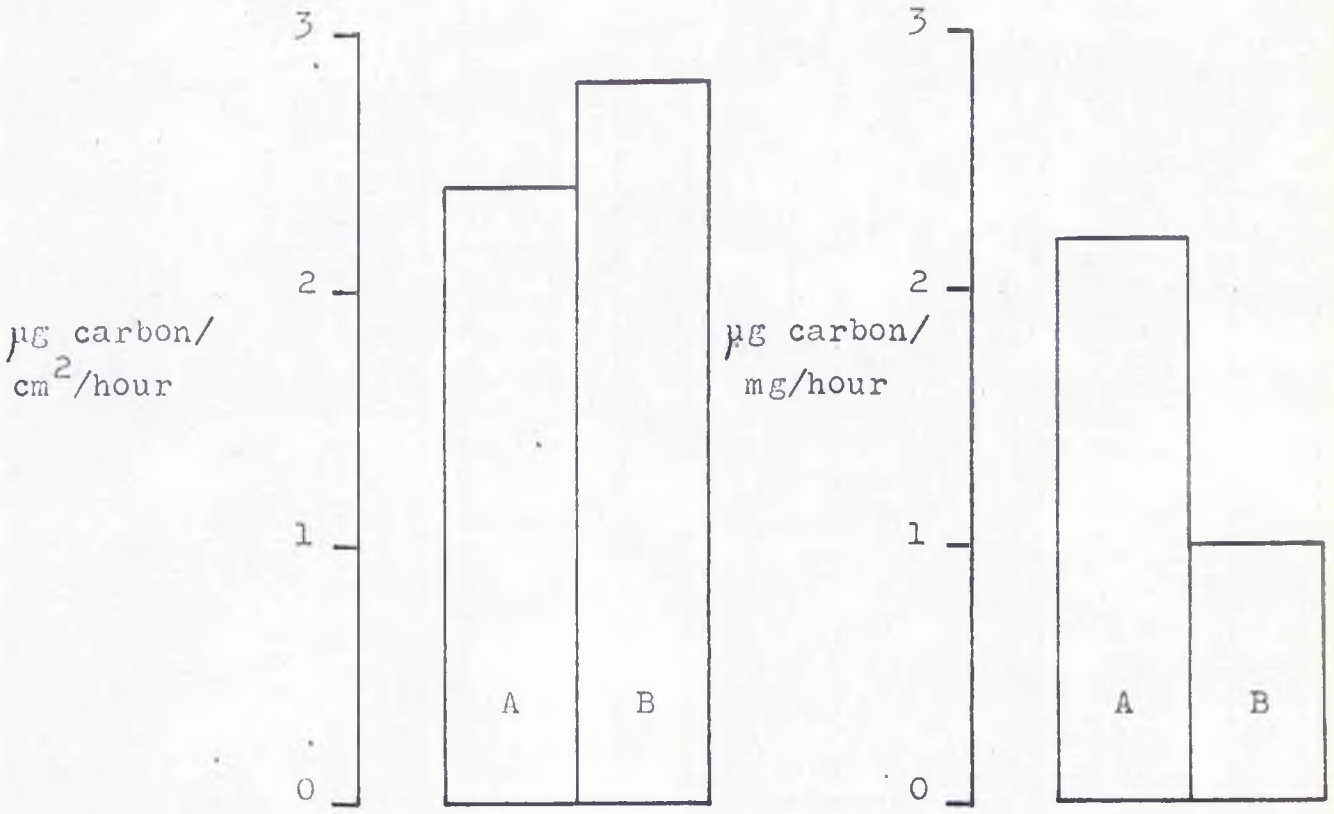
in the loch. The C-14 activity of the algae in the incubation bottles was estimated after filtration onto Whatman GF/C glass fibre filters and the results are histogrammed with those of P.obtusifolius in Figure 3.5. It thus appears that not only is the primary productivity of the P.obtusifolius leaves increased upon the addition of phosphate to the loch water but also that of the planktonic algae.

Loch Uanagan

Three experiments were carried out in this loch in July 1970 to determine the relative carbon fixation rates of P.perfoliatus and P.praelongus. The first experiment was carried out between 1040 hours and 1400 hours and the second between 1345 hours and 1700 hours. Both experiments were incubated at a water depth of 2.5 m where, in this loch, the zone of P.praelongus and P.perfoliatus overlap, being the deep limit for P.praelongus and the shallow limit for P.perfoliatus. Results are presented in Table 3.7. Bearing in mind the errors attached to the estimates there seems little evidence to support any great variation between the carbon fixation rates of the two species.

Figure 3.6 presents the data of Table 3.7 in the form of a histogram and the same rates of carbon fixation expressed on a leaf weight basis.

FIGURE 3.6



Mean rates of carbon fixation by leaves of Potamogeton perfoliatus (A) and P.praelongus (B) measured in Loch Uanagan on 15.7.70 (µg carbon/cm^2 leaf area/Hour and µg carbon/mg leaf dry weight/ hour). Incubation depth was 2.5 metres and water temperature 12.5°C.

TABLE 3.7

Loch Uanagan 15.7.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus and P.praelongus (µg carbon/cm^2 leaf area/hour).

	Experiment A (morning)	Experiment B (afternoon)
P.perfoliatus	2.87 ± 0.46	3.29 ± 0.64
P.praelongus	2.24 ± 0.33	2.31 ± 0.72
Number of replicates	4	
Incubation depth	2.5 m	
Water temperature	12.5°C	

The productivity of both species as measured on a leaf area basis is similar but when one takes into account the SLA (0.77 for P.perfoliatus, 0.35 for P.praelongus) P.perfoliatus, the deeper ranging species, is in fact almost twice as effective in increasing its organic matter per unit leaf weight.

The third primary productivity experiment carried out in Loch Uanagan was with P.perfoliatus and consisted of incubating bottles at two water depths; 2.5 m as in the previous experiments, at the start of the P.perfoliatus beds and 4 m, at the depth limit for this species. Results are presented in Table 3.8.

TABLE 3.8

Loch Uanagan 16.7.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus incubated at 2.5 m and 4 m depth of water with loch water and loch water + 20 µg phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

	2.5 m depth	4 m depth
Loch water	2.32 ± 0.24	1.76 ± 0.07
Loch water + phosphate	4.10 ± 1.00	2.51 ± 0.46

Number of replicates 6

Water temperature 12.5°C

The mean rate of carbon fixation of leaves incubated at 2.5 m was greater than that of those incubated in the deeper water. In both cases the addition of phosphate to the loch water stimulated the rate of carbon fixation.

Lochs Borralie, Croispol and Lanlish

The largest series of C-14 productivity studies was carried out in the three limestone lochs (Borralie, Croispol and Lanlish) near Durness, Sutherland. Investigations were originally concentrated on Loch Croispol and then extended to cover the other two, more inaccessible lochs.

In the light of the previous investigations in other lochs, the first experiment in Loch Croispol was designed to measure and compare the extent of any influence of additions of different forms of phosphate to the loch water upon the rate of carbon fixation by leaves of P. perfoliatus. The rates of carbon fixation of the P. perfoliatus leaves are given in Table 3.9. Under the conditions of the experiment the addition of phosphate to the loch water did not significantly alter the mean rate of carbon fixation of the P. perfoliatus leaves.

Table 3.10 presents the results from two further experiments in Loch Croispol. These experiments were carried out in the morning and in the afternoon. The mean rates of carbon fixation by the leaves in the two experiments are not significantly different. The addition of phosphate to the loch water did, however, produce an increase in these mean rates.

TABLE 3.9

Loch Croispol 4.8.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus when incubated with loch water and loch water + 20 µg of phosphate; the phosphate being added in three different forms (µg carbon/cm^2 leaf area/hour).

Loch water	Loch water + KH_2PO_4	Loch water + K_2HPO_4	Loch water + NaH_2PO_4
1.90 ± 0.83	2.09 ± 0.81	0.91 ± 0.23	2.05 ± 1.00

Number of replicates 4

Incubation depth 4.5 m

Water temperature 15°C

TABLE 3.10

Loch Croispol 5.8.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus when incubated with loch water and loch water + 20 µg phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

	Experiment A 0915-1245 hours	Experiment B 1245-1645 hours
Loch water	1.39 ± 0.28	1.83 ± 0.55
Loch water + phosphate	6.91 ± 0.86	4.19 ± 0.48

Number of replicates 5

Incubation depth 4.5 m

Water temperature 15°C

One further experiment was designed to compare the mean carbon fixation rate of leaves of P.perfoliatus from Loch Croispol with that of leaves of P.praelongus from Loch Lanlish. The incubation was carried out in Loch Croispol, specimens being collected from Loch Lanlish immediately prior to the experiment and kept in dim light until required.

TABLE 3.11

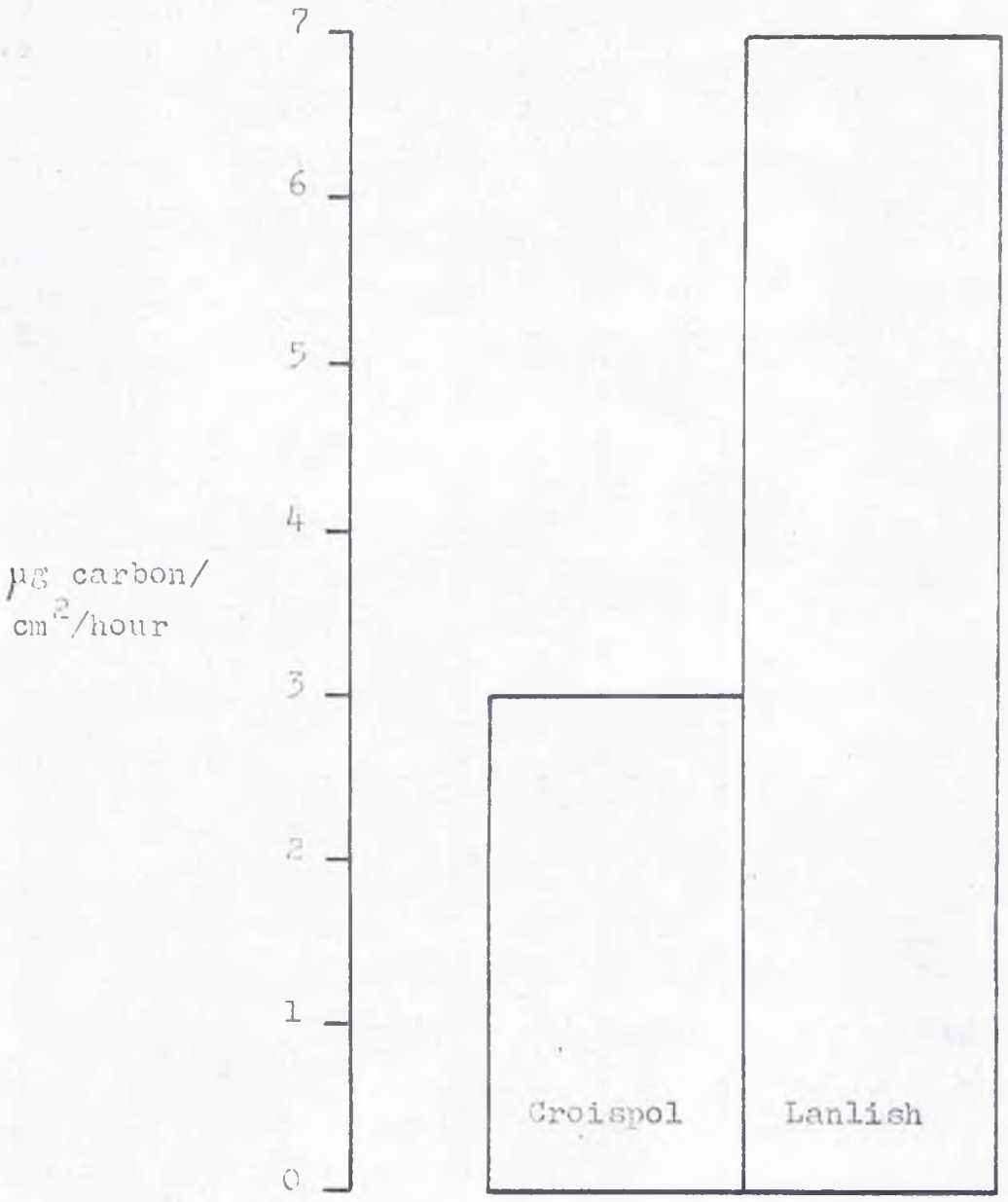
Loch Croispol 6.8.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus from Loch Croispol and P.praelongus from Loch Lanlish when incubated with water from Loch Croispol and Loch Lanlish (μg carbon/cm^2 leaf area/hour).

	P.perfoliatus	P.praelongus
Loch Croispol water	2.40 ± 0.21	2.82 ± 0.35
Loch Lanlish water	1.91 ± 0.06	6.87 ± 0.83
Number of replicates	4	
Incubation depth	4.5 m	
Water temperature	15°C	

The results presented in Table 3.11 and Figure 3.7 illustrates that the two loch waters induced a significantly different rate of carbon fixation in both species though, for each species, the highest mean rate was recorded from the "parent"

FIGURE 3.7



Mean rates of carbon fixation by leaves of Potamogeton praelongus measured in Loch Croispol on 6.8.70, when incubated with water from Loch Croispol and water from Loch Lanlish, where the specimens were collected (µg carbon/cm^2 leaf area/hour). Incubation depth was 4.5 metres and water temperature 15°C.

loch water. P.praelongus produced mean rates of carbon fixation greater than those of P.perfoliatus but this difference was non-significant in the case of the incubations in Croispol water. There is here, however, a situation similar to that already described in Loch Uanagan, where the two species have different SLA values. Table 3.12 presents the rates of carbon fixation in terms of µg carbon fixed per mg leaf dry weight.

TABLE 3.12

Loch Croispol 6.8.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus from Loch Croispol and P.praelongus from Loch Lanlish when incubated with water from Loch Croispol and Loch Lanlish (µg carbon/mg leaf dry weight/hour).

	P.perfoliatus	P.praelongus
Loch Croispol water	2.53	1.54
Loch Lanlish water	2.01	3.75

Although, on the basis of comparison, the leaves of P.praelongus still possess the higher rate of carbon fixation in Lanlish water, leaves of P.perfoliatus in Loch Croispol water are increasing their organic matter content at a faster rate than those of P.praelongus incubated in the same water. This rate is not as far removed from the rate

of P.praelongus in Lanlish water as is suggested by the comparison on a leaf area basis.

Three in situ experiments with P.perfoliatus were carried out in Loch Croispol in September 1970. Two experiments were carried out in the afternoon and one in the morning. The afternoon experiments are reported in Table 3.13.

TABLE 3.13

Loch Croispol 16.9.70 and 17.9.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus when incubated with loch water and with loch water + 20 µg phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

	Experiment A 16.9.70	Experiment B 17.9.70
Loch water	7.32 ± 0.62	2.71 ± 0.60
Loch water + phosphate	20.72 ± 1.03	12.27 ± 1.49
Number of replicates	6	
Incubation depth	3.5 m	
Water temperature	11°C	

In both experiments the rate of carbon fixation of the leaves was stimulated by the addition of phosphate to the loch water, although climatic conditions during the two incubations were vastly different, there being much more sunshine on 16.9.70 than 17.9.70.

The one morning experiment is reported in Table 3.14. The same pattern is seen here as in the previous two experiments. A greater rate of carbon fixation was found in the leaves incubated in the loch water with the addition of phosphate

TABLE 3.14

Loch Croispol 17.9.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus when incubated with loch water and loch water + 20 µg phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

Loch water	Loch water + phosphate
3.70 ± 0.94	9.83 ± 1.96

Number of replicates 6

Incubation depth 3.5 m

Water temperature 11°C

One experiment was carried out in Loch Croispol in October. Plant material was collected from five metres but the weather conditions made it necessary to incubate the leaves in shallow water (0.5 m). Results are presented in Table 3.15. Again stimulation of the carbon fixation rate of the leaves was induced by the addition of phosphate to the loch water.

TABLE 3.15

Loch Croispol 29.10.70

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus when incubated in loch water and loch water + 20 µg phosphate as K_2HPO_4 (µg carbon/cm^2 leaf area/hour).

Loch water	Loch water + phosphate
0.99 ± 0.08	2.42 ± 0.33

Number of replicates 6

Incubation depth 0.5 m

Water temperature 5°C

No *in situ* experiments were carried out in the Durness lochs over the winter months but in March three laboratory experiments were undertaken with freshly collected plant material.

In the first of these experiments leaves of *P.praelongus* from Loch Lanlish were incubated in the field laboratory, with loch water and with various added quantities of phosphate. The rates of carbon fixation recorded in Table 3.16 are low when compared to some previous estimates. When the incident light intensity is taken into account, however, then the basic rates are in the region of 1 µg C/cal. Underwater light measurements in relation to primary productivity are discussed in Chapter 5. Only the addition of 390 mg/l

K_2HPO_4 to the loch water significantly influenced the rate of carbon fixation of the leaves.

TABLE 3.16

Laboratory 25.3.71

Mean rates of carbon fixation by leaves of Potamogeton praelongus from Loch Lanlish when incubated with loch water and loch water + various concentrations of phosphate as K_2HPO_4.

Addition of phosphate to loch water (as mg/l K_2HPO_4)	Carbon fixation rate (µg carbon/cm² leaf area/hour)
0	0.31 ± 0.07
0.39	0.43 ± 0.09
3.90	0.25 ± 0.10
39.00	0.42 ± 0.08
390.00	0.92 ± 0.17

Water temperature 6°C

The second experiment was with P.praelongus collected from Loch Borralie and involved the measurement of the carbon fixation rate of leaves of this species when incubated in their natural loch water and in loch water with additions of phosphate, nitrate and of both these nutrients, in the concentrations present in Gorham's medium (Hughes et al. 1958, and Table 7.2 in Chapter 7).

TABLE 3.17

Laboratory 26.3.71

Mean rates of carbon fixation by leaves of Potamogeton praelongus from Loch Borralie when incubated in loch water, loch water + 0.496 g/l $NaNO_3$, loch water + 0.039 g/l K_2HPO_4 and loch water + both these nutrients in these concentrations (µg carbon/cm^2 leaf area/hour).

Loch water	0.18 ± 0.04
Loch water + nitrate	0.35 ± 0.05
Loch water + phosphate	0.39 ± 0.09
Loch water + nitrate and phosphate	0.39 ± 0.06

Number of replicates 4

water temperature 6°C

The nutrient bioassay treatments stimulated primary productivity but there was no significant difference between the rates of carbon fixation of the leaves when in loch water plus phosphate, plus nitrate and when in loch water with the addition of both of these nutrients.

The third experiment carried out in the field station at Durness during March 1971 was designed to investigate, by a slight modification of the basic technique, the relative productivity of the different submerged macrophytes from Loch Borralie. In order to compare carbon fixation rates of different species from Loch Borralie, the plant

tissue was quickly killed at the conclusion of the incubation period by immersion in cold 80% ethanol. Ethanol extracts of the plant tissue were made by washing three times in hot 80% ethanol over a 24 hour period. These extracts were made up to aconstant volume, a known aliquot evaporated to dryness on a planchet and counted for radioactivity. The extracted plant tissue was oven dried and weighed. The results in Table 3.18 permit the comparison of the carbon fixation rates of the different species of varied morphology.

TABLE 3.18

Laboratory 29.3.71

Relative mean rates of carbon fixation by Potamogeton praelongus, Hippuris vulgaris, Myriophyllum spicatum and Nitella species from Loch Borralie (cpm x 10^{-2}/mg ethanol extracted tissue).

P.praelongus	42.62 ± 5.34
Hippuris	18.53 ± 1.21
Myriophyllum	10.93 ± 1.27
Nitella	3.97 ± 0.30
Number of replicates	6
Water temperature	6°C

P.praelongus was by far the most productive of the species

investigated in terms of the amount of ethanol-soluble, extracted-carbon, fixed per unit tissue weight.

The final series of *in situ* primary productivity experiments were carried out in June 1971 and consisted of three experiments involving all three lochs and two species of Potamogeton (*P.perfoliatus* and *P.praelongus*).

The first experiment was carried out in Loch Croispol and consisting of incubating leaves of *P.perfoliatus*, from that loch, with loch water and with loch water enriched with 39 mg/l K_2HPO_4 and 390 mg/l K_2HPO_4. One additional series of bottles contained leaves in Gorham's medium and Gorham's medium without the phosphate component. The carbon fixation rates are given in Table 3.19. The addition of phosphate to the loch water induced both a depression (at 39 mg/l K_2HPO_4) and an enhancement (at 390 mg/l K_2HPO_4) in the rate of carbon fixation of the *P.perfoliatus* leaves over their basic rate in loch water. Rates of carbon fixation in culture medium were similar to those in the natural loch water.

The second experiment was carried out in Loch Borralie, using species collected in that loch. Leaves of *P.perfoliatus* and *P.praelongus* were incubated *in situ* with their natural loch water and with additions of 39 mg/l K_2HPO_4 and 390 mg/l K_2HPO_4. One other series of bottles contained water from the adjoining Loch Croispol. Results are presented in Table 3.20.

TABLE 3.19

Loch Croispol 17.6.71

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus when incubated with loch water, with loch water + 39 mg/l K_2HPO_4, with loch water + 390 mg/l K_2HPO_4, with Gorham's medium and with Gorham's medium without phosphate.

Phosphate addition to loch water (mg/l K_2HPO_4)	Rate of carbon fixation (µg carbon/cm^2/leaf area/hour)
0	1.72 ± 0.19
39	0.97 ± 0.12
390	3.77 ± 0.44
Gorham's medium	1.72 ± 0.36
Gorham's medium - phosphate	1.34 ± 0.12

Number of replicates 6

Incubation depth 5 m

Water temperature 15°C

TABLE 3.20

Loch Borralie 19.6.71

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus and P.praelongus from Loch Borralie when incubated with their natural loch water; Loch Croispol water and the natural loch water enriched with phosphate.

Phosphate addition (mg/l K_2HPO_4)	Rate of carbon fixation (mg carbon/cm^2 leaf area/hour)	
	P.perfoliatus	P.praelongus
0	1.79 ± 0.30	1.86 ± 0.26
39	2.43 ± 0.13	2.05 ± 0.23
390	5.09 ± 1.00	5.38 ± 0.61
Loch Croispol water	2.11 ± 0.45	2.32 ± 0.24

Number of replicates 6

Incubation depth 3 m

Water temperature 14°

The mean rates of carbon fixation for both species in Loch Borralie were similar and the addition of phosphate produced some degree of enhancement of these rates. The addition of both 39 and 390 mg/l K_2HPO_4 to the loch water produced significant increases in the carbon fixation rate of P.perfoliatus leaves, but only the larger quantity significantly increased the carbon fixation rate of P.praelongus leaves.

The carbon fixation rates of the two species in Loch Croispol water were not significantly different from each other and the rates are comparable with those in their natural loch water.

The last of the primary productivity experiments carried out in this study was designed to test the effect of incubation of leaves of both species in all three loch waters and a "neutral" solution; 0.01 M Warburg buffer No. 11 (95% $KHCO_3$: 5% Na_2CO_3). Incubation was carried out in Loch Croispol. The plant material and water from the other lochs were collected on dives just prior to the start of the experiment. Results are presented in Table 3.21.

P.praelongus from Loch Lanlish produced the significantly highest rate of carbon fixation in all the bathing solutions and only in one case (P.praelongus from Borralie in Croispol water) did any other specimen approach these rates which, among themselves, were not significantly different. There was

TABLE 3.21

Loch Croispol 21.6.71

Mean rates of carbon fixation by leaves of Potamogeton perfoliatus from Lochs Borralie and Croispol and P.praelongus from Lochs Borralie and Lanlish when incubated in water from Lochs Borralie, Croispol and Lanlish and with 0.01 M Warburg buffer No.11 (µg carbon/cm^2 leaf area/hour).

	Buffer	L. Borralie water	L. Croispol water	L. Lanlish water
P.perfoliatus (Borralie)	1.12 ± 0.16	1.16 ± 0.21	1.50 ± 0.33	1.50 ± 0.01
P.praelongus (Borralie)	1.90 ± 0.17	1.28 ± 0.36	2.04 ± 0.51	1.93 ± 0.19
P.perfoliatus (Croispol)	0.89 ± 0.21	1.62 ± 0.12	1.65 ± 0.17	1.83 ± 0.23
P.praelongus (Lanlish)	2.49 ± 0.06	2.40 ± 0.19	2.50 ± 0.15	2.54 ± 0.08

Number of replicates 4

Incubation depth 3.5 m

Water temperature 14°C

no species which consistantly produced low rates of carbon fixation in all waters, though P.perfoliatus (Croispol) produced the lowest overall rate (in buffer). P.perfoliatus (Borralie) produced similar mean rates of carbon fixation in buffer and Borralie water and higher rates in Croispol and Lanlish waters, although only the Lanlish-water rate was significantly higher. P.praelongus (Borralie) produced mean rates of carbon fixation in all waters which, like P.praelongus (Lanlish) were not significantly different from each other. P.perfoliatus (Croispol) produced similar rates of carbon fixation in all but Warburg buffer where it was significantly the lowest producer of all.

DISCUSSION

As far as the present investigations have served to measure the natural primary productivity of aquatic macrophytes it is concluded that the productivity of submerged aquatic vegetation, like all other types of vegetation is variable, productivity being largely dependent upon the conditions under which it is measured. Table 3.22 summarises the results from the *in situ* experiments carried out under near-natural conditions.

The estimates of the primary productivity of *Potamogeton perfoliatus* range between 0.49 and 7.32 ug carbon/cm^2 leaf area/hour, *P. praelongus* 1.28 to 5.87 and *P. obtusifolius* 1.15 - 9.37. The standard errors attached to such estimates are, by some standards, relatively high but they are within the range reported from other C-14 productivity studies of aquatic macrophytes (Wetzel, 1964a, 1964b). Such errors must not only reflect inaccuracies involved in the numerous manipulations, operations and calculations of the method employed but, though care was exercised in chosing plant material, must also reflect natural variation, such as may be attributable to age and vitality, within the tissue of specimens chosen for experimentation.

Hartman and Brown (1965), Salageanu and Tipa (1967) and Goulder (1970) have described intrinsic diurnal patterns

TABLE 3.22

Mean rates of carbon fixation by leaves of Potamogeton species measured under near-natural conditions (µg carbon/ cm^2 leaf area/hour).

Date	Incubation depth (m)	Rate ± standard error
	P.perfoliatus	
29.6.70	4.0	0.49 ± 0.13
15.7.70	2.5	2.87 ± 0.46
15.7.70	2.5	3.29 ± 0.64
16.7.70	2.5	2.32 ± 0.24
16.7.70	4.0	1.76 ± 0.07
4.8.70	4.5	1.90 ± 0.83
5.8.70	4.5	1.39 ± 0.28
5.8.70	4.5	1.83 ± 0.55
6.8.70	4.5	2.40 ± 0.21
12.8.70	3.0	5.66 ± 0.02
16.9.70	3.5	7.38 ± 0.62
17.9.70	3.5	2.71 ± 0.60
17.9.70	3.5	3.70 ± 0.94
17.6.71	5.0	1.72 ± 0.19
19.6.71	3.0	1.79 ± 0.30
21.6.71	3.5	1.16 ± 0.21
21.6.71	3.5	1.65 ± 0.17
	P.praelongus	
15.7.70	2.5	2.24 ± 0.33
15.7.70	2.5	2.31 ± 0.72
6 .8.70	4.5	6.87 ± 0.83
19.6.71	3.0	1.86 ± 0.26
21.6.71	3.5	1.28 ± 0.36
21.6 71	3.5	2.54 ± 0.08
	P.obtusifolius	
12.8.70	3.0	6.13 ± 2.51
13.8.70	3.0	1.90 ± 0.20
19.10.70	1.5	2.89 ± 0.42
17.12.70	0.5	1.15 ± 0.16
3. 7.70	2.0	9.37 ± 1.90

of photosynthetic activity in several species of aquatic macrophytes. Bearing in mind the errors attached to the various measurements required to substantiate such a claim, no evidence has been obtained in the present investigations which can be said to support this idea.

Assuming that the *in situ* measurements reported here were carried out under conditions which reflect the broad spectrum of those prevailing in the habitats studied, and that the mean productivity estimates recorded can be taken to represent the mean productivity of the species investigated, then the productivity of these plants must be in the region of 3 µg carbon/cm^2 leaf area/hour. Whether this figure holds for other species of submerged macrophyte is largely unknown. Results from one experiment (Table 3.18) suggest that, on a weight basis at least, this is probably not the case but that species of *Potamogeton* may have a high rate of production relative to some other submerged species.

In Loch Wanagan *P.perfoliatus* and *P.praelongus* have been shown to have similar rates of carbon fixation when compared on a leaf area base (Table 3.7). Similar results were reported from experiments with the same species in Lochs Borralie, Croispol and Lanlish. Experiments in Loch of the Lowes could not differentiate between the productivity of *P.perfoliatus* and *P.obtusifolius* and, from the present

data, it must be concluded that on the basis of carbon fixed per unit leaf area per hour no distinction can be made between the productivity of different Potamogeton species in any one loch.

Wetzel (1964a) reports rates of carbon fixation of 1.16 mg C/g/hour for Elodea canadensis and 0.88 mg C/g/hour for Ceratophyllum demersum. Taking a mean SLA of 1 for Potamogeton species then these figures of Wetzel are somewhat lower than the mean values reported here. Hartman (1967) has, however, found rates of about 3 mg C/g/hour for Elodea canadensis which seem more in line with the present mean figures.

Direct comparison of productivity estimates carried out on different days and in different lochs can only be made by way of the records of incident light energy measured during experimental incubations (see Chapter 5). Even when primary productivity can be expressed on the basis of µg carbon fixed per calorie of incident light, comparisons are still complicated by differences in variables of unknown influence, such as temperature. From the present limited investigations no conclusive statements regarding differences in the productivity of freshwater macrophytes in different lochs can be made with any great confidence. In several experiments where sufficient light data were available the primary productivity of the macrophytes approximated to

1 µg carbon/calorie. This and several other such estimates are discussed in relation to photosynthetic efficiency in Chapter 5 of this thesis.

Wetzel (1965) concluded that there is little correlation between biomass determinations and C-14 estimates of primary productivity. This is perhaps not surprising as the biomass of the vegetation represents an integration of environmental variables over a much longer period of time than any C-14 productivity estimate. In the present studies there also seems no obvious correlation between the biomass of the vegetation in any loch and the carbon fixation rates of its component species. This is most noticeable in Loch Leven. The sparsity of the vegetation in this loch (Table 1.1B) is hardly consistant with a productivity estimate of nearly 10 µg carbon/cm^2 leaf area/hour (Table 3.4). It is possible in Loch Leven that, due to turbulence and wave action, macrophytes are prevented from rooting in the shallow water which is the only depth zone in which they can photosynthesise. The penetration of light in this loch (Chapter 5) is very low, partly the result of almost continuous blooms of planktonic algae. It is also possible that, in this loch, the algae are successfully competing with the macrophytes for the available nutrients (Figure 3.9).

A great deal of information has accumulated on the relative rates of carbon fixation of Potamogeton species

when incubated in different loch waters and in nutrient enrichments of these waters. According to Goldman (1960) an increased rate of primary productivity should accompany the incubation of a nutrient-limited phytoplankton population with that nutrient which is in short supply. A similar criterion has been assumed to hold in the present investigations of aquatic macrophytes when a stimulation of the carbon fixation rates of leaves has been achieved when they were incubated with a nutrient-enriched loch water or with water from another loch. This postulate may be questioned, however, on the grounds that the addition of nutrients, such as phosphate, to loch water alters the pH value (Table 3.23) and this changes the proportions of bicarbonate and free CO_2 in the water and, hence, possibly releases more carbon for fixation by photosynthesis. In the lowland lochs which have been studied this possibility cannot be excluded, but is unlikely in the highly buffered, limestone waters (Table 3.24).

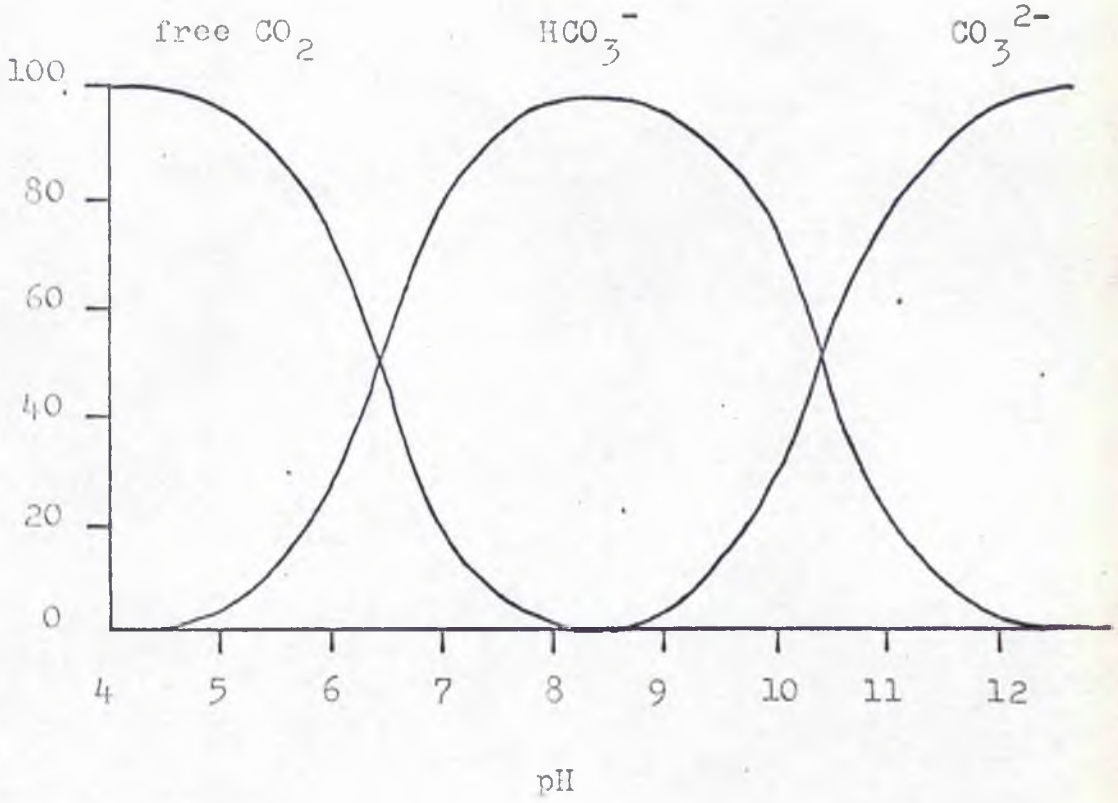
In the present studies, the degree of enhancement of the carbon fixation rate by leaves incubated with additional phosphate has not been constant, but varied from non-significant to a ten-fold increase. Although many studies have been reported in which the enrichment bioassay technique has been used to assess the nutritional factors limiting algal productivity, no previous studies appear to

TABLE 3.23

Changes in the pH values of loch waters upon the addition of 20 µg phosphate as K_2HPO_4.

Loch	Original pH	pH after phosphate addition
Croispol	8.10	7.94
Lanlish	8.70	8.65
Leven	7.60	7.55
Lowes	6.30	6.30
Uanagan	7.48	7.38

FIGURE 3.8



Relationship between pH and the proportions of free carbon dioxide, bicarbonate and carbonate in loch water. Redrawn from Hem (1970).

TABLE 3.24

Some chemical properties of the waters of Loch Croispol, Loch Lanlish, Loch Leven, Loch of the Lowes and Loch Wanagan. (Some of this data was kindly provided by Mr.A.V.Holden, Freshwater Fisheries Laboratory, Pitlochry).

	Croispol	Lanlish	Leven	Lowes	Wanagan
pH	8.1-8.8	8.7	7.6-7.8	6.3-6.8	7.0-7.5
conductivity (μmhos)	-	-	206	-	79
alkalinity (meq HCO_3^-)	2.70	1.60	0.70-1.30	0.27-0.32	0.62-0.71
Cl^- (meq)	-	-	0.31-0.46	-	0.33
Ca^{++} (meq)	1.35	1.20	0.80-1.10	0.41	0.70
Mg^{++} (meq)	1.83	0.37	0.50-0.83	0.23	-
Na^+ (meq)	0.91	1.26	0.28-0.33	0.24	0.24
K^+ (meq)	0.04	0.02	0.03-0.05	0.02	-
PO_4-P (μg/l)	8	17	2-25	18	-

have been concerned with aquatic macrophytes. Without a knowledge of all the numerous factors which simultaneously influence primary productivity, any interpretation of the present data must be cautious. It can only be concluded therefore that, in the majority of enrichment bioassay experiments reported here, the productivity of aquatic macrophytes appears to have been limited by phosphate availability.

It is unlikely that phosphate is the only nutrient limiting carbon fixation, as the one experiment with added nitrate testifies, or that such nutritional factors operate independently of other environmental variables, such as temperature, turbulence and light intensity, the importance of which are, to a large extent, unknown. Enrichment bioassay has, however, a great potential as an effective tool, not only for investigating nutritional, production-limiting factors, but also for monitoring pollutants and their possible nutritional or toxic effects on the growth of aquatic macrophytes. The accurate interpretation of such studies, however, must be made in cognizance of the other numerous factors which may be simultaneously influencing growth.

The primary productivity estimates and enrichment bioassay experiments reported here were carried out with detached leaves. It is therefore possible that the plants as a whole are not as nutrient limited as is suggested from these investigations. The question as to whether a supply

of nutrients can be transported to the leaves from other, non-photosynthetic, parts of the plant and whether the roots of submerged macrophytes can function in nutrient absorption, and, hence, help alleviate such deficiencies, are discussed in Chapter 6 of this thesis.

The productivity of leaves of different species from the same loch, when incubated with loch water or phosphate-enriched loch water, have generally been similar (Table 3.2). This, coupled to the fact that specimens from different lochs responded differentially to different loch waters (Table 3.11), suggests some inherent ecotypic productive capacity, induced by the conditions in their natural habitats. In so far as this is linked to phosphate availability and the response of specimens from different lochs to phosphate enrichment, the matter is raised in Chapter 7 of this thesis, which discusses the levels of the enzyme alkaline phosphatase in several species of aquatic macrophyte in relation to their primary productivity.

In concluding this chapter it is thought worthwhile to compare the findings from the present studies on the productivity of submerged macrophytes with published figures from similar studies on other types of vegetation. Figure 3.9 presents such a comparison. While allowing for the fact that it is difficult to make an accurate extrapolation from a few short-term experiments to a daily production rate;

FIGURE 3.2

(Opposite)

Comparison of the primary productivity (µg carbon/cm^2 tissue area/hour) of different plant communities measured under near-natural conditions. Data from Drew (pers.com.) and recalculated from Verduin (1952, 1953) and Altman and Dittmer (1964).

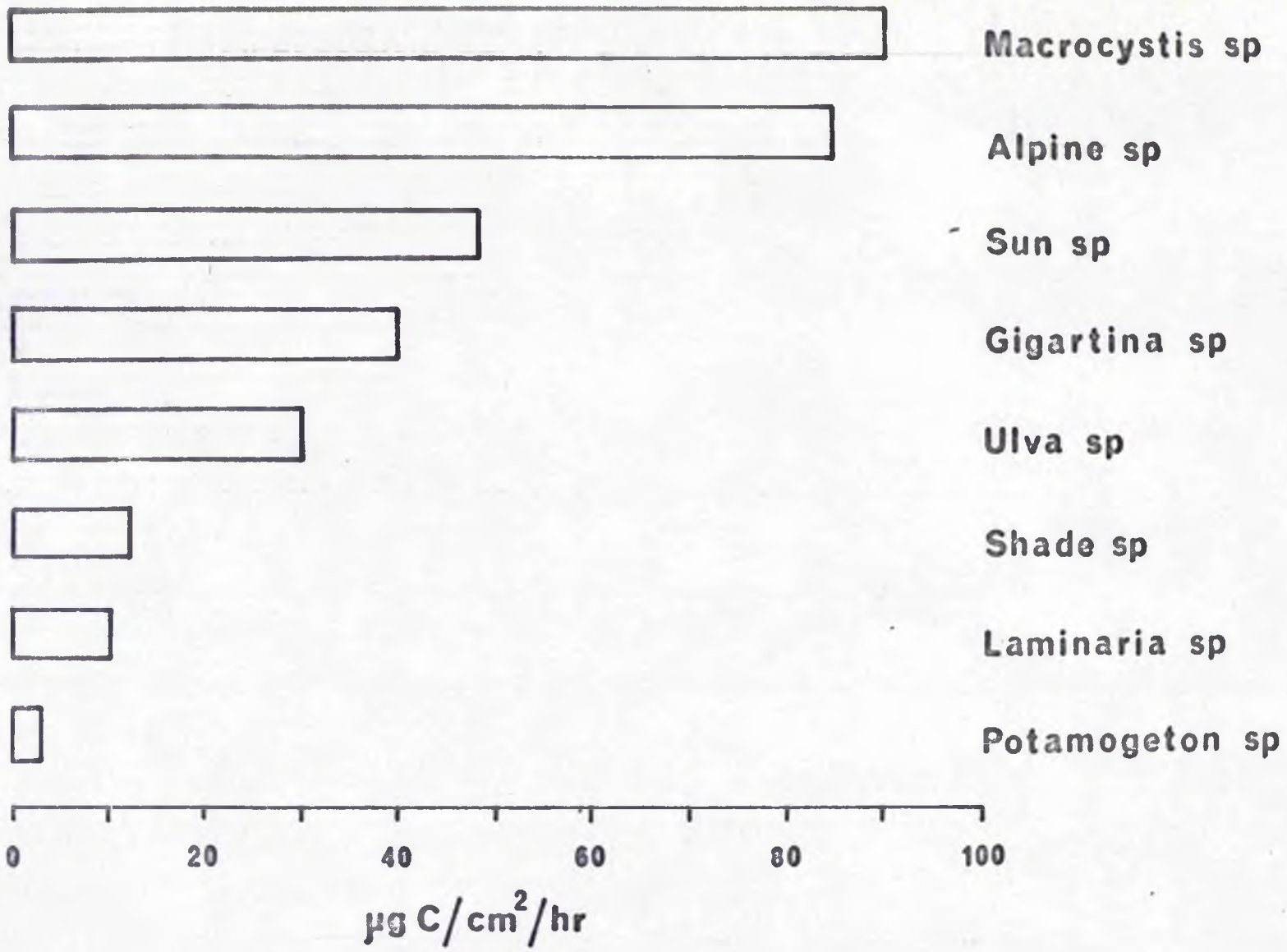

Macrocystis sp
Alpine sp
Sun sp
Gigartina sp
Ulva sp
Shade sp
Laminaria sp
Potamogeton sp
0
20
40
60
80
100
µg C/cm²/hr

FIGURE 3.9

assuming an eight hour day, utilising 3 µg carbon/cm^2 leaf area/hour as a mean figure for the primary productivity of Potamogeton species (Table 3.22) and taking 4 as a possible LAI (Table 1.13), then the productivity of those species may approach 1 g of carbon/m^2 loch floor/day. This figure is similar to that determined by Wetzel (1964a) for the macrophytes of Borax lake, California (982.2 mg carbon/m^2/day) but is, however, only a fraction of some of those published for terrestrial species and smaller than those often reported from freshwater periphyton and phytoplankton communities. It is, therefore, cautiously concluded that the productivity of the submerged macrophytes in Scottish freshwater lochs is relatively low.

Additional Discussion

The viewpoints of Strickland (1960) and Wetzel (1964a), as reported on pages 104 and 105, regarding the purpose of incubating dark, control bottles in C-14 experiments designed to estimate primary productivity are apparently not accepted by all workers. The processes involved and hence estimated in such incubations are thought by some to be entirely active metabolic carboxylations and not both these and isotopic exchange within tissue free space as stated. Similarly, the conclusions reached by Steeman-Nielsen (1957), Strickland (1960) and Vollenweider and Nauwerck (1961) from phytoplankton studies, the criterion adopted in macrophyte investigations by Wetzel (1965), supported by Hartman and Brown (1967) and recommended in a current IBP manual by Vollenweider (1969), and hence applied in the present investigations, that in short-term C-14 incubations the rate of photosynthesis measured approximates to the net rates, is not generally recognised. It is beleived by some workers that in such experiments C-14 will most certainly be lost in respiration and a portion of this fixed again in photosynthesis.

The experimental procedure adopted to estimate the primary productivity of aquatic macrophytes has involved the incubation of detached leaves in glass bottles with 25 ml

of loch water, while the enrichment bioassay experiments involved the inclusion of an additional nutrient to this volume. It is possible that firstly, due to the unnatural detachment of the leaves and their subsequent enclosure, the measured response to incubation with C-14 reflects, in part, this artificial situation and not a true rate of primary production. Such artifacts, arising from the enclosure of plants in bottles, have been discussed, with regard to phytoplankton by Vollenweider and Nauwerck (1961) and deemed unimportant in short-term incubations lasting only three or four hours. It is also possible, in these experimental incubations, that a carbon limitation for photosynthesis may arise through the maintenance of a high rate of fixation over an extended period of time in such a small volume of loch water. Such an occurrence could also influence the results of the enrichment bioassay experiments where the increased rates of photosynthesis, arising from the removal of a nutrient limitation, might well bring about a carbon limitation.

Utilising the pertinent water chemistry data on pH and alkalinity given in Table 3.24 (page 140) and using the conversion factors from the tables of Saunders et al (1962), derived and applied as detailed in Appendix 1A, the minimal total carbon contents of the various loch waters have been computed. These are compared, in Table 3.25, with the

maximal natural *in situ* rates of carbon fixation recorded from each locality. It will be recalled that these rates of macrophyte carbon fixation were determined during incubations of no longer than four hours and with leaves of no greater than four square centimetres. Calculating the maximal carbon fixation rates and expressing the carbon content of the loch water as µg C/25 ml, the volume used in the incubations, the maximal percentage of the total carbon of the loch water in an incubation bottle fixed by an incubated leaf can be readily determined. These data are also given in Table 3.25.

Viewing the data in Table 3.25 on the percentage of carbon utilised it will be noted that in Lochs Croispol, Lanlish and Uanagan the maximal rates of carbon fixation recorded come well within the minimal total carbon content of the loch water present in the incubation bottles and even including the results from the phosphate enrichment experiments in these lochs only one recorded rate would utilise as much as 40% of the minimal carbon. This latter rate of 20.72 ± 1.03 µg C/cm^2 leaf area/hour (Table 3.13, page 121) being recorded from a bioassay experiment with *P.perfoliatus* carried out in Loch Croispol on 16/9/71. It is therefore unlikely that during the primary productivity determinations carried out in these three lochs the incubated tissues were carbon-limited.

Turning now in Table 3.25 to the data from Lochs Leven and Lowes, a different picture of carbon utilisation emerges, where the maximal rates of carbon fixation would require more than half of the calculated minimal carbon content of the loch water in the incubation bottles and, in the case of the Lowes data, nearly all of this amount. Therefore, due to the technique employed in the experiments, a consideration of a carbon limitation to the measured in situ rates of primary productivity by the submerged macrophytes in these lochs cannot be entirely precluded from discussion. The increased rates of carbon fixation detected in the nutrient enrichment bioassay experiments in these lochs are similarly subject to the same considerations.

TABLE 3.25

The maximal measured rates (μg C/cm^2/hr) of *in situ* carbon fixation by leaves of *Potamogeton* species recorded in Lochs Croispol, Lanlish, Leven, Lowes and Uanagan; the date of the determinations; the loch water temperature (^oC), pH and alkalinity (ppm $CaCO_3$); the computed minimal total carbon content of the loch water (mg/l); the total carbon content (μg) of the 25 ml of loch water of an incubation bottle and the percentage of this which would be utilised by a leaf of 4 cm^2 area maintaining the maximal recorded carbon fixation rate over a four hour period.

Loch	Date	Species	µg C/cm^2/hr	µgC/4cm^2/4hr	°C	pH	Alkalinity	mgC/l	µg C/ 25 ml	% total C fixed
Croispol	16.9.70	P.perf	7.32 ± 0.62	117	11.0	8.8	135.0	32.40	810	14
Lanlish	6.8.70	P.prae	6.87 ± 0.83	110	15.0	8.7	80.0	19.20	480	23
Leven	3.7.70	P.obt	9.37 ± 1.90	150	14.0	7.8	35.0	8.75	219	68
Lowes	12.8.70	P.obt	6.13 ± 2.51	98	17.0	6.8	13.5	4.59	115	85
Uanagan	15.7.70	P.prae	3.29 ± 0.64	53	12.5	7.5	31.0	8.06	202	26

P.perf = P.perfoliatus

P.prae = P.praelongus

P.obt = P.obtusifolius

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CARBOHYDRATES

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CHAPTER 4

CARBOHYDRATES

STORAGE COMPOUNDS

Introduction

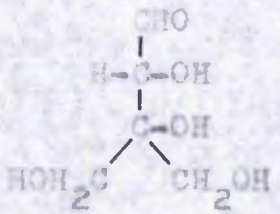
The majority of workers in the field of primary productivity are content to express their results in terms of oxygen evolved or carbon fixed. Few are interested in the chemical products of photosynthesis which, from the viewpoint of efficiency of the process can be important. For this reason a limited investigation into the major alcohol-soluble carbohydrate constituents of several species of aquatic macrophytes has been carried out using the technique of gas-liquid chromatography (GLC). The general principals of GLC for the detection of soluble carbohydrates in extracts of plant tissue has recently been reviewed by Holligan (1971) while Holligan and Drew (1971) give much comparable data on the separation and estimation of soluble sugars and polyols from a variety of plant tissue.

McClure (1970) reviews the literature concerning the secondary constituents of aquatic angiosperms from the

viewpoint of phytochemical phylogeny and refers to the work of Duff (1965), McClure and Alston (1966), van Beusekom (1967) and Bate-Smith (1968) all of whom have investigated the occurrence of the best known branched-chain sugar, apiose (Figure 4.1), in various species of aquatic macrophytes.

Figure 4.1

Structure of the 5 carbon branched sugar, Apiose.



Apart from the Lemnaceae, according to van Beusekom (1967), the only aquatic species in which apiose occurs are those members of the families Hydrocharitaceae, Potamogetonaceae and Posteraceae which inhabit marine or brackish-water environments. Duff (1965) also found that, as well as apiose, members of the Lemnaceae contained appreciable quantities of glucose and xylose.

Very few other studies appear to have been concerned with the carbohydrate constituents of aquatic macrophytes, though Drew (pers.com.) is presently investigating the alcohol-soluble carbohydrates in several genera of marine

angiosperms.

Methods

Plant material was collected either from natural locations or from laboratory culture and, following the procedure of Holligan (1971), was immediately killed by immersion in a small volume of cold 99% ethanol. Over a period of 24 hours extraction of the plant material was completed with three additions of hot 80% ethanol. Aliquots of this extract were then taken to prepare the volatile derivatives for GLC analysis. This initial study was purely qualitative and no attempt was made to determine the absolute quantities of carbohydrate present in the plant material. Results are presented in relative terms only.

Suitable aliquots of the alcohol extracts were dried at 40°C in quickfit flasks under vacuum using a rotary film evaporator and the dried material was redissolved in 1 ml of pyridine. The volatile derivatives of the soluble carbohydrates (trimethyl disilazane ethers) were prepared by the immediate addition to this pyridine solution of 0.2 ml of hexamethyldisilazane (HMDS) followed by 0.1 ml of trimethylchlorosilane (TMCS). The reaction flasks were tightly stoppered and shaken rapidly, usually overnight on a mechanical shaker, until analysed.

Samples were analysed using a Pye series 104 analytical gas chromatograph with a glass column and flame ionisation detector coupled to a chart recorder and ~~electronic~~ disc integrator. Column packings were prepared as described by Holligan (1971) and all samples were analysed on a five feet, 2% SE 52 column (acid washed, silanised Diatomite C as solid support and methyl phenyl silicone gum as stationary phase). Oxygen-free nitrogen was employed as the carrier gas and flow was maintained at 40 ml/min. Samples were injected into the column with a 10 µl syringe fitted with a 4.5 inch stainless steel needle. All analyses were carried out with an oven temperature programme of 140 - 275°C, at 4°C/min.

Unknown carbohydrates were identified by reference to samples of known composition, prepared from biochemical grade reagents and reacted in a similar way to the unknown; also by the use of internal standards, and by their characteristic relative retention times with respect to alpha-glucose and sucrose.

Results and Discussion

Figure 4.2 illustrates the retention times for a series of known standard compounds, while Figure 4.3 illustrates traces from the analysis of samples of Potamogeton praelongus and P.perfoliatus. Table 4.1 lists the major alcohol soluble carbohydrates identified from several species of Potamogeton.

TABLE 4.1

Major alcohol soluble carbohydrates identified in members of the genus Potamogeton.

Species	Carbohydrates identified
P.crasineus	glucose, sucrose.
P.obtusifolius	fructose, glucose, sucrose.
P.perfoliatus	glucose, sucrose.
P.polygonifolius	sucrose.
P.praelongus	fructose, glucose, sucrose.
P.schweinfurthii (Benn)	glucose, sucrose.

Figure 4.4 presents GLC traces obtained from analysis of the three rosette species, (a) Littorella uniflora, (b)

Note: the following figures are individually captioned and the peak labels are not specific to any one compound throughout.

FIGURE 4.2

(opposite)

GLC separation of carbohydrates as their TMS derivatives. S, solvent peak; 1, alpha-glucose; 2, mannitol; 3, beta-glucose; 4, sucrose.

FIGURE 4.2

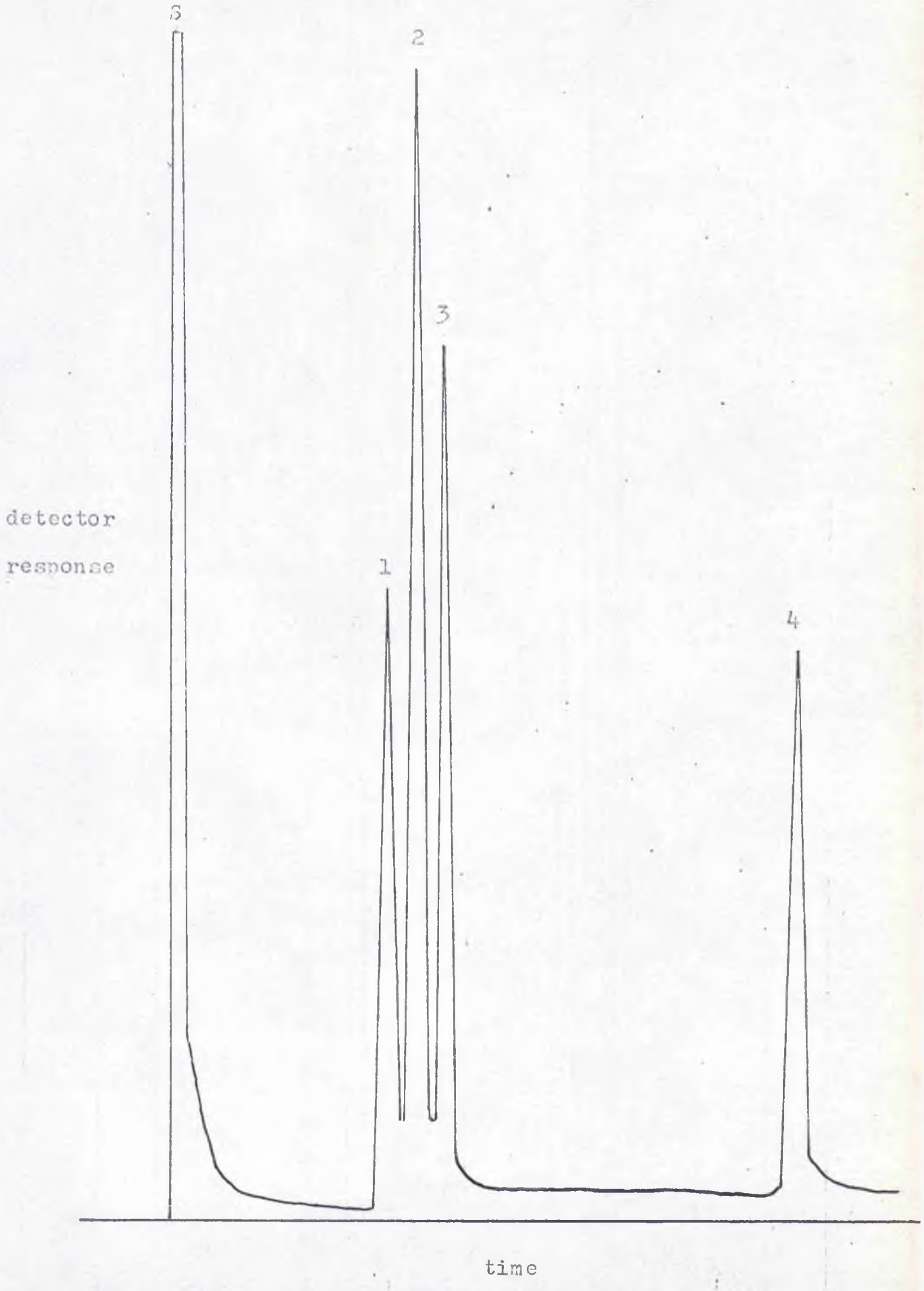


FIGURE 4.3

(opposite)

GLC separation of soluble carbohydrates from:
A, Potamogeton praelongus, rhizomes; B, P.perfoliatus, turions; C, P.praelongus, leaves; D, P.perfoliatus, leaves. S, solvent peak; 1, fructose; 2, alpha-glucose; 3, unknown; 4, beta-glucose; 5, sucrose.

FIGURE 4.3

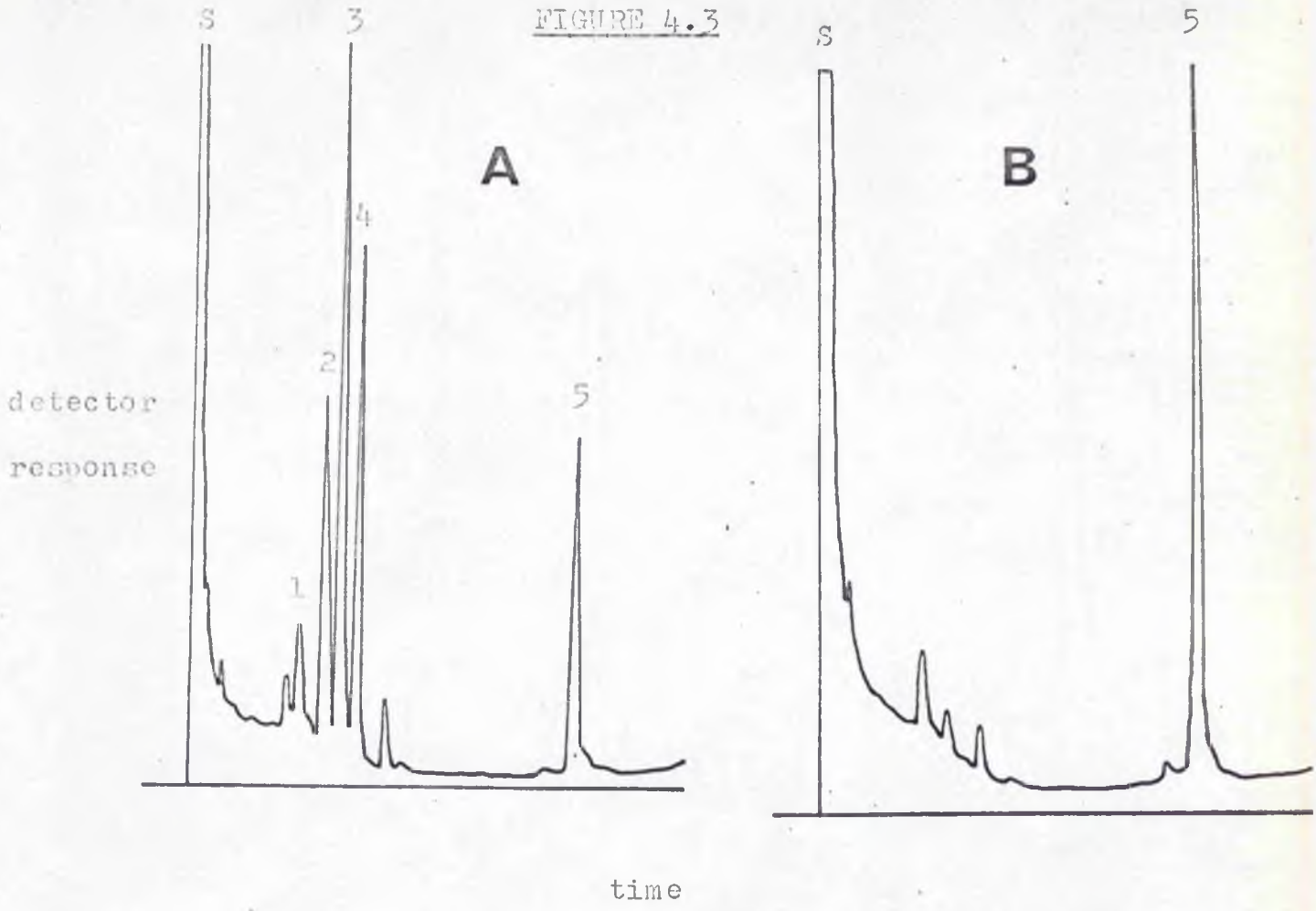


FIGURE 4.4

(opposite)

GLC separation of soluble carbohydrates from:
A, *Littorella uniflora*; B, *Isoetes lacustris*;
C, *Lobelia dortmanna*, shoots; D, *Lobelia dortmanna*,
roots. S, solvent peak; 1, fructose; 2, alpha-
glucose; 3, unknown; 4, beta-glucose; 5, sucrose.

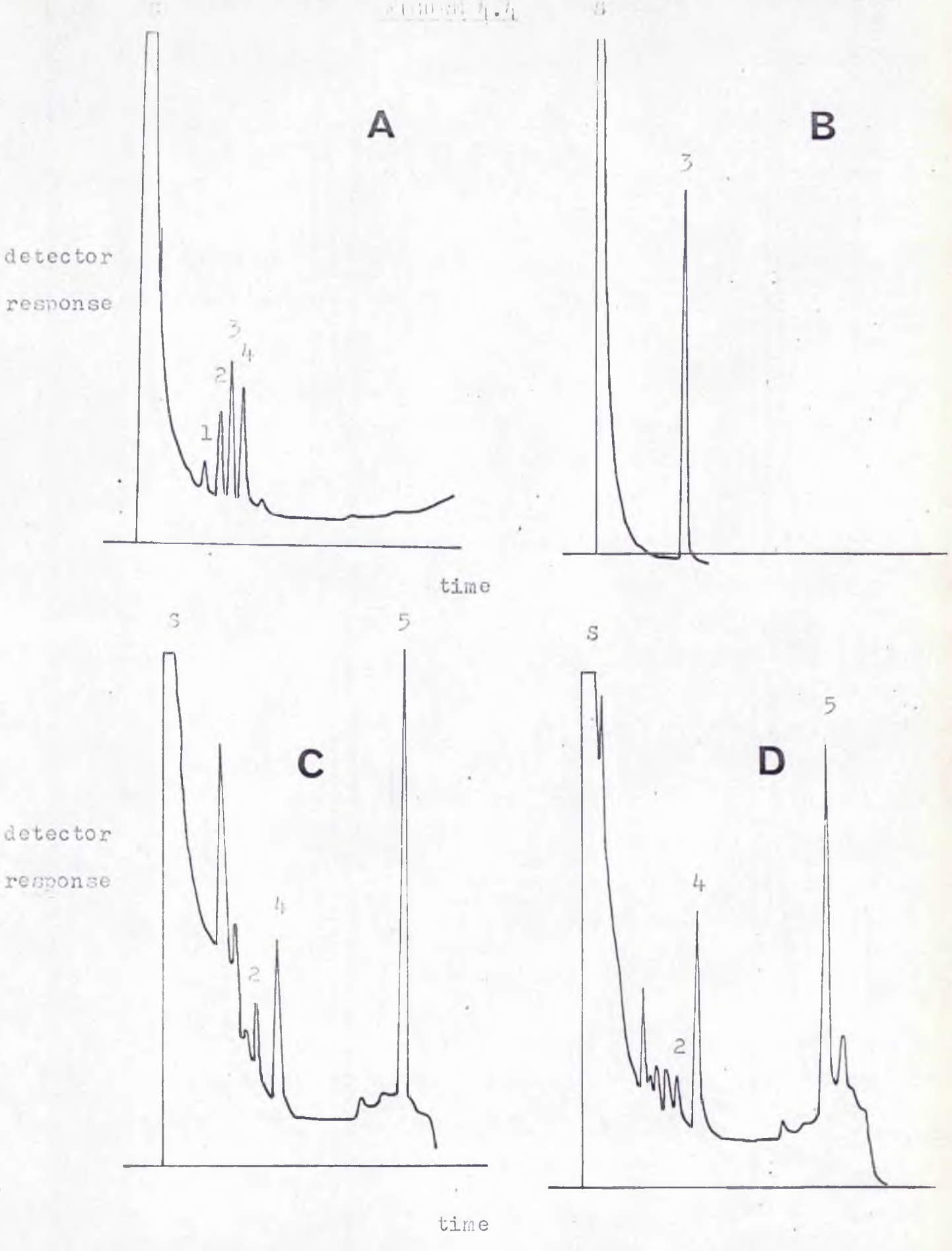

A
B
C
D
detector response
time
S
1
2
3
4
5

FIGURE 4.5

(opposite)

GLC separation of soluble carbohydrates from:

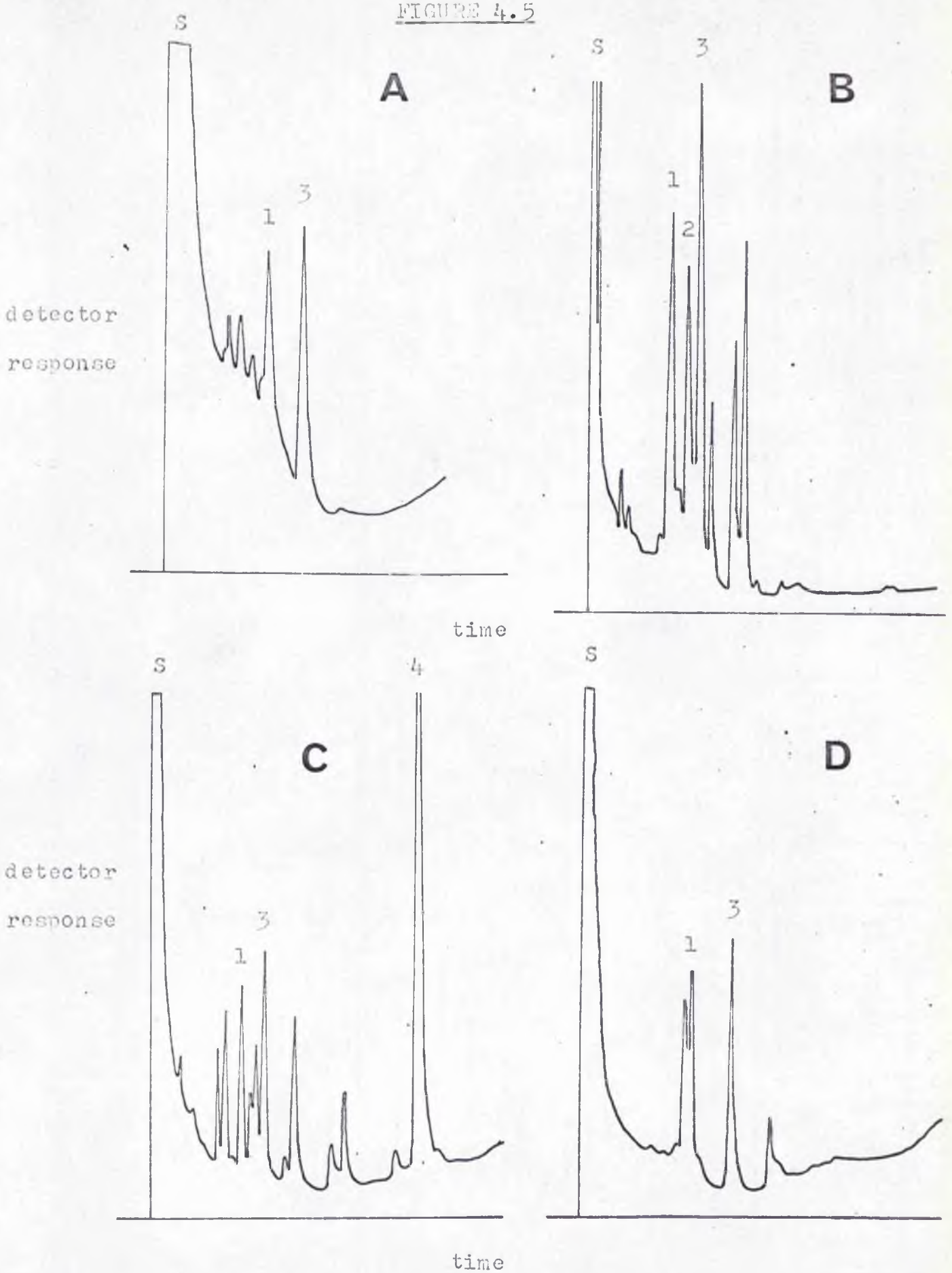
A, *Elodea canadensis*; B, *Myriophyllum alterniflorum*;

C, *Chara aspera*; D, *C.papillosum*. S, solvent peak;

1, alpha-glucose; 2, unknown; 3, beta-glucose;

4, sucrose.

FIGURE 4.5


A
S
1
3
B
S
1
2
3
detector response
time
C
S
1
3
4
D
S
1
3
detector response
time

(b) Isoetes lacustris and (c&d) Lobelia dortmanna. Figures 4.5a and 4.5b present the traces obtained from GLC analysis of extracts of Elodea canadensis and Myriophyllum alterniflorum, while Figures 4.5c and 4.5d illustrate the diverse pattern of carbohydrates present in two species of the macrophytic algal genus Chara, Chara aspera and Chara papillosum.

From the data presented in the figures and table it would appear that there are relatively few major alcohol soluble carbohydrate compounds present in aquatic angiosperms, these being fructose, sucrose, glucose and one other. This last compound has a similar relative retention time to that of mannitol but it is not in fact, mannitol, since co-chromatography with an internal standard containing mannitol produced either two peaks, or else one peak with a pronounced shoulder, indicating the presence of two distinct but similar chemical fractions. From the data of Holligan and Drew (1971) the relative retention time of this unknown carbohydrate indicates that it is probably a six-carbon sugar, or a polyol.

Though the composition of the carbohydrate constituents changes little in the specimens investigated, there was some variation in the relative proportions present both between the species, as might be expected, and within the tissues of the same species. This tissue difference is

most noticeable when comparing the turions and leaves of Potamogeton perfoliatus. Large quantities of sucrose were present in the turions relative to glucose whereas the leaves contained more glucose than sucrose. In this context it is interesting to note the findings discussed by Hillman (1961), that turion development in Lemna species is promoted in both the light and the dark by the addition of sucrose to the medium while glucose was effective only in the light. Hemsen (1954) had previously noted that fructose and maltose also promoted turion development, suggesting that under conditions of abundant sugar availability some is channelled into the storage organs, the turions. A similar situation may occur in the pondweeds where, for the specimens of P.perfoliatus analysed, sucrose was the main storage substance in the turions.

Though sucrose was present in all the species of Potamogeton investigated (Table 5.1) and occurs in large amounts in relation to the other alcohol-soluble carbohydrates in Lobelia dortmanna (both roots and shoots) and Chara aspera it was noticeably absent in samples of Littorella uniflora, Isoetes lacustris, Elodea canadensis, Myriophyllum alterniflorum and Chara papillosum which were analysed, though all of these plants, except Isoetes lacustris, contained several other carbohydrates. The samples of Isoetes lacustris which were analysed only contained

appreciable quantities of the unknown carbohydrate which has a relative retention time of 1.1 and which eluted from the GLC column at a similar time to that of mannitol.

Vigorously growing specimens of Potamogeton species, collected either from the field or from stock cultures, contained, in relation to their total alcohol-soluble carbohydrates, appreciable amounts of sucrose in their leaves. Specimens which were less vigorous, and producing turions or vegetative organs for overwintering, possessed leaves with relatively little sucrose while the overwintering organs contained, in relative terms, abundant sucrose. It is thus possible that the variation in carbohydrate composition detected in the present study can, in part, be explained by a seasonal rebalance of the carbohydrates in these plants, brought about by different stages in their growth cycle. Any critical study on the carbohydrate chemistry of aquatic macrophytes should therefore take into account this possible variation in relative carbohydrate composition of different plant tissues which may be due solely to the stages in their life cycle. At present the regular collection of specimens of several species is being undertaken from several lochs and it is hoped that a quantitative carbohydrate analysis will be performed at some later date.

PRIMARY PRODUCTS OF PHOTOSYNTHESIS

Introduction

Though the GLC analyses reported previously gave information on the total carbohydrate composition of some aquatic macrophytes and hence some of the final products of photosynthesis, no information was obtained on the primary products of photosynthesis or on the form in which sugars are transported from the leaves to other parts of the plant. These problems have been studied briefly in Potamogeton perfoliatus using a combination of chromatographic and isotopic techniques.

Methods

A specimen of P.perfoliatus was sealed in the apparatus described in Chapter 6 and used to study the movement of P-32 in aquatic plants, so that the roots and shoots were contained in separate solutions. The roots were kept completely dark by wrapping the jar in which they were contained with aluminium foil. Both roots and shoots were bathed in 0.01 M Warburg buffer, the shoot solution being labelled with 100 µCi of C-14 sodium bicarbonate. The plant was illuminated by three Gro-lux lamps for a period

of four hours while maintained at a temperature of 20°C in a constant-temperature water-bath. After the period of illumination the specimen was removed from the container and separated into three portions, (1) roots and rhizomes (never in contact with C-14), (2) stems and (3) leaves. Each portion was immediately killed by immersion in hot 95% ethanol and alcohol extracts made from each portion as described for GLC analysis.

Each alcohol extract was concentrated to a small volume by evaporation under vacuum at 40°C in a rotary film evaporator and spotted on a paper chromatogram (Whatman No.1) following the standard procedures (Feinburg and Smith, 1962). A marker spot of sucrose and C-14 labelled glucose was used to aid identification of the unknown substances in the tissue extracts.

A descending chromatogram was run overnight at room temperature using a solvent system of iso-propanol:n-butanol: water (14:2:4), (Smith, 1962). The chromatogram was then removed and allowed to dry, before being placed in contact with a piece of Kodak crystallex X-ray film in a light-tight packet and left for 14 days. The chromatogram was then removed and the film developed in D 19 developer, following the procedure described in Chapter 6.

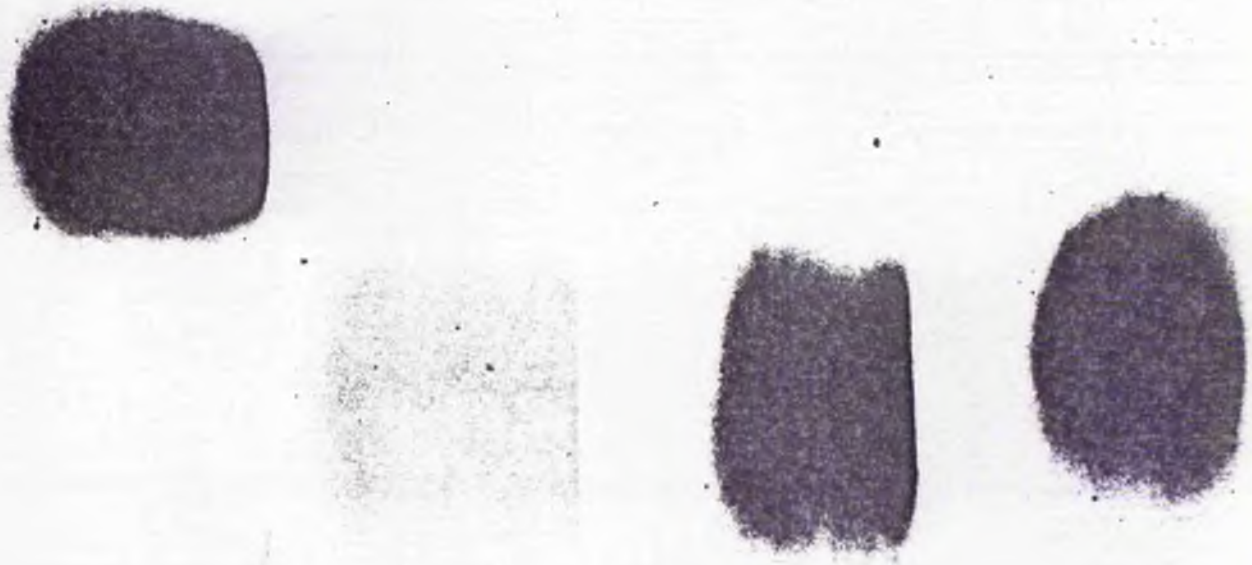
The paper chromatogram was cut into strips parallel to the direction of solvent flow and centred on the original

spots. These strips were scanned in turn for the presence of radioactivity using a Nuclear Chicago Actigraph, Model III coupled to an integrating chart recorder. Finally the carbohydrates on the chromatograms were developed chemically by dipping in a solution of silver nitrate in acetone, drying in air and then spraying with alcoholic sodium hydroxide (Trevlyan, Proctor and Harrison, 1950), which produced brown spots on a pale background. The spots were made permanent, and the background was removed, by quickly dipping the paper in concentrated ammonia, then washing with tap water for 30 minutes.

Results and Discussion

Figure 4.6 presents the autoradiogram of the chromatograms, indicating the presence of the same single C-14 labelled substance in all three extracts (root, stem and leaves). This substance is not glucose as it runs slightly slower than the marker C-14 glucose on the paper chromatogram. This finding is confirmed from the scans of the actigraph (Figure 4.7) where the single peaks again indicate the presence of only one radioactive substance in the alcohol extracts. Using the known marker spots and by comparing the developed paper chromatograms (Figure 4.8) with the autoradiograph and with the actigraph scans, this substance

FIGURE 4.6



Autoradiograph of a paper chromatogram spotted with an ethanol extract of C-14 labelled photosynthesate of Potamogeton perfoliatus. 1, glucose marker; 2, root extract; 3, stem extract; 4, leaf extract.

FIGURE 4.7

(opposite)

Actigraph traces of a paper chromatogram spotted with an ethanol extract of C-14 labelled photosynthesate of Potamogeton perfoliatus. 1, glucose marker; 2, leaf extract; 3, stem extract; 4, root extract.

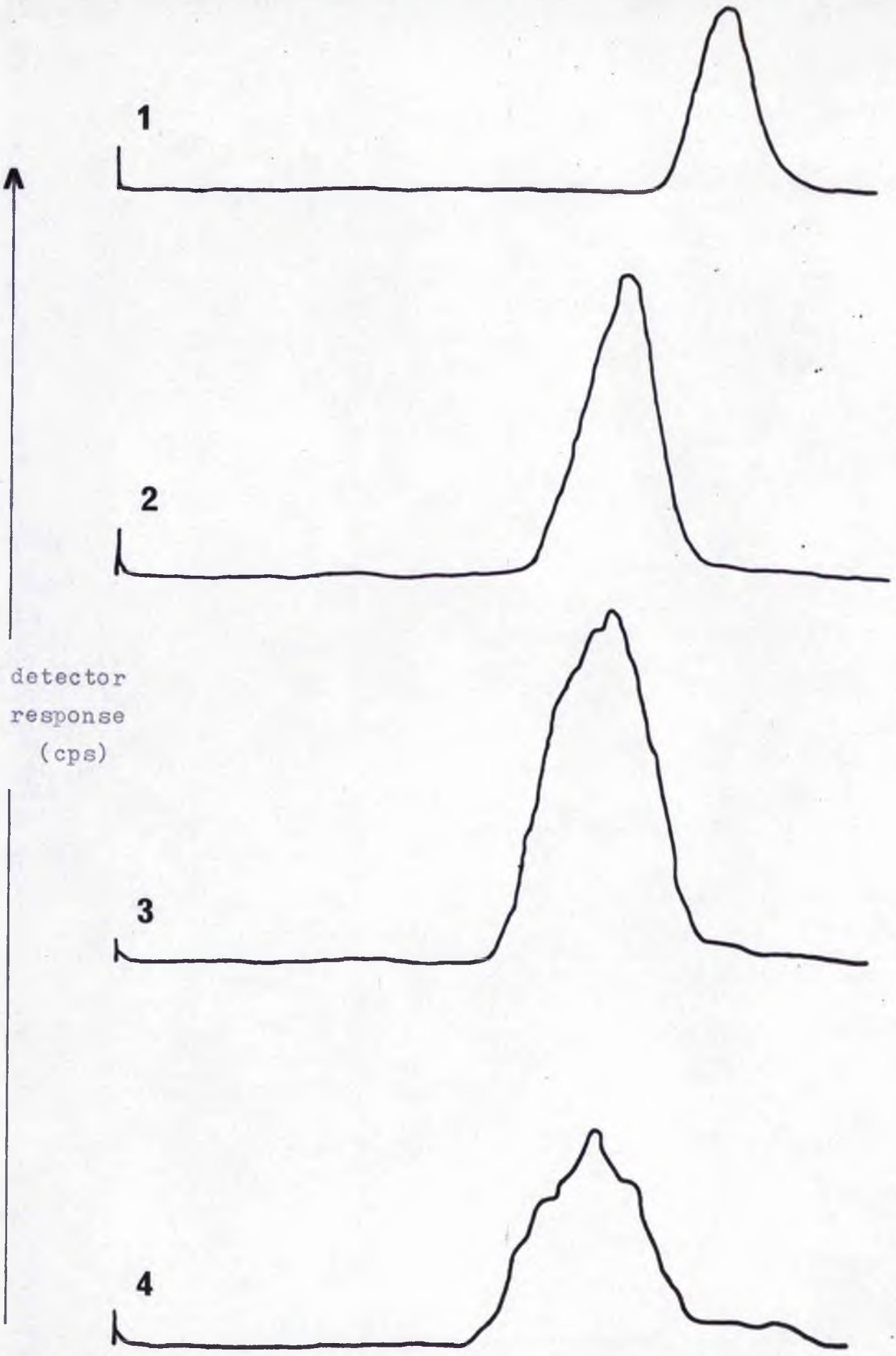
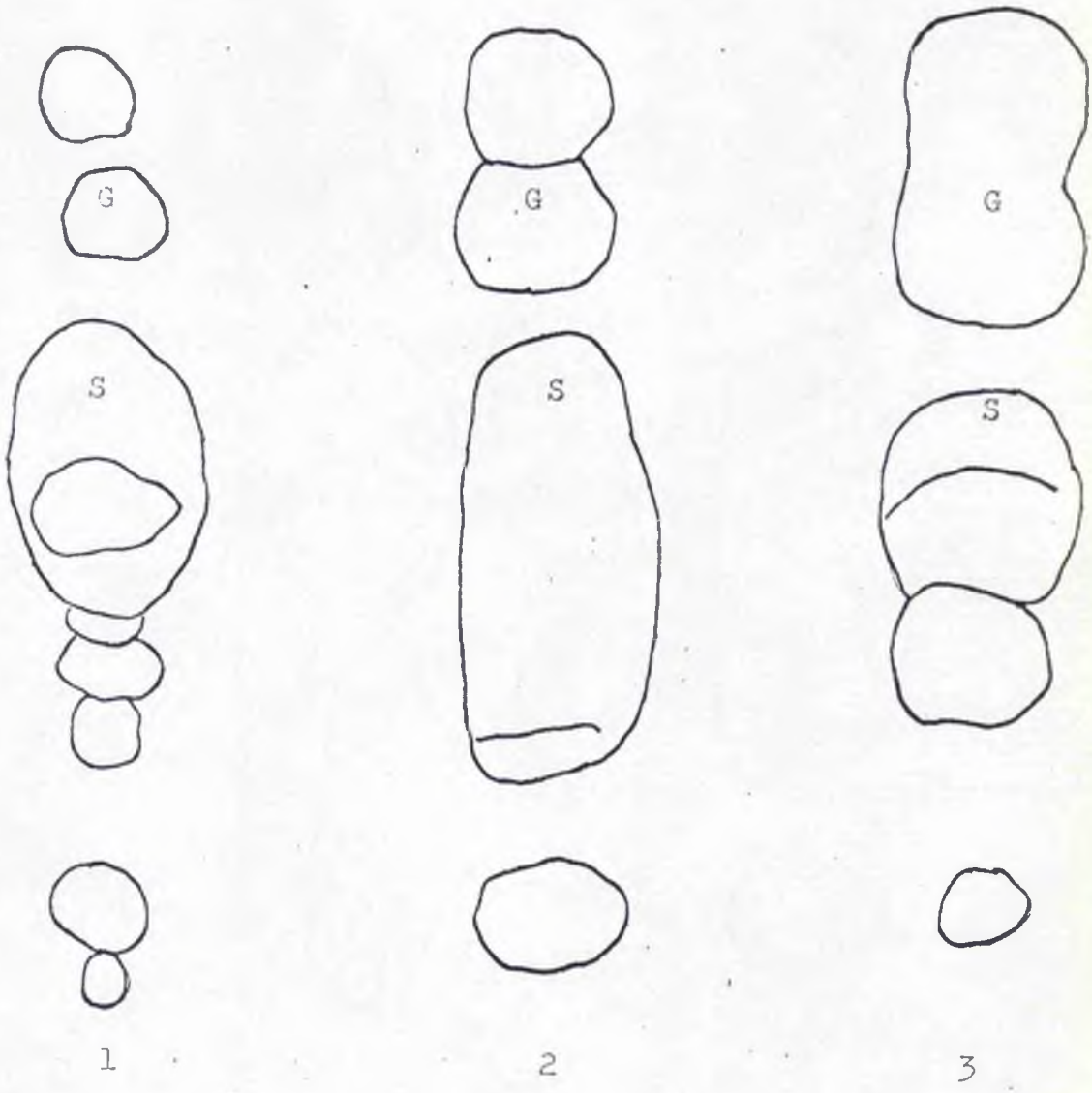

1
2
3
4
detector
response
(cps)
relative retention time on paper chromatogram

FIGURE 4.8



Diagrammatic representation of a paper chromatogram spotted with an ethanol extract of C-14 labelled photosynthesate of Potamogeton perfoliatus. 1, leaf extract; 2, stem extract; 3, root extract. S, sucrose; G, glucose.

is confirmed as sucrose which has travelled exactly the same distance along the marker chromatogram as have the C-14 labelled spots in the plant extracts. Thus, under the conditions and duration of the experiment, the only major alcohol-soluble product of photosynthesis of P.perfoliatus was sucrose. This was found labelled in the leaves, stems, roots and rhizomes and thus ~~must~~ also, by the same reasoning, could be ~~the~~ a primary transport sugar.

As mentioned previously (in discussion of GLC results) there are generally other carbohydrates present in the leaves of P.perfoliatus besides sucrose. This finding is also substantiated from the paper chromatograms, Figure 4.8, which indicate the presence of glucose and several other compounds besides sucrose. As these were all unlabelled with C-14 they must have been synthesised prior to the experiment.

In an endeavour to discover the rate at which these other carbohydrate fractions are formed, the previous experiment was repeated. This time, however, only detached leaves were used as in the in situ primary productivity experiments, and the incubation period was 36 hours instead of 4. A paper chromatogram of an ethanol extract of leaf tissue was spotted and run as before, an autoradiograph was prepared and the chromatogram was chemically developed. No actigraph trace was made but an aliquot of the ethanol

extract was analysed by GLC using the method previously described. The autoradiograph indicated that again only one fraction contained C-14 label (sucrose), though the picture was somewhat confused due to heavy loading of one of the initial spots and consequent streaking of the chromatogram. The resulting trace from the GLC analysis of an aliquot from the same sample indicated that only two alcohol-soluble carbohydrates were present in large amounts in the tissue, namely sucrose and the unidentified carbohydrate with a retention time similar to mannitol. Thus, even after 36 hours, sucrose is the only alcohol soluble carbohydrate which can be clearly detected as being a product of photosynthesis of P.perfoliatus leaves. During the in situ experiments that have been carried out, it is therefore likely that for P.perfoliatus at least, sucrose is the only storage substance synthesised. Further, since appreciable quantities of sucrose relative to all other alcohol-soluble carbohydrates occurred in all species of Potamogeton investigated by GLC, the same substance may well be the initial product of photosynthesis in this genus. Sucrose may also be the main storage carbohydrate though further investigations of this topic are required.

EXTRACELLULAR PRODUCTS OF PHOTOSYNTHESIS

Introduction

The extracellular release by algal cells of photo-assimilated compounds as dissolved organic carbon (DOC) has in recent years received considerable attention. Wetzel (1969a, 1969b) appears to be the only worker to investigate this phenomenon in aquatic macrophytes with his work on *Najas* species, though such excretion was suspected to represent a significant portion of gross photosynthesis (Wetzel, 1965). Excreted organic compounds from macrophytes might well be utilised by epiphytic bacterial, and algal populations. Wetzel (1969b) amassed data to demonstrate the interactions of photosynthesis and excretion of DOC, typical of a submerged macrophyte inhabiting marl lakes. Though presenting such information Wetzel has published no data regarding the chemical components of such excreted organic products.

In the study reported here no measure of rate or quantity of DOC excreted by aquatic macrophytes has been attempted but a brief attempt has been made to identify some of the substances excreted.

The standard and widely-used method for determining DOC in water was developed by Menzel and Vaccaro (1964) and involves the combustion-oxidation of samples freed of

inorganic carbon: the released CO_2 passing through an infra-red gas analyser enabling quantitative measurement to be made. Wetzel (1969a) determined the photosynthetic rates of carbon fixation of macrophytes by the uptake and incorporation of C-14 labelled bicarbonate of known radioassay. He monitored the excreted DOC by combining the techniques for the analysis of total DOC, with radioanalysis for the C-14 in the gas phase. These techniques permitted the measurement of the rate of excretion of DOC to be studied under varying environmental parameters. None of the apparatus used by Wetzel (1969a) was available for the present study.

Methods

In the present investigations whole shoots of P.perfoliatus were collected from stock cultures and washed free of obvious algal contaminants. Cut ends of the shoots were sealed by immersion in molten eicosane wax, and a number of shoots were incubated for six hours at 20°C under three Grolux lamps in a solution of 0.01 M Warburg buffer labelled with C-14 sodium bicarbonate. At the end of the incubation period the plant material was removed and quickly killed in hot 95% ethanol. Aliquots of the bathing solution were removed from the incubation flasks and evaporated to dryness

at 40°C under vacuum in a rotary film evaporator, and then taken up in a small volume of 80% ethanol. An aliquot of this alcohol extract was evaporated to dryness as above, taken up in 1 ml of pyridine and analysed for carbohydrates by GLC as described previously. Another aliquot of the ethanol extract was spotted on Whatman No.1 chromatography paper and a descending paper chromatogram was run, following the methods previously used. An autoradiograph of this chromatogram was prepared as before. An alcohol extract of the plant material was also made and this ran on a paper chromatogram with the extract of the bathing solution. The plant extract was also analysed for carbohydrates by GLC.

Results and Discussion

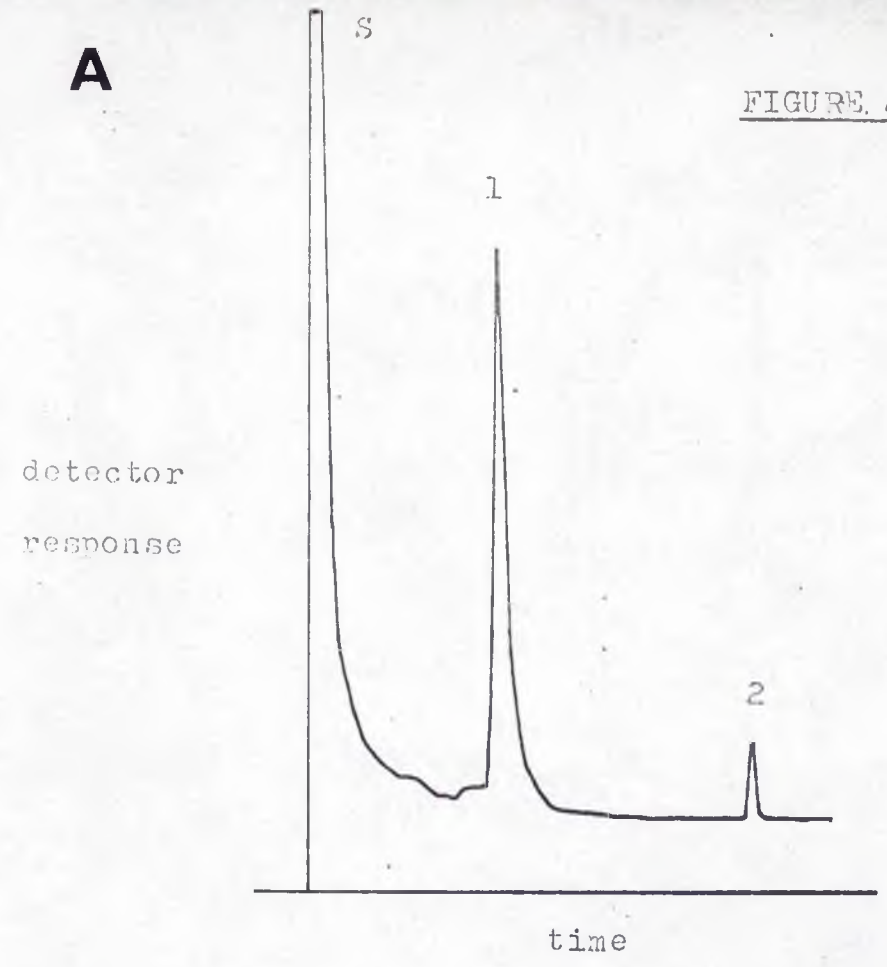
As in previous experiments on the carbohydrate products of photosynthesis of *L.perfoliatus*, the only labelled sugar obtained from the paper chromatography of the ethanol extract was sucrose while the GLC analysis (Figure 4.9a) indicated that only two major carbohydrates were present in the experimental plant material: sucrose and the unidentified carbohydrate with a relative retention time of 1.1. Therefore if any ethanol soluble carbohydrates were excreted during photosynthesis they were probably

FIGURE 4.9

(opposite)

A, GLC separation of soluble carbohydrates from leaves of Potamogeton perfoliatus; B, GLC separation of soluble carbohydrates excreted by leaves of P. perfoliatus. S, solvent peak; 1, unknown; 2, sucrose.

FIGURE 4.9



these substances.

Figure 4.9b illustrates the GLC trace obtained from the ethanol extract of the bathing solution. The concentration of any carbohydrates, if present, in this solution would be very low so the chromatogram was run with a very high degree of amplification of the detector signal; this accounts for the rather peculiar trace which has arisen, due to "column bleed". There is, however, a distinct, though small, peak, corresponding exactly to sucrose, indicating the presence of this substance in the extract. Similarly, the other small but less distinct peak corresponds to the unknown carbohydrate, also present in the plant tissue. The autoradiograph of the paper chromatogram produced a slight fogging in the area corresponding to where the sucrose would be, again indicating the presence of small quantities of this sugar in the alcohol extract of the bathing solution.

Thus the evidence presented from the few preliminary experiments carried out indicates that sucrose may be one of the substances excreted from actively photosynthesising pondweeds. The experiments were, however, crude and though the ends of the cut stems were sealed no check of the effectiveness of the seal could be made. The experiments were also carried out only with one species and thus no general postulates can be safely declared, especially as

axenic conditions were not absolutely ensured and some of the resulting findings could well stem from algal or bacterial metabolism.

Though glycolic acid is thought to be the major organic exudate from phytoplankton (Fogg, 1965), carbohydrates have also been identified as important, natural, extracellular products in several species of freshwater phytoplankton. Watt (1966) identified ribose, ribulose, glucose, maltose and glycerol from their mobility on a paper chromatogram while a large polysaccharide fraction was characterised by its non-mobility. Extracellular release of carbohydrates has been reported for a number of algae under cultural conditions (Watt and Fogg, 1966). Mollebust (1965) found that polysaccharides were the major extracellular product of most of the algae which he studied. Though polysaccharide release can, in part, be explained by the breakdown of the muco-polysaccharide sheaths of algae, Tolbert and Zill (1956) have reported that, at low pH values, sucrose is excreted by Chlorella pyrenoidosa. The phenomenon of carbohydrate excretion has also been reported for some marine flagellates (Guillard and Wangersky, 1958) and has tentatively been correlated with population age, carbohydrate accumulation being greatest in the media of old cultures. Wetzel (pers.com.) has used thin layer chromatography to identify glucose, sucrose, fructose and xylose as excretory

products in axenic cultures of Najas flexilis. Thus, the extracellular release of sucrose by P.perfoliatus is plausible in the light of the findings from other investigations. This, however, by no means excludes the possibility of the excretion of such substances as glycolic acid and other amino acids not detected by the present methods.

Little is known of the qualitative and quantitative significance of the excreted DOC of aquatic macrophytes to the surrounding bacterial, planktonic or even macrophytic populations and its role in ecosystem metabolism, though a distinct functional role has been postulated (Wetzel, 1969b).

The literature on substrate assimilation and heterotrophy in the algae reviewed by Danforth (1962) indicates a wide range of organic substances which can be utilised by phytoplankton, among them sucrose. The use of sucrose as a source of organic carbon for phytoplankton growth has been demonstrated by Fogg and Belcher (1961) for Tribonema aequale and by Samejima and Myers (1958) for Scenedesmus; and though heterotrophy has not been directly demonstrated for aquatic macrophytes certain species of Lemna are able to grow in darkness in the presence of sucrose (Hillman, 1961), and in general sucrose, glucose and fructose promote the growth of Lemna under a sub-optimal light condition and thus a functional role for the dissolved organic matter excreted by aquatic macrophytes cannot be precluded; yet

at the same time cannot be justifiably postulated without further thorough investigations.

CHAPTER 5

LIGHT

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C H A P T E R 5

LIGHT

The study of light in freshwaters has been undertaken as a joint project by Professor D.H.N.Spence, Mrs. .M. Campbell and myself and full results will be published shortly in the Journal of Freshwater Biology. Only those parts of this work specifically related to the present study are reported here.

Observations on the light climate of submerged freshwater macrophytes have been undertaken for two purposes:-

(1) To determine during the *in situ* primary productivity experiments the quantity of light energy available for photosynthesis.

(2) To gather information on the quantitative and qualitative changes in the passage of light through freshwater and its possible influence on the submerged vegetation.

MEASUREMENT OF UNDERWATER IRRADIANCE DURING IN SITU PRIMARY PRODUCTIVITY EXPERIMENTS

There have been few studies in freshwater which report measurements of the incoming light energy made in conjunction with primary productivity estimates. In these, the calculation of energy fluxes to the plants are generally approximated from light transmission curves, a process which, though tedious, still provides only approximate values (Vollenweider, 1969). Simpler procedures have been advocated by Sauberer (1962) and Vollenweider (1969) but these still produce results which are approximations of unknown accuracy, due mainly to the fact that light is measured above the surface of the water and usually by an instrument which does not measure light energy directly and thus requires cross calibration.

During several of the in situ primary productivity experiments described in Chapter 3 an estimate of the incoming, underwater irradiance was made by frequently scanning the underwater visible spectrum (400 - 750 nm) with a direct-reading spectroradiometer. The instrument used was a Model SR ISCO Spectroradiometer attached to a Model SRR Programmed Scanning Recorder. These instruments enabled a continuous spectrum scan to be recorded automatically

at 15 minute intervals throughout the course of an experiment.

Underwater records were made by attaching to the instrument a two metre fibre-optic cable terminating in a teflon diffusing screen. This screen ensured that the directional response of the instrument was proportional to the cosine of any angle of incident light, except when near horizontal. This approximate conformance to Lambert's Cosine Law eliminated any need for precise aiming of the detector and is important because natural incident light is utilised for photosynthesis regardless of its direction of propagation. During all observations the teflon diffusing head was held facing upwards on a horizontal plane.

The electronics of the instrument provide an extremely narrow electrical noise bandwidth and hence a stable meter response to the incoming light beam. The light beam is interrupted before it reaches the light sensing planar photodiode by an electromechanical chopper. The monochromator is an interference wedge and the wavelength is varied by lengthwise movement of this filter between the light entrance slit and the measuring photocell. The bandwidth of the instrument is 15 nm over the range 380 - 750 nm.

The spectroradiometer was calibrated at two monthly intervals against a standard light source at a constant current in an ISCO calibrator. The calibrating standard

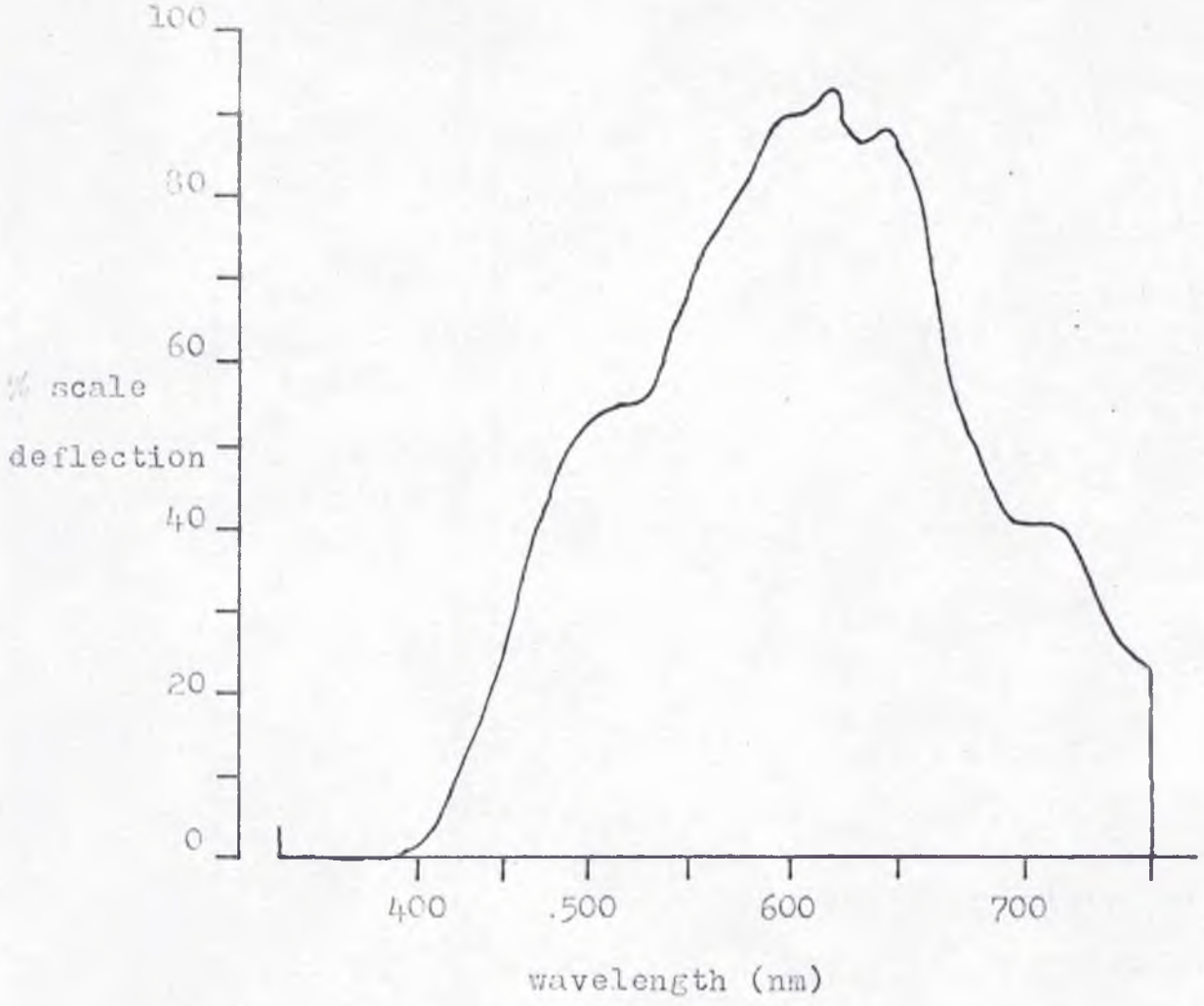
was a ribbon filament tungsten lamp (Type 18A/F10/2P-6V) which in turn was standardised against a Canadian Research Council calibrated colour-temperature and luminous intensity lamp (ISCO No.123). Calibration charts were prepared by determining at successive wavelengths the ratios of actual spectral intensity to measured spectral intensity. The correction of observations taken at any wavelength was made by multiplying the observed values by the calibration factor of that particular wavelength.

The instrument reads directly in μwatts/cm^2/nm and the chart recorder plots the percentage full scale deflection of this reading against wavelength. Accuracy of the instrument is 30 - 40% but inaccuracy is reduced to 8 - 10% by the calibration described above. The greater part of this remaining error is due to uncertainty in the calibration standard itself.

The area under each recorder plot (Figure 5.1) was integrated for each 25 nm waveband, the appropriate correction factor applied and the figures summed to give a value for the total irradiance (400 - 750 nm). All records obtained during the course of any one experiment were pooled and an average value for the spectral intensity measured throughout the incubation period computed.

The majority of the <u>in situ</u> experiments were carried out at depths in excess of two metres. Due to the limited

FIGURE 5.1



Typical chart record of a spectroradiometric scan of underwater irradiance (400 - 750 nm).

Note: Figures 5.4 A, B, C following p. 182 present underwater irradiance data in terms of $\mu w/cm^2$ plotted against wavelength.

length of the underwater probe no records of underwater light could be obtained at these depths. Estimates of the light energy received by the plants during these deep incubated experiments were made by applying an attenuation coefficient (Westlake, 1965c) to records of spectral intensity made in shallow water (usually at a depth of 50 cm). This method was thought preferable to measuring the incoming radiation above water and then applying some correction factor to allow for water surface reflection and refraction (Sauberer, 1962; Westlake, 1964) the values of which are uncertain (Vollenweider, 1969).

Measurements of attenuation coefficients were made with the spectroradiometer and recorder placed on a dexion platform standing in at least 1.25 m depth of water with the detector held horizontally, facing upwards, under water. Under as uniform weather conditions as possible, scans of the underwater visible spectrum (400 - 750 nm) were made at 10 cm depth intervals down to the limit of reach of the glass-fibre, optic probe and sensor head. The recorder scans were processed as described previously and values calculated for the spectral intensity recorded at each depth. These values were then plotted semi-logarithmically against depth and only those plots that were sensibly linear retained. on-linearity of the plot arose through changing overhead light conditions during the time of the experimental

observations. Straight lines were computed by applying the linear regression option in the conversational mode, statistical package programme of Cole and Campbell (1969) and the irradiance value at zero depth extrapolated. This diffuse irradiance (Io) was equated with 100% subsurface transmission and the percentage diffuse irradiance (Im) at 1 m depth calculated, where m is the vertical depth or distance between planes of measurement, in metres.

From these figures the vertical attenuation coefficient E' (Westlake, 1965c) was derived using $\log_e$ in the expression (Westlake, loc.cit.):-

$$I_m^H = Io(\log.base)^{-E'm}$$

where I_m^H is the diffuse irradiance measured by a horizontal detector facing upwards at m metres after passing through a scattering medium, Io the initial irradiance for a diffuse source (divergent and deflection losses being insignificant) and E' is the attenuation or extinction coefficient for the diffuse radiation in limiting situations related to m in metres. E' is the angle of slope in the plot of $\log_e I_m$ against depth in metres (m) where I_m is the irradiance at depth m in metres.

The attenuation coefficients calculated by the methods described above were computed from observations taken in less than two metres of water. There is, however, other

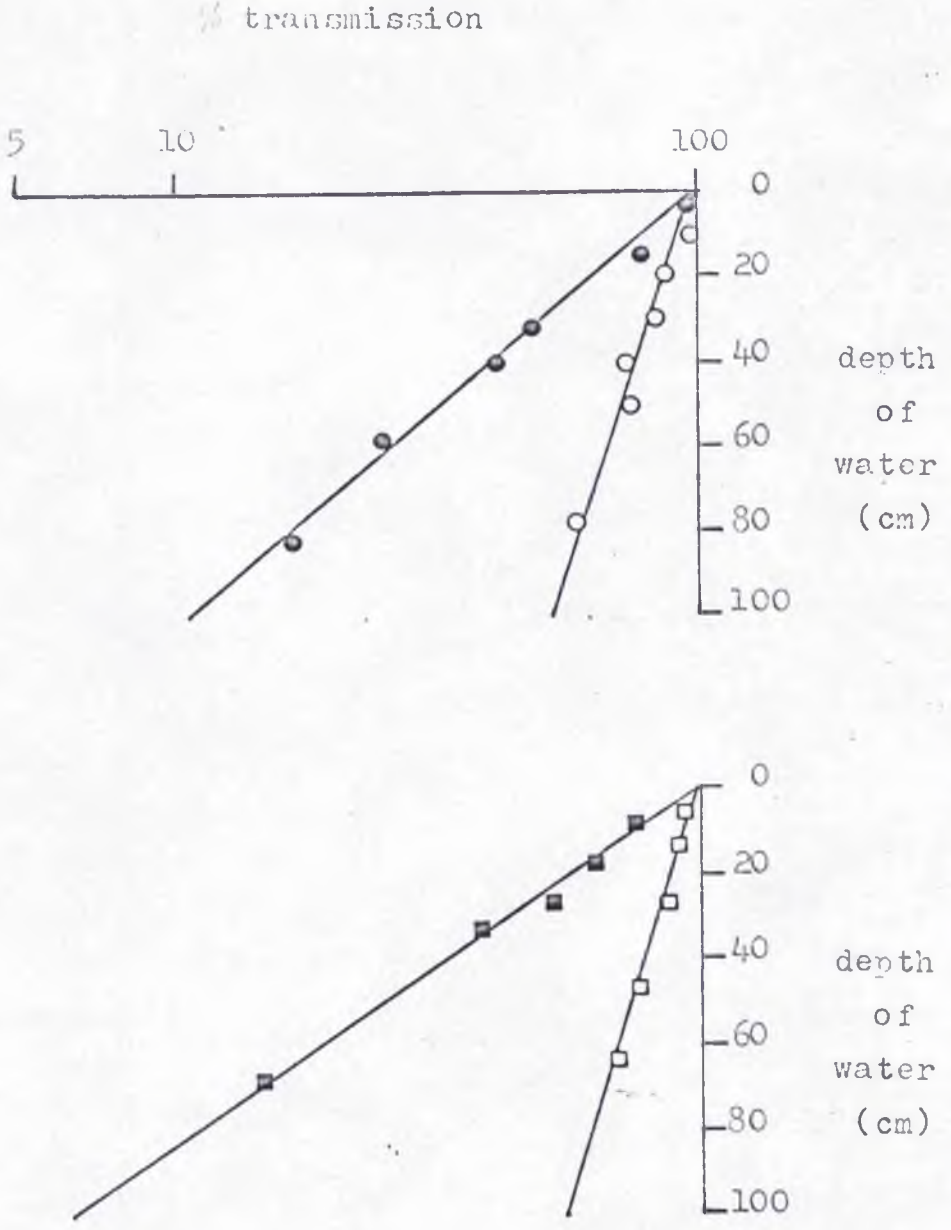
evidence from measurements made with selenium photocells (Spence et al., in press) to suggest that such observations hold good, in some lochs at least, down to a depth of eight metres. In the present study this observation is assumed valid for all the lochs investigated. Attenuation coefficients calculated by the methods outlined above are presented in Table 5.1 while they are graphically illustrated in Figure 5.2. The variation in the values of these coefficients are discussed in a later section of this chapter.

TABLE 5.1

Attenuation of light in Loch Croispol, Loch Leven and Loch of the Lowes, each measured on three separate dates during 1970

Loch	Date	Attenuation Coefficient (E_e)
Croispol	19/6/70	0.56
	4/8/70	0.55
	16/9/70	0.57
Leven	4/5/70	1.30
	2/7/70	2.86
	14/8/70	2.90
Lowes	30/4/70	0.76
	14/5/70	1.20
	18/8/70	1.30

FIGURE 5.2



Attenuation of diffuse down-welling irradiance ($\mu w/cm^2$) over the range 400 - 750 nm, in relation to depth of water, computed as lines of best fit and expressed as percentages of subsurface (0 cm) irradiance.

- ● Loch Uanagan 15.7.70
- ■ Loch Leven 2.7.70
- ○ Loch of the Lowes 30.4.70
- □ Loch Croispol 4.8.70

The percentage of light transmitted to the incubation depth of the plant tissue in a productivity experiment was calculated by applying the attenuation coefficients derived as above. The value of the light energy was found with reference to the chart records of underwater spectral intensity by simple proportionality and thus the carbon fixed by the leaves per unit area, per unit time was related to the light energy.

The topics of terminology and units for use in the measurement of light underwater have been reviewed by Strickland (1958) and by Westlake (1965c). The spectro-radiometer used in the present studies records in units of $\mu w/cm^2/nm$ or $\mu w/cm^2/waveband$. One watt is equivalent to one joule per second and thus the light energy can be easily expressed in terms of joules as is advocated by Westlake (loc.cit.). The use of the joule or Kilojoule would also, strictly, require the adoption of the square metre as the unit of area but it is felt that the results of primary productivity experiments based on units of square metres of leaf area would be difficult to interpret. The calorie has been adopted (1 Kcal = 4186 joules). By transforming $\mu w/cm^2$ to $cal/cm^2/hour$ (by multiplying by 8.604) the units of light energy and units of primary productivity ($\mu g\ C/cm^2$/hour) are then compatible and hence carbon fixation can be expressed in terms of $\mu g\ C/cal$. Appropriate conversions

can, however, be used to transform these units into those which may be more acceptable though, perhaps, in this study, less meaningful.

QUANTITATIVE AND QUALITATIVE CHANGES IN THE PASSAGE OF LIGHT THROUGH FRESHWATER

The underwater light field is characterised by two vectors (Vollenweider, 1969):-

(1) A vertical gradient (attenuation or extinction)- a quantitative change.

(2) A shifting of the radiation spectrum - a qualitative change.

These photic conditions underwater are a combination of the effects of the incident irradiance, the absorption of this by the water and its scattering by the suspended matter of the water. A quantitative change with depth brought about by such parameters is termed vertical attenuation and is merely a diminution of light with depth. The measurement of this diminution has been delt with in the first part of this chapter. Current terminology favours avoiding the term extinction and replacing it with attenuation though for all practical purposes they are synonomous (Westlake, 1965c). The influence of intensity and spectral composition of light on the distribution of aquatic vegetation has been discussed previously by Shirley (1935, 1945) and Gessner (1955).

Quantity

The effect of light intensity upon submerged aquatic vegetation are two fold:-

(a) The induction in several species of a degree of morphological adaption.

(b) The effect of light intensity upon photosynthesis.

Both these points can be related to the natural distribution of the species concerned.

Effects on morphology

Spence and Chrystal (1970) have shown that the ability of several Potamogeton species to produce "sun" and "shade" adapted leaves under laboratory conditions of varying light intensity was directly related to their natural zonation with depth of water, so that habitually deep growing species were easily induced to produce "shade" leaves while shallow plants produced "sun" leaves. These workers also compared the photosynthetic response of these sun and shade adapted leaves, concluding that the photosynthesis of shade species, from deep water, could be limited at higher irradiances by an inability to alter their leaf morphology and hence increase their capacity to absorb light. They were able to photosynthesise in deep water, at low light intensities

due to a lowered respiration rate per leaf area, which could in part result from a lower ratio of leaf weight to leaf area, itself an adaption to absorb more light per unit leaf weight.

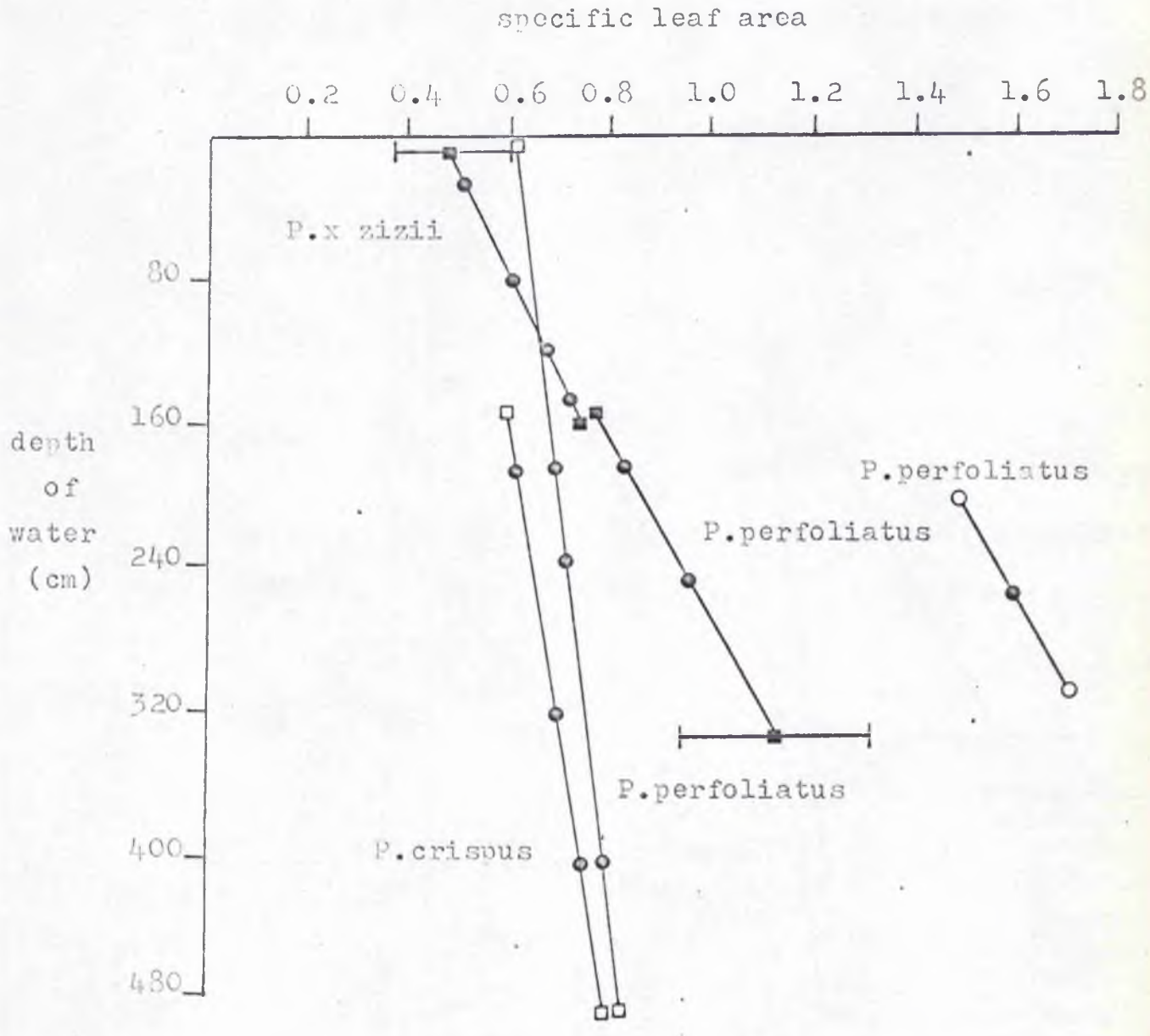
In a recent joint study, not reported here in full, Spence, Chrystal and Campbell (1971, in press) have investigated the leaf morphology of several species of Potamogeton, in Scottish lochs, throughout a period of nearly two years, finding that measurements of specific leaf area (SLA: cm^2 leaf area/mg leaf dry weight) corroborate the laboratory findings of Spence and Chrystal (loc.cit.), that light is important in controlling the distribution of submerged freshwater macrophytes. Figure 5.3 shows that with no overlap in rooted depth there was no overlap in SLA for two species in one loch and in another loch inclusive range in rooted depth for two species was accompanied by inclusive specific leaf areas. Two further observations can be made from the SLA data:-

(i) It would appear that, regardless of species, the relationship of SLA and depth is similar for any loch.

(ii) P.perfoliatus in three lochs has mutually exclusive ranges in SLA.

The computed regression lines of SLA upon depth for P.x zizii and P.perfoliatus in Loch Uanagan are practically

FIGURE 5.3



The lines of a least squares analysis of specific leaf area (cm^2 leaf area/mg leaf dry weight) upon depth of water (cm) for; Potamogeton crispus and P.perfoliatus in Loch Baille na Ghobhainn (□); P.x zizii and P.perfoliatus in Loch Uanagan (■); P.perfoliatus in Loch of the Lowes (○). The least and greatest error mean square are indicated and rooting depths denoted (●).

continuous, implying a fixed relationship between depth and SLA regardless of species. This implication is further strengthened by the data from Loch Baille na Ghobhainn. The regression line for P.perfoliatus in Loch of the Lowes is practically parallel to the Loch Uanagan line for the same species. These lochs have similar light attenuations (Table 5.1) while Loch Baille na Ghobhainn is a clear water loch similar to Loch Croispol. It is suggested that the rate of increase in SLA is inversely related to the mean light attenuation while absolute values may be influenced by other parameters.

The ranges in SLA exhibited by P.perfoliatus are interesting in terms of zonation and limits of colonisation. P.perfoliatus can achieve a lower SLA than it does in Loch Uanagan where P.x zizii and P.praelongus occupy the shallower water where its theoretical SLA should permit it to grow. This restriction in rooting range could arise from competition or perhaps substrate factors. In Loch Baille na Ghobhainn P.perfoliatus does not grow as deep as its potential SLA should permit. Here the limiting factor could be that the very steep and unstable marl slope is preventing rooting or that the opacity of the mesolimnion is drastically reducing the penetration of light (Spence, 1971).

Effects on photosynthesis

There are a number of reports on laboratory studies of the photosynthesis of submerged aquatic macrophytes carried out under varying light conditions (Spence and Chrystal, 1970) but few field studies. Meyer et al.(1943) studied the relative rates of apparent photosynthesis in several species of aquatic macrophytes at depths ranging to ten metres, well in excess of their natural depth limit. In all species tested the rate of apparent photosynthesis decreased less rapidly with depth of immersion than did the light intensity. Similar results have been reported by Wuttner (1963) from even greater depths and in the present studies primary productivity experiments carried out at different depths have been reported and discussed in Chapter 3. With regard to photosynthesis and water depth, many limnologists have shown that the depth of penetration of light into water can vary greatly with the time of year (Sculthorpe, 1967) and it is thus possible that at some periods photosynthesis is possible under a great depth of water yet, on other occasions, is not. Such a seasonal variation in the attenuation of light has been recorded in Table 5.1. Over the period of measurements, Loch Croispol had the lowest attenuation (i.e. the deepest penetration of light) and this varied least. Loch of the

Lowes had a low attenuation in March but this rose during the summer as did that of Loch Leven, though the highest value recorded in Loch of the Lowes corresponded with the lowest value from Loch Leven. These differences in the attenuation of light, especially in Loch Leven, are, most probably, a direct reflection upon the variations in the phytoplankton populations of the lochs and upon the quantity of colloidal and particulate matter. Lochs Baille na Ghobhainn, Borralie and Lanlish are phytoplankton-poor, limestone lochs with clear water like Loch Croispol while Lochs Achtriochtan and Uanagan are comparable to Loch of the Lowes. Similar attenuation values were recorded in Loch Uanagan as are reported for Loch of the Lowes.

The primary productivity of macrophytes is ultimately dependent upon light, thus as light is attenuated in water there is a theoretical depth at which photosynthesis cannot compensate for respiration and growth becomes impossible. As noted in Chapter 1, Sculthorpe (1967) concludes that it is this limited penetration of light in freshwaters which confines the rooted, vascular plants of lakes to the uppermost ten metres or less. Regarding the depth limits of colonisation recorded for Scottish lochs (Table 1.17) and the estimates of light attenuation given in Table 5.1 a similar relationship seems apparent, deepest colonisation being recorded in the clear-water, calcareous lochs,

shallower limits in the brown water lochs and least colonisation in the one plankton-rich, eutrophic loch which was investigated.

If primary productivity is dependent upon light then it might be expected that the lochs with the lowest average values for light attenuation would have the highest productivity. As noted in Chapter 3, no conclusions regarding the productivity of different lochs could be reached from the *in situ* C-14 experiments. The biomass estimates reported in Chapter 1 and the energy contents given in Chapter 2 might, however, be of use to compare the production of the different lochs, as they represent an integration of the total light climate. As pointed out in Chapter 1 caution must be exercised in the interpretation of biomass data and in the present comparison it must be remembered that the vegetation biomass is not only a reflection upon the light available for its growth but on several other parameters such as temperature and nutrient availability. Bearing these points in mind, Loch Croispol appears to be a productive loch, the bulk of the vegetation, however, consists of the perennial algae *Chara aspera* and *Chara nabilloana*. When only the *Potamogeton* species are compared Loch Hanagan is the most productive, but here the evergreen *P.praelongus* contributes a great deal and overshadows the mean annual standing crop value of 10 g/m^2 (Table 1.17) for

P.perfoliatus in Loch Croispol. Thus a true comparison is difficult and, perhaps, not valid from the limited data available, if even possible at all.

The overall importance of light quantity in the aquatic habitat therefore cannot be neglected, yet at the same time from the present studies with submerged macrophytes, some of its effects are difficult to judge.

Quality

Introduction

The effect of changes in light quantity have on the distribution and productivity of submerged macrophytes has been discussed in the first part of this Chapter. A second feature of the light properties of freshwater lochs which may reflect limitations upon distribution and productivity is the variation in light quality between different water bodies, brought about by the differential transmission and absorption of light of different wavelengths and expressed qualitatively in the water colour. These water colours extend from the clear, usually blue-green waters of the limestone lochs through dark-green, enriched, eutrophic waters to dark-brown, peaty-water. Little is

known of the extent to which variations in light quality effects macrophyte photosynthesis and hence limits of downward colonisation.

The majority of work published on the qualitative changes in underwater spectral intensity have been carried out using matched selenium photocells and a series of coloured, broad-band filters (Westlake, 1965c), few results have so far been published of underwater spectral intensity measured directly by a spectroradiometer such as has been employed in studies reported here. The majority of data stems from the marine environment and has been well reviewed by Tyler (1965). Using a Scripp's submersible spectroradiometer described by Tyler and Smith (1966) these authors have published accounts of the optical properties of Crater Lake, Oregon, U.S.A. (Tyler, 1965; Tyler and Smith, 1967), while in this country Talling (1970) using an instrument similar to that employed in the present study has reported briefly on observations made in Blelham Tarn.

As with all the studies reported here observations have been made in a number of lochs which, based upon a previous survey (Spence, 1967), probably approach the extremes of the optical conditions found in Scotland.

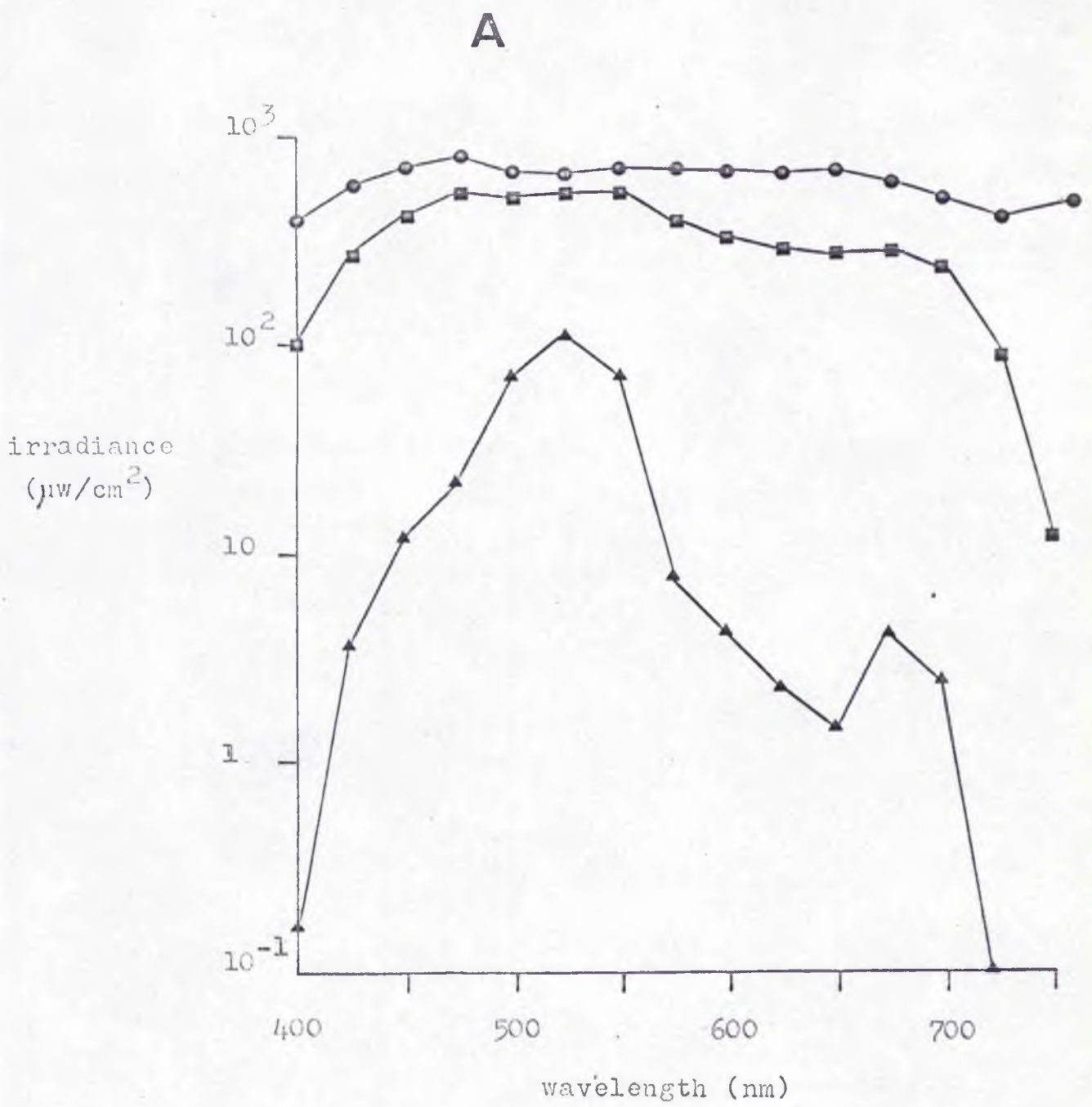
Methods

Utilising the same observations on light intensity and depth used previously to calculate attenuation coefficients (Table 3.1), attenuation coefficients were calculated for every 25 nm waveband of the visible spectrum (400 - 750 nm) by the same methods as applied to the whole spectrum. For each depth record the percentage composition of the spectrum (400 - 750 nm) was calculated in 25 nm wavebands so that changes in spectral composition with depth could be easily recognised.

Results

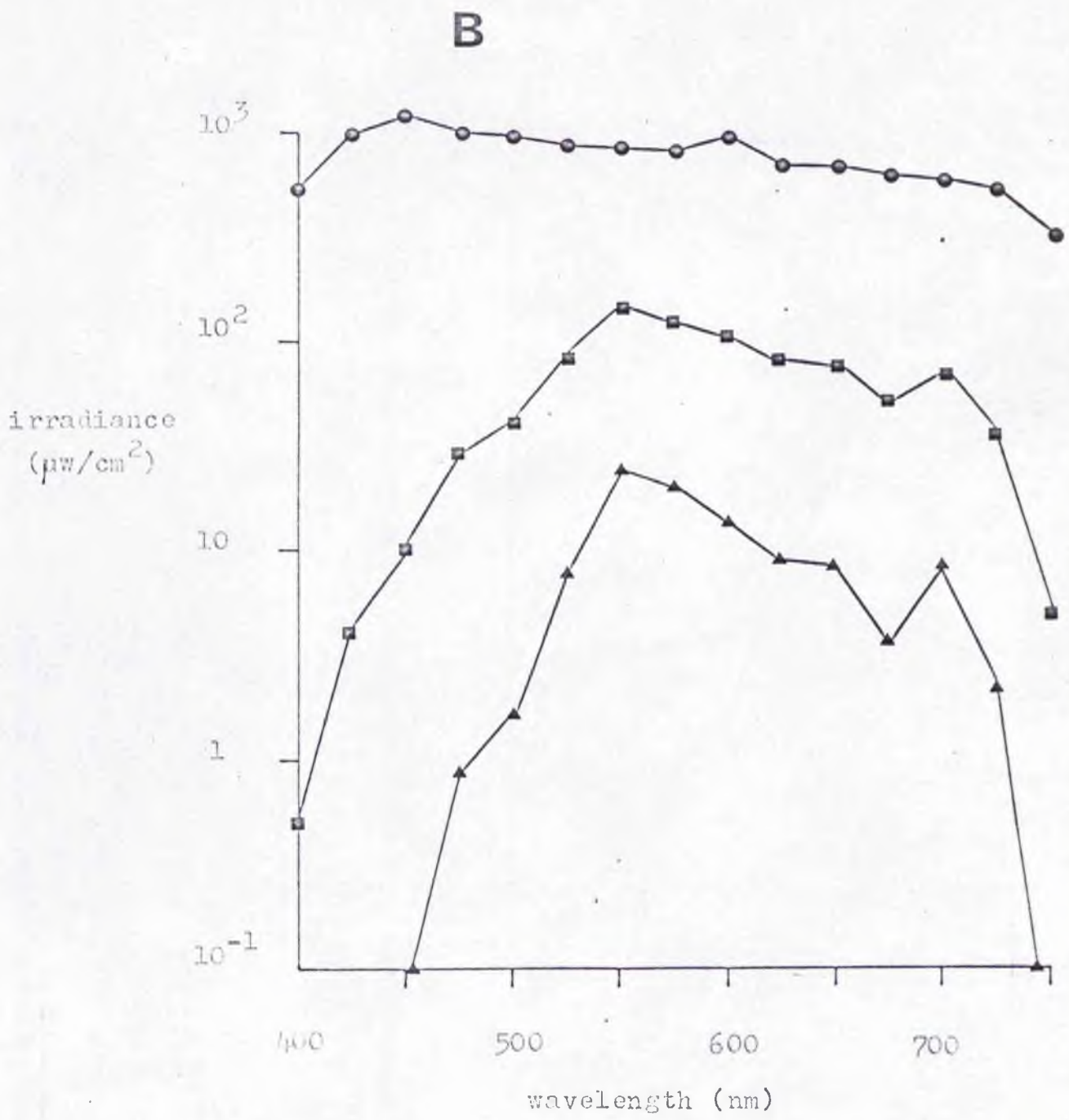
Figure 5.4 presents data on the diffuse downwelling irradiance ($\mu w/cm^2$) in relation to wavelength (400 - 750 nm) at subsurface (0 m) and at 1 m depth of water, derived from the calculated lines of best fit of $\log_{10}$ irradiance/25 nm upon depth, and serve to illustrate the changes in spectral intensity with depth in Lochs Croispol, Leven and Uanagan. Data is also presented for a depth of 6 m in Loch Croispol, the depth limit of all but sparce colonisation by rooted macrophytes, and for 2 m in Loch Leven which probably represents the maximum colonised depth in that loch. Figure 5.5 presents data on the percentage composition of

FIGURE 5.4



Diffuse down-welling irradiance in Loch Croispol ($\mu w/cm^2$) in relation to wavelength (400 - 750 nm) at subsurface (0 m, ●), at one metre (■) and at six metres (▲), the depth limit of colonisation by rooted macrophytes. Computed from lines of best fit of $\log_{10}$ irradiance per 25 nm waveband upon water depth, measured over one metre depth on 4.8.70.

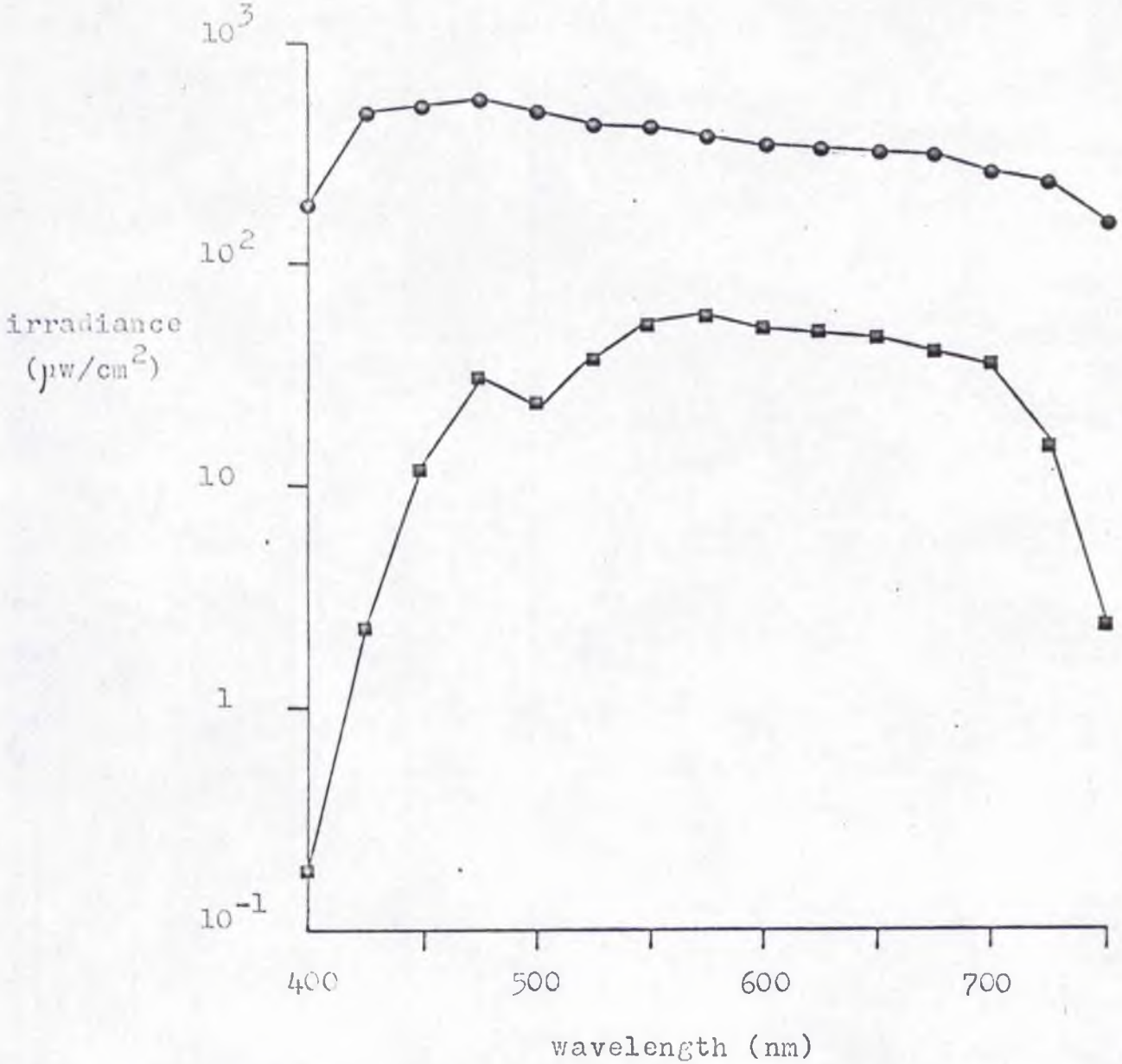
FIGURE 5.4



Diffuse down-welling irradiance in Loch Leven ($\mu w/cm^2$) in relation to wavelength (400 - 750 nm) at subsurface (0 m, ●), at one metre (■) and at two metres (▲) the depth limit of colonisation by rooted macrophytes. Computed from lines of best fit of $\log_{10}$ irradiance per 25 nm waceband upon water depth, measured over one metre depth on 2.7.70.

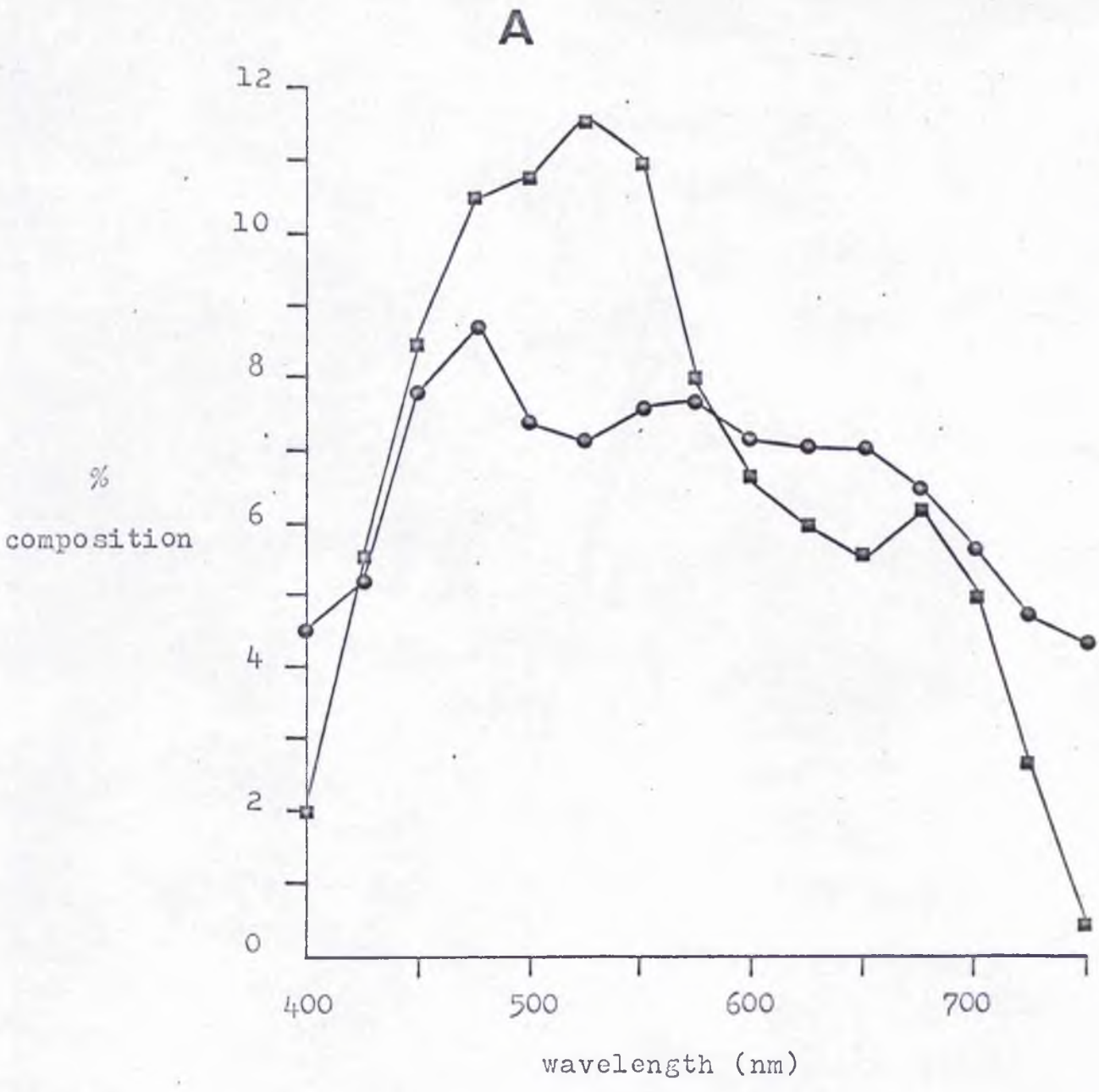
FIGURE 5.4.

C



Diffuse down-welling irradiance in Loch Uanagan ($\mu w/cm^2$) in relation to wavelength (400 - 750 nm) at subsurface (0 m, ●) and at one metre (■). Computed from lines of best fit of $\log_{10}$ irradiance per 25 nm waveband upon water depth, measured over one metre depth on 15.7.70.

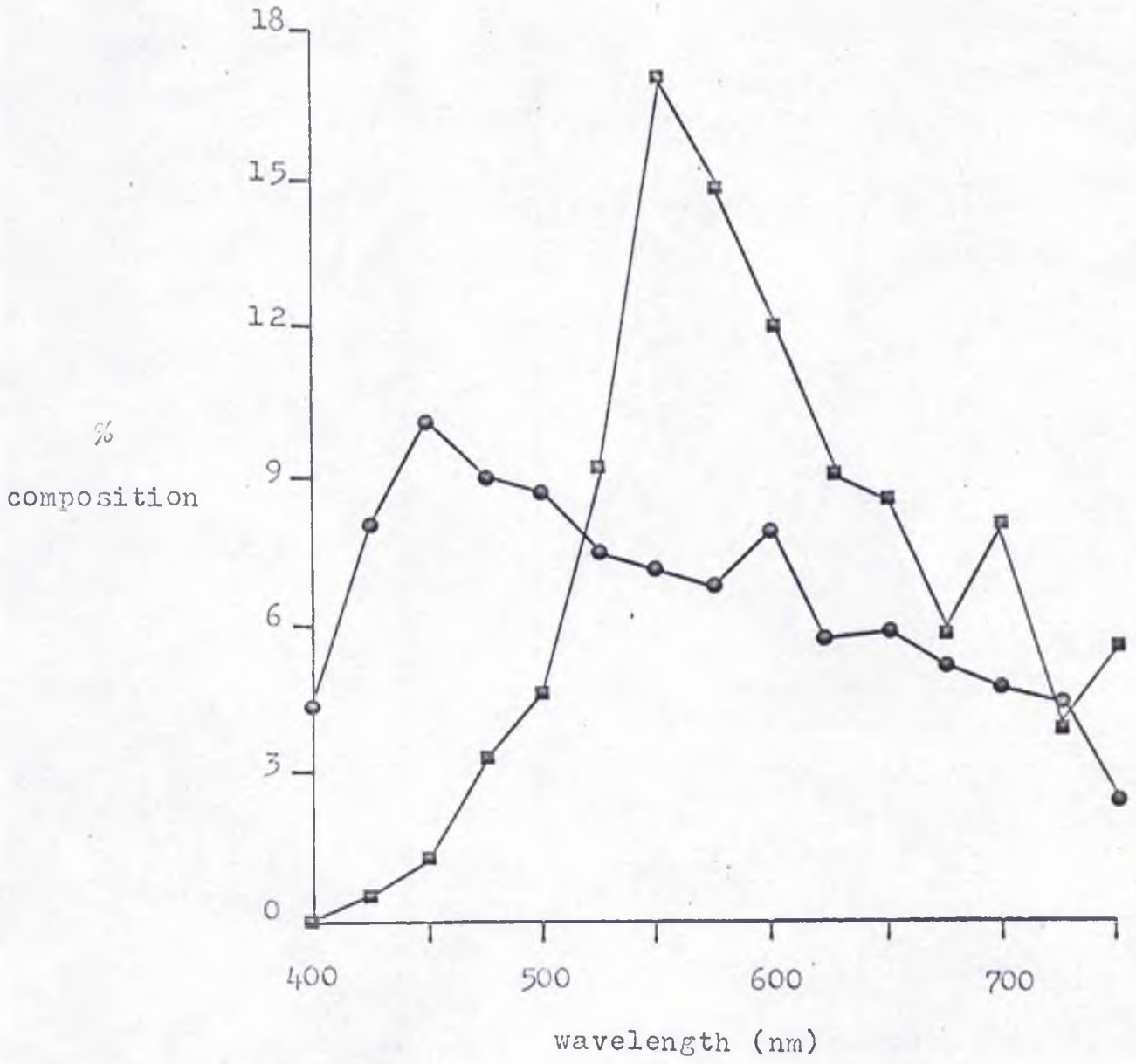
FIGURE 5.5



Percentage composition of spectral intensity (400 - 750 nm), in 25 nm wavebands, at one metre depth of water (■) and at subsurface (0 m, ●). Measured in Loch Croispol on 4.8.70.

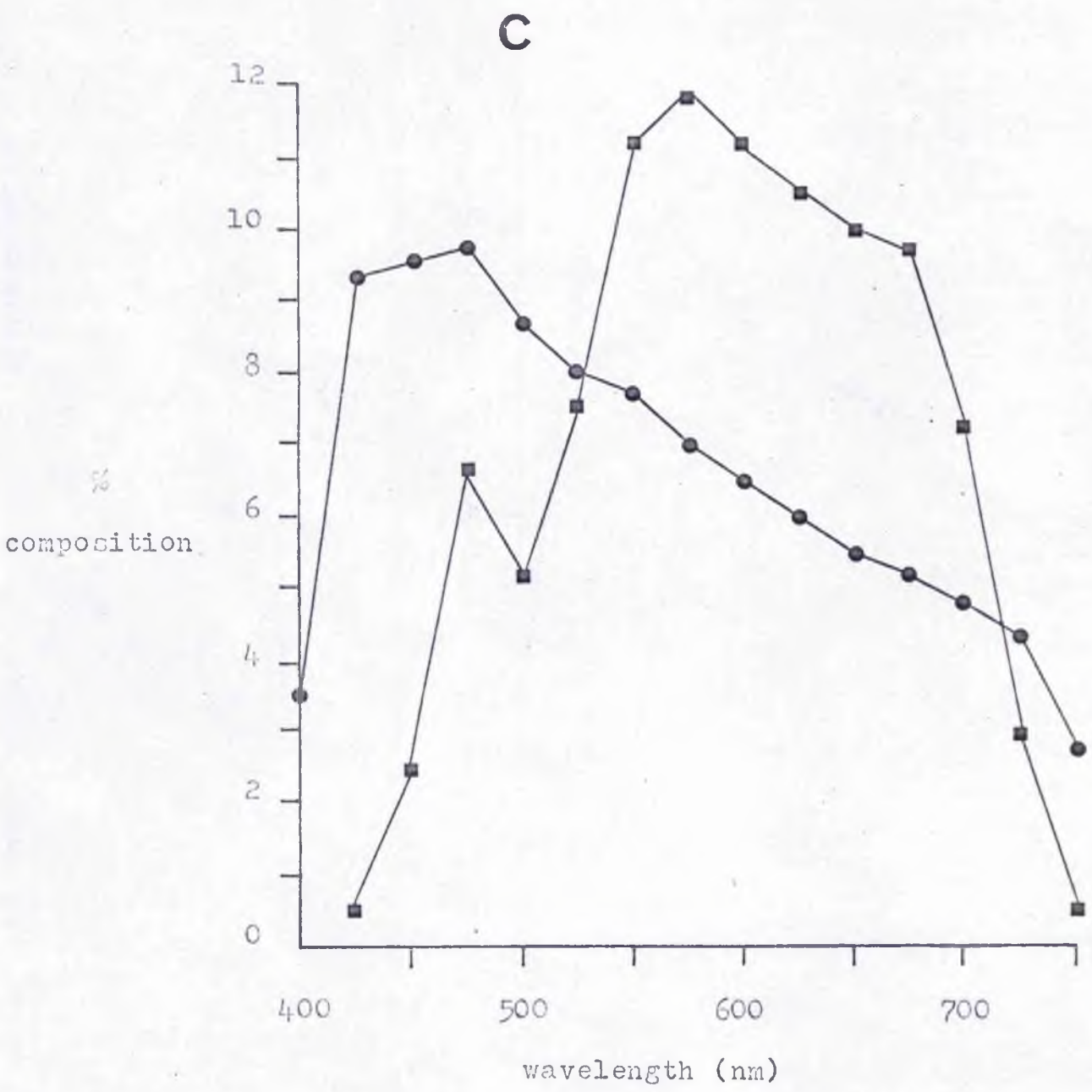
FIGURE 5.5

B



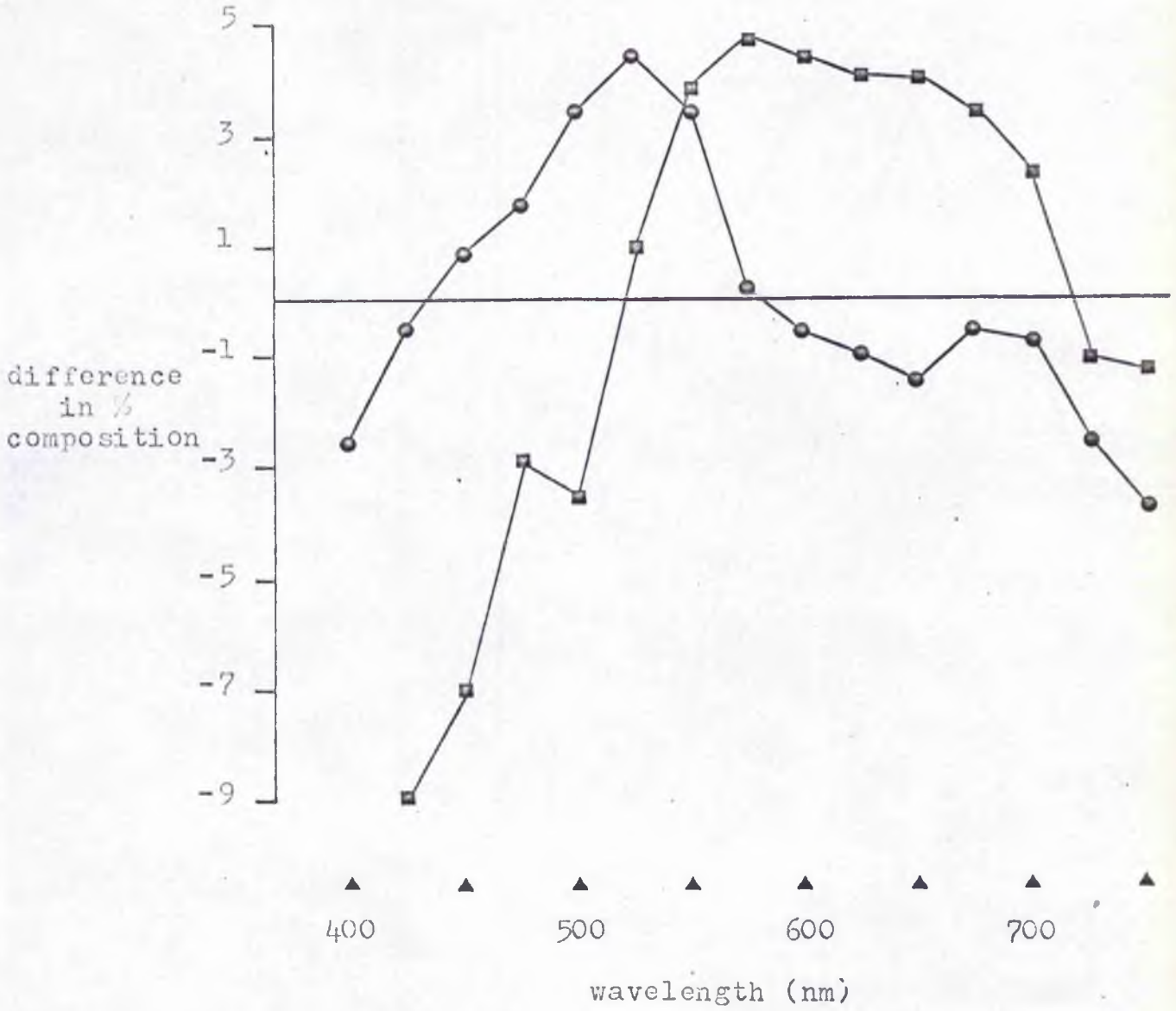
Percentage composition of spectral intensity (400 - 750 nm), in 25 nm wavebands, at one metre depth of water (■) and at subsurface (0 m, ●). Measured in Loch Leven on 2.7.70.

FIGURE 5.5



Percentage composition of spectral intensity (400 - 750 nm), in 25 nm wavebands at one metre depth of water (■) and at subsurface (0 m, ●). Measured in Loch Uanagan on 15.7.70.

FIGURE 5.6



Difference in spectral composition (400 - 750 nm), in 25 nm wavebands, between one metre depth of water and subsurface (0 m). Values for Loch Croispol (●) on 4.8.70 and Loch Uanagan (■) on 15.7.70. Positive percentage figures indicate a greater proportion of a particular waveband at one metre than at subsurface.

the spectral intensity in 25 nm wavebands in these three lochs at 1 m as compared with that at the subsurface, both calculated from extrapolation of the appropriate attenuation coefficients. Figure 5.6 illustrates the difference in these composition patterns in Loch Croispol and Loch Uanagan.

Discussion

The spectral composition diagrams illustrate clearly the proportional increase in short-wave radiation in the blue-green, limestone loch Croispol at 1 m compared with the relative increase in long-wave radiation over the same depth range in the brown coloured Loch Uanagan, which, since water of this quality is the commonest in Scottish lochs (Spence, 1964), represents qualitatively the most typical pattern in Scottish lochs. The figures for the yellow-green Loch Leven fall between these two extremes.

Ultimately, photosynthesis is a quantum reaction; the total amount of energy is unimportant. This is also true of other photochemical reactions due to the fact that light quanta, like other chemical reactants, participate in the reaction stoichiometrically (Richardson, 1964). According to Einstein's law of photochemical equivalence, in chemical reactions resulting directly from the absorption

of light, one quantum of light activates one molecule of a substance. The energy (E') in one quantum of radiation of frequency (v) is given by E' = hv ergs (Einstein's law) where h = Plank's constant (6.56×10^{-27}) and is the amount of energy absorbed per molecule. The energy (E) absorbed per mole is therefore Nhv where N is Avagadro's Number, the number of molecules per mole ($= 6.02 \times 10^{23}$), E' is termed one einstein of radiation:-

$$E' = Nhv \text{ ergs}$$

$$= \frac{Nhc}{\lambda} \text{ ergs}$$

where $v\lambda = c$ = the velocity of light ($= 3 \times 10^{10}$ cm/sec), λ = wavelength. Thus the energy per mole may be calculated by substituting in the above equation:-

$$E' = \frac{6.02 \times 10^{23} \times 6.56 \times 10^{-27} \times 3}{\lambda \times 4.18}$$

Thus the energy contained per mole or quanta of light at any wavelength can be calculated and Table 5.2 shows that an einstein or quantum of blue light (401 - 492 nm) has a greater energy content than an einstein of red light (621 - 720 nm) and hence photosynthesis, since it is a quantum reaction, proceeds more efficiently in terms of total energy at the red end of the light spectrum than at the blue end.

TABLE 5.2

Values of the einstein for visible radiation

Wavelength (nm)	E (Kcal/einstein)
400	71.45
500	57.18
600	47.65
700	40.84

Bearing in mind the quantum nature of photosynthesis and the differential attenuation of the light spectrum in freshwaters it can be concluded that if the major portion of the transmitted light energy is at the blue end of the spectrum, where one quantum has a high energy content, then less quanta will be available for photosynthesis were an equivalent light energy transmitted at the red end of the spectrum, hence the rate of photosynthesis may be light limited in a light spectrum which is predominantly blue. Calculating figures for the average energy content of light quanta at the depth limits of colonisation in Loch Croispol and Loch Uanagan from the irradiance data in Figure 5.5 and from the equations given above, figures of 55.64 Kcal (230.6 Kjoule)/einstein and 46.23 Kcal (196.0 Kjoule)/einstein are obtained, reflecting little difference in the number of quanta which would be available from the same

amount of energy at these depths.

With regard to the estimates of the quantitative changes in the passage of light through different loch waters expressed as attenuation coefficients (Table 5.1), there is a four-fold difference in the maximum values of these coefficients for Loch Croispol (E_0 = 0.57) and Loch Managan (E_0 = 2.30). Comparing Loch Leven (E_0 = 2.90) with Loch Croispol there is nearly a six-fold difference in the depth of water which would receive the same total irradiance whereas, in comparison, there is only some slight difference in the depth receiving the same number of quanta/sec/cm^2 due solely to the difference in the spectral composition of the light. Thus it would seem that any effect of variations in light quality upon the distribution and productivity of aquatic macrophytes are probably very slight when compared to the overall importance of light intensity.

This conclusion is, however, drawn from field observations on light quality and intensity at the lower limit of colonisation and thus before any hard and fast conclusions can be drawn a great deal more field observations must be gathered and the problem must also be investigated under the controlled conditions of the laboratory. Data are still required on the pigment composition of plants growing in waters of contrasting optical properties and on the

action spectra of the leaves of the submerged species. It is possible, however, that conditioning effects similar to those discovered for Ulodea species when grown in red and blue light (Döring and Simonis, 1936) may operate, or, as was found in Marsilea species by Gaudet (1963), morphological changes are induced by changes in the spectral composition of light. In the absence of such knowledge any speculations on the relative importance of light quality and quantity in influencing the distribution, zonation and productivity of submerged aquatic macrophytes must be made with caution.

EFFICIENCY OF PRIMARY PRODUCTIVITY

It will be recalled from Chapter 1 that the production of freshwater macrophytes in Scottish lochs was, on the basis of biomass estimates, concluded to be small. Chapter 3 of this thesis found that the rates of primary productivity of several Potamogeton species were also small when compared to those of plants from other habitats (Figure 3.9). As stated in that account, Ikusima (1966) has concluded that the low productivity of submerged aquatic plants may be ascribed, at least in part, to a lower photosynthetic capacity. This claim will now be discussed in relation to the efficiency of carbon fixation.

There has been much discussion over the theoretical minimum quantum requirement for photosynthesis i.e. the maximum number of light quanta required to initiate the fixation of one carbon dioxide molecule. It has been found by bomb calorimetry (Richardson, 1964) that one mole of hexose sugar ($C_6H_{12}O_6$) has a calorific content of 689 Kcal and hence approximately 115 Kcal of energy must be stored per mole of carbon dioxide fixed. Red light (41.5 Kcal/einstein at 670 nm) is particularly effective in promoting photosynthesis and thus the number of quanta of red light required to fix one mole of carbon dioxide is 115/41.5 = 3 (by definition quanta cannot be divided).

Therefore the theoretical quantum requirement is 3 (Richardson, loc.cit.)

Experimental determinations on the quantum requirement for photosynthesis have given varying results ranging between 1 and 12, depending upon the conditions of the determination, and the value is now thought to lie between 8 and 12 and generally averaged at 10 (Kok, 1970). This is some three to four times the theoretical value and results from the fact that though the light energy is absorbed and is essential to photosynthesis, it does not appear in the final products but is lost as heat and thus only one quarter to one third of the light absorbed is eventually "stored" as carbohydrate. With a quantum requirement of 10 the theoretical maximum efficiency in white light of equal irradiance is 21%; with a quantum requirement of 8, it is 35%; with 4, 70% and with 3, 90%. It is interesting to note that the figures reported from determinations carried out under natural conditions are seldom above 3% (Richardson, loc.cit.)

Photosynthetically useful light is generally held to extend from 380 - 710 nm, but irradiance records taken during the C-14 *in situ* incubations of *Potamogeton* species leaves were measured over 400 - 750 nm and, as no data are available on the action spectra of leaves or on the proportion of radiation which was actually absorbed, then any determinations of photosynthetic efficiency are

approximations.

Because of the optical properties of water previously discussed, the spectral distribution underwater differs from that of air and therefore the energy available for carbon fixation in terms of Kcal or Kjoule/einstein is not the same. The average einstein of radiation (400 - 750 nm) underwater may contain more, or less, energy than the average einstein measured over the same wavelengths in air.

During one three hour experiment in Loch Leven, carried out in July 1970 (Table 3.4) the mean irradiance (400 - 750 nm) recorded at a depth of one metre approximated to one cal/cm^2/hour while the rate of carbon fixation of the leaves of Potamogeton obtusifolius incubated in natural loch water at the same depth was 9.4 µg/cm^2/hour. In Chapter 4 it was concluded that sucrose is probably the only major product of photosynthesis in such short incubation periods. The calorific content of one gram of sucrose is 3.94 Kcal and as there are 0.42 g of carbon per gram of sucrose then the 9.4 µg of carbon fixed by P.obtusifolius leaves represents an energy store of 0.08 cal or an efficiency of energy conversion of 8%*. In many of the other C-14 experiments reported in Chapter 3, rates of carbon fixation in natural loch waters approximated to 1 µg C/cal which represents an efficiency of energy storage of 0.9%. Calculations from several of the experiments carried out

* Note: strictly, calorific content equals energy content only where no osmotic work is done at all.

with *P. perfoliatus* in Loch Croispol give efficiency values of between 6 and 9% for leaves bathed in their natural loch water while phosphate enrichment nearly doubled this efficiency and brought it to near the maximum possible.

Though bearing in mind the limitations of the efficiency estimates calculated above, the figures are not appreciably different from those quoted by Richardson (*loc. cit.*) derived from other studies under natural environmental conditions, and it must be concluded that low efficiencies of energy conversion and low photosynthetic capacity are probably not the main cause of the observed poor productivity of aquatic macrophytes. The overall effect of the rapid attenuation of light in water, and the consequent reduction in light intensity available for photosynthesis, is thought more important than the actual efficiency of the carbon fixation process, in limiting productivity.

It has been shown in Chapter 3 that the productivity of submerged freshwater macrophytes may, in part, be limited by a shortage of available nutrients as well as by a reduction in the light energy required for photosynthesis. The remainder of this thesis is concerned with nutrient relationships of aquatic plants and attempts to synthesise the total findings of this research project to help explain their distribution and productivity.

CHAPTER 6

ROLE OF THE ROOTS

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CHAPTER 6

ROLE OF THE ROOTS

INTRODUCTION

A controversy still remains concerning the role of roots in the biology of submerged aquatic macrophytes. The idea perpetuated in the textbooks, which give but a cursory account of aquatic angiosperms, is that all submerged plants absorb their nutrients via their leaves, their roots being insignificant and functionless, except for anchorage (Olsen, 1953). This idea is based upon the many valid, experimental demonstrations that there is a relatively free movement of ions between the leaves of submerged plants and their bathing medium (Winter, 1961). These experiments have, however, not shown that the roots of submerged plants are incapable of nutrient absorption.

In view of the possible nutrient limitations upon the productivity of submerged macrophytes, and hence the importance of root absorption and translocation, the idea of a wholly foliar nutrient absorption pattern has been reviewed. Data are presented from personal observations, from an examination of the literature and from a series of field

and laboratory experiments.

OBSERVATIONS AND EXAMINATION OF THE LITERATURE

Morphology and Extent of Underground Organs

In his recent text on the biology of aquatic vascular plants, Sculthorpe (1967) summarises the range in morphology exhibited by underground organs. In general, hydrophytes possess a ramifying, rhizomatous root system often accompanied by the development of adventitious roots which help to bind and stabilise soil particles, especially in the shallow water sediments subject to periodic wind and wave action and subsequently erosion. Species with poorly developed root systems are generally found only in the deeper or sheltered water, less subject to wind and wave action. The delicate nature of the slender rhizomes of several species such as Potamogeton perfoliatus, P.obtusifolius and Myriophyllum species is overcome by a tendancy to form extensive mats by repeated branching of the rhizome and the production of copious fibrous roots at the nodal regions. McCully and Dale (1961) report rhizome growth to be sympodial in Hippuris vulgaris and this seems also to be the general case (Sculthorpe, loc.cit.), though in the present studies

rhizomes of P. praelongus showed a notable monopodial growth.

Rosette species such as Littorella uniflora and Lobelia dortmanna possess a root stock which is slender and soft while species of Alisma exhibit a bulbous and succulent root stock. Isoetes lacustris has a root stock which is hard and corm like. Additional anchorage in this latter genus is provided by adventitious roots arising from furrows near the base of the corm, while in most other rosette species they arise from the leaf bases. Extensive swards of rosette species can be formed by the production of runners and stolons which root at their nodes. This is especially true of Littorella uniflora and has also been reported for Lobelia dortmanna (Sculthorpe, 1967). These two rosette species along with such as Zannichellia species, Myriophyllum species and some Potamogeton species produce twisting spirals of corkscrew roots, a morphological feature which must aid firm anchorage in the substratum. Species of Ranunculus which colonise swiftly flowing streams anchor themselves in shallow substrata with profuse clusters of fine roots arising from the stem nodes.

Westlake (1965b) calculated biomass figures for the roots of several emergent macrophyte species, concluding that they form an important contribution to the standing crop of the vegetation and cannot be neglected in production estimates. Little information is available in the

literature on the biomass of the roots of submerged plants, as in many production studies they have been completely neglected (Edwards and Owens, 1960; Forsberg, 1960). Some species e.g. Ceratophyllum demersum and Utricularia vulgaris certainly have no roots and Sorutskii (1950) has estimated that only 2.6% of the weight of Elodea canadensis is root, yet species such as Littorella uniflora can have more than 50% of their biomass as roots as has been reported in Chapter 1. Burkholder et al.(1959) have estimated that for the marine angiosperm Thalassia testudinum between 75 - 85% of the biomass may be underground. Westlake (1965b) suggests that for many submerged species the contribution of the roots to the biomass is less than 10%

Production of Root Hairs

Supporters of the view of wholly foliar nutrient absorption by submerged aquatic plants seldom present data on the occurrence of root hairs. It is frequently argued that submerged plants do not require to increase their root surface area for absorption in this way. Snell (1908) and MacDougal (1941) state that no root hairs are produced unless the roots are underground, while Goebel (1891) states that some species possess long hairs on roots which do not enter the substratum.

Shannon (1953) investigated 209 species from 54 families of plants which grow naturally rooted in mud, silt or sand at the bottom of shallow water. He found that 93.3% of the species examined produced root hairs and only 2.4% produced glabrous roots. Roots of emergent and floating-leaved plants produce profuse root hairs (Sculthorpe, 1967). Bloch (1943) was able to detect the prospective hair-producing cells in Phalaris species by their high anthocyanin content. In the present studies root hairs have been detected in several Potamogeton species.

The absence of root hairs in several submerged species, such as Elodea canadensis, when grown in water culture, is apparently due to the cuticularisation of the root epidermis (Arber, 1920). Root hairs are, however, produced in this species in the dark in either water or soil and in the light, in water, if chlorophyll formation is prevented, the latter resulting in the production of only a thin cuticle, easily penetrated by the root hairs (Cormack, 1937). Root hairs are produced readily in Elodea canadensis in darkness and in the light if high carbon dioxide concentrations can be maintained, preventing the oxidation of unsaturated fatty acids as a film of cuticle on the epidermis (Dale, 1951). Cormack, (1949, 1962) believes that it is unlikely that the O_2 concentrations of substrates are sufficient to permit the formation of a true cuticle and thus root hairs

are allowed to develop. An unresolved, anomalous situation occurs in several free-floating rosette species which, in the light and in normal carbon dioxide concentrations, produce profuse root hairs although the roots hang freely in the water. One of these genera, Hydrocharis, is in fact widely used experimentally for the study of root growth and protoplasmic streaming (Sculthorpe, 1967).

The widespread occurrence of root hairs in submerged plants seems unlikely to be a non-functional attribute, though their absence would not per se indicate the inability of the roots to absorb nutrients.

Vascular Anatomy

The vascular anatomy of submerged angiosperms, as a whole, differs from that of the typical land plant in being reduced (Sculthorpe, 1967). The roots, however, closely resemble those of land plants and exhibit the least vascular reduction though a wide range of differentially developed systems occurs. Potamogeton natans (floating-leaved species) has a pentarch system of bundles in the central root stele where all the xylem elements, including the vessels, are thickened (Arber, 1920) while in P. pectinatus (submerged) the five protoxylem elements are absent at maturity and this stele is composed merely of an axial spirally thickened

vessel. Callitriche species have a simple root stele of two protoxylem bundles each comprising of only a single tracheid, separated by a metaxylem element and with a single sieve tube and companion cell. Vallisneria species have only a central channel surrounded by a ring of three sieve tubes and companion cells while in Najas species the phloem is more conspicuous than the xylem.

The stems of the submerged species have a condensed vascular system represented by a single strand devoid of secondary thickening and in which individual bundles cannot be readily distinguished. The centralisation of the vascular tissue, similar to the stele of roots, is thought to be an adaption to the pulling forces encountered in the aquatic medium (Sculthorpe, 1967). The reduced xylem elements are poorly lignified and the spiral or annular vessels which are present in the apex may be destroyed during growth and persist only at the nodes, as in Potamogeton lucens and Hannichellia palustris, while in Elodea canadensis the tracheidal thickening does not persist at all after internode elongation, leaving xylem lacunae (Cheadle, 1942). As in their roots, the members of the genus Potamogeton exhibit a range of vascular reduction in their stems, showing differing degrees of concentration of the components of the central vascular cylinder (Arber, 1920). Callitriche species have a ring of xylem surrounded by phloem but no

cambium, while Hippuris species have an external phloem and internal xylem of true vessels forming, in the centre of the old stems, pith-like structures of mature xylem parenchyma. These are composed of cauline elements similar to the reduced xylem of Myriophyllum species (Arber, 1920). Ceratophyllum species represent the extreme stage in stem simplification in the dicotyledons, with a central duct surrounded by cellulose-thickened walls and a broad zone of phloem. The phloem is generally better developed than the xylem and often has the advanced characteristics of sieve tubes and oblique walls (Esau, 1953). Arisz (1958) maintains that inorganic ions as well as organic compounds could be transported through this system.

The presence of an endodermis in both root and shoot of many species of aquatic macrophyte coupled with the knowledge that this structure is involved in regulating the transfer of water and nutrients to and from the stele in the roots of terrestrial species, supports the idea of a functional role for the vascular system (Sculthorpe, 1967).

Plates 2A and 2B in Appendix II illustrate the vascular anatomy of Potamogeton perfoliatus.

Absorption and Transport of Nutrients

Pond (1905) showed that a variety of plants produced more luxurient growth when they were grown on organically rich mud rather than sand. Brown (1913), however, illustrated that the difference in growth rate found between rooted and non-rooted specimens could be eliminated by bubbling carbon dioxide through the water. In the light of the findings of Cormack (1937) it is interesting to postulate the importance of the cuticle and also root hairs. More recently Spence and Denny (unpublished) and Denny (1966), working with tropical species have substantiated the findings of Misra (1938) and others, that aquatic plants when rooted in organic soils grow better than plants rooted in sand, and Denny (pers.com.) has shown that this effect is correlated with the habit of the leaves.

Pearsall (1918, 1920, 1921) in a series of papers on the biology of the English Lakes produced evidence that the edaphic conditions were the fundamental habitat factors controlling the distribution of submerged vegetation. His conclusions, though based on a very meagre number of observations (discussed by Spence, 1967), have been perpetuated in several texts e.g. Hansley (1949), while other workers, Misra (1938), Matthews (1914), Pickett (1921, 1924), Veach (1933) and Wilson (1937, 1939, 1941) have drawn

similar conclusions though unfortunately from equally sparse observations. Spence (1964, 1967) is more cautious in the interpretation of his findings from a survey of Scottish lochs, concluding that the possibility of the edaphic factors controlling zonation is feasible but admitting that no species, even in a single lake, has been shown to be causally correlated with one particular substrate type. A similar conclusion was reached from the studies reported in Chapter 1.

Though no intimate plant-substrate association may exist it is possible, as Spence (1964) has rightly stated, that the situation may arise when nutrients in the water can be depleted and ion absorption from the substratum by the roots through contact exchange, as described by Gonzalez and Jenny (1958), may be an important function. As roots are able to grow deep in anaerobic muds the respiratory energy for this process would appear to be available. The network of aerenchyma which runs throughout all submerged species of angiosperms (Sculthorpe, 1967) presumably retains sufficient oxygen tensions for root growth and metabolism deep in the substrate though, as has recently been demonstrated by Hartman and Brown (1966, 1967), the composition of the gases in the internal atmosphere of several hydrophytes is partly methane.

Several workers have attempted to demonstrate a flow of water and nutrients from the roots of submerged species

to their shoots. Most of the early experiments are described by Arber (1920) who lends support to the idea while Sculthorpe (1967), having critically reviewed some later accounts, remains unconvinced. A transpiration current relying on an evaporating surface, as in terrestrial vegetation, clearly cannot exist in submerged species. An upward stream could, however, be powered by exudation pressures such as have been demonstrated by Thut (1932), and Hohn and Ax (1961). Movement of eosin dye in Elodea canadensis and in some Potamogeton species has been demonstrated by Snell (1908) while Pond (1905) followed the movement of lithium nitrate in Ranunculus species. Wilson (1947) points out that water could be exuded from stems, due, not only to its transport from the roots, but also through a rebalance, made necessary by the changes in such gas pressure flows as could arise in photosynthesis. He demonstrated a direct relationship between rates of water uptake by shoots of Ranunculus fluitans and the ionic concentration of the bathing medium, while noting that the whole process could be inhibited in the presence of cyanide.

Through the precise significance of Wilson's (1947) findings is not clear and most experiments have so far been carried out using cut stems and not intact plants, Stocking (1956) concludes that a slowly moving stream of water does exist in submerged plants. His conclusion depends upon

the build up of root pressures and the excretion of water through pores or hydrathodes. These structures, according to Sculthorpe (1967) are not, however, of universal occurrence in aquatic angiosperms and when they are present are often blocked by brownish deposits.

Several American laboratories have initiated a new series of studies on the transport systems of submerged aquatic plants as part of a programme designed to evaluate the application of herbicides for the control of aquatic weeds in commercial waterways. Evaluation of herbicide translocation in submerged plants has, however, been limited due to the difficulty of separating the root and shoot environment of the plant. An effective barrier is required which will prevent passive movement of chemicals to the partitioned root and shoot yet still allow for the normal physiological functions of the plant.

Aldrich and Otto (1959) attempted to trace the movement of carboxyl-labelled 2-4-dichlorophenoxyacetic acid in Potamogeton pectinatus but their findings are somewhat in doubt due to the questionable effectiveness of the latex membrane used to partition roots and shoots. Sunderburk and Lawrence (1963) traced the movement of C-14 labelled simazine and fennac in Heteranthera dubia using a polythene bottle immersed in a graduated cylinder. The roots of the plant extended through a small opening at the top of the

bottle sealed with silicone grease while the shoots extended into the cylinder. Using the technique of gross autoradiography they found not only that the specimens exposed to the labelled herbicide for one week had accumulated activity, when either shoot or root had been exposed, but also that, in the case of the simazine treated plants, a limited amount of herbicide movement to the untreated part, both shoot to root and root to shoot, had occurred. Frank and Hodgson (1964) reported an elegant and effective method for separating roots and shoots. They used a low melting point wax as a barrier in the neck of a filter funnel which separated the shoot, in the upper portion, from the root in a bottle supporting the funnel. From their findings it appears that Potamogeton pectinatus can absorb the herbicide fennac through either its roots or shoots and accumulate activity in the nodal regions. They reported little or no basipetal translocation of the herbicide and limited acropetal movement, this being reduced by the removal of the root tubers prior to herbicide application.

Frank, Hodgson and Comes (1963) investigated the effectiveness of herbicide applied to the soil for control of weeds in irrigation canals and found that 21 out of 91 trial compounds were absorbed by the roots of test plants, P.pectinatus and P.nodosus, to a degree where deleterious effects could be observed. Sutton and Bingham (1967)

investigated the translocation patterns of C-14 labelled simazine in P.crispus. Incorporation was detected in both root-treated and shoot-treated portions, but no movement from the roots was recorded even after 48 hours exposure and no tissue damage was apparent. Foliar treatment resulted in basipetal movement but no label was detected in the roots.

McRoy and Barsdate (1970) have studied the absorption of phosphate by eelgrass (Zostera marina L.) using P-32. Absorption was found to be greatest in the light and occurred through both leaves and roots, and the absorbed phosphorus was transported rapidly to all parts of the plant. More recently, Bristow and Whitcombe (1971) have carried out similar studies with three species of freshwater vascular plants. They cultured rooted stems in a two-compartment apparatus applying P-32 to the bottom compartment, containing the roots. At the end of a ten day growth period the specific activity of phosphate was determined in axillary shoots which had developed during the course of the experiment from buds in the upper compartment. The results indicated that most of the phosphate in these shoots was not absorbed from the ambient medium but was derived from the rooted-stem base in the lower compartment.

EXPERIMENTAL INVESTIGATIONS

Introduction

The experimental investigations reported in this section were carried out prior to the publication of the papers by McRoy and Barsdate (1970) and Bristow and Whitcombe (1971).

One nutrient known to be of great importance in plant metabolism, yet occurring in many inland waters in only minute concentrations, is phosphorus (Hem, 1970). In view of the possible implications of the shortage upon the productivity of aquatic macrophytes as illustrated in Chapter 3, the uptake and translocation patterns of this element have been investigated using the radionuclide P-32.

The plant specimens used in these studies were collected from the field and cultivated in the laboratory until required for experimentation. Root stocks and rhizomes of Potamogeton species were first buried in a mixture of sand and loam at the bottom of glass tanks and this covered with a layer of washed sand. Water was carefully introduced into the tanks using a sheet of polythene to prevent the disturbance of the substrate and the tanks filled to the top with tap water. The layer of sand was used to prevent undue exchange of nutrients between the water and the garden

loam. The tanks were placed either in a glasshouse or in controlled environment chambers where the air temperature was maintained at 20°C and the daylength regulated at 16 hours. A bank of Sylvania Gro-lux wide spectrum lamps provided the light in these growth chambers. The rhizomes readily produced young shoots which could be cropped and used for experiments. Whole plants of Littorella uniflora and Lobelia dortmanna were planted as above and maintained until required for experiments.

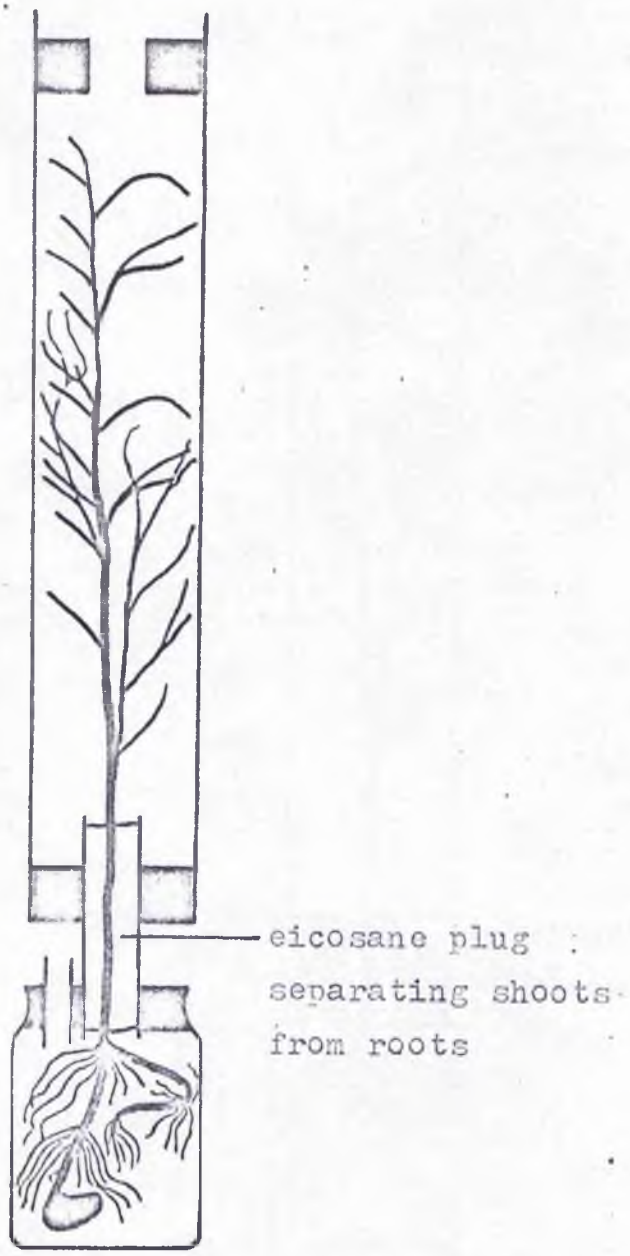
A technique similar to that employed by Frank and Hodgson (1964) was used to separate the roots and shoots of plants and hence permit the investigation of the nutrient absorption capacity of both shoots and roots. The specimens to be investigated were removed from their culture tanks and any adhering algae or mud carefully washed away. A plant was gently threaded through a glass tube inserted in a rubber stopper (No. 25) so that only the roots remained on one side. Using a Pasteur pipette, aliquots of molten eicosane wax were gently dropped into the glass tube enclosing the plant stem while a finger blocked the bottom. As the surface of the wax hardened the stopper was plunged into cold water for several minutes causing the plug to solidify, completely sealing the stem of the specimen in the stopper. The stopper and plant were then inserted into a glass tube so that the shoots were enclosed in the tube and the roots

dangled from the stopper. The tube was gently filled with distilled water and clamped upright over an opaque glass jar, also containing distilled water, into which the roots penetrated. The tube was lowered until the stopper just touched the surface of the water in the jar so that every part of the plant was in touch with water except that portion sealed in the stopper (Figure 6.1). The apparatus was left for a few hours to check for leakage through the seal before proceeding with the experiment.

Eicosane wax ($CH_3(CH_2)_8CH_3$) proved an excellent sealing material, as has been found by Frank and Hodgson (1964). This low melting-point compound was not only a successful seal but its use caused no obvious damage to the plant. The sealing procedure was quickly carried out, the plants being out of water for no more than one or two minutes. This is essential for any such manipulation if wilting of the delicate tissue of aquatics is to be prevented.

When it had been ascertained that the seals were effective, the distilled water bathing the plant was replaced with the nutrient solutions to be used in the experiment.

FIGURE 6.1



Apparatus used to study nutrient absorption and translocation in submerged aquatic macrophytes.

Root and Shoot Absorption of Nutrients by *Potamogeton perfoliatus*

Shoot and root incorporation were investigated independently in *Potamogeton perfoliatus*. High specific activity P-32 as orthophosphate in dilute hydrochloric acid was injected as tracer, 10 μCi being given as a root treatment and 2.5 μCi as a shoot treatment. The shoots were bathed in 150 ml of culture medium (Table 6.1) and the roots[1] in 450 ml. The complementary solutions to those treated with P-32 contained no phosphorus.

TABLE 6.1

Culture medium used in the studies of phosphorus absorption and translocation in aquatic macrophytes. Salt concentrations in grams per litre (Forsberg, 1965).

$CaNO_3$	0.08 g	Zn (as chloride)	0.10 mg
$MgSO_4,7H_2O$	0.10 g	Mn "	2.00 μg
Na_2CO_3	0.02 g	Co "	2.00 μg
Na_2SiO_3	0.01 g	Cu "	4.00 μg
KCl	0.03 g	B (H_3BO_3)	0.40 mg
K_2HPO_4	0.56 g	Mo (Na salt)	0.10 mg
Fe (as chloride)	0.40 g	NTA	20.00 mg
TRIS	0.50 g		

pH adjusted to 7.0 with 1 N HCl.

1. In this context "roots" signifies the entire root/rhizome system.

four containers not holding plants but with a wax plug separating the top and bottom compartments were also set up to test the permeability of the plug. In two containers the top solution was labelled with P-32 and in the other two the bottom solution was labelled. The containers were allowed to stand for 48 hours in a water bath kept at 20°C and received light from a bank of three Gro-lux lamps programmed on a 16 hour day.

At the end of the experimental period the plants were removed from their containers. The roots were cut off just below the rubber stopper, then the solution which bathed the shoots was poured off, the stopper was removed and the wax plug was melted with the heated blade of a scalpel so that the shoots could be withdrawn. All plant parts were washed separately in running tap water for five minutes, blotted, wrapped in aluminium foil, placed in an oven and dried at 105°C under vacuum for 24 hours, then cooled in a desiccator and weighed. Each sample was then acid-digested with a mixture of sulphuric and nitric acids, neutralised and the digest was made up to a standard volume of 25 ml with distilled water. Three aliquots, each of 0.1 ml were pipetted from the extract onto aluminium planchets using a micro-syringe. The planchets were placed on a warm hot-plate and allowed to evaporate slowly to dryness. The radioactivity on the dried planchets was measured using a

Panax solid scintillation counter. Each sample was counted at least three times and each to a set count of at least 1000. Activity was expressed as counts per minute per mg tissue dry weight (Tables 6.2 and 6.3).

TABLE 6.2

Radioactivity (cpm/mg tissue) in extracts of shoot-treated plants and ratio of activity in treated : non-treated parts.

All counts corrected for background.

Shoots	Roots	Incorporation ratio S/R
1402	94	14.9
625	220	2.8
847	114	7.4

TABLE 6.3

Radioactivity (cpm/mg tissue) in extracts of root-treated plants and ratio of activity in treated : non-treated parts.

All counts corrected for background.

Shoots	Roots	Incorporation ratio R/S
122	42×10^3	344
208	75×10^3	360
398	70×10^3	175

Before the plants were removed from their containers, three samples each of 0.1 ml were taken from each root- and shoot-bathing solution, placed on a planchet and the radioactivity was measured as above (Table 6.4).

TABLE 6.4

Radioactivity (expressed as cpm) of solutions where the (a) top compartment (shoots) had been treated with P-32 and (b) bottom compartment (roots) had been treated with P-32.

(a) shoot solution treated with P-32		(b) root solution treated with P-32	
shoot solution	root solution	shoot solution	root solution
957	42	51	3,170
684	48	44	4,243
689	39	38	3,549

background = 49

Similar aliquots from the solutions with no plants were also assayed for the presence of radioactive phosphorus which had leaked through the seal (Table 6.5). Background radiation was estimated from 0.1 ml aliquots of the stock nutrient solution.

The data presented in Table 6.5 indicates that during the course of the experiment no leakage of radioactivity through the eicosane seal was likely to occur. Data in

Table 6.4 confirms this finding.

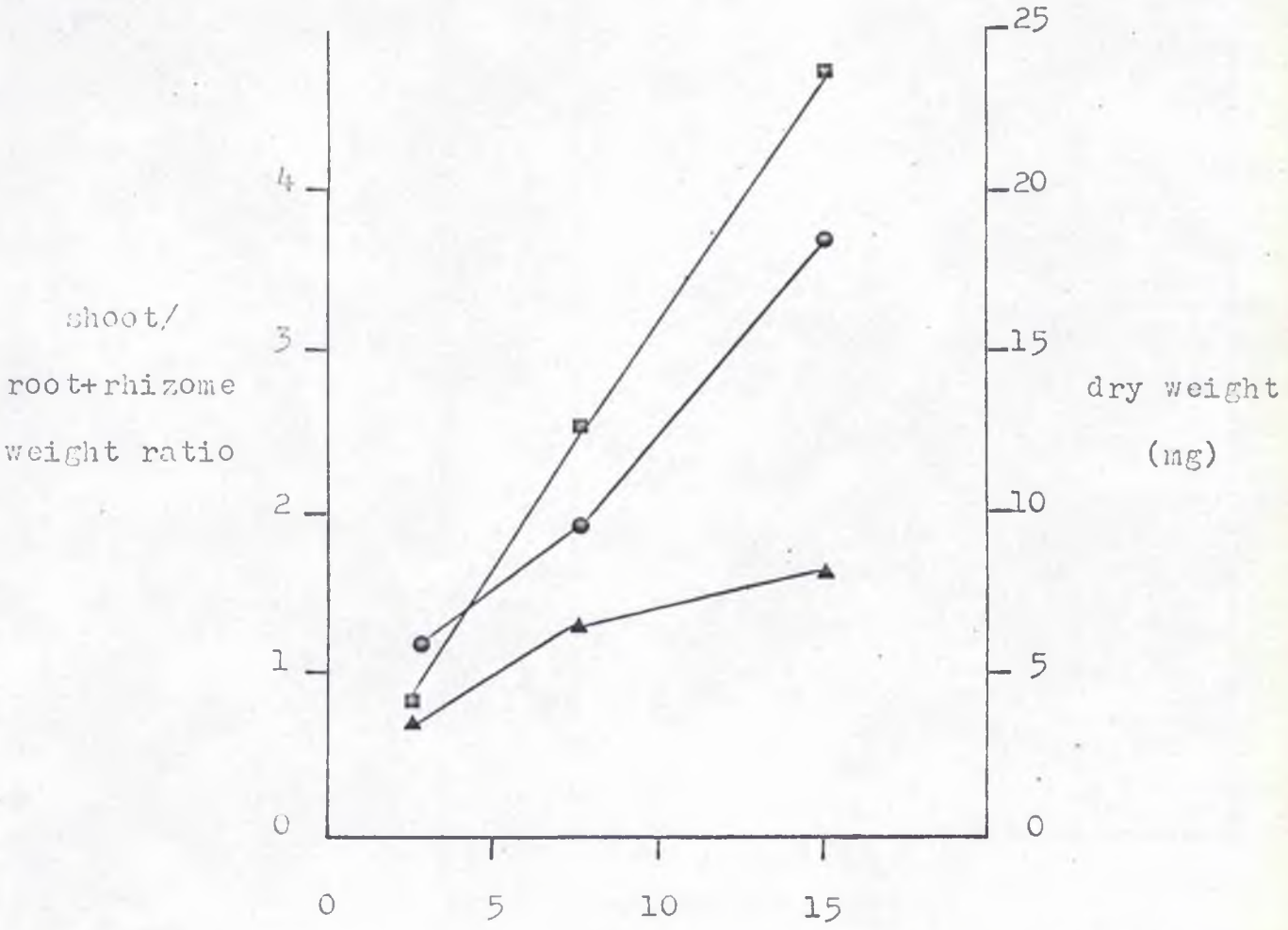
TABLE 6.5

Radioactivity (expressed as cpm) of solutions from containers used to test the seal. Based upon three 0.1 ml samples each counted to at least 1000 counts three times.

Top solution treated with P-32		Bottom solution treated with P-32	
Top solution	Bottom solution	Top solution	Bottom solution
609	42	42	2,640
731	42	45	3,051

From the data in Table 6.2 it would appear that the shoots have incorporated a quantity of radioactive phosphorus and, as some activity also appears in the roots, some of this phosphorus has been translocated into the root. Since Table 6.4 showed that no leakage through the wax plug had occurred, the shoots must be the source of this P-32 in the roots. Table 6.2 also shows the incorporation ratio, i.e. the ratio of radioactivity in the shoots to that in the roots. While this ratio is not constant, ranging from 2.8 to 14.9 in the three experimental treatments, it does, however, serve to illustrate that more P-32 was incorporated into the shoots than was translocated to the roots. If these ratios are now plotted against the weight of the shoots

FIGURE 6.2



Ratio of radioactivity (cpm/mg tissue dry weight) in shoots : radioactivity in roots + rhizomes of specimens of Potamogeton perfoliatus, kept for 48 hours with their root systems in P-32, plotted against; a, shoot dry weight (■); b, shoot :· root + rhizome dry weight ratio (●); c, dry weight of roots + rhizomes (▲).

as in Figure 6.2 a straight line relationship emerges. Thus it would appear that the distribution of the P-32 per unit weight between the shoot and the root is governed more by the weight of the shoot than by the weight of the roots, or even by the ratio of these weights. Considerable radioactivity has been concentrated in the roots of the plants but in comparison only a small proportion was found in the shoots. Thus from the results of this experiment it would appear that both the shoots and the roots of Potamogeton perfoliatus have a capacity to absorb nutrients but that the rate of translocation is slow, especially from the roots to the shoots. The remaining experiments described here were designed to investigate this translocation pattern in this and other species of aquatic macrophytes.

Autoradiography

Many workers have used the technique of gross autoradiography to illustrate the pathway of movement of radioactive tracer nutrients through the vascular tissue of plants. The general methods are reviewed by Duggar (1957). Gross autoradiography has also been used to study the movement of products of photosynthesis arising from the initial labelling of carbon with carbon - 14 and also for many other studies on the movement of metabolites and chemicals through the plants.

The methods employed in the present study were as follows. After a plant had been treated with the labelled nutrient using the apparatus previously described, and the plant parts separated and washed as before, the specimen was prepared for autoradiography. Crafts and Yamaguchi (1960, 1964) recommend freeze-drying under vacuum to kill a plant before autoradiography but no apparatus was available to carry this procedure out and so the labelled specimen was treated as a normal herbarium specimen and dried between sheets of paper in a plant press and then mounted on stiff card. A sheet of Kodak Crystallex (estar base) X-ray film was placed over the specimen and the assembly returned to the plant press. Care was taken not to tighten the press to the degree where the specimen might produce pseudoautoradiograms caused merely by the pressure of the plant on the film. Where more than one specimen was in the press, spacers, made from cardboard covered with aluminium foil, were used to separate them and prevent radiation from one specimen impinging on the X-ray film of another. The film was allowed to stay in contact with the specimen for a few days, the time varying upon the intensity of labelling as estimated before hand by a Geiger-Muller rate-meter.

The film used was of two sizes and of two package types. The sizes were 4 x 10 inches (10.2 x 25.4 cm) and 6 x 15 inches (15.2 x 38.1 cm), the size used being dependent

upon the size of the specimen under investigation. The two package types were:-

(1) Folder-wrapped film.

(2) Envelope-packed film.

The folder-wrapped film was enclosed in a simple folder which was not light-tight and therefore could only be handled in the dark room using a brown safelight (Wratten series 6B). As dark room facilities initially were limited and departmental regulations prevented the removal of the radioactive material from the designated isotope laboratory, each piece of film was completely wrapped in black light-proof polythene. This enabled exposures to be carried out in the light and hence in the isotope laboratory, where no dark room facilities were available. The envelope-packed film was much more expensive than the folder-packed and came in light-tight envelopes ready for use.

All autoradiograph exposures were carried out with either a sheet of polythene or card between the radioactive specimen and the X-ray film. This method proved satisfactory with the strong beta-radiation by P-32 and served to prevent excessive fogging of the film, though long exposure times (one week) were usually necessary. This technique was also successful in preventing the plants from producing pseudoautoradiographs due to natural labile chemicals.

The exposed film was developed in the photographic dark room using Kodak D.19 developer which is a high contrast, quick acting, developer. Films were processed in developer at 20°C for five minutes, with gentle shaking every minute, after which they were rinsed for two minutes with running, filtered water and transfered to sodium hypochlorite for a further ten minutes, again with gentle shaking every minute. They were finally washed for approximately ½ minutes in running, filtered water after which time the film was hung up to dry in a dust-free room.

The autoradiographs reproduced here in Appendix II were photographed alongside their source specimens against a white background using standard photographic techniques.

Translocation in Littorella uniflora and Lobelia dortmanna

Littorella uniflora is a small rosette plant of relatively shallow water (Chapter 1) and colonises rocky, muddy and sandy substrates; it also has a land form. Littorella uniflora often forms swards on the sides of reservoirs (Grainger, 1947) and when the water level drops in the summer months the plants are exposed and frequently flower. Littorella uniflora does not flower underwater. The fact that this species can survive out of water lends support to the idea of root absorption of nutrients and

this species was selected for initial experiments on nutrient translocation along with Lobelia dortmanna, another frequent colonisor of shallow water and often found in association with Littorella uniflora (Chapter 1). In contrast to Littorella uniflora this species has not been shown to survive for long out of water.

Specimens of both species, grown submersed in tanks, were selected for uniformity and then the root and shoot environments were separated by the wax plug technique as described previously. Both roots and shoots were bathed in the same nutrient solutions as before but for any one specimen only one compartment was labelled with high specific activity P-32. Plants were exposed to the isotope solutions for 16 hours in continuous light and then removed, cut, washed and autoradiographs prepared as described previously. Three aliquots (0.5 ml) of each bathing solution were pipetted on to planchets and assayed for radioactivity. Results indicated that no leakage of activity had occurred through the wax plugs as the count rates of the unlabelled solutions were not significantly different from the background radiation.

The autoradiographs were left for seven days and developed by the methods set out previously. In all cases where either the shoots or roots of either species were bathed in the radioactive solution fogging on the autoradiograph indicated a degree of nutrient absorption. The

roots of both species appeared relatively more heavily labelled than the shoots. No fogging was detected on the autoradiographs which would have corresponded with P-32 having been translocated from either the shoots or the roots. It must therefore be concluded that either no translocation occurs or else the conditions of the experiment were such that movement of the absorbed phosphate did not take place, or if so at a very slow rate which was not detected. Nutrient absorption was, however, found to take place into both the root and the shoots of Littorella uniflora and Lobelia dortmanna.

Being primarily interested in the possibility of root absorption and the translocation of nutrients from the roots to the shoots, another series of Littorella uniflora plants was prepared as in the last experiment, but in this experiment only the root solutions were labelled with P-32. The plants were maintained at 22°C in a constant environment chamber programmed on a 16 hour day. Two plant specimens were removed and autoradiographs prepared every day for five days at approximately 24 hour intervals so that in all ten autoradiographs were made. The autoradiographs were again left for seven days and developed as before. The results, indicated by the fogging on the developed autoradiograph, illustrated a progressive increase in the density of the fogging around the roots of the plants with increasing

exposure time to the isotopic solution. Plants taken after 24 hours showed no fogging corresponding to translocation to the shoots, but plants collected after 48 hours exposure showed increasing amounts of labelled phosphate present in the shoots. The intensity of fogging due to the shoots of the specimens collected after five days, however, was not as great as that due to the roots after only 24 hours exposure to the labelled nutrient solution.

Assay of samples of the bathing solutions again indicated that no leakage occurred, as no count rates were significantly greater than the background radiation. It is concluded that phosphate was incorporated into the roots of the Littorella uniflora plants and that some degree of translocation of this phosphate occurred, so that the phosphate taken in by the roots eventually appeared in the shoots. The degree of this translocation was, however, slow and, as the shoots of this species have also been shown to absorb phosphate, the nutritional significance of such translocation rates is questionable. For this species, when submerged anyway, it is possible that root and shoot absorption of nutrients occurs independently of one another so that, in effect, the roots and shoots are self-sufficient with regard to their nutritional requirements. The fact that Littorella uniflora can exist when its shoots are out of water would suggest that either a store of nutrients is held in the shoots

or else a translocation of root absorbed nutrients occurs. Unless the translocation rate of the exposed plants is markedly different from that found in the present investigation with submerged shoots it is thought unlikely that the latter process would supply sufficient nutrients to maintain active growth. It is possible, however, that, as a result of transpiration, the rate of nutrient transport from the roots to the shoots may be considerably increased when the plants grow out of water.

To measure the ability of plants to absorb and translocate only one nutrient and then to make general postulates would be unfounded. For this reason the last experiment with Littorella uniflora was repeated using exactly the same procedure but with radioactive sulphate instead of phosphate. The autoradiographs of these plants again indicated the ability of the roots of this species to absorb nutrients and to slowly translocate these absorbed nutrients to the shoots. It is thus possible that this may be the general pattern of nutrient absorption in this species.

Translocation in Potamogeton species

The majority of the primary productivity measurements reported in Chapter 3 were carried out with species of Potamogeton and it was the carbon fixation rate of the leaves of those species which was shown to be phosphate limited. The pattern of translocation of both phosphate and sulphate was therefore further investigated in several of the species of this genus, using the autoradiograph technique to judge the degree of absorption and translocation. A series of plates in Appendix II (Plates 4 to 7) at the end of this thesis illustrate some of the results of this study.

Absorption of nutrients into both the shoots and the roots of several species was shown to be both fast and accumulative. Label was detected in autoradiographs after two hours exposure to the isotopic solutions while plants kept exposed for longer periods showed progressive degrees of incorporation. Translocation from both shoots to rhizomes and from roots to rhizomes and shoots was recorded but, as with Littorella uniflora, this was a slow process.

In the shoot-treated specimens no label was ever detected in the actual roots but actively accumulated in the nodes of both rhizomes and stems. No shoot-to-rhizome translocation was detected in less than 24 hours exposure although the process, as for Littorella uniflora, was

accumulative. Labelled phosphate applied to the bathing solution of one stem was found to be incorporated and, in 48 hours, translocated along the rhizome to the leaves of another stem kept in a phosphate-free solution. No translocation occurred in the same period if the shoot was kept in a solution with phosphate. Where both stems, rhizomes and roots were kept in the same labelled-phosphate solution, activity tended to accumulate in the leaves and in the stem nodes rather than in the roots.

As with the shoots, the roots of all species investigated readily took up label and accumulated the radioactivity, producing dense fogging on many of the autoradiographs. As with stems, the nodal regions of the rhizomes accumulated the labelled phosphate. The results from the translocation experiments were mixed. In not all cases, even after long exposure times, was translocation from the roots to the shoots detected, even though the roots appeared to accumulate great quantities of phosphate and the shoots were kept phosphate-free. In some experiments, translocation to the stems but not the leaves was detected, and, in others, evidence was obtained to illustrate the presence of phosphate in the leaves of plants, the roots of which had been exposed to P-32 for only 24 hours. As with the shoot-treated plants, when translocation of either phosphate or sulphate occurred the nutrients tended to accumulate in

the nodal regions.

Thus it has been shown that several species of aquatic macrophyte have the capacity to absorb nutrient ions through their shoots and through their roots and that, under certain conditions, translocation from one to the other can occurr. This translocation is, however, slow, with nutrients tending to accumulate in the nodal regions and in the actively growing areas of the rhizomes and stems which suggests preferential demand. This low rate of translocation could be the result of some degree of nutritional independence for both shoots and roots, as was postulated for Littorella uniflora. From the present results it is questionable whether luxuriant growth could be maintained on the quantities of phosphate which are translocated from the roots if those in the water were unduly depleted.

Polyphosphates

Jeffrey (1964) noted a low translocation rate of phosphate from the roots to tops of Banksia ornata, an Australian heath plant which grows successfully in an environment which is low in soil phosphorus. Earlier investigations (in Jeffrey, 1964) on the same species indicated an apparently seasonal rhythm in the form of phosphorus within the plant. In the growing season much

of the plant phosphorus was extractable in cold trichloroacetic acid (TCA) but for the remainder of the year TCA-insoluble fraction predominated. Jeffrey (1964, 1967, 1968) was able to correlate previous findings and explain his own observations on low rates of translocation by demonstrating that the components of the large TCA-insoluble fraction of phosphorus in the roots of Banksia ornata and in several heath species exhibited several features which indicated that long-chain polyphosphates were present. He postulated that this phenomenon of long-chain polyphosphate formation may be of ecological importance in a low phosphorus environment. Polyphosphates are known to exist in microorganisms (Harold, 1966) but apart from the work of Jeffrey (1964, 1967, 1968) there have been few reports of their occurrence in higher plants (Hardin, 1892; Miyachi, 1961) though they may easily exist but have escaped detection.

The role generally attributed to these long-chain compounds is one of storage (Harold, loc.cit.) but this is by no means certain. Harold (loc.cit.) pointed out that the available phosphate content of natural environments is low due mainly to the insolubility of calcium phosphate (note the low levels of phosphate present in limestone Loch Croispol) and that it would appear reasonable that some organisms should have evolved a means to accumulate a phosphorus reserve. The low rates of translocation recorded in

the previous experiments could thus be due to the build up of long chain polyphosphate molecules as described above. As an initial step in investigating the possibility the following experiment was carried out.

Four specimens of Potamogeton perfoliatus were removed from their tank and washed clean of adhering soil particles and obvious epiphytes and partitioned in the apparatus previously described, so that the roots and shoots could be bathed in different solutions. The plants were left in continuous light for 24 hours while both roots and shoots were bathed in distilled water. After that pretreatment the solutions were changed. The roots of two specimens received 500 ml 3×10^{-6} M sodium orthophosphate while their shoots had the distilled water replaced. The treatment was reversed for the other two specimens, the shoots being bathed in 100 ml of the phosphate solution. The root treated plants were given a 10 μCi dose of P-32 as orthophosphate while the shoots received 2 μCi. The plants were left for 48 hours in continuous light supplied by a bank of three Gro-lux tubes and kept at 20°C in a water bath. After that period the plants were removed, washed and air dried. Fresh weights were recorded.

Two 0.5 ml aliquots were taken from each solution, placed on to a planchet and evaporated. Radioactivity was counted as before. The count rates ascertained that

no leakage of isotope through the seal had occurred. The plant material was bulked into four portions:-

(1) Shoots of shoot-treated specimens.

(2) Roots of shoot-treated specimens.

(3) Shoots of root-treated specimens.

(4) Roots of root-treated specimens.

Each portion was then ground up in a chilled mortar and pestle with a volume of 10% TCA and left to extract in the refrigerator at 5°C for 60 minutes. The material was filtered through fine muslin, washed with more TCA and the extracts made up to a standard volume of 25 ml. The remainder of the plant material was then digested by a nitric acid/hydrochloric acid mixture, neutralised and again made up to a standard volume of 25 ml.

From each extract three 0.5 ml samples were pipetted onto planchets, evaporated to dryness on a hot plate and counted to 1000 counts. Counts were expressed as mean cps/sample (Table 6.6).

The results indicate that firstly, under the experimental conditions and the extraction procedures used, not all the phosphorus taken up by the plants in the 48 hour period was readily soluble in TCA and secondly, that the movement of phosphorus from shoots to roots and roots to shoots was slight, only 4.8% of the total radioactivity in the extracts being found in the roots of shoot-treated plants and only

TABLE 6.6

Proportions of radioactive phosphate extracted from *Potamogeton perfoliatus* plants after 48 hours exposure to P-32 (based on 0.5 ml aliquots of 25 ml extracts).

(a) Distribution of radioactivity

(1) Shoots treated with P-32

	Fresh weight of tissue	cpm in TCA extract	cpm in acid extract
Shoots	376.5	4,101.6	1,142.5
Roots	352.8	99.5	89.1

(2) Roots treated with P-32

Shoots	999.2	72.1	163.2
Roots	939.1	15,422.5	10,210.6

(b) Relative distribution of radioactivity between extracts

	% cpm in TCA extract	cpm in acid extract
Shoots treated with P-32	77.3	22.7
Roots treated with P-32	60.3	39.7

(c) Relative distribution of radioactivity between tissues

	% cpm in shoots		% cpm in roots	
	TCA extract	acid extract	TCA extract	acid extract
Shoots treated with P-32	97.6	92.8	2.4	7.2
Roots treated with P-32	0.9	1.0	99.5	99.0

0.75 % in the shoots of the root-treated plants.

It would be premature, from the results of one experiment, to postulate the universal occurrence of polyphosphates in aquatic macrophytes. From the evidence, however, of low phosphate-solubility in TCA and the fact that several rhizomes of Potamogeton species have been shown to stain with toluidine blue, the presence of polyphosphate may perhaps be suggested to account for the low translocation rate of absorbed phosphate in some species. One of the postulated uses of polyphosphate (Harold, 1966) is a phosphate store for growth initiation. As the turions of P.perfoliatus, and many other species of Potamogeton, originate from the rhizome, the phosphate absorbed by the roots might well be channelled into polyphosphate for turions, or for the initiation of fresh growth from the rhizomes. While further investigations on this topic are required, such a process would certainly account for the low rates of phosphate translocation recorded in the present studies.

Note: in this section on polyphosphates "roots" signifies the entire root/rhizome system.

Nutrient Absorption under Natural Conditions.

Two experiments were carried out to examine the possibility of root uptake of nutrient ions under natural conditions.

A perspex tube one metre long and 10 cm inside diameter was used to collect a plant specimen from its natural habitat. Using SCUBA diving equipment the tube was lowered over a specimen of Potamogeton perfoliatus and the tube pressed down firmly into the substratum to a depth of about 30 cm. A No. 92 rubber stopper was then inserted in the top of the tube and pressed home. The tube now acted as a pipette and it was possible to withdraw the tube from the mud along with the intact plant and its immediate habitat. The tube was brought ashore and some of the mud removed from inside the bottom of the tube to allow another stopper to be inserted, and thus secure the sample. In this way a portion of the loch was transported back to the laboratory.

The perspex tube had been previously drilled with fine holes along its length, to allow the passage of a No. 10 hypodermic needle, and these holes were sealed with selotape. It was thus possible to inject through one of these holes into the substratum surrounding the plant roots. Using this technique, on two occasions approximately 20 μCi of

high specific activity P-32 orthophosphate was injected into the substratum surrounding the specimens of P.perfoliatus collected from Loch of the Lowes. After 24 hours the plants were removed and autoradiograms prepared by the method already described. On one occasion no tracer isotope was apparent in the plant and on the other occasion a slight fogging on the autoradiograph indicated some isotope accumulation in a nodal region of a rhizome.

To test if any leakage or exchange of tracer occurred through the mud/water interface, samples of the water above the mud were taken after six hours. These were plancheted out, evaporated and counted as before. The count rates indicated that no significant radioactivity was present in the water above the mud. A sample of fine mud was also removed from one of the serum-cap holes and counted as above. This gave a considerable count rate indicating the presence of the tracer phosphate in the mud, just below the water surface.

The lack of heavy label in the plant tissue could be explained by the fact that no root absorption occurs in natural conditions or if so at a rate which was hardly detectable by the techniques used in the experiment. Another explanation, however, is that the tracer nutrient never reached the surrounding area of the roots but was bound up by the soil particles and rendered unavailable for uptake.

Nutrient Absorption and Epiphytic Micro-organisms

Barber and Loughman (1967) have compared the uptake of phosphorus by the roots of barley plants (*Hordeum vulgare* var. Mavis Badger) grown under sterile and non-sterile conditions, concluding that if special precautions are not taken to exclude micro-organisms their activity in or on plant roots can greatly modify the absorption pattern, especially at low external concentrations. These workers have shown that when the external concentration of phosphorus is limiting, phosphorus is accumulated by micro-organisms at the expense of the plant. This is reflected in a reduced translocation rate to the shoots of non-sterile plants. It is now generally accepted that results from nutrient uptake experiments carried out under non-sterile conditions must be viewed with caution. It therefore seems advisable in the present investigations, designed to clarify the role of the roots of aquatic plants in nutrient uptake, to compare the results of experiments carried out under sterile and non-sterile conditions. It also seems necessary, in the light of the findings of Barber and Loughman (*loc.cit.*), to ensure that the observed accumulation of phosphorus by the roots of aquatic macrophytes is, in fact, due to nutrient uptake and not due to its absorption by epiphytic organisms.

Though such experimental work has been carried out

with Lemna species (Hillman, 1961) and the free-floating fern Marsilea species (Allsopp, 1952), in sterile culture, few accounts report the successful maintainance of rooted submerged macrophytes in axenic conditions. Forsberg (1965, 1966) describes a technique by which he successfully germinated seeds of several species of rooted aquatics in sterile culture. Wetzel and McGregor (1968) were unable to germinate seeds in sterile conditions employing the methods of previous workers (including Forsberg) but did, however, succeed, though not without some difficulty, in obtaining axenic cultures of Najas flexilis L.

In the present study a combination of the methods that have been utilised in previous investigations have been used in an attempt to obtain sterile cultures of Potamogeton species, Myriophyllum species and Lobelia dortmanna.

Seeds of the following species were collected in the late summer of 1969 and again in 1970. Lobelia dortmanna (from Loch Achtriochtan and Loch Uanagan), Potamogeton natans (from Loch Achtriochtan and Loch Tarff), P.pectinatus (from Loch Croispol), P.polygonifolius (from Loch Achtriochtan and Loch Tarff) and P.praelongus (from Loch Lanlish, Loch Tarff and Loch Uanagan). Turions of P.perfoliatus and P.obtusifolius were collected from glasshouse grown material as were propagules of Myriophyllum spicatum and

M.alterniflorum. The seeds were stored until required in the dark in a cool cupboard as were the turions, while the propagules were selected just prior to treatment.

Fleshy seeds were first shaken with sand and water to remove the fleshy parts and then the sand removed by passing the mixture through a soil sieve. The seeds were then surface sterilised by shaking for 15 minutes with 80% ethanol followed by 10% sodium hypochlorite for one hour. Following the method of Forsberg (1965) some of the seeds were embedded in test tubes containing 0.5% agar, with the addition of 0.5% beef extract, 0.1% peptone and 0.1 g/l TRIS buffer, while others were placed directly in test tubes of tapwater. Other embryos were exposed by dissection (Spence et al., 1971) and then placed in either agar, as before, or in a test tube containing tap water. Approximately 20 seeds were placed in each tube. All tubes had cotton plugs in their mouth and foil covers. All glassware, media and instruments were autoclaved at 15 lb/15 minutes before use and all operations were carried out in a culturing room which had been previously sprayed with 1% thymol in 80% ethanol. Cultures were placed in an incubator previously sprayed with 1% thymol in 80% ethanol and programmed at 25°C and a 16 hour day light.

Germination rates were poor for Potamogeton species under all conditions and in no case exceeded 10% after 20

days, while Lobelia dortmanna seeds germinated well under all conditions. Viability tests with tetrazolium (2.5 g of 2,2,5-triphenyl-2H tetrazolium chloride per litre and 24 hours in the dark), which reacts with respiratory hydrogen to form a deep red stain, were applied to seeds and indicated that the low rates of germination were not due to detrimental effects of the sterility treatment but were inherent in the seed population or its pretreatment.

Germinated seedlings were transfered individually to 100 ml conical flasks containing Gorham's medium (Hughes et al., 1953) and allowed to develop. Algal and bacterial contamination had, however, not been completely eliminated by the methods employed and proved difficult to control by subculture and no plants grew under completely axenic conditions to a size suitable for experimentation.

Propagules of Myriophyllum species and turions of P. perfoliatus were washed in 80% ethanol for five minutes, shaken with 5% sodium hypochlorite for 30 minutes and then transfered to 250 ml autoclaved culture flasks containing 100 ml Gorham's medium. All cultures grew well under the incubation conditions (as above) and turions germinated in about two days. Contrary to the findings of Franks (1966) no pretreatment was necessary, probably because the present experiments were carried out in the spring, which is their natural germinating time. Unfortunately, again, it proved

impossible to raise plants to a suitable size for experimentation under algal-free conditions and the sterile culture programme was regretfully abandoned. Fitzgerald (1969) reports the same problem. He found that contaminating algae cover sprigs of Myriophyllum species in as little as three weeks when grown in a full culture medium.

No experiments to compare the ability of the roots of sterile and non-sterile plants to absorb and translocate nutrients have therefore been carried out.

The question as to whether nutrients are absorbed into or onto the roots of aquatic macrophytes has been investigated by microautoradiography. The following method was employed to obtain microautoradiographs of the distribution of P-32 in the roots of several Potamogeton species.

After the P-32 treated material had been washed in running tap water for 10 minutes, conveniently sized pieces were cut and inserted between slices of carrot, which proved an adequate support for hand cut razor sections. The slides on which the sections were to be mounted were treated in the following way:-

(1) The slides were cleaned by soaking in the following solution of chromic acid until they were perfectly wetted by tap water

Potassium dichromate	100 g
H_2SO_4 (concentrated)	100 ml
Distilled water to make	1000 ml

The potassium dichromate was dissolved in the water and the sulphuric acid added slowly with constant stirring.

(2) The acid treated slides were then washed in running water and dipped in the following solution at about 20°C

Gelatin	5.0 g
Chrome alum	0.5 g
Water to make	1000 ml

and without further treatment they were placed in racks to dry.

This "subbing" procedure (coating with gelatin) was necessary to ensure good wet-adhesion of the autoradiograph emulsion.

The fresh cut sections were mounted on the dry, subbed slides and a piece of fine lens tissue placed on top. This tissue not only prevented the sections from floating off but also served as an inert layer between the section and film and thus cut down chances of pseudoautoradiographs being produced.

The Kodak AR10 stripping film plate was used as the autoradiographic emulsion and pieces sufficient to cover the slides were cut from the sheets and floated, emulsion side downwards, in a dish of a 10% solution of the following reagents at 20°C:-

Sucrose	200 g
Potassium bromide	0.1 g
Distilled water to make	1000 ml

The film was allowed to float for two to three minutes and then a slide of specimen sections was introduced underneath it, and drawn from the solution at an angle of about 30° from the horizontal, so that the emulsion draped itself over the slide and the solution drained away. The emulsion was freed of wrinkles by gently brushing with a damp camel hair brush, then the slides, with their sections and emulsion coating, were dried in a stream of cold air from a hair drier and placed about 1 cm apart in a light-tight box for exposure. Correct exposure time was estimated by exposing a series of slides for varying periods from two days. Twenty days exposure usually proved adequate.

Kodak D 19 developer was used to develop the emulsion. Developing time was five minutes at 20°C with gentle shaking every minute. The slides were then rinsed gently in running water for two minutes and fixed in a solution of Kodak "metafix" powder for ten minutes at 20°C, again with gentle shaking every minute. The preparation was washed for five minutes in gently running water and dried in a dust-free atmosphere. Apart from slide preparation and section cutting, the procedure was carried out in a photographic darkroom using a Kodak safelight, Wratten series

No. 1 (red), with a 25 watt lamp at a distance no less than four feet from the emulsion.

Results from these microautoradiograph studies indicated that some label accumulated round the outside of the roots and rhizomes but a greater degree of fogging was apparent within the plant tissue in the region of the vascular tissue. The clarity of the autoradiographs was, however, poor and no attempts at any quantitative analyses were made. It was thus apparent, however, that in the nutrient absorption experiments reported previously some of the labelled phosphorus was in fact adhering to the outside of the plant tissue and probably was metabolised by micro-organisms. This amount was, however, likely to be small in comparison to that actually absorbed into the tissue of the plant.

Translocation of Metabolites

It has been demonstrated that the roots of aquatic plants have some capacity to absorb nutrients and that under certain conditions these nutrients may be translocated to the shoots. The rate of this translocation process has, however, been shown to be relatively slow. In Chapter 4 it was shown that sucrose, metabolised in photosynthesis, is transported from the leaves to the roots and rhizomes. It is possible that this process also occurs at a slow rate

and that one feature of aquatic macrophytes is a slow transport system for both nutrients and photosynthetic metabolites. Two experiments were carried out to investigate this possibility.

A specimen of Potamogeton perfoliatus was partitioned as in the nutrient absorption experiments. A nutrient solution was used to bathe the roots while the same solution, but with added C-14 sodium bicarbonate, was used to bathe the shoots. The plant was left for six hours in continuous light at 20°C and an autoradiograph of the specimen prepared. This was exposed for two weeks before being developed (weak beta emission of C-14). The fogging on the autoradiograph indicated firstly, a high degree of photosynthetic activity in the topmost leaves and secondly, the transport of C-14 labelled products down the plant stem to other young shoots which were not in contact with C-14 and which were kept in the dark. This autoradiograph (Plate 8) is presented in Appendix II.

To try and obtain some quantitative information on the rate of movement of carbohydrates produced during photosynthesis, one further experiment was carried out. Six specimens of P. perfoliatus were sealed in containers so that their roots and shoots were separated. Each shoot solution was treated with 10 μCi of C-14 sodium bicarbonate while the roots received only the nutrient medium. The

plants were maintained at 20°C in a water bath under a bank of three Gro-lux lamps programmed on a 16 hour day. After 3, 6 and 22 hours, two specimens were removed, divided into leaves, stems and roots and rhizomes and killed in 80% ethanol. The specimen removed after 22 hours had received 14 hours of light while the others had been in continuous light. Ethanol extracts of the plant tissues were made following the method previously described in Chapter 3. The radioactivity in three 1 ml aliquots of each 25 ml extract was assayed for radioactivity as before, but using a Nuclear Chicago gas-flow detector and scaler. Each sample was counted for three ten minute periods and results expressed as cpm/mg extracted-tissue dry weight. Table 6.7 presents the relative percentage distribution of the total count rate between the different plant tissues.

TABLE 6.7

Percentage distribution of ethanol-soluble carbohydrate products of photosynthesis of Potamogeton perfoliatus based on the relative distribution of cpm/mg tissue.

length of exposure to C-14	% activity cpm/mg leaves	stems	rhizomes & roots
3	96.9	2.5	0.6
	95.6	3.4	1.0
6	95.2	4.5	0.3
	95.4	4.2	0.3
22	94.1	5.7	0.2
	94.6	5.3	0.1

It is apparent that much activity has accumulated in the leaves and that only relatively little of this has been transported to the non-photosynthetic tissues. Even after a period of eight hours darkness 94% of the alcohol-soluble carbohydrate products of photosynthesis still remained in the leaves while in none of the samples did there appear to be more than 1% of this photosynthesate in the roots and rhizomes. Thus, although a movement of photosynthetic metabolites did occur from the leaves of the specimens under investigation, this was only small and comparable to that reported from some of the nutrient transport studies reported earlier in this chapter.

CONCLUSIONS

Far from being insignificant it would appear that in many cases the roots of submerged aquatic macrophytes form a considerable portion of their biomass. Undoubtedly they function in anchorage and are often highly adapted for this purpose but such dispute over their possible role in nutrient absorption still exists.

The fact that root hairs are generally present is often overlooked and though the vascular system is simplified the uptake of nutrients should not be effected, as this process is not directly related to the reduction in the extent of the xylem. Similarly the transport of nutrients should not be unduly hampered since, assuming that water and dissolved nutrients are carried through their cavities, the functional efficiency of the xylem elements is not proportional to their degree of liguification. The presence of an endodermis in both the roots and the shoots of many species, coupled with the knowledge that this structure is involved in regulating the transfer of water and nutrients to and from the stele in the roots of terrestrial species, provides strong evidence in favour of a functional role for the vascular system.

Evidence from the incorporation of herbicides into both shoot and root lends support to the idea of nutrient

absorption by both organs but there is contrasting evidence from the same sources as to the degree of movement capable of taking place within the plant. Two groups of workers have recently, and independently, demonstrated the uptake of a nutrient ion by the roots of a submerged plant and its subsequent transport throughout the plant.

Experimental investigations carried out as part of the present studies have confirmed the ability of roots of several species of aquatic macrophyte to absorb nutrients. Nutrients have been shown to accumulate in the roots of Potamogeton species and a limited upward transport via the vascular system has been noted. Phosphorus has been shown to accumulat in the nodal regions of both the shoots and the rhizomes. The low rate of translocation of phosphorus may, in part, result from an accumulation of polyphosphates as a phosphate store, to enable the initiation of new growth. Although the transport pathways throughout the plants have been shown to be extensive, no nutrient entering the shoots of the plant was ever detected in the roots, the roots being apparently self-sufficient for their nutrient demands. No direct, conclusive, field evidence for this possibility was, however, obtained. As well as a low rate of acropetal and basipetal nutrient translocation, a low rate of movement of the metabolites of photosynthesis was also noted, and it is possible that such slow transport mechanisms are

characteristic of aquatic macrophytes.

The fact that the roots of submerged plants have been shown to be capable of nutrient absorption re-opens the question of correlations between the distribution of these plants and the nutritional status of the substratum. While there is still no direct evidence of a causal correlation between the distribution of any species and the nutrients of the substrate, the ability of the roots to absorb nutrients does explain the numerous reports of luxuriant macrophyte growth on rich mud as opposed to nutrient-poor sand.

It is possible that in cases of chronic limitation of nutrients in the water, the substrate, via the roots, may provide an alternative supply. It is doubtful, from the evidence of translocation rates reported here, whether the rate of supply of such nutrients would be sufficient to meet the demands of growth, if other conditions were optimal. Such may be the case in Loch Croispol where the clear waters provide near-ideal light conditions, yet the primary productivity of Potamogeton perfoliatus appears to be limited by the low quantity of phosphorus available in the water. The primary productivity experiments were, however, carried out with detached leaves and it is possible that their potential productivity has been reduced due to the severing of the connection from their nutrient supply. Chapter 7 of this thesis deals with other measurements of the nutrient

status of aquatic macrophytes which aid in the interpretation of possible nutrient limitations upon productivity.

With respect to the investigations reported in this chapter, it must be pointed out that the interpretation of the results of experiments involving the use of radioactive tracers to gauge the rate of nutrient translocation is somewhat complicated by the fact that the rate of translocation may not be given by the apparent movement of the radioactive ion where losses or gains into storage may occur and where the source concentrations, as in the present studies, are low. This situation is further complicated in the use of autoradiography to gauge such movement, where the exposure times may be too short to produce suitable autoradiographs of the absorbing zone are generally not sufficient to record the presence of the tracer ion in the receiving and translocating tissues.

CHAPTER 7

NUTRIENT AVAILABILITY

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CHAPTER 7

NUTRIENT AVAILABILITY

TISSUE ANALYSIS

Introduction

In many investigations concerning the distribution and abundance of macrophytes in lakes and streams, it has been a routine task to measure the concentrations of several chemical components of the vegetation and the lake water. The full potential of this information is often not realised. Perhaps the two most useful measures are those of phosphorus and nitrogen, two elements which are generally recognised to be critical for plant growth.

Bould *et al.* (1960) review the technique of leaf analysis, a method by which the nutritional status of crop plants can be assessed by measuring the concentration of certain elements in their leaf tissue and comparing this with the range of nutrient levels over which plant growth has been found, experimentally, to be dependent. Evidence indicates that when other growth requirements are sufficient, plants with different nutrient levels give similar yields so long

as those nutrient levels are well above the, so-called, critical level. Tissue contents below the critical concentrations are associated with deficiencies and result in poor yields. Tissue contents above the critical level have no effect on yield and are termed luxury consumption.

The assumption on which the critical levels of nutrients are determined is that only one nutrient is limiting growth at any one time. This may not be so and it is possible that an interaction of nutrient factors controls the growth of aquatic macrophytes, so that the critical concentration of one nutrient varies with the abundance of another. This is, however, difficult both to establish and to analyse and so far as the present studies are concerned, the critical levels idea, as proposed by Mould et al. (loc.cit.), is considered to hold.

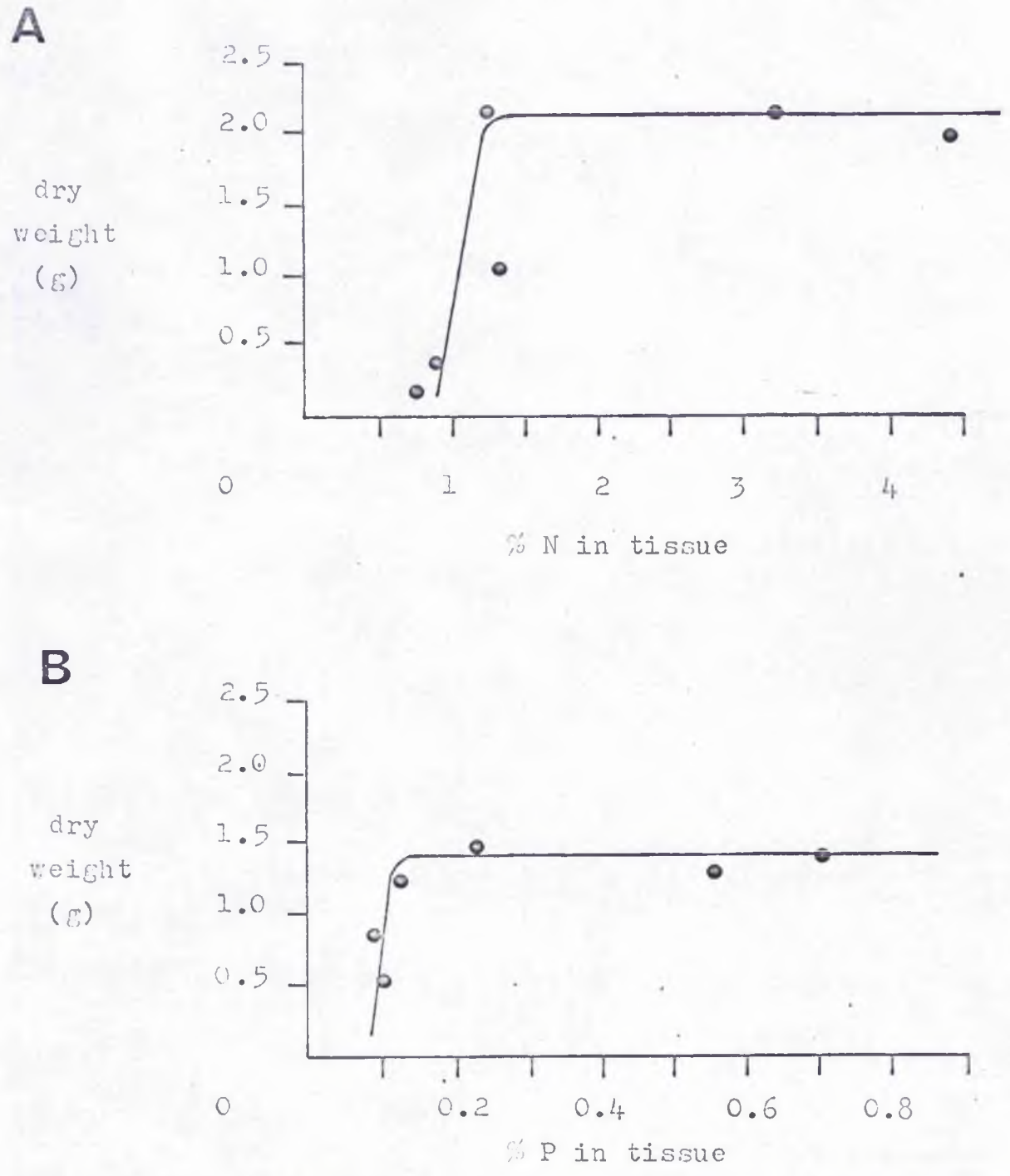
Tissue analysis appears adaptable for evaluating the availability of nutrients for aquatic plants in lakes and rivers and forms an alternative approach to that of relating the nutrient concentrations of the water to plant growth, as reviewed by Fogg (1965) for algae. Gerloff and Skoog (1954, 1957) have successfully used the tissue analysis technique to evaluate the nutrient status of Microcystis aeruginosa, a bloom producing blue-green alga, while Gerloff and Krombholz (1966) have adapted the technique for use with aeruginosa aquatic macrophytes and were successful in establishing the

critical levels of nitrogen and phosphorus for a number of species.

To determine the critical levels of these elements for aquatic plants, Gerloff and Krombholz (loc.cit.) were able to establish cultures in sterile conditions, over a range of nitrogen and phosphorus concentrations. Algal-free cultures are essential in such studies otherwise the abundant algal growth under high levels of nutrient enrichment make the interpretation of the results difficult. As has been previously explained it proved impossible in the present study to obtain algal-free cultures of macrophytes and therefore attempts to obtain critical concentrations of nutrients have not been successful. It is, however, probable that, unlike crop plants, there will be little variation in the critical levels of nutrients for aquatic macrophytes due to their lack of woody tissue and to the similarity of their structure. This postulate is borne out by the findings of Gerloff and Krombholz (loc.cit.). With the lack of personal findings the data of these workers have therefore been taken as a rough guide to the critical concentrations of nitrogen (1.3%) and phosphorus (0.13%) for aquatic macrophytes (Figure 7.1).

The phosphorus content of several species of aquatic plants, collected on one sampling date in early spring 1970, have been estimated. These values are discussed in relation

FIGURE 7.1



Relationship between dry weight of Vallisneria americana and tissue (A) nitrogen content and (B) phosphorus content (% dry weight), when cultured in nutrient media containing various concentrations of these nutrients (from Gerloff and Krombholz, 1966).

to levels of phosphorus and nitrogen in aquatic macrophytes reported in the literature, in an attempt to gain an idea of the general availability of these nutrients for the growth of angiosperm aquatic plants.

Methods

Whole plants of various species were collected by wading in shallow water or by aqualung diving in deeper water, washed free of adhering algae and sediments, divided into roots, rhizomes and shoots, and oven dried. Specimens were required not only free of soil particles and epiphytic organisms but also free of any inert surface deposits which might contain unavailable resources of phosphorus and hence distort the observations of tissue content.

Each sample to be analysed generally consisted of more than one individual plant which were first ground in a mortar and pestle. Approximately 0.1g of dried, powdered plant material was accurately weighed into a 30 ml micro-kjeldahl flask, 3 ml of concentrated sulphuric acid were added followed by 5 ml of nitric acid. The flask was heated on an electric mantle and allowed to boil until all the brown fumes were evolved, no plant material remained and the digest was clear. When the reaction was finished the flask was cooled and the contents poured into a 250 ml

conical flask. The digestion flask was washed out with distilled water and the washings transfered to the conical flask. Washing continued until the conical flask held approximately 50 ml of solution. A filter funnel was then placed in the neck of the flask and the flask placed on a hot plate and allowed to boil for about 15 minutes. This procedure allowed conversion of any pyro- and meta- phosphate to orthophosphate. The digest was cooled, a drop of phenolphthalein added and the solution neutralised with dilute sodium hydroxide. The contents of the conical flask were then poured into a 100 ml volumetric flask, the conical flask washed out several times with distilled water and the washings transfered to the volumetric flask which was made up to volume with distilled water. Phosphorus was measured by the new six-second method of Chamberlain and Shapiro (1969), a method based on the fact that the rate of formation of molybdenum blue by the reduction of molybdophosphoric acid is dependent upon acidity.

Reagents:- Basic ammonium molybdate - 22 g of ammonium molybdate were dissolved in 900 ml of distilled water, the pH adjusted to 8 with concentrated ammonium hydroxide solution and the solution made up to one litre with distilled water. Acid stannous chloride - 125 mg of stannous chloride were dissolved in 100 ml 5.5 N sulphuric acid. ($2.4\ N\ H_2SO_4$) prepared by adding 667 ml of concentrated acid to

333 ml of distilled water.

Procedure:- 50 ml of neutral plant extract and 2 ml of basic ammonium molybdate were mixed in a 250 ml conical flask. A 250 ml beaker containing a magnetic stirring bar and 2 ml of acid stannous chloride was placed on a magnetic stirrer and rapid stirring started. At a noted time the prepared sample was poured into the beaker and, after six seconds, 4 ml of 24 N sulphuric acid were added from a blow-out pipette. The optical density of the sample was read at 510 nm after approximately five minutes using a 1 cm cell in an SP 600 spectrophotometer. The phosphorus values were read from a calibration graph prepared from standard solutions, and analysed by the same procedures as above.

Results and Discussion

Table 7.1 lists the concentration of phosphorus measured in the tissues of species collected from Loch of the Lowes in the spring of 1970. The values for Myriophyllum alterniflorum compare well with those found by Caines (1965) for this species in a similar loch, Loch Choin, and are in the region of the critical concentration of Gerloff and Krombholz (1966). Values for Littorella uniflora are slightly lower than those of Caines (loc.cit.) and the roots (mean = 0.23% P) were found to contain more phosphorus than

TABLE 7.1

Total phosphorus concentrations (% P dry weight) of plants collected in Loch of the Lowes in April and May, 1970.

Shoot	Root	Whole plant
Littorella uniflora		
0.11	0.24	0.20
0.14	0.20	0.19
0.13	0.26	0.20
0.13	0.21	0.18
Lobelia dortmanna		
0.16	0.13	0.15
0.14	0.12	0.15
0.17	0.12	0.15
Myriophyllum alterniflorum		
0.10	0.11	0.11
0.14	0.16	0.15

Potamogeton obtusifolius

Leaves	Stems	Roots	Whole plant
0.61	0.71	0.60	0.65
0.98	0.36	1.03	0.96

Note: in this context "roots" signifies the entire root/rhizome system.

the shoots (mean = 0.14% P). Shoot levels approached the critical concentration. Analyses of Lobelia dortsanna indicated levels of phosphorus only just above the critical concentration and compared well with the analysis of this species by Oerloff and Krombholz (loc.cit.) and by Caines (loc.cit.). The species with the highest individual level of phosphorus (1.03%) was Potamogeton obtusifolius. Though the results were variable for this species, levels were generally higher than the proposed critical values and no conclusive picture of the distribution of phosphorus throughout the plant can be gained. Thus, in the one loch, there is variation in tissue phosphorus within and between species. Similar variations would probably also be encountered in a mixture of land plants from any one site and probably reflects the different plant capacities to accumulate this nutrient.

The analyses reported in Table 7.1 were carried out in early spring, the start of the growing season, and should therefore indicate to some degree the basic fertility of the loch, as there was not yet a luxuriant plant growth. Before any generalisations regarding the fertility of the loch can be made, however, marked differences in the distribution and growth habits of the various species must be taken into account. This, coupled with the observations reported in Chapter 6 on the nutrient-absorption ability of roots, could easily result in the situation in which some

species (as in Table 7.1) are apparently supplied with adequate nutrients while others are not.

Harper and Daniel (1934) present data from ponds and lakes near Stillwater, Oklahoma, giving figures ranging between 1.4% and 2.5% for the nitrogen concentrations of several species of Potamogeton and, 1.1% to 3.0% for phosphorus. They explain a variation in the phosphorus content of a single species in terms of luxury consumption of this element by plants growing in fertile sites. Forsberg (1960) quotes figures of 2.0% phosphorus in Ceratophyllum demersum and Myriophyllum verticillatum growing in Osbysjon, Sweden, while Stake (1967) gives average figures of 2.6% nitrogen in Potamogeton species growing in central Sweden. Bernatowicz (1969) produced data on the nitrogen and phosphorus content of several species of aquatic macrophyte from Lake Warniak (Poland). The nitrogen content of the plants analysed varied from 1.18% to 2.82% while the phosphorus content varied little, Ceratophyllum demersum having 0.61% phosphorus and Elodea canadensis 0.59% phosphorus. Mulligan and Baronowski (1969), in carrying out glasshouse experiments to determine the optimal-levels of nitrogen and phosphorus for the growth of aquatic plants, obtained maximum tissue levels of 0.80% phosphorus (in Elodea canadensis) and 3.41% nitrogen (in Potamogeton crispus). Even at low levels of nutrients in the bathing media no

nitrogen or phosphorus values in the plant tissues approached the critical levels of Gerloff and Krombholz (loc.cit.). Holden (1954) gives data from investigations carried out in some Scottish hill lochs indicating natural phosphorus concentrations of about 0.4% in the tissues of several Potamogeton species and 0.2% in Myriophyllum spicatum.

The literature offers little information on the seasonal variation of mineral nutrients in higher aquatic plants, though the problem has been touched upon by Owens and Edwards (1962), Arnemo (1964) and Koelling and Lucerna (1965). Gerloff and Krombholz (loc.cit.) provide some information for a few species in several Wisconsin lakes.

Haines (1965) gives an account of the extent of the normal seasonal fluctuations in the phosphorus contents of aquatic plants in a Perthshire loch, as well as detailed investigations into phosphorus distribution in the tissues and the effects of fertilisation. His results demonstrate a seasonal cycle in the concentration of phosphorus present in Potamogeton praelongus and Myriophyllum alterniflorum, the two species which he investigated. The phosphorus content in the growing tips of P.praelongus varied between 0.3% (September) to 0.9% (May) and in the rhizome between 0.1% (May) and 0.3% (November). Specimens of Myriophyllum alterniflorum, though generally having a tissue concentration of about 0.1% phosphorus, displayed a seasonal variation

ranging between 0.05% and 0.4% phosphorus. The growing tips averaged 0.3% and ranged between 0.2% and 0.6% phosphorus.

From the majority of the data quoted above it would appear that the levels of nitrogen and phosphorus in aquatic plants lie above the critical levels ascertained by Gerloff and Krombholz (loc.cit.). Where analytical data has been obtained from the growing tips of plants, concentrations of nitrogen and phosphorus were generally much greater than the proposed critical values. In few data quoted here, and elsewhere, have the levels of nitrogen in the tissues of aquatic macrophytes been recorded below the critical level of 1.5%, though low levels of phosphorus have frequently been recorded here and elsewhere (Stake, 1968), which fall well below the proposed critical level of 0.1%. It can thus be tentatively suggested that phosphorus may be a more important nutrient than nitrogen in limiting the productivity of aquatic macrophytes. The results of Gerloff and Krombholz (loc.cit.) also indicated that, in all but one of the lakes they investigated, phosphorus was more likely to limit the growth of higher plants than was nitrogen.

This idea is in complete contrast to the findings of Gerloff and Skoog (1954, 1957) who concluded that the nitrogen supply was the most likely limiting factor for the growth of the blue-green alga Microcystis aeruginosa. In the same context, Forsberg (1964), has reported that high

concentrations of phosphorus (greater than 20 µg/l) are inhibitory to the growth of several members of the Characeae and proposes phosphorus to be a maximum factor limiting and restricting their growth. It is thus possible that the balance between the growth of algae and aquatic macrophytes may rely upon the available concentrations of nitrogen and phosphorus in the water and the differing critical levels of these nutrients required for successful growth. Fitzgerald (1969b) has reported a study of some factors influencing the competition between algae and aquatic macrophytes. He found, from nutritional studies in the laboratory, that cultures of macrophytes remained relatively free of epiphytes and competing phytoplankton when the cultures were nitrogen-limited. He proposes that the aquatic macrophytes can prevent the growth of contaminating algae by successful competition for the limited nitrogen compounds available. A simpler solution would appear to be that of a smaller demand required to satisfy thecritical level. No studies appear to have been published dealing with the relative phosphorus demands of algae and aquatic macrophytes and many fruitful studies could well be centred around this topic.

These speculations about possible controlling influences on the competition of algae and aquatic macrophytes are, however, based only upon the determinations, by one group

of workers, of the critical concentration levels of nitrogen and phosphorus in a few species. It is by no means certain that these levels are applicable to all species. It is unfortunate that, in the present studies, attempts to measure the critical levels of nutrient requirements have failed because of an inability to maintain algal-free cultures. Tissue analysis, however, remains important in evaluating nutrient supplies in natural waters.

BIOASSAY FOR LIMITING OR SURPLUS NUTRIENTS

Introduction

Fitzgerald and Nelson (1966) have adopted a different approach to the problem of assessing the nutrition of aquatic plants. They have described a technique by which they were able to assess the changes in the phosphate status of plants by using a simple, extractive procedure to detect surplus, luxury-consumed phosphorus and an enzymic bioassay for conditions of phosphorus limitations. Limiting or surplus nitrogen was detected by measuring the rate of NH_4 nitrogen absorption in the dark.

As pointed out by Gerloff and Krombholz (1966), tissue analysis can be used to assess some of the factors involved in the nutrition of plants, but tests which are based on an analysis of particular chemical fractions, or enzymic reactions which reflect limiting or surplus conditions of a nutrient are more meaningful than whole plant analysis. It is on this point that the work of Fitzgerald and his co-workers differs from that of Gerloff and his group. Though much of Fitzgerald's work has been concerned with algae (both planktonic and attached) the methods hold good for the higher aquatic plants and he has presented some limited data on Myriophyllum species, Ceratophyllum species, Lemna

species and Potamogeton species.

Boiling-water extracts of plants leaches out any surplus phosphate and this surplus, or luxury phosphate, can be measured as orthophosphate. The ease with which this phosphate is released indicates that it must be stored in a highly labile form. Boiling-water extracts of plants growing in conditions of phosphate limitation should contain little or no orthophosphate.

Following on from the work with Escherichia coli, in which Torriani (1960) found high levels of activity of the enzyme, alkaline phosphatase, in cells growing on a medium that had become exhausted of phosphate, Fitzgerald and Nelson (1966) and Fitzgerald (1969a) demonstrated that the same holds true for algae and aquatic macrophytes. They developed a simple bioassay to assess the phosphorus nutrition of aquatic plants. Plants starved of phosphate accumulated alkaline phosphatase, and contained a large excess of this enzyme compared with plants which were not phosphorus-limited.

Phosphatases are important enzymes because of their ability to hydrolyse phosphate esters or to transfer phosphate from one organic group to another. The most common phosphatases are phosphomonesterases which hydrolyse monoesters of phosphoric acid.

$$RO\text{-}PO_3H_3 + H_2O \rightarrow ROH + H_3PO_4$$

Other phosphatases hydrolyse diesters of phosphoric acid, pyrophosphates or metaphosphates. Provasoli (1958) has reviewed the data on algal phosphatases which permit many species of algae to obtain phosphorus from esters in order to sustain growth in the absence of orthophosphate. Kuenzler and Perras (1965) found that many species of marine algae produce alkaline phosphatase when they become phosphorus deficient. This enzyme was shown to hydrolyse dissolved phosphate esters on cell walls and in the tissue bathing solution; the cell then absorbed the phosphate ion, leaving the organic moiety in the medium.

In the present study a series of enzymic analyses for limiting and surplus phosphate in aquatic macrophytes has been carried out, following the techniques of Fitzgerald and Nelson (1966).

Methods

Enzymic Bioassay for Alkaline Phosphatase Activity

The basis of the method relies upon the fact that the compound p-nitrophenol phosphate is colourless but, upon hydrolysis of the phosphate group, the yellow salt of p-nitrophenol is liberated (absorption maximum = 410 nm). Thus the substrate is itself an indicator of its utilisation and hence is a measure of the phosphatase activity as given in Figure 7.2.

FIGURE 7.2

Chemical reaction of alkaline phosphatase

p-nitrophenyl phosphate

+

H_2O

(colourless)

|

phosphatase

↓

p-nitrophenol + H_3PO_4

(coloured when alkaline)

One unit of alkaline phosphatase is defined as the amount of

enzyme which liberates 1 mμ mole of nitrophenol/hour under the specified conditions (1 mμM = 0.1391 mg).

The experimental procedure used in the present studies was a slight modification of that used by Fitzgerald and Nelson (loc.cit.). The alkaline phosphatase activity in the aquatic plants was determined by suspending a piece of washed tissue in 32 ml of Gorham's medium (Table 7.2) minus the phosphate component, within a 100 ml conical flask.

TABLE 7.2

Composition of Gorham's medium (g/l)

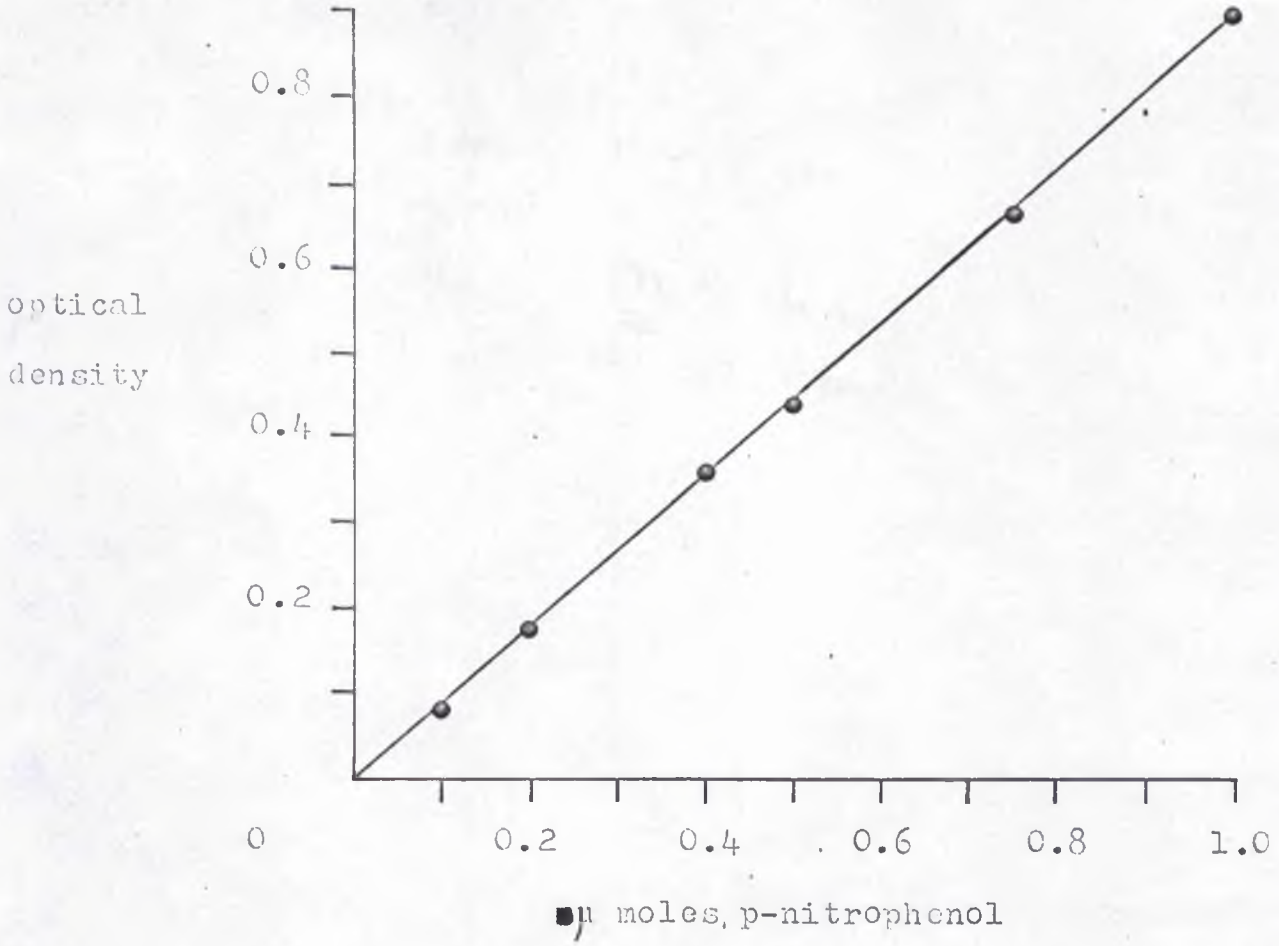
$NaNO_3$	0.496	Na_2CO_3	0.020
K_2HPO_4	0.039	Ferric citrate	0.006
$MgSO_4.7H_2O$	0.075	Citric acid	0.006
$CaCl_2.2H_2O$	0.036	EDTA	0.001
Na_2SiO_3	0.058	pH	8.5

4 ml of TRIS buffer were added (1 M TRIS in 0.01 M $MgCl_2$, adjusted to pH 8.5 with acetic acid) followed by 4 ml p-nitrophenyl phosphate solution (30 mg/100 ml distilled water). The suspension was incubated at 36°C in a constant-temperature shaking bath. After 30 minutes, 10 ml of solution were removed by pipette and added to a test tube containing 10 mg orthophosphate in 0.5 ml of NH_2PO_4. After a further five minutes the optical density (at 410 nm) of the solution was measured in an SP 600 spectrophotometer

using a 1 cm cell. The tissue was removed from the flask, blotted dry, placed in an aluminium foil cup and dried overnight in a vacuum oven at 105°C, allowed to cool in a desiccator and weighed. Incubation times ranged up to two hours and enzyme rates remained constant over this time scale. Controls to take account of any changes in the optical density of Cotham's medium due to the extraction of any plant material and to measure any natural changes in the substrate solution were always incubated with the test samples.

Phosphatase enzyme activity was expressed in terms of the rate of substrate utilisation which was indicated by the production of the yellow p-nitrophenol. A calibration graph was prepared by adding varying quantities of a p-nitrophenol solution of a known concentration (Sigma 104 - 1) to a standard volume of 0.02 N NaOH and reading the optical density on a spectrophotometer at 410 nm. The calibration graph is given in Figure 7.3. The enzyme activity was expressed as μ moles p-nitrophenol liberated per mg tissue dry weight per hour. This method of expressing results was also adopted by Fitzgerald and Nelson (loc.cit.). No measure of the surface area of the plant tissue through which the substrate may penetrate or the enzyme be present upon has been taken into account and this, along with the activity of the irremoveable, epiphytic microflora may have some influence upon the observed values.

FIGURE 7.3



Calibration graph for determining alkaline phosphatase activity.

A. Farmer.

I think this graph is wrong. Bottom scale should read.

m. moles = p-nitrophenol.

Results

Levels of phosphatase activity were first measured in a series of laboratory stock-culture material and then in material subjected to various phosphorus treatments. A second set of observations was made on phosphatase activity of the leaves of plants growing under natural conditions in a series of lochs known to contain low concentrations of available phosphorus. These latter experiments were carried out simultaneously with *in situ* measurements of carbon fixation under near-natural conditions.

Levels of phosphatase activity present in Littorella uniflora and Lobelia dortmanna plants

Specimens were selected from laboratory cultures which were growing under similar conditions of substrate, water chemistry, light and temperature. All obvious adhering mud and algae were removed and the plants were separated into roots and shoots and incubated as described above, to determine the levels of enzyme activity. Two replicates of each sample were assayed and the results are presented in Table 7.3. Table 7.3 also presents the results of a parallel series of incubations carried out in acid solution using citric acid buffer (pH 4.8) to replace the TRIS.

TABLE 7.3

Phosphatase enzyme activities in cultures of Littorella uniflora and Lobelia dortmanna. (mμ moles p-nitrophenol/mg tissue dry weight/hour)

	Littorella uniflora		Lobelia dortmanna	
	Acid	Alkaline	Acid	Alkaline
Shoots	4.5	8.3	2.7	13.1
	4.1	7.5	3.2	14.2
Roots	10.5	12.1	14.3	51.1
	11.0	12.9	13.9	51.3

Levels of phosphatase activity in specimens of Potamogeton schweinfurthii

Specimens of P.schweinfurthii were removed from the culture tanks and washed clean in gently running tap water. Using the apparatus and methods described previously, for studying translocation in aquatic plants, specimens were placed in containers so that the roots and shoots were kept in different solutions. The roots of one set of plants were kept in full Gorham's medium while those of another series were kept in Gorham's medium without the phosphorus component. The shoots of all specimens were kept in Gorham's medium without the phosphorus component. The plants were maintained at 20°C in the above conditions for 48 hours in

continuous light as supplied by three Sylvania Gro-lux lamps. The plants were removed, washed in distilled water, and the activity of the phosphatase enzyme was measured in various plant parts; roots, rhizomes, basal leaves and apical leaves. The enzyme rates were determined as before and compared (Table 7.4) with those of similar specimens at the start of the experiment. The rate of carbon fixation of the leaves was also determined by the methods set out in Chapter 3.

TABLE 7.4

Levels of alkaline phosphatase activity in Potamogeton schweinfurthii (mμ moles p-nitrophenol/mg tissue dry weight/hour).

Plant part	Treatment	Rate	
Rhizome	original	6.8	7.0
	- PO_4 48 hour	12.5	13.2
	+ PO_4 48 hour	5.2	4.8
Root	original	7.4	7.8
	- PO_4 48 hour	11.5	12.0
	+ PO_4 48 hour	4.2	3.9
Basal leaves	original	10.3	11.1
	root - PO_4	12.9	13.7
	root + PO_4	8.7	8.3
Apical leaves	original	22.0	25.1
	root - PO_4	33.3	36.4
	root + PO_4	17.4	16.6

Phosphatase activity in leaves of Potamogeton schweinfurthii after culturing in a series of phosphate concentrations

A number of apical leaves of P.schweinfurthii specimens were detached from healthy growing cultures and washed gently under tap water. A series of cultures were then prepared so that one leaf was placed in each flask of Gorham's culture medium which formed a series of four phosphate concentrations in the range 3.3, 13.6, 26.6 and 39 mg/l K_2HPO_4. All other nutrients were as in Gorham's medium. All cultures consisted of 100 ml of autoclaved medium and were maintained for seven days in a 16 hour daylength at 20°C in a constant environment chamber when the leaves were removed, washed in Gorham's medium minus phosphate and the phosphatase activities determined as before. Again enzyme rates were compared with those measured in similar specimens at the start of the culture period and are reported in Table 7.5.

Phosphatase activity in leaves of Potamogeton perfoliatus

Apical leaves of P.perfoliatus were collected from laboratory cultures and assayed for phosphatase activity after 48 hours culture in Gorham's medium plus varying amounts of phosphate. Cultures were maintained in a 16 hour day at 20°C. Results were compared (Table 7.6) with the assay

TABLE 7.5

Phosphatase activity of leaves of Potamogeton schweinfurthii after one week of culture in Gorham's medium with varying phosphate concentrations (mμ mole p-nitrophenol/mg tissue dry weight/hour).

Level of phosphate (mg K_2HPO_4/l)	Rate (1)	(2)
original material	25.2	29.3
3.375	400.0	386.9
13.600	270.1	260.3
26.600	97.1	104.8
39.000	59.7	62.4

TABLE 7.6

Phosphatase activity of leaves of Potamogeton perfoliatus after culturing for 48 hours in Gorham's medium with a range of phosphate concentrations (mμ mole p-nitrophenol/mg tissue dry weight/hour).

Level of phosphate (mg K_2HPO_4/l)	Rate (1)	(2)	(3)
original material	36.9	31.8	29.8
3.375	135.6	122.5	
13.600	107.3	100.0	
26.600	46.2	54.7	
39.000	38.7	42.8	

of similar leaves carried out at the start of the culture period. Table 7.7 presents the original levels of the enzyme measured under acid and alkaline conditions.

Levels of phosphatase activity in leaves of Potamogeton perfoliatus and P. praelongus collected from the three limestone lochs near Durness.

By aqualung diving, leaves were collected from the apices of healthy growing plants in all three lochs and, after washing them free of adhering matter, the activity of the alkaline phosphatase enzyme was measured as before. Measurements of the carbon fixation rate of similar plants carried out parallel with the phosphatase determination have been presented in Chapter 3 (Tables 3.19, 3.20, 3.21). Table 7.8 gives the levels of alkaline phosphatase activity recorded while Figure 7.4 represents these data graphically and serves to illustrate the steady rate of enzyme reaction measured over a 90 minute period.

TABLE 7.7

Levels of acid and alkaline phosphatase activity in Potamogeton perfoliatus from laboratory cultures (mμ mole p-nitrophenol/ mg tissue dry weight/hour).

	Rate	
	Acid	Alkaline
Leaves	97.4	29.8
	71.8	36.3
	74.8	31.8
	97.1	
Stems	8.5	5.0
	5.0	4.8

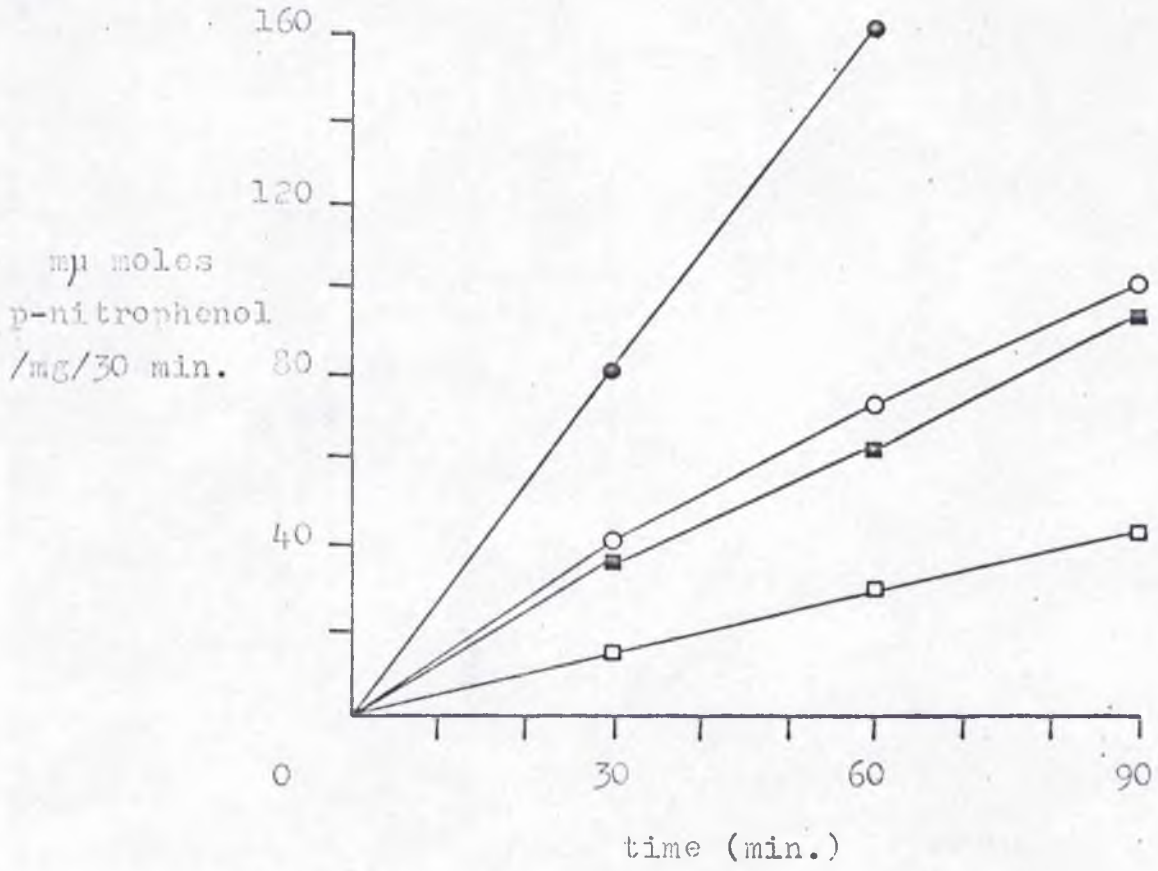
TABLE 7.8

Natural levels of phosphatase in apical leaves of Potamogeton perfoliatus and P.praelongus (mμ mole p-nitrophenol/mg tissue dry weight/hour).

Locality	P.perfoliatus		P.praelongus	
Loch Croispol	162 ± 4	164 ± 2	-	
Loch Borralie	83 ± 4	72 ± 1	61 ± 3	66 ± 5
Loch Lanlish		-	29 ± 2	30 ± 1.5

Each value represents the mean rate determined on one of two separate occasions in June, 1971. P.perfoliatus does not occur in Loch Lanlish and P.praelongus does not occur in Loch Croispol.

FIGURE 7.4



Levels of alkaline phosphatase, as measured on 21.6.71, in leaves of Potamogeton perfoliatus from Loch Croispol (●) and Loch Borralie (○) and in leaves of P.praelongus from Loch Borralie (■) and Loch Lanlish (□). (mμ moles p-nitrophenol/ mg leaf dry weight/30 minutes).

DISCUSSION

Results from the investigations on the rosette species Littorella uniflora and Lobelia dortmanna revealed a higher alkaline phosphatase activity in their roots than in their shoots. The results of the phosphorus analyses reported earlier in this chapter (Table 7.1) indicated that the roots of these species contained higher concentrations of phosphorus than the shoots. These latter analyses were few, and on specimens from only one loch, but it is possible that there is a differential critical level of phosphorus for roots and shoots and that this is related to the differential rates of enzyme activity determined in this instance. The fact that the roots of both species also had greater rates of acid phosphatase activity, known to be associated with phosphorus uptake (Woolhouse, 1969), partly bears out this hypothesis of a greater phosphorus demand by the roots than by the shoots.

Results from the first of the experiments with P. schweinfurthii indicate the metabolic function of the roots and rhizomes in phosphorus nutrition. Both roots and rhizomes responded to phosphorus starvation and availability over a 48 hour period, the difference between the starved and non-starved being greater than 100%. The most interesting observation from these experiments, however, was made

on the leaves. Not only did the phosphatase activity of the root and rhizome alter when they were subjected to phosphorus starvation and addition, but also that of the basal and apical leaves. The changes were best reflected in the apical leaves being less marked in the basal leaves. Comparing the original phosphatase activities of the root and shoot, the levels in the apical leaves were more than twice those of the roots and rhizomes and basal leaves, indicating a high demand for phosphorus in the growing point.

This high phosphorus demand is clearly reflected in the results from the phosphatase assays performed after the leaves of P.schweinfurthii had been kept in culture for one week. All enzyme activities were higher than the values recorded before the cultures were started and an almost ten-fold difference existed between the activities found in the cultured material. This difference, however, accompanied differences in available phosphorus in the culture medium.

The difference in phosphatase metabolism brought about by differences in available phosphorus in the culture media is again shown in experiments with leaf cultures of P.perfoliatus, though such a wide range of activities was not found. The experiments were, however, not carried out over so long a time scale and little differences from the original rates were found in the cultures with the high concentrations of available phosphorus.

The functional role of the leaves of P.perfoliatus in nutrient absorption is indicated by the high acid phosphatase activity presented in Table 7.7, whereas the stems appear to have only 1/10 of this activity. Similarly the stems have only 1/6 of the alkaline phosphatase activity of the leaves.

Enzyme rates measured in specimens from the three Burness, limestone lochs were not widely different from those recorded from laboratory cultures of the same species kept in varying phosphate concentrations. P.perfoliatus from Loch Croispol, which from other evidence, contains the least available phosphate of the three (Table 3.24), had the highest rates of phosphatase enzyme activity while specimens of P.praelongus from Loch Lanlish had the least. Plants from Loch Borralie fell in between and provided the linking species in an interesting, discontinuous distribution in three lochs, so spatially close and in many respects, so similar.

With regard to phosphatase activity being a guide to the nutrient limitation of primary production; in the one experiment carried out with P.schweinfurthii in a partitioned environment (Table 7.4), an interesting relationship emerged between phosphorus nutrition, phosphatase enzyme activity and carbon fixation. It has been shown that when the roots were kept in conditions of either surplus or limited available phosphate then not only was the phosphatase activity

of the roots and rhizomes effected but also that of the basal and apical leaves, which had not been in contact with the phosphate solutions. This fact lends further support to the idea of a nutrient transport system within aquatic macrophytes. The fact that the carbon fixation rate of leaves of plants having their roots in a phosphate-free solution was reduced compared to that of plants having their roots in a solution of surplus available phosphate illustrates how the nutritional status of the roots can influence the productivity of the plant (Figure 7.5).

The relationship between alkaline phosphatase activities and primary productivity is again seen by comparing data on the phosphatase activities of P.perfoliatus and P.praelongus, from the Burness lochs, both with the estimates of their primary productivity and with the influence of added phosphate upon those carbon fixation rates (Chapter 3). Comparing the primary productivity estimates in Table 3.21 (Chapter 3) with the estimates of phosphatase activity of similar specimens, the rate of carbon fixation of the different species from the different lochs when incubated in Warburg buffer appears to bear a relationship to their phosphatase activities (Figure 7.6). Although the relationship is not linear, it illustrates a trend of low rates of carbon fixation accompanying high rates of alkaline phosphatase metabolism. It will be recalled from Chapter 3 that P.praelongus from

FIGURE 7.5

(opposite)

A. Relative increase in the alkaline phosphatase activity of roots, rhizomes, basal and apical leaves of Potamogeton schweinfurthii, when kept in phosphate-free solution for 48 hours.

B. Difference in the carbon fixation rate of phosphate-starved leaves of P. schweinfurthii, when the roots were kept in a phosphate-rich solution for 48 hours.

C. Relative decrease in the phosphatase activity of phosphate-starved roots, rhizomes, basal and apical leaves of P. schweinfurthii, when the roots were kept in a phosphate-rich solution for 48 hours.

FIGURE 7.5

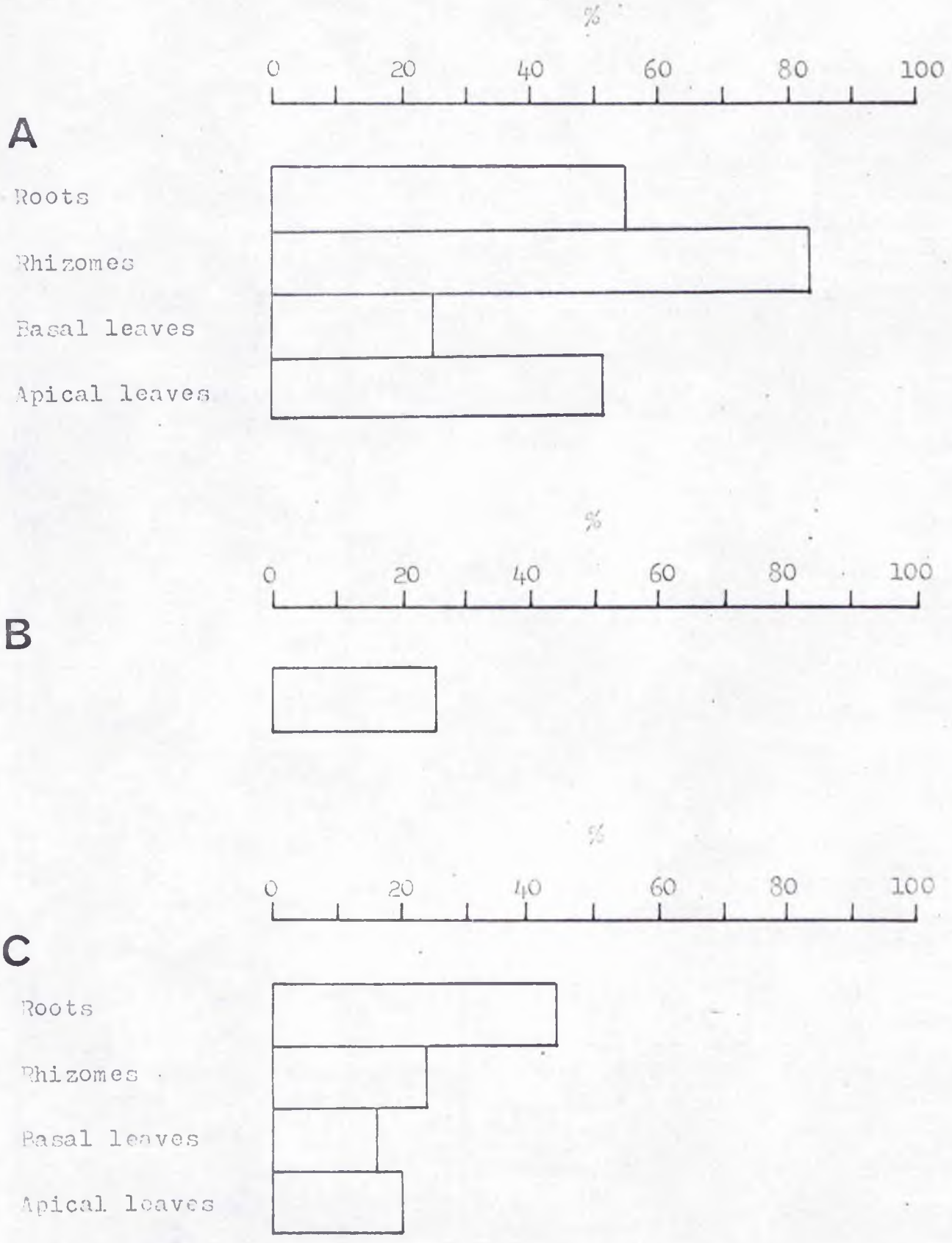
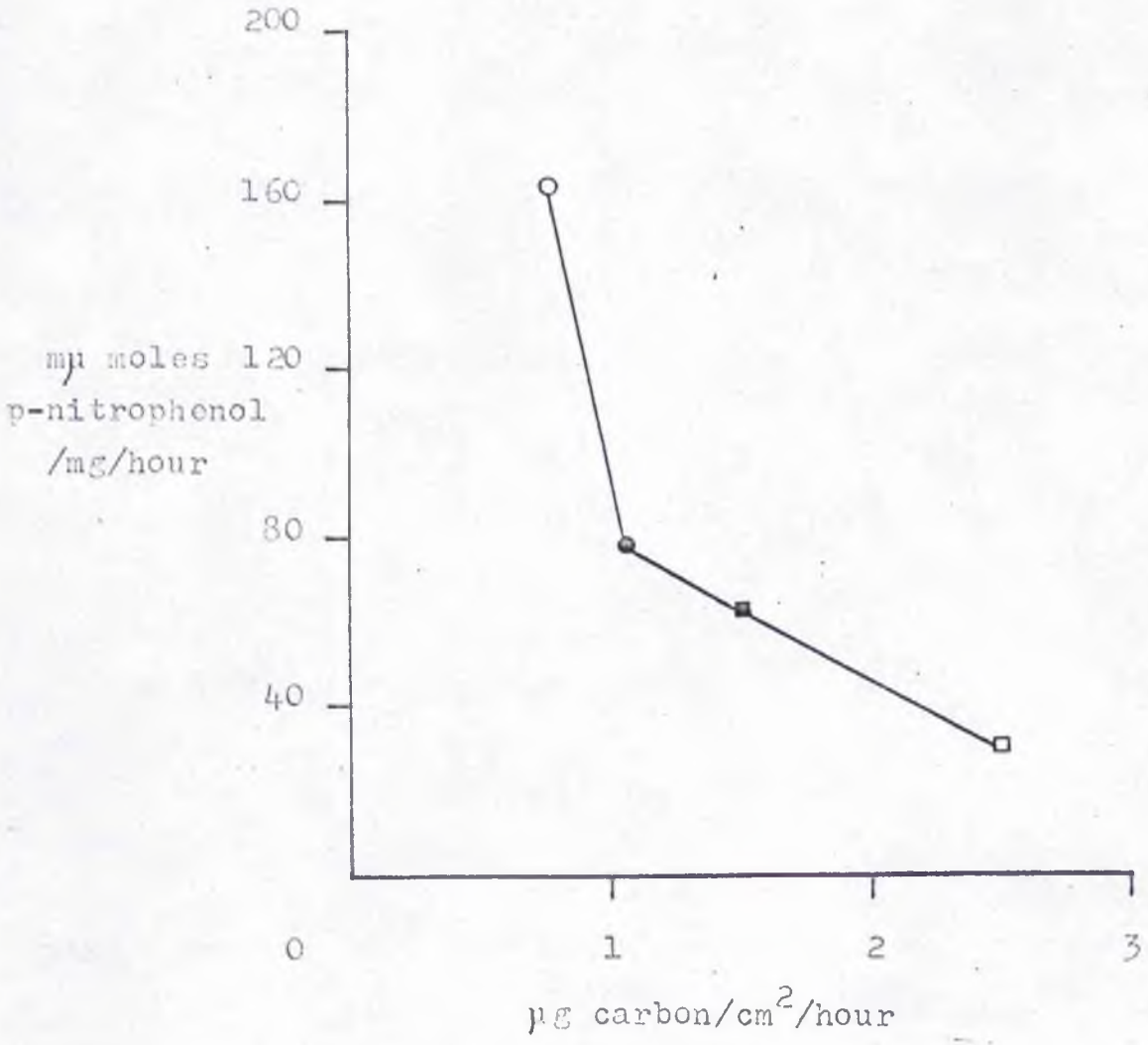

%
0
20
40
60
80
100
A
Roots
Rhizomes
Basal leaves
Apical leaves
%
0
20
40
60
80
100
B
%
0
20
40
60
80
100
C
Roots
Rhizomes
Basal leaves
Apical leaves

FIGURE 7.6



Relationship, as measured on 21.6.71, between carbon fixation rate (μg carbon/cm^2 leaf area/hour) and alkaline phosphatase activity (mμ mole p-nitrophenol/mg leaf dry weight/hour) of leaves of Potamogeton perfoliatus from Loch Borralie (●) and Loch Croispol (○) and P.praelongus from Loch Borralie (■) and Loch Lanlish (□). Carbon fixation rates were determined by incubating leaves with 0.01 M Warburg buffer No.11 at a depth of 3.5 metres in Loch Croispol.

Loch Lanlish, here shown to have a low rate of alkaline phosphatase activity, produced the highest rate of carbon fixation in all the loch waters, while P.perfoliatus from Loch Croispol, with the highest phosphatase activity, produced the lowest carbon fixation rate.

The results from the enrichment bioassay experiments (Tables 3.19, 3.20 Chapter 3) with additional phosphorus appears contradictory to the phosphatase data presented in this chapter. A greater increase in the carbon fixation rate was recorded from plants in Loch Borralie than was recorded in Loch Croispol experiments. The results themselves were, however, somewhat confusing both an inhibition and an enhancement of carbon fixation being recorded. Besides this there is the probability that the response of the tissue to the nutrient bioassay is dependent upon several other environmental variables (Chapter 3).

The phosphatase data from the Durness lochs does fit in with the water chemistry of the lochs (Table 3.24, Chapter 3) from which it is known that Loch Lanlish is chemically richer than Loch Croispol. No data are available for Loch Borralie though it is assumed to fall, somewhat, between the other two and perhaps from the primary productivity data of Chapter 3 and the alkaline phosphatase estimates reported here, this hypothesis can be upheld.

Stewart, Fitzgerald and Burris (1967) suggested that

there is a great need for simple, inexpensive methods for studying the nutritional status of algae in their natural environments. The same need is also true for higher aquatic plants. The phosphatase bioassay technique used in the present studies to evaluate the phosphorus nutrition of Potamogeton species growing in Scottish lochs is emphasised as a simple, reliable method. The technique has produced valuable information to confirm the idea of a translocation of nutrients from the roots of aquatic plants and to illustrate the effect that the nutritional status of the roots may have on primary productivity. Information has also been obtained which lends support to the hypothesis that the low primary productivity of aquatic macrophytes in some lochs at least, may, in part, be attributable to a lack of nutrients, the most important of which may be phosphate.

SUMMARY AND CONCLUSIONS

This thesis has attempted to describe some of the distribution patterns of submerged aquatic macrophytes in Scottish lochs. Effort has been directed towards prescribing a precise meaning to productivity with respect to these plants, to its measurement, and to investigating possible influencing factors.

Using aqualung diving techniques, biomass estimates have been made in several lochs. These results have been viewed in two ways, (a) qualitatively and (b) quantitatively.

Qualitative

It has been shown that, in any one loch, there is a zonation of species with depth of water. Generally, Littorella uniflora/Lobelia dortmanna communities were dominant in the shallower water (up to one metre), while Potamogeton species occupied the deeper areas (up to seven metres). Of the substrate variables investigated, no parameters have been shown to be causally correlated with the distribution of any particular species, although the interpretation of such factors was complicated by a high degree of variance in the biomass estimates of vegetation on fairly uniform substrates.

Distribution patterns of species between lochs have

also been noted. The members of the Characeae were only found in the limestone lochs of high pH and alkalinity, while Lobelia dortmanna and Isoetes lacustris were confined to the more base-poor waters. Littorella uniflora was found in all the lochs studied. Only two species of broad-leaved, submerged pondweeds were found in the limestone lochs but, while these were not restricted to such habitats, other species of the genus Potamogeton were. No one factor has been shown to underlie this distribution pattern though, in the case of the Characeae, it would appear that the high alkalinity of the water is related to the predominance of some members of this family in the limestone lochs, where they can produce their calcium carbonate exoskeletons. It is also possible that the low concentration of phosphorus in the limestone waters, shown by others to be a maximum factor in the growth of the Characeae, is also important, while the general low nutrient concentrations may exclude other species from these areas.

Quantitative

The biomass of the submerged vegetation in all lochs was shown to vary with depth. No one depth in all lochs has been found to support the largest biomass, though this was generally attained within the first two metres of water, but in all cases there was, after this peak, a general reduction in the biomass with increasing water depth. The

maximum depth at which rooted vegetation was recorded was seven metres (in Loch Croispol).

The interpretation of biomass data has been shown to be complicated by many factors, and such measures, as presently gauged, may serve only to indicate the production of a loch in a very approximate manner. Although bearing such limitations in mind, it is concluded that the biomass of macrophytic, submerged, freshwater vegetation is small when compared to similar measures from marine and terrestrial environments.

The calorific values of several macrophyte species were determined by bomb calorimetry. Using biomass estimates and these measured calorific equivalents, computations produced figures of 3,000 Kcal/m^2 for the energy stored in the Chara beds of Loch Croispol. The energy bound in Littorella uniflora/Lobelia dortmanna swards ranged between 120 and 600 Kcal/m^2 loch floor for different lochs, while three stands of Potamogeton species were calculated to be equivalent to 42, 200 and 290 Kcal/m^2.

An in situ C-14 technique, developed to estimate the carbon fixation rate of leaves of submerged macrophytes, has been described and discussed. The technique has been used to estimate the primary productivity of three Potamogeton species in several lochs. No distinction could be made between the primary productivity of different Potamogeton

species in any one loch, when measured simultaneously, although the productivity of species of this genus of aquatic macrophyte is possibly greater than those of several others. Insufficient data were available for accurate interspecific comparisons on a seasonal basis. Little correlation has been established between the biomass of the vegetation in a loch and its primary productivity as measured in short-term experiments.

From experiments carried out in near-natural conditions the mean productivity of submerged, broad-leaved Potamogeton species was estimated at 3 µg carbon/cm^2 leaf area/hour, and a ~~possible~~ daily productivity of 1 g carbon/m^2 loch floor computed from a ~~probable~~ leaf area index measurement. These figures were relatively low when compared to productivity estimates of other vegetation as given in the literature.

The carbohydrate storage compounds of several species of aquatic macrophyte have been investigated by gas-liquid chromatography. Fructose, glucose and sucrose have been identified as ~~two major~~ ethanol-soluble carbohydrate constituents of all Potamogeton species examined, though one major compound remains unidentified. The glucose/sucrose ratio in the leaves of Potamogeton species was found to change with the development of turions, which stored large quantities of sucrose in relation to any other ethanol-soluble carbohydrate. Paper chromatography and autoradiography

of C-14 labelled ethanol-soluble photosynthesate of Potamogeton perfoliatus indicated that, even after 36 hours of active photosynthesis, sucrose was the only isotopically labelled carbohydrate present in detectable quantities. Sucrose has also been identified as a ~~major~~ probable transport sugar in this species and is ~~possibly~~ excreted during photosynthesis.

Measurements have been made of diffuse attenuation coefficients of light (E') in water and of underwater spectral intensity (400 - 750 nm) in several lochs. E_e ranged from 0.55 in Loch Croispol to 2.9 in Loch Leven. Data on spectral intensity ($\mu w/cm^2/25$ nm) at one metre depth relative to subsurface, showed a proportional increase in short wave radiation in the blue-green Loch Croispol; the converse applied to Loch Ganagan. It has been calculated that the colour range in depths colonisable by macrophytes is equivalent to 48.2 to 55.6 Kcal/einstein. The depth zonation of several Potamogeton species was shown to be linked with their ability to adapt their leaf morphology to the "sun" and "shade" conditions of shallow and deep water while the depth limits of colonisation were related to the overall penetration of light; deepest colonisation being recorded in the clearest water.

Comparing Loch Croispol and Loch Leven there was nearly a six-fold difference in the depth of water which received the same total irradiance, whereas there was only some slight

difference in the depth which received the same number of quanta/sec/cm^2 due solely to differences in the spectral composition of light. Any effects of variations in light quality upon the distribution and productivity of aquatic macrophytes are thus ~~probably~~ thought to be slight when compared to the overall importance of light intensity.

Intrinsic low efficiencies could result in the observed low rates of primary productivity, however, in one experiment with Potamogeton obtusifolius in Loch Leven an efficiency of energy fixation of 11%, or 50% of the possible maximum, was recorded. Calculations from data collected in other experiments with P.perfoliatus and P.praelongus gave figures of between 6 and 9%. From a knowledge of other values of efficiency of energy fixation it is concluded that freshwater macrophytes are not unduly inefficient producers of organic matter but are, rather, ~~possibly~~ limited in their productivity by low, underwater, light intensities and by the availability of nutrients.

Enrichment bioassay experiments have indicated that the availability of phosphate in waters is ~~probably~~ limiting macrophyte production in several lochs. It is thought unlikely that phosphate is the only nutrient factor limiting growth and that such nutritional factors operate independently of each other, or of other environmental variables.

Using isotopic tracers of phosphate and sulphate and an

autoradiographic, technique the shoots and roots of several species of submerged macrophyte have been shown to be capable of nutrient absorption. Although, under the experimental conditions both acropetal and basipetal nutrient transport was observed no labelled nutrient absorbed by the shoots appeared in the roots. It is postulated that the roots and shoots of these plants ~~may be~~ are, to a large extent, self-reliant with regard to their nutritional requirements, although it has been shown, in one experiment, that phosphate absorbed by the roots can stimulate carbon fixation in phosphate-starved leaves. Translocated phosphate accumulated in the nodes of both rhizomes and shoots and in all actively growing areas. The rate of translocation was slow and is thought to be the result of the intrinsic inefficiency of all transport processes within these plants. The low transport rate of phosphate from roots to shoots could also, in part, result from an accumulation of polyphosphates.

Techniques for assessing the nutritional status of aquatic plants have been discussed. Tissue analysis of several specimens confirmed that phosphate is limiting productivity, while the hypothesis was suggested that the balance of growth between algae and macrophytes might lie with the availability of nitrogen and phosphorus in the water, and the differential critical levels of these nutrients required for growth. Algae are thought to be primarily limited by nitrogen, while macrophytes are ~~probably~~

more susceptible to phosphorus limitation.

Estimates of the activity of the enzyme alkaline phosphatase in several laboratory cultures have confirmed that this is a simple, yet useful, method for assessing the phosphate nutrition of aquatic plants. Results have confirmed the idea of the translocation of nutrients from the roots of aquatic plants, and illustrated the effect that the nutritional status of the roots may have on primary productivity. Measurements of enzyme activity carried out in conjunction with _in situ_ primary productivity estimates and enrichment bioassay experiments have given support to the hypothesis that the observed low productivity of aquatic macrophytes may, in part, be attributable to a shortage of available nutrients, among them phosphorus.

The investigations on which this thesis is based have succeeded in accumulating, what Westlake (1969b) terms, "basic data" concerning aquatic macrophytes and some of the conditions of their natural habitats. Although several hypotheses, based on these data, have been put forward to partly explain some of the determined distribution patterns and measured productivity values, the clarification of these postulates awaits further, carefully integrated field and laboratory studies.

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APPENDIX I

Calculation of the Results of the Primary Productivity Experiments

In the C-14 tracer experiments, calculations of the carbon fixation rate by detached leaves requires a knowledge of three factors.

(1) The uptake of radioactive carbon by the leaf.

(2) The total available radioactive carbon.

(3) The total available inorganic carbon in the loch water.

$$P = \frac{c \times g \times I}{C}$$

where P = primary productivity in µg C/leaf area.

c = cpm in leaf tissue.

g = C-14 added.

C = cpm in the loch water.

I = ratio of C-14 to inorganic carbon in the loch water.

In one experiment a leaf of Potamogeton praelongus of area 3.52 cm^2 had an absolute count rate of 2,21[illegible] cpm after incubation with C-14 for 3.3 hours, while a 5 ml aliquot of

the loch water precipitated with $Ba(OH)_2$ gave a count rate of 4,924 ± 9 cpm. The weight of the precipitate was 1.1 mg and the area 2.545 cm^2.

∴ with reference to the self-absorption curve (Figure 3.3) this count rate is 94.8% of the true activity and as the total volume of loch water was 24.5 ml then the total count rate of the loch water:-

$$= \frac{4,924 \pm 9 \times 100 \times 4.9}{94.8}$$

$$= 25,451 \pm 52 \text{ cpm}$$

As the tissue had a total count rate of 2,201 ± 36 cpm then the total radioactivity present:-

$$= 27,652 \pm \text{ cpm}$$

Now 0.2545 µg of C-14 were added, and as the total inorganic carbon content of the loch water was 0.33 mg, then the ratio of C-12 : C-14 = 1,296.66, thus the carbon fixed by the leaf; P

$$= \frac{2,201 \pm 36 \times 0.2545 \times 1,296.66}{27,652 \pm} \text{ µg C}$$

$$= \frac{726,235.20}{27,652 \pm}$$

= 26.27 µg C/3.52 cm^2/3.3 hours

= 2.26 µg C/cm^2/hour

The method of calculation of the total carbon in the loch water is as described on page 93.

It has been brought to my attention that the method of calculation I have adapted from Strickland (1960, p.83) and Vollenweider (1969, p.72) may be open to a degree of inestimable potential error. The method as used does not account for the fact that there is also some unlabelled, carrier carbon in the isotope samples as supplied from Amersham, this, however, is small and does not directly affect the present considerations. The ratio of C-12 to C-14 is, however, large and it is possible that the application of Amersham specifications in such cases, and where the samples of C-14 are diluted with what is probably slightly acidic distilled water, may be ill advised. Recalculation of the given example by the alternative method of utilising the specific activity of the loch water as directly estimated from the corrected count rate of the barium carbonate precipitate (25,451 cpm/330 µg C) and thus calculating the carbon fixed by the leaf by simple proportions $\frac{330 \times 2.201}{25,451}$ yields an answer of 2.46 µg C/cm^2/hour, a figure within 10% of that previously obtained. Bearing in mind the other errors inherent in the use of C-14 to estimate primary productivity it is felt that this discrepancy, though it may vary, is unlikely to influence the results to a significant degree for

them to be dismissed out of hand, but rather viewed with the caution already advocated.

APPENDIX 1A

The rationale for the table of conversion factors devised by Saunders *et al* (1962) to derive values of the total concentration of dissolved inorganic carbon in lake water from a knowledge of pH, alkalinity and temperature is as follows:-

The CO_2 dissolved in water is in equilibrium according to the equation:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^{=} \qquad (1)$$

When a total alkalinity titration is made to the methyl orange end point (pH 4.6) the carbonates and bicarbonates are converted to carbonic acid and free CO_2; as below:

$$CO_3^{=} + 2H^+ + HCO_3^- + H^+ \rightarrow 2H_2CO_3 \qquad (2)$$

It thus requires two acid equivalents for each mole of carbonate and one for each mole of bicarbonate. Therefore if c = the molar concentration of the carbonates and b = the molar concentration of the bicarbonates, it thus requires b + 2c molar equivalents of acid to reach the methyl orange end point when titrating one litre of solution. Standard alkalinity titrations are, however, generally carried out with 100 ml water samples and 0.02 N sulphuric acid. If t ml of acid are used in such a titration then:

$$0.02t = 100(b + 2c)$$

$$t = 5000(b + 2c) \qquad (3)$$

In the standard calculations the total alkalinity, expressed as ppm $CaCO_3$, is numerically determined by multiplying the acid titre by 10. If T = the total alkalinity then:

$$T = 10t$$

$$= 50,000(b + 2c) \qquad (4)$$

If a = the combined molar concentration of carbonic acid and free CO_2 and b and c are as defined previously then, from equation (1), the total molar concentration of carbon atoms = (a + b + c), since each radical has just one carbon atom per molecule. Thus if C = the total carbon present in the water sample titrated, expressed as mg/l, then, as there are 12,000 mg of carbon in one mole:

$$C = 12,000(a + b + c) \qquad (5)$$

The total alkalinity can now be related to the carbon content by dividing equation (5) by equation (4) so that:

$$\frac{C}{T} = \frac{12,000(a + b + c)}{50,000(b + 2c)}$$

or

$$C = 0.24T\frac{(a + b + c)}{(b + 2c)} \qquad (6)$$

Assuming in equation (1) that for fresh waters the activity coefficients are unity then the ionisation constant (K_1) for the dissociation:

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

can be written as:

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

while that (K_2) for the dissociation:

$$HCO_3^- \rightleftharpoons CO_3^{=} + H^+$$

can similarly be written as:

$$K_2 = \frac{[H^+][CO_3^{=}]}{[HCO_3^-]}$$

where, in both cases $[H^+]$ is the hydrogen ion concentration of the water.

The terms a, b and c can thus be substituted in equation (6) as:

$$K_1 = \frac{b\,[H^+]}{a} \qquad \text{and} \qquad K_2 = \frac{c\,[H^+]}{b}$$

or $$a = \frac{b\,[H^+]}{K_1} \qquad \text{and} \qquad c = \frac{b.K_2}{[H^+]}$$

thus $$C = 0.24T \frac{\frac{b\,[H^+]}{K_1} + b + \frac{b.K_2}{[H^+]}}{\frac{2b.K_2}{[H^+]^2} + b}$$

or $$C = T\left(\frac{0.24\,[H^+]^2 + 0.24K_1.[H^+] + 0.24K_1.K_2}{0.24K_1.[H^+] + 0.48K_1.K_2}\right) \qquad (9)$$

where the expression in brackets is known as the "conversion factor".

Since the equilibrium constants K_1 and K_2 are temperature dependent (Table A) the appropriate constants were substituted in equation (9) in compiling the values of the

conversion factors given in Table B.

In using this table the appropriate conversion factor for the pH and temperature of the water sample is read off and this multiplied by the total alkalinity value as ppm $CaCO_3$ (= 50 x meq/l) (Spence, 1967)) to obtain the value for the total carbon content expressed as mg/l.

Example. Let pH = 7.7, temperature = 15°C and total alkalinity = 68 ppm $CaCO_3$, then from Table B the conversion factor = 0.25 and hence the carbon content of the water = 0.25 x 68 = 17 mg/l.

TABLE A

Temperature dependence of the first (K_1) and second (K_2) ionisation constants of carbonic acid. $pK = -\log K$

Temperature °C	0	5	10	15	20	25
pK_1	6.58	6.52	6.46	6.42	6.38	6.35
pK_2	10.63	10.56	10.49	10.43	10.38	10.33

from Golterman, H.L. (1970), Methods for Chemical Analysis of Freshwaters. IBP, London.

TABLE B

Factors for the conversion of total alkalinity (ppm $CaCO_3$) to mgC/l using data on pH and temperature ($^{\circ}C$).

pH	Temperature $^{\circ}C$				
	0°	5°	10°	15°	20°
6.8	0.38	0.37	0.35	0.34	0.33
6.9	0.35	0.34	0.33	0.32	0.31
7.0	0.33	0.32	0.31	0.30	0.30
7.1	0.31	0.30	0.29	0.29	0.29
7.2	0.30	0.29	0.28	0.28	0.28
7.3	0.29	0.28	0.27	0.27	0.27
7.4	0.28	0.27	0.27	0.26	0.26
7.5	0.27	0.26	0.26	0.26	0.26
7.6	0.27	0.26	0.26	0.25	0.25
7.7	0.26	0.26	0.25	0.25	0.25
7.8	0.25	0.25	0.25	0.25	0.25
7.9	0.25	0.25	0.25	0.25	0.25
8.0	0.25	0.25	0.25	0.25	0.24
8.1	0.25	0.25	0.24	0.24	0.24
8.2	0.24	0.24	0.24	0.24	0.24
8.3	0.24	0.24	0.24	0.24	0.24
8.4	0.24	0.24	0.24	0.24	0.24
8.5	0.24	0.24	0.24	0.24	0.24
8.6	0.24	0.24	0.24	0.24	0.24
8.7	0.24	0.24	0.24	0.24	0.24
8.8	0.24	0.24	0.24	0.24	0.23

From Saunders *et al* (1962)

APPENDIX II

Plates

PLATE 1 A

(opposite)

Underwater photograph of *Chara* spp. beds in Loch Croispol. Depth 2 metres.

PLATE 1 B

(opposite)

Underwater photograph of *Potamogeton perfoliatus* in Loch Croispol. Depth 4 metres.

PLATE 2 A and PLATE 2 B

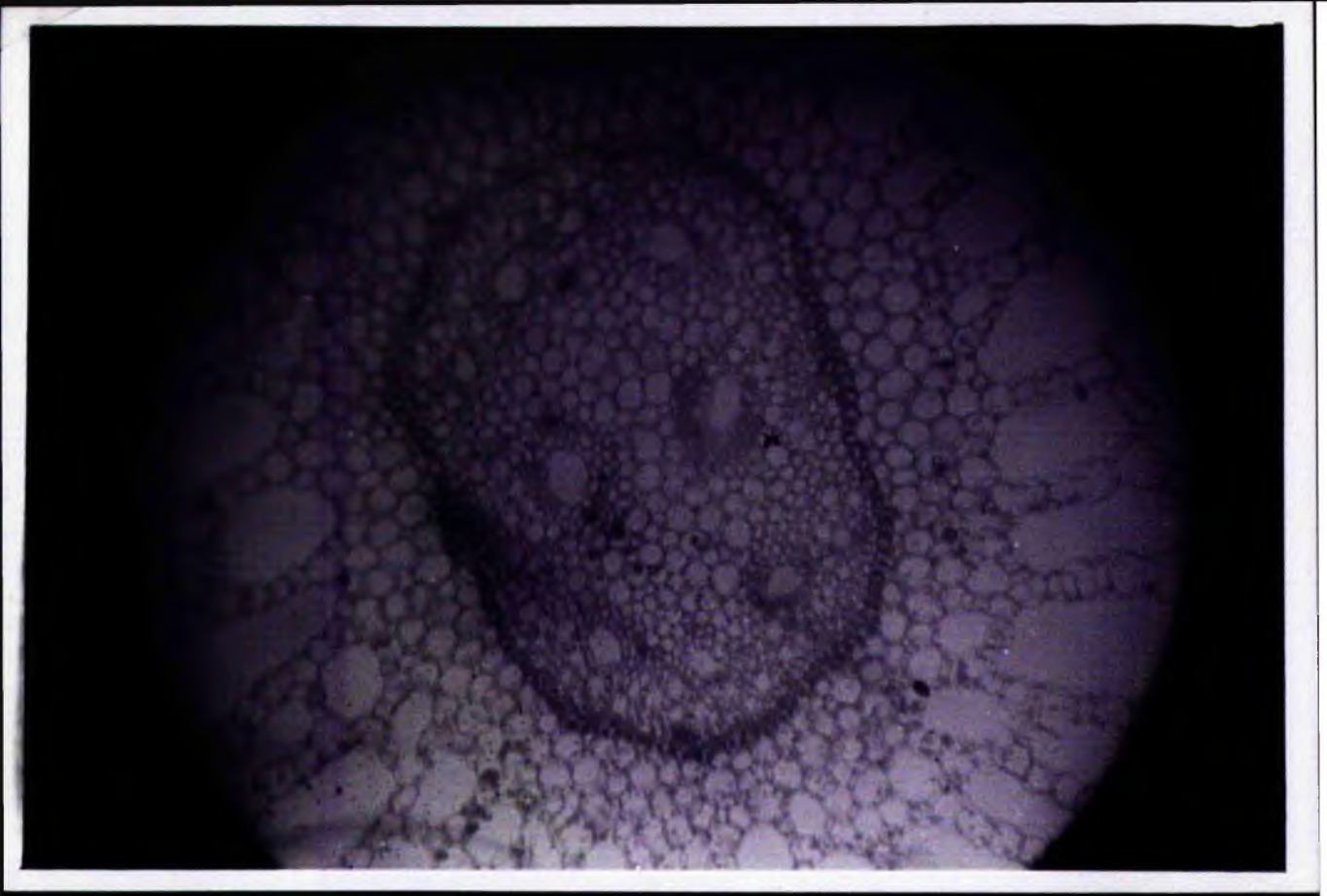
(opposite)

Underwater photographs of racks of incubating bottles, as used to study the *in situ* primary productivity of aquatic macrophytes.

PLATE 3 A and PLATE 3 B

(opposite)

Photomicrographs of a section through the rhizome of *Potamogeton perfoliatus*, cut by freezing microtome.



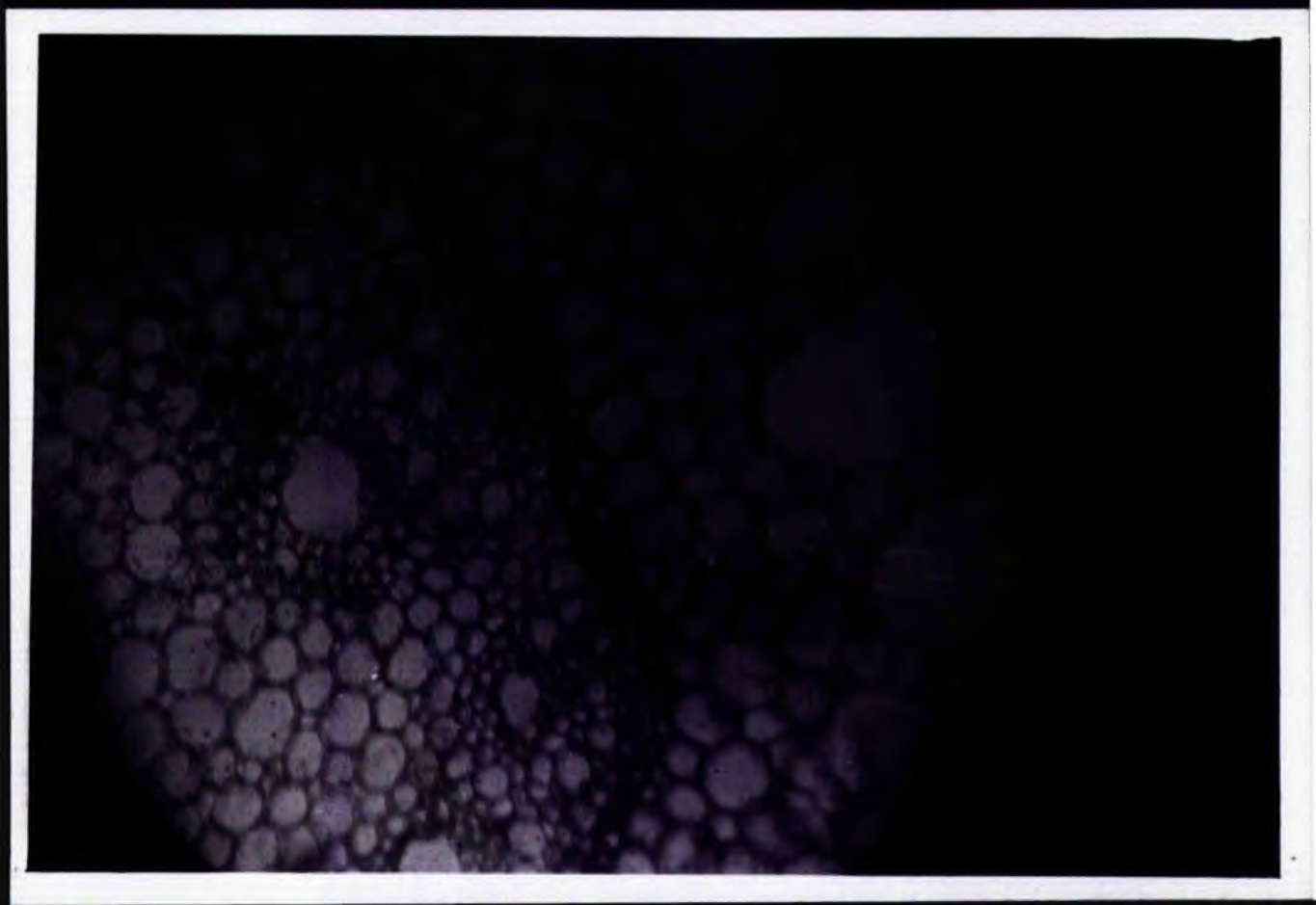


PLATE 4

(opposite)

Autoradiograph of a runner of Potamogeton perfoliatus after incubation for 6 hours in a nutrient solution containing P-32. Note the accumulation of radioactivity in the nodal regions and the growing points. The plant specimen is mounted on the left and the autoradiograph is on the right.

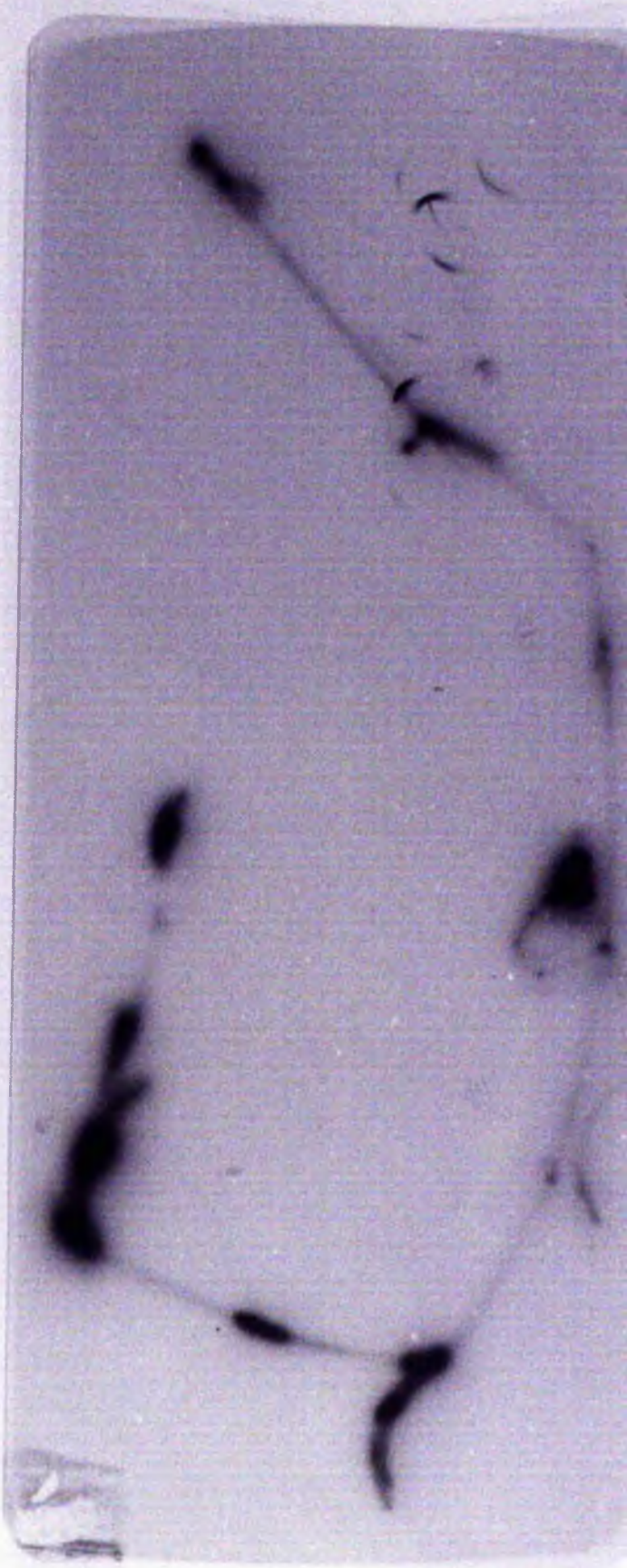


PLATE 5

(opposite)

Autoradiograph of specimens of Potamogeton obtusifolius which had been incubated for 12 hours with their roots in a nutrient solution containing P-32. Note the accumulation of radioactivity in the roots but no trace in the shoots. The plant specimens are mounted on the left and the autoradiograph is on the right.

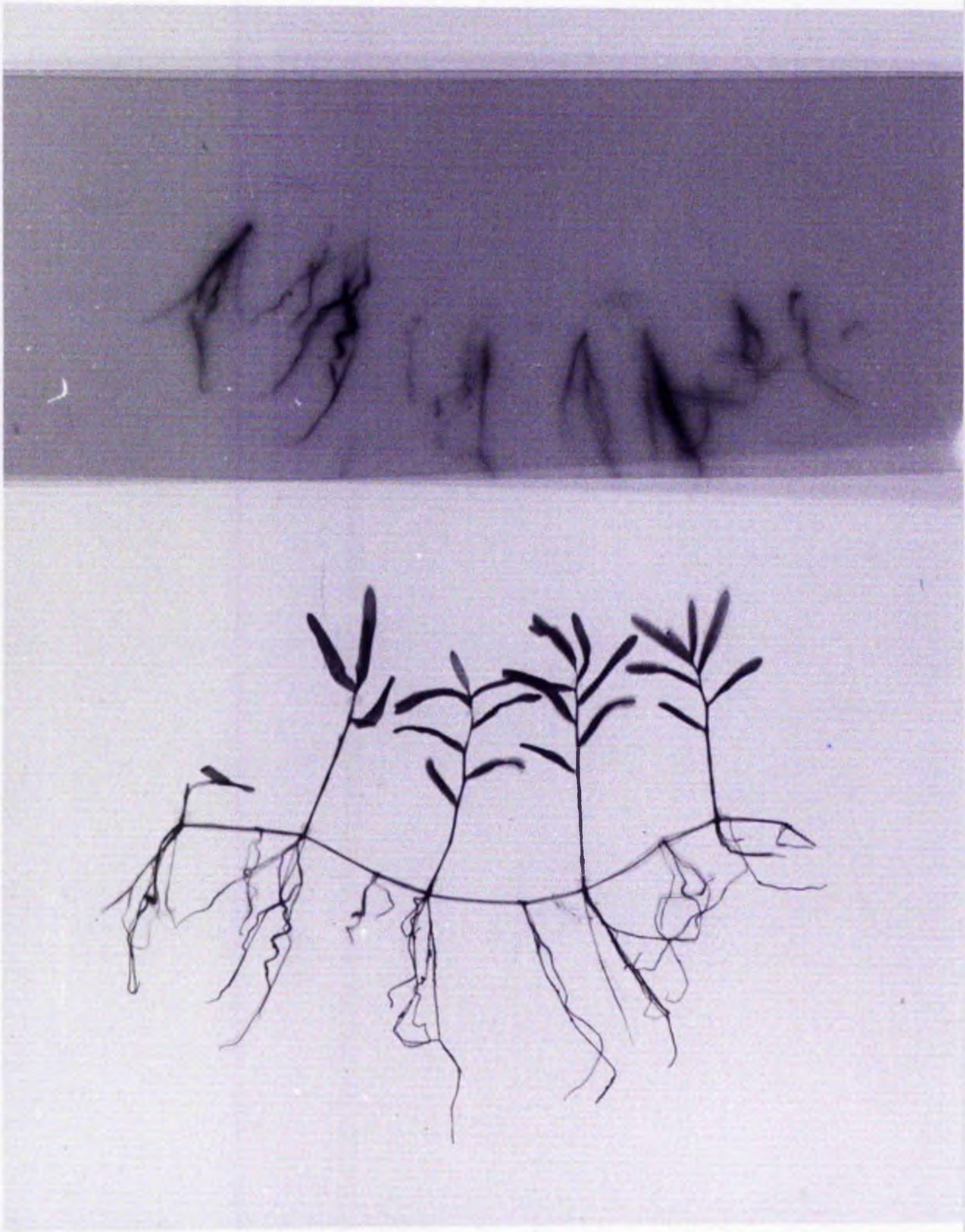


PLATE 6

(opposite)

Autoradiograph of a specimen of Potamogeton obtusifolius which had been incubated for 48 hours with its roots in a nutrient solution containing P-32 and its shoots in a separate phosphate-free solution. Note the accumulation of radioactivity in the roots and translocation into the shoots. The plant specimen is mounted on the left and the autoradiograph is on the right.

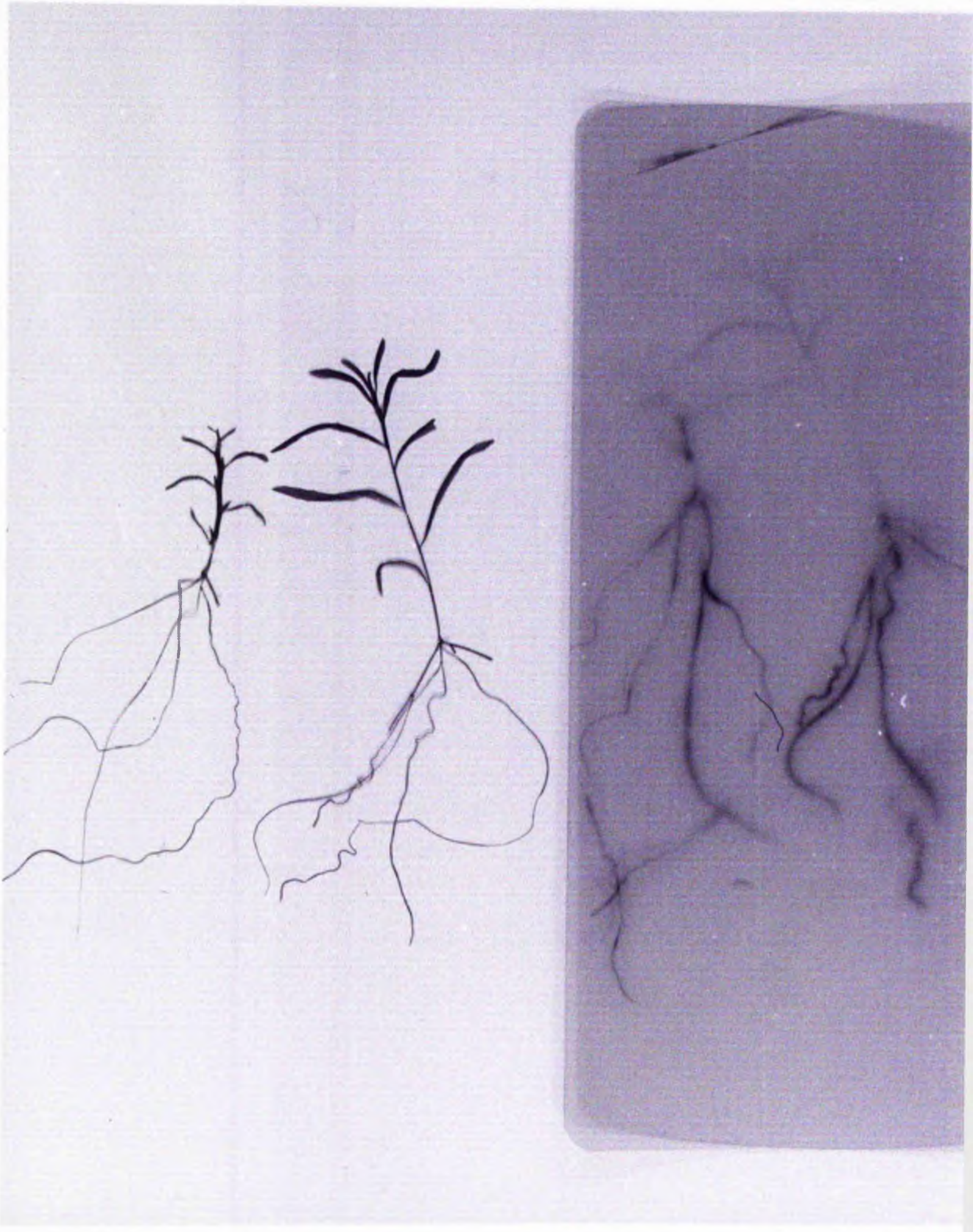


PLATE 7

(opposite)

Autoradiograph of a specimen of Potamogeton perfoliatus which had been incubated for 48 hours with its shoots in a nutrient solution containing P-32 and its roots in a separate phosphate-free solution. Note the movement of absorbed phosphate down the stem to the rhizome, accumulating in the nodes, but not appearing in the lower leaves or in the roots. The plant specimen is mounted on the left and the autoradiograph is on the right.

PLATE 8

(opposite)

Autoradiograph of a specimen of Potamogeton perfoliatus after the upper part of the shoot had been incubated in a nutrient solution containing C-14, for 6 hours in the light, while the rest of the plant was kept in a separate solution in the dark. Note the accumulation of C-14 activity in the apical leaves and the movement of carbon fixed in photosynthesis to other parts of the plant; even to young leaves which had been kept in the dark.