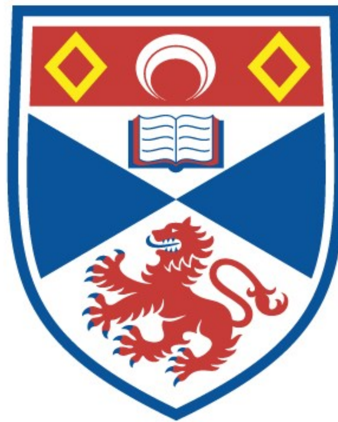
THE NATURE OF COMPETITION BETWEEN MACROPHYTES AND PHYTOPLANKTON IN FRESHWATERS

Stephen C. Maberly

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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by

Stephen C. Maberly

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

Department of Botany
University of St. Andrews

September 1981

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ABSTRACT

In field experiments designed to induce dense phytoplankton crops by phosphate and nitrate additions to enclosures in a bed of *Potamogeton filiformis* in Loch Fitty, the anticipated phytoplankton were not produced. Bioassays showed that phytoplankton were limited by phosphorus and nitrogen. No evidence for an allelopathic effect was found. Macrophyte uptake was responsible for removing 36% of the nitrate added, and the sediment responsible for a part of the phosphate uptake. Some phytoplankton uptake was inferred from the increased zooplankton numbers in enclosures receiving phosphate and nitrate. Nutrient additions had no effect on macrophyte standing crop, as predicted, because the sediment provided an adequate nutrient supply.

With decay of macrophytes and nutrient release, phytoplankton increased in certain enclosures, but not others, probably as a result of large increases in zooplankton numbers and hence grazing pressure. The filamentous alga *Rhizoclonium* became abundant at the end of the season in enclosures receiving phosphate and nitrate, but did not appear to harm the macrophytes. Epiphytes were only visibly obvious in one enclosure.

Failure to produce dense phytoplankton crops in the field led to a laboratory study of the effects of phytoplankton-induced carbon competition on macrophytes. Phytoplankton species were shown to have a smaller ~~total~~ resistance to CO_2 fixation than macrophytes and hence greater photosynthetic rates under most CO_2 concentrations. The boundary layer was the largest component of the total resistance in macrophytes,

suggesting that the thin leaves of many macrophytes were a response to this rather than an aid to diffusion. The linear leaves of other species could be adaptations to reduce the boundary layer thickness.

A pH-drift technique confirmed that the best phytoplankton species were more efficient at carbon removal than any macrophyte shoots. The macrophytes were even less efficient when the whole plant was considered. The carbon compensation point was shown to rise under the low light conditions that would be found under a dense phytoplankton crop. Macrophytes showed seasonal changes in carbon extractive ability, but the range was less than published data for phytoplankton from a lake, probably because the latter consists of a series of populations, which are closely adapted to the prevailing conditions. Different leaf types of heterophyllous macrophytes had different CO_2 compensation points and one leaf type could use HCO_3^-. A growth experiment confirmed that carbon competition with phytoplankton could have a detrimental effect on macrophytes.

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DECLARATION

I declare that this thesis is a record of my own work and that it has not been previously presented in application for a higher degree.

Stephen C. Maberly

St. Andrews, September 1981

CERTIFICATE

I certify that Stephen C. Maberly has spent 12 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.N. Spence,

St. Andrews, September 1981.

ACKNOWLEDGEMENTS

I should like to thank Professor D.H.N. Spence for his enthusiasm and encouragement over the last three years. Mr. J. Glowa gave much useful advice on water chemistry and helped on numerous field trips, while Miss Patricia Chambers kindly dived for me on several occasions and collected most of the macrophytes from L. Drumore.

I thank the owner and staff of L. Fitty for permitting enclosures to be placed in the lake and Mr. Porteous was always extremely helpful in providing boats.

Mr. R. Harriman of the Freshwater Fisheries Laboratory, Pitlochry allowed me to use some of his unpublished water-chemistry data for L. Fitty.

The receipt of a Natural Environment Research Council Studentship is greatly acknowledged.

When numbered pieces of toast and marmalade were dropped on various samples of carpet arranged in quality, from coir matting to the finest Kirman rugs, the marmalade-downwards-incidence ($\mu \delta$ I) varied indirectly with the quality of the carpet (Qc) - the Principle of the Graduated Hostility of Things.

Paul Jennings.

Battle within battle must be continually recurring with varying success; and yet in the long-run the forces are so nicely balanced that the face of nature remains for long periods of time uniform, though assuredly the merest trifle would give the victory to one organic being over another.

Charles Darwin.

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LIST OF SYMBOLS

SYMBOL	MEANING
CHL	chlorophyll
C_{chl}	CO_2 concentration in the chloroplasts
C_o	CO_2 concentration in the bulk solution
CO_2^*	free carbon dioxide (dissolved gas + H_2CO_3)
CP	compensation point
C_T	total carbon
D	diffusion coefficient of CO_2 in water
l	internal diffusive pathway
l/D	internal diffusive resistance
PFAD	photon flux area density (μmol m^{-2} s^{-1}) (Bell & Rose 1981) refering here to wavelengths 400-700 nm
Γ	CO_2 compensation point
δ	boundary layer thickness
ϕ net	net photosynthetic rate
1/k	chemical resistance
1/P	diffusive resistance
1/k + 1/P	total resistance
δ/D	boundary layer resistance

LOCATION OF COLLECTION SITES

Black Loch, Fife	(56° 20'N, 3° 12'W)
Loch Borralie, Sutherland	(58° 34'N, 4° 47'W)
Loch Caladail, Sutherland	(58° 34'N, 4° 46'W)
+Loch Croispol, Sutherland	(58° 34'N, 4° 47'W)
Loch Drumore, Perthshire	(56° 44'N, 3° 24'W)
Loch Fitty, Fife	(56° 9'N, 3° 12'W)
Loch Lindores, Fife	(56° 20'N, 3° 12'W)
Loch Kilconquhar, Fife	(56° 12'N, 3° 50'W)
Loch Long, Perthshire	(56° 32'N, 3° 9'W)
+Loch of Lowes, Perthshire	(56° 34'N, 3° 32'W)
Loch Na Craige, Perthshire	(56° 35'N, 4° 49'W)
Loch Na Uala, Sutherland	(58° 24'N, 5° 0'W)
St. Andrews, pond, Fife	(56° 20'N, 2° 47'W)
+Loch Uanagan, Inverness	(57° 7'N, 4° 42'W)
+White Loch, Wigtown	(54° 54'N, 4° 58'W)

+ collected by Black (1973) and data used in Chapter 4.

SPECIES USED IN PHOTOSYNTHETIC EXPERIMENTS

MACROPHYTES

Chara sp.

Elodea canadensis Michx.

Hippuris vulgaris L.

Littorella uniflora (L.) Ascherson

Myriophyllum spicatum L.

Nuphar lutea (L.) Sm.

Polygonum amphibium L.

Potamogeton crispus L.

P. filiformis Pers.

P. lucens L.

P. natans L.

P. perfoliatus L.

P. polygonifolius Pourr.

P. praelongus Wulfen

P. x zizii Roth

Ranunculus sp.

PHYTOPLANKTON

Anabaena cylindrica Lemmerman (C.C.A.P. 1403/2A)

Chlamydomonas reinhardtii Dangeard (C.C.A.P. 11/32C)

Cosmarium botrytis Meneghini (C.C.A.P. 612/5)

Scenedesmus quadricauda (Turpin) Brebisson (C.C.A.P. 276/4A)

CHAPTER 1

INTRODUCTION

Five major types of plants are responsible for the primary production of a lake: the submerged macrophytes, the phytoplankton, the attached communities consisting of epiphytic, epipelic, epipsammic and epilithic algae, filamentous algae which may become loosely attached to macrophytes and benthos at certain times, and pleustonic species within the water such as Ceratophyllum. While emergent macrophytes are commonly present in lakes, their photosynthetic activity occurs largely in the terrestrial environment as does that of floating pleustonic species such as Lemna and macrophytes with floating leaves. The case of the latter is complicated, as certain species of macrophyte produce both submerged and floating leaves on the same individual.

This thesis considers the interaction between two of the above groups of primary producers; the submerged macrophytes and the phytoplankton. The submerged macrophytes (henceforth called macrophytes) are multicellular plants with a complicated structure which normally grow below the water surface, and are rooted in the sediment and so are rhizophytes in the sense of Raven (1981). They include representatives from the Charophyceae (a class of green alga), Hepaticeae, Musci, Lycopodinae, Filicinae and Angiospermae. The Angiosperms are normally predominant (Spence 1981) and include monocotyledons e.g. Potamogeton and dicotyledons e.g. Ranunculus, Myriophyllum and Elodea. The phytoplankton are algae (in the broad sense

to include blue-green 'algae' or cyanobacteria) in suspension in the water, and may be unicellular, bicellular or colonial. They include representatives from the Cyanophyceae (or Myxophyceae), Chlorophyceae, Cryptomonadinaceae, Chrysophyceae, Bacillariophyceae, Dinophyceae and Euglenineae.

Table 1.1 compares some of the general features of macrophytes and phytoplankton. Of particular ecological significance is the fact that macrophytes can obtain their nutrients from the sediment and the water (see review by Denny 1980) while phytoplankton are restricted to one primary division of the biosphere, namely the water. Because of their short generation time, phytoplankton often undergo a temporal succession over a growing season, while the distribution of macrophytes is (in the short term) spatial, resulting in a zonation of species with water depth. Both types of distribution probably result in part from competition between different species within a group (and possibly in certain cases from competition between macrophytes and phytoplankton Spence 1981). Within-group competition is likely to be intense (Darwin 1859) but is unlikely to cause the absence of either macrophytes or phytoplankton as a group, but rather to determine which species flourish in a given situation.

Grime (1973) defines competition between plants as "the tendency of neighbouring plants to utilize the same quantum of light, ion of a mineral nutrient, molecule of water or volume of space"; to this may be added competition for a molecule of CO_2. The definition implies that competition only occurs when plants deplete a resource in their environment to the detriment of others. Competition for space *per se* is

unlikely to occur between macrophytes and phytoplankton as they inhabit different ecological niches. In an unproductive lake supporting small populations of macrophytes and phytoplankton, any form of competition between these two groups is unlikely.

In productive lakes with a large potential availability of nutrients in the water, dense phytoplankton crops could occur and it is under these conditions that macrophyte/phytoplankton competition may take place. If phytoplankton are favoured, macrophytes may be eliminated, or considerably reduced as a result. However, macrophytes may be excluded from, or uncommon in, a lake because of physical factors such as greatly fluctuating water levels (Quennerstedt 1958), extreme wave action in the photic zone (e.g. Jupp & Spence 1977b), unsuitable substrate, or turbid water resulting from abiotic factors such as silt; or some combination of these.

The following hypotheses were tested:

1) Under certain conditions competition can occur between macrophytes and phytoplankton in freshwaters. However, being limited to the macrophyte growing season, such competition is unlikely to exist as a result of early-season diatom blooms, and therefore to directly involve silicon depletion.

2) Instead, phytoplankton growth is assumed to be mainly limited over the macrophyte growing season by the availability of phosphorus and nitrogen in the water.

3) Because of their additional supply from the sediment, macrophytes are less likely to be limited by the availability of P & N in the water.

4) When high levels of P & N do exist in the water, sustained dense crops of phytoplankton are possible, and under

these circumstances macrophytes may become adversely affected by phytoplankton competition.

5) This competition takes the form of:

a) depletion of photosynthetic carbon

b) reduction of the PFAD[⊛] available to the macrophytes.

PLAN OF THESIS

Chapter 2 presents the results of an enclosure experiment in L. Fitty which attempted to induce high phytoplankton crops in a weedbed by the addition of phosphate and nitrate and to study the effects of carbon depletion and shading (5 a & b above) on the macrophytes. In the event, large phytoplankton crops were not produced until the macrophytes started to decay. The cause of the low crops was investigated, and the possible effects of allelopathy studied (Chapter 3).

The failure to produce dense phytoplankton crops in the field and the difficulties encountered in interpreting complicated multi-factorial field data led to a study of macrophyte/phytoplankton competition under controlled laboratory conditions. These results (Section B) concentrate on the photosynthetic attributes of the two types of plants, some of which are determined by the general characteristics of macrophytes and phytoplankton (e.g. size, Table 1.1). Chapter 4 compares the resistance to CO_2 fixation of macrophytes and phytoplankton, and the effect of the unstirred or boundary layer on this resistance is estimated. Chapter 5 compares CO_2^* and HCO_3^- CP's of a range of different species of macrophytes and phytoplankton using a pH-drift technique developed by Allen

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⊛ see list of symbols, page 1.

& Spence (1981). The combined effect on phytoplanktonic carbon depletion and shading on macrophyte photosynthesis was studied by investigating the effect of low PFAD's on CO_2^* and HCO_3^- CP's (Chapter 6). The possibility of seasonal changes in CO_2^* and HCO_3^- CP's are followed in Chapter 7. Chapter 8 presents results on CO_2^* CP's and HCO_3^- use in the different leaf types of various heterophyllous macrophytes, arising from this Department's interest in heterophylly. Finally, Chapter 9 presents the results of a competition experiment between macrophyte and phytoplankton species under conditions where inorganic carbon is continually renewed, or allowed to become depleted through photosynthetic uptake. This permitted the ideas based on physiological experiments in Chapters 4 & 5 to be tested.

A literature review of the relevant topic is given in each Chapter.

TABLE 1.1

Comparison of the relative characteristics of submerged macrophytes and phytoplankton.

Characteristic	Macrophyte	Phytoplankton
Evolutionary position	generally advanced	primitive
Structural organisation	complicated	simple
Size	large	small
Generation time	long	short
Within lake distribution	spatial (zonation)	temporal (succession)
Ecological position	sediments & water	water
Nutrient source	sediments & water	water

SECTION A

NITROGEN AND PHOSPHORUS COMPETITION, GRAZING AND ALLELOPATHY

INTRODUCTION

Section A is concerned with the factors which may prevent phytoplankton crops from developing. Chapter 2 presents the results of a field experiment in L. Fitty where phosphate and nitrate were added to enclosures in a weedbed to try to induce phytoplankton crops in order to study the effects of these on the macrophyte performance. No phytoplankton crops were produced however, and the work consequently changed emphasis to find out why.

Chapter 3 presents the results of an investigation into the possibility of a direct, non-competitive allelopathic effect of macrophytes on phytoplankton.

CHAPTER 2

ENCLOSURE EXPERIMENTS

2.1 INTRODUCTION

Reports at the turn of the century (West 1910) describe Loch Leven, Kinross as having clear water and a diverse macrophyte flora with nineteen species recorded, including eight species of Potamogeton. P. perfoliatus was particularly abundant, as was Elodea canadensis (=Anacharis alsinastrum) and the vegetation grew to a depth of 4.6m. Today, the macrophytes are scarce and ten species are known to have disappeared (Morgan 1970), while the phytoplankton have increased and large blooms of blue-green algae are commonly produced in the summer. The maximum colonisable depth is 1.5m (Jupp, Spence & Britton 1974). This change has been associated with artificial enrichment of the lake by agricultural fertilizers, sewage from the towns of Kinross, Milnathort, and Kinnesswood, and waste from a woollen mill in Kinross, (Holden & Caines 1974).

A nearby lake, L. Fitty, Fife also visited by West (1910) had a diverse and abundant macrophyte flora. Today the macrophytes are still abundant and phytoplankton crops are low. This lake is about 3 km west of Cowdenbeath, 3 km north of Dunfermline, and 18 km south of L. Leven. The lake is generally shallow (Table 2.1) and macrophytes colonise about half the area of the lake in summer. The water is brown in colour as indicated by the high attenuation coefficient for blue light (Table 2.1), because the inflow passes through an area of bog prior to discharge into the lake, and because of the high macrophyte crops which decompose each year.

Figure 2.1 gives an outline map of L. Fitty with approximate bathymetric contours, derived from a survey made in 1905 by James Murray (Murray & Pullar 1910). Water deeper than about 1m is dominated by P. perfoliatus which grows to a depth of about 2.2m. P. x zizii is also present in the deeper water, as is P. pectinatus. In water less than about 1m in depth, P. filiformis dominates, with P. crispus, P. pusillus, Callitriche sp., E. canadensis, Myriophyllum spicatum, Hippuris vulgaris and Chara sp. also present in small amounts. Figure 2.2 gives a depth biomass profile recorded at the time of maximum biomass (1.viii.79).

In the autumn of 1978, rotenone was used to kill all the fish in the lake. Restocking with brown trout (Salmo trutta) and rainbow trout (Salmo gairdneri) took place in the spring as the lake is used for angling. The fauna is diverse and abundant with many insect larvae, including the following orders: Ephemeroptera, Odonata, Plecoptera, Trichoptera and Diptera. Snails and Crustaceans are common in the littoral region. The bird life is not unusually extensive, mute swans (Cygnus olor) nest at L. Fitty, coots (Fulica atra) are fairly common, and the great crested grebe (Podiceps cristatus) is also present. Gulls are not usually seen in any numbers. General information on L. Fitty is shown in Table 2.1.

The aim of this research was to try to induce high phytoplankton crops in L. Fitty, similar to those found in L. Leven, by adding nutrients to small enclosures in a weedbed, and to study the effect of these phytoplankton crops on the macrophyte population, particularly with respect to shading and carbon depletion.

2.2 MATERIALS AND METHODS

2.2.1 Construction of enclosures

I built eight enclosures 180 cm square and 155 cm tall out of wood and polythene as shown in Figure 2.3. In the workshop at St. Andrews joints were cut and holes drilled in the wood to allow easy passage of the brass screws used to hold the enclosures together. The enclosures were constructed on the shores of L. Fitty by first assembling the wooden frames except the diagonal struts (Fig. 2.3). A single length of polythene (250 µm thick) was wrapped around the outside of each enclosure and carefully wound round the upper and lower bars before securing with staples. A length of polythene of about 90 cm was left over to allow a seal to be made between the two ends of the polythene. This was done by stapling the ends together, and then tightly wrapping the remaining length on itself before stapling to the frame. The side of the enclosure with the polythene join was always positioned so that it would not face the lake to minimise any damage and leakage. A strip of "Netlon" c. 90 cm wide was attached to the inside of the enclosure uprights on the side facing the open lake to protect the polythene from being ripped by waves (see photograph 1). The diagonal struts were attached last. When all eight enclosures had been built they were positioned in the lake at a pre-determined site in a bed of P. filiformis (see Fig. 2.1). Care was taken not to tread on the area to be enclosed. The enclosures were sunk into the substrate by gently 'tapping' with a sledge hammer until the top of the lower bar was below the sediment level. "Netlon" was wrapped around the entire block of enclosures to protect the polythene

from damage by boats and waves. Large stakes were driven into the sediment around the enclosures with the sledge hammer to provide anchorage. Finally a thin piece of wood was screwed to the upper half of the enclosures linking them together and securing the polythene further. Photograph 2 shows the completed enclosures.

2.2.2 Nutrient addition and treatments

Phosphate was added as $K_2\ HPO_4$ and nitrate as $NaNO_3$ at the ratio of 15N:1P which is similar to the proportions found in plant tissue A weighed amount of salts was dissolved in distilled water, made up to a known volume and divided equally into five volumes and placed into small plastic bottles the day before the additions were to be made. The solutions were scattered over the entire surface of an enclosure, and the bottle washed several times in the enclosure. Nutrients were added weekly over a thirteen week period from 31.v - 12.ix.79. Dates, amount added per m^{-2}, and concentration assuming a volume of 2.3 m^3 are given in Table 2.2. The higher nutrient addition rate if continued over a year would give a loading of 1.84 g m^{-2} yr^{-1} for PO_4-P, and 27.60 g m^{-2} yr^{-1} for NO_3-N, which is nine times the minimum amount of P and five times the amount of N which Vollenweider (1968) considers likely to cause eutrophication; and slightly greater than the loading to L. Leven (Holden & Caines 1974).

The enclosures were positioned in the lake on 9.i.79 and left for about two weeks before the first nutrient addition on 31.v.79. Perching by birds and deposition of guano on the sides and into the enclosures was minimal.

The following treatments were carried out:

Enclosure: I all macrophytes removed (30.v.79) and subsequently on regrowth

II $+PO_4$-P

III $+PO_4$-P, $+NO_3$-N

IV control

V $+PO_4$-P, $+NO_3$-N

VI $+PO_4$-P, $+NO_3$-N

VII $+PO_4$-P, $+NO_3$-N

VIII $+NO_3$-N

weedbed = further control.

It was originally intended to use the four $+PO_4$-P, $+NO_3$-N treatments for different purposes once dense phytoplankton crops had been produced. As these did not occur (see later) no further treatments (e.g. re-establishment of air-equilibrium) were appropriate.

2.2.3 Measurements

Fortnightly measurements were made in the eight enclosures, the weedbed and occasionally in open water in the centre of the lake.

a) Physical and chemical

i) Water temperature - using a mercury thermometer just below the water surface

ii) Water depth - with a meter rule in the centre of the enclosures. The remaining parameters were measured from a water sample in the laboratory taken from the subsurface in "Azlon" bottles of c. 1.25 l capacity. Samples were collected before the weekly nutrient addition. Bottles were washed out once in the water before filling, and lids were screwed on underwater to prevent trapping air.

iii) Conductivity - measured immediately on return to the

laboratory using a Lock meter (BC1), with a cell constant determined by calibration against a standard KCl solution. Conductivity readings were corrected to 25°C using the tables in Golterman, Clymo & Ohnstad (1978).

iv) pH - measured immediately on return to the laboratory using a Pye (290) meter and combined electrode calibrated against two buffers. Temperature correction was carried out using the meter control.

v) Air-equilibrium pH - pH measured as above after bubbling air through c. 100 cm^3 of water until a stable pH was reached.

vi) Alkalinity - phenolphthalein and total alkalinity determined following Golterman et al. (1978) by titration against standardised c. 0.02 mol l^{-1} H_2SO_4 to end points detected using phenolphthalein and methyl orange, and analysed in triplicate. Proportions of HCO_3^-, $CO_3^=$ and OH^- were calculated from Standard Methods (American Public Health Association 1971).

vii) NO_3-N determined on 0.45 µm millipore-filtered water using the U.V. method in Standard Methods (American Public Health Association 1971). Aluminium hydroxide was used to reduce interference by organic matter.

viii) PO_4-P - determined on 0.45 µm millipore-filtered water using reaction with molybdate, and reduction by ascorbate following Golterman et al. (1978). The hexanol extraction method was used to increase sensitivity.

b) Biological

i) Phytoplankton chlorophyll - following Golterman et al. (1978) using extraction in 90% acetone at 4°C in the dark for 24h. Absorbance read at 663 nm and 750 nm, the latter to correct for turbidity. Degradation products were not estimated.

ii) Phytoplankton types - 250 cm^3 of lake water was placed in a measuring cylinder and preserved and caused to settle by addition of c. 3 cm^3 of Lugol's iodine solution. After one week, the supernatant was siphoned off, and the process repeated in a 50 cm^3 measuring cylinder, before storage in labelled tubes. Phytoplankton were examined in a Lund counting chamber (Lund 1959, 1962).

iii) Zooplankton numbers and types - concentrated as above, counting under a binocular microscope, in a cylindrical perspex counting wheel.

iv) Macrophyte biomass - enclosures and the weedbed were sampled fortnightly, but on alternate weeks. Four quadrats were sampled per frame, one from each of the four quadrats of the enclosure (Fig. 2.4), on each sampling date. A sampling frame was made of "Dexicon" across which string was tightly tied to divide it into fifteen equal areas of 25 x 15 cm, (Fig. 2.4). The frame was placed over the quadrat by positioning one corner on a central post and lining up its edges with the sides of the enclosure. A quadrat of size 14 x 7 cm, so giving an area of 98 cm^2, was placed centrally within one of the sampling areas (Fig. 2.4) and kept in place with two pins which stuck into the substrate. Random number tables determined the position of the quadrat. Over the season, twenty eight quadrats were sampled from each enclosure which is equivalent to 9.1% of the total area of the enclosure, and 10.3% of the total macrophyte area (i.e. total frame area minus the standing area, see Fig. 2.4).

In the weedbed, a larger quadrat (10 x 25 cm) was used giving an area of 250 cm^2. The sampling position was determined by random tables and located by walking the

appropriate distance. Again, four samples were collected on each occasion.

A snorkel, mask and wet-suit were used to facilitate sampling. All the macrophytes rooted within a quadrat were removed. Only the above ground parts or standing crop were sampled as it would have been virtually impossible to remove all the below ground parts.

On return to the laboratory, the macrophytes were washed under a running tap, non-green material removed, and the shoots dried at 90°C for 24h before cooling in a desiccator and weighing.

Occasional measurements were made of the following:

i) Diffuse downwelling attenuation coefficients for red, green and blue light were made with matched F.B.A. selenium cells at the surface and at a range of depths (below 0.5m). Three broad band filters were used, Chance red OR, green OG, and blue OB.

ii) Sediment - a sample was taken near to the enclosures from c. 0.5m depth of water with a perspex sediment corer at the begining of the growing season (5.iii.81). An interstitial water sample was taken by placing a glass and perspex probe into the sediment and sucking. Coarse particles were trapped in glass-fibre in the probe and analysis was carried out on 0.45 μm millipore-filtered water. PO_4-P and NO_3-N were analysed as described in the previous section (excluding the extraction step for PO_4-P). NH_4-N was also analysed by the phenol-hypochlorite method of Solorzano (1969). All analyses were triplicated. pH was measured with a calibrated combination electrode carefully placed directly in the sediment. Percent water content was calculated from loss in weight of sediment

samples after drying at 90°C for 24h. A measure of the organic content was gained by measuring the loss of weight on ignition of oven dried samples in a furnace at 550°C for 4.5h.

iii) Macrophyte biomass changes with depth at time of maximum biomass - sampled on 1.vii.79 offshore from the enclosures. Duplicate samples were taken at 0.5, 1.0, 1.5, 2.0m and one sample at 2.2m, the approximate limit for macrophyte colonisation. Roots and shoots were removed from the area within a cylindrical metal corer which was lowered into position from buoys at the surface. A net bag placed over the top of the corer prevented loss of any material. The corer had a diameter of 39.5 cm and so enclosed an area of 0.113 m^2. The plants were placed in labelled plastic bags and then in the laboratory they were washed, separated into roots, and shoots of individual species, dried at 90°C for 48h and weighed after cooling in a desiccator.

iv) Macrophyte tissue content - dried samples were digested in H_2SO_4 according to the technique in Golterman et al. (1978) and phosphorus analysed as described for the water (excluding the extraction step) and NH_4-N as described for the sediment interstitial water.

2.2.4 Nutrient bioassay

Lake water was taken and used in a bioassay to test if a nutrient was limiting the phytoplankton standing crop. Either 75 cm^3 in 100 cm^3 conical flasks, or 200 cm^3 in 250 cm^3 conical flasks of homogenised lake water was used. The flasks were plugged with cotton wool and shaken either in a water bath at 18°C or on an orbital shaker at room temperature. A 16h light : 8h dark photoperiod was used with a PFAD of 190

μmol m^{-2} s^{-1}. Position effects were minimised by moving the flasks every day. The experiment was run until good growth had occurred in some of the flasks, usually about fourteen days. Chlorophyll concentration of initial and final treatments were estimated by extraction with 90% acetone as in 2.2.3.

PO_4-P was added as K_2HPO_4, NO_3-N as $NaNO_3$, iron as ferric citrate and citric acid, and carbon as $NaHCO_3$. Nutrients were added as 1-5 cm^3 of concentrated stock.

The treatments used were:

Control	no additions
+P	PO_4-P 50 μg l^{-1}
+N	NO_3-N 1-1.3 mg l^{-1}
+Fe	Fe citrate 3 mg l^{-1}, citric acid 3 mg l^{-1}
+C	air bubbled initially, +$NaHCO_3$ 2 mmol l^{-1}
-zooplankton	filtering through six layers of muslin
+P+N	as for single additions
+P+N -zooplankton	" " " "
+P+N+Fe+C	" " " "

Additions of K^+ or Na^+ as chlorides, in the same concentration as in the other treatments were tested on one occasion, and had no effect on the final chlorophyll concentration.

2.2.5 Sediment uptake of phosphorus

On 19.ix.79, two sediment cores were taken in shallow water (30 cm) near to the enclosures, using a perspex corer of internal diameter of 8 cm and hence area of 50.3 cm^2. The bottom of the tube was pushed into the sediment, the top tightly stoppered with a rubber bung, and the tube carefully

removed from the sediment, a rubber bung being placed in the bottom as soon as possible. This enabled fairly undisturbed sediment cores to be obtained.

On return to the laboratory, the lake water was carefully siphoned off and a disc of polythene placed over the sediment surface to minimise disturbance. The lake water was replaced by 2 l of K_2HPO_4 solution added slowly via a thin polythene tube. The polythene disc was carefully removed, and the tubes then placed in a light-free box to discourage algal growth.

A control was run using the PO_4-P solution but no sediment. Both control and experimental tubes were duplicated. The initial PO_4-P was c. 80 µg l^{-1}, and 20 cm^3 samples were removed periodically for phosphate analysis as for the water chemistry, but without an extraction step.

2.3 RESULTS

2.3.1 Changes in environmental parameters

The changes in water depth, water temperature, conductivity and air-equilibrium pH were similar for all enclosures and so are shown as an average for a particular sampling date (Fig. 2.5). The weedbed was often slightly warmer than the enclosures, possibly because it had access to the warm shallow water. The open water ($>$ 2m) was generally colder than either the weedbed or the enclosures.

Figure 2.6 shows changes in total alkalinity, and the components of this, HCO_3^-, $CO_3^{=}$, and OH^-, expressed as mg l^{-1} of $CaCO_3$. Enclosures with macrophytes showed a marked mid-summer reduction in total alkalinity, presumably as a result of precipitation of $CaCO_3$ caused by elevated pH values (see Fig. 2.7), as a result of macrophyte photosynthesis. In

confirmation, a white precipitate was visible on the leaves of P. filiformis and very noticeable on the adaxial surface of the leaves of P. perfoliatus, a species which was rarely present in the enclosures but dominant in the deeper water of the weedbed. Enclosure I with macrophytes removed had a slight reduction in alkalinity, but less than the other enclosures. Only a slight summer decline in alkalinity occurred in the weedbed, even though a carbonate precipitate was visible on the macrophyte leaves. Replenishment from undepleted open water may have caused this.

More generally marked than the reduction in alkalinity was the reduction in HCO_3^-, a photosynthetic carbon source for P. filiformis, and P. perfoliatus (also P. x zizii broad leaves, Myriophyllum spicatum, and Elodea canadensis, all present in small amounts in L. Fitty; see Chapter 5). This decline was caused in part by the reduction in total alkalinity, but mainly by the increase in pH as a result of macrophyte photosynthesis, causing $CO_3^=$ to predominate. In enclosure III (+P+N), the HCO_3^- was depleted virtually to zero and OH^- became an important part of the alkalinity. This enclosure had a pH of 10.45 at the height of the summer (Fig. 2.7). In the enclosure with macrophytes removed, only a slight reduction in HCO_3^- occurred.

The data presented in Figure 2.7 can be divided into two periods. The first extended from the begining of June to the begining of August, when macrophyte biomass was increasing, causing an increase in pH as a result of carbon uptake. During this period, nitrate declined to low levels as did phytoplankton chlorophyll. The nutrient additions had disappeared by a week later. The enclosure with macrophytes

removed had lower pH values than the others, but showed a similar decline in NO_3-N and phytoplankton chlorophyll.

In the second period from mid-August to late September, the macrophytes declined, as did the pH. The nitrate concentration began to rise in most of the enclosures probably as a result of release from the decaying macrophytes. The heavy rainfall during this period (causing an increase in water level, Fig. 2.5) may also have caused some input of NO_3-N. In certain enclosures, (III and V particularly) large pulses of NO_3-N of between 2-3 mg l^{-1} were found after the macrophyte decline began (Fig. 2.7). PO_4-P remained at below 10 µg l^{-1} throughout the experiment, apart from one peak at 19 µg l^{-1} in enclosure VII (+P+N). During this period of macrophyte decline, phytoplankton chlorophyll increased to some extent in all the enclosures, and particularly in enclosure VIII (+N) with a maximum of 41 µg l^{-1}, II (+P) with a maximum of 37 µg l^{-1}, I (- macro) and III (+P+N), both with a maximum of 33 µg l^{-1}. In most enclosures a slight increase in pH occurred in early September, probably as a result of phytoplankton photosynthesis. The major phytoplankton in these late summer maxima was the colonial blue-green alga Gloeotrichia echinulata P. (Richt.) (see Table 2.3). The exception was enclosure III (+P+N) where large amounts of cryptomonads occurred, largely Cryptomonas sp and Rhodomonas sp. In this enclosure, a dense epiphyte population developed on the macrophytes; no epiphytes were visibly obvious in any other enclosure.

The filamentous alga Rhizoclonium hieroglyphicum (Agardh) developed in some of the enclosures to form floating mats during the period of macrophyte decline (Table 2.4).

It first appeared in late August well after macrophyte growth had ceased, and was at a maximum in early September. Enclosures receiving +P+N had the largest amounts, estimated on a percentage cover basis (Table 2.6) the enclosure with no macrophytes had none. The weedbed also contained some <u>Rhizoclonium</u> but this did not form floating mats, possibly because of the greater exposure.

Zooplankton (Fig. 2.7) were scarce in the enclosure without macrophytes, and relatively scarce in the control enclosure and the weedbed. Slightly greater numbers were found in enclosure II (+P), and considerably greater in enclosure VIII (+N). Three of the four enclosures with P+N additions (V, VI, VII, but not III) had high zooplankton crops, with a maximum in enclosure VIII of 884 l^{-1}. Again enclosure III was the exception, where zooplankton were low and large phytoplankton crops developed. Significantly greater numbers of zooplankton were found in enclosures with added nitrogen (+N and +P+N) (Table 2.6).

Macrophyte biomass appeared unaffected by the addition of P+N, either singly or in combination, presumably because of their ability to obtain nutrients from the sediment, (Table 2.6). Results in 2.3.4 show that the P content at about 3.1% in control and enriched enclosures was well above the critical level of 0.13% suggested by Gerloff & Krombholz (1966). The N content was lower at about 2.5% in an enriched enclosure, and only 1.2% in the control. Gerloff & Krombholz (1966) suggest that N is limiting when less than 1.3% of the dry weight, so N may have been limiting in the control enclosure. As the dry weight of the plants may have included a proportion of marl, the % N on an ash-free dry weight basis was probably

greater than the critical value. Also, the plants were sampled at the end of the growing season in mid-summer when plant nutrient levels are often at their lowest (Gerloff & Krombholz 1966, Boyd 1969, Nichols & Keeney 1976a). Self-induced carbon depletion was probably more likely to limit their biomass in this lake in shallow water.

An attempt was made to gain a better understanding of what was occurring in the enclosures by averaging the results from all the enclosures. Figure 2.8 shows the average change in various important parameters over the macrophyte growing season. During the period of increase in macrophyte standing crop, the phytoplankton declined, as did the NO_3-N and HCO_3^-. As the macrophytes stopped growing the NO_3-N and HCO_3^- concentrations increased, as did the phytoplankton, then by late August a reduction in NO_3-N and HCO_3^- concentration occurred at the time of phytoplankton maximum. Zooplankton changed little until the end of the phytoplankton peak, when they increased, and then decreased sharply when the phytoplankton declined in late September. The change in PO_4-P changed little over the season (not shown).

2.3.2 Analysis of events

A more critical analysis was attempted by plotting two variables against each other. If they increased and declined together a line of positive slope was obtained (Fig. 2.9); while if one declined as the other increased or vice versa, a line of negative slope was produced (Fig. 2.9). This analysis also allowed the determination of which variable changed first, enabling some clues to be gained as to cause and effect.

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Figure 2.10 gives plots of phytoplankton chlorophyll against macrophyte standing crop. The general slope is negative, indicating interaction so that high macrophyte crops occur when phytoplankton crops are low and vice versa, while the direction of change indicates that the macrophytes changed first. (This is not true for the weedbed, but here samples for macrophyte standing crops were not taken on the same day as the water samples, so comparisons are probably not valid).

Figure 2.11 shows plots of changes in the concentration of HCO_3^-, PO_4-P, NO_3-N and zooplankton numbers against phytoplankton chlorophyll or macrophyte standing crop. Zooplankton changes do not appear to be correlated with either macrophytes or phytoplankton, on an average basis. Macrophyte increase is correlated with decrease in HCO_3^- and NO_3-N, and from the direction of change slightly precedes them, suggesting that macrophyte uptake is responsible for the decrease of these nutrients. Decrease in phytoplankton crops occur with, but just after, decrease in NO_3-N and HCO_3^-, suggesting that this may be the cause of their reduction. Changes in concentration of PO_4-P do not appear to correlate with phytoplankton chlorophyll, but macrophyte standing crop does, with high PO_4-P concentrations occurring with high macrophyte standing crops. The direction of change indicates that PO_4-P changes slightly before the macrophytes. The reason for this is unclear, but may be a result of an increasing NO_3-N shortage.

2.3.3 Bioassays

The preceding analyses indicate that HCO_3^- and/or NO_3-N could be the cause of the marked phytoplankton decline.

Bioassays of the natural phytoplankton were undertaken to determine if nutrient deficiency was the cause, rather than a result of zooplankton grazing, a toxicity from allelopathic compounds secreted by the macrophytes, or from some other toxicity, e.g. heavy metals. Figure 2.12 shows that both P & N were limiting to weedbed phytoplankton populations on two occasions, since neither P nor N alone produced an increase in standing crop. Addition of Fe or C had no effect, while zooplankton removal increased phytoplankton crops in July, but not in August; the difference possibly lying in the slightly lower zooplankton density on the latter date (Fig. 2.7). On 24.viii.79, a bioassay was carried out on phytoplankton from all the enclosures, the weedbed and the open water. It showed (Fig. 2.13) that addition of P & N either singly or in combination always produced a large phytoplankton crop. An allelopathic effect is unlikely, unless it is operating by removing P or N. Phytoplankton from enclosure II (+P) were N-limited, while phytoplankton from enclosure VIII (+N) were P-limited. Apart from this, there did not appear to be any pattern as to which nutrient was limiting. The Cryptomonads of enclosure III were strongly P-limited, as they showed a massive increase in chlorophyll on PO_4-P addition, much larger than any of the other treatments. It is probable that different species react in different ways to nutrient additions, as suggested by Jordan & Bender (1973).

2.3.4 Cause of nutrient loss

The PO_4-P and NO_3-N added each week was not detected the following week when the water was analysed. Several causes of loss are possible:

i) Leakage; this is unlikely to have caused all of the loss

as other water chemistry data (Fig. 2.6, and 2.7) suggest that each enclosure was different from each other and from the weedbed.

ii) Sediment uptake; uptake of PO_4-P by the sediment was tested on an undisturbed sediment core in the laboratory. The uptake rate was 9 mg m^{-2} wk^{-1} for the first 50 hours, and about 5 mg m^{-2} wk^{-1} for the rest of the week (Fig. 2.14). This gives an average of about 6.7 mg m^{-2} wk^{-1} for the week which is less than the 35 mg m^{-2} wk^{-1} at which PO_4-P was added to the enclosures for most of the experiment. The sediment uptake represents 14-25% of that added, with an average of 20% over the week.

iii) Phytoplankton uptake; some phytoplanktonic uptake of P & N is likely, particularly during the period of macrophyte decline when increased phytoplankton crops occurred. The increased zooplankton numbers in response to nutrient addition suggests an increased phytoplankton production even though the standing crop remained low, which would indicate that some nutrient uptake did occur.

iv) Rhizoclonium uptake; this would only be important near the end of the experiment, but could account for uptake of the nutrients released by the decaying macrophytes.

v) Co-precipitation; Otsuki & Wetzel (1972) have shown phosphate to co-precipitate with $CaCO_3$, and this could have been an important loss of PO_4-P from the water, as precipitation of marl was considerable.

vi) Macrophyte uptake; this could be a major source of loss for both PO_4-P and NO_3-N. Duplicate analyses were made of oven dried material collected on 25.vii.79 when biomass was near maximal. In the control enclosure (IV) the P content was

31.9 mg P g dw^{-1}, similar to 30.8 mg P g dw^{-1} for macrophytes from enclosure VI (+P+N). However, there were differences in the nitrogen content as 12.0 mg N g dw^{-1} for the control, and 24.5 N g dw^{-1} for the +P+N enclosure. At this time there was an above ground crop of 73.86 g dw m^{-2} in enclosure VI, representing an accumulation of 0.92 g m^{-2}, or 2.67 g N for the enclosure. Up to this period, a total of 7.35 g N had been added to the enclosure, so that macrophyte uptake represents 36% of the amount added.

2.3.5 Comparison of phytoplankton and zooplankton in the weedbed and open water.

On occasions when the open water was studied, it contained generally higher amounts of phytoplankton chlorophyll and smaller numbers of zooplankton than did the weedbed (Fig. 2.15).

2.4 DISCUSSION

2.4.1 Factors responsible for the low phytoplankton crops in early summer.

The nutrient additions to the enclosures failed to produce the large phytoplankton crops that were anticipated, probably as a result of several causes. First, the enclosures were placed in an area of L. Fitty where dense beds of P. filiformis grew. In the spring, rapid growth of P. filiformis shoots from underground turions caused NO_3-N to be removed and inorganic carbon to be depleted. The water was shallow (c. 0.7m) and so once the shoots had reached the surface, shading of any phytoplankton present could also occur. Several workers have found a reduction in phytoplankton biomass and/or production in stands of emergent macrophytes such as Phragmites, or Glyceria which they attributed to

shading, as nutrients are taken up by the roots, and CO_2 is taken up by the shoots above the water (Dokulil 1973, 1975, Dvorak 1970, Straskraba & Pieczynska 1970). Dense beds of submerged macrophytes have also been shown to reduce phytoplankton production. This has been attributed to shading (Postolkova 1967), shading with some carbon depletion (Brandl, Brandlova & Postolkova 1970), and combined effects of shading, carbon depletion and nutrient depletion (Goulder 1969). Brammer (1979) found smaller amounts of phytoplankton chlorophyll in a dense bed of Stratiotes aloides than in open water, and the decrease in chlorophyll was correlated with decreases in conductivity, alkalinity, and concentrations of calcium, potassium and sodium; PO_4-P was also low. Brammer concluded that the observed decline in phytoplankton was a result of changes in the ionic composition of the water and competition for nutrients, although unfortunately no tests such as bioassays were made. Marshall (1947) found that fertilisation of a sea loch in spring failed to produce a phytoplankton bloom; he attributed this to nutrient uptake by benthic seaweeds which showed a resulting increase in growth. Boyd (1971) suggested that macrophytes gain an advantage over phytoplankton by nutrient depletion of the water by luxury consumption of nutrients early in the growing season.

Nutrient depletion was probably the main cause of the low phytoplankton crops in the enclosures and the weedbed. The reason for the low phytoplankton crops in the macrophyte-removed enclosure (I) is unclear, but decreased macrophyte shading could have allowed benthic algae to develop to a greater extent than in the other enclosures, and these could have been responsible for some uptake of nutrients from the

water. With hindsight, an enclosure with macrophytes removed and +P+N additions would have been useful. Bioassays of all field treatments indicated that the phytoplankton were limited by both P & N, and no evidence for an allelopathic effect was observed (see Chapter 3). Analysis of the macrophyte tissue showed that shoot uptake was responsible for a significant part of the N lost from the water, but possibly not the P. Co-precipitation with $CaCO_3$ and sediment uptake could have caused some of the loss of P. This agrees with most of the literature which suggests that macrophytes gain most of their P from the sediment (Barko & Smart 1979, 1981; Bole & Allen 1978; Carignan & Kalff 1979, 1980) although other workers have found shoot uptake to also be important (Best & Mantai 1978; Peverly & Brittain 1978). The nitrogen supply is believed to come from both the water and the sediment (Nichols & Keeney 1976b). The greater N content of macrophytes from an enclosure with +P+N and the order-of-event analysis fits in with this. An experiment in the laboratory (Chapter 9) suggested that competition between macrophytes and phytoplankton for N was important.

The rapid photosynthetic removal of inorganic carbon by the macrophytes (Fig. 2.6) may also have had a detrimental effect on the phytoplankton growth. Even though phytoplankton are often more efficient users of carbon than macrophytes (Chapters 4 & 5), photosynthetic rates will be depressed at the low inorganic carbon levels caused by macrophyte photosynthesis. The bioassays indicated that C was not limiting the phytoplankton crop, but it could still limit the rate of production of the biomass (O'Brien 1972).

The zooplankton populations were probably partly responsible for the low phytoplankton crops early in the season in all the enclosures and the weedbed. Zooplankton numbers increased in treatments with added N or P & N (Table 2.6) (except enclosure III) indicating an increased phytoplankton productivity as a result of nutrient addition, but not an increased crop because of grazing pressure. The dominant zooplankton were mainly cladocerans (Table 2.5) which are believed to be efficient phytoplankton grazers (Porter 1977). Cyclopoid copepods, many of which eat other zooplankton, were generally low, apart from an early outburst on 22.vi.79 (Table 2.5). O'Brien & DeNoyelles (1972) have suggested that high pH, resulting from photosynthetic carbon uptake, may result in zooplankton mortality. Zooplankton numbers were generally high in the enclosures, even though the pH was greater than 10.0 in most of the enclosures for several weeks. However, of the four enclosures receiving +P+N only one (III) had low zooplankton numbers (Fig. 2.7) and this enclosure had the highest pH (10.45) of any of the enclosures. Rotifers declined as the macrophytes developed (Table 2.5) but it is noticeable that their decline was less marked in the enclosure with the macrophytes removed (I). This may have been because the pH changes were less great (Fig. 2.7). Hasler & Jones (1949) found that dense beds of Elodea in small ponds suppressed rotifers but not crustacean zooplankton; a pH effect may explain this observation.

The relationship between herbivorous zooplankton and phytoplankton has been established by a number of workers. Postolkova (1967) found that the removal of fish predation caused an increase in zooplankton numbers and reduced

phytoplankton production. In pond enrichment experiments, Losos & Hetesa (1973) found that in ponds without fish fry, phytoplankton crops were low and Daphnia was dominant. In those ponds with fish fry, Daphnia numbers were suppressed, carnivorous copepods increased and the phytoplankton productivity and biomass increased greatly. Figure 2.15 suggests that higher phytoplankton crops occur in the open water compared to the weedbed as a result of lower zooplankton numbers. This could be caused by greater predation of the zooplankton by fish in the open water. The weedbed was very dense, particularly in the shallow P. filiformis bed, and fish may not have gained easy access. Also, zooplankton would be less visible here than in the open water. This agrees with Northcott (1979) who found a greater density and biomass of zooplankton in a dense bed of Elodea canadensis compared to the open water in a gravel pit containing roach and perch. Mitchell & Wetzel (1980) in bioassay tests, found grazing by Daphnia to reduce winter phytoplankton productivity. The bioassay carried out on L. Fitty weedbed phytoplankton on 27.vii.79 showed zooplankton removal to be beneficial once high crops were produced by adding P & N.

Leah, Moss & Forrest (1980) found that zooplankton (largely Cladocerans) increased, the phytoplankton decreased, and the submerged macrophytes increased in a small broad where a reduction in fish numbers had occurred probably as a result of heavy predation. Nilssen (1978) and Shapiro (1980) suggest a similar link between fish, zooplankton and phytoplankton. All three papers point out that increased grazing pressure by zooplankton will favour less edible or inedible forms such as large colonial phytoplankton, many of which are

blue-greens. In the enclosures in L. Fitty, the late summer phytoplankton bloom was largely made up of Gloeotrichia, a large colonial blue-green alga.

In L. Leven, Daphnia were abundant in 1890 and the zooplankton were rich. Daphnia were also present in 1954 but by 1966 they were extinct in L. Leven and a carnivorous copepod was dominant (Refs. Morgan 1970). Between 1958 and 1964, L. Leven was polluted with the insecticide dieldrin (Morgan 1970, Holden & Caines 1974). Cladocerans are known to be extremely sensitive to insecticides (Refs. in Shapiro 1980), and this could have caused the extinction of Daphnia (Morgan 1970). The removal of this grazer could have allowed the phytoplankton to increase causing a reduction in the submerged macrophytes.

2.4.2 Factors responsible for the loss of nutrients

NO_3-N and PO_4-P concentrations had returned to control levels a week after additions had been made. Similar rapid reductions from fertilised lakes have been reported by Brook & Holden (1957), Smith (1969), Schindler, Armstrong, Holmgren & Brunski (1971), Schindler, Kling, Schmidt, Prokopowich, Frost, Reid & Capel (1973); and in small ponds or enclosures, Ryan, Reimer & Toth (1972), Mulligan, Baronowski & Johnson (1976).

Uptake of PO_4-P by the sediment was estimated to account for 14-25% of that added, similar to the 10-30% found by Brook & Holden (1957) in L. Kinardochy. The uptake rates of 5-9 mg P m^{-2} wk^{-1} are similar to those of 3-4 mg P m^{-2} wk^{-1} calculated from data of Brook & Holden, and Holden (1961) by assuming a starting concentration of 80 μg l^{-1} in the water. This is also similar to 4 mg P m^{-2} wk^{-1} found by Kamp-Nielsen

(1975) for sediment from a depth of 3m. The causes of the sediment uptake is not known, but it could include chemical adsorption to ferric compounds (Mortimer 1941) as well as biological uptake by bacteria and benthic algae.

Brook, Holden & Caines (1965) found that certain submerged macrophytes, e.g. *Myriophyllum spicatum*, *M. alterniflorum*, *Potamogeton praelongus* and *P. gramineus* took up PO_4-P, as shown by an increase in P content of their tissue, as a result of fertilisation. Other species, e.g. *Lobelia dortmanna* and *Littorella uniflora* did not show such an increase. *P. filiformis* shoots from the control enclosure did not have a greater P content than shoots from an enclosure which had received P & N additions. This indicates that shoot uptake of P probably did not occur, although shoot uptake and subsequent translocation to roots, rhizomes and turions cannot be ruled out (Harrison & Mann 1975).

NO_3-N appeared to be taken up by the shoots of *P. filiformis* and uptake was estimated to represent 36% of the added N. Benthic algae may have been responsible for some uptake, but no measurements were made of this component of the ecosystem. Reduction in NO_3-N concentration also occurred in enclosure I where macrophytes had been removed, and unless large leaks were occurring, which is unlikely as the pH and carbon system differed from the surroundings (Fig. 2.6), uptake by benthic algae and phytoplankton are the likely causes of loss. A phytoplankton uptake in those enclosures receiving P & N can be inferred from the larger zooplankton density, indicating a greater phytoplankton productivity.

2.4.3 Factors responsible for the increase in phytoplankton in late summer.

At the end of the macrophyte growth period in the begining of August, a slight increase in phytoplankton chlorophyll was observed in many of the enclosures (Fig. 2.7) although this was not seen in the weedbed. The reduction in macrophyte growth probably reduced the macrophyte demand for C and N. Photosynthetic studies on P. filiformis in the succeeding year at L. Fitty showed very low photosynthetic rates in early August (Chapter 7). The increasing inorganic carbon is shown by the falling pH (Fig. 2.7) and increasing HCO_3^- (Fig. 2.6). A high rainfall in August could have increased the NO_3-N input, while the rising water-level may have improved the light climate for the phytoplankton as the macrophytes were no longer at the water surface.

Macrophyte decay is associated with considerable release of nutrients (Kistritz 1978, Hill 1979, Howard-Williams & Davies 1979 and Landers 1979). Hill (1979) found that 60% of the maximum summer biomass was lost in the first eight days which he attributed to leaching of soluble material. In certain enclosures, considerable peaks of NO_3-N were found, with a maximum of about 3 mg l^{-1} in enclosure III (+P+N). Pulses of high PO_4-P concentration related to macrophyte decay were found by Landers (1979), and NO_3-N and NH_4-N pulses were also recorded. NH_4-N was not measured in the enclosures, and it was probably not present in any extent. On 5.iii.81, when nutrient levels were probably high as the macrophyte growing season had not started, NO_3-N was 1.08 mg l^{-1} while NH_4-N was only 0.019 mg l^{-1}. This probably decreased to zero as the growing season progressed, however, some NH_4-N may have been released as a result of macrophyte decay.

The increased availability of nutrients caused a large increase in phytoplankton in certain enclosures. A similar phytoplankton response to macrophyte decay has been found by Surber (1954) and Landers (1979). In enclosures V, VI and VII, the phytoplankton increase was not as great as in enclosures IV, II, VIII and III, probably because in the former, zooplankton density was high. In enclosures V and VI furthermore, the filamentous alga Rhizoclonium became abundant (Table 2.4) probably also in response to macrophyte nutrient release and may have acted as competitor for nutrients and light with the phytoplankton. The greatest cover of Rhizoclonium occurred in the +P+N treatments (Table 2.4 and 2.6) which could support the contention that macrophytes were responsible for the uptake of the added nutrients, which were released when they decayed. The large Rhizoclonium crops were found after the macrophyte growth period, and so probably did not have a detrimental effect on them. This is substantiated by historical evidence; West (1910) found abundant Cladophorae at L. Fitty, and their presence does not appear to have harmed the macrophytes over the last seventy years.

2.4.4 Conclusions

These results highlight the difficulties of interpretation of field experiments where many factors interact. Large numbers of variables have to be analysed on a regular basis without the certainty that an important component (e.g. benthic algae in this Chapter) is being studied. However, as individual components will not necessarily respond in isolation in the way that they will when in a complicated ecosystem (Lane & Levins 1977), some form of field experimentation is necessary. Moss (1976) points out some of the advantages and disadvantages

of field experiments.

These results, particularly those of the bioassays, confirm the initial hypothesis that phytoplankton production is limited by the amount of P & N available in the water. What was previously unclear was the extent to which zooplankton grazing could control the phytoplankton standing crop, particularly under conditions where predation by fish is minimal. The macrophytes appear to aid the development of zooplankton partly by allowing refuge from fish predation, and probably partly by providing shelter. Macrophytes also appear to outcompete phytoplankton directly by taking nutrients, particularly N, out of the water. The nutrient additions did not aid the macrophytes, suggesting that the sediment supplied most of their nutrients. Any shoot uptake probably represents luxury consumption, and although its purpose may not be phytoplankton suppression, this is its effect. Thus, macrophytes are probably able to prevent nutrient levels in the water from increasing as long as the loading is below a critical rate. Once this critical loading has been exceeded, nutrients availability in the water will increase and allow the development of phytoplankton crops. Such a threshold in nutrient loading was found by Moss (1976) in replicated experimental ponds. Lakes with a large macrophyte biomass may be expected to withstand a greater nutrient input before nutrient levels in the water increases, and phytoplankton crops develop.

Factors causing a loss of nutrients from the water such as chemical adsorption by aerobic sediments or uptake by benthic algae and bacteria will favour macrophytes, while loss of nutrients from the sediment because of wind-disturbance (e.g.

Moller-Andersen 1974) or anaerobic conditions (Mortimer 1941 and 1971), will favour phytoplankton.

PHOTOGRAPH 1: Two nearly constructed enclosures

PHOTOGRAPH 2: The completed enclosures in position

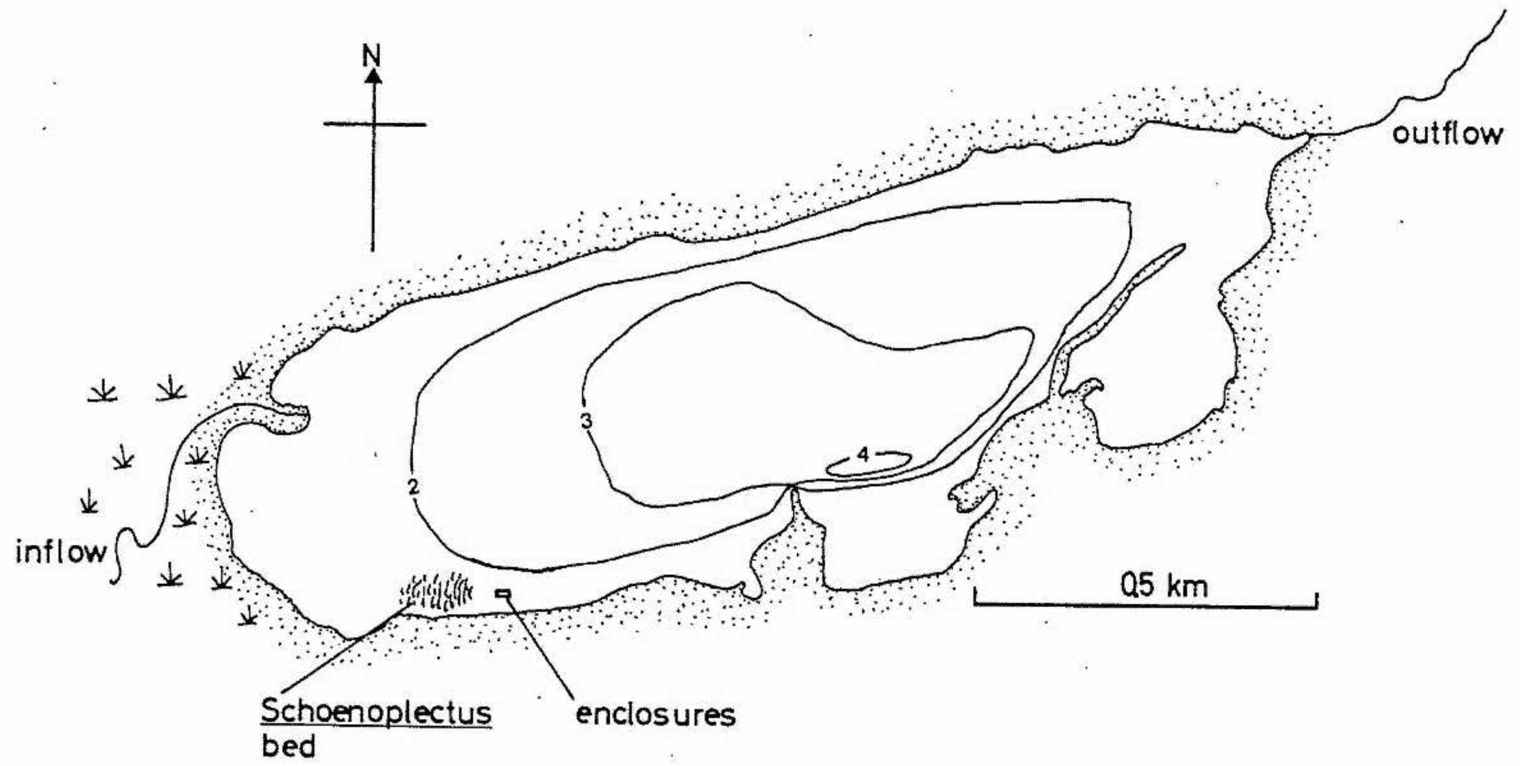


FIGURE 2.1 Outline map of Loch Fitty, Fife, based on a 6" Ordnance Survey map. Depth contours (m) from Murray & Pullar (1910). Position of enclosures shown.

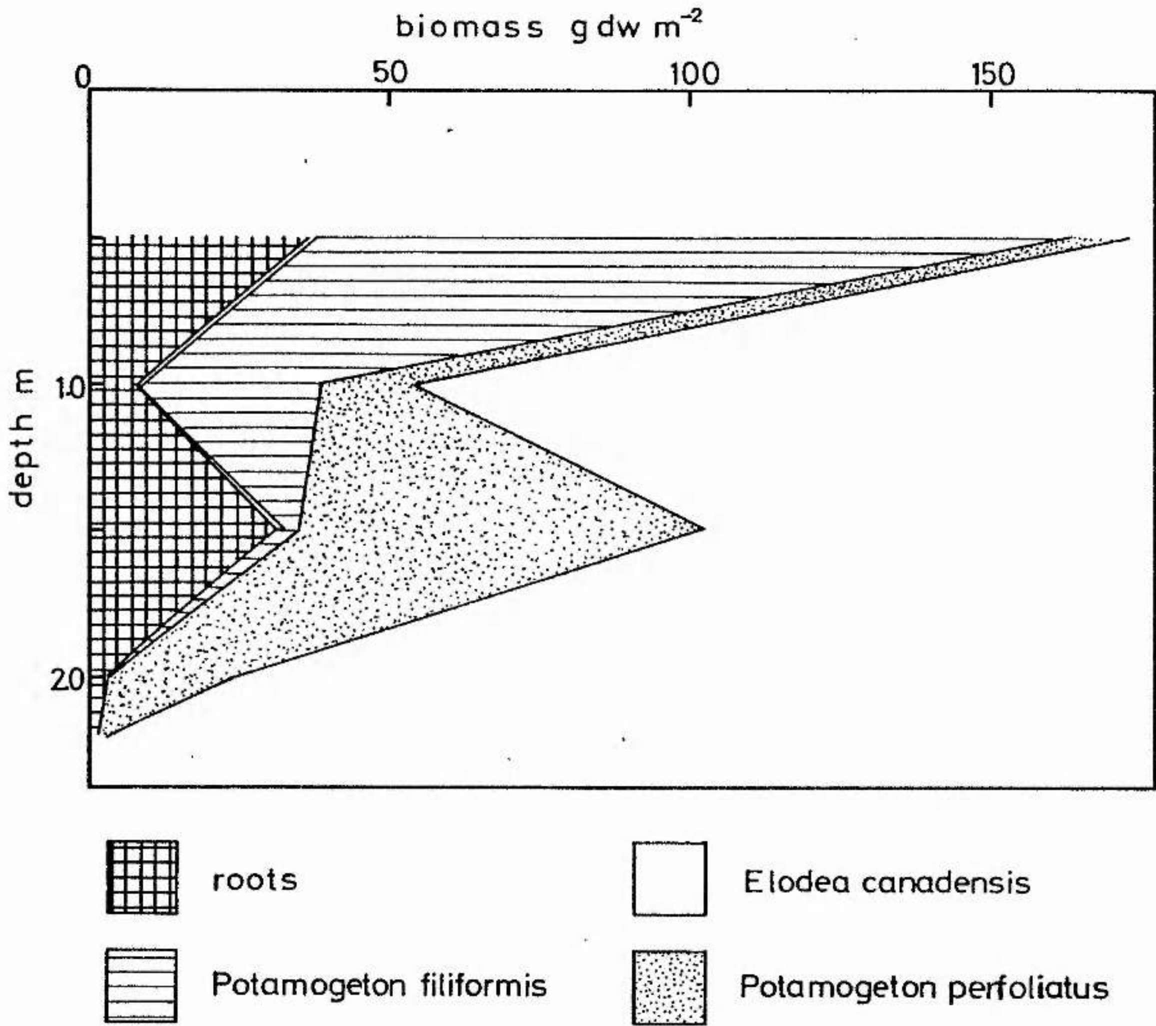


FIGURE 2.2 Depth distribution of macrophyte biomass at time of seasonal maximum (1.viii.79). Sampled offshore from enclosures.

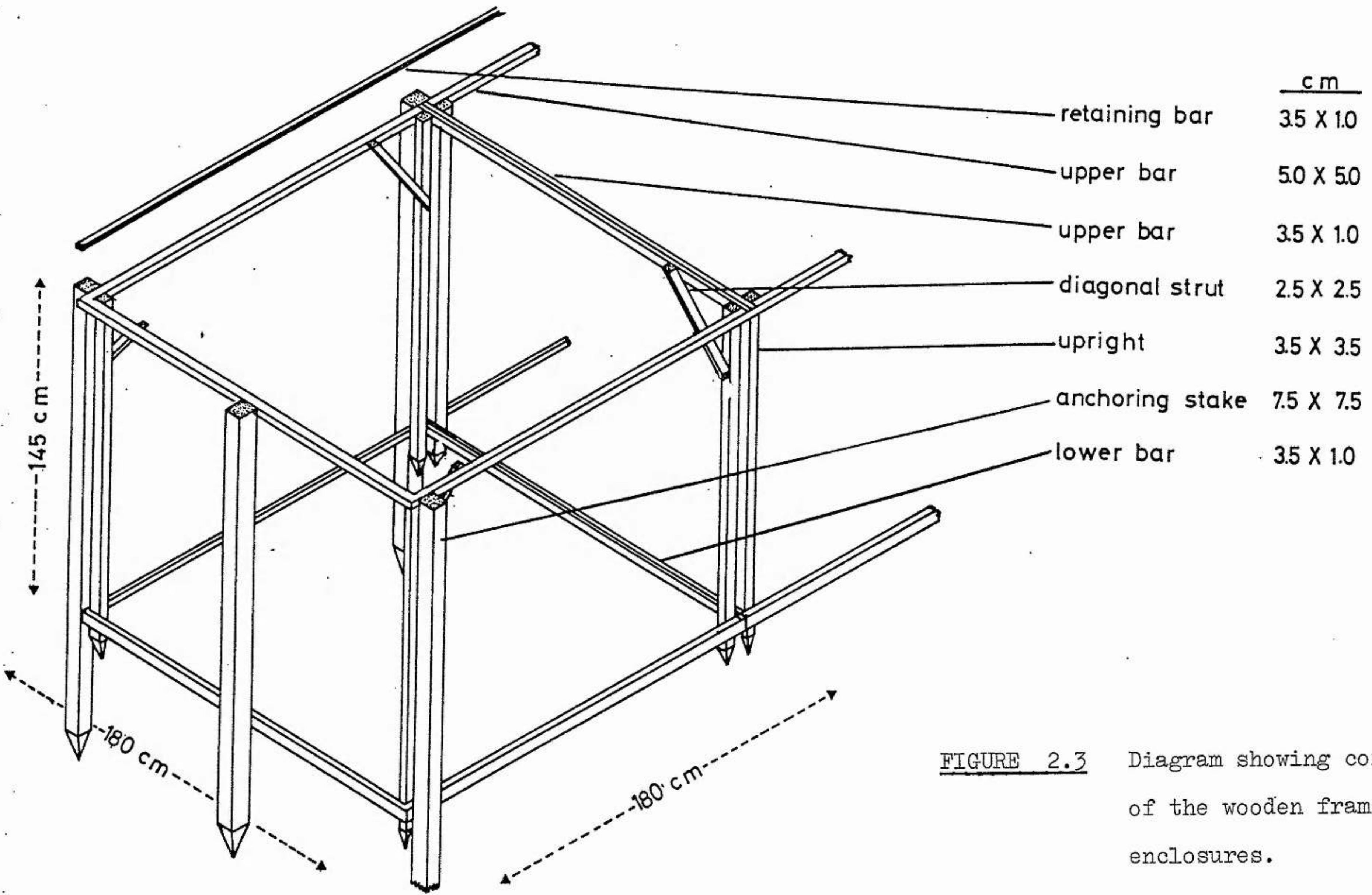


FIGURE 2.3 Diagram showing construction of the wooden frame of the enclosures.

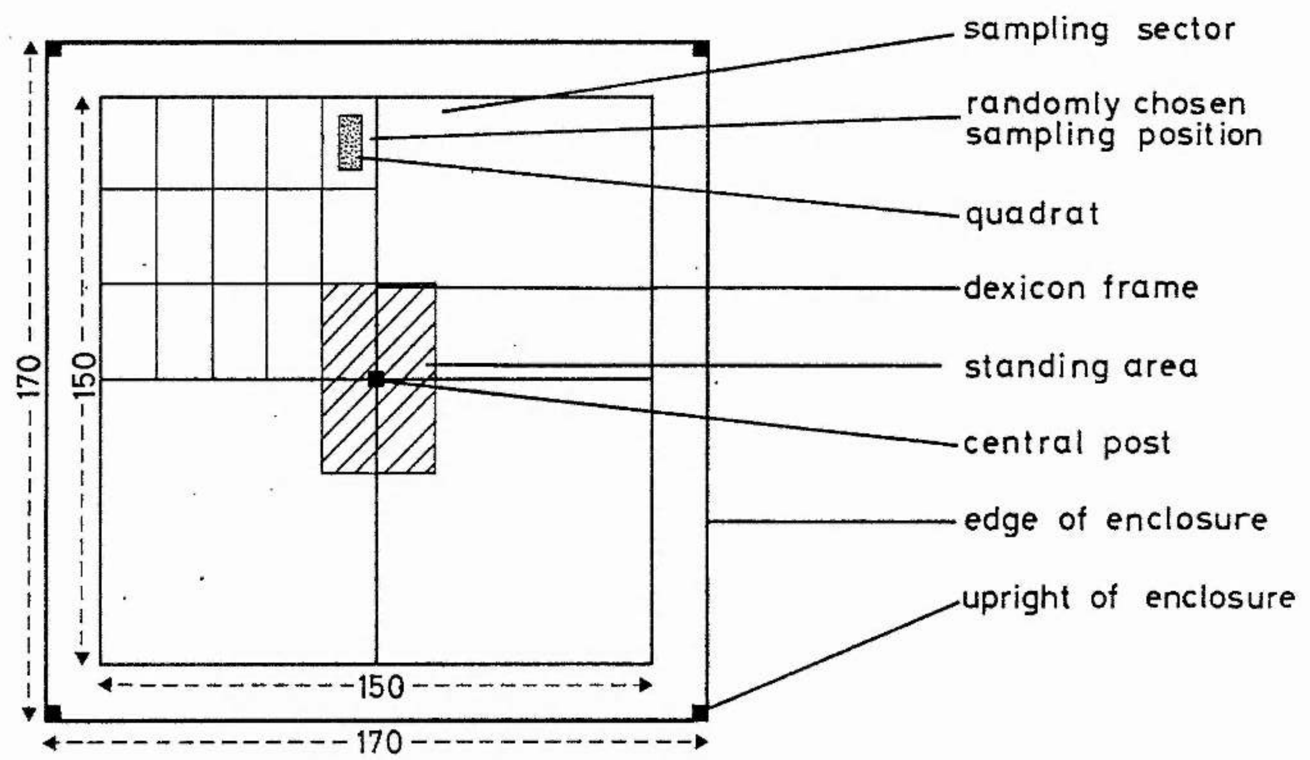


FIGURE 2.4 Aerial plan of an enclosure, showing sampling positions. Distance in cm.

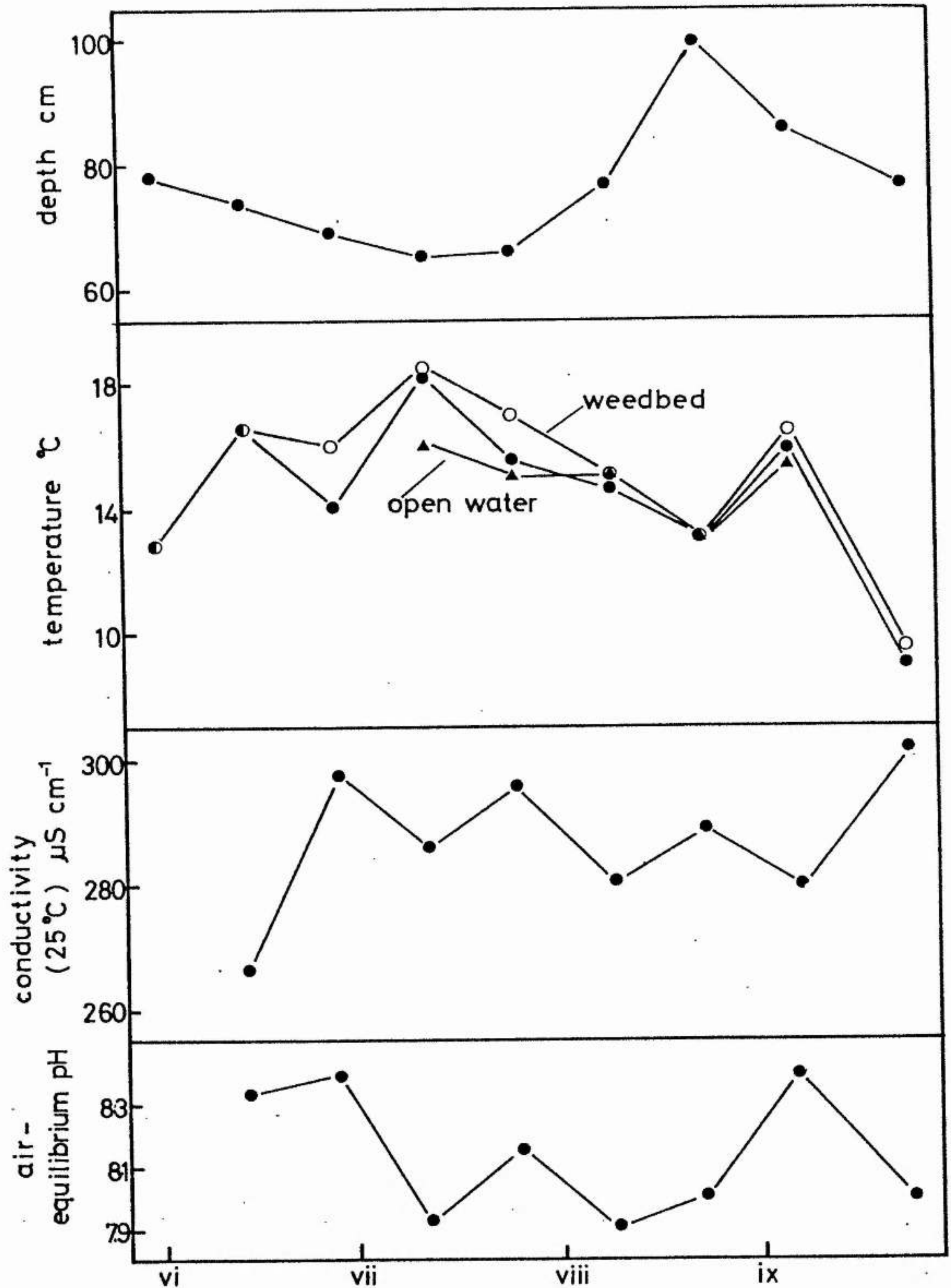
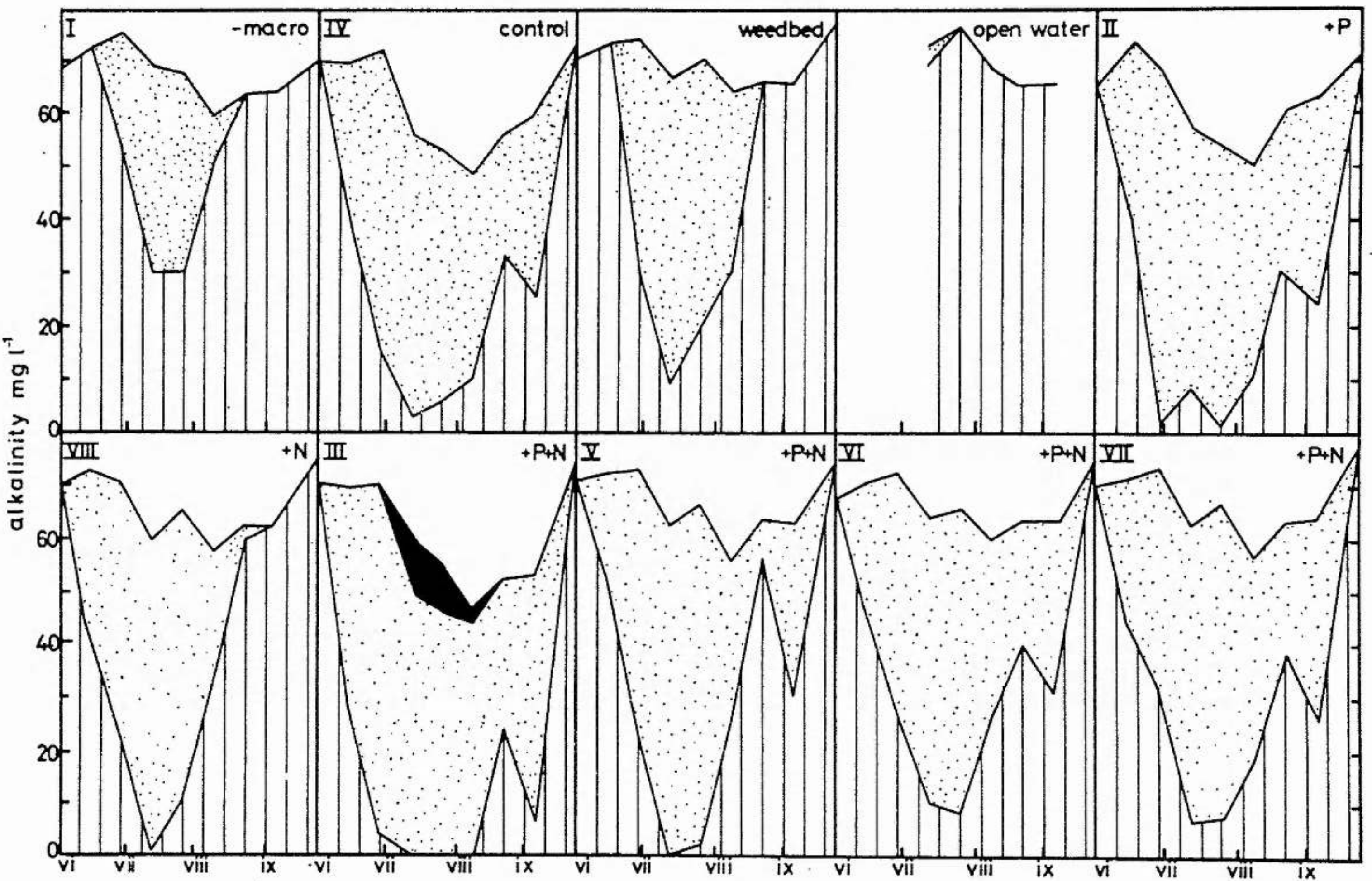


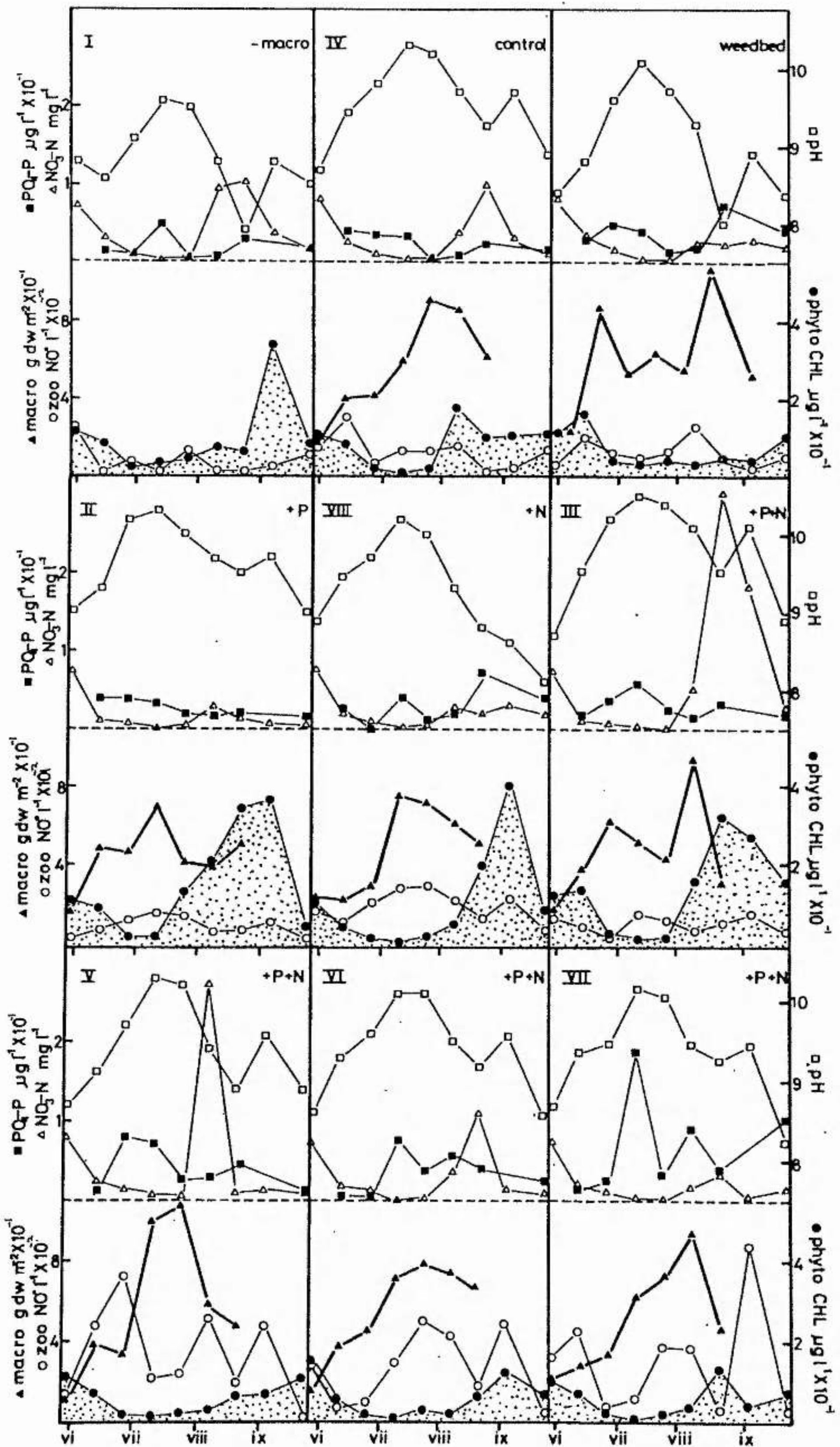
FIGURE 2.5 Average seasonal changes in depth, temperature, conductivity (25°C) and air-equilibrium pH for the eight enclosures, (●). Also temperature changes for the weedbed (o) and open water (▲).

FIGURE 2.6 Seasonal changes in total alkalinity, and its component species, for the eight enclosures, the weedbed, and the open water.



I
-macro
IV
control
weedbed
open water
II
+P
VIII
+N
III
+P+N
V
+P+N
VI
+P+N
VII
+P+N
alkalinity mg l⁻¹
0
20
40
60
VI
VII
VIII
IX

FIGURE 2.7 Seasonal changes in PO_4-P (■), NO_3-N (△), pH (□), macrophyte standing crop (▲, heavy lines), phytoplankton chlorophyll (● , stippled), and zooplankton numbers (○), for the eight enclosures and the weedbed.



I
- macro
IV
control
weedbed
II
+ P
VIII
+ N
III
+ P + N
V
+ P + N
VI
+ P + N
VII
+ P + N
■ PO4-P µg l-1 X10-1
△ NO3-N mg l-1
▲ macro g dw m-2 X10-1
○ zoo NO l-1 X10-2
□ pH
● phyto CHL µg l-1 X10-1
vi
vii
viii
ix

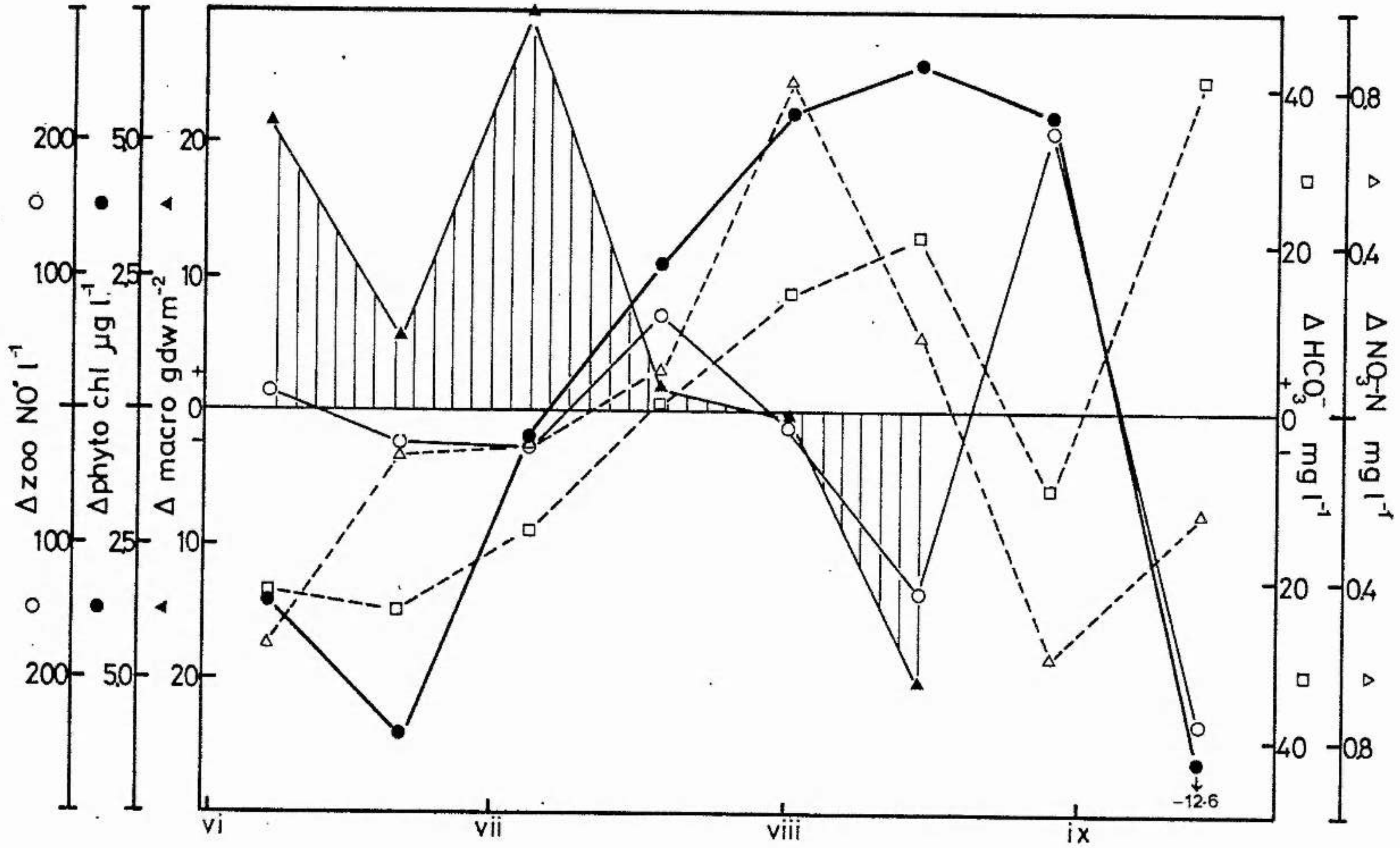


FIGURE 2.8 Seasonal variation in changes in macrophyte standing crop (▲, shaded), phytoplankton chlorophyll (●, heavy line), zooplankton numbers (○), NO_3-N (△), and HCO_3^- (□), as an average for the eight enclosures.

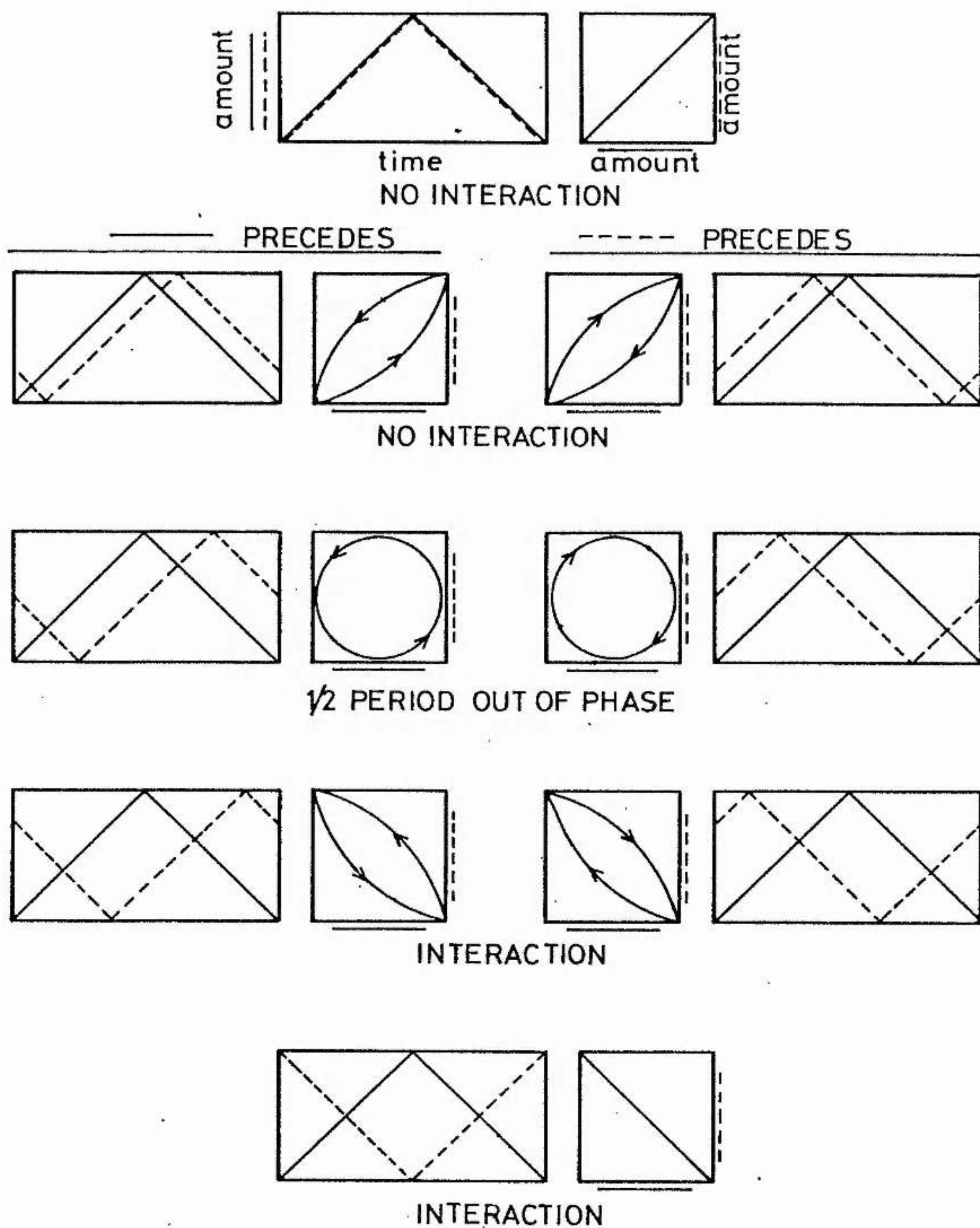


FIGURE 2.9 Theoretical analysis of the change of two variables against time, and against each other.

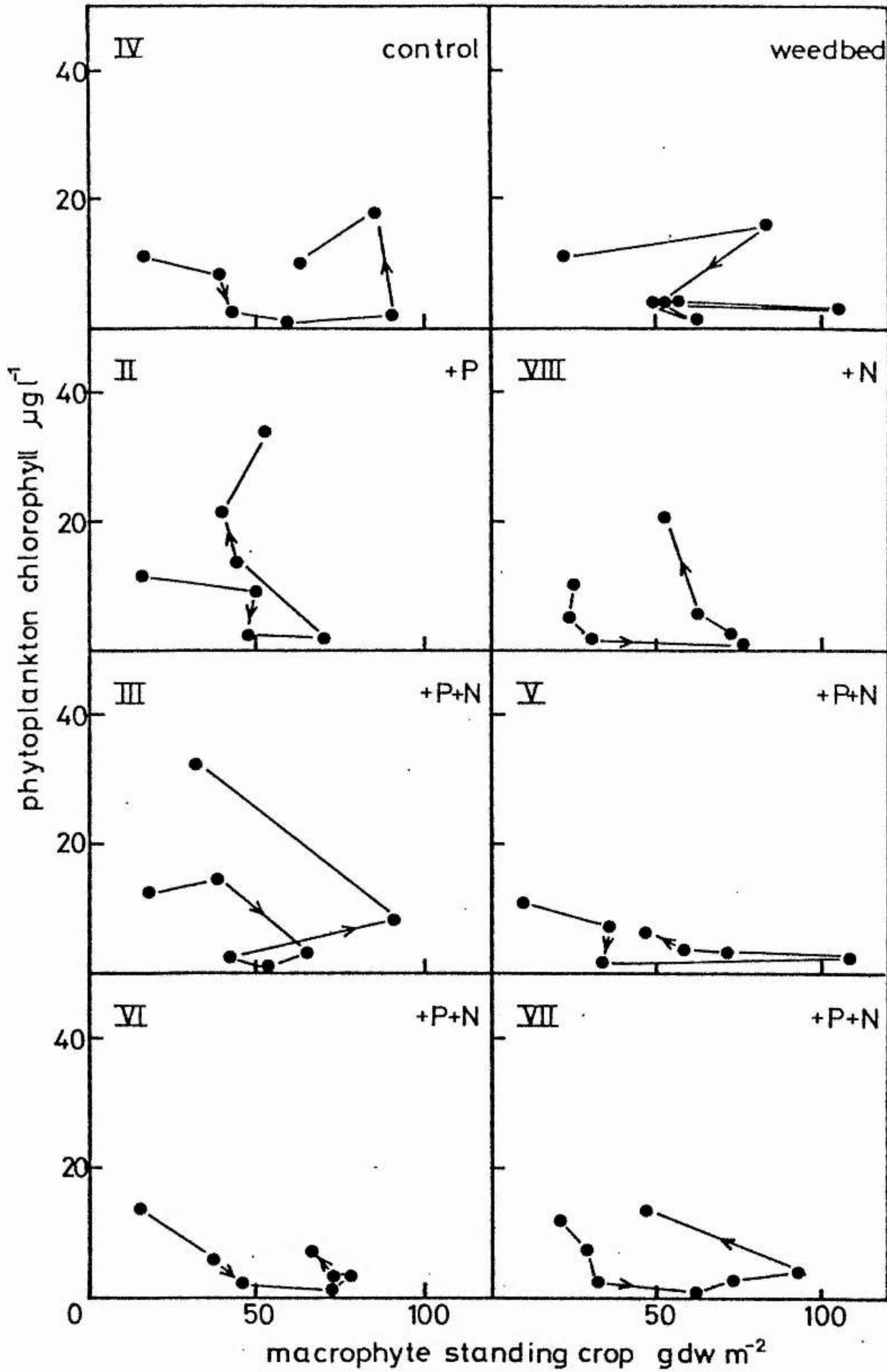


FIGURE 2.10 Change in phytoplankton chlorophyll against macrophyte standing crop for the eight enclosures and the weedbed.

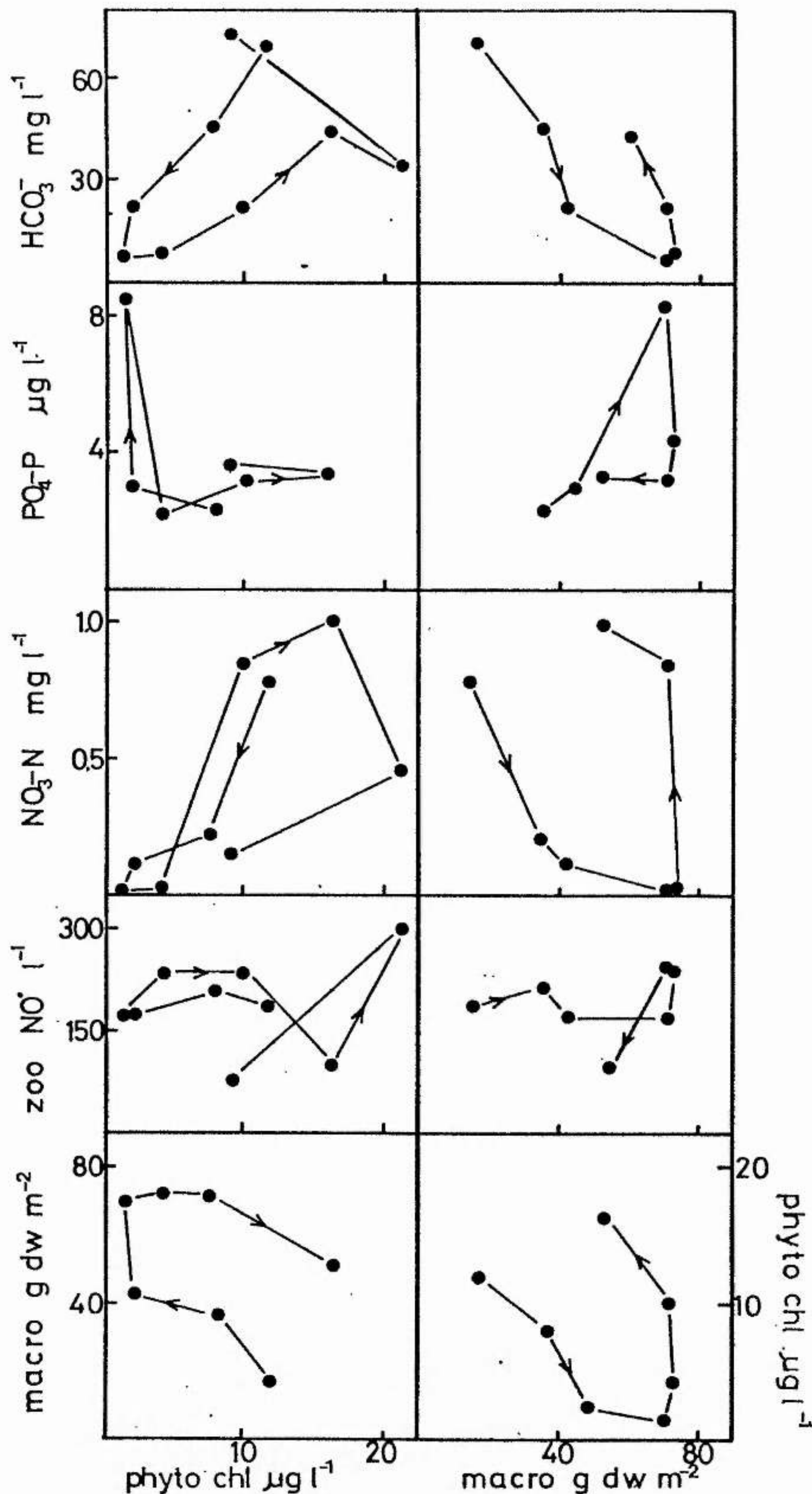
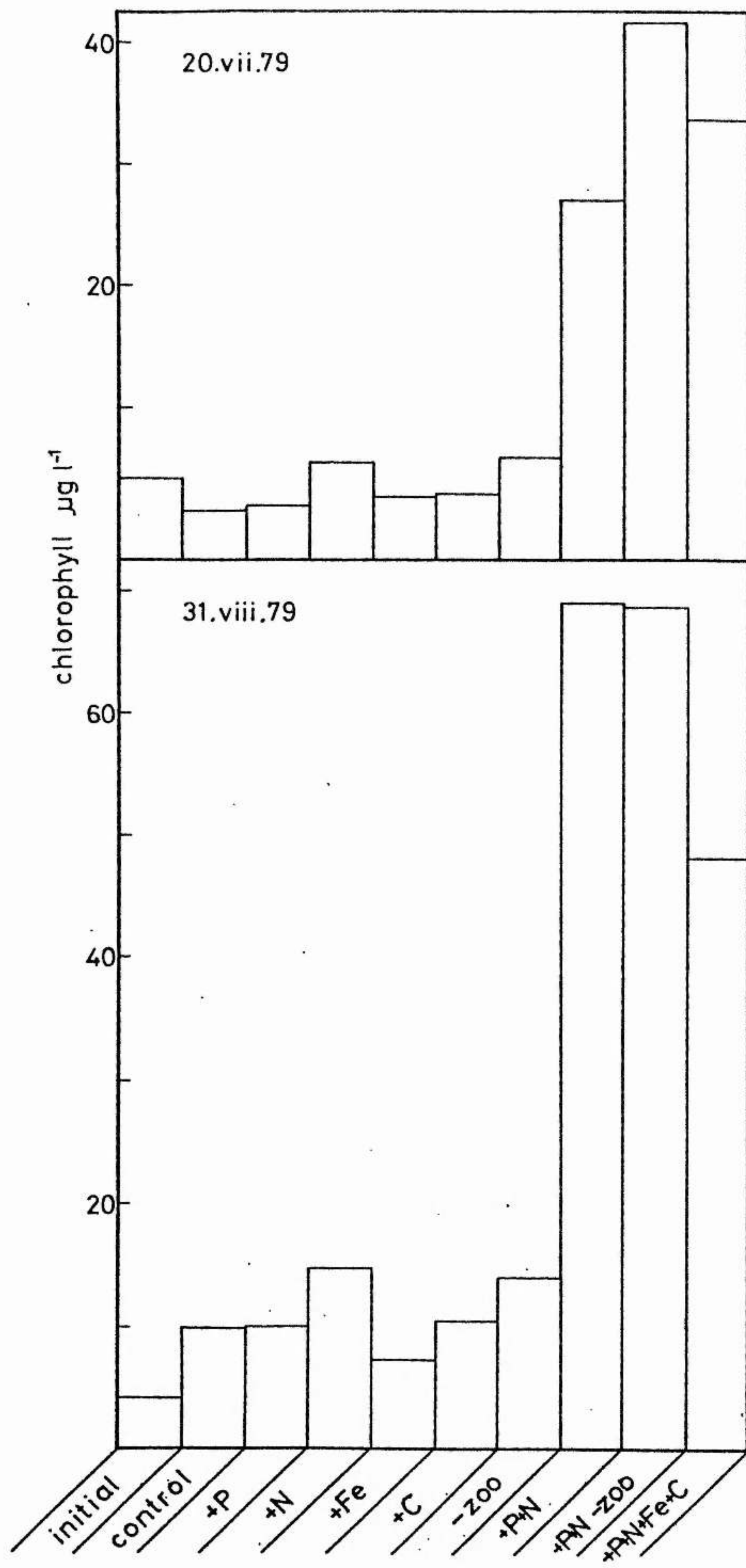


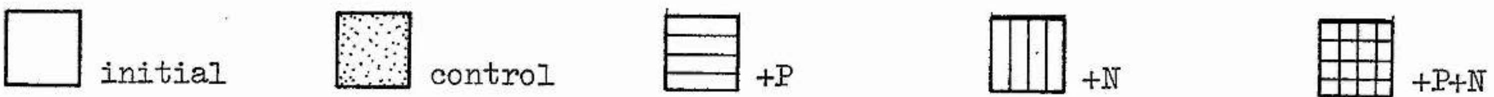
FIGURE 2.11 Change in HCO_3^-, PO_4-P, NO_3-N, zooplankton numbers, macrophyte standing crop and phytoplankton chlorophyll against phytoplankton chlorophyll or macrophyte standing crop. Average for the eight enclosures.

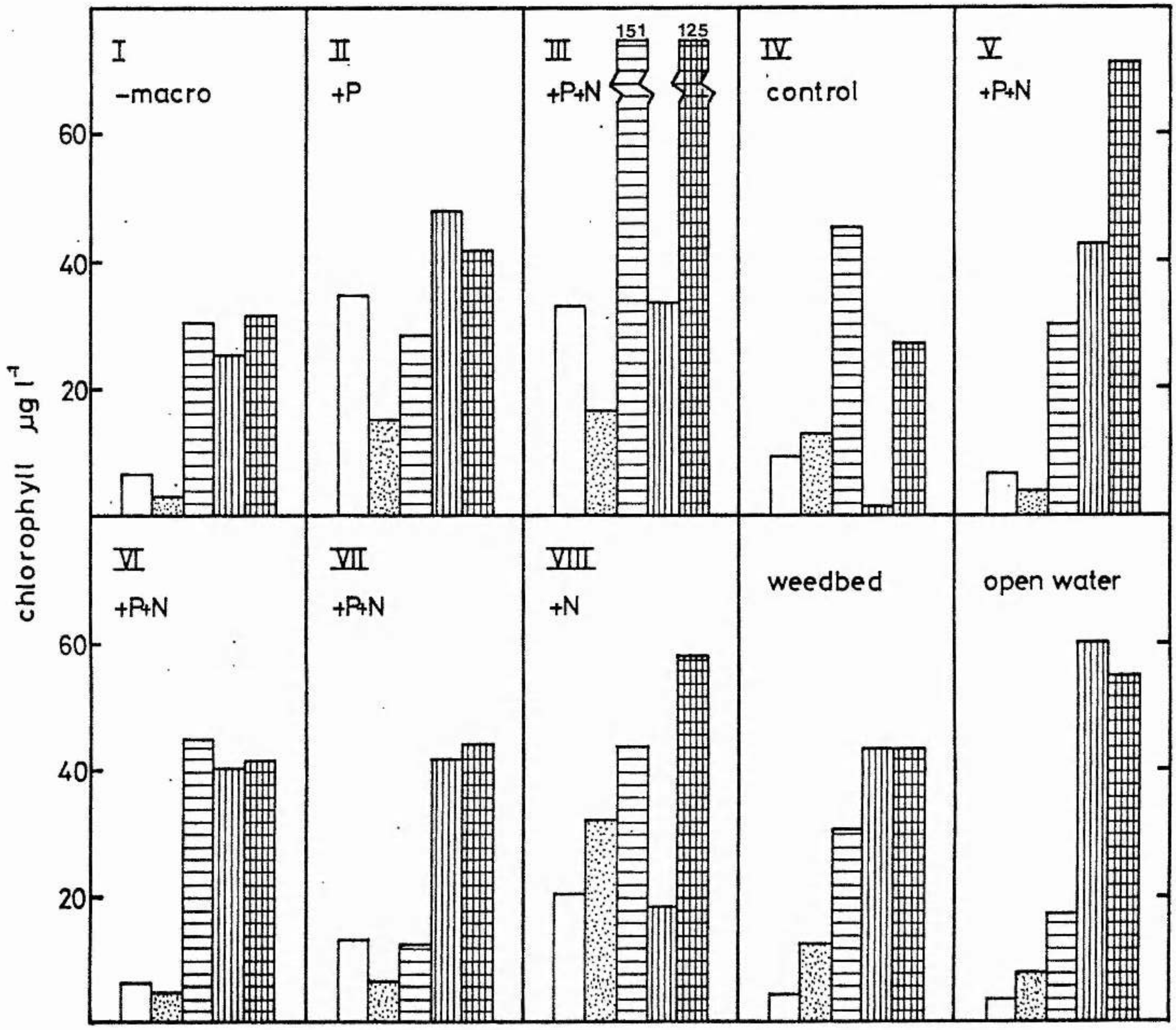
FIGURE 2.12 Nutrient bioassay of natural phytoplankton populations from the weedbed on two occasions.



20.vii.79
31.viii.79
chlorophyll µg l⁻¹
40
20
60
40
20
initial
control
+P
+N
+Fe
+C
-zoo
+P+N
+P+N -zoo
+P+N+Fe+C

FIGURE 2.13 Nutrient bioassay of natural phytoplankton populations from the eight enclosures, the weedbed and the open water on 24.viii.79.





I
-macro
II
+P
III
+P+N
151
125
IV
control
V
+P+N
VI
+P+N
VII
+P+N
VIII
+N
weedbed
open water
60
40
20
60
40
20
chlorophyll μg l⁻¹

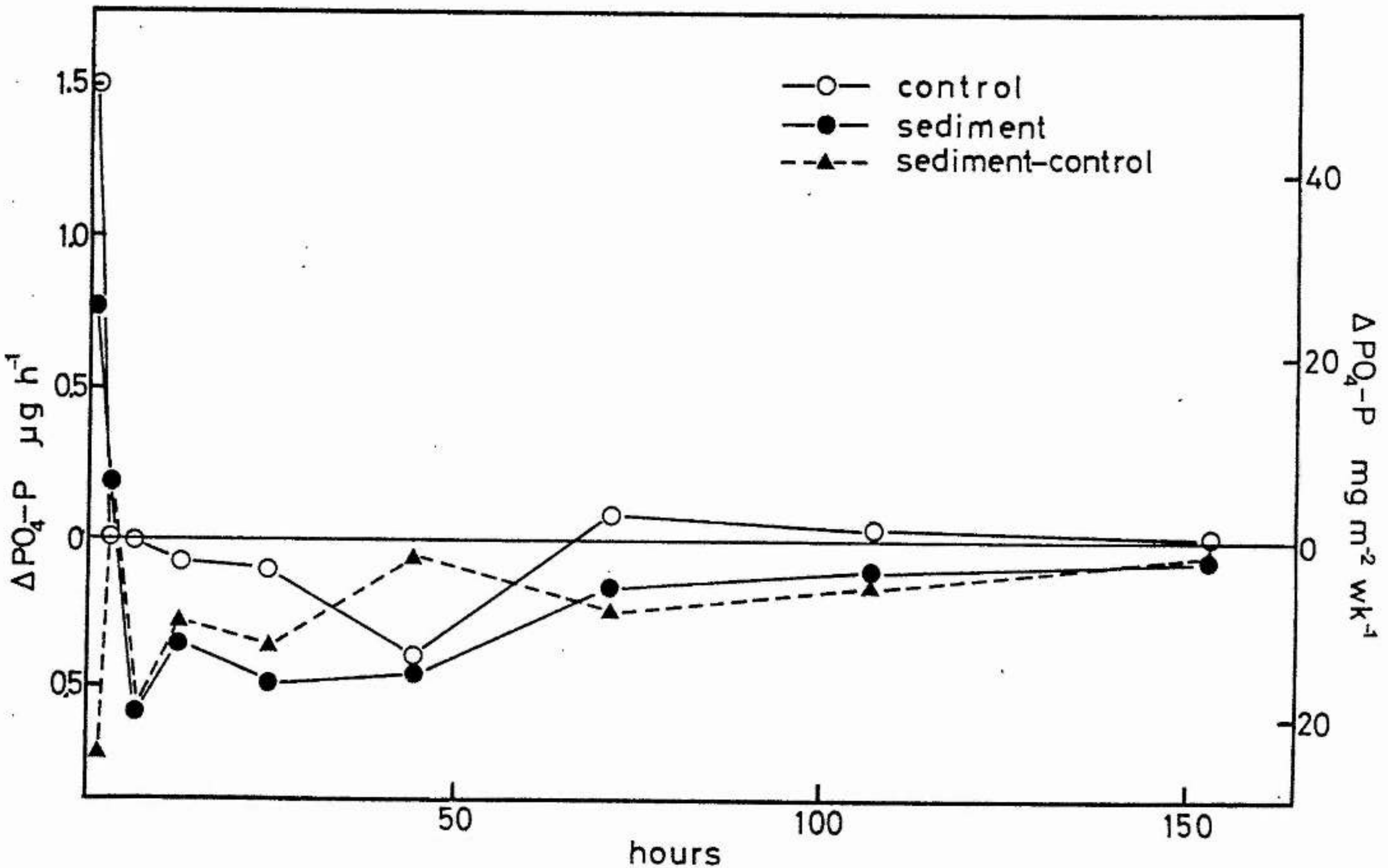


FIGURE 2.14 Change in PO_4-P as μg h^{-1} and mg m^{-2} wk^{-1} in perspex tubes containing water only (control) or sediment and water, over a week, Difference between them also shown. Samples duplicated.

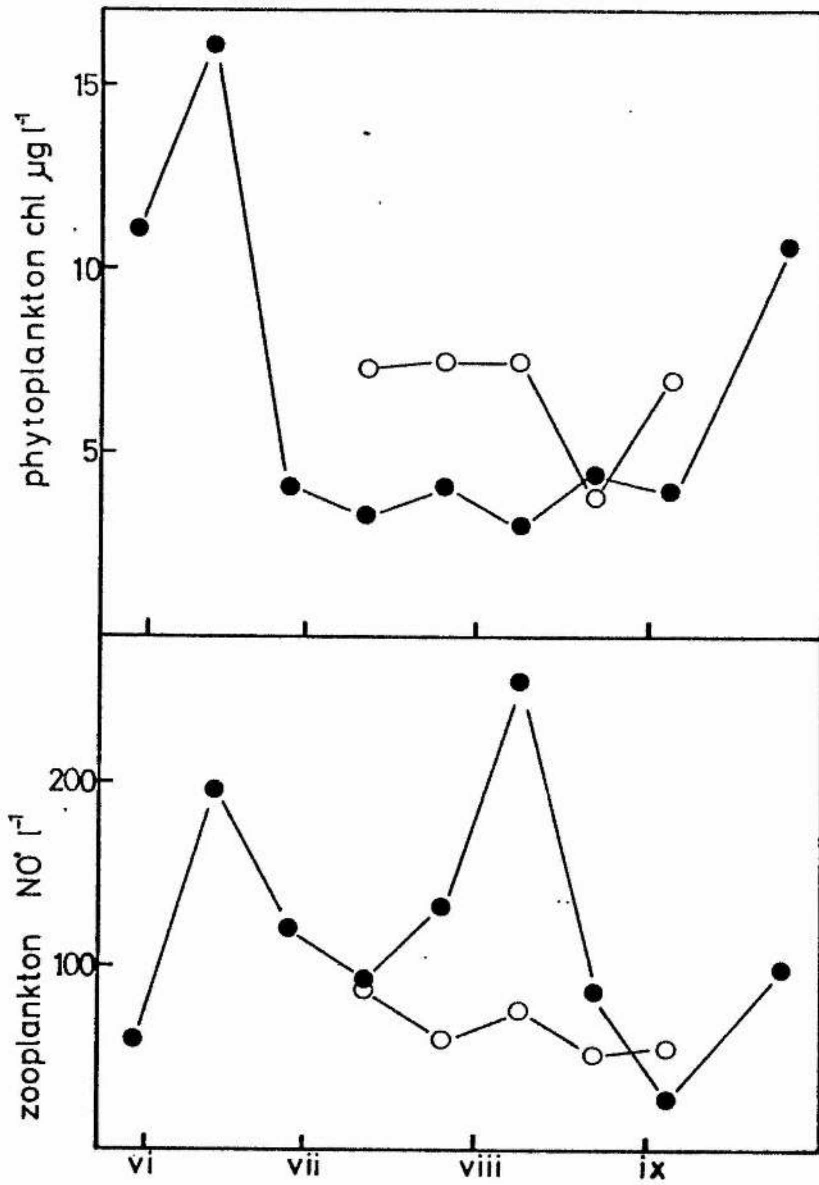


FIGURE 2.15 Comparison of seasonal changes in phytoplankton chlorophyll and zooplankton numbers in the weedbed (●) and open water (o).

TABLE 2.1

Background information on L. Fitty, Fife.

	+ height above sea level	126 m
	+ length	1.45 km
	+ area	0.57 km^2
	+ maximum depth	4.88 m
	+ mean depth	2.26 m
	+ volume	1.3 x 10^6 m^3
	‡ catchment area	14 km^2
light attenuation (1.v.80, when phytoplankton chl 6 μg l^{-1})	K_B	3.14 ln m^{-1}
	K_G	1.30 ln m^{-1}
	K_R	1.12 ln m^{-1}
water	conductivity (25°C)	290 μS cm^{-1}
	alkalinity	70 mg l^{-1} as $CaCO_3$
	* Ca^{++}	26-42 mg l^{-1}
	* Mg^{++}	7-13 mg l^{-1}
	* Na^+	10-15 mg l^{-1}
	* K^+	1.7-8.7 mg l^{-1}
	* SiO_2	0.8-7.4 mg l^{-1}
sediment 0.3 m 5.iii.80	water content	28%
	loss on ignition	4.4%
	interstitial water PO_4-P	0.32 mg l^{-1}
	interstitial water NO_3-N	0.20 mg l^{-1}
	interstitial water NH_4-N	4.50 mg l^{-1}
	interstitial water pH	6.94

+ Murray & Pullar (1910)

‡ from Ordnance survey map

* Mr. R. Harriman, Freshwater Fisheries Laboratory, Pitlochry.

TABLE 2.2

Additions of PO_4-P and NO_3-N to the enclosures as weekly loading (mg m^{-2} wk^{-1}) and initial concentration (µg l^{-1}) assuming a volume of 2.3 m^3.

DATE	PO_4-P		NO_3-N	
	mg m^{-2} wk^{-1}	µg l^{-1}	mg m^{-2} wk^{-1}	µg l^{-1}
31.v	9.1	11.4	94	119
6.vi	9.1	11.4	94	119
14.vi	9.1	11.4	94	119
20.vi	18.2	22.9	188	237
27.vi	39.7	50.0	595	750
4.vii	39.7	50.0	595	750
11.vii	39.7	50.0	595	750
18.vii	39.7	50.0	595	750
25.vii	39.7	50.0	595	750
1.viii	39.7	50.0	595	750
8.viii	39.7	50.0	595	750
16.viii	39.7	50.0	595	750
30.viii	39.7	50.0	595	750
12.ix	39.7	50.0	595	750

TABLE 2.3

Number of *Gloeotrichia echinulata* colonies per litre for the eight enclosures and the weedbed on the nine sampling dates.

ENCLOSURE AND TREATMENT	$NO^o\ l^{-1}$								
	31.v	14.vi	27.vi	11.vii	25.vii	8.viii	22.viii	5.ix	24.ix
I -macro	4	12	0	16	0	32	136	312	4
IV control	0	20	4	24	44	36	112	152	0
weedbed	0	24	8	0	0	12	36	4	0
II +P	4	12	24	52	268	732	696	528	0
VIII +N	8	32	4	4	44	80	520	460	0
III +P +N	0	36	8	32	4	16	0	0	0
V +P +N	0	52	4	0	40	16	168	52	0
VI +P +N	0	32	0	4	16	36	152	140	0
VII +P +N	8	56	0	4	60	40	268	12	0

TABLE 2.4

Percentage cover of *Rhizoclonium hierglyphicum* for the eight enclosures on the two sampling dates when it was abundant.

ENCLOSURE AND TREATMENT	% COVER	
	5.ix	24.ix
I -macro	0	0
IV control	10	<1
II +P	<1	<1
VIII +N	<1	0
III +P +N	25	<1
V +P +N	50	30
VI +P +N	25	5
VII +P +N	3	0

TABLE 2.5

Percentage composition of the zooplankton population for the eight enclosures, the weedbed and the open water on the nine sampling dates. Dominant form for a given date underlined.

		SAMPLING DATE								
		31. v	14. vi	27. vi	11. vii	25. vii	8. viii	22. viii	5. ix	24. ix
I	Rotifers	98	86	61	50	0	17	25	0	86
	Cladocerans	0	0	33	0	86	33	0	8	7
	Cyclopoids	0	0	6	25	9	17	0	8	0
	Calanoids	0	0	0	0	0	17	0	50	0
	Ostracods	0	0	0	0	0	0	0	17	4
	Mites	2	14	0	25	5	0	75	17	4
	Others	0	0	0	0	0	17	0	0	1
Weed -bed	Rotifers	74	86	3	9	0	0	0	0	4
	Cladocerans	0	0	83	52	79	40	24	17	16
	Cyclopoids	5	2	7	13	3	15	14	33	12
	Calanoids	0	0	0	0	3	4	10	0	4
	Ostracods	0	0	0	0	0	18	10	17	40
	Mites	21	12	7	22	12	18	43	33	24
	Others	0	0	0	4	3	6	0	0	0
Open water	Rotifers				4	0	0	15	0	
	Cladocerans				74	53	47	23	0	
	Cyclopoids				9	40	21	8	11	
	Calanoids				0	0	11	0	22	
	Ostracods				0	0	5	15	11	
	Mites				13	7	16	38	56	
	Others				0	0	0	0	0	

		SAMPLING DATE								
		31. v	14. vi	27. vi	11. vii	25. vii	8. viii	22. viii	5. ix	24. ix
IV	Rotifers	97	87	0	0	0	0	0	0	59
	Cladocerans	0	1	67	91	90	66	0	33	0
	Cyclopoids	0	0	25	9	10	13	0	0	3
	Calanoids	0	0	0	0	0	0	0	11	3
	Ostracods	0	0	0	0	0	0	0	22	6
	Mites	3	12	8	0	0	21	100	33	28
	Others	0	0	0	0	0	0	0	0	0
III	Rotifers	89	91	0	0	0	0	0	0	33
	Cladocerans	0	0	67	41	68	26	2	0	0
	Cyclopoids	3	4	25	36	23	5	3	21	33
	Calanoids	0	0	0	0	0	0	0	2	0
	Ostracods	0	0	0	0	0	0	50	12	14
	Mites	8	4	8	23	9	68	45	64	19
	Others	0	0	0	0	0	0	0	0	0
V	Rotifers	92	56	1	0	0	0	0	0	13
	Cladocerans	0	0	94	74	88	46	16	11	0
	Cyclopoids	7	17	2	9	7	15	2	1	0
	Calanoids	0	0	0	0	2	3	2	4	0
	Ostracods	0	0	0	0	0	0	67	11	38
	Mites	1	27	2	17	3	36	13	73	50
	Others	0	0	0	0	0	0	0	0	0
VI	Rotifers	92	90	0	0	0	0	0	0	0
	Cladocerans	0	0	21	83	89	35	33	17	61
	Cyclopoids	7	5	63	5	2	19	0	1	8
	Calanoids	0	0	0	3	1	11	0	0	15
	Ostracods	0	0	0	0	0	0	44	17	8
	Mites	1	5	17	8	9	34	22	75	8
	Others	0	0	0	0	0	0	0	0	0

		SAMPLING DATE								
		31. v	14. vi	27. vi	11. vii	25. vii	8. viii	22. viii	5. ix	24. ix
VII	Rotifers	95	92	0	0	0	0	0	0	6
	Cladocerans	0	1	33	90	84	50	35	13	50
	Cyclopoids	0	1	29	7	1	9	6	3	6
	Calanoids	0	0	0	0	0	0	0	0	0
	Ostracods	0	0	0	0	0	15	42	14	11
	Mites	5	6	10	3	6	28	18	71	28
	Others	0	0	29	0	0	0	0	0	0
II	Rotifers	90	95	0	5	0	0	5	0	18
	Cladocerans	0	0	67	75	94	81	5	24	9
	Cyclopoids	0	0	30	5	3	6	0	10	9
	Calanoids	0	0	3	0	0	0	15	17	0
	Ostracods	0	0	0	0	0	0	0	24	0
	Mites	10	5	0	15	3	13	75	34	64
	Others	0	0	0	0	0	0	0	0	0
VIII	Rotifers	85	92	0	0	0	0	0	0	33
	Cladocerans	0	0	7	88	76	18	0	52	44
	Cyclopoids	4	0	89	1	5	4	3	7	0
	Calanoids	0	0	0	0	5	2	6	7	0
	Ostracods	0	0	0	0	0	67	41	10	11
	Mites	10	4	4	11	13	11	50	25	11
	Others	0	4	0	0	0	0	0	0	0

TABLE 2.6

Results of Students' t-test on average results from enclosure experiments; for macrophyte standing crop, zooplankton numbers and *Rhizoclonium* cover.

	t	df	signif.
a) Seasonal average macrophyte standing crop:			
+P+N (III,V,VI,VII) vs rest (II,IV,VIII)	0.739	5	NS
+N,+P+N (III,V,VI,VII,VIII) vs rest (II,IV)	0.300	5	NS
+P,+P+N (II,III,V,VI,VII) vs rest (IV,VIII)	0.318	5	NS
b) Seasonal average zooplankton numbers:			
+P+N (III,V,VI,VII) vs rest (I,II,IV,VIII)	2.387	6	$P > 0.10$
+N,+P+N (III,V,VI,VII,VIII) vs rest (I,II,IV)	2.716	6	$P > 0.05$
+P,+P+N (II,III,V,VI,VII) vs rest (I,IV,VIII)	1.290	6	NS
c) Average *Rhizoclonium* cover on 5.ix and 24.ix:			
+P+N (III,V,VI,VII) vs rest (I,II,IV,VIII)	2.430	14	$P > 0.05$
+N,+P+N (III,V,VI,VII,VIII) vs rest (I,II,IV)	1.630	14	NS
+P,+P+N (II,III,V,VI,VII) vs rest (I,IV,VIII)	1.653	14	NS

CHAPTER 3

ALLELOPATHY

3.1 INTRODUCTION

Many workers have observed that high submerged macrophyte populations have a suppressing action on phytoplankton (Embody 1928, Postolkova 1967, Brandl, Brandlova & Postolkova 1970, Goulder 1969, Brammer 1979). In many of the above cases this has been attributed to shading and/or depletion of nutrients or inorganic carbon from the water. However, Hasler & Jones (1949) quote Langhans as suggesting in 1928 that macrophytes secrete antibiotics which inhibit the growth of planktonic algae. Hasler & Jones (1949) also found a suppression of phytoplankton growth, but came to no conclusion as to the mechanism. Allelopathic effects have been suggested subsequently by other workers (Hogetsu, Okanishi & Sugawara 1960, Guseva & Goncharova 1965, and Kogan & Chinnova 1972), while Sand-Jensen (1977) suggested that epiphyte populations may also be controlled by substances released by their host.

Low phytoplankton crops were observed in the weedbed and enclosures in L. Fitty during the summer of 1979, and this experiment was designed to test whether or not, *Potamogeton perfoliatus*, the dominant submerged macrophyte in L. Fitty, had an allelopathic effect on *Scenedesmus quadricauda*, a test phytoplankton species present in L. Fitty.

3.2 MATERIALS AND METHODS

P. perfoliatus shoots from L. Fitty were grown in pots

containing a mixture of compost:sand, 1:1, with a thick (c. 1 cm) layer of washed sand at the top and bottom to reduce nutrient release from the compost. The shoots were grown in a glass container of de-ionised water for three weeks when the shoots were actively growing, then removed, the container cleaned and any dead leaves removed. The shoots were then placed in new de-ionised water for three weeks before the start of the experiment. No epiphytes were visible and phytoplankton chlorophyll was less than 1 $\mu g\ l^{-1}$.

A unialgal culture of S. quadricauda was grown in Bold's basal medium with micronutrients and vitamin B_{12} for five days when the culture was growing rapidly (visual observation). 1.5 cm^3 of culture was added to a 100 cm^3 conical flask containing the appropriate culture medium to give a final volume of 75 cm^3. The flasks were stoppered with long-fibre cotton wool bungs, covered with muslin, and continually agitated on an orbital shaker. They received continuous illumination from four 20W fluorescent tubes at a PFAD of 190 $\mu mol\ m^{-2}\ s^{-1}$. Temperature was not controlled, but was about 25°C.

Three different media bases were used; distilled water, water from the macrophyte growth container, and water from the macrophyte growth container which had been autoclaved at 20 lb in^2 for 20 minutes. All media bases were filtered through Whatman's GF/C glass fibre filter paper to remove particulate matter. Three strengths of nutrients were used based on Bold's basal medium in which S. quadricauda grew well, namely; full strength nutrients (= high nutrients), half strength nutrients (= medium nutrients) and quarter strength nutrients (= low nutrients). This gave nine treatments,

and all treatments were triplicated.

Every two days (except between days 20 and 27 when no measurements were made) 10 cm^3 of algal suspension was removed from each flask with a pipette in a sterile room, and placed in a numbered tube for counting. 10 cm^3 of the appropriate nutrient solution replaced that taken out. A Lund counting chamber, (Lund 1959, 1962) was used for counting cell numbers. Fifty fields (of 100 µm side) were counted since preliminary counts had shown this to give a good estimate of cell numbers.

The experiment lasted for 27 days.

3.3 RESULTS

The results presented in Figure 3.1 strongly suggest that there was no difference between the different media in their ability to support different amounts of S. quadricauda cells. The different nutrient levels did support different cell numbers although the initial increase in numbers was the same. There is a slight indication that the two types of water from the macrophyte container allowed better growth than did distilled water in the initial growth period.

The two way analysis of variance on the final cell numbers (Table 3.1) indicates no difference between the different media, but differences (at $P > 0.01$) between the two nutrient levels tested. The high nutrient level was not tested as one of the treatments became contaminated with blue-green algae.

3.4 DISCUSSION

In the enclosure experiment (Chapter 2), very low

phytoplankton crops were found within the P. filiformis bed during the period of macrophyte growth. Bioassays were carried out which suggested that increased crops could be obtained by adding PO_4-P and NO_3-N either singly or in combination. This suggested that an allelopathic effect of the macrophytes on the phytoplankton was not responsible for the low phytoplankton crops. Further, control flasks to which no nutrients were added did not show a marked increase, discounting a short-lived allelopathic effect. These conclusions are supported by the experiment presented in this Chapter with P. perfoliatus, the dominant submerged macrophyte in L. Fitty.

Conflicting evidence exists in the literature as to whether or not allelopathy is responsible for suppression of phytoplankton by macrophytes. Of the two submerged macrophytes used by Guseva & Goncharova (1965), Myriophyllum spicatum suppressed the growth of Anabaena, while P. perfoliatus stimulated it. Kogan & Chinnova (1972) found that Ceratophyllum demersum suppressed the growth of three Anabaena species when grown together, but a water extract from this macrophyte stimulated growth. Perhaps the strongest evidence for an allelopathic effect is that of Hogetsu et al. (1960) who found an increase in the growth of Chlorella in lake water in which macrophytes were dominant only when nutrients had been added, and the water heat-treated. No growth occurred if only one of these treatments was carried out. This suggests that part of the inhibition was the result of nutrient deficiency, but a heat-labile effect is also apparent. Fitzgerald (1969) found that under N-limitation, cultures of macrophytes suppressed the growth of phytoplankton and epiphytes. However, he also

found some evidence for another suppressing effect with the filamentous green alga Pithophora oedogonium in Chlorella pyrenoidosa and Microcystis aeruginosa, but not Ankistrodesmus sp. Tests suggested that this was caused by the presence of bacteria-sized organisms which had a selective toxicity to some of the algae.

The experiments on allelopathy reported to date between macrophytes and phytoplankton do not contain sufficient tests to show conclusively that any suppression of phytoplankton is not caused by for example, nutrient depletion. Bioassays would be useful here, rather than an analysis for P & N in the water as usually employed. Sterile conditions have not been attained (including the experiment reported here) and so any "allelopathic" effect may be the result of a more complex interaction between macrophytes, phytoplankton and bacteria or fungi as found by Fitzgerald (1969). The exception to the unsterile conditions is that of Berglund (1969) who found that organic compounds excreted by Enteromorpha linza stimulated the growth of two species of marine phytoplankton. This author was able to extract the organic compounds involved, and this procedure would be helpful in further experiments on allelopathy between macrophytes and phytoplankton.

Phillips, Eminson & Moss (1978) put forward a hypothesis to explain the decline of submerged macrophytes in waters receiving increasing loadings of nutrients. They attributed the decline to an initial increase in epiphytes and filamentous algae on the macrophytes, lowering the performance of the latter so that phytoplankton populations could become established and further outcompete the macrophytes for light. This hypothesis rests on the secretion of allelopathic

compounds by the macrophytes which suppress the phytoplankton but not the epiphytes. The only evidence they give for an allelopathic effect is that of Hogetsu et al. (1960). Recent work by Moss & Eminson (1979) and Moss (1981) has shown that certain species of algae are present and undergo cell division within both the plankton and the epiphyte community. It appears unlikely that a given species would be susceptible to an allelopathic compound when growing as a component of the plankton, but not when growing as an epiphyte. Further, as an epiphyte is close to the source of the secretions, it must experience higher concentrations than the plankton, particularly as the epiphytes probably grow within the unstirred or boundary layer surrounding the macrophyte, (Chapter 4).

Before an allelopathic effect of macrophytes on phytoplankton can be accepted, unequivocal evidence has to be presented both in the field and the laboratory. It is suggested that bioassays are a useful means of assessing whether or not nutrient competition can explain a low phytoplankton crop, and sterile conditions in the laboratory would enable bacterial effects to be eliminated.

It should be borne in mind that different species of macrophyte and phytoplankton may differ in their abilities to secrete and tolerate any allelopathic compounds. Macrophytes in unproductive lakes with low phytoplankton crops, as a result of nutrient deficiency, would be less likely *a priori* to secrete allelopathic compounds.

FIGURE 3.1 The effect on the growth of *Scenedesmus quadricauda* of media made in distilled water (○); water from a dense culture of macrophytes (△); and water from the dense macrophyte culture which had been autoclaved (□); at three nutrient levels. Each point is the mean of three.

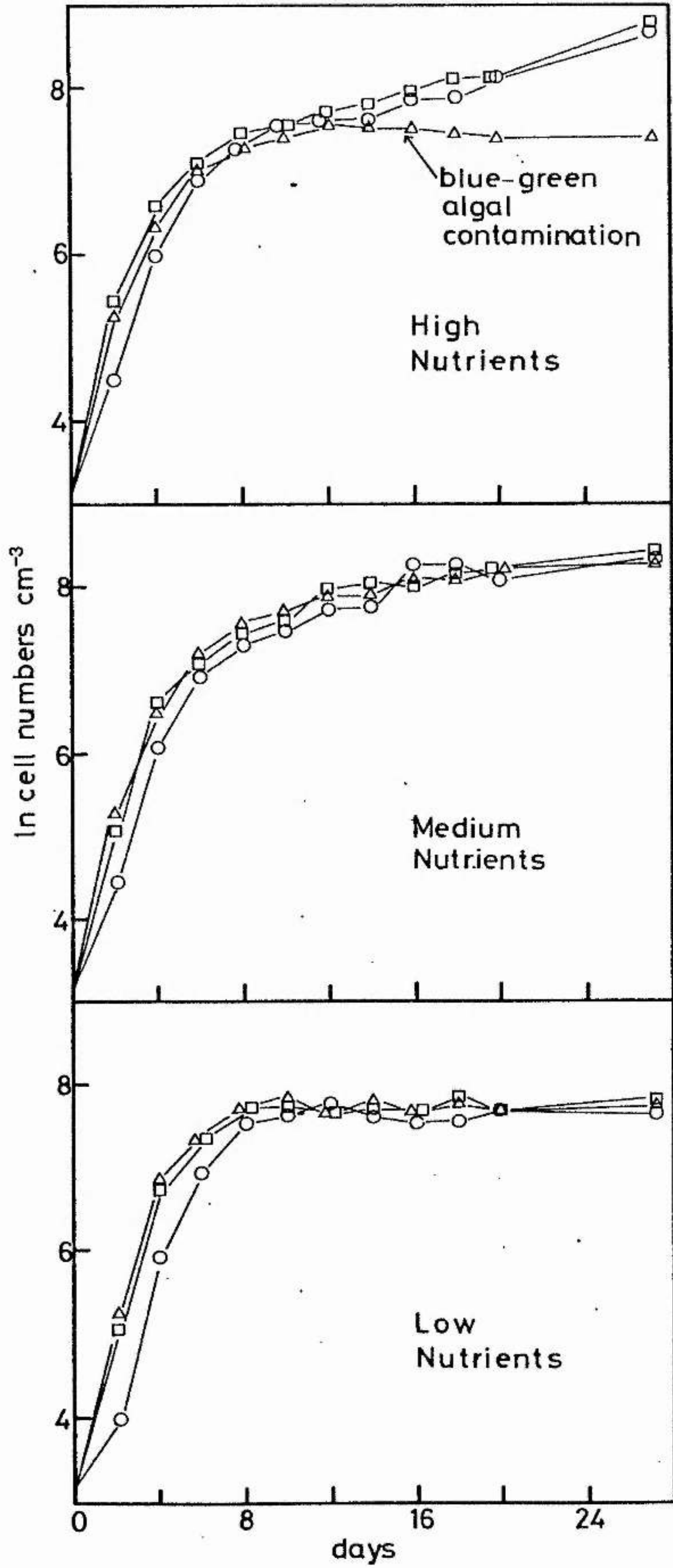

blue-green
algal
contamination
High
Nutrients
Medium
Nutrients
Low
Nutrients
ln cell numbers cm^{-3}
days
0
8
16
24
4
6
8

TABLE 3.1

2-way analysis of variance of cell numbers mm^{-3} on the final day (day 27). High nutrient level excluded because of contamination in one treatment.

Total sum of squares	=	5.36
Nutrient sum of squares	=	5.14
Media sum of squares	=	0.17
Interaction sum of squares	=	0.05

Nutrient F	=	184.1 df 1,2	$P > 0.01$
Media F	=	2.9 df 2,2	not significant

SUMMARY

The dense beds of submerged macrophytes in L. Fitty suppressed the growth of phytoplankton largely because of N and P deficiency (shown by bioassays of the natural phytoplankton populations). This was caused largely by uptake of nutrients (particularly NO_3-N) by the macrophytes (Chapter 2). The sediment is also responsible for some nutrient loss, and any benthic algae will also remove nutrients from the water. Shading and carbon depletion by the macrophytes may have reduced the rate of production of the phytoplankton, but bioassays showed this not to be important in causing the low phytoplankton crops.

High zooplankton densities in the weedbed were probably also responsible for reducing the phytoplankton crop. Enclosures receiving N and N & P (apart from one) showed increased zooplankton crops, indicating that phytoplankton production may have been increased in response to the nutrient additions, but the crop remained low as a result of the increased zooplankton. The open water, where predation of zooplankton by fish was more likely, had lower zooplankton densities and higher phytoplankton crops.

At the end of the macrophyte growing season, phytoplankton crops increased in most of the enclosures, probably as a result of nutrient release from the decaying macrophytes. In certain enclosures, the filamentous alga _Rhizoclonium hieroglyphicum_ became abundant at this time, again probably in response to nutrient release by the decaying macrophytes. This was not considered to be detrimental to the macrophytes as the growth of _Rhizoclonium_ occurred after the macrophyte growing season had finished.

The macrophyte standing crop was not affected by the nutrient addition, and any nutrient uptake from the water was probably a form of luxury consumption. The different enclosures positioned in an apparently homogenous weedbed, showed a large variability in response which was presumably caused by slight initial differences between the enclosures, changing the subsequent course of events.

No evidence for an allelopathic effect was found between the natural populations of macrophytes and phytoplankton in the enclosures, or between *P. perfoliatus* and *S. quadricauda* in the laboratory (Chapter 3).

SECTION B

CARBON COMPETITION

INTRODUCTION

Water in equilibrium with air at 15°C and normal pressures, has a CO_2 concentration of about 0.62 mg l^{-1} which is equivalent to about 14 µmol l^{-1}. The total amount of carbon in solution is usually greater, and at air-equilibrium approximately equals the alkalinity, which may range from 0.01-4.40 m equivalents l^{-1} (0.5-220.0 mg l^{-1}) in British lakes.

This may suggest that lakes have favourable inorganic carbon supplies for photosynthesis, but this is not so, for several reasons. First, a large amount of the total inorganic carbon may be in the form of HCO_3^- or $CO_3^=$ which is not available to all aquatic plants for photosynthesis. Second, rapid photosynthesis can quickly reduce the amount of inorganic carbon to below air-equilibrium levels and more importantly, shift the position of the carbonate system (see Chapter 5) so that the free CO_2 is virtually zero. In some species, HCO_3^- use allows photosynthesis under these conditions. Third, the diffusion of CO_2 in water is 10^4 times slower than in air, and the length of the diffusion pathway is comparatively long as a result of a boundary or unstirred layer which exists around every object in water. This may reduce photosynthetic rates under all but very high free CO_2 concentrations.

A controversy existed in the early seventies over the role of carbon in the 'eutrophication' of lakes (e.g. Kuentzel 1969, King 1970, Lange 1970, Kerr et al. 1972 and Schindler 1971). While it is unlikely that carbon is the major factor limiting plant yields in lakes, it may limit the rate of photosynthesis in those lakes with a high availability

of P & N, as carbon depletion in the field has been found by several workers (e.g. Talling 1976, Deuser 1970). Talling (1976) points out that under certain conditions, such as a short growing season and also where interspecific differences in carbon uptake properties occur, yield and rate limitation may amount to the same thing. Having established the existence of differences in uptake properties between macrophytes and certain phytoplankton species, Allen & Spence (1981) suggested that reduced macrophyte biomass may result from carbon competition with phytoplankton.

The work on the field enclosures (Chapter 2) was intended to study the effects of dense sustained crops on macrophytes, particularly with respect to carbon competition. In the event, large phytoplankton crops were not produced in the field, so the response of macrophytes to phytoplankton induced carbon competition was studied under laboratory conditions.

Estimates of the total resistance to CO_2 fixation in macrophytes and phytoplankton and the importance of the boundary layer in this resistance are presented in Chapter 4. The CO_2 and HCO_3^- compensation points of a range of macrophytes and phytoplankton are compared in Chapter 5, and the effects of a low PFAD (as would occur when dense phytoplankton crops are present) on these compensation points are examined in Chapter 6. Effects of season and leaf type on the compensation points of macrophytes are given in Chapters 7 and 8 respectively. Finally in Chapter 9, results are presented of a competition experiment which was designed to show whether or not phytoplankton are able to reduce macrophyte growth as a result of carbon competition.

CHAPTER 4

PHOTOSYNTHETIC RESISTANCES & RATES

4.1 INTRODUCTION

When different species or types of plants are competing for a resource which is in short supply, the type that can use the limiting resource most rapidly will be at an advantage. Carbon dioxide is used by macrophytes and phytoplankton in photosynthesis and can be depleted by their uptake to low levels which limit or prevent further net photosynthesis (see Chapters 5 and 9). Thus, differences in maximum uptake rates and resistances to CO_2 uptake will be an important factor in macrophyte/phytoplankton competition. The total resistance to CO_2 fixation will include chemical and diffusive components at subsaturating $[CO_2]$, whereas, at CO_2 saturation, the photosynthetic rate will be determined by the chemical resistance only.

There are few published measurements of total resistance to CO_2 fixation during photosynthesis in freshwater macrophytes or phytoplankton, let alone estimates of the relative importance of the diffusive and chemical components of such resistance. This situation exists in spite of two obvious differences between the aquatic and aerial environments for photosynthesis. First, an unstirred layer extends from the cell membrane to the bulk solution around the leaf or cell, where flow is laminar and solute transport diffusional. Even in well stirred water, the thickness δ, of such a layer must be greater than it is for a similar photosynthetic area in well mixed air.

Second, CO_2 diffuses 10^4 times more slowly in water than in air. These two facts suggest that in water, rates of CO_2 uptake will be low, particularly when boundary layers are thick, as occurs under poorly-stirred conditions. The boundary layer can develop to a far greater thickness around a large area compared with a small area and so macrophytes are more likely to be affected by its presence than the smaller phytoplankton.

Early work on resistances has been carried out on the Charophyta. Dainty & Hope (1959) showed that the diffusive resistance of the unstirred layer of water was large compared with that of the membranes in Chara australis, and Dainty (1963) noted the general importance of unstirred layers where solute absorption was rapid. Collander (1954) working with Nitella mucronata and Dainty & Grizburg (1964) with N. translucens and Chara corallina, demonstrated by different methods that up to 2-fold corrections to permeability constants were needed for some non-electrolytes (alcohols) because of the resistance of unstirred layers.

Allen & Spence (1981) have shown that the larger apparent K_m values for CO_2 displayed during photosynthesis by a range of submerged macrophytes relative to microalgae studied in the same conditions, were correlated with larger diffusion pathways and hence diffusive resistance to CO_2 fixation. Browse, Dromgoole & Brown (1979) examined photosynthesis by the macrophyte Egeria densa in well-stirred solutions, and concluded that the unstirred layer is the dominant resistance to CO_2 uptake. Smith & Walker (1980) reached the same conclusion for several other macrophytes from a re-assessment of published experimental evidence, particularly of Lucas (1975). Talling (1976) obtained

a value for total resistance to CO_2 uptake for the phytoplankter *Asterionella formosa* but did not separate diffusive and chemical resistances.

Black (1973) carried out photosynthetic ^{14}C uptake experiments by well-stirred leaf discs of four *Potamogeton* species at saturating irradiance. From her data for rates in low-CO_2 solutions, estimates have recently been made of total resistance to CO_2-fixation, and of the relative importance of the chemical and diffusive components of this resistance (Black, Maberly & Spence 1981). In the present study, using an O_2-electrode technique, similar measurements were made on whole leaves of two species of macrophyte and a suspension of cells of two phytoplankton species. Values for photosynthetic rates of macrophytes and phytoplankton under saturating light and CO_2 are compared, using values obtained from this thesis and the literature.

4.2 MATERIALS AND METHODS

4.2.1 ^{14}C uptake by leaf discs of four *Potamogeton* species

An outline only of this method is given, greater detail may be found in Black (1973) or Black, Maberly & Spence (1981).

Perennating parts of *P. lucens*, *P. perfoliatus*, *P. polygonifolius* and *P. praelongus* were grown in a greenhouse in St. Andrews. Plants were rooted in a soil-sand mixture and their above-ground parts submerged in regularly changed tap-water. Plants were grown under mercury vapour lamps, receiving 16h light every 24h.

Healthy plants provided the leaves used in every experiment which were freshly picked so that the rates of photosynthesis obtained were as near as possible to those that would have been

achieved by attached leaves. Tissue variability was reduced by using leaves of similar appearance and position on the plant.

Distilled water was flushed well with N_2 gas for about 30 minutes to reduce concentrations of O_2 and CO_2^*. The initial $[CO_2^*]$ was only considered in relation to solutions with very low concentrations of added CO_2. Carbon was added to the solution as $KHCO_3$. CO_2^* solutions were prepared by acidifying bicarbonate solutions to pH 4.3 with 1.0 mol l^{-1} HCl. At this pH and 20^oC, the temperature at which all experiments were run, only 1% HCO_3^- is in equilibrium with CO_2^*, so all the added carbon was assumed to be in the form of CO_2^*. 0.1 mmol l^{-1} $CaCl_2$ was added to all solutions, since according to Steemann-Nielsen (1947), it enhances photosynthesis in $KHCO_3$ solutions.

Photosynthesis was measured by determining the rate of uptake of ^{14}C by well-stirred, light-saturated leaf discs in acidified $KHCO_3$ solutions to which $NaH^{14}CO_3$ had been added. The leaves were cut into discs of 0.6 cm diameter and area for both sides of 0.56 cm^2. Measurements of leaf thickness of each species were made from transverse sections of leaf laminae. A 100 cm^3 conical flask was used as a reaction vessel. 20 leaf discs were placed in 100 cm^3 of bathing solution within the flask and allowed to equilibriate for 20 minutes before the radioactive solution was added. Photosynthesis was allowed to proceed for 1 hour, as initial experiments had established that photosynthetic carbon uptake was more or less linear over this period. The discs were stirred continuously with a magnetic stirrer. Light was supplied by 5 Osram spotlights each of 130 W, arranged around the thermostated reaction vessel. A saturating irradiance (400-700 nm) for leaves of all the species was found to be not more than 120 W m^{-2} (approximately 560 µmol m^{-2} s^{-1} PFAD),

and this was the level of irradiance used in the experiments. A dark fixation rate was obtained by treating 20 leaf discs in an identical manner but enclosing the reaction vessel in black polythene. Net carbon uptake rates were obtained by subtracting dark fixation rates from the total. Dark fixation rates were always less than 5% of the light fixation rates.

At the end of the photosynthetic period, discs were removed, rinsed in distilled water, stuck onto planchets and covered with 10% acetic acid to remove volatile carbon and dried. Radioactive emissions were counted in a Panax solid scintillation counter. Self-absorption by one leaf thickness was found to be negligible and so was ignored. Carbon uptake was calculated by knowing the specific activity of the bathing solution and taking into account that the rates of uptake of the two isotopes are not identical (Van Norman & Brown 1952).

4.2.2 O_2 Evolution by whole leaves of macrophytes or suspensions of phytoplankton

Hippuris vulgaris was obtained from L. Kilconquhar and grown in a greenhouse in St. Andrews receiving normal daylight. They were rooted in a soil-sand mixture and submerged in aerated tapwater. *Potamogeton polygonifolius* was collected from L. Na Craige, and grown for two weeks in 0.2 strength Bold's basal medium with 2.0 mmol l^{-1} $KHCO_3$ and bubbled continually with air. They received 24h lighting.

Both *Scenedesmus quadricauda* and *Chlamydomonas reinhardtii* were grown in Bold's basal medium with added micronutrients and 2.5% soil extract, and were bubbled with air continually. Cells were used from cultures at the end of the log phase of growth (visually estimated). The PFAD was 200 μmol m^{-2} s^{-1}, and a 16h light, 8h dark photoperiod was used.

Distilled water was deoxygenated and the $[CO_2^*]$ reduced by bubbling with oxygen-free nitrogen which was first bubbled through two solutions of 5.0 mol l^{-1} NaOH followed by bubbling through 1.0 mol l^{-1} H_2SO_4 and finally distilled water. A saturated solution of CO_2 was prepared by bubbling previously N_2-purged distilled water with CO_2 for 5-10 minutes in a measuring cylinder at 20°C. The concentration of CO_2 in solution was calculated from the barometric pressure using the equations in Allen (1977, p. 71). This saturated solution was diluted with low CO_2, (N_2-bubbled) distilled water to give an 80% saturated solution, and this was used to carefully fill the reservoir of an automatic bur ette (Radiometer ABU 1b).

Photosynthesis proceeded in a perspex chamber with inbuilt magnetic-stirrer designed by Allen (1977). Leaves, or a washed suspension of phytoplankton cells were placed in the chamber in the low-CO_2, deoxygenated distilled water with a pH adjusted to 6.0 with 1.0 mol l^{-1} HCl. O_2 evolution was measured with a polarographic O_2 sensor (Beckman 39553) connected to a meter (Beckman Field lab.) and recorded on a chart recorder (Heath-Schlumberger SR-255 A/B). The leaves or cells were kept at 20°C and received a PFAD of 500 μmol m^{-2} s^{-1} (400-700 nm). Once a steady trace had been achieved indicating no net photosynthesis, a small volume of the 80% CO_2 solution was injected into the chamber using a syringe needle passing through a suba-seal near the base of the chamber. A similar needle at the top of the chamber allowed the excess volume to be released. Once a steady photosynthetic rate had been attained, a further increment of CO_2 solution was added and the photosynthetic rate again recorded. This was repeated until further additions did

not increase the photosynthetic rate.

At the end of the experiment, leaf area was measured by photocopying and weighing the cut-out paper leaves. Area was obtained by comparing with a prepared standard curve. Areas are expressed for both sides of the leaf, as CO_2 was assumed to be taken up by both sides. Transverse sections of laminae were made to measure thickness. Total area of phytoplankton cells was measured by counting cells in a Lund chamber (Lund 1959, Lund 1962). The dimensions of 10 cells were measured with an eyepiece graticule to calculate the area of 1 cell.

4.2.3 CO_2 Kinetic curve using pH-drift

Elodea canadensis was collected from a pond in St. Andrews, and Myriophyllum spicatum originally collected in L. Fitty but grown in a greenhouse in St. Andrews receiving normal daylight, rooted in a sand-soil mixture and submerged in aerated tap-water. Chlamydomonas reinhardtii was grown in an aerated culture as described in the previous methods section.

The pH-drift technique was used to construct a CO_2^*-kinetic curve, full details of the pH-drift technique are given in Chapter 5. Varying $[CO_2]$ were produced by allowing the pH to drift between pH 6.9-7.5 in solutions of varying alkalinity: 1.0, 4.0 and 10.0 mmol l^{-1} $KHCO_3$ in the case of the macrophytes; 1.0 and 2.0 mmol l^{-1} $KHCO_3$ in the case of the phytoplankton. PFAD was saturating at 500 µmol m^{-2} s^{-1}, temperature constant at 20°C, and a high stirring speed used.

At the end of the experiment, chlorophyll was extracted in boiling 90% methanol by the method in 5.2.6.

4.3 RESULTS

4.3.1 Calculation of resistances

The diffusion of CO_2 into a photosynthesising cell will

obey Fick's first law, which relates the flux of a substance across an area to the diffusion coefficient of the substance, and to the steepness of the concentration gradient:

$$\underline{J} = \underline{D}\,\frac{\underline{C}}{\underline{L}} \qquad (1)$$

$\underline{J}$ = flux (pmol cm^{-2} s^{-1})

$\underline{D}$ = diffusion coefficient (cm^2 s^{-1})

$\underline{C}$ = concentration difference (pmol cm^{-3})

$\underline{L}$ = diffusion distance (cm)

In terms of photosynthesis (Raven 1970):

$$\underline{\emptyset}_{net} = \frac{\underline{D}(\underline{C}_o - \underline{C}_{chl})}{\underline{L}} \qquad (2)$$

where $\underline{\emptyset}_{net}$ = net rate of photosynthetic uptake of CO_2 (pmol cm^{-2} s^{-1})

$\underline{C}_o$ = CO_2 outside diffusion layer, i.e. in bulk phase of bathing medium (pmol cm^{-3})

$\underline{C}_{chl}$ = CO_2 at the site of carboxylation (pmol cm^{-3})

$\underline{D}$ = diffusion coefficient for CO_2 in water (0.16×10^{-4} cm^{-2} s^{-1})

The area term for the photosynthetic rate is that area across which diffusion is occurring; that is the leaf or cell surfaces.

Raven (1970) derives the following equation from (2) to calculate the total resistance to CO_2 uptake ($1/\underline{k} + 1/\underline{P}$):

$$\emptyset_{net} = \frac{\underline{C}_o}{1/\underline{k} + 1/\underline{P}} \qquad (3)$$

where $1/\underline{k}$ = chemical or reaction resistance, the reciprocal of the first order rate constant $\underline{k}$ (s cm^{-1})

$1/\underline{P}$ = diffusive resistance, the reciprocal of the permeability constant $\underline{P}$ (s cm^{-1})

This relationship holds true for values of $\underline{C}_o$ less than that value needed to half-saturate the photosynthetic rate, and only these values have been used here.

In steady state photosynthesis, the photosynthetic rate is equal to the concentration of CO_2 at the site of carboxylation ($\underline{C}_{chl}$) multiplied by the rate-constant ($\underline{k}$):

$$\underline{\phi}_{net} = \underline{k}.\underline{C}_{chl} \qquad (4)$$

The appropriate photosynthetic rate here is strictly the gross rate, rather than the net rate which $^{14}CO_2$ uptake appears to have measured and which O_2 evolution measures. There are no estimates of rates of photorespiration although the low ambient oxygen concentrations mean that these were probably low. Use here of net photosynthetic rates means that $1/\underline{k}$ will have been overestimated but $1/\underline{k}$ cannot be precisely calculated anyway, because values for $\underline{C}_{chl}$ are themselves based on estimates:

$$1/\underline{k} = \frac{\underline{C}_{chl}}{\underline{\phi}_{net}} \qquad (5)$$

$$1/\underline{P} = \frac{\underline{C}_o - \underline{C}_{chl}}{\underline{\phi}_{net}} \qquad (6)$$

$$1/\underline{P} + 1/\underline{k} = \frac{\underline{C}_o}{\underline{\phi}_{net}} \qquad (7)$$

The diffusive resistance term, $1/\underline{P}$, can be divided into two resistances: that caused by diffusion through the unstirred or boundary layer outside the plasmalemma of the leaf epidermis or the phytoplankton cell, and that caused by diffusion through the plant to the site of carboxylation. These are related in the following equation:

$$1/\underline{P} = \underline{L}/\underline{D} = \underline{\delta}/\underline{D} + \underline{l}/\underline{D} \qquad (8)$$

where $\underline{\delta}$ = length of diffusive pathway through the boundary layer

$\underline{l}$ = length of diffusive pathway within the leaf.

The concentration gradient in the boundary layer will be linear but, within the plant, photosynthetic CO_2 uptake will cause the concentration gradient to be curved (Fig. 4.1). The actual shape of the curve will depend on which of the terms $1/\underline{k}$ and $1/\underline{P}$ is the larger. For the leaves, the calculation of the average diffusive pathway inside the leaf ($\underline{l}$) was performed assuming that this distance is a quarter of the leaf thickness, or halfway into the leaf from each side. For phytoplankton, the average internal pathlength was calculated to be a quarter of the cell width, for each face of the cell, weighted for the contribution of each face to the total area of the cell. This will only be correct if the CO_2 concentration gradient within the leaf or cell is linear which is not true, particularly when $1/\underline{k} < 1/\underline{P}$ (Fig. 4.1). This effect may be offset by cytoplasmic streaming within the cells which would tend to equalise $[CO_2]$ internally. As the data show that the internal

diffusive resistance is a small part of the total resistance (1.0-4.3%) slight errors in the estimation of this pathlength will only have a small effect on the values obtained.

In estimating the values of $1/\underline{P}$, no adjustment has been made for the diffusive resistance to CO_2 of the cell membranes; water and CO_2 are held to penetrate the membranes readily (Nobel 1970, and see discussion and references in Raven 1970).

The average $[CO_2]$ at the site of carboxylation ($\underline{C}_{chl}$) cannot be measured directly but a value for this term is needed to solve equations (5) and (6). Its range is set at the upper limit by C_o, the concentration of CO_2^* in the bathing solution, and at the lower limit by $\underline{\Gamma}$, the CO_2 compensation point. The relative importance of $1/\underline{k}$ and $1/\underline{P}$ to the total resistance will determine whether $\underline{C}_{chl}$ is near the upper or lower regions of its possible range (Fig. 4.1 a,b & c). An idea of the range of possible values of $1/\underline{k}$ and $1/\underline{P}$ can be obtained by making $\underline{C}_{chl}$ equal the lowest and highest possible value and thus calculating $1/\underline{k} + 1/\underline{P}$ from equations (5) and (6). The lowest $\underline{\Gamma}$ value is taken to equal 3×10^3 pmol cm^{-3} (66.4 $\mu l\ l^{-1}$) for the leaf discs of the four Potamogeton species. This is within the range of values for terrestrial C3 plants and similar to values found for freshwater macrophytes (Black 1973, Bowes et al. 1977, Raven & Glidewell 1978 and Allen & Spence 1981).

4.3.2 Measurements of total resistance are given in Tables 4.1a and 4.1b. Tables 4.2a and 4.2b present values for the total resistance $1/\underline{k}$ and $1/\underline{P}$ as an average of the values in Tables 4.1a & b. Also given are values for $\underline{l}$ and the internal diffusive resistance ($\underline{l}/\underline{D}$), calculated from measurements of leaf or cell thicknesses. Estimates of $1/\underline{k}$, $1/\underline{P}$ and $\delta/\underline{D}$ are calculated for different values of $\underline{C}_{chl}$ and expressed as actual amounts and

as a percentage of the total resistance. Calculated values for the boundary layer thickness (δ), and mean leaf or cell width are also given.

Table 4.3 gives values for $1/k$, estimated using equation (10), for whole leaves of H. vulgaris and P. polygonifolius and a suspension of cells of C. reinhardtii. Table 4.4 presents rates of net photosynthesis at saturating CO_2 and light, on a chlorophyll basis for various species of macrophyte and phytoplankton obtained from the literature and this thesis.

4.4 DISCUSSION

4.4.1 Methodology

^{14}C uptake and O_2 evolution techniques have been used to measure net photosynthetic rates. However, Black (1973) found net O_2 evolution measured using the Winkler technique (Golterman 1969) and ^{14}C uptake to be alike (at 16.9 and 17.1 pmol cm^{-2} s^{-1} respectively) during a field experiment under natural light intensities. In the present experiment with macrophytes involving O_2 measurements, the leaf discs used by Black (1973) were replaced by whole leaves to allow a better estimate of the diffusive resistances involved in comparisons with phytoplankton cells.

4.4.2 Total resistance

The mean total resistance to CO_2 fixation for leaf discs of four Potamogeton species ranged from 3.7×10^3-5.9×10^3 s cm^{-1} for P. praelongus and P. perfoliatus respectively. P. lucens (4.7×10^3 s cm^{-1}) and P. polygonifolius (4.4×10^3 s cm^{-1}) had intermediate values (Table 4.2a); the average total resistance was 4.7×10^3 s cm^{-1}. The latter species is only able to use CO_2 while the three other species are capable of

using HCO_3^-, but at higher pH values than those used here (Black 1973). The total resistance of whole leaves of P. polygonifolius was found to be 11.2×10^3 s cm^{-1} (Table 4.2b). This difference will be caused in part by the greater width of the whole leaves, (1.4 compared to 0.6 cm diameter for the leaf discs), so increasing the boundary layer thickness. The different techniques used, and the fact that the plants for the whole-leaf experiments may not have been very photosynthetically active as they were collected in December, may also play a part. Browse, Dromgoole & Brown (1979) estimated the total resistance in water for Egeria densa at 4.0×10^2 s cm^{-1} while they found a value of 4.7×10^2 s cm^{-1} if photorespiration was taken into account; these are substantially less than the values reported here. A part of the discrepancy may be accounted for by these workers' apparent underestimation of resistance, because they used 2-3 shoots of 7 nodes each in their experiments, but calculated photosynthetic rate on a leaf area basis and ignored possible stem uptake.

The total resistance of 3.7×10^3-11.2×10^3 s cm^{-1} found for submerged macrophytes is much greater than values found for terrestrial macrophytes. For example, Jones & Slatyer (1972) estimated the total resistance of Gossypium hirsutum to be 3-4 s cm^{-1}, while Nobel, Zaragoza & Smith (1975) found the total resistance to be 16-59 s cm^{-1} for Plectranthus parvifolius. Total resistance in Impatiens parviflora ranged from 16-98 s cm^{-1} calculated using data from Rackham (1966). Snelgar, Green & Wilkins (1981) working with six lichen species found a total resistance of 30-70 s cm^{-1} at intermediate thallus water contents. These rose to about 300-400 at low and high thallus

water contents. The total resistance of the two phytoplankton species studied were 3.3×10^2 s cm^{-1} for S. quadricauda and 2.3×10^2 s cm^{-1} for C. reinhardtii (Table 4.2b). This is similar to 3.1×10^2 s cm^{-1} found by Talling (1976) for Asterionella formosa. These are slightly greater than for terrestrial macrophytes, but much less (11-50 times) than for aquatic macrophytes.

Therefore, when $[CO_2]$ determines the photosynthetic rate of both macrophytes and phytoplankton, the latter will have a greater photosynthetic rate on a surface area basis.

4.4.3 Separation of $1/k$, $1/P$, δ/D, & l/D

The difference in total resistance between macrophytes and phytoplankton could be caused by differences in the chemical resistance ($1/k$), or the internal (l/D) and boundary layer (δ/D) resistances which make up the diffusive resistance ($1/P$); therefore an attempt has been made to partition the total resistance into its component parts. Table 4.2a shows that in the case of Potamogeton leaf discs, $1/P$ is always greater than $1/k$ for the possible range of values for C_{chl}, making up on average 99.5%, 94.7%, and 74.0% of the total resistance where $C_{chl} = \Gamma$, $10 \times \Gamma$, and $50 \times \Gamma$ respectively. The highest C_{chl} taken was slightly greater than the lowest $[CO_2^*]$ used, so for the lowest concentrations at least, C_{chl} must have been less than this value. This is less clear for the whole leaf data (Table 4.2b) but even here, $1/P$ makes up 90-97% of the total resistance if C_{chl} is near the compensation point. The values of $1/P$ for S. quadricauda and C. reinhardtii are much lower than those for the macrophytes, but make up a similar proportion of the total.

It is suggested that an exact measurement of $1/\underline{k}$, and hence of the other resistances, may be made at the CO_2 compensation point. Here, by definition, there is no net photosynthesis and therefore no net influx of CO_2. As a result, no diffusion resistances ($\underline{\delta}/\underline{D}$ and $\underline{l}/\underline{D}$) are involved, and equation (7) is reduced to:

$$1/\underline{k} = \underline{C}_o / \not{\phi}\text{net} \tag{9}$$

At the CO_2^* CP ($\underline{\Gamma}$), $\underline{C}_o = \underline{C}_{chl} = \underline{\Gamma}$, and strictly the photosynthetic rate is the gross rate, which at $\underline{\Gamma}$ equals the respiration rate. Therefore:

$$1/\underline{k} = \underline{\Gamma}/\underline{R} \tag{10}$$

where: $\underline{\Gamma} = CO_2^*$ CP (pmol cm^{-3})

$\underline{R}$ = respiration rate (pmol cm^{-2} s^{-1})

Using the O_2 evolution data, and dark respiration rates obtained at the end of the experiments, $1/\underline{k}$ has been calculated (Table 4.3). As no estimate of photorespiration was made, any contribution of this to total respiration will have resulted in an overestimation of $1/\underline{k}$. The values for $1/\underline{k}$ produced by this method are lower than the values in Table 4.2 a & b as a result of using gross rather than net photosynthesis. They suggest however, that the true value for $1/\underline{k}$ lies near the lower limit of the possible range of values in Tables 4.2 a & b, and therefore the average $\underline{C}_{chl}$ is close to the CO_2^* CP. It follows from this that $1/\underline{k}$ only makes up a small part of the total resistance, the bulk resulting from the boundary

layer resistance δ/D.

Estimates of $1/k$ for Potamogeton leaf discs ranged from 19-1501 s cm^{-1}, with the most reasonable estimate being a mean of the values for the two lower possible values for C_{chl}, namely 133 s cm^{-1}. Using equation (10), whole leaves of H. vulgaris had a similar $1/k$ at 125 s cm^{-1}, while P. polygonifolius had a higher $1/k$ at 519 s cm^{-1} (Table 4.3), possibly because it was winter tissue. Browse et al. (1979) calculate $1/k$ to be 38 s cm^{-1} for E. densa, lower than that found in this study. Terrestrial macrophytes have lower chemical resistances; Jones & Slatyer (1972) give 0.2-0.3 s cm^{-1} for Plectranthus parviflora. Collins & Farrar (1978) estimate $1/k$ as 48 s cm^{-1} for the lichen Xanthoria parietina, although this value has been criticised as being too high (Snelgar et al. 1981). The two phytoplankton species studied here had values for $1/k$ intermediate between terrestrial and aquatic macrophytes at an average of 50 s cm^{-1} for the two lower values of C_{chl} (Table 4.2b). This may be an overestimate of $1/k$ as, using equation (10), C. reinhardtii has a $1/k$ of 7.5 s cm^{-1}, considerably less than for the macrophytes.

Rackham (1966) concludes that for Impatiens parviflora, $1/k$ is greater than $1/P$, and this conclusion is supported by Raven (1970) for terrestrial C_3 plants. Browse et al. (1979) found $1/P$ to constitute 90% of the total resistance in E. densa and they further concluded that diffusion through the boundary layer made the largest part of $1/P$. This finding is supported by the results presented here which indicate that even in well-stirred conditions, $1/P$ makes up 66-99% of the total resistance for leaf discs of macrophytes and the internal diffusive resistance only makes up 2.6-4.4% of the total, the remainder of

$1/\underline{P}$ is made up by the boundary layer resistance. These conclusions agree with the recent review by Smith & Walker (1980). For the two phytoplankton species studied it also appears that, again as a result of the boundary layer resistance (δ/D), $1/P$ is larger than $1/k$, although the magnitude of the boundary layer resistance is much smaller. Again, the internal diffusive resistance is only a small percentage of the total resistance.

4.4.4 Boundary layer thickness

The thickness of the boundary layer of leaf discs ranged from 210-459 µm (Table 4.2a). The average value for the middle C_{chl} is 340 µm. This was greater for whole leaves of H. vulgaris and P. polygonifolius at an average of 440 µm. These values are larger than 30-150 µm found for Chara by Walker, Beilby & Smith (1979) in well-stirred conditions and, using an optical method, by Green & Otori (1970) working with rabbit cornea, who found a thickness of 65 µm under well-stirred and 350 µm under still conditions. Browse et al. (1979) give a value of 46-51 µm for δ in E. densa; however, this value may be affected by the arrangement of the shoot system they used, and by their apparent overestimation of the photosynthetic rate (see earlier comment, p. 88). Different methods of stirring may partly explain the discrepancies found between their and these estimates of δ. Wheeler (1980) showed the boundary layer to vary with both water speed and distance from the leading edge of a blade of the giant kelp Macrocystis pyrifera. For a position 25 cm along the blade, δ was estimated to be 200 µm at high current speeds (7 cm s^{-1}) but as thick as 800 µm at lower current speeds (2 cm s^{-1}).

δ is a function of the area and shape of a leaf as well as

the degree of water movement. One can compare the diameter of the leaf discs used here to the shortest linear dimension, the width, of whole leaves of the four Potamogeton species recorded in nature. The widths range from 1.0-3.0 cm in *P. polygonifolius*, 1.0-4.0 cm in *P. perfoliatus*, 2.0-4.5 cm in *P. praelongus*, and 2.5-6.0 cm in *P. lucens* (Clapham, Tutin & Warburg 1962), so the disc diameter of 0.6 cm lies between 10% and 60% of the greatest and least recorded leaf width of any of these species. If the resistance varies as the square of the leaf width (Zelitch 1971), and an average of 340 µm is taken for δ, then for leaf widths of 1-6 cm, δ would be about 440 and 1090 µm respectively. These are considerably greater than the δ values estimated for the phytoplankton with an average of 22 µm for *S. quadricauda*, and 14 µm for *C. reinhardtii*, (average of the lower 2 values for ϱ_{chl}), Table 4.2b.

4.4.5 Photosynthetic rates at light and CO_2 saturation

At saturating CO_2 concentrations, the rate of diffusion across the boundary layer and within the plant no longer limits the photosynthetic rate, so the only resistance in operation is the chemical resistance $1/k$. Table 4.4 indicates that net photosynthetic rates for a unit of chlorophyll are slightly higher for phytoplankton than macrophytes, as would be expected from the lower $1/k$ values for the former (given equivalent chlorophyll/ surface area ratios). Fig. 4.2 also shows the greater photosynthetic rate at saturating CO_2. At low $[CO_2^*]$ (less than 0.3 mmol l^{-1}) the differences between rates are great, presumably as a result of the greater boundary layer resistance in macrophytes. This agrees with data in Allen & Spence (1981) in indicating that the saturating $[CO_2^*]$ and $K_{\frac{1}{2}}$ concentration is greater in

macrophytes than in phytoplankton as a result of a greater diffusion resistance, but disagrees with Lloyd et al. (1977) whose techniques have been criticised by Browse et al. (1979). Since low $[CO_2^*]$ are more common than very high $[CO_2^*]$ in lakes, phytoplankton would be able to attain considerably higher rates than macrophytes under these conditions.

4.4.6 Ecological considerations

Westlake (1967) has shown for several aquatic angiosperms, and Wheeler (1980) for macroalgae, that compared with still conditions, water flow increases photosynthetic rates. Macrophyte beds in swift flowing rivers may represent well-mixed conditions, but these will not always exist for macrophytes in lakes, particularly on those shores where macrophyte colonisation extends below the wave-mixed zone (Spence 1981). It is at such depths, as much as 6m in clear water (Spence 1976) that species like P. praelongus habitually root and photosynthesise for much of the year, while evergreen plants of low stature, such as some species of Chara and Nitella, exist there all year. In this environment, the relative importance of boundary layer resistance as a rate limiting factor in photosynthesis will increase. Several mitigating factors may exist. First, some enhancement of photosynthetic rates by HCO_3^- uptake may occur at sufficiently high alkalinity and pH (Allen & Spence 1981). Second, depletion of CO_2 below air-equilibrium is unlikely to occur in deep water because of low photosynthetic demand at low light intensities, and possible respiratory release of CO_2^* from the sediments. Third, at very low light intensities such as may be found near the depth limits of macrophyte colonisation in lakes, photosynthetic rates may be so low that diffusion through the boundary layer is not limiting.

Under most conditions it would appear that for submerged macrophytes $1/\underline{P}$ is greater than $1/\underline{k}$. The leaves of submerged macrophytes are thin, varying from 120 µm in sun leaves of P. polygonifolius down to 26 µm in shade leaves of P. obtusifolius (Spence & Chrystal 1970). These are well below that for a typical shade leaf of a terrestrial species, e.g. 170 µm in Fraxinus excelsior (Gabrielsen 1948). Submerged plants also have large specific leaf areas; a low value like 475 cm^2 g^{-1} in sun leaves of P. polygonifolius (Spence & Chrystal 1970) compares with 350 cm^2 g^{-1} in sun leaves of Impatiens parviflora (Coombe 1966), while the highest, 2050 cm^2 g^{-1} in shade leaves of P. obtusifolius, compares with 1450 cm^2 g^{-1} in shade leaves of I. parviflora.

The idea that the thin leaves of certain aquatic macrophytes represent an adaptation to aid diffusion of CO_2 and HCO_3^- (Hutchinson 1975) appears unlikely because, even if the leaves of the four Potamogeton species studied were twice as thick, the total resistance would be only about 3% greater. Since, moreover, even submerged sun leaves are relatively thin and have relatively high specific leaf areas, these characteristics are unlikely to result solely as a response to shade. Chlorophyll content on a leaf area basis (1 sided) is also very low in submerged leaves; compare 24 and 13 µg cm^{-2}, respectively for sun leaves of P. polygonifolius and shade leaves of P. obtusifolius (Spence & Chrystal 1970) with 66 and 44 µg cm^{-2} in sun and shade leaves of F. excelsior (Gabrielsen 1948) or 45 µg cm^{-2} in Nicotiana tabacum var. John William's Broadleaf (Okabe, Schmid & Straub 1977).

A more likely interpretation of this combination of thin leaves, high specific leaf area and low chlorophyll content per

unit leaf area is that, as diffusion through the boundary layer causes the greatest resistance to photosynthesis, a large photosynthetic capacity per unit leaf area is unnecessary and would presumably carry a high metabolic cost to maintain. In deep water, low light intensities are coupled with diffusion through large boundary layers, providing an environment with a very low supply of the two primary photosynthetic substrates, light and carbon. Linear leaved associes, common in shallow water, are normally viewed as a response to the turbulent environment in which they grow. However, these species, plus myriophylloid forms such as Myriophyllum spicatum also have a shape of leaf, which by virtue of its small width, will have a thinner boundary layer than that of a wide leaf.

The boundary layer may also affect other processes. For instance, most macrophytes are able to obtain large quantities of nutrients from the sediment; however the water is also a potential source of nutrients and shoot uptake has been shown to occur (Denny 1980); particularly for nitrate-nitrogen (Nichols & Keeney 1976). The boundary layer around the shoot will cause a resistance to the uptake of nutrients in the same way that it does for CO_2.

Shoots covered with a dense epiphyte population will probably have a different thickness of boundary layer from an epiphyte-free shoot. The epiphyte population may either increase the boundary layer by slowing the movement of water above the macrophyte, or decrease it by causing turbulence, as Wheeler (1980) found for leading edge spines on blades of Macrocystis pyrifera. In either case a significant proportion of the epiphytes will be situated within the boundary layer of the macrophyte. This will surely affect macrophyte-epiphyte

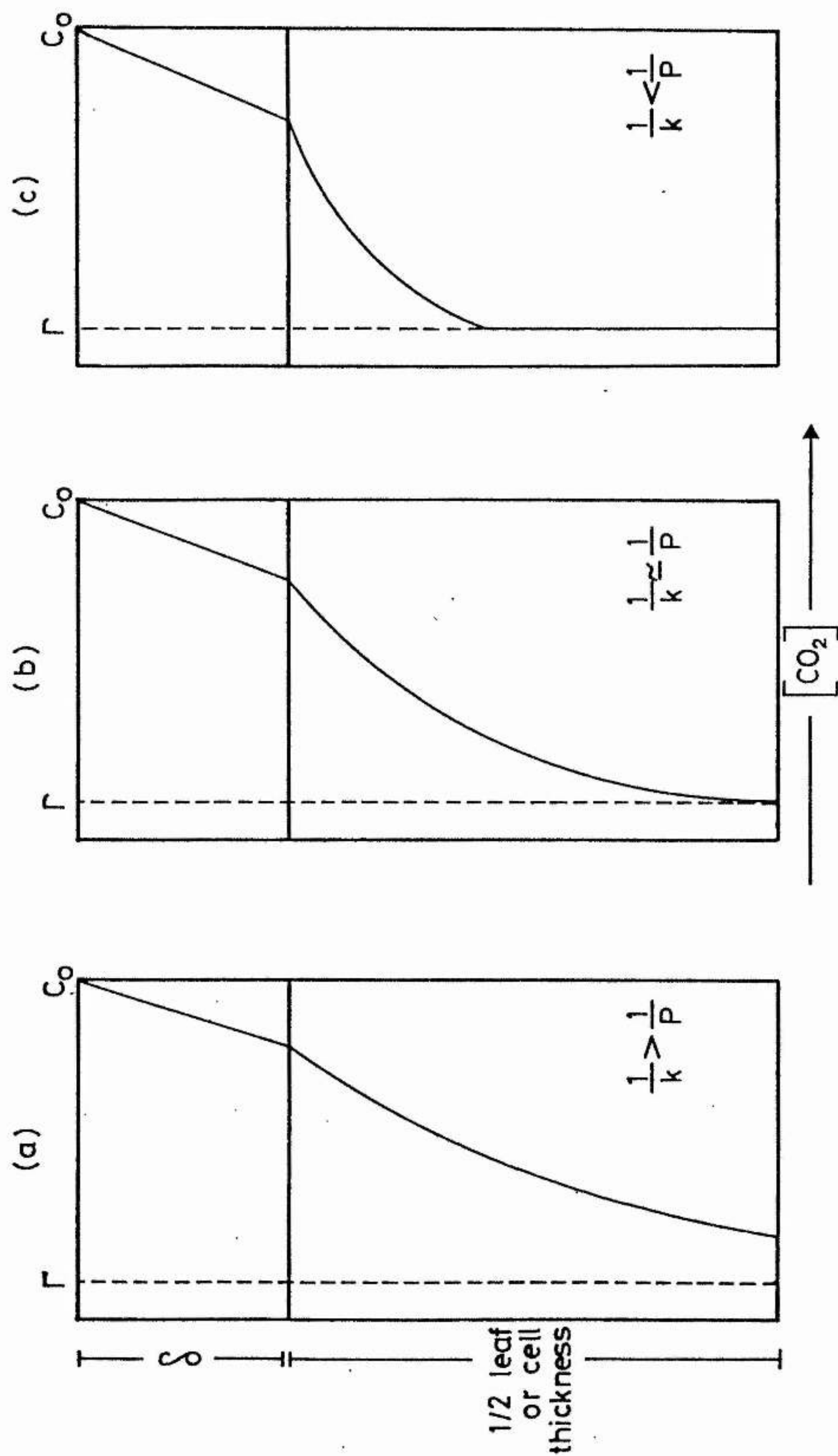
interactions particularly with respect to competition for carbon, nitrogen and phosphorous.

Thus, regardless of the higher affinity some phytoplankton species have for HCO_3^- as an alternative carbon source (Chapter 5), phytoplankton are likely to be able to outcompete macrophytes for carbon because of their relatively low resistance to CO_2-uptake, caused largely by a smaller boundary layer through which CO_2 has to diffuse.

FIGURE 4.1 Diagram to illustrate possible variation in the CO_2 concentration gradient in a leaf or cell, as diffusive or chemical resistance predominates. Only a $\frac{1}{2}$ leaf or cell thickness is shown.

(a) $1/k > 1/P$; (b) $1/k \simeq 1/P$; (c) $1/k < 1/P$; $C_0 = [CO_2]$ in bathing solution

$\Gamma = CO_2$ compensation point.



(a)
(b)
(c)
Co
Γ
δ
1/2 leaf or cell thickness
[CO2]
1/k > 1/P
1/k ≃ 1/P
1/k < 1/P

FIGURE 4.2 Photosynthetic rate as a function of CO_2^* at 20°C and saturating PFAD, 500 μmol m^{-2} s^{-1} and fast-stirring for a named species of phytoplankton and two named species of macrophyte. Results obtained from pH-drift experiments at varying alkalinity. Arrow indicates $[CO_2^*]$ when water is in equilibrium with air. Dotted lines indicate $K_{\frac{1}{2}}$.

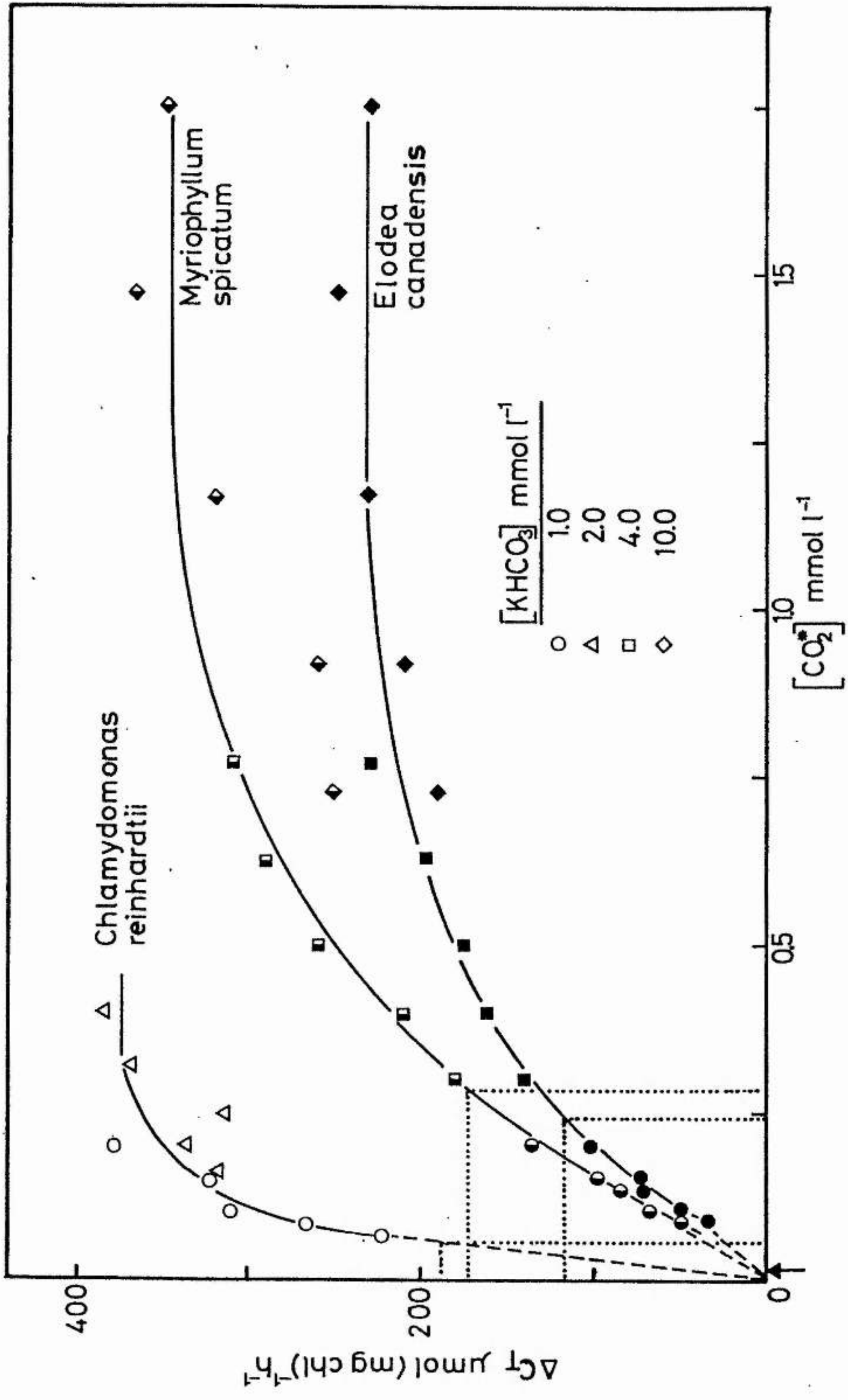

Chlamydomonas reinhardtii
Myriophyllum spicatum
Elodea canadensis
[KHCO3] mmol l-1
1.0
2.0
4.0
10.0
[CO2*] mmol l-1
0
0.5
1.0
1.5
ΔCT µmol (mg chl)-1h-1
0
200
400

TABLE 4.1a

Rate of net photosynthetic carbon uptake (± s.e.) in solutions of varying $[CO_2^*]$ at pH 4.3 by leaf-discs of four *Potamogeton* species, and calculated values of total resistance ($1/\underline{k} + 1/\underline{P}$). Leaf discs 0.6 cm diameter; area refers to both sides of the leaf. Under well-stirred conditions, with irradiance (400-700 nm) of 120 W m^{-2} ($\simeq$ 560 µmol m^{-2} s^{-1}) and temperature 20°C. Calculated from data of Black (1973).

species	$[CO_2^*]$ pmol cm^{-3} x 10^{-4}	rate of C uptake pmol cm^{-2} s^{-1}	$1/\underline{k} + 1/\underline{P}$ s cm^{-1}
P. lucens	100	194.3 ± 5.8	5147
	80	189.0 ± 9.0	4233
	50	124.4 ± 5.0	4019
	30	64.7 ± 3.6	4637
	10	19.0 ± 1.4	5263
P. perfoliatus	100	172.4 ± 13.9	5800
	80	146.4 ± 6.1	5464
	50	77.2 ± 6.1	6476
	10	8.8 ± 1.1	(11364)
P. polygonifolius	80	171.0 ± 20.8	4678
	50	144.4 ± 12.2	3763
	40	85.1 ± 6.8	4700
	30	80.1 ± 6.5	3745
	10	19.0 ± 1.4	5263
P. praelongus	100	327.5 ± 13.1	3053
	80	272.5 ± 18.8	2936
	50	96.5 ± 9.2	5181
	30	80.6 ± 11.1	3722
	10	26.9 ± 2.8	3717

TABLE 4.1b

Rate of net photosynthetic oxygen evolution in solutions of varying $[CO_2^*]$ at pH 6.0 by whole leaves of two macrophyte species, or suspensions of two phytoplankton species, and calculated values of total resistance (1/k + 1/P). Well-stirred conditions, PFAD (400-700 nm) 310 µmol m^{-2} s^{-1}, and temperature 20°C.

Species	CO_2^* pmol cm^{-3} x 10^{-4}	rate of O_2 evolution pmol cm^{-2} s^{-1}	$1/\underline{k} + 1/\underline{P}$ s cm^{-1}
Hippuris vulgaris	6.7	8	8400
	13.3	16	8300
Potamogeton polygonifolius	6.7	5	13400
	13.3	15	8900
Scenedesmus quadricauda	6.6	200	300
	13.1	400	330
	19.7	600	330
Chlamydomonas reinhardtii	0.9	49	180
	2.0	74	270

TABLE 4.2a

Average total resistance ($1/\underline{k} + 1/\underline{P}$) for leaf discs of four Potamogeton species. $1/\underline{k}$, $1/\underline{P}$, $\underline{l}/\underline{D}$ and $\delta/\underline{D}$, expressed as resistances and percentages of $1/\underline{k} + 1/\underline{P}$, calculated for a range of possible values of C_{chl}. The CO_2^* CP (Γ) is taken to equal 3×10^3 pmol cm^{-3} (66.4 $\mu l\ l^{-1}$). Values are also given for $\underline{l}$, the internal diffusive pathlength, and δ, the boundary layer thickness, both for one side of a leaf.

species	value taken for C_{chl}	C_{chl} (pmol $cm^{-3} \times 10^{-3}$)	average total resistance $1/\underline{k} + 1/\underline{P}$ (s cm^{-1})	s cm^{-1}				% $1/\underline{k} + 1/\underline{P}$				µm	
				$1/\underline{k}$	$1/\underline{P}$	$\underline{l}/\underline{D}$	$\delta/\underline{D}$	$1/\underline{k}$	$1/\underline{P}$	$\underline{l}/\underline{D}$	$\delta/\underline{D}$	$\underline{l}$	δ
P. lucens	$\Gamma \times 1$	3	4660	25	4625	196	4439	0.5	99.5	4.2	95.3	15.7	355
	$\Gamma \times 10$	30	4660	254	4406	196	4210	5.5	94.5	4.2	90.3	15.7	337
	$\Gamma \times 50$	150	4660	1268	3392	196	3196	27.2	72.8	4.2	68.6	15.7	256
P. perfoliatus	$\Gamma \times 1$	3	5913	23	5890	159	5731	0.4	99.6	2.7	96.9	12.7	459
	$\Gamma \times 10$	30	5913	227	5686	159	5527	3.8	96.2	2.7	93.5	12.7	442
	$\Gamma \times 50$	150	5913	1136	4777	159	4618	19.2	80.8	2.7	78.1	12.7	369
P. polygonifolius	$\Gamma \times 1$	3	4430	30	4400	113	4287	0.7	99.3	2.6	96.7	9.0	343
	$\Gamma \times 10$	30	4430	300	4130	113	4017	6.8	93.2	2.6	90.6	9.0	321
	$\Gamma \times 50$	150	4430	1501	2929	113	2816	33.9	66.1	2.6	63.6	9.0	225
P. praelongus	$\Gamma \times 1$	3	3722	19	3703	159	3544	0.5	99.5	4.3	95.2	12.7	284
	$\Gamma \times 10$	30	3722	187	3535	159	3376	5.0	95.0	4.3	90.7	12.7	270
	$\Gamma \times 50$	150	3722	933	2789	159	2630	25.1	74.9	4.3	70.7	12.7	210

TABLE 4.2b

Average total resistance ($1/k + 1/P$) and $1/k$, $1/P$, l/D, and δ/D expressed as resistance and percentages of $1/k + 1/P$, calculated for a range of possible values of C_{chl}. The CO_2^* CP (Γ) is derived from pH-drift data, (see Chapter 5). Values are also given for l, the internal diffusive pathlength, and the boundary layer thickness, both for one side of a leaf; and mean leaf or cell width.

				s cm^{-1}				% $1/k + 1/P$						
SPECIES	value taken for C_{chl}	C_{chl} pmol cm^{-3} x 10^{-3}	average $1/k + 1/P$ (s cm^{-1})	$1/k$	$1/P$	l/D	δ/D	$1/k$	$1/P$	l/D	δ/D	l	δ	leaf width (mm) or cell width (µm)
Hippuris vulgaris	Γ x 1	3	8350	250	8100	113	7987	3	97	1	96	18	639	
	Γ x 10	30	8350	2500	5850	113	5737	30	70	1	69	18	459	2 mm
	Γ x 20	60	8350	5000	3350	113	3237	40	60	1	59	18	259	
Potamogeton polygonifolius	Γ x 1	10.7	11150	1070	10080	93	10173	10	90	1	89	15	813	14 mm
	Γ x 10	107	11150	10900	450	93	357	96	4	1	3	15	29	
Scenedesmus quadricauda	Γ x 1	3.4	330	9	321	13	308	3	97	4	93	2	25	
	Γ x 10	34	330	85	245	13	232	26	74	4	70	2	19	6 µm
	Γ x 20	68	330	170	160	13	160	52	48	4	44	2	12	
Chlamydomonas reinhardtii	Γ x 1	0.6	225	10	215	3	212	5	95	1	94	1	17	
	Γ x 10	6	225	96	129	3	126	43	57	1	56	1	10	2 µm
	Γ x 20	12	225	195	30	3	30	87	13	1	12	1	2	

TABLE 4.3

Chemical resistance ($1/\underline{k}$) estimated from dark respiration rate (R) and CO_2^* CP (Γ) using: $1/\underline{k} = \Gamma/\underline{R}$ (equation 10).

Species	Γ pmol $cm^{-3} \times 10^{-3}$	$\underline{R}$ pmol cm^{-2} s^{-1}	$1/\underline{k}$ s cm^{-1}
Hippuris vulgaris	3.0	24.0	125
Potamogeton polygonifolius	10.7	20.6	519
Chlamydomonas reinhardtii	0.6	80.0	7.5

1. Badger, Kaplan & Berry (1980)
2. Birmingham & Colman (1979)
3. Browse, Dromgoole & Brown (1979)
4. Findenegg (1976)
5. Kaplan (1981)
6. Lloyd, Canvin & Bristow (1977)
7. Lloyd, Canvin & Culver (1977)
8. Maberly, this thesis (1981)
9. Osmond, Valanne, Haslam, Uotila & Roksandic (in press)
10. Sondergaard (1979)
11. Van, Haller & Bowes (1976)
12. Weber, Tenhunen, Yocum & Gates (1979)

TABLE 4.4

Rates of net photosynthesis at saturating light and $[CO_2^*]$ for a variety of macrophytes and phytoplankton.

species	net photosynthesis µmol (mg chl)$^{-1}$ h^{-1}	temperature °C	measurement	references
Berula erecta	95-100	15	O_2 electrode	9
Callitriche stagnalis	140-175	15	O_2 electrode	9
Ceratophyllum demersum	58	30	I.R.G.A.	11
Egeria densa	100	20	I.R.G.A.	3
Elodea canadensis	180	26	O_2 electrode	12
" "	235	20	pH-drift	8
Hippuris vulgaris	40	15	O_2 electrode	9
Hydrilla verticillata	54	30	I.R.G.A.	11
Littorella uniflora	84	8	gas chromotography	10
Myriophyllum spicatum	20	25	I.R.G.A.	6
" "	51	30	I.R.G.A.	11
" "	345	20	pH-drift	8
Potamogeton amplifolius	20	25	I.R.G.A.	6
Anabaena variabilis	380	30	O_2 electrode	5
Chlamydomonas reinhardtii	380	20	pH-drift	8
" "	320+	25	O_2 electrode	1
Chlorella pyrenoidosa	125	25	I.R.G.A.	7
Navicula pellicosa	280	25	I.R.G.A.	7
Scenedesmus obliquus	320	25	O_2 electrode	4
"Variety of F.W. algae"	40-250	20-28	flame ionisation	2

CHAPTER 5

CO_2^* AND HCO_3^- COMPENSATION POINTS

5.1 INTRODUCTION

5.1.1 Chemical aspects

Lakes contain salts in solution, the most abundant of which are carbonates, such as $CaCO_3$ and $MgCO_3$. The total base concentration of lake water is termed the alkalinity, and this can vary widely e.g. from 0.01-4.40 m equivalents l^{-1} in British lakes. The aqueous carbon present in a lake is derived from carbonates dissolved from rocks, and CO_2 dissolved from the air. The aqueous carbon can take the form of four chemical species linked by the following chemical equilibria:

$$CO_2 \text{ (aqueous)} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^= + 2H^+ \quad (1)$$

The free CO_2 consists of CO_2 (aqueous) which is dissolved CO_2 gas, and H_2CO_3. Since H_2CO_3 only makes up $1/650^{th}$ of the free CO_2 (Stumm & Morgan 1970), the term CO_2^* is used following Allen (1977), unlike Stumm & Morgan who used $H_2CO_3^*$.

The total carbon (C_T) is made up of CO_2^*, HCO_3^-, and $CO_3^=$, and when the lake water is in equilibrium with the air, the concentration of these species is about the same as the alkalinity. At increasingly alkaline pH, an increasing proportion of the alkalinity is made up of OH^- with a consequent decrease in C_T as described by:

$$\text{Alkalinity} = \left[HCO_3^-\right] + 2\left[CO_3^=\right] + \left[OH^-\right] - \left[H^+\right] \quad (2)$$

The pH also controls the position of the equilibrium between the different aqueous carbon species (1), by changing the ionisation fractions (Stumm & Morgan 1970):

$$\alpha_0 = \left(\frac{K_1}{[H^+]} + 1 + \frac{K_1 \ K_2}{[H^+]^2} \right)^{-1} \qquad (3)$$

$$\alpha_1 = \left(\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]} \right)^{-1} \qquad (4)$$

$$\alpha_2 = \left(\frac{[H^+]^2}{K1 K_2} + 1 + \frac{[H^+]}{K_2} \right)^{-1} \qquad (5)$$

where: α_0 = ionisation fraction for CO_2^*

α_1 = ionisation fraction for HCO_3^-

α_2 = ionisation fraction for $CO_3^=$

$K_1 = [H^+] \ [HCO_3^-] \ / [CO_3^-]$

$K_2 = [H^+] \ [CO_3^=] \ / [HCO_3^-]$

the values for K_1 and K_2 are corrected for the total ionic strength of the solution.

The $[C_T]$ is calculated from the following equation:

$$[C_T] = \frac{\text{Alkalinity} \ - \ [OH^-] + [H^+]}{\alpha_1 + 2\ \alpha_2} \qquad (6)$$

Therefore, equations 3-6 allow the calculation of the concentrations of C_T and all its component species, from a knowledge of the pH, alkalinity, temperature and total ionic strength of the solution. Allen (1977) has calculated this for solutions of $KHCO_3$ of alkalinity ranging from 0.1-10.0

mmol l^{-1}, and these detailed tables have been used in all calculations from pH-drift data.

5.1.2 Physiological and biochemical aspects

The aquatic environment differs from the terrestrial in having three forms of inorganic carbon which may be available for photosynthesis; namely CO_2^*, HCO_3^- and $CO_3^=$. In addition, carbamino carboxylic complexes may occur in alkaline water, and Smith, Tatsumo & Hood (1960) have shown this to be a photosynthetic carbon source for some phytoplankton . All the aquatic plants that have been studied use CO_2^* as a carbon source. In addition, certain species have also been shown to use HCO_3^-, reviewed by Raven (1970) , including both phytoplankton e.g. Scenedesmus quadricauda (Österlind 1950) and macrophytes e.g. Myriophyllum spicatum (Steemann-Nielsen 1947). The aquatic bryophytes as a group appear to be unable, or poorly able, to use HCO_3^-, e.g. Fontinalis antipyretica (Steemann-Nielsen 1947). Among the 20 species of bryophytes tested by Bain & Proctor (1980), only Anthoceros husnotii showed slight HCO_3^- use. Allen & Spence (1981) suggest that the moss Eurhynchium rusciforme is also able to use HCO_3^-, and show that a gradient exists between HCO_3^- users and non-users. Felföldy (1960) suggested that the two phytoplankton species Coelastrum microsporum and Chlorococlaster terrestris may also be able to use $CO_3^=$, although this idea has not been confirmed by other workers. Indeed, Lucas (1975) has suggested that high concentrations of $CO_3^=$ may inhibit HCO_3^- uptake.

The mechanism of carbon fixation in aquatic plants appears to be basically by the C_3 pathway. This includes all the algae tested, which were from the Chlorophyceae,

Cyanophyceae, Chrysophyceae, and Rhodophyceae, (references in Raven 1970). Although Raven & Glidewell (1978) found physiological characteristics similar to C_4 plants in _Hydrodictyon africanum_, this species fixes carbon via the C_3 pathway. Most of the macrophytes studied are also C_3, (Hough & Wetzel 1972, Stanley & Naylor 1972, Browse, Dromgoole & Brown 1977, Winter 1978). In photosynthetic ^{14}C uptake experiments with _Elodea canadensis_, De Groote & Kennedy (1977) found a significant amount of labelled C_4 acids, but pulse and chase experiments showed that they were not produced by the normal C_4 pathway. However, Holaday & Bowes (1980) suggest that _Hydrilla verticillata_ does not fit into either C_3 or C_4 category. Keeley (1981) and Keeley et al. (1981) have found that two species of _Isoetes_ show dark fixation and diurnal malic acid fluctuations similar to the crassulacean acid metabolism of terrestrial plants. In general though, C_3 fixation appears to predominate (Smith & Walker 1980).

5.1.3 Ecological aspects

At 15°C and normal air pressures, water in equilibrium with air containing 0.003% CO_2 by volume, will have a concentration of CO_2^* of about 0.62 mg l^{-1} which is about 14 µmol l^{-1}. However, as a result of rapid photosynthetic uptake by phytoplankton and/or macrophytes, carbon deficits can occur, causing a rise in pH and a decrease in $[CO_2^*]$. This depletion is unlikely in an unproductive lake or in a lake with a high alkalinity and hence a large buffering capacity. In lakes of high productivity, but relatively low buffering capacity, the greatest carbon depletion may occur (Allen 1977). When the $[CO_2^*]$ is low, HCO_3^- uptake would obviously be advantageous.

Carbon competition between macrophytes and phytoplankton may be important in determining the relative success of these two groups under conditions of carbon depletion.

This chapter presents data on CO_2 and HCO_3^- use by a range of macrophyte and phytoplankton species to supplement that already obtained by Allen & Spence (1981). A few of the results presented here are from pH-drift experiments used for other purposes e.g. effect of light intensity on carbon-compensation points, which may be found in Chapters 6-8.

5.2 MATERIALS AND METHODS

5.2.1 Collection sites

Myriophyllum spicatum (23.viii.80), Potamogeton filiformis (6.vi.80), P. perfoliatus (16.vii.80), P. x zizii broad leaves (11 & 22.viii.80) and linear leaves (24.viii.80) were collected from L. Fitty. Chara sp. (23.vi.80), Elodea canadensis (28.i.81 & 10.vi.81), P. crispus (27.vi.80 & 28.i.81) and P. praelongus (30.v.80) were collected from L. Drumore. Littorella uniflora (13.viii.80) and P. polygonifolius (11.viii.80) were collected from L. Na-Uala. Submerged shoots of Hippuris vulgaris (26.viii.80) were collected from L. Kilconquhar. Submerged leaves of Nuphar lutea (28.viii.80) were collected from Black Loch. Linear and broad submerged leaves of P. natans (20 & 22.ix.80) were collected from L. Caladail. Ranunculus sp. (14.viii.80) were collected from Long Loch (14.viii.80).

5.2.2 Growth conditions

P. filiformis was occasionally grown from turions, and H. vulgaris from small underground buds. The former species was grown in liquid culture containing 1.0 mmol l^{-1} $KHCO_3$ and 0.2 x Bold's basal medium. The medium was bubbled continually

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and kept at 20°C. Lighting was 16h out of 24h at a PFAD of 270 µmol m^{-2} s^{-1}. Three week old shoots were used. H. vulgaris was rooted in a sand-compost mixture and the shoots placed in regularly changed, aerated tap-water. They were grown in a greenhouse receiving normal daylight.

Phytoplankton were grown in Bold's basal medium with added micronutrients and 2.5% soil extract. They were aerated continually, unless stated otherwise. They received 16h light every 24h at a PFAD of 200 µmol m^{-2} s^{-1} and were grown at room temperature.

The species used were: Anabaena cylindrica, Chlamydomonas reinhardtii, Cosmarium botrytis and Scenedesmus quadricauda.

5.2.3 Photosynthetic measuring apparatus

All measurements were made in the perspex chamber designed by Allen (1977), details of which can be found in Allen & Spence (1981). Briefly, this consisted of two vertical concentric cylinders of perspex with a magnetic bar at the base to provide stirring. Two ports allowed entry for pH and oxygen electrodes. The total volume of the chamber was 170 cm^{3}. A constant temperature of 20°C was maintained by placing the chamber in a cylindrical perspex water-bath supplied with water pumped from an external constant temperature bath containing a cooler and thermostated heater, and allowed to flow back by gravity. One to five 150 W tungsten reflector bulbs positioned vertically above the chamber provided the lighting. The PFAD could be altered by varying the number of bulbs turned on, and by interposing neutral-density filters between the lights and the chamber. A small fan was used at the height of the lights to help disperse the heat produced by the bulbs. Aluminium foil was placed at a distance of about 25 cm around the chamber to

increase PFAD and to produce a more uniform light field. Above this, black polythene was used to prevent interference from external light sources. This was particularly important for work at low PFAD's, as presented in Chapter 6. PFAD was measured outside the chamber at the base with a Macam quantum meter sensitive to photosynthetically available wavelengths of 400-700 nm (Macam Q 101). PFAD was high and saturating or near-saturating at 220-500 µmol m^{-2} s^{-1}.

pH was measured with a combination pH electrode (Radiometer GK 2403C) connected to a Radiometer specific ion meter (PHM 53) and recorded on a chart recorder (Heath-Schlumberger SR-255 A/B) or occasionally on a digital integrator (Doric Digitrend 2000). On occasions, dissolved oxygen was measured with an oxygen sensor (Beckman 39553) connected to a Beckman Fieldlab oxygen meter (1008), and recorded on the chart recorder. The pH electrode was calibrated against two buffers of approximately pH 7.0 and pH 9.2 in the chamber at 20°C with the appropriate temperature corrections used for the buffers.

5.2.4 Photosynthetic measurements

Solutions of $KHCO_3$ were made up prior to use from a stock of 125 mol l^{-1} $KHCO_3$ in N_2-bubbled distilled water to reduce the oxygen concentration. The starting pH could be lowered by addition of a small volume (generally less than 1 cm^3) of the appropriate strength of $KHCO_3$ solution which had been bubbled with CO_2 for several seconds.

No pretreatment was given to macrophytes or phytoplankton. Only healthy macrophyte tissue with no visible epiphyte contamination was used. The shoots were rinsed gently under a running tap to remove any debris, shaken dry, and placed in a small volume of the appropriate strength $KHCO_3$ solution, shaken

dry again, and placed in the $KHCO_3$ solution in the chamber. The phytoplankton suspension was concentrated as necessary by gently centrifuging (1,000-2,000 r.p.m.) one or several times. After the last supernatant had been discarded, an appropriate solution of $KHCO_3$ was added and the phytoplankton centrifuged, the supernatant discarded and the phytoplankton added to the chamber.

Plant material was left for about 15 minutes in dim light (laboratory lighting) to allow equilibriation of both plant and temperature. The chart recorder was turned on and a steady or slightly declining trace was produced. The lights were then turned on and the pH change recorded until a final pH was reached. This was determined when no increase in pH had occurred for at least one hour. Once this had been achieved, the pH was normally stable for many hours. Occasionally an immediate decrease in pH occurred; in which case the experiment was discarded. At the end of a successful experiment the plant material was removed from the chamber and the amount estimated as either dry weight (90°C for 24h) or as chlorophyll, (5.2.6).

5.2.5 Calculation of C_T uptake rate and CO_2^* and HCO_3^- CP's

C_T uptake rates were calculated from the record of pH change over time by measuring the time taken for the pH to increase by 0.1 pH unit. The tables in Allen (1977) allowed the calculation of the change in $[C_T]$ and therefore a photosynthetic uptake rate could be obtained from a knowledge of this, the chamber volume, and the amount of plant material present. This uptake rate was plotted against the $[C_T]$ at which the rate occurred. This plot takes two general forms; first, a straight line, which Allen & Spence (1981) have suggested indicates CO_2^* use only, the interception of the

x-axis representing the CO_2^* CP; and second a two-phase plot consisting of an initial linear portion which can be extrapolated to yield a CO_2^* CP, and then a further section of different slope, which Allen & Spence (1981) take to be the section of the plot denoting HCO_3^- use. The intersection of this section with the x-axis represents the apparent HCO_3^- CP.

5.2.6 Determining of chlorophyll

The methanol extraction method of Golterman et al. (1978) was used. Part or all of the macrophyte, or a known volume of the phytoplankton suspension which was filtered on Whatman's GF/C glass fibre paper, was ground in a pestle and mortar in a small volume of 90% methanol with a little acid-washed sand and Mg CO_3 to prevent acidity. The slurry was washed quantitatively into a glass vial to give a final volume of approximately 10 cm^3. This mixture was brought to boiling for 10 seconds in a water-bath in dim light, and then removed and allowed to extract and cool in the dark for 10 minutes. The exact volume was noted and the suspension filtered through the GF/C filter paper and the absorbance read in a Beckman GB/GD spectrophotometer at 663 nm and 750 nm (turbidity correction). A factor of 13.9 recommended by Talling & Driver (1963) was used to calculate chlorophyll concentrations.

5.2.7 Discussion of pH-drift technique

The pH-drift technique relies on the fact that any carbon (e.g. CO_2 or HCO_3^-) taken up by the plant is replaced by OH^- in order to maintain a constant alkalinity. This causes the pH to rise, shifting the equilibrium position between the various forms of aqueous carbon. Therefore, a plant photosynthesising in a closed system experiences a decline in $[C_T]$, and an accompanying change in the proportions of CO_2^*, HCO_3^- and $CO_3^=$.

This technique appears to give consistent CO_2^* CP values. Allen & Spence (1981) report similar CO_2^* CP's for E. canadensis and Fontinalis antipyretica in $KHCO_3$ solutions of 0.5, 1.0, and 2.0 mmol l^{-1}. A second technique, producing curves of net photosynthesis against pH in a solution of constant total carbon concentration, give CO_2^* CP's in close agreement with those obtained from the pH-drift results (Allen & Spence 1981). In E. canadensis and Chlorella emersonii, both of which use HCO_3^-, the results from pH-drifts at different alkalinities gave similar estimations of the apparent HCO_3^- CP.

The technique could be criticised on the grounds of the increasing $[O_2]$ produced as carbon is taken up. However, this is the situation found in the field, when extensive carbon depletion occurs as a result of rapid photosynthesis (Allen 1977). Thus, CO_2^* and HCO_3^- CP's measured at this high $[O_2]$ are likely to be relevant to the performance of a species under natural conditions.

5.3 RESULTS

Figures 5.1-5.3 show plots of C_T uptake rates against concentrations of CO_2^*, HCO_3^-, $CO_3^=$, and C_T for a CO_2^* user, (L. uniflora), a very poor HCO_3^- user, (winter grown E. canadensis), and a very good HCO_3^- user (S. quadricauda). The normal plot of uptake against $[C_T]$ gives a straight line for L. uniflora (Fig. 5.1), indicating CO_2^* use only. This is validated by the plot against $[CO_2^*]$ which is also linear. The plot against $[HCO_3^-]$ shows a very rapid reduction in uptake for a very small decrease in $[HCO_3^-]$ suggesting that this carbon species is not being used directly. The $[CO_3^=]$ is very low throughout the pH range studied and so is unlikely to be important.

The plot of uptake against $[C_T]$ gives a two phase graph for E. canadensis (Fig. 5.2) indicating overlapping CO_2^* and HCO_3^- uptake. This interpretation is supported by the plot against $[CO_2^*]$ which shows that at low $[CO_2^*]$, faster photosynthetic rates occurred than would be predicted if only CO_2^* was being used. This argument is similar to that of the classic method used to determine HCO_3^- uptake, namely a comparison of photosynthetic rates at low and high pH at the same $[CO_2^*]$. If greater rates are obtained at high pH, this is attributed to additional HCO_3^- uptake. The pH-drift allows a more sophisticated way of showing this, as a complete record of photosynthetic rates can be gained over a range of pH values. The plot against $[HCO_3^-]$ shows a rapid reduction in photosynthetic rate for a small change in concentration, which represents the CO_2^* uptake portion of the graph. This is followed by a fairly constant HCO_3^- uptake rate over a range of HCO_3^-.

The results for S. quadricauda (Fig. 5.3) give even more convincing evidence for HCO_3^- uptake. A rapid reduction in uptake rate occurs over a range of $[CO_2^*]$ followed by a large increase in rate at $[CO_2^*]$ at or near zero. This increase in rate is most likely to be the result of HCO_3^- uptake. At the high pH values which S. quadricauda may attain in 1.0 mmol l^{-1} $KHCO_3$ solutions, the $[CO_3^=]$ is high, with a maximum of about 0.3 mmol l^{-1}. It is possible that this high concentration does inhibit HCO_3^- uptake; however at very high pH, the rate of uptake decreases rapidly at a pH when the $[CO_3^=]$ is also decreasing. The usefulness of the C_T uptake / $[C_T]$ plot partly results from the fact that between pH 6-8, which is the region of CO_2^* uptake, the change in $[C_T]$ is largely a result of changing $[CO_2^*]$. At pH values greater than about 8, the reduction in $[C_T]$ is

paralleled by a reduction in $[HCO_3^-]$. Further validation of the technique is given in Figure 4.2 where CO_2^* kinetic curves, similar to those produced by Allen & Spence (1981) using a different technique, were obtained from pH-drift experiments between pH 6.9-7.5 at different alkalinities.

Figures 5.4-5.6 show rates of C_T uptake against $[C_T]$ for twelve species of macrophyte and four species of phytoplankton. In many of the results for macrophytes, only the HCO_3^- portion of the uptake plot has been obtained, and therefore no measure of their CO_2^* CP can be given. In general, HCO_3^- users can be identified by their ability to reduce the $[C_T]$ to below the alkalinity concentration, given on each graph. It is interesting to note that the four species of Potamogeton which use HCO_3^- (Fig. 5.4), all have a maximum HCO_3^- uptake rate at a $[C_T]$ of about 0.45 mmol l^{-1} which is at a pH of about 9.2 at this alkalinity.

In macrophyte/phytoplankton competition, the final $[C_T]$ will be of importance. Table 5.1 summarises pH-drift data from this thesis with additional results from Allen (1977) and Talling (1976). The species are ranked according to their ability to remove carbon from solution, which is expressed as the ratio $[C_T]$ / alkalinity. A low ratio shows that little C_T remains after photosynthetic uptake. It is evident that there is a middle portion of the ranking where the uptake abilities of macrophytes and phytoplankton overlap. However, the four most effective species at removing C_T are phytoplankton, while the eight least effective species are macrophytes. For some HCO_3^- users CO_2^* CP's have been calculated from intercept data, and these are given in Table 5.2 in order of increasing compensation point. No overall differences between macrophytes

and phytoplankton appear, as would be expected since in these HCO_3^- users, the HCO_3^- CP would be more critical in competition.

Figure 5.7 shows HCO_3^- uptake curves (shaded) for two species of macrophyte and two species of phytoplankton. These are calculated from the difference in rate between the C_T uptake rate and the extrapolated CO_2^* uptake rate where HCO_3^- and CO_2^* uptake rates overlap, and the C_T uptake rate in the HCO_3^- "tail" portion of the graph. The extent of the overlap is fairly small at a $[C_T]$ of about 0.05 mmol l^{-1} for the two macrophytes, 0.085 mmol l^{-1} for C. botrytis and 0.02 mmol l^{-1} for S. quadricauda. At the alkalinity of 1.0 mmol l^{-1} used, this overlap occurs in the following pH ranges: 7.7-8.6 for E. canadensis; 8.1-8.8 for P. filiformis; 7.7-9.0 for C. botrytis; and 8,2-8.6 for S. quadricauda. This fairly rapid change from CO_2^* to HCO_3^- fixation is in disagreement with Findenegg (1976) who found HCO_3^- uptake even in acidic medium with high $[CO_2^*]$ in S. obliquus. Only in S. quadricauda is the HCO_3^- uptake rate appreciable; if one assumes a maximum CO_2^* and light saturated rate of 380 μmol (mg chl)$^{-1}$ h^{-1} (see Table 4.4 and Fig. 4.2) then the maximum HCO_3^- rate is 38% of this rate, compared to 7% for C. botrytis, a relatively poor HCO_3^- user (Table 5.1). If a maximum CO_2^* and light saturated CO_2^* uptake rate for macrophytes is taken as 235 μmol (mg chl)$^{-1}$ h^{-1}, as found for E. canadensis (Table 4.4, Fig. 4.2) then the HCO_3^- uptake rates for E. canadensis and P. filiformis represent 1% and 4% of this rate respectively.

The effect of $[O_2]$ on the CO_2^* CP is presented in Table 5.3 for a submerged shoot of Hippuris vulgaris grown in a greenhouse in St. Andrews. The $[O_2]$ was varied by starting at different pH values, and therefore different amounts of oxygen were

evolved before a final pH was reached. The results show an approximate linear increase in CO_2^* CP with increasing $[O_2]$, evidence for the occurrence of photorespiration. As pointed out in the Methods section (5.2.7), high $[O_2]$ normally accompany low CO_2 in the field and therefore, by allowing these to develop in the pH-drift experiments, conditions similar to those where carbon competition occurs in the field will be created.

In the field, macrophytes consist of both the shoots used in the present experiments and the roots and rhizomes in the substratum, which will cause a respiratory burden to the whole plant. Figure 5.8 shows the results of a pH-drift experiment with P. filiformis, consisting of shoot, root and turion, or shoot alone. The photosynthetic rate expressed on a shoot dry weight basis, shows that the whole plant has a lower rate and higher CO_2^* and HCO_3^- CP's.

Figure 5.9 shows a possible case of adaptation to HCO_3^- in Potamogeton crispus collected from L. Drumore in winter when the pH was 7.55 and the alkalinity 0.61 m equivalent l^{-1}. Under these conditions, HCO_3^- uptake would probably not occur. The pH-drift result suggests that as photosynthesis proceeded and the CO_2^* was depleted to near the CO_2^* CP, of 6.5 µmol l^{-1}, a small net respiration rate occurred, lasting for about 7 hours. After this time, photosynthetic HCO_3^- uptake proceeded until an apparent HCO_3^- CP of 0.68 mmol l^{-1} was reached, giving a C_T/alkalinity ratio of 0.830 in the 1.0 mmol l^{-1} solution used. This is greater than the ratio of 0.417 attained by summer grown plants (Table 5.1). A similar induction period has been reported to be necessary before HCO_3^- uptake will proceed in other species (e.g. Smith 1968). Chapter 7 presents more

detailed data on the seasonal variation in CO_2^* and HCO_3^- CP's in two species of macrophyte.

Different CO_2^* and HCO_3^- CP's are found for S. quadricauda grown in aerated and non-aerated culture (Fig. 5.6(d), Table 5.1). The aerated cells had a CO_2^* CP of 5.5 μmol l^{-1} compared to 1.5 μmol l^{-1} for the non-aerated cells, while HCO_3^- CP varied from 10 μmol l^{-1} for the aerated to 60 μmol l^{-1} non-aerated cells respectively.

5.4 DISCUSSION

The CO_2^* CP's of macrophytes ranged from 1.4 μmol l^{-1} (31 μl l^{-1}) for broad and linear leaves of P. x zizii, to 22.9 μmol l^{-1} (513 μl l^{-1}) for L. uniflora. The CO_2^* CP for the phytoplankton varied from 0.5 μmol l^{-1} (11 μl l^{-1}) for C. reinhardtii to 8.7 μmol l^{-1} (195 μl l^{-1}) for A. cylindrica. This range is much greater than that found in the terrestrial environment. Certain of the aquatic species have lower CO_2^* CP's at low pH than those found for terrestrial C_3 plants (Krenker, Moss & Crookston 1975) despite their putative C_3 fixing mechanism. This may be a result of a CO_2 concentrating mechanism (Raven & Glidewell 1978), not operating by HCO_3^- uptake.

One third of the species for which pH-drift data are available (Table 5.1) were unable to use HCO_3^- under the test conditions. Of the macrophytes, these fall into two broad groups; first, those such as H. vulgaris and N. lutea which are able to obtain CO_2 from the air via emergent shoots or floating leaves; and second, those such as L. uniflora, P. polygonifolius and P. natans which grow in unproductive lakes where CO_2^* depletion to below air-equilibrium levels is unlikely, and so HCO_3^- uptake unnecessary. The latter two species may also

possess floating leaves. Fontinalis antipyretica is an exception as it may be found in lakes which experience carbon depletion (e.g. Esthwaite Water pers. obs.) and aerial CO_2 is not available to it. F. antipyretica is a species of low stature, and Spence (1967) has suggested that it may benefit from locally high $[CO_2^*]$ as a result of respiratory release from the sediment. This may also apply to Nitella flexilis, which is also of low stature, and further tends to grow in lakes of high alkalinity which are consequently well buffered against carbon depletion.

Two-thirds of the species in Table 5.1 were able to use HCO_3^-, and for these, the CO_2^* CP is probably not of importance in macrophyte/phytoplankton competition. HCO_3^- uptake is considered to be an active, light-activated, process involving photosystem 2 (Raven 1968). HCO_3^- ions are taken up, dehydroxylated in the cytoplasm to form CO_2 and OH^-, then the former is assimilated and the latter excreted. Thus HCO_3^- uptake acts as a mechanism which allows photosynthesis to proceed even when the CO_2^* has dropped to extremely low levels. As rapid photosynthesis can cause carbon depletion, HCO_3^- use will be an important characteristic determining the outcome of any competition between macrophytes and phytoplankton for carbon.

For HCO_3^- users, the final pH allows the calculation of the apparent HCO_3^- CP for a given alkalinity. It is possible that this is not a true CP, but that it is affected by pH and/or $CO_3^=$. Allen & Spence (1981) found a similar final HCO_3^- for E. canadensis in 1.0 and 2.0 mmol l^{-1} $KHCO_3$, and for Chlorella emersonii in 0.5 and 1.0 mmol l^{-1} $KHCO_3$. The latter species

had a slightly higher final HCO_3^- in 2.0 mmol l^{-1} which Allen (1977) suggests is a result of the harmful effect of high pH, in this case 11.23. This is unlikely to be a $CO_3^=$ effect, as at this alkalinity the maximum $CO_3^=$ occurs at pH 10.64, where the concentration is 0.71 mmol l^{-1} compared to 0.40 mmol l^{-1} at the higher pH. Table 5.1 gives results for <u>P. x zizii</u> (broad leaves) at two alkalinities. The apparent HCO_3^- CP is higher in the higher alkalinity at 138 µmol l^{-1} compared to 60 µmol l^{-1} for the lower alkalinity, resulting in a higher C_T/ alkalinity ratio of 0.43 in the 1.0 mmol l^{-1} solution compared to 0.32 in the 0.5 mmol l^{-1} solution. This less efficient carbon use may be caused by the higher pH needed to achieve the same HCO_3^- CP.

As most of the phytoplankton were studied here at an alkalinity of 1.0 mmol l^{-1}, and many of the macrophytes at 0.5 mmol l^{-1}, a comparison between the two will probably overestimate the ability of those macrophytes, run at the lower alkalinity, to remove carbon. Nevertheless, it is evident from Table 5.1 that the most efficient phytoplankters are considerably more efficient than the best macrophytes. Conversely, the least efficient carbon users are all macrophytes. It is interesting to note that the two most efficient carbon users in Table 5.1 are blue-green algae, both of which commonly form blooms in enriched lakes. <u>S. quadricauda</u> is also found in eutrophic lakes (e.g. Hickling Broad Moss 1981) and <u>Scenedesmus</u> species and other small Chlorophytes became dominant in a small hyper-eutrophic prairie lake to which large amounts of nitrogen fertilisers had been added, causing a suppression of blue-green algae, (Barica, Kling & Gibson 1980). De Noyelles and O'Brien

(1978) found that N and P enrichment of experimental ponds, creating eutrophic conditions, caused a change in the dominant phytoplankton from Chrysophyta to Chlorophyta, particularly S. quadricauda and Cyanophyta, particularly M. aeruginosa. This change was associated with high pH values of above 10.5, and so severe carbon depletion can be inferred. Shapiro (1973) has suggested that blue-green algae become dominant in eutrophic waters because of their greater ability to use carbon at low levels compared to other types of algae. Certain chlorophyta e.g. S. quadricauda and Chlorella emmersonii also have this ability as a result of efficient HCO_3^- uptake mechanisms.

Bloom forming algae appear to be particularly efficient at using carbon. Under these conditions, macrophytes are likely to suffer from the dense phytoplankton crops, and therefore carbon competition will probably be important. In general however, the differences between macrophytes and phytoplankton appear to be less marked than Allen & Spence (1981) suggested from the data available to them.

In the field, phytoplankton are in competition with macrophytes, which consist of roots, rhizomes and turions, as well as the shoots used in these experiments. Figure 5.8 shows that the whole plant has lower uptake rates, and shows an increase in CO_2^* CP (from 5.5 to 8.8 µmol l^{-1}) and an increase in the final C_T/alkalinity ratio (from 0.388 to 0.595) compared to the shoot alone. This may be offset to some extent by CO_2 uptake from the roots, shown for Lobelia dortmanna (Wium-Anderson 1971) and for L. uniflora (Sondergaard & Sand-Jensen 1979). Both of these species are typical of unproductive lakes where little carbon depletion would be expected. Bodkin (1979)

found no evidence for CO_2 uptake by the roots of Hippuris vulgaris, a species more commonly found in productive lakes. The dark fixation of CO_2, similar to that found for terrestrial CAM plants in two species of Isoetes (Keeley 1981, Keeley et al. 1981) may be a response to the higher ambient CO_2^* found at night. Again, Isoetes is commonly found in unproductive lakes and it would be interesting to investigate whether or not this type of fixation occurs in macrophytes growing in lakes that experience carbon depletion as a result of intense phytoplankton activity. The role of the roots in obtaining carbon for the macrophytes growing in these lakes would also be worth studying.

In Chapters 6, 7 and 8, the pH-drift technique is used to study the effects of light, season and heterophylly, on CO_2^* and HCO_3^- CP's.

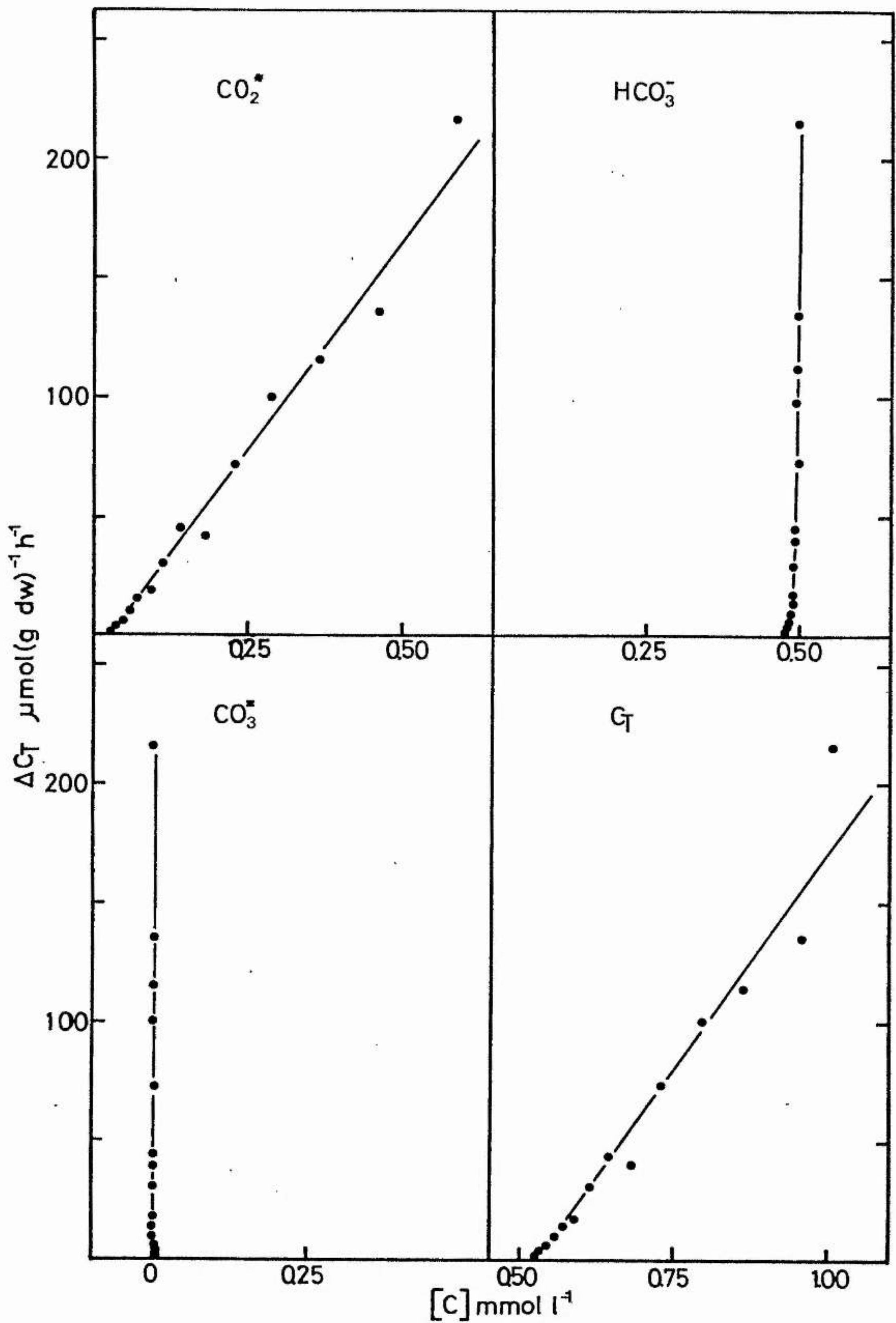


FIGURE 5.1 Change in photosynthetic carbon uptake rate of Littorella uniflora in relation to concentrations of CO_2^*, HCO_3^-, $CO_3^=$, and C_T. 20°C; 0.5 mmol l^{-1} $KHCO_3$; 220 µmol m^{-2} s^{-1}.

Substitute

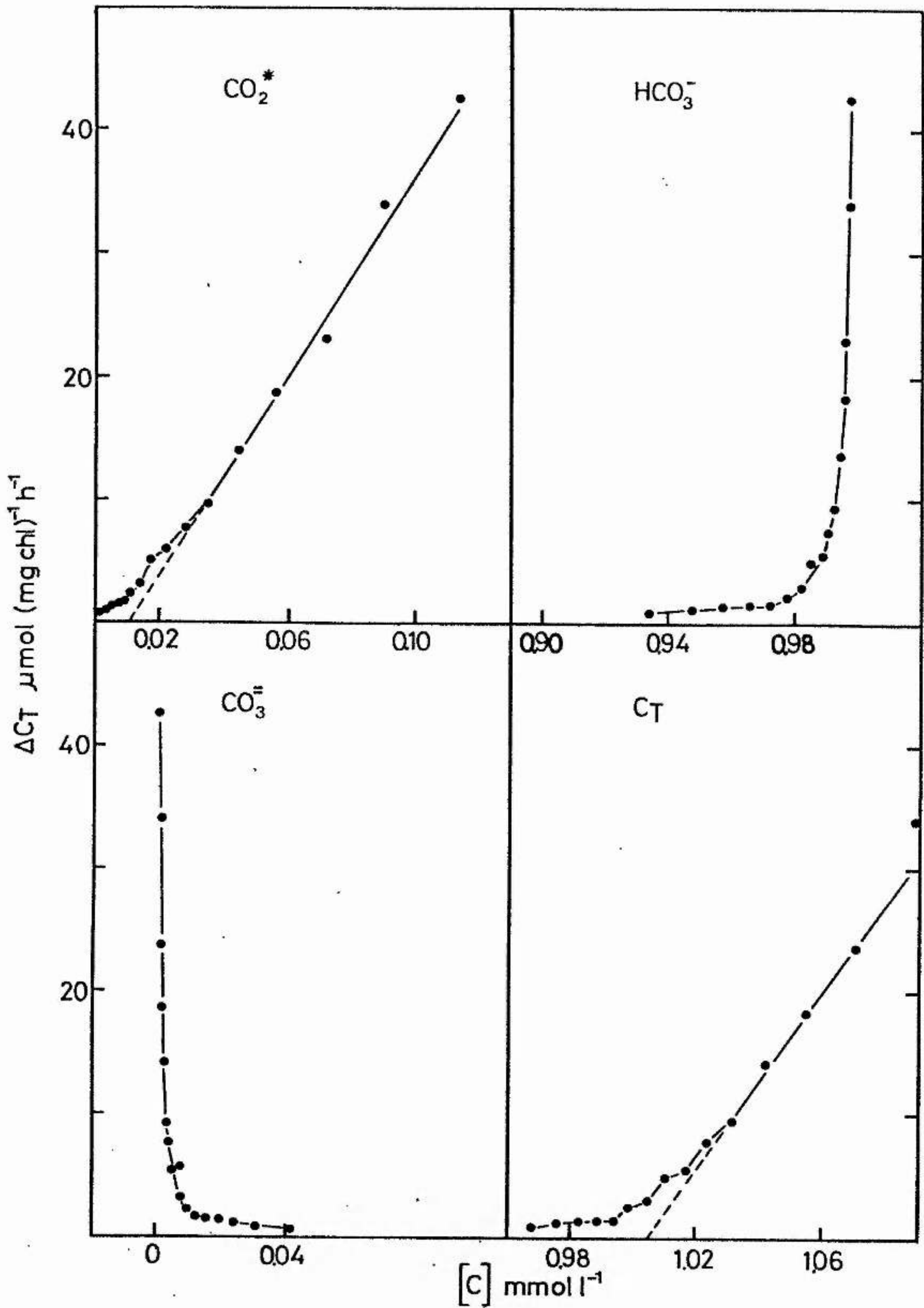


FIGURE 5.2 Change in photosynthetic carbon uptake rate of Elodea canadensis (28.i.81) in relation to concentrations of CO_2^*, HCO_3^-, $CO_3^=$, and C_T. 20°C; 1.0 mmol l^{-1}; 500 µmol m^{-2} s^{-1}.

Substitute Fig 5.5 - c & recalc. lines in graphs!

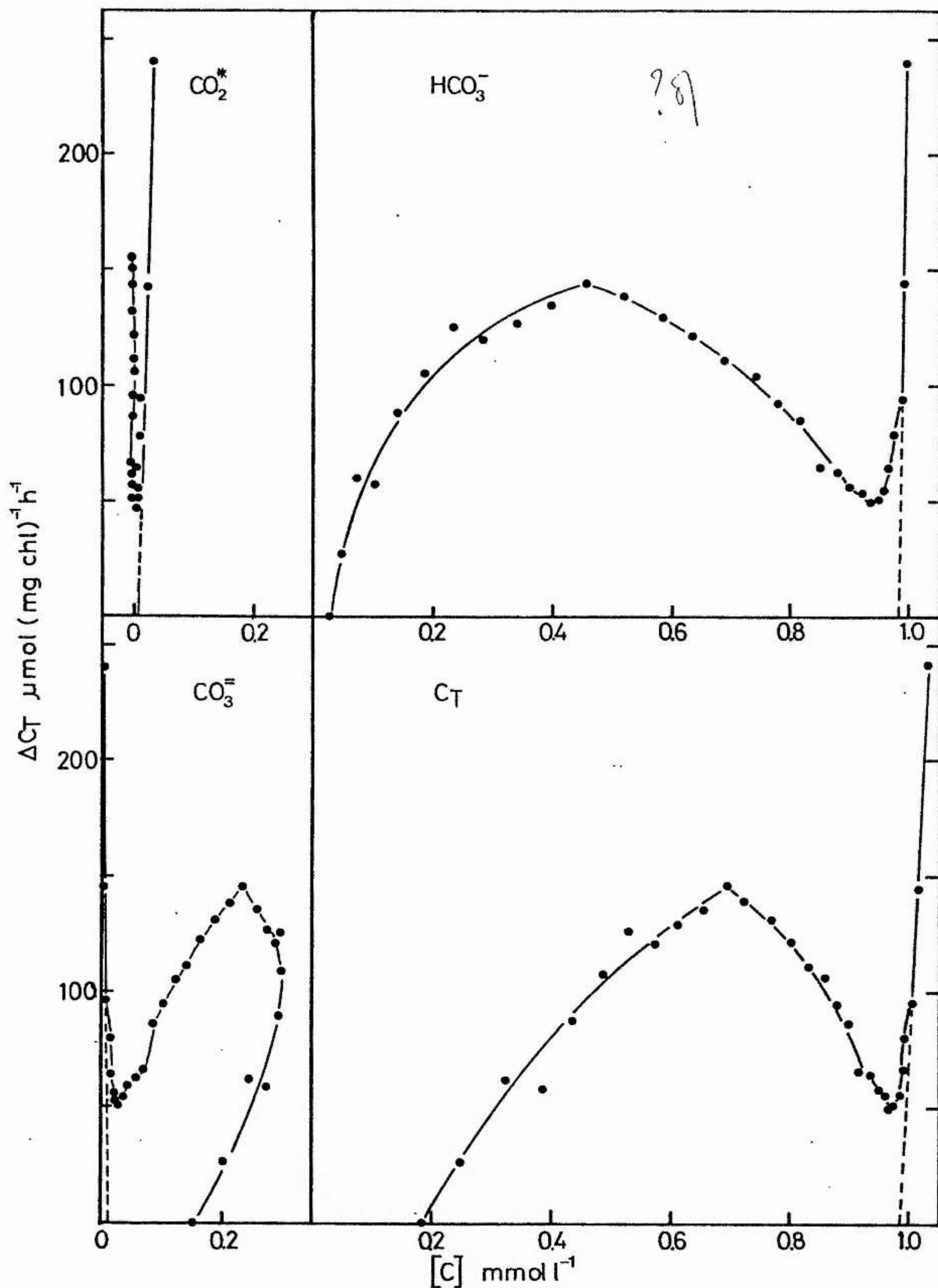
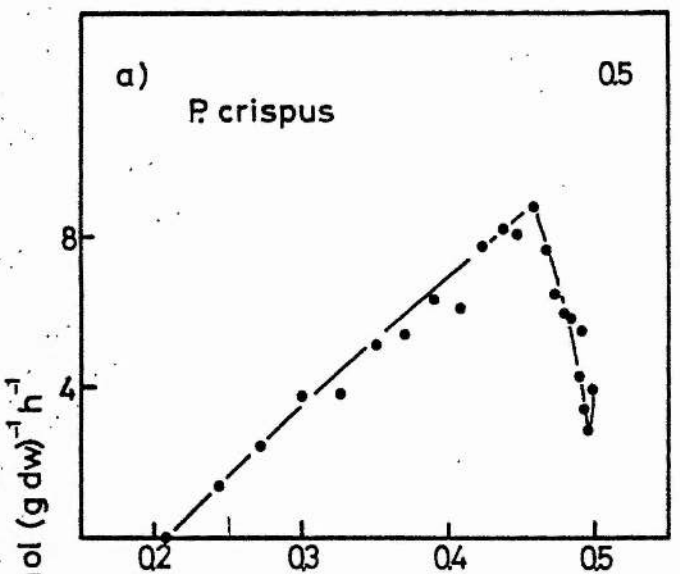
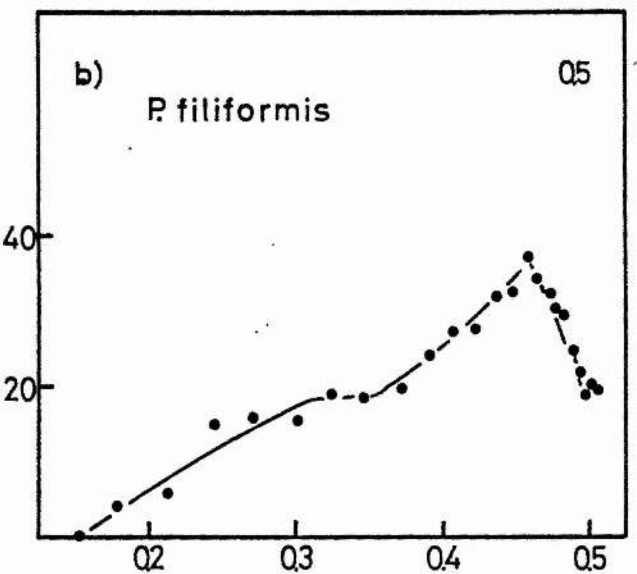


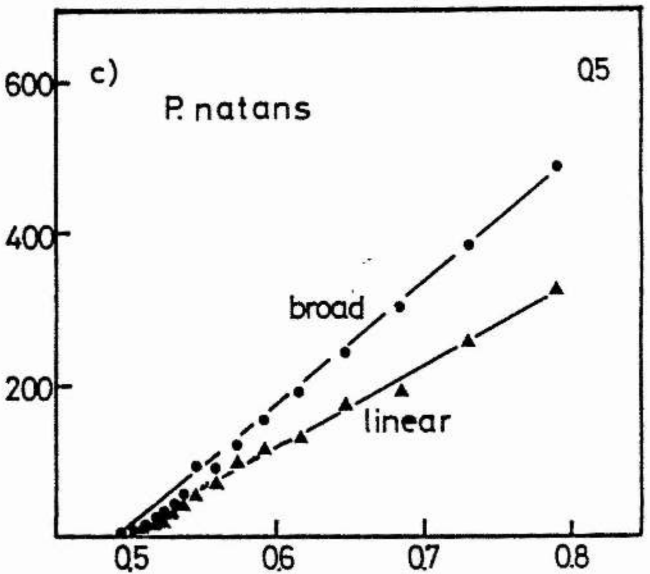
FIGURE 5.3 Change in photosynthetic carbon uptake rate of Scenedesmus quadricauda in relation to concentrations of CO_2^*, HCO_3^-, $CO_3^=$, and C_T. 20°C; 1.0 mmol l^{-1} $KHCO_3$; 500 μmol m^{-2} s^{-1}.

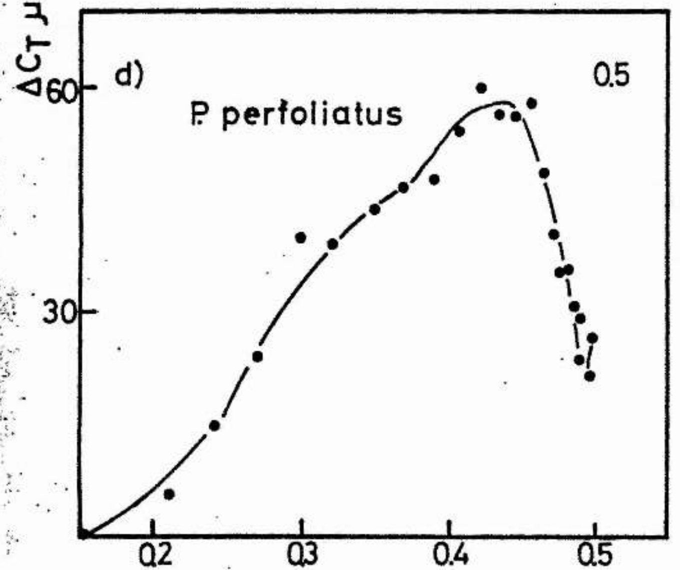
<u>FIGURE 5.4</u> Photosynthetic carbon uptake rate against C_T for six *Potamogeton* species. Alkalinity (mmol l^{-1} $KHCO_3$) indicated. $20^{\circ}C$; high or saturating PFAD (220 or 500 µmol m^{-2} s^{-1}).

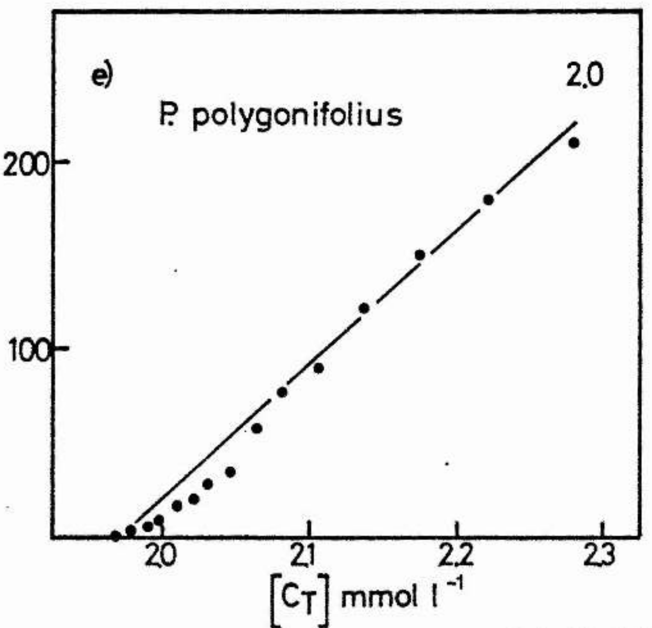
Example of uptake shapes of curves produced by 5 Pot. spp. shown


a)
P. crispus
0.5
8
4
0.2
0.3
0.4
0.5


b)
P. filiformis
0.5
40
20
0.2
0.3
0.4
0.5


c)
P. natans
0.5
broad
linear
600
400
200
0.5
0.6
0.7
0.8


d)
P. perfoliatus
0.5
ΔCT µmol (g dw)-1 h-1
60
30
0.2
0.3
0.4
0.5


e)
P. polygonifolius
2.0
200
100
2.0
2.1
2.2
2.3
[CT] mmol l-1

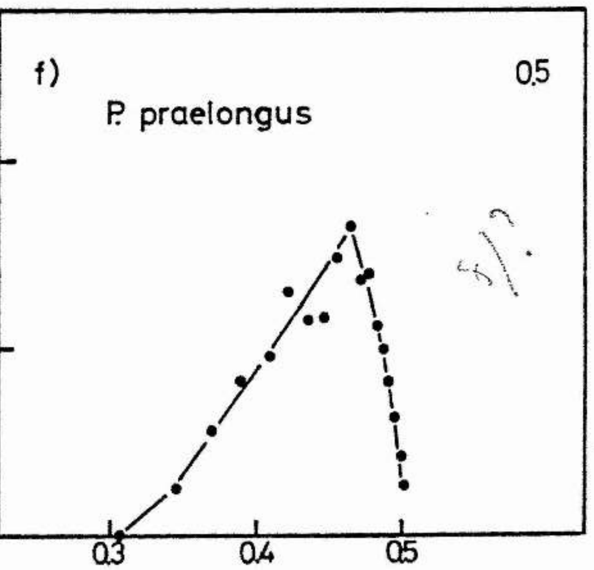
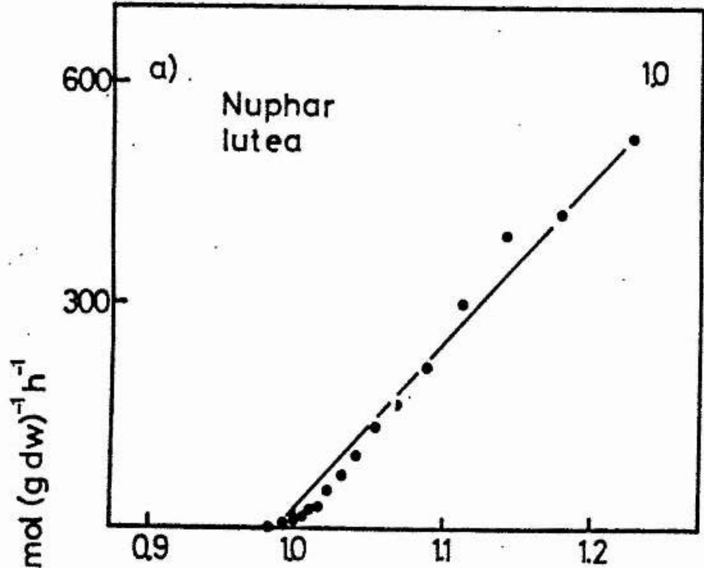
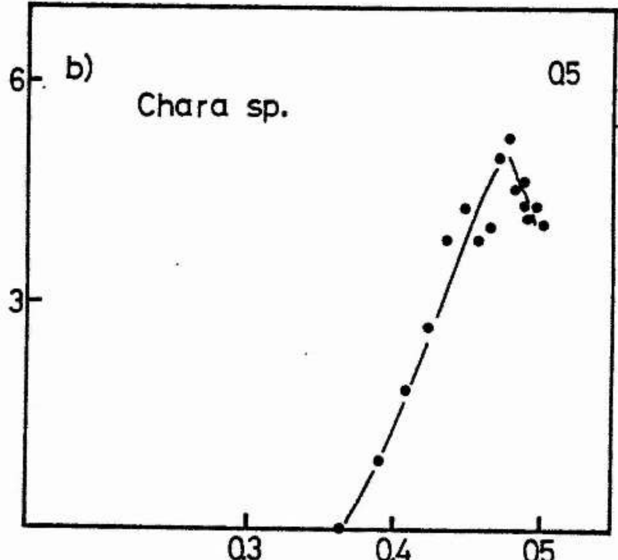
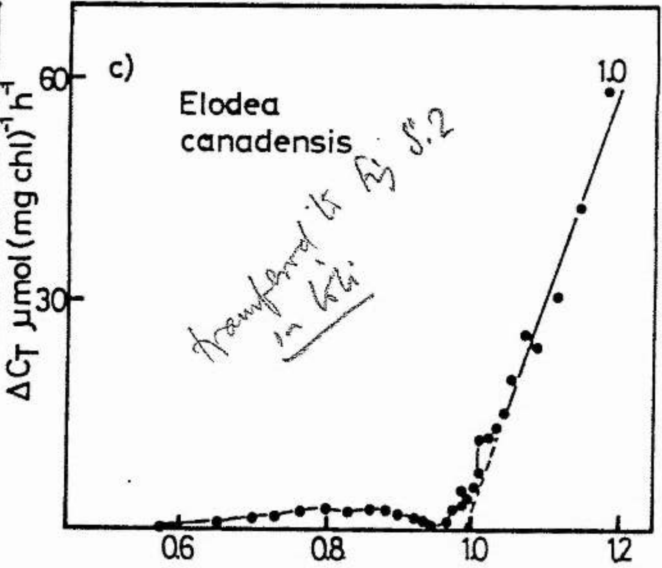
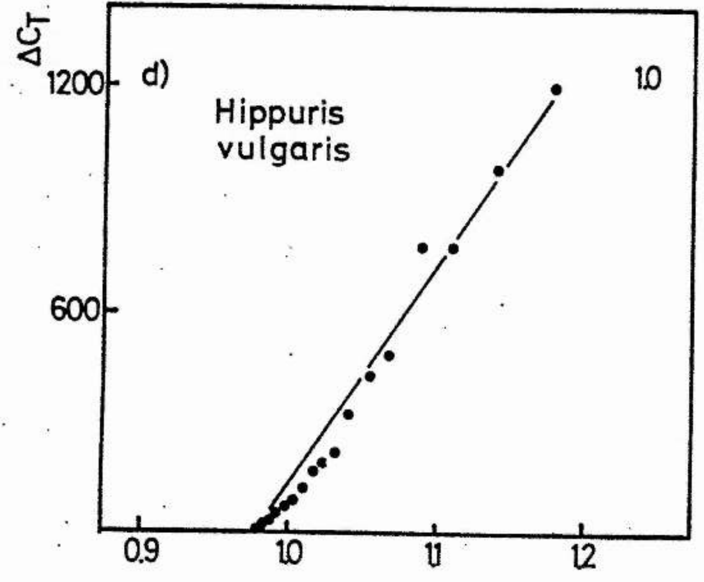

f)
P. praelongus
0.5
0.3
0.4
0.5

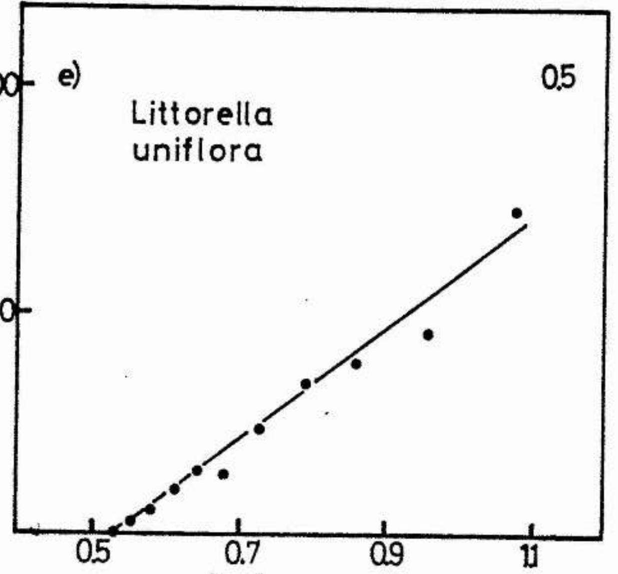
FIGURE 5.5 Photosynthetic carbon uptake rate against C_T for six species of macrophyte. Alkalinity (mmol l^{-1} $KHCO_3$) indicated. 20°C; high or saturating PFAD (220 or 500 μmol m^{-2} s^{-1}).


a)
Nuphar lutea
1.0
600
300
0.9
1.0
1.1
1.2
ΔC_T µmol (g dw)$^{-1}$h^{-1}


b)
Chara sp.
0.5
6
3
0.3
0.4
0.5


c)
Elodea canadensis
1.0
60
30
ΔC_T µmol (mg chl)$^{-1}$h^{-1}
0.6
0.8
1.0
1.2


d)
Hippuris vulgaris
1.0
1200
600
0.9
1.0
1.1
1.2


e)
Littorella uniflora
0.5
300
150
0.5
0.7
0.9
1.1
$[C_T]$ mmol l^{-1}

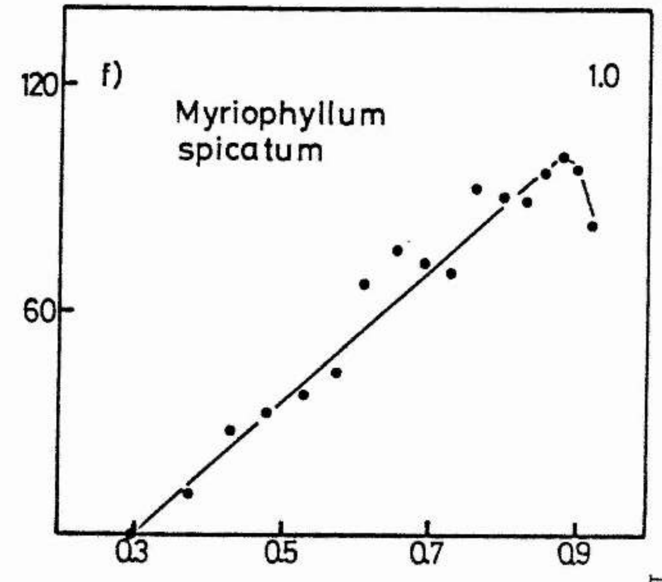
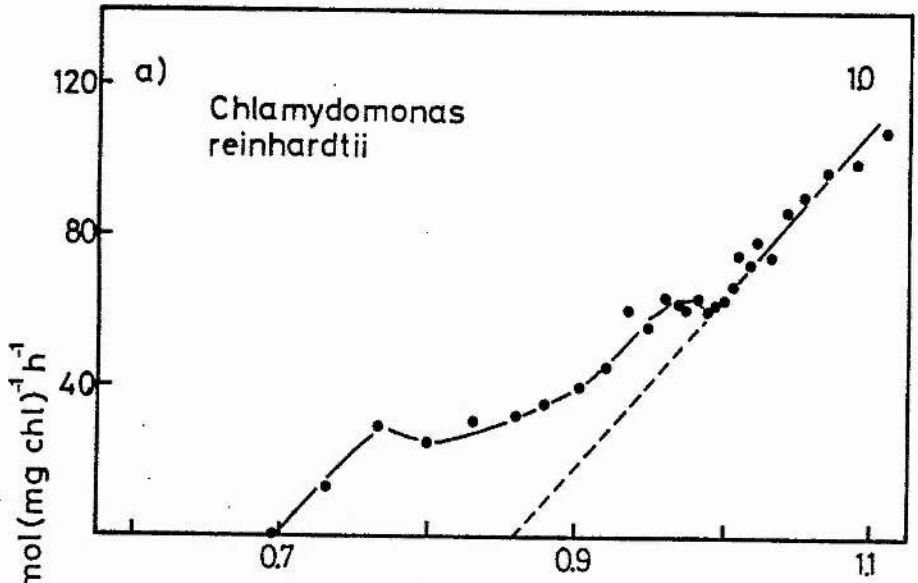
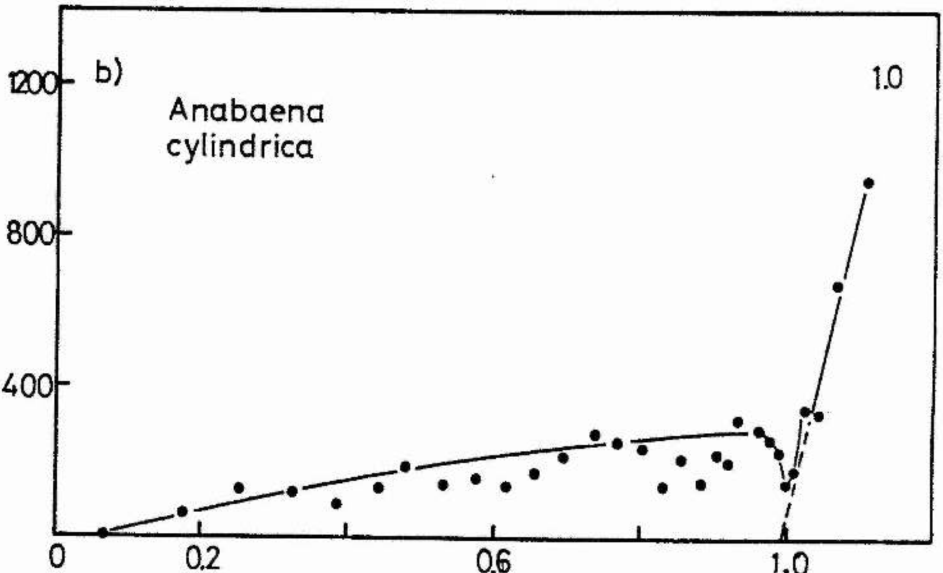
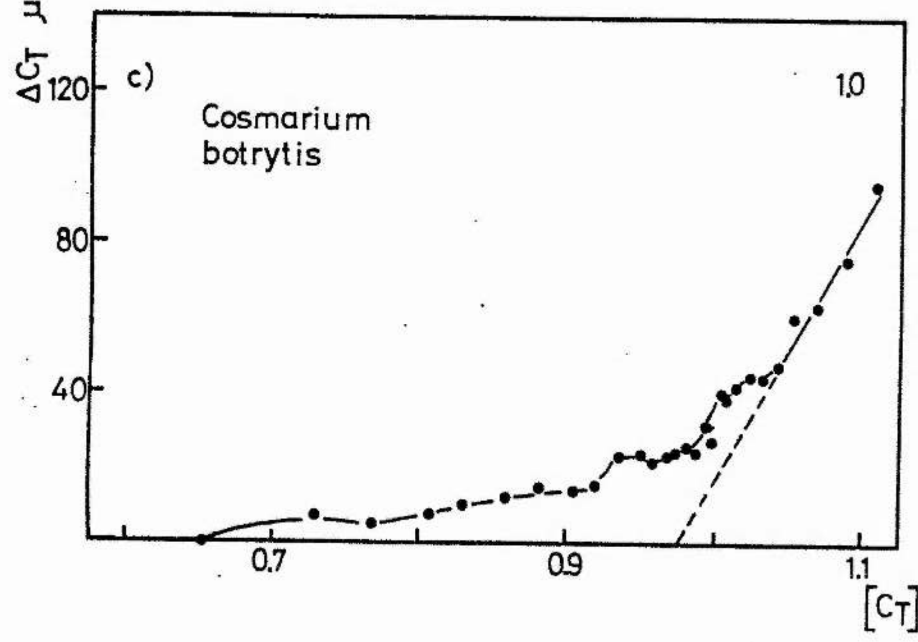

f)
Myriophyllum spicatum
1.0
120
60
0.3
0.5
0.7
0.9

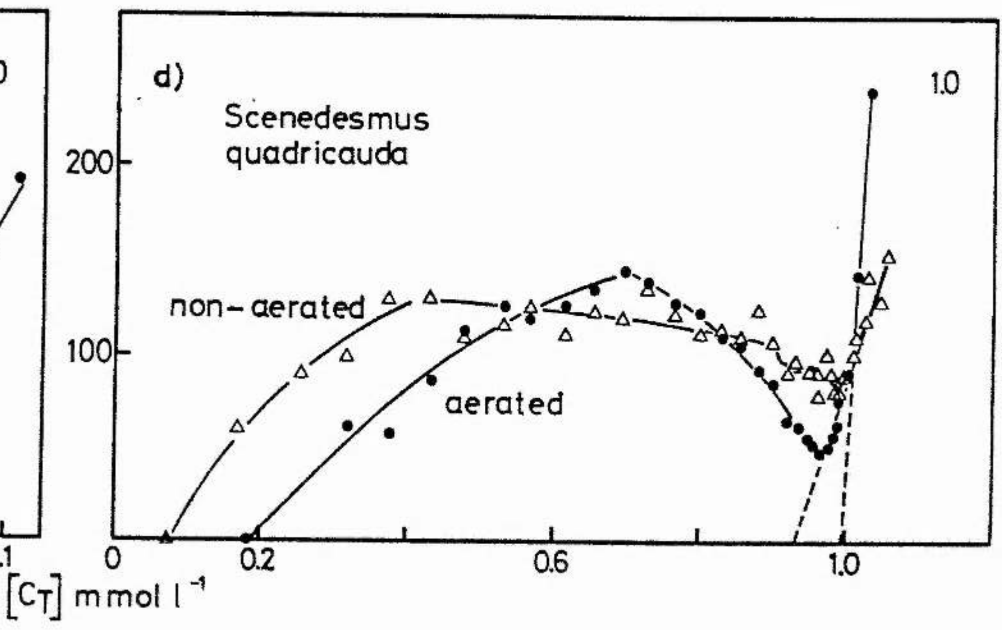
FIGURE 5.6 Photosynthetic carbon uptake rate against C_T for four phytoplankton species. Alkalinity (mmol l^{-1} $KHCO_3$) indicated. $20^{\circ}C$; 500 µmol m^{-2} s^{-1}.

[Lines drawn by eye from intercept determined ~~by~~ from by eye / ? or second linear regression based on larger points of (points) [CO_2] not [C_T].


a)
Chlamydomonas reinhardtii
1.0
120
80
40
0.7
0.9
1.1
ΔC_T µmol (mg chl)$^{-1}$ h^{-1}


b)
Anabaena cylindrica
1.0
1200
800
400
0
0.2
0.6
1.0


c)
Cosmarium botrytis
1.0
120
80
40
0.7
0.9
1.1


d)
Scenedesmus quadricauda
1.0
non-aerated
aerated
200
100
0
0.2
0.6
1.0
$[C_T]$ mmol l^{-1}

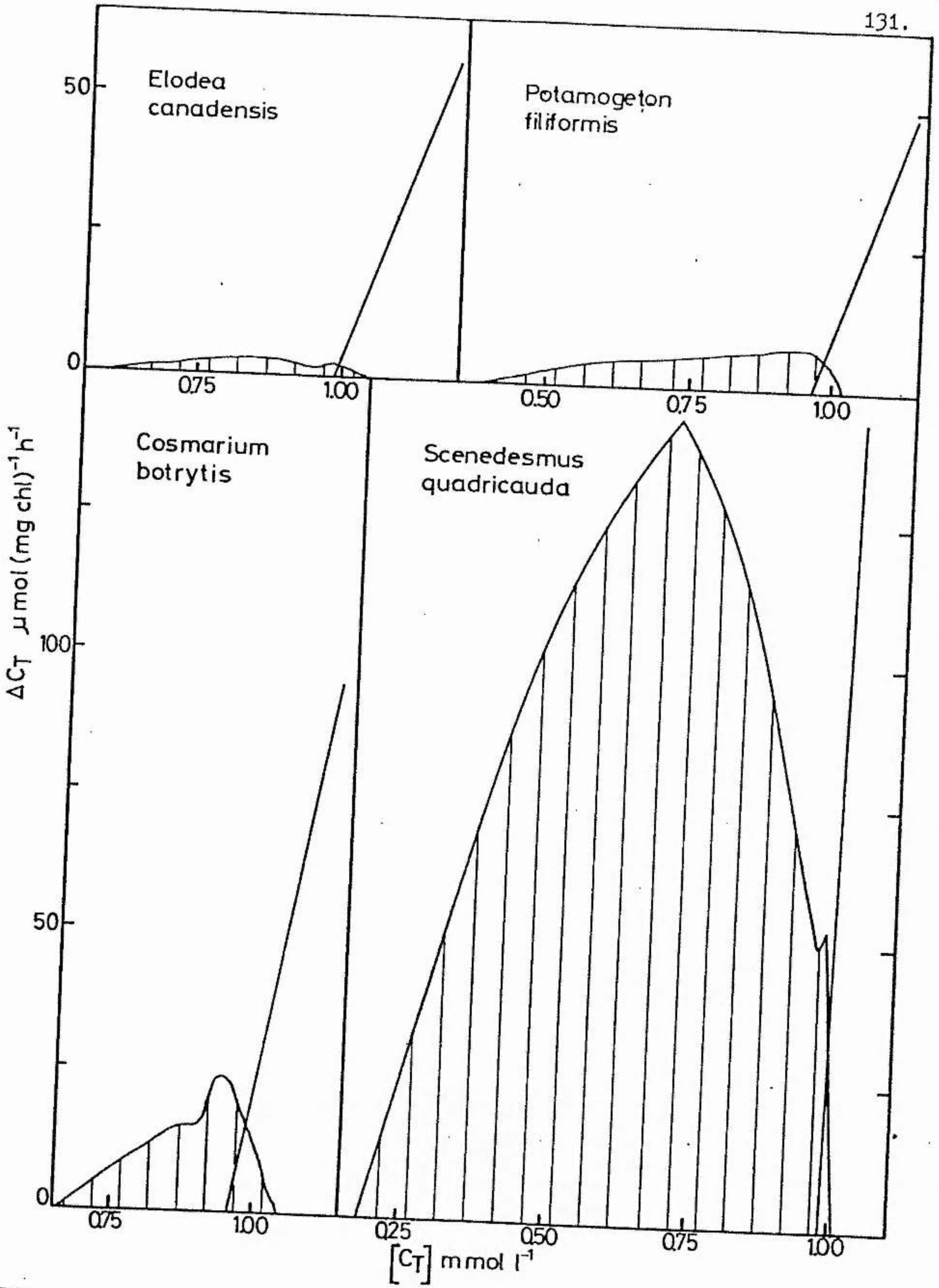
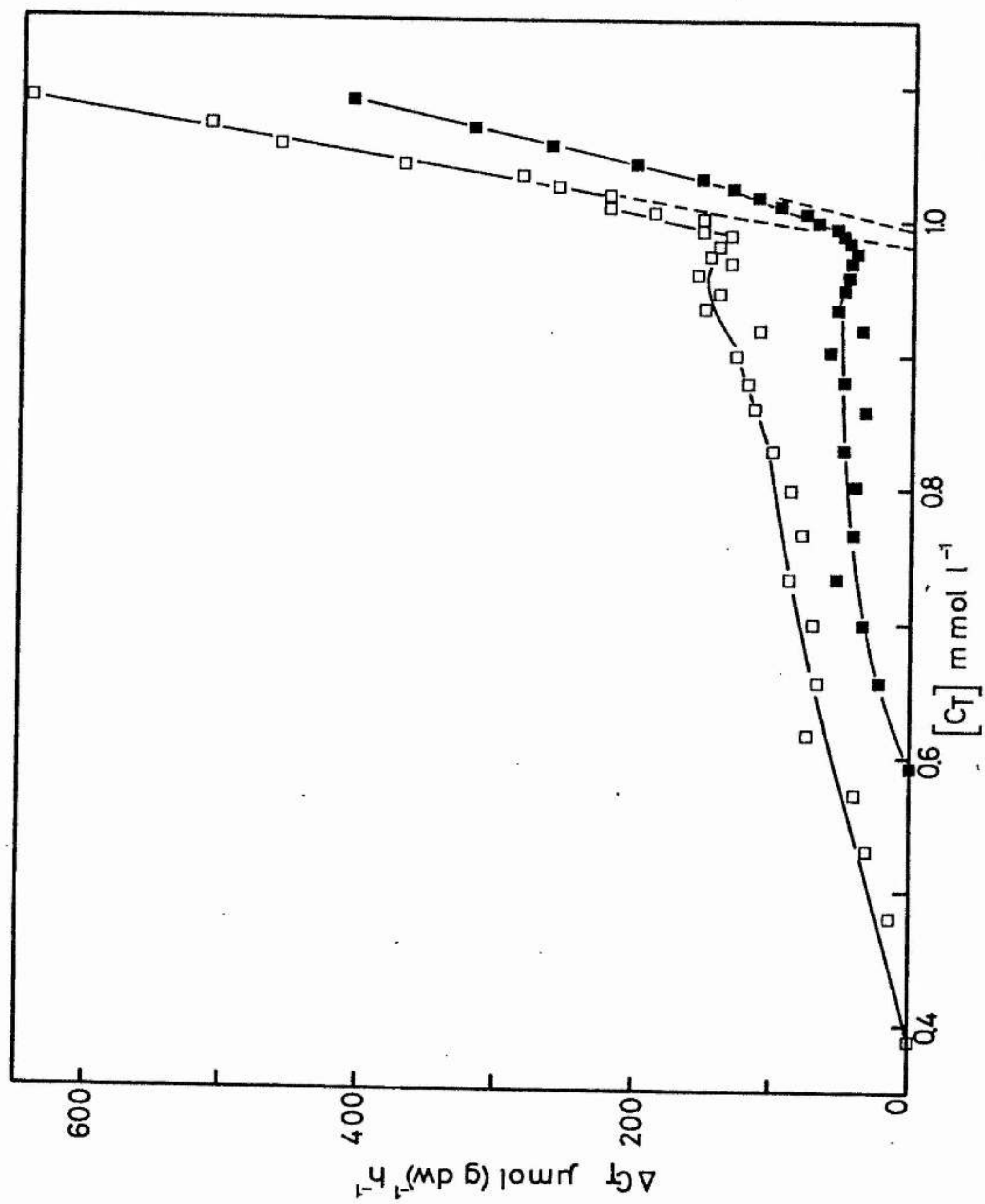


FIGURE 5.7 Photosynthetic carbon uptake rate of CO_2^* (straight line) and HCO_3^- (shaded area) for two species of macrophyte and phytoplankton. HCO_3^- uptake rate calculated from difference between extrapolated CO_2^* uptake rate and C_T uptake rate. 20°C; 1.0 mmol l^{-1} $KHCO_3$; 500 µmol m^{-2} s^{-1}.

FIGURE 5.8 Photosynthetic carbon uptake rate against C_T for *Potamogeton filiformis* seedlings (■) or shoot only (□). Dashed line yields extrapolated CO_2^* CP. 20°C; 1.0 mmol l^{-1} $KHCO_3$; 500 µmol m^{-2} s^{-1}.

transfer HCO_3-CP's to [illegible] table as 'shoot' [illegible] plant'



600
400
200
0
ΔCT μmol (g dw)-1 h-1
0.4
0.6
0.8
1.0
[CT] mmol l-1

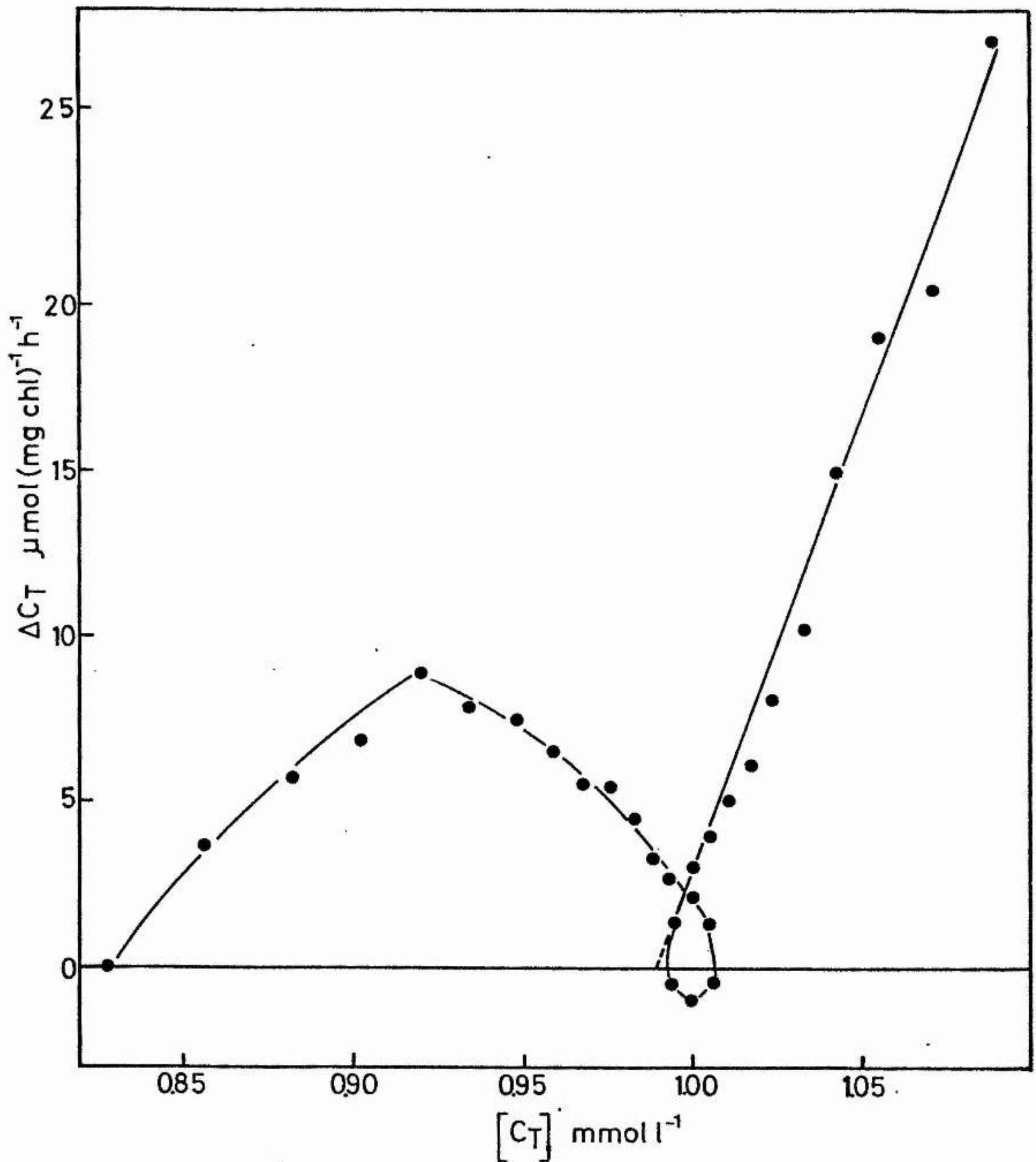


FIGURE 5.9 Photosynthetic carbon uptake rate against $[C_T]$ for Potamogeton crispus collected from L. Drumore (28.i.81), showing possible HCO_3^- adaptation. $20^{\circ}C$; 1.0 mmol l^{-1} $KHCO_3$; 500 µmol m^{-2} s^{-1}.

TABLE 5.1

Final pH and $\left[C_T\right]$, with calculated HCO_3^- and CO_2^* CP's for named species of macrophyte (o) and phytoplankton (•) at given alkalinity. Ranked in order of decreasing C_T/alkalinity ratio, which gives an estimate of a species ability to remove carbon from solution. + data of Allen (1977), ‡ data of Talling (1976) [Fig. 19].

M or P	SPECIES	ALK mmol l^{-1}	FINAL pH	$[C_T]$ mmol l^{-1}	HCO_3^- CP µmol l^{-1}	CO_2^* CP µmol l^{-1}	CT/ALK
•	Anabaena cylindrica	1.0	11.11	0.067	7	–	0.067
• ‡	Microcystis aeruginosa	1.0	11.10	0.077	10	–	0.077
•	Scenedesmus quadricauda (non-aer.)	1.0	11.10	0.077	10	–	0.077
•	Scenedesmus quadricauda (aerated)	1.0	10.99	0.182	60	–	0.182
• +	Chlorella emersonii	0.5	10.90	0.093	27	–	0.186
o	Myriophyllum spicatum	1.0	10.84	0.296	65	–	0.296
o	Potamogeton perfoliatus	0.5	10.57	0.150	53	–	0.299
o	Potamogeton filiformis	0.5	10.56	0.154	56	–	0.308
o	Potamogeton x zizii (brd.)	0.5	10.54	0.161	60	–	0.323
o	Potamogeton crispus	0.5	10.41	0.209	92	–	0.417
• ‡	Fragilaria crotonensis	0.4	10.40	0.170	76	–	0.425
o	Potamogeton x zizii (brd.)	1.0	10.61	0.430	138	–	0.430
o	Elodea canadensis	1.0	10.30	0.573	281	–	0.572
o	Potamogeton praelongus	0.5	10.07	0.308	195		0.615
•	Cosmarium botrytis	1.0	10.11	0.651	390	–	0.651
•	Chlamydomonas reinhardtii	1.0	10.00	0.695	457	–	0.695
o	Chara sp.	0.5	9.82	0.366	276	–	0.731
o +	Myriophyllum alterniflorum	2.0	9.80	1.550	1110	–	0.775
• ‡	Asterionella formosa	0.4	9.70	0.311	250	–	0.778
o	Potamogeton x zizii (lin.)	0.5	8.88	0.479	–	1.4	0.958
o +	Eurhynchium rusciforme	1.0	8.91	0.960	920	–	0.960
• ‡	Melosira italica	0.4	8.80	0.386	–	1.4	0.965
o +	Nitella flexilis	2.0	8.84	1.930	–	6.0	0.965
o	Hippuris vulgaris (subm.)	1.0	8.80	0.968	–	3.4	0.968
o	Ranunculus sp.	1.0	8.77	0.971	–	3.7	0.971
o	Potamogeton natans (lin.)	0.5	8.72	0.486	–	2.1	0.973
o	Nuphar lutea (subm.)	1.0	8.66	0.979	–	4.8	0.979
o	Potamogeton natans (brd.)	0.5	8.58	0.491	–	2.9	0.983
o	Potamogeton polygonifolius	2.0	8.60	1.965	–	10.7	0.983
o +	Fontinalis antipyretica	1.0	8.45	0.990	–	7.9	0.990
o	Littorella uniflora	0.5	7.70	0.521	–	22.9	1.043

L P. fil. shoot + whole plant

apply qualif^n on Table 5.1 to this Table ~ 5.1 (which will be reversed)

TABLE 5.2

CO_2^* CP's of macrophytes (o) and phytoplankton (●) calculated from intercept $[C_T]$ and alkalinity. Ranked in order of increasing compensation point. + data of Allen (1977).

M or P	SPECIES	ALK mmol l^{-1}	INTERCEPT $[C_T]$ mmol l^{-1}	CO_2^* CP µmol l^{-1}
●	Chlamydomonas reinhardtii	1.0	0.855	0.5
o	Potamogeton x zizii (brd.)	0.5	0.480	1.4
●	Scenedesmus quadricauda (non-aer)	1.0	0.930	1.5
o +	Myriophyllum spicatum	2.0	1.830	2.1
o	Potamogeton filiformis	1.0	0.960	2.6
●	Cosmarium botrytis	1.0	0.975	4.3
o +	Eurhynchium rusciforme	1.0	0.980	5.5
o +	Ranunculus aquaticus	2.0	1.950	8.4
●	Anabaena cylindrica	1.0	0.994	8.7
●	Scenedesmus quadricauda (aer)	1.0	0.994	8.7
o +	Chara vulgaris f. contraria	2.0	1.963	10.8

P. fil. whole plant

when ~~mentiong~~ mentioning effect of $[O_2]$ on CO_2-CP (as general other of possible cause of var^n);

TABLE 5.3

Effect of oxygen concentration on the CO_2^* CP of a submerged shoot of Hippuris vulgaris originally collected from L. Kilconquhar, Fife and grown in a greenhouse in St. Andrews. The same shoot was used for the oxygen concentration range in the order shown. 1.0 mmol l^{-1} $KHCO_3$; 20°C; 310 µmol m^{-2} s^{-1}.

STARTING pH	FINAL pH	$[O_2]$ mg l^{-1}	O_2% SATURATION	CO_2^* CP mmol l^{-1}
8.79	9.38	5.5	60	0.8
7.10	9.29	8.0	87	1.0
6.30	8.96	15.5	169	2.3
9.05	9.40	4.3	47	0.7

CHAPTER 6

LIGHT EFFECTS ON COMPENSATION POINTS

6.1 INTRODUCTION

About 10% of the light reaching the surface of a lake is reflected; the remainder enters the lake where water molecules, salts in solution, and suspended or colloidal matter such as silts, clay or gelbstoff, absorb and scatter light. These factors cause both a reduction in the total irradiance and a selective attenuation of certain wavelengths, particularly those in the red and blue regions of the spectrum. This results in a lake being an environment where PFAD is low, especially at depth.

A dense phytoplankton population can be a major factor responsible for light attenuation in a water column. Jewson (1977) showed that the euphotic zone in L. Neagh varied from 1-3 m as a result of changes in algal crops of between 26-92 μg chlorophyll a l^{-1}. Kirk (1975 a & b) showed how suspensions of cells or colonies of green or blue-green algae affect the attenuation of light in water, and concluded that large sized particles (e.g. colonies) attenuate light less than small particles (e.g. unicells) for a given amount of pigment. The mean spectral coefficient for a unit of chlorophyll also varies with the colour of the water, the colour of the phytoplankton, and the depth of water (Atlas & Bannister 1980).

In the absence of detailed measurements of PFAD and macrophyte photosynthetic rates in response to PFAD in the field over a season, it is difficult to determine what direct

shading effects a phytoplankton population will have on macrophyte performance. Jupp & Spence (1977) found a correlation between high phytoplankton crops and reduced biomass of Potamogeton filiformis in L. Leven. This was attributed largely to shading, but the effect of phytoplanktonic carbon depletion was unknown.

In terrestrial plants such as Lactuca sativa, the CO_2 CP is increased at low PFAD's (Heath & Meidner 1967). When a phytoplankton population is in competition with a submerged macrophyte population, the macrophytes are likely to suffer from both light and carbon depletion. Therefore the effect of low PFAD's on CO_2 and HCO_3^- CP's was determined using the pH-drift technique.

The term C_T CP refers to the C_T at the end of a pH-drift experiment in a solution of given alkalinity. For a CO_2^* user, this will represent the CO_2^* CP, for a HCO_3^- user, the HCO_3^- CP. These CP's can be calculated from the C_T and the alkalinity of the solution.

6.2 MATERIALS AND METHODS

6.2.1 Collection sites

Hippuris vulgaris was obtained from L. Kilconquhar (3.ix.80), Elodea canadensis from a small pond in St. Andrews (13.iii.80), Potamogeton polygonifolious from L. Na Craig (11.viii.80), P. crispus (27.vi.80) and P. praelongus (14.v.80) from L. Drumore, and P. x zizii (24.vii.80), P. perfoliatus (16.vii.80) and P. filiformis (on 4 occasions) from L. Fitty.

6.2.2 Photosynthetic measurements

Light effects on CO_2^* and HCO_3^- CP's were studied in three ways. First a pH-drift experiment was run at a high or saturating PFAD

as outlined in Chapter 5. Once a final pH value had been reached, the PFAD was reduced and the effect on pH recorded. If the pH remained unchanged for about one hour, successively lower PFAD's were used until a new, lower equilibrium pH value was obtained. This sequence was repeated until a PFAD below the light compensation point was reached, or until the lowest PFAD (6 µmol m^{-2} s^{-1}) was used. Secondly, some complete pH-drift experiments were run at two different PFAD's to obtain a complete record of carbon uptake over a range of C_T. This allowed the determination of both CO_2^* and HCO_3^- CP's at different PFAD's. pH-drifts were run at 20°C in 1.0, or 0.5 m mol l^{-1} $KHCO_3$ prepared in N_2-bubbled distilled water. Thirdly, the changes in CO_2 or HCO_3^- CP's were related to the light-photosynthesis curve. This was produced using the same equipment as for the pH-drift technique, but photosynthesis was measured by following oxygen evolution with a polarographic O_2 sensor (Beckman Instruments 39553) and a Beckman Fieldlab oxygen meter (1008) connected to a chart recorder. The plant material was allowed to photosynthesise in a 10.0 mmol l^{-1} $KHCO_3$ solution made up in N_2-bubbled distilled water to which some CO_2 had been added to give a pH of approximately 7, so ensuring non-limiting carbon concentrations. After an initial period of about 15 minutes in dim light, to allow temperature and plant equilibriation, the photosynthetic rate was measured at a series of increasing PFAD's. These were produced by using a combination of different numbers of 150W incandescent lamps and neutral density filters. The photosynthetically available radiation (400-700 nm) was measured outside the chamber with a Macam Q101 quantum meter, at a position near the chamber base, and so was probably an overestimation of the light

received by the plant. Small amounts of phytoplankton and macrophytes were used to reduce self-shading effects. A respiration rate was obtained after the highest PFAD had been run by turning the lights off and wrapping the water bath jacket with aluminium foil.

6.3 RESULTS

Figure 6.1 shows that high PFAD's, generally above 100 $\mu mol\ m^{-2}\ s^{-1}$, had no effect on the CO_2^* or HCO_3^- CP's in the seven macrophyte species studied. At low light intensities there was an interaction between light and both CO_2^* and HCO_3^- CP's, with higher compensation points at lower PFAD's. The asymptote to the x-axis represents the carbon compensation point, while the asymptote to the y-axis represents the light compensation point.

This interaction varied seasonally in P. filiformis collected from L. Fitty. Plants collected early in the season (May and June) had HCO_3^- compensation points that were sensitive to light only at low PFAD's, while the HCO_3^- compensation point of plants from July and August appeared to be sensitive to all the PFAD's used (Fig. 6.2). This could reflect a seasonal change in response to light, or more likely, a greater respiratory burden in the older plants. The latter may be inferred from data in Chapter 7 which shows a seasonal change in net photosynthetic rates at light saturation, associated with changes in the HCO_3^- CP's.

Figures 6.3-6.5 show results from complete pH-drifts at two different PFAD's, presented as net carbon uptake rates over a range of $[C_T]$ for P. crispus, a HCO_3^- user; H. vulgaris, a putative CO_2^* user; and the alga Scenedesmus quadricauda, a

HCO_3^- user. In each case, the final $[C_T]$ was highest at the lowest PFAD. These represent HCO_3^- CP's of 0.206 m mol l^{-1} for 117 µmol m^{-2} s^{-1} and 0.432 m mol l^{-1} for 22 µmol m^{-2} s^{-1} in the case of P. crispus. The CO_2^* CP appears to be identical for both PFAD's at 7.0 µmol l^{-1}. Similarly for S. quadricauda at 500 µmol m^{-2} s^{-1}, the HCO_3^- CP was 0.0223 m mol l^{-1}, and 0.0902 m mol l^{-1} at 34 µmol m^{-2} s^{-1}. Again, the CO_2^* CP was identical at 3.4 µmol l^{-1}. Submerged leaves of H. vulgaris have been shown to be non-users of HCO_3^- at an alkalinity of 1.0 m mol l^{-1} by Allen & Spence (1981). The results of Figure 6.4 suggest a slight HCO_3^- use, particularly at the higher PFAD. The CO_2^* CP's appear slightly different at 1.0 and 2.0 µmol l^{-1} for 500 and 22 µmol m^{-2} s^{-1} respectively, while the apparent HCO_3^- compensation points were 0.8767 and 0.9281 m mol l^{-1} for the high and low PFAD's. The H. vulgaris used in these experiments were grown in non-aerated culture and so carbon-depletion would occur as photosynthesis proceeded. This would result in low concentrations of CO_2^* and it is possible that the apparent HCO_3^- use is an adaptation to this.

At limiting concentrations of CO_2^* the differences between the actual photosynthetic rates for the two PFAD's are small as light is presumably not the major limiting factor. In both P. crispus and S. quadricauda, the rate of HCO_3^- uptake remains relatively constant over a range of $[C_T]$ at the low PFAD whereas at saturating light there is a peak rate of HCO_3^- uptake near the centre of the HCO_3^- "tail", followed by a rapid decline in rate to the compensation point.

Figures 6.6, 6.7 and 6.8 show changes in C_T CP's with varying PFAD, in relation to the light-photosynthesis curve for three species of macrophytes and four species of phyto-

plankton. In general, the increase in C_T CP's occur at PFAD's less than half that needed to saturate photosynthesis, which is the portion of the light-photosynthesis curve where light is limiting. The $K_{\frac{1}{2}}$ values for macrophytes and phytoplankton are similar ranging between 75-110 μmol m^{-2} s^{-1} and 60-80 μmol m^{-2} s^{-1} respectively.

6.4 DISCUSSION

Figures 6.3 and 6.4 show that at low carbon concentration, differences in photosynthetic rates between saturating and low PFAD's are less at limiting carbon than when carbon, principally CO_2^*, is high or saturating. As $[C_T]$ below air-equilibrium values are common (Allen 1977, Talling 1976) particularly in productive lakes, photosynthetic rates of macrophytes in these lakes may be determined largely by concentrations of CO_2^* and HCO_3^-.

Heath & Meidner (1967) demonstrated an interaction between light and the CO_2 compensation point in Lactuca sativa, with increased CO_2 compensation points at low light, and increased light compensation points at low $[CO_2]$. A similar relationship has been shown for Nicotiana tabacum (Okabe, Schmid & Straub 1977), Phaseolus vulgaris (Catsky & Ticha 1979), Populus curamericana (Furukawa 1973) Pinus sylvestris (Golomazova & Kaverzina 1977) and Pelargonium zonale (Meidner & Glinka unpublished in Heath 1969). Figures 6.1-6.7 show that this effect is also apparent in aquatic macrophytes and phytoplankton, and that both CO_2^* and HCO_3^- CP's are affected. Figures 6.3 and 6.5 suggest that it is the C_T CP that is affected - the CO_2^* CP for a CO_2^* user or the HCO_3^- CP for a HCO_3^- user.

Fair, Tew & Cresswell (1974) working with Hordeum vulgare

found increasing CO_2 CP's with increasing light from 30,000-100,000 lux. These are probably equivalent to 600-2,000 µmol m^{-2} s^{-1}, much higher than the PFAD's used in this study or that of Heath & Meidner (1967), although Catsky & Ticha (1979) and Okabe et al. (1977) approached these values and found no increase in compensation point. Smith, Tolbert & Ku (1976) found no increase in CO_2 CP at high light intensities, but showed the compensation point to be very sensitive to temperature. The increasing CO_2 CP with increasing light found by Fair et al. (1974) could be caused by inadequate temperature control in their experiments.

Figures 6.6, 6.7 and 6.8 show that in both macrophytes and phytoplankton, the first increase in C_T CP's occurred at about the PFAD required to half-saturate photosynthesis. The depth at which this PFAD occurs will vary with surface PFAD's, attenuation properties of the water, and any adaptation by the macrophytes to low light conditions (Spence & Chrystal 1970 a & b). The $K_{\frac{1}{2}}$ varied between 70 and 110 µmol m^{-2} s^{-1}. By interpolation of Spence, Campbell & Chrystal's (1971) data (Table 4), these light levels were found at depths of about 2.5m in the clear L. Croispol on a bright but hazy day (4.viii.70; 16:30-16:55) where the depth limit of attached macrophytes was at 6m. In the brown water of L. Uanagan, where the depth limit of attached macrophytes was at 4m, 100 µmol m^{-2} s^{-1} occurred at 0.5m on an overcast day (15.viii.70; 11:40-12:00). In L. Borralie, a clear limestone lake, Bodkin (1979) measured a PFAD of 100 µmol m^{-2} s^{-1} at 6m at noon on a bright sunny day in July. This was the maximum depth for angiosperms in this lake, and the light intensity probably represented the maximum received at this depth. These examples suggest that for much

of the time, submerged macrophytes are growing at PFAD's at which light and C_T compensation points interact.

When macrophytes are in competition with a dense phytoplankton crop, they will be both shaded and carbon depleted. Under saturating light conditions, many phytoplankton species, particularly "bloom-forming" species (Chapter 5, Allen & Spence 1981, Talling 1976) have lower HCO_3^- compensation points than any macrophytes. Under the reduced PFAD's that would result from phytoplankton shading, the C_T compensation points of macrophytes are increased so that they are less efficient competitors. Figures 6.1, 6.2 and 6.6 show that at low $[C_T]$ the light compensation point was increased, so any detrimental effect caused by shading would be intensified. Although these phenomena are also shown by phytoplankton (Figs. 6.7 & 6.8) they are likely to be less important for two reasons. Firstly, water movement in the lake will circulate the phytoplankton through the water column and so bring them periodically to higher light levels. Ward & Wetzel (1980) have shown this to be important in light adaptation in phytoplankton. Secondly, many "bloom-forming" species are blue-green algae, most of which are able to regulate their bouyancy by means of gas-vacuoles (Walsby 1972), enabling favourable light conditions to be maintained. It is worth noting that this alleviation is not available to the macrophytes' epiphytic competitors.

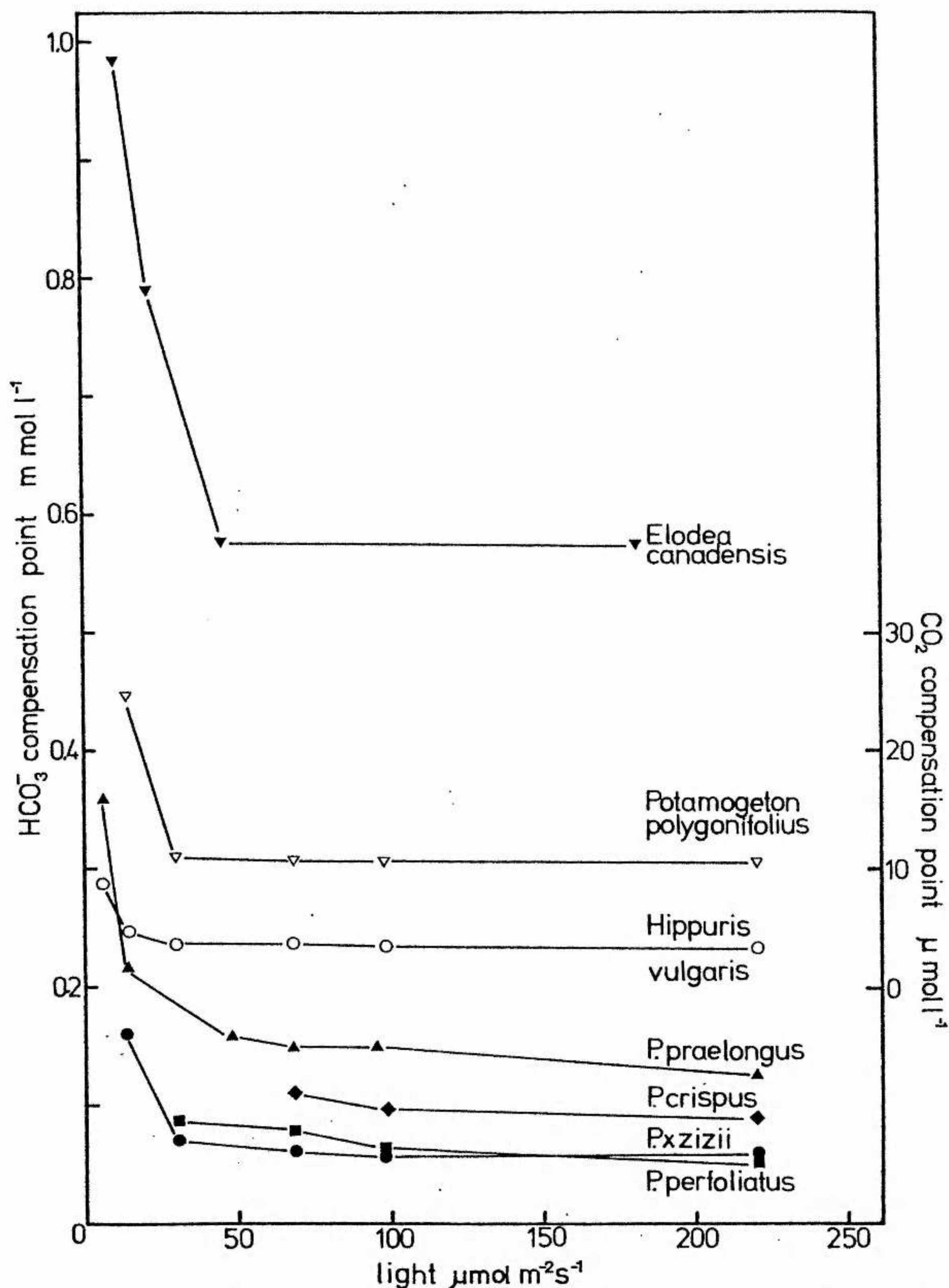


FIGURE 6.1 Effect of PFAD on CO_2^* and HCO_3^- CP's for seven species of macrophytes. 20°C. Alkalinity (mmol l^{-1} $KHCO_3$): 2.0 P. polygonifolius; 1.0 E. canadensis; and 0.5 other Potamogeton species. Closed symbols HCO_3^- CP, open symbols CO_2^* CP.

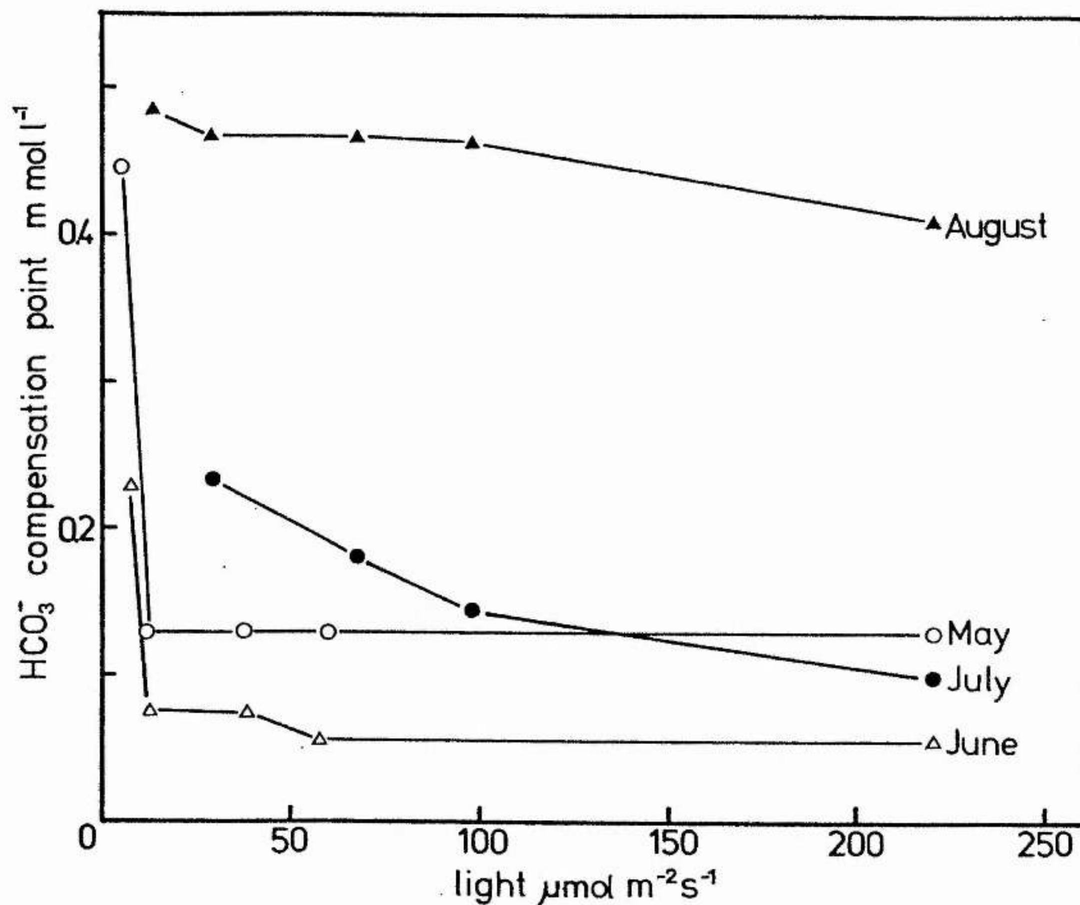


FIGURE 6.2 Seasonal changes of the effect of PFAD on HCO_3^- CP's for Potamogeton filiformis collected from 0.5m depth in L. Fitty. Collection dates: 1.v., 6.vi., 3.vii. and 14.viii.80. 20°C except May which was run at the lake temperature of 10.6°C. Alkalinity, 0.5 mmol l^{-1} $KHCO_3$.

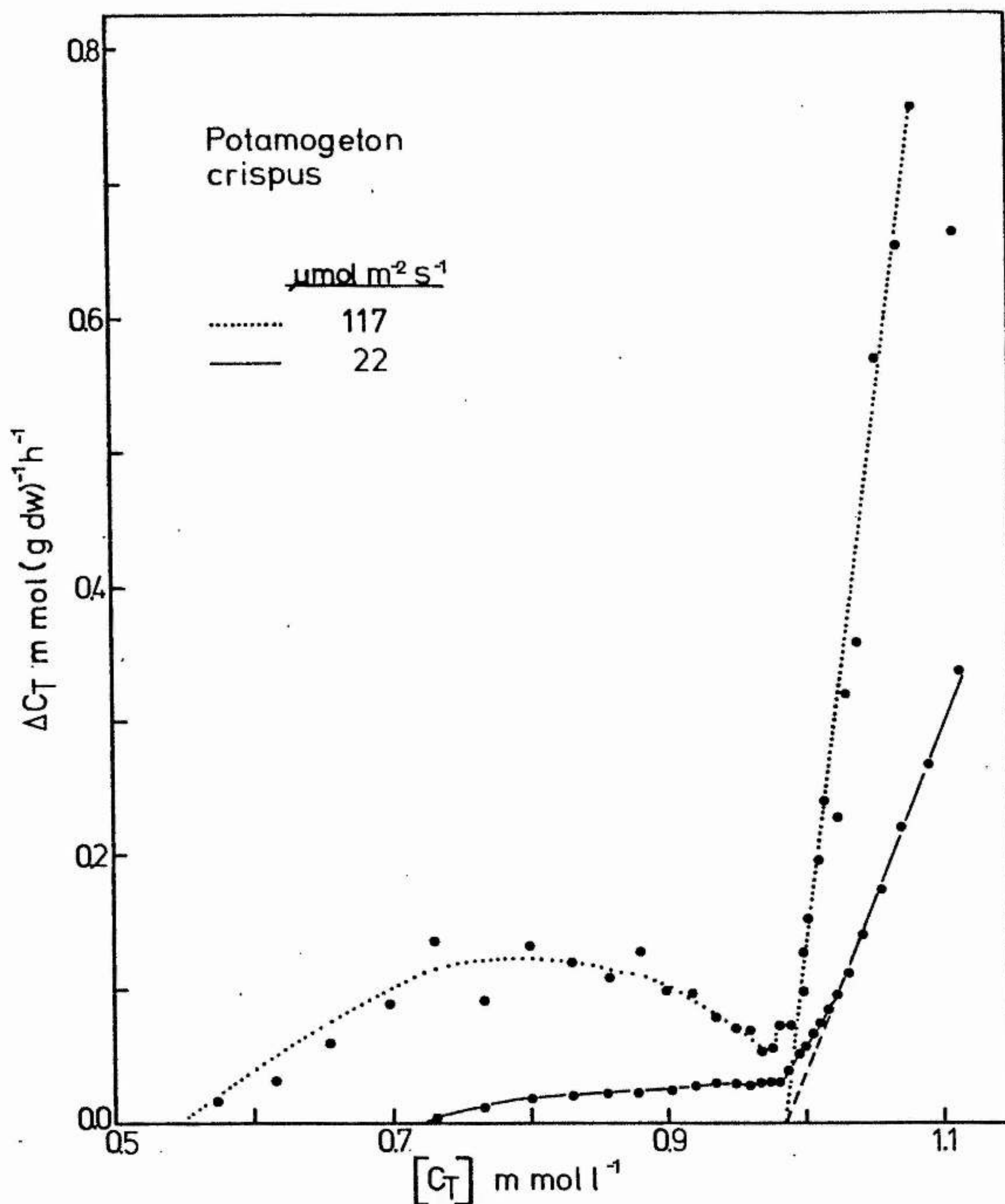


FIGURE 6.3 Changes in net C_T uptake rates over a range of $[C_T]$ for Potamogeton crispus at 117 µmol m^{-2} s^{-1} and 22 µmol m^{-2} s^{-1} (100% and 46% CO_2 + light saturated rate respectively. Extrapolation yields CO_2^* CP; final C_T yields HCO_3^- CP. 20°C. Alkalinity 1.0 mmol l^{-1} $KHCO_3$.

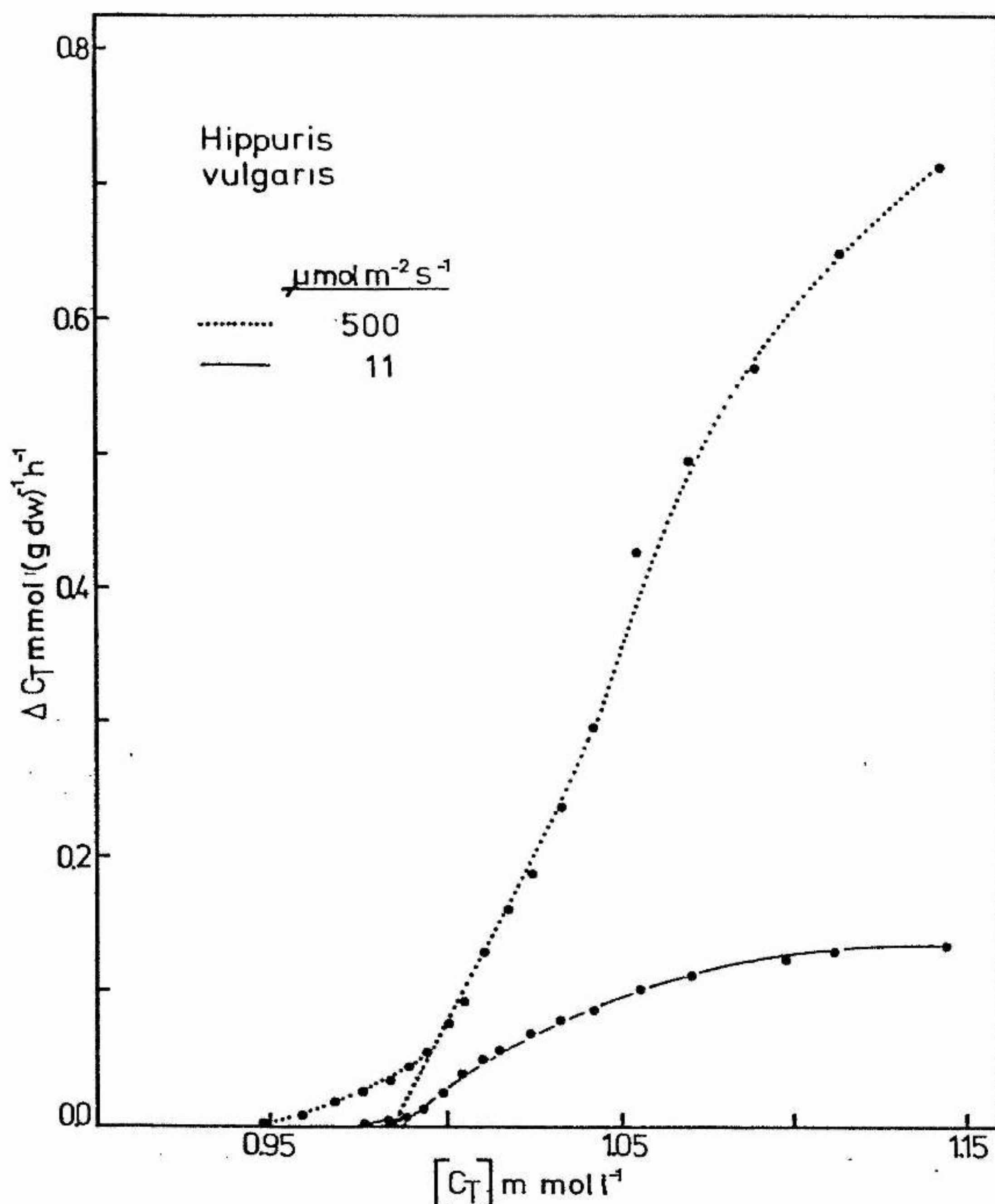


FIGURE 6.4 Changes in net C_T uptake rates over a range of $[C_T]$ for Hippuris vulgaris at 500 μmol m^{-2} s^{-1} and 22 μmol m^{-2} s^{-1} (100% and 11% light and CO_2^* saturated rate respectively). Extrapolation yields CO_2^* CP; final C_T yields HCO_3^- CP. 20°C. Alkalinity 1.0 mmol l^{-1} $KHCO_3$.

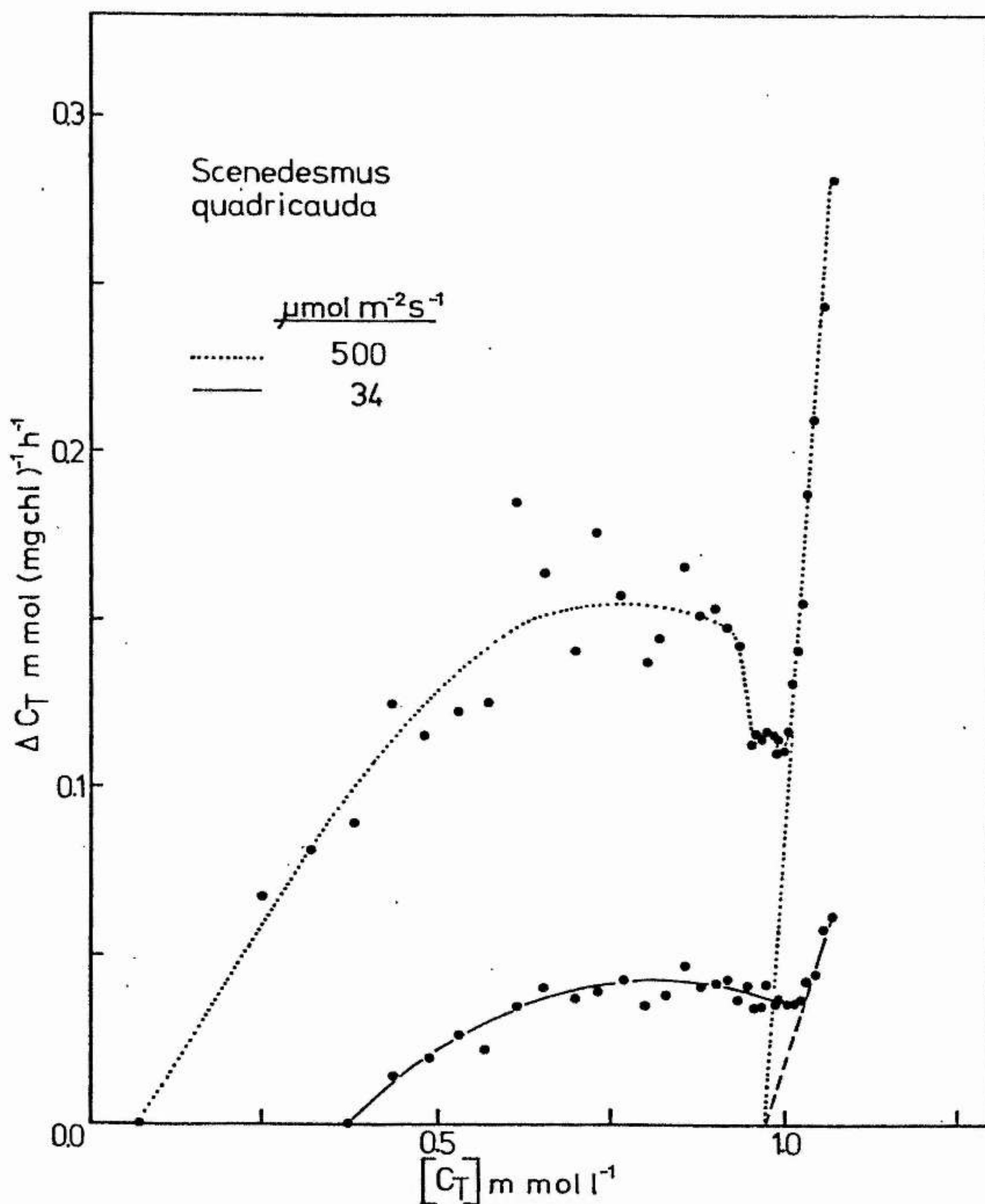


FIGURE 6.5 Changes in net C_T uptake rates over a range of $[C_T]$ for Scenedesmus quadricauda at 500 µmol m^{-2} s^{-1} and 34 µmol m^{-2} s^{-1} (100% and 45% CO_2 and light saturated rate respectively). Extrapolation yields CO_2^* CP; final C_T yields HCO_3 CP. 20°C. Alkalinity 1.0 mmol l^{-1} $KHCO_3$.

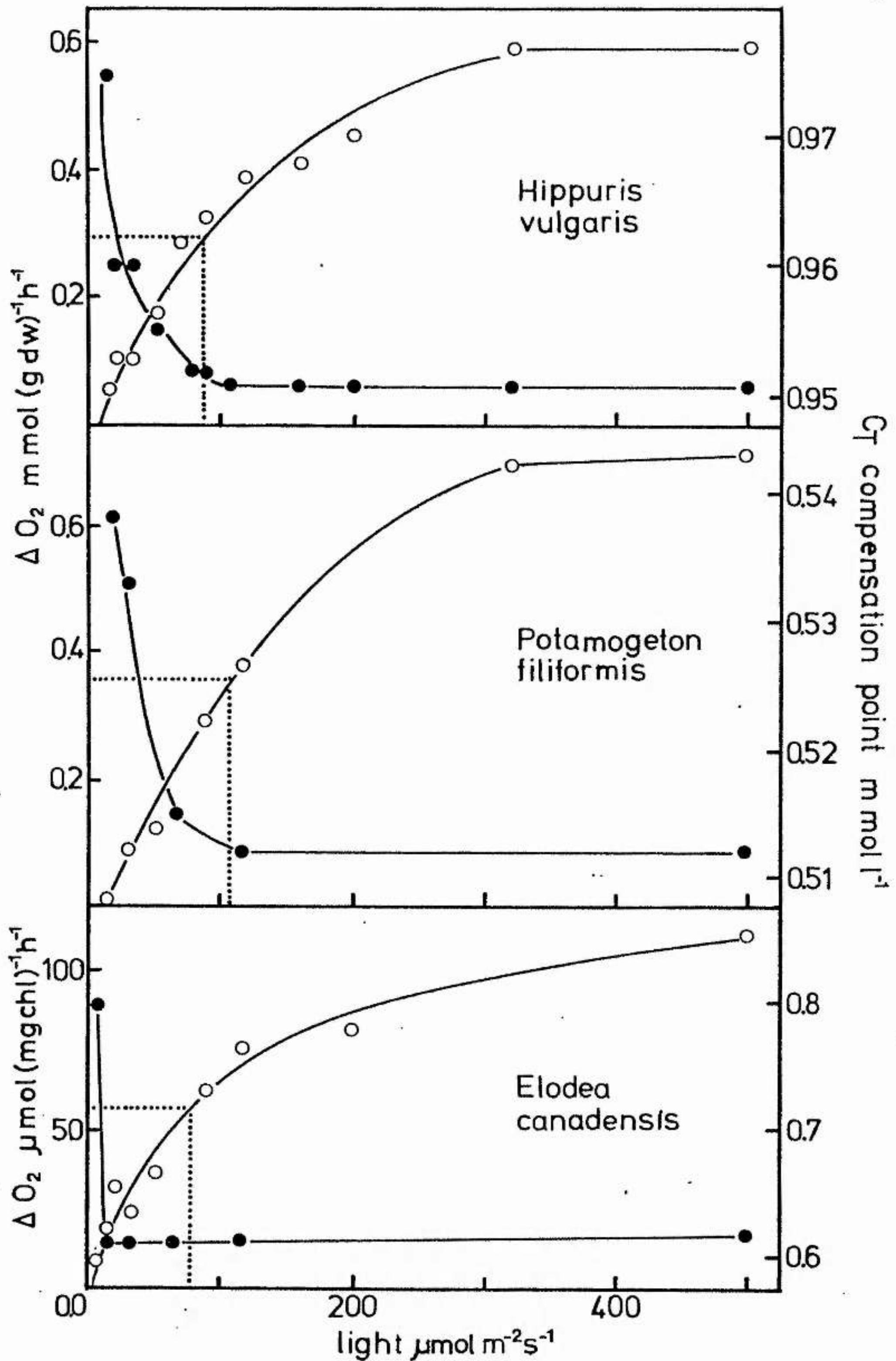


FIGURE 6.6 Changes in net photosynthetic rate (o) and C_T CP (•) against PFAD for three named macrophyte species. $K_{\frac{1}{2}}$ PFAD indicated by dotted line. 20°C. Alkalinity (mmol l^{-1} $KHCO_3$) 0.5 *P. filiformis*; 1.0 H. vulgaris; 2.0 *E. canadensis*; for compensation point data.

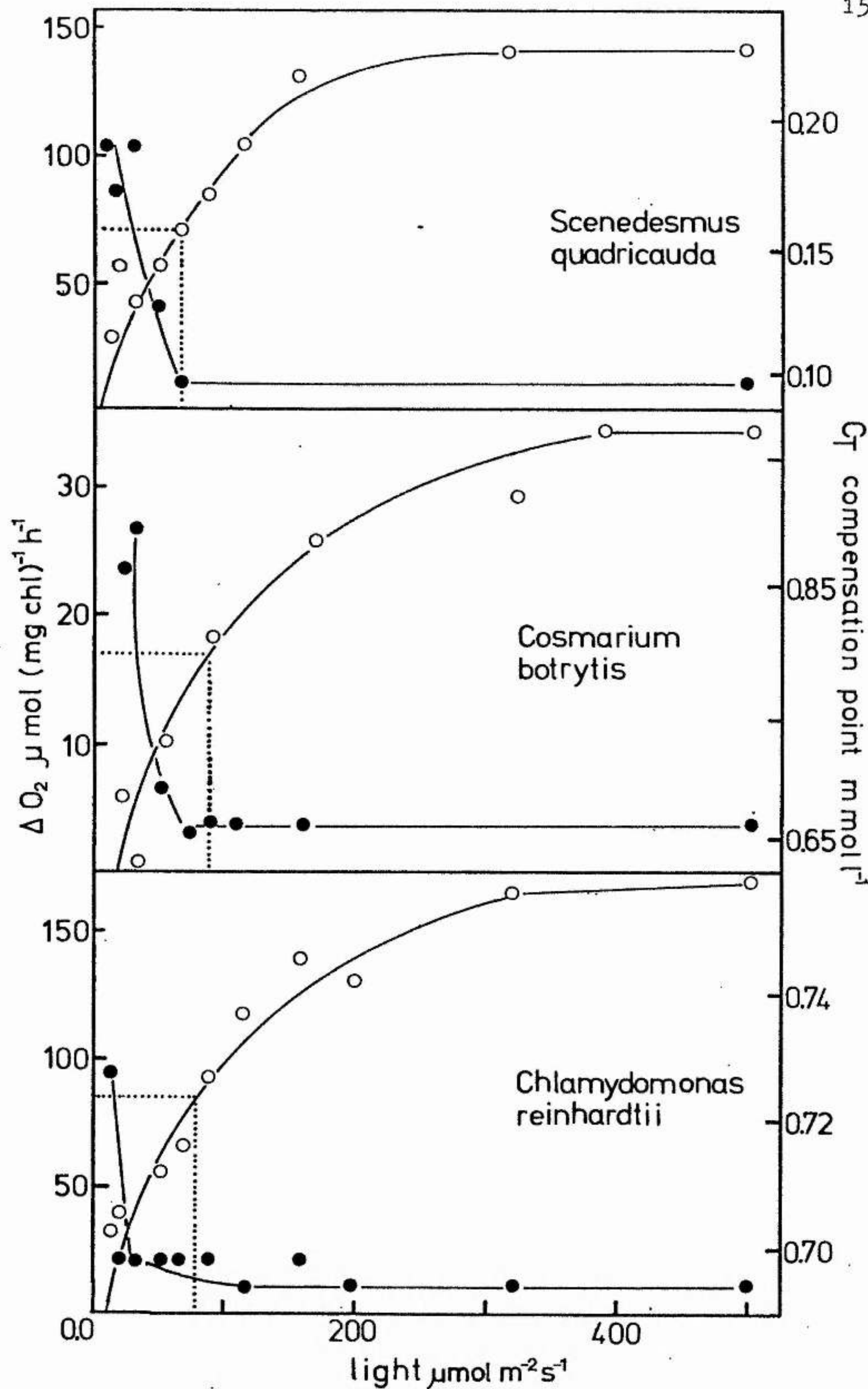


FIGURE 6.7 Changes in net photosynthetic rate (o) and C_T CP (•) against PFAD for three named phytoplankton species. $K_{\frac{1}{2}}$ PFAD indicated by dotted line. 20°C. Alkalinity 1.0 mmol l^{-1} for compensation point data.

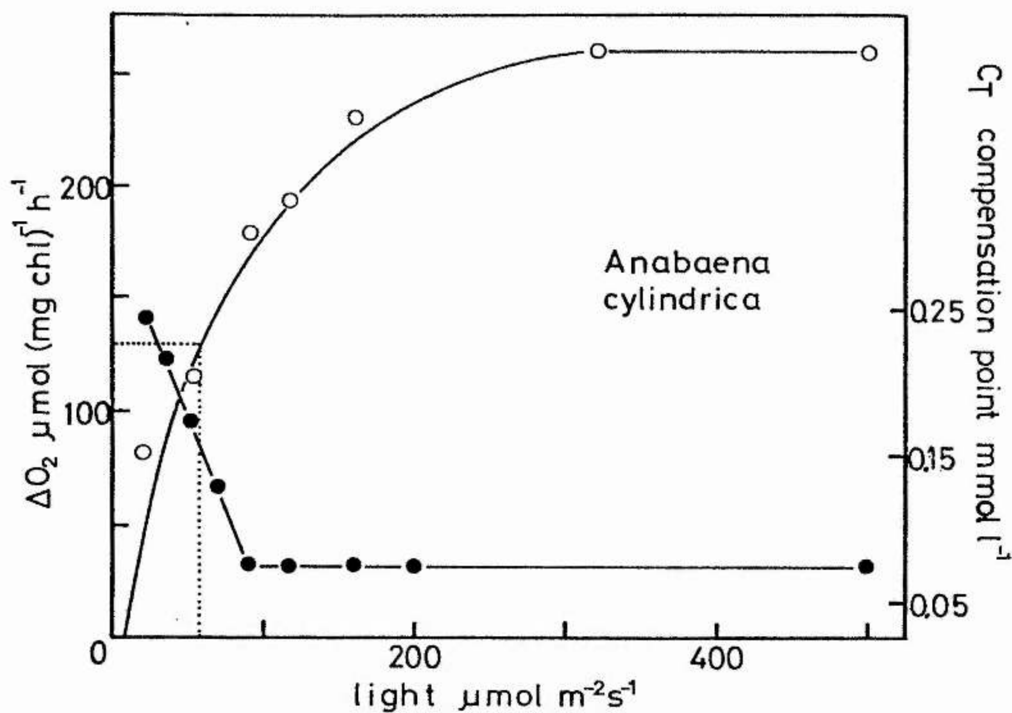


FIGURE 6.8 Changes in net photosynthetic rate (o) and C_T CP (•) against PFAD for Anabaena cylindrica. $K_{\frac{1}{2}}$ PFAD indicated by dotted line. 20°C. Alkalinity 1.0 mmol l^{-1} for compensation point data.

CHAPTER 7

SEASONAL CHANGES IN CO_2^* AND HCO_3^- COMPENSATION POINTS

7.1 INTRODUCTION

CO_2^* CP's have been determined for a number of macrophytes and phytoplankton species (Allen & Spence 1981, Brown & Tregunna 1966, Lloyd, Canvin & Bristow 1977, Lloyd, Canvin & Culver 1977, Talling 1976, and this thesis, Chapter 5), while Allen & Spence (1981) also estimated HCO_3^- CP's. Obviously, in macrophyte/phytoplankton competition, relative carbon compensation points could be an important factor determining the success of these two groups of plants.

Competition can occur throughout the macrophyte growing season, and during this time a succession of different phytoplankton populations will be present (Hutchinson 1967). Talling (1976) has shown that the seasonal succession of phytoplankton in Lake Windermere and Esthwaite Water is associated with changes in the abilities of the different populations to remove carbon from the water. Furthermore, a given phytoplankton species has been shown to alter its carbon uptake properties, depending on the availability of inorganic carbon in laboratory cultures e.g. Chlamydomonas reinhardtii (Berry, Boynton, Kaplan & Badger 1976; Badger, Kaplan & Berry 1980; Kaplan & Berry 1981) and Scenedesmus obliquus (Findenegg 1976). Some terrestrial macrophytes such as Glycine max and Phaseolus vulgaris have been shown to have a seasonally variable CO_2^* CP (Smith, Tolbert & Ku 1976). Aquatic macrophytes also apparently have a variable CO_2^* CP. Sondergaard (1979) found a variation in Elodea

canadensis, but not in *Littorella uniflora*, while Bowes, Holaday, Van & Haller (1977) have shown a seasonal variation in the CO_2^* CP of *Hydrilla verticillata*.

In the case of those macrophytes that are able to use HCO_3^-, seasonal changes in HCO_3^- CP's and net photosynthetic rates are more likely to be relevant to their performance than is seasonal variation in CO_2^* CP's when in competition with phytoplankton for carbon.

Seasonal variations in carbon compensation points were followed using the pH-drift technique for *Potamogeton filiformis* in 1980 and *Elodea canadensis* in 1981. Attempts were made to measure the response of phytoplankton populations from the same site as *E. canadensis* during 1981.

7.2 MATERIALS AND METHODS

7.2.1 Collection sites

Shoots of *P. filiformis* were collected from L. Fitty, from a water depth of about 0.5m on four occasions (1.v, 6.vi, 3.vii and 14.viii.80). Apical shoots of *E. canadensis* were collected from L. Drumore from plants rooted in 2.5-3.0m of water on four occasions (28.i, 11.iii, 21.iv and 10.vi.81).

7.2.2 Photosynthetic measurements

Plant material was used as soon as possible after collection from the field, duplicate samples of *E. canadensis* were run. The results from the second run (stored for about 24h in the laboratory) usually had lower rates and higher compensation points, so these data were not used.

Photosynthetic rates and CO_2^* and HCO_3^- CP's were measured using the pH-drift technique outlined in Chapter 5. In the

case of P. filiformis, measurements were made at 20°C (with the exception of 1.v.80 which was run at lake temperature, 10.6°C) and 220 µmol m^{-2} s^{-1} (400-700 nm) in 0.5 mmol l^{-1} $KHCO_3$. E. canadensis was run at 20°C µmol m^{-2} s^{-1} in 1.0 mmol l^{-1} $KHCO_3$. Photosynthetic rates were expressed on a dry weight basis for P. filiformis by drying the plant material at 90°C for 24h, and on a chlorophyll basis for E. canadensis by extracting the plant material in boiling 90% methanol as outlined in Chapter 5, (5.2.6).

On several occasions in 1981, phytoplankton from L. Drumore was concentrated by allowing lake water to pass through Whatman's GF/A filter paper by gravity, and the filtered material re-suspended in a small volume of lake water. Unfortunately no photosynthetic results were obtained, because the lake contained insufficient amounts of phytoplankton during the study period, even when concentrated.

7.3 RESULTS

Figure 7.1 shows a seasonal change in final $\left[C_T\right]$ for P. filiformis. This occurs in the HCO_3^- uptake section of the C_T uptake curves of Chapter 5, and so represents a seasonal change in HCO_3^- CP. Unfortunately, no information on CO_2^* CP's can be obtained, as the pH-drift was not started at a low enough pH. The data for E. canadensis presented in Figure 7.2 shows that both HCO_3^- and CO_2^* CP's vary seasonally in this species.

Figure 7.3a shows an inverse correlation between the HCO_3^- CP and the maximum HCO_3^- uptake rate for different times of the year for P. filiformis. Figure 7.3b for E. canadensis shows that this relationship does not hold for this species,

both CO_2^* and HCO_3^- CP's decline from winter to early summer, but this is not reflected in large changes in the photosynthetic rate for CO_2^* use at a given $[CO_2^*]$, except for an unusually high rate in April. This difference between the two macrophytes may be a result of their different life cycles; P. filiformis overwinters as turions which germinate in April-May, grow rapidly in mid-summer and start to die back in August-September, while E. canadensis is an evergreen species.

The data for E. canadensis show a much greater variability in the HCO_3^- CP (280-910 µmol l^{-1}) than in the CO_2^* CP (3.5-10.0 µmol l^{-1}), resulting in the C_T CP varying from 573-974 µmol l^{-1} in the 1000 µmol l^{-1} $KHCO_3$ solution used. In the 500 µmol l^{-1} solution used for P. filiformis experiments, the C_T CP ranged from 154-448 µmol l^{-1}, again as a result of seasonal variability in the HCO_3^- CP of between 55 and 410 µmol l^{-1}.

7.4 DISCUSSION

Seasonal variation in CO_2^* CP's occur in terrestrial macrophytes (Smith et al. 1976) and aquatic macrophytes (Sondergaard 1979, Bowes et al. 1977). Certain phytoplankton species are able to vary their CO_2^* CP in response to their carbon supply (Berry et al. 1976). Furthermore, Talling (1976) has demonstrated a seasonal change in CO_2^* and HCO_3^- use for a succession of phytoplankton species in a lake. The results in Figures 7.1-7.3 show that P. filiformis has a seasonally variable HCO_3^- CP and E. canadensis a seasonally variable CO_2^* and HCO_3^- CP. No information is available for P. filiformis on the CO_2^* CP. The range of 3.5-10.0 µmol l^{-1} for the CO_2^* CP is similar to the 3-6 µmol l^{-1} found by Sondergaard (1979) for

this species. In relation to macrophyte/phytoplankton competition, seasonal variation in the HCO_3^- CP will be of greater importance than variation in the CO_2^* CP for HCO_3^- users, as the former determines the amount of C_T a species is able to remove.

The experiments were all run at 20°C (with the exception of one run at ambient lake temperature). It could be argued that the higher CO_2^* and HCO_3^- CP's found early in the growing season for P. filiformis and E. canadensis could be caused by 20°C being too high a temperature for these plants which are accustomed to cooler water. However, preliminary results on the effect of temperature on net photosynthetic rates for winter shoots of Hippuris vulgaris from L. Kilconquhar (collected from under ice) showed 15-20°C to be optimal (results not presented). Two further lines of evidence confirm this. First, the low photosynthetic rate and high HCO_3^- CP of August-collected shoots of P. filiformis (Fig. 7.1) could not be caused by a temperature effect, as lake temperatures were only slightly lower than the 20°C used in the experiments. Second, a detrimental effect of 20°C on winter grown E. canadensis is not found, as the photosynthetic rate at a CO_2^* of 100 µmol l^{-1} is virtually the same for January and June material (Figures 7.2 and 7.3b).

There are various possible causes of the seasonal variation. Figure 7.3a suggests a link between photosynthetic uptake rates and HCO_3^- CP's in P. filiformis; the fastest rates being associated with the lowest compensation points. A compensation point represents a balance between respiration and photosynthesis, and therefore an increased photosynthetic rate would allow a lower compensation point. This link

between photosynthetic rate and compensation point is not apparent in E. canadensis. Bowes et al. (1977) found a seasonally variable CO_2^* CP in Hydrilla verticillata which they suggested was caused by seasonal variation in the activity of two carboxylating enzymes, ribulose-bisphosphate carboxylase and phosphoenolpyruvate carboxylase, the latter predominating in summer. E. canadensis is taxonomically similar to H. verticillata so it is possible that this mechanism also occurs in this species.

A population of macrophytes in a lake may experience a large range of carbon conditions over a growing season, to which they appear to adapt to some extent. This takes the form of lower CO_2^* and HCO_3^- CP's in the summer, when carbon depletion is the most likely. The change in the C_T/alkalinity ratio (see Chapter 5) over a season will give an indication of the ability of a species to adapt to changing carbon conditions. The C_T/alkalinity ratio varies from 0.95-0.57 in E. canadensis and 0.90-0.31 in P. filiformis. Talling (1976) has studied populations of phytoplankton from Esthwaite Water in the English Lake District. The diatom Melosira italica subsp. subartica is common between November and April, at which time of the year, little or no carbon depletion is found in the lake, and this species has a high C_T/alkalinity ratio of 0.97 indicating poor carbon use. In the summer in Esthwaite, carbon depletion can be extensive, with pH values exceeding 10. At this time of the year, the blue-green alga Microcystis aeruginosa is common, which has a C_T/alkalinity ratio of 0.08 indicating that almost all of the inorganic carbon in the lake was available to it. The range of 0.97-0.08 for the phytoplankton from Esthwaite is much greater than

the ranges of 0.95-0.57 and 0.90-0.31 for the two macrophytes, E. canadensis and P. filiformis respectively, (the former species is present in Esthwaite pers. obs.).

Both the macrophyte species were able to use HCO_3^- even in winter or early spring, when little carbon-depletion occurs, and so HCO_3^- uptake confers no advantage. This inability to completely "turn-off" the HCO_3^- uptake mechanism must involve a metabolic cost. The phytoplankton from Esthwaite do not bear this cost, as M. italica is unable to use HCO_3^-, and its use would not be necessary at the time of year this species is found. M. aeruginosa however is a very efficient HCO_3^- user. However, winter grown P. crispus from L. Drumore did show a phenomenon which could be interpreted as an adaptation to the presence of HCO_3^- (see Chapter 5).

Thus, by virtue of their seasonally changing populations, the phytoplankton as an entity is more adaptable than a given species of macrophyte to the changing carbon conditions found over a season in a lake. This would probably also be true for other photosynthetic characteristics such as temperature optima, and light saturation and compensation points.

In competition between macrophytes and phytoplankton, the phytoplankton, comprising of many species, is at an advantage since each population can be more closely adapted to the prevailing conditions than a macrophyte population of few species, whose individuals are present throughout the growing season.

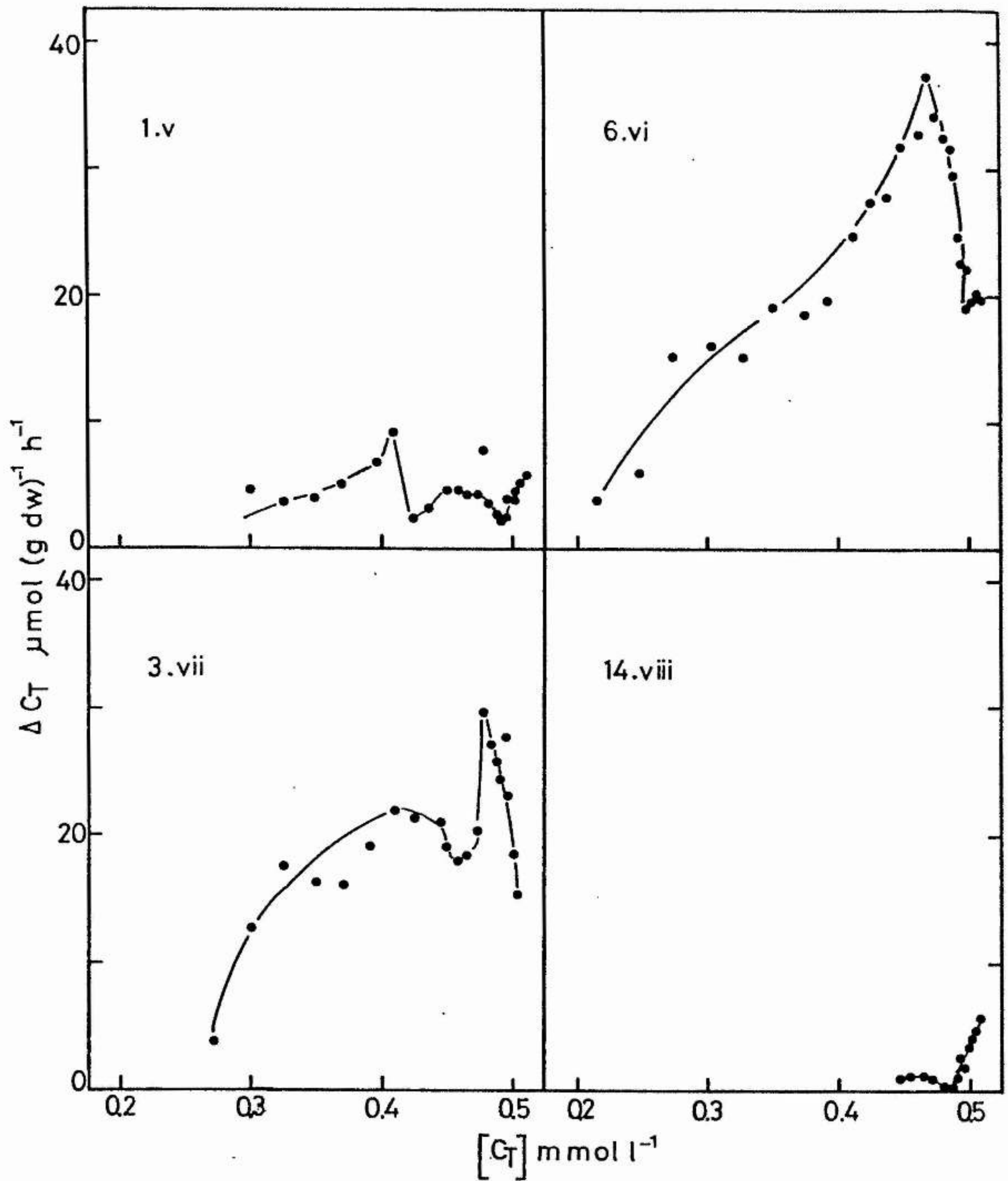


FIGURE 7.1 Seasonal variation in photosynthetic rate and final $[C_T]$ for Potamogeton filiformis collected from L. Fitty. 0.5 mmol l^{-1} $KHCO_3$; 220 µmol m^{-2} s^{-1}; 20°C with the exception of 1.v run at lake temperature of 10.6°C.

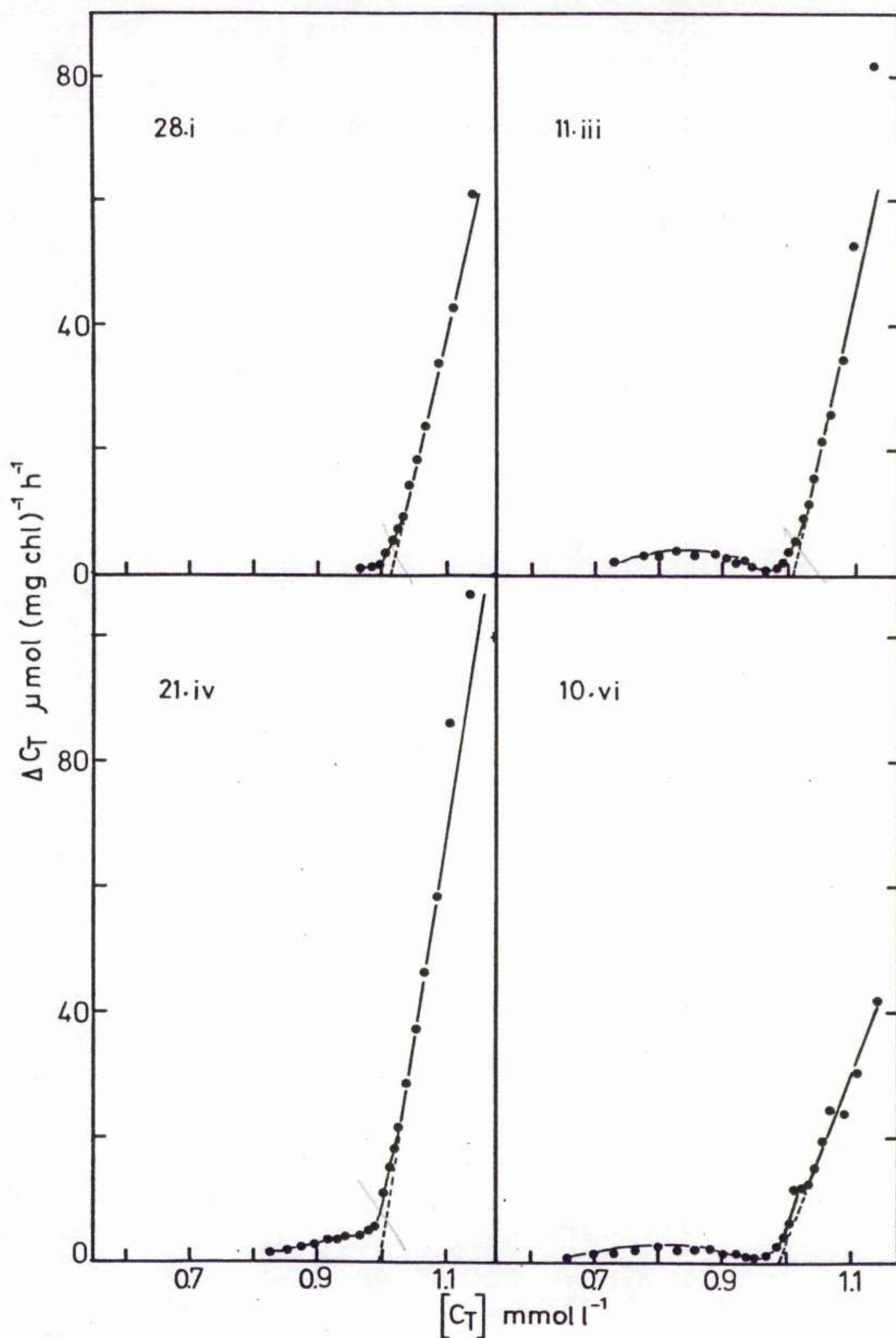
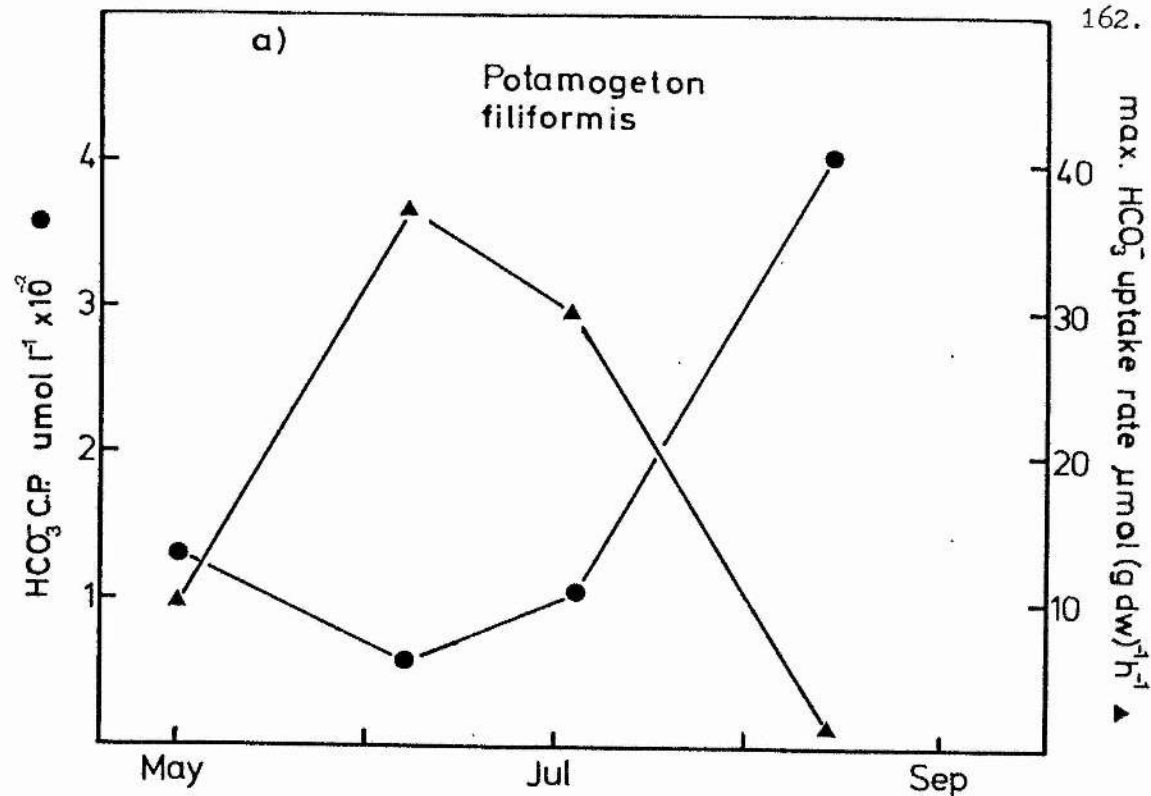


FIGURE 7.2 Seasonal variation in photosynthetic rate, final $[C_T]$ and CO_2^* CP for Elodea canadensis collected from L. Drumore. 1.0 mmol l^{-1} $KHCO_3$; 500 µmol m^{-2} s^{-1}; 20°C.

? diff. depending on populations here, rather than by age, for calculations in Fig 7.3b



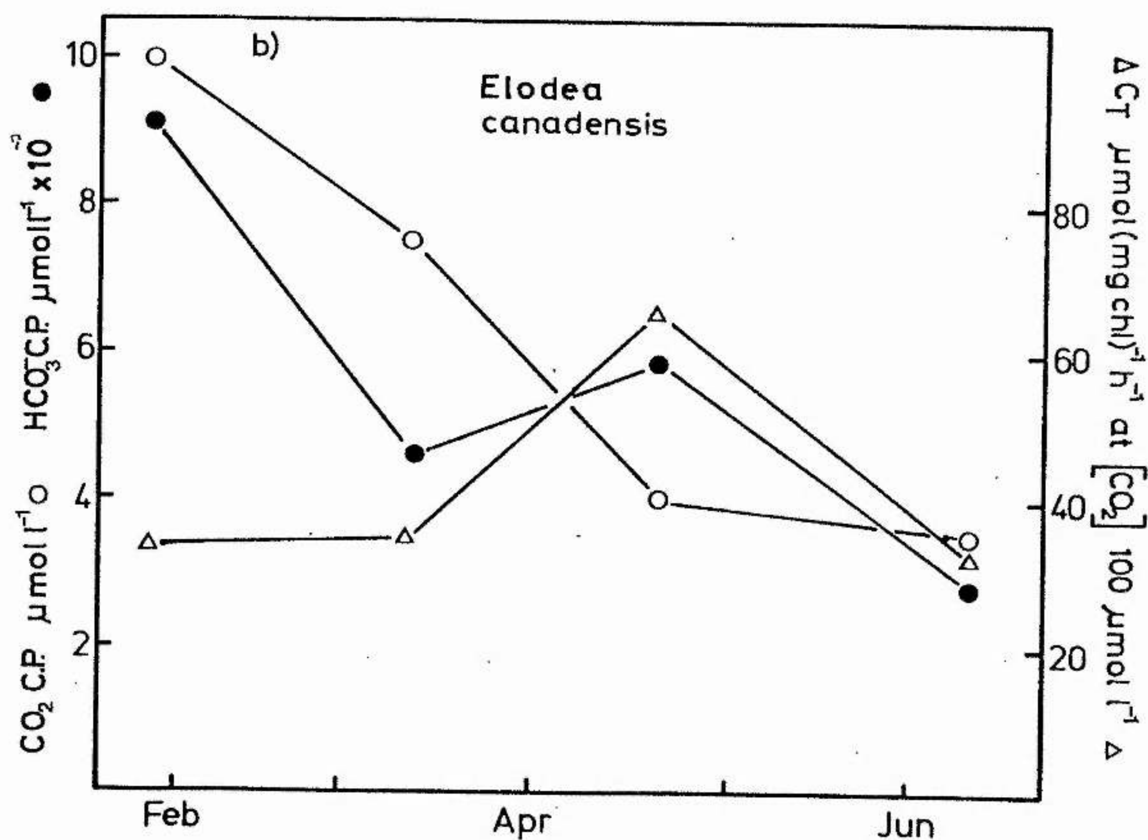


FIGURE 7.3 Seasonal variation in photosynthetic rates and HCO_3^- and CO_2^* CP's derived from pH-drift data for (a) Potamogeton filiformis and (b) Elodea canadensis.

CHAPTER 8

HETEROPHYLLY

8.1 INTRODUCTION

Heterophylly is a common feature of many freshwater macrophytes (Sculthorpe 1967, Hutchinson 1975). In some species such as *Potamogeton x zizii* different leaf types are produced underwater, whereas in most heterophyllous species, the different morphological leaf forms are usually associated with a change from a submerged to a floating or emergent habit.

In certain species of macrophytes, aerial-type leaves are produced underwater. These either remain permanently submerged as in *Hippuris vulgaris*, or later reach the water surface as is the case for floating leaves of *Nuphar lutea*, while both occur in *P. natans*.

The problems of obtaining inorganic carbon in the aquatic environment are different from those in the terrestrial one, so one would expect the leaves to be adapted to the environment in which they grew. This appears to be the case for characteristics such as leaf and cuticle thickness and possession of stomata (Hutchinson 1975). Physiological differences may also occur, such as different CO_2^* CP's and the ability to use HCO_3^-, a photosynthetic carbon source unavailable in the terrestrial environment.

Little work has been published on the effect of heterophylly on the carbon uptake characteristics of macrophytes. Lloyd, Canvin & Bristow (1977) used shoots of heterophyllous macrophytes held in water-saturated air, but their results are open to criticism on the grounds of the harmful effects this

could have on submerged leaves, (Browse, Dromgoole & Brown 1979). Also any HCO_3^- uptake could obviously not be studied. Kadono (1980) found that submerged leaves of Potamogeton distinctus were able to use HCO_3^- while the floating leaves were not, but his method did not allow the estimation of CO_2^* or HCO_3^- CP's. Osmond et al. (in press) using a carbon isotopic discrimination technique found different $\delta^{13}C$ values in submerged, floating, or emergent leaves of heterophyllous macrophytes. This could be a result of HCO_3^- use in the submerged leaves, although boundary layer resistance effects could also cause this. The analysis is further complicated by possible utilization of CO_2^* derived from decomposition of plant material, and the use of carbohydrates stored in rhizomes or turions.

In the terrestrial plant, Zea mays, both C_3 and C_4 photosynthetic characteristics have been found on a single plant, the lower leaves were C_3 while the upper leaves were C_4 (Crespo, Frean & Cresswell 1979). However, Bauer & Bauer (1980) were unable to find differences in the CO_2 CP between adult and juvenile leaves of Hedera helix, although they did show sun and shade differences.

In lakes experiencing C-depletion as a result of the photosynthetic activities of a phytoplankton population, differences in CO_2^* CP's or the ability to use HCO_3^- between the different leaves of a heterophyllous macrophyte will determine which leaf type is the major contributor to the growth of the plant, and may also determine the success of the individual.

This chapter presents values for CO_2^* CP's and photosynthetic rates obtained using different types of leaves or shoots from

five aquatic heterophyllous angiosperms, and investigates their ability to use HCO_3^-.

8.2 MATERIALS AND METHODS

8.2.1 Collection sites

Aerial and submerged shoots of H. vulgaris were obtained from L. Kilconquhar (26 & 27.viii & 3 & 5.ix.80), while aerial-type shoots produced underwater were obtained from L. Borralie (16.ix.80). Floating, submerged and floating leaves found underwater, of Nuphar lutea were gathered from Black Loch (28 & 29. viii & 4 & 6.ix.80). Linear and broad leaves of Potamogeton natans, found both underwater and on the surface in the latter case, were collected from L. Caladail (20.ix.80). Linear and oblong-lanceolate leaves of P. x zizii, a putative hybrid between P. gramineus and P. lucens, were obtained from L. Fitty (24 & 25. viii.80). Both of these leaf types were produced underwater, and no floating leaves were found. Floating leaves and terrestrial shoots of Polygonum amphibium were collected from L. Kilconquhar (31.viii.80) and terrestrial shoots were also collected from L. Lindores (i.ix.80).

8.2.2 Photosynthetic measurements

A continuous record of photosynthetic rate over a range of C_T was obtained using the pH-drift technique of Allen & Spence (1981) as outlined in Chapter 5, and CO_2 compensation points and HCO_3^- use were determined from the data. Macrophytes were used as soon as possible after collection from the field; no pretreatment was given. A constant temperature of 20°C was used with a PFAD of 310 $\mu mol\ m^{-2}\ s^{-1}$ (400-700 nm). pH-drifts were carried out in N_2-purged solutions of $KHCO_3$; 1.0 $mmol\ l^{-1}$ for H. vulgaris, N. lutea and Polygonum amphibium; 0.5 $mmol\ l^{-1}$ for the two Potamogeton species. The plant material was dried

at 90°C for 24 hours, and photosynthetic rates expressed on a dry weight basis.

8.3 RESULTS

The different leaf types of H. vulgaris, N. lutea, and Polygonum amphibium had different CO_2^* CP's (Table 8.1). In the case of H. vulgaris, the submerged shoots had an average CO_2^* CP of 3.5 µmol l^{-1}, compared to an average of 25.9 µmol l^{-1} for the aerial shoots. An aerial shoot produced underwater had a CO_2^* CP very similar to that for the normal submerged shoot. The lower CO_2^* CP were generally associated with faster photosynthetic rates. Submerged leaves of N. lutea had an average CO_2^* CP of 4.2 µmol l^{-1} compared to that of 63.3 µmol l^{-1} for a floating leaf (Table 8.1). A floating leaf produced underwater had a similar CO_2^* CP to a normal floating leaf, although the photosynthetic rate was eight times lower. The submerged leaf had a photosynthetic rate nearly ten times greater than a normal floating leaf. No leaves used HCO_3^- at this alkalinity. A terrestrial shoot of Polygonum amphibium from L. Lindores had a very high CO_2^* CP of 227.8 µmol l^{-1}. A terrestrial shoot from L. Kilconquhar, the rhizome of which was submerged, had a CO_2^* CP of 53.5 µmol l^{-1} whereas a CO_2^* CP of 25.5 µmol l^{-1} was found for a floating leaf from L. Kilconquhar (Table 8.1). The decrease in compensation points were associated with increase in photosynthetic rates. None of the leaves used HCO_3^- at this alkalinity.

The three different leaf types of P. natans had similar CO_2^* CP's ranging from 2.1 to 2.9 µmol l^{-1}. The photosynthetic rates were of a similar order of magnitude. None of the leaves used HCO_3^- at this alkalinity, (Table 8.1).

P. x zizii produces both broad oblong-lanceolate leaves, and narrow linear leaves. Intermediate forms with a broad base and exerted midrib have also been found in the field. Table 8.1 and Figure 8.1 show that both leaf types had a CO_2^* CP of 1.4 $\mu mol\ l^{-1}$. However in an alkalinity of 0.5 $mmol\ l^{-1}$, the broad leaf raised the pH to 10.54 compared to pH 8.88 for the linear leaf. This high pH was caused by HCO_3^- uptake (Fig. 8.1), with an apparent compensation point of 60.0 $\mu mol\ l^{-1}$.

8.4 DISCUSSION

The CO_2^* CP's of 3.5 $\mu mol\ l^{-1}$ (78 $\mu l\ l^{-1}$) and 4.1 $\mu mol\ l^{-1}$ (92 $\mu l\ l^{-1}$) for the submerged parts of H. vulgaris and N. lutea respectively, are similar to values for terrestrial C_3 plants whose CO_2 CP's range from 35-70 $\mu l\ l^{-1}$, Krenzer, Moss & Crookston (1975). The three leaf types of P. natans had CO_2^* CP's from 2.1-2.9 $\mu mol\ l^{-1}$ (47-65 $\mu l\ l^{-1}$) also within the normal terrestrial C_3 range.

The broad and linear leaves of P. x zizii had CO_2^* CP's of 1.4 $\mu mol\ l^{-1}$ (31 $\mu l\ l^{-1}$), slightly lower than for terrestrial C_3 plants. Nevertheless, aquatic macrophytes appear to fix carbon by the C_3 pathway, (Stanley & Naylor 1972, Hough & Wetzel 1972, Browse, Dromgoole & Brown 1977 and Winter 1978). In photosynthetic ^{14}C uptake studies with Elodea canadensis De Groote & Kennedy (1977) found a significant amount of labelled C_4 acids, but pulse and chase experiments suggested they were not being produced by the normal C_4 pathway. Raven & Glidewell (1978) found C_4 photosynthetic characteristics, including low CO_2^* CP's in the complex alga Hydrodictyon africanum, despite the C_3 fixation pathway. They attributed this to a CO_2 concentrating mechanism which could also be present in P. x zizii.

This would not seem to operate by HCO_3^- uptake at the plasmalemma, one of the possible mechanisms discussed by Raven & Glidewell (1978) , as the linear leaves do not use HCO_3^- (Fig. 9.1).

The aerial shoots or leaves of the heterophyllous macrophytes had higher CO_2^* CP's than the submerged parts (Table 8.1). The aerial shoots of H. vulgaris and Polygonum amphibium had the lowest CO_2^* CP's of the aerial parts at 25.9 $\mu mol\ l^{-1}$ (580 $\mu l\ l^{-1}$) and 25.5 $\mu mol\ l^{-1}$ (571 $\mu l\ l^{-1}$) respectively. Floating leaves of N. lutea and terrestrial forms of Polygonum amphibium had very high CO_2^* CP's. This probably results from drowning, so greatly increasing the diffusive resistance normally experienced by the leaves; other effects are probably also responsible.

The aerial-type shoot of H. vulgaris found underwater in L. Borralie was unusual as it had a CO_2^* CP virtually identical to the morphologically submerged shoot, and only a slightly lower photosynthetic rate. In this species, the leaves are small and in whorls around the stem, and so the depth at which they are initiated is approximately the depth at which they photosynthesise. These results suggest that although morphologically aerial, they were physiologically and biochemically aquatic, and the light trigger involved in the change from submerged to aerial shoots (Bodkin, Spence & Weeks 1980) does not affect their photosynthetic properties. This was not the case for a floating type leaf of N. lutea found underwater as it had a similar CO_2^* CP to the normal floating type. However, except for those produced at the end of the growing season, floating leaves are destined to reach the surface where they normally photosynthesise. The photosynthetic rate of this particular leaf was very low, and it is probable that its growth

was largely dependent on the reserves of the rest of the plant at this stage of its development.

The terrestrial leaves of Polygonum amphibium obtained from L. Lindores and L. Kinconquhar differed in their CO_2^* CP's. Turesson (1961) found that different clones of this species respond differently to aquatic and terrestrial conditions, and it is possible that is also true of photosynthetic characteristics.

Lloyd, Canvin & Bristow (1977) found a variation in CO_2^* CP between aerial and submerged parts of Potamogeton amplifolius and Myriophyllum spicatum. The differences between the aerial and submerged parts of M. spicatum were similar to those found in this study and that by Kadono (1980) in that the submerged parts had the lowest CO_2^* CP's; however the reverse was true for P. amplifolius. The photosynthetic rates of the aerial leaves were twelve and two times faster than the submerged leaves for P. amplifolius and M. spicatum respectively, (on a chlorophyll basis). Kadono (1980) found that the aerial leaves of Potamogeton distinctus had a photosynthetic rate four times slower than that of the submerged leaves (on a dry weight basis). In this study, aerial leaves had a lower photosynthetic rate than submerged ones (on a dry weight basis). This study and that of Kadono (1980) measured photosynthesis in water. The system of Lloyd et al. (1977) involved holding leaves in a water-saturated atmosphere which Browse, Dromgoole & Brown (1979) found to be damaging to submerged leaves, and this may account for the low photosynthetic rates found by Lloyd et al. (1977) for these leaf types.

In order to compare properly the photosynthetic rates and CO_2^* CP's of aerial and submerged parts of heterophyllous

macrophytes, two different methods are needed, appropriate to the environment in which the leaf or shoot normally grows. In the case of H. vulgaris, N. lutea and P. natans, floating or emergent parts are sometimes found underwater and so in these cases, it is valid to measure their photosynthetic characteristics in water so long as it is realised that their response in air will be different.

In an aquatic environment, carbon-depletion to below air-equilibrium levels can occur when photosynthetic carbon uptake is faster than replenishment from the atmosphere or sediment. In the presence of a dense phytoplankton crop, carbon depletion can be acute (Talling 1976) and any aerial leaves of macrophytes that are underwater (e.g. as a result of high water levels, or because they are in transit to the surface) are likely to be severely affected. If the $[CO_2^*]$ is below their compensation point, aerial leaves will represent an energy drain on the rest of the plant.

Under conditions of carbon-depletion, which result in elevated pH-values, $[CO_2^*]$ is low and HCO_3^- is the predominant form of inorganic carbon. Any leaf able to take up HCO_3^- will be at an advantage under these conditions. The only leaf type showing HCO_3^- uptake was the broad leaves of P. x zizii (Table 8.1, Fig. 8.1) which was the only species studied which normally produces different types of leaves underwater. The linear leaves of this plant were basal and so produced at the begining of the season when carbon-depletion is less likely. The broad leaves produced for most of the growing season may experience carbon-depletion and so HCO_3^- uptake would be an advantage.

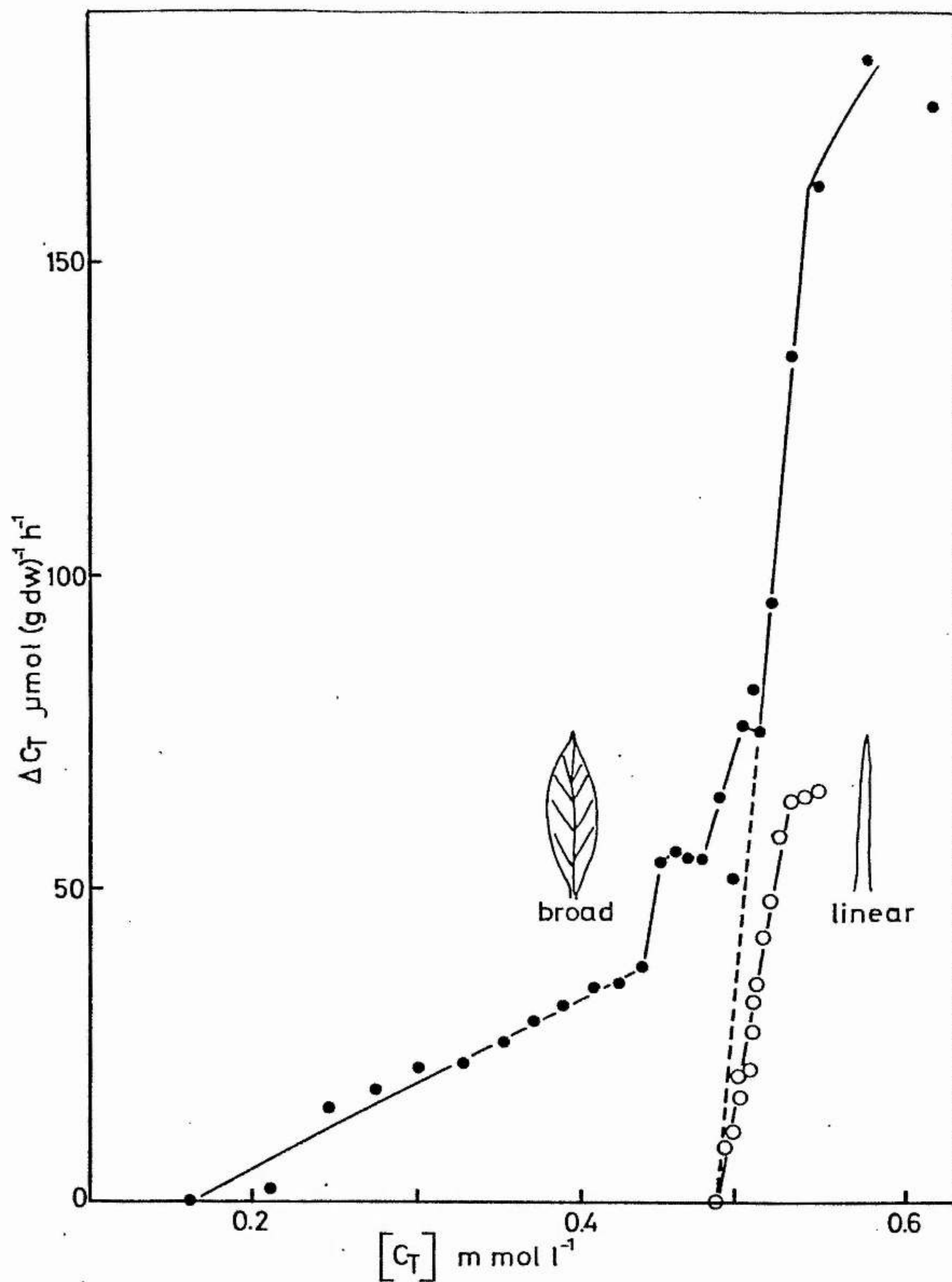


FIGURE 8.1 Net C_T uptake rate against $[C_T]$ for broad and linear leaves of Potamogeton x zizii. PFAD 220 µmol m^{-2} s^{-1}. 20°C. Alkalinity 0.5 mmol l^{-1} $KHCO_3$.

TABLE 8.1

CO_2^* compensation points, HCO_3^- compensation points and photosynthetic rates for 5 heterophyllous macrophytes. Derived from pH-drift data at 0.5 or 1.0 mmol l^{-1} $KHCO_3$. Final pH given. 20°C; 310 µmol m^{-2} s^{-1}.

				C-COMP. POINTS		C_T UPTAKE RATES	
SPECIES	FORM	mmol l^{-1} ALKALINITY	pH	CO_2^* µmol l^{-1}	HCO_3^- µmol l^{-1}	CO_2^* µmol l^{-1}	ΔC_T µmol (g dw)$^{-1}$ h^{-1}
	aerial	1.0	7.89	29.2	-	455	130.3
		1.0	8.00	22.6	-	455	308.1
Hippuris vulgaris	submerged	1.0	8.80	3.4	-	455	890.6
		1.0	8.79	3.5	-	455	2747.1
	aerial/under-water	1.0	8.81	3.3	-	455	696.9
	floating	1.0	7.44	83.1	-	455	51.9
		1.0	7.72	43.4	-	/	/
Nuphar lutea	submerged	1.0	8.66	4.8	-	455	490.2
		1.0	8.78	3.6	-	/	/
	floating/underwater	1.0	7.45	81.2	-	455	6.4
	terrestrial	1.0	7.00	227.8	-	455	21.6

CHAPTER 9

CARBON COMPETITION GROWTH EXPERIMENTS

9.1 INTRODUCTION

Chapters 4 & 5 have shown that in carbon competition, phytoplankton have two major advantages over macrophytes. First, because of their smaller size, they are surrounded by a much smaller unstirred, or boundary layer than are macrophytes, whose photosynthetic rates are, as a result, lower than those of phytoplankton at most $[CO_2^*]$ found in the field. Second, phytoplankton are generally more efficient users of carbon (lower C_T/alkalinity ratio) than macrophytes, particularly those phytoplankton species which commonly form blooms. These are the conditions when any detrimental effect of phytoplankton on macrophytes is likely to be at its most intense.

The results of Chapters 4 & 5 are obtained from physiological experiments. This Chapter attempts to test the conclusions derived from these experiments by growing macrophytes and phytoplankton together under conditions where carbon competition is either prevented by continually renewing the carbon supply, or allowed to proceed by restricting replenishment from the atmosphere. High PFAD's are used to ensure that any effects are the result of carbon competition.

9.2 MATERIALS AND METHODS

9.2.1 Hippuris/Scenedesmus experiment

The experiment was carried out in the apparatus shown in Figure 9.1. Chromo-jars (Shandon) were filled with 2.5 l

of 0.2 x strength Bold's basal medium with added micronutrients and 1.0 mmol l^{-1} $KHCO_3$, which was autoclaved at 15 lb in^2 for 15 minutes. The 0.2 x Bold's basal medium consisted of: (mg l^{-1}) $NaNO_3$ -50, KH_2PO_4 -35, K_2HPO_4 -15, $MgSO_4$ -15, $CaCl_2$ -5 and NaCl -5. The chromo-jar lids were flat pieces of clear glass with a stopcock grease seal. A temperature of 20°C was maintained by a cooler and thermostated heater. Three sources of lighting were used: (i) two 40W white, and two 40W Gro-lux fluorescent tubes vertically above the water surface, (ii) two 150W tungsten reflector lamps above this bank of tubes, (iii) two 20W fluorescent tubes at the side of the tank. The down-welling PFAD at the shoot height was 450 µmol m^{-2} s^{-1} (400-700 nm) with a red/far red ratio of 1.22. Light measurements were made with a Macam quantum sensor. A 24 hour photoperiod was used.

Dormant buds of Hippuris vulgaris were collected from L. Kilconquhar and cleaned by removing silt and any loose scales. The buds were agitated in 70% methanol for 5 seconds and then rinsed in sterile distilled water to try and reduce algal contamination. They were blotted dry, fresh-weighed, and allocated to the experiment using random number tables. Shoots of similar appearance were used for a given position for all four treatments.

A plastic tube was filled with Bold's basal medium (full strength macronutrients) in 3% agar and formed into a ring. Holes were made 15 mm apart in the top of the ring, and the shoots carefully placed in these numbered holes. In a sterile room, the entire ring and shoots were placed in a shallow tray containing sodium hypochlorite with approximately 3% available chlorine (stock solution diluted four times) and left for 5

seconds. Ring and shoots were then removed and placed in a tray of sterile distilled water and rinsed well before they were lowered into position in a chromo-jar. The tubes were suspended by thin nylon threads, and as the shoots grew, the tubes were lowered to keep the shoot tip at a constant height of 1-2 cm below the water surface so that they received the maximum amount of light.

A 60 cm^3 inoculum of Scenedesmus quadricauda was taken from an actively growing aerated culture in Bold's basal medium, and introduced to the chromo-jars 2 days before the shoots of H. vulgaris were added to allow a dense algal suspension to be produced.

Four treatments were used:

(a) H. vulgaris + S. quadricauda + CO_2 renewed

(b) H. vulgaris + S. quadricauda + CO_2 unrenewed

(c) H. vulgaris + CO_2 renewed

(d) H. vulgaris + CO_2 unrenewed

For the first 4 days carbon renewal was by bubbling with cotton-wool filtered air. However, in treatment (a) the rapid algal photosynthesis raised the pH above air-equilibrium, so for the rest of the experiment, carbon renewal was provided by bubbling with cotton-wool filtered 5% CO_2 in air. This gave a $[CO_2^*]$ of approximately 0.7-1.4 mmol l^{-1}. The bubbling also caused stirring, so the unrenewed treatments were stirred with a magnetic stirrer in order to equalise boundary layer thicknesses.

Every six days, seven shoots were removed from the same position in each treatment; choice of shoots being decided from random number tables. Shoots were rinsed in distilled water, dried at 90°C for 24h, and cooled in a desiccator before weighing.

Every 2 days a small volume (approx. 10 cm^3) of liquid was removed from each treatment and a combination pH electrode and meter (Pye 293) calibrated against a buffer at pH 9.2 used to measure pH. Readings were taken to the nearest 0.1 pH unit. Every 6 days cell counts were made of S. quadricauda in treatments (a) and (b) using a Lund counting chamber. Initial dry weights of each shoot was estimated from the freshweight/dry weight ratio obtained for 10 shoots of similar appearance to those used in the experiment. This enabled the change in dry weight of individual shoots to be estimated.

A nutrient bioassay of the S. quadricauda suspension was made on day 12 of the experiment.

9.2.2 Potamogeton/Scenedesmus experiment

The method and apparatus used was similar to that for the previous experiment. Chromo-jars containing 2.5 l of autoclaved 0.2 x strength Bold's basal medium with added micronutrients and 1.0 mmol l^{-1} $KHCO_3$ was maintained at 20°C by a cooler and thermostated heater. Lighting was provided by four 40W white fluorescent tubes vertically above the water surface, and two 20W white fluorescent tubes at an angle of about 45° to the water surface. This gave a downwelling PFAD at the plant height of 270 μmol m^{-2} s^{-1} (400-700 nm) and a red/far red ratio of 10.80. A 16h light: 8h dark photoperiod was used.

Potamogeton filiformis turions were obtained from L. Fitty, and dead outer material and rhizome removed. In a sterile room, the turions were shaken in 7% calcium hypochlorite for ten minutes and then rinsed in sterile distilled water. After blotting dry, they were fresh weighed in a sterile balance (sprayed down with 90% methanol), washed in

70% methanol for two minutes and allowed to air dry in position in the experiment. The turion shoots were threaded through holes in an inverted plastic plant pot, and kept in place with a wedge of autoclaved muslin in the centre of the pot. The pot was attached to a glass rod placed vertically in the chromo-jar by means of small plastic clamps. In the case of bubbled treatments, the glass rod was the stem of the sintered glass bubbler. The shoots were positioned 1-2 cm below the water surface and as they grew the pot was lowered by moving the clamps. Each hole was numbered and matched turions were allocated to a position using random number tables.

S. quadricauda was taken from an actively growing culture in aerated Bold's basal medium, and a 60 cm^3 inoculum added to the appropriate treatments two days before P. filiformis was introduced.

There were four treatments:

(a) P. filiformis + S. quadricauda + CO_2 renewed

(b) P. filiformis + S. quadricauda + CO_2 unrenewed

(c) P. filiformis + CO_2 renewed

(d) P. filiformis + CO_2 unrenewed

On introduction of P. filiformis, all treatments were bubbled with cotton-wool filtered 5% CO_2 in air for about an hour which brought the pH to 6.5, so that all treatments started with the same carbon supply. After this only treatments (a) and (c) were bubbled, treatments (b) and (d) were stirred.

Every two days, a small volume of liquid (approx. 10 cm^3) was removed and the pH measured and cell counts made on treatments (a) and (b). Every eight days, six shoots were removed from the same position in each treatment, determined using

random number tables, rinsed in distilled water, dried at 90°C for 24h, cooled in a desiccator and weighed. Initial dry weights of shoots were estimated from fresh weight/dry weight ratios of eight turions of similar appearance to those used in the experiments.

A nutrient bioassay of the S. quadricauda suspension was made at the end of the experiment (day 24).

9.3 RESULTS

The previously performed pH-drift experiments with the species used (Chapter 5), indicate that S. quadricauda will be a better carbon competitor than either H. vulgaris or P. filiformis (Fig. 9.2). On a chlorophyll basis, the rate of carbon uptake by the alga is greater than that of either of the macrophytes in the CO_2^* region of uptake, probably as a result of smaller boundary layers (see Chapter 4). In addition, S. quadricauda is an efficient carbon user, and can deplete the C_T to low levels as a result of efficient HCO_3^- uptake. P. filiformis shoots are the third best HCO_3^- users of the macrophytes tested (Table 4.1). In this experiment, turions were used, and these have been shown to be less efficient users than shoots alone (Fig. 4.8). Figure 9.2 shows the rate of HCO_3^- uptake to be much lower than that of S. quadricauda. H. vulgaris is one of the least efficient carbon users (Table 4.1) and is probably restricted to CO_2^* only.

Results of the competition experiment between H. vulgaris and S. quadricauda are shown in Figure 9.3. The CO_2-bubbled treatments had a fairly steady pH of about 6.2-6.5 with and without algae. In the stirred conditions where no carbon

renewal was possible, the treatment with S. quadricauda showed a very rapid increase in pH to 10.9, close to the maximum pH attained by this species in pH-drift experiments (Chapter 5). At this pH, the $[CO_2^*]$ is extremely low, at about 1.4 nmol l^{-1} and would not allow H. vulgaris to photosynthesise. The pH in the stirred container without algae rose slowly until days 16-18 when a rapid increase occurred as a result of algal contamination at the end of the experiment.

The number of S. quadricauda cells in the stirred experiment with poor H. vulgaris growth, was slightly greater at the end of the experiment (x 1.6 greater) than where H. vulgaris growth was good in the CO_2 bubbled treatment. By day 12, the S. quadricauda was observed to be browning in the latter treatment, which is normally a sign of nutrient deficiency in ageing cultures. To test this, a nutrient bioassay was performed. Stimulation of growth was noted by a change in colour from brown to green. Of the four treatments; control (no addition), +P, +N, and +P+N, only the latter two turned green, strongly indicating that nitrogen was limiting.

H. vulgaris only failed to grow when in carbon competition with S. quadricauda. The best growth was when CO_2 was bubbled, as would be expected. The $[CO_2^*]$ of 0.7-1.4 mmol l^{-1} is probably close to saturating for macrophytes, (Allen & Spence 1981, this thesis Chapter 4). The CO_2 bubbled H. vulgaris grew slightly better without S. quadricauda than with it, although the difference was not significant (Table 9.1). This may have been caused by a slight shading effect.

Figure 9.4 shows the result of a similar experiment between P. filiformis and S. quadricauda. In both treatments bubbled with 5% CO_2, a pH of 6.1-6.5 is maintained. In the

stirred treatments with S. quadricauda a pH of 10.9 was quickly reached which declined slowly towards the end of the experiment, possibly as the culture aged. The stirred treatment without S. quadricauda showed a slow pH increase which had reached pH 9.9 by the end of the experiment. At this pH and alkalinity, the $\left[C_T\right]$ is about 0.83 mmol l^{-1} and so P. filiformis would be using HCO_3^- at this stage, (Fig. 9.2). The pH of 10.9 caused by S. quadricauda is at a $\left[C_T\right]$ of about 0.19 mmol l^{-1}, well past the final $\left[C_T\right]$ which P. filiformis can reach (Fig. 9.2).

The number of S. quadricauda cells in the unstirred container where poor growth of P. filiformis occurred, was 5-6 times greater than in the CO_2-bubbled container at the end of the experiment. The CO_2-bubbled culture was brown near the end of the experiment, and a nutrient bioassay showed greening only when nitrogen was added indicating a deficiency of this nutrient as in the similar treatment of the previous experiment.

In all treatments, the turions showed a large initial decrease in dry weight in the first 8 days, presumably as a result of a high respiration rate associated with breaking of dormancy. By the end of the experiment on day 24, both groups of turions that were bubbled with 5% CO_2 had grown well; the presence of algae had no effect (Fig. 9.4, Table 9.1). The unbubbled plants did not put on any net growth over the period of the experiment. However, the plants in the absence of S. quadricauda had put on significantly more growth than those with the competitor (Table 9.1) and showed an increase in dry weight between sampling days 16 and 24. The plants in competition with S. quadricauda showed a decline throughout the growth period.

9.4 DISCUSSION

Although laboratory experiments on competition between different species of phytoplankton have been reported (e.g. Jorgensen 1956, Kroes, 1971, 1972, 1973; Tilman 1977, Lam & Silvester 1979), little work has been published on laboratory interactions between macrophytes and algae. Mulligan & Baranowski (1969) studied the yields of three species of macrophyte grown in greenhouses, each of which received different amounts of inorganic N & P. Yields were decreased under the highest nutrient concentrations, which the authors attributed to an increased phytoplankton population of 24-40 $\mu g\ l^{-1}$ chlorophyll as compared to 2-3 $\mu g\ l^{-1}$ in the control or low fertiliser experiments. As no attempt was made to prevent phytoplankton growth it is not possible to determine whether or not the reduced growth was caused by the phytoplankton. As to the possible mechanism of any phytoplankton interaction, it is impossible to conclude whether or not the reduced yields result from shading, carbon depletion, allelopathy, or some other effect.

On the basis of field observations, supported by laboratory experiments, Fitzgerald (1969) suggested that macrophytes have an antagonistic effect towards phytoplankton and epiphytes by acting as a nitrogen sink. Laboratory cultures of macrophytes in Gorham's medium rapidly became contaminated with epiphytes and phytoplankton, while nitrogen-limited or nitrogen-free cultures did not. Fitzgerald also concluded that the action of bacteria-sized organisms with a selective toxicity to certain algae may be important.

Eminson & Philips (1978) carried out growth experiments on seedlings of <u>Najas marina</u> rooted in sediment from Upton

Broad, and grown in enriched or unenriched water from the same site. Slight increases in both phytoplankton and epiphytes occurred in enriched tanks, correlated with a significant ($P > 0.5$) reduction in total macrophyte biomass. The cause of the reduction is unclear; the authors attribute it to shading by epiphytes, but carbon depletion by epiphytes and/or phytoplankton is also possible as no aeration system was used to maintain air-equilibrium levels of CO_2^*.

The results in this Chapter attempt to present unequivocal evidence either for or against carbon competition between macrophytes and phytoplankton. Growth of two macrophyte species, H. vulgaris a CO_2^* user and P. filiformis a HCO_3^- user, with a phytoplankter S. quadricauda a very efficient HCO_3^- user, under conditions where no carbon renewal was possible, demonstrated that an actual reduction in biomass, or a reduction in growth rate occurs in the macrophytes. No significant difference between macrophyte growth was observed in the presence of S. quadricauda if an adequate carbon supply was provided.

When growing with macrophytes which grew well as a result of a good carbon supply, S. quadricauda turned brown, and bioassays showed this to be a result of nitrogen deficiency. This is in agreement with Fitzgerald (1969), who showed algal growth to be suppressed when in nitrogen competition with macrophytes. As macrophytes can obtain a portion of their supply by shoot uptake from the water (e.g. Nichols & Keeney 1976), nitrogen competition may be an important mechanism allowing macrophytes to outcompete phytoplankton in certain situations. Nitrogen-fixing blue-green algae will not be affected to such an extent.

In previous experiments that demonstrated a detrimental effect of phytoplankton on macrophytes (Mulligan & Baronowski 1969, Eminson & Philips 1978) no efficient bubbling system was used, so that carbon competition could account for a part, or all of the effects attributed to shading. It is worth noting that when rapid phytoplankton photosynthesis occurs, bubbling with air alone may not be sufficient to maintain air-equilibrium of pH and CO_2^* .

The growth experiments reported in this Chapter reinforce the physiological results of Chapters 4 & 5 by demonstrating a reduction in macrophyte growth as a direct result of carbon competition with phytoplankton.

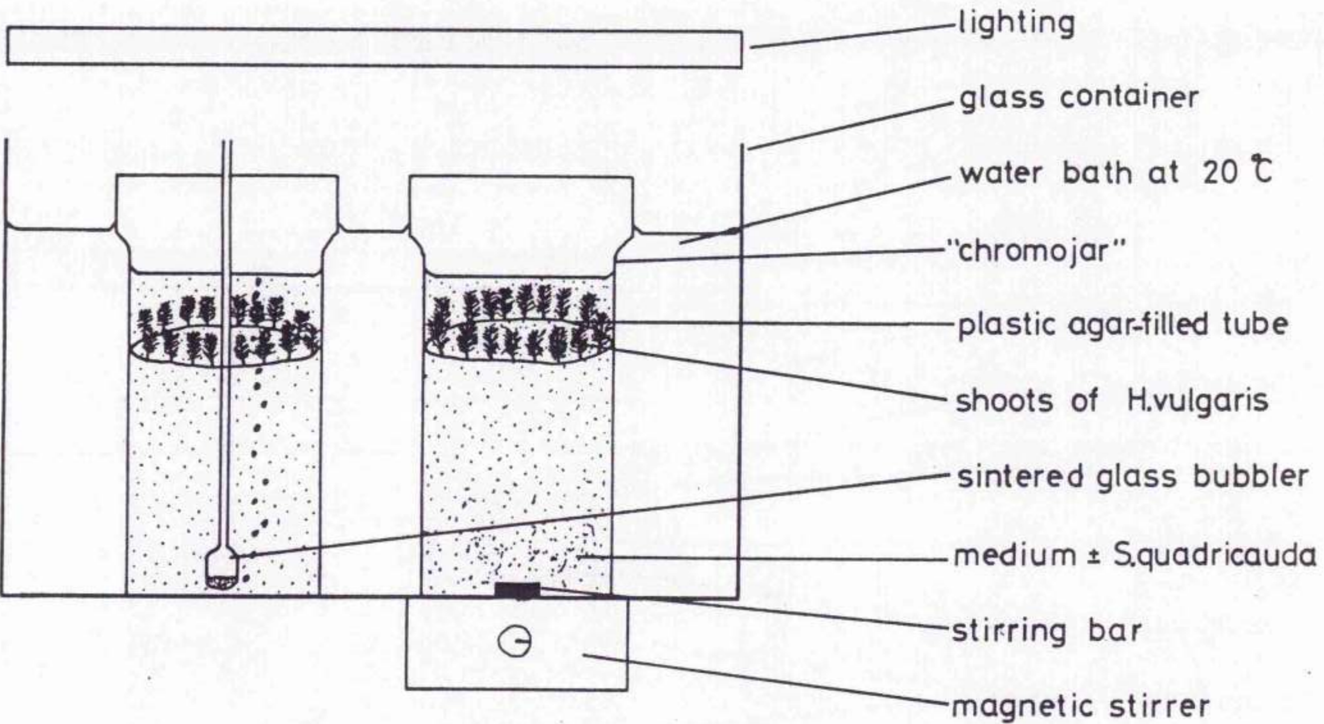


FIGURE 9.1 Diagram of apparatus used in carbon competition experiments.

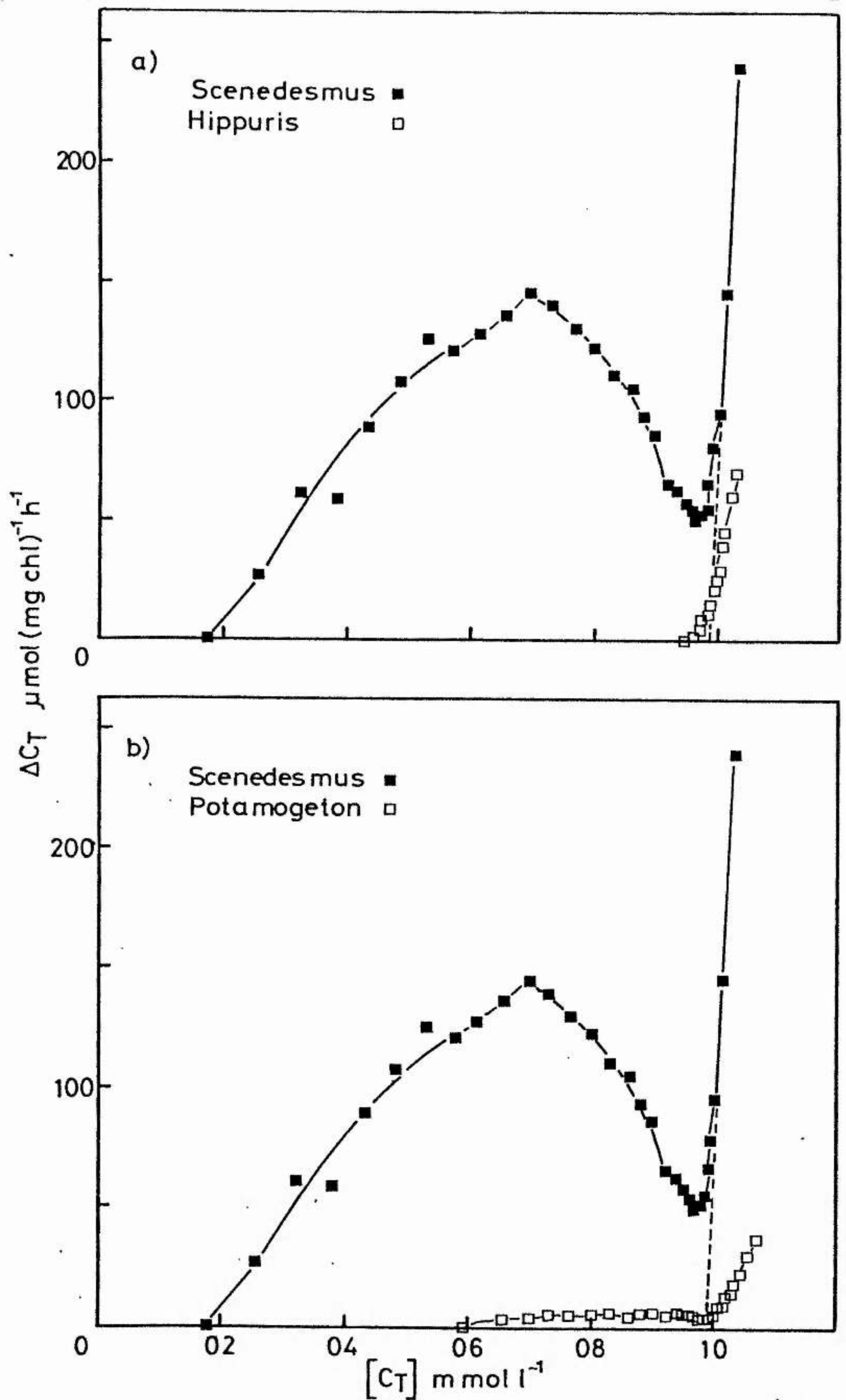


FIGURE 9.2 Photosynthetic carbon uptake rates against $[C_T]$ of the species used in carbon competition experiments.
(a) Scenedesmus quardicauda and Hippuris vulgaris
(b) Scenedesmus quadricauda and Potamogeton filiformis.
$20^{o}C$; 1.0 mmol l^{-1} $KHCO_3$; 500 μmol m^{-2} s^{-1}.

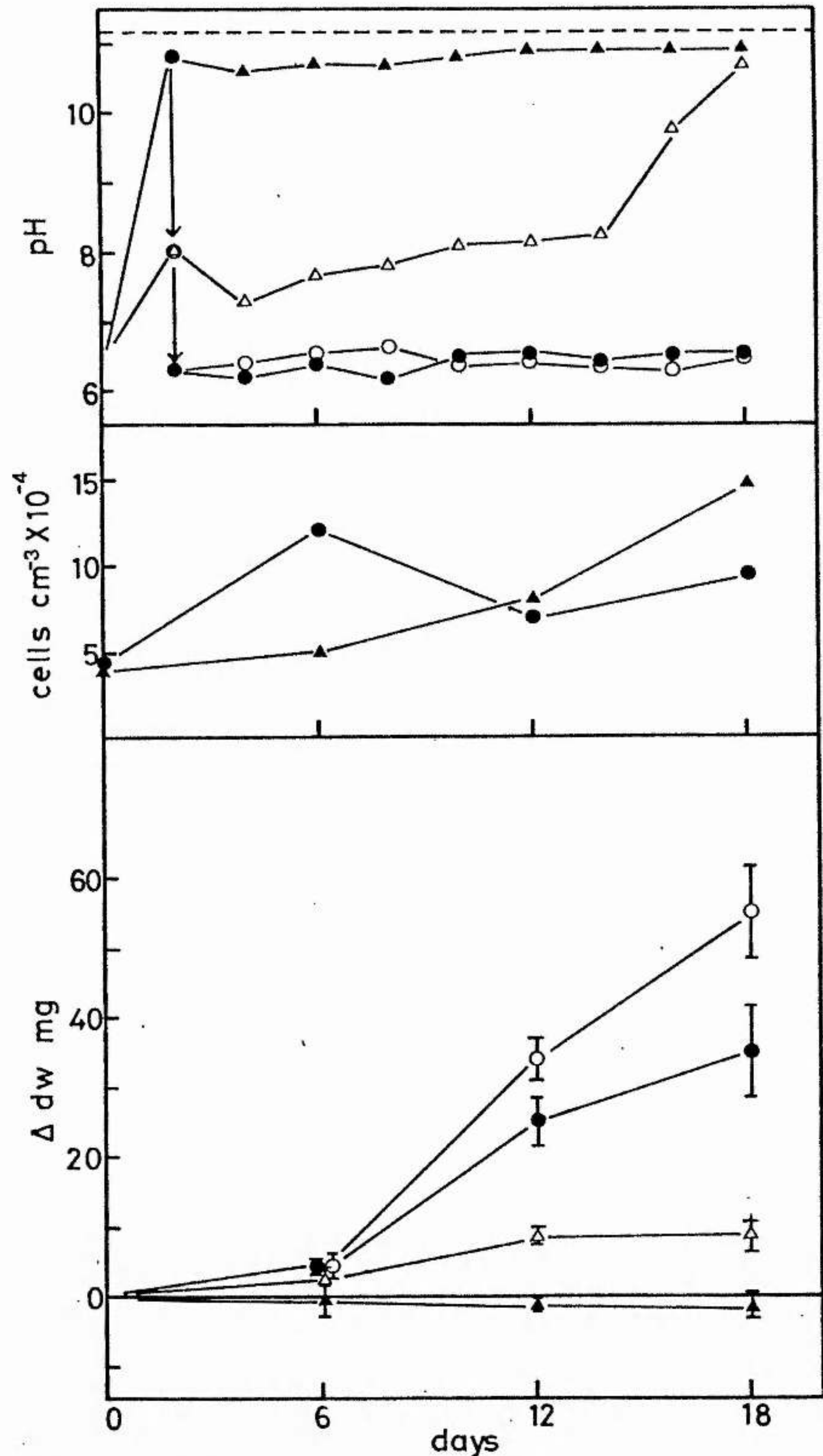


FIGURE 9.3 Carbon competition between Scenedesmus and Hippuris showing change in pH, cell numbers of Scenedesmus and dry weight of Hippuris with calculated standard errors. With Scenedesmus (● ▲); without Scenedesmus (○ △); 5% CO_2 (● ○); stirred only (▲ △).

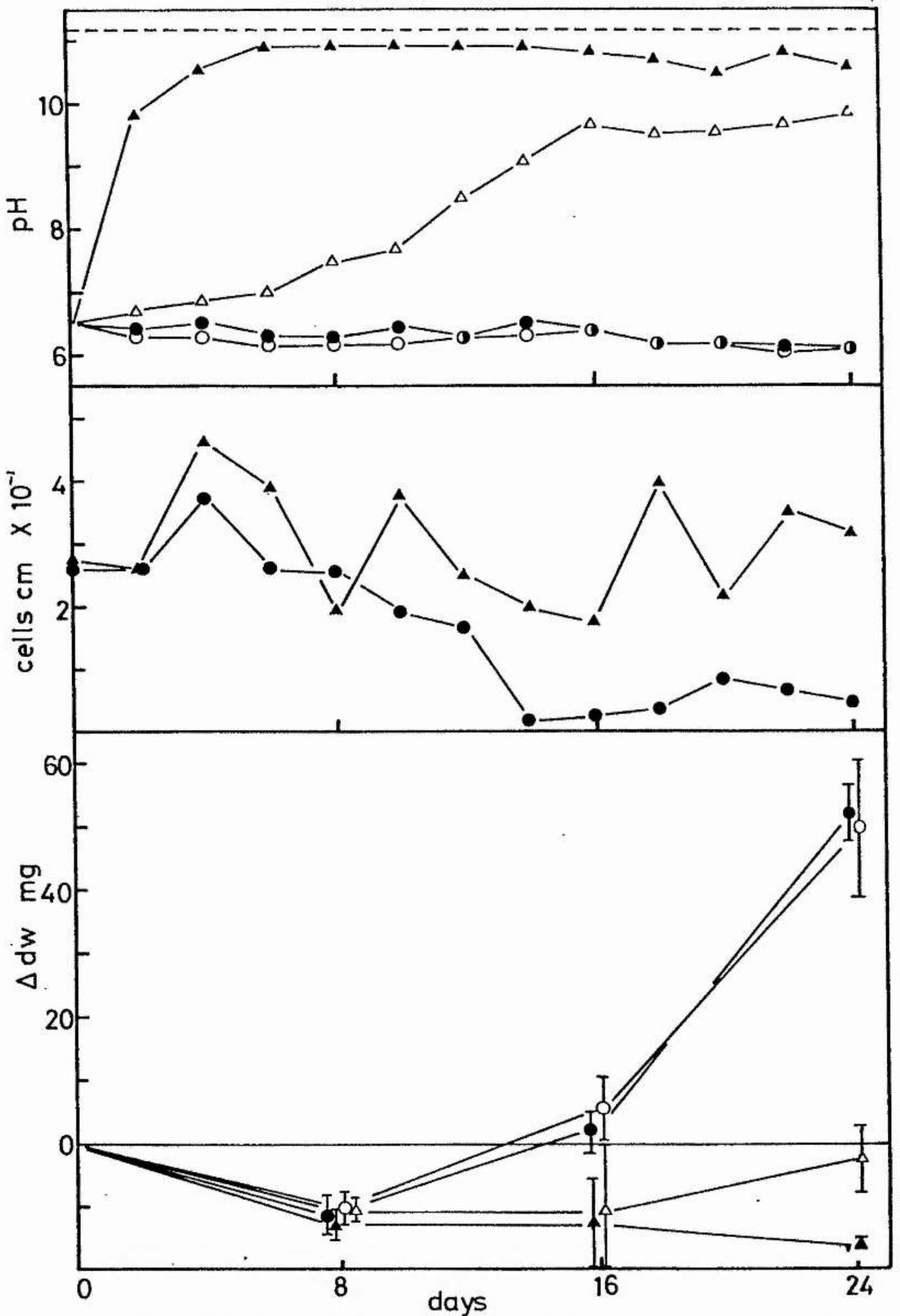


FIGURE 9.4 Carbon competition between Scenedesmus and Potamogeton showing change in pH, cell numbers of Scenedesmus, and dry weight of Potamogeton with calculated standard errors. With Scenedesmus (● ▲); without Scenedesmus (○ △); 5% CO_2 (● ○); stirred only (▲ △).

TABLE 9.1

Results of t-tests on change in dry weight over total growth period (18 days for H. vulgaris; 24 days for P. filiformis).

HIPPURIS/SCENEDESMUS

Comparison	Result
+5% CO_2 ± Scenedesmus	not significant
stirring ± Scenedesmus	$P > 0.01$
+Scenedesmus ± 5% CO_2/stirring	$P > 0.01$
−Scenedesmus ± 5% CO_2/stirring	$P > 0.001$

POTAMOGETON/SCENEDESMUS

Comparison	Result
+5% CO_2 ± Scenedesmus	not significant
+stirring ± Scenedesmus	$P > 0.05$
+Scenedesmus ± 5% CO_2/stirring	$P > 0.001$
−Scenedesmus ± 5% CO_2/stirring	$P > 0.01$

SUMMARY

Macrophytes show lower photosynthetic CO_2^* uptake rates compared with phytoplankton at saturating PFAD and $[CO_2^*]$ (Chapter 4). At subsaturating $[CO_2^*]$, the difference is even greater as a result of greater diffusive resistances in the macrophytes. Macrophytes have a greater resistance to CO_2^* uptake than do phytoplankton largely as a result of a large boundary layer resistance (δ/D) caused by a thicker boundary layer around the larger macrophyte leaves compared with the small phytoplankton cells. δ/D is also the largest component of the total resistance for phytoplankton, but is much smaller in magnitude than that of the macrophytes. The internal diffusive resistance (l/D) is a small part of the total resistance in macrophytes. This suggests that the thin leaves, high SLA's and low chlorophyll content per unit surface area of many macrophytes are not adaptations to reduce the internal diffusive pathlength, but are responses to the large diffusive resistance which makes a large photosynthetic capacity unnecessary on an area basis. The linear or myriophylloid leaves of many macrophytes may represent an adaptation to reduce the thickness of the boundary layer (δ). The smaller resistances of phytoplankton will enable them to outcompete macrophytes for carbon when other conditions are favourable for their growth (e.g. nutrients in the water), even if the CO_2^* and HCO_3^- CP's of the macrophytes and phytoplankton are identical.

The best phytoplankton species are better than any macrophyte at using HCO_3^-, and can remove almost all of the inorganic carbon from solution, thus preventing macrophyte photosynthesis (Chapter 5). It is suggested that species

which commonly form "blooms" in nutrient rich waters are those species which are efficient at removing inorganic carbon from water. The respiratory burden caused by the non-photosynthetic underground parts (roots, rhizomes and turions), of macrophytes is shown to reduce the photosynthetic rate and increase their compensation point. This will reduce their competitive ability further under conditions of carbon depletion.

Large crops of phytoplankton are likely to both shade and carbon-deplete macrophytes. It is shown that low PFAD's of below about that required to half saturate photosynthesis, caused an increase in the CO_2^* and HCO_3^- CP's of macrophytes thus decreasing their performance, (Chapter 6). It is likely that macrophytes grow for much of the time at PFAD's where there is an interaction between light and C_T compensation points. Phytoplankton also show this effect, but it may not be so detrimental to them, as turbulence in the water column will bring them periodically into good light conditions, and certain blue-green algae are able to adjust their buoyancy so that they can maintain themselves in a favourable light climate.

CO_2^* and HCO_3^- CP's (E. canadensis) and HCO_3 CP's (P. filiformis) have been shown to change over the growing season (Chapter 7). The range in C_T/alkalinity ratios in the final solution is less than that found for phytoplankton in Eshwaite Water by Talling (1976), probably because phytoplankton consist of seasonally changing populations, therefore each can be more closely adapted to the prevailing conditions than can a given macrophyte.

Different leaf types from heterophyllous macrophytes often show different CO_2^* CP's (Chapter 8). In addition, P. x zizii

has broad leaves which use HCO_3^- and linear leaves which use CO_2^* only. Both leaf types had the same CO_2^* CP.

A growth experiment demonstrated that at high PFAD's macrophytes only showed a reduced growth in the presence of phytoplankton when inorganic carbon was allowed to become depleted as a result of photosynthetic carbon uptake (Chapter 9). When a good carbon supply was provided, allowing macrophyte growth, the test alga S. quadricauda showed a nitrogen deficiency presumed to be caused by uptake by the macrophyte. This is in agreement with data in the literature and in Section A.

CHAPTER 10

DISCUSSION

10.1 COMPARISON WITH THE ORIGINAL HYPOTHESIS

The results obtained in this thesis generally support the original hypothesis. The standing crop of phytoplankton in a weedbed has been shown to be limited by the availability of P & N in the water as predicted; this was true even when nutrients were added to the water. Macrophytes shoots were shown to be responsible for a part of the nitrate lost from the water, and this uptake appeared to be luxury consumption. Luxury uptake of nutrients from the water by macrophytes may be an important mechanism reducing phytoplankton crops. It is worth noting that Brook & Holden (1957) found that after phosphate addition to a small lake, Lobelia dortmanna and Littorella uniflora did not take up any P, while Myriophyllum spicatum, Potamogeton praelongus and P. gramineus did. The two former species are generally confined to nutrient poor waters and so a shoot uptake mechanism would be unnecessary both as a means of obtaining nutrients and as a way of controlling phytoplankton.

The sediment was shown to take up some phosphate and this may have been chemical or biological. Factors causing a loss of nutrients from the water, such as a dense population of benthic algae, would probably favour macrophytes and disfavour phytoplankton. As a macrophyte-dominated ecosystem is usually a detritus based system (Pieczynska & Ozimek 1976) one may expect bacterial populations to be high. Yull-Rhee (1972) has shown under laboratory conditions that bacteria can

outcompete phytoplankton for phosphate, and this may also be important in the field.

Zooplankton were not considered in the original hypothesis, but results from this thesis and the literature clearly show that they can significantly reduce phytoplankton crops, particularly where efficient filter feeders such as Cladocerans are dominant. A low predation pressure from fish favours zooplankton in general and Cladocerans in particular, and helps to produce clear water favouring macrophyte growth.

No convincing evidence for an allelopathic effect of macrophytes upon phytoplankton has been shown, and low phytoplankton crops in weedbeds may be explainable in terms of depletion of light, inorganic carbon, phosphorus and nitrogen.

Nutrient addition to the enclosures in the weedbed did not affect the macrophyte standing crop, confirming the original hypothesis that the growth of macrophytes was not limited by P & N. In unproductive lakes with nutrient-poor sediments, macrophytes may be limited by P & N as found by Weatherley & Nicholls (1955) who obtained an increase in macrophyte growth as a result of adding fertilizer bags to the sediment surface of an unproductive lake. However, in most cases macrophytes are generally not limited by P & N (Gerloff & Krombholz 1966, Peltier & Welch 1969, 1970). Light and carbon may be more likely to limit their standing crop as a result of limiting photosynthetic rates in the relatively short growing season of most macrophytes.

Carbon competition between macrophytes and phytoplankton which resulted in a reduction of macrophyte growth rates or actual reduction of biomass has been demonstrated in this thesis.

This results from two facts. First, because of the small size of most phytoplankton, the boundary layer around them is much smaller than that around the larger macrophytes, causing a smaller total resistance to CO_2 fixation and hence greater rate of CO_2 uptake. Second, the most efficient carbon extractors are certain phytoplankton species particularly those that are found under bloom conditions. These can remove almost all of the inorganic carbon from lake water as a result of an efficient HCO_3^- uptake mechanism. None of the macrophytes studied were as efficient, even though only shoots have been used in experiments. It was demonstrated in this thesis that the respiratory burden of the below-ground parts of macrophytes reduced the photosynthetic rates and increased the carbon compensation points, rendering the macrophytes less efficient carbon competitors. In the field, carbon competition would be most intense in poorly buffered (low alkalinity) lakes which had a high nutrient availability as the supply of inorganic carbon would be low, and the photosynthetic demand would be high. In highly buffered (high alkalinity) lakes a high photosynthetic demand for inorganic carbon would be offset by a large inorganic carbon supply, and in most poorly buffered lakes the productivity is low, thus causing no carbon depletion even though the supply may be small.

Lakes commonly undergo a seasonal succession of different phytoplankton populations and thus any one population may be better adapted to the prevailing conditions than the macrophyte population which consists of the same individuals throughout the growing season. Results were presented in this thesis which demonstrated a seasonal change in HCO_3^- CP for two species of macrophyte. The range of uptake abilities was less than that

found for phytoplankton in a productive lake by Talling (1976). It is probable that seasonal populations of phytoplankton are better adapted than the macrophytes to other seasonally changing factors such as light and temperature conditions.

Although reduction of light by phytoplankton crops could clearly reduce macrophyte photosynthesis, no direct work has been done on this aspect in the present study because of the large number of variables involved (e.g. fluctuations in light levels at the surface, changes in phytoplankton chlorophyll, water depth, macrophyte adaptation etc.). Instead, the effect of low PFAD's on carbon compensation points was investigated in the laboratory. At PFAD's below that required approximately to half-saturate photosynthesis, decreasing PFAD's caused an increase in both CO_2^* and HCO_3^- CP's. When dense phytoplankton crops develop, both carbon depletion and shading is likely to occur. Under these conditions there will probably be an interaction between light and carbon CP's which results in higher PFAD's and inorganic carbon concentrations being required in order to maintain the macrophyte at a compensation point, compared to a situation where only one of these photosynthetic substrates is in short supply.

10.2 TIMING OF COMPETITION

It is probable that the effect of phytoplankton will vary depending on the time of year that it occurs. It was argued earlier in this thesis that the large amounts of Rhizoclonium produced in certain enclosures when the macrophytes were declining would not have a detrimental effect. In the terrestrial environment, a critical period for weed infestation of crop plants has been shown (Nieto, Brondo & Gonzalez 1968, Dawson

1970) which occurs in the major growth period of the crop. One would expect that macrophytes would be most susceptible to the effects of phytoplankton competition in their early growth period. As the young shoots of most species will be near the bottom of the lake, competition for light will be at its most intense, and any carbon depletion will also greatly reduce the macrophyte growth rate. High crops of phytoplankton found in the spring which continued into the early summer as at L. Leven in 1963 (Morgan 1970) and 1973 (Jupp & Spence 1977a) or in experimental tubes in the Norfolk Broads (Moss 1981) may be particularly harmful to macrophytes.

10.3 RELATIVE EFFECTS OF PHYTOPLANKTON AND EPIPHYTES ON MACROPHYTES

It is difficult to generalise about those conditions under which phytoplankton competition will be detrimental to macrophytes compared to epiphytes and vice versa, as slight differences in conditions might allow one of these competitors to develop and not the other. However, some indication of when each of these two groups of competitors may become important can be suggested.

In rivers, or in small lakes with a large flow-through of water, one would expect epiphytes to be more likely to have a detrimental effect on macrophytes than phytoplankton as the latter would tend to be washed away (Brook & Woodward 1956). Epiphytes may also be more important in lakes with low nutrient availability in the water, as epiphytes may be able to use nutrients leaked from the macrophytes which were originally derived from the sediment (Jorgensen 1957, McRoy & Goering 1974). However, epiphyte populations are unlikely to be very

dense under these conditions and so may not be greatly detrimental to their hosts.

In shallow water, epiphyte shading is possible, and phytoplankton shading is less likely to be important, while in deeper water phytoplankton shading could obviously be important and epiphyte development is less likely. Carbon depletion by epiphytes and phytoplankton can occur in deep and shallow water, but epiphytes have two disadvantages compared to phytoplankton in this respect. Firstly, they will be surrounded by a much larger boundary layer than that around phytoplankton, even if they are not within the boundary layer of the macrophyte. If they are within the host's boundary layer they will be photosynthesising in a zone of carbon (and nutrient) depletion caused largely by the host. Secondly, their carbon compensation points will probably be affected by low PFAD's as is the case for phytoplankton and macrophytes. The buoyant phytoplanktonic blue-green algae will not suffer from this, and the phytoplankton in general will probably benefit from water currents moving them periodically into brighter light conditions.

Rapid macrophyte growth appears to be able to outstrip epiphyte growth (Bell & Eaton 1976) and thus a macrophyte can continually produce new photosynthetic tissue which is free from epiphytes (Sand-Jensen 1977). With regard to the time of competition, if the early growth phase of the macrophyte is the most critical one, epiphyte competition is less likely to be important than is phytoplankton competition as the rapidly growing macrophyte shoots will probably be epiphyte free, particularly in the case of those species which over-winter underground as turions or buds on rhizomes as is the

case for many Potamogeton species.

Spence (1981) has suggested that epiphyte competition is more a feature of small shallow lakes while phytoplankton competition is more likely in larger deeper lakes. Both epiphyte and phytoplankton competition with macrophytes can occur and when they are acting together will be probably more harmful than when acting singly.

10.4 PLANKTONIC BLUE-GREEN ALGAE

The best competitors with macrophytes would appear to be certain blue-green algae as they have several characteristics which give them an advantage over macrophytes and also over other phytoplankton species. Firstly, bloom-forming species (e.g. Anabaena and Microcystis) are very good HCO_3^- users and can remove almost all of the inorganic carbon from lake water. Secondly, they can often adjust their buoyancy, allowing them to photosynthesise in the most favourable light conditions. Dense macrophyte beds may promote settling of phytoplankton by reducing water current speeds, and the buoyancy of the blue-green algae would overcome this. Thirdly, many species are able to fix molecular nitrogen, so low inorganic nitrogen availability in the water caused by macrophyte shoot uptake is not so important for them as for other types of algae. Fourthly, the large colonies that many of them form, gives them an advantage when large zooplankton populations are present.

10.5 SPECULATIONS

Macrophytes are complicated plants with relatively long generation times and can obtain nutrients from the sediment

and the water. They are normally present in undisturbed or slightly disturbed environments. In contrast, the phytoplankton are simple plants with short generation times but are restricted to the water for their nutrients. They can quickly respond to disturbances in the environment particularly in the form of a nutrient input to the water.

Margalef (1975) regards an oligotrophic lake as the 'climax' in lake succession, while a eutrophic lake is regarded as a regression caused by a disturbance (e.g. a large nutrient input). This idea has an attraction since a eutrophic lake with a large phytoplankton population has a simple structure compared to the 'climax' vegetation type of a terrestrial community or a lake dominated by macrophytes with a low availability of nutrients in the water. Also, the phytoplankton show characteristics of 'r-selection' rather than the 'k-selected' characteristics of macrophytes and, again in the terrestrial environment, the 'climax' vegetation shows features of 'k-selection'. The aquatic environment may be less stable than the terrestrial one because the 'climax' vegetation (macrophytes) is able to be shaded by the 'sub-climax' vegetation (the phytoplankton).

10.6 CONCLUSIONS

When nutrient availability in the water is low, macrophytes may be able to grow because of their nutrient supply in the sediment; phytoplankton crops will be low. A small increase in the amount of nutrients entering the lake water may not lead to greater availability for the phytoplankton as macrophyte shoots and the sediment are capable of some nutrient uptake. When the nutrient input to the water

increases further, phytoplankton productivity may increase, although the standing crop may not change significantly if the zooplankton population grazing the phytoplankton is able to control them. A large fish population preying on the zooplankton may remove this check on phytoplankton growth. A large fish population, or an even greater nutrient availability in the water could lead to the development of large phytoplankton crops which could be detrimental to the macrophyte population, particularly if the phytoplankton are dense in the main macrophyte growth period of late Spring.

The rooted nature of submerged macrophytes causes them to be susceptible to shading by phytoplankton, particularly those populations in deep water. Phytoplanktonic carbon depletion is also possible particularly in lakes of low or moderate alkalinity. Competing epiphyte populations may develop on the macrophytes under certain conditions, but although they could reduce the performance of the macrophyte, they may not be so detrimental to them as dense phytoplankton crops, except perhaps in very shallow water where phytoplankton shading would be insignificant.

The large shoot size of macrophytes compared to phytoplankton cells and colonies, allows relatively large boundary layers to develop around them reducing their photosynthetic rate at most concentrations of CO_2^*. Bloom forming species of phytoplankton are able to remove almost all the inorganic carbon from lake water, while macrophyte shoots are less efficient. The macrophyte roots which are an advantage when nutrient levels in the water are low, are a disadvantage under conditions where competition for light and carbon is occurring, as they decrease the net photosynthetic

rate and increase the carbon (and presumably the light) compensation point. Dense phytoplankton crops that both shade and carbon deplete submerged macrophytes are probably particularly harmful, as under these conditions, the photosynthetic compensation point of the macrophyte is increased above that that would occur if only one of these photosynthetic substrates was in short supply.

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