An Ecophysiological Study of the *Elodea nuttallii*–Epiphyton Association

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ABSTRACT

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John Iwan Jones

It has been suggested previously that a nutrient-mediated increase in epiphyton is the cause of the loss of submerged plants that often accompanies advanced eutrophication in shallow waterbodies. The aim of this study was to investigate the nature of the interaction between epiphyton and host plant and hence assess any ability of epiphyton to deleteriously affect the plants' performance. In order to do this the work was divided into two parts. First, the physiology of the chosen plant, *Elodea nuttallii* (Planch) St. John, was investigated in epiphyte-free conditions to establish a baseline detailing the performance of *E. nuttallii* under varied conditions. This provided a foundation for the second part of the work, an assessment of the interactions with epiphyton.

All the variables tested in the laboratory exerted some influence on the physiology of *E. nuttallii*, namely oxygen, pH, dissolved inorganic carbon (DIC) and the boundary layer surrounding the leaves. Whilst increased photorespiration occurred with increased oxygen, photosynthesis responded most to changes in the supply of free carbon dioxide (CO_2*), either through pH or DIC. An increased boundary layer thickness also affected photosynthesis though a reduction in CO_2* supply.

It was found that the plants could counteract low CO_2* availability by utilising HCO_3^-. Crystalline $CaCO_3$ (marl) was deposited on the adaxial leaf surface in a predictable fashion, consistent with the plants utilising HCO_3^- by the polar leaf mechanism. *E. nuttallii*, and the closely related *E. canadensis* Michx., were able to switch on this mechanism under low CO_2* conditions within a few days, far more rapidly than previously thought. However, the growth rate of plants utilising HCO_3^- was very low. The cost of construction, maintenance and running of the HCO_3^- utilisation mechanism was calculated to be 60 $\mu mol\ m^{-2}\ s^{-1}$.

In the field, upper layers of weedbeds were found to experience a diurnal cycle of oxygen accumulation and CO_2* depletion, with the majority of photosynthesis occuring in the morning. In the afternoon the extremely low CO_2* concentrations arrested photosynthesis. The changes in the lower parts of the bed were less extreme. It was concluded that photosynthesis in the field was restricted by a balance of light and CO_2* supply, dependent on which was less available.

Due to the extremely small scale of the epiphyton-plant association, measurements taken in the bulk fluid would misrepresent the nature of any interaction. It was necessary to develop and construct microelectrode apparatus, with probes ≈5 µm tip diameter and sensitive to 0·01 pH, to investigate the pH climate within and immediately surrounding the association. This apparatus was used to examine epiphyte-free plants, artificial plants with epiphyton and *E. nuttallii* with epiphyton, providing a detailed description of the association. Epiphyton layers of different densities were produced by culture under different nutrient regimes. The effect of epiphyton on the boundary layer thickness and CO_2* availability to the underlying leaf were characterised, but it was concluded that in this study the performance of the plants used was affected by epiphyton shading.

It is suggested here that epiphyton can only "invade" a system dominated by submerged macrophytes if the macrophytes' growth rate is first reduced through carbon limitation. This is likely to occur as a result of increased productivity in the waterbody. Once the rate of macrophyte shoot extension is lowered, epiphyton can establish in sufficient densities to affect the plants' performance through shading and disruption of CO_2* supply.

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Chapter 1.

Introduction.

1.1 Introduction.

The influence of man on natural and semi-natural habitats has become a matter of grave concern in recent decades. For most habitats the greatest threat, along with outright destruction, is an anthropogenic increase in nutrients and nowhere more so than wetlands (Dugan, 1993). Such an increase in nutrient availability, termed eutrophication, leads to increased productivity and changes in community structure.

In the case of standing waters, nutrient poor, or oligotrophic, habitats are typified by clear water conditions, with few species and slow-growing macroscopic plants colonising to great depths. As nutrients become more abundant, typically the number of macrophyte (vascular plants and charophytes) species present increases, as does macrophyte growth, becoming luxuriant in eutrophic conditions, but the depth to which the macrophytes colonise decreases (Lachavanne, 1982 & 1985). If nutrients increase still further from an already eutrophic condition, a switch of dominance from macrophytes to phytoplankton occurs. The resultant hypertrophic system will typically have very turbid water, with huge populations of algae developing then collapsing in unstable cycles (Kerfoot & DeAngelis, 1989). Here plants, if present, are restricted to a fringe of marginal vegetation, with submerged species virtually absent (Lachavanne, 1982 & 1985; Vadineanu *et al.*, 1992).

The key point in this process of community change is the switch from plant to phytoplankton dominance, which has severe consequences for many of the organisms which live in the aquatic ecosystem. Plant loss leads to a loss of habitat for feeding, oviposition and refuge of fish, amphibians and invertebrates (de Nie, 1987). Birds are similarly affected by this loss of habitat (de Nie, 1987). Whilst the number of piscivorous fish tends to decline and planktivorous fish become dominant (Townsend, 1988; Maceina *et al.*, 1992), their populations become stunted and small, young fish predominate (Townsend, 1988; Perrow & Irvine, 1992). Since the habitat available becomes less diverse and species are lost, food webs become shorter and less complex (Kerfoot &

DeAngelis, 1989). As those species which tolerate change tend to be the weedy ones common in many altered habitats and rare species tend to be associated with more pristine communities, this change has severe consequences for conservation (De Nie, 1987; Holmes, 1990). Also, as many of the charactistics of hypertrophic water bodies render them unfavourable as a water resource for human exploitation (Rast & Holland, 1988) there are changes in the potential use. Fisheries decline, with the commercially important coregonid and salmonid stocks most affected (Johansson & Persson, 1986; Müller, 1992). If used as a potable water supply the toxins sometimes produced by cyanobacteria, often the dominant group in hypertrophic waterbodies, can have serious consequences for human health (Attwood, 1992). Less dramatic but more common are such problems as increased filtration costs and foul odour and taste.

It appears that there can be a point where, for the same nutrient loading, it is possible for a waterbody to be dominated by either macrophyte or phytoplankton populations to the exclusion of the other (Scheffer, 1990). Evidence supporting this idea of alternative dominance has been recorded for communities separated spatially (Moss, 1990) and temporally (Moss, 1990; Blindow, 1992a; Blindow *et al.*, 1993) within lakes. In one of these circumstances (Blindow, 1992a; Blindow *et al.*, 1993) temporary variations in the water level of the lakes studied may have forced the change, but the resultant communities were stable for long periods.

There are several features of both plant and phytoplankton dominated communities which have a strong stabilising influence, conferring a high degree of resistance to change on the communities. Aquatic plants are to a certain extent able to deplete nutrients from the water column by luxury consumption (Balls *et al.*, 1989; Ozimek *et al.*, 1993), thus denying supplies to phytoplankton. Once nutrients have been totally depleted from the water column the plants can continue growing by utilising nutrients from the sediment (Denny, 1972; Raven, 1981; Barko *et al.*, 1991; van Donk *et al.*, 1993). The plants may also harbour communities of large-bodied cladocera which efficiently graze phytoplankton

keeping their populations low (Timms & Moss, 1984). Allelopathy, the release from the plants of chemical agents inhibitory to phytoplankton, has been suggested as a further mechanism whereby phytoplankton populations are controlled (Phillips *et al.*, 1978). However, evidence is poor, being based on the effect of fractions isolated from plant macerations (Anthoni *et al.*, 1980; Planas *et al.*, 1981; Wium-Andersen *et al.*, 1982, 1983 & 1987; Elankovitch & Wooten, 1989; Pip, 1992) which does not necessarily indicate release by intact plants, or concentrations *in vivo* which are biologically active. Evidence from field and laboratory investigations has been equivocal (Fitzgerald, 1969; Hootsmans, 1991) or contradictory (Godmaire & Planas, 1983; Hootsmans, 1991), or simply lacked adequate control (Crawford, 1979). Further, in a very careful field investigation of *Chara*, repeatedly quoted as possessing allelopaths (Hutchinson, 1975; Crawford, 1979; Anthoni *et al.*, 1980; Wium-Andersen *et al.*, 1982; Hootsmans, 1991), no negative effects were found (Forsberg *et al.*, 1990). The presence of grazing deterrents in aquatic plants has been both shown (Jefferies, 1990) and inferred (Otto & Svenssen, 1981; Otto, 1983; Ostrofsky & Zettler, 1986) and in the presence of relatively high grazing pressure (Sand-Jensen & Madsen, 1989; Lodge, 1991; Jacobsen & Sand-Jensen, 1992; Creed & Sheldon, 1993; Lauridsen *et al.*, 1993) is a far more likely explanation for the many alkaloids, phenolics and other compounds which have been isolated (Anthoni *et al.*, 1980; Planas *et al.*, 1981; Wium-Andersen *et al.*, 1982, 1983 & 1987; Ostrofsky & Zettler, 1986; Elankovitch & Wooten, 1989; Pip, 1992).

On the other hand, phytoplankton populations are stabilised by having an advantageous position with respect to light and populations often increase in early spring before macrophytes become established. Phytoplankton have proportionally less non-photosynthetic tissue than macrophytes, giving them a competitive advantage with respect to carbon uptake if light or carbon is limiting. Cyanobacteria in particular possess a carbon uptake mechanism which is very efficient, especially at high pH (Olofsson, 1980; Shapiro, 1990a). Carbon limitation of macrophytes has been shown to restrict their field distribution (Kadono, 1980 & 1982; Hough & Fornwall, 1988), and growth (Adams *et al.*, 1978;

Hough & Putt, 1988; Madsen & Maberly, 1991; Rattray *et al.*, 1991; Maberly, 1993) and such limitation is potentially the basis of great competition between plants and phytoplankton. In the absence of other food sources, large-bodied cladocera are heavily predated upon by fish, largely relieving the phytoplankton of grazing pressure. The high productivity associated with phytoplankton dominance results in heavy organic loading of the benthos, producing soft, unstable sediment which is easily disturbed by wind or benthic feeding fish, thus increasing turbidity to the detriment of macrophytes (Meijer *et al.*, 1990; Tátrai *et al.*, 1990; Wright & Phillips, 1992). The instability (see Moss & Timms, 1989) of this mud may also prove difficult for macrophyte rooting, especially for sparse populations. Also when submerged plants are scarce, grazing by waterfowl, such as coots (*Fuilca atra* L.), can keep populations low and delay recolonisation of the waterbody (Lauridsen *et al.*, 1993).

The action of these stabilising mechanisms has been inferred from field results, where an increase or reduction of nutrient inputs has not produced the change in community dominance predicted from nutrient levels in other lakes (Balls *et al.*, 1985 & 1989; Irvine *et al.* 1989; Jeppesen *et al.*, 1990; Moss, 1990), and their existence has serious consequences for lake management, making it far harder to reverse the change from plant to plankton dominance once it has occurred than to prevent it from happening. Unfortunately the mechanism which drives the switch is not known, and hence predicting when it will occur is difficult, if not impossible. In the past the first indication that there was something wrong with the system often came when people noticed the development of large algal populations and loss of submerged plants (Mason & Bryant, 1975). It is therefore of great importance to elucidate what drives this switch from plant to phytoplankton dominance, so that the agent(s) forcing it can be monitored and preventative action taken before it is too late. This understanding would also greatly enhance our knowledge of the aquatic ecosystem and of the way in which functional groups from the same trophic level interact.

There are many reports of a decline in aquatic plant populations as a result of factors other than eutrophication which in some circumstances lead to increased phytoplankton densities. Examples of such factors include changes in the introduced, herbivorous black swan populations (Mitchell *et al.*, 1988; Mitchell, 1989), increased inorganic turbidity of inflows (Mitchell *et al.*, 1988), clearance with herbicides or biological control (Richard *et al.*, 1984; Maceina *et al.*, 1992) and disturbance by boat traffic (Murphy & Eaton, 1983; Vermaat & De Bruyne, 1993). In these cases the direct cause of the decrease has been identified and once this pressure has been alleviated, the plants readily regrow (Mitchell *et al.*, 1988). What is of importance to the present study is the nutrient-driven change from plants to phytoplankton that is part of the process of eutrophication and is of biological origin.

Several theories have been proposed to explain the decline of submerged plants with eutrophication. The first and simplest, is that as phytoplankton populations increase, so the light climate for the plants becomes poorer and they are shaded out. However, the tall elodeid plants typical of eutrophic waters grow rapidly to the water surface where light is strongest, so if phytoplankton affect them through light attenuation, this must be mainly early in the season before the plants become tall. Many species have tubers or rhizomes which store large quantities of carbohydrate used to support the establishment phase and enable plants to grow up to where the light is stronger. Further, plants have been known to disappear from lakes where the lake bed is within the theoretical euphotic zone (ie. >1% surface irradiance) (Phillips *et al.*, 1978). Palaeolimnological evidence from shallow lakes suggests that, at least in some cases, phytoplankton populations do not flourish until the plants have gone (Phillips *et al.*, 1978: Moss, 1988), indicating that competition for light is not what is forcing the change. In deep lakes, where macrophytes do not have the potential to occupy the majority of the water column, the change may be gradual rather than stepwise

and shading by phytoplankton may play an important role (Moss, 1990; Scheffer *et al.*, 1993).

Secondly, the loose, and frequently anoxic, mud that is typical of highly productive lakes has also been suggested as a cause of the decline of submerged plants, creating an unfavourable environment for root growth (Bye, 1966). However, the lower parts of weedbeds often become anoxic without any obvious detrimental effect to the plants (Frodge *et al.*, 1990), and both emergent (Dacey, 1980 & 1987; Dacey & Klug, 1982; Schröder *et al.*, 1986; Armstrong *et al.*, 1992) and submerged (Sand-Jensen *et al.*, 1982; Caffrey & Kemp, 1991) plants have been shown to release oxygen from their roots which would reduce potential toxicity. Further, those experiments which showed a negative effect of organic matter on plant growth used nutrient-poor organic matter additions (Barko & Smart, 1983), whereas those conducted with nutrient-rich lake sediment produced the opposite effect (Lauridsen *et al.*, 1993). Although sediment of this nature is common in phytoplankton-dominated lakes, it would have to be produced prior to plant loss to be causative.

A third theory is that phytoplankton oust macrophytes through superior competition for carbon dioxide. Large populations of phytoplankton, particularly cyanobacteria, commonly raise the pH of the water greatly as a result of photosynthesis, and thereby reduce CO_2 to very low levels. Many species of submerged macrophytes can still photosynthesise under these conditions by utilising bicarbonate (Maberly & Spence, 1983; Spence & Maberly, 1985; Madsen & Sand-Jensen, 1991), which remains plentiful at high pH, but they are apparently less efficient at this process than phytoplankton (Olofsson, 1980; Maberly & Spence, 1983; Peslova *et al.*, 1990). Restrictions on the supply of CO_2 can determine the distribution of submerged macrophyte species (Kadono, 1980 & 1982; Hough & Fornwall, 1988; Hough *et al.*, 1989) and cause reduced growth (Adams *et al.*, 1978; Hough & Putt, 1988; Madsen & Maberly, 1991; Rattray *et al.*, 1991; Maberly, 1993). However, cyanobacteria, which are extremely effective competitors for carbon

(Olofsson, 1980; Shapiro, 1990a) and high numbers of phytoplankton in general, are not usually found until the plants have disappeared and, once again, cannot be causative.

There are also several theories which implicate agents other than the phytoplankton to produce the switch of dominance away from macrophytes.

The removal or reduction of zooplankton populations by agricultural insecticide run-off has been proposed, but palaeolimnological evidence linking the cladocera decline with insecticide residues is not strong, the residues having obviously moved within the sediment (Stansfield *et al.*, 1989). Further, the loss of plants by some other mechanism would result in a similar loss of plant associated cladocera and it is possible that other invertebrates, such as snails which graze epiphyton (see below), were more severely affected by the pesticides.

Changes in the fish community, which becomes dominated by cyprinids with eutrophication (Johansson & Persson, 1986), have also been suggested as the cause of the switch. Laboratory studies have shown that in the absence of other food, grazing of *Elodea canadensis* Michx. by the omnivorous rudd (*Scardinius erythrophthalamus* L.) can release nutrients into the water column making them available to phytoplankton (Hansson *et al.*, 1987), but omnivorous fish and plants often coexist in large numbers in the field. The removal of large populations of benthivorous fish, mostly bream (*Abramis abrama* L.), from a shallow gravel pit with loose sediments, produced significant reductions in inorganic turbidity, previously caused by their feeding activity stirring up sediment, together with vast increases in the number of plants (Wright & Phillips, 1992). This effect has been noted in other lakes where benthivorous fish have been removed (Meijer *et al.*, 1990; Tátrai *et al.*, 1990) and depends on the firmness of the sediment (Wright & Phillips, 1992). Bream and the omnivorous rudd and roach (*Rutilus rutilus* L.), all of which readily feed on zooplankton, are often removed or reduced during the biomanipulation of European lakes, in an attempt to reduce predation on zooplankton and thus reduce phytoplankton populations (eg. Moss *et al.*, 1986; Jeppesen *et al.*, 1990b; Meijer *et al.*, 1990; Tátrai *et al.*,

1990; van Donk *et al.*, 1993). The effects on water clarity are often good, either as a result of a decrease in phytoplankton or inorganic turbidity (Shapiro, 1990b), and in several cases large populations of submerged plants have re-appeared, but have sometimes proved unstable and reverted back to plankton dominance (Shapiro, 1990b; Van Donk *et al.*, 1993). Although this illustrates a potential management tool for the reversion of lakes to a plant-dominated state, it is not necessarily indicative of the mechanism by which plant dominance was lost in the first place.

The final theory for the change from plant to plankton dominance to be considered here concerns the community of algae which, together with bacteria, fungi, protozoans and inorganic matter, grow over the surfaces of submerged plants and is collectively called epiphyton (Wetzel, 1983a). This community has been shown to respond far more rapidly to nutrient increases than phytoplankton (Sand-Jensen & Søndergaard, 1981) and palaeolimnological evidence indicates that epiphytic algae increase in numbers before their planktonic counterparts (Phillips *et al.*, 1978; Moss, 1988). It has been suggested that excessive growths of epiphyton are detrimental to the host plant beneath, either by shading its surfaces or by reducing the exchange of dissolved gases (Phillips *et al.*, 1978), with both these effects being shown in the laboratory for the marine plant *Zostera marina* L. (Sand-Jensen, 1977). Whilst field studies have shown that shading by epiphyton is almost certainly responsible for the decreased depth distribution of isoetid plants that occurs as oligotrophic lakes become more nutrient rich (Sand-Jensen & Søndergaard, 1981; Sand-Jensen & Borum, 1984; Sand-Jensen, 1990), such deleterious effects have only been suggested for the tall elodeid species typical of eutrophic waters (Phillips *et al.*, 1978). These elodeids have fast growth rates and apical growing points (*cf.* basal in isoetids and *Zostera*), with the majority of photosynthesis concentrated near the shoot tips. This growth form may enable them to outgrow epiphyton colonisation, and hence escape at least some of the detrimental effects (Birch, 1990). There is, however, good evidence that epiphyton can deleteriously affect submerged macrophytes (Eminson & Phillips, 1978). In two

circumstances where epiphyton grazing has been altered, both experimentally (Underwood, 1991a; Underwod *et al.*, 1992) and/or naturally (Irvine *et al.*, 1993; Bales *et al.*, 1993), submerged macrophyte growth has increased. In the case of Hickling Broad, as a result of increased epiphyton grazing by a mysid shrimp, the plant populations increased without any change in phytoplankton populations (Bales *et al.*, 1993). A continuation of this idea is that winter-kills of piscivorous fish allow more tolerant, molluscivorous fish populations to expand, reducing the number of snails which in turn allows epiphyton to increase, to the disadvantage of the submerged macrophytes (Brönmark & Weisner, 1992).

Loosely attached filamentous algae such as *Cladophora* and *Spirogyra*, more accurately described as metaphyton than epiphyton (Wetzel, 1983a), have also been implicated in plant loss (Simpson & Eaton, 1986; Phillips *et al.*, 1978; Ozimek & Kowalczewski, 1984; Ozimek *et al.*, 1991). Experimental manipulations have shown that such algae can, under certain conditions, have a competitive advantage over *E. canadensis* with respect to carbon acquisition (Simpson & Eaton, 1986), and can negatively affect growth of this species (Ozimek *et al.*, 1991). Excessive growths of *Spirogyra* sp. may explain the loss of *Najas marina* L. in Upton Broad, Norfolk (Phillips *et al.*, 1978), where the loss roughly matched the area afflicted by algae. Submerged plants would be affected by these algae in the same way that they are affected by epiphyton, ie. by decreased light, reduced carbon dioxide concentration and by decreased flow.

It is the fundamental question of how macrophyte performance is affected by epiphyton, and how this may lead to plant loss and associated habitat degradation, that is the central theme of this work.

In order to investigate the macrophyte-epiphyton interaction the work was divided into two parts. First, using a plant typical of eutrophic waters in Europe, *Elodea nuttalli* (Planch.) St John (Mériaux, 1982; Simpson, 1984) as the subject, this study aimed to

initially characterise the effect of changing environmental parameters on its physiology in epiphyte-free conditions. The way in which the plant could counteract any epiphyton-mediated constraints on its performance was also investigated and field data collected so that the restrictions on photosynthesis under natural conditions could be assessed. In this way a base-line, detailing the performance of *E.nuttallii* under varied conditions, was established as a foundation for the second part of the work, an assessment of the interactions with epiphyton.

The work of Allanson (1973) and others (Luttenton *et al.*, 1986; Patterson & Wright, 1986; Birch, 1990) has described the extremely close spatial proximity that exists between the host plant and its epiphyton, an association so close that small-scale, possibly symbiotic, recycling loops between the plant and epiphyton (Allen, 1971; Wetzel, 1983b) and within the epiphyton (Riber & Wetzel, 1987) have been proposed, particularly for bacteria. Hence, to understand properly the nature of the interaction between epiphyton and host macrophyte it was necessary to probe the micro-environment surrounding the plant's leaves, where the conditions may be very different to those in the bulk fluid. In order to do this a microelectrode system, with probes of much finer resolution than any previously used (electrode tip diameter ≈ 5 μm), was developed to investigate the pH conditions in the water immediately adjacent to the leaves of *E. nuttallii* (within 2·5 mm). The second part of this work will thus describe the construction and use of this microelectrode apparatus.

Once the pH climate surrounding epiphyton-free leaves had been described, epiphyton was introduced into the system and the resultant effects on the performance of the plant determined. The effect of increased epiphyton was ascertained, in isolation and with the complicating factor of the plant's photosynthesis. This was done by culturing epiphyton on both artificial (plastic) and real plants at different nutrient levels and then investigating the conditions within the association with the microelectrode apparatus. The effect of increasing epiphyton on plant performance was also determined.

The final section will be the synthesis of this work. This will conclude by a discussion of the factors governing the growth of *E. nuttallii*, and submerged macrophytes in general in the field and the potential for epiphyton to grossly interfere with their performance. The relevance of this work to lake management and restoration will also be considered in this final discussion.

Elodea nuttalli was ideally suited to this type of study because:

i) a plentiful field supply was available all year round,

ii) the plants were easy to collect and work with,

iii) the plants respond well to culture conditions, and have been grown in such a manner sucessfully in the past (Birch, 1990),

iv) a microalgal epiflora does develop on its surfaces, often becoming quite dense,

v) although some initial studies have been undertaken on both the physiology of this species and its interaction with epiphyton much still remains to be done.

Chapter 2.

Standardisation of Methodology.

2.1 Collection of Material from the Field.

2.1.1 Plants.

The plants were collected using a grapnel, from the tow-path side of a section of the Leeds and Liverpool Canal between Old Roan Bridge (7D), and Blue Anchor Bridge (8) (SJ371991 - 378985) and returned to the laboratory in buckets filled with canal water. There plants were carefully cleaned of periphytic filamentous algae and detritus by hand and intact, healthy shoots selected for later use. These shoots were stored in a bucket filled with twice-filtered canal water (see following section), in a temperature-controlled growth tank at 15 °C ± 2 °C , recieving a light intensity of 100 μ mol (PAR) $m^{-2}s^{-1}$ on a 16:8 light:dark cycle.

For experiments involving both *E. nuttallii* and *E. canadensis,* an alternative collection site was used, namely the Lancaster Canal at Stainton (SD523854), as the two species grow together at this site. The plants were collected, cleaned and stored in the same manner as those from the Leeds and Liverpool Canal.

2.1.2 Water.

Water was collected from the Leeds and Liverpool Canal at Melling Stone Bridge (11), Maghull (SD384004) in cleaned 25 l plastic carboys and returned to the laboratory, where it was twice filtered through 25 TI 35, 40 x 40 μm mesh plankton netting to remove algae and suspended solids. Filtration through Whatman GFC filter paper was also used as a further step when heavy blooms of the blue-green alga *Oscillatoria* occurred. The water was finally stored in the laboratory in clean plastic carboys for up to several weeks. The effectiveness of the filtration was evident from the length of time it was possible to store the water without significant algal growth.

2.1.3 Sediment.

The sediment used to root the plants was collected with a Petersen Benthic Grab from the bed of the Leeds and Liverpool Canal at Maghull (SD375019). This site was chosen as the dual-carriageway Northway Bridge (12A) provides strong shading and thus prevents the growth of plants, which otherwise make collection of the sediment difficult, but the canal is not narrowed, as it is at the original bridges where the flow is increased and silt scarce. A growth test showed that this sediment produced better growth of *Elodea* plants than soaked John Innes Seed Compost Number 1 (appendix 1).

2.2 Laboratory Analyses.

2.2.1 Photosynthetic Determinations.

In all cases photosynthetic and respiratory rates were measured as oxygen evolution and uptake, respectively, at 15 °C using a Clarke-type oxygen electrode (Hansatech, King's Lynn, UK), with a tungsten slide projector bulb providing incident light of 290 μmol m^{-2} s^{-1} PAR, sufficient to saturate photosynthesis (Birch, 1990), or darkened with close fitting aluminium foil and black polythene. Some workers (Jahnke *et al.*, 1991) have reported that photosynthesis in *E. nuttallii* does not become saturated until a far higher level of illumination, 1,100 μmol m^{-2} s^{-1} , but self-shading was probably high in this study due to their use of a large number of leaves (10) in a small volume of medium (2 cm^3). Since *Elodea* has an caulescent growth form, the leaves increase in age with distance from the apex. If leaves from different plants are selected from the same point on the stem, they should be of a similar age, though variability in growth rate between shoots cannot be accounted for. It was decided following the work of Birch (1990) and Simpson (1981) to use leaves taken 3 cm from the shoot apex, as these leaves are fully expanded, activity is maximal and epiphyte density is low. For determinations, unless otherwise stated, one complete whorl (three leaves) was carefully excised from the stem with a scalpel, then brushed clean of epiphytes and marl (crystalline $CaCO_3$) with a soft paint brush, before being placed in the electrode reaction chamber containing 1·5 cm^3 of Forsberg II (Forsberg, 1965) medium, modified by omission of Na_2SiO_3 and carbon sources. This technique of epiphyte removal was found by Simpson (1981) to be quick and efficient, without damaging the leaves. The solution used for the determinations was maintained at pH 7, unless otherwise stated, by the addition of Tris buffer at a concentration of 50 mM, and adjusted to pH 7 using a weak HCl solution before use. The temperature was maintained at 15 °C by use of a water jacket fed from a temperature-controlled bath. In each case photosynthesis was initiated by the injection of 0·1 cm^3 of $NaHCO_3$ solution into the reaction chamber to give a final concentration of 2·4 mM, the natural concentration of

dissolved inorganic carbon (DIC) found in the Leeds and Liverpool Canal. Preliminary studies showed that no significant pH change occurred on addition of the $NaHCO_3$.

To reduce the effects of photorespiration on photosynthesis (Simpson *et al.*, 1980), all photosynthetic rates were determined within 1 mg O_2 l^{-1} amplitude change, in solutions containing 9 mg O_2 l^{-1} (±100% saturation), being sparged when necessary with either O_2 or N_2 to achieve this concentration before immersion of the leaves and measurements began. Photosynthetic rates were determined from the measured rate of change in oxygen concentration in the reaction chamber and calculated as rate of oxygen change per unit chlorophyll.

2.2.2 Chlorophyll Determination.

The chlorophyll content of leaves was determined by the method of Arnon (1949). The leaves were first ground in a few drops of ice-cold 90% v/v acetone with a pestle and mortar, then the solution was transferred into a centrifuge tube, and centrifuged at 3,000 R.P.M. for 5 minutes. After decanting the supernatant into a 5 cm^3 graduated flask, the tube was refilled with acetone, the pellet resuspended and the centrifugation procedure repeated. The graduated flask was then topped up to 5 cm^3. Finally, the absorbance of the solution was measured in a 1 cm glass cell at 645 and 663 nm (abs_{645} and abs_{663} respectively), using a Pye-Unicam SP8-100 spectrophotometer (Cambridge). These values were then substituted into the equation of Arnon (1949) to calculate total (a + b) chlorophyll per whorl in µg.

$$\text{Total Chlorophyll a + b} = 5 \times [(20{\cdot}20 \times abs_{645}) + (8{\cdot}02 \times abs_{663})]$$

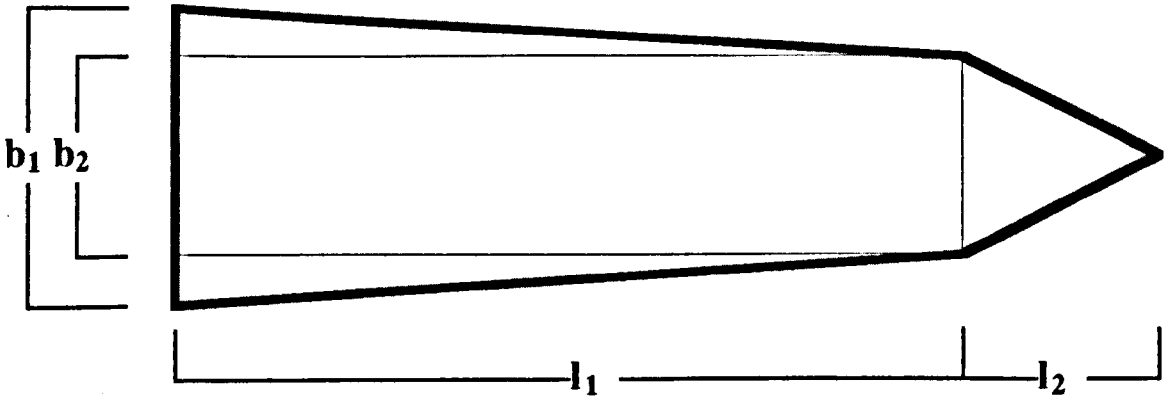
Although recently alternative adsorption constants have been suggested for chlorophyll (Porra, 1991), those used in the Arnon equation were the best available at the start of the project. The equation of Porra (1991) gives values averaging 11·5% less than

those of Arnon, but varying with the ratio of chlorophyll a:b, which was not determined in this study. It was therefore decided, for the sake of consistency, to continue using the Arnon equations, despite the possible overestimate in chlorophyll content and hence an equivalent underestimate of photosynthesis and respiration per unit chlorophyll.

2.2.3 Measurement of Leaf Area.

The leaves of *E. nuttallii* closely approximated to the simple geometric pattern outlined below, ie. one rectangle and three triangles. Four measurements were taken for each leaf with a dissection microscope fitted with an eyepiece micrometer, namely; the width of the leaf at its base (b_1), the width of the leaf at the shoulder, near the apex (b_2), the length of the leaf from its base to the shoulder (l_1) and the length from the shoulder to the apex (l_2). The "shoulder" was determined by eye as the point where the leaf began to sharply taper towards the apex. Repeat measurements indicated that the shoulder point was easily and reliably identifiable. The area was then calculated using the formula:

$$\text{area} = [b_2 \times l_1] + [2 \times (b_1 - b_2)/4 \times l_1] + [b_2/2 \times l_2]$$



Since the leaves are consistently two cell layers thick, a measure of leaf area such as this can be used as an accurate estimate of leaf size and hence for comparison between different leaves.

2.2.4 Scanning Electron Microscopy.

A simplified method of dehydration, as developed by Veltcamp *et al.* (in press), was used to prepare the samples for scanning electon microscopy (SEM). The sample was put into a closed container filled with absolute ethanol which had been cooled to circa -15 °C for the previous 24 hours, then immediately transferred to 4 °C for a further 24 hours. After this time the sample was transferred to a second container of cooled ethanol for a further 24 hours as before. Use of this technique resulted in dehydration of the often fragile sample with very little handling or distortion as compared to the normal dehydration series.

Following dehydration, the samples were dried using a Polaron E3000 critical-point dryer, mounted on an aluminium stub and coated with a 60% gold-palladium alloy (Polaron E5100 sputter coater) before examination with a Phillips 501B SEM.

2.3 Standardisation of Physiological Determinations.

2.3.1 Methods.

2.3.1.1 Cumulative mean.

A cumulative mean test was used on repeat photosynthetic determinations to establish the number of samples required for statistically adequate replication in further analysis.

2.3.1.2 Leaf size and chlorophyll content.

An estimate of the chlorophyll per unit area of leaf was made by measuring leaf size and chlorophyll content of single leaves from whorls 3cm from the tip of replicate shoots as outlined above. By taking leaves from the same point on the stem, potential variation in chlorophyll content with leaf age was largely eliminated

2.3.1.3 Effect of chlorophyll content on physiology.

Repeat measurements of photosynthesis and respiration were taken, as outlined above, on the leaves from a number of stems and the effect of variations in chlorophyll on oxygen exchange plotted.

2.3.1.4 Variation due to changing the solution within the reaction chamber.

Since the Clarke-type oxygen electrode only measures the concentration of oxygen within the reaction chamber, which is then extrapolated to absolute amounts, the volume of medium within the chamber is very important. The error involved in the medium changing procedure was estimated from weight differences of repeated filling and draining of the reaction chamber.

2.3.1.5 Sectioning of shoots.

This experiment was to determine if removing the leaves from the stem adversely affected their physiology over the limited period in which measurements of photosynthesis and respiration were to be made and also to assess the role of the stem, and especially the lacunae within the stem, in photosynthesis. According to some workers (Sorrell & Dromgoole, 1986, 1987, 1988 & 1989; Sorrell, 1991) submerged aquatic plants use these lacunae as gas storage organs, building up carbon dioxide within them during periods of net respiration, for later use in photosynthesis, the converse occurring with oxygen.

Determinations of photosynthesis and respiration were made on three replicate sections cut from randomly selected shoots in the following ways;

i) intact whorl, one half internode of stem above and below, lacunae cut.

ii) intact whorl, one whole internode of stem above and below, lacunae intact.

iii) intact whorl, one whole internode of stem above only, lucunae intact.

iv) intact whorl, only sufficient stem attached to hold leaves together, no lacunae

v) three separate leaves no stem attached, no lacunae.

vi) stem only, one complete internode, two nodes, lacunae intact.

vii) stem only, two half internodes, one node, lacunae cut.

By sectioning the shoots in this manner, it was hoped to distinguish the effect of the lacunae on gas exchange with the medium into the following categories;

a) leaves with attached lacunae and diphragms intact - strongest effect of lacunae (section types ii and iii).

b) leaves with lacunae exposed to the medium and gas exchange much greater than above - slight effect of lacunae (section type i).

c) leaves with no lacunae attached - no effect of lacunae (section types iv and v).

d) effect of lacunae on stem photosynthesis and respiration (section types vi and vii).

Each determination was repeated four times for each of the three replicates and a mean for each shoot calculated. Structuring of the experiment in this manner allowed the results to be analysed using a nested analysis of variance for those samples containing leaves.

The morphology of the gas lacunae was investigated using scanning electron microscopy in conjunction with this experiment.

2.3.1.6 Effect of storage of plants after collection.

A selection of about 150 healthy shoots taken from the field and carefully cleaned as described above was stored in a bucket filled with twice filtered canal water (5 l approximately), in a temperature-controlled tank at 15 °C ± 2 °C, light intensity 100 μmol m^{-2} s^{-1} on a 16:8 (light:dark) cycle. Shoots were randomly taken from this stock container at intervals and leaf photosynthesis and respiration measured as above. Any plants which were obviously unhealthy or damaged were rejected and removed from the stock. The areas of the leaves used in the determinations were measured, together with their chlorophyll contents.

2.3.1.7 Diurnal changes in physiology.

To determine whether the diurnal influence on oxygen exchange rates reported from the field by many workers (Goulder, 1970; Brown *et al.*, 1974; Van *et al.*, 1976; Pokorny *et al.*, 1984) was exogenous (caused by environmental stimulii), or endogenous, measurements of photosynthetic and respiratory rates were repeated under constant conditions at intervals during one day. Preliminary experiments over the whole 16 hour photoperiod, as well as one hour before, and after, seemed to indicate that photosynthesis was maximal prior to the onset of illumination and dropped in the first hour of light, agreeing with findings from the field (Goulder, 1970; Hough, 1974; Pokorny *et al.*, 1984). However, rates were low and variable when compared with other experiments. A further

experiment concentrated on a shortened time scale, from one hour before the lights came on until six hours after and each reading was treated as a discrete point. This was repeated over three successive days.

These experiments were carried out in early summer, when day length in the field was similar to that for the stored plants, with the time that the lights turned on and off corresponding approximately with dawn and dusk.

The stock plants from which samples were taken were stored in twice-filtered canal water at 15 °C ± 2 °C, light intensity 100 μmol m^{-2} s^{-1} on a 16:8 (light:dark) cycle, in a number of separate, glass jars (capacity 2 l) and only one random sample was taken from each container.

2.3.2 Results and Discussion.

2.3.2.1 Cumulative mean.

The calculated mean rate of photosynthesis became stable with relatively few replicates when measured both per unit chlorophyll (Figure 2.1) and per unit area (Figure 2.2). However, the variation between replicates, and hence standard errors, was large (Figure 2.3). A total of 4 replicates was required to bring the standard error to less than 20% and 8 replicates per unit area, or 9 per unit chlorophyll, to bring it below 10%. Even at high replication (12 replicates per unit chlorophyll) the standard error could be increased by one differing measurement, without any apparent effect on the mean (Figure 2.3).

It was decided to use 4 replicates for each treatment in subsequent experiments, since this level of replication can be expected to give a reliable mean and a standard error of less than 20%. As each determination takes 30 minutes, this does not unduly lengthen the experiments. To use replication at a level where the standard error is significantly lower (8-12 replicates for a standard error of less than 10% - Figure 2.3) would introduce a time variable into the experiments. High variability, though unwelcome, is typical of *Elodea* species (Birch, 1990; Pizarro & Montecino, 1992) and submerged aquatic plants in general (Spence & Crystal, 1970a; Owttrim & Colman, 1989).

2.3.2.2 Relationship between leaf size and chlorophyll content.

Regression lines fitted to the data from single leaves taken 3cm from the tip of twelve replicate shoots showed a significant linear relationship between leaf area and total chlorophyll content (from t-test, $p = 0{\cdot}0021$, Figure 2·4), but no significant relationship between leaf area and chlorophyll concentration (from t-test, $p = 0{\cdot}547$, Figure 2·5), indicating little variation in chlorophyll concentration for all sizes of similarly-aged leaves. Using the equation fitted to the plot of leaf area against chlorophyll content, an estimate of $18{\cdot}48 \pm 4{\cdot}26$ µg chl cm^{-2} is obtained, which, considering the degree of extrapolation (a

Figure 2.1 Change in mean net photosynthesis per unit chlorophyll of *E. nuttallii* leaves taken 3 cm from the shoot tip with increasing replication.

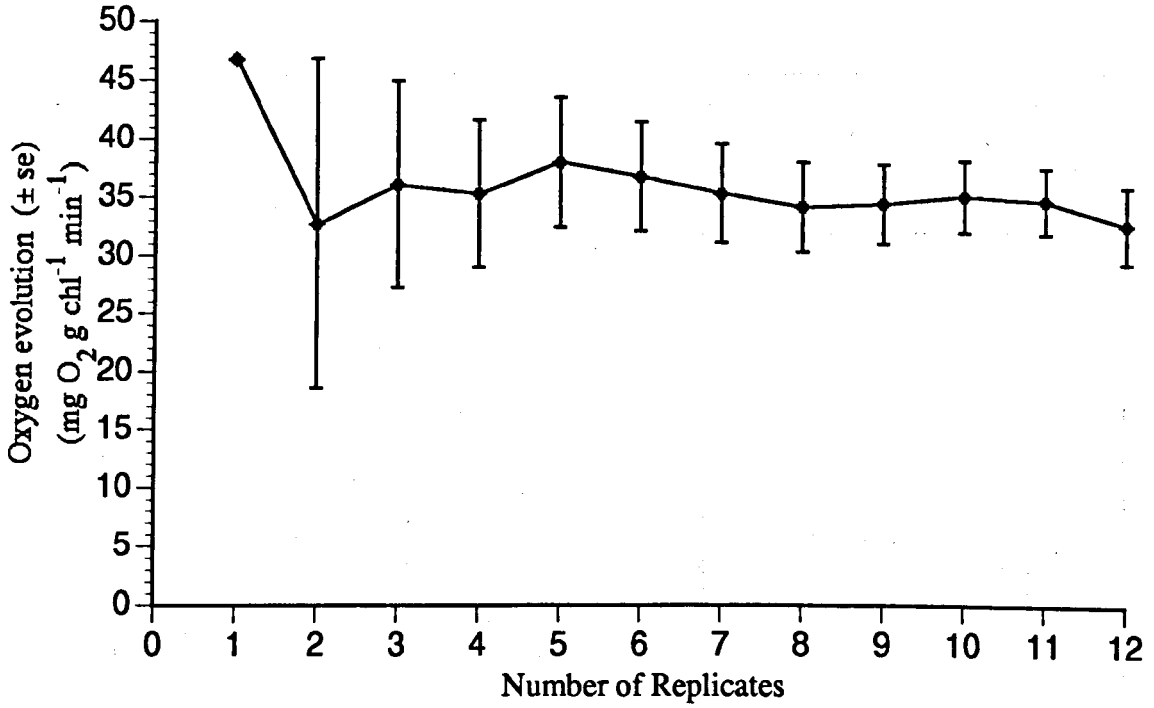


Figure 2.2 Change in mean net photosynthesis per unit area of *E. nuttallii* leaves taken 3 cm from the shoot tip with increasing replication.

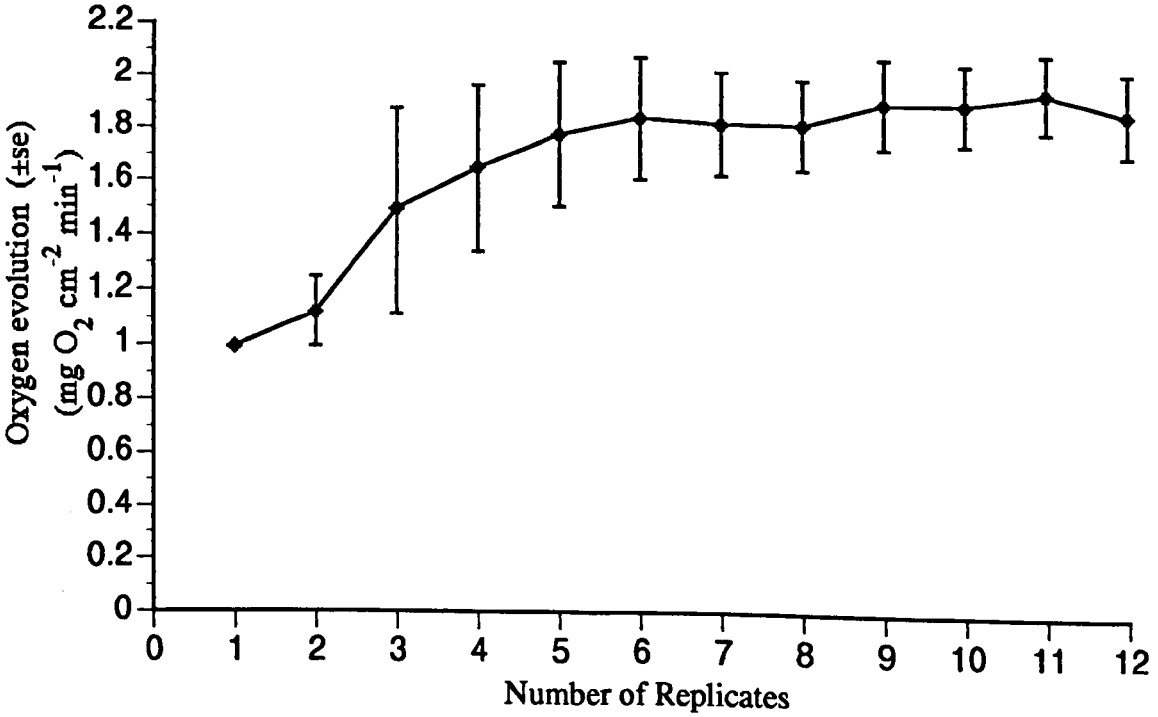


Figure 2.3 Change in the standard error of net photosynthesis of *E. nuttallii* leaves as a percentage of the mean value with increasing replication.

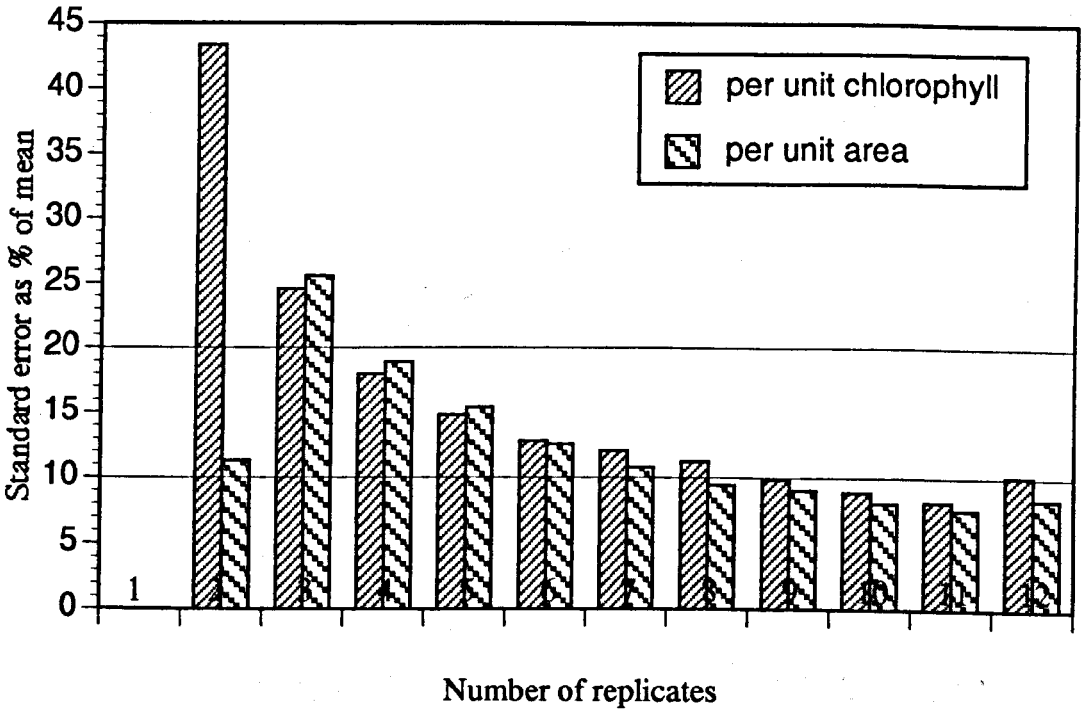
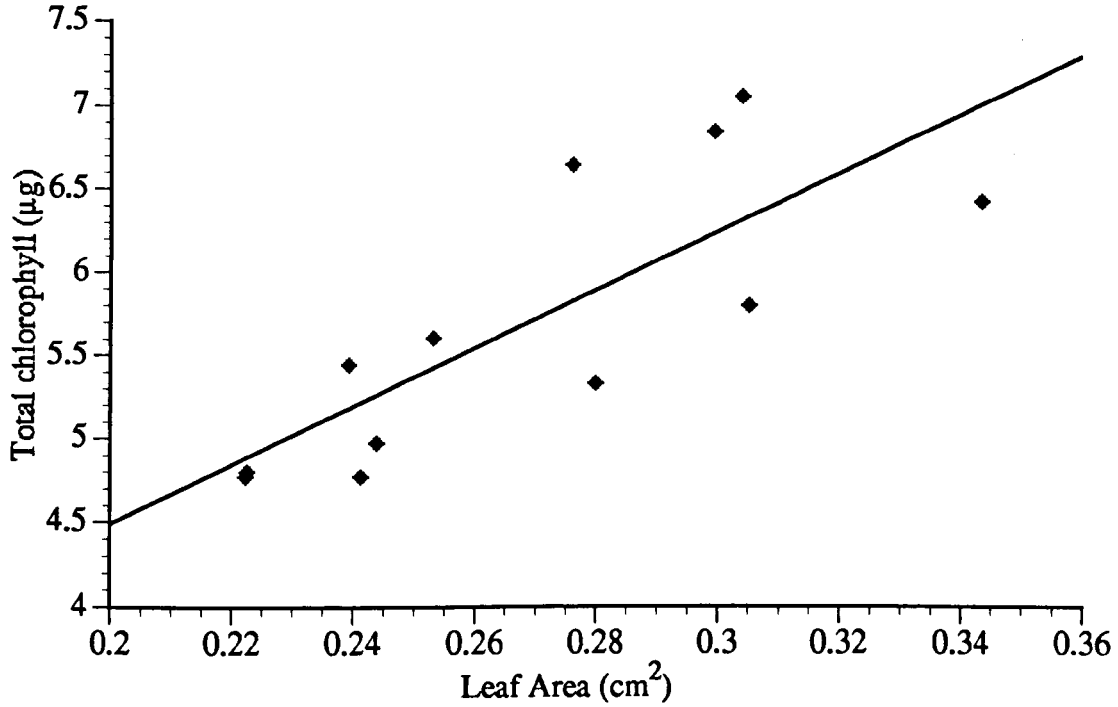
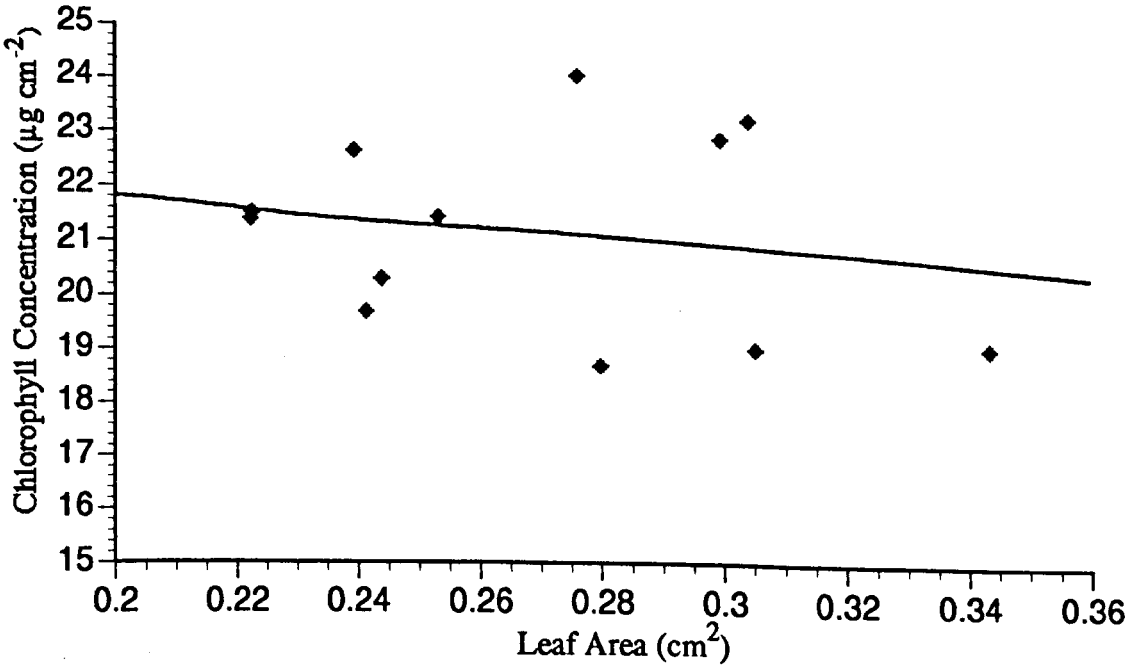


Figure 2.4 Relationship between leaf area and total chlorophyll content of the leaves of *E. nuttallii*.



The equation for this line is, $y = 0.995 + 17.481x \pm 4.26$ $R^2 = 0.627$, $p = 0.002$

Figure 2.5 Relationship between leaf area and chlorophyll concentration of the leaves of *E. nuttallii*.



The equation for this line is, $y = 23.64 - 9.12x$, $R^2 = 0.037$, $p = 0.547$

statistically dangerous procedure), is not greatly different from the mean chlorophyll concentration established from these twelve replicate shoots, *viz.* 21·18 μg cm^{-2} (s.e.= 0·521, Figure 2·4). The variation about this mean was relatively low, the standard error being only 2·46% of the mean (s.e. = 0·521, n = 12), considerably lower than that found for the physiological determinations (s.e. = 10·10%) with the same level of replication.

These levels of chlorophyll per unit area are about four-fold high than the 5·58 ± 0·59 μg cm^{-2} (± s.e., n = 5) found for the first mature leaves of the closely related *E. canadensis* growing in Denmark (Nielsen & Sand-Jensen, 1989), and in fact completely outside the range of values these workers found for fourteen species of aquatic macrophytes with submerged leaves, max = 13·36 ± 0·25 μg cm^{-2} (± s.e., n = 5) for *Sparganium erectum*, min = 3·31 ± 0·10 (± s.e., n = 5) for *Sparganium emersum*. Simpson (1981), however, reported a concentration of 21 ± 0·9 μg chl cm^{-2} (± s.e., n = 9) in leaves taken 5 cm from the tips of *E. canadensis* shoots, when this was the dominant macrophyte in the Leeds and Liverpool Canal, with chlorophyll concentration decreasing towards the shoot apex to within the range quoted by Nielsen & Sand-Jensen (1989), indicating that the difference is largely one of leaf age.

The mean concentration reported here is near to the value the Danish workers quote for terrestrial sun plants (ie. 22·1 μg cm^{-2}), and is not comparable to that of terrestrial shade plants (ie. 36·3 μg cm^{-2}), therefore supporting their conclusion that the frequent notion of submerged leaves being typically shade leaves (e.g. Bowes, 1985) is not entirely correct (Nielsen &Sand-Jensen, 1989). Whilst photosynthesis is light-saturated at low photon flux densities (Simpson & Eaton, 1986; Birch 1990; Madsen *et al.*, 1991; Maberly, 1993) in common with shade-plants, the high ratio of chlorophyll a:b is more typical of sun-plants (Peñuelas *et al.*, 1988). Leaves of submerged plants are typically thin, like those of shade plants, often only 2-3 cell layers thick (Sculthorpe, 1985; Triest, 1982), with chloroplasts present in the epidermal layer of many species (Sculthorpe, 1985),

reducing the amount of light absorbing tissue that must be traversed to reach the chloroplasts. In the case of aquatic plants the reduction in the diffusion path-length of the dissolved gases involved in photosynthesis may be a far more important consequence of this adaptation.

2.3.2.3 Effect of chlorophyll content on physiology.

A t-test showed that the regression lines fitted to the plots of chlorophyll content versus photosynthesis and respiration per unit chlorophyll, were not significantly different to lines of zero slope ($p = 0{\cdot}525$ and $p = 0{\cdot}399$ repectively, Figure 2.6) indicating that the total chlorophyll content of the leaves has no significant effect on standardised measurements of their physiology. The mean rate of photosynthesis for these leaves was 27·25 mg O_2 g^{-1} chl min^{-1} (se = 1·81, n = 12), and dark respiration was 2·83 mg O_2 g^{-1} chl min^{-1} (se = 0·09, n = 12), within the range found by other workers for *Elodea* species (Simpson, 1981; Nielsen & Sand-Jensen, 1989; Birch, 1990) and other submerged plants (Nielsen & Sand-Jensen, 1989).

In the absence of any statistical advantage of either basis of measurement, leaf area or chlorophyll, over the other, it was decided to use chlorophyll in the subsequent experiments for the following reasons;

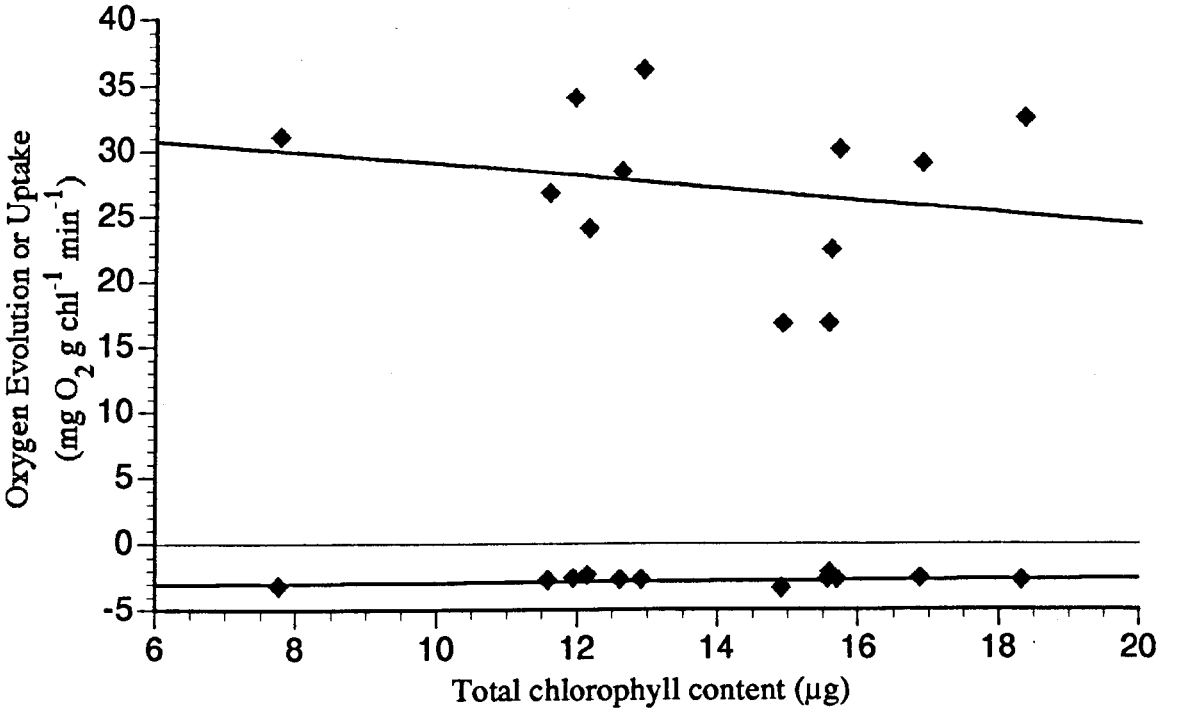
i) for healthy leaves chlorophyll content was proportional to leaf area (Figure 2.4) and, correspondingly, area had no significant effect on chlorophyll concentration (Figure 2.5).

ii) measurement of chlorophyll gives some indication of the physiological condition of the leaf.

iii) by using chlorophyll, a comparison can be made with the results of other workers (Simpson, 1981; Birch 1990) who have worked on *E. nuttallii* or closely related species.

iv) it was quicker and easier to measure chlorophyll content than leaf area.

Figure 2.6 The relationship between total chlorophyll per whorl of *E. nuttallii* leaves taken 3 cm from the shoot tip and oxygen evolution in the light and uptake in the dark, per unit chlorophyll.



The equations describing the lines are:

photosynthesis	$y = 33.39 - 0.445x$, $R^2 = 0.042$, $p = 0.525$
respiration	$y = -3.23 + 0.029x$, $R^2 = 0.071$, $p = 0.399$

2.3.2.4 Error involved in changing the solution in the reaction chamber.

The error involved in changing the solution within the reaction chamber was very small. The mean weight of 15 repeat changes of solution was 1·603g, an error of 0·003g or 0·188%, and the standard deviation was 0·022 (1·37 %). The maximum error was +0·07g, which is equal to 4·37%. These slight errors were obviously not responsible for the large degree of variation found in the physiological determinations.

2.3.2.5 Sectioning of shoots

Although gas-filled lacunae are a common structural feature of plants growing in the aquatic environment (Sculthorpe, 1985), their functions appear to be various. In emergent plants and plants of waterlogged soils they conduct air from the atmosphere to support the metabolism of submerged parts and roots growing in anaerobic sediment (Braendle & Crawford, 1983; Armstrong *et al.*, 1992; Crawford 1992), in nyphaeids the pressure caused by temperature gradients (thermal transpiration) force air from the leaves through the lacunae to the roots and rhizomes for the same purpose (Dacey, 1980 & 1987; Dacey & Klug, 1982; Schröder *et al.*, 1986; Crawford 1992), and in low growing isoetids the lacunae conduct a flow of carbon dioxide in the other direction, from the sediment surrounding the roots to the photosynthesising leaves (Wium-Andersen 1971, Søndergaard & Sand-Jensen 1979). In submerged elodeid species their role is less clear and it has been suggested that they serve in root aeration (Caffrey & Kemp, 1991; Sorrell, 1991), buoyancy (Sculthorpe, 1985), excretion of oxygen by bubbling via lenticels (Sculthorpe, 1985) and gas storage for photosynthesis and respiration (Sorrell & Dromgoole, 1986, 1987, 1988 & 1989).

The results from an experiment whereby physiological determinations were made on leaves, together with varying amounts of stem, showed that no significant difference in subsequent activity between the methods of sectioning the plant (photosynthesis $F = 1·17$, respiration $F = 1·57$, $F_{0.05(1),4,15} = 3·06$), whilst the differences within the treatments, ie.

Table 2.1 The effect of different methods of sectioning the shoots of *E. nuttallii*; the mean of four repeat determinations per shoot (replicate), total chlorophyll content of the section used, and the mean for the three shoots used per treatment (treatment) are shown. See text for explanation of section types.

Section Type	Chlorophyll Content	Replicate Shoots Photosynthesis	Respiration	Treatments Photosynthesis	Respiration
i	9.07	31.90	7.26	24.97	5.72
	10.67	22.66	3.96		
	9.56	20.34	5.93		
ii	11.70	26.90	4.50	26.33	5.98
	5.11	29.75	8.55		
	14.79	22.34	4.90		
iii	10.72	31.51	6.71	23.06	6.04
	11.41	19.75	7.39		
	15.09	17.88	4.01		
iv	12.82	25.67	6.16	24.02	5.80
	10.28	21.23	5.73		
	12.87	25.16	5.50		
v	9.54	29.39	4.65	21.79	4.74
	13.31	15.06	4.34		
	13.27	20.93	5.23		
vi	2.48	0.00	4.10	0.00	—
	0.08	0.00	171.26		
	0.00	0.00	—		
vii	1.31	0.00	11.46	-6.25	45.75
	0.32	-18.75	68.25		
	0.88	0.00	57.55		
	(μg)		(mg O_2 g chl^{-1} min^{-1})		

between the plants, were highly significant (photosynthesis F = 11·51, respiration F = 15·01, $F_{0.05(1),10,15}$ =4·42). Of the variation found, none was attributable to the treatments, but 89·04% was attributable to between-plant variation, and the remaining 10·96% was described by the variation between replicate measurements on the same shoot. This indicates that either the lacunae play no role in photosynthesis or that their role is a subtle one and the variability between plants masked any effect they had (Table 2.1).

No significant change in the gas exchange characteristics of the leaves was seen over the two hours following excision, which was required to make the four replicate measurements in this experiment.

The stem-only sections were capable of compensatory photosynthesis when illuminated at saturating light intensity, with one exception out of 24 readings taken on six samples (Table 2.1). Chlorophyll content was not a good parameter to standardise the measurements taken on these stem sections. Even though dark respiration was only 25-33% that of the leaves when absolute rates of oxygen uptake were compared, the exceedingly small amounts of chlorophyll within the stem distorted the readings to very high levels (Table 2.1).

Investigation of the lacunae with the SEM showed that, unlike some other members of the Hydrocharitaceae (Triest, 1982), lacunae were lacking within the leaves (Plate 1; see also Plates 20 & 21) and that the connections between the leaf blades and the lacunae within the stem were poor, involving many small cells (Plate 1). If the distance dissolved gases must travel by diffusion from the leaf blade to the lacunae, up to 15 mm from the very tip, is compared to the thickness of the boundary layer, 1-2 mm, it seems highly unlikely that the lacunae do act as gas storage vessels for leaf photosynthesis and respiration in this species. Søndergaard (1979) also concluded that, due to the small size of the lacunal sytem in *E. canadensis*, storage and refixation of respired CO_2 was unimportant. The idea of excretion of photosynthetically-dereived oxygen by bubbling from lenticels in the stem is

Plate 1 Longitudinal section of an *E. nuttallii* shoot showing leaf primordia, leaves and lacunae with nodal diaphragms. Scale bar shown at the base of the shoot = 10 μm.

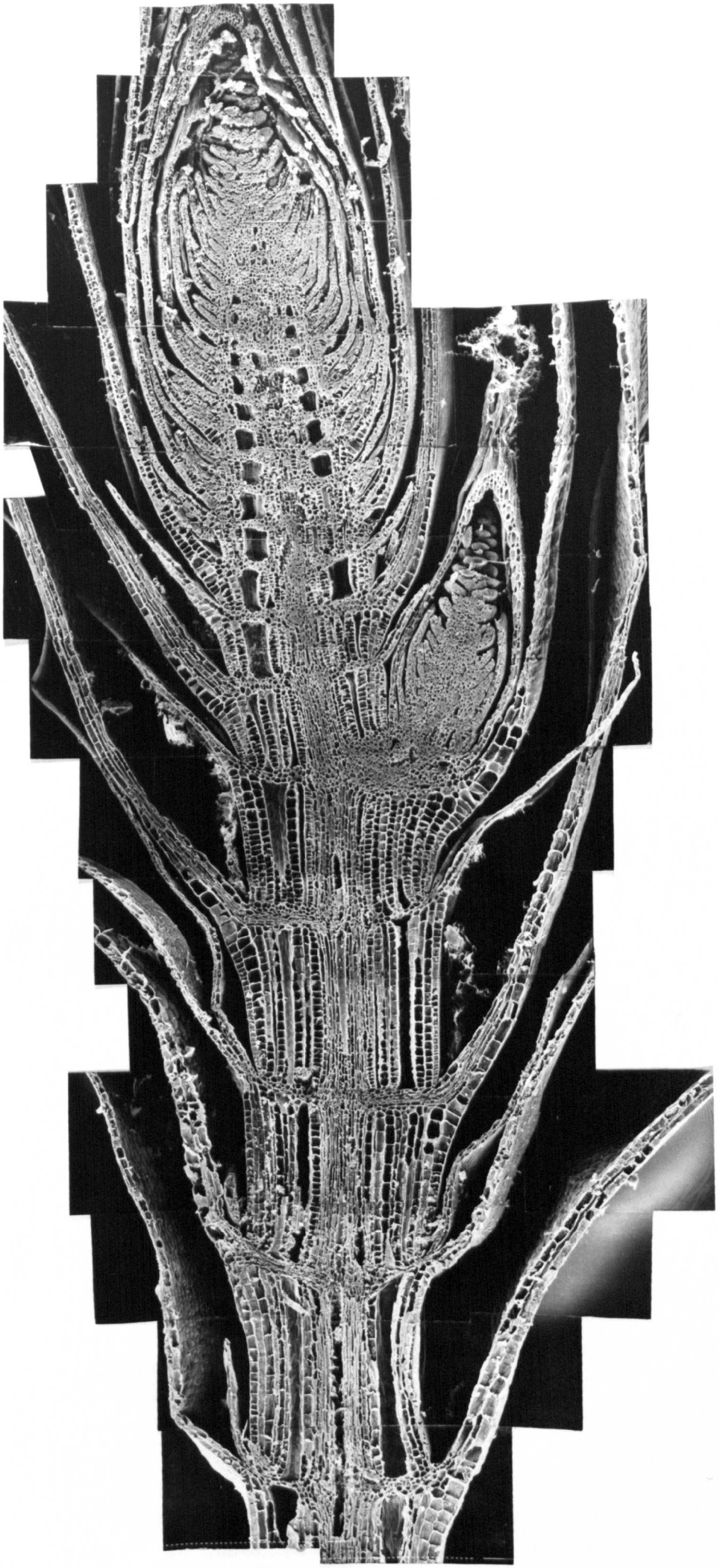
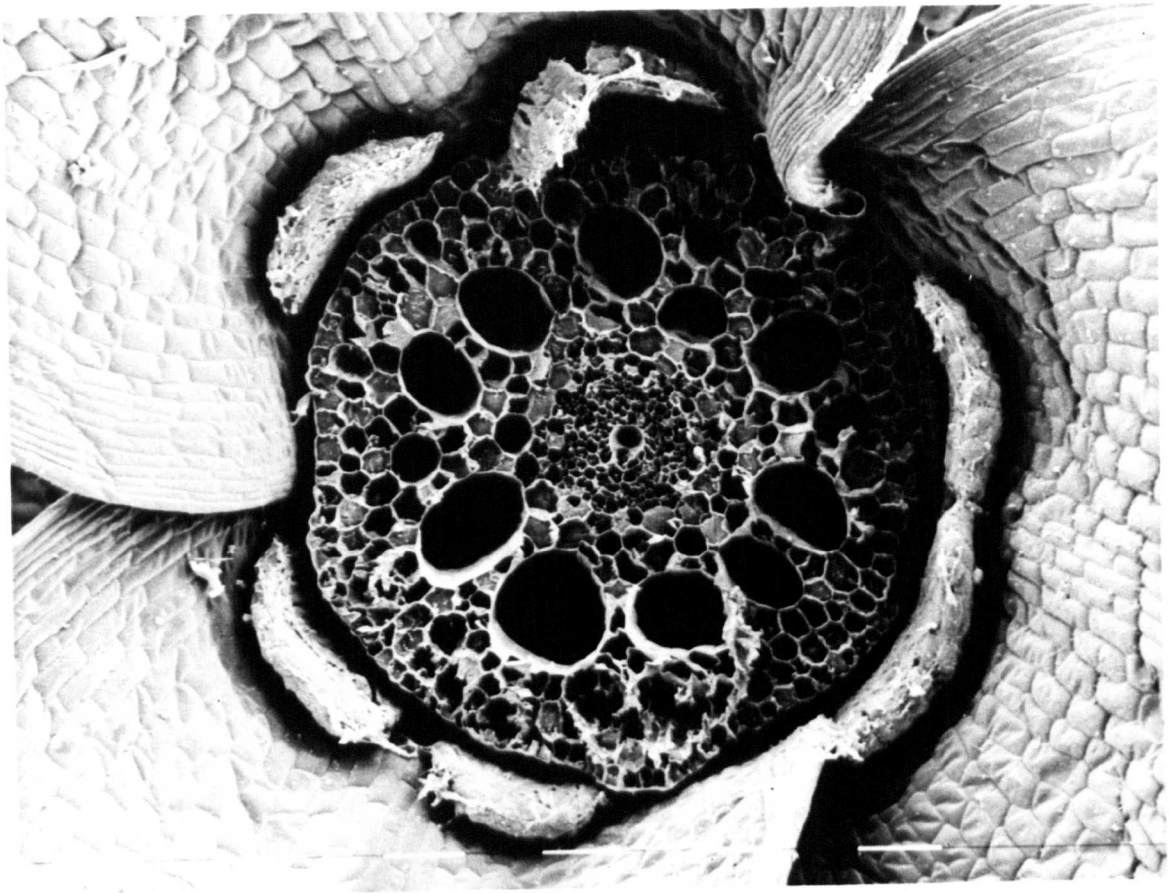


Plate 2 Transverse section of an *E. nuttallii* stem showing the position and number of the lacunae surrounding a central vascular bundle. Scale bar = 100 μm.

Plate 3 View down a lacuna showing the nodal diaphragm at the end of a lacunal chamber. Note the pores between the cells. Scale bar = 10 μm.



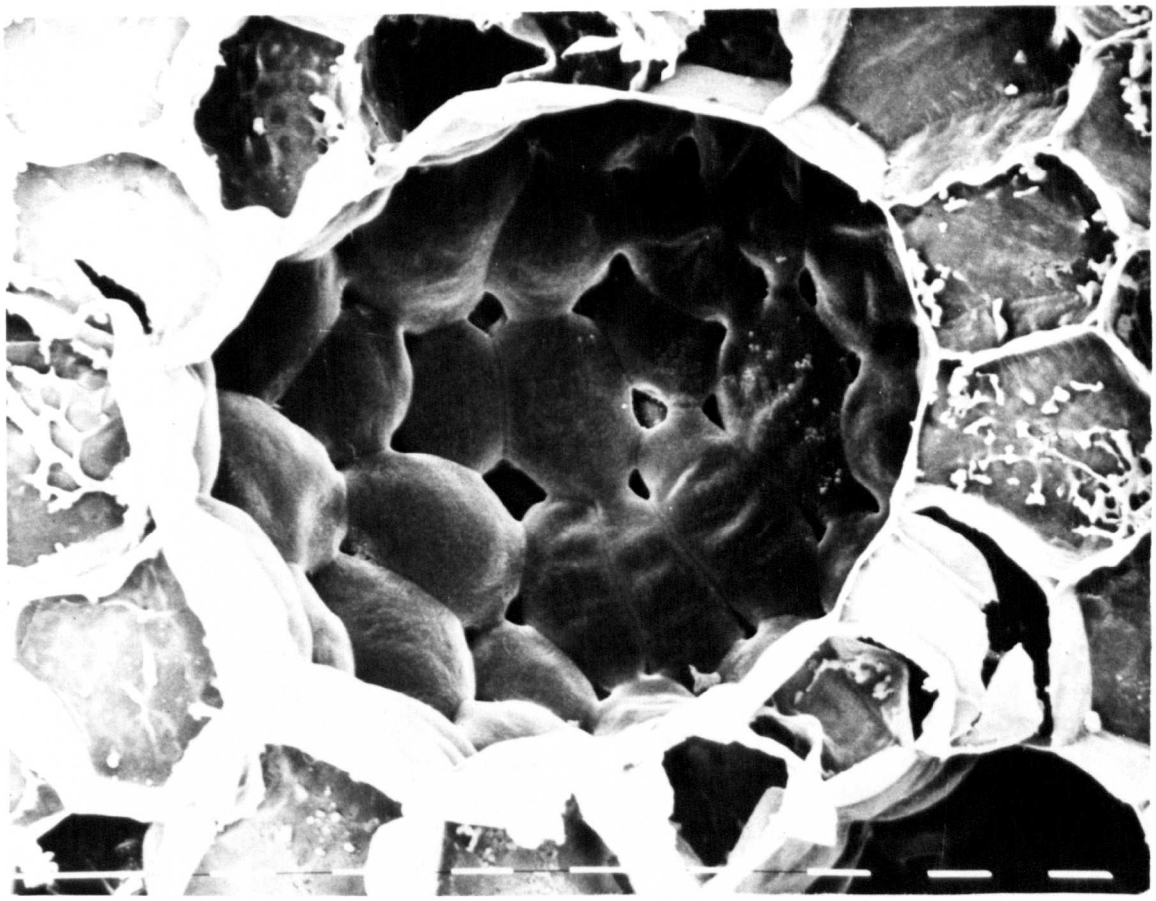
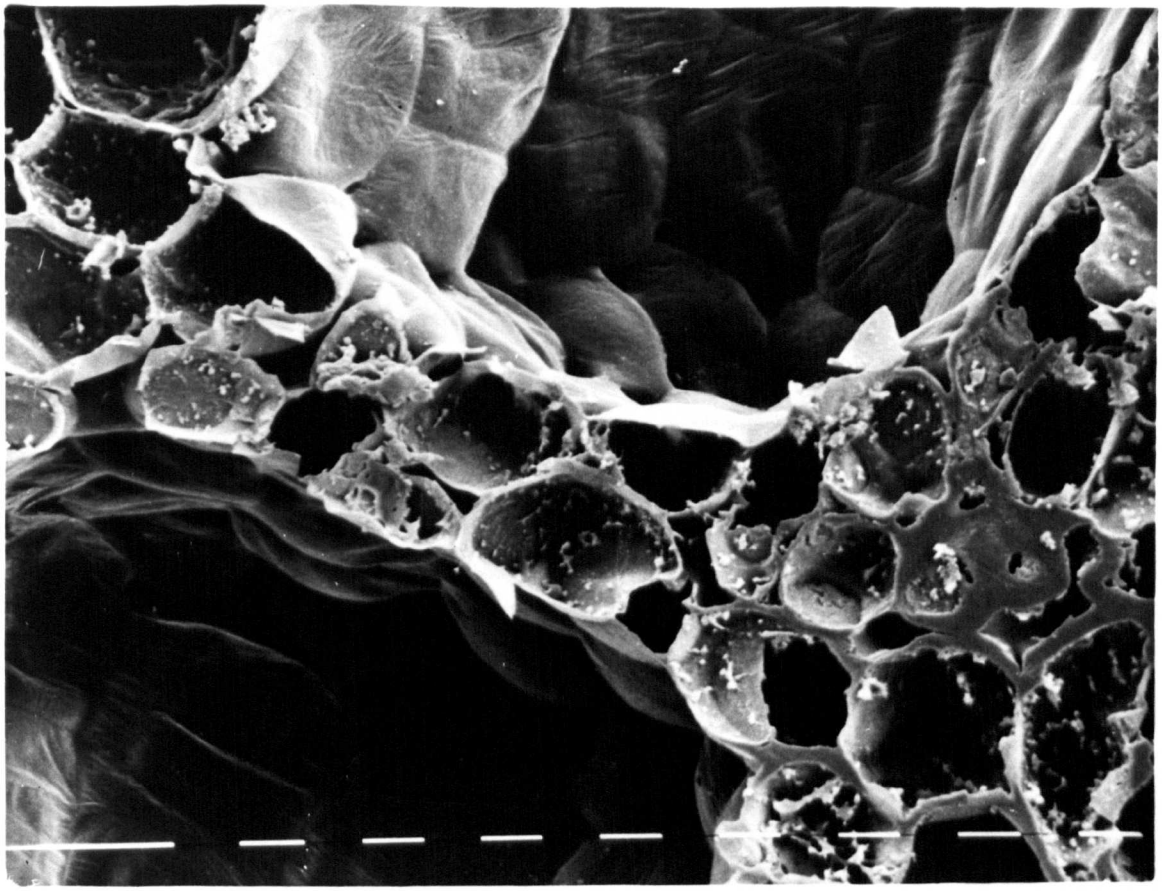
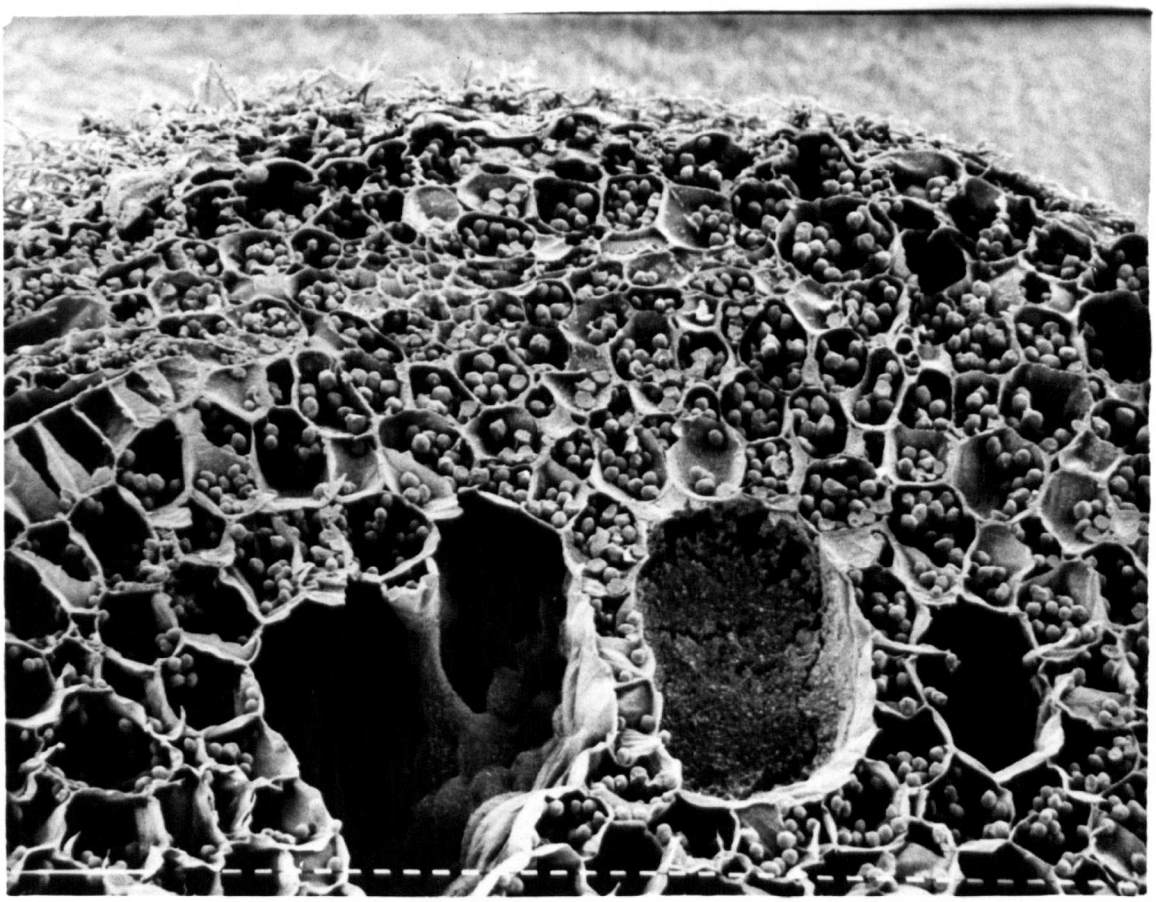


Plate 4 Longitudinal section of the nodal region of an *E. nuttallii* stem showing the two cell layers which constitute the diaphragm and the pores between the cells. Scale bar = 10 μm

Plate 5 Transverse section of an *E. nuttallii* stem, showing a large number of starch amyloplasts within the cells. Scale bar = 10 μm.





well dealt with by Sculthorpe (1985), where he argued that the proportion of oxygen lost in this way was exceedingly small when compared to that retained or lost by diffusion.

Whilst the lacunae constituted a large proportion of the stem volume (Plate 2), the connection between individual lacunae appeared limited (Plates 3 & 4). A two cell thick diaphragm was present at each node (Plates 3 & 4), separating the lacunae from each other, and, on occasion, the connections were not in a direct one-to-one fashion (Plate 1). The small size of the pores within these diaphragms protects the lacunae from waterlogging if breakage occurs (Sculthorpe, 1985), but also causes a great resistace to the flow of gases. However, release of oxygen has been recorded from the roots of *Potamogeton pectinatus* L., *P. crispus* L., *P. friesii* Rupr. and *Sparganium simplex* (Sand-Jensen *et al.*, 1982) and although this was a small proportion of total oxygen production (2-4%), it is sufficient to supply root respiration. Release of oxygen into the rhizosphere is also important for nutrient uptake, and has been shown to occur from the roots of *P. perfoliatus* L. (Caffrey & Kemp, 1991). Given that the both the roots and the lacunal system of *E. nuttallii* are not as extensive as those of *P. pectinatus*, *P. crispus*, *P. friesii*, *P. perfoliatus* or *S. simplex*, it is apparent that the structures required for root aeration are present, with the stem (which is generally capable of compensatory photosynthesis - Table 2.1), perhaps together with the basal part of the leaves, providing the oxygen. Increased pressures, which could push gases into the roots, have been recorded within the lacunae of another member of the Hydrocharitaceae, *Egeria densa* Planch. (Sorrel & Dromgoole, 1987 & 1988; Sorrel, 1991), though these may be of a transient nature. In *Halophila ovalis* (R.Br.) Hook F., a further member of the Hydrocharitaceae which has a very similar system of stem lacunae to *E. nuttallii*, gas escapes from the cut roots if the leaves are pressurised (Roberts *et al.*, 1984). The lacunae must also play a role in flotation by increasing the plants buoyancy. Further, the stem can act as a storage organ, accumulating starch under favourable conditions (Plate 5).

As the stem appeared to have no role in the gas exchange of the leaves, it was decided to use three leaves separated from the stem in all following determinations, to allow free movement of the leaves within the reaction chamber, thereby maximising mixing and minimising shading effects between the leaves.

2.3.2.6 Effect of storage.

A rapid decline in the activity of aquatic plants brought into laboratory culture has been described by some workers (Brown *et al.*, 1974), whilst others have found that plants can be retained for some time without being affected (Simpson, 1981). The present study found that plants could be retained for up to thirteen days in a bucket of filtered canal water at 15 °C ± 2 °C, illuminated with 100 μmol m^{-2} s^{-1} PAR on a 16:8 (light:dark) cycle without any adverse effect on the plants' physiology (Figure 2.7 to 2.9). After this time there was a sudden drop in the chlorophyll content of the plants (Figure 2.7), together with a decline in the rate of photosynthesis and an increase in respiration (Figure 2.8 & 2.9). This change in activity between days 13 and 16 was observed when the determinations were standardised both against chlorophyll content (Figure 2.8) and leaf area (Figure 2.9), indicating that the changes were a consequence of real alterations in the physiological condition of the plants and not an "artefact" caused by the change in chlorophyll content. When measured on a per unit area basis, the plants were incapable of compensatory photosynthesis on day 16 and, although they showed some recovery after this time (both as per unit chlorophyll and per unit area), it was not to the levels found prior to day 13. Respiration also changed markedly between day 13 and day 16 increasing 10-fold when measured on a unit chlorophyll basis (Figure 2.8) and 3-fold on a unit area basis (Figure 2.9), the difference being caused by the drop in chlorophyll content, which emphasised the increase in respiration of parts of the leaf other than the chloroplasts. Variability between determinations of photosynthesis and respiration was much greater on day 16 than at other times.

Figure 2.7 Effect of storage in culture at 15 °C, 100 μmol m^{-2} s^{-1} on the chlorophyll concentration (± s.e.) of *E. nuttallii* leaves.

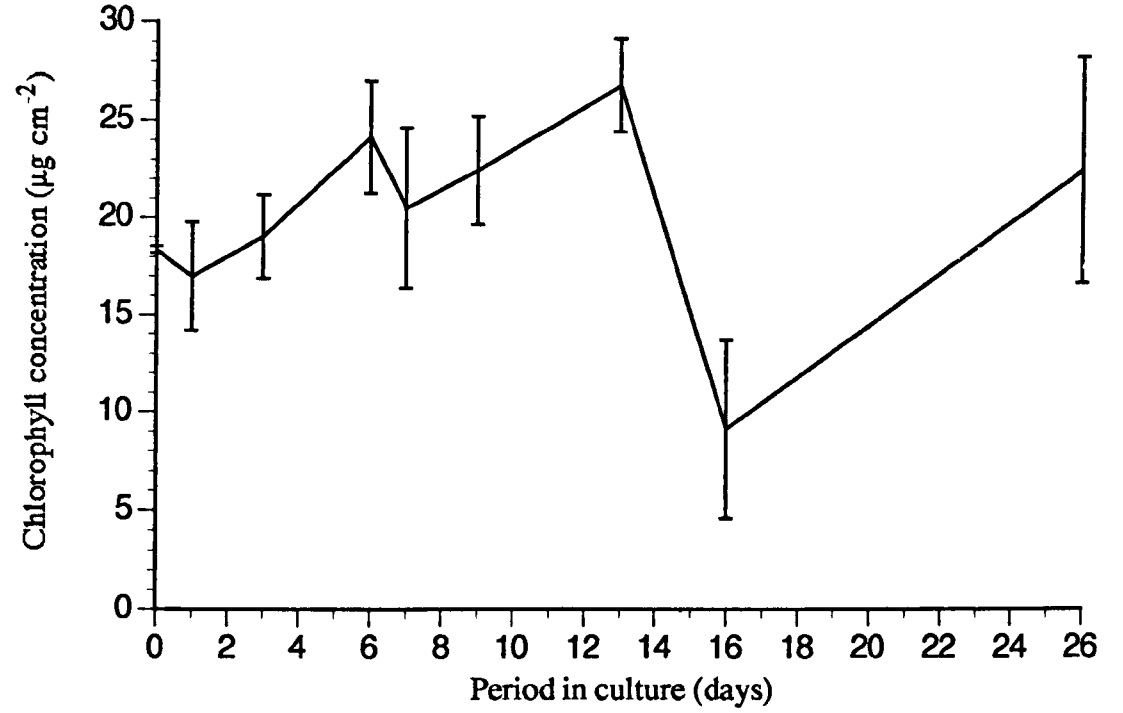


Figure 2.8 Effect of storage in culture at 15 °C, 100 μmol m^{-2} s^{-1} on oxygen evolution in the light and uptake in the dark - per unit chlorophyll (± s.e.) of *E. nuttallii* leaves.

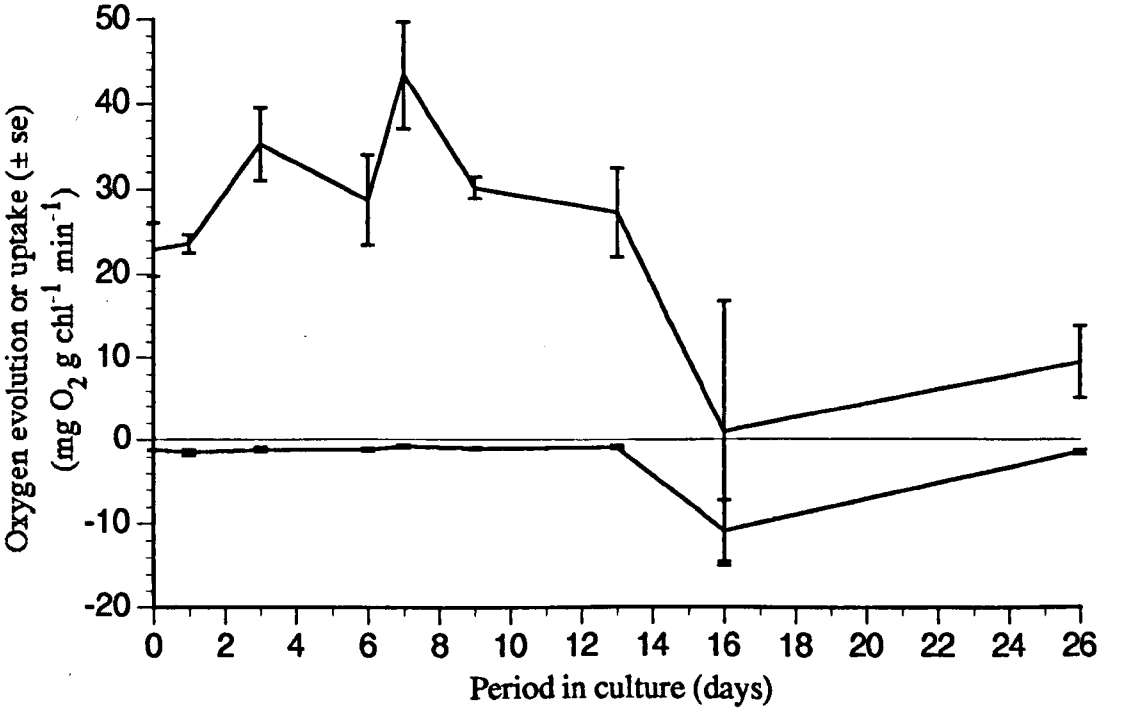
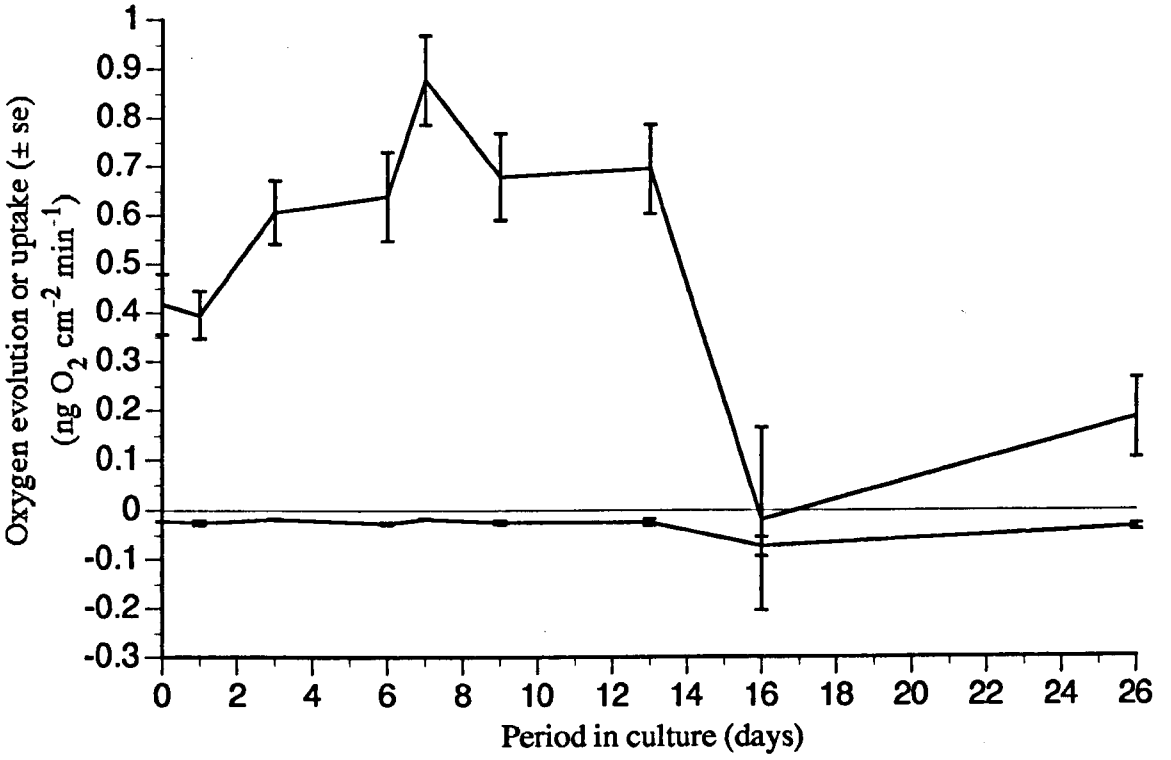


Figure 2.9 Effect of storage in culture at 15 °C, 100 µmol m^{-2} s^{-1} on the oxygen evolution in the light and uptake in the dark - per unit area (± s.e.) of *E. nuttallii* leaves.



After day 16 there was widespread death amongst the plant material. The samples taken on day 26 were from those shoots which had survived and in some cases formed new growth and do not, therefore, indicate an actual recovery of the original plants' activity. A likely cause of plant death is nutrient limitation. Those plants which survived to day 26 may have utilised nutrients released by the breakdown of dead tissue from predecessor plants. This hypothesis is supported by the fact that when plants were grown rooted into sediment (later experiments) they could survive for much longer periods.

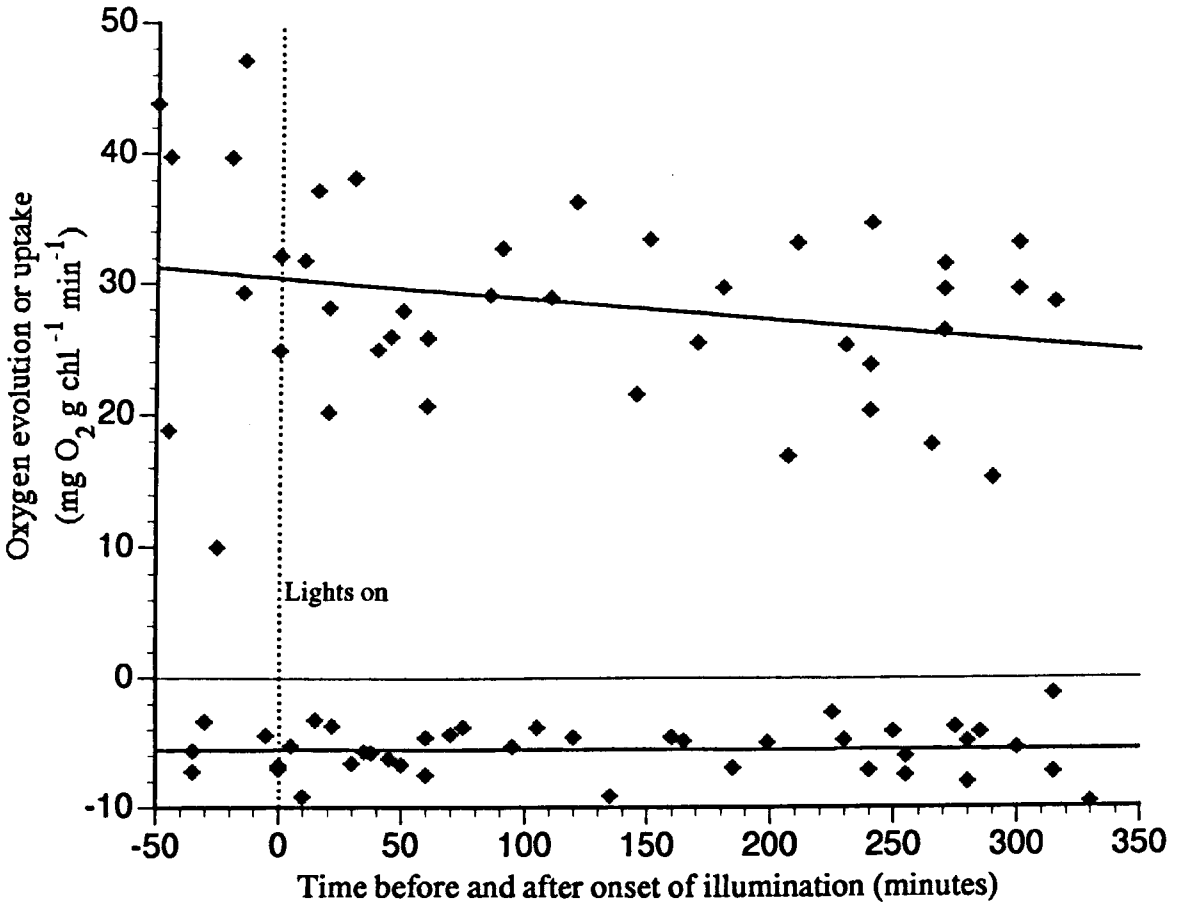
From this investigation it was decided to use only those plants which had been stored in culture for up to one week in subsequent experiments.

2.3.2.7 Diurnal changes in physiology.

In the field, rates of oxygen exchange have been reported to fluctuate with time of day, with peak output occurring in the early morning and rates reducing in the afternoon (Goulder, 1970; Unni, 1972; Brown *et al.*, 1974; Hough, 1974; Van *et al.*, 1976; Pokorny *et al.*, 1984). Whilst the diurnal change in irradiance has been shown to play the expected role (Sculthorpe, 1985), it has been suggested that this does not explain the variation fully, and that the plants' photosynthetic potential has a fixed diurnal rhythm (Simpson, 1981). Preliminary investigations into whether this was true for *E. nuttallii* or not, showed that rates of photosynthesis fluctuated with no apparent pattern in relation to diurnal cycle (Appendix 2). However, these experiments appeared to show that photosynthesis, although low when compared to other experiments, was maximal prior to the onset of illumination and dropped in the first hours of light, agreeing with other workers (Goulder, 1970; Hough, 1974; Van *et al.*, 1976; Pokorny *et al.*, 1984). It was therefore decided to concentrate on a shortened time period, from one hour before the lights came on until six hours after.

By repeating the measurements three times over three successive days it was possible to build up sufficient determinations to visualise the variation in rates of oxygen

Figure 2.10 The effect of time of day on the measured oxygen evolution in the light and uptake in the dark of *E. nuttallii* over a morning period.



The equations for the regression lines fitted to the data are;
photosynthesis, $y = 30.444 - 0.0157\ x$, $R^2 = 0.057$, $p = 0.1311$
respiration, $y = -5.5745 + 0.00022\ x$, $R^2 = 0.000$, $p = 0.9284$

exchange between the plant samples (Figure 2.10). Fitted regression lines showed a slight change with time, but a t-test showed that the slopes did not deviate significantly from zero and hence there was no significant effect of time on photosynthesis or respiration ($p = 0{\cdot}131$ and $0{\cdot}928$ respectively - Figure 2.10) over this 7 hour period. There was no significant difference between fitted lines when each day was treated as separate. It was concluded that there was no fixed diurnal cycle of photosynthesis or respiration and, as each sample had been previously undisturbed, there was no great build-up of oxygen or carbon dioxide within the tissues. An alternative explanation of the diurnal rhythms found in the field is that they are driven by changes in physico-chemical parameters within the water column, ie. a build up of oxygen, increase in pH and decline of carbon dioxide with illumination, the opposite happening during darkness (see Chapter 5).

It is noted that those samples taken in the dark and therefore blind, were the most variable of the samples taken, indicating some degree of selection by the author in the normal procedure.

Although no diurnal effects on oxygen exchange characteristics were found, the subsequent determinations were all carried out in the middle eight hours of the photoperiod.

One of the main features of this series of experiments and of the subsequent investigations, was the great variability in activity between plants (see Figures 2.1 to 2.3), a surprising finding considering that *E. nuttallii* is entirely clonal in Britain and possibly only a single clone, with only the male plant occurring (Simpson, 1984 & 1986). No single factor could be found to explained this variation, which appeared to be inherent in the plants brought in from the field. In view of the phenotypic plasticity of this species in response to environmental variables (Simpson, 1988) and later investigations into physiological plasticity, this seems entirely plausible (see Chapter 4), differences between plants being caused by adaptation to the different micro-environments in which they were growing within the canal and these adaptations persisting for some time after collection. A

high degree of variability has also been recorded for other submerged macrophytes collected from the field (Spence & Crystal, 1970a; Owttrim & Colman, 1989; Birch, 1990; Pizarro & Montecino, 1992).

2.4 Standardised methodology

From the above, the following standardised methods were established.

Plants collected from the field were stored under standard culture conditions for a maximum of one week before use.

One complete whorl of leaves (3) taken 3 cm from the shoot apex of a stem was used for each determination of photosynthesis and respiration, and all determinations were standardised against chlorophyll content of the leaves.

The three leaves used in the oxygen electrode were separate from each other, with no stem attached.

Each treatment level was replicated 4 times, since this level of replication can be expected to give a stable mean and a standard error of less than 20%. As each determination takes 30 minutes this does not unduly lengthen the subsequent experiments. Replication was increased for experiments with few treatment levels.

Determinations were all carried out in the middle eight hours of the photoperiod.

Chapter 3.

The Effect of Changes in the Bulk Medium on the Physiology of *E. nuttallii.*

3.1 Introduction.

This section of the study is concerned with how the conditions in the bulk fluid surrounding *E. nuttallii* can affect its physiology, establishing a baseline characterising the response of the plant to those variables most likely to be affected by epiphyton. Hence, the effect of increasing epiphyton on the plant's performance can be hypothesised, especially once the conditions altered by an increasing layer of epiphyton have been determined (Chapter 6).

Elodea nuttallii, one of three introduced species of the genus *Elodea* now growing naturalised in British waterbodies, is a much later introduction into Europe than the closely related *E. canadensis* Michx. (*E. nuttallii* first introduced into Europe in 1939 *cf. E. canadensis* 1836 - Simpson, 1984) and consequently has received far less investigation into its biology. Although these two species are closely related and difficult to separate by the untrained eye, it cannot be assumed that they will behave similarly, especially as they are known to have a sympatric distribution in their native North America (St. John, 1965). Further, *E. nuttallii* has replaced *E. canadensis* in many British and European waterbodies (Mériaux, 1982; Simpson, 1984 & 1990).

Constraints on the growth of a submerged aquatic plant are very different to those of the terrestrial environment. The light experienced by a submerged plant is greatly reduced by absorbance of the water itself (Hutchinson, 1957) and, to a lesser extent, by reflection at the air-water interface (Wetzel, 1983a; Roos & Meulmans, 1987). Further attenuation of light is caused by dissolved substances and by particles suspended in the water column, especially phytoplankton which absorbs most strongly in the photosynthetically active wavelengths (Hutchinson, 1957). Epiphyton (Sand-Jensen, 1977 & 1990; Sand-Jensen & Søndergaard, 1981; Losee & Wetzel, 1983; Sand-Jensen & Borum, 1984; Birch, 1990) silt and marl deposits (Wetzel, 1960; Schiemer & Prosser,

1976; Losee & Wetzel, 1983) are final barriers to light reaching macrophyte leaves. For the taller species, which grow in weed-beds, self-shading can compound these problems further (Westlake, 1964 & 1966; Sculthorpe, 1985; Van der Bijl *et al.*, 1989; Madsen & Maberly, 1991; Wychera *et al.*, 1993). Also, due to the differential absorbance at different wavelengths, the quality of the light changes with depth, typically from white to predominantly green or brown, as the blue and red (photosynthetically active) wavelengths are preferentially absorbed.

It is, therefore, no surprise to find that submerged macrophytes show many physical features typical of terrestrial plants from low light environments and that their photosynthesis becomes light saturated at low irradiances (Spence & Crystal, 1970a; Simpson, 1981; Nielsen & Sand-Jensen, 1989; Birch, 1990; Maberly, 1993; See Section 2.3.2.2). The irradiance required to saturate photosynthesis of submerged macrophytes has been shown to decrease with rooting depth (Maberly, 1993), with distance from the shoot apex (Simpson, 1981; Birch, 1990), and in winter (Simpson, 1981) which all correspond with decreasing light availability. The light compensation point varies in similar fashion (Simpson, 1981; Maberly, 1985a; Birch, 1990; Maberly, 1993). Variations in pigment composition with decreasing irradiance has been reported for the stonewort *Chara hispida* L., but not for vascular plants (Andrews *et al.*, 1984a). These physiological changes serve to increase photosynthesis at low irradiances, but other morphological changes can also contribute (Maberly, 1993).

The proportion of structural tissue (often revealed as a high fresh:dry weight ratio) in submerged parts is generally low, significant support being provided by the water and the amount of non-photosynthetic tissue is often reduced to a minimum. This results in the leaves having a very high specific leaf area (area per unit weight), comparable to that of shade-plants (Spence & Crystal, 1970a). The specific leaf area typically varies with incident light, especially in species from deep water (Spence & Crystal, 1970a). It decreases with depth (Spence & Crystal, 1970b; Spence *et al.* 1973; Spence & Dale, 1978;

Maberly, 1993) and with seasonal decreases in irradiance (Spence & Crystal, 1970b). The internodal cells of *C. hispida* also decrease in weight per unit area with reduced light intensity, due either to increased depth, or to self shading within the stand (Andrews *et al.* 1984a). It is likely that the availability of light is the major environmental factor determining the maximum depth of colonisation by macrophytes (Spence, 1982; Chambers, 1987; Chambers & Kalff, 1987; Blindow, 1992b).

As with other plant responses, some species are stress-tolerators and others are stress avoiders. To gain maximal light, many species have evolved growth strategies where leaves float or protrude from the surface of the water. Totally submerged species typical of highly productive habitats, where competition for light is high, produce most of their photosynthetic tissues in a highly branched pattern near the water surface (Kunni, 1984; Machena *et al.*, 1990; Lillie & Budd, 1992; Wychera *et al.*, 1993), with the most photosynthetically active parts of the plant near the shoot apices (Simpson, 1981; Birch, 1990). By extension of internode length, plants from deep water can extend into a more favourable light climate (Tobiessen & Snow, 1984; Maberly, 1993). Such plants tend not to produce as many side-branches as ones from higher light environments (Maberly, 1993).

Water is a far more viscous medium than air and movement by molecular diffusion within it some 10,000 times slower. As the physical presence of the plant stems and leaves within a weed-bed gives structure to the water column, leading to reduced turbulent mixing and conditions of very low flow (Madsen & Warncke, 1983; Losee & Wetzel, 1988 & 1993; Machata-Wenninger & Janauer, 1992), movement of dissolved gases is largely by the slow process of diffusion. Because of this, the water surrounding actively photosynthesising plants can undergo huge changes in quality as dissolved gases build up or are depleted, typically supersaturation of oxygen, high pH, and a decreased availability of carbon dioxide (Goulder, 1970; Unni, 1972; Brown *et al.*, 1974; Van *et al.*, 1976;

Beeton & Sikes, 1978; Bowmer *et al.*, 1984; Pokorny *et al.*, 1984; Frodge *et al.*, 1990; Chapter 5). The reduced mixing also permits the development of raised temperatures in the upper parts of the water column during the day (Dale & Gillespie, 1978; Chapter 5).

Increased oxygen concentration can lead to elevated levels of photorespiration, a phenomenon reported for many aquatic macrophytes (Brown *et al.*, 1974; Hough, 1974; Lloyd *et al.*, 1977; Bowes *et al.*, 1978; Simpson *et al.*, 1980; Søndergaard & Wetzel, 1980; Salvucci & Bowes, 1981; Simpson & Eaton, 1986; Hough & Putt, 1988; Owttrim & Colman, 1989; Bowes & Salvucci, 1989; Maberly & Spence, 1989; Sand-Jensen, 1989) caused by the dual nature of RuBPcarboxylase, which can fix oxygen as well as carbon dioxide (Ogren & Bowes, 1971; Bowes *et al.*, 1971; Bowes & Ogren, 1972; Andrews *et al.*, 1973; Lorimer *et al.*, 1973). In the light, this enzyme combines CO_2 with ribulose 1,5-bisphosphate to produce 3-phosphoglycerate as the first step of the Calvin cycle (Bassham & Calvin, 1957), but can also act as RuBPoxygenase, combining O_2 with ribulose 1,5-bisphosphate, producing phosphoglycolate which is subsequently further metabolised with the ultimate release of CO_2 via the glycolate pathway (Tolbert, 1971). Both CO_2 and O_2 compete for the same active site on the enzyme (Ogren & Bowes, 1971; Bowes & Ogren, 1972; Laing *et al.*, 1973 & 1974). The rate at which metabolism of CO_2 and O_2 proceeds is largely dependant on their relative concentrations at the active site, with high O_2 and low CO_2 favouring glycolate synthesis and photorespiration (Lorimer, 1981). High temperatures and irradiances also encourage photorespiration (Lorimer, 1981).

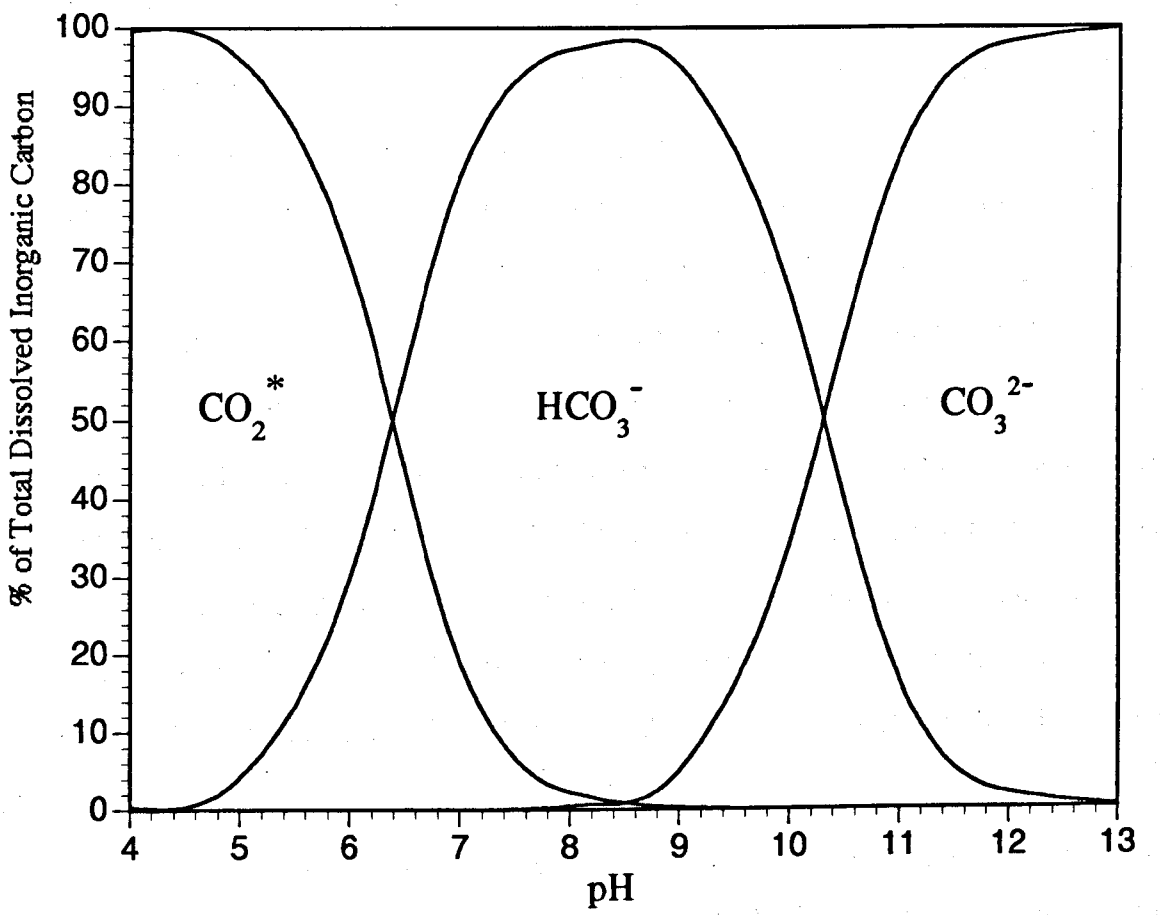
The photosynthesis of epiphytic algae will increase the concentration of oxygen immediately adjacent to the plants photosynthetic surfaces. This will in turn lead to increased levels of photorespiration for the host plant.

The disequilibrium of charge between the H and O atoms in the covalent water molecule gives water many of the properties of a charged ion, such as bringing into solution ionic crystals or other polar molecules, for example CO_2 which is far more soluble in water than it is in air. The attraction of charge by the O atom is so great that the water

molecule can split an H^+ ion from another molecule, water or otherwise. The concentration of H^+ ions (actually H_3O^+) in the water is described numerically as pH (= -log[H^+]) and determines the equilibrium position of the reactions of many of the substances dissolved within it, such as CO_2. When dissolved in water, some CO_2 molecules react to form H_2CO_3 (carbonic acid) which in turn dissociates into HCO_3^- (bicarbonate) and CO_3^{2-} (carbonate) in quantities largely determined by the pH of the water (Figure 3.1). [CO_2 is so readily interconvertible with H_2CO_3 that hereafter they are together referred to as CO_2^* - free carbon dioxide. It should be stressed that this reaction is relatively slow when compared with the ionic reactions of DIC, see p211.] However, CO_2^* is a vital part of the biological processes of organisms, being taken up by photosynthesis and released during respiration, which changes both the concentration of total dissolved inorganic carbon (DIC) and, more importantly, the pH of the water, which in turn affects the proportions of the carbon species. Hence, dependant on the initial DIC concentration and the pH, the concentration of CO_2^* can vary from zero to several mmol l^{-1}, in marked contrast with the aerial environment where CO_2 concentration is relatively constant.

Although CO_2^* is the "preferred" carbon species for the photosynthesis of all submerged macrophytes investigated to date (Steeman-Nielsen, 1947; Stanley & Naylor, 1972; Lloyd *et al.*, 1977; Brown *et al.*, 1974; Simpson *et al.*, 1980; Bowes, 1983; Maberly & Spence, 1983; Sand-Jensen, 1983; Pokorny *et al*, 1984 & 1989; Spence & Maberly, 1985; Sand-Jensen & Gordon, 1986), some species can, to varying extents, utilise the HCO_3^- ion (Steeman-Nielsen, 1947; Raven, 1970; Prins *et al.*, 1982a & b; Bowes, 1983; Maberly & Spence, 1983; Raven *et al.*, 1985; Spence & Maberly, 1985; Sand-Jensen & Gordon, 1986; Newman & Raven, 1993). Neither HCO_3^- nor H_2CO_3 can diffuse through bilipid membranes sufficiently rapidly to support significant photosynthesis (Gutknecht *et al.*, 1977), so use of HCO_3^- must in some way involve active uptake, and hence incur some energy cost. The CO_3^{2-} ion is completely excluded from photosynthesis (Raven,

Figure 3.1 Effect of pH on the relative proportions of the different species of dissolved inorganic carbon in water. (Calculated from the equations of Mackereth, Heron & Talling, 1978; after Wetzel, 1983a)



1970). As pH is the major determinant of the proportions that the different carbon species take, it has a profound effect on photosynthesis, especially in the region pH 7 to pH 9, where CO_2^* becomes reduced to negligible proportions and HCO_3^- becomes the predominant species (Figure 3.1). Increased photorespiration may also occur under the influence of high pH due to the low availability of CO_2^*.

One way of escaping the constraints of gaseous diffusion in water is to produce floating leaves or emergent parts with stomata (Sculthorpe, 1985). A few species avoid carbon restriction by exploiting alternative sources of CO_2^* for photosynthesis. *Fontinalis antipyretica* growing in the eutrophic Esthwaite Water has a low stature and grows in a zone near to the lake bed where respiration in the mud greatly increases the CO_2^* concentration of the overlying water (Maberly, 1985a & b). It is likely that *Callitriche hermaphroditica* L. survives in Rosthern Mere, Cheshire, where the pH of the lake water frequently exceeds pH 9 (Carvalho, 1993), by growing in such a manner. The taxonomically disparate *Lobelia dortmanna* L., *Littorella uniflora* (L.) Asch. and *Isoetes spp.* all utilise CO_2^* produced in the sediment by conducting it from their roots via lacunae to the leaves (Wium-Andersen 1971, Søndergaard & Sand-Jensen 1979). The latter two also possess a form of Crassulacean Acid Metabolism which enables them to utilise CO_2^* fixed during the night in photosynthesis (Keeley, 1981 & 1990; Keeley & Bowes, 1982; Raven *et al.*, 1985; Madsen, 1987; Robe & Griffiths, 1992). Some species which lack any physiological or morphological modifications continue to thrive in alkaline waters by simply colonising sites where the flow is rapid and the supply of CO_2^* thus increased (Raven, 1970; Sand-Jensen, 1983; Raven *et al.*, 1987; see below).

Since pH of a water is heavily influenced by photosynthesis, the presence of a layer of metabolically active algae growing over a plant's surfaces has the potential to be a severe problem for the plant. The algae are in a superior position with regards to the supply of CO_2^* and could, due to their photosynthesis, greatly reduce the availability of CO_2^* to the plant. The low rate of exchange with the bulk water (see below) would only serve to worsen the pH climate for the plant, and hence reduce photosynthesis further.

Figure 3.2 Formation of laminar and turbulent boundary layers over the surface of a flat plate.
(redrawn from Leighton, 1975)

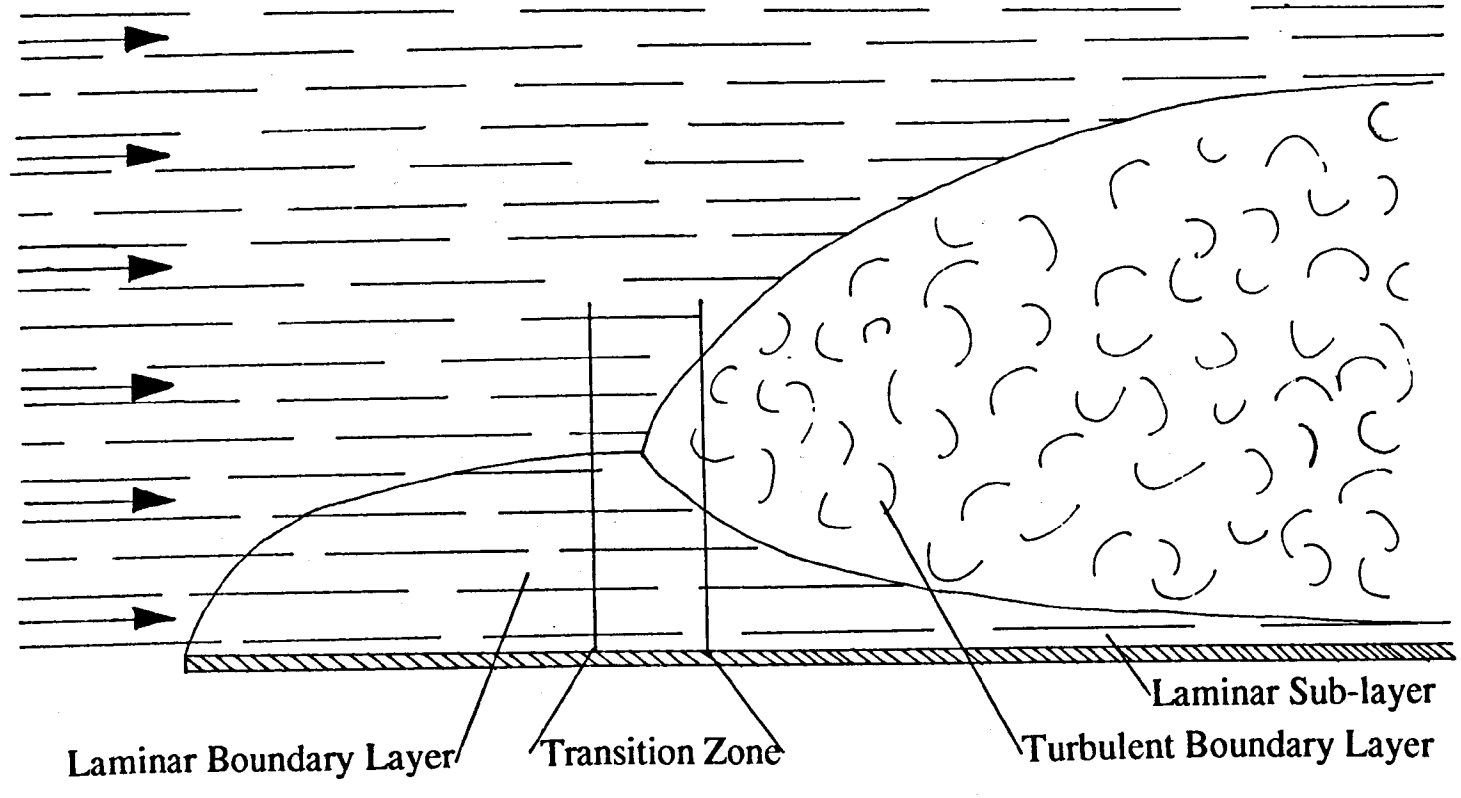
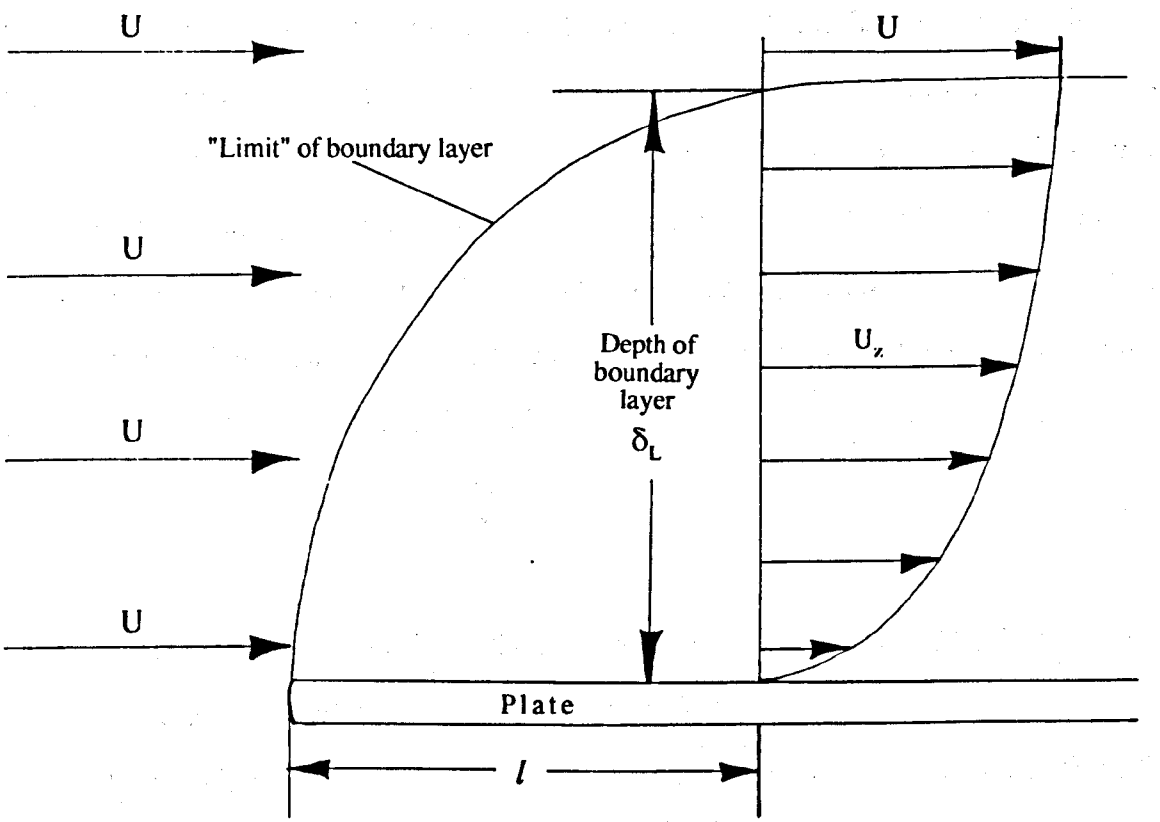


Figure 3.3 The growth of the laminar boundary layer over a flat plate, with a velocity profile indicated at a distance l from the leading edge.

(adapted from Leighton, 1975)



Free stream velocity = U. Velocity of fluid with the boundary layer at a distance z from the surface of the plate = U_z. The depth of the boundary layer at a distance l from the leading edge = δ_L.

The viscosity of water, in addition to causing the low diffusion constants, has another serious physical affect on the plants growing submerged within it, that of increased boundary layer thickness. Due to friction, a shear stress exists between the surface of an object and a moving fluid in which it is immersed. This leads to a steady decrease in the velocity of the fluid as the surface is approached, the fluid immediately adjacent to the object being virtually static. The layer of fluid which is strongly influenced by the object is termed the boundary layer. Put simply, for a fluid moving over a smooth flat surface, the flow within the boundary layer can be of two types, one being laminar, where the fluid moves in sheets parallel to one another, and the other turbulent, where vertical mixing is present but a thinner sub-layer of fluid abutting the object exists where flow is entirely laminar. A boundary layer with laminar flow is formed nearest the leading edge of the plane surface, becoming turbulent further from the edge (Figure 3.2). The transition from laminar to turbulent flow can be predicted from the ratio of inertial to viscous forces known as the Reynolds' number, R_e and defined by

$$R_e = \frac{Ul}{v}$$

where

v = the kinematic viscosity of the fluid (absolute viscosity divided by density)

U = the velocity of the fluid relative to the object

l = the characteristic length dimension of object, typically the distance from the leading edge

The distance from the edge, or more strictly the zone, where transition from laminar to turbulent flow occurs is determined when the R_e exceeds some critical value. This varies with the turbulence of the free-stream and the roughness of the surface. For a smooth, plane surface and low free-stream turbulence, transition does not occur until the R_e exceeds about 3×10^6, but when the free-stream is turbulent this figure is about 5×10^5 (Leyton, 1975).

The thickness of the laminar boundary layer (δ_L), if we define it as the region where flow is 99% or less of the free-stream rate, can be calculated as:

$$\delta_L \approx 5\,(v\mathit{l}/U)^{1/2} \text{ or } \delta_L \approx 5\mathit{l}\,(R_{eL})^{-1/2}$$

Where R_{eL} is the Reynolds' number at the distance l from the leading edge (Figure 3.3). The numerical factor 5 must be treated as a working approximation; depending on the assumptions made in solving the problem, other slightly different values have been suggested, but the rest of the equation remains unaffected (Leyton 1975). From this it can be seen that δ_L increases with the square root of l, and inversely with the square root of U.

If the flow within the boundary layer is turbulent, the equation required to calculate the boundary layer thickness (δ_T) is:

$$\delta_T = 0{\cdot}376\, \mathit{l}_T\, (R_{e\mathit{l}T})^{-1/5}$$

where l_T is the distance from the begining of turbulence, and $R_{e\mathit{l}T}$ is the Reynolds' number based on $U\, \mathit{l}_T / v$. The turbulent boundary layer therefore increases in depth with $\mathit{l}_T^{1/5}$, and inversely with $U^{1/5}$, ie. more rapidly than the laminar boundary layer (δ_L).

The thickness of the laminar sub-layer within this boundary layer (δ_s) is largely determined by the roughness of the surface, but can be approximated as:

$$\delta_s \approx \frac{58\,\mathit{l}_T}{(R_{e\mathit{l}T})^{0.9}}, \text{ or very approximately as: } \delta_s \approx \frac{58v}{U}$$

As transport of dissolved substances is much more rapid when turbulence is present (eddie transport *cf.* diffusion) and the region of laminar flow decreases inversely with the free-stream velocity, it would be very advantageous to a submerged plant to have a turbulent boundary layer. Hypothetically, assuming that submerged leaves act as smooth plates and using figures typical of plants growing in streams (Hynes, 1970; Whitton, 1975; Westlake, 1975; Raven, 1981), it is possible to calculate what size they must be before the boundary layer becomes turbulent. From Table 3.1 it is seen that this is only of concern to plants of very fast flowing environments, such as members of the Podostemaceae and encrusting or mat-forming bryophytes, lichens and algae (Whitton, 1975; Westlake, 1975;

Table 3.1 Distances from the leading edge of a flat plate that the flow within the boundary layer changes from laminar to turbulent.

Free-stream flow	Temperature	Mean free-stream velocity		
		0·05 m s^{-1}	1·00 m s^{-1}	5·0 m s^{-1}
Smooth	10 °C	78·6 m	3·93 m	0·78 m
	20 °C	60·6 m	3·03 m	0·60 m
Turbulent	10 °C	13·1 m	0·66 m	0·13 m
	20 °C	10·1 m	0·51 m	0·10 m

Although the distances are given here as points, the transition from laminar to turbulent flow is not wholly abrupt, but occurs over some finite length known as the transition zone (see Figure 3.2). The values given correspond to a Reynolds' number of 3×10^6 for free-stream flow with low turbulence and 5×10^5 for free-stream flow with high turbulence, figures which approximately define the position of the transition zone (Leyton 1975). The values for free-stream velocity are typical of plants living in flowing water, with only encrusting and thalloid forms at the highest velocity (Westlake, 1975; Whitton, 1975; Raven, 1981).

Raven, 1981; Jenkins & Proctor, 1985), or species with large submerged parts, for example certain bryophytes (Jenkins & Proctor, 1985) and water crowfoots, which, due to their "streamer-like" action, deviate from the theoretical flat plane surface and induce eddies and turbulent flow along their length (see frontispiece). It has also been noted that the nodal papillae along the filaments of the red alga *Lemanea mamillosa*, typical of fast flowing waters, will increase turbulence over the thallus (Raven *et al.*, 1985).

Although the principles are the same, this type of boundary layer, surrounding a submerged object, should not be confused with that associated with the bed of a flowing water-body such as a stream, for which a different suite of calculations exist (Smith, 1975). The calculations describing this latter type of boundary layer are often used by biologists working with benthic invertebrates (Carling, 1992) and sediment resuspension (Huttula, 1992).

Since the flow within the boundary layer is laminar, substances must travel across it solely by diffusion, an exceedingly slow process in water, creating a great physical resistance to gas movement and therefore restricting exchange of materials during photosynthesis (Raven, 1970 & 1981; Smith & Walker, 1980; Black *et al.*, 1981; Madsen & Sand-Jensen, 1991). This resistance can be reduced by increasing the flow of the water around the plant, which increases the rate of photosynthesis, provided that the flow is not overly vigorous (Westlake, 1967; Smith & Walker, 1980; Black *et al.*, 1981; Madsen & Søndergaard, 1983; Søndergaard, 1988). Submersed macrophytes of low-flow environments typically have highly dissected or lanceolate, thin (2 to 3 cells thick), flexible leaves, which reduce the thickness of the boundary layer (Boston *et al.*, 1989). Highly dissected leaves are also typical of plants from high-flow habitats where they reduce the damage caused by drag and the risk of uprooting considerably (Boston *et al.*, 1989). It seems that if the plants are well rooted and flows moderate, growth increases with flow (Boeger, 1992), but if the substrate is poor or the flow is too great, then growth is inversely proportional to flow (Chambers *et al* , 1991; Boeger, 1992). This reduced

growth may be due to physical damage or to a reduction of photosynthesis caused by increased physical stress (Madsen *et al.*, 1993). The latter phenomenon has only been shown in laboratory studies under conditions non-limiting to photosynthesis, where plants were subjected to flows of up to 8 cm s^{-1} (Madsen *et al.*, 1993). The tendency for aquatic plants to form clumps or weed-beds (see frontispiece), which reduces the flow within the bed to about 10% of the free-stream velocity (Madsen & Warncke, 1983; Losee & Wetzel, 1988 & 1993; Machata-Wenninger & Janauer, 1992), and limitation on the availability of dissolved gases in the field may largely counteract this effect in the field.

It is apparent that the resistance to gas movement across the laminar boundary layer is the rate limiting step of photosynthesis, even at moderate flows (Smith & Walker, 1980; Black *et al.*, 1981; Søndergaard, 1988), with changes in the thickness of this layer causing large changes in the rate of photosynthesis and respiration (Owens and Maris, 1964; Westlake, 1967; Sculthorpe, 1985; Smith & Walker, 1980; Black *et al.*, 1981; Madsen & Søndergaard, 1983; Søndergaard, 1988; Madsen & Sand-Jensen, 1991). It has been proposed that the presence of a layer of epiphyton over the plants photosynthetic surfaces will increase the width of the boundary layer, and hence the distance that CO_2^* must travel by diffusion (Sand-Jensen, 1977; Phillips *et al.*, 1978). As a result, the rate of photosynthesis of the plant will be reduced.

3.2 Methods.

3.2.1 The effect of changes in oxygen concentration on the physiology of *E. nuttallii*.

Physiological determinations were made as outlined before (Section 2.2.1), with the starting oxygen concentration in the medium adjusted before the immersion of the leaf sample or injection of the $NaHCO_3$ solution, with oxygen or nitrogen gas to one of several levels (1, 2, 4, 7, 10, 13, 16, 20 mg l^{-1}), from near 0% to in excess of 200% saturation with air, selected at random. Points were plotted as the mid point of 1 g l^{-1} maximum change in dissolved oxygen. A total of seven replicate determinations were made at each level, due to the high degree of variability between plants.

3.2.2 The effect of changes in pH on the physiology of *E. nuttallii*.

Physiological determinations were made with a Clarke-type oxygen electrode as outlined in Section 2.2.1, in medium containing buffer components varied to provide the required pH range. Tris was used at a concentration of 50 mmol l^{-1}, adjusted with dilute HCl to give the range pH 7 to pH 9, 50 mmol l^{-1} Na-citrate - NaH_2PO_4 for pH 5 to pH6. A Student's t-test showed that that there was no significant difference between the rates measured at the junction (pH7) of the two buffers ($p > 0{\cdot}2$). This experiment was repeated using *E. nuttallii* and *E. canadensis* samples collected from the Lancaster Canal at Stainton.

Since some workers have reported that buffers interfere with the uptake of HCO_3^- by submerged aquatic plants (Prins *et al.*, 1982a & c; Prins & Helder, 1985; Elzenga & Prins, 1987) and lead to incorrect interpretation of the pH-photosynthesis relationship, a further experiment was conducted using buffer-free Forsberg medium. Here the pH was adjusted using dilute NaOH or HCl to a range of values from pH 4 to pH 10. The pH of the medium was measured within the reaction chamber both at the start, once $NaHCO_3$ had been added, and end of the illumination period using a Lazar PHR-146 microcombination

pH electrode, tip diameter = 3 mm (Lazar Research Laboratories Inc., Los Angeles, California).

Carbon dioxide concentrations were calculated by substituting known values of pH, dissolved inorganic carbon concentration and temperature into the equations given by Mackereth *et al.* (1978).

3.3.3 The effect of changes in dissolved inorganic carbon on the physiology of *E. nuttallii*.

Again determinations were made as outlined in Section 2.2.1, in modified Forsberg medium buffered to pH 7, but the concentration of $NaHCO_3$ solution injected into the reaction chamber was adjusted to give a range of final concentrations from 0·15 to 24 mmol l^{-1}. In determinations at higher $NaHCO_3$ concentrations, the buffering capacity of the added $NaHCO_3$ solution was compensated for by the addition of predetermined amounts of 0·2 mol l^{-1} HCl. Prior to use, the medium was vigorously sparged with nitrogen gas to remove all carbon dioxide, either dissolved or in complex with the tris buffer as carbamate. Oxygen gas was then used to return the solution to slightly below 10 mg O_2 l^{-1} and the determinations conducted within 1 mg O_2 l^{-1} change. The efficiency of this procedure was shown as leaves were unable to photosynthesise unless $NaHCO_3$ solution was added.

The experiment was repeated in full with a separate collection of plants from the Leeds and Liverpool Canal and the dissolved oxygen concentration adjusted to give mean levels of 2 and 16 mg O_2 l^{-1}.

3.3.4 The effect of changes in boundary layer thickness on the physiology of *E. nuttallii*.

In this experiment, each determination was made using an intact whorl of leaves attached to a small length of stem, ~1 mm either side of the node. Two Clarke-type electrodes were used consecutively, one with the stirrer speed set at maximum and the

leaves wedged near the base of the reaction chamber, the other with the stirrer speed set at minimum and the leaves wedged near the top. Both electrodes were calibrated with 2 cm^3 total volume with the $NaHCO_3$ solution adjusted to give the normal final concentration of 2·4 mmol l^{-1}.

As the thickness of the unstirred boundary layer surrounding an object is inversely proportional to the speed of flow of the medium in which it is immersed (see above), two treatment levels were produced, one with a thin boundary layer, the other thick. Although the rates of flow, and hence actual thicknesses, are unknown, they could be visualised as "fast" and "slow" by the injection of small quantities of dye. Each pair of determinations was replicated ten times, the order of electrodes being allocated at random. The results were analysed using a paired t-test.

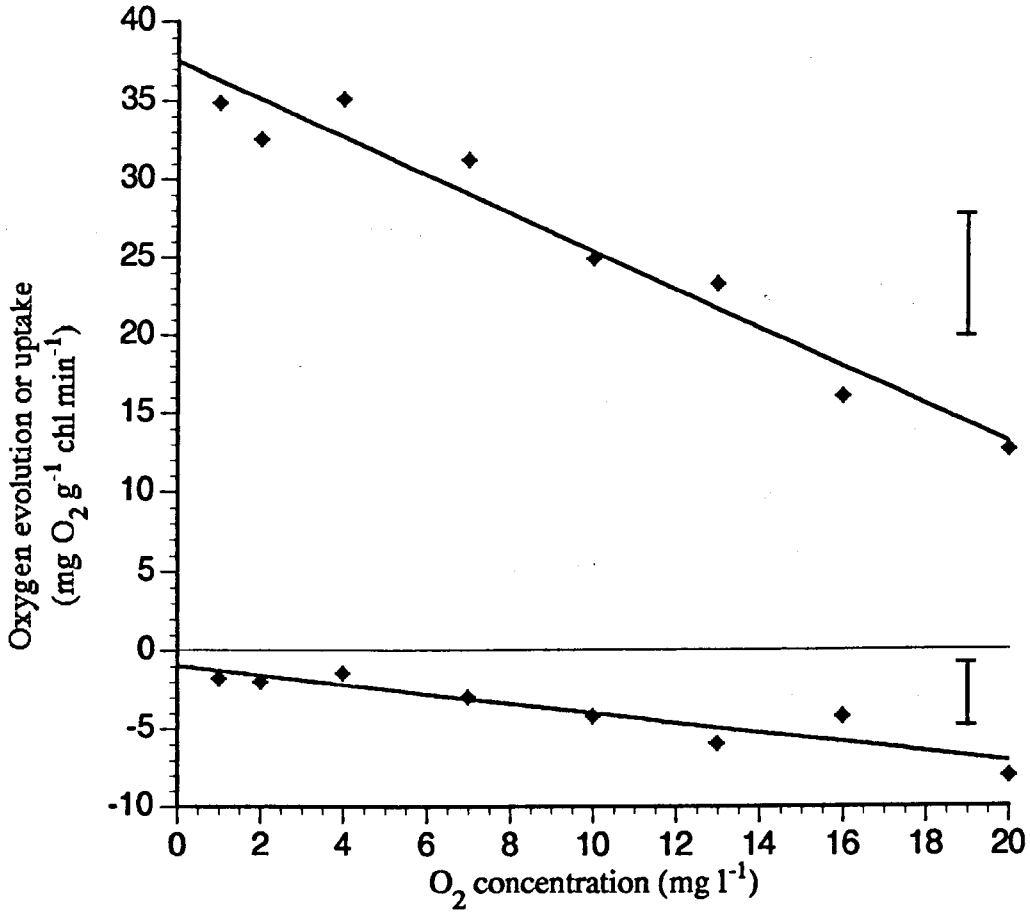
3.4 Results and Discussion.

3.4.1 The effect of changes in oxygen concentration on the physiology of *E. nuttallii*.

Under constant light and DIC, photosynthetic oxygen evolution decreased linearly with increasing oxygen concentration in the surrounding medium (Figure 3.4). The problems caused by the formation of bubbles at higher concentrations reported by other workers (Brown *et al.*, 1974; Dromgoole, 1978; Simpson *et al.*, 1980) were never encountered. Dark respiration also increased linearly with oxygen concentration, but at a much slower rate than the equivalent decrease in photosynthesis. An analysis of variance showed that the effect of increasing oxygen was highly significant for both photosynthesis and respiration ($p = 0{\cdot}0001$ in both cases) and provided the error mean square necessary for calculating the least significant difference with Tukey's Test (7·71 for photosynthesis, 3·82 for respiration).

Dark respiration in the aerial parts of plants is unaffected by atmospheric O_2 concentrations above 2% (Jackson & Volk, 1970), and O_2 availability does not generally limit respiration, although there are some occasional exceptions (Crawford, 1992). The rate of O_2 uptake of submerged aquatic plants, however, has been shown in this study and by others to be affected by the O_2 content of the surrounding water over a wide range of environmentally relevant concentrations (1-17 mg l^{-1}, Owens & Maris, 1964; 1-20 mg l^{-1}, Sculthorpe, 1985 pp130; 1-10 mg l^{-1}, Simpson *et al.*, 1980; 1-20 mg l^{-1}, this study), an effect attributed to the low diffusivity of O_2 in water. Owens & Maris (1964) stressed the similarity between the effect of oxygen concentration on respiration and an equation which describes a system where simultaneous diffusion and chemical reaction occurs. This type of equation also explains the relationship between photosynthesis and CO_2^* concentration (Smith & Walker, 1980; Madsen, 1984; Madsen & Sand-Jensen, 1991; see Section 3.4.2). In the light, dark respiration of submerged macrophytes, measured by $^{14}CO_2$ release,

Figure 3.4 Effect of oxygen concentration in the incubating medium on net oxygen evolution in the light and uptake in the dark of *E. nuttallii.*



For photosynthesis, $y = 37.726 - 0.969x$, $R^2 = 0.638$, $p = 0.0001$

For respiration, $y = -0.922 - 0.311x$, $R^2 = 0.476$, $p = 0.0001$

Error bars show least significant difference for any pairwise comparison calculated from Tukey's test

appears to be supressed (Hough, 1974; Søndergaard, 1979), agreeing with findings for terrestrial plants (Jackson & Volk, 1970; Zelitch, 1971; Graham, 1980). It has been argued (Hough, 1974) that the slow rate of diffusive loss of photorespired CO_2 to the water could lead to extensive refixation, a situation which has been shown for certain species with thick cuticles and extensive lacunal systems (Søndergaard, 1979; Søndergaard &Wetzel, 1980) and hence to an underestimate of respiration in the light. The leaves of *Elodea* species do not have lacunae and air spaces are few (Plate 1; see also Plates 20 & 21), and it has been suggested that refixation is probably minor in the closely related *E. canadensis* (Søndergaard, 1979). Whether the respiration in the dark is equal to or greater than that in the light, the increased rates with increasing O_2 could not wholly explain the apparent inhibition of photosynthetic O_2 evolution and another mechanism must be active to produce this effect. It is generally accepted that the stimulation of photorespiration is the most important effect of increased O_2 concentration in the light (Zelitch, 1971; Lorimer, 1981).

3.4.2 The effect of changes in pH on the physiology of *E. nuttallii*.

Under constant dissolved inorganic carbon (2·4 mmol l^{-1}), a decline in light-saturated photosynthesis with rising pH was seen above pH 7, reaching very low rates at pH 9. Similarly the rate decreased sharply below pH 6 (Figure 3.5). Dark respiration is high and stable between pH 5 and pH 7 but at a low level between pH 8 and pH 9, the change-over occurring in the pH 7-8 range. This decline in photosynthetic activity at the upper end of the pH scale parallels the decline in CO_2^* as the DIC equilibrium shifts towards HCO_3^- (Figure 3.1). In all species of submerged macrophytes investigated to date, CO_2^* is the preferred carbon source, and a reduction in the rate of photosynthesis above pH 7 is typical (Steeman-Nielsen, 1947; Stanley & Naylor, 1972; Lloyd *et al.*, 1977; Brown *et al.*, 1974; Simpson *et al.*, 1980; Bowes, 1983; Maberly & Spence, 1983; Sand-Jensen, 1983; Pokorny *et al*, 1984 & 1989; Spence & Maberly, 1985; Sand-Jensen & Gordon, 1986), though the extent of the reduction differs between species depending on their ability to utilise HCO_3^- (Kadono, 1980; Sand-Jensen, 1983; Sand-Jensen & Gordon, 1984 & 1986; Madsen & Sand-Jensen, 1987). According to Prins *et al.* (1982a & c), the mechanism of HCO_3^- use in the Potamogetonaceae and Hydrocharitaceae involves the creation of acidified regions on the surface of the leaf cells, where the conversion of HCO_3^- to CO_2^* occurs. Use of strong buffers in the medium would be expected to inhibit this process, and they have a negative effect on apparent photosynthesis at high pH (Prins *et al.*, 1982a & c; Prins & Helder, 1985; Elzenga & Prins, 1987). A further experiment in pH-adjusted, unbuffered media showed a very similar decline above pH 7 (Figure 3.6), indicating that *E. nuttallii* growing in the Leeds and Liverpool Canal was not using HCO_3^- at this time. Caution is required when interpreting this result, since *Elodea* species have been shown both here (Chapter 4) and by others (Sand-Jensen & Gordon, 1986; Madsen & Sand-Jensen, 1987; Adamec & Ondok, 1992; Adamec 1993) to have a high degree of adaptability to conditions of low CO_2.

Figure 3.5 Effect of pH in the incubation medium on the net oxygen evolution in the light and uptake in the dark of *E. nuttallii.*

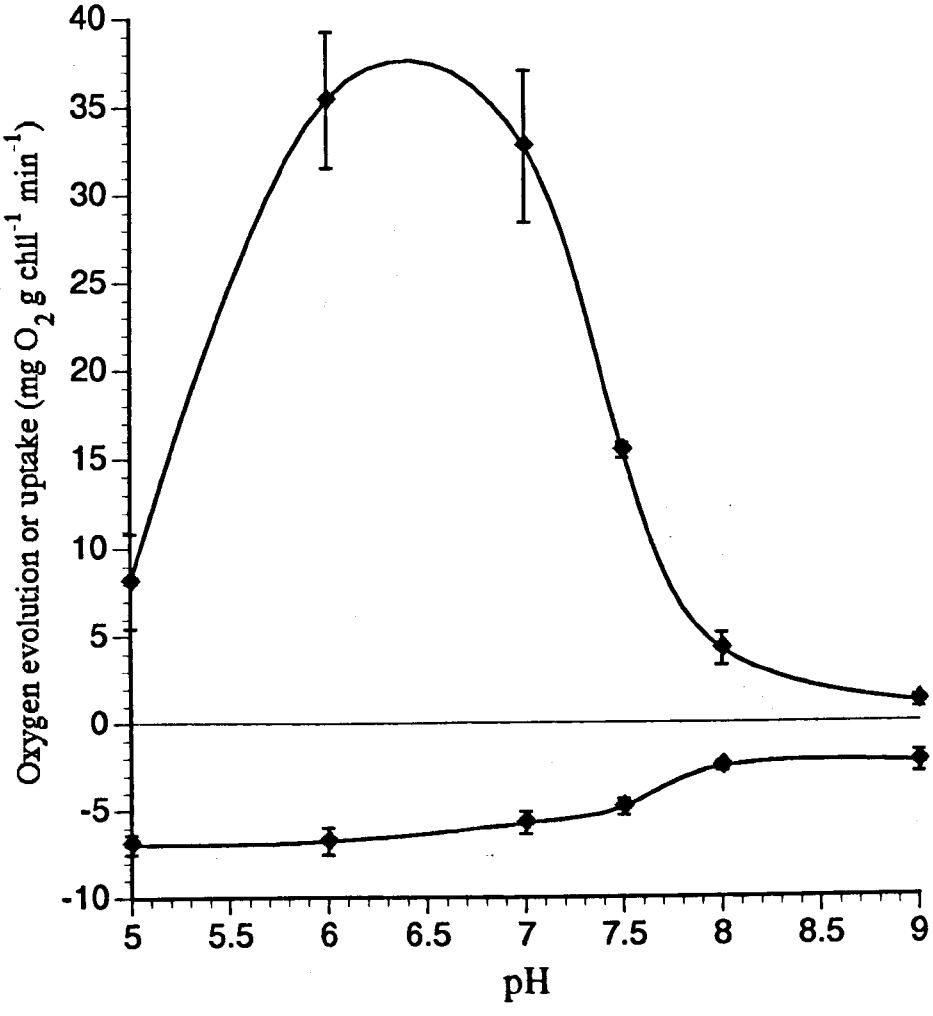
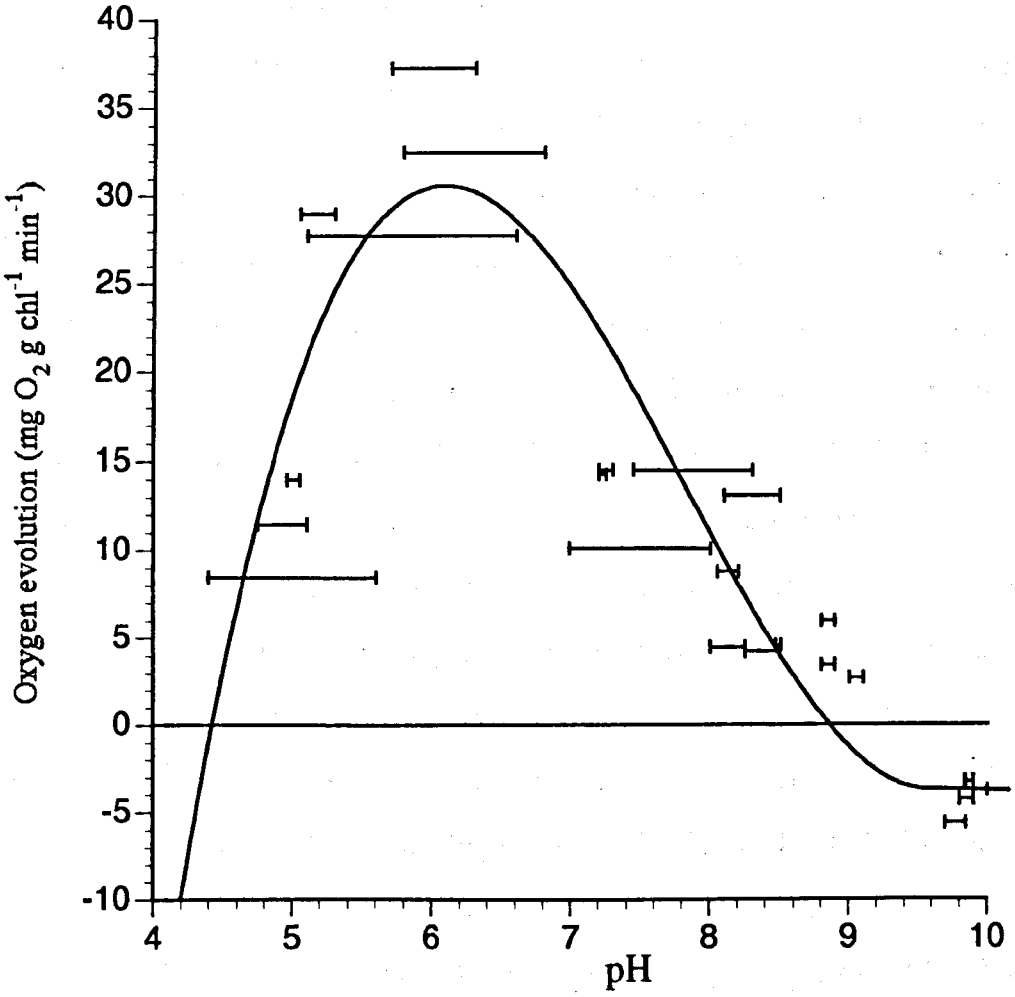


Figure 3.6 Relationship between pH and net photosynthesis of *E. nuttallii* measured in unbuffered media. Individual points plotted as oxygen evolution versus pH range, curve fitted by eye.



The low rate of photosynthesis at pH 5 is somewhat less readily explained. This decline was not caused by an increase in respiration, nor by the Na-citrate - NaH_2PO_4 buffer, since photosynthesis and respiration did not differ significantly when measured in the two different buffers (t-test, $p > 0.2$). When plants from the Lancaster Canal were tested, *E. nuttallii* and *E. canadensis* appeared to react similarly to pH changes in the bathing medium, apart from at pH 5 (Figure 3.7), indicating that it was a very specific effect. Further, a CO_2^* effect was ruled out by later experiments (Section 3.4.3) in which *E. nuttallii* was exposed to higher concentrations without adverse effects. An "acid" effect on photosynthesis has also been described for *Myriophyllum spicatum* L. (Owttrim & Colman, 1989), and on the reproduction of *Najas flexilis* Rostk. & Schmidt and *Vallisneria americana* Michx. (Titus & Hoover, 1993). This may explain the CO_2^* inhibition reported for high concentrations when CO_2^* is altered through pH manipulation (Sand-Jensen, 1987). This finding gives rise to a note of caution when using a comparison of photosynthetic rates at high and low pH to predict the extent of HCO_3^- utilisation, without prior investigation into the effect of pH on the physiology of the plant, as has regretably been done for several species of submerged macrophytes (Van *et al.*, 1976; Holaday & Bowes, 1980), including *E. nuttallii* (Eighmy *et al.*, 1987; Jahnke *et al.*, 1991).

The effect of CO_2^* availability is shown clearly when photosynthesis is plotted against the CO_2^* concentration at the different pH levels (Figure 3.8). It has been argued that the shape of this curve owes more to diffusive resistance than to enzyme kinetics (Smith & Walker, 1980; Madsen & Sand-Jensen, 1991), being less gradual than anticipated from the Michaelis-Menten equation. The rate of photosynthesis is proportional to carbon concentration for a wider range of low concentrations than would be expected, and the transition from rate-limitation to rate-saturation is more sudden (Smith & Walker, 1980; Madsen, 1984; Madsen & Sand-Jensen, 1991). Given that submerged macrophytes generally have thin leaves with high concentrations of chloroplasts in the epidermis, the most likely cause of this deviation is the resistance of the boundary layer. Diffusion

Figure 3.7 Effect of pH in the incubation medium on the oxygen evolution in the light and uptake in the dark of *E. nuttallii* and *E. canadensis* collected from the Lancaster Canal.

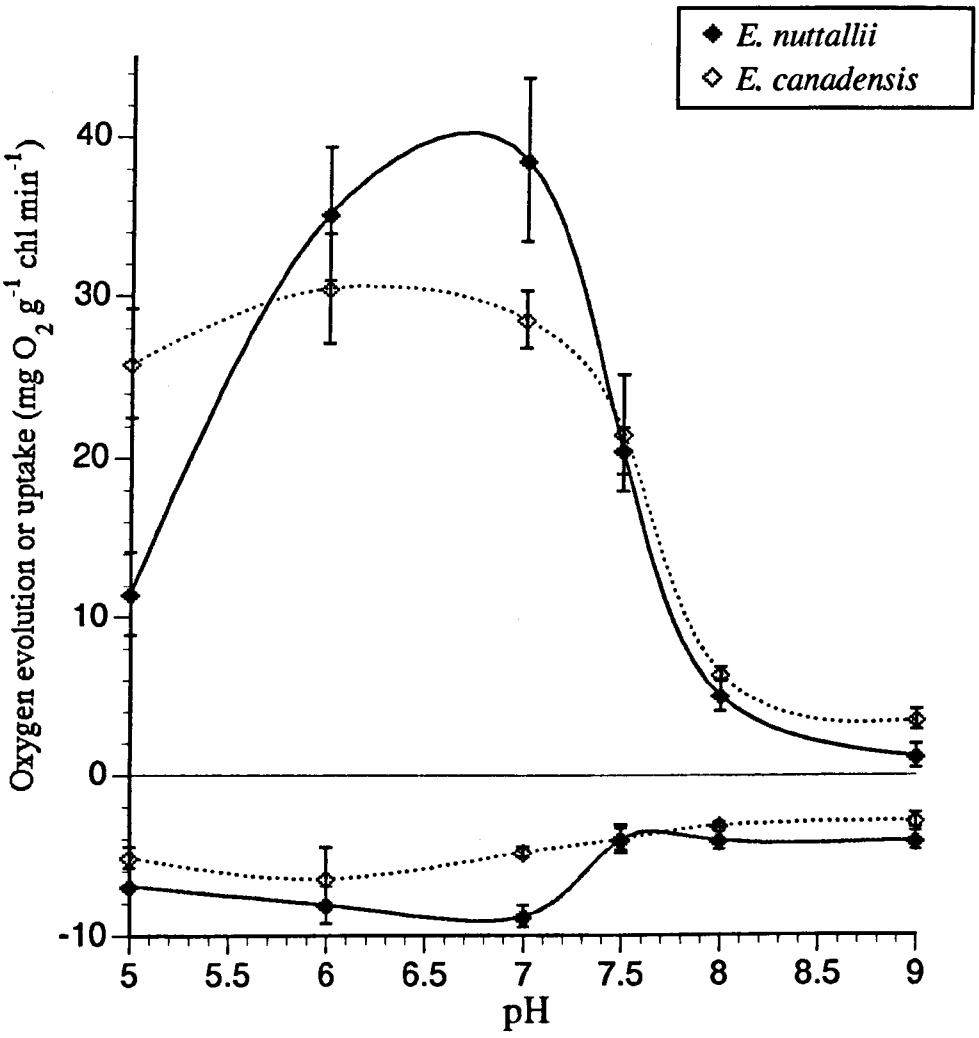


Figure 3.8 Relationship between CO_2* concentration in the incubating medium at the different pH levels used and net photosynthesis of *E. nuttallii*.

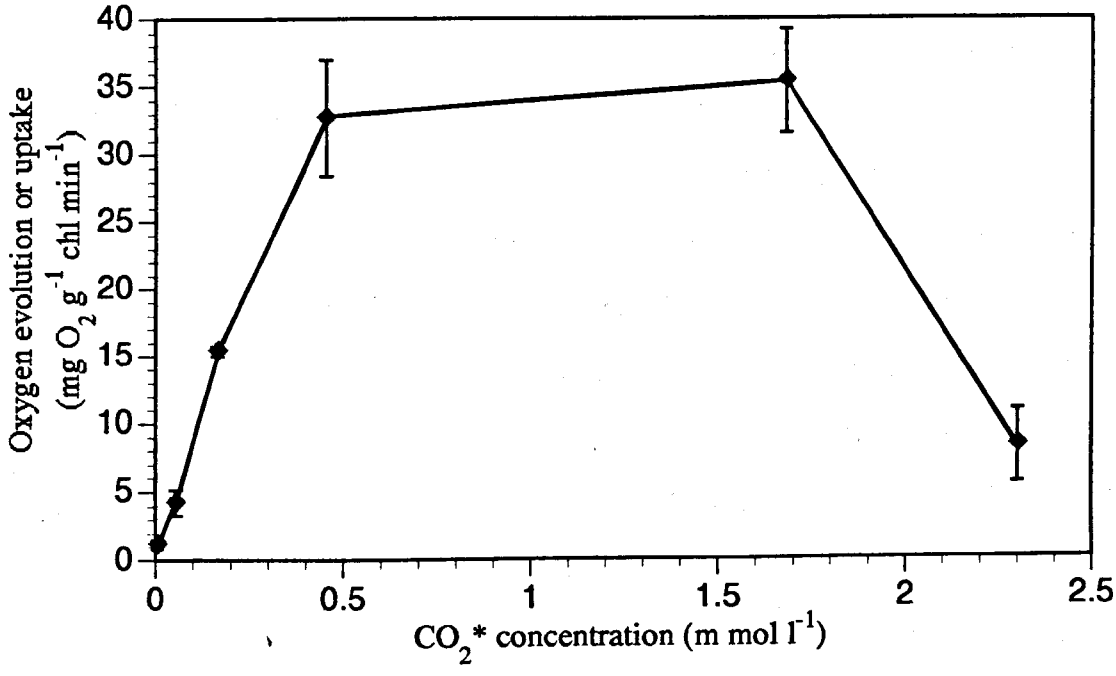
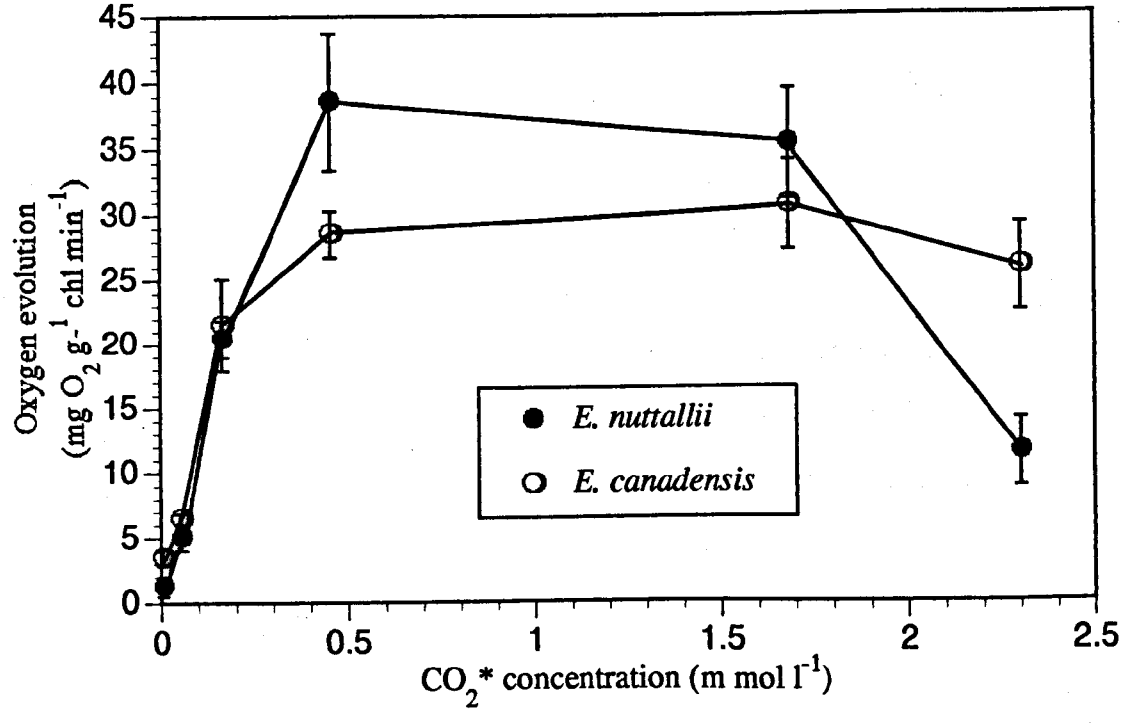


Figure 3.9 Relationship between CO_2* concentration in the incubating medium at the different pH levels used and net photosynthesis of *E. nuttallii* and *E. canadensis* collected from the Lancaster Canal.



through the boundary often constitutes more than 90% of the total resistance to carbon fixation, whereas biochemical resistance constitutes only a few percent (Browse *et al.*, 1979; Black *et al.*, 1981; Madsen, 1984). Models using the Whittingham-Hill equation (1955), which is a combination of Fick's first law (governing diffusion) and the Michaelis-Menten equation for a one-substrate enzyme catalyst reaction, have shown that the relationship at low CO_2^* concentration changes from a hyperbolic pattern to a more linear response as the boundary layer resistance becomes significant (Madsen & Sand-Jensen, 1991). This deviation from Michaelis-Menten kinetics is reflected in the apparent half saturation constant, which increases with the boundary layer resistance (Smith & Walker, 1980; Madsen & Sand-Jensen, 1991). Eliminating or greatly reducing boundary layer effects by measuring photosynthesis in water-saturated air has been shown to increase the affinity for CO_2^* to far above that determined in water (Lloyd *et al.*, 1977; Salvucci & Bowes, 1982). Unfortunately, due to the complex nature of the Wittingham-Hill equation and the low number of levels used in this experiment, a curve could not be fitted accurately to these data, but the shape is very much what would be expected from such a relationship.

In this experiment, the intercept with the x-axis appeared to be greater than zero (Figure 3.8), indicating that HCO_3^- was not used, but caution is stressed once more since the buffer may have disrupted the uptake mechanism and physiological plasticity has been shown in *Elodea* (Sand-Jensen & Gordon, 1986; Adamec & Ondok, 1992; Adamec 1993; Chapter 4).

When data from the experiment using both *E. canadensis* and *E. nuttallii* from the Lancaster Canal were plotted against CO_2^* concentration (Figure 3.9), it was seen that at low CO_2^* levels *E. canadensis* appeared to have a slightly higher rate of photosynthesis than *E. nuttallii*. Since the lines could not be straightened accurately this was not tested statistically, but may explain why *E. canadensis* continues to grow at this site (alkalinity = 0·82 m equil l^{-1}), when it is usually outcompeted by *E. nuttallii*.

3.4.3 The effect of changes in dissolved inorganic carbon on the physiology of *E. nuttallii*.

When $NaHCO_3$ concentration was varied, the rate of photosynthesis followed a curve similar to that described by the Whittingham-Hill equation (Figure 3.10). Respiration was reduced at the lowest concentrations, with a steady state above 1 mmol l^{-1} $NaHCO_3$ (Figure 3.10). Using the calculated CO_2^* concentrations available at the different DIC levels, the CO_2^* compensation point was calculated to be 16·3 µmol l^{-1} (Figure 3.11), which compares well with the reported values for various submerged macrophytes including members of the Hydrocharitaceae (0·7 to 25 µmol l^{-1} – Van *et al.*, 1976; Søndergaard, 1979; Bain & Proctor, 1980; Søndergaard & Wetzel, 1980; Allen & Spence, 1981; Salvucci & Bowes, 1981; Maberly & Spence, 1983; Sand-Jensen, 1983; Bowes & Salvucci, 1989; Madsen 1991). The CO_2^* compensation point, however, is very sensitive to HCO_3^- utilisation by the plant and in such circumstances can appear negative (Sand-Jensen & Gordon, 1984 & 1986; Madsen & Sand-Jensen, 1987).

When the experiment was repeated at both increased and reduced oxygen concentrations the effect of photorespiration was clearly seen, ie. the increased CO_2^* compensation point (Figure 3.12), together with what appears to be a direct effect of oxygen. The maximum rate of photosynthesis was reduced under increased oxygen, even though the plants were experiencing near-saturating DIC concentrations (10-25 mmol l^{-1}) and a competitive effect of oxygen would not be expected (Figure 3.12). Such oxygen inhibition at CO_2 saturation has previously been found in some species of marine macroalgae (Cook & Colman, 1987; Surif & Raven, 1989). Dark respiration also increased with higher oxygen. To linearise the relationship, the data were transformed to log $[CO_2^*]$ and regression lines fitted (Figure 3.13). A t-test showed that the fitted lines were parallel (for slopes $t = 0{\cdot}206$, $t_{0.05(2)50} = 2{\cdot}009$), but that their intercepts were different ($t = 7{\cdot}083$, $t_{0.05(2)50} = 2{\cdot}009$), the difference being caused by photorespiration. From these fitted lines, CO_2^* compensation points were calcuated as 6·0 µ mol l^{-1} at 2 mg

Figure 3.10 Relationship between DIC concentration in the incubation medium and oxygen evolution in the light and uptake in the dark of *E. nuttallii* measured at 10 mg O_2 l^{-1}.

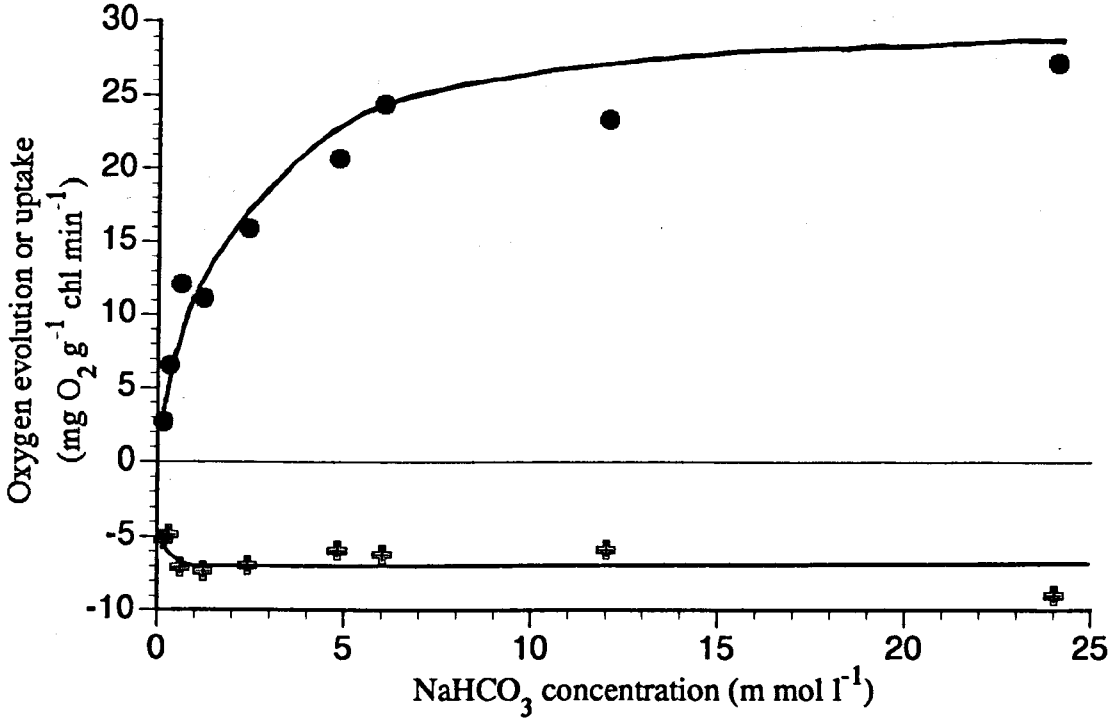
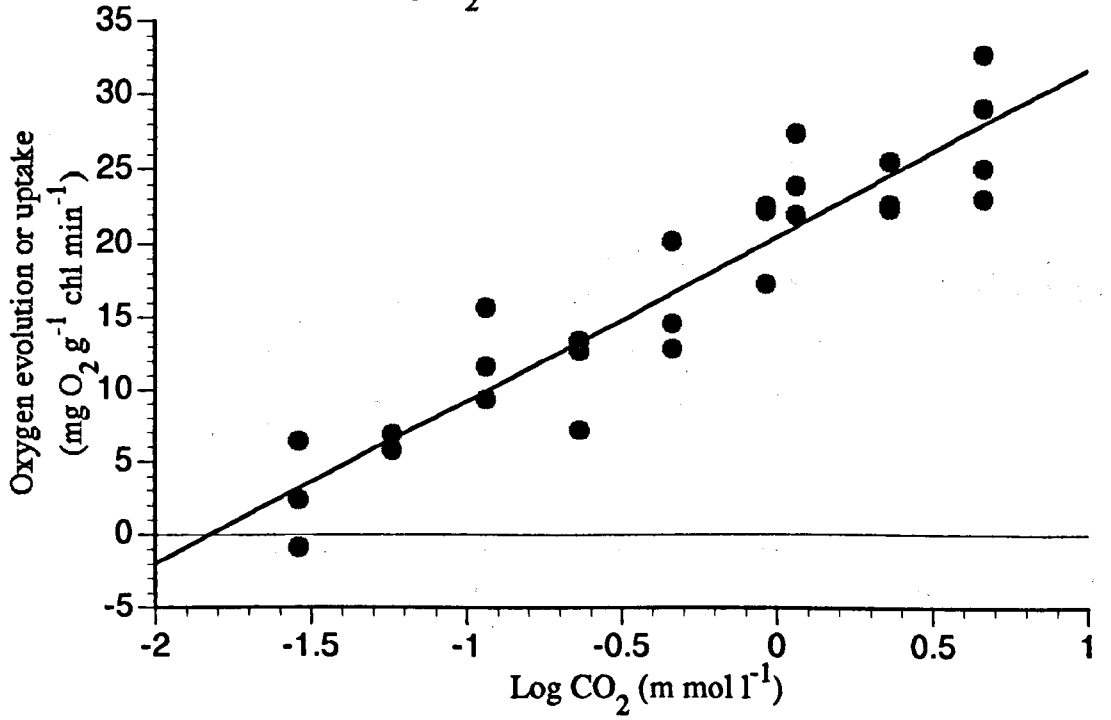


Figure 3.11 Relationship between CO_2^* concentration in the incubation medium and oxygen evolution in the light and uptake in the dark of *E. nuttallii* measured at 10 mg O_2 l^{-1}.



$y = 11.069 \log(x) + 20.761$, $R^2 = 0.882$, $p = 0.0001$ compensation point = 13 μ mol CO_2 l^{-1}

Figure 3.12 Relationship between DIC concentration in the incubation medium and oxygen evolution in the light and uptake in the dark of *E. nuttallii* measured at 2 and 16 mg O_2 l^{-1}.

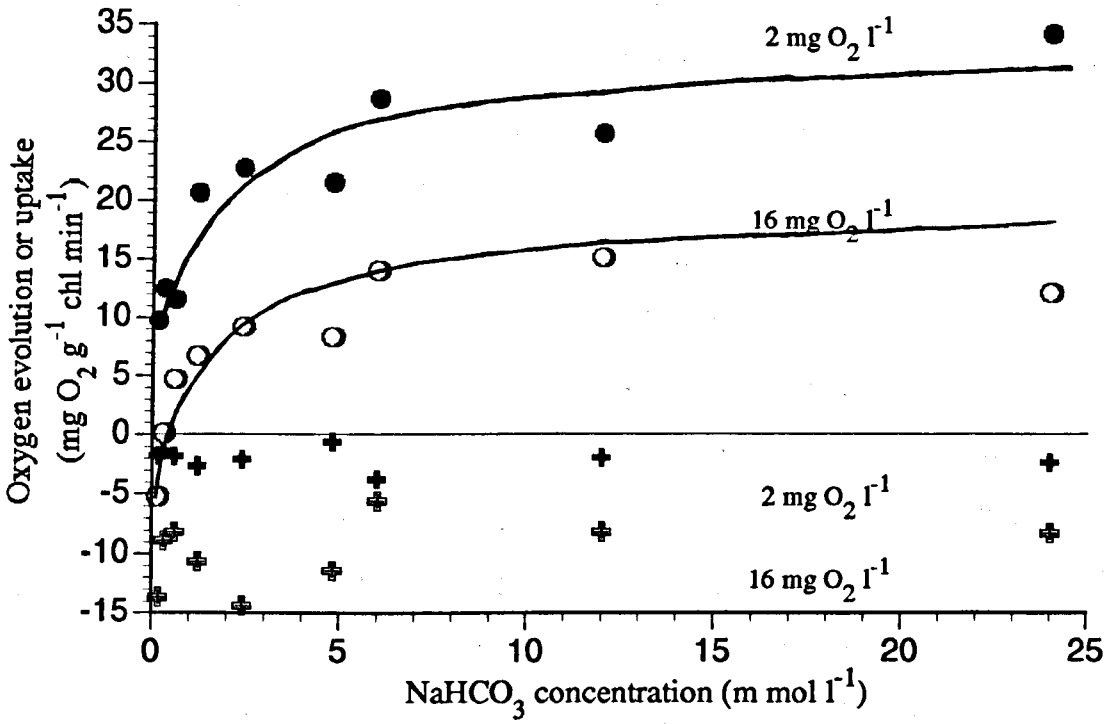
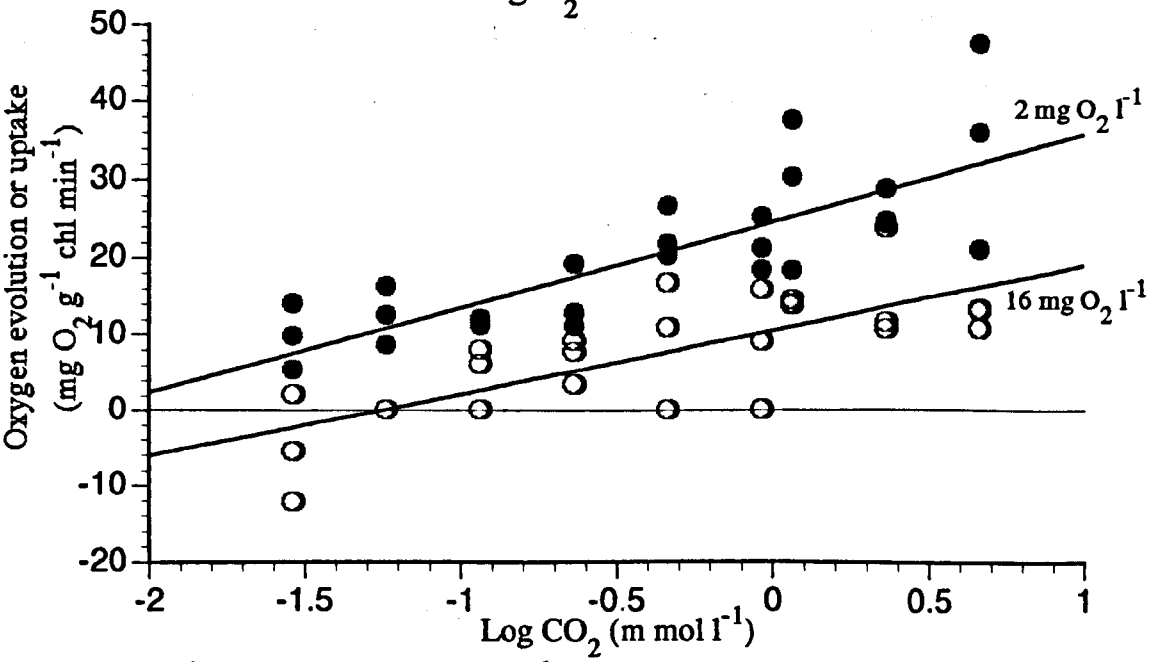


Figure 3.13 Relationship between CO_2^* concentration in the incubation medium and oxygen evolution in the light and uptake in the dark of *E. nuttallii* measured at 2 and 16 mg O_2 l^{-1}.



For 2 mg O_2 l^{-1}, $y = 10.979\log(x) + 24.434$, $R^2 = 0.642$, $p = 0.0001$ compensation point = 6 μ mol CO_2 l^{-1}

For 16 mg O_2 l^{-1}, $y = 8.283\log(x) + 10.457$, $R^2 = 0.576$, $p = 0.0001$ compensation point = 55 μ mol CO_2 l^{-1}

O_2 l^{-1} and 54·6 μ mol l^{-1} at 16 mg O_2 l^{-1}. A shift in compensation point with increased oxygen has been reported for many species of macrophyte and is commonly used as an indicator of active photorespiration (Van *et al.*, 1976; Søndergaard, 1979; Salvucci & Bowes, 1981; Holaday *et al.*, 1983; Bowes &Salvucci, 1989). Having a relatively high CO_2^* compensation point, which was O_2 sensitive, the plants used here must have been in the high-PR state of Bowes (1983).

It was noted that the CO_2^* compensation point and maximum rate of photosynthesis from the previous experiment, where rates were determined for a separate collection of plants at a concentration of 10 mg O_2 l^{-1}, were far closer to those measured for 2 mg O_2 l^{-1} than for 16 mg O_2 l^{-1}. This is rather unexpected since photorespiration increased linearly with oxygen concentration (Figure 3.4). Seasonal changes in CO_2 compensation points have been found previously in plants collected from the field (Bowes *et al.*, 1978 & 1979; Søndergaard, 1979; Maberly & Spence, 1983; Maberly, 1985a; Spencer & Bowes, 1985; Robe & Griffith, 1992). These have been attributed to changes in specific environmental variables (Bowes & Salvucci, 1989), especially stress conditions of low CO_2^*, high temperatures and long photoperiods (Bowes *et al.*, 1978; Holaday & Bowes, 1980; Barko & Smart, 1981; Salvucci & Bowes, 1981 & 1983; Holaday *et al.*, 1983; Spencer & Bowes, 1985), though these conditions often occur simultaneously in the field.

3.4.4 The effect of changes in boundary layer thickness on the physiology of *E. nuttallii.*

A paired t-test showed that photosynthesis was significantly lower under the slower flow (mean = 18·64 mg O_2 g^{-1} chl min^{-1}) than under the higher one (mean = 27·31 mg O_2 g^{-1} chl min^{-1}; t = 4·1685, $t_{0.05(2),9}$ = 2·262). Dark respiration, on the other hand, was not significantly affected by changes in the speed of flow in the reaction chamber (mean high = 9·02 mg O_2 g^{-1} chl min^{-1}, low 6·80 mg O_2 g^{-1} chl min^{-1}; t = 1·1807, $t_{0.05(2),9}$ = 2·262). There are two possible causes for this difference between photosynthesis and respiration. The effect of the increased boundary layer in the low flow treatment on photosynthesis could have been caused by a reduced supply of CO_2^*. From the experiments above it can be seen that CO_2^* only affects respiration at very low concentrations, and an increased boundary layer would only serve to increase the CO_2^* within the leaf in the dark (reduced rate of loss), so no effect would be seen. Alternatively the increased boundary layer could reduce the rate of O_2 loss, causing a build-up within the leaf tissue and hence stimulate increased photorespiration. As respiration responds much less rapidly than photosynthesis to changes in O_2 concentration, the change in boundary layer thickness, and hence reduced O_2 supply in the dark, may have not have been sufficient to produce a significant effect on respiration. Given that CO_2^* has a far greater effect on photosynthesis than O_2 (Sections 3.4.1 & 3.4.3), it is likely that the former explanation is the more likely, but both mechanisms will be operating at the same time. Together with the finding that increased flows stimulate photosynthesis (Westlake, 1967; Smith & Walker, 1980; Søndergaard, 1988) up to moderate levels (Madsen & Søndergaard, 1983), several workers have reported that faster flows increase respiration (Owens & Maris, 1964; Sculthorpe, 1985; Westlake, 1967; Madsen *et al.*, 1993). However in the case of *Ranunculus penicillatus ssp. pseudofluitans* (Syme) S. Webster, this was only significant when O_2 concentrations were low and not when they were near saturation (Westlake, 1967).

Smith and Walker (1980) and Black *et al.* (1983) argued that the response of aquatic macrophytes to CO_2* is largely determined by the resistance to diffusion caused by the unstirred boundary layer and that the concentration required to saturate photosynthesis is controlled by the flow around the plant, and hence the thickness of the boundary layer. Boundary layers surrounding macrophyte leaves in well stirred, laboratory conditions (Raven, 1970; Smith & Walker, 1980; Walker *et al.*, 1980; Madsen, 1984; Walker, 1985) or in the field (Losee & Wetzel, 1993) have been calculated to be hundreds of micrometers thick . Actual measurements in the laboratory (Sand-Jensen *et al.*, 1985; Sand-Jensen & Revsbech, 1987; this thesis Chapter 6) agree with these calculated values and are ample enough to be the major controlling process of photosynthesis (Raven, 1970; Smith & Walker, 1980; Black *et al.*, 1983; Madsen, 1984; Søndergaard, 1988; Madsen & Sand-Jensen, 1991).

3.5 Interactions with Epiphyton.

All the variables investigated were able to influence the physiology of *E. nuttallii* and, if modified by epiphyton, could deleteriously affect the perfomance of the plant. Oxygen will be produced by the epiphyton in the light as a result of photosynthesis, providing that the community is not predominantly heterotrophic. High levels of oxygen have been recorded within layers of epiphyton (Sand-Jensen *et al.*, 1985; Carlton & Wetzel, 1985; Sand-Jensen & Revsbech, 1987), where the concentration was at maximum about 20 mg O_2 l^{-1}. This was recorded in a relatively thick (1·1 mm) layer of epiphyton on *Potamogeton crispus* L. (Sand-Jensen *et al.*, 1985), where the algae and the leaf beneath were both releasing oxygen into the water. Nevertheless the rate of photosynthesis of *E. nuttallii* at such a raised concentration, although reduced, is still about 65% that at the ambient concentration of 10 mg O_2 l^{-1} (Figure 3.4).

As well as increasing O_2, epiphyton photosynthesis will reduce the CO_2^* available to the plant, both through increased pH and through depletion of the total DIC. The former is the more important mechanism in all but the softest waters. The extent to which photosynthesis changes the pH will depend on the buffering capacity, usually the DIC, of the water (Talling, 1985), but in waters of moderate alkalinity, such as the medium used here, photosynthetically driven pH changes in the range 7–9 are common (Talling, 1985; see Chapter 5). A pH change of this extent produced a drastic change in the rate of photosynthesis of *E. nuttallii* (Section 3.4.2) and, if driven by epiphyton photosynthesis, is likely to affect the plant's performance severely. Since this effect is mediated through the effect of pH on the concentration of CO_2^*, the response of *E. nuttallii* to CO_2^* is probably more useful when predicting the effect of increased epiphyton (Section 3.4.3).

However, the shape of the relationship between photosynthesis and CO_2^* concentration is, in part, determined by the thickness of the boundary layer (Raven, 1970; Smith & Walker, 1980; Black *et al.*, 1983; Madsen & Sand-Jensen, 1991). This boundary layer surrounding the leaf is, in turn, increased by the presence of a layer of epiphyton on

the leaf surface. An increased resistance to diffusion will cause the concentration of CO_2^* required to saturate photosynthesis to increase and, at CO_2^* concentrations lower than this value, lead to reduced rates of photosynthesis (Madsen & Sand-Jensen, 1991).

In conclusion, it is likely to be through interference with the supply of CO_2^* to the plant's photosynthetic surfaces that epiphyton exerts a negative influence. It is suggested that although an increased rate of plant photorespiration will occur, its influence will be secondary. The role of light should not be forgotten, especially as epiphyton shading has been shown to have a negative effect on isoetid plants (Sand-Jensen & Søndergaard, 1981; Sand-Jensen & Borum, 1984; Sand-Jensen, 1990). However, these plants do not utilise CO_2^* from the water column and therefore epiphyton can only affect the light climate.

By utilising HCO_3^- in photosynthesis, *E. nuttallii* may, to a large extent, be able to counteract the negative effects of epiphyton on CO_2^* supply. This will be investigated in the following chapter.

Chapter 4.

Bicarbonate Utilisation.

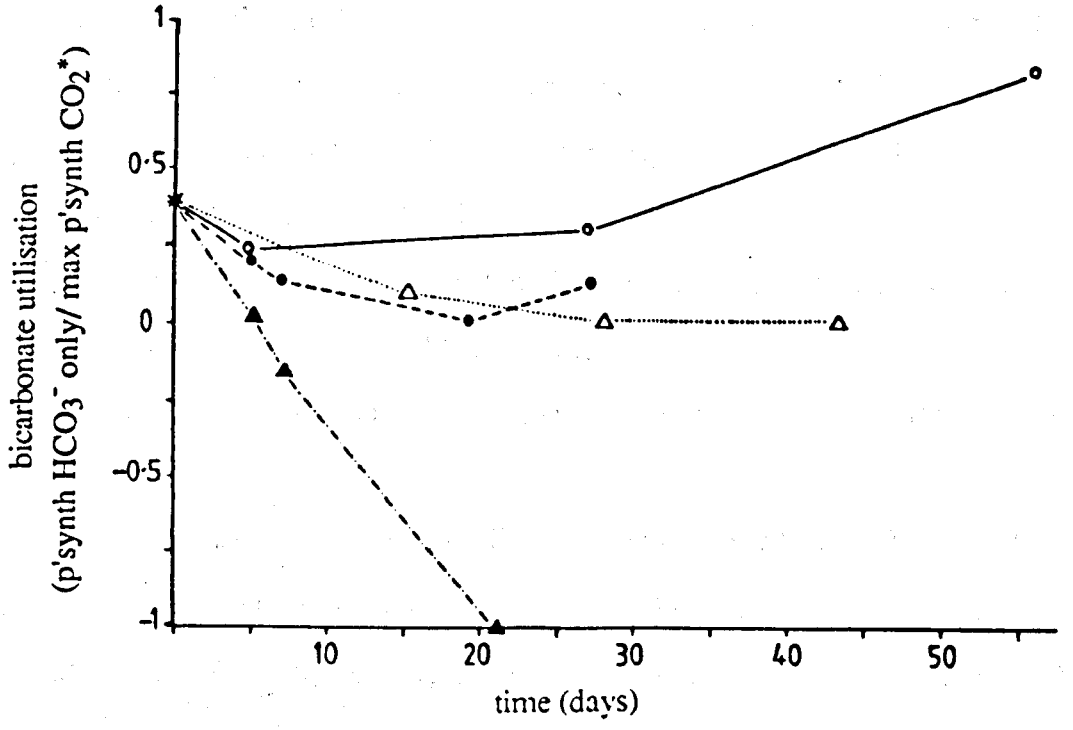
4.1 Introduction.

Dissolved inorganic carbon (DIC) is present in natural waters in different ionic forms, which are freely interconvertible. These consist of CO_2^*, HCO_3^- and CO_3^{2-}, in proportions largely determined by pH, with the equilibrium shifting towards CO_3^{2-} with increasing pH. Many productive waters have pH values in the range 7 to 8, where concentrations of CO_2^* are low and can be limiting as a photosynthetic carbon source. This is exacerbated in still conditions where replenishment is retarded by the slow diffusion of dissolved gases in the aquatic environment (Raven, 1970; Smith & Walker, 1980; Black *et al.*, 1981). Plants growing in such waters often experience adverse conditions as photosynthesis raises oxygen levels and reduces CO_2^* to very low concentrations, severely restricting further photosynthetic assimilation and inducing conditions for increased photorespiration (Hough, 1974; Van *et al.*, 1976; Simpson, *et al.*, 1980; Ondok *et al.*, 1984). There is also the possibility that a nutrient-mediated increase in epiphyton will lead to a further degradation of conditions. These adverse conditions can occur both as part of rapidly changing diurnal cycles (Goulder, 1970; Unni, 1972; Brown *et al.*, 1974; Van *et al.*, 1976; Ondok *et al.*, 1984; Pokorny *et al.*, 1984; Frodge *et al.*, 1990; see Chapter 5) and as much longer-term seasonal changes (Bindloss, 1976; Talling, 1976; Howard *et al.*, 1984; Frodge *et al.*, 1990). Any mechanism which enables a plant to overcome such limiting conditions will confer a competitive advantage, reducing photorespiration and maintaining net positive photosynthesis and growth when other species are under stress. One such mechanism is the ability to use bicarbonate as a photosynthetic carbon source. This ability has been described for many species common in eutrophic and hard waters (Prins *et al.*, 1982b, Maberly & Spence, 1983; Raven *et al.*, 1985; Spence & Maberly, 1985; Madsen & Sand-Jensen, 1991) and has been used to explain the field distribution of some species (Kadono, 1982; Adams, 1985).

Other species of aquatic plant which lack the ability to use bicarbonate, reduce the problem of low CO_2^* supply in the surrounding water by using other sources. Many species have access to atmospheric CO_2 through floating or emergent leaves (Salvucci & Bowes, 1982; Maberly & Spence, 1989; Madsen & Sand Jensen, 1991), others exploit the CO_2^* produced in the sediment either by conducting it from the roots to the leaves through a system of lacunae (Wium-Andersen, 1971; Søndergaard & Sand-Jensen, 1979; Boston *et al.*, 1989), or simply by low stature (Maberly, 1985 a & b). Others grow in flowing waters where replenishment reduces the build up of adverse conditions, and thinner boundary layers surrounding the plant lead to an increased supply of CO_2^* (Pannier, 1960; Sand-Jensen, 1983a; Jenkins & Proctor, 1985; Raven *et al.*, 1987).

To take full advantage of changing conditions, a plant must be able to adapt its physiology accordingly and use HCO_3^- by active uptake only "when necessary", since this process involves energy usage to gain carbon, whilst the uptake of CO_2^* by simple diffusion does not (Raven & Lucas, 1985). Such an adaptive capability has previously been reported for *Elodea canadensis* (Sand-Jensen & Gordon, 1986) and suggested for *Ranunculus peltatus* Schrank (Madsen, unpublished, referred to in Madsen & Maberly, 1991), with the former reporting that an increase of HCO_3^- use took 56 days to develop (though loss of the ability to use HCO_3^- occurred over a much shorter period, see Figure 4.1). This extended time scale of 56 days is only sufficient to allow the plant to adapt to seasonal changes, or to movements between water bodies as a result of vegetative spread. It will not allow phenotypic adaptation to short-term changes in carbon availability, such as those which might arise during periods of hot, calm weather or algal blooms. If *E. nuttallii* can adapt rapidly to such adverse conditions then the adverse effects of epiphyton on the gaseous exchange of the host plant will be greatly reduced. There is also the possibility that epiphyton could force the plant to turn to HCO_3^- as a source of photosynthetic carbon. This work, part of

Figure 4.1 Change in bicarbonate utilisation of *E. canadensis* incubated under four different CO_2 concentrations.
(redrawn from Sand-Jensen & Gordon, 1986)



14.8 µmol l^{-1} CO_2 o——o
37 µmol l^{-1} CO_2 ●– – –●
1,000 µmol l^{-1} CO_2 △· · ·△
2,200 µmol l^{-1} CO_2 ▲—▲

which has been reported elsewhere (Jones *et al.*, 1993), was undertaken to investigate the mechanism by which *E. nuttallii* uses HCO_3^- as a carbon source to determine how it will interact with an increased epiphyton load. This ability could be crucial to the success of this submerged plant in the changeable conditions of lentic eutrophic waters.

In charophytes the deposition of marl (crystalline $CaCO_3$ precipitated at high pH) is generally considered to be a result of the HCO_3^- uptake mechanism and is associated with the alkaline bands involved in the process (Raven *et al.*, 1986). It has been suggested that the formation of marl is more of an integral part of the process than a byproduct (McConnaughey, 1991; McConnaughey & Falk, 1991). For submerged angiosperms it has been suggested variously that:

i) as photosynthesis raises the pH within the water column, marl forms and precipitates on to the leaf surfaces (Moss, 1988);

ii) that marl is a byproduct of epiphyton photosynthesis (Wetzel, 1960; Wetzel, 1983a);

iii) that marl is a consequence of HCO_3^- uptake by a polar leaf mechanism (Raven, 1970), through which the pH at the adaxial surface (facing towards the growing tip) is raised as a result of net OH^- efflux, while the pH at the abaxial surface is lowered by efflux of H^+ (Prins *et al.*, 1982c).

An investigation was made to determine whether marl is deposited on the leaves in a polar fashion, which would indicate that HCO_3^- is taken up by the polar leaf mechanism in *E. nuttallii*.

The rate at which *E. nuttalli* can adapt its carbon uptake characteristics to changing conditions was also investigated, as was the effect of this adaptation on the response of photosynthesis to pH.

Since the utilisation of HCO_3^- is an active process, with energy being spent to gain carbon (Raven & Lucas, 1985), it must involve some cost to the plant. However, a vast reserve of carbon will be open to exploitation by the plant, which may lead to

improved growth. An experiment was undertaken to compare the growth of *E. nuttalli* plants utilising HCO_3^- with that of plants using CO_2^* only.

4.2 Methods.

4.2.1 Mechanism of marl deposition.

Twelve 10 cm long, healthy shoots of *E. nuttallii*, collected in June 1991 from the Leeds and Liverpool Canal, were gently brushed clean of filamentous algae and loosely tied to glass rods using cotton thread. The plants were then arranged randomly, six in normal, vertical orientation and six turned through 180° (ie. grown upside down), in a glass tank with blackened sides, containing approximately 30 l of filtered canal water, (2.4 mmol l^{-1} DIC, pH 8.1, $[Ca^{2+}]$ =1.5 mmol l^{-1}). The rods were supported by insertion into a layer of acid-washed sand. These plants were then grown for 10 days at 15 ± 2 °C, 100 µmol m^{-2} s^{-1} PAR, by which time a substantial layer of marl had developed on the leaves. The water was neither aerated nor mixed during this period. The plants were subsequently examined using a binocular dissecting microscope (Kyowa, Tokyo). Some were also prepared for examination by S.E.M.

4.2.2 Induction of bicarbonate utilisation.

Brown, opaque plastic beakers, height 7.5 cm, diameter 7 cm, were filled with canal sediment and covered with discs of black polythene. Into each beaker five 10 cm long shoots, collected in July 1991 from the canal, were inserted through small slits in the plastic, thus providing the plants with access to natural substrate, but largely isolating the CO_2-producing mud from the water body. Five plants were used to ensure an adequate supply of material for the physiological determinations. Each planted pot was carefully placed in a glass jar containing 2 l of twice-filtered canal water and 24 such jars were incubated at each of the two temperatures, 25 °C and 15 °C, illuminated with 100 µmol m^{-2} s^{-1} PAR in a 16h light 8h dark cycle. The water was aerated by bubbling with air adjusted in one of the following three ways, eight jars per treatment;

i) **low CO_2^*** - air previously passed through soda lime at a rate which reduced the CO_2^* in the water to about half ambient,

ii) **ambient CO_2^*** - untreated air,

iii) **high CO_2^*** - untreated air, with small quantities of dilute hydrochloric acid added each evening, as required, to the water to reduce the pH to about 7·5. The amount of acid added had a negligible effect on the conductivity of the water.

To prevent any contaminants from the air supply (compressor oil, dust, soda lime) reaching the growth vessel it was bubbled through tap water before entering the jars for all these and subsequent treatments.

Jars were randomly removed at different times and the ability of four of the five plants in each to use bicarbonate as a carbon source was determined as below. Conductivity and pH were measured each morning and a water sample was taken from each jar to determine total alkalinity, by titration to pH 4·5 with 0·01 mol l^{-1} HCl, on the day it was sampled. Total DIC and the proportions of its constituent species in the water samples were calculated according to Mackereth, Heron and Talling (1978). Bicarbonate utilisation by eight plants freshly collected from the field was also determined at the start of the experiment, to establish a baseline.

In order to assess bicarbonate utilisation, the photosynthetic rate of leaves was measured as outlined previously (Section 2.2.1), but at 20 °C in modified Forsberg media adjusted to pH 6·5 and pH 9 (unbuffered-pH adjusted to be pH 9 after the addition of $NaHCO_3$) successively, and bicarbonate utilisation was expressed as the ratio of photosynthesis in these two media (9·0:6·5). At pH 6·5, CO_2^* is plentiful, being about 40% total DIC (= 0·98 mmol l^{-1}) and any limited change in pH produces little change in photosynthetic rate (see section 3.2). At pH 9, CO_2^* is only 0.3% total DIC (= 5 µmol l^{-1}) and the plant can therefore only carry out significant photosynthesis if it can utilise HCO_3^-. Use of the ratio removes effects due to variations in absolute rates between plants. To reduce the effects of photorespiration on photosynthesis, all

photosynthetic rates were determined within 1 mg O_2 l^{-1} amplitude change, in solutions containing 9 mg O_2 l^{-1} (100% saturation), being sparged when necessary with either O_2 or N_2 gas to achieve this concentration before measurements began.

To ensure that any effects found were not specific to *E. nuttallii*, a second experiment using *E. nuttallii* and *E. canadensis* collected from the Lancaster Canal in August 1991 and grown at 25 ± 2 °C, was conducted in the same way, but with only two treatment levels, namely reduced CO_2* and ambient CO_2*.

In conjunction with these experiments, plants were collected from the Leeds and Liverpool Canal site, and after preparation, examined by S.E.M., together with plants from the laboratory cultures.

4.2.3 Effect of bicarbonate utilisation on response to pH.

Cleaned 10 cm long shoots of *E. nuttallii* and *E. canadensis* collected from the Lancaster Canal site, and *Lagarosiphon major* (Moss) Ridley collected from the Leeds and Liverpool Canal site in April 1992 were placed in covered, sediment-filled, brown plastic beakers as outlined above and grown in glass jars containing 2 l of filtered canal water for 21 days at 15 ± 2 °C, illuminated with 100 µmol m^{-2} s^{-1} PAR in a 16h light 8h dark cycle. Each jar contained five shoots of one species and was bubbled with either untreated air or air that had been passed over soda lime to reduce the CO_2, giving two treatment levels, namely reduced CO_2* and ambient CO_2*. The treatments were replicated six times per species, divided evenly between three blocks. At the end of the growth period, the activity of four out of the five plants in each jar was determined in weakly buffered Forsberg medium adjusted to one of a series of pH levels from pH 6 to pH 10 (0·5 mmol l^{-1} Tris-HCl for pH7 to pH10, 0·5 mmol l^{-1} NaCitrate-NaH_2PO_4 for pH 6), attributed randomly to each plant. Due to the large number of determinations that had to be made, the plants were harvested blockwise, one per day over three days.

4.2.4 Metabolic cost of bicarbonate utilisation.

Six cleaned, 10 cm long shoots of *E. nuttallii* collected from the Leeds and Liverpool Canal were planted into covered, sediment-filled, brown plastic beakers as outlined above, and grown in glass jars each containing 2 l of filtered canal water at 20 ± 1 °C, illuminated with 100 μmol m^{-2} s^{-1} PAR in a 16h light 8h dark cycle. Each of the jars was bubbled with either untreated air or air that had passed through soda lime to reduce the CO_2 content, with the treatments arranged in a 4 x 4 Latin square, giving a total of eight replicate jars per treatment.

At the start of the experiment the ability of eight randomly selected *E. nuttallii* plants from the stock collection to utilise bicarbonate was assessed by measuring photosynthesis at pH 6·5 and pH 9 as above. A further twenty shoots were cut to 10 cm length and used to measure blotted wet weight and, after drying to constant mass at 60 °C, dry weight.

At intervals during the growing period, the pH of the water within all the jars was measured using a field pH meter (CD66, WPA Scientific Instruments, Saffron Walden, Essex). At the same time approximately 100 cm^3 of water was removed using a narrow-necked, screw-top bottle from three randomly selected jars per treatment, and the concentrations of CO_2^* and total DIC measured by Gran titration with 0·1 mol l^{-1} HCl, according to Mackereth, Heron & Talling (1978). To compensate for this removal, and for evaporation, the jars were regularly topped up with twice-filtered canal water. On day three a small quantity of 0·1 mol l^{-1} HCl was added to the jars bubbled with unadjusted air in order to bring the water to below pH 8, increasing the concentration of CO_2^* and reducing the total DIC.

After 21 days one plant was removed from each replicate jar and its ability to utilise bicarbonate assessed as above. The following day the remaining five plants in each jar were harvested. Each plant was carefully brushed clean of epiphyton with a soft paint brush and the following parameters measured: length of the main shoot, total

length of the main shoot and all the side branches, number of side branches, blotted wet weight and, after drying to constant mass at 60 °C, dry weight.

4.3 Results and Discussion.

4.3.1 Mechanism of marl deposition.

A Mann-Whitney U test showed that inversion of plants had no significant effect on the pattern of marl deposition ($U' = 25.5$, $U_{0.05,6,6} = 31$, Table 4.1), with all leaves examined having marl deposits on the adaxial surface, irrespective of their orientation during the experiment. This consistent development of marl on adaxial rather than abaxial leaf surfaces of the inverted plants is evidence that its production is not simply a general precipitation from the water above the leaves. Neither is it a product of epiphyton photosynthesis, since SEM examination of leaves from the inverted plants showed heavy epiphytic development on abaxial surfaces, with no marl, whereas marl on the adaxial surfaces had no epiphytes amongst it (Plate 6). Where epiphyton developed it was invariably found on the upward facing surface of the leaf. Instead it confirms that marl is produced by the leaves in a polar fashion and that this polarity is retained after inversion. In the few cases where marl was found on the abaxial surface, in both normally orientated and inverted plants, its presence was likely to have resulted from dislodgement from the upper surface of the next leaf during handling, or by interaction between the leaves where they are positioned very close together.

These findings do not support the acid-alkali banding theory of HCO_3^- uptake suggested for *E. nuttallii* by Eighmy and co-workers (Eighmy *et al.*, 1987; Fagerberg *et al.*, 1991), though no investigation was made into whether HCO_3^- uptake is an energy-requiring process utilising a proton pump mechanism, as they concluded (Eighmy *et al.*, 1991). Rather the results indicate that marl is produced in a polar fashion by *E. nuttallii*, as a result of the differentiated physiology of the leaf, a finding consistent with the polar leaf mechanism of HCO_3^- utilisation proposed by Prins *et al.* (1982a & c).

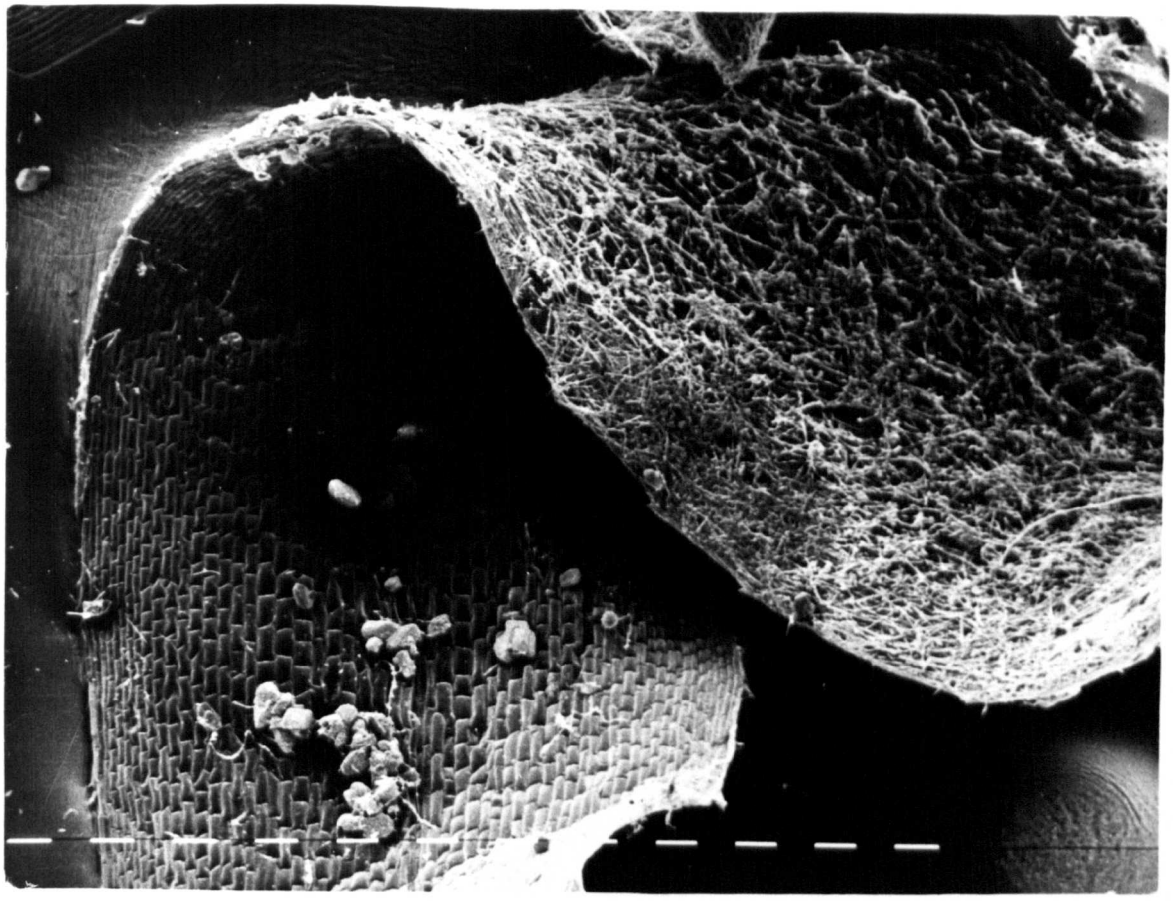
Table 4.1. Effect of orientation on the distribution of marl deposits on the leaves of *E. nuttallii.*

Orientation of shoots	No. leaves counted	No. leaves marled on abaxial surface	(%)
normal	32	3	(9.4)
	45	5	(11.1)
	14	3	(21.4)
	31	2	(6.5)
	20	2	(10.0)
	47	0	(0.0)
inverted	68	3	(4.4)
	69	2	(2.9)
	60	8	(13.3)
	38	1	(2.6)
	22	0	(0.0)
	56	5	(8.9)

The Mann-Whitney U Test showed there was no difference ($p = 0{\cdot}05$) between the pattern of marl deposition on the abaxial surface of normal and inverted plants. Results: $U' = 25{\cdot}5$, $U_{0.05,6,6} = 31$. All leaves examined had marl deposits on the adaxial surface.

Plate 6 A leaf from an inverted *E. nuttallii* shoot, with marl on the downward facing adaxial leaf surface and epiphyton on the upward facing abaxial leaf surface. Scale bar = 100 μm. 15 KV

Plate 7 Another leaf from an inverted *E. nuttallii* shoot with deposits of marl on its adaxial leaf surface. Note the absence of marl from above the leaf mid-rib. Scale bar = 100 μm.



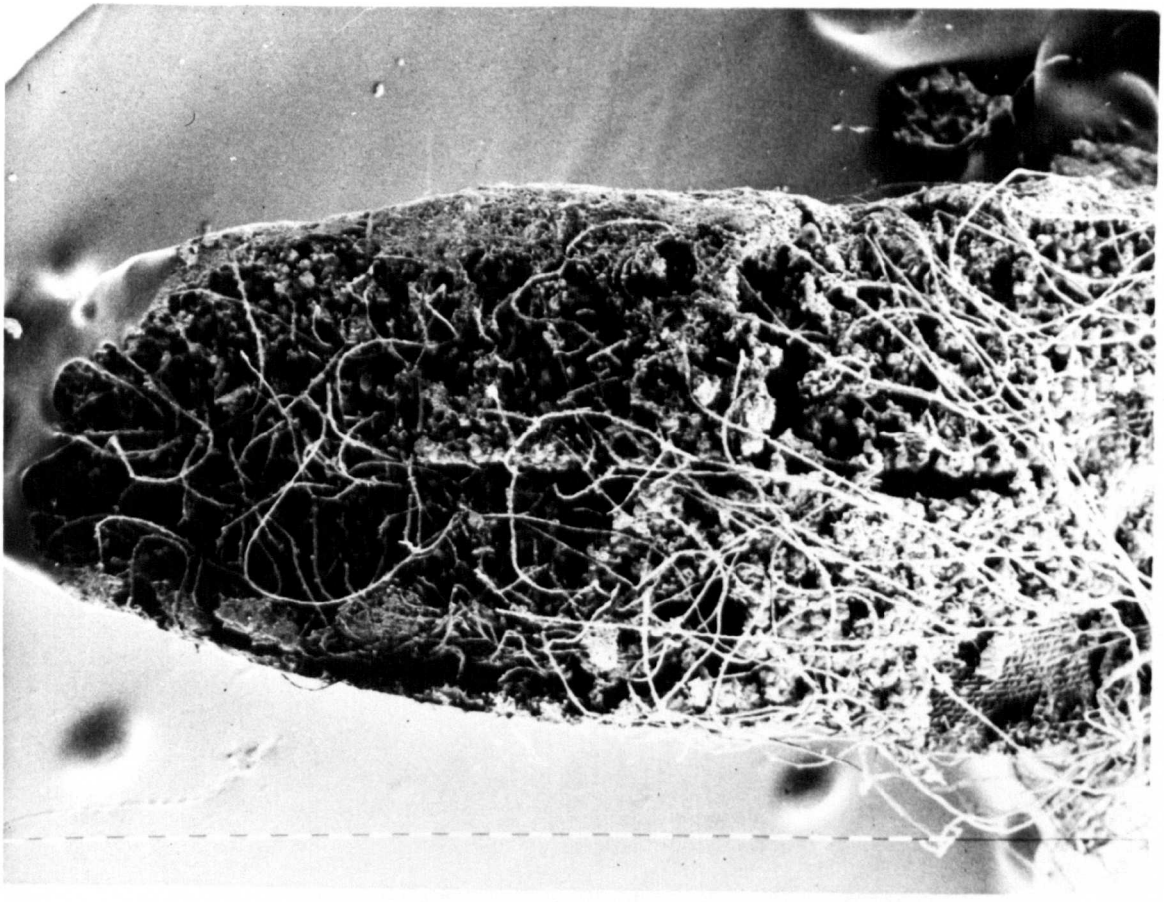
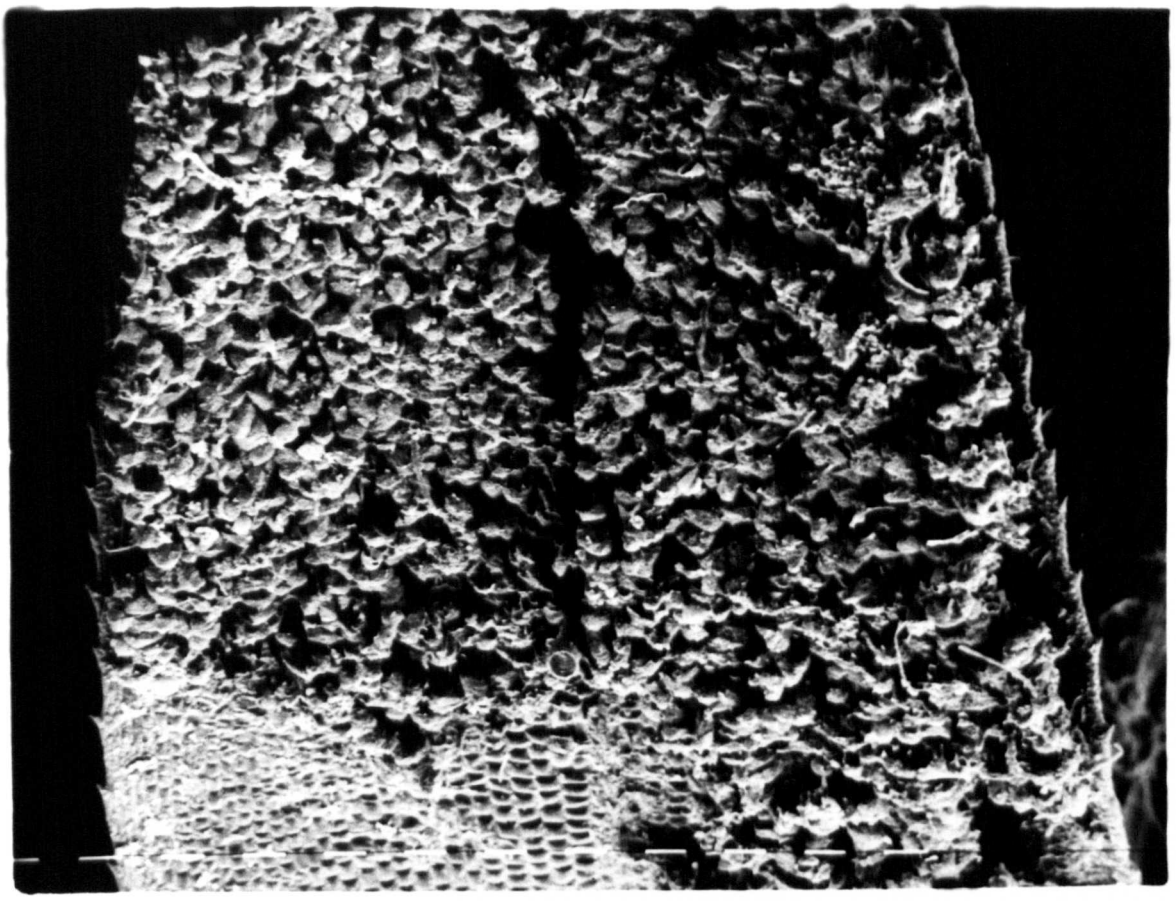


Plate 8 An *E. canadensis* leaf from laboratory culture showing heavy marl deposits; note again absence of marl from above the leaf mid-rib. Scale bar = 100 μm.

Plate 9 An *E. nuttallii* leaf from the field showing marl deposits over the adaxial leaf surface but with the leaf mid-rib marl free. Scale bar = 100 μm.



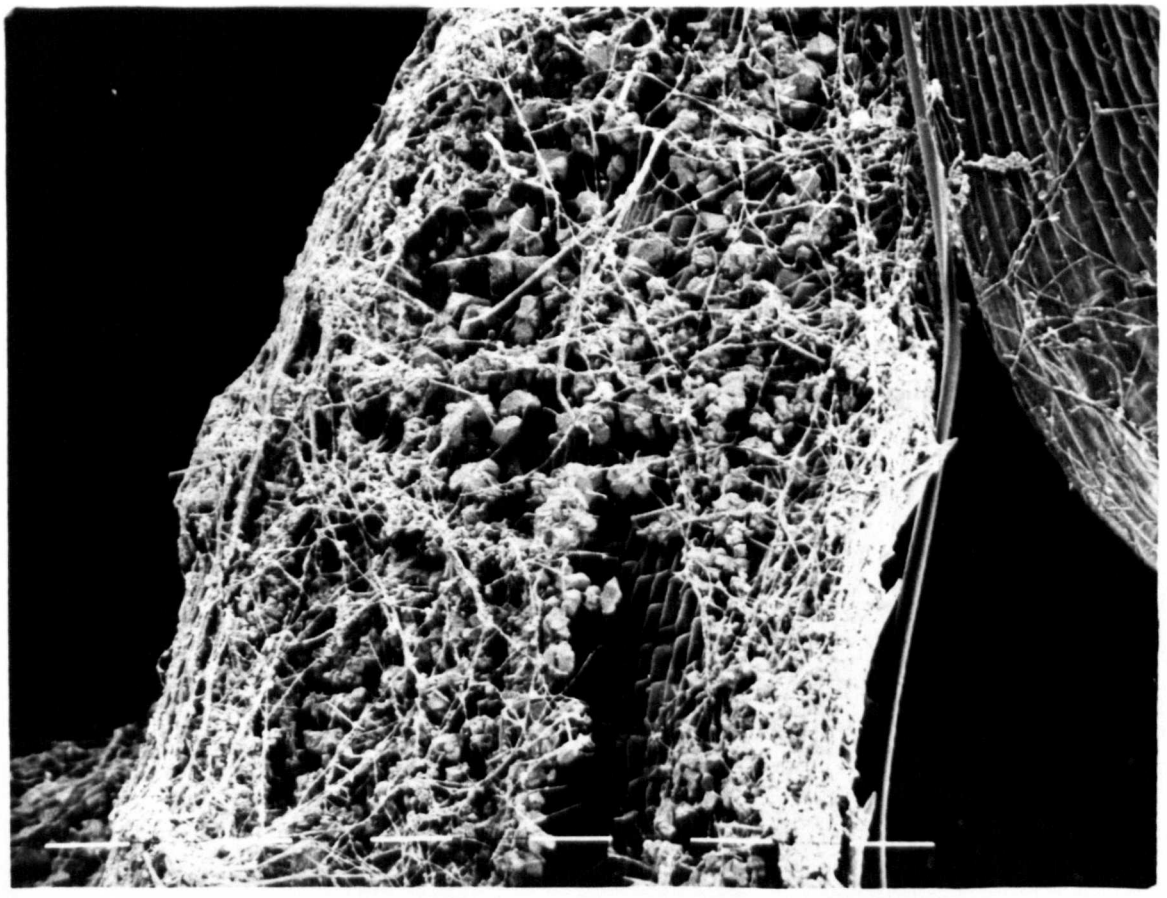
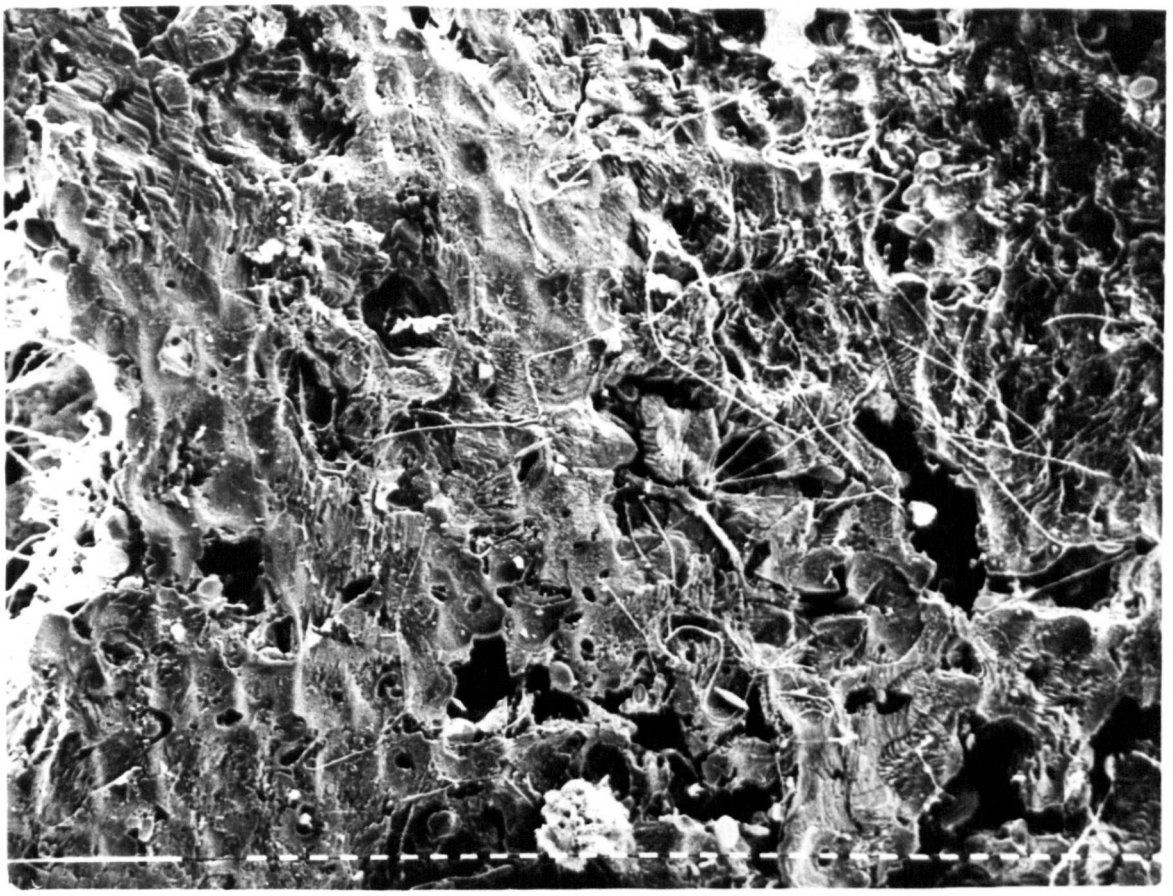


Plate 10 The underside of a layer of marl dislodged from an *E. nuttallii* leaf showing an impression of the leaf surface. Note the completeness of the marl layer and the extremely close association that must exist between the leaf and marl for such an impression to be made. Scale bar = 10 μm.

Plate 11 Early stage of marl development on an *E. nuttallii* leaf showing that marl deposits to some extent follow the pattern of the underlying cells, growing over the cell surface but lacking from above the vertical cell walls (most obvious towards the top right-hand corner). Scale bar = 10 μm.



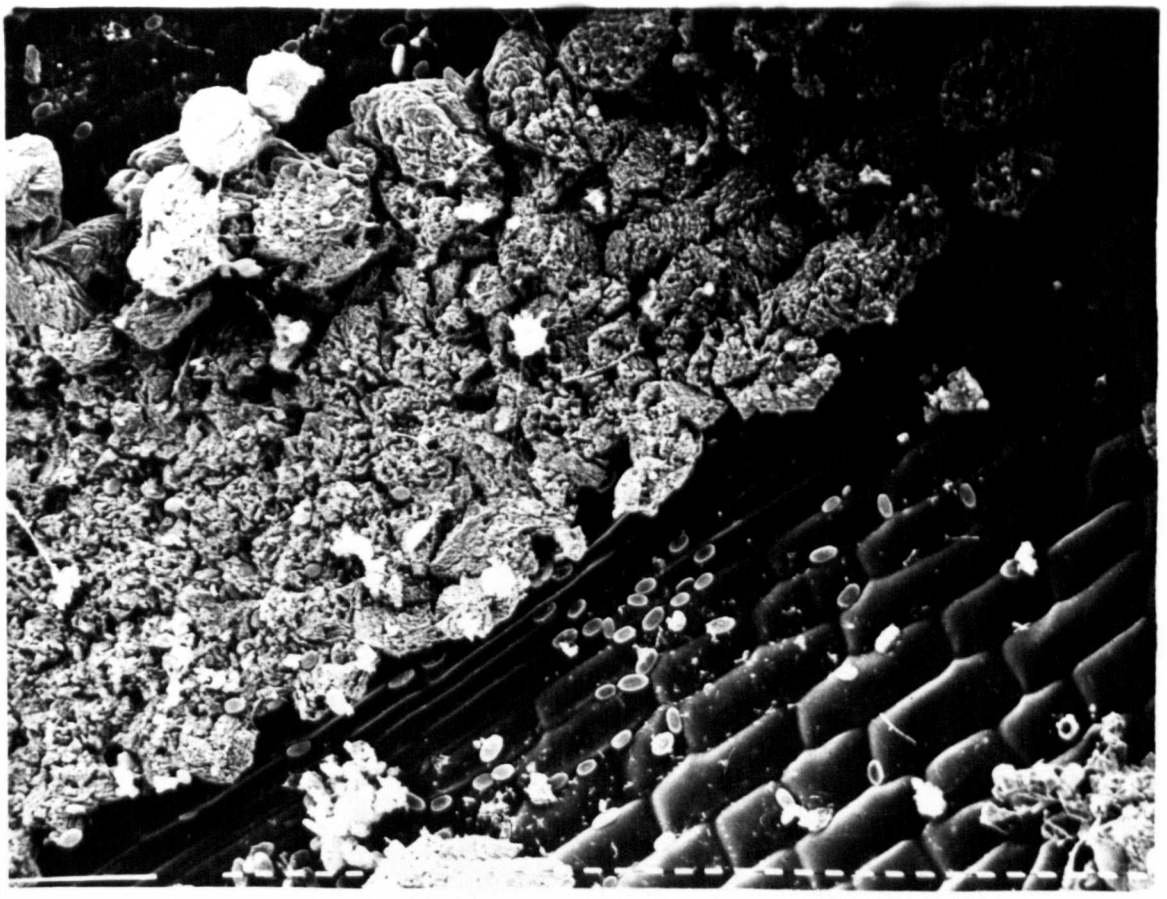
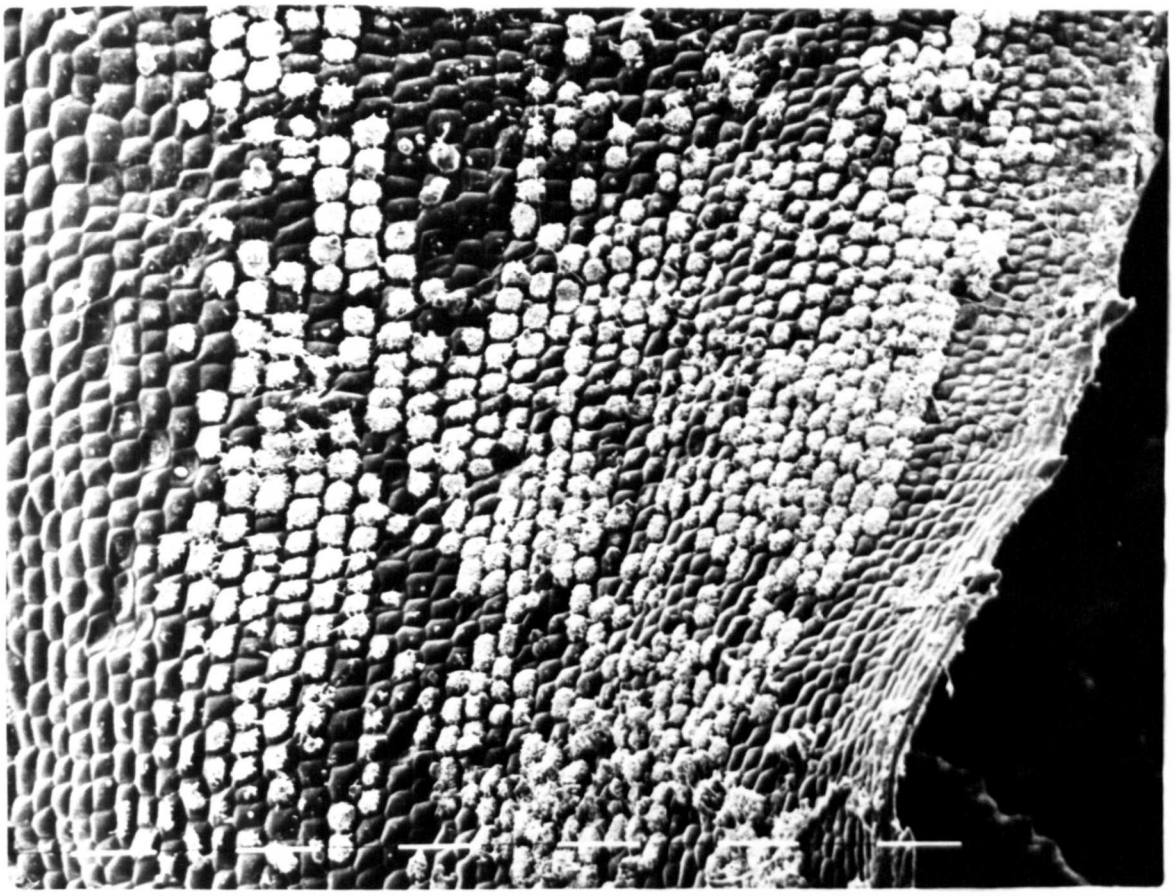


Plate 12 The early stages of marl deposition on the adaxial leaf surface of field collected *L. major* showing a very close relationship between the marl deposits and the underlying cells. Scale bar = 100 μm.

Plate 13 The early stages of marl deposition on the adaxial leaf surface of field collected *L. major* again showing a very close relationship between the marl deposits and the underlying cells. Scale bar = 10 μm.



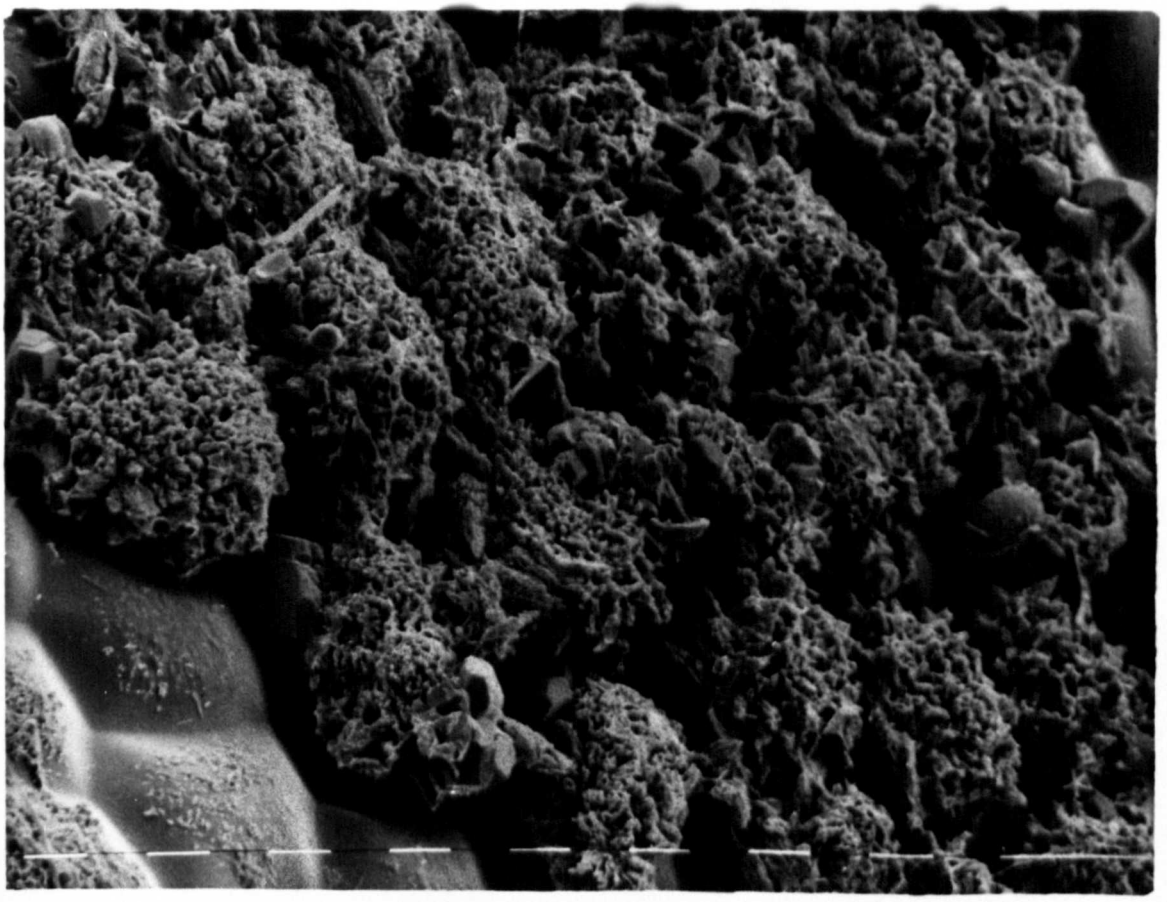
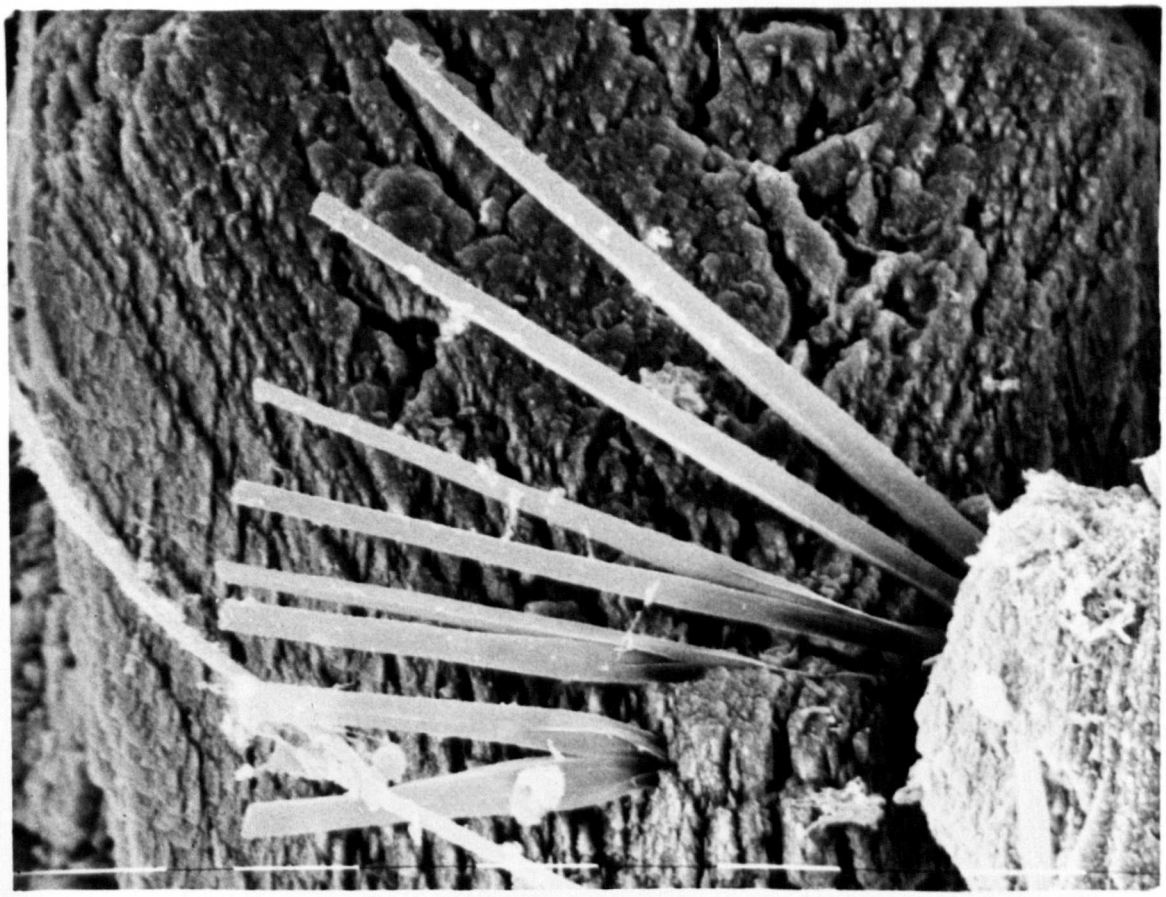


Plate 14 The early stages of marl deposition on the adaxial leaf surface of laboratory cultured *L. major* showing a very close relationship between the marl deposits and the underlying cells. The area above the vertical walls of the leaf cells is free of marl. Scale bar = 10 μm.

Plate 15 Diatoms trapped and deformed by a developing marl crystal. Scale bar = 10 μm.



The detailed arrangement of the marl crystals on the leaf surface, closely associated with the photosynthesising cells of the leaf blade, but absent from the transport cells of the midrib and the single row of cells which forms the edges of the leaf (Plates 7, 8 & 9), is further circumstantial evidence that leaf photosynthesis is the cause of marl accumulation. In *Lagarosiphon major* (Ridley) Moss (collected from the field) and to some extent in *E. nuttallii* and *E. canadensis*, this patterning is even more exact, the marl being positioned in the centre of each cell and absent (at least initially) from the area above the vertical walls (Plates 11, 12, 13 & 14). This patterning is consistent with a locally high pH, resulting from the active parts of those cells involved in photosynthesis extruding OH^- ions into the water, causing the precipitate. The difference between *Lagarosiphon* and *Elodea* could be due to the former, a much more vigorous and active plant (Howard-Williams, 1993), extruding OH^- ions more rapidly than the latter. Such detailed spatial heterogeneity is consistent with the $CaCO_3$ being laid down predominantly adjacent to the cell surface even in the presence of a thick layer of marl, as suggested for charophytes (McConnaughey & Falk, 1991). This could be the result of cations diffusing through the leaf from below, either via the vertical cell walls or the protoplasm (Lucas, 1983; Smith, 1985; Prins & Helder, 1985; McConnaughey & Falk, 1991; McConnaughey, 1991); CO_2 diffusing from the cells (McConnaughey & Falk 1991, McConnaughey 1991); OH^- pumping from the cell (Prins *et al.* 1982b & c); or a combination of these mechanisms.

The SEM investigation showed that epiphytic algae grew on the upward facing leaf surface, regardless of whether this was the ab- or adaxial surface (Plate 6). This may be due to proximity to light, or more simply the tendency of propagules to settle out of the water column on to such surfaces. A poorly developed layer of epiphyton on the lower leaf surface is in agreement with the findings of Bell (1976), Birch (1990) and Patterson & Wright (1986). Since carbon is taken up through the abaxial surface of polar leaves the effect of epiphyton (growing on the adaxial surface) on gaseous

exchange will be negligible, but shading of the plant's photosynthetic surfaces will occur.

These results do not preclude the formation of marl by the photosynthetic activity of epiphyton in the absence of a polar leaf, as was found on the petioles of *Nymphaea* and *Nuphar* and on other species (*Scirpus, Decodon* and *Pontederia*) which had access to aerial CO_2 (Wetzel, 1960). Nor does it deny the possibility that marl may precipitate from the water column onto the plants and bed below when such phenomena as lake-whitening occur (Goldman & Horne, 1983). It is here stated only that when growing in an alkaline, calcium-rich water-body, a plant taking up HCO_3^- by the polar-leaf mechanism will cause a localised pH increase greater than that caused by photosynthesis alone. This will cause $CaCO_3$ to precipitate (together with $MgCO_3$) on the adaxial leaf surface in a predictable pattern, before the extreme conditions are reached under which general physicochemical precipitation of marl occurs (even though they may be of biological origin).

Super-saturation with $CaCO_3$ is a situation often found in natural waters (Howard *et al.*, 1984; Duston, 1986), but need not necessarily lead to precipitation as crystal poisons (eg. inorganic phosphate, humic acid and other organic materials) can inhibit marl formation (Simkiss, 1964; Howard *et al.*, 1984). Further, a seed particle may be required to initiate crystal formation (Howard *et al.*, 1984). Therefore, marl formation may be a relatively slow process, deposits taking some time to build up on the surface of a leaf. This will leave the oldest, and hence least active leaves, with the thickest deposits. This may explain the finding of Wetzel (1960) that marl increased with depth and (since he used sub-samples from individual *Potamogeton gramineus* L. shoots) plant length, and leaf age.

The amount of marl precipitated during HCO_3^- utilisation can be huge, forming a very large proportion of the total dry weight of uncleaned plants (Wetzel, 1960; Kelly & Ehlmann, 1979), often over 50% (Pentecost, 1984; Blindow, 1992a), and is,

therefore, an important component of sediments (deposition rate at Malham Tarn = 0·28 kg m^{-2} a^{-1} – Pentecost, 1984; see also eg. Moss, 1988; Wetzel, 1970; Dudston *et al*, 1986). Its presence can be used to give some information of previous activity within a basin, ie. that communities dominated by certain plant groups were present and, as marl deposits are often not uniformly distributed (Wetzel, 1970; Dudston *et al.*, 1986), where plant beds were in the lake basin.

Whilst marl is being precipitated, the Ca^{2+} concentration of the water can be severely affected, both near to the plants and lake-wide, for example Malham Tarn where *Chara vulgaris* during the growing season causes Ca^{2+} in the open water to be reduced to about 66% of its winter level (Pentecost, 1984).

The presence of marl causes adsorption of PO_4 (Kitano *et al.*, 1978) and/or precipitation (due to the high pH and Ca^{2+}), typically as apatite or carbonato-apatite (Stumm & Morgan, 1981). These processes will not only occur within the marl deposits on the leaves (McConnaughey & Falk, 1991), but also in the sediment, leading to reduced release of PO_4 even under conditions of anoxia (Boström & Pettersson, 1982; Löfgren & Ryding, 1985a & b). This is of advantage to the plants in that PO_4 in the water column is reduced keeping phytoplankton populations low. The plants, having access to sediment nutrients through their roots, are not as disadvantaged (Denny, 1972; Carignan & Kalff, 1980; Raven, 1981; Moeller *et al.*, 1988). Further, if conditions in the centre of the weed-bed do lead to PO_4 release from the sediment, the PO_4 will tend to be retained within the bed because of adsorption/precipitation within the marl on the surrounding leaves.

There are other possible advantages for a plant to have a seemingly cumbersome layer of marl over its photosynthetic surfaces. Firstly, calcium carbonate absorbs light with very little selectivity over the photosynthetically active wavelengths and has a much lower absorbance than epiphytic algae (Losee & Wetzel, 1983). These authors suggest that the crystals could provide a conduit for light across the epiphytic layer.

However the presence of a marl layer may also inhibit the growth of epiphytes due to the associated high pH, or physically, by crystals growing over the algae (Plate 15). The high pH could also shift the composition of the epiphyton to a community of less light absorptive blue-greens (Losee & Wetzel, 1983), since these algae are reported to be favoured by high pH (Olofsson, 1980; Shapiro, 1990a). Also, as it is presumed that a plant taking up HCO_3^- is under a climate of excess light (Sand-Jensen & Gordon, 1986; Madsen & Maberly, 1991), a layer of calcium carbonate will reduce the intensity of incident light and hence reduce to some extent the demand for photosynthetic substrate, when carbon is limiting.

In charophytes the deposits may act to stabilise the position of the alkaline bands (Smith, 1985), but this does not appear necessary for plants using the polar-leaf mechanism, as the alkaline region is apparently always on the upper surface (Prins *et al.*, 1982a & b; Elzenga & Prins, 1987).

Finally, the layer of marl is far less well attached than an equivalent layer of epiphyton and is easily shed when a leaf is disturbed, removing both marl and epiphytes and leaving the leaf very clear.

Alternatively, the marl may be of no advantage to the plant whatsoever, and even a hindrance, but the gains made by being able to tap HCO_3^- as a source of photosynthetic carbon, allowing photosynthesis to continue under conditions where it would otherwise have been arrested, far outweigh the losses caused by the presence of the precipitate.

4.3.2 Induction of bicarbonate utilisation.

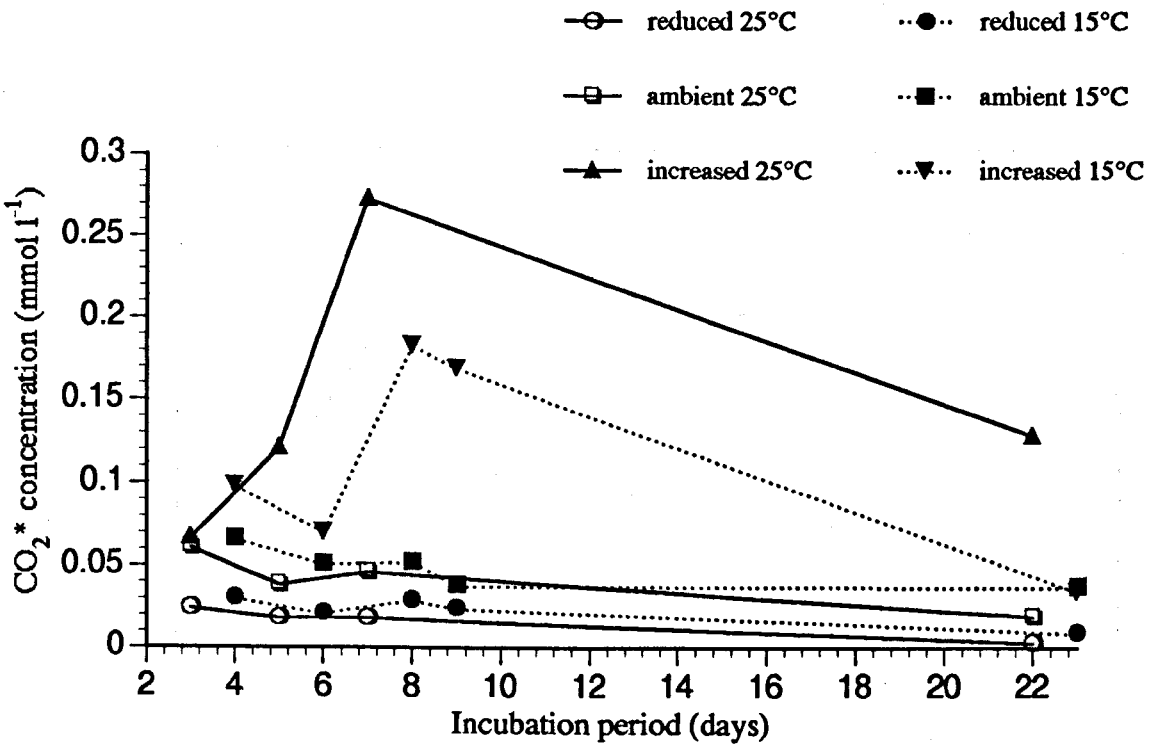
The method of manipulating the CO_2^* concentration of the water within the jars, although unsophisticated, did produce the three required levels, roughly described as reduced, ambient and increased (Figure 4.2a). These concentrations did not remain constant throughout the growing period, since a reduction of total DIC generally occurred (Figure 4.2b) as carbon was taken up by the plants, precipitated as carbonate, or absorbed by the air passing through the water. The mean values of pH taken daily over the whole growing period, together with the mean DIC concentrations are shown in Table 4.2. In order to simulate field conditions, a "natural" water was used and not a laboratory-prepared medium, so less precise control was possible over the levels than is customary in physiological investigations. However, the manipulations did produce marked differences in the physiology of the plants.

The rate of initiation of HCO_3^- utilisation, measured as the ratio of photosynthesis at pH 9 to that at pH 6·5, remained low in plants grown under both ambient and increased CO_2^* conditions, but increased in those grown under reduced CO_2^* conditions (Figure 4.3). A statistically significant increase in HCO_3^- use was detectable after only five days at 25 °C, mean $[CO_2^*] = 16\ \mu mol\ l^{-1}$, and eight days at 15 °C, mean $[CO_2^*] = 23\ \mu mol\ l^{-1}$. The least significant difference used is the standard error of the mean from an analysis of variance of the data (for 25 °C, se = 0·05 and for 15 °C, se = 0·085). The pH range over which this switch occurred was very small, the difference between the low and ambient treatments being about half a pH unit (Table 4.2).

A further, similar experiment using both *E. nuttallii* and *E. canadensis* collected from the Lancaster Canal, (total alkalinity = 0·82 mequil l^{-1}, field pH 8·26, equilibrium pH 7·63) showed that bicarbonate utilisation increased very rapidly in both species (Figure 4.4), becoming statistically significant in both species after only 2 days of incubation (for *E. nuttallii* se = 0·052 and for *E. canadensis* se = 0·039). The

Figure 4.2 Concentration of carbon species in the experimental vessels containing *E. nuttallii* from the Leeds and Liverpool Canal under different conditions of CO_2 and temperature.

a) CO_2* Concentration



b) Total dissolved inorganic carbon

Legend as above

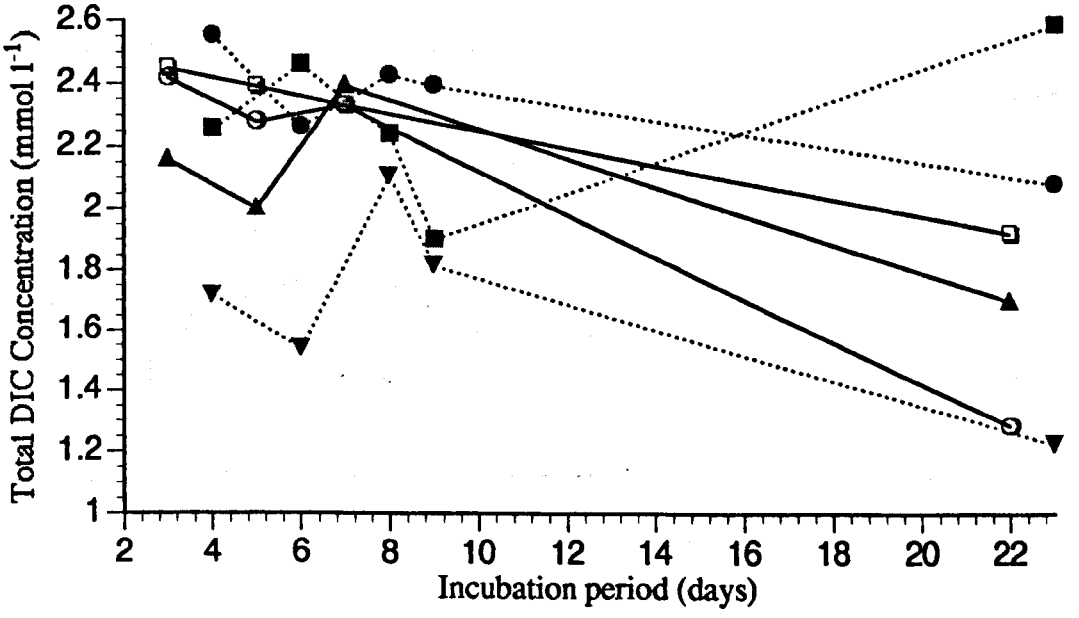
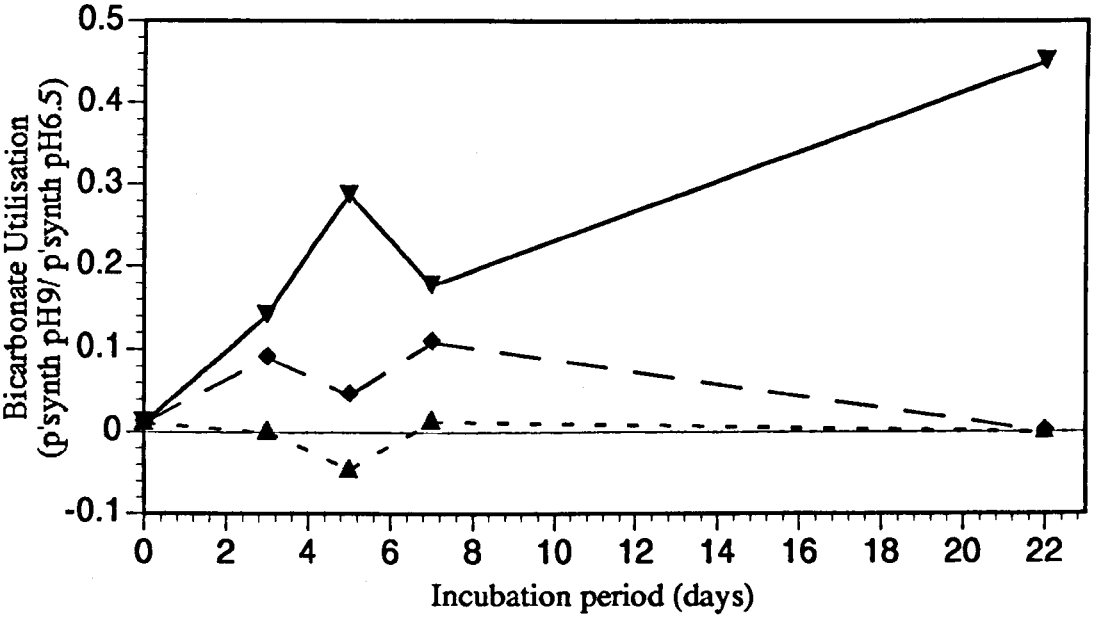


Figure 4.3 Changes in bicarbonate utilisation of *E. nuttallii* collected from the Leeds and Liverpool Canal when grown under different CO_2* conditions, at two temperatures.

Low CO_2* ▼ , Ambient CO_2* ◆ , Increased CO_2* ▲ .
SE from Anova, at 25 °C = 0.05, at 15 °C = 0.085.

a) Incubated at 25 °C



b) Incubated at 15 °C

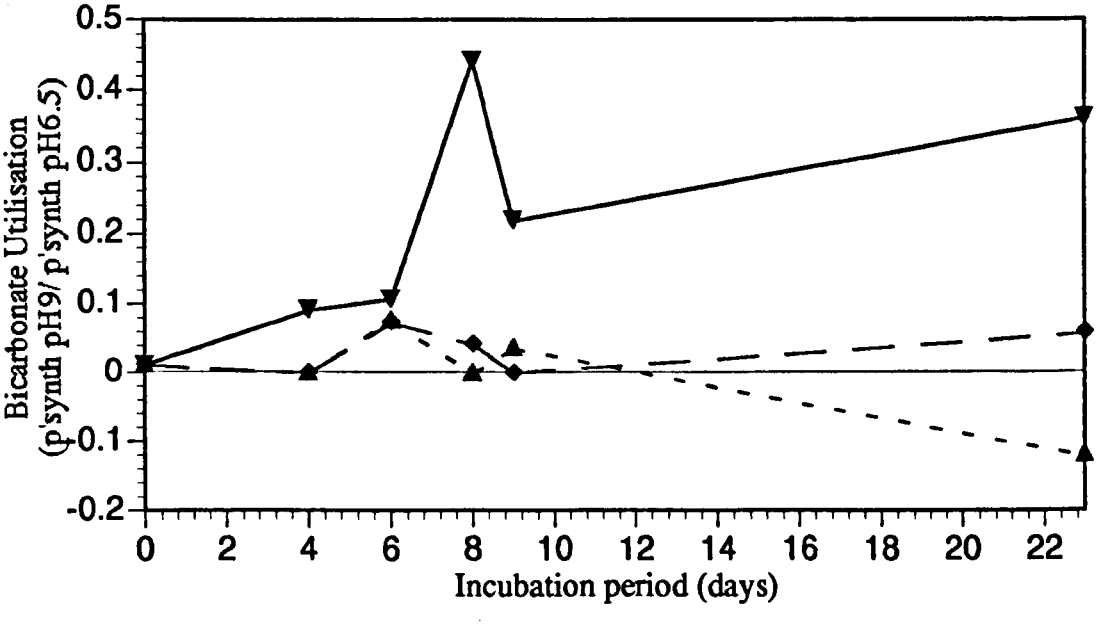


Table 4.2. Concentrations of dissolved inorganic carbon and CO_2* (means of six measurements taken on the day the vessel was sampled), and pH (means from measurements taken daily during the experiment, n>100) during the experiment. Also shown are mean values for the Leeds and Liverpool Canal (Saednia 1980).

Temperature (°C)	Treatment	CO_2* (µmol l^{-1})	DIC (mmol l^{-1})	pH
25	reduced	16	2·08	8·42
	ambient	39	2·27	7·90
	increased	147	2·06	7·42
15	reduced	23	2·35	8·38
	ambient	49	2·29	7·87
	increased	111	1·69	7·40
	L & L Canal	44·6	2·40	8·10

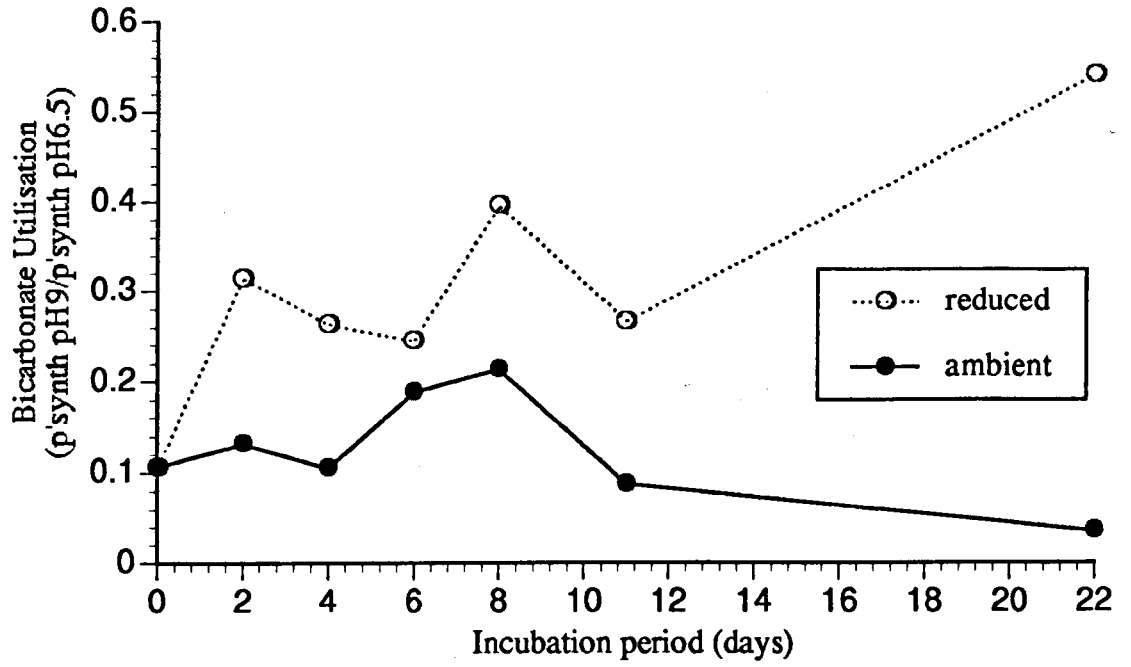
concentrations of CO_2^* and DIC in this experiment showed similar trends to those in the previous one (Figure 4.5 *cf.* Figure 4.2).

When compared with the 56 days reported by Sand-Jensen and Gordon (1986) for an increase in bicarbonate use (Figure 4.1), the rates reported here are rapid, and are clearly sufficient to allow plants to adapt to short term changes in the water body. The experiment using both *E. nuttallii* and *E. canadensis* taken from another site showed that faster induction found here was not due to a specific or a clonal difference, but was likely to be the result of a difference in the physiological states of the plants at the start of the experiments. At the time of collection, all the plants used in the present experiments had very little affinity for HCO_3^-, whereas the Danish ones were already utilising HCO_3^- at a substantial rate and any further increase above this could take a considerable time to develop. The concentration of CO_2^* in the "reduced" treatments used here (Table 4.2, Figures 4.2 & 4.5) were similar to that used by Sand-Jensen and Gordon (14·8 μmol l^{-1}) to produce an increase in HCO_3^- use, and it is likely to be this low CO_2^* concentration, rather than the pH of the water, which triggers the switch to HCO_3^- utilisation in both cases.

Together with between-waterbody variations in the ability of plants to use HCO_3^- (Maberly & Spence, 1983; Sand-Jensen & Gordon, 1986; Madsen & Sand-Jensen, 1987), seasonal changes within a waterbody have been reported, with plants collected from the field having a tendency to show increased affinity in summer (Maberly & Spence, 1983; Sand-Jensen & Gordon 1986). This may be the result of periods when productivity and temperature are high and CO_2^* correspondingly low (eg. June-July for *Chara hispida* in Wellbank quarry, Andrews *et al.*, 1984b). These conditions are often associated with periods of low water movement and high light intensity, which accentuate the problem of carbon supply. However, it has been noted that HCO_3^- uptake is unlikely to occur under conditions of light limitation (Sand-Jensen & Gordon, 1986; Madsen & Maberly, 1991), and as low light conditions are

Figure 4.4 Changes in bicarbonate utilisation of plants collected from the Lancaster Canal when grown under different conditions.

a) *E. nuttallii.* (se = 0.052)



b) *E. canadensis.* (se = 0.039)

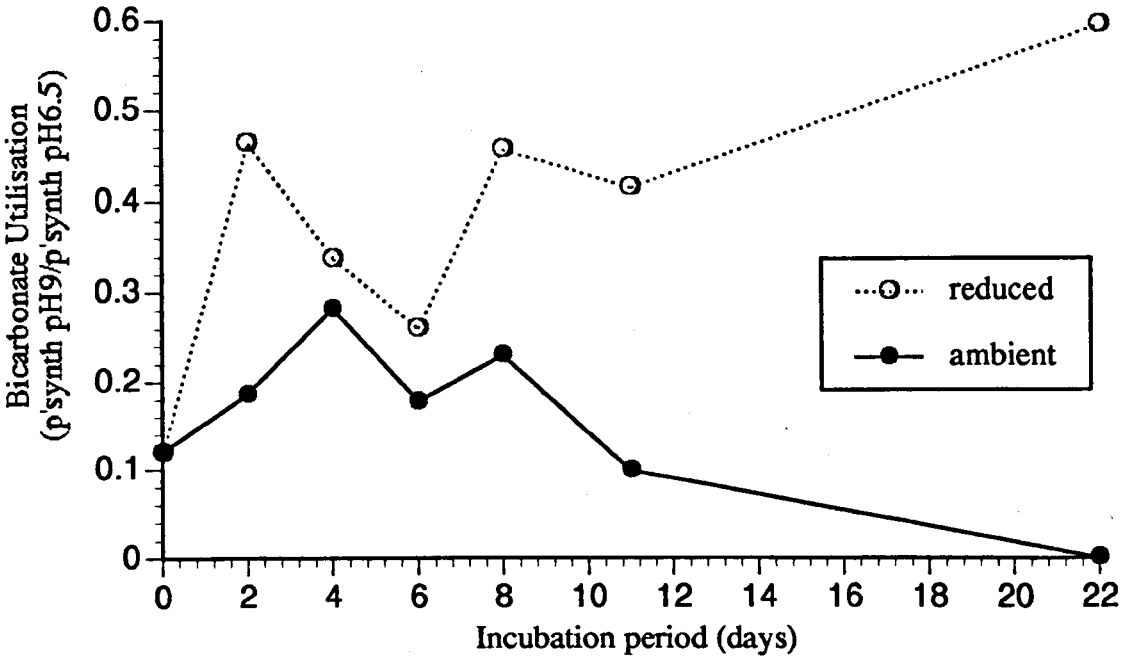
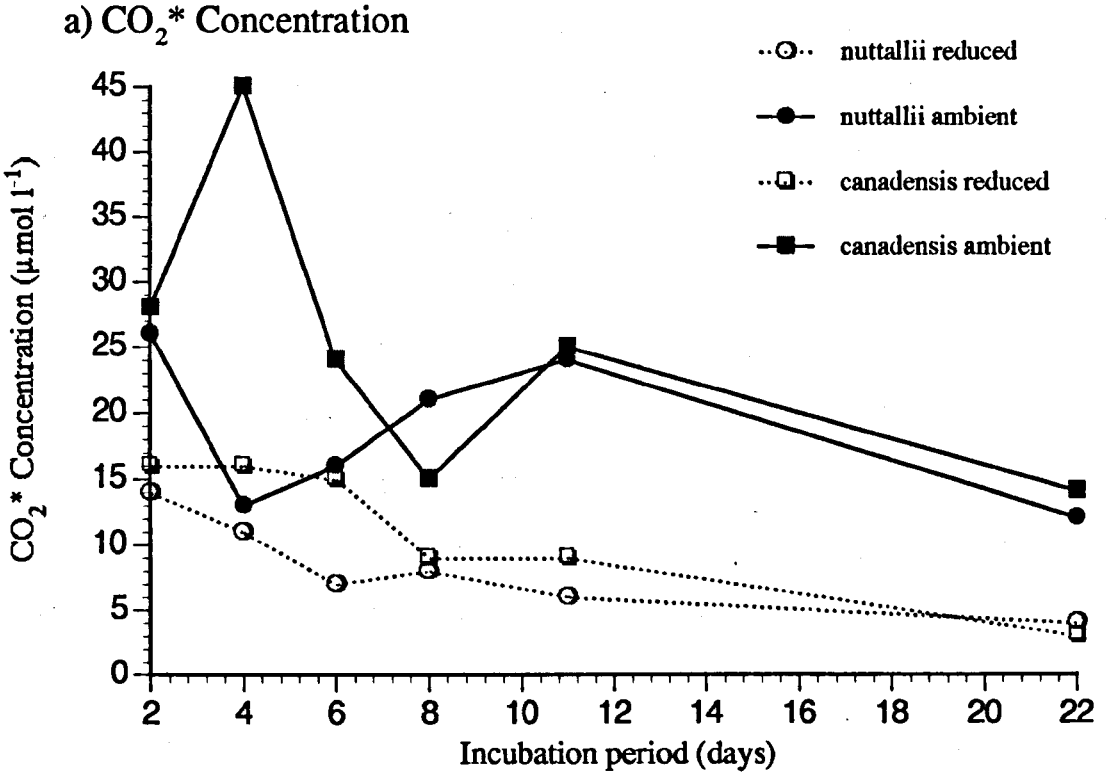
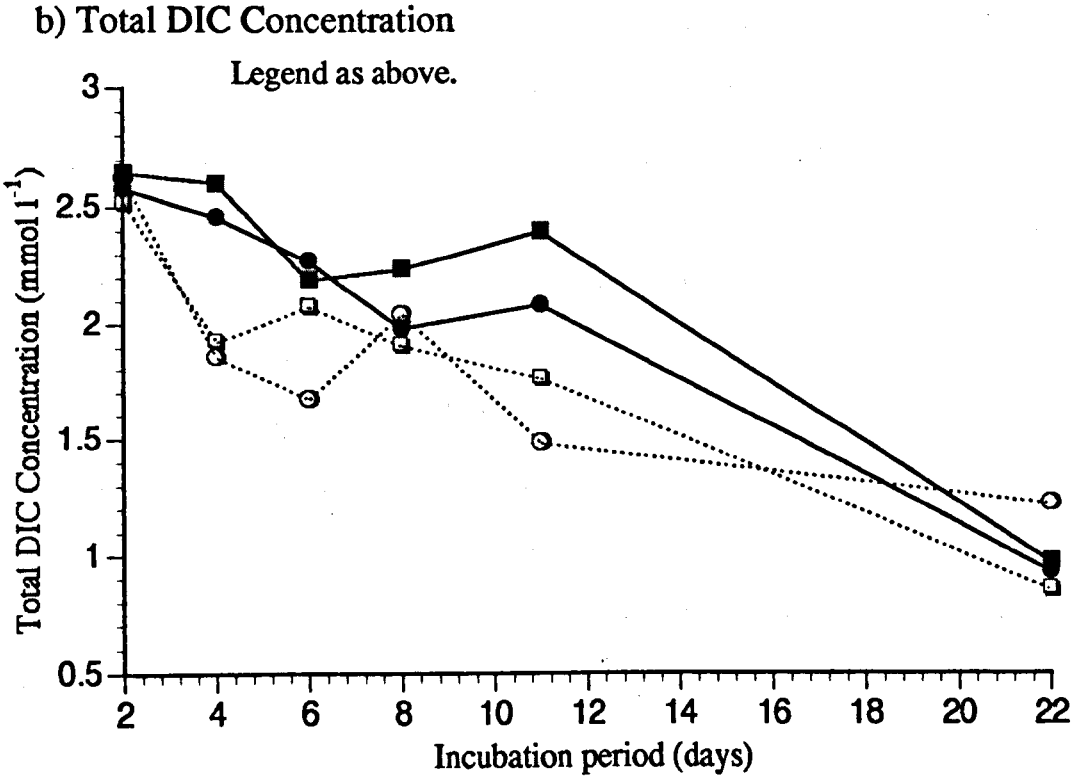


Figure 4.5 Concentration of carbon species in the experimental vessels containing plants from the Lancaster Canal incubated under different conditions.





more likely to predominate in winter, the affinity for HCO_3^- may be reduced at this time (Sand-Jensen & Gordon, 1986).

It has been demonstrated for some species that CO_2^* compensation points are reduced under conditions similar to those that induce HCO_3^- utilisation, ie. conditions of low CO_2^*, long photoperiods, high irradiance and high temperatures (Bowes *et al.*, 1978 & 1979; Holaday & Bowes, 1980; Barko & Smart, 1981; Salvucci & Bowes, 1981 & 1983; Holaday *et al.*, 1983; Spencer & Bowes, 1985), inducing what is termed a low-PR state (Bowes *et al.*, 1978). Further, the PR state has been shown to vary seasonally in the field (Bowes *et al.*, 1978 & 1979), the low-PR state also being induced under summer conditions. Many of the features of the low-PR state, ie. low CO_2^* compensation points; low photorespiration; an ability to photosynthesise at a high pH; an inter-cellular carbon concentrating mechanism (Bowes & Reiskind, 1987) and induceability under conditions of CO_2^* stress (Bowes *et al.*, 1978; Holaday & Bowes, 1980; Salvucci & Bowes, 1981; Holaday, *et al.*, 1983; Bowes & Salvucci, 1984 & 1989), are also typical of plants that are utilising bicarbonate (Prins *et al.*, 1982b; Maberly & Spence, 1983; Raven *et al.*, 1985; Sand-Jensen & Gordon, 1986; Madsen & Sand-Jensen, 1987 & 1991; Sand-Jensen, 1987; Prins & Elzenga, 1989). Further, the species which display these characteristics are not taxonomically isolated from each other [eg. *Hydrilla* (high/low-PR) and *Elodea* (HCO_3^- use by polar leaf) are both members of the Hydrocharitaceae]. So it seems likely that these mechanisms are one and the same. In support of this suggestion are the findings of Prins, O'Brien & Zanstra (1982) indicating that *Hydrilla verticillata* (L.fil.) Royle (the species most studied with regards to the high/low-PR state) has a polar leaf. The mechanism by which *Myriophyllum spicatum* L. (dicotyledon – Haloraginaceae) achieves low-PR does, however, appear to be distinct and, although it utilises HCO_3^-, does not use the polar leaf mechanism (Prins *et al.*, 1982b), apparently relying on a carbonic anhydrase (Salvucci & Bowes, 1983). Carbonic anhydrase is also important for the

photosynthesis of *Ranunculus penicillatus* spp. *pseudofluitans* (Syme) S.Webster (dicotyledon – Ranunculaceae) at high pH (Newman & Raven, 1993).

Potamogeton pectinatus, from *Potamogeton* subgenus *Coleogeton* Reichb. (Stace, 1991), utilises HCO_3^- by a different mechanism (Steemann-Nielsen, 1947). This species has a very different leaf structure to those *Potamogeton* species which possess the polar leaf mechanism, and are all from subgenus *Potamogeton.* It has been suggested that the leaves of *P. pectinatus* may have evolved from the submerged petioles of a *P. natans*-like ancestor. *P. filiformis* is also from subgenus *Coleogeton* and utilises HCO_3^- (Maberly & Spence, 1983), but the mechanism has not been investigated.

As HCO_3^- uptake is unlikely to occur under conditions of light limitation and the underwater light climate is very variable, the plants within a waterbody may be utilising HCO_3^- to varying degrees dependant on the incident light (Sand-Jensen, 1983a; Madsen & Maberly, 1991), and the plants in weedbeds have a wide variety of affinities for HCO_3^- depending on their position and the amount of shading from above (Madsen & Maberly, 1991). Differences in the availability of CO_2^* caused by the presence of other shoots either rapidly photosynthesising or respiring (dependant on the incident light – see Chapter 5), and the proximity to CO_2^*-producing mud at the bottom of the water column (*cf.* Maberly, 1985b) will accentuate this effect further. This may explain, to some extent, the high variability found in the rate of photosynthesis of the plants used in Chapter 3.

Recent work has given contradictory reports regarding the switching on and off of HCO_3^- utilisation, with the same workers finding that HCO_3^- utilisation in *E. canadensis* was switched off after a brief exposure (≥30 minutes) to high CO_2^* concentrations (1·0 mmol l^{-1}, pH 6·37) in the laboratory (Adamec 1993), but not when grown outside in soft water (≈100-50 μmol l^{-1}, pH 6·7-7·1), over a longer period (Adamec & Ondok, 1992). Little or no change occurred in *Ceratophyllum demersum*

L. under either treatment. What we may be seeing here is the effect of light on the switch mechanism, as this worker comment that when incubated under natural light after a brief of exposure to high CO_2^* in the laboratory, the *E. canadensis* plants did not switch to CO_2^* use (Adamec, 1993). It could be that in the laboratory light was limiting, and a switch to CO_2^* use would be advantageous, whereas under natural light carbon was limiting and such a switch pointless. HCO_3^- utilisation in soft waters has been previously reported for *E. canadensis* (Madsen & Sand-Jensen, 1987), where low carbon concentrations may still cause limitation. The factors inducing the switch may well be different in *C. demersum* which is quite taxanomically distinct (a dicotyledon from the Ceratophyllaceae).

It is not known if an increased layer of epiphyton can induce the subtending leaf to use HCO_3^-, but it is suggested that the reduction in light available to the host leaf by such a layer would tend to counteract any reduced CO_2^* availability. A more likely scenario is that a nutrient-mediated increase in productivity of both macrophytes and epiphyton from other parts of the shoot (together with phytoplankton and metaphyton) would lead to carbon limitation of the whole stand, and thus force the switch to HCO_3^- use.

4.3.3 Effect of bicarbonate utilisation on response to pH.

It has been shown above that *E. nuttalli* can switch from using CO_2^* to HCO_3^- as a substrate for photosynthesis. As CO_2^* declines with increasing pH, with HCO_3^- becoming the predominant ion, it would be expected that this switch would be reflected in the response of photosynthesis to pH. This was the case in all three species studied, those plants which had been grown under reduced CO_2^* being apparently more active at the higher end of the pH scale than those grown under ambient CO_2^* conditions (Figures 4.6, 4.8 & 4.10). Whilst a factorial analysis of variance showed the effect of treatment to be significant in *E. nuttallii* and *E. canadensis* ($p<0.05$), there was large

Figure 4.6 Photosynthesis of *E. nuttallii* as a function of pH at constant DIC, after 21 days of growth at different CO_2* levels.

LSD comparing between means at a given pH from Bonferroni's test = 12.64

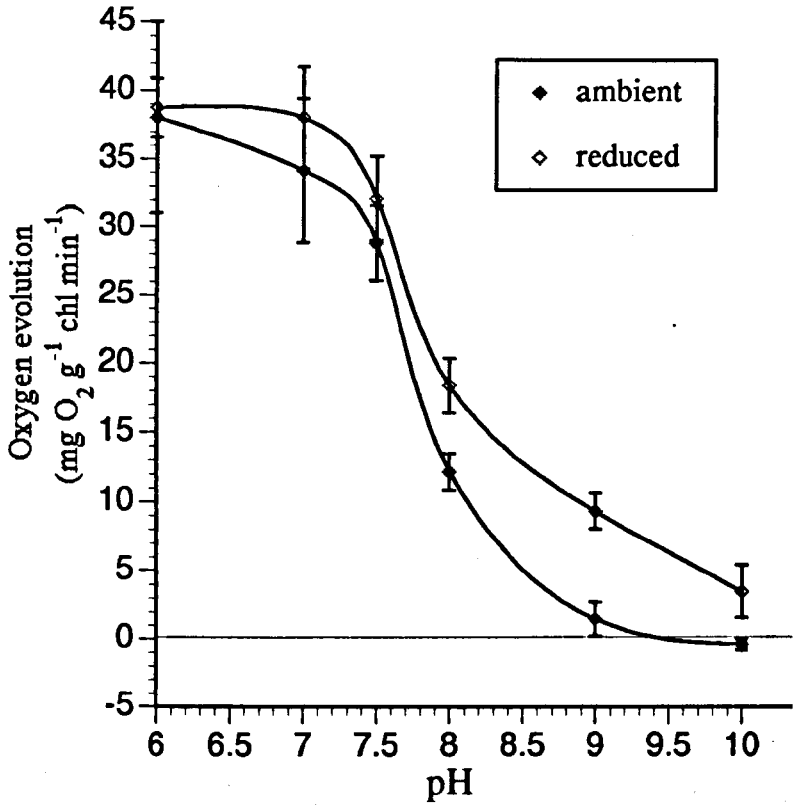


Figure 4.7 Photosynthetic rates of cultured *E. nuttallii* as a function of CO_2* available at different pH levels

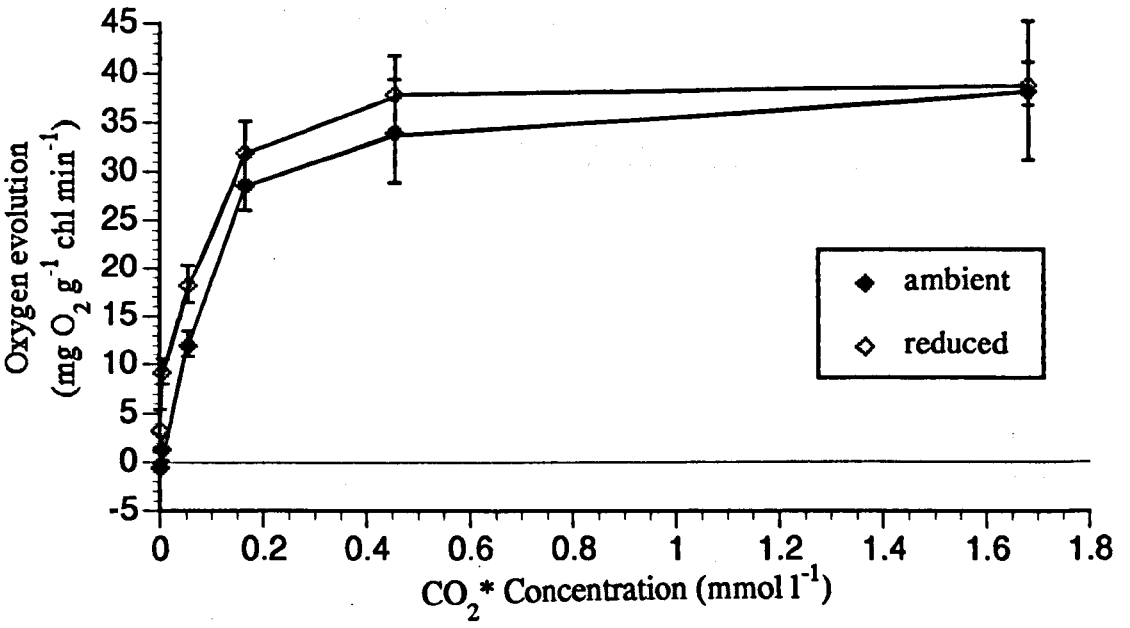


Figure 4.8 Photosynthesis of *E. canadensis* as a function of pH at constant DIC, after 21 days of growth at different CO_2* levels.

LSD comparing between means at a given pH from Bonferroni's test = 13.32

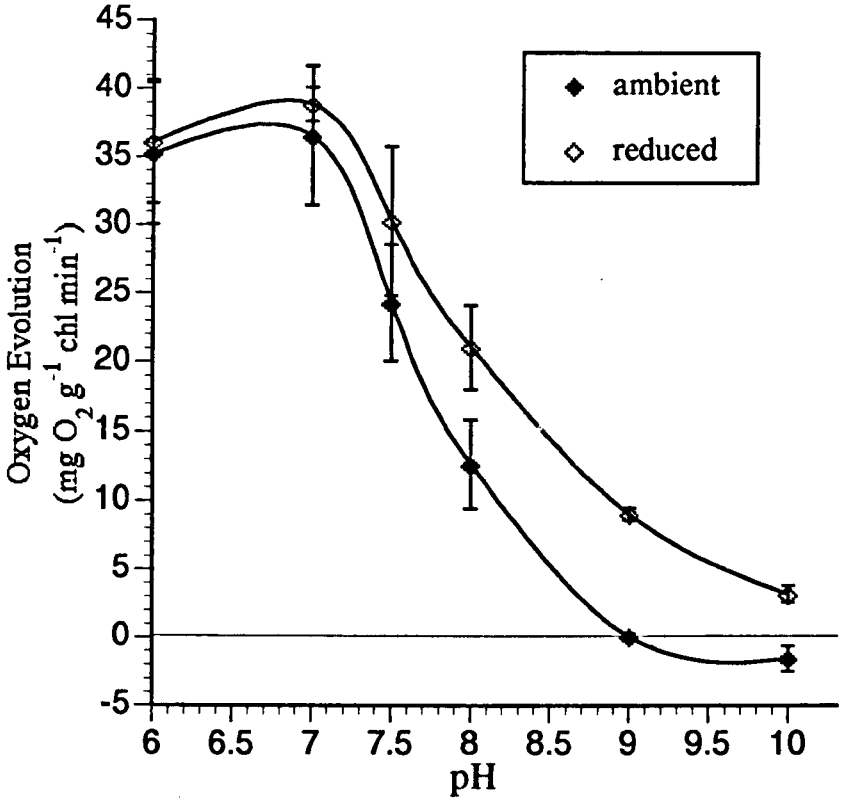


Figure 4.9 Photosynthetic rates of cultured *E. canadensis* as a function of CO_2* available at different pH levels

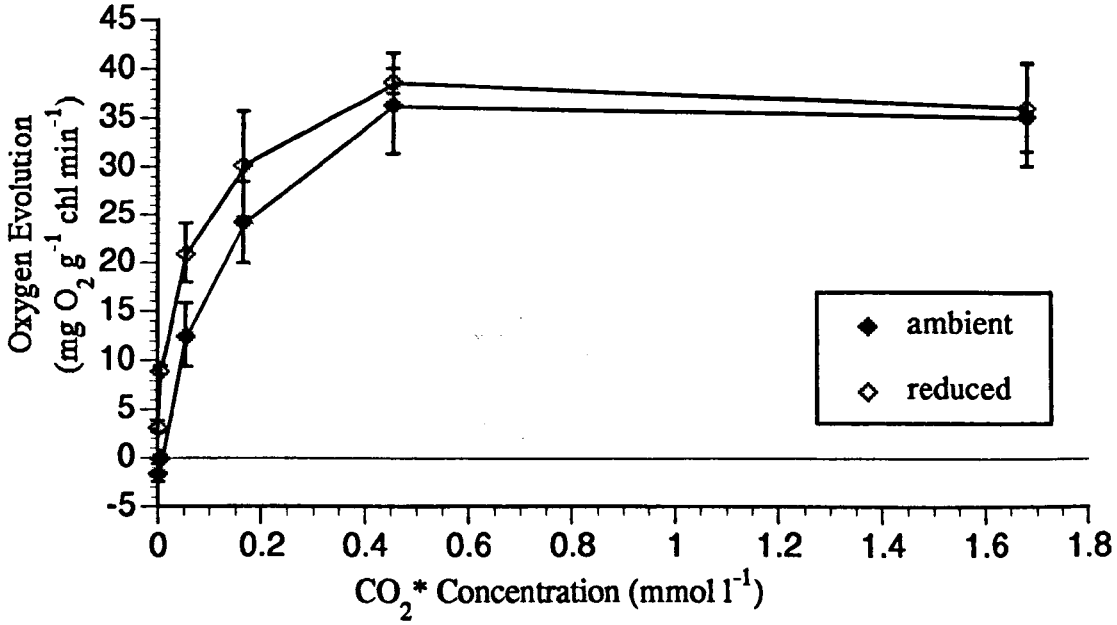


Figure 4.10 Photosynthesis of *L. major* as a function of pH at constant DIC, after 21 days of growth at different CO_2* levels.

LSD comparing between means at a given pH from Bonferroni's test = 28.48

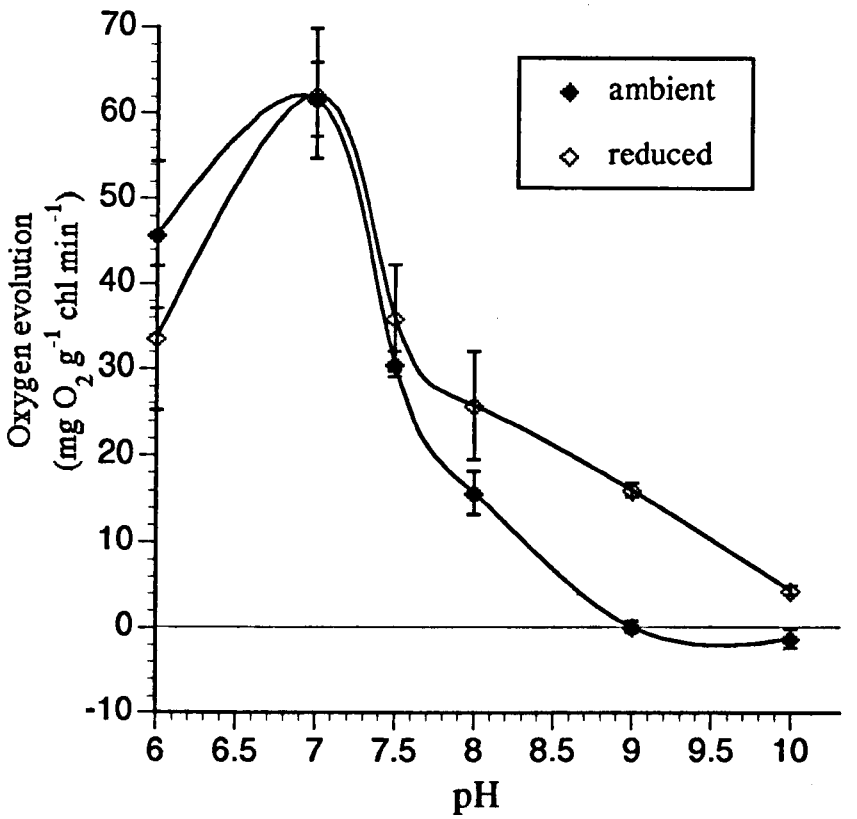
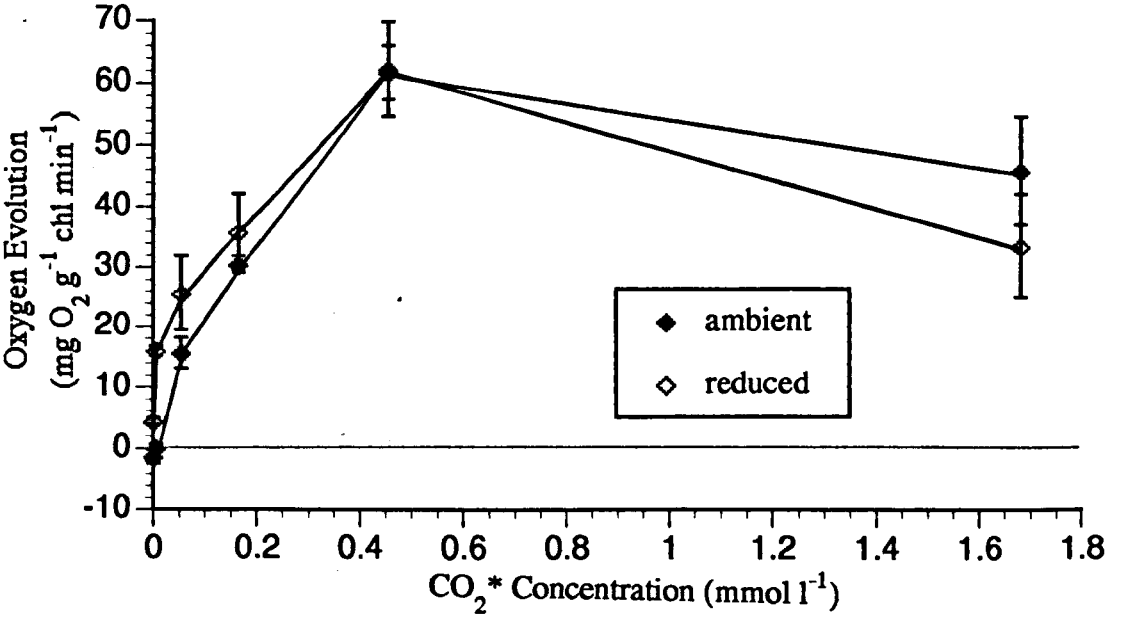


Figure 4.11 Photosynthetic rates of cultured *L. major* as a function of CO_2* available at different pH levels



variation between replicate shoots. For all three species, Bonferroni's test showed that the rates of photosynthesis were never significantly different for a given pH (Figures 4.6 to 4.11). However, Bonferroni's test is somewhat more conservative than the anova, and less powerful at detecting differences. The buffers used in this experiment to maintain pH may have reduced the efficiency of the HCO_3^- uptake mechanism (Prins *et al.*, 1982b &c; Prins & Helder, 1985; Prins & Zanstra, 1985; Elzenga & Prins, 1987; Prins & Elzenga, 1989), which seems rely on external conversion to CO_2^* (see Chapter 6). Plants taken from waterbodies of varying DIC concentration and utilising HCO_3^- utilisation to different extents, have displayed similar changes in response to pH (Sand-Jensen & Gordon, 1986).

If the rate of photosynthesis at each pH value is plotted against the available CO_2^* at that pH (Figures 4.7, 4.9 & 4.11), and the point for the lowest CO_2^* concentration (pH 10) is ignored, it can be seen that the intercept with the y-axis of the reduced air plants appears greater than zero, indicating that HCO_3^- is being used (Sand-Jensen, 1983a & 1987; Sand-Jensen & Gordon, 1984 & 1986; Madsen & Sand-Jensen, 1987). The reduced rate at the lowest CO_2^* concentration (pH 10) is probably caused by the pH of the water changing less for a given quantity added of H^+ ions, which reduces the plants capability to acidify the water at the abaxial leaf surface, and hence convert HCO_3^- to CO_2^* (Prins *et al.*, 1982b &c; Prins & Helder, 1985; Prins & Zanstra, 1985; Elzenga & Prins, 1987; Prins & Elzenga, 1989, see also Chapter 6). The y-axis intercept of the plants from the ambient CO_2^* treatment is near to or below zero (Figures 4.7, 4.9 & 4.11), indicating reliance solely on CO_2^* (Sand-Jensen, 1983a & 1987; Sand-Jensen & Gordon, 1984 & 1986; Madsen & Sand-Jensen, 1987).

4.3.4 Metabolic cost of bicarbonate utilisation.

This experiment was undertaken to determine the effect that the switch to HCO_3^- utilisation had on the growth of *E. nuttallii*. First the plants were grown under conditions which would force the switch, together with control plants which continued to use CO_2^* only. Then the extent to which the plants were utilising HCO_3^- was determined, and finally several measures of the growth of the plants taken.

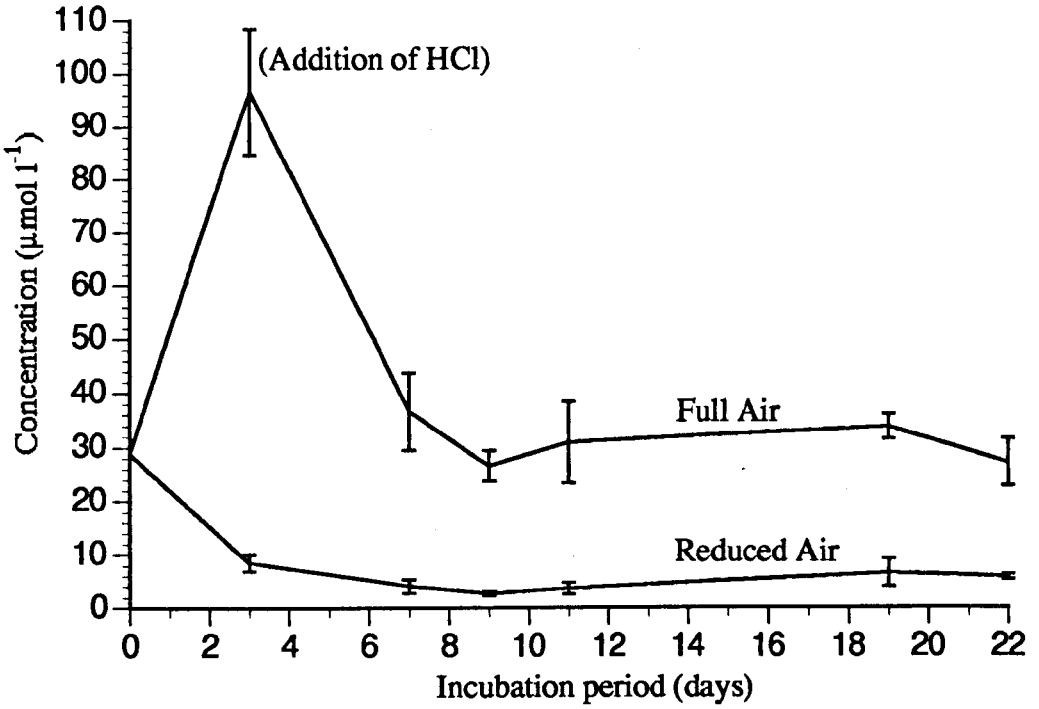
4.3.4.1 Physiology.

By bubbling the jars with untreated air, or air that had passed through soda lime two levels of CO_2^* were produced (Figure 4.12a), namely full and reduced. Whilst the pH of these treatments remained relatively constant, except when HCl was added to the full air treatment, the total DIC concentration decreased over the first 7 days and then levelled off (Figure 4.13). As with all the experiments manipulated in this way the pH was higher, and the concentration of CO_2^*, lower in the reduced air treatment at all times. This had a marked effect on the physiology of the plants with almost all those under reduced CO_2^* relying heavily on HCO_3^- utilisation after 21 days (Table 4.3). However, the plant sampled from one jar did not appear to be using HCO_3^- to any great extent, perhaps due to inadequate aeration. This replicate was removed from all subsequent calculations, with the statistical tests adjusted for unequal replication accordingly. The plants which had been subjected to the higher CO_2^* concentration showed a significant decrease in HCO_3^- utilisation, when compared to the starting population ($p < 0{\cdot}05$, Table 4.3). After 21 days of incubation, the full air plants were unable to use HCO_3^- as a carbon source for photosynthesis (Table 4.3).

Together with the changes in HCO_3^- utilisation, other changes occurred during the growth period. Both sets of plants showed a significant reduction in the total chlorophyll content of the leaves during the incubation period. Changes in chlorophyll content inversely proportional to irradiance have been shown for *Elodea potamogeton*

Figure 4.12 Concentration of carbon species in the experimental vessels bubbled with either full air or CO_2–reduced air.

a) CO_2^* (± se)



b) Total dissolved inorganic carbon (dashed line) and HCO_3^- (± se).

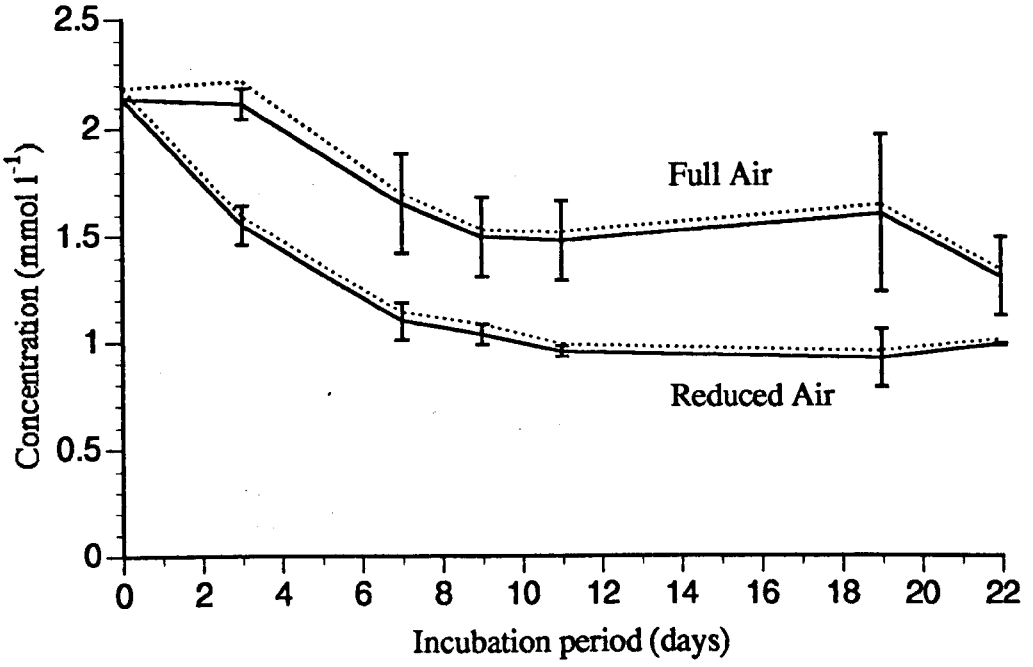


Figure 4.13 Mean pH (± se) in the experimental vessels bubbled with either full air or CO_2–reduced air.

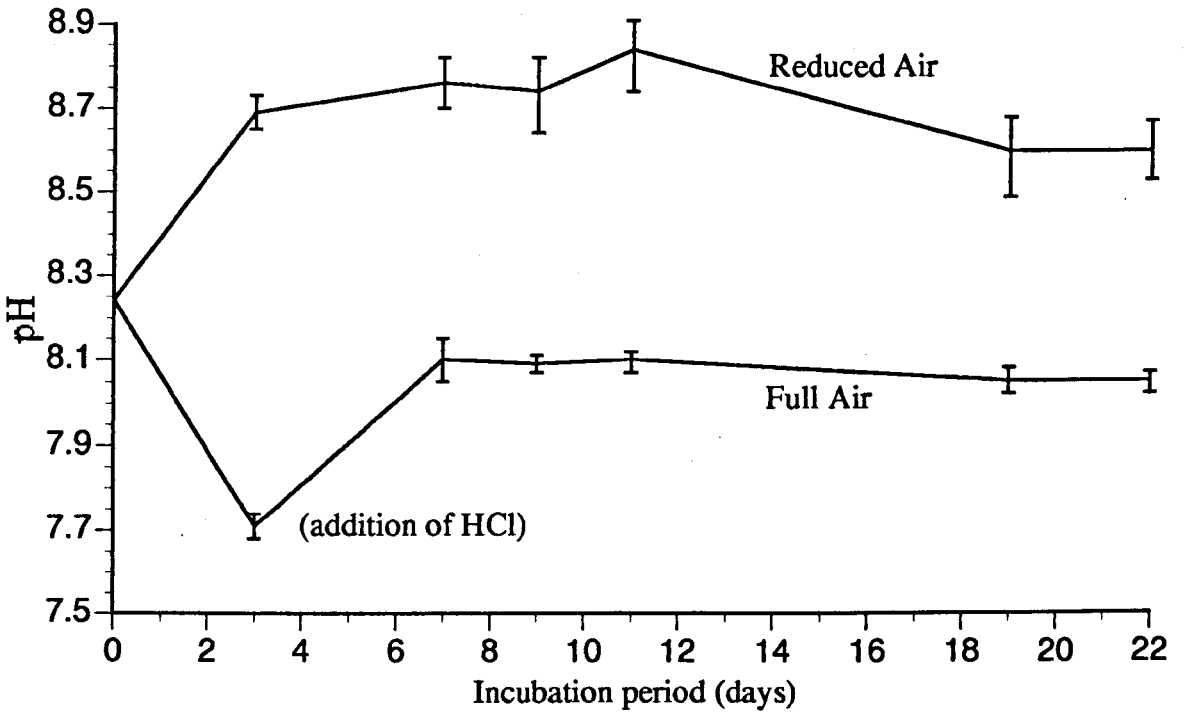


Table 4.3 Photosynthetic characteristics of *E. nuttallii* plants collected from the field at the start of the experiment and also of plants which have been grown under different culture conditions for 21 days.

The units of measurement used are: Total Chlorophyll = µg, Maximum Photosynthesis (measured as photosynthesis at pH 6·5) = mg O_2 g^{-1} chl min^{-1}. Figures shown in brackets are standard errors.

Start			Full Air			Reduced Air		
Total Chl.	Max P'synth	$\frac{HCO_3^-}{CO_2}$	Total Chl.	Max P'synth	$\frac{HCO_3^-}{CO_2}$	Total Chl.	Max P'synth	$\frac{HCO_3^-}{CO_2}$
14·99	28·80	0·178	13·56	16·79	0·000	12·40	32·26	0·308
11·59	8·96	0·095	8·37	19·92	0·000	6·48	32·25	0·566
10·29	18·88	0·000	6·42	23·34	-0·137	12·58	40·53	0·349
12·20	24·92	0·137	8·27	32·67	0·090	12·80	36·02	0·139
12·92	29·79	0·062	9·98	32·35	0·000	8·03	40·55	0·488
13·85	27·04	0·115	4·45	40·17	0·000	8·84*	20·12*	0·076*
13·36	19·32	0·093	7·57	12·62	0·038	5·47	40·25	0·282
12·88	17·73	0·157	6·32	17·29	-0·038	6·32	48·32	0·335
12·76	21·93	0·105	8·12	24·39	-0·006	9·15†	38·60†	0·352†
(0·51)	(2·49)	(0·020)	(0·97)	(3·40)	(0·023)	(1·25)	(2·14)	(0·053)

One replicate (marked *) was apparently not utilising HCO_3^- to any great extent and was therefore not used for the calculation of the means (marked †), standard errors, and all subsequent calculations. The mean values and standard errors including this replicate are 9·12 (1·08), 36·29 (2·96), 0·318 (0·057) for total chlorophyll, maximum photosynthesis and HCO_3^-/CO_2 respectively.

The following differences between the treatments were shown by a modified Tukey's test for unequal replication to be significant at p = 0·05 level. Those means joined by the same lines are not significantly different, descending order.

Chlorophyll	START	REDUCED	FULL
Maximum Photosynthesis	REDUCED	FULL	START
Bicarbonate utilisation	REDUCED	START	FULL

(Pizarro & Montecino, 1992) and *E. canadensis* (Barko & Filbin, 1983), which would indicate that the plants were under a more favourable light climate than they were experiencing in the field. The fact that the plants switched to HCO_3^- use would support the suggestion that they were not light limited (see section 4.3.2).

Another interesting finding was an increase in the maximum rate of photosynthesis in the plants from the reduced CO_2^* treatment, ie. in the plants utilising HCO_3^-. A change must have occurred in the photosynthetic apparatus of these plants to increase the apparent efficiency of chlorophyll (against which the measurements were standardised). Changes in the enzymes which fix carbon have been recorded when plants shift from the high- to low-PR state (Bowes *et al.*, 1978; Holaday & Bowes, 1980; Salvucci & Bowes, 1981; Ascensio & Bowes, 1981; Holaday *et al.*, 1983; Bowes & Salvucci, 1984), with levels in the cytosol of PEPCarboxylase and other enzymes of the C_4 cycle greatly increasing. In this low-PR state the production of malate and aspartate appears to constitute a large proportion of carbon fixation, in what is termed "C_4-like" photosynthesis (Brown *et al.*, 1974; DeGroote & Kennedy, 1977; Browse *et al.*, 1979 & 1980; Holaday & Bowes, 1980; Salvucci & Bowes, 1983). Turnover of these acids can be either very slow (DeGroote & Kennedy, 1977; Browse *et al.*, 1979 & 1980) or, for plants known to be in the low-PR state, very rapid (Holaday & Bowes, 1980; Salvucci & Bowes, 1983). Further support is given to the argument that the low-PR state is part of the polar-leaf mechanism (see above) by the fact that the species used in these investigations of low-PR state/C_4-like photosynthesis are all members of the Hydrocharitaceae (*Egeria densa* Planch. (Brown *et al.*, 1974; Browse *et al.*, 1979 & 1980) *Lagarosiphon major* (Brown *et al.*, 1974), *Hydrilla verticillata* (Bowes *et al.*, 1978; Holaday & Bowes, 1980; Salvucci & Bowes, 1981; Ascensio & Bowes, 1983; Holaday *et al.*, 1983; Bowes & Salvucci, 1984) and *Elodea canadensis* (DeGroote & Kennedy, 1977)) with *Egeria densa*, *Hydrilla verticillata* and *Elodea canadensis* utilising HCO_3^- by the polar leaf mechanism (so far only found in

the Hydrocharitaceae and Potamogetonaceae - Prins *et al.*, 1982a & b). As *E. nuttallii* utilises HCO_3^- by the polar-leaf mechanism, the change in photosynthetic efficiency may reflect an increase in the enzymes of the C_4-like pathway. Given that *E nuttallii* has a polar leaf it is highly unlikely that the uptake of HCO_3^- involves a process similar to that of *Myriophyllum spicatum* (as suggested by Janke *et al.*, 1991), which involves a carbonic anhydrase (Salvucci & Bowes, 1983).

The significance of the extensive fixation of carbon into malate and aspartate during C_4-like photosynthesis is as yet largely unknown (Bowes & Salvucci, 1989), as it does not appear to be true Crassulacean Acid Metabolism (CAM) with CO_2^* fixation occurring predominantly at night (Holaday & Bowes, 1980). Malate has, however, been suggested to play a role in maintaining electroneutrality and regulating pH within the cell (Helder & Van Harmelen, 1982), two extremely important functions when considering the ion fluxes involved in the polar-leaf mechanism (see Chapter 6). C_4-acids may also play a role in the prevention of back-diffusion of CO_2^* from the leaf to the water (see Chapter 6).

A process biochemically similar to true CAM, but lacking the structural features typical of CAM plants, has been reported in several species in the evolutionary convergent isoetid group. Night fixation of CO_2^* and diel fluctations in acid titratability have been found in several *Isoetes* species and *Littorella uniflora* (L.) Aschers (Keeley, 1982, 1985 & 1990; Keeley & Bowes, 1982; Madsen, 1987; Raven *et al.*, 1985; Robe & Griffiths, 1992). The only aquatic species that has so far been found to possess classic C_4 metabolism, including Kranz anatomy, is *Eleocharis vivipara* Link (Ueno *et al.*, 1988), an amphibious species from the Cyperaceae, which only displays this character in the emergent form.

4.3.4.2 Growth.

Although there were significant differences in the physiology of the plants from the different treaetments, no differences were found between any of the growth parameters measured (Table 4.4). However, the plants from the full air treatment were restricted to CO_2^* as a substrate for photosynthesis (Table 4.3), whereas the plants from the reduced air treatment were utilising HCO_3^- at about a third of their maximum rate (Table 4.3). Considering that CO_2^* was only available to the full air plants at quite low concentrations (about 30 μmol l^{-1} over most of the growing period, Figure 4.12a) which would have been severely restricting to photosynthesis (see Section 3.4.2), whilst the level of HCO_3^- in the reduced treatment was about 30-40 fold higher (Figure 4.12b), the similarities in growth are remarkable. If we accept that the switch to HCO_3^- utilisation does not occur under conditions of light limitation (Sand-Jensen & Gordon, 1986; Madsen & Maberly, 1991) and that a decline in chlorophyll concentration without a decline in activity indicates an increase of incident light (Barko & Filbin, 1983; Pizarro & Montecino, 1992), then light limitation of growth can be ruled out. There are, therefore, only two possible explanations for the lack of difference in growth between the two sets of plants. Either, even with increased photosynthesis at high pH, the overall cost involved in the utilsation of HCO_3^- is very large, or the growth of the plants was limited by some nutrient other than carbon.

Since rooted macrophytes take up the majority of their nutrients via their roots (Denny, 1972; Carignan & Kalff, 1980; Raven, 1981; Moeller *et al.*, 1988; Barko *et al.*, 1991), and the experimental plants were provided with a rich organic canal sediment, it is very unlikely that they experienced nutrient limitation. Both sets of plants rooted well into the mud, which was taken from a stretch of the Leeds and Liverpool canal where *E. nuttallii* grows plentifully. Although nitrogen has been shown to be taken up from the water by *E. nuttallii* plants (grown without a rooting medium - Ozimek *et al.*, 1993) and can be depleted in the water column within dense

Table 4.4 Final growth characteristics of *E. nuttallii* plants grown for 22 days under ambient or reduced carbon dioxide conditions.

All figures are mean values with the standard error shown in brackets. The probabilities were calculated using a factorial analysis of variance. For full air treatment n = 40, and for reduced CO_2* air n = 35, as one jar was removed from the calculations.

Treatment	Length (mm)	Total Length (mm)	No. Shoots	Wet Weight (g)	Dry Weight (g)
Full Air	136·8 (3·1)	200·8 (6·8)	1·93 (0·18)	1·099 (0·48)	0·063 (0·003)
Reduced Air	135·3 (4·2)	228·7 (18·9)	2·11 (0·25)	0·979 (0·69)	0·062 (0·005)
	p = 0·766	p = 0·148	p = 0·536	p = 0·149	p = 0·886

For 20 plants collected from the field at the begining of the experiment these parameters were as follows:

	100	100	none	0·255 (0·011)	0·0144 (0·0009)

stands of macrophytes (Van Donk *et al.*, 1983), it is to a large extent supplied from the sediment (Barko *et al.*, 1991). The water may have become depleted in this nutrient, as it was not changed throughout the growth period, but, unless conservation of nitrogen was the ultimate goal, it is unlikely that a switch in carbon substrate would occur. However, an indication that nitrogen (and phosphate) was not limiting was given by a later similarly designed experiment where moderate loadings of nitrate (and phosphate) did not produce a significant increase in plant growth (Section 6.3.2). Nevertheless, it is possible to measure the cost to the plant of a biochemical pathway in units of nitrogen (Osmond, 1987), as well as photons of light (Raven & Lucas, 1985). Hence, if the plants were nitrogen limited it would not be unreasonable to expect to see a reflection of these costs in the growth of the plants (Raven *et al.*, 1985).

If we accept that the limited access to carbon as a photosynthetic substrate was the major constraint on growth, then the lack of difference in the growth between the plants must be an indication of the great metabolic cost involved in the construction, maintainance and running of the polar-leaf mechanism in *E. nuttallii.* These costs must be very great since the plants utilising HCO_3^- will not have been suffering the loss to photorespiration experienced by the full air plants as a result of the low CO_2/O_2 concentration ratios (Raven & Lucas, 1985). There are two other lines of evidence indicating that carbon accumulation in aquatic macrophytes is metabolically very costly. First is the finding that, even when supplied with saturating DIC and light, the maximum rate of photosynthesis of submerged macrophytes taking up HCO_3^- is far below that when relying solely on CO_2^* (Lucas, 1983; Maberly & Spence, 1983; Raven & Lucas, 1985; Sand-Jensen & Gordon 1986; Madsen & Sand-Jensen, 1987 & 1991), typically about 50% for species possessing a polar-leaf mechanism (Maberly & Spence, 1983; Spence & Maberly, 1985; Sand-Jensen & Gordon, 1986; Madsen & Sand-Jensen, 1987 & 1991). (This may not be true of microphytes (Maberly & Spence, 1983) which apparently use far more energetically efficient mechanisms to

accumulate carbon (Raven, 1970; Olofsson, 1980; Lucas, 1983; Shapiro, 1990a)). Second is the very low concentration of CO_2^* which is required before a plant will switch from CO_2^* to HCO_3^- use (Sand-Jensen & Gordon, 1986; Results above). Teleologically speaking, why should a plant wait until HCO_3^- concentrations are so many times greater (30-40) than CO_2^* before tapping it as a resource, when photosynthesis is quite clearly restricted at such low CO_2^* concentrations (see Figure 3.10).

Although apparently able to photosynthesise better at high pH (see above), the costs of constructing, maintaining and running this polar leaf mechanism are obviously very high and it is only used under conditions of extreme carbon limitation, where photosynthesis would otherwise be virtually arrested. Under these conditions the polar-leaf mechanism could be described as a survival strategy for periods of exteme hardship.

Here we have visualised the costs involved, but to quantify them precisely is somewhat more difficult. If we assume that carbon is being fixed at the maximum, rate with photorespiration reduced to a minimum (Raven & Lucas, 1985), then any energetic cost due to the polar-leaf mechanism will be equivalent to a reduction in photosynthesis from this maximum value. We must also assume the plants are equally efficient either side of the switch from CO_2^* to HCO_3^-. Then, using a known value for photosynthesis at the CO_2^* concentration found in the full air treatment, we can approximately estimate the cost of the polar leaf mechanism.

From Figure 3.3 we can estimate maximum photosynthesis without photorespiration (P_{max}Free) as 50 % greater than that at equilibrium with air. Using the mean value of maximum photosynthesis at ambient O_2 (P_{max}Air) for the full air plants (24·39 mg O_2 g^{-1} chl min^{-1}, Table 4.3), this gives us a likely value of 36·59 mg O_2 g^{-1} chl min^{-1} for P_{max}Free. The value of photosynthesis at the CO_2^* concentration found in the full-air treatment (about 30 µmol l^{-1} over most of the growing period, Figure

4.11a) and ambient concentration of O_2 (P_{air}) can be obtained from Figure 3.1, as 3·9 mg O_2 g^{-1} chl min^{-1}. The overall cost of the polar-leaf mechanism in terms of reduction of photosynthesis is therefore,

$$\text{Overall Cost} = P_{max}\text{Free} - P_{air} = 32{\cdot}69 \text{ mg } O_2 \text{ g}^{-1} \text{ chl min}^{-1}.$$

To remove the effect of night-time respiration we must take into account the proportion of time spent in darkness each day (8 hours out of 24).

$$= \frac{32{\cdot}69 \times 16}{24} = 21{\cdot}79 \text{ mg } O_2 \text{ g}^{-1} \text{ chl min}^{-1}.$$

Assuming a 1:1 ratio of CO_2^* fixed to O_2 released and using values for RuBISCarboxylase for cyanobacteria with air-equilibrated O_2 in the cell as as an estimate, ie. 1 mole of CO_2^* fixed to carbohydrate equates to 25 moles of photons (Raven & Lucas, 1985):

$$= 17{\cdot}02 \text{ mmol photons g}^{-1} \text{ chl min}^{-1}$$

As chlorophyll was found in field populations to be present in the leaves at a concentration of 21·18 µg cm^{-2}, this can be converted to incident light,

$$= 60 \text{ µmol photons m}^{-2} \text{ s}^{-1}$$

This value, an irradiance commonly experienced by submerged macrophytes in the field, can be used as an estimate, below which HCO_3^- use would not be expected.

A simple working cost, from which the gains of decreased photorespiration are excluded, is given by

$$\text{Working Cost} = \frac{P_{max}O_2 - P_{air}}{P_{air}} \times 100 = 84\ \%$$

Using this figure we can predict that a switch to HCO_3^- use will occur and be metabolically efficient once photosynthesis is reduced below 16 % (100 – 84) of its maximum value by carbon limitation. Since the cost of production is a once only cost, then the cost to the plant of maintainance and running the mechanism will be slightly below the figure given. The return switch to use of CO_2^* only is therefore not likely to occur under the same conditions, but depend on how costly the mechanism is to produce.

This method of calculating the costs of this mechanism does, obviously, include the errors involved in comparing populations of plants taken at different times, and relies on several large assumptions. If HCO_3^- use by the polar-leaf mechanism works through external conversion to CO_2^* (see Chapter 6), the rate of CO_2^* production, and hence photosynthesis, will depend on the capacity of the water to buffer pH changes at the abaxial leaf surface. This effect will also incur a cost which is changeable, dependent on the composition of the water and its pH (Prins *et al.*, 1982c; Prins & Zanstra, 1985). Nevertheless, the method used does give a value for the overall cost of the mechanism of 60 μmol photons m^{-2} s^{-1}, a figure which appears realistic and can be tested experimentally. It would be interesting to see how this figure compares with irradiances experienced by *E. nuttallii* plants utilising HCO_3^- in the field.

The most important conclusion to be drawn from this part of the study is that, when reliant on the polar-leaf mechanism for photosynthesis, the growth of *E. nuttallii* is very slow. An estimate of 16% of the rate when supplied with plentiful CO_2^* has been made. Under such conditions, the rate of shoot extension will be very much reduced and the plant will lose its advantage over epiphyton colonisation as described by Birch (1990), ie. new tissue will be produced at a slower rate and dense coverings

of epiphyton will be expected to occur further up the shoot. Some algal groups are apparently far more efficient at utilising HCO_3^- (Talling, 1976; Maberly & Spence, 1983), particularly cyanobacteria whose photosynthesis appears to be favoured by high pH (Olofsson, 1980; Shapiro, 1990a), and as such are not so restricted by carbon limitation. The presence of dense growths of epiphyton in the vital upper part of the shoot, where the majority of photosynthesis takes place, is likely to severely affect the performance of the plant through shading. Hence the advantage that the polar leaf mechanism gives to the plant, ie. insensitivity to epiphyton effects on gaseous exchange, will be counteracted if the rate of epiphyton development is high.

Chapter 5.

Field Measurements.

5.1 Introduction.

The aim of this part of the work was to investigate the changes in water quality that can occur within stands of *E. nuttallii* growing in a lowland, eutrophic canal of moderate flow, and thus determine what was controlling plant photosynthesis. Once the factors which most affect the plants' activity had been identified, a more accurate assessment of the effect of epiphyton could be made. Also, by measuring the extent to which environmental variables changed it was possible to verify that the ranges used in the laboratory experiments were relevant to the plant in a field situation.

As described in the previous chapter, photosynthesis of submerged plants is affected by water flow around their leaves, but, conversely, the physical presence of the plant affects the flow of the water around it. The affect on flow is accentuated when, as is often the case, the plants are growing in a weedbed. In such circumstances the flow within the bed is very much reduced compared to that immediately upstream, with the majority of the flow diverted around the bed. The bed then acts in much the same way as a solid object, with a surrounding boundary layer. The extent of resistance to flow and hence the reduction in water velocity, is determined by architecture of the plants and the shoot density (Losee & Wetzel, 1993; Dodds, 1991). Often different parts of a bed act in different ways, with the leafless parts allowing far more flow around them than the leafy parts (Dale & Gillespie, 1978; Losee & Wetzel, 1988 & 1993). This effect on flow occurs whenever there is water movement near submerged plants and has been recorded for weedbeds growing in lakes (Losee & Wetzel, 1988 & 1993), as well as in streams and rivers (Madsen & Warnke, 1992; Machata-Wenninger & Janauer, 1992). Flow diversion by weedbeds can easily be visualised in fast flowing streams with abundant beds of watercrowfoot (see frontispiece) or water starwort (Sand-Jensen & Madsen, 1992).

As a consequence of reduced flow within weedbeds there is decreased mixing and, since there is enormous potential biological activity in these plant stands, the water quality

can be greatly altered compared to that outside the bed. An additional aspect of the physical structuring of the vertical water column is reduced transmission of infra-red radiation, the surface becoming somewhat warmer and the bottom cooler than when mixing occurs (Dale & Gilespie, 1978). Further, as the plants greatly absorb the light incident on them, there exists a strong gradient of light through the bed (Westlake, 1964). Obviously the growth form of the plant is highly important in this phenomenon, with floating leaved forms attenuating light principally near the surface (Frodge *et al.*, 1990) and submerged forms mostly at greater depths where the biomass is concentrated (Van der Bijl *et al.*, 1989; Madsen & Maberly, 1991; Wychera *et al.*, 1993). This increased attenuation of light adds to the already strong effects of the water itself.

These gradients and the reduced mixing lead to rapid vertical and diurnal changes in physico-chemical parameters, which have often been recorded. Diurnal changes in pH, O_2 and temperature have been variously recorded in beds of *Ceratophyllum demersum* L., *E. canadensis* mixed with *Callitriche hermaphroditica* and *Potamogeton berchtoldii* (Goulder, 1970), *Najas minor* and *Nelumbo* (Unni, 1972), *Lagarosiphon major* and *Egeria densa* (Brown *et al.*, 1974), *Hydrilla verticillata* (Van *et al.*, 1976), and *E. canadensis* (Pokorny *et al.*, 1984). Vertical gradients of these parameters have been described for beds of *Najas minor* and *Nelumbo* (Unni, 1972), *Myriophyllum heterophyllum* (O'Niel-Morin & Kimball, 1983), *Brasenia schreiberi* Gmel. and mixed *Ceratophyllum demersum* L. and *Myriophyllum exalbescens* (Fern.) Jeps. (Frodge *et al.*, 1990) . Seasonal changes in the O_2 profiles through stands of *B. schreiberi* and mixed *C. demersum* and *M. exalbescens* have also been recorded (Frodge *et al.*, 1990). Additionally, comparisons between weedbeds and open water have shown large differences in pH, O_2, and conductivity (Goulder, 1969; Frodge *et al.*, 1990) as a result of the structuring of the water and high metabolic activity of the plant stand.

5.2 Methods.

Investigations were made on 5th August 1992 and 17th/18th August 1993. On both occasions, water samples were taken from within weedbeds composed almost entirely of *E. nuttallii* (with occasional plants of *Myriophyllum spicatum*), situated on the towpath side of the Leeds and Liverpool Canal at Aintree (SJ371990).

On 5th August 1992 three profiles were taken from near the middle of an *E. nuttallii* bed approximately 5 m long and 1·5 m wide, with plants filling the water column. When the first was measured, at 10:30 h, the sun was obscured by haze. This cleared soon afterwards and the rest of the day was warm and bright with light winds. Unfortunately this investigation was curtailed by the passage of a boat along the canal (not usually trafficked), which severely disturbed the structure of the water column and the weed bed.

During the second investigation, profiles were taken from near the middle of a bed 3·5 m long and 2 m wide, again with plants reaching to the surface, at 10:00 h, 13:00 h, 15:00 h, 20:00 h, 24:00 h and 07:30 h (all times G.M.T.). These times corresponded to midmorning – bright sun, midday – bright sun, midafternoon – first shadows cast on the water from the far bank, evening – sun low in the sky, midnight – total darkness, and first light the next day respectively. Again the weather was warm and bright and the winds light. A profile was also measured from an adjacent area of open water slightly upsteam (≈15 m from the bed) and in full sunlight at 13:30 h, a time when gradients would be expected to be near maximum. No submerged plants were growing within 5 m upstream or downstream of this site.

Small water samples from known depths were collected with a remote sampler consisting of a 60 cm^3 hypodermic syringe without needle and attached to a long, stiff graduated pole. The syringe was attached so that its intake protruded 5 cm beyond the lower end of the pole. A strong length of nylon twine was attached to the syringe plunger which, when pulled, enabled the operator to fill the syringe. Using a 8 foot long plank

extended over the weedbed, with the weight of an assistant as a counterbalance, it was possible to take water samples from several depths through the water column. Once the water sample was removed, a 15 guage needle was fitted to the syringe and a 25 ml, nitrogen-filled bottle was carefully filled with the collected water. This sample was then stabilised with 0·25 cm^3 each of Winkler's reagents I and II and stored under water until return to the laboratory, where the Winkler titration was completed (Mackereth *et al.*, 1978). The remainder of the water was used to fill a 30 ml beaker with minimum disturbance and the pH measured with a WPA CD66 field meter (WPA Scientific Instruments, Saffron Walden, Essex). In the second investigation an OXI 196 oxygen meter probe (Wissenschaftlich Technische Werktätten, Wiellheim, Germany), with the guard removed, was attached to a second graduated pole and used to measure the temperature, oxygen concentration and percentage saturation. Each profile took about 10 minutes to complete, with the approximate midpoint used to indicate when it was taken. The conductivity was measured at approximately 5 cm depth with a dip cell (Conductivity Meter, Simac Instrumentation Ltd., Walton-on-Thames, Surrey) and the results standardised to 25 °C. A water sample was also removed from near to the surface at 07:30 h on 18th August 1993 and the total DIC measured by Gran Titration (Mackereth *et al.*, 1978).

A paired t-test showed that there was no significant difference between the results from the Winkler titration and those from the OXI 196 oxygen probe ($p = 0{\cdot}165$), even though they were taken from slightly different parts of the weedbed to obtain readings from undisturbed water columns. The readings from the OXI 196 meter were used to plot the graphs, eliminating the chance of worker-induced error and providing values of percentage saturation.

A brief investigation of plants collected from the weedbed with a binocular microscope (Kyowa, Tokyo, Japan) showed that epiphyton was scarce, and marl deposits lacking. Also, there was little evidence of filamentous algae within the weedbed, indicating

that the changes that occurred over the diurnal cycle were as a result of the plants alone (predominantly *E. nuttallii*).

5.3 Results and Discussion.

The first investigation, though brief, confirmed that the water quality in weedbeds of *E. nuttallii* was capable of rapid changes. A high degree of structuring of the water column was apparent from the steep vertical gradients of the variables measured. At the surface the O_2 concentration increased three-fold in 5 hours (Figure 5.1), whilst the pH increased from 7·6 to nearly 8·8 over the same timescale (Figure 5.2). Deeper in the water column, where the light intensity would be far lower, the changes were less dramatic, the photosynthetic activity of the bed being concentrated in the upper 20 cm.

The second, more detailed, investigation again showed a highly structured water column, with very steep gradients through the weedbed and a concentration of photosynthetic activity near to the surface. The temperature profiles (Figure 5.3) showed the water warming up as the day progressed, particularly in the upper layers, reaching a maximum of 22·6 °C at 15:00 h. The water then experienced a period of cooling, again most rapidly in the upper layers, with the minimum temperature at 07:30 h after a whole night of cooling. Remarkably, the temperature of the surface water at this time was over 1 °C less than that of the deeper layers. Since the density of water over 4 °C is inversely proportional to its temperature, the upper layer of water should sink by convection. The resistance to flow caused by the plants evidently slowed the movement of the water so much that mixing could not counteract heat loss. An alternative explanation could be that the water was mixing freely but that rapid heat loss through the surface was cooling the water as it circulated, but vertical differences in other parameters show that this was unlikely. This inverted temperature gradient illustrates the greatly reduced flow that occurs

Figure 5.1 Oxygen concentration profiles (mg l^{-1}) taken vertically through a weedbed taken at three different times over 5 hours.

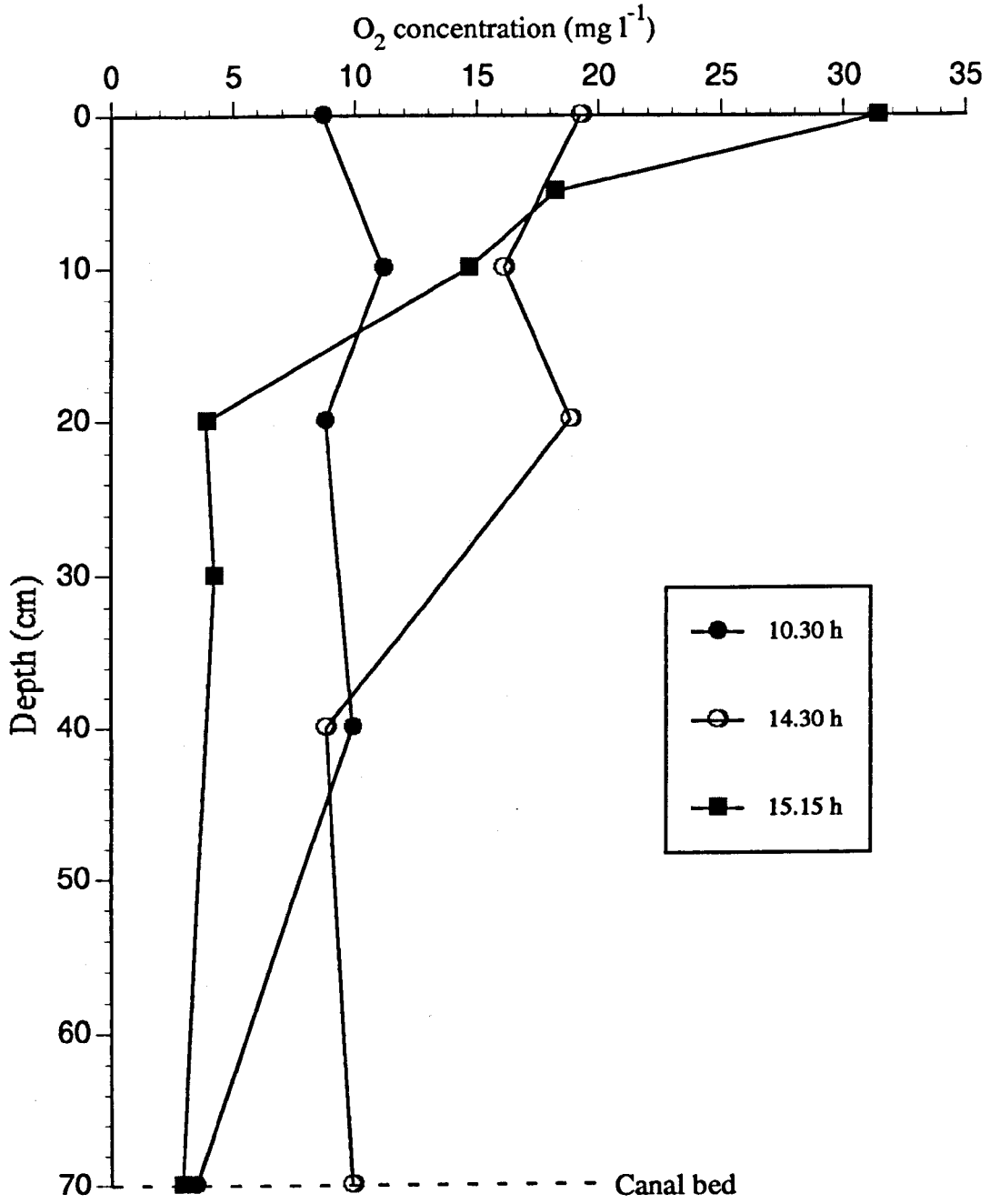


Figure 5.2 pH profiles taken vertically through a weedbed at three different times over 5 hours.

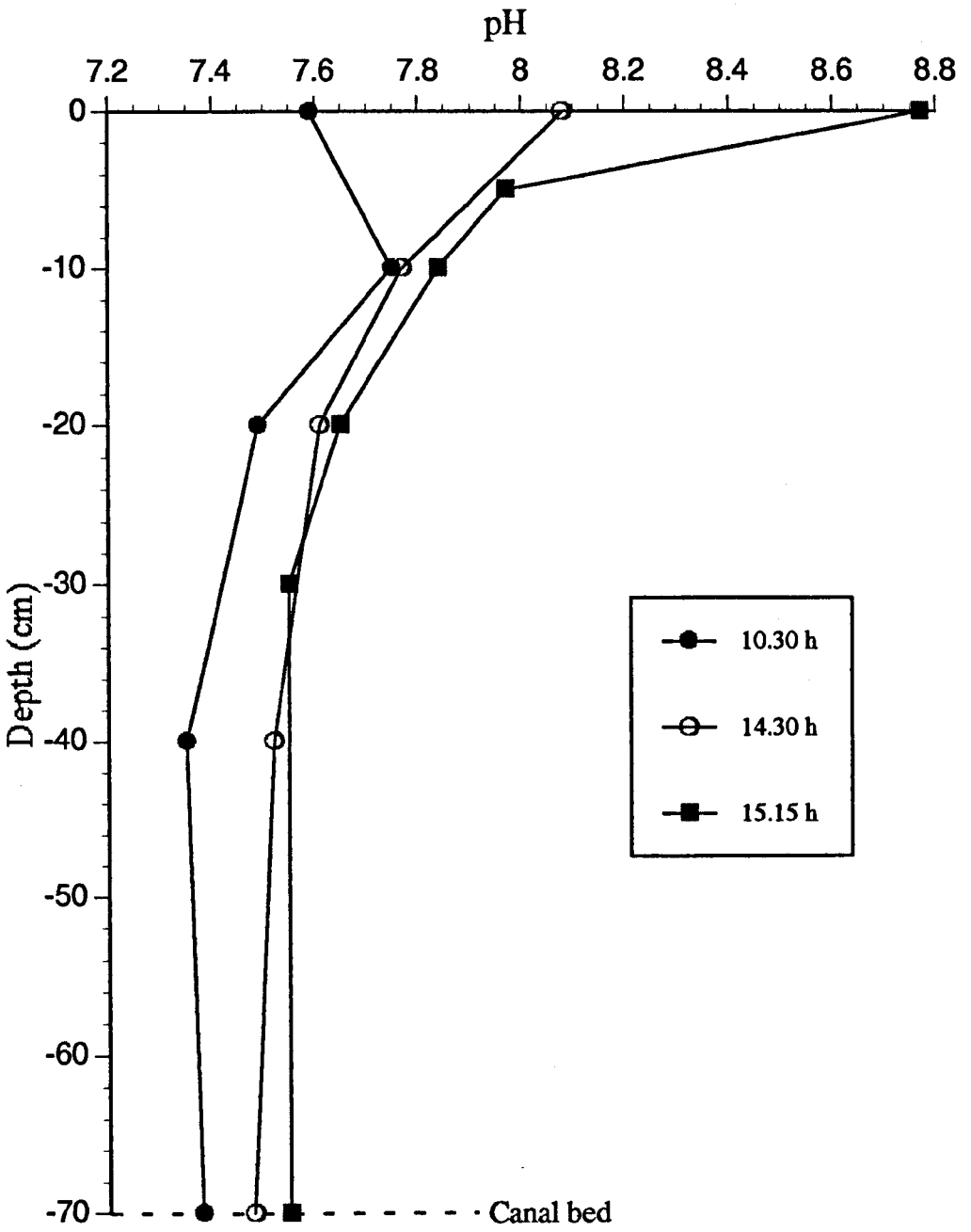


Figure 5.3 Temperature profiles through a weedbed at different times over 24 hours. Also shown is the profile through open water at 13:30 h.

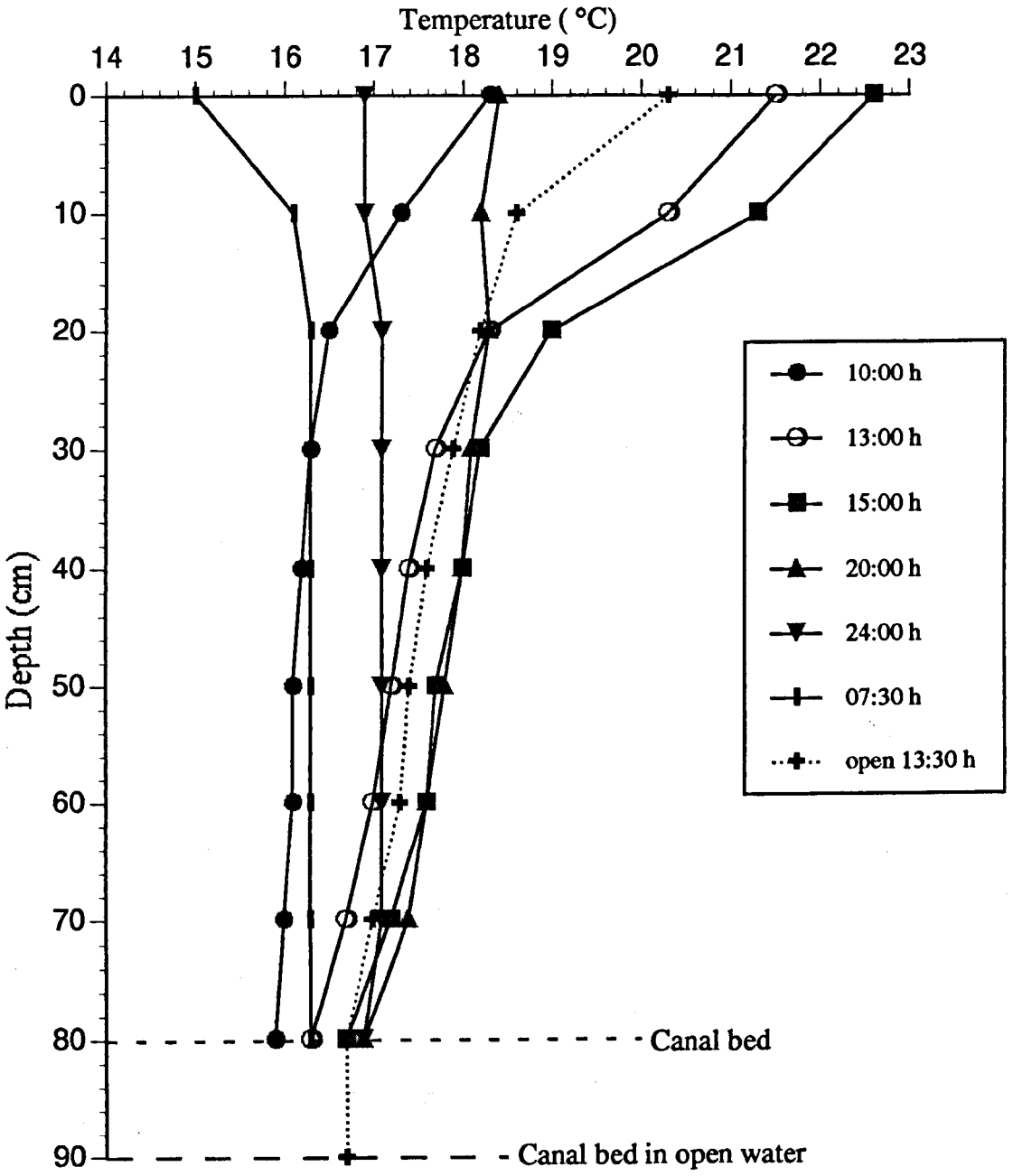


Figure 5.4 Diurnal changes in temperature at different depths through a weedbed of *E. nuttallii.*

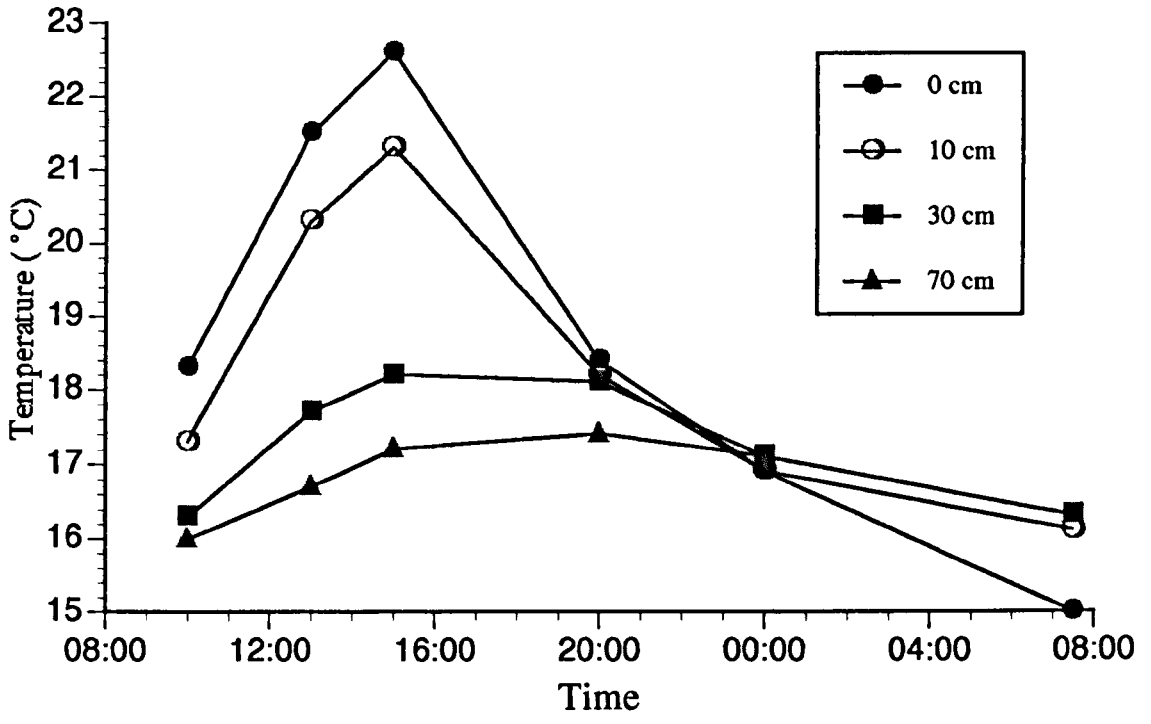
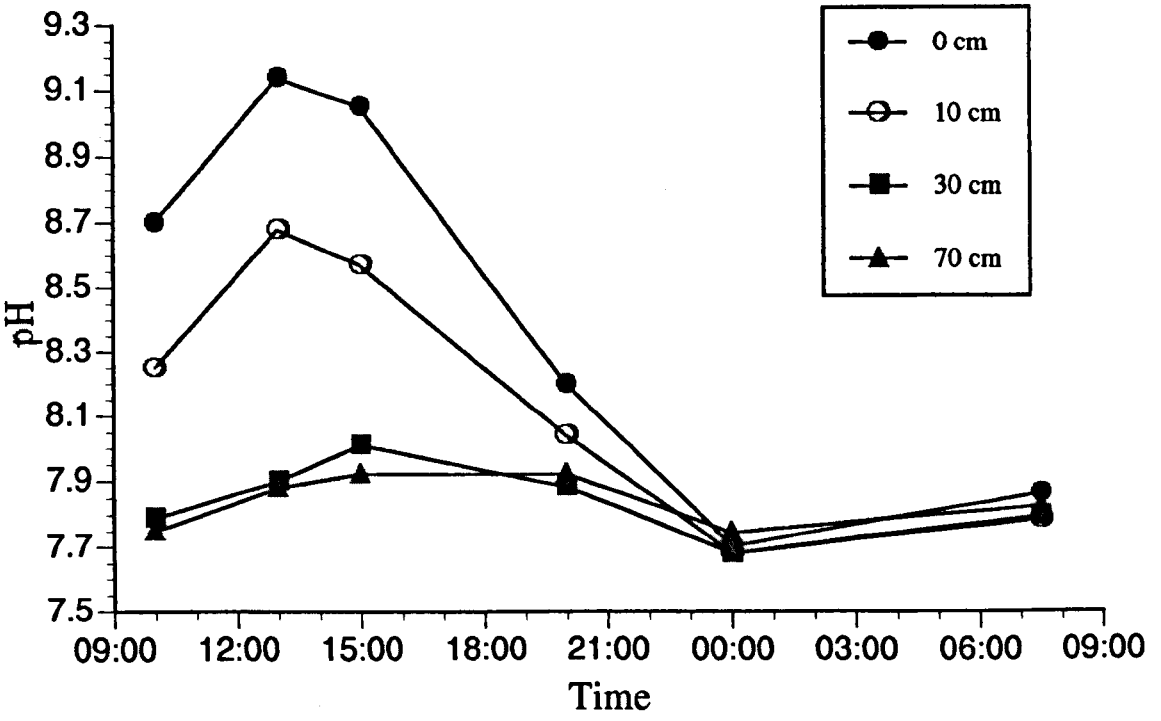


Figure 5.5 Diurnal changes in pH at different depths through a weeedbed of *E. nuttallii.*

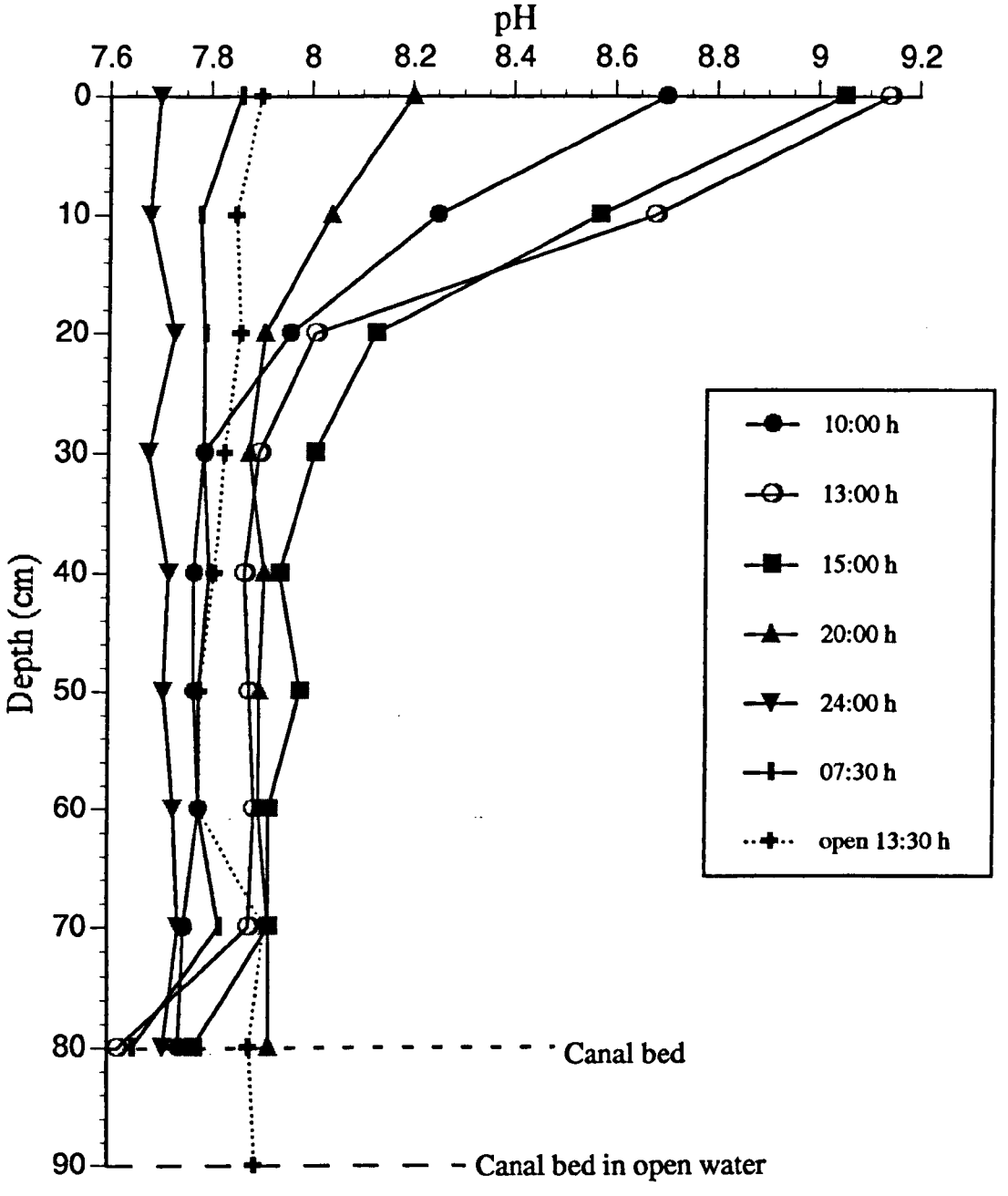


within the weedbed. Over a wide range of conditions the flows experienced within weedbeds in two North American lakes (surface area = 4·7 and 822 ha) ranged from 0·03 cm s^{-1} to 0·46 cm s^{-1}, whilst the flow outside the beds, measured simultaneously, ranged from 0·09 cm s^{-1} to 30 cm s^{-1} (Losee & Wetzel, 1993). Bioturbation by fish is probably of little significance to overall flow since it occurs so infrequently (Losee & Wetzel, 1993).

Also plotted is the temperature profile measured of the water column slightly upstream of the weedbed where plants were absent. This profile was measured at 13:30 h and, if compared to the that of the water in the weedbed measured thirty minutes earlier, showed a less marked gradient through the water column. Whilst the surface layers of water, less than 20 cm depth, were more than 1 °C colder at the open site, the water below this point was slightly warmer than in the weedbed. This effect is likely to be due to a reduction of water movement, and of vertical penetration of infra-red radiation into the weedbed. The diurnal variation in temperature at four selected depths through the weedbed (Figure 5.4), shows clearly that the surface layers were more prone to much larger fluctuations than water at depth.

The pH profiles also followed a pattern of increase during the day and decrease at night, with maximum change again in the surface layers (Figure 5.6). The maximum was attained at the surface at 13:00 h, dropping slightly by 15:00 h when the temperature was at its highest. Minimum values, except those found near the highly anaerobic mud at the canal bottom, were at midnight. pH in a situation such as this is primarily altered by the uptake or release of the acidic gas CO_2^* during photosynthesis and respiration, and as such can be used as an indicator of biological activity. Hence most of the photosynthesis of the weedbed was carried out in the morning, particularly in the upper parts and even though the surface was receiving light in the afternoon, photosynthesis in the top 20 cm had essentially stopped. In the deeper parts, 20 cm and below, the pH continued to rise until 15:00 h, as a result of continued photosynthesis. Once light had stopped entering the water, respiration took over as the predominant process and the pH fell to a minimum at 24:00 h. The

Figure 5.6 pH profiles through a weedbed at different times over 24 hours. Also shown is the profile through open water at 13:30 h.



slightly increased pH at 07:30 h, especially at the surface, indicate that the cycle was starting again the following day. The sun had risen about an hour and a half earlier. This cycle of change is shown for different depths in Figure 5.5, the peak pH for the upper layers being clearly at 13:00.

When compared to the open water site, the pH differences are enormous. Apart from a slight increase near the canal bottom, perhaps due to epipelic algal activity, the open water was pH 7·8 – 7·9 throughout its depth, compared to pH 9·15 at the surface and pH 7·9 at 70 cm depth within the weedbed (Figure 5.6). Since light penetrated to the canal bottom at the plant-free site, this indicates an almost complete lack of photosynthetic organisms in the open water. Differences in the pH between weedbeds and open water have been recorded in the Leeds & Liverpool Canal before (Saednia, 1980; Bell, 1976), and at several other sites studied (Goulder, 1969; Beeton & Sikes, 1978; Dudston *et al.*, 1986; Frodge *et al.*, 1990).

Oxygen, as percentage saturation (Figure 5.7) and as concentration (Figure 5.8), followed much the same pattern as pH, increasing to a maximum at the surface at 13:00 h, then declining. The diurnal fluctuations were again greatest in the upper layers (Figures 5.9 & 5.10), with little variation at depth. The low diurnal variation in oxygen in the deeper parts of the bed (Figures 5.9 & 5.10) indicate that they were contributing very little to overall productivity. The levels of oxygen in deeper parts of this weedbed were not as low as has been described for fully developed beds of *M. exalbescens* and *B. Schreberi* (Frodge *et al.*, 1990), perhaps due to greater penetration of light. Respiration was the predominant process under the interlocking, floating leaves of *B. Schreberi* (Frodge *et al.*, 1990) and *Nelumbo* (Unni, 1972), where the foliage probably cut out almost all the incident light. The profile taken at 15:15 h in the 1992 investigation showed reduced oxygen concentrations in the deeper parts of the bed, even though the surface was obviously supersaturated (Figure 5.2), but no measurement of the biomass distibrution of the bed was taken. Changes in oxygen profiles as plant beds developed were followed by

Figure 5.7 Oxygen profiles (% saturation) through a weedbed at different times over 24 hours. Also shown is the profile through open water at 13:30 h.

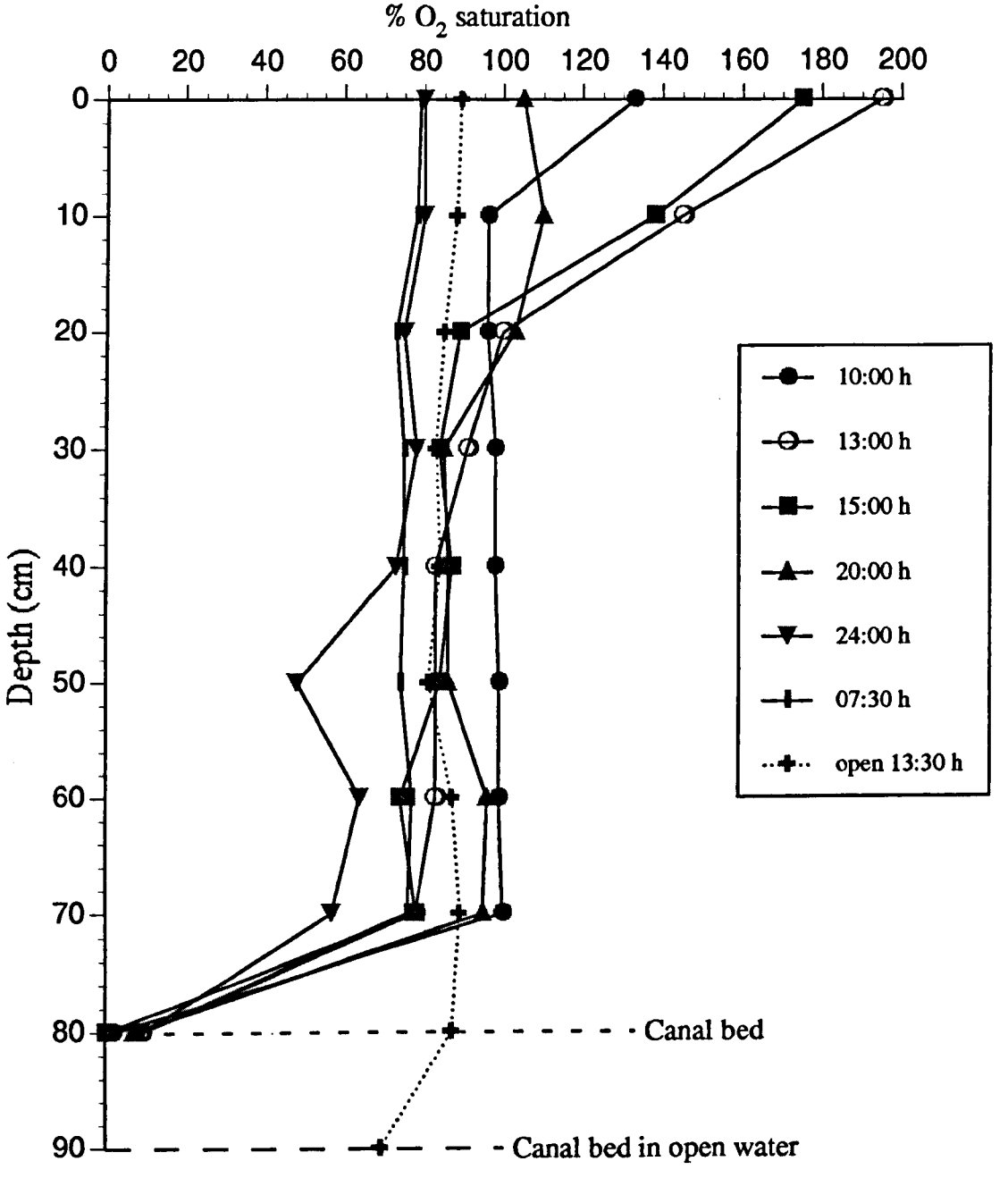


Figure 5.8 Oxygen concentration profiles (mg l^{-1}) through a weedbed at different times over 24 hours. Also shown is the profile through open water at 13:30 h.

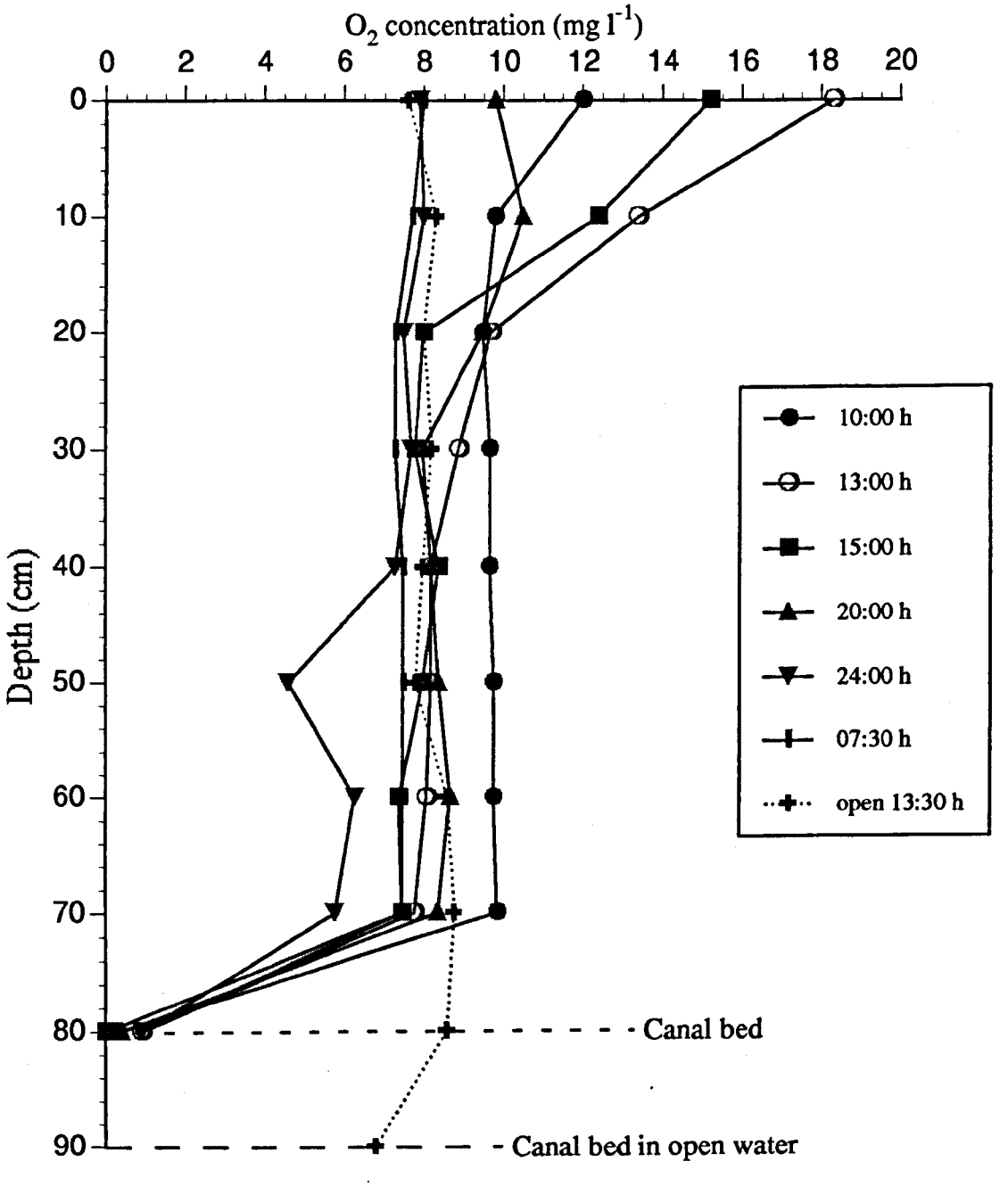


Figure 5.9 Diurnal changes in % oxygen saturation at different depths through a weedbed of *E. nuttallii*.

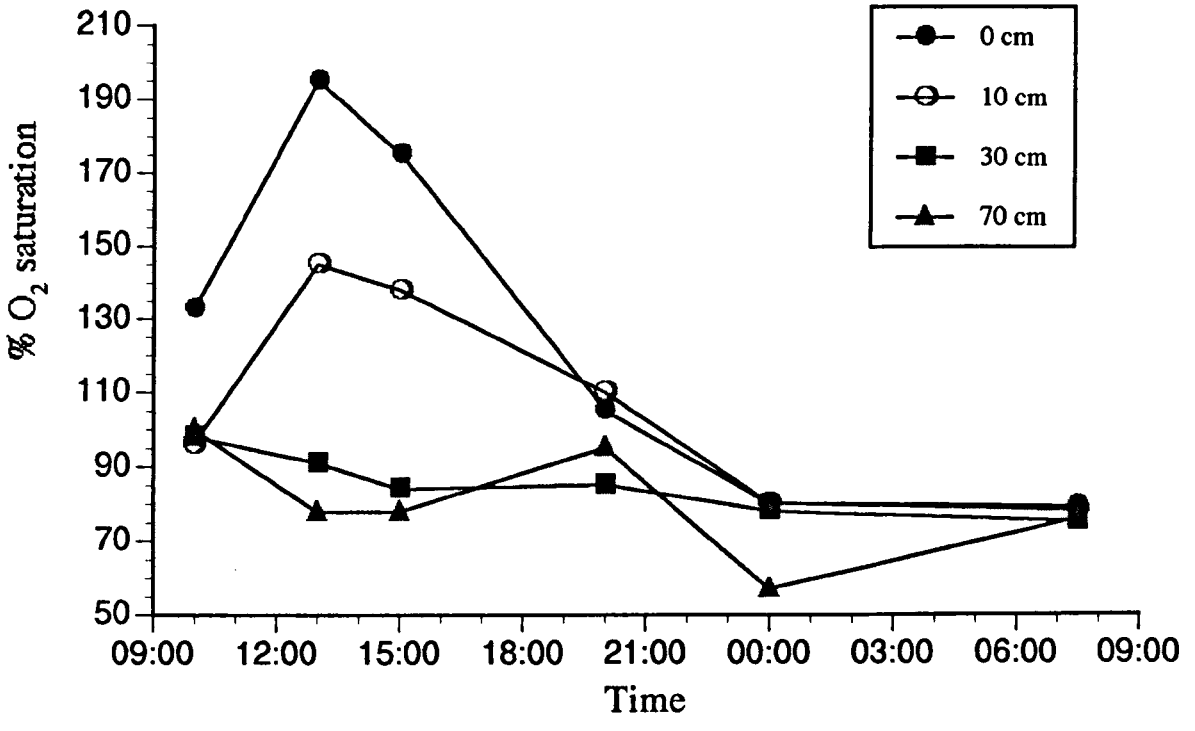
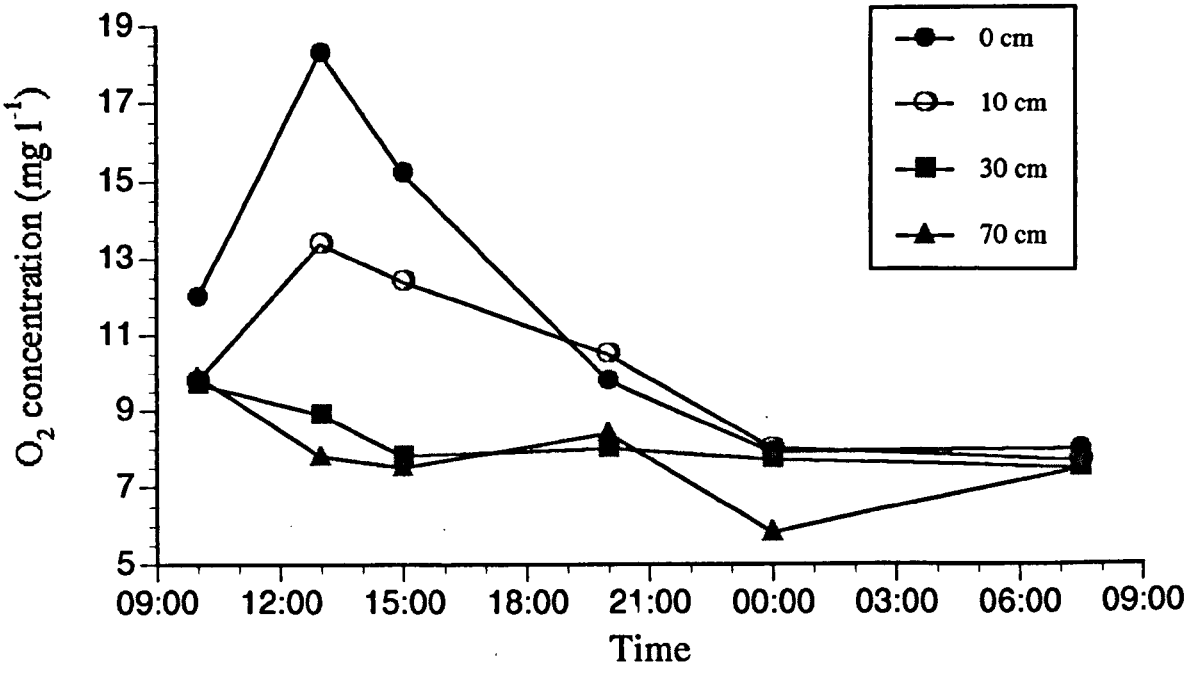


Figure 5.10 Diurnal changes in oxygen concentration (mg l^{-1}) at different depths through a weedbed of *E. nuttallii*



Frodge, Thomas and Pauley (1990), who showed that the oxygen conditions were largely determined by the structure of the weedbed. Obviously, the architecture of the plants involved and the weedbed as a whole, play an important role in determining its functioning. The high respiratory activity of the mud on the canal bottom was evident from the very low oxygen (Figure 5.7 & 5.8) and decreased pH (Figure 5.6) found at the benthos. At night respiration reduced the oxygen to below 100% saturation throughout the water column (Figure 5.7), though rarely below 70%.

As with pH the oxygen profile through the open water site (below 100% throughout its depth – Figure 5.7), showed little sign of photosynthetic activity. Again such differences in oxygen between weedbeds and nearby open water have been previously recorded (Frodge *et al.*, 1990).

Since there was no difference between the oxygen values measured by the OXI196 meter and Winkler titration (paired t-test, $p = 0{\cdot}165$), the use of the pH and the temperature together to calculate CO_2^* was justified, even though they were measured in slightly different parts of the weedbed. The values of CO_2^* concentration were calculated by substituting values for, the total alkalinity (2·449 meq l^{-1}) measured for surface water at 07:30 h; the conductivity (527 $\mu S\ cm^{-1}$) measured at the same time; the temperatures measured by the OXI 196 meter, and the pH, into the equations of Mackereth, Heron and Talling (1978). Although the total DIC concentration was measured by Gran Titration as 2·549 mmol l^{-1}, this figure was not used in the calculations since it is prone to variation due to the uptake or release of CO_2^* during photosynthesis and respiration. Alkalinity, on the other hand, is much more stable in the presence of photosynthesis and respiration (Stumm & Morgan, 1980). The uptake of ions by plants can alter the alkalinity of the water, but the extent is generally negligible (Stumm & Morgan, 1980), unless marl ($CaCO_3$) precipitation occurs as a result of HCO_3^- uptake. The almost constant conductivity throughout the diurnal cycle (Figure 5.12), measured at the water surface where HCO_3^- utilisation would be expected to be greatest, indicated that no such precipitation occurred. A careful

Figure 5.11 Calculated carbon dioxide concentration profiles (μmol l^{-1}) through a weedbed at different times over 24 hours. Also shown is the profile through open water at 13:30 h.

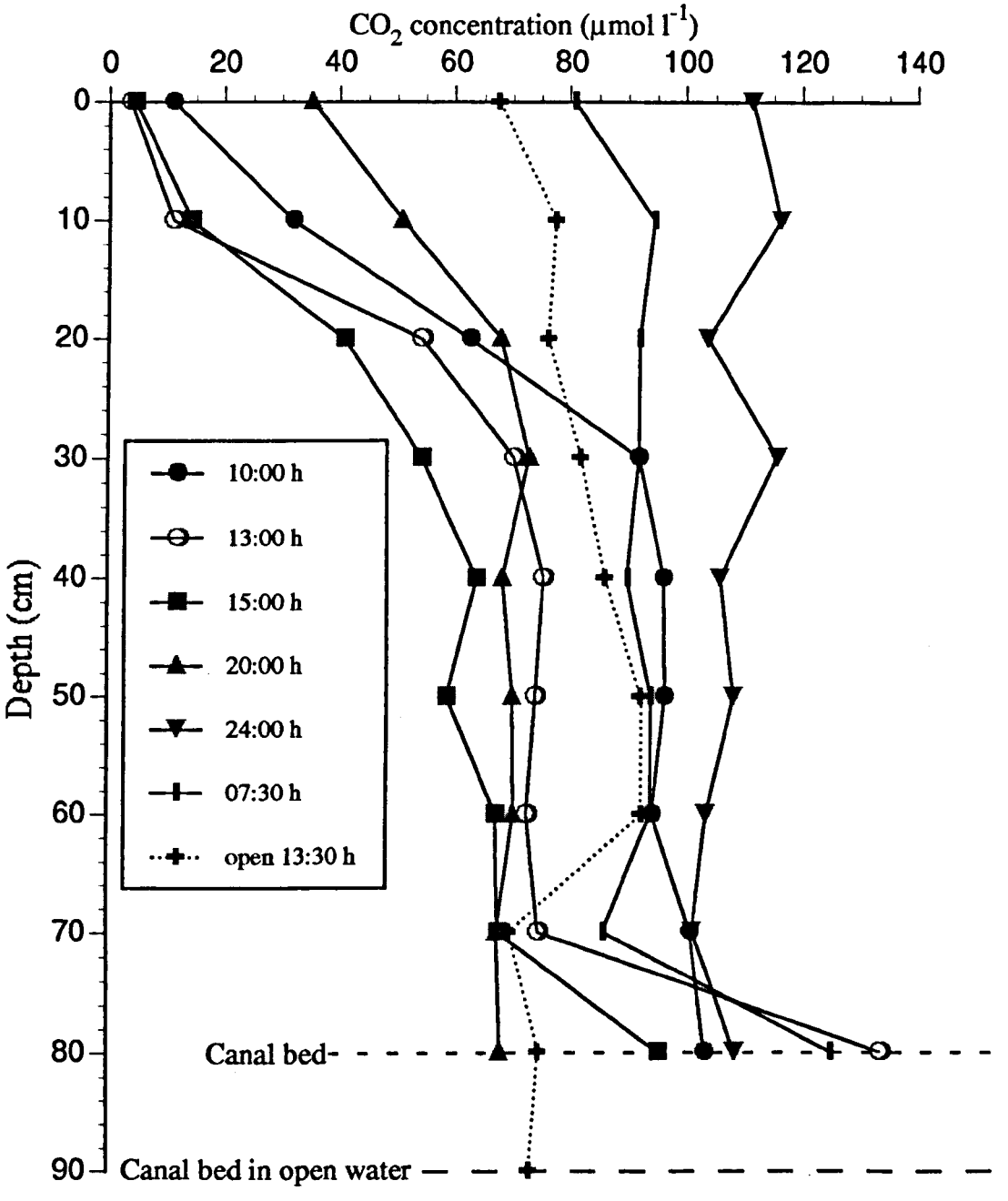


Figure 5.12 Diurnal changes in temperature-corrected conductivity measured at 5 cm depth.

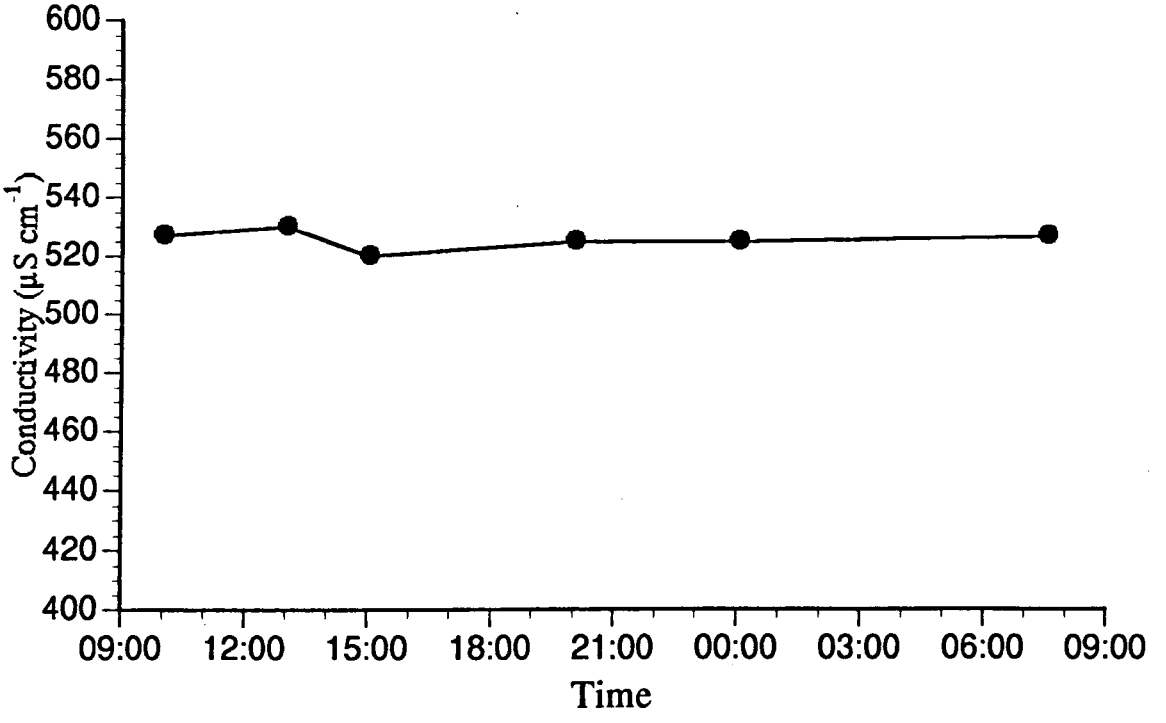
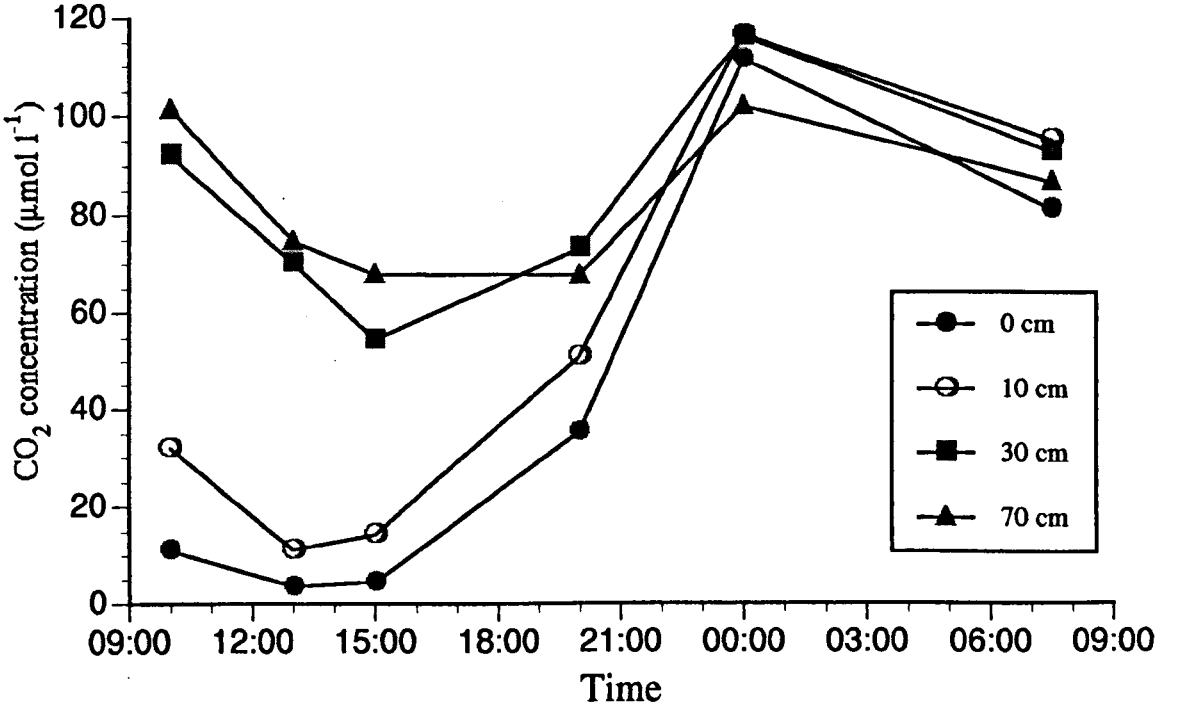


Figure 5.13 Diurnal changes in calculated carbon dioxide at different depths through a weedbed of *E. nuttallii.*



inspection of the plants from the surface of the weedbed studied showed no evidence of marl deposition on the leaves, so it was unlikely that the plants were utilising HCO_3^- (see Section 4.3.1). The contribution which the carbon system makes to the alkalinity, which is pH dependent, can be calculated (Mackereth *et al.*, 1978). Having taken the precaution of using alkalinity in these calculations, the difference between the results and those obtained using total DIC were negligible.

The CO_2^* concentrations showed maximum depletion in the upper layers with a minimum of 3·6 μmol l^{-1} occurring at the surface at 13:00 h (Figure 5.11). By 15:00 h the concentration at the surface had only increased to 4·5 μmol l^{-1}. When compared to laboratory studies on the photosynthesis of *E. nuttallii* at similar concentrations (Section 3.4.2), it is obvious that the plants in the upper parts of the bed were experiencing extreme carbon limitation over most of the daylight hours. The afternoon decrease in photosynthesis found in previous field studies has been attributed to increased photorespiration due to elevated oxygen concentrations (Hough, 1974), but the extremely low values of CO_2^* found in the surface layers of this carbon rich water indicate that, although exacerbated by high oxygen, it was depletion of CO_2^* which caused this effect. Since CO_2^* was more plentiful in the deeper parts of the weedbed (Figure 5.11) it is not surprising that photosynthesis here continued further into the day than near the surface. The diurnal changes of CO_2^* illustrate this, minimum concentrations for the upper layers being found at 13:00 h and the deeper ones at 15:00 h (Figure 5.13). It is likely that light was more limiting for the deeper parts of the bed and CO_2^* for the upper parts, as suggested for *Callitriche cophocarpa* and *Ranunculus peltatus* (Madsen & Maberly, 1991), but it is also apparent that the upper parts of the bed are more active and contribute more to productivity. It is possible that CO_2^* was transferred to the upper from the lower parts of the bed by diffusion or by water movement caused by rising bubbles, but the former is extremely slow and the latter insufficient to affect the gradients which had established. A daily cycle of CO_2^* depletion in the morning and replenishment at night was seen,

especially in the upper layers (Figure 5.13), and is consistent with previous findings that the majority of photosynthesis occurs in the morning (Goulder, 1970; Hough, 1974; Van *et al.*, 1976). A comparison between the profiles taken at 24:00 h and 07:30 h (Figure 5.11) indicate that although the sun had not long risen, the cycle had started once more, particularly at the water surface.

The effect of this daily cycle of high and low CO_2^* on the switch to HCO_3^- uptake is not known, previous investigations having relied on constant conditions (Sand-Jensen & Gordon, 1986; Chapter 4). Since such a cycle is inevitable in the field and plants from this stretch of canal have been found to have the potential to utilise HCO_3^- (Chapter 4), it is probably exposure to low CO_2^* concentrations for longer periods which induces the switch. Although difficult to manipulate, it would be interesting to test this experimentally, since this is likely to be important to the plants in the field.

When compared with the CO_2^* profile from the weedbed, the one from open water was markedly different (Figure 5.11). The CO_2^* concentration at the surface of the open water at 13:30 h was over twenty times that in the weedbed at 13:00 h and was higher at almost every other depth. There was a small indication of epipelic activity at the open site however. Although the weedbeds were depleted of CO_2^* compared with the open water, previous detailed studies of the physico-chemistry of the Leeds and Liverpool Canal have shown that even the open water along this plant-dominated stretch was well below saturation during the summer months (Saednia, 1980; Howard *et al.*, 1984). This stretch of canal is also regularly supersaturated with calcium carbonate at the same time, but crystal poisons prevent precipitation (Saednia, 1980; Howard *et al.*, 1984). Carbon limitation of photosynthesis has been recorded on several occassions (Adams *et al.*, 1978; Hough & Fornwall, 1988; Hough & Putt, 1988; Madsen & Maberly 1991; Rattray *et al.*, 1991; Maberly, 1993) and is typically associated with bed formation (Adams 1985).

As well as the plants, other organisms are affected by the physico-chemical changes in the weedbeds. The extremely reduced flow and mixing must affect non-motile phytoplankton by increased sinking (Lohammar, 1966; Schiemer & Prosser, 1976; Blindow *et al.*, 1993), which has been shown for clay particles (Rowan *et al.*, 1992). This effect may go some way to explaining the reduced levels of plankton found in weedbeds which are often attributed to allelopathy (Hasler & Jones, 1949; Fitzgerald, 196) although no allelopathic mechanism has been found. The high pH climate can adversely affect zooplankton such has *Daphnia longispina*, which actively avoid beds of *Chara*, *Potamogeton lucens* and *E. nuttallii* in the light but not in the dark and do not avoid *P. pectinatus* or plastic plants under either condition (Dorgelo & Heyroop, 1985). Since the former three species all utilise HCO_3^- by acid-alkali banding or polar leaves and the latter do not (Steeman-Nielsen, 1947), it is probably localised extreme pH changes over short distances which cause this behavioural response. Fish, on the other hand apparently benefit from the increased oxygen levels, which counteract the adverse effects of high pH, allowing them to remain and feed within weedbeds (Serafay & Harrell, 1993). It seems that the refuge theory of Timms & Moss (1984) which regarded all plant stands as the same, is too sweeping a generalisation. Rather, it appears that there are several differently functioning groups. Floating leaved plants like water-lilies reduce light, oxygen and pH beneath them (Unni, 1972, Frodge *et al.* 1990) and are avoided by fish (Caffrey, 1993). Elodeids reduce mixing to a greater extent and light to a lesser extent, and increase oxygen and pH (and reduce CO_2^*) in the upper parts. Elodeids in turn can be split on a scale of decreasing effects on pH into three groups; those that utilise HCO_3^- by localised pH increase/decrease (greatest effect), those that use some other mechanism and those that cannot use HCO_3^- (least effect) It is expected that with this scale there will be a corresponding decrease in avoidance by planktonic cladocera. The reduced oxygen concentrations and low light under water-lilies may reduce predation of planktonic cladocera by fish (Timms & Moss, 1984). Further, low resistance to flow of water-lily

petioles may allow greater exchange with the open water , and enhanced the effect of filtration by attached crustacea, such as *Sida* and *Simocephallus*. In conclusion, perhaps it is the reduced mixing, as much as the increased grazing, which leads to reduced phytoplankton numbers in lakes dominated by submerged plants.

It is apparent that at this time the major constraint on the photosynthesis of *E. nuttallii* growing in the Leeds & Liverpool Canal was reduced availability of CO_2^*. The extremely low water movement within the weedbed enabled such conditions to develop, and will have caused the plants to have large boundary layers. Although the deeper parts of the bed were less affected by CO_2^* depletion, and probably limited by light, they contributed little to overall production. This situation is likely to be typical of elodeid plants growing in moderately hard, slow-flowing or standing waters, and as such it is through adversely affecting the supply of CO_2^* that epiphyton is most likely to exert its influence. Further, if the plants are growing in a compact weedbed, diffusion distances are likely to be great and epiphyton increased boundary layers of reduced importance. When the plants are short relative to the water column, and at a low density, both the increase in shading and boundary layer thickness caused by epiphyton will become of increased importance.

Chapter 6.

Microelectrode Investigations.

Whereas previous chapters have all dealt with changes affecting the plants at a macroscopic level, the close association between submerged macrophytes and their epiphyton (Allanson, 1973) dictates that interactions will occur on a far smaller scale (Allen, 1971; Wetzel & Allen, 1972; Wetzel, 1983b). To fully understand the nature of these interactions, it is necessary to investigate changes that occur within the boundary layer. The effect that epiphyton has on these conditions is vitally important and needs to be characterised so as to understand the nature of the interaction.

This chapter will report on a series of investigations using a microelectrode apparatus, with probes of approximately 5 μm tip diameter and a resolution of 0·01 pH unit, specially constructed to probe the boundary layers surrounding the leaves of *E. nuttallii*. The chapter is divided into three sections, dealing with:

1. the construction and development of the microelectrode apparatus,
2. the boundary layers surrounding epiphyte-free leaves of *E. nuttallii*,
3. the modifications to the boundary layer caused by an increasing layer of epiphyton.

Thus a largely descriptive account of the conditions immediately surrounding the plant on its own are separated from a more analytical investigation into the effect of a nutrient-mediated increase in epiphyton. In this way a detailed picture will be presented of the previously unknown conditions within the boundary layer, before epiphyton is introduced into the system. The technical details are dealt with in the first section.

Section 1.

Development and Construction of the Microelectrode Apparatus.

6.1.1 Introduction

In recent years there has been a growing concern over the question of scale in ecology, especially in aquatic systems (Giller *et al.*, 1994) where the water has long been considered to provide a homogeneous environment. This has led to investigations being made at the microscopic level into systems which had previously only been looked at macroscopically.

The problem of slow diffusion in water, not offset by the often smaller diffusion boundary layer thicknessin water than in air, has led to several investigations into the water immediately surrounding, and within, algal layers and sediments. The pioneering work in this field was conducted by Niels Peter Revsbech, Bo Barker Jørgensen and co-workers, who developed microelectrodes sensitive to oxygen (based on the Clark-type design – tip diameter ≈ 5 μm), sulphide (tip diameter ≈ 100 μm) and pH (pH glass extended tip – diameter ≈ 50 μm, length of sensitive area ≈ 200 μm). With these electrodes, various chemical gradients caused by photosynthesis and respiration have been measured in mats of cyanobacteria (Jørgensen *et al.*, 1979; Revsbech *et al.*, 1983; Jørgensen *et al.*, 1983; Revsbech & Ward, 1984), sulphur bacteria (Jørgensen & Revsbech, 1983; Revsbech & Jørgensen, 1986), hot-spring algae (Revsbech & Ward, 1983), epiphyton (Sand-Jensen *et al.*, 1985; Sand-Jensen & Revsbech, 1987) and benthic marine algae (Revsbech & Jørgensen, 1983 & 1986). Marine and estuarine sediments have also been investigated (Revsbech *et al.*, 1980a &b; Revsbech *et al.*, 1981; Revsbech, 1983; Jørgensen & Revsbech, 1985; Revsbech & Jørgensen, 1986; Revsbech, 1989), as has the rhizosphere of certain submerged macrophytes (Caffrey & Kemp, 1991). Also of note is the work by Carlton & Wetzel (1987), who used oxygen microelectrodes of the Revsbech-Jørgensen modified Clark-type to investigate conditions within freshwater epiphytic, epilithic and epipelic algal communities. So far all these investigations have been conducted with field samples brought into the laboratory, though there are moves to adapt such apparatus for field use (D. Thomas, pers comm.).

Of particular interest to this project are three studies performed on the epiphyton communities growing on submerged aquatic plants (Sand-Jensen, *et al.* 1985; Carlton & Wetzel 1987, Sand-Jensen & Revsbech 1987). Here the oxygen profiles surrounding individual whole leaves with attached algae, excised from field grown plants collected at different times of the year, were measured under different light intensities, at different times after the onset of illumination (Sand-Jensen *et al.*, 1985; Carlton & Wetzel, 1987; Sand-Jensen & Revsbech, 1987). The transfer of substances between the bulk water and layers of periphytic algae of varying thickness attached to glass slides has also been investigated using radioactive tracers (Riber & Wetzel, 1987).

The changes within the boundary layers surrounding aquatic macrophytes without attached epiphyton have been studied very little. There is merely one specific physiological study of pH (Lucas *et al.*, 1983), using a recessed-tip microelectrode (Thomas, 1978) on *Chara corallina* and two brief descriptive studies of oxygen using *Zostera marina* L. with epiphytes removed (Sand-Jensen *et al.*, 1985) and of *Scirpus subterminalis* uncolonised by algae (Carlton & Wetzel, 1987). There remains much work to be done on epiphyte-free submerged macrophytes.

Although these works have provided a valuable and beautiful, insight into the micro-environment in which aquatic plants and their associated epiphyton live (Sand-Jensen *et al.*, 1985; Carlton & Wetzel, 1987; Sand-Jensen & Revsbech, 1987), workers have not taken advantage of microelectrode technology to investigate in a unique way the largely unexplained nature of the interaction between the plant and epiphytic algae.

6.1.2 The Microelectrode System.

6.1.2.1 Theory.

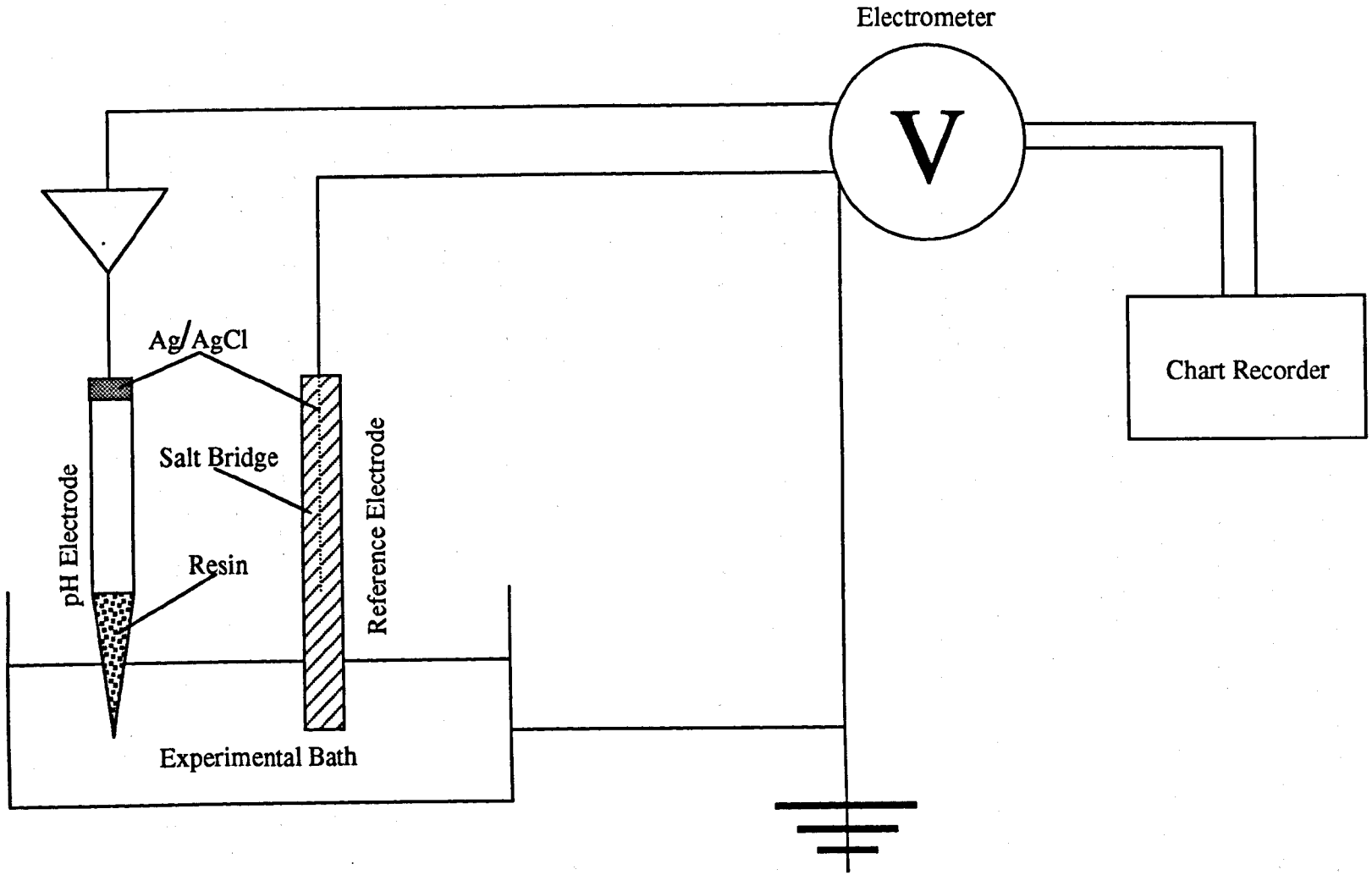
The theory of ion-selective microelectrodes, which have for some time been used in intra-cellular studies, is dealt with by Koryta (1981) and the practical design and application by Thomas (1978) and Thomas & Moody (1980). Since the publication of these articles, however, the technology has advanced somewhat and the ion selective resins now available are more responsive, less sensitive to interference from other ions and far easier to use (Fluka, 1988). The result is that improved electrodes represent an extremely powerful tool for use in plant physiology (Felle & Bertl, 1986).

6.1.2.2 Components.

Put simply, the microelectrode system consists of an earthed electrical circuit where the largest resistance occurs across a resin contained within a glass capillary (Figure 6.1). The resistance of this resin, hydrogen-ion ionophore II cocktail A (Fluka Chemicals, Buchs, Switzerland), changes with the concentration of H^+ ions to which it is exposed (57·1 ± 0·8 mV per pH unit – manufacturers specifications). For pH determination purposes this change is measured by an electrometer (Dual Differential Electrometer FD 223, World Precision Instruments, Hastings) and recorded on a flat-bed chart recorder (CR500, JJ Instruments, Southampton).

A current, which must be exceedingly small so as not to cause disruption of the preparation ($\approx 10^{-15}$ A, supplied by the electrometer), must be applied to the circuit before the potential difference can be measured. This current is far too small for the electrometer to measure the voltage accurately, so a preamplifier, which amplifies the current and not the voltage (± 0·1 %, manufacturers specification), is built into the probe supplied with the electrometer to which the electrode is attached. By "taking the amplifier to the electrode" the need for long, noisy cables carrying minute currents, where potential can be lost, is

Figure 6.1 Circuit diagram of Microelectrode Apparatus.



obviated. The connection between the ion-sensitive electrode (the capillary-containing resin) and an earthed reference electrode is bridged by the bathing medium, completing the circuit (see Figure 6.1).

The WPI dual differential electrometer is designed for intracellular use, with two matched input probes, supplied with the electrometer, one set up to measure the membrane potential, the other measuring both the membrane potential and the effect of changing ion concentration. The difference between these two measurements is calculated within the equipment to compute the ion concentration. With the equipment set up as described to measure extracellular ion concentrations, no membrane potential is encountered and so only one probe and channel is required. With the settings properly adjusted, the equipment is perfectly suited to such use (WPI FD 223 Instruction Manual, page 6).

6.1.2.3 Microelectrode Construction.

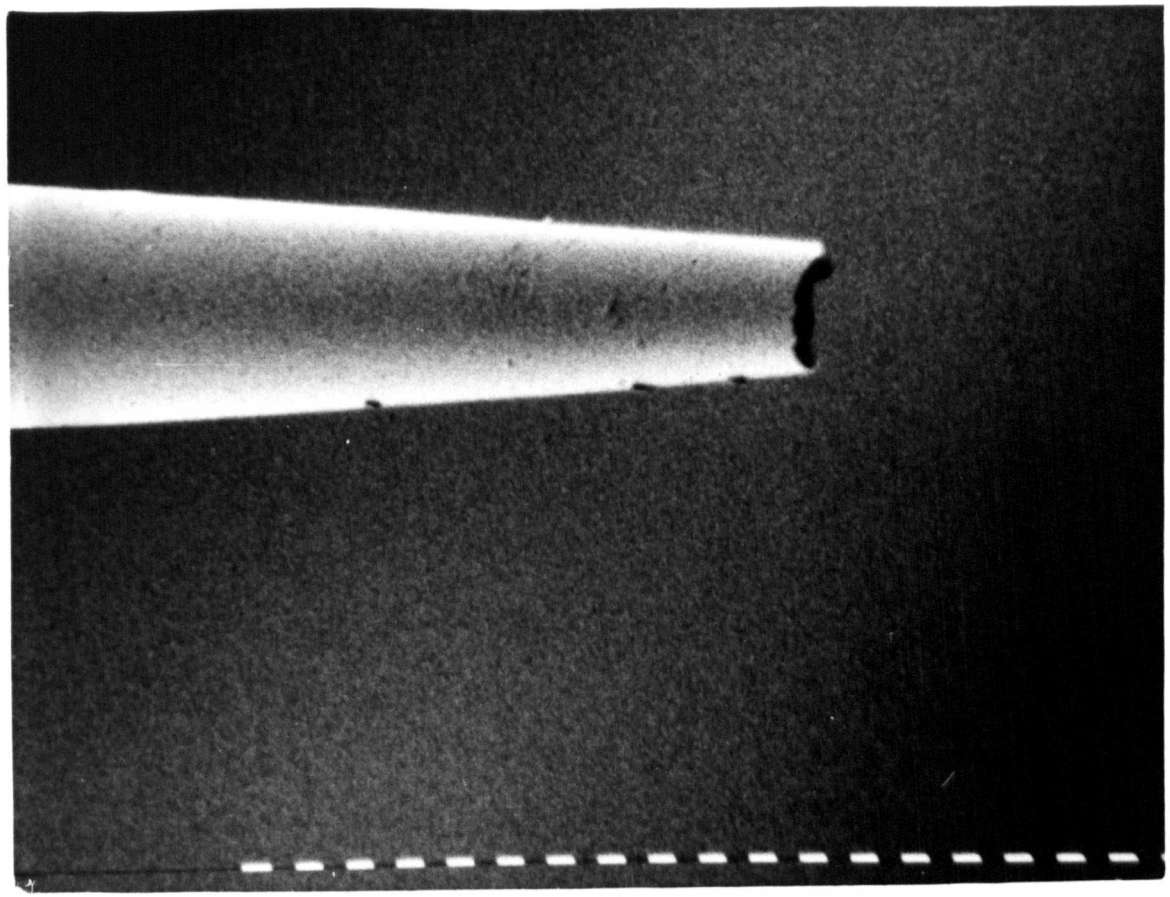
The microelectrodes were constructed from glass micropipettes shaped and treated in the laboratory then filled with the appropriate ion-sensitive resin thus.

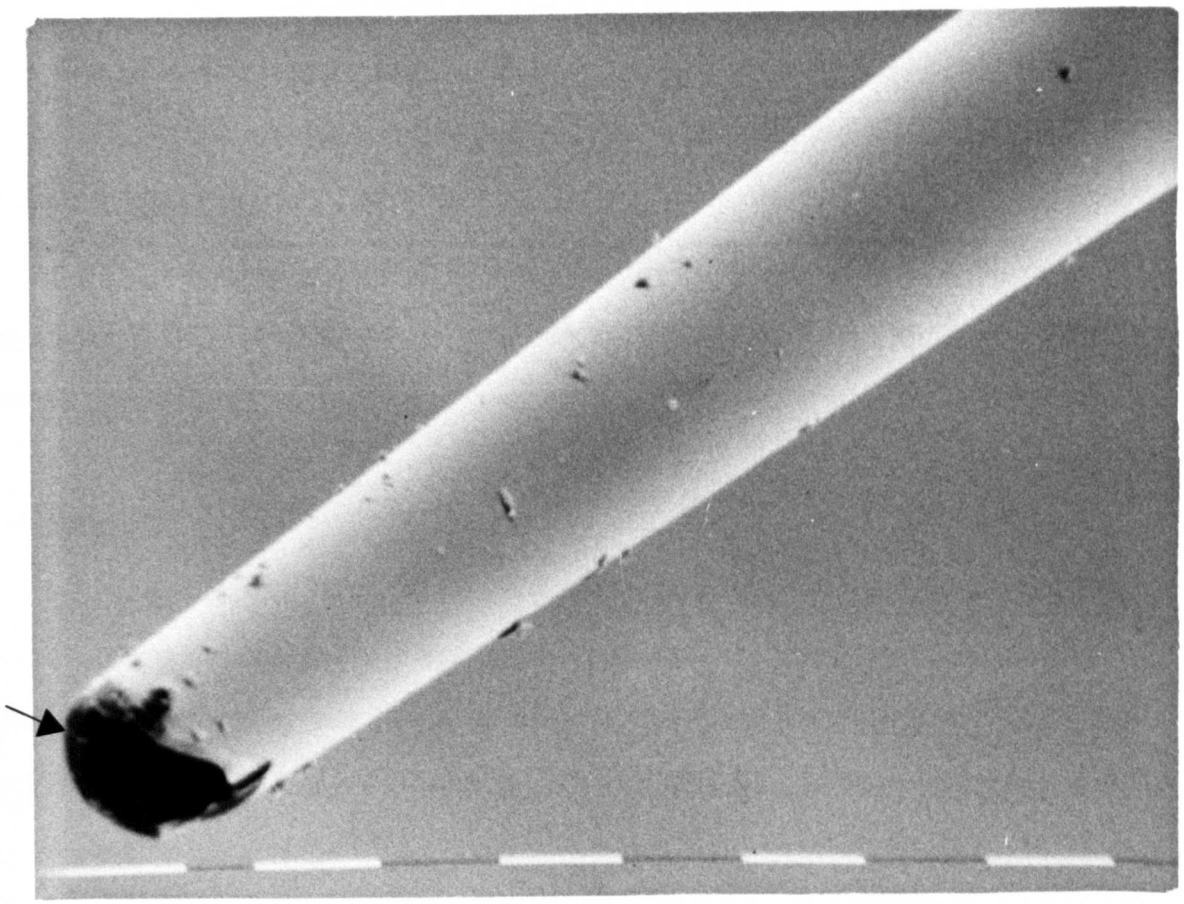
i) Pulling.

The electrodes were made from 1 mm external diameter borosilicate glass capillaries containing a solid filament. These were pulled to a fine point using an Industrial Science Associates (Ridgewood, N.Y., USA) M1 horizontal, two-stage, magnetic puller, with nichrome wire as a heating element. Adjustments were made to the strength of the pull by trial and error, until a tip of appropriate size and shape was achieved as verified by light microscopy. The ideal shape for a micropipette was a body of unaltered capillary 1 mm external diameter, 40 mm long for ease of filling and back-filling (see below), followed by a rounded shoulder and a shank 7-10 mm long, tapering to the tip. This shank, produced by pulling, was not to be too thin and hence too flexible, nor too short. The puller settings

Plate 16 An unfilled microelectrode tip. Scale bar = 1 μm.

Plate 17 An unfilled microelectrode tip showing the glass filament (marked) which extended to the tip of the micropipette. Scale bar = 5 μm.





were modified when glass from a different source was used, but all capillaries used produced equally good micro-pipettes.

The size of some of the electrode tips was checked with the SEM, which showed that the tip diameter was of the order of 5 μm (Plates 16 & 17).

ii) Salinization.

A glass petri-dish, containing two glass microscope slides on top of each other, was put into an oven within a fume-cupboard and the micro-pipettes were carefully placed on top of the slides at right angles to the long axis, about 20 at a time. A small (≈ 15 mm long) aluminium boat was also placed in the petri-dish. The oven was then allowed to heat up to 200 °C, with the lid of the petri-dish in the oven but the dish not closed. After 1 hour at 200 °C, to ensure complete dehydration of the micro-pipettes, the lid was put on the petri-dish. Then, one side of the petri-dish lid was gently lifted and a long pasteur pipette was used to fill the aluminium boat with dimethyldichlorosilane, without allowing much of the vapour to escape. The petri-dish lid was replaced and the oven left at 200 °C for a further 30 minutes allowing the dimethyldichlorosilane vapour to deposit Si onto all the free radicals in the glass, thus rendering it hydrophobic. The oven was then turned off and allowed to cool.

The hydrophobic glass gripped the resin, which was also hydrophobic, far better than untreated glass and encouraged the resin to move right to the tip of the capillary (Munoz *et al.*, 1983).

iii) Filling.

The micro-pipettes were gripped with a pair of clamp forceps, bent so that there was sufficient room between the teeth to hold a capillary without breaking it, and filled with the ion-exchange resin by injection from a hypodermic syringe equipped with a 40 mm long, 30 gauge needle. The needle was used to inject the resin as far as possible into the

micro-pipette, usually to the shoulder where the glass narrowed. The micro-pipettes were then stored, tip downwards, in a desiccator and the resin was allowed to slip into the tip of the electrode by gravity. The glass filament, already attached inside the capillary, aided in this process, acting in much the same way as a cat's whisker, encouraging the resin downwards and air bubbles upwards. The glass filament continued right to the tip of the micro-pipettes (Plate 17), a good sign indicating that the resin would fill completely to the tip.

The bent clamp-forceps were used at all times when working with the electrodes, making manpulation of them easier and reducing the risk of dropping them and breaking the tips, this being the largest cause of loss.

iv) Storage.

Two methods of storage were used. After pulling and before salinization the micro-pipettes were stored in a petri-dish with strips of foam rubber attached to both the base and lid, such that they were firmly gripped between the foam when gentle pressure was put on the petri-dish (by hand or a rubber-band). This allowed the micro-pipettes to be carried easily to and from the oven. Once they had been salinized, the micro-pipettes were held vertically to the side of a glass beaker with Blu-Tack (Bostik, Leicester), tip downwards, and stored inside a desiccator until filling. After filling with resin they were similarly secured and stored until required.

v) Backfilling and mounting.

When required, a number of resin-filled micro-pipettes were taken from the desiccator and back-filled with 0·1 mol l^{-1} KCl solution, using a hypodermic syringe equipped with a 40 mm long, 30 gauge needle, making sure that the solution was in contact with the resin and that there were no bubbles in the lumen of the capillary. The micro-pipettes were again stuck to the side of a beaker with Blu-Tack, and the beaker filled with

distilled water so that the tips were submerged. The beaker was covered with aluminium foil to prevent dust entering and left overnight (at least) for the electrodes to mature before use.

The electrodes were connected to the amplifier, and hence the recording equipment, with a purpose built holder (MEH3SF, WPI, Hastings). This consisted of a perspex cylinder with an approximately 1 mm bore into which the blunt end of the electrode was inserted. At one end of the bore was a silver-silver chloride plug attached to a copper electrical connection (which clips onto the amplifier); at the other was a rubber gasket which gripped the electrode. A threaded nut was supplied to compress the gasket, tightening the grip, but this was not used as in trials it tended to pressurise the electrode and force resin from the tip, deleteriously affecting the signal from the electrode. When mounting the electrode, the holder was completely filled with 0·1 mol l^{-1} KCl solution with a hypodermic syringe. The rear of the electrode was then inserted into the bore, care being taken to ensure that no bubbles were trapped anywhere. As a precaution to ensure that no dirt was present in either the holder or the electrode, both were rinsed throughly with clean KCl solution before assembly.

Once the holder was attached to the amplifier, the electrode was ready for use.

vi) Tip diameter and resistance.

The electrometer was equipped with a test facility, which allowed the resistance of an electrode to be determined by passing a current of approximately 1pA through the system. This produced a shift in the voltage reading of 1 mV per GΩ. Stabilization of the signal took some time, dependent on the resistance. Bubbles or other blockages within the electrode, which caused the resistance to be enormous, could be detected in this way. Such affected electrodes had a severely impaired response and were discarded . For good electrodes this test allowed an approximate measure of the tip size, as the resistance of

electrodes is inversely proportional to the tip diameter. Only electrodes with a resistance greater than 10 GΩ, corresponding to a tip diameter of 5 μm, were used.

6.1.2.4 Reference Electrode Construction.

The reference electrode completes the circuit between the electrode, the medium and the electrometer (see Figure 6.1), and as it is made of siver/silver chloride is reversible. This means that current can flow in either direction through the circuit, a vital condition for the ion-sensitive electrode to function properly (Thomas, 1978). The reference electrode was connected to the medium via a salt bridge and was constructed as follows.

A short (2-3 cm) piece of 0·25 mm diameter silver wire (Clark Electromedical, Pangbourne, Berks.) was soldered to a female, medium-sized, jack-plug connector. After gentle cleaning with emery paper, the silver wire was chlorided by electrolysis as follows. Two jack-plug/silver wires were attached by means of short wires to a 9 V battery, one to the + terminal and the other to the – terminal. Both silver wires were then dipped into a weak solution of KCl (≈ 0·01 mol l^{-1}), taking care not to wet the solder or the copper of the connector. After 1-2 minutes the wires were removed from the KCl, the attachments to the battery were exchanged, thereby reversing the current, and the wires returned to the KCl for a further 1-2 minutes. This process was repeated until a layer of grey silver chloride had built up on the wires.

The salt-bridge was made from Agar with 5% KCl, which whilst molten was drawn into a 1 mm lumen diameter polyethylene tube with a glass syringe. The chlorided silver wire was then fully inserted into this tube and excess molten agar/KCl mix, which had been previously drawn into the syringe, was forced down the tube, expelling all bubbles. Any agar which had extruded from the tube was gently removed once it had hardened, and the tube was fixed in place with epoxy resin (Araldite Rapid, Ciba-Giga Plastics, Cambridge). The polyethylene tube was cut to the required length, leaving about 1-2 cm from the end of the silver-silver chloride wire to the tip. The electrode was then

ready for use, but was found to be more effective if aged for a day or two with the tip in distilled water prior to use. Having a female connector attached to the reference electrode and a male jack-plug attached to the linking wire allowed easy change of electrodes. When not in use, the reference electrodes were stored with their tips dipped in distilled water.

This form of electrode, though referred to as the reference electrode, is equivalent to the bath electrode used by workers using microelectrodes for intracellular studies (*cf.* Thomas, 1978). Since in the present study no membranes were pierced, no transmembrane potentials were involved, and investigations with an electrode filled with KCl showed that no surface potentials were present, a "true" reference electrode of the type described by Thomas (1978) was not required.

6.1.2.5 Oxygen Microelectrode.

A concerted effort was made to construct an oxygen microelectrode following the miniature Clark-type design of Revsbech (1989), but problems were encountered in the final assembly of the constructed components. Since so little research had been done with pH microelectrodes in aquatic situations, and from pH measurements it is possible to infer much about the limitations on photosynthesis, it was decided to concentrate on these electrodes, which had been successfully built and were working well.

6.1.2.6 Faraday Cage and Experimental System.

As the electrodes used had resistances in the Gigaohm range (Ω^{12}), they were extremely sensitive to static charge. A vibration-free environment was necessary in view of the delicate equipment and precise positioning required for the flexible electrode tips. Hence, the equipment was set up in a vibration-free Faraday cage, in a quiet basement room. The cage was constructed from a square, 26 mm thick steel baseplate, with sides 75 cm long, onto which was screwed a square, 1 m high box of 1mm thick steel. The front of the box had hinged, lockable doors. Whilst the baseplate was not painted to improve

earthing, the rest of the interior surfaces were painted matt black to reduce reflection of light and the outside surfaces painted to prevent rusting.

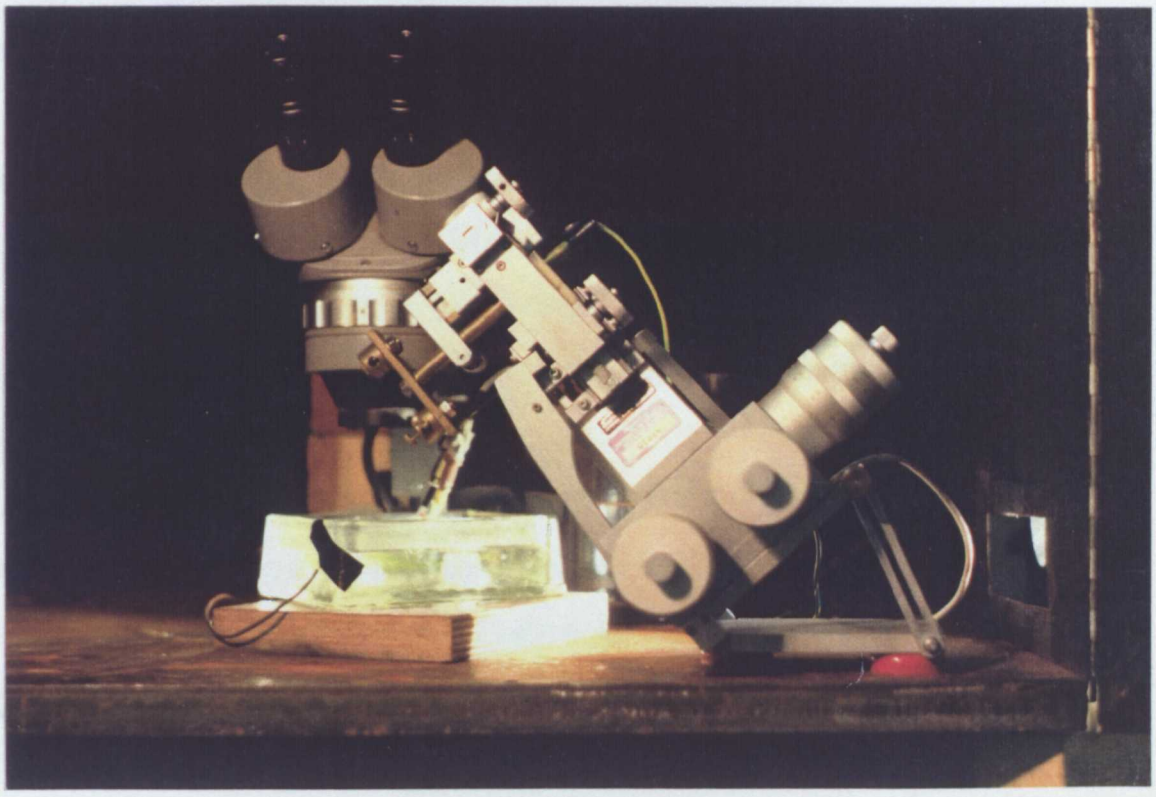
To the inside back wall of the cage a heavy brass bar (15 cm x 3 cm x 0·6 cm), fitted with brass terminals for connecting wires, was attached by means of two large brass screws. This was then connected to earth, and every item within the cage efficiently earthed to it, including the baseplate and the walls of the cage itself. If a component consisted of more than one part which could possibly have been isolated from one another, then each component was connected to earth separately.

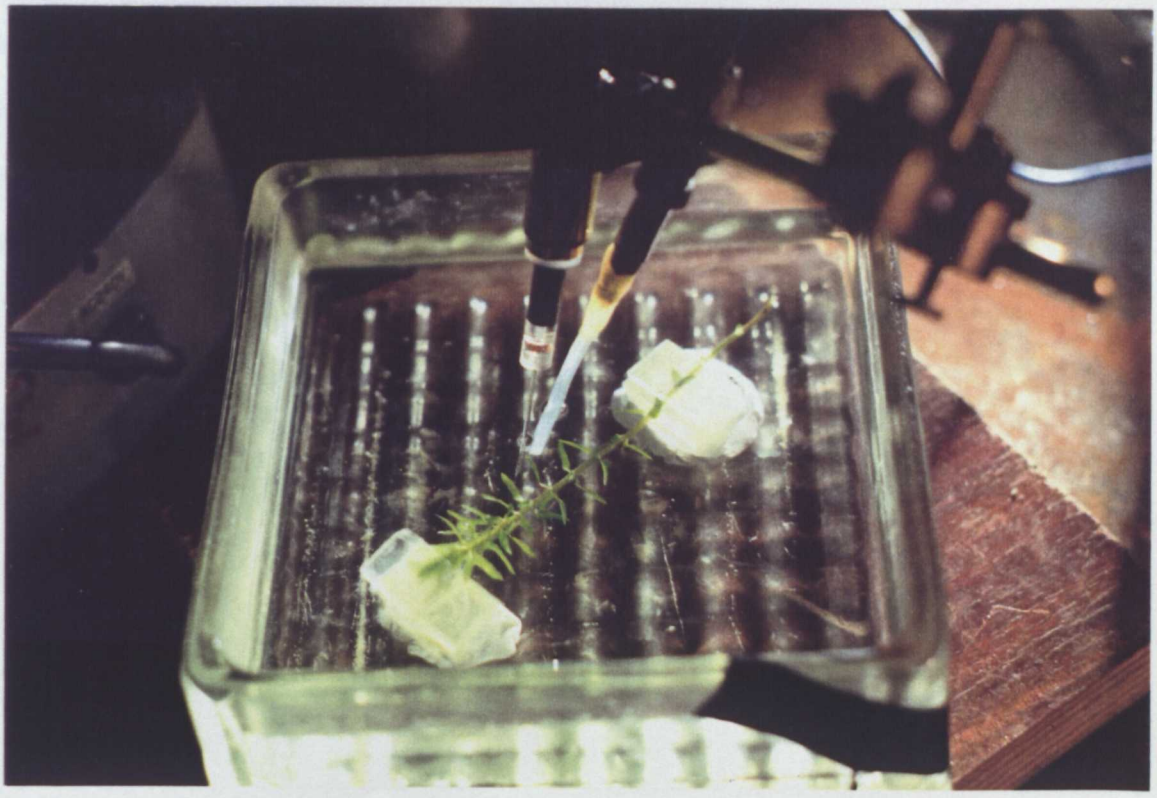
Two holes were cut in the cage walls. A small round one (≈ 3 cm diameter), allowed passage of the leads connecting the two electrodes to the electrometer and a large oblong one (16 cm x 5 cm) enabled the experimental bath to be illuminated from an external source. A projector was used as a light source. Since swiching the projector, or any other appliance connected to the ring circuit in the room, on or off caused extreme disturbance to the microelectrode apparatus, taking a minute or more to return to equilibrium, a light-proof, black polythene flap was attached to the outside of the cage covering the oblong hole. This could be raised to allow illumination of the plant and lowered to provide darkness, with no need to switch the projector on or off.

Inside the cage was a glass bath filled with twice-filtered canal water, in which the plants for investigation were incubated. This was positioned so as to recieve an incident irradiance of approximately 300 μmol m^{-2} s^{-1} PAR. The plants were gripped gently but firmly in home-made, acrylic holders, 1-2 cm tall. Silicone vacuum grease (Glisseal, Borer Chemie A.G., Zuchwil, Switzerland) was used to keep the holders closed and to stick them to the base of the bath. A binocular dissecting microscope (Kyowa, Tokyo, Japan) was positioned over the bath for observation and to allow careful positioning of the electrode. The pH and reference electrodes were attached to a short brass rod by means of a specially designed grip and Blu-Tack respectively, and the rod fastened to a Huxley-type, 3-direction manual micromanipulator with an adjustable angle stage (Campden Instruments Ltd.,

Plate 18 Microelectrode apparatus in working position, showing the binocular microscope, experimental bath, micromanipulator holding electrode, steel base-plate and the hole through the side of the cage to provide illumination.

Plate 19 An *E. nuttallii* shoot clamped in the experimental bath with the acrylic holders, the pH microelectrode and reference electrode in position for taking a measurement.





London). The micromanipulator was connected firmly to the baseplate by means of magnetic feet.

It was decided not to attempt to control the temperature of the bath containing the experimental plant material for the following reasons,

i) the friction caused by cooling/heating water flowing through a tube induces static to gather which would have had to have been controlled,

ii) it would have required the design and construction of a special temperature controlled bath,

iii) the temperature in the basement room used was very stable: in a test, 6 hours of illumination with the projector caused an increase of only 1 °C from a starting 20 °C.

The temperature in the bath was monitored with a mercury thermometer during experiments to detect any major variations.

Once all the components were positioned in the cage, three taxi-tyre inner-tubes were carefully positioned under the cage, which was already raised on wooden blocks. The inner-tubes were then inflated with a hand-pump until the cage lifted free from the blocks. A process of deflation, repositioning and reinflation was repeated until the baseplate was horizontal at equilibrium (positioned with a spirit level). The working position is shown in Plate 18. The shear weight of the cage (>75 kg), supported pneumatically, removed virtually all vibrations transmitted through the floor or supporting bench.

6.1.3 Calibration.

Since no work of this nature had been done before, a standard protocol for the calibration of electrodes had to be established. It was also necessary to test that the electrodes functioned well in the filtered canal water, a complex ionic solution which was used as the experimental medium.

6.1.3.1 Method of Calibration.

A special piece of apparatus was constructed to assist in the calibration of electrodes. This consisted of a funnel attached to an S-shaped glass tube, which was attached to the side of a beaker (Figure 6.2). During calibration, the pH and reference electrode were positioned within the funnel and the funnel was filled with the required buffer. When the time came for the buffer to be changed, the new buffer was added dropwise to the funnel until the S-bend overtopped and all the liquid siphoned into the beaker. This was repeated several times with the new buffer, to remove all traces of the previous one, before filling the funnel without overtopping it. The electrode was left in each buffer for approximately 10 minutes to ensure that the signal was stable, as seen on the chart recorder, and the reading from the digital display was then noted (Figure 6.3). The beaker was emptied of waste buffer at intervals. All the components of this apparatus were connected to the brass earthing bar.

In order to reduce interference due to static during the calibration process, the doors of the cage were closed once the buffer had been changed.

6.1.3.2 Results of Calibrations.

The manufacturers of the ion selective resin recommend that calibration is undertaken in a fairly concentrated solution, with tris buffer present at a concentration of 0·01 mol l^{-1} (Fluka, 1988). Hence, the first calibration of an electrode was done in strong buffer solutions, made up according to the Rothamsted specifications (Table 6.1). These

Figure 6.2 Apparatus for calibration of Microelectrodes.

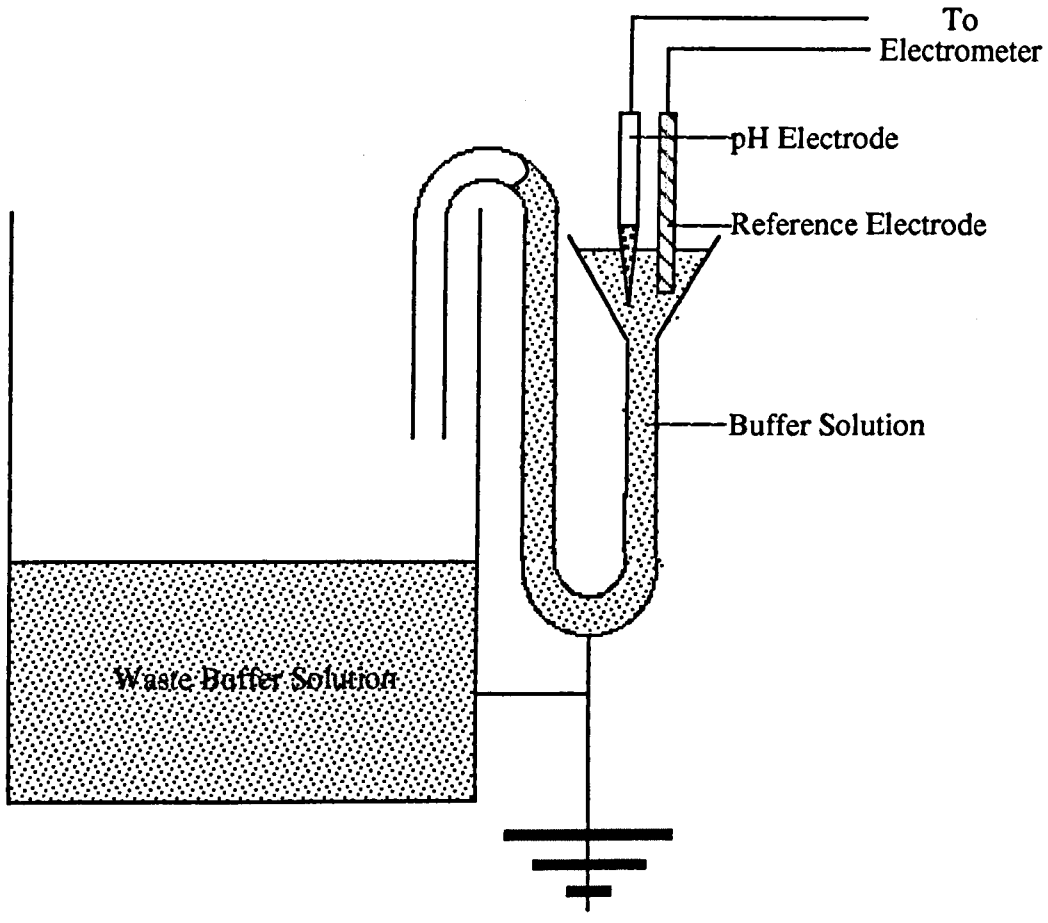


Figure 6.3 A typical calibration trace using the Rothamsted solutions diluted thirty times.

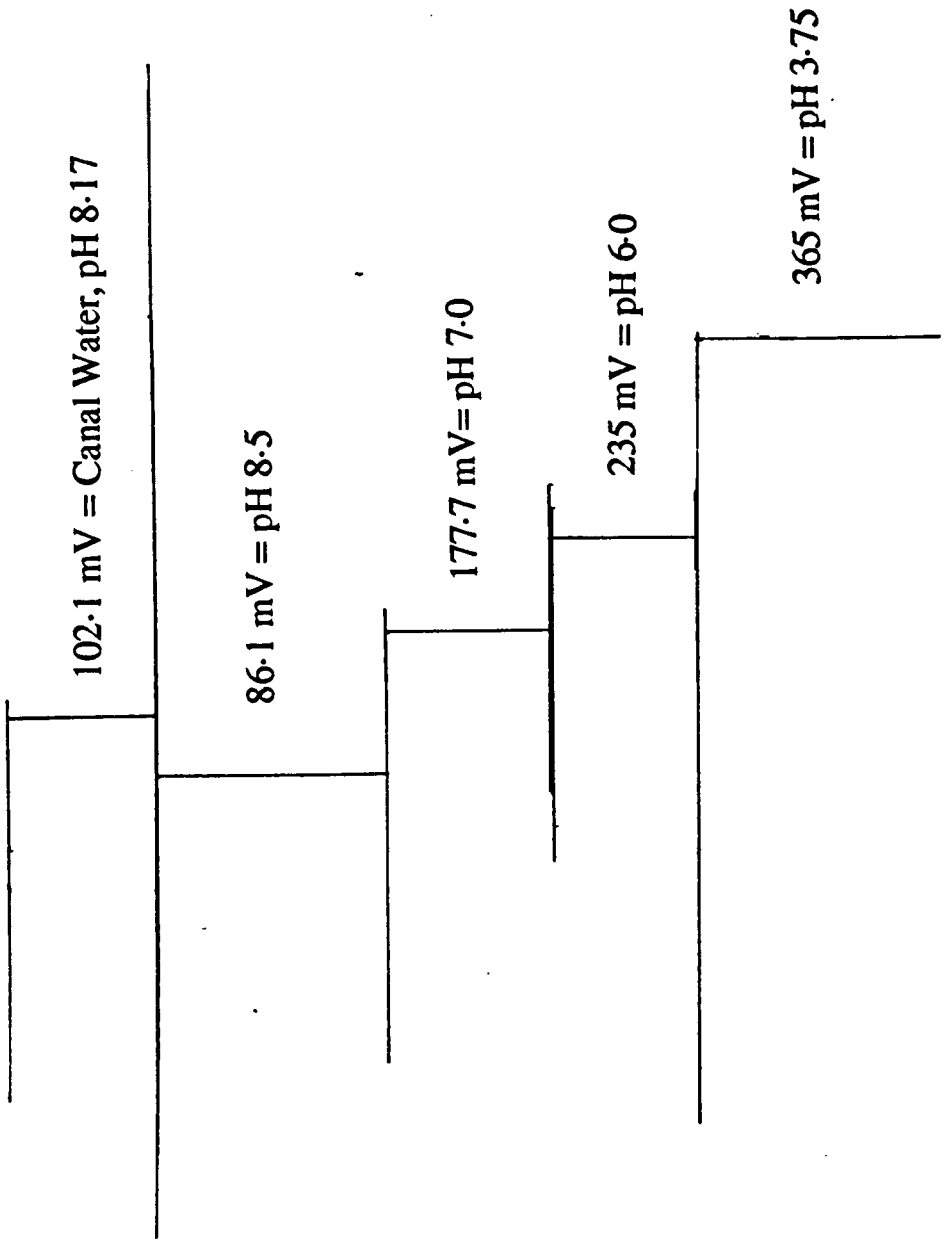


Chart recorder paper speed = 2 mm min^{-1} and full scale deflection = 1 V

Equation calculated from this trace for the diluted Rothamsted solutions

is y = 584·05 – 58·35x, R^2 = 1·00

Back calculated for filtered canal water

is y = 578·84 – 58·35x

Table 6.1 Composition of Buffer Solutions.

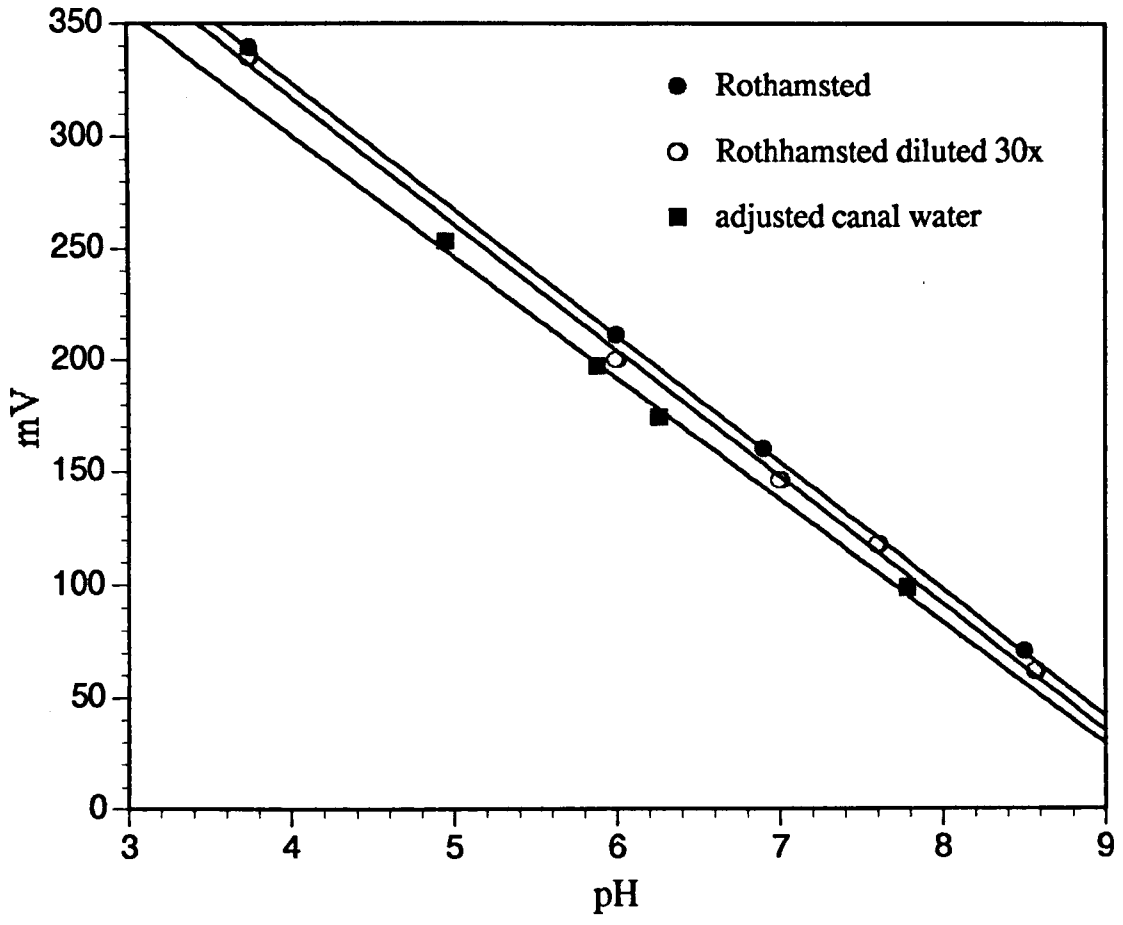
The recipe used for making these solutions is based on that used by A.G. Miller and co-workers at Rothamsted Experimental Station (pers. comm.). Concentrations are in mmol l^{-1}.

	pH 3·75	pH 6·00	pH 7·00	pH 8·50
Buffer (type)	MES	MES	HEPES	TRIS
(concentration)	20	20	20	20
KCl	120	120	120	120
$NaH_2PO_4.2H_2O$	10	10	10	10

Once mixed, the pH of the solutions was adjusted to the appropriate level with HCl or NaOH solutions.

The solutions corresponding to these recipes are referred to in the text as Rothamsted solutions. However, as the ionic activity of these solutions was far greater than that of canal water, they were diluted by thirty times after mixing to give a working concentration. The pH of the solutions was always checked immediately before use, and corrected if necessary.

Figure 6.4 Calibration curves of an electrode in different buffer solutions



The equations for the lines are,

Rothamsted,	$y = 551.18 - 56.65x$, $R^2 = 1.00$
Rothamsted diluted 30x,	$y = 544.50 - 56.61x$, $R^2 = 1.00$
adjusted canal,	$y = 518.94 - 54.43x$, $R^2 = 1.00$

From analysis of covariance there is no difference between the slopes,

$F = 2.77$ $F_{0.05(1),2,7} = 4.74$

but there is a significant difference between the intercepts,

$F = 88678.6$ $F_{0.05(1),2,9} = 4.29$

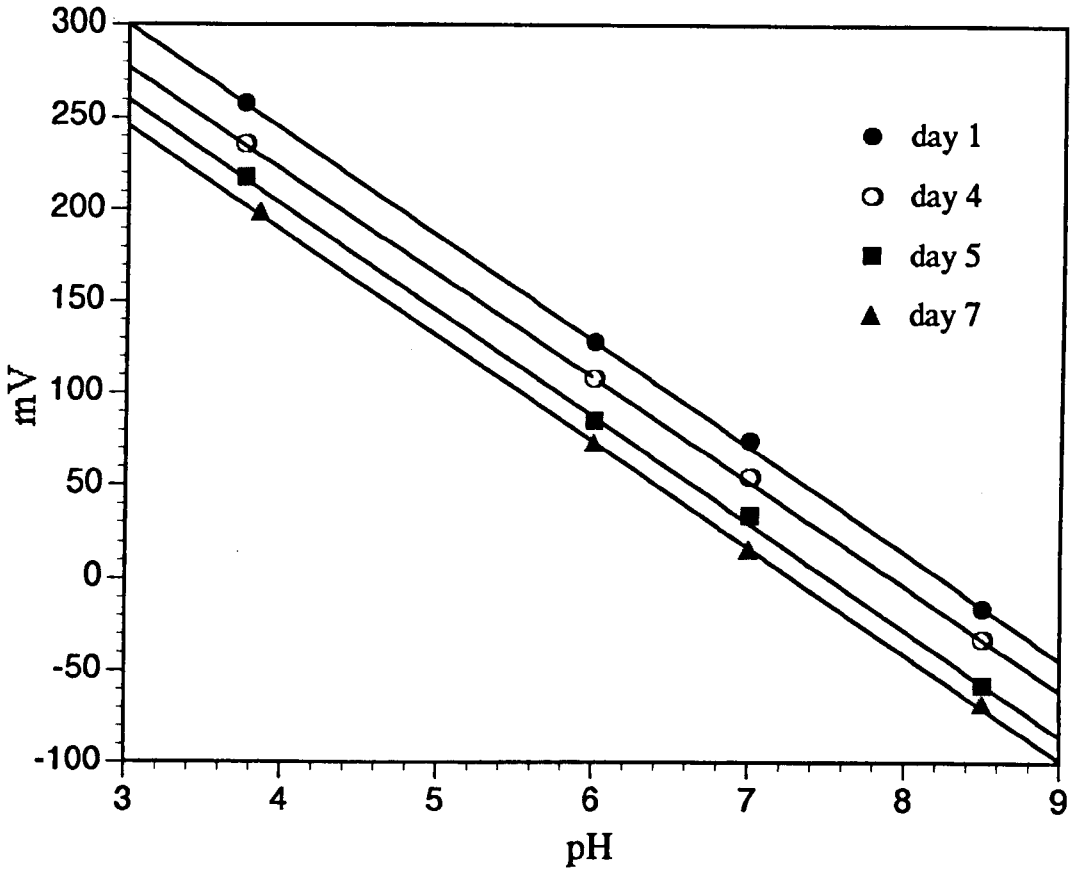
solutions produced a calibration curve with a gradient of 56·65 mV pH^{-1} (Figure 6.4). However, the ionic activity of the solution used is known to affect the output of microelectrodes such as these (Thomas, 1978). As it was intended to use filtered canal water as the experimental medium these Rothamsted solutions were diluted 30 times, giving them a conductivity, used here as a simple measure of ionic activity, similar to that of the filtered canal water. A calibration curve with a gradient of 56·61 mV pH^{-1} was obtained, but with a different intercept, 544·5mV (Figure 6.4). A final calibration was made in filtered canal water with the pH adjusted by small additions of weak HCl or NaOH solutions (Figure 6.4). Here the gradient was 54·43 mV pH^{-1} and the intercept 518·94 mV.

It is obvious from these three calibrations that large changes in the ionic activity of the buffer solutions, either due to changes of concentration or composition, lead to changes in the intercept determined by the overall resistance of the circuit ($p < 0·05$ from analysis of covariance), but not in the gradient determined by the response of resin to H^+ ($p > 0·05$ from analysis of covariance).

The adopted protocol was to calibrate each electrode in 30-times diluted Rothhamsted solutions, which gave the gradient of the equation. Then, to rinse the electrode and dip it into the filtered canal water in the experimental bath, whilst measuring the pH with a portable field meter (CD66, WPA Scientific Instruments, Saffron Walden, Essex). Using this measured value of pH for the water, together with the calculated gradient and the readout from the electrometer with the electrode in filtered canal water, the intercept was back-calculated. Also, as the projector or any other electrical appliance caused a drain on the ring circuit to which the apparatus was connected which affected the output from the electrometer, the projector was switched on during calibration.

The response to H^+ of this particular ion-selective resin is very rapid, ≈0·6 s to 90% response (Fluka, 1988). An example of a typical calibration trace, indicating the rapid response, is given in Figure 6.3. Since a large pH range was covered, the chart recorder

Figure 6.5 The effect of age on the response of an electrode.



The equations of the lines, fitted by regression, are,

Day 1	$y = 472.75 - 57.42x$	$R^2 = 1.00$
Day 4	$y = 446.48 - 56.37x$	$R^2 = 1.00$
Day 5	$y = 432.86 - 57.62x$	$R^2 = 1.00$
Day 7	$y = 417.81 - 57.39x$	$R^2 = 1.00$

From analysis of covariance there is no difference between the slopes,

$F = 0.801$ $\quad F_{0.05(1),3,8} = 4.07$

but there is a difference between the intercepts,

$F = 497.14$ $\quad F_{0.05(1),3,14} = 3.34$

was set at 1·0 V full-scale-deflection during calibration, but for experimental determinations was used at 100 mV full-scale-deflection for increased accuracy.

The effect of ageing on the electrodes was seen by recalibrating the same electrode, at the same time each day, over a number of days. A steady decline in the intercept was seen ($p < 0{\cdot}05$ from analysis of covariance), but again the gradient was very stable ($p > 0{\cdot}05$ from analysis of covariance – Figure 6.5).

From the above work it can be seen that four factors controlled the potential difference over the circuit, namely:

i) the response of the ion-selective resin to H^+ concentration,

ii) the medium used, both its composition and concentration,

iii) the resistance of the components within the system (especially the tip diameter of the pH electrode and the length of the salt bridge of the reference electrode),

iv) the age of the electrode used.

Of all these parameters, the effect of changing H^+ concentration was most dynamic and considered to be by far the most important. Although changes in other ions do alter the potential difference, their effect on the resin is slight, it being highly selective for H^+ ions. To detect any effects of ageing or damage, each electrode was calibrated immediately before and after use to determine if there had been a shift in the gradient/intercept. The mean of the two readings was used if this was the case.

6.1.4 Measurements of pH.

6.1.4.1 Method

A bright green, healthy *E. nuttallii* shoot carefully selected from a recent collection of plants, was clamped at tip and base to the glass bath by the acrylic holders. The bath was then put in place, earthed and filled with filtered canal water. The pH electrode was positioned near to a leaf such that the manipulator controlled approach would be at right angles to the leaf surface, by viewing through the binocular microscope and using the coarse adjustments and angled base of the micromanipulator. The criteria for selecting a particular leaf were:

i) it was inserted about 3 cm, and definately within 6 cm, from the shoot apex (unless otherwise stated),

ii) easy access to it without the need to disturb the shoot or other leaves,

iii) it was angled to the stem in such a way that an advance, perpendicular to the surface, could be made towards it,

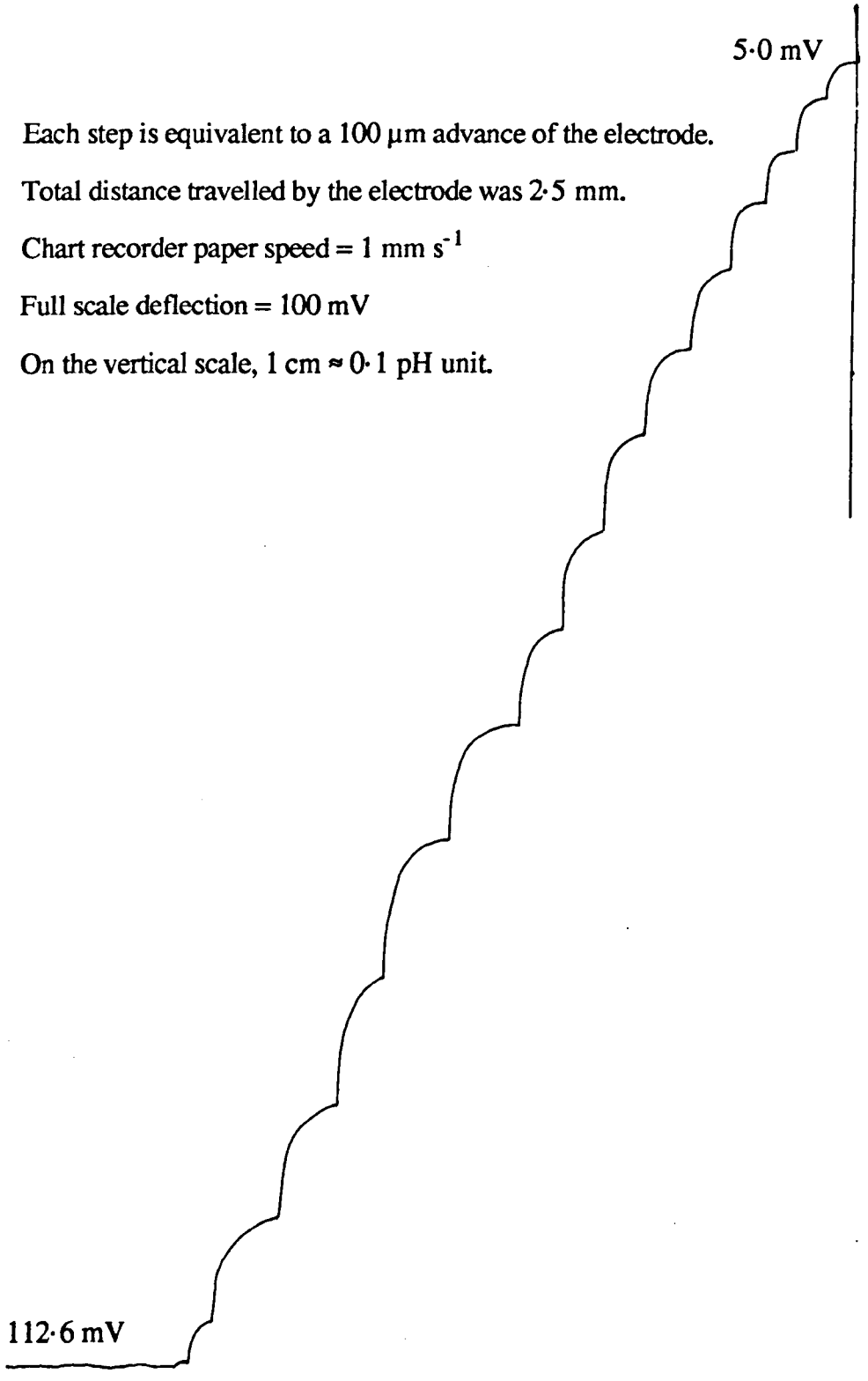
iv) it was, as far as possible, flat across its width and length,

v) it was healthy and undamaged.

Where possible, to ensure the maximum travel on the micromanipulator, the electrode was positioned abutting the leaf with the fine adjustment fully extended, and then fully retracted ready to make the experimental approach. Once this was done, the plant was left for some time (from 1 hour to overnight) before the experimental approach was made. The reference electrode was positioned so as not to disturb the plant during the approach.

Each approach was made by rapidly advancing the fine adjustment of the micromanipulator through 100 µm by hand and waiting for the chart recorder (paper speed 1 mm s^{-1}) to equilibrate before advancing the next step. This resulted in a series of stepped readings, from which the potential difference and hence pH, could be calculated (see Figure 6.6). The point where the tip of the electrode made contact with the leaf, marked by a

Figure 6.6 A typical chart recorder trace from an advance towards a leaf, used to calculate pH.



sudden peak, was recorded from the reading on the micromanipulator. Each complete approach took 1-2 minutes to complete.

The chart recorder, set at f.s.d. = 100 mV, was calibrated using buffers and the output from the digital display of the electrometer. In this way it was found that the chart recorder responded at a rate of 0·55 mV per mm, equivalent to about 0·01 pH units per mm (dependant on the calibration curve of the electrode) and considered to be the limit of sensitivity for the system. The electrometer was capable, within the range +199·9 to –199·9 mV, of recording a change of 0·1 mV.

Section 2.

The pH Climate Surrounding Epiphyte-Free Leaves of *E. nuttallii.*

6.2.1 The pH climate surrounding epiphyte-free leaves.

The following investigations were made using unepiphytised *E. nuttallii* leaves, enabling a detailed picture of the pH climate surrounding a shoot to be built up. Previous studies have been few and brief, with more attention being paid to the epiphyton growing on the plants' surfaces than the to plants themselves (see section 6.1.1). Further, elodeids have never been investigated in this manner and for those species that have been used the variations around the shoot have been ignored.

This study was therefore designed to investigate:

i) the change in pH at the leaf surface with time,

ii) the establishment of pH gradients around the leaf after the onset of illumination,

iii) the effect of close proximity of an investigated leaf to other leaves,

iv) the differences in pH climate due to the distance of the leaf from the shoot apex,

v) the variations in pH climate around the leaf,

vi) the CO_2^* climate that the leaves experience.

Although none of these measurements were replicated in a statistically rigorous manner, due to difficulties in taking them, they do give a full descriptive account which can be applied to other shoots.

6.2.2 Changes in the pH at the leaf surface with time and illumination.

6.2.2.1 Method.

A shoot was set up as above (section 6.1.4.1) and the pH electrode positioned so that it was close to an appropriate leaf inserted approximately 3 cm from the shoot tip, then left overnight in total darkness. The next morning, in low light (ie. not illuminated by the projector), the electrode was advanced carefully until it was abutting the leaf at a point about a third of the way down from the tip of the leaf to the junction with the stem, and about a third of the way across the width of the leaf. With the chart recorder set on a slow paper speed, the plant was left in darkness for some time, illuminated and then darkened again. The pH at the leaf surface was recorded during this process.

This was repeated using another shoot, investigating both the adaxial and abaxial surfaces.

6.2.2.2 Results and Discussion.

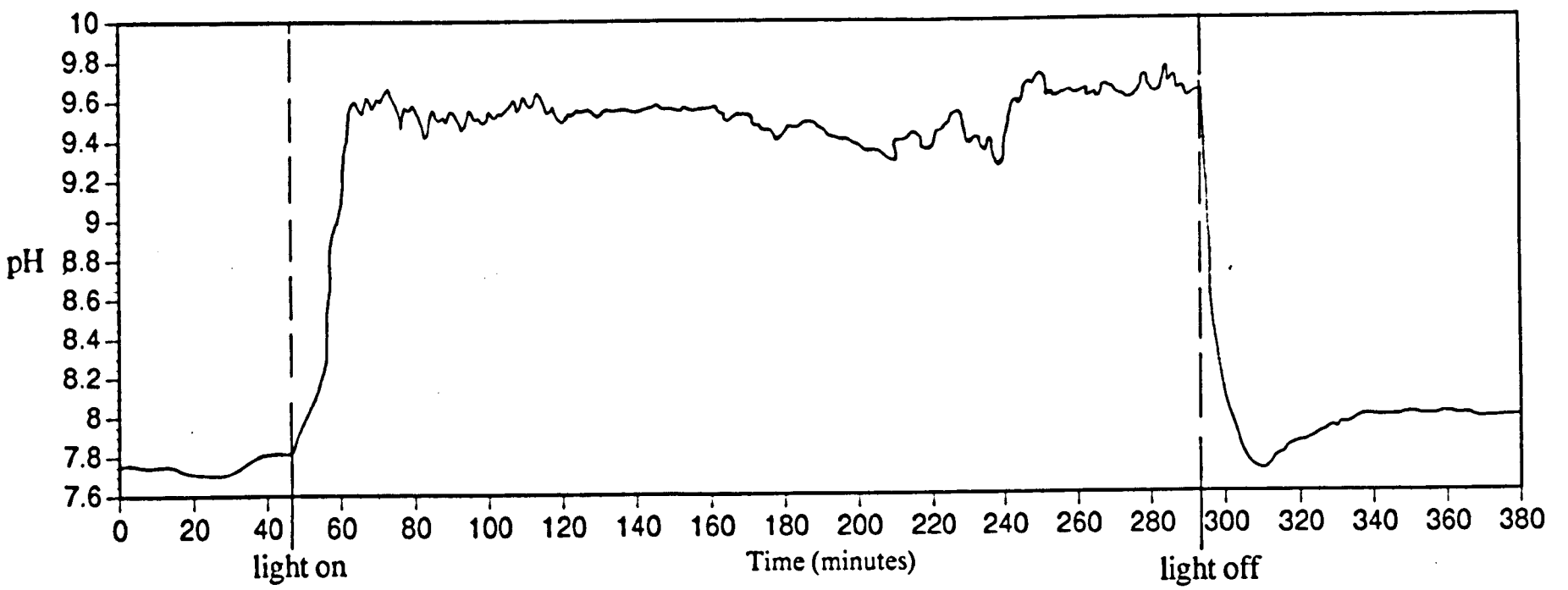
From Figure 6.7 it can be seen that, both for the adaxial and abaxial surfaces, there are two possible responses of a leaf to illumination.

i) The pH at the adaxial surface increases rapidly from pH 7·8 up to about pH 9·6 and stays approximately stable (Figure 6.7a), whilst at the corresponding abaxial surface the pH increases only slightly (to pH 8·2 – Figure 6.7b).

ii) The pH at the adaxial surface has a more two-stepped increase, the first step lasting only 5 to 10 minutes, the second, more rapid increase, pushes the pH to very high levels, pH >10 (Figure 6.7c). The two-stepped change in pH can be seen far more easily on the abaxial surface, where an initial slight increase in pH, lasting for 5 to 10 minutes, is followed by a precipitous decline, to pH 6 – 6·4 (Figure 6.7d).

Figure 6.7 Changes in pH at the leaf surface with time and illumination.

a) Shoot 1, adaxial leaf surface.



b) Shoot 1, abaxial leaf surface.

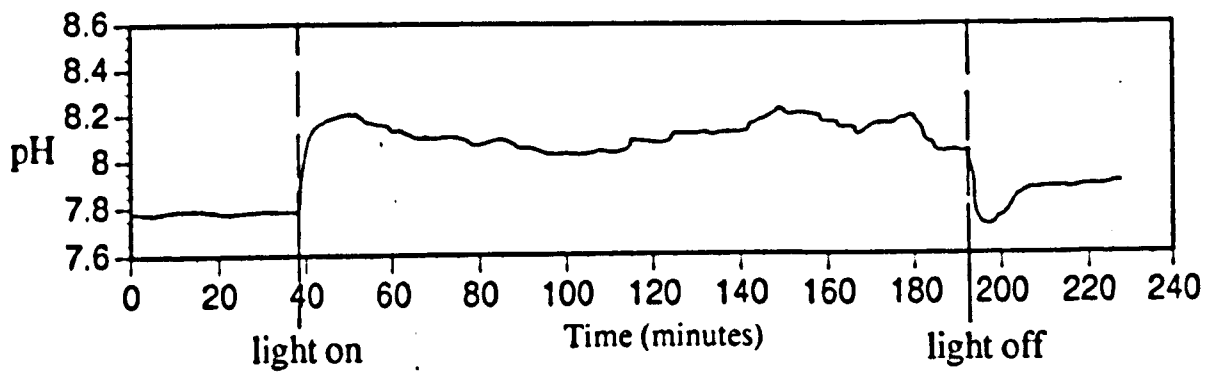
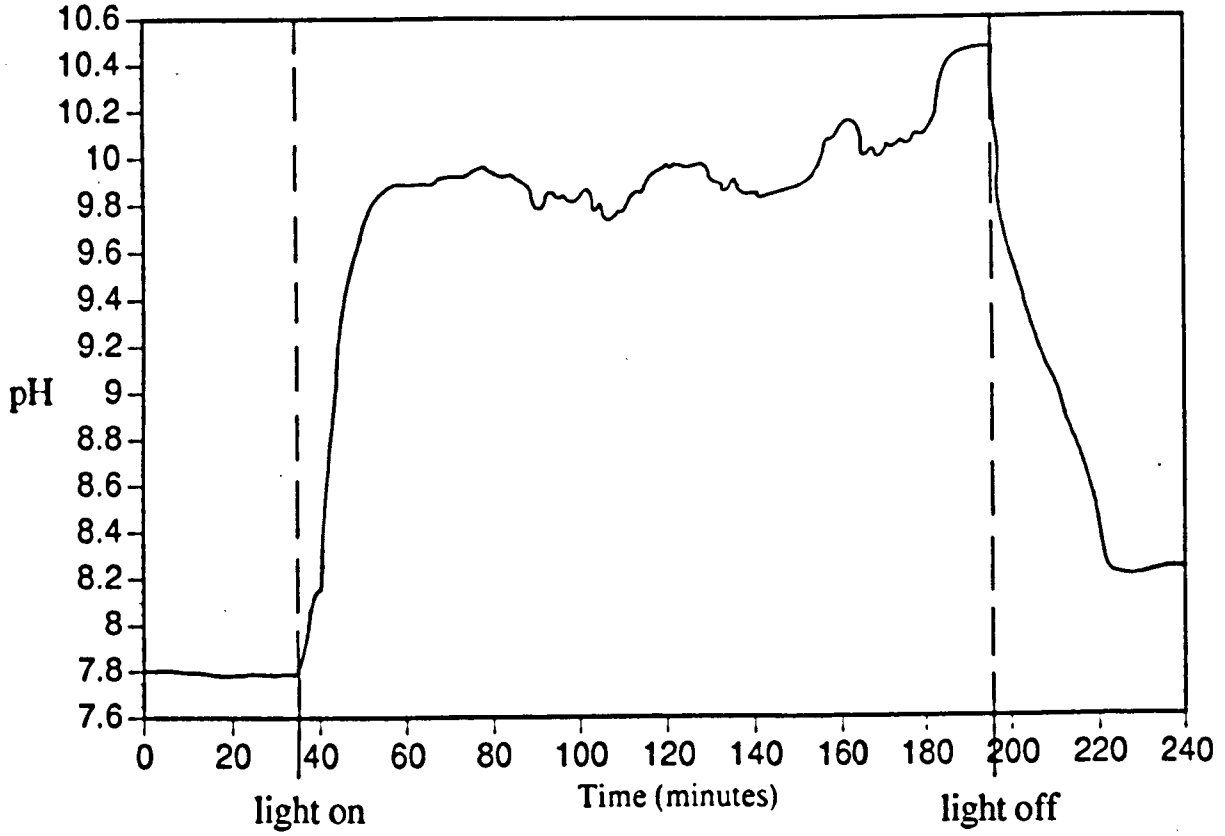
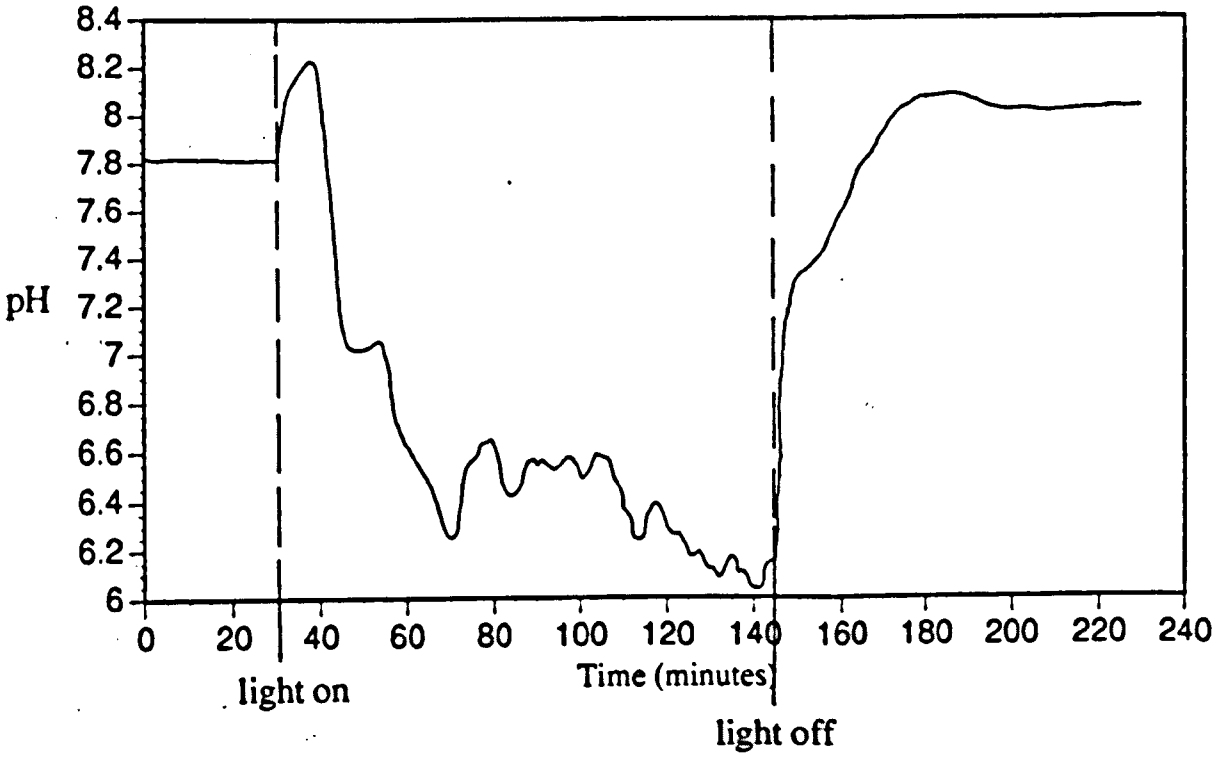


Figure 6.7 Changes in pH at the leaf surface with time and illumination. (continued)

c) Shoot 2, adaxial leaf surface.



d) Shoot 2, abaxial leaf surface.



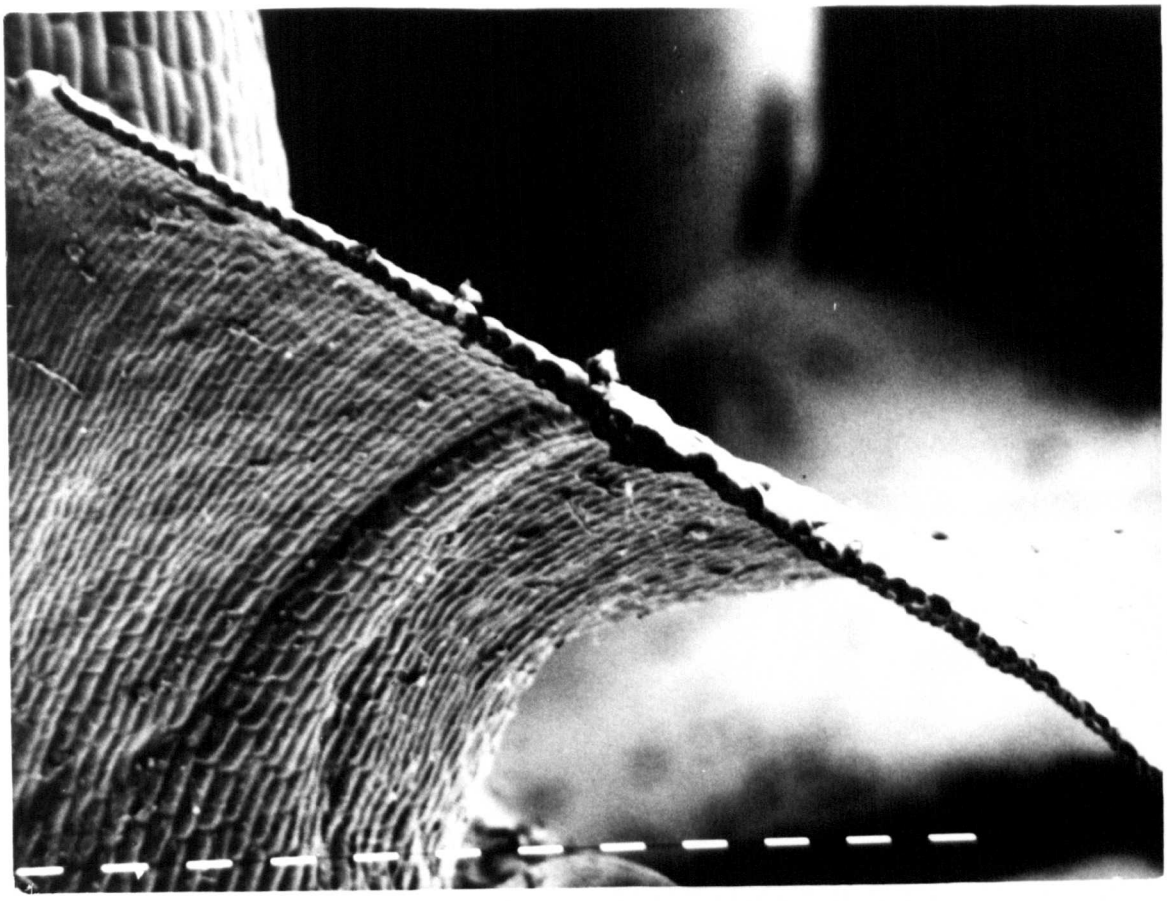
According to Prins *et al.* (1982b), who measured pH change with relatively large electrodes ≈2 mm tip diameter, these two patterns of response relate to carbon utilisation, the plant utilising only CO_2^* as a photosynthetic substrate and the plant utilising HCO_3^- respectively. Although previous reports have classed species as having either one or other of these responses (Prins *et al.* 1982b), *E. nuttallii* has here been shown to be able to adapt its physiology to utilise either carbon species as a photosynthetic substrate, dependent on environmental conditions (Chapter 4). Since the only difference between the plants used to generate the data for Figures 6.7a – d was their time of collection from the Leeds and Liverpool Canal, this can be taken as further evidence of substantial changes in HCO_3^- utilisation in the field.

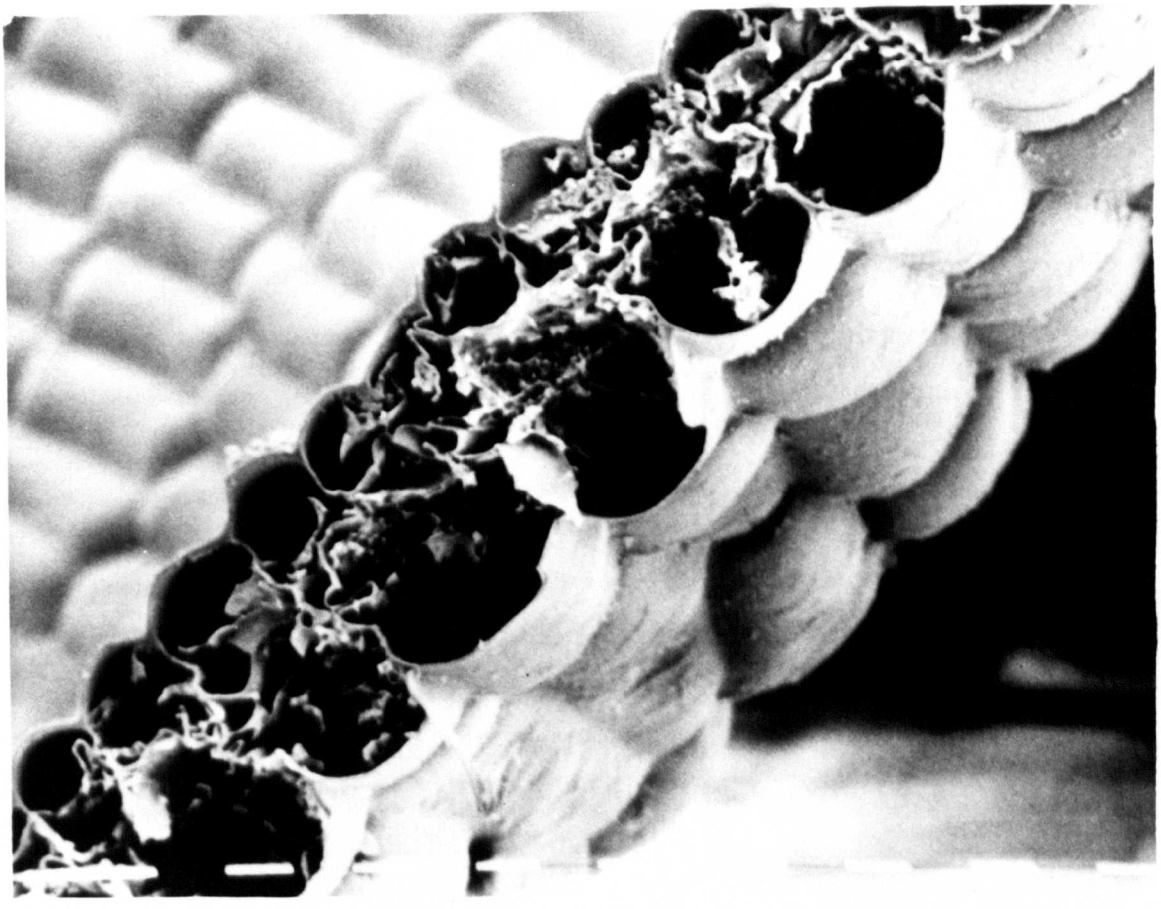
In the first of these responses, the change in pH is mediated through the photosynthetic uptake of CO_2^*. The underside of the leaf appears somewhat less active (Figure 6.7b), an effect not due to shading from the upper part, since the same response was found when the shoot was illuminated from below (Prins *et al.*, 1982b). A morphological differentiation between the upper and lower cell layers of the leaf is apparent, there being a noticeable size difference between the two layers (Plate 21), such as has been noted in *Potamogeton lucens* L. (Miedema & Prins, 1991). It is possible that this differentiation is important in the uptake of HCO_3^- by the polar leaf mechanism.

When the plant is utilising HCO_3^- (Figure 6.7c & d), the initial change in pH after the onset of illumination (first 5-10 minutes) is due to the photosynthetic uptake of CO_2^*. There then occurs a rapid increase at the adaxial surface, together with a decrease at the abaxial surface, as the polar-leaf mechanism takes over. This mechanism entails, effectively, the pumping of H^+ ions from the abaxial surface of the leaf and extrusion of OH^- ions from the adaxial surface (see Chapter 4). Hence the pH at the adaxial surface is increased above that driven by CO_2^* fixation alone, whilst that at the abaxial is reduced, enhancing the conversion of HCO_3^- to CO_2^*. The absolute levels of pH are determined by the rate of ion pumping and the capacity of the water to buffer the change in H^+

Plate 20 A leaf of *E. nuttallii* cut transversely. Scale bar = 62·5 μm.

Plate 21 An inverted leaf of *E. nuttallii* cut transversely showing the differentiation in size between the cells of the adaxial layer (right) and those from the abaxial layer (left). Scale bar = 10 μm.





concentration. The difference between the absolute pH values found here and those previously reported (pH 12 upper – pH 4 lower) is not notable, since a natural, alkaline water with complex buffer system was used here, as compared to relatively unbuffered experimental solutions used by Prins and co-workers (Prins *et al.*, 1982a, b & c; Prins & Zanstra, 1985; Prins & Helder, 1985; Elzenga & Prins, 1987; Prins & Elzenga, 1989). However, those reported here are more comparable to those found in the field. It is likely that it is not the pH but the concentration of CO_2^* which determines the rate of photosynthesis. Later results (see section 6.2.7) were used to calculate the concentration of CO_2^* in the water surrounding the leaves.

6.2.3 The establishment of gradients with illumination.

6.2.3.1 Method.

A shoot was set up as above (section 6.1.4.1) and left overnight in total darkness. The following day repeat approaches were made towards an appropriate leaf, inserted approximately 3 cm from the tip of the shoot, so that the point of contact was about a third of the way down from the leaf tip and about a third of the way across its width. Approaches were made during darkness and at measured time intervals after the start of illumination, with those measured up to 15 minutes after the start of illumination made in the reverse direction, ie. away from the leaf. In this way the electrode would follow the direction of pH change as the gradient became established and the effect of changes during the time taken to complete the approach would be minimised.

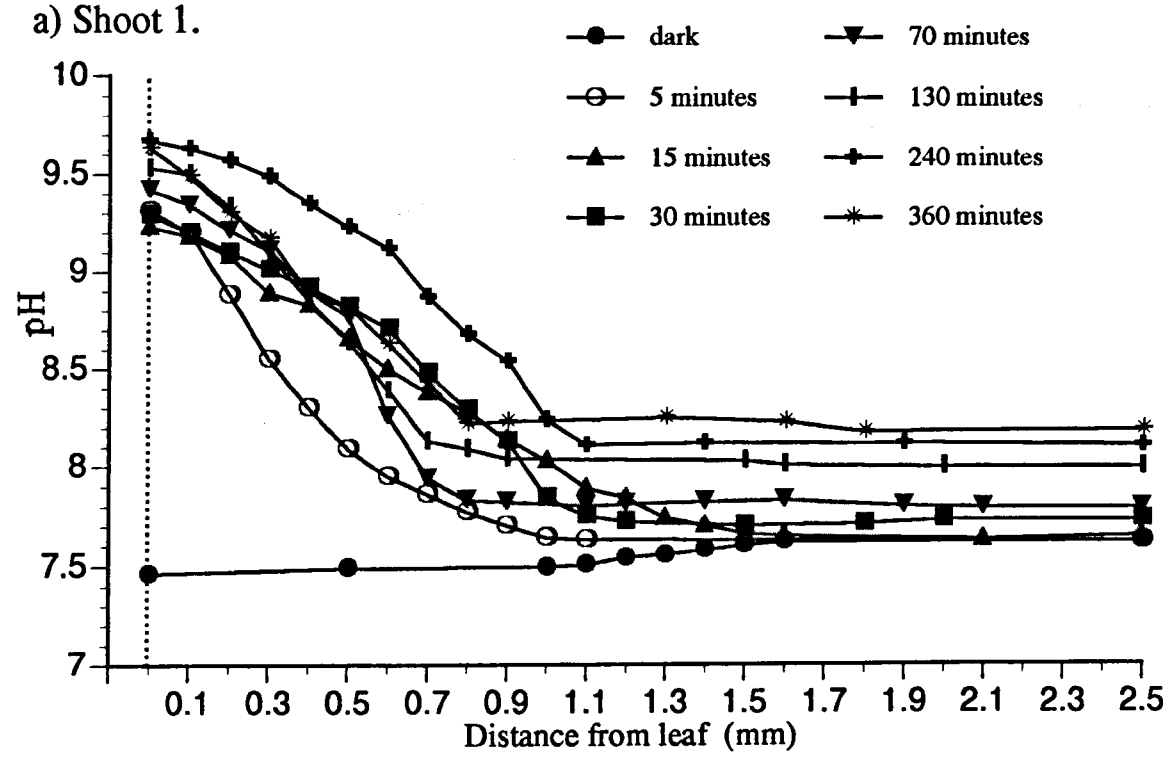
This was repeated with four different shoots, approaching either the adaxial and abaxial surface of the selected leaf.

6.2.3.2 Results and Discussion.

The gradients approaching the adaxial surface of the leaves at different times after the onset of illumination are shown in Figure 6.8a. At this surface, an approach in darkness showed a very slight gradient from the bulk water to the leaf surface, with a release of respiratory CO_2^* presumably the cause of a slightly lower pH at the leaf surface. Once the plant was illuminated, there was a rapid and marked change in pH, as photosynthesis removed CO_2^*. Even after five minutes illumination the pH gradient was steep (7·65 – 9·3 over 1·1 mm), with an easily definable limit to the diffusion boundary layer at 1·1 mm, this being the point beyond which there was no detectable difference between the pH and that of the bulk water. For the gradients measured later than five minutes after the onset of illumination, there was a continued increase of pH at the leaf surface, but the extent was small and somewhat variable. Also, due to the plant's

Figure 6.8 pH gradients approaching the upper surface of a leaf at different time intervals after the start of illumination.

a) Shoot 1.



b) Shoot 2.

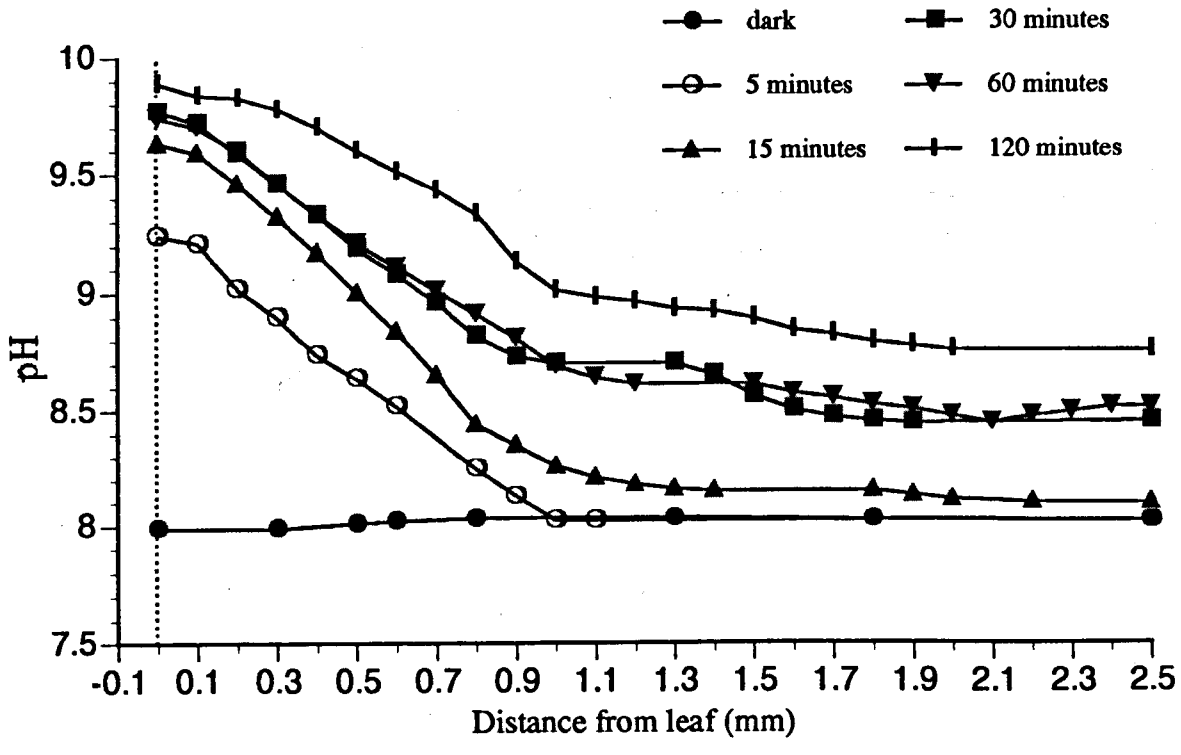
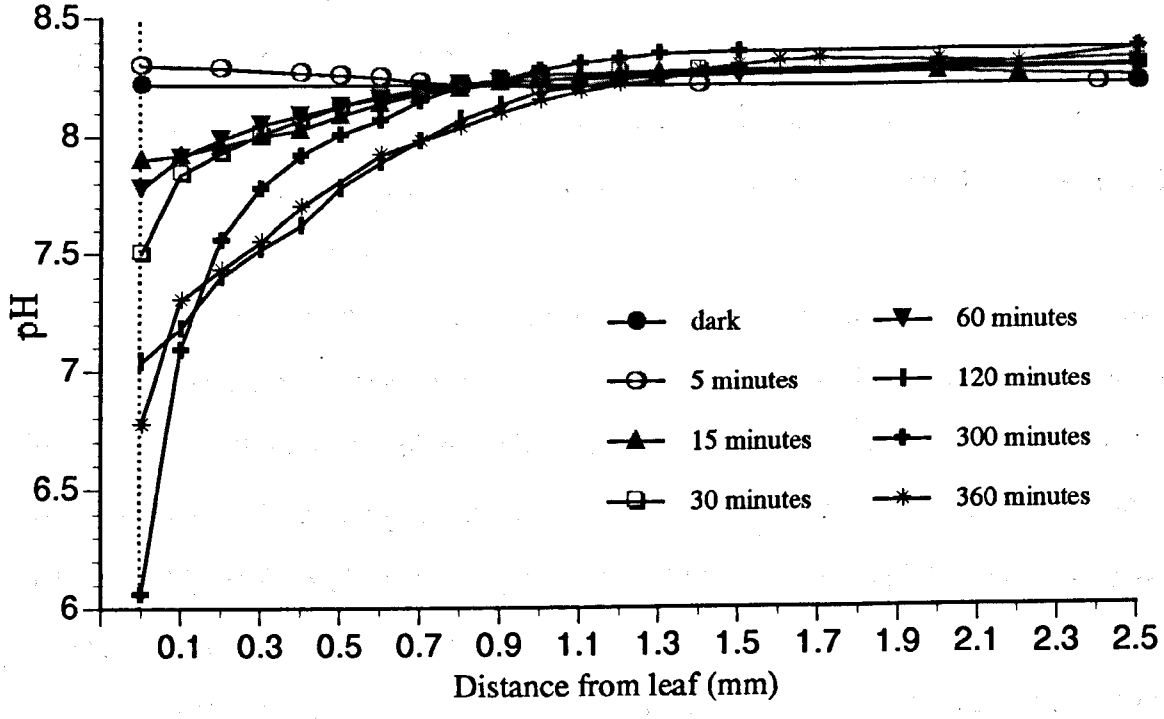
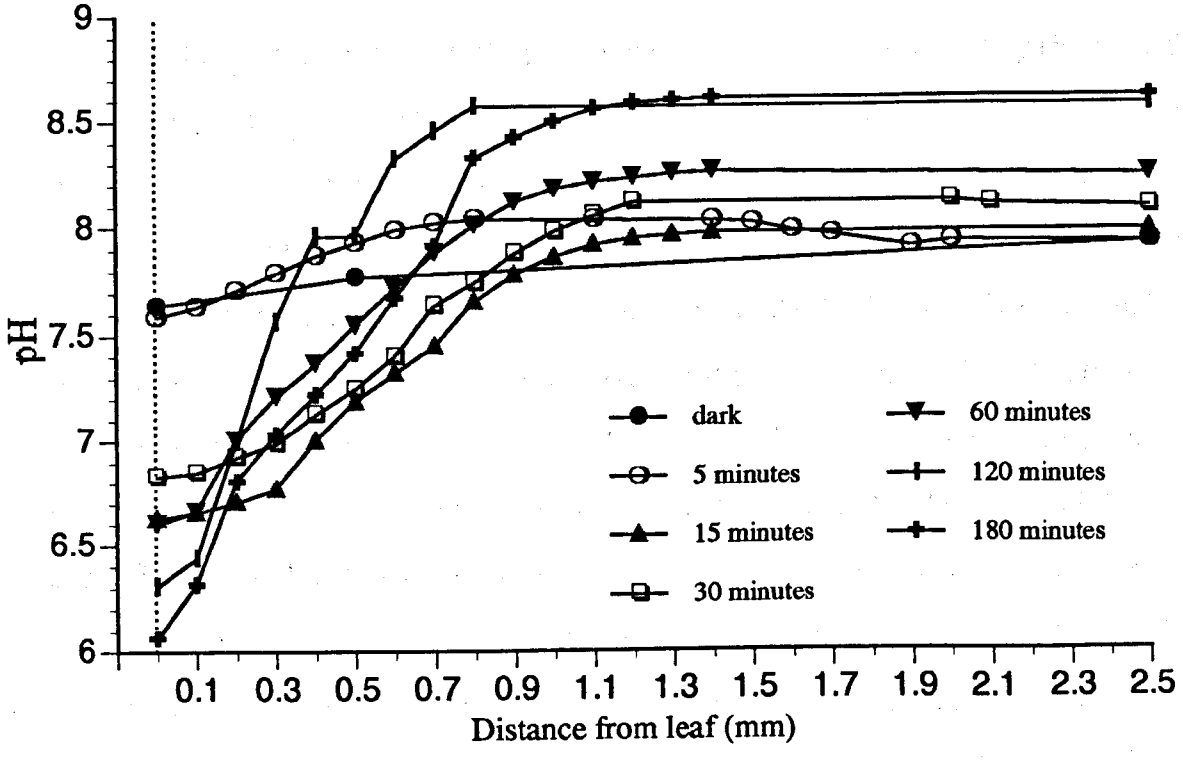


Figure 6.9 pH gradients approaching the underside of a leaf at different times after the onset of illumination.

a) Shoot 3



b) Shoot 4



photosynthesis, the pH of the bulk water increased with time. However, the diffusion boundary layer remained at approximately constant thickness, here 0·9–1·2 mm and probably equivalent to the physical boundary layer, whose limit is determined by the extent of water movement around the plant. Water movement, which was visible through the microscope, was probably driven by convection and was apparently very slow (see section 6.2.6). Convection currents have been observed in similar circumstances where a quantity of illuminated water was not intentionally mixed (Carlton & Wetzel, 1987), with stable boundary layers being established.

In a second investigation the results followed the above pattern in all aspects except that the diffusion boundary layer was greater (Figure 6.8b). In the case of this second shoot, the outer limit of the physical boundary layer was about 1·9–2·0 mm from the leaf surface. The much shorter gradient, measured 5 minutes after the onset of illumination (to ≈1·0 mm, Figure 6.8b), had probably not reached the limit of the physical boundary layer due to the slow rate of diffusion in water.

The pH gradient approaching the abaxial surface was similar in darkness to that recorded for the adaxial surface, but on illumination showed a very different reponse (Figure 6.9). After five minutes, the pH at the leaf surface increased slightly (Figure 6.9a), then subsequently decreased to a minimum of less than pH 6·1 in both shoots tested, but being generally higher in shoot 3 than in shoot 4 (Figure 6.9a & b). The gradient measured after five minutes for shoot 4 was taken at a slightly later stage in the process than for shoot 3 and shows the initial stages of the decline of pH at the leaf surface, with the pH first increasing then decreasing with proximity to the leaf surface (Figure 6.9b). The time taken to make the approach may have influenced the shape of this gradient, which was not in a steady state. The limit to the boundary layer surrounding these two leaves was 1·3 mm for shoot 3 and 1·2–1·4 mm for shoot 4. It should be stressed that these results do not correspond to the upper and lower surfaces of the same leaves, and that those leaves which were used to investigate the adaxial surface were not necessarily taking up HCO_3^-.

The relatively rapid establishment of a gradient across the boundary layer has previously been reported for epiphyton growing on either plants or inactive substrata (Sand-Jensen *et al.*, 1985), with steady state conditions achieved after 15 – 30 minutes. That time scale is much the same as that found here. The fluctuations seen here once steady state conditions had been established were attributed to small changes in the conditions such as flow and differences in the instantaneous rates of photosynthesis and respiration.

It was decided from this work to illuminate the plants for a minimum of 1 hour, to allow stable conditions to develop, before measuring pH gradients in subsequent investigations.

6.2.4 The effect of proximity to other leaves on the pH gradient approaching a leaf.

6.2.4.1 Method.

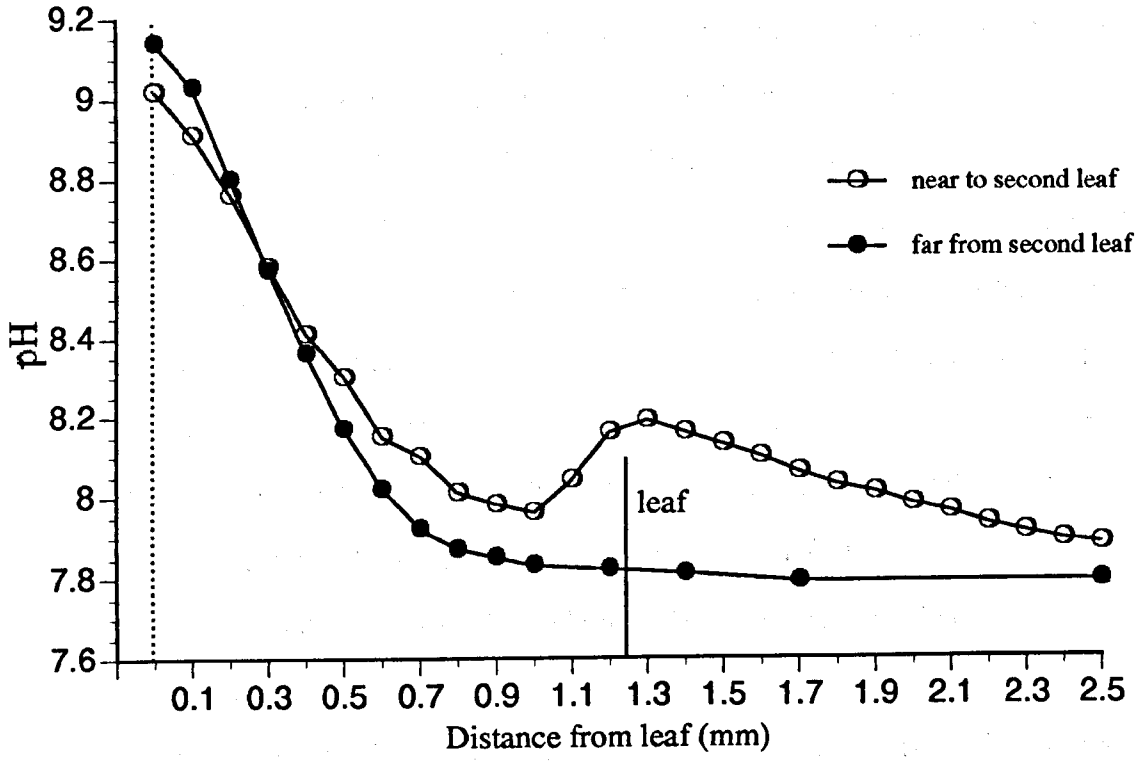
A shoot was set up in the experimental bath as before (section 6.1.4.1) and illuminated for 2 hours. The target leaf selected for making the approach was at one point, due to strong contortion of the leaves from the next whorl, within 2mm of another leaf. Two approaches were made towards the selected leaf, one where the leaves were in close proximity to each other made so that the electrode only just missed the second leaf and one some distance from this point.

6.2.4.2 Results and Discussion.

From the results in Figure 6.10 it can be seen that the leaves, when they were in close proximity, could interact, causing perturbations to the pH gradient. The pH at the leaf surface investigated was not greatly affected by the proximity of another leaf, but the reduced water movement caused by the presence of other leaves resulted in increased boundary layer thickness.

As most of the extension of an *E. nuttallii* shoot occurred within 1-2 cm of the apex (see Section 6.2.5 and Birch 1990) and the whorls after this point are typically about 0·5–1 cm apart, the leaves from the main part of the shoot are rarely close enough to interact significantly, even under still conditions. The leaves which were closer together, ie. those near to the apex, were of lower activity than those further from the apex (Section 6.2.5) reducing the potential for interaction. Further, as the three leaves of each whorl were not produced directly in rank, each whorl being offset by an effective one ninth rotation in relation to the one below, a line perpendicular to the surface of one leaf passed by two whorls before "hitting" another leaf (pers obs.). Only one other case of interaction of pH

Figure 6.10 Effect of the close proximity of one leaf on the pH gradient approaching a second, the target leaf.



gradients between leaves was observed (Figure 6.18), and this when an advance was made towards a leaf near to the shoot apex.

Nevertheless, *E. nuttallii* is noted for its occurrence in a form with strongly recurved leaves, as are certain other species in the Hydrocharitaceae, eg. *Lagarosiphon major*, although the closely related *E. canadensis* does not show such recurvature (Simpson, 1988). Perhaps there is a functional significance to this recurved form. If a leaf is taking up HCO_3^- by the polar-leaf mechanism, any interaction between leaves would be highly detrimental, the high pH of the adaxial surface interfering with the reduced pH of the abaxial surface where CO_2^* is taken up. Strong recurvature produces a situation where the adaxial surfaces are the ones most likely to interact, and creates a small hoop between the leaf and stem where the pH is reduced. As plants utilising HCO_3^- are not light limited (see Chapter 4) the reduced capacity to absorb light by being recurved is of no great significance to the plant. In support is a rare report of weakly recurved *L. major* noted for plants growing in a lake of very low DIC (Rattray *et al.*, 1991) where HCO_3^- utilisation was unlikely. Testing this idea with the microelectrodes proved rather difficult due to obstruction of access to the abaxial leaf surface. However, the investigations undertaken here showed that plants without recurved leaves were utilising HCO_3^-, so the recurved form is not prerequiste for the operation of the polar leaf mechanism in this species.

6.2.5 The effect of position on the stem on the pH climate surrounding individual leaves.

6.2.5.1 Method.

An unbroken, long, straight shoot without side-branches was clamped in the experimental bath such that sections of shoot received unshaded light of approximately the same intensity (>300 $\mu mol\ m^{-2}\ s^{-1}$). Approaches were made towards the adaxial surface of appropriate leaves along the selected sections after illumination for 3 hours. The number of fully formed whorls from the shoot apex to each leaf used was recorded. Once all the approaches had been made, the distance from the shoot apex to the selected leaves was also measured with a ruler. Each whorl of leaves was then removed, one at a time and in a random sequence, and the gas exchange characteristics determined at 20 °C using a Clark-type oxygen electrode (Hansatech, King's Lynn) as outlined previously (Section 2.2.1). Whilst the determinations were being made, the shoot, with remaining whorls attached, was stored at 20 °C in illuminated canal water.

6.2.5.2 Results and Discussion.

This investigation was not replicated and, whilst the shoot used appeared representative, the findings may not be applicable to the whole of the population. It was decided not to replicate for the following reasons.

i) The time involved in replicating a minimum of three times would have been prohibitive (at least 4 days), each leaf requiring one hour of illumination before steady state conditions could be assured. Using different leaves along the length of three shoots constituted pseudoreplication.

ii) A similar, fully replicated, experiment was conducted on *E. nuttallii* from the Leeds and Liverpool Canal by Birch (1990), where the rates of photosynthesis and respiration of leaves from different positions on the stem were measured using a Clark-type oxygen

Figure 6.11 Relationship between whorl number and distance from the shoot apex.

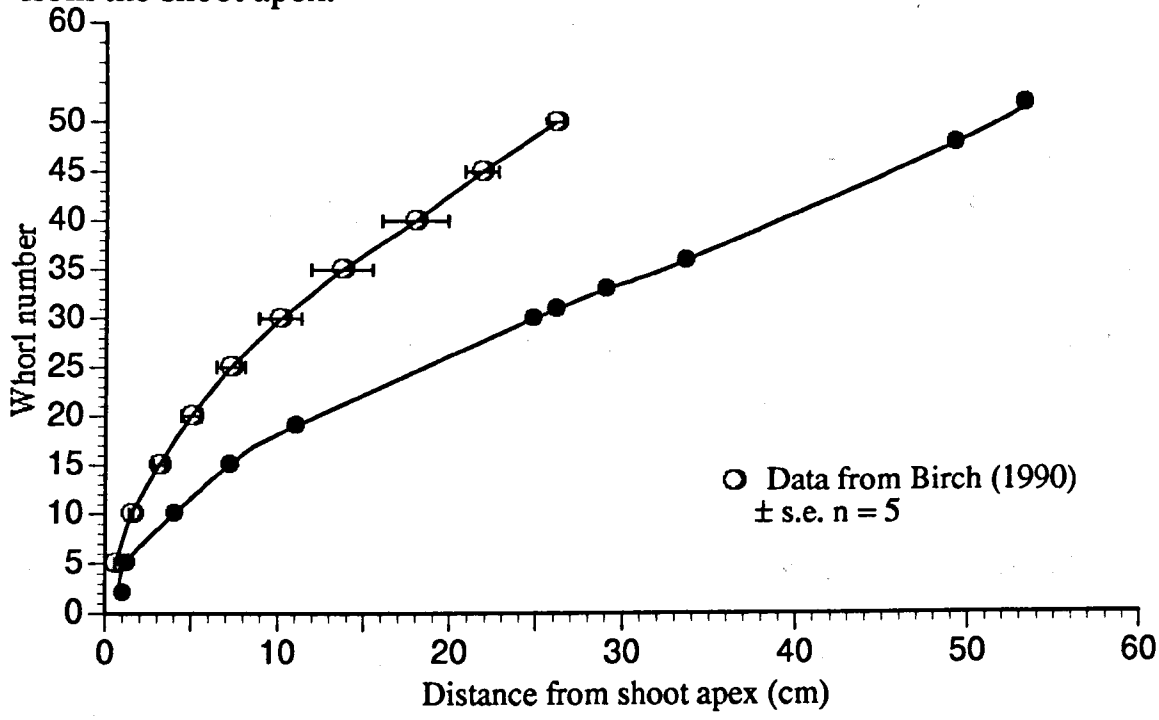


Figure 6.12 Relationship between total chlorophyll of the three leaves in the whorl and whorl number.

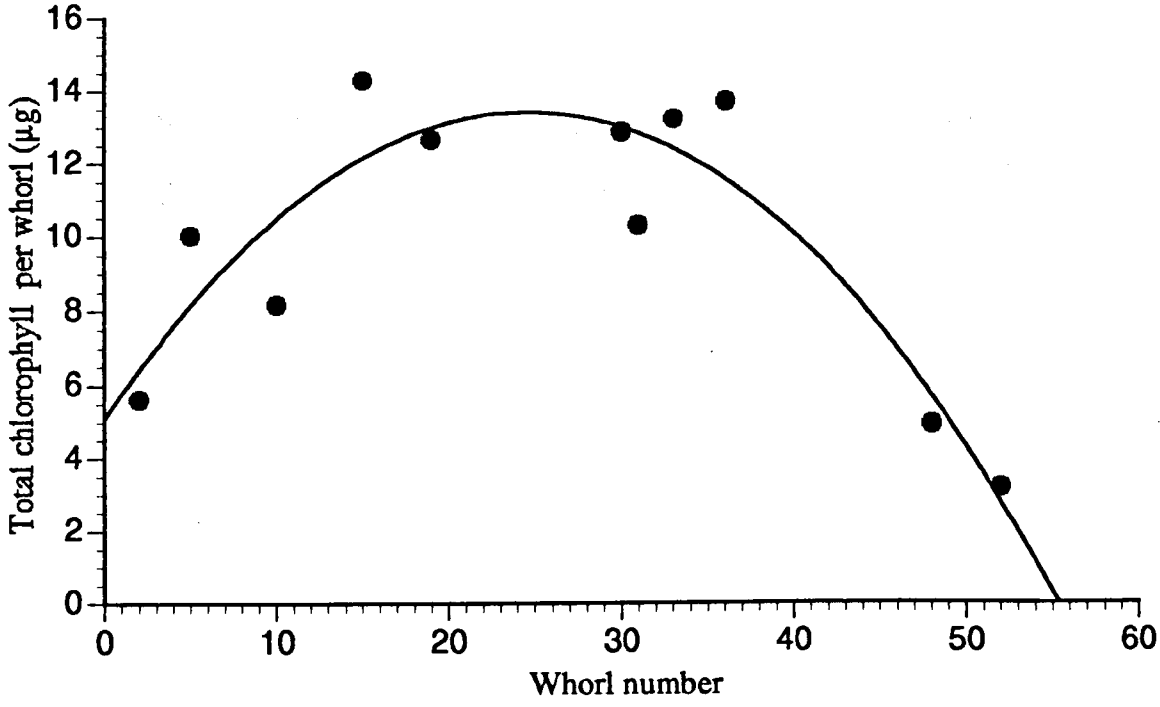


Table 6.2 Comparison between the growth characteristics of the *E. nuttallii* shoots studied by Birch (1990) and those of the shoot studied here.

	— Present investigation—		—Birch (1990)—	
	Whorl Number	Distance (cm)	Whorl Number	Distance (cm)
Limit of extension	19	10·1	21	5
Maximum chlorophyll	25	19–25	30–35	10–13·5
Maximum photosynthesis	0–13	0–5	15	3
Zero photosynthesis	57*	58*	50	26

The point of zero net photosynthesis (marked *) was estimated by extrapolation as all the leaves investigated were capable of positive photosynthesis.

electrode. Since the results from the present investigation followed the same pattern as that found by Birch, it was decided that no further replication would be needed.

iii) One shoot was sufficient to provide the required descriptive information about the conditions surrounding the shoot.

The region of extension of the selected *E. nuttallii* shoot was clearly restricted to the upper 10 cm of the shoot and mainly to 1·5 cm of the shoot apex (Figure 6.11). This region of extension corresponded to about the first 20 whorls of the shoot, as was found by Birch (1990), but does not correspond in length (10 cm *cf.* 5 cm). Although plants were collected from field in both cases, the shoots used by Birch had much shorter internodes than that used here (Figure 6.11), which was collected in the early spring when all the plants were growing very straight, vertically from the bed of the canal and the water was very clear (canal bed visible). Visual differences in the growth and morphology of *E. nuttallii* have previously been noted between plants from different sites and from the same site at different dates (Simpson, 1986; Birch, 1990). However, many of the features of the plant investigated here, compare favourably with those used by Birch (1990) on a whorl number basis (Table 6.2).

The total chlorophyll content of the leaves varied with distance from the apex, being initially low, increasing to a peak at whorl 25 (≈20 cm) and declining again beyond this point (Figure 6.12). Net photosynthesis, measured as rate of oxygen production, followed a similar curve, with a peak about 18 – 23 whorls (≈15–20 cm) from the apex (Figure 6.13). But, when standardised against the chlorophyll content of the leaves, the rate of photosynthesis was greatest at the apex and declined increasingly with distance from this point (Figure 6.14). When measured as rate of oxygen use, respiration showed no obvious relationship with distance (Figure 6.15), but if standardised against chlorophyll followed a U shaped pattern (Figure 6.16), with the highest rates near the apex and base of the shoot. This pattern is very much the opposite of that described by chlorophyll (Figure 6.12) and appears to be an artefact caused by variation of respiration of the leaves

Figure 6.13 Relationship between net photosynthesis, as rate of oxygen production, and whorl number.

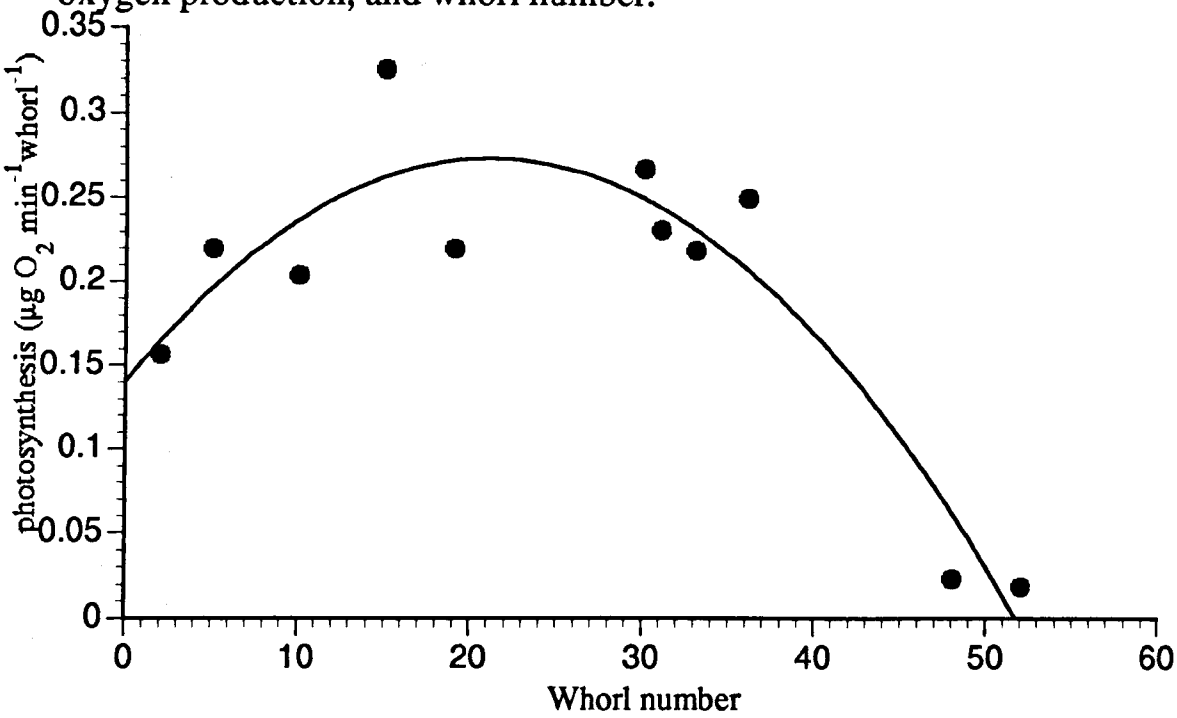


Figure 6.14 Relationship between net photosynthesis, as rate of oxygen production per unit chlorophyll, and whorl number.

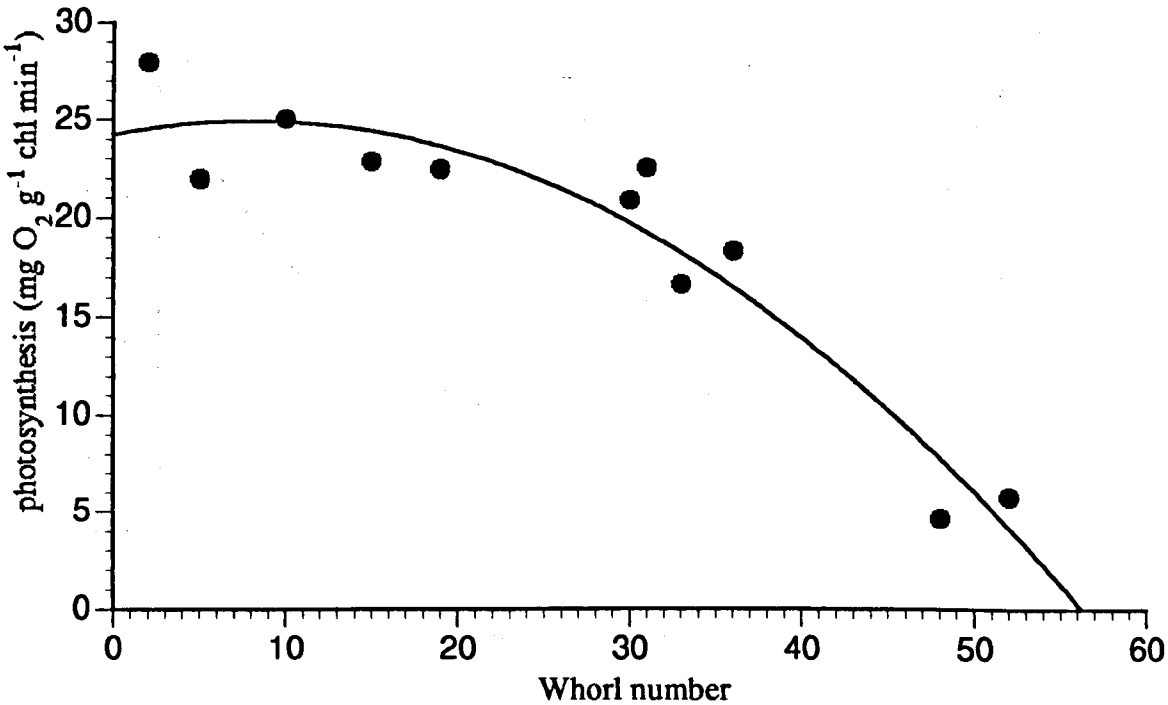


Figure 6.15 Relationship between dark respiration, as rate of oxygen use, and whorl number.

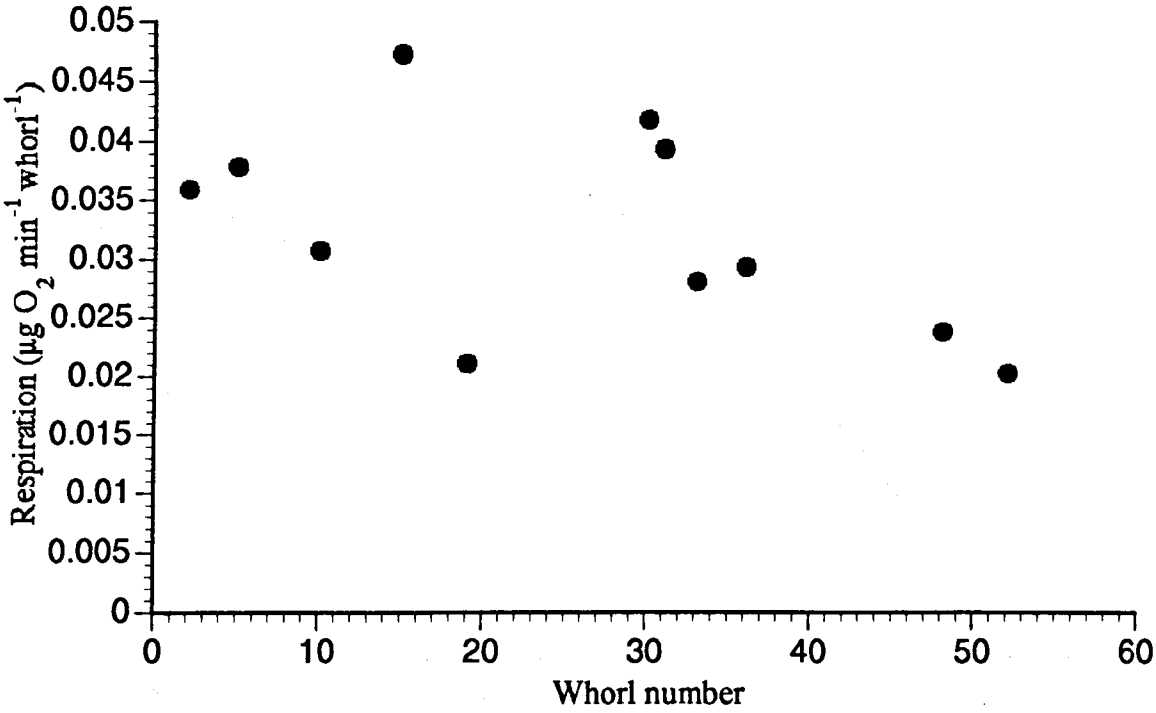


Figure 6.16 Relationship between dark respiration, as rate of oxygen use per unit chlorophyll, and whorl number.

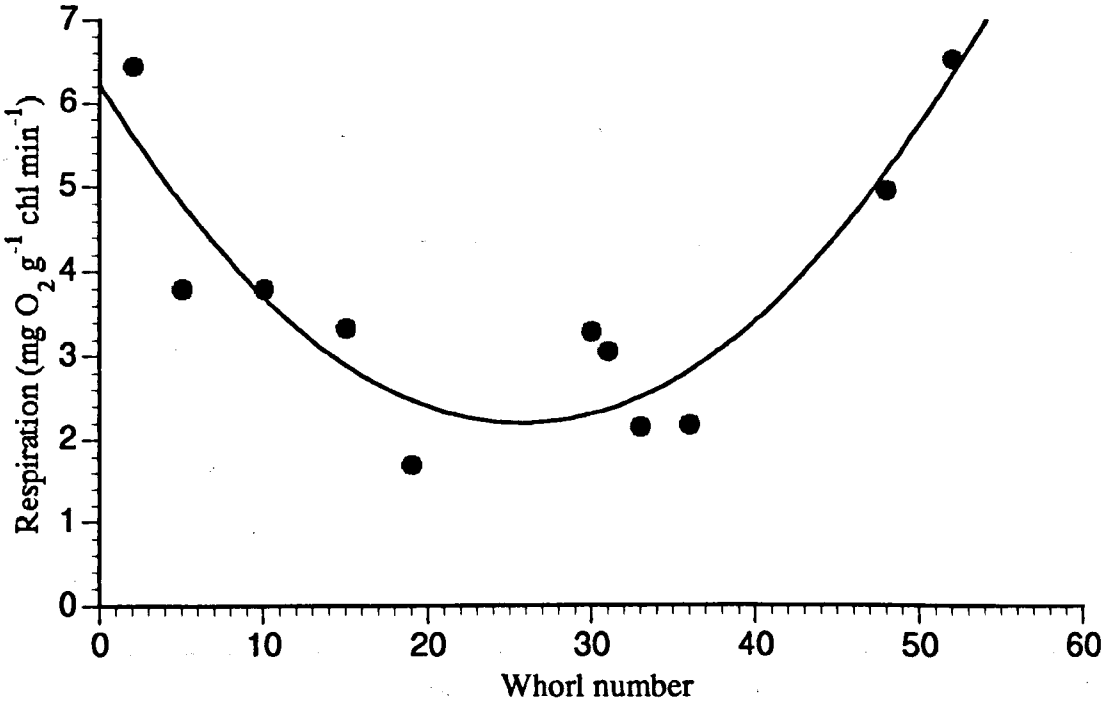


Figure 6.17 Relationship between pH at the adaxial leaf surface and whorl number.

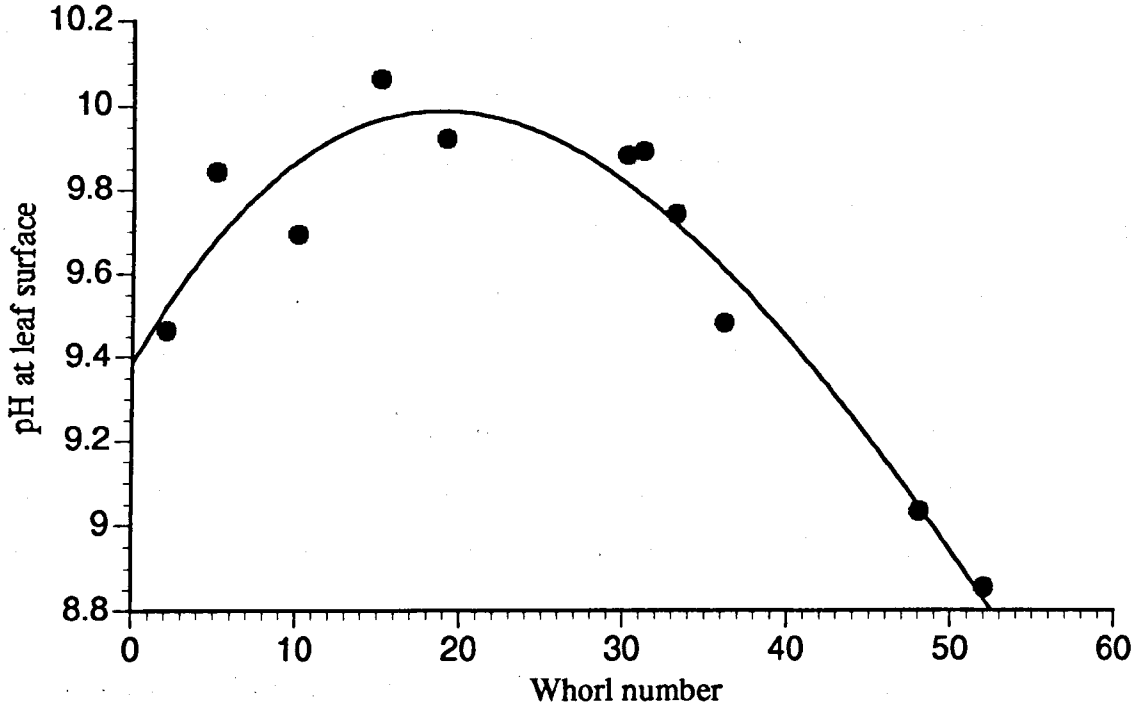
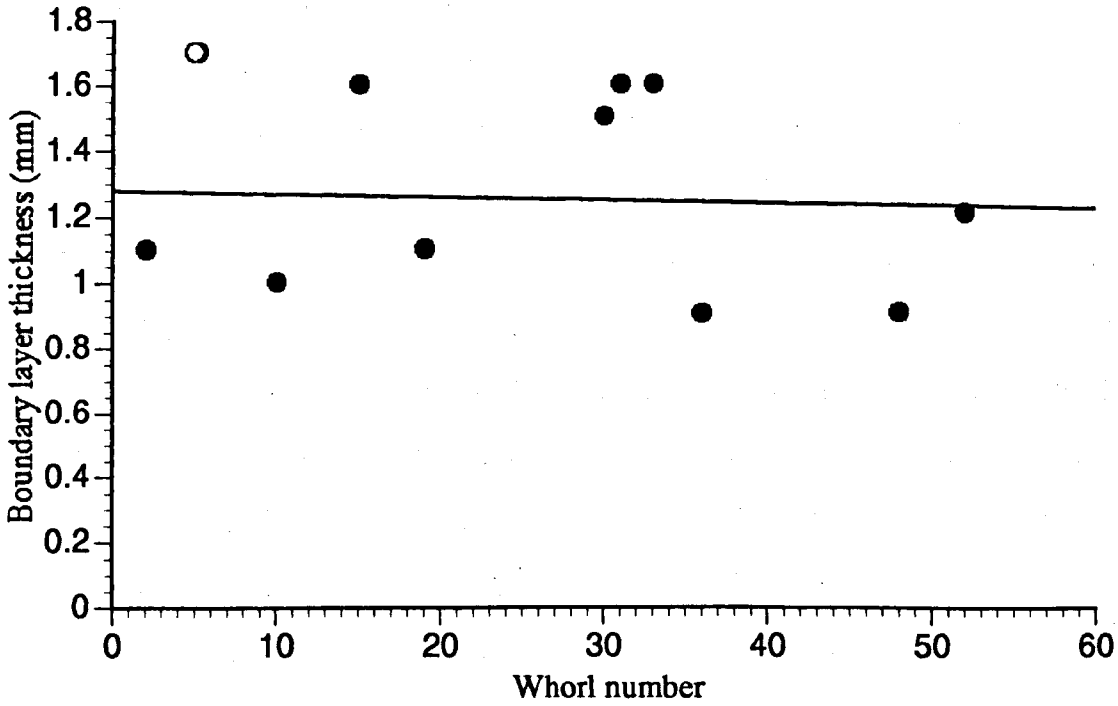


Figure 6.18 Relationship between boundary layer thickness and whorl number.



The gradient for one point (O) was affected by the close proximity of other leaves.

independent of their total chlorophyll content. Since all the above parameters followed the patterns described by Birch (except respiration as rate of oxygen use, which here showed no overall pattern *cf.* a slow decline with distance from the apex), especially on a whorl number basis (Table 6.2), it was concluded that this was a typical *E. nuttallii* shoot.

The pH at the adaxial leaf surface followed much the same pattern as the net photosynthesis, measured as oxygen production, being low near the apex, increasing to a maximum around whorls 17 – 21 (14 cm), then declining again (Figure 6.17). This is not surprising as photosynthesis, through depletion of CO_2^* in the water leading to disassociation of HCO_3^- to CO_2^* and OH^-, is the main factor controlling pH changes in aquatic systems. Uptake of other ions, such as NO_3^- and NH_4^+, from the water does affect its pH and alkalinity, but the effect is small when compared with that caused by photosynthesis (Stumm & Morgan, 1981). The thickness of the boundary layer, a physical effect of the flow of water around the leaf, did not appear to be related to the position of the leaf on the shoot (Figure 6.18). However, at one point where the leaves were close together, ie. near the apex, they did interact with each other (Figure 6.18) and this may have caused less water movement and thicker boundary layers.

Photosynthesis showed a linear relationship with pH at the leaf surface, both as rate of oxygen production ($p = 0{\cdot}0001$, Figure 6.19) and when standardised the against chlorophyll content of the leaves ($p = 0{\cdot}0048$, Figure 6.20). Although each leaf was not totally independent, all being from the same shoot, this result is exactly what would be expected, with photosynthesis causing a pH increase at the leaf surface. Since the change in pH is proportional to the amount of CO_2 removed, it is not surprising that the absolute rate of photosynthesis, uncorrected for chlorophyll, described the pH more effectively than the corrected values, which in part describe the efficiency of the chlorophyll within the leaf. If the leaves were taking up HCO_3^- by the polar leaf mechanism, a similar pattern would be expected, the rate of OH^- ions pumped from the adaxial surface being proportional to the photosynthetic activity of the leaf. Further, since the relationship between position on

Figure 6.19 Relationship between net photosynthesis, as rate of oxygen production, and pH at the adaxial leaf surface of *E. nuttallii.*

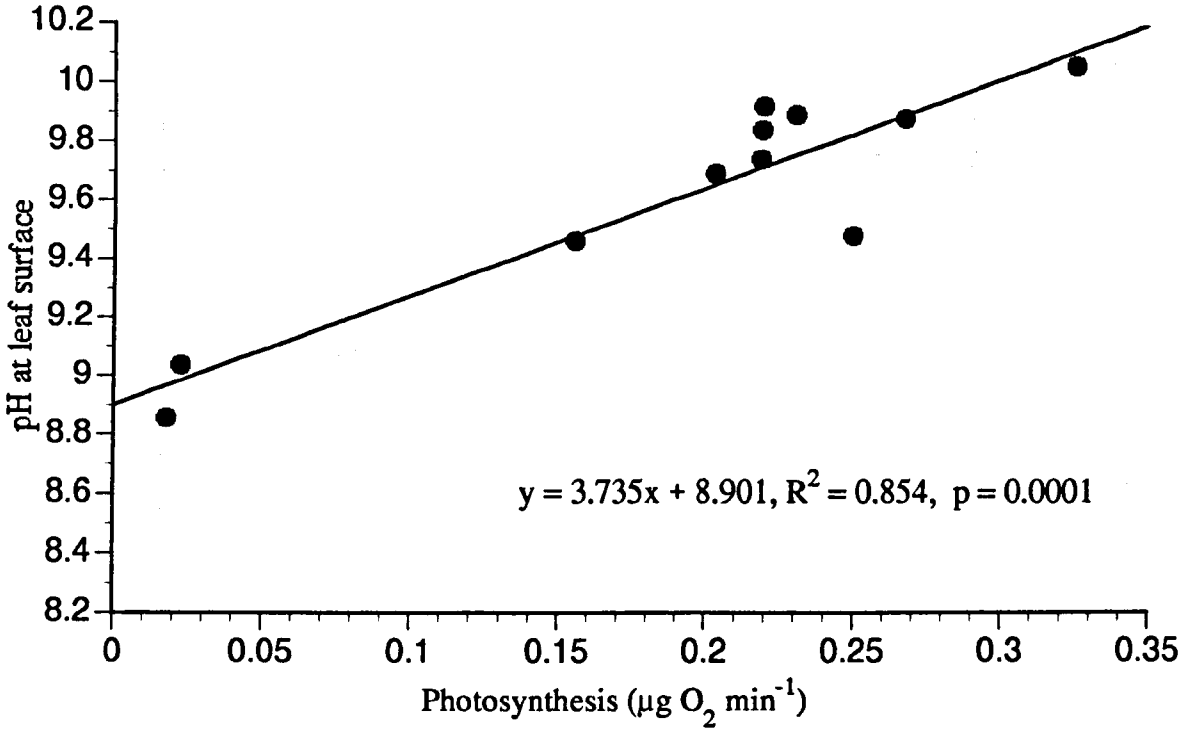


Figure 6.20 Relationship between net photosynthesis, as rate of oxygen production per unit chlorophyll, and pH at the adaxial leaf surface of *E. nuttallii.*

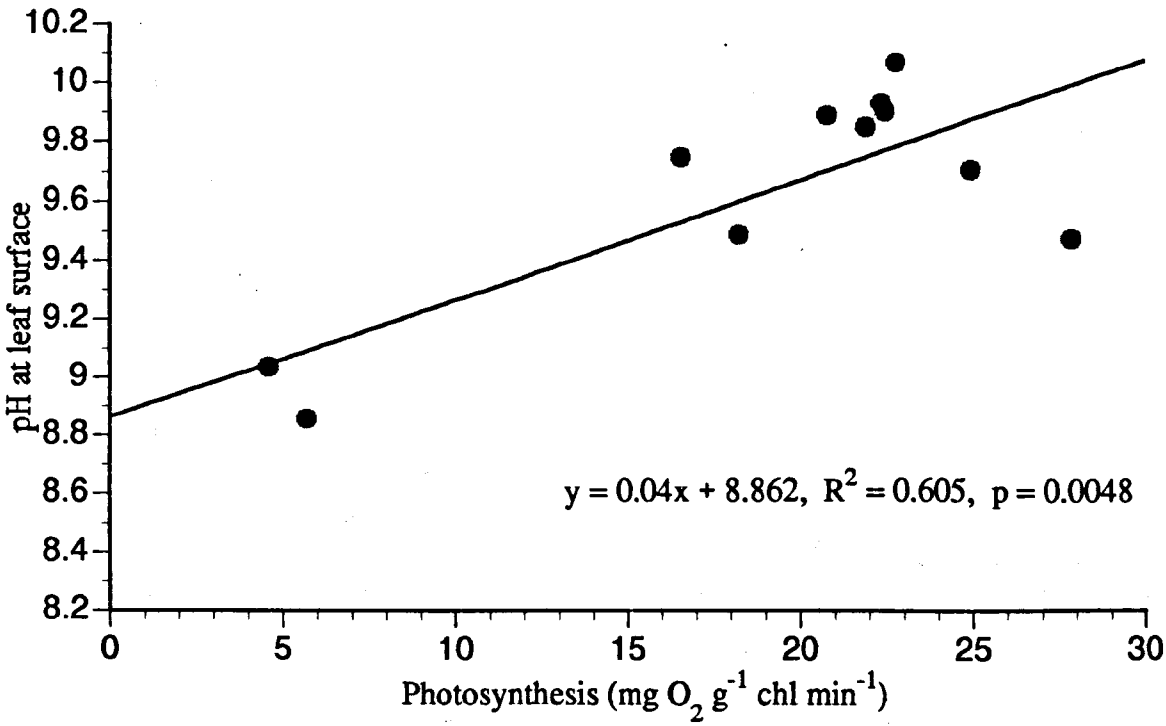


Figure 6.21 Relationship between total chlorophyll content per whorl and pH at the adaxial leaf surface of *E. nuttallii.*

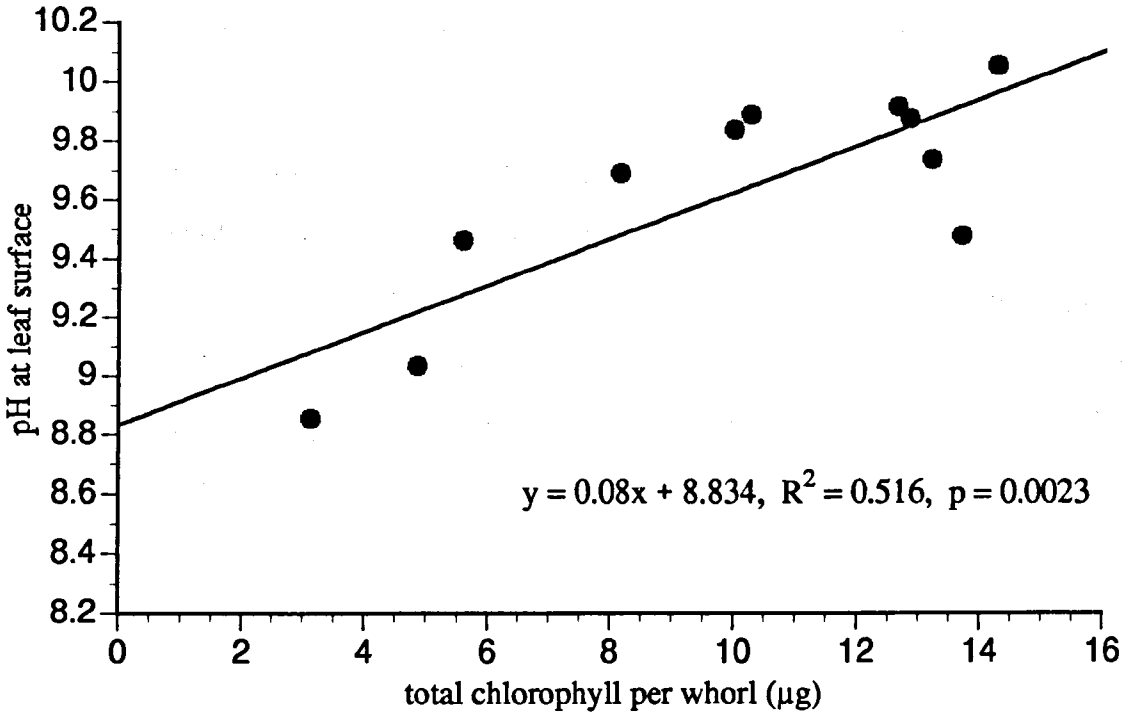
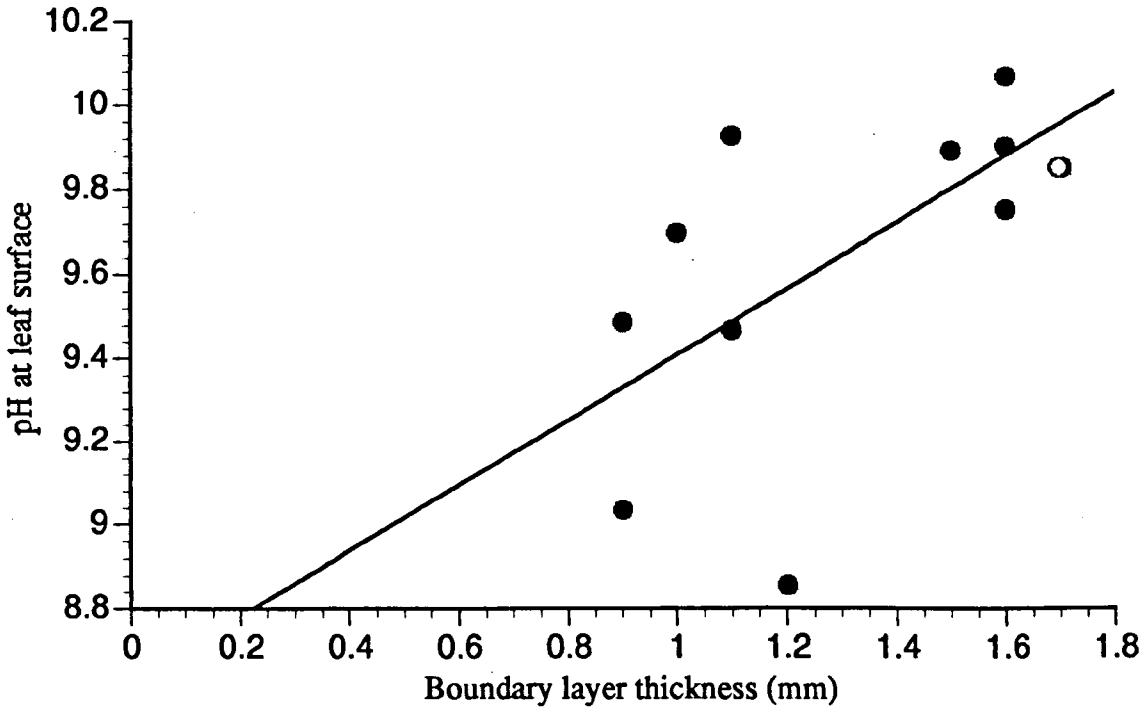


Figure 6.22 Relationship between boundary layer thickness and pH at the adaxial leaf surface of *E. nutallii.*



The equation of this line, including one point (marked O) where there was an interaction from neighbouring leaves, is,

$y = 0.732x + 8.677$, $R^2 = 0.351$, $p = 0.137$

the stem and chlorophyll content of the leaves was much the same as that for position on the stem and photosynthesis, and to a large extent determines it, the total chlorophyll of the leaves described the expected linear relationship with the pH at the leaf surface ($p = 0{\cdot}023$, Figure 6.21).

In contrast the thickness of the boundary layer was not related to the pH at the leaf surface ($p = 0{\cdot}137$, Figure 6.22). Boundary layer thickness is a physical feature determined by the movement of water around the leaf rather than its metabolic activity.

As the number of whorls per unit length was skewed towards the upper part of the shoot, plotting the above parameters against distance from the shoot apex would shift the lines towards the left.

6.2.6 The pH climate surrounding epiphyte free leaves.

6.2.6.1 Methods.

A shoot was set up in the experimental bath as above and illuminated for three hours. Repeat advances were made towards a straight, flat leaf, about 3–5 cm from the apex at different points on and around the leaf blade. Transects were made crossing both the adaxial and abaxial surfaces in a transverse and longitudinal (near to the midrib) fashion. The position of the microelectrode on the leaf, with tip touching the leaf blade, was recorded using either the coarse, vernier (accuracy 0·1 mm) or the fine, micrometer (accuracy 1 μm) scales on the micromanipulator, together with the microscope to determine the edges, tip, and base of the shoot.

The distances from the leaf where the gradient crossed certain pH boundaries were calculated from individual plots of each measured pH gradient. By connecting these points between advances and knowing the position of the advances relative to the leaf it was possible to plot the iso–pH contours surrounding the leaf.

6.2.6.2 Results and Discussion.

The pH of the water surrounding the leaves is determined by a number of highly variable factors (eg. velocity and turbulence of bulk fluid, instantaneous photosynthesis), and these factors are in part unique to each leaf on the shoot. As such the objective was not to categorise the pH climate exactly, but to build a general, descriptive picture which could be applied to other leaves.

The iso–pH plots for the longitudinal transects are shown in Figures 6.23 a – c (adaxial surface) and 6.23 d (abaxial surface), with the plots for the transverse transects shown in Figures 6.24 a – b (adaxial surface) and 6.24 c – e (abaxial surface). All the abaxial surfaces investigated were actively involved in HCO_3^- uptake, as shown by the reduced pH in the adjacent water.

Figure 6.23 Iso-pH lines surrounding leaves of *E. nuttallii*, drawn from longitudinal transects from leaf tip to point of insertion on stem.

a) Shoot 1, adaxial leaf surface. (Position of leaf indicated by the dark bar.)

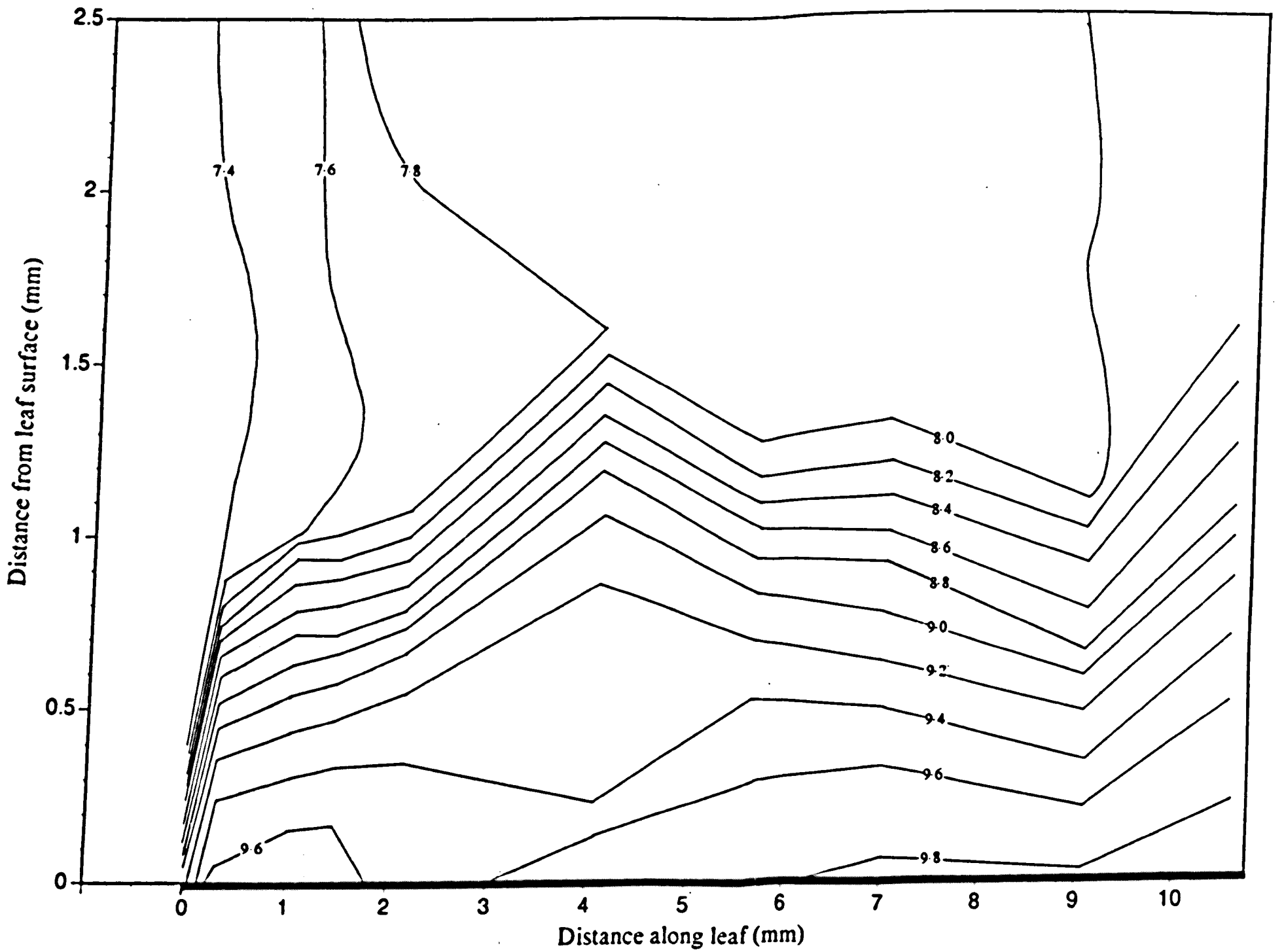


Figure 6.23 Iso-pH lines surrounding leaves of *E. nuttallii*, drawn from longitudinal transects from leaf tip to point of insertion on stem. (continued)

b) Shoot 2, adaxial leaf surface. (Position of leaf indicated by the dark bar.)

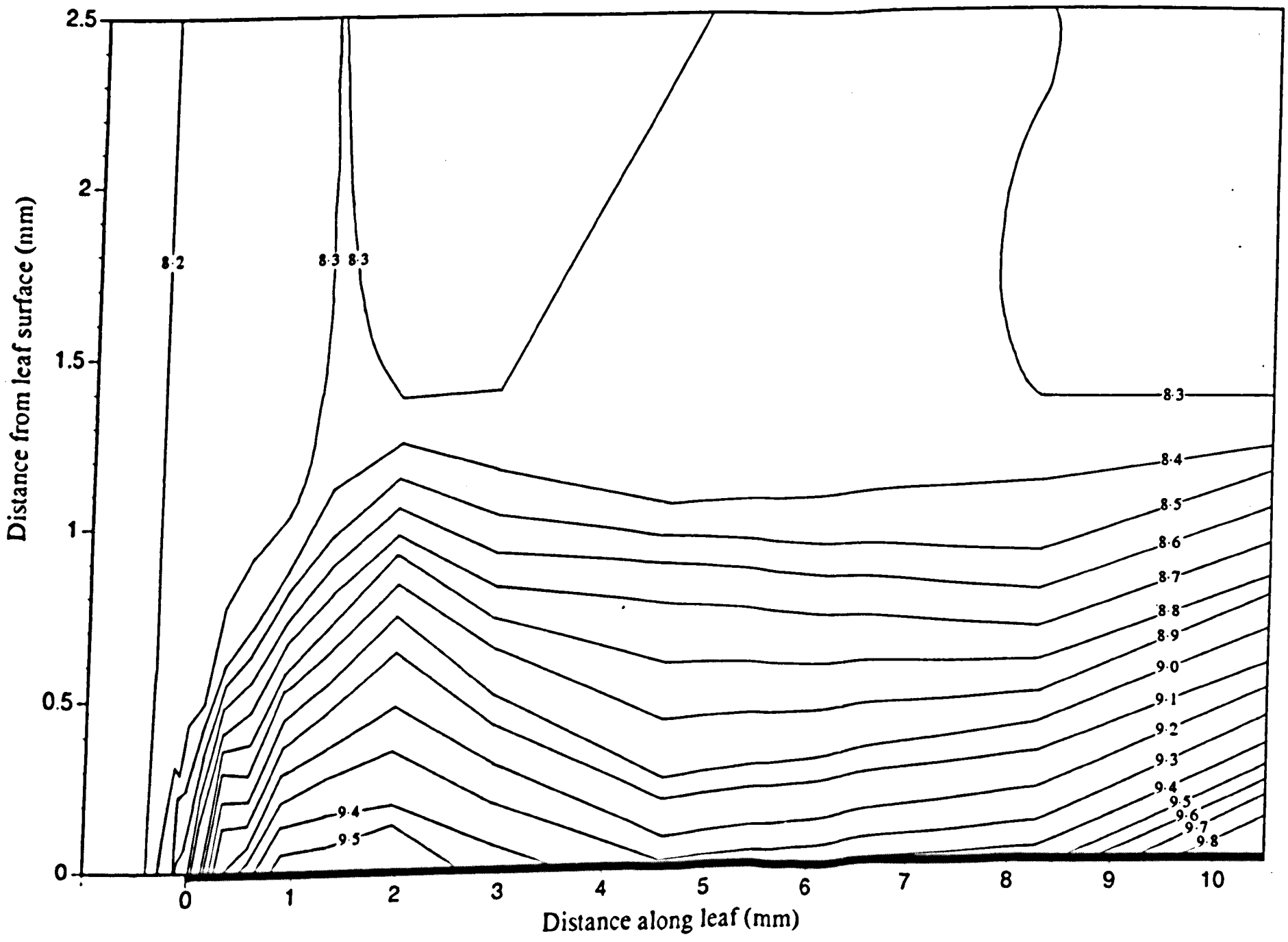


Figure 6.23 Iso-pH lines surrounding leaves of *E. nuttallii*, drawn from longitudinal transects from leaf tip to point of insertion on stem. (continued)

c) Shoot 3, adaxial leaf surface. (Position of leaf indicated by the dark bar.)

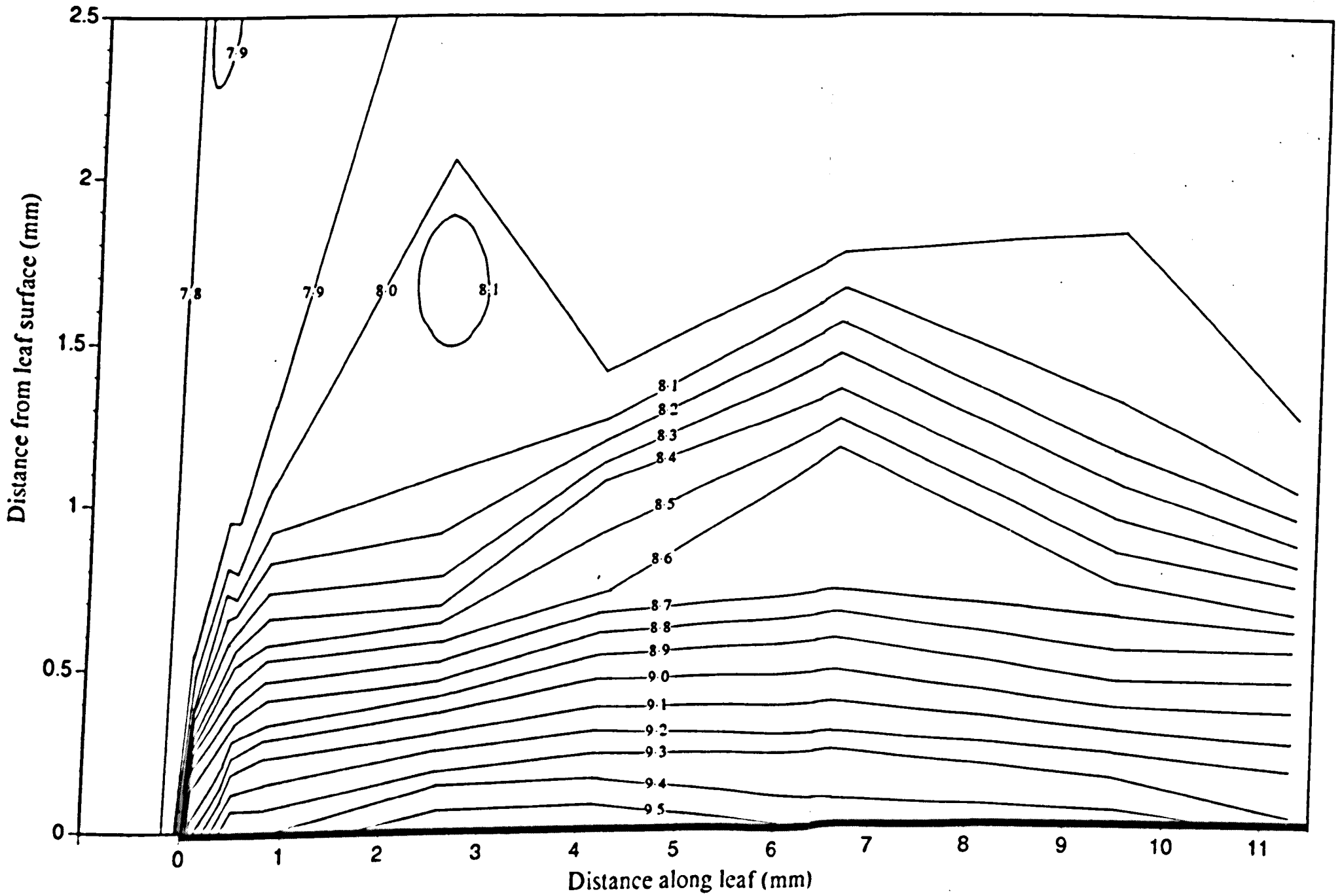


Figure 6.23 Iso-pH lines surrounding leaves of *E. nuttallii*, drawn from longitudinal transects from leaf tip to point of insertion on stem. (continued)

d) Shoot 4, abaxial leaf surface. (Position of leaf indicated by the dark bar.)

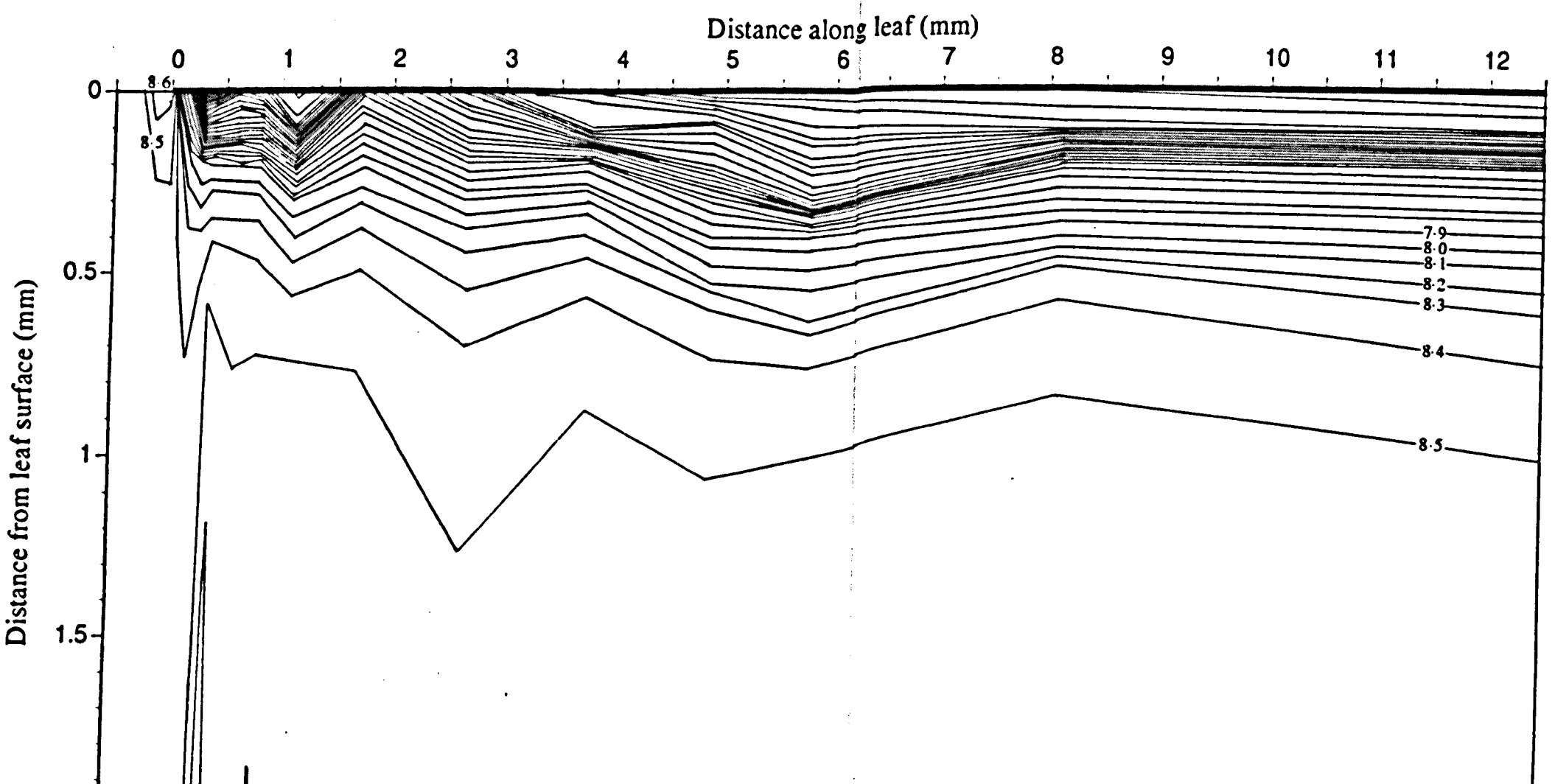
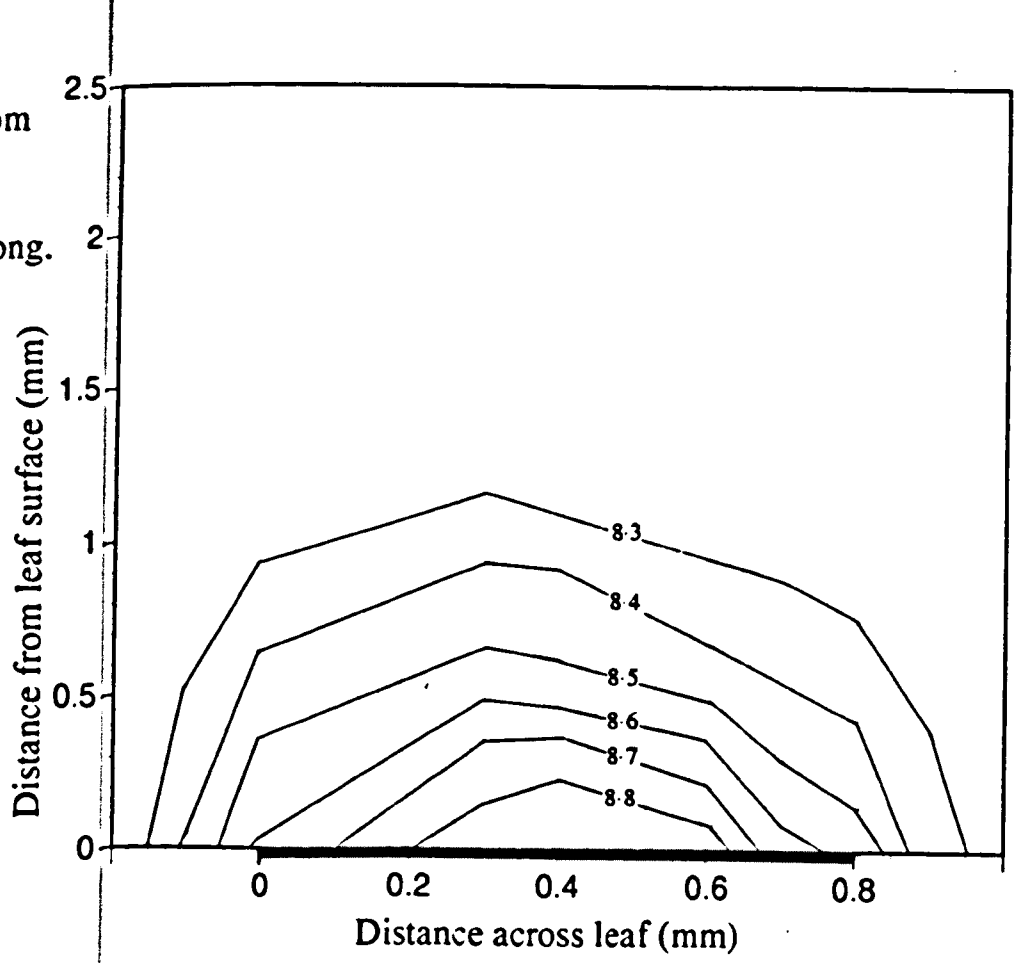


Figure 6.24 Iso-pH lines surrounding the leaves of *E. nuttallii* drawn from transverse transects at different positions on the leaf.

a) Shoot 1, adaxial leaf surface. 0·3 mm from the tip of a leaf 10·7 mm long.



b) Shoot 1, adaxial leaf surface. 5·7 mm from the tip of a leaf 10·7 mm long.

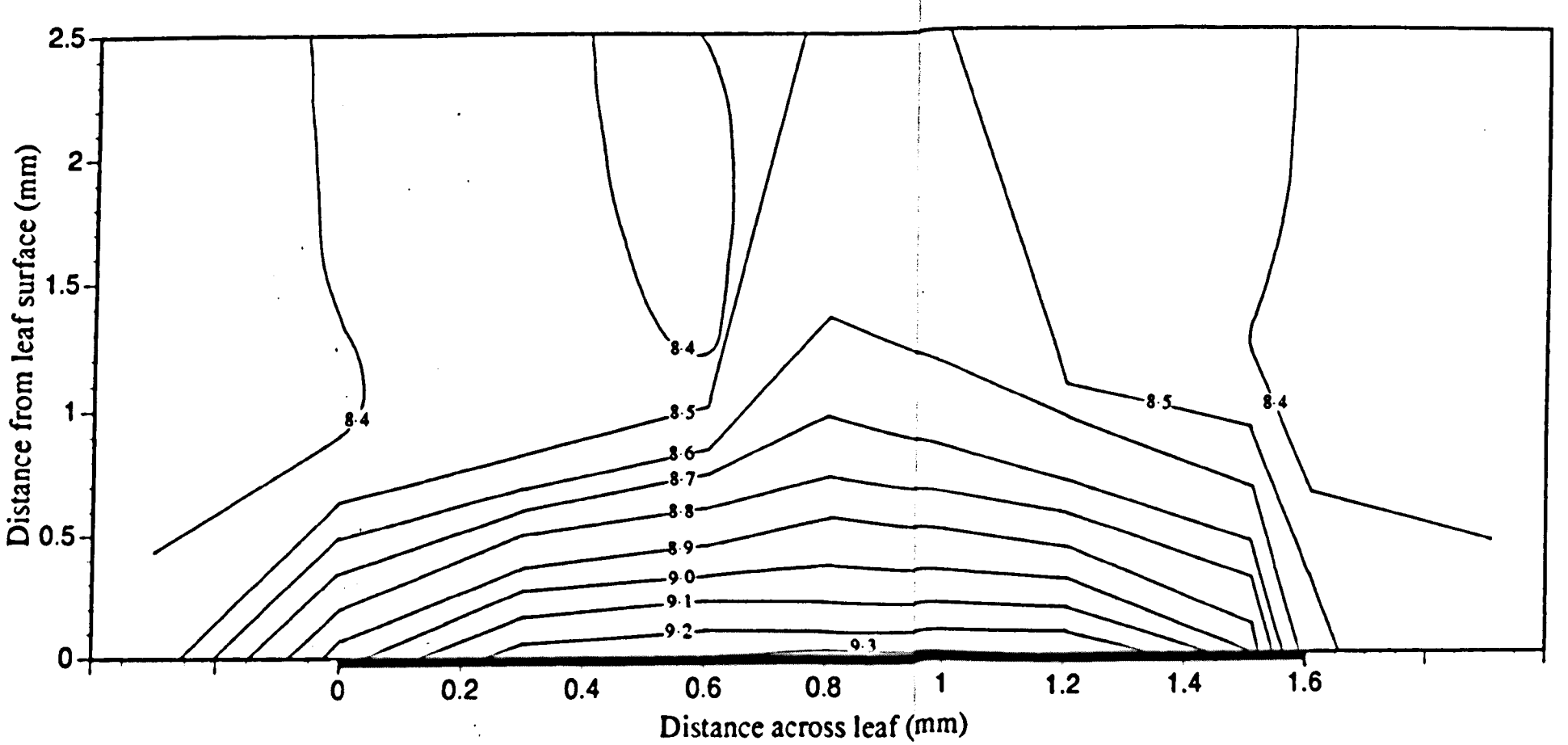
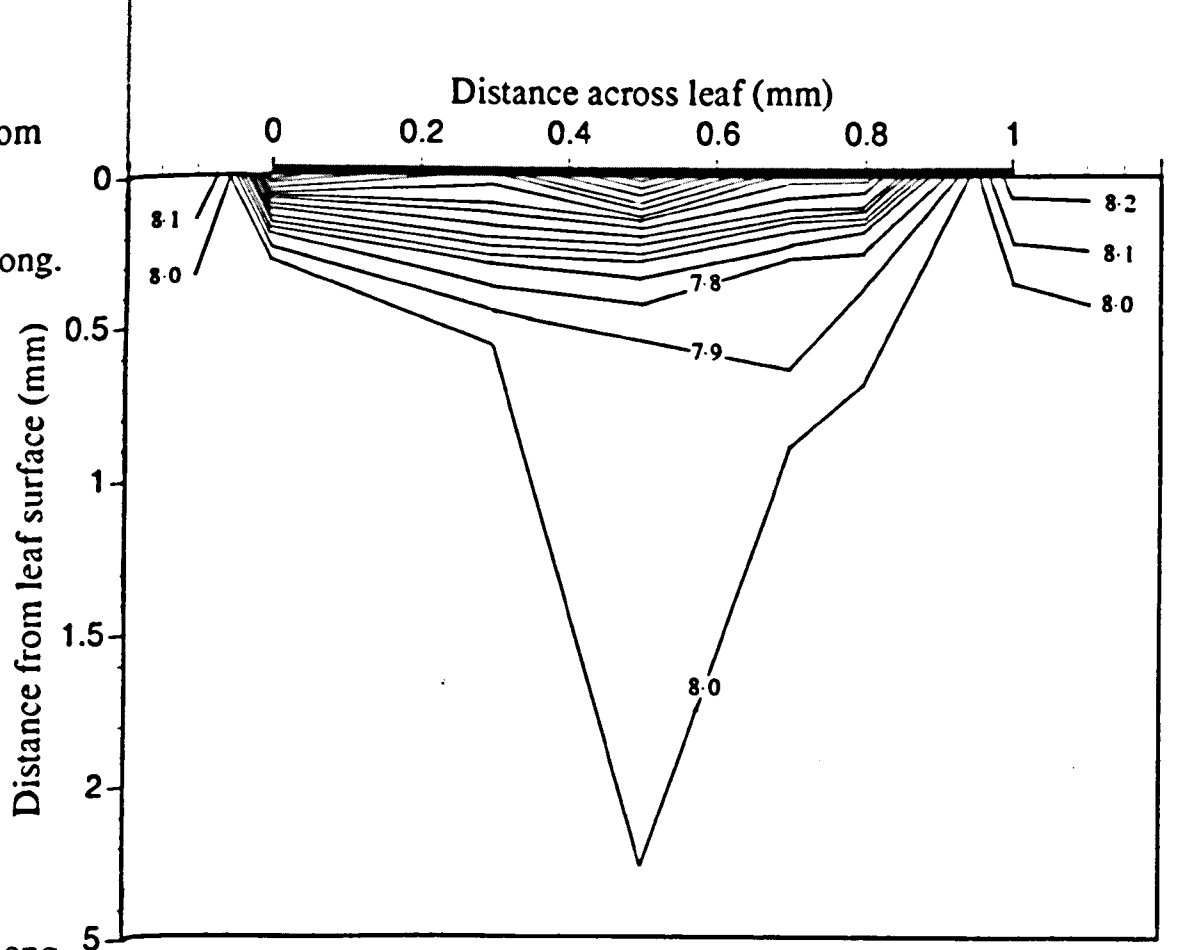


Figure 6.24 Iso-pH lines surrounding the leaves of *E. nuttallii* drawn from transverse transects at different positions on the leaf. (continued)

c) Shoot 2, abaxial leaf surface. 0·5 mm from the tip of a leaf 11·8 mm long.



d) Shoot 2, abaxial leaf surface. 6·3 mm from the tip of a leaf 11·8 mm long.

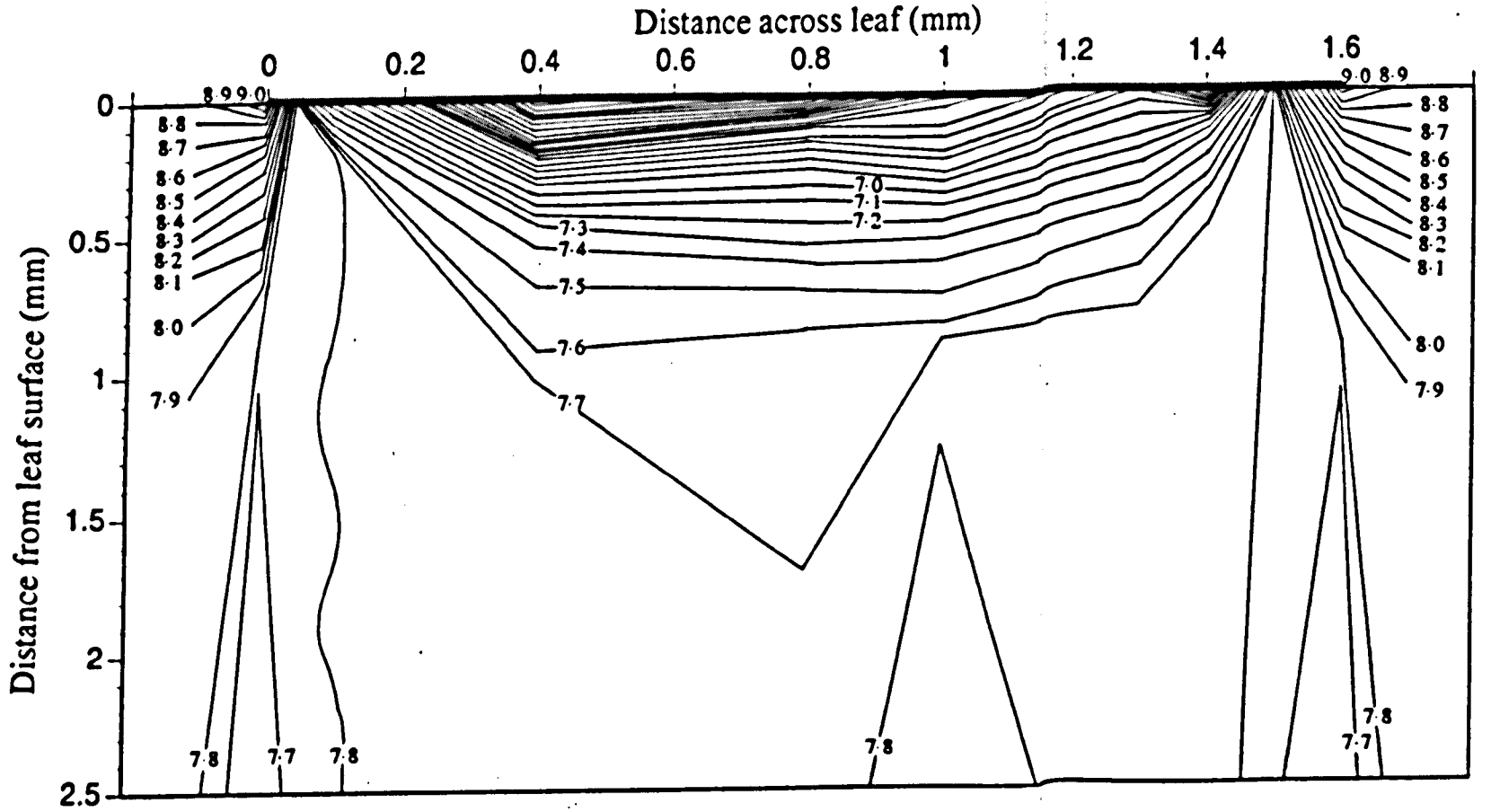
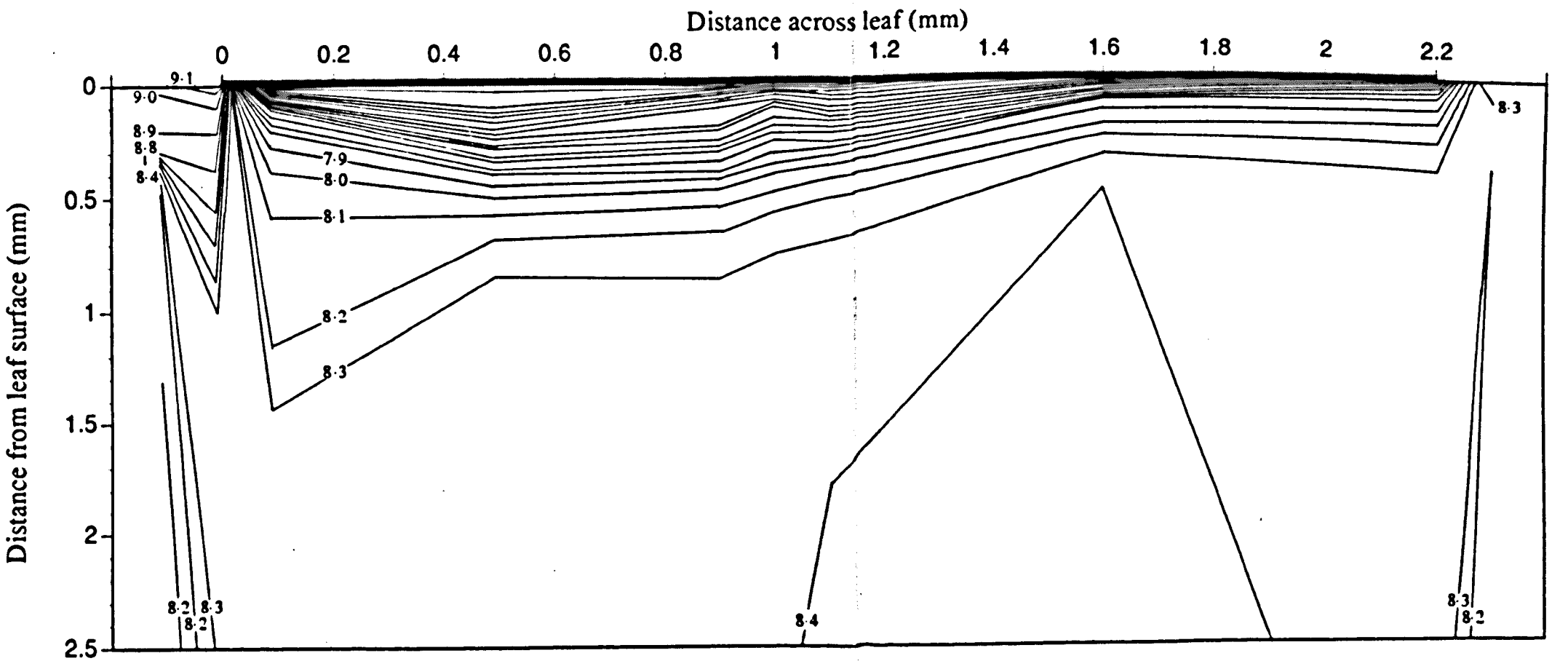


Figure 6.24 Iso-pH lines surrounding the leaves of *E. nuttallii* drawn from transverse transects at different positions on the leaf. (continued)

e) Shoot 2, abaxial leaf surface. 10·5 mm from the tip of a leaf 11·8 mm long.



The longitudinal transects along the adaxial surface (Figures 6.23 a – c) show the largest increase in boundary layer thickness near to the leaf tip and, once established at a width of ≈1·5mm, relatively little increase with distance along the leaf. This is the typical shape of the boundary layer over a flat plate, with the largest growth in boundary layer at the leading edge with respect to the flow (Leyton, 1975). Compared to the boundary layer the leaf is very thin (Plates 20 & 21) and would be expected to act in the same way as a flat plate. As it was the furthest point from the stem, the tip of the leaf was be exposed to the greatest flow and would act as the leading edge. Over most of the leaf surface, the pH was 9·5 – 9·8. No consistent pH change was found near to the stem, nor on a perpendicular approach, supporting the earlier finding that the stem plays little part in photosynthesis (Section 2.3.2.5). Presumably there was a boundary layer around the stem, but as there was no pH change the method used here, which infers flow conditions from changes in pH, was unable to detect it.

An interesting finding is that the whole shoot, as well as its constituent leaves, had a boundary layer enveloping it. This was seen in that the water above the leaves, but outside the leaf boundary layer, was at a slightly higher pH than the bulk fluid (Figures 6.23 a – c). The transverse transect from nearer to the leaf base showed this also (Figure 6.24 e).

The boundary layer from the longitudinal transect along the abaxial surface followed much the same shape, with growth concentrated near the leaf tip (Figure 6.23 d). This leaf was obviously taking up HCO_3^- by the polar leaf mechanism, as shown by the reverse gradient. The pH was less than pH 6 over most of the leaf surface, with a layer of water ≈200 μm thick at less than pH 7 surrounding the leaf. When compared with the theory of Prins and co-workers (Prins *et al.*, 1982a, b & c; Prins & Zanstra, 1985; Prins & Helder, 1985), ie. that this mechanism relies on the conversion of HCO_3^- to CO_2^* mainly within the cell wall or invaginations of the cell membrane, it is obvious that external conversion in the water surrounding the plant will supply plentiful CO_2^* for

photosynthesis. This finding more than satisfies the prediction of Walker *et al.* (1980) for external conversion to be the primary mechanism supplying carbon and not a H^+/HCO_3^- symporter as suggested by Lucas *et al.* (1983; Lucas, 1983 & 1985) and others (Eighmy *et al.*, 1987; Fagerberg *et al.*, 1991). In the only other similar investigation, Lucas *et al.* (1983), using recessed-tip microelectrodes and *Chara corallina*, did not find such steep gradients. Occasionally in the present study the resin receded from the tip of the micropipette and, although the electrode was still functional, the response became slow and underestimated pH change. This was probably a result of the distance from the open tip to the sensitive area where transport of ions must be by diffusion. This problem would hamper recessed tip electrodes in much the same way, and it is suggested that although taxonomically distant, *Chara corallina* may also rely on external conversion. Recessed-tip electrodes are commonly used in intracellular measurements where turgor pressure apparently forces the protoplasm into the recess of the electrode, overcoming the problem described above (Thomas, 1978).

At the tip of the leaf there is a slight increase in pH to 8·5 – 8·6, which appears to be an "overspill" from the increased pH at the adaxial surface. Such overspills were also seen at the leaf edges of the abaxial transverse transects (Figures 6.24 c – e).

The transverse transects across the adaxial surface showed that the shape of the boundary layer followed the same pattern as the longitudinal one, with the largest change in width at the edges (Figures 6.24 a – b). Since the leaves have two edges, the boundary layer has a rounded appearance, with a flat middle section if the leaf is sufficiently wide (Figures 6.24 b). The pH over most of the leaf blade is constant and appears to increase with distance from the leaf tip. The leaf mid-rib was not marked by a decline in pH, although earlier investigations had found that marl was not deposited in this region (Plates 7, 8 & 9). The absence of marl from the leaf edges can be explained by the lower pH in this region, but the same relationship evidently did not apply at mid-rib. Perhaps the suggestions that Ca^{2+} ions are transported through the leaf, via either the symplast or

apoplast (Prins *et al.*, 1982c; Lucas, 1983 & 1985; Prins & Helder, 1985; Smith, 1985; Miedema & Prins, 1991), do have some basis, and it is a disruption of this process which prevents marl deposition over the mid-rib. As the polar-leaf mechanism appears to involve close coupling of the upper and lower layer of cells, those of the mid-rib, where the two layers are separated by the vascular bundle, were probably not involved in HCO_3^- uptake. The pH is more likely to be raised near to the mid-rib by the activity of nearby cells.

The abaxial surfaces again show the expected shape of boundary layer (Figures 6.24 c – e). The overspill from the upper surface was quite pronounced in some cases (eg Figure 6.24 d). If the cells of the leaf edge are not involved in HCO_3^- uptake, then this region will have no ability to counteract the increased pH, which may go some way to explain how such overspills occur. Again a substantial layer of water is of sufficiently low pH to allow external conversion to be the principal source of CO_2^*.

Since the leaves were essentially acting as thin, flat plates it was possible to use the equations derived from fluid dynamics theory to calculate an approximate velocity of the bulk fluid in the experimental bath. Using a value of 1·6 mm (the distance from the leaf beyond which there was no further detectable pH change) as the thickness of the physical boundary layer 0·2 mm from the edge the leaf (Figure 6.24a), and defining the limit of this layer as 99% of the free stream velocity we can rearrange the equation given in Section 3.1 to give a convection driven velocity.

$$\text{Free Stream Velocity } U \approx \frac{25\,\nu l}{\delta_L^{\,2}}$$

$$\approx \frac{25 \times 10{\cdot}1 \times 10^{-7} \times 0{\cdot}0002}{0{\cdot}0016^2}$$

$$\approx 1{\cdot}97 \text{ mm s}^{-1}$$

The actual velocity of the water will be slightly faster near to the tip of the leaves than near to the stem, where the stem and leaves will serve to trap water and reduce

movement. As such, this figure of ≈2 mm s^{-1} is probably a good estimate of the bulk fluid velocity, but it is only an approximation, the actual value will vary with time and between shoots. This calculated value is at the upper end of the range of measured, mainly wind-driven flow within weedbeds growing in lakes (0·3 – 4·6 mm s^{-1}, Losee & Wetzel, 1988 & 1993), but at the lower end of the range for plants growing in streams (3 – 34 mm s^{-1}, Madsen & Warnke, 1983). *E. nuttallii* is typically a lake, pond or slow-flowing river plant and as such the description of the boundary layer and its dimensions given here approximate to the conditions in the field. Further work is required on the effect of flow on the boundary layers surrounding the leaves of submerged plants.

6.2.7 CO_2^* climate within the boundary layer calculated from the measured pH.

The primary object of this work is to elucidate the conditions within the boundary layer and here the CO_2^* concentration is of great importance. In the bulk fluid the CO_2^* concentration can be calculated from the pH and the concentration of total DIC using equations based on the dissociation constants of the various reactions involved (Mackereth *et al.* 1978). Within the boundary layer, where conditions are maintained in a state of disequilibrium by the constant removal of CO_2^* by photosynthesis, it must first be shown that this removal is not causing a serious depletion of the total DIC before applying these equations.

Hence;

the maximum rate of photosynthesis at CO_2^* and O_2 concentration found in the bulk fluid

$$= 5 \text{ mg } O_2 \text{ g}^{-1} \text{ chl min}^{-1}$$

$$= 0{\cdot}002604 \text{ mmol g chl}^{-1} \text{ s}^{-1}$$

Since chlorophyll is present in the leaves at $21{\cdot}18 \ \mu g \ cm^{-2}$

$$= 0{\cdot}0005516 \text{ mmol m}^{-2} \text{ s}^{-1}$$

$$= 0{\cdot}5516 \ \mu mol \ m^{-2} \ s^{-1}$$

The distance (x) that CO_2^* can diffuse in 1 sec can be calculated from Poisson's equation

$$x^2 \approx 4Dt$$

where D is the diffusivity (taken from International Critical Tables as $1{\cdot}8 \times 10^{-9} \ m^2 \ s^{-1}$) and t is the time taken, ie 1 second

$$x \approx \sqrt{7{\cdot}2 \times 10^{-9}}$$

$$\approx 0{\cdot}000085 \text{ m or } 85 \ \mu m$$

For a sheet of water surrounding the plant 85 μm thick and 1 m^2 in area

$$\text{Volume} = 0{\cdot}085\ 1$$

and this layer contains, at a total DIC concentration of 2·4 mmol l^{-1}

$$\text{DIC} = 204\ \mu\text{mol}$$

which at pH 9·6 gives

$$CO_2^* = 0{\cdot}095\ \mu\text{mol}$$

Of the CO_2^* used by photosynthesis 17·2% can be replaced by diffusion.

The other pathway by which CO_2^* can be replaced is by dissociation from HCO_3^-, which is a complex process with the reactions

$$2H^+ + CO_3^{2-} = H^+ + HCO_3^- = H_2CO_3 = H_2O + CO_2 \qquad (1)$$

and

$$HCO_3^- = CO_2 + OH^- \qquad (2)$$

acting in parallel.

Overall, this reaction has a pH-dependent time constant of 3·15 sec at pH 9·6 (calculated from Walker *et al.*, 1980). Reaction (2) is the primary one at the high pH's relevant here, but total DIC must be used to calculate how much CO_2 is potentially replenished within this layer in 1 second.

$$CO_2 = \frac{204}{3{\cdot}15}$$

$$= 64{\cdot}76\ \mu\text{mol}$$

When this is compared with the 0·5516 μmol used by photosynthesis, it can be seen to be more than sufficient to replace that lost. Dissociation was concluded to be very important in the supply of CO_2 for photosynthesis by Helder (1985), in his theoretical work on boundary layers.

The total DIC present in the layer, 204 μmol, is also very much greater than the amount used in photosynthesis, with 0·3% of the total used per second. The diffusivity of

HCO_3^- = 1·185 10^{-9} m^2 s^{-1}, and CO_3^{2-} = 0·923 10^{-9} m^2 s^{-1}, somewhat slower than CO_2, but the far greater concentrations (especially of HCO_3^-) would increase the flux and enable that used up to be rapidly replaced. It is possible that $CaCO_3$ precipitation occurred at the adaxial leaf surface, but again diffusion and the relatively high DIC concentration would probably have counteracted any losses.

Therefore, use of the equations of Mackereth *et al.* (1978) is valid, with neither a serious depletion of total DIC nor CO_2 occurring. If serious depletion did occur however, then these equations will overestimate the CO_2 concentration, and hence the calculated values should be regarded as maximum estimates.

6.2.7.1 Results and Discussion.

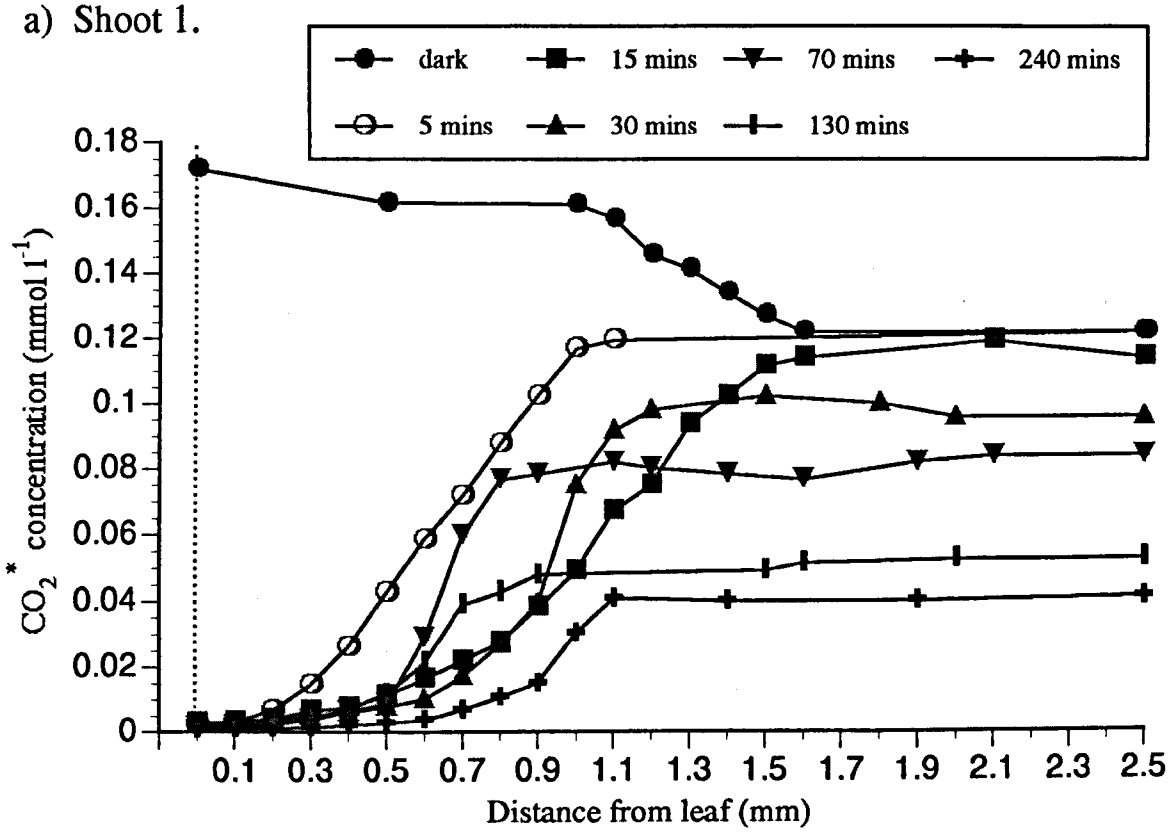
The CO_2^* concentrations were calculated for the results from Section 6.2.3, repeat advances towards leaves at different times after the onset of illumination, and Section 6.2.6, advances made towards different points on the leaf blade.

i) Time after onset of illumination.

From the results of these calculations on the approaches towards the adaxial surfaces (Figure 6.25 a & b), it is obvious that there was a massive reduction in CO_2^* in the water abutting the leaf within minutes of the start of illumination. At the start, in darkness, the CO_2^* concentration at the leaf surface was higher than in the bulk fluid due to net respiration of the leaf, but after five minutes the concentration was less than 5 µmol l^{-1}. The concentration at the leaf surface remained below this low CO_2^* concentration for the whole period of illumination, indicating that fluctuations in pH seen previously (Figure 6.8) were insignificant and could easily be caused by small variations in the instantaneous rate of photosynthesis. Even though there was a difference in the initial concentration of

Figure 6.25 Calculated CO_2^* gradients approaching the upper surface of leaves at different times after the onset of illumination.

a) Shoot 1.



b) Shoot 2.

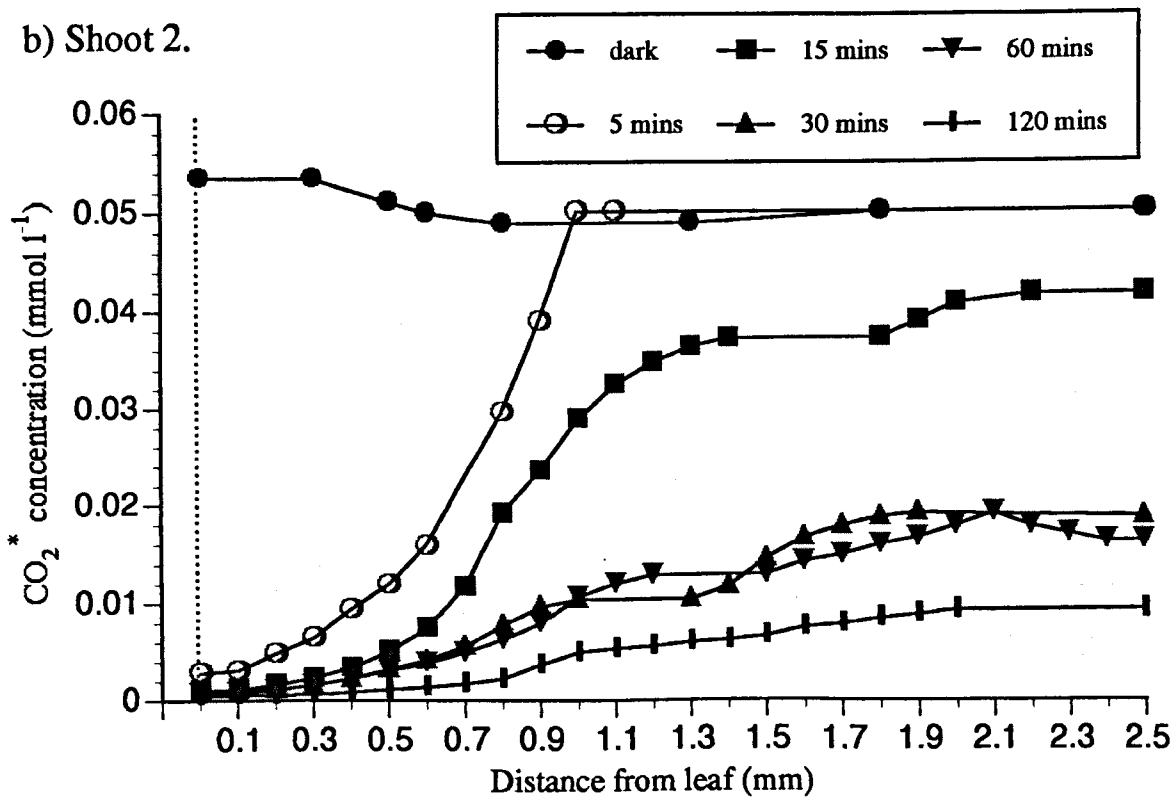
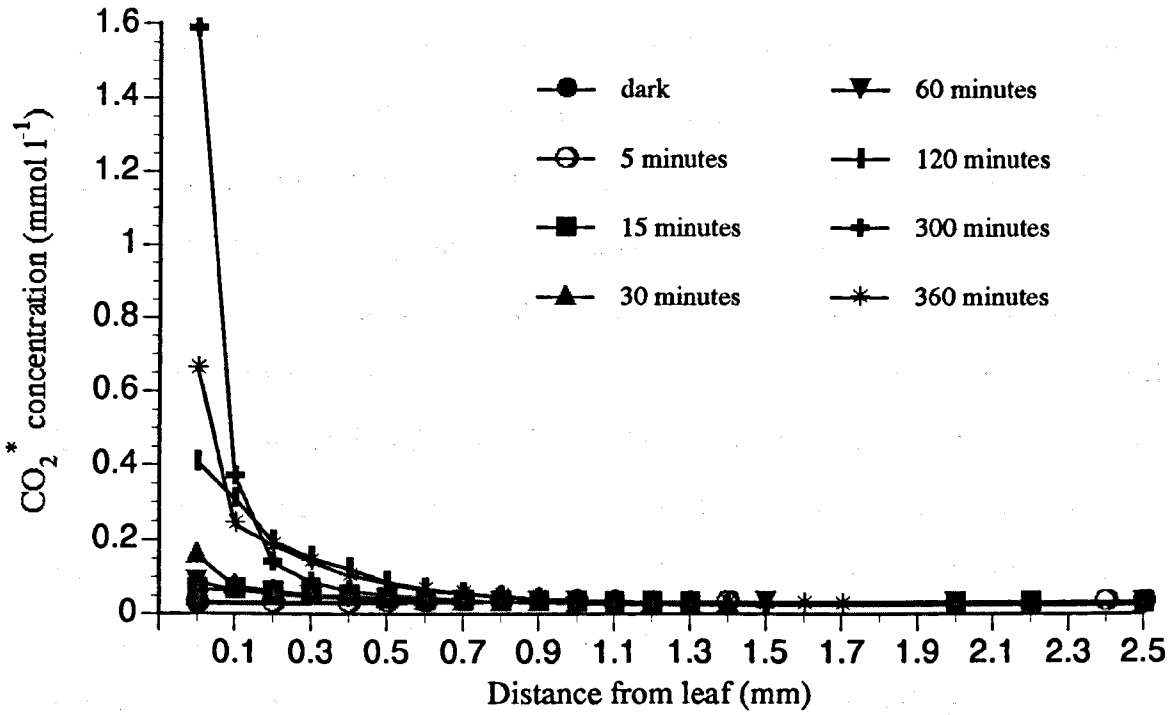
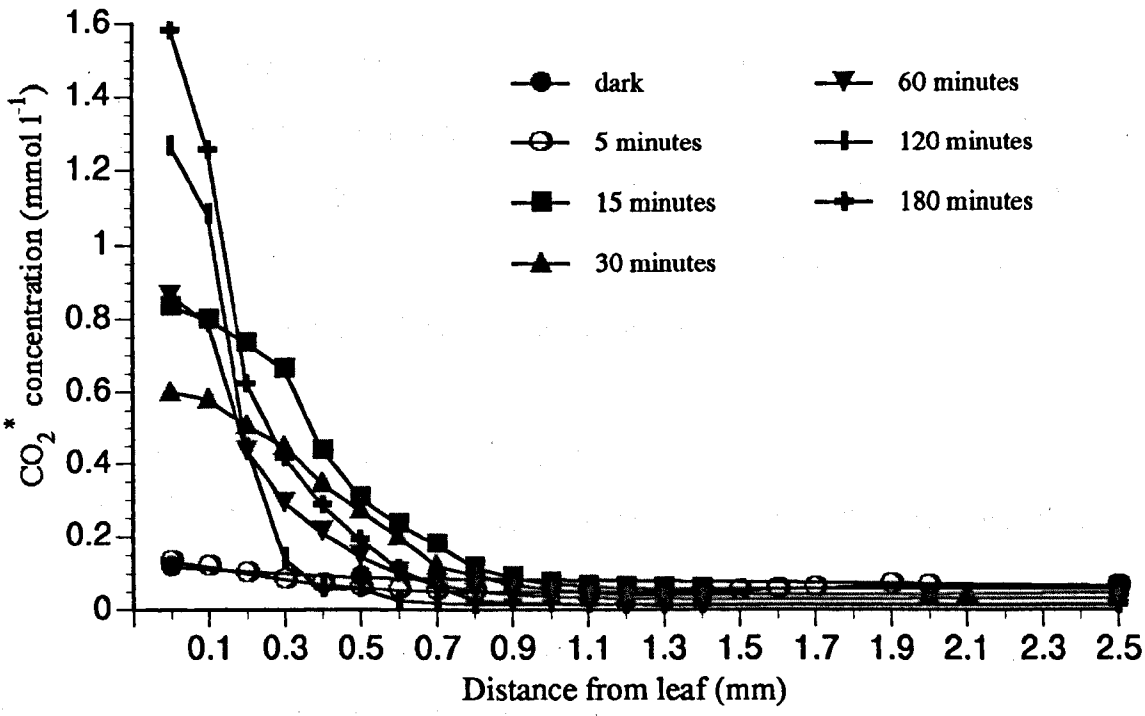


Figure 6.26 Calculated CO_2^* gradients approaching the underside of a leaf at different times after the onset of illumination.

a) Shoot 1.



b) Shoot 2.



CO_2^* in the bulk fluid between these two investigations (≈ 0·05 mmol l^{-1} *cf.* ≈ 0·12 mmol l^{-1}), the concentration at the leaf surface was very similar.

In both cases, the edge of the boundary layer was clearly identifiable after a short period of illumination, but became less clear as the CO_2^* within the bulk fluid was depleted (Figure 6.25 a & b). The shapes of the CO_2^* gradients approaching the leaves differed markedly from the pH gradients, with maximum change towards the outer part of the boundary layer and little change in concentration near to the leaf.

At the abaxial leaf surface, the reduced pH caused large increases in CO_2^* availability (Figure 6.26 a & b), though the absolute concentrations were somewhat variable. When compared to the bulk fluid, CO_2^* was far more concentrated near to the leaf surface, up to 10 times more, which presumably translates into increased fixation. The cost involved in running the H^+ pump must be large for the plant not to turn this high concentration of CO_2^* into an increased growth rate (see Section 4.3.4), even though only half the leaf surface is involved in carbon uptake. The variations in CO_2^* concentration at the leaf surface were presumably attributable to variations in the rate of H^+ pumping by the leaf.

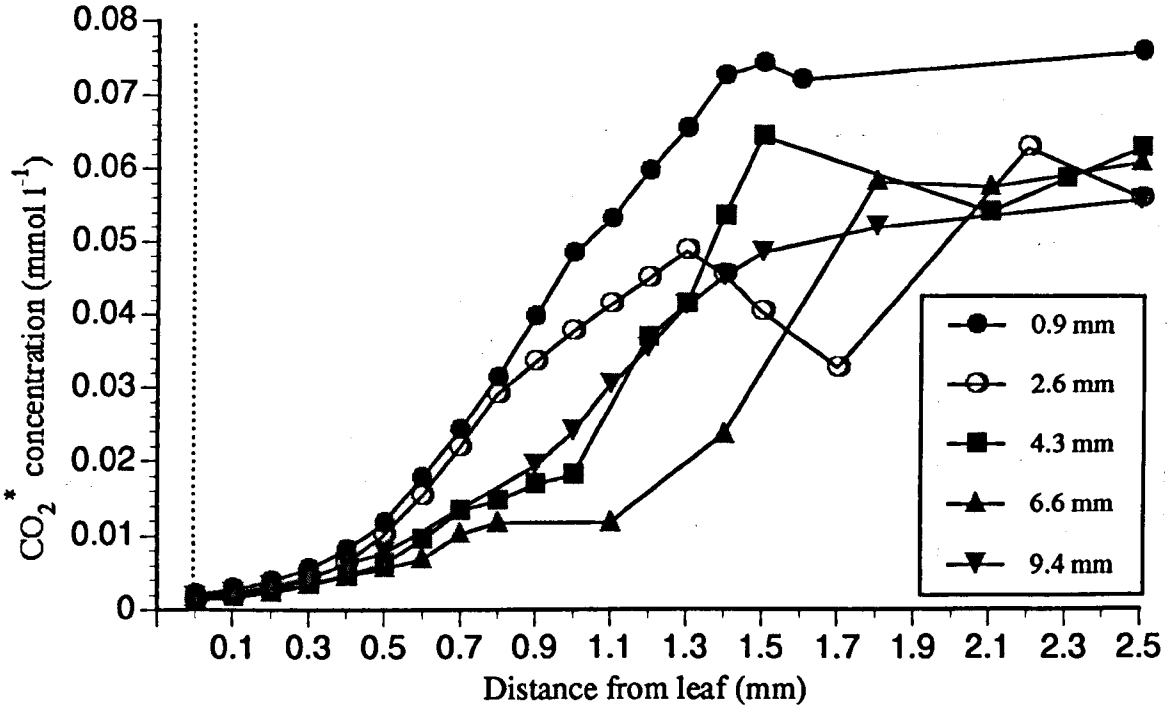
The shapes of the CO_2^* gradients were determined largely by the fact that the abaxial surfaces were effectively acting as a source rather than as a sink for CO_2^*, and were very different from those from the adaxial leaf surface. Most of the change in concentration occurred near to the leaf and hence it is difficult to determine the outer limit of the boundary layer from these plots (Figure 6.26 a & b).

ii) Different positions on the leaf blade.

Data from four investigations were used to calculate the CO_2^* concentrations over large parts of the leaf blade, two from the longitudinal transects (pH data shown in Figures 6.23 c & d) and two from the transverse transects (pH data shown in Figures 6.24 b &e). Data from the edges and tip of the leaves were not used.

Figure 6.27 CO_2^* concentrations calculated from longitudinal transects data, lines represent gradients at different points from the leaf tip.

a) Adaxial leaf surface, pH data shown in Figure 6.23c.



b) Abaxial leaf surface, pH data shown in Figure 6.23d.

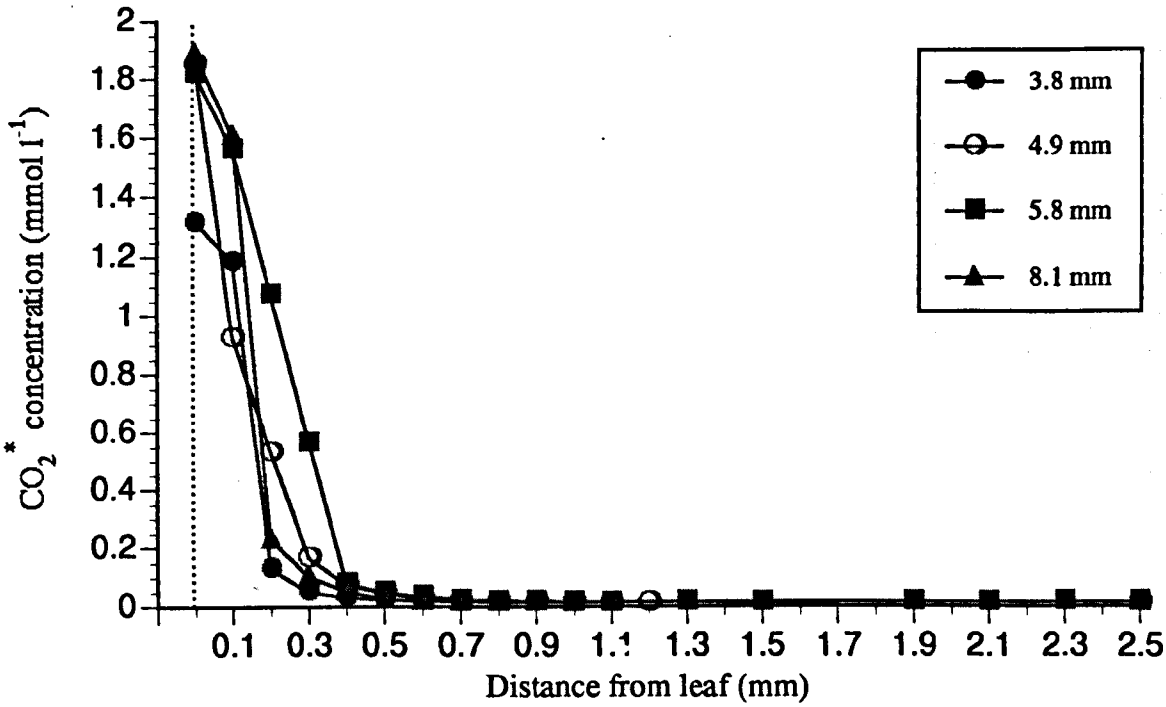
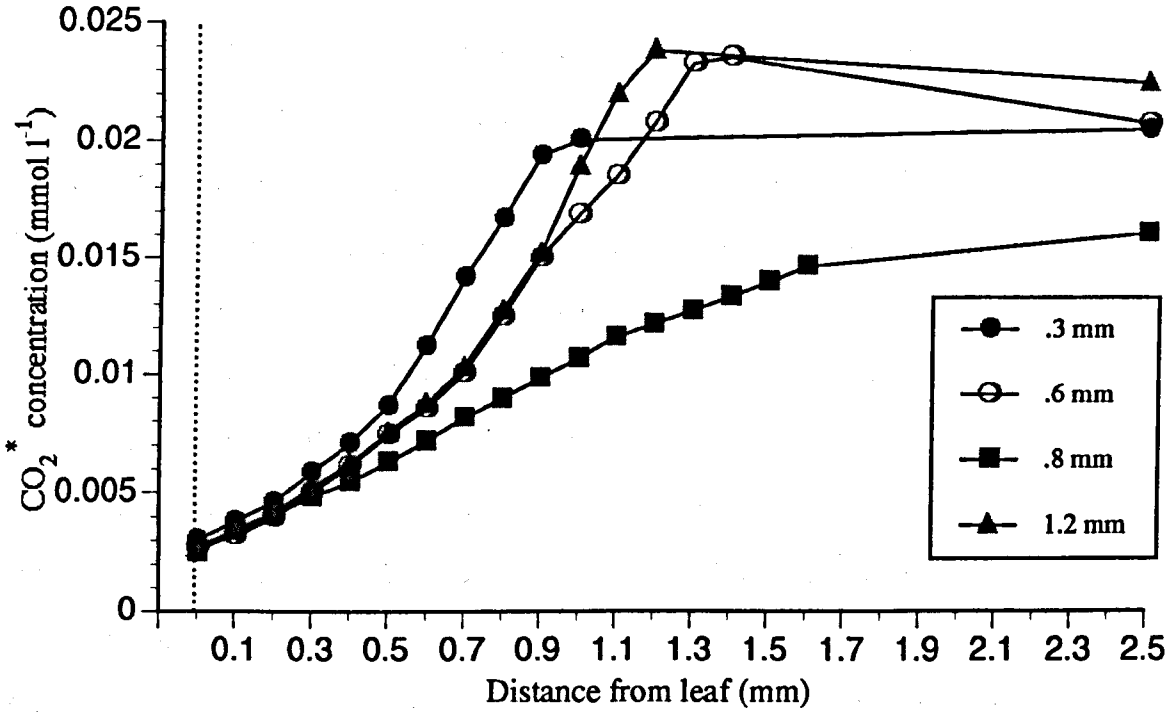
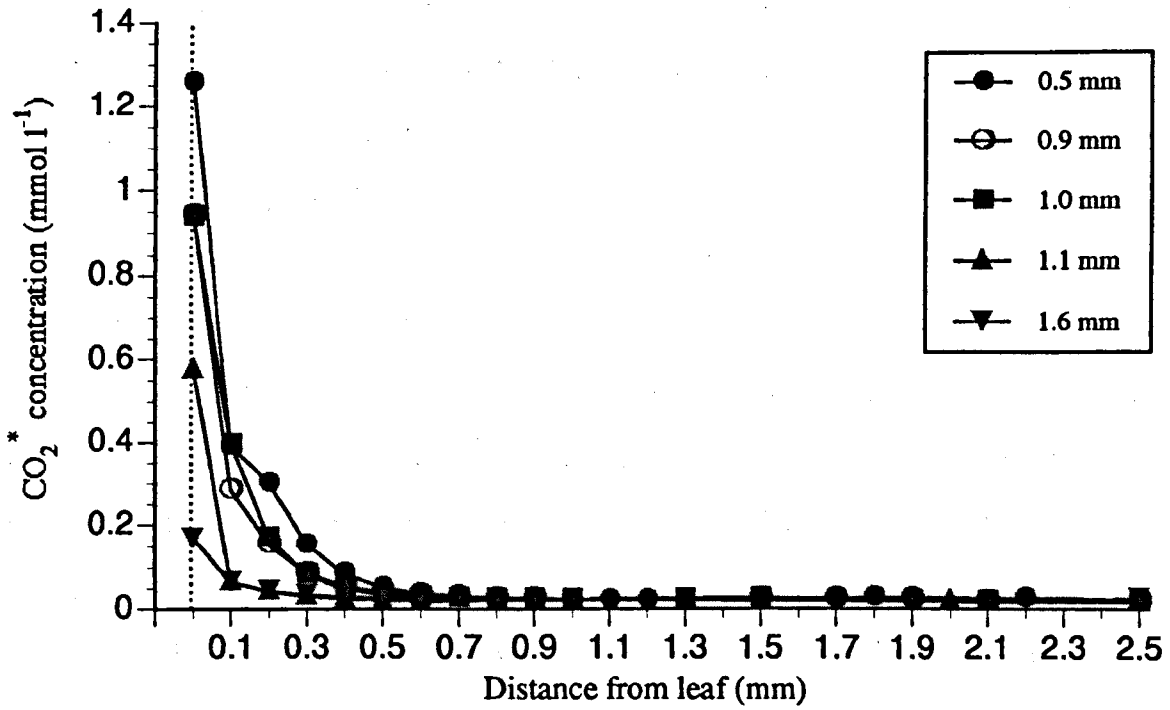


Figure 6.28 CO_2^* concentrations calculated from transverse transects data, lines represent gradients at different points from the leaf edge.

a) Adaxial leaf surface, pH data shown in Figure 6.24b.



b) Abaxial leaf surface, pH data shown in Figure 6.24e.



The gradients from the longitudinal (Figures 6.27 a) and transverse (Figures 6.28 a) transects over the adaxial surface showed similar characteristics. In both cases, the CO_2^* at the leaf surface was less than 5 μmol l^{-1}, even though the bulk fluid concentration was different. The gradients were also of a similar shape, with easily definable outer limits and again resembled those from the previous data (Figure 6.25). Of noteworthy interest was the gradient measured 2·6 mm from the leaf tip, where a zone of higher pH water was in the process of being lifted from the boundary layer, presumably by turbulence in the bulk water. This demonstrates how the boundary layer is very much dependent on the bulk flow and how variations in this flow can cause large alterations to the boundary layer.

The longitudinal (Figure 6.27 b) and transverse (Figure 6.28 b) transects over the abaxial leaf surfaces again showed remarkable similarity, even though they were also taken from different leaves and from different shoots. The CO_2^* concentration at the leaf surfaces was about 10 times higher than in the bulk fluid, except at one point (Figure 6.28b, 1·6 mm) which was close to the edge of the leaf, and may not have been representative of the rest of the leaf blade. The shape of these gradients was once more very consistent, with a layer of water of high CO_2^* concentration about 200 μm thick abbuting the leaf. This, like the pH data, more than satisfied the predicted conditions whereby bicarbonate utilisation would function through external conversion of HCO_3^- into CO_2^* (Walker *et al.*, 1980).

When considering the large time-scales and relatively large areas of leaf that have been covered in these two investigations, the overall impression is one of remarkable uniformity. It appears that the CO_2^* concentration is rapidly reduced to less than 5 μmol l^{-1} over most of the leaf surface as soon as the leaf is illuminated. This concentration is not much greater than the CO_2^* compensation point for *E. nuttallii*. Since the compensation point is the lowest concentration at which the plant can successfully show net positive photosythesis, it is not surprising to find that it is unable to reduce the CO_2^* concentration

below this level. The flux of carbon into the cells, determined by the length and steepness of the concentration gradient from the bulk fluid to the point of fixation in the chloroplast, will be a major determinant of the rate of photosynthesis. Since the concentration at the leaf surface is not much greater than the compensation point, the internal resistance to photosynthesis cannot contribute greatly to the overall resistance, a conclusion in agreement with other workers (Smith and Walker, 1980; Black *et al.*, 1981).

The CO_2^* concentration at the abaxial surface of an illuminated leaf showing polar activity is again largely consistent, but very much increased, about ten times, when compared to the bulk fluid. This will probably translate into a very much increased CO_2^* concentration within the leaf chloroplasts, and an increased rate of fixation.

Section 3.

Microelectrode Investigations of the Epiphyton – Macrophyte Association.

6.3.1 The interaction between *E. nuttallii* and its epiphyton.

Since the microelectrode apparatus was only capable of detecting changes in pH resulting from the balance between photosynthesis and respiration and not the agent(s) causing this effect, it was decided to work initially on the epiphyton alone, grown on an inert substrate, before investigating both epiphyton and plant together. By separating the system into its components in this way, it was hoped that the nature of the interaction between *E. nuttallii* and its epiphyton could be elucidated more fully.

The use of the word epiphyton for the algal community and other associated matter and organisms growing on the plastic plants is not technically correct (Wetzel, 1983a), but is used here as it is a more descriptive term than the correct title of periphyton, a general term for attached algae. It was hoped that the community growing on the plastic plants would be typical of epiphyton growing on *E. nuttallii* and other submerged macrophytes.

6.3.2 Epiphyton on plastic plants.

6.3.2.1 Methods.

The thickness of a boundary layer is in part determined by the distance from the leading edge of the object (Leyton, 1975), as well as the flow of water around the object. In order to simulate, as far as possible, natural dimensions and flow conditions it was decided to use artificial, plastic plants as a substrate for growing epiphyton. Of the artificial aquarium plants available the one which most closely resembled *E. nuttallii* was plastic *Egeria densa*, which was of similar size and shape but had five leaves in each whorl.

i) Culture.

Three such plastic plants, each 20 cm long, were attached to a fishing weight by 50 cm lengths of braided nylon thread. A fourth thread, approximately 3 m long, was also attached to the weight. In total nine plastic plants were set up in this fashion, on three paternoster rigs. These rigs were then thrown into a weed-bed situated on the offside of the Leeds and Liverpool Canal at Aintree (SJ374987) in a fan pattern, with the plastic plants suspended in approximately 1 m of water (Figure 6.29). The weed-bed was composed mainly of *E. nuttallii*, but small quantities of *Myriophyllum spicatum* L. and *Potamogeton trichoides* Cham. & Schlecht. were also present. The long thread was secured to a stake driven into the canal bed. Once in this position, the rigs were left for 3 weeks, from the 6th to the 27th of July 1993, allowing the plastic plants to be seeded with a "natural" epiphyton community.

Once retrieved from the canal the plastic plants were carefully placed into a bucket of canal water for transport back to the laboratory. Upon return, the threads attaching the plastic plants to the weights were cut, leaving 5 cm for the attachment of a plastic screw valve to act as a weight during culture and one plant was carefully placed in each of nine glass jars containing 2·5 l of twice-filtered canal water. These jars were then cultured for

Figure 6.29 Diagram of paternoster rig with plastic plants in position in the canal during seeding.

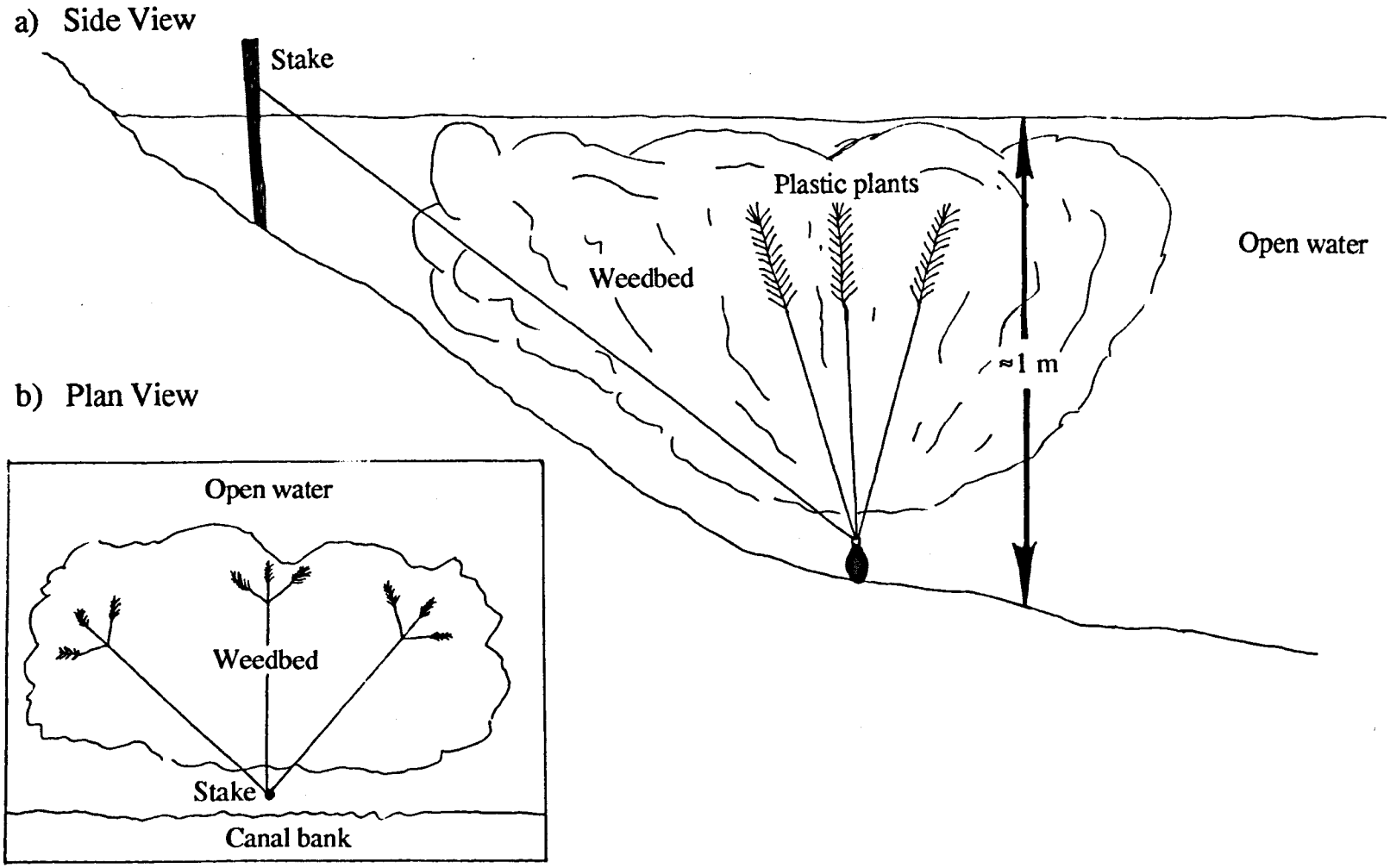


Plate 22 The plastic plants with associated epiphyton in the glass jars during the period of culture under three different loading regimes.

Plate 23 *E. nuttallii* and associated epiphyton in the glass jars during the period of culture under three different loading regimes.


8
6


7

21 days at 20 °C, receiving illumination of 100 μmol m^{-2} s^{-1} PAR on a 16 hour light : 8 hour dark cycle and bubbled with untreated air (Plate.22). Whilst in culture, the jars were loaded weekly with nutrients in one of three ways:

i) with 2 mg l^{-1} Si (as $Na_2SiO_3 \cdot 5H_2O$),

ii) with 2 mg l^{-1} Si (as $Na_2SiO_3 \cdot 5H_2O$) + 2 mg l^{-1} N (as $Ca(NO_3)_2 \cdot 4H_2O$) + 100 μg l^{-1} P (as K_2HPO_4),

iii) or with 2 mg l^{-1} Si (as $Na_2SiO_3 \cdot 5H_2O$) + 6 mg l^{-1} N (as $Ca(NO_3)_2 \cdot 4H_2O$) + 300 μg l^{-1} P (as K_2HPO_4),

referred to hereafter as low, medium and high loading. It was hoped that loading nutrients in this way would encourage epiphyton to grow at three different levels. Silicate was added to ensure that diatom growth would not be hindered. The treatments were arranged in a 3 x 3 latin square, with each treatment having one plant from each rig. At the end of the culture period the plastic plants with associated epiphyton were investigated using the microelectrodes.

ii) Microelectrodes.

On the twenty-second day after the plastic plants were retrieved from the field, each jar was randomly selected in turn from culture and the plastic plant carefully removed. Using the acrylic holders, the plastic plant was clamped to the base of the glass bath and the bath carefully filled with 450 ml of twice-filtered canal water (as in Section 6.1.4). The plastic plant with associated epiphyton was then illuminated with 300 μmol m^{-2} s^{-1} PAR for 1 hour. After this time, three advances were made towards an appropriate leaf between 3 and 5 cm from the shoot apex.

Each advance was made towards a different randomly selected point, about one third of the way across and one third the distance down the leaf. The outer limit of the epiphyton layer was estimated, to the nearest 100 μm, as the point when the tip of the microelectrode was no longer visible when viewed through the microscope. The point of

contact with the plastic leaf surface was marked by a sudden peak in the readout. All the plastic plants were fully analysed on the same day. Advances made towards a clean plastic plant which had been soaked overnight in filtered canal water, showed that the plant had no effect on the pH of the surrounding water.

After all three advances had been made, the selected leaf was carefully removed from the stalk with a scalpel and prepared in freezing alcohol for SEM. The epiphyton was removed from the other four leaves in the whorl by gentle brushing with a soft paint brush, then ground with sand in iced 90% acetone to extract the chlorophyll from the algae. The absorbance of the acetone, after centrifugation, was measured as outlined before (Section 2.2.2) except that the absorbance was measured at 750, 663, 645 and 630 nm, following the SCOR-UNESCO protocol (1964). The 750 nm absorbance was used to correct for turbidity and subtracted from the readings at the other wavelengths. These corrected values were then substituted into the following equation, to give the total chlorophyll-a in µg:

$$\text{Total Chlorophyll-a} = 5 \times [(11{\cdot}64 \times Abs_{663}) - (2{\cdot}16 \times Abs_{645}) + (0{\cdot}10 \times Abs_{630})]$$

(SCOR-UNESCO, 1964). This procedure has been shown to give consistent results with epiphyton communities of different species composition and hence different secondary pigments (Birch, 1990). No detectable colour was released when a clean plastic leaf was ground with sand in iced 90% acetone and the absorbance measured. The epiphyton from the ten leaves in the whorls above and below the selected leaf was also removed by brushing, and then washed onto a dried, pre-weighed filter disk (Whatman GFC). This disk was then dried to constant weight at 60 °C and weighed to four significant figures.

The area of the leaves was estimated by weight, as the mean of ten traced onto waterproof paper of known weight per unit area. The method used for estimating the area of the real plants was not applicable here, as the sides of the leaves were markedly curved.

6.3.2.2 Results.

i) Leaf area.

The leaves from these moulded, plastic plants were of a consistent area with a mean size of 0·6063 cm^2 (se = 0·0071, n = 10), which was used to calculate the chlorophyll-a content and dry weight of the epiphyton on an areal (= per unit area of leaf surface) basis. This standardisation allowed comparison with the results from the leaves of the real plants, which were of varying size. As well as having more leaves per whorl than the real plants (5 *cf.* 3), each plastic leaf was approximately 2 times larger, giving a far greater area for colonisation by the algae.

ii) Epiphyton characteristics – community composition.

The seeding procedure appeared successful in its aim to produce a epiphyton-like community, composed of a variety of algal species growing on the plastic (Table 6.3), which could, in the subsequent culture period, be manipulated to produce differing amounts of algae by nutrient loading. A large number of studies have used artificial substrata of different types to sample periphyton communities (see Gibeau & Miller, 1989; Aloi, 1990; Cattaneo & Amireault, 1992) and there has been some debate as to the extent to which they represent realistic communities (Allen, 1971; Brown, 1976; Cattaneo, 1978; Cattaneo & Kalff, 1978, 1979 & 1981; Gough & Gough, 1981; Rogers & Breen, 1983; Wetzel, 1983b; Luttenton *et al.*, 1986; Blindow, 1987; Hudon *et al.*, 1987; Lethbridge *et al.*, 1988; Aloi, 1990; Cattaneo & Amireault, 1992). To a large extent, these studies have been concerned with comparisons between the communities on the introduced, artificial, and the already established, natural substrata in an attempt to standardise field sampling of periphyton.

Questions have also been raised regarding the role that the (real) plant plays in determining the composition of the epiphyton growing over its surfaces (Jørgensen, 1957;

Prowse, 1959; Allen, 1971; Wetzel & Allen, 1972; Allanson, 1973; Harlin, 1973 & 1975; McRoy & Goering, 1974; Gough & Woelkerling, 1976; Cattaneo & Kalff, 1978, 1979 & 1981; Eminson & Moss, 1980; Penhale & Thayer, 1980; Gough & Gough, 1981; Carignan & Kalff, 1982; Delbecque, 1983; Riber *et al.*, 1983; Rogers & Breen, 1983; Wetzel, 1983b; Blindow, 1987; Lethbridge *et al.*, 1988; Burkholder & Wetzel, 1989; Underwood *et al.*, 1992). The general opinion appears to be that artificial substrata must be left *in situ* for a long time (>60 days) before they begin to resemble the relatively stable, epilithic assemblages that grow on submerged stones, whereas the more ephemeral substrate offered by submerged macrophytes is to a large extent reproduced in a shorter time period. However, there do appear to be some consistent differences in species composition between artificial and real substrata (see Cattaneo & Amireault, 1992). In the case of epiphytic algae, it has been suggested that this difference may only apply in waters of lower nutrient concentration, where the plant may play a more important role in nutrient supply, the epiphytes relying on the bulk water for nutrients in more productive situations (Eminson & Moss, 1980). Nevertheless, substantial differences have been found between the epiphytes on *Potamogeton pectinatus* L. and charophytes from a shallow eutrophic lake (Blindow, 1987). To understand such situations it is necessary to consider ways in which the plants can affect the conditions for the epiphyton other than by supplying nutrients. A likely contender is the pH microclimate, especially in those species which take up HCO_3^-. Many charophytes possess an alkaline-acid band mechanism (much the same as the polar leaf mechanism but here the pH varies between adjacent hoops along the internodal cells) whereas *P. pectinatus* uses a different mechanism without areas of high/low pH (Steeman-Nielsen, 1947). Freshwater diatom species appear to be poorer utilisers of HCO_3^- than greens and blue-greens (Maberly & Spence, 1983), and blue-green algae are said to be most efficient at high pH (Shapiro, 1990a). Hence species composition and density differences could be established by the precipitation of marl by the plant during HCO_3^- uptake and the associated high pH (eg. Cattaneo, 1978), with marl deposition not being an

external factor as suggested by Eminson & Moss (1980). If marl deposition occurs in response to the physiology of the plant, as argued earlier (Section 4.3.1), then the suggestion by Vermaat & Hootsmans (1991a) that marl deposition becomes similar on all substrata under eutrophic conditions is also erroneous. This would only occur if the marl formed in the water column precipitated uniformly onto all objects.

This study did not aim to assess whether the communities growing on plastic plants accurately represented the species composition of epiphytic assemblages growing on the real plants, but to produce communities which were architecturally and functionally representative of epiphyton from different nutrient regimes.

The architecture of the constituent organisms is an extremely important factor in determining the composition of attached aquatic communities, both for macro- and microphytes (Den Hartog, 1982; Meulmans & Roos, 1985; Duarte & Roff, 1991). For epiphyton there is the added factor of time. As the host plant is perpetually growing and producing new tissue, so the epiphyton is colonising these new surfaces, with an ensuing succession in the epiphyton community as the host tissue ages. This succession typically follows the sequence shown in Figure 6.30 of

bacteria,
adpressed diatoms, particularly *Cocconeis placentula*,
stalked diatoms and encrusting green algae,
filamentous forms,

with the community becoming progressively denser and more three-dimensional as time progresses (Siver, 1978; Robinson, 1983; Meulmans & Roos, 1985; Paterson & Wright, 1986; Otten & Willemse, 1988; Birch, 1990). Since most of the early colonisers remain within the community (Siver, 1978; Rogers & Breen, 1983; Paterson & Wright, 1986), this is not a succession in the true sense of the word, though the extent to which these basal layers are dead (Meulmans & Roos, 1985; Burkholder & Wetzel, 1989) or alive (Hudon *et al.*, 1987) is debated. The primary colonisers do fill an important structural role in the

Figure 6.30 Diagram of the main stages in the development of epiphyton.

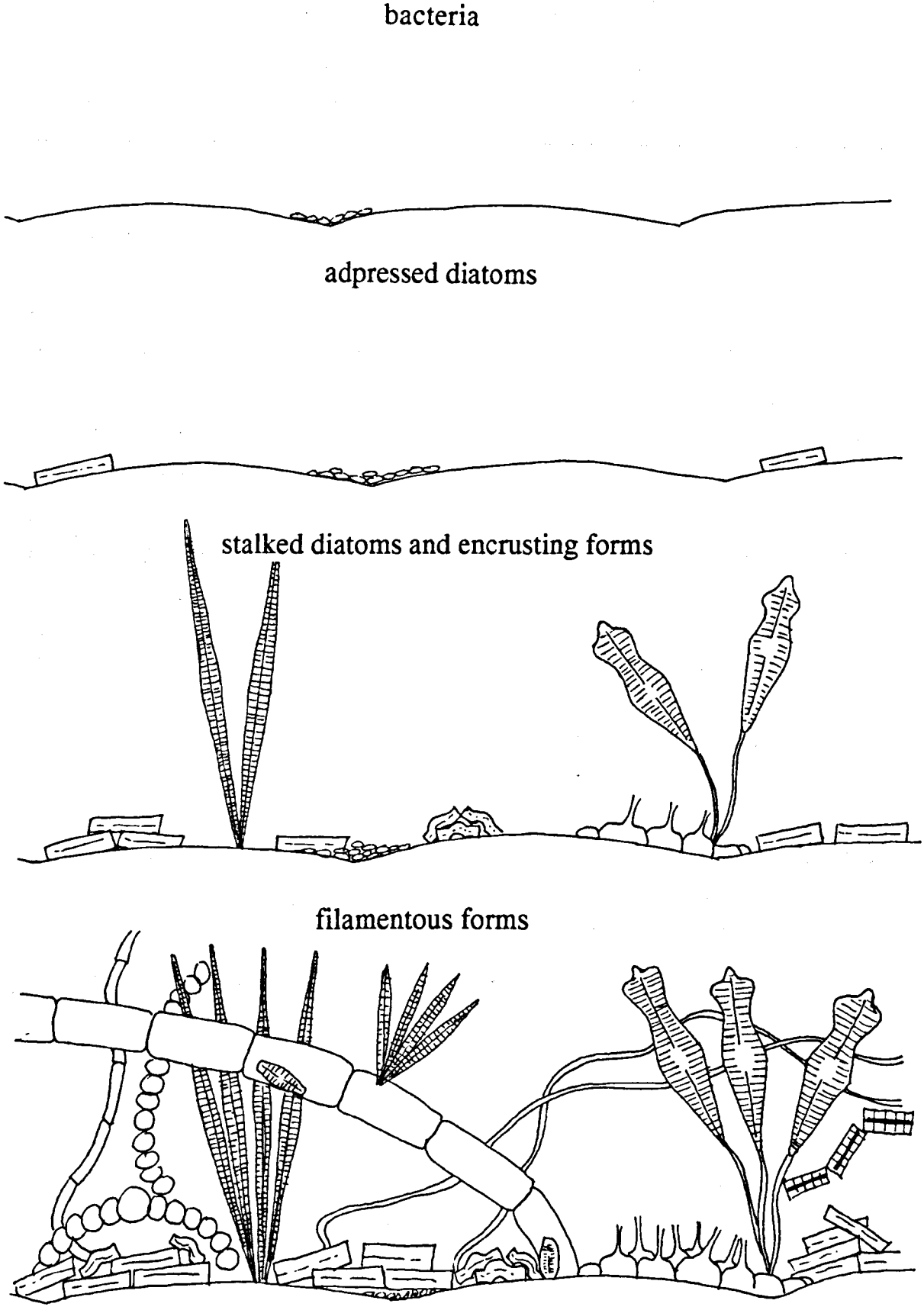


Table 6.3 Algae found growing epiphytically on the leaves of the plastic plants and *E. nuttallii* at the end of the laboratory culture period.

Taxa	Plastic Plants	*E. nuttallii*	Typical Habit
Cyanophyta			
Anabaena spp.	*	*	
Chamaesiphon (incrustans ?)		*	epiphytic/attached
Chlorophyta			
Zygnema sp.	*	*	epiphytic/attached
Oedogonium sp.	*	*	epiphytic/attached
Aphanochaete sp.		*	epiphytic/attached
Scenedesmus quadricauda		*	planktonic
Chrysophyta			
Cocconeis placentula	*	*	epiphytic/attached
Achnanthes minutissima	*	*	epiphytic/attached
Achnanthes exigua var constricta		*	
Epithemia zebra	*		epiphytic/attached
Amphora (ovalis ?)	*		
Synedra ulna		*	planktonic/attached
Nitzschia palea	*	*	
Gomphonema constrictum	*	*	epiphytic/attached
Fragilaria sp.		*	epiphytic/attached
Stephanodiscus astraea	*		planktonic

Presence of a species is indicated with an asterix.

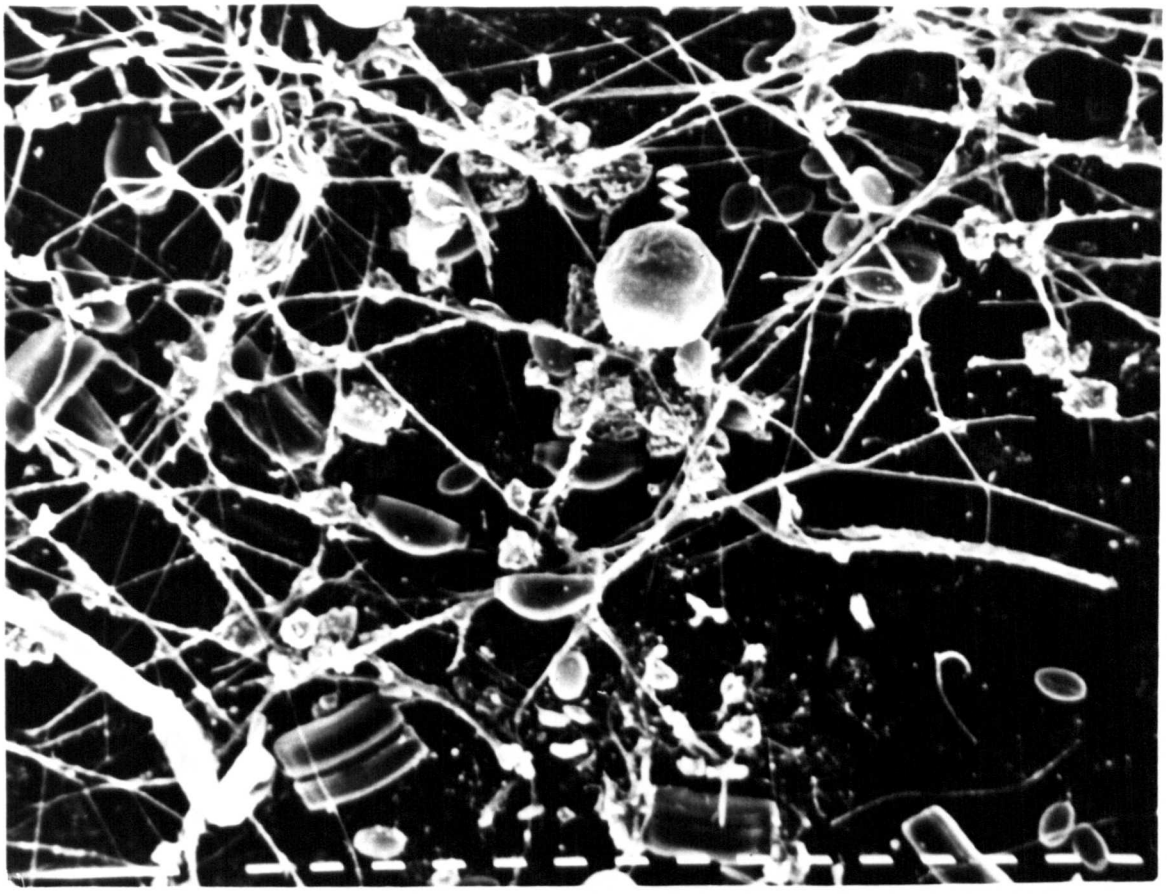
community, supporting a superstructure of other species, with some species dependent on the presence of others which they can subsequently epiphytise (Roos *et al.*, 1981).

Other factors do affect epiphyton community structure and composition, such as coloniser effects and deposition from the seston (Cover & Harrel, 1978; Moss & Leah, 1982); flow (Luttenton *et al.*, 1986; Otten & Willemse, 1988); position within the weedbed (Roos *et al.*, 1981; Kairesalo, 1983; O'Niel-Morin & Kimball, 1983); season (Jørgensen, 1957; Eminson, 1978; Gons & van Keulen, 1983; Jones & Mayer, 1983; Roos, 1983; Haines *et al.*, 1987; Meulemans, 1988; Burkholder & Wetzel, 1989; van Dijk, 1993); light (O'Niel-Morin & Kimbal, 1983; Sand-Jensen, 1983b); grazing (Allanson, 1973, Bell & Eaton, 1976; Hunter, 1980; Rogers & Breen, 1983; Orth & van Montfrans, 1984; Kairesalo & Koskimies, 1987; Brönmark, 1989; Mazzella & Russo,1989; Brönmark & Weisner, 1992; Underwood *et al.*, 1992; Irvine *et al.*,1993; Neckles *et al.*, 1993; Underwood in press); nutrient availability (Moss, 1976; Eminson, 1978; Eminson & Phillips, 1978; Eminson & Moss, 1980; Sand-Jensen & Søndegaard, 1981; Cattaneo, 1987; Daldorph & Thomas, 1991); and other physicochemical factors (Sand-Jensen, 1983b, amongst others).

With so many interacting factors epiphyton communities are potentially very variable, so it was decided that the plastic plants should have examples of communities representative of each of the algal stages (ie. not the solely bacterial stage), from the colonisation sequence growing on them. This was achieved, with the increased nutrient levels appearing to speed up the succession (Plates.24 to 27). It was assumed that, since many typically epiphytic taxa were present (Table 6.3), these communities would function in essentially the same way as true epiphyton.

Plate 24 A sparse covering of adpressed diatoms and filamentous green algae growing on a plastic plant. Scale bar = 10 μm.

Plate 25 Epiphyton growing on a plastic plant showing adpressed diatoms, stalked diatoms and filamentous green and blue-green algae. Scale bar = 10 μm.



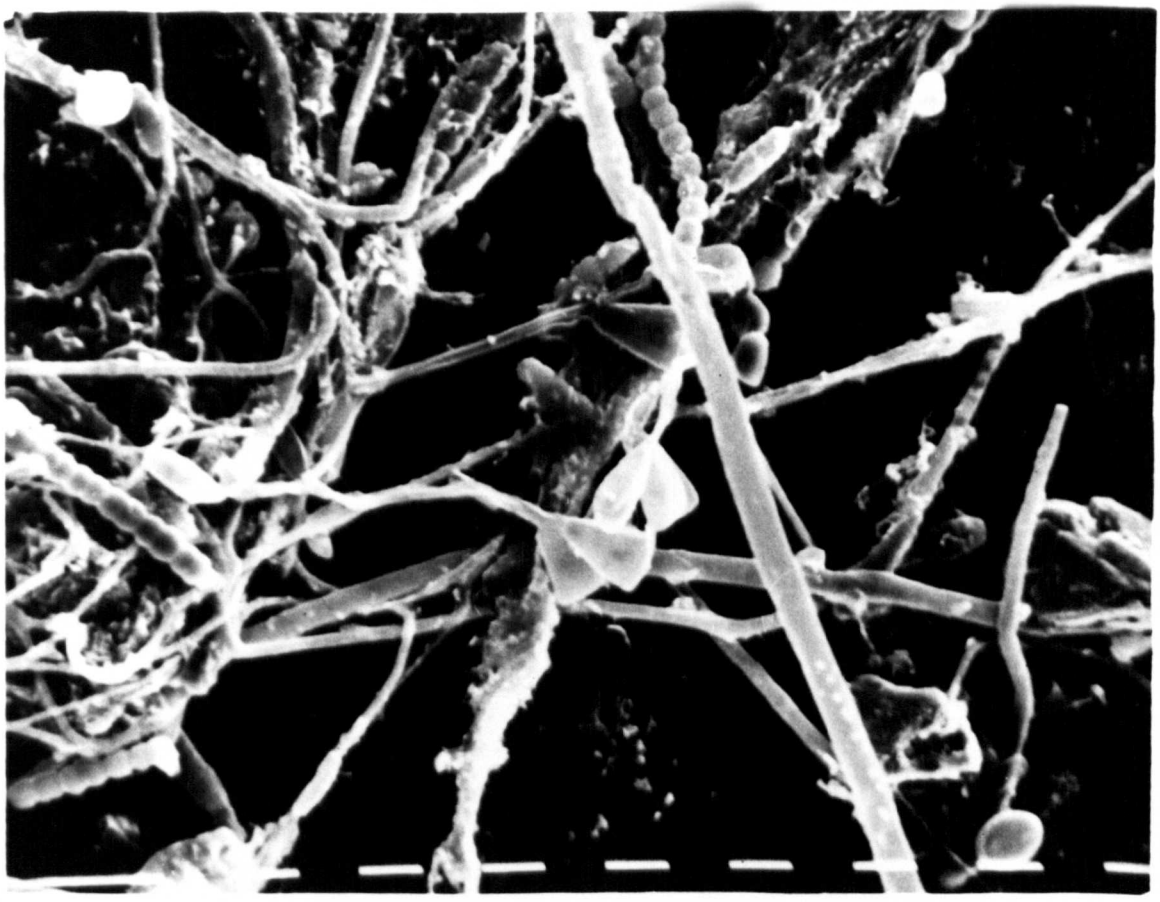


Plate 26 A dense layer of diatoms, particularly *Achnanthes minutissima*, growing together with filamentous blue-green algae on a plastic plant. Scale bar = 6·25 μm.

Plate 27 An extremely dense layer of epiphyton with large numbers of the filamentous blue-green alga *Anabaena* sp. growing on a plastic leaf. Scale bar = 10 μm.

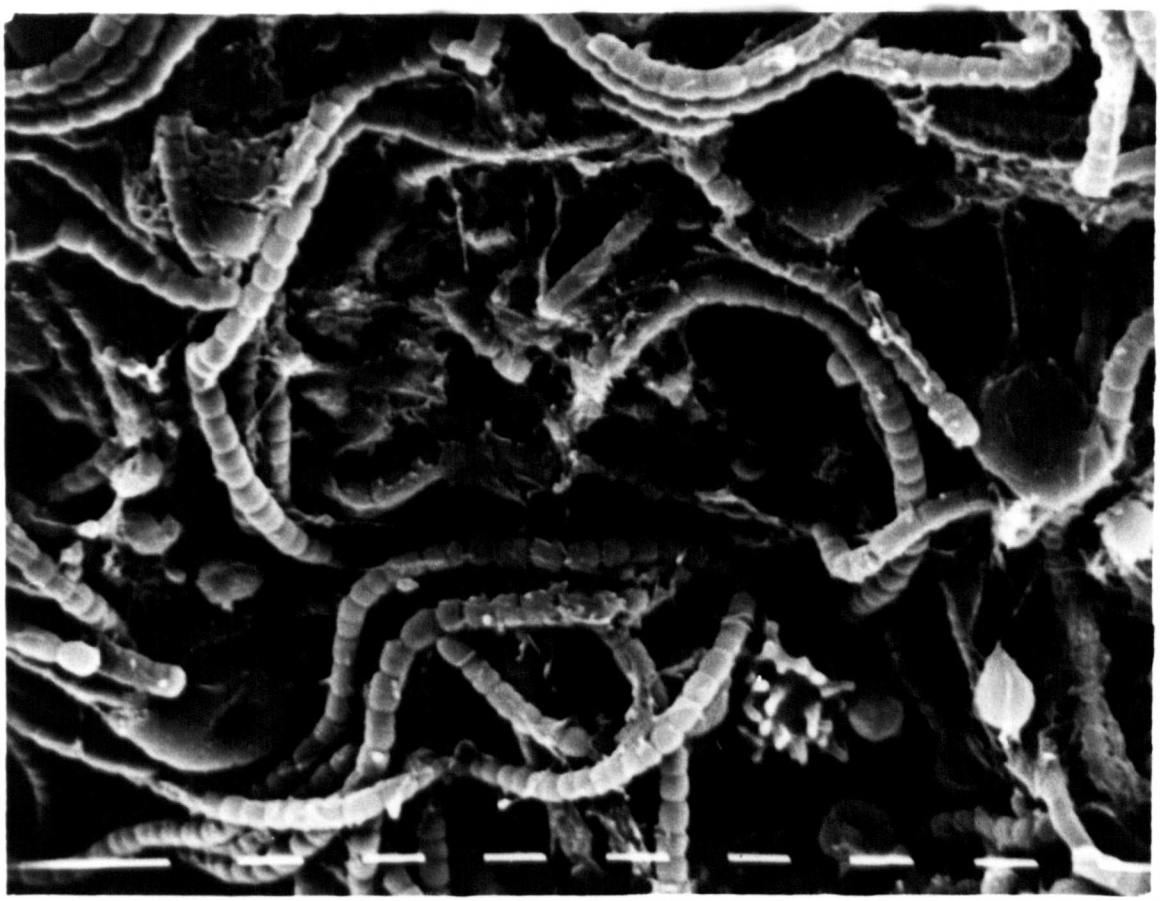


Plate 28 Sparse epiphyton growing on *E. nuttallii*, showing the adpressed diatom *Cocconeis placentula* and an encrusting green alga. Scale bar = 10 µm.

Plate 29 Stalked and basally attached diatoms growing on *E. nuttallii.* Scale bar = 10 µm.

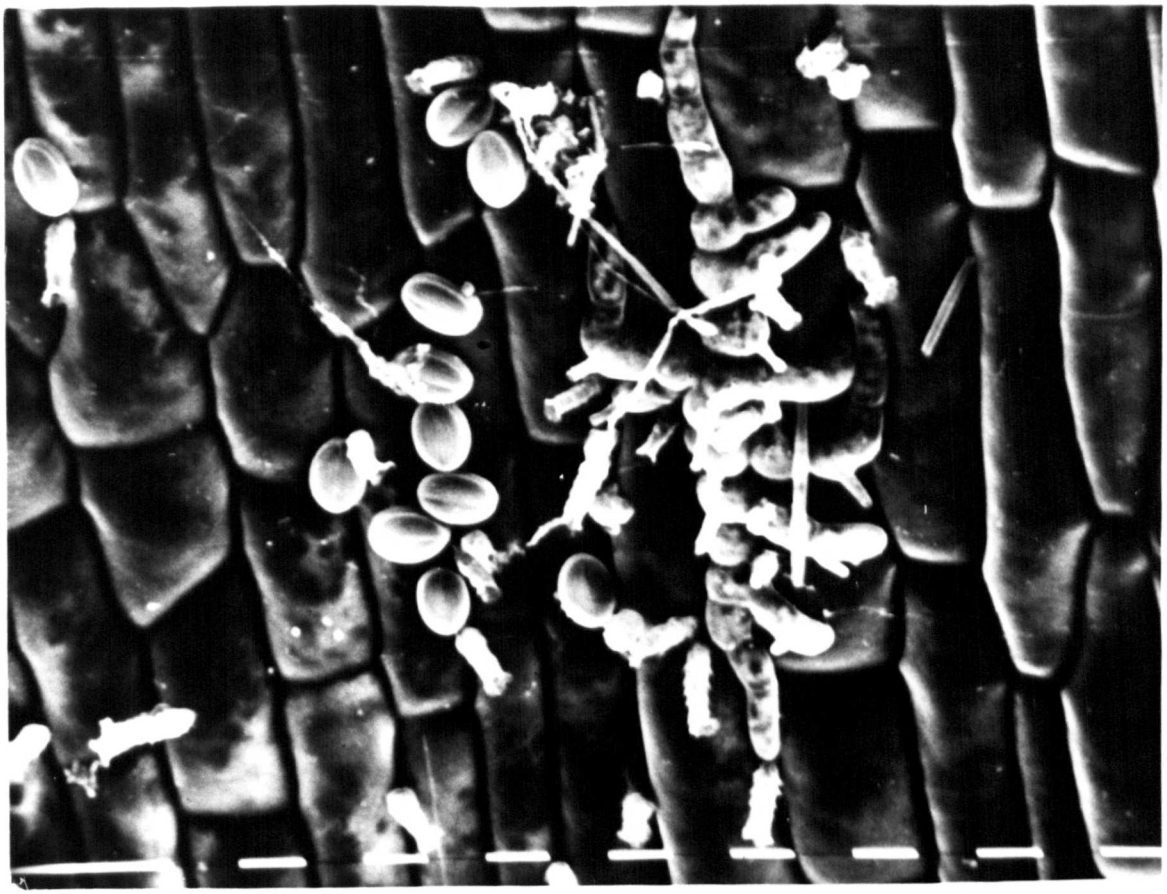
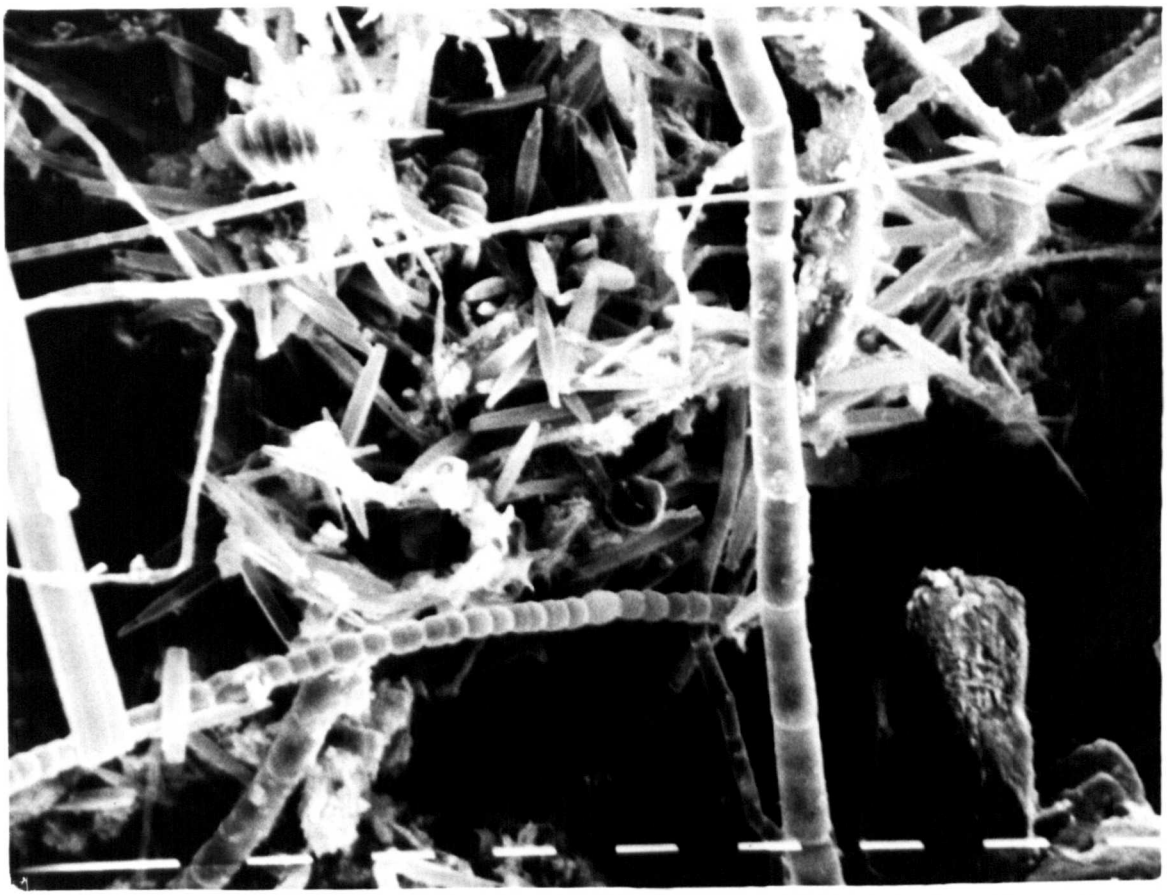


Plate 30 A dense layer of diatoms, filamentous green and blue-green algae and marl on an *E. nuttallii* leaf. Scale bar = 10 μm.

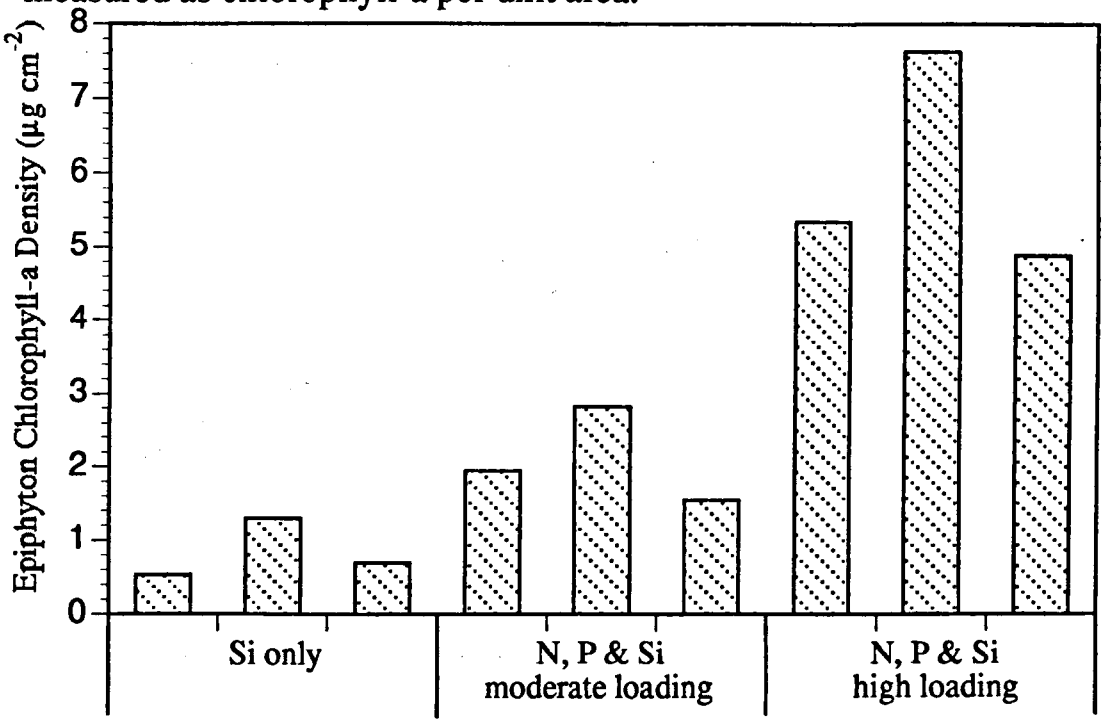
Plate 31 An extremely dense layer of epiphyton, particularly the filamentous blue-green alga *Anabaena* sp., growing on *E. nuttallii.* Scale bar = 10 μm.



iii) Epiphyton characteristics – the effect of nutrient loading.

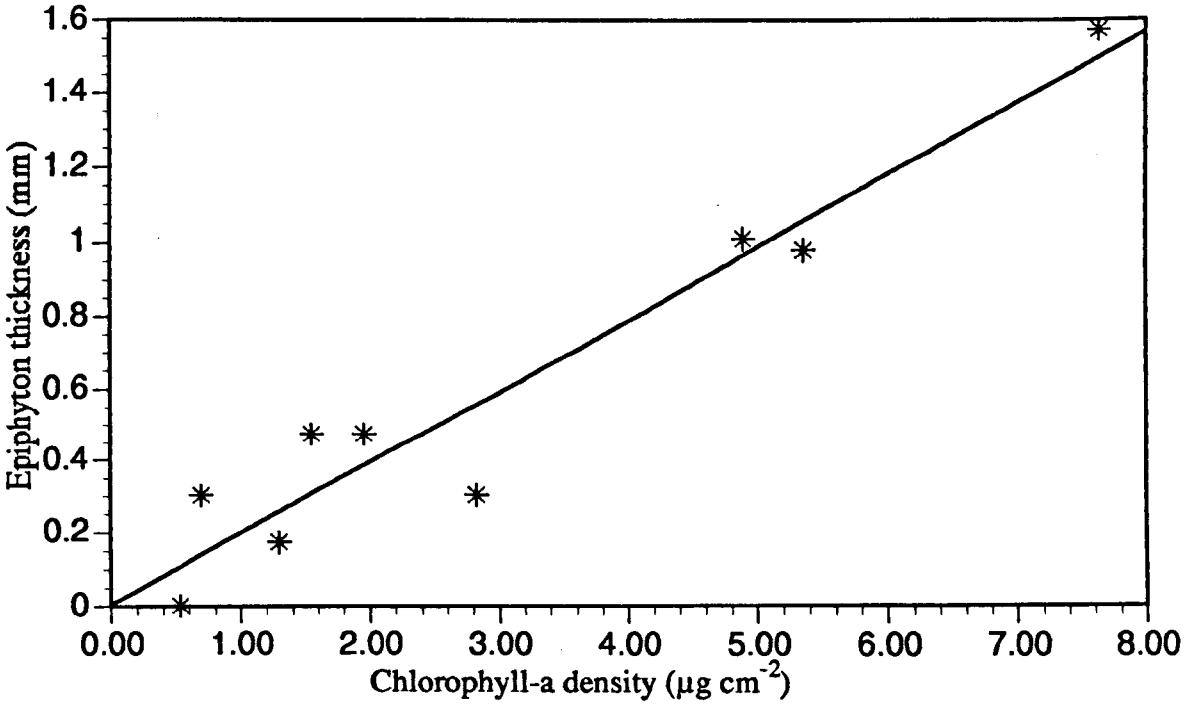
The increase in nutrient loading, as expected, produced a significant increase in the amount of algae growing on the plastic plants measured, as areal chlorophyll-a density (Figure 6.31, from anova p = 0·0015). Tukey's test, a somewhat conservative measure, distinguished the mean chlorophyll-a density from the high loading as being significantly different from the other two (LSD = 2·446). One of the replicates at each level appeared to have more algae growing on it than the other two which may have been due to their position within the weedbed during seeding since all these replicates were taken from one paternoster rig (see Section 6.3.2.1). Position within the weedbed has been shown to have substantial effects on epiphton development (Roos *et al.*, 1981; Kairesalo, 1983; O'Niel-Morin & Kimball, 1983). The chlorophyll-a density, for epiphyton from the other leaves in the whorl apart from the one investigated with the microelectrodes, described the thickness of the layer of epiphyton very well (p = 0·0001, Figure 6.32). However, the other way of estimating of the amount of epiphyton, the dry weight of the layers removed from the whorl above and below the leaf investigated, described a somewhat poorer relationship with epiphyton thickness (p = 0·0838, Figure 6.33). It is possible that variations in the amount of inorganic material which had settled onto the leaves caused this difference. This finding that chlorophyll-a per unit area was the best estimate of epiphyton density is in contradiction to the suggestion of Vermaat & Hootsmans (1991a) that epiphyton chlorophyll is unpredictable in epiphyton communities of changing species composition and thickness, even though both experiments involved laboratory studies. The values of epiphyton dry weight per unit area growing on these artificial substrata were within the range of published values (Vermaat & Hootsmans, 1991a), supporting the suggestion that the cultured communities were representative of natural communities. There was also a tendency, though not strong (p = 0·0918), for the epiphyton to increase in chlorophyll-a per unit weight ($\mu g\ g^{-1}$) with increasing chlorophyll-a per unit area ($\mu g\ cm^{-2}$), ie. as the epiphyton increased, the proportion of chlorophyll-a relative to other

Figure 6.31 Effect of nutrient loading on epiphyton development, measured as chlorophyll-a per unit area.



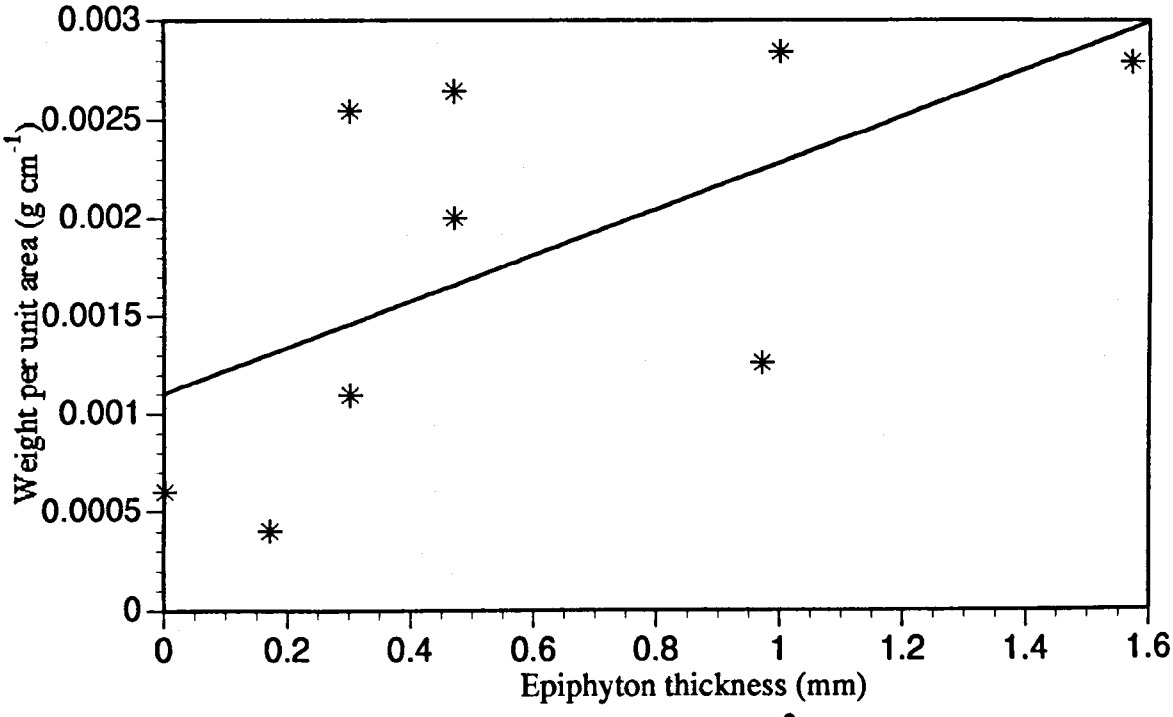
From anova, loading has a significant effect on epiphyton chlorophyll, p = 0.0015
From Tukey's test LSD = 2.4461. LOW MEDIUM HIGH

Figure 6.32 Relationship between epiphyton chlorophyll-a per unit area and thickness of the epiphytic layer.



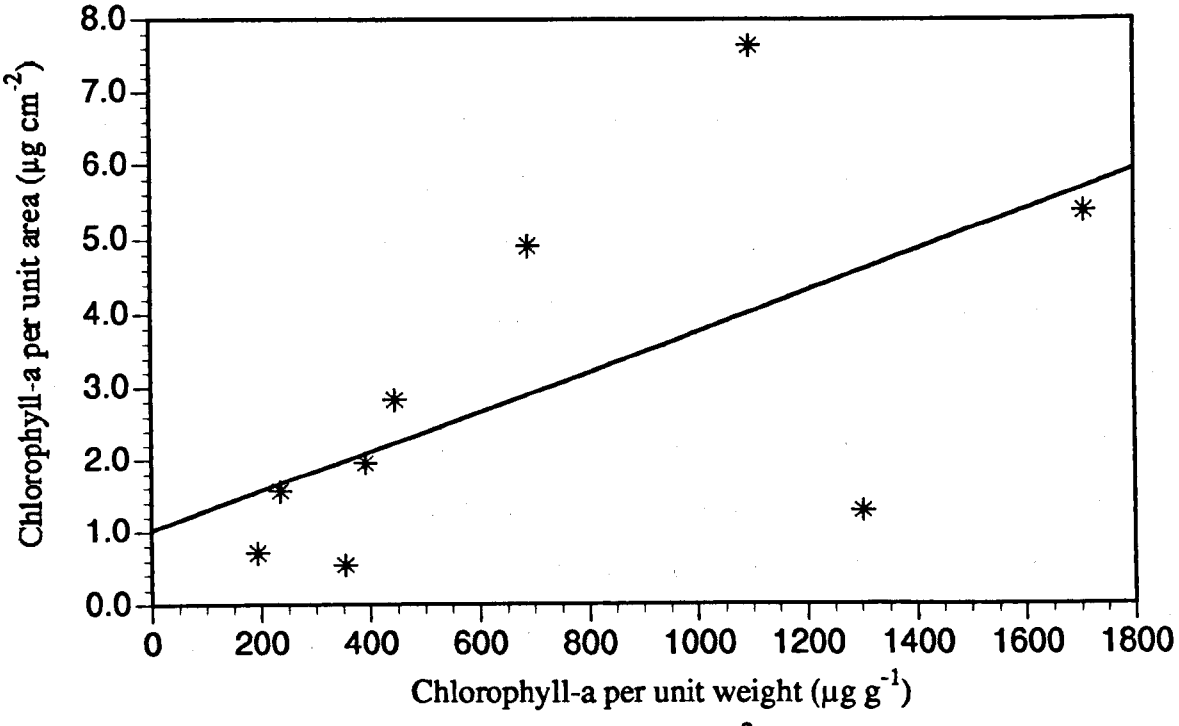
The equation for this line is $y = 0.195x + 0.00466$, $R^2 = 0.921$, $p = 0.0001$

Figure 6.33 Relationship between dry weight per unit area and thickness of epiphyton.



The equation for this line is $y = 0.00118x + 0.0011$, $R^2 = 0.367$, $p = 0.0838$

Figure 6.34 Relationship between chlorophyll-a per unit area and per unit weight.



The equation for this line is $y = 0.0027x + 1.024$, $R^2 = 0.353$, $p = 0.0918$

substances increased (Figure 6.34). This trend is concurrent with the change of dominance from diatoms at low nutrient loading to filamentous green and blue green algae at higher loading, but may also be an indication that lower layers become shade-adapted, and hence chlorophyll-rich, as the epiphyton thickened (Hudon *et al.*, 1987). The chlorophyll-a content of the organic component of field-grown periphyton from a eutrophic lake was found to range from 1–2 mg g^{-1} ash free dry weight (van Dijk, 1993) which, although a direct comparison was not possible, do correspond to those found here for the higher nutrient loadings, ie. 1–1·8 mg g^{-1} dry weight. Since the inorganic component was not compensated for in this study, the low ratios at low nutrient loadings, where diatoms dominated, may be attributable to the higher inorganic fraction arising from their silca frustules. Laboratory cultures of almost pure algal periphyton have been found to have chlorophyll–a densities of 5–10 mg g^{-1} ash free dry weight (Vermaat & Hootsmans 1991a). Perhaps it is these differences in the proportion of inorganic matter in the epiphytic algae which create the poor correlation between dry weight and epiphyton thickness (Figure 6.33).

iv) pH profiles.

Of all the 27 individual advances made towards the plastic leaf surfaces with the microelectrodes, only 3 showed a decrease in pH in the deeper layers of epiphyton. In all three of these cases the pH at the leaf surface was still above that in the bulk medium. These were not all from the same replicate plant, but from two replicates grown with medium nutrient loading. In these three replicates, respiration must have been the predominant process at the leaf surface, either due to shading or more likely to a predominance of heterotrophic organisms (fungi, bacteria or protozoans). The heterogeneity of the community was illustrated by the fact that other parts of these leaves were predominantly autotrophic throughout the depth of the epiphyton. No spontaneous loss of epiphyton was seen, which is not surprising since this effect has been attributed to

the death and decay of the basal layers (Meulemans & Roos, 1985), and little evidence of heterotrophic activity was found. Perhaps it was the more aged epiphyton community growing on the dead reed stems in the Meulemans & Roos study, >1 year *cf.* 6 weeks, which caused the reported effect. In the replicates with thicker layers, the pH gradient flattened somewhat towards the leaf surface indicating reduced activity, an effect attributed to shading from the upper layers.

Since the only published microelectrode profiles measured in epiphyton are for dissolved oxygen, comparison with the results presented here can only be qualitative. These published reports showed that photosynthesis of the natural epiphyton communities used, attached to both plants and inert substrate, was saturated at light intensities of 100 – 300 µmol m^{-2} s^{-1} and that heterotrophic activity was negligible under high light in all the communities investigated (Sand-Jensen *et al.*, 1985; Sand-Jensen & Revsbech, 1987; Carlton & Wetzel, 1987). Obviously as light intensity was reduced so respiration ultimately became the predominant process. Light compensation points of 22 and 37 µmol m^{-2} s^{-1} were calculated for the communities growing on *Potamogeton crispus* in early June and late August respectively (Sand-Jensen & Revsbech, 1987). It was also shown that in the thickest epiphyton layers (≈1 mm), photosynthetic activity was concentrated in the upper parts of the layer (Sand-Jensen *et al.*, 1985; Sand-Jensen & Revsbech, 1987). This was also the case for the periphyton attached to a rock investigated by Carlton & Wetzel (1987), but the irradiance used for this study was low (40 µmol m^{-2} s^{-1}). Some pH profiles, though much less detailed than the ones reported here, have on the other hand been reported for benthic algal mats, where photosynthesis has again been shown to be concentrated in the uppermost layers (Jørgensen *et al.*, 1983; Revsbech *et al.*, 1983; Revsbech & Jørgensen, 1986). This finding corresponds with the results from O_2 microelectrode studies on such mats (Jørgensen *et al.*, 1979; Jørgensen *et al.*, 1983; Revsbech & Jørgensen, 1983 & 1986; Revsbech & Ward, 1983; Revsbech *et al.*, 1983). However, the presence of sediment beneath such layers of benthic algae with a high

respiratory demand did make a significant impact on the shape of the pH and O_2 microprofiles.

As only one measure of chlorophyll-a and dry weight was made for each replicate plant, to plot this against the pH from each advance would have represented pseudoreplication. The mean values from the three advances was used instead, to remove some of the effects of heterogeneity within the epiphyton but not bias the results statistically. The standard errors of these means are shown to indicate the extent of heterogeneity, but were not used in the statistical analyses. As each pH gradient had a corresponding thickness of epiphyton measured at the same point, features from the gradient could be plotted independently and used in statistical analyses.

Figure 6.35 shows the pH at the plastic leaf surface summarised from all the advances, in relation to the epiphyton chlorophyll-a density measured on an areal basis. This relationship is described by a logarithmic curve, which, after linearisation ($\log_{10}(x)$ transformation), proved to be a highly significant fit (R^2 = 0·815, p = 0·0009, Figure 6.37). The pH at the surface of the epiphyton also followed a logarithmic curve when plotted against areal chlorophyll-a density (Figure 6.36) and again was significant when transformed (R^2 = 0·629, p = 0·0108, Figure 6.38). From the gradients of these two lines (1·012 *cf.* 0·628), it can be seen that they were increasingly divergent with increasing epiphyton, the difference between them being due to pH increases within the layer of epiphyton.

The pH at the plastic leaf surface also described a logarithmic curve with increasing epiphyton dry weight (Figure 6.39), but the fit was less good (R^2 = 0·473, p = 0·0406, Figure 6.41). This is consistent with the dry weight being a poorer indicator of the amount of epiphyton measured as thickness (Figure 6.33). There was no relationship between chlorophyll-a per unit weight and pH at the leaf surface (Figure 6.40).

Figure 6.35 Relationship between epiphyton chlorophyll-a per unit area and pH at leaf surface (mean from 3 advances ± se).

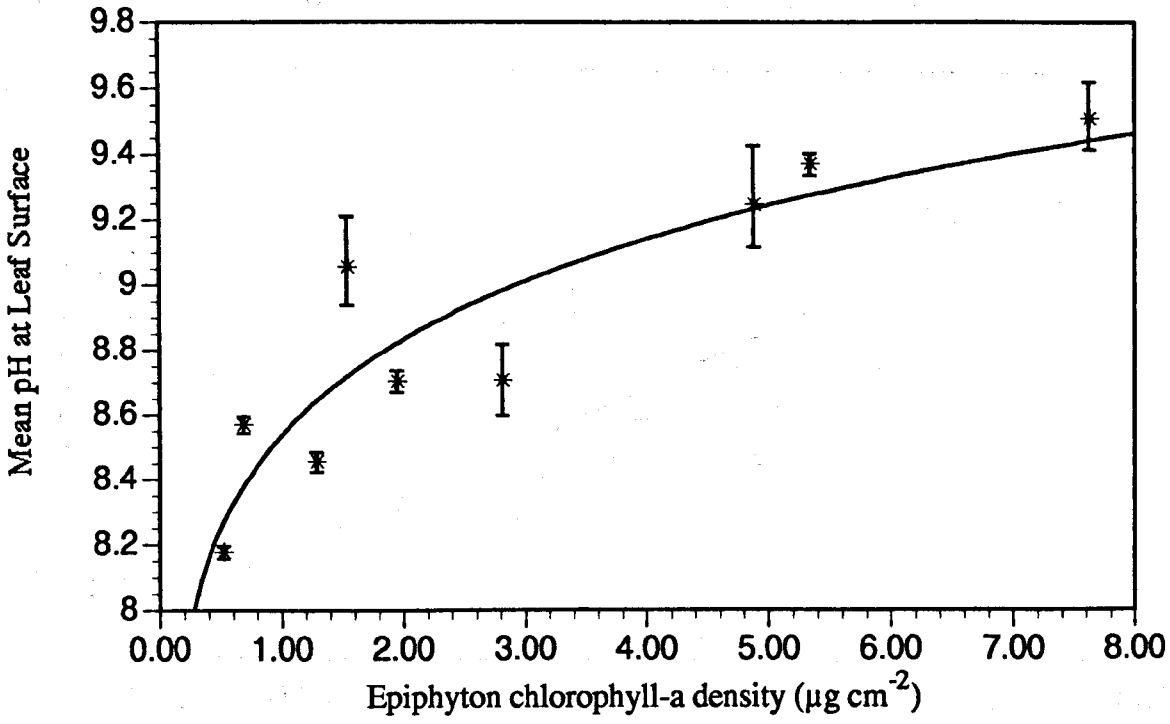


Figure 6.36 Relationship between epiphyton chlorophyll-a per unit area and pH at epiphyton surface (mean of 3 advances ± se).

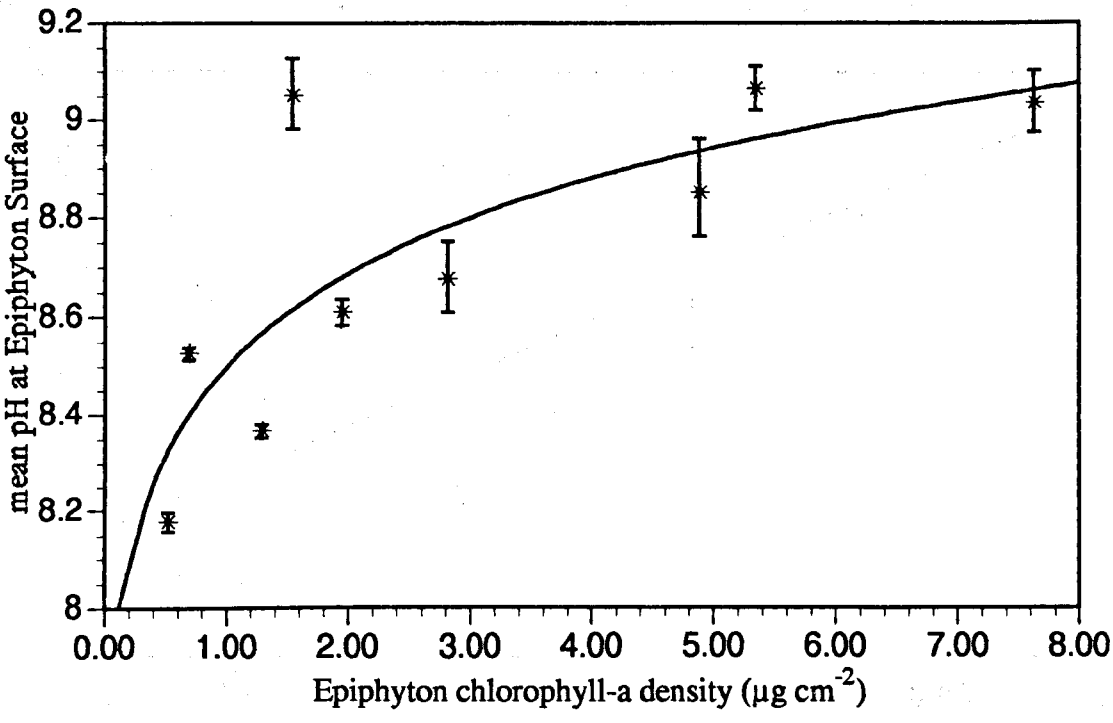
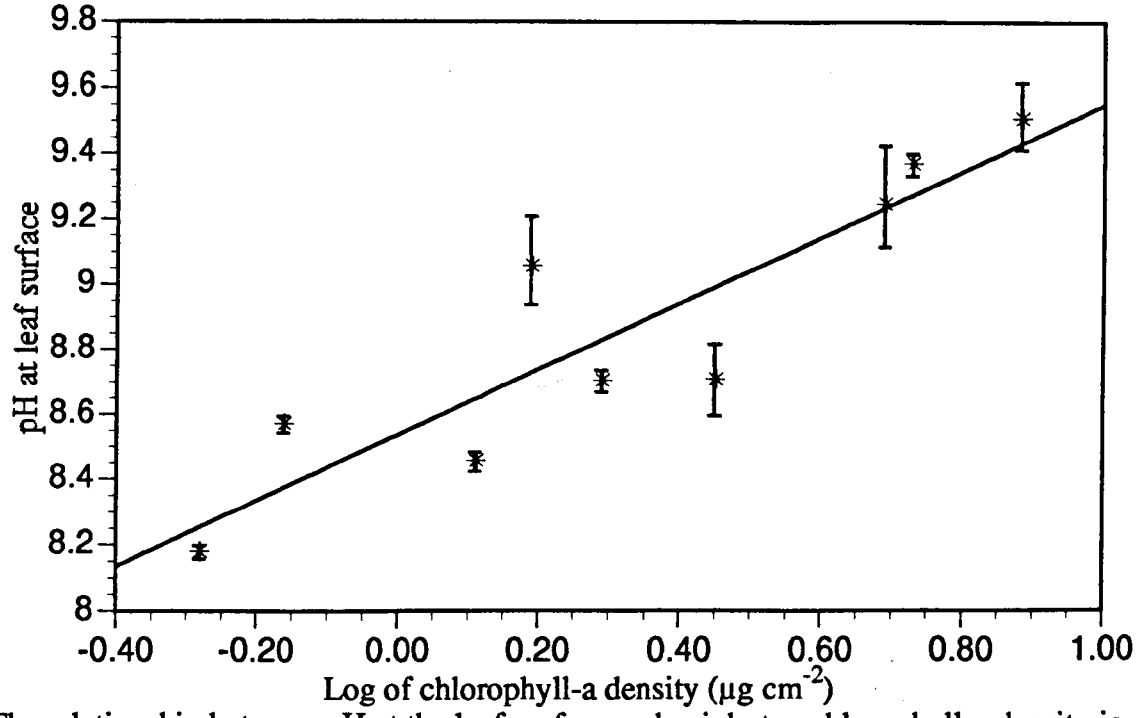
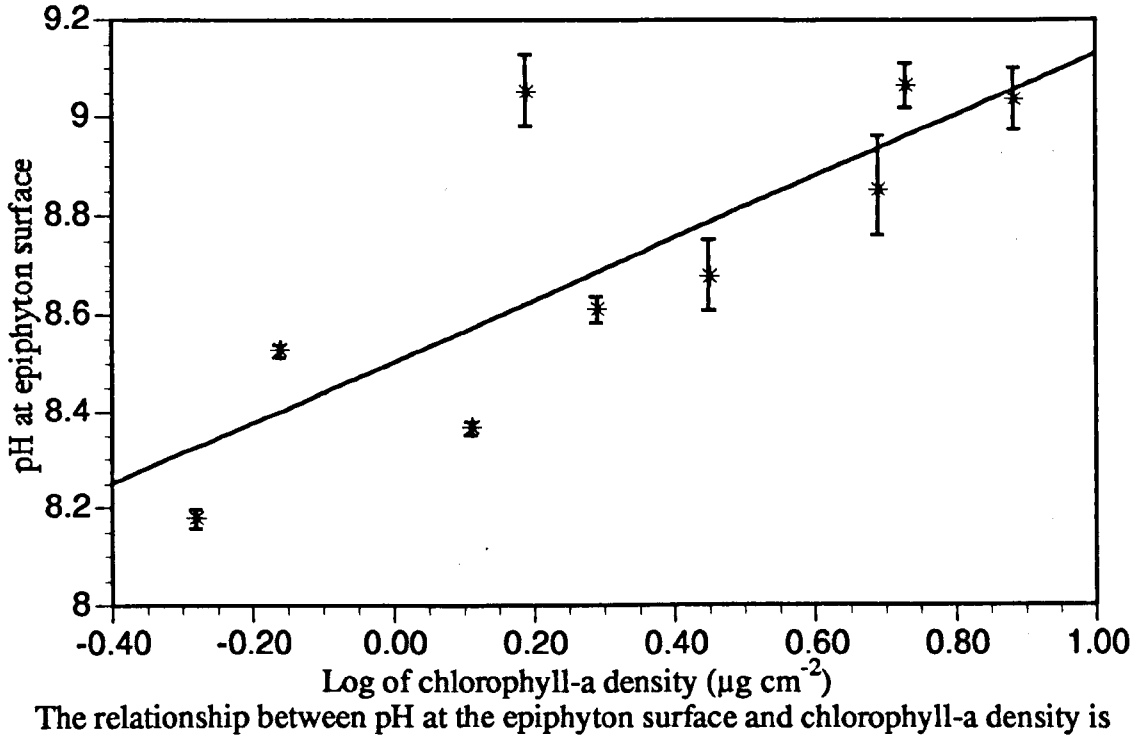


Figure 6.37 Relationship between Log_{10} of epiphyton chlorophyll-a density and pH at the leaf surface (mean of 3 advances ± se).



The relationship between pH at the leaf surface and epiphyton chlorophyll-a density is $y = 1.012 \log(x) + 8.539$, $R^2 = 0.815$, $p = 0.0009$

Figure 6.38 Relationship between Log_{10} of epiphyton chlorophyll-a concentration and pH at the epiphyton surface (mean of 3 advances ± se).



The relationship between pH at the epiphyton surface and chlorophyll-a density is $y = 0.628 \log(x) + 8.502$, $R^2 = 0.629$, $p = 0.0108$

Figure 6.39 Relationship between epiphyton dry weight and pH at the leaf surface (mean of 3 advances ± se).

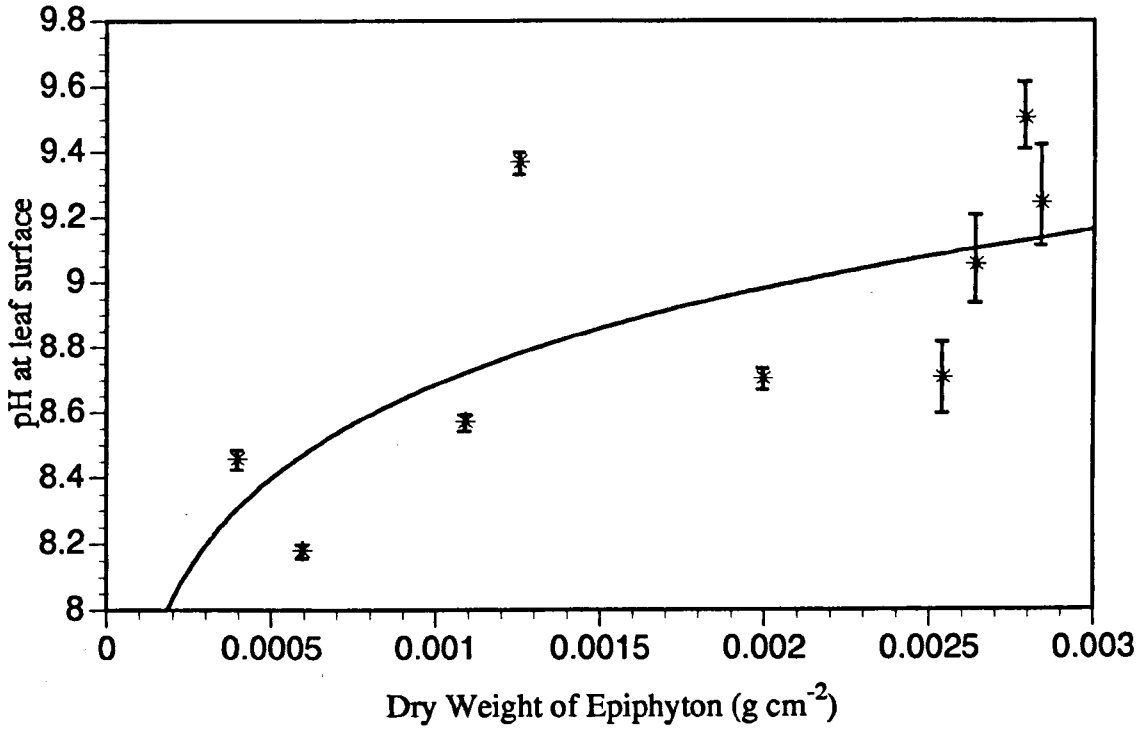


Figure 6.40 Relationship between chlorophyll-a per unit weight and pH at leaf surface (mean of 3 advances ± se).

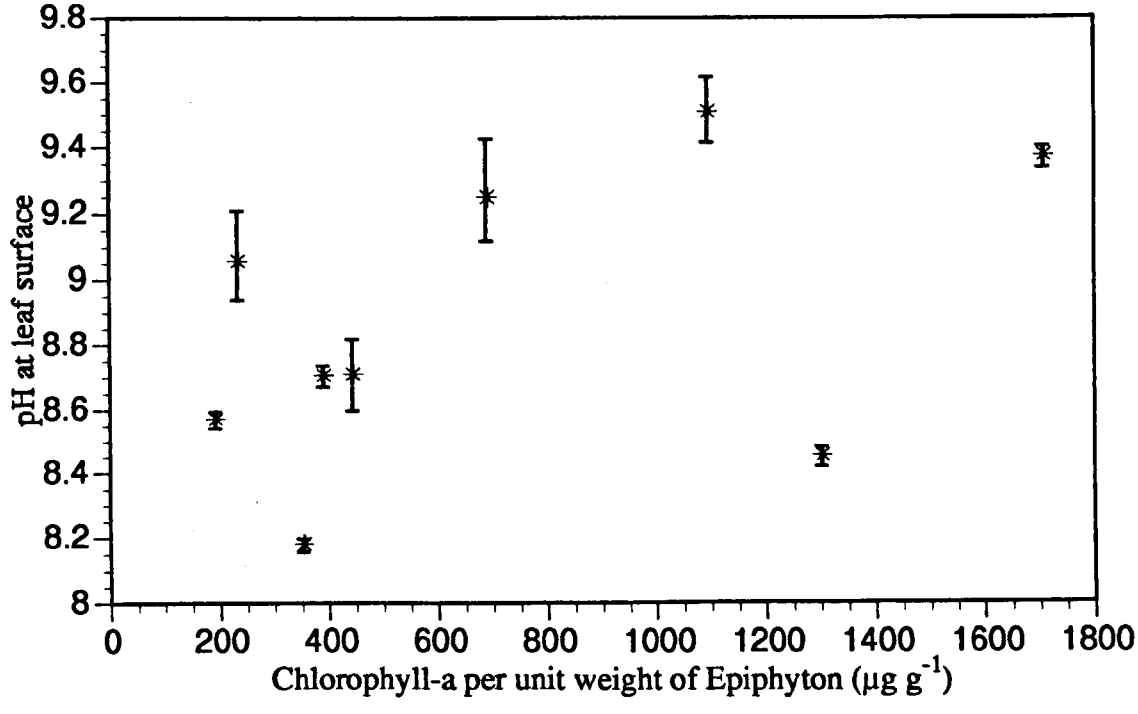
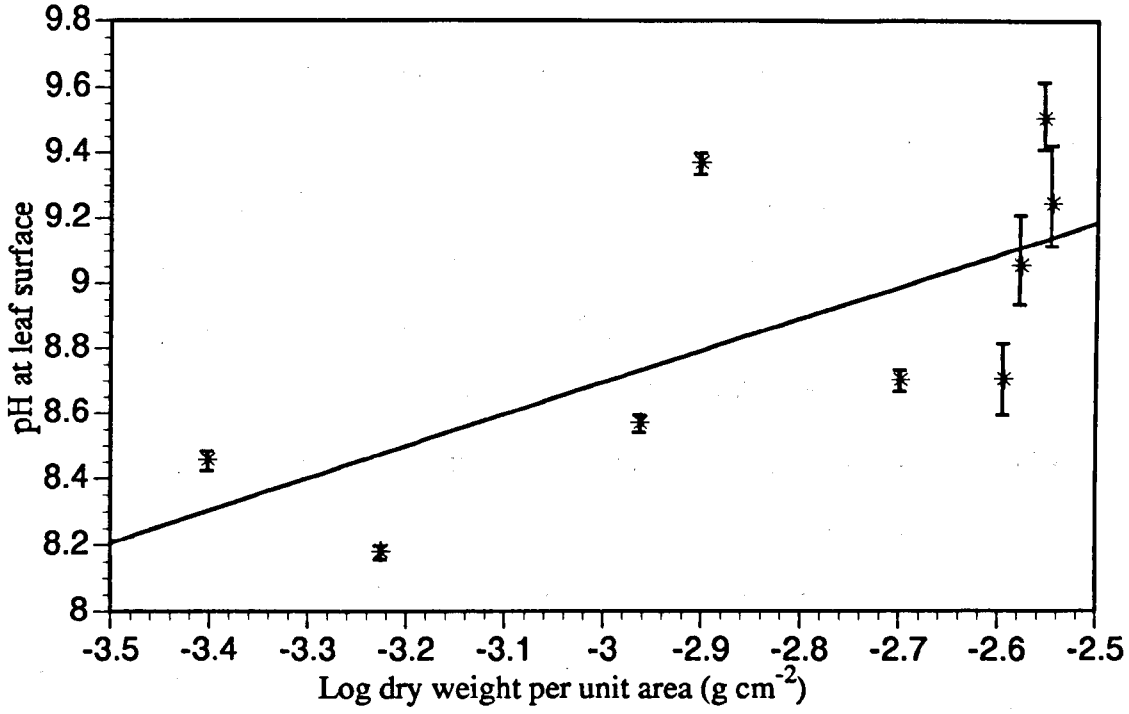


Figure 6.41 Relationship between Log_{10} of epiphyton dry weight per unit area and pH at the leaf surface (mean of 3 advances ± se).



The relationship between pH and epiphyton dry weight per unit area is
$y = 0.981 \log x + 11.64$, $R^2 = 0.473$ $p = 0.0406$

As with the other measures of epiphyton, a logarithmic relationship was found between the epiphyton thickness and the pH at the leaf surface (Figure 6.42). A $\log_{10}(1+x)$ transformation had to be used to linearise this data so as to include layers of zero thickness (ie. very sparse algae). Once this had been performed, the regression line gave a very good fit ($R^2 = 0{\cdot}748$, $p = 0{\cdot}0001$, Figure 6.43).

Tentatively using the model described previously (Section 6.2.7), the pH values were converted to CO_2^* concentrations, indicating that the algae were capable of depleting CO_2^* to very low levels, less than 2 μmol l^{-1} (Figures 6.44 & 46). The relationship between increasing epiphyton and CO_2^* concentration was shown to be an negative exponential curve by plotting the natural logarithm of the calcuated CO_2^* concentrations against the chlorophyll-a density ($p = 0{\cdot}0011$) or the epiphyton thickness ($p = 0{\cdot}0001$), which resulted in straight line relationships (Figures 6.45 & 47). Hence, the effect of increasing epiphyton on CO_2^* concentration was greatest when the epiphyton was sparse. It is also apparent that the thickest layers of epiphyton were capable of reducing CO_2^* to levels below the compensation point for many submerged macrophytes (0·7 to 25 μmol l^{-1} – Van *et al.*, 1976; Søndergaard, 1979; Bain & Proctor, 1980; Søndergaard & Wetzel, 1980; Allen & Spence, 1981; Salvucci & Bowes, 1981; Maberly, 1983; Sand-Jensen, 1983a; Bowes & Salvucci, 1989; Madsen, 1991), and would thus effectively arrest photosynthesis in such plants.

It has been predicted that an increasing layer of epiphyton will result in an increase in the width of the boundary layer as a result of reduced water movement within the epiphyton (Chapter 2). Since this is the result of a physical process the directly measured epiphyton thickness was used in the regression analysis (Figure 6.48) giving a highly significant linear relationship ($R^2 = 0{\cdot}697$, $p = 0{\cdot}0001$). The slope of this line, 1·282, was not significantly different from 1, indicating that the surface of the epiphytic layer effectively replaced the plastic leaf surface as the outer limit of the object. The interstitial water of the epiphyton would therefore be static. The intercept of this line is the thickness

Figure 6.42 Relationship between epiphyton thickness and pH at leaf surface.

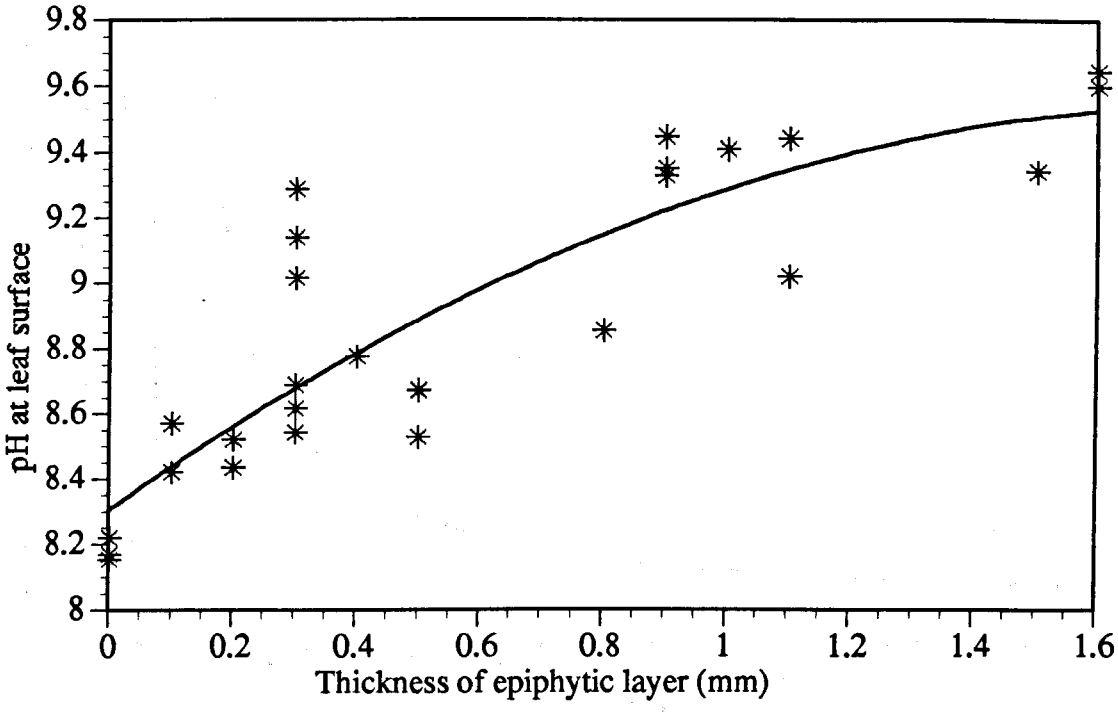
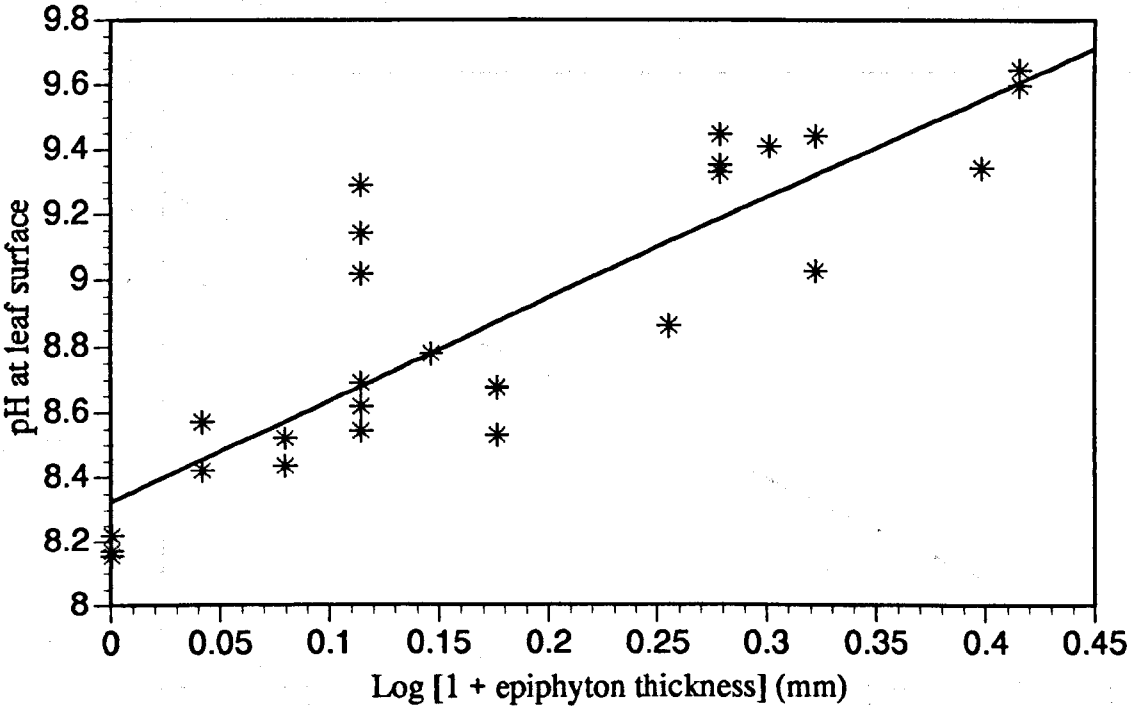


Figure 6.43 Relationship between Log_{10} of epiphyton thickness +1 and pH at the leaf surface (from individual advances).



The relationship between pH at the leaf surface and epiphyton thickness is
$y = 3.081 \log(1+x) + 8.326$, $R^2 = 0.748$, $p = 0.0001$

Figure 6.44 Relationship between epiphyton chlorophyll-a per unit area and CO_2^* at the leaf surface (calculated from mean pH of 3 advances).

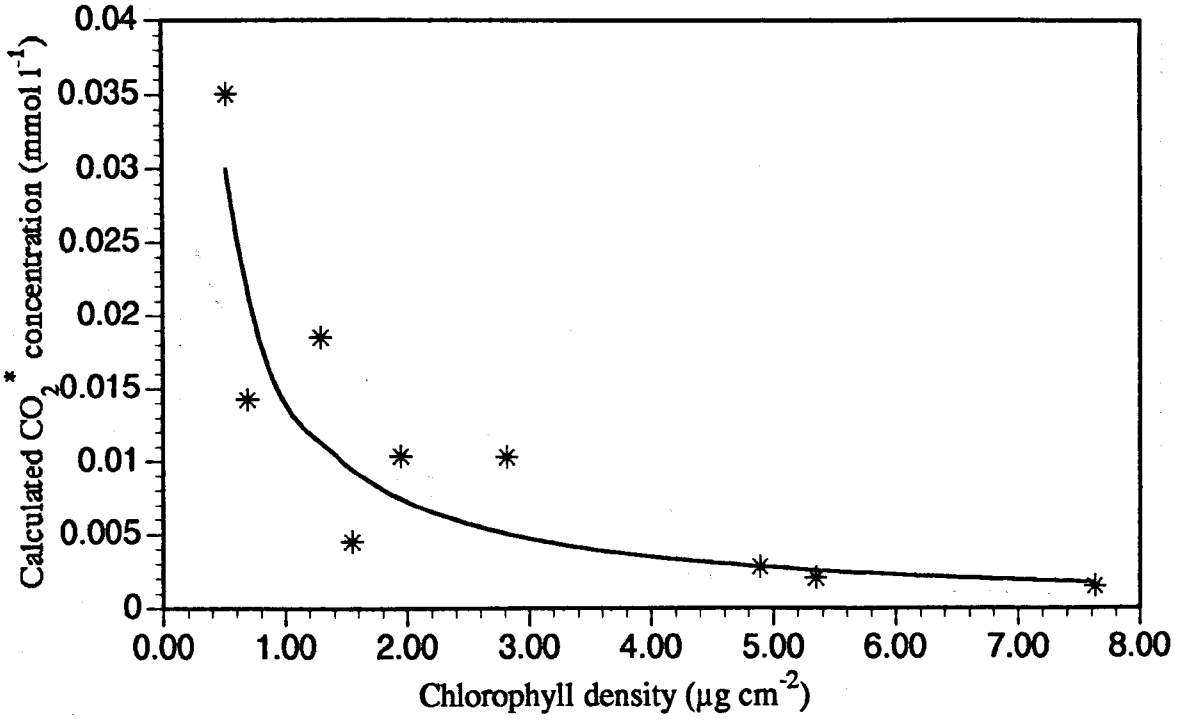
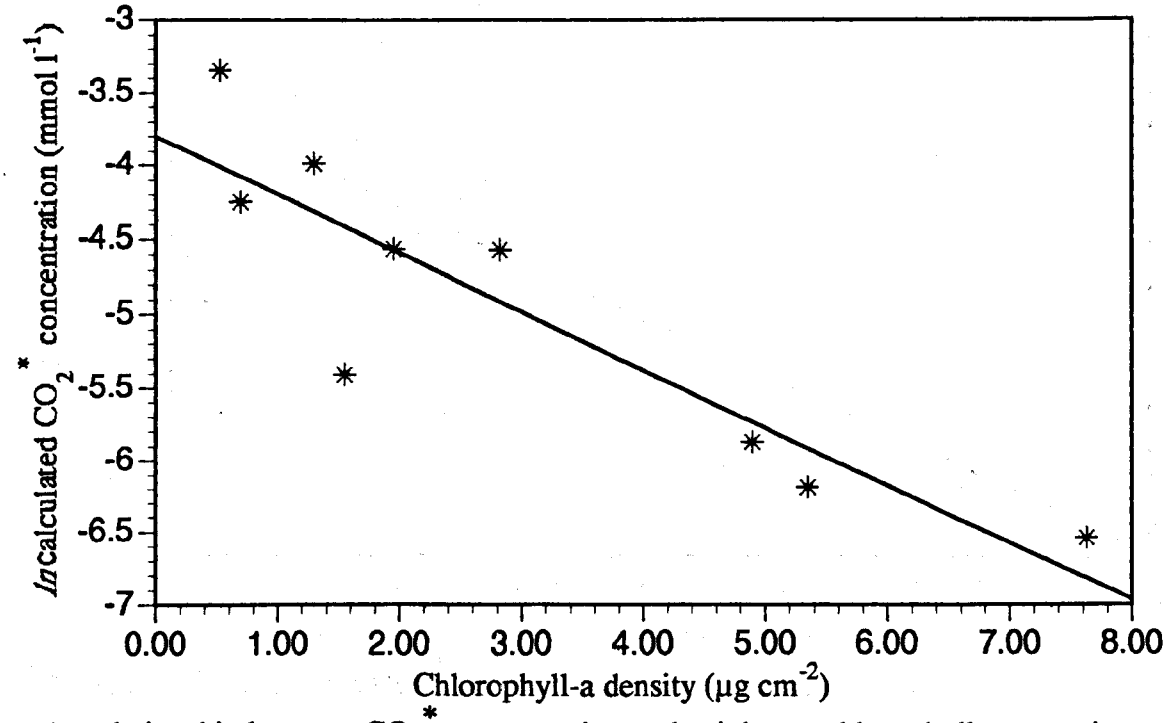


Figure 6.45 Relationship between epiphyton chlorophyll-a per unit area and *ln* CO_2^* at the leaf surface (calculated from mean pH of 3 advances).



The relationship between CO_2^* concentration and epiphyton chlorophyll-a per unit area is

$y = e^{-0.395x - 3.803}$, $R^2 = 0.803$, $p = 0.0011$

Figure 6.46 Relationship between epiphyton thickness and CO_2^* at the leaf surface (calculated from pH of individual advances).

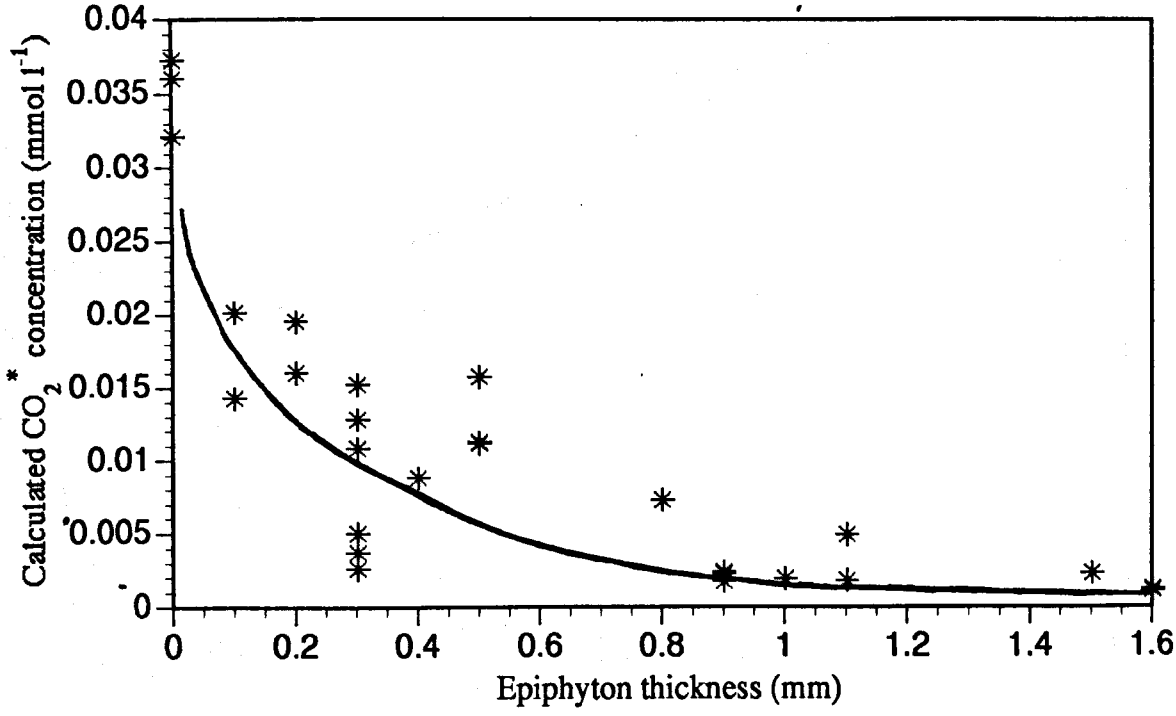
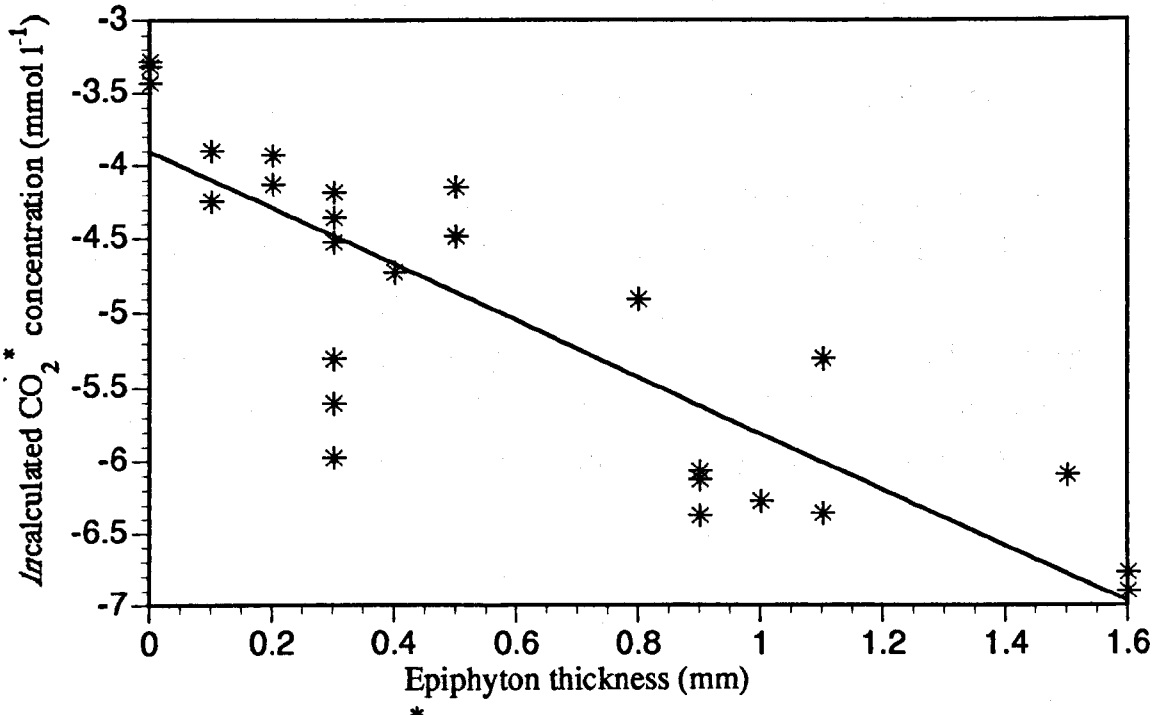


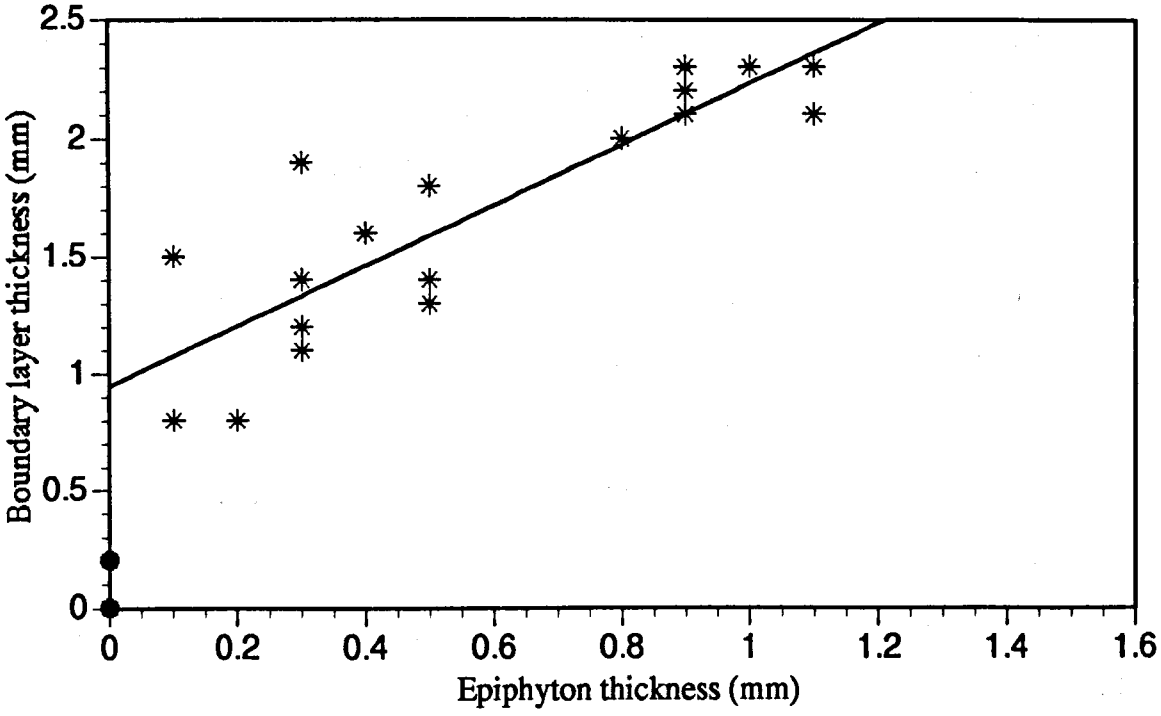
Figure 6.47 Relationship between epiphyton thickness and *ln* of CO_2^* at the leaf surface (calculated from pH of individual advances).



The relationship between CO_2^* concentration epiphyton thickness is

$y = e^{-1.914x - 3.906}$, $R^2 = 0.723$, $p = 0.0001$

Figure 6.48 Effect of increasing epiphyton thickness on boundary layer thickness.



The equation of the line fitted by regression is,

$y = 1.282x + 0.948 \quad R^2 = 0.697 \quad p = 0.0001$

with 95% confidence intervals of the slope at 0.876 and 1.688

A total of six points (two plants) were omitted from this analysis for the following reasons,

i) for the thickest layer of epiphyton the limit of the boundary layer was greater than 2.5 mm from the leaf surface and could not be determined accurately (points not shown).

ii) for the thinest layer of epiphyton the effect of the algae on pH was very small and the gradient slight. In this case it is unlikely that the diffusion-boundary layer was equal to the physical boundary layer (shown as ●)

of the boundary layer surrounding the plastic leaves in the absence of epiphyton, ie.0·948 mm. The scatter of the points around the line was quite large ($R^2 = 0·697$), which could be due to three factors; the impossibility of defining the outer edge of the epiphyton layer accurately due to its fuzziness, variations in flow around the leaves, or "dead zones" where water is trapped between taller regions of epiphyton (see Riber & Wetzel, 1987).

From Fick's First Law, the flux rate of a substance moving by diffusion is inversely proportional to the distance traversed. Hence, the flux of CO_2^* across the boundary layer will decrease linearly with increasing thickness of the layer of epiphyton and (from Figure 6.32) the areal chlorophyll-a density.

A total of six points were excluded from this regression analysis, three advances each from one replicate plastic plant at the upper and one at the lower end of the range. For the plant with the thickest layer of epiphyton (1·5-1·6 mm), the boundary layer was greater than 2·5 mm and hence outside the operating limit of the apparatus. The thinnest layer of epiphyton had such a sparse covering of algae that its effect on pH was very small and the measured gradients were slight or absent. Hence the edge of the boundary layer was impossible to detect with this method, which relies on changes in pH to infer conditions of flow.

v) Summary.

In summary, an increase in the thickness of the epiphyton layer results in:

i) a logarithmic increase in pH at the leaf surface and an exponential decrease in CO_2^* concentration to exceeding low levels (>2 $\mu mol\ l^{-1}$), with the fastest rate of CO_2^* decrease during the early stages of colonisation,

ii) a linear increase in boundary layer thickness, the limit of which increases in unitary proportion to the thickness of the epiphyton layer.

6.3.3 Epiphyton on real plants.

6.3.3.1 Methods.

i) Culture.

A collection of *E. nuttallii* plants growing in the Leeds and Liverpool Canal was made on the 3rd of August 1993, one week after the plastic plants had been retrieved, from the same weedbed in which the plastic plants had been seeded (SJ374987). On return to the laboratory, the plants were carefully sorted from dead material and metaphyton. Five 10 cm long shoots were then rooted into one uncovered plastic beaker (height 4·5 cm, diameter 7 cm), filled with canal sediment and the beaker placed into a glass jar filled with 2·5 l of twice-filtered canal water. Nine such jars were filled with plants in this way. These jars were then maintained in the same way as those containing the plastic plants, namely for 21 days at 20 °C, recieving illumination of 100 μmol m^{-2} s^{-1} PAR on a 16 hour light : 8 hour dark cycle, and bubbled with untreated air (Plate.23). Whilst in culture the jars were loaded weekly with nutrients in one of three ways, as described for the plastic plants (Section 6.3.2.1i), and again referred to as low, medium and high loading. As with the plastic plants, the treatments were arranged in a 3 x 3 latin square, and investigated with the microelectrodes at the end of the culture period.

ii) Microelectrodes.

Twenty-two days after collection, each jar was randomly selected in turn and one of the five shoots carefully removed. This shoot, with associated epiphyton, was then mounted and investigated with the microelectrode apparatus as described for the plastic plants (Section 6.3.2.1ii).

After all three advances had been made, the selected leaf was carefully removed from the stalk with a scalpel and prepared in freezing alcohol for SEM. The epiphyton was

removed from the other two leaves in the whorl by gentle brushing with a soft paint brush, then ground with sand in iced 90% acetone to extract the chlorophyll from the algae. The absorbance of the acetone, after centrifugation, was measured in the same way as for the epiphyton growing on the plastic plants (Section 6.3.2.1ii). The photosynthesis of these two cleaned leaves was determined in modified Forsberg medium adjusted to pH 7 with a Clark-type electrode as outlined previously (Section 2.2.1).

The epiphyton from the six leaves in the whorls above and below the selected leaf was also removed by brushing, and the dry weight measured as described for the epiphyton grown on the plastic plants (Section 6.3.2.1ii). Once cleaned, the areas of 3 of these leaves, selected randomly, were measured as outlined previously (Section 2.2.3), and the mean used to calculate the leaf area for standardisation of chlorophyll-a and dry weight of epiphyton.

All these plants were fully analysed on the same day. The following day the remaining four plants were removed from each jar and all the leaves carefully brushed, and washed clean of epiphyton. The roots were also washed clean of sediment. All four plants from each jar were then dried in an aluminium weigh boat to constant weight at 60 °C and weighed to four decimal places. In this way the final biomass of the plants in each treatment was measured to assess their performance under the different nutrient regimes.

6.3.3.2 Results.

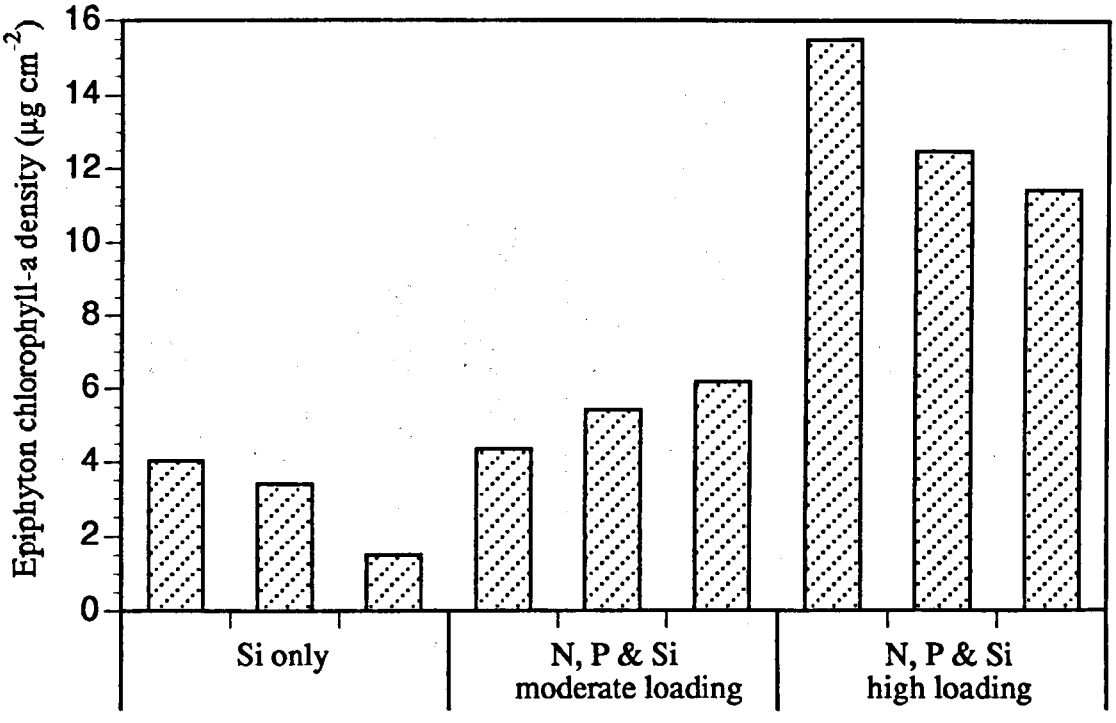
i) Epiphyton and plant characteristics.

The nutrient loading produced a significant increase in the chlorophyll-a per unit area of the epiphyton growing on the leaves of the real *E. nuttallii* plants (Figure 6.49), from anova p = 0·0004), with the mean from the high loading being significantly different from the other two (from Tukey's LSD = 3·9143). These areal densites were greater than those from the plastic plants under the same nutrient loading, perhaps indicating a larger initial population of algae growing on the real plants when they were collected from the field. The actual values are similar to those previously reported in the literature (Sand-Jensen & Søndergaard, 1981).

The nutrient loading also caused a significant difference in the final dry weight of the cleaned plants (Figure 6.50, from anova p = 0·0186). The medium loading produced the highest biomass of plants and the high loading the lowest, with the low loading in between (Figure 6.50). A Tukey's test indicated that there was a significant difference between the means from the medium and high loadings (LSD = 0·3630). The growth rate of floating stems of *E. nuttalli* has been shown to increase with moderate increases in nitrogen loading, but high nitrogen loading (5 mg l^{-1}) was deleterious (Ozimek *et al.*, 1993). However, the reason for this deleterious effect was not investigated (Ozimek *et al.*, 1993). The differences found here in plant biomass and hence area will have had an effect on the epiphyton density, as the area of colonisable plant surface either increased (medium loading) or decreased (high loading) with a corresponding dilution or concentration of epiphyton.

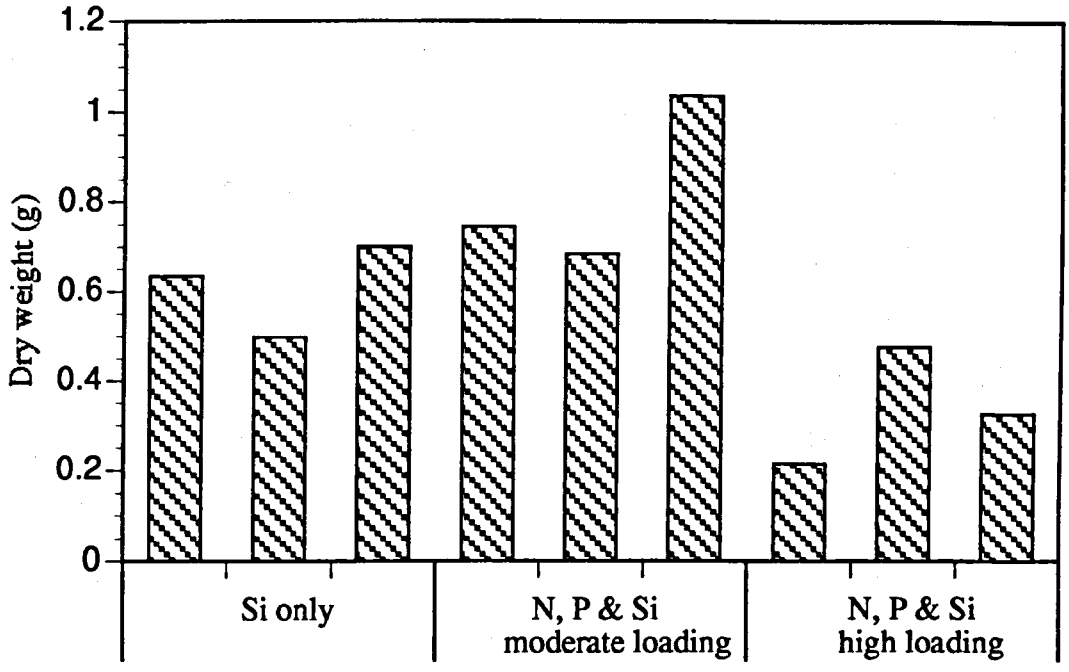
There appeared within the range of treatments, as with the plastic plants, to be communities representative of all algal stages of epiphyton development (Plates.28 to 31). The epiphyton growing on the real and the artificial plants did appear similar in architecture, with the typical adpressed, encrusting, erect, stalked and filamentous growth forms of

Figure 6.49 Effect of nutrient loading on epiphyton development on the leaves of *E. nuttallii*, measured as chlorophyll-a per unit area.



From anova there is a significant effect of loading on epiphyton chlorophyll-a per unit area, p = 0.00004. From Tukey's test LSD = 3.9143.
For means LOW MEDIUM HIGH

Figure 6.50 Final dry weight of plants from the different nutrient loading regimes.



An anova showed that there was a significant effect of loading on the dry weight of the plants p = 0.0186. Tukey's test distinguished a difference between the mean values of the high and medium treatment, with the low treatment between the two. LSD = 0.363.

MEDIUM LOW HIGH

epiphytic algae (see Plates 24 to 31). The species composition of the *E. nuttallii* epiphyton was similar to that from the plastic plants, except for the notable frequent presence on the real plants of the diatom *Synedra ulna* and occasional chaetophoralean green algae, and a lack of the diatom *Epithemia zebra* (Table 6.3). These differences could be due to intrinsic features of the artificial and real plants, or to coloniser effects with the communities only representing those species which were brought in from the field. The age of the plant material when it was brought into culture was not known, but may have been more than the 3 weeks that the plastic plants spent in the canal, hence allowing greater and more varied colonisation. However, the plants had all grown visibly and the leaves sampled were all taken from sections that had formed during the culture period and were therefore less than 3 weeks old. These leaves must have been colonised from the epiphyton growing on the original 10 cm lengths. Also, the difference in time of collection may have influenced the species composition, mass migrations of epiphytic diatoms (*Synedra* and *Diatoma elongatum*) into and out of the plankton having been reported previously (Moss, 1981). Another obvious difference between the real and artificial substrata is that the real plants were growing and continually producing new surfaces for the epiphyton to colonise. The age of the communities on the real plants and hence the degree of development, will depend on the position of the leaf on the stem and the growth rate of the plants. The differences in plant dry biomass from the three treatments must be the result of differences in growth rate, which translates into rate of production of leaves. The leaves, which were taken from the same point on the stems of plants with different growth rates, will have been of different ages, with those from the high nutrient treatment being the oldest and their associated epiphyton the most developed. This effect may in part explain the greater epiphyton density on these leaves, but it is very difficult to control this experimentally and it is part of the natural interaction between submerged plants and their epiphyton. If epiphyton is considered to have a negative effect on plant growth, then this interaction acts as a positive feedback. When the plant's growth rate is high, the epiphyton load is reduced,

encouraging further plant growth; when the plant's growth rate is low, the epiphyton load is increased and plant growth is further reduced. The differences in density and architecture of the epiphyton from the plastic plants, which obviously did not grow, were due to differences in nutrient loading and followed the same trends as those from the real plants.

ii) pH profiles.

The pH at the surface of the real leaves increased logarithmically with increasing epiphyton chlorophyll-a per unit area ($p = 0{\cdot}0182$, Figures2 6.51 & 6.52), in a similar fashion to the pH at the surface of the plastic leaves. Oddly the dry weight of the epiphyton appeared to be related to the pH at the leaf surface in an linear fashion ($p = 0{\cdot}011$, Figure 6.53), but as detailed in section 6.3.2.2ii the epiphyton dry weight was a less good predictor of epiphyton than was chlorophyll-a. As was the case with epiphyton growing on the plastic plants, chlorophyll-a per unit weight of epiphyton did not show any relationship with pH (Figure 6.54).

The measured photosynthesis of the leaf subtending the epiphyton did not appear to be related to the pH measured at the leaf surface when the epiphyton was present (Figure 6.55). Although the oxygen exchange of these leaves was measured under different conditions, where photosynthesis would be near to maximum, one would expect the pH to follow the carbon extraction capability of the leaf. Such a relationship between maximum photosynthesis and pH at the leaf surface has been shown before (Section 6.2.5.2), as has the relationship between the pH of the water in an enclosed vessel and the carbon extraction capability of the plant incubated within it (Maberly & Spence, 1983; Adamec & Ondok, 1992). Since the epiphyton was removed from the leaves before their photosynthesis was measured, it can be assumed that it was the presence of this layer which caused a breakdown of the relationship with pH. Further, as the pH was proportional to the epiphyton chlorophyll-a density, it can be stated that it was the epiphyton and not the plant

Figure 6.51 Relationship between epiphyton chlorophyll-a density and pH at *E. nuttallii* leaf surface.

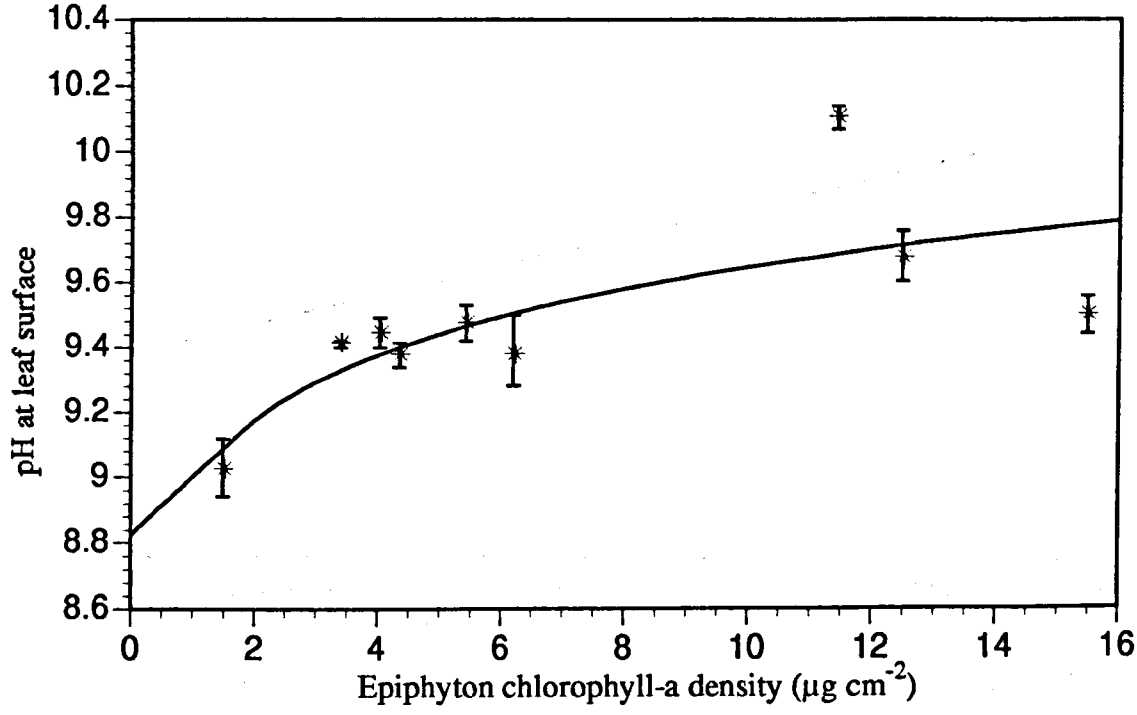
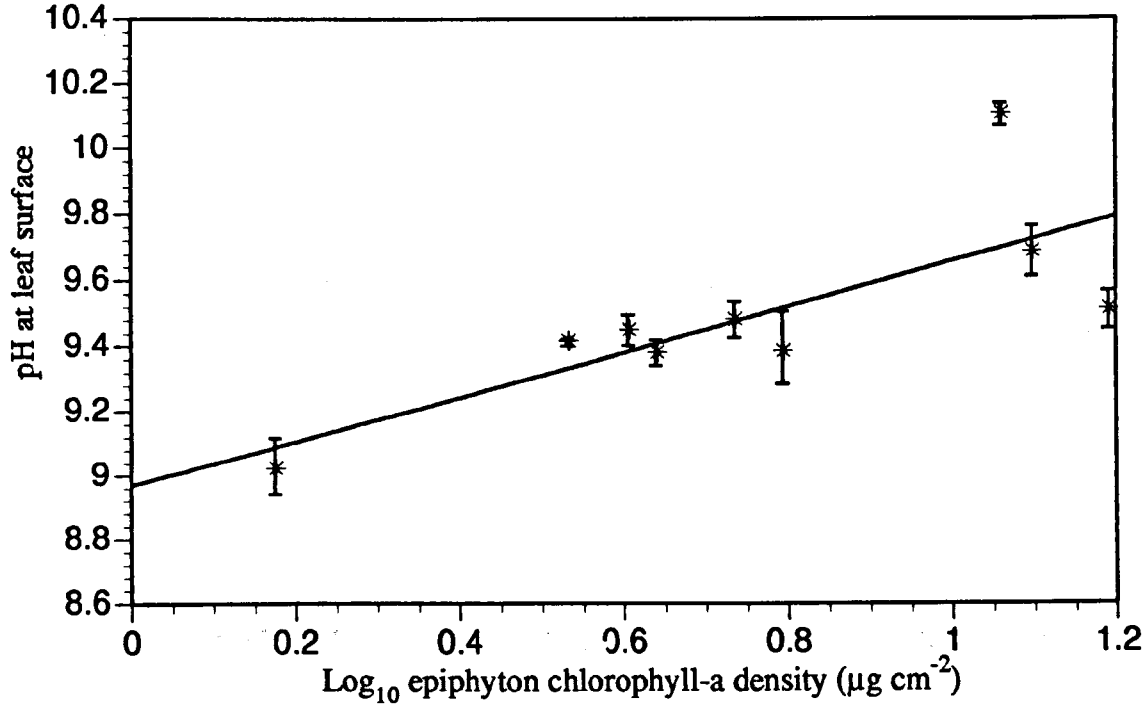
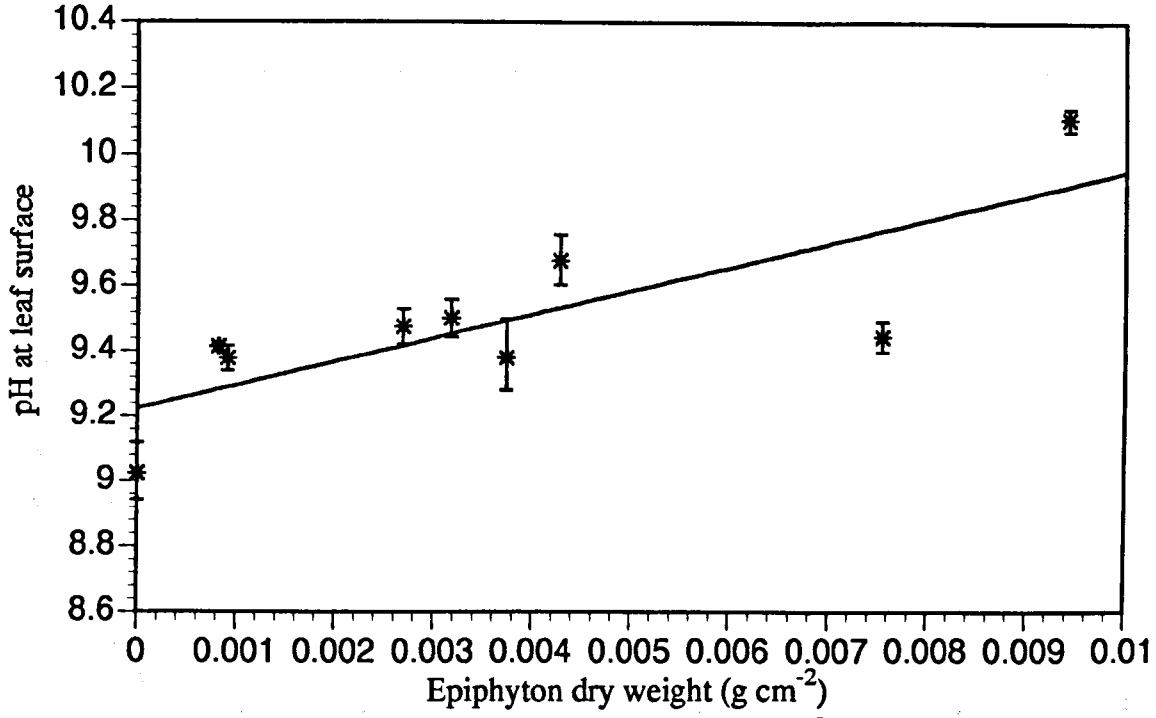


Figure 6.52 Relationship between Log_{10} of chlorophyll-a density and pH at the *E. nuttallii* leaf surface (mean from 3 advances ± se).



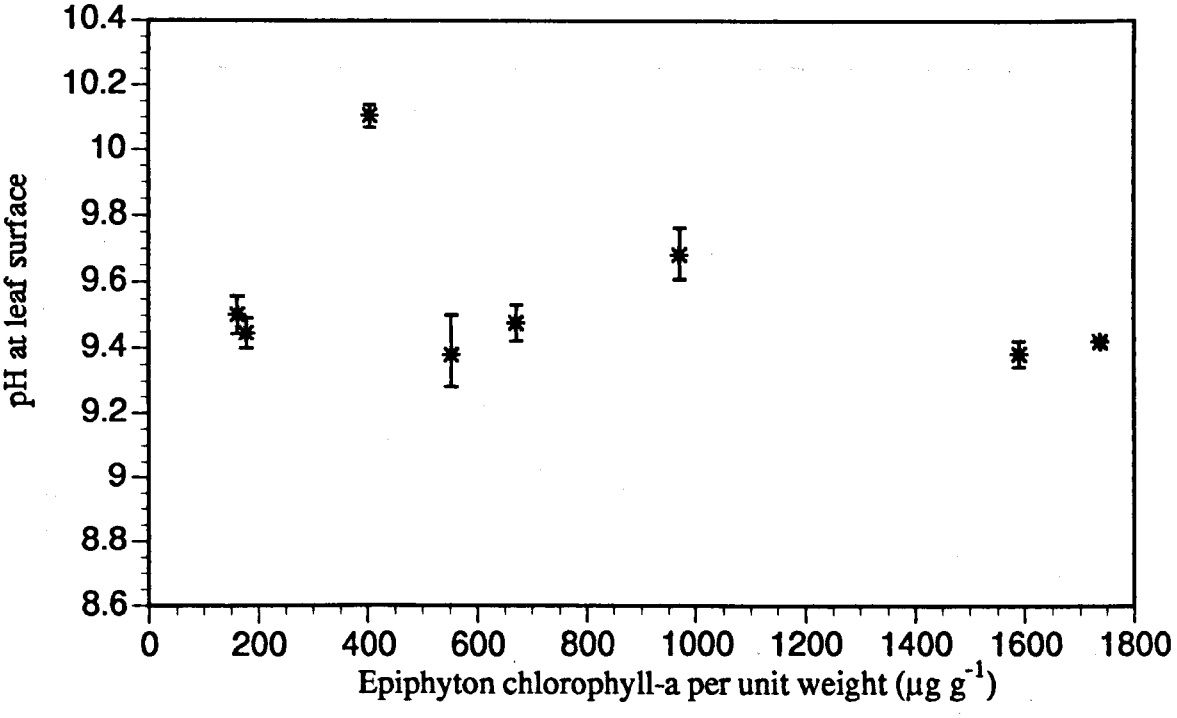
The relationship between epiphyton chlorophyll-a and pH is $y = 0.680\log(x) + 8.971$, $R^2 = 0.573$, $p = 0.0182$

Figure 6.53 Relationship between epiphyton dry weight per unit area and pH at *E. nuttallii* leaf surface (mean from 3 advances ± se).



The equation for this line is $y = 72.553 + 9.223$, $R^2 = 0.627$, $p = 0.011$

Figure 6.54 Relationship between chlorophyll-a per unit weight and pH at *E. nuttallii* leaf surface (mean from 3 advances ± se).



The value of chlorophyll-a per unit dry weight from one replicate was not used in this analysis since the epiphyton dry weight of this replicate was not detectable by the method used.

Figure 6.55 Relationship between *E. nuttallii* photosynthesis and pH at the leaf surface (mean of 3 advances ± se).

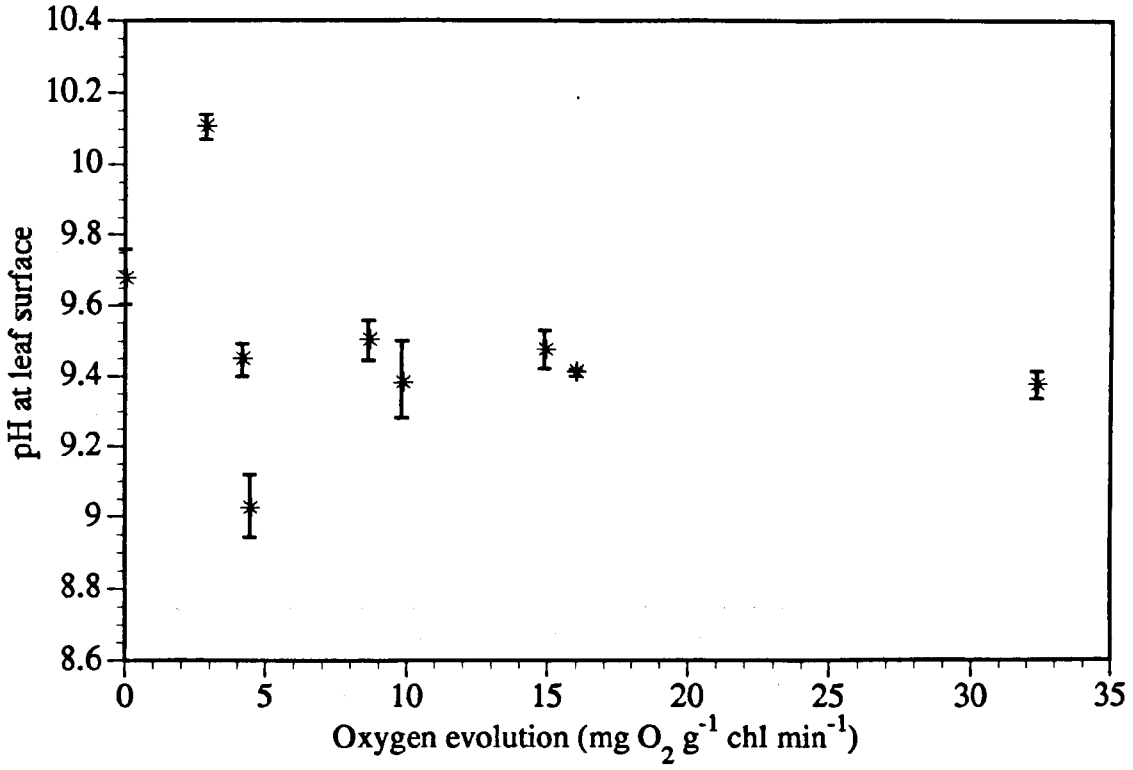
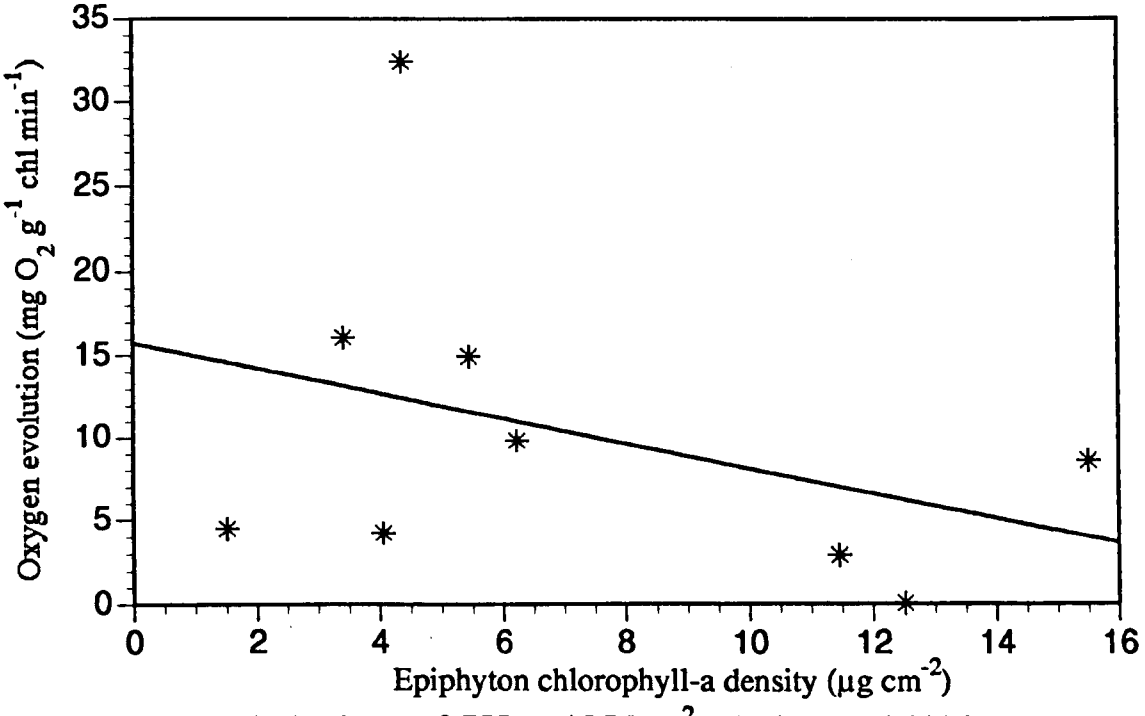
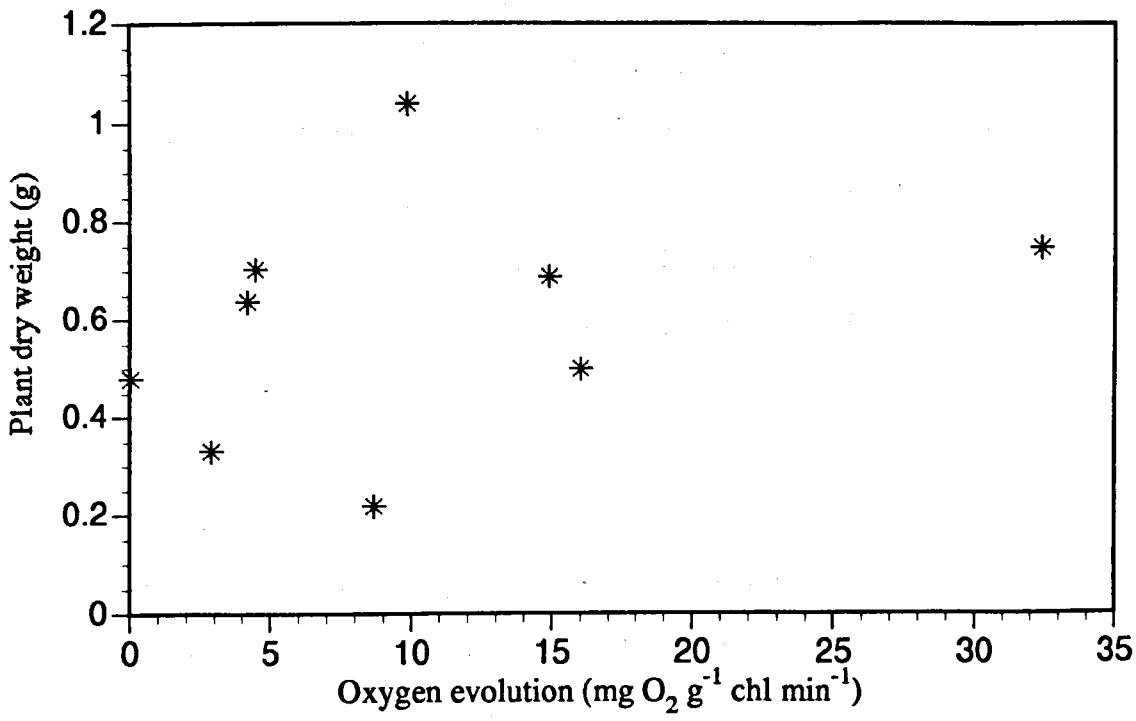


Figure 6.56 Relationship between epiphyton chlorophyll-a density and photosynthesis of the leaves from that plant.



The equation for this line is $y = 0.755x + 15.75$, $R^2 = 0.135$, $p = 0.3306$

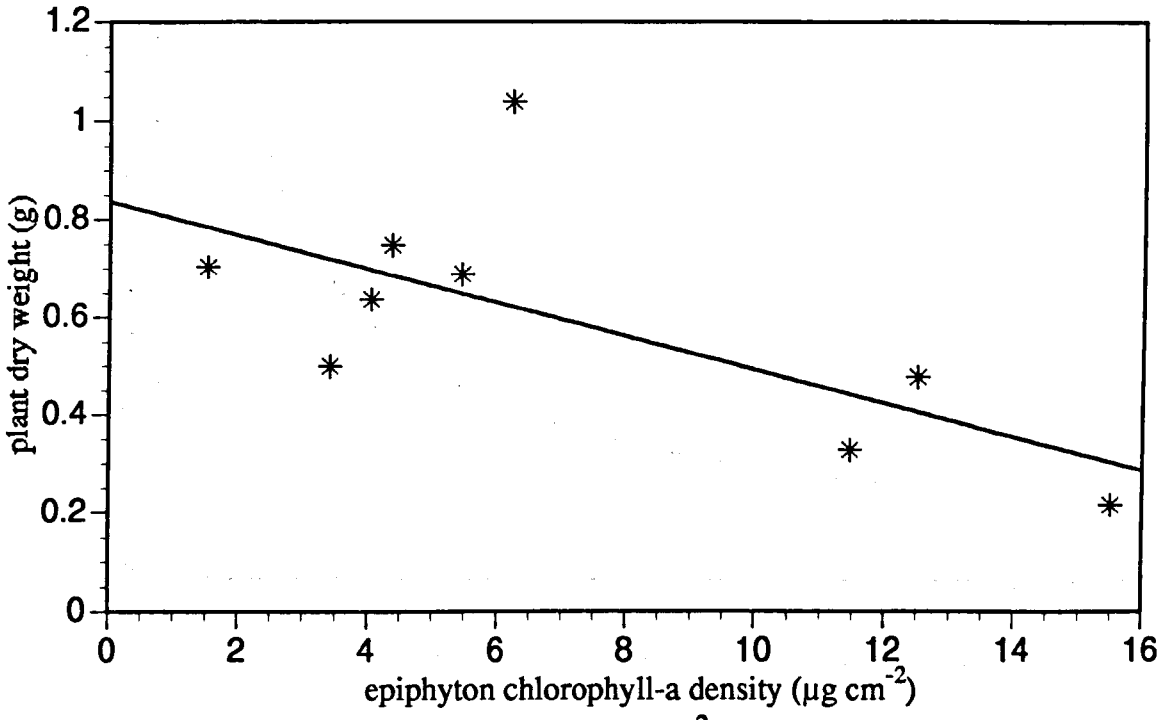
Figure 6.57 Relationship between photosynthesis of the plant used and the dry weight of the other plants from the jar.



which was controlling the pH at the leaf surface. The effect of the epiphyton on the leaves must have been reversible, since the photosynthesis, measured after cleaning the leaves, was not proportional to the amount of epiphyton ($p = 0{\cdot}3306$, Figure 6.56). Epiphyton has been suggested to affect plant leaves non-reversibly through the encouragement of necrosis-inducing bacteria populations which invade the plant cells (Howard-Williams *et al.*, 1978; Rogers & Breen, 1981 & 1983), but this can be discounted in this study since no non-reversible effects were found. Underwood (1991b) found that bacteria only invaded the cells after senescence had occurred and were not causative. The plant leaves here were not in a scenescent state.

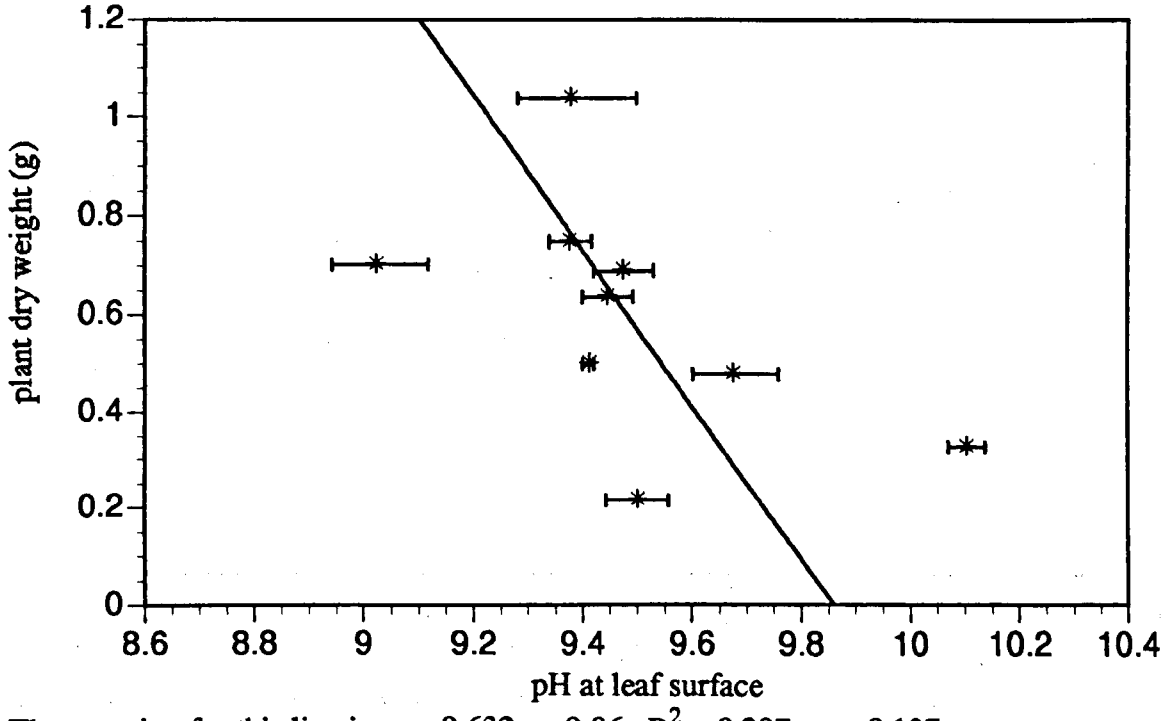
The decrease in plant growth at the high nutrient loading was related to the density of epiphyton chlorophyll-a on the leaves measured ($p = 0{\cdot}0478$, Figure 6.58). Although these measurements of epiphyton were taken from only one point on one plant from each jar, this relationship would indicate that they were either typical of the whole plant or in a critical position with regard to its growth. From the work of Birch (1990) and that reported earlier (Section 6.2.5) on the distribution of activity along the shoots of *E. nuttallii*, the point where the measurements were taken was expected to be well within the peak of photosynthetic activity for the shoot and hence critical for its growth. On current theory and in the absence of any other satisfactory explanation for this decline in plant growth, it can be assumed that it was caused by the increase in epiphyton (Figure 6.58). However, when plant dry weight was plotted against pH at the leaf suface there was no apparent relationship ($p = 0{\cdot}245$, Figure 6.59). This is somewhat surprising when the effect of increasing epiphyton on the calculated CO_2^* concentration at the leaf surface is considered (Figure 6.60), but again there was no relationship between these values and the dry weight of the plants ($p = 0{\cdot}245$, Figure 6.61). It was therefore concluded that the epiphyton must be acting on the plant through some mechanism other than by CO_2^* depletion. There are two likely candidates for this mechanism, namely,

Figure 6.58 Relationship between epiphyton chlorophyll-a density and plant dry weight.



The equation for this line is $y = -0.034x + 0.836$, $R^2 = 0.45$, $p = 0.0478$

Figure 6.59 Relationship between pH at leaf surface (mean of 3 advances ± se) and plant dry weight.



The equation for this line is $y = -0.632x + 9.86$, $R^2 = 0.287$, $p = 0.137$

Figure 6.60 Relationship between CO_2^* concentration at the leaf surface (calculated from mean pH) and epiphyton chlorophyll-a density.

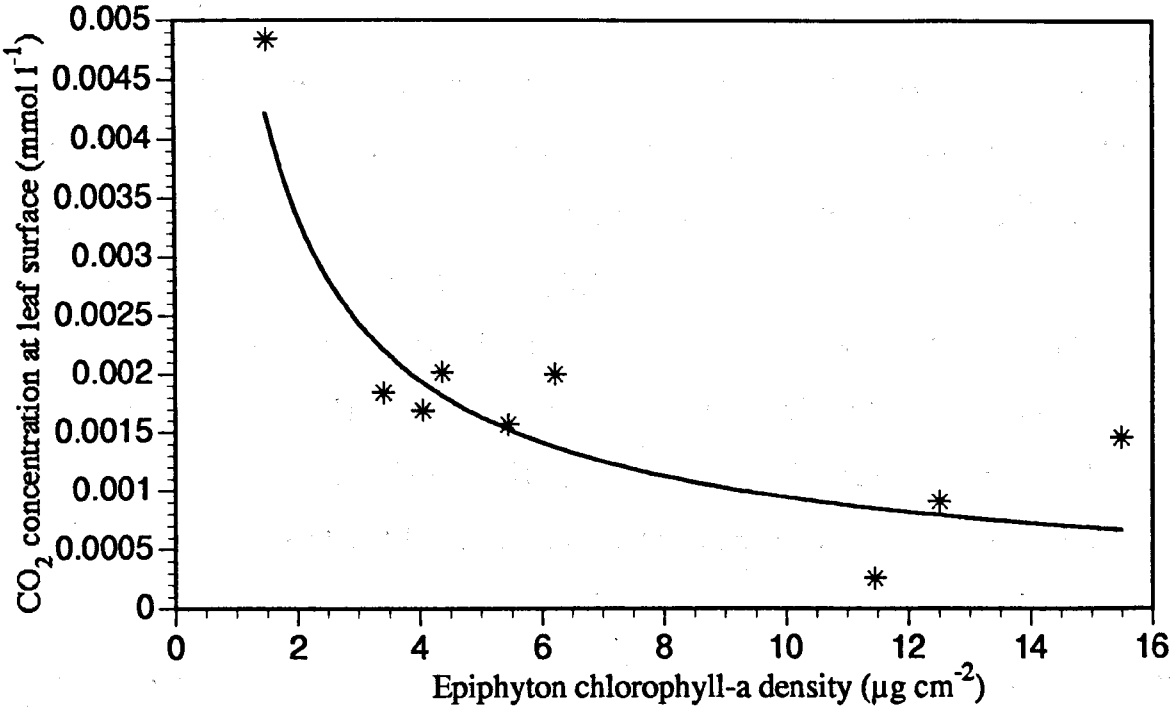
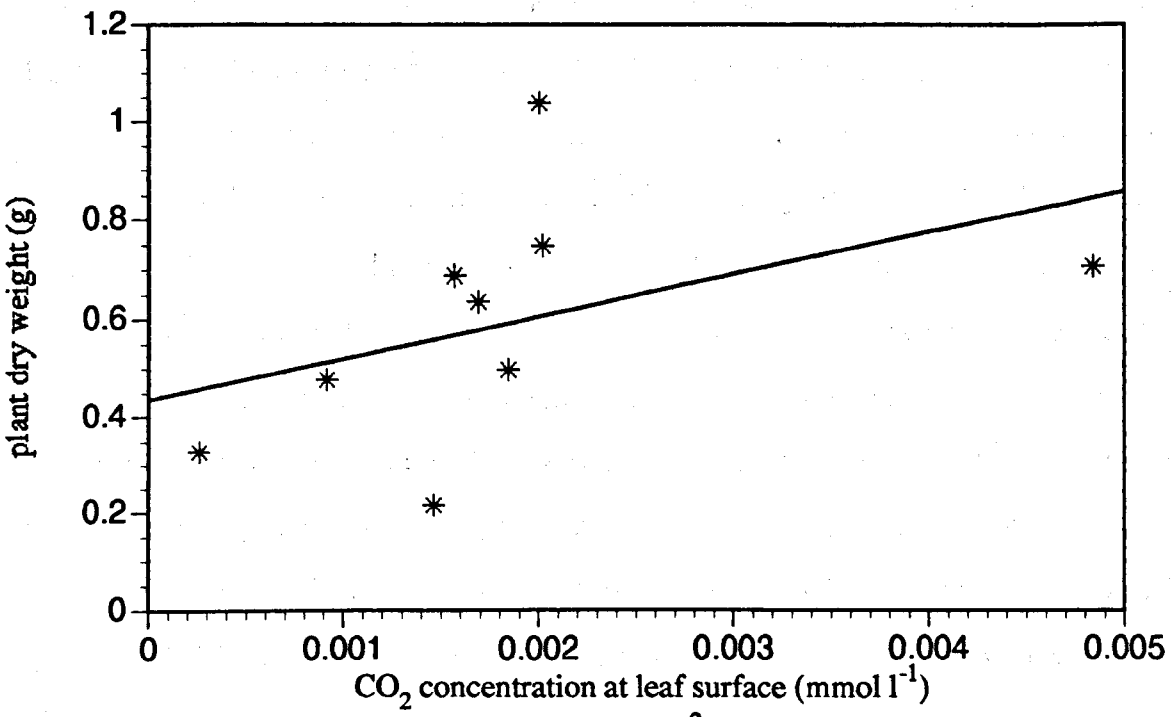


Figure 6.61 Relationship between CO_2^* concentration at the leaf surface (calculated from mean pH) and plant dry weight.



The equation for this line is $y = 83.999x + 0.437$, $R^2 = 0.187$, $p = 0.245$

i) as shown from the work with the plastic plants (Section 6.3.2.2iii), increasing the boundary layer thickness and hence the distance over which CO_2^* must diffuse, or,

ii) by shading the plants photosynthetic surfaces.

iii) notwithstanding earlier well-substantiated suggestions that nutrients other than C are generally obtained from the sediment by elodeids, it is also possible that nutrient uptake from the bulk water phase may have been limited by the increased diffusion distance and interception of nutrients by the epiphyton.

Unfortunately the method of investigation used here was not capable of separating the effect of increased resistance to diffusion from that of shading, as the thickness of the epiphyton was proportional to its chlorophyll content and hence shading potential.

The work of Sand-Jensen (1977) on the marine plant *Zostera marina* L. showed that at low light intensities epiphyton reduced the plants photosynthesis by shading, but with increasing light this effect decreased, becoming constant at the point where the epiphyton was restricting the supply of carbon (7·2 mW cm^{-2}, $[HCO_3^-]$ = 1·7 meq l^{-1}). Using a conversion factor of 46 to convert mW cm^{-2} to µmol m^{-2} s^{-1} (Hall *et al.*, 1993) the point where carbon restriction of *Z. marina* photosynthesis occurred was calculated to be 331 µmol m^{-2} s^{-1}. When the HCO_3^- concentration of the bulk fluid was varied at constant light intensity (14·7 mW cm^{-2} ≈ 676 µmol m^{-2} s^{-1}), saturating for both the epiphytes and *Z. marina*, the inhibition decreased with increasing carbon concentration to a very low level at $[HCO_3^-]$ = 2·55 meq l^{-1}. Although the levels of epiphyton used by Sand-Jensen were approximately constant and given no further description than "a homogeneous diatom crust of average developmental stage compared with the other leaves", it is noteworthy that at the same light and carbon levels, a difference in % inhibition of photosynthesis occurred between these two experiments. This was attributed to differences in the epiphyte load (Snad-Jensen, op. cit.). Another important point is that this study on *Z. marina* was conducted in water of a pH similar to that used here (7·8 – 8·1), but the salinity was far greater (20 ‰), which would slightly reduce the available CO_2^* (Stumm & Morgan, 1981).

Other investigations have either used isoetid plants which take up CO_2^* from the sediment via their roots and not from the bulk water, thus the only effect epiphyton could have is through shading (Sand-Jensen & Søndergaard, 1981; Sand-Jensen & Borum, 1984; Sand-Jensen, 1990), or have ignored the potential for epiphyton to disrupt the supply of carbon to the plant (Bulthius & Woelkerling, 1983).

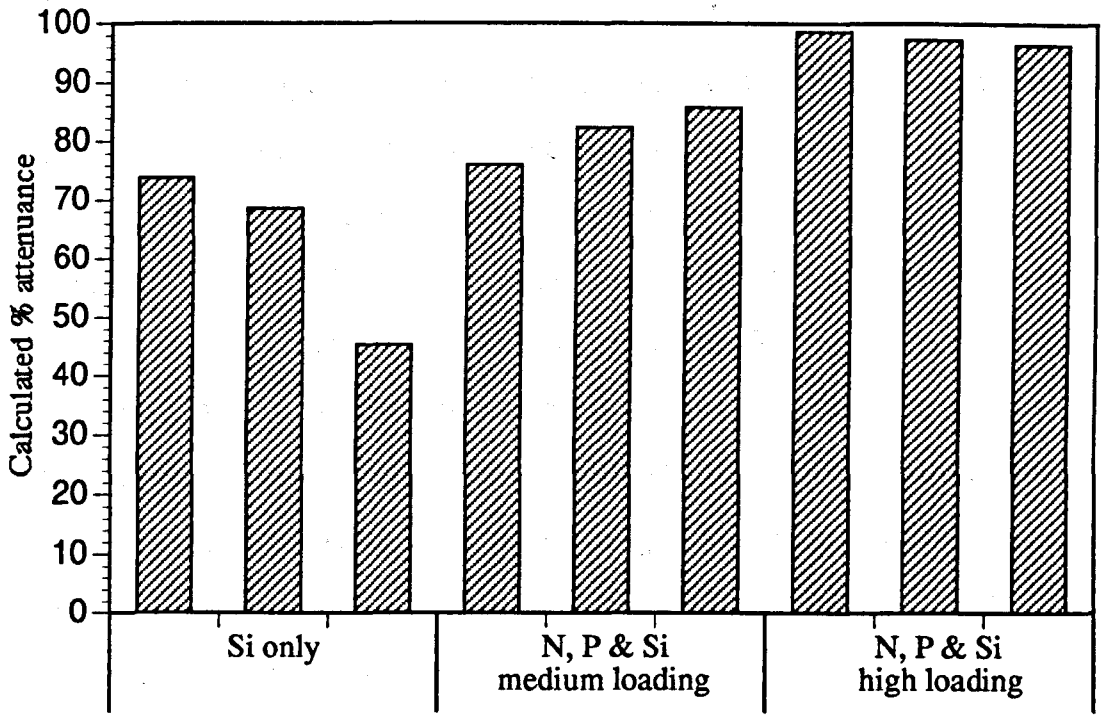
The microelectrode work reported here was carried out at an irradiance of 300 μmol m^{-2} s^{-1}, sufficient to saturate the photosynthesis of both epiphyton (Sand-Jensen *et al.*, 1985; Sand-Jensen & Revsbech, 1987) and *E. nuttallii* (Birch, 1990). Even at 100 μmol m^{-2} s^{-1}, the irradiance used to grow the plants in culture, epiphyton photosynthesis would have been saturated (Sand-Jensen *et al.*, 1985; Sand-Jensen & Revsbech, 1987) and the epiphyton was presumably unable to increase the pH above the levels found in the microelectrode investigation. Despite this no relationship was found between the pH and the final biomass of the plants.

There are two lines of evidence which would suggest that it is through the shading of the leaves' photosynthetic surfaces that the epiphytes exerted their influence in this study. Firstly *E. nuttallii* is capable of utilising HCO_3^- by the polar leaf mechanism, whereby the pH is lowered at the abaxial leaf surface, encouraging the conversion of HCO_3^- to CO_2^*, which is then utilised by the plant. The upper surface, where the epiphytes grow, is not involved in carbon uptake. Hence any effect of the epiphyton on carbon supply at the upper surface will have little relevance to the *E. nuttallii* plant. The polar leaf mechanism is often associated with the precipitation of marl ($CaCO_3$), which was seen on the surfaces of the some of these plants (Plate 30). It is possible that by growing on the underside of leaves utilising HCO_3^- by the polar leaf mechanism epiphyton could disrupt the influx of CO_2^*. The pH at the abaxial surface, although very much controlled by the plant, is favourable for photosynthesis and an epiphyton-mediated increase in pH could severely reduce the gain that the host plant achieves by this mechanism. However,

this surface was effectively epiphyte free in the leaves studied here, and the presence of epiphyton on this surface in the field is infrequent (Paterson & Wright, 1986; Birch, 1990).

The second piece of evidence comes from the published equations relating to light attenuation and epiphyton chlorophyll-a. Four such equations are available, from epiphyton growing on *Littorella uniflora* (L.) Aschers. in two eutrophic and two oligotrophic lowland, Danish lakes (Sand-Jensen & Søndergaard, 1981). The differences between these equations were attributed to changes in species composition of the epiphyton between the lakes and hence differences in the secondary pigments, as the equations use only chlorophyll-a as a variable. Differences in the amount of inorganic matter trapped within the community may also have contributed. These equations were calculated from suspensions of epiphyton and may represent a significant underestimate when compared to the intact community (Vermaat & Hootsmans, 1991a), but are the best available in the literature. The attenuation by epiphyton for each replicate *E. nuttallii* plant was calculated from all four equations and the mean value is shown in Figure 6.62. Other equations for this relationship are given in the literature (Bulthius & Woelkerling, 1983; Birch, 1990), but appear to be related only to low epiphyton densities as they are linear. The true relationship is described by either a negative exponential or a rectangular hyperbola (Vermaat & Hootsmans, 1991a, who give no equation for their dataset). Using periphyton grown on glass slides suspended in a shallow eutrophic lake, van Dijk (1993) showed that a significant part of the attenuation in the field could be attributed to inorganic matter deposited on the slides, and that laboratory grown cultures attenuated less light. Because of this the equations used may represent an over-estimate. However, it is obvious that any significant development of epiphyton greatly attenuated the incident light, and that the plants from the high loading treatment may have been receiving less than 10 % of the light incident on the outer surface of the epiphyton (Figure 6.62). This will have been severely inhibiting to photosynthesis, the light compensation point of *E. nuttallii* being about 20 $\mu mol\ m^{-2}\ s^{-1}$ (Birch, 1990). In the study by Sand-Jensen (1977), where the bulk fluid

Figure 6.62 Percentage attenuation of light by epiphyton, mean calculated from chlorophyll density (μg cm^{-2}) using the 4 equations of Sand-Jensen & Søndergaard (1981).



CO_2^* concentration will have been somewhat lower than the present study, carbon supply was not limiting until an irradiance greater than ≈ 330 µmol m^{-2} s^{-1} was experienced, which is greater than the light intensity used here. There is the potential for plants experiencing higher light intensities than those used here to be limited by carbon supply.

The CO_2^* compensation point of *E. nuttallii* has been shown to be variable and dependent on the O_2 concentration in the medium (Section 3.3.3). Nevertheless, if a figure of 6 µmol l^{-1}, the lowest value for the CO_2^* compensation point found in this study, was substituted into the equations derived from the relationship between epiphyton grown on the plastic plants and CO_2^* concentration (Figures 6.45 & 6.47), then plant photosynthesis would be arrested at an epiphyton density greater than 3·32 µg chl-a cm^{-2} or a thickness of 0·632 mm. As argued above, the plant would have to be experiencing a high light intensity to counteract the shading effect of the epiphyton before such limitation occurred. But under such conditions, the effect of increased oxygen concentrations and an increased CO_2^* compensation point at low irradiance (Maberly, 1983) would lead to CO_2^* limitation at lower epiphyton densities.

iii) General summary.

It can be seen that plant species which can utilise CO_2^* only will be limited by the disruption to carbon supply, especially CO_2^* depletion by algal photosynthesis, at a much lower epiphyton density than those species with polar leaves. The total DIC concentration of the water is important, the effect of epiphyton on the supply of CO_2^* being increased in the presence of low DIC. Also, plants exposed to greater incident light will be affected less by shading, until they reach a point where carbon supply becomes limiting.

To extrapolate these findings to the field is very difficult and the exact mechanism(s) acting on the plant will vary with the prevalent conditions, especially of light and DIC availability. Account must also be taken of the work by Vermaat & de Bryne (1993) on the relative contributions of the epiphyton and the water column to light

attenuation, which varies with the rooting depth and the length of the plant, and also the growth rate of the plant which has a positive feedback on epiphyton density. We should know the buffering capacity of the water, both as DIC and as other buffers, and the flow around the plant which will affect the supply of carbon. Also of great importance are the findings reported earlier on the polar leaf mechanism of *E. nuttallii* and other members of the Hydrocharitaceae and Potamogetonaceae. This mechanism is only employed at low CO_2^* concentrations (Sand-Jensen & Gordon, 1986; Chapter 4), and the maximum growth rate of plants utilising it is very much reduced, when compared to plants growing in plentiful CO_2^* (Section 4.3.4). From this earlier work, a limiting light intensity of 60 $\mu mol\ m^{-2}\ s^{-1}$ was calculated below which the HCO_3^- uptake mechanism would not be expected to be operative in *E. nuttallii.* A further complication in estimating the effect of epiphyton on carbon availability comes from the findings of Maberly (1983), that the carbon compensation point of aquatic plants increases with decreasing light at low irradiances (typically <50 $\mu mol\ m^{-2}\ s^{-1}$). This is of great importance to the plant, since the epiphyton decreases both the light and carbon availability.

Other species lack the ability to utilise HCO_3^- (eg. *Callitriche spp.*) or utilise other mechanisms such as acid-alkali banding (eg. *Chara spp.*), carbonic anhydrase (eg. *Myriophyllum spicatum*), or use of sediment CO_2^* (eg. *Littorella uniflora*). These mechanisms have their relative advantages and disadvantages with respect to the potential deleterious effects of epiphyton. Those species which only use CO_2^* will be affected by the limitation on carbon supply at much lower epiphyton densities and light intensities than those which can use HCO_3^-. The acid bands on the internodal cells of charophytes are prone to epiphyton colonisation which could severely hamper the uptake of carbon. Species which use carbonic anhydrase will be mainly affected by shading and the increase in boundary layer thickness, whilst those which use CO_2^* from the sediment can only be affected by shading.

iv) Conclusions.

It was concluded that in this study the principal interaction between epiphyton and the host *E. nuttallii* plants was one of competition for light. If the light intensity had been raised sufficiently, however, the epiphyton would start to interrupt the supply of carbon through an increased diffusion pathlength and decreased CO_2^* availability. Due to the high DIC concentrations used in this study this is likely to occur only at very high irradiances. At lower DIC, the effect of epiphyton on CO_2^* supply will occur at lower densities or at lower irradiances. It must be noted, however, that waters with lower DIC are often of lower pH also, which will result in a compensating higher CO_2^* concentration. The effect of reduced carbon supply will affect those species which rely solely on CO_2^* from the bulk fluid as the source of photosynthetic carbon at lower epiphyton densities and irradiances than those which exploit alternative sources.

E. nuttallii can largely avoid the effects of epiphyton on carbon supply through the uptake of HCO_3^- by the polar leaf mechanism, but the resultant reduced growth rate would seem likely to encourage increased epiphyton density and shading. In an earlier investigation using *E. nuttallii*, Birch (1990) suggested that this species concentrated its photosynthetic activity near to the shoot apex and had the ability to outgrow epiphyton colonisation/succession. It is suggested here that this only applies to ideal growth conditions and that if the growth rate is reduced, either by high pH and low CO_2^*, or by a switch to HCO_3^- use, then the epiphyton can and will become established at greater densities further up the shoot. This will have serious consequences for further growth of the plant.

Chapter 7.

Discussion.

7.1 Discussion.

The overall aim of this project was to investigate how epiphyton affected the performance of the submerged macrophyte *E. nuttallii.* This involved an initial characterisation of the basic physiology of epiphyte-free *E. nuttalli* under a range of environmental variables. Then the way in which epiphyton modified these variables was investigated, together with the effect of epiphyton on the physiology and performance of the host plant. Thus it was hoped to understand how epiphyton can invade a stabilised, plant dominated state in the field and precipitate a switch to phytoplankton dominance, together with all the associated problems of eutrophication.

As this work draws on several, apparently disparate fields of investigation, the discussion will be divided into separate sections dealing with, the physiology of epiphyton-free *E. nuttallii* under laboratory and field conditions, the interactions between the plant and epiphyton, and the functioning of macrophytes in shallow aquatic ecosystems, respectively. The final section will be a synthesis of the findings on the plant-epiphyton interaction.

7.1.1 The ecophysiology of epiphyton-free *E. nuttallii.*

It was evident that all the variables studied in the laboratory experiments exert an influence on the physiology of *E. nuttallii* leaves, but that the supply of carbon dioxide is paramount to photosynthesis (Chapter 3). When this is disrupted, particularly through increasing pH in the bathing medium, photosynthesis is severely curtailed (Section 3.4.2). This situation appears to be common to all submerged macrophytes, with pH severely affecting photosynthesis above pH 7 (Steeman-Nielsen, 1947; Stanley & Naylor, 1972; Brown *et al.*, 1974; Lloyd *et al.*, 1977; Simpson *et al.*, 1980; Bowes, 1983; Maberly & Spence, 1983; Sand-Jensen, 1983a; Pokorny *et al*, 1984 & 1989; Spence & Maberly, 1985; Sand-Jensen & Gordon, 1986) as CO_2^* becomes limiting. There is an inverse linear

relationship between oxygen concentration and photosynthesis, presumably due to stimulation of photorespiration, which over the range used (approximately 0 – 200% saturation) resulted in a 50% reduction in net photosynthesis. Whilst this increase in photorespiration should not be ignored, it is not the main determinant of the rate of photosynthesis. Flow was also very important and, through its effect on the boundary layer, largely determines the shape of the response to increasing CO_2^* (Smith & Walker, 1980; Black *et al.*, 1981; Madsen & Sand-Jensen, 1991). Variations in the thickness of the boundary layer affect the rate of supply of CO_2^* to the plant's photosynthetic surfaces.

E. nuttallii is at times able to utilise HCO_3^- as a carbon source by the polar leaf mechanism proposed by Prins *et al.* (1982 a & c), which at high levels of pH and low CO_2^* concentrations can reduce the effect of carbon limitation on photosynthesis. From the results reported here it was concluded that the acid-alkali banding theory suggested for *E. nuttallii* by Fagerberg *et al.* (1991) is erroneous. The polar leaf mechanism will effectively increase the concentration of CO_2^* at the site of fixation in the chloroplasts and hence reduce the oxidase activity of RuBisCO and photorespiration. It is suggested that the polar leaf mechanism and the low-PR state described for several members of the Hydrocharitaceae and Potamogetonaceae are one and the same.

Under laboratory conditions both *E. nuttallii* and *E. canadensis* can switch on the HCO_3^- utilisation mechanism within 2–8 days (Section 4.3.2), far more rapidly than previously thought (Sand-Jensen & Gordon, 1984). The mechanism can also be switched off rapidly, at least under certain laboratory conditions (Sand-Jensen & Gordon, 1986; Adamec & Ondok, 1992; Adamec, 1993). Although HCO_3^- use can ameliorate the effect of very low CO_2^* availability it is progressively less effective with increasing bulk water pH (Prins *et al.*, 1982c; Sand-Jensen, 1983a; Prins & Zanstra, 1985), since it depends on reducing the pH of the external medium to stimulate conversion of HCO_3^- to CO_2^*. The mechanism, however, is not very efficient even under favourable conditions, with the rate of photosynthesis at most only about 60% of the maximum rates achieved by the plants

when utilising CO_2^* by diffusion. A similar situation has been reported for all other species with polar leaves (Maberly & Spence, 1983; Spence & Maberly, 1985; Sand-Jensen, 1987; Madsen & Sand-Jensen, 1991). The cost of the polar leaf mechanism in *E. nuttallii* was calculated here to be equivalent to 60 μmol m^{-2} s^{-1}. Field evidence indicates that the use of bicarbonate will only occur when CO_2^* and not light is limiting to photosynthesis (Sand-Jensen & Gordon, 1986) and, as light is progressively attenuated with depth, HCO_3^- use will vary dependent on the position of the active plant surface in the water column (Madsen & Maberly, 1991). Hence, HCO_3^- use will be highest near the water surface and lowest near the bottom, with an estimated switch to CO_2^* use when the light intensity is below 60 μmol m^{-2} s^{-1}. The change in dependence on HCO_3^- will be exaggerated by CO_2^* concentrations, being lower near the surface, due to higher light, temperature and photosynthesis (see Chapter 5) and higher near the bed, where there is lower light, temperature and photosynthesis, and CO_2^*-producing mud (see Chapter 5).

Using a specially constructed microelectrode apparatus, with probes of about 5 μm tip diameter to detect fine changes in pH, it was possible to define the shape of the boundary layer surrounding the leaves of *E. nuttallii* and to show that it followed the shape predicted from experiments with thin, flat plates (Leyton, 1975). A further boundary layer surrounding the whole shoot was also found. Large pH differences between the bulk fluid and the water within the boundary layer were found, often of 1–2 pH units. These differences were caused by the photosynthesis of the plant and resulted in a massive depletion of CO_2^* from the water in the boundary layer. The very low CO_2^* concentration, close to the compensation point, at the leaf surface indicated, in agreement with other workers (Smith & Walker, 1980; Black *et al.*, 1981), that the majority of the resistance to photosynthesis was due to slow diffusion through the boundary layer. The boundary layers found here were approximately 1,500 μm thick, which corresponds to a flow of ≈ 2 mm s^{-1}, using equations derived from experiments with flat plates (Leyton, 1975). Flows of this velocity are at the higher end of the range found in weedbeds

growing in lakes (Losee & Wetzel, 1993). The polar-leaf mechanism was found to reduce the pH of the water within the boundary layer of the abaxial leaf surface to very low levels (< pH 6). This will catalyse the conversion of HCO_3^- to CO_2^*, which is subsequently used in photosynthesis. Although conversion of HCO_3^- must occur within the cell walls of the leaf, as suggested by Prins *et al.* (1982c), the amount of CO_2^* produced in this way will have been small when compared with that from the relatively thick (≈ 200 μm) layer of low pH water that subtended the leaf.

From the field data it was apparent that the photosynthesis of *E. nuttallii* followed a diurnal cycle, with the majority occuring in the morning. In the afternoon, photosynthesis was arrested by severe CO_2^* limitation. The high afternoon levels of oxygen will have exacerbated the low CO_2^* concentrations by increasing photorespiration, but the high oxygen concentrations alone were insufficient to halt photosynthesis. In the deeper parts of the weedbed, CO_2^* concentrations never fell as low as those found in the upper 20 cm and it is likely that light limitation predominated with increasing depth (Westlake, 1966; Madsen & Maberly, 1991). It was apparent that the *E. nuttallii* plants within the weedbed were not utilising HCO_3^- at this time. If they had been, light and temperature alone would have determined the rate of photosynthesis. The fact that flow within weedbeds is reduced to about 10% of the unaltered velocity (Madsen & Warnke, 1983; Losee & Wetzel, 1988 & 1993; Machata-Wenninger & Janauer, 1992) plays an important role in the afternoon lull of photosynthesis by allowing the establishment of harsh conditions. In a slowly-flowing system like a canal, where the potential for wave-induced mixing is low, the within-weedbed flow was likely to have been extremely small and vertical mixing was minimal (Chapter 5). Under such conditions, movement of dissolved gases will have been predominantly by diffusion and boundary layers will be large. If flow through the weedbed was greater, such extreme conditions would not establish.

7.1.2 The interactions between *E. nuttallii* and its epiphyton.

To understand the nature of the interactions between *E. nuttallii* and its epiphyton more fully, it was necessary to determine the conditions within the association on an extremely small-scale. By using the microelectrode apparatus to investigate the epiphytic communities developing on artificial plants it was possible to show that an increased layer of epiphyton did affect the flux of carbon to the "leaf" surface in two ways.

i) The diffusion boundary layer was increased by an amount equivalent to the thickness of the epiphyton layer, ie. the surface of the epiphyton replaced the leaf surface as the functional edge of the plant.

ii) Algal photosynthesis reduced the availability of CO_2^* in an exponential fashion with increasing epiphyton chlorophyll density.

These findings confirm and characterise the suggestion of Sand-Jensen (1977) that epiphyton interferes with the supply of carbon to the plant. Previous workers have found elevated oxygen concentrations within the epiphyton (Sand-Jensen *et al.*, 1985; Sand-Jensen & Revsbech, 1987; Carlton & Wetzel, 1987) and have postulated increased photorespiration. Although this will exacerbate the problem of low carbon, it is unlikely to be the main determinant of photosynthesis of submerged plants in the field.

When the effect of increasing epiphyton density on real *E. nuttallii* plants was investigated, a negative relationship was found (Figure 6.58). No irreversible changes in leaf activity occurred as a result of increased epiphyton, supporting the suggestion that bacteria do not invade the leaf cells until the leaves have started to senesce (Underwood, 1991b). The microelectode investigation showed that the epiphyton on the real plants interfered with the supply of CO_2^* through the same mechanisms as epiphyton growing on the artificial plants. It was apparent that it was the epiphyton, and not the plants, which controlled the pH at the leaf surface, highlighting the potential for increased epiphyton to detrimentally affect photosynthesis of the host plant. However, neither the pH nor the CO_2^* concentration bore a significant relationship with plant biomass. It was therefore

concluded that some other factor must have caused the decrease in plant growth, with the most likely candidate being shading by the epiphyton. It has been argued before that there is a balance between light and carbon limitation in the field (Sand-Jensen & Gordon, 1986; Madsen & Maberly, 1991; Maberly, 1993; Chapter 5), which must also apply to submerged plants covered with epiphyton (Sand-Jensen, 1977). Hence, if a higher light intensity or lower total DIC had been used, then the epiphyton would have been expected to exert its influence through carbon limitation, with the balance of influence dependent on light intensity, DIC concentration and epiphyton density. The situation is again complicated by the ability of *E. nuttallii* to take up HCO_3^- through the abaxial leaf surface and thus reduce CO_2^* limitation of photosynthesis; this will in turn favour light limitation. The ability to utilise HCO_3^-, however, is likely to switch off when the epiphyton reduces the available light sufficiently. Under such conditions the plants will be severely light limited and unable to counter epiphyton-mediated pH increases. To the plant's further disadvantage, it has been shown that the CO_2^* compensation point increases at low light intensities (Maberly, 1983), adding further to the difficulty of achieving net photosynthesis.

7.1.3 Macrophytes and the shallow aquatic ecosystem.

A high correlation between available light and maximum rooting depth of macrophytes has been found by many workers (Spence, 1982; Chambers & Kalff, 1987; Jeppesen *et al.*, 1990b; Blindow, 1992b), indicating that at low irradiances light is limiting to growth. Several experimental manipulations of the light incident on macrophytes have shown that growth can be impaired by strong shading (eg. van Dijk & van Vierssen, 1991). However, at higher irradiances there is little evidence to indicate that light is the primary determinant of growth. Naturally there will always be a balance between light and carbon limitation dependent on which is more available.

Carbon limitation of photosynthesis has previously been described for the upper parts of beds of *Potamogeton obtusifolius* Mert. & Koch growing in a lake (Maberly, 1993) and *Callitriche cophocarpa* Sendtner and *Ranunculus peltatus* Schrank growing in flowing water (Madsen & Maberly, 1991), with light limitation restricted to deeper parts of the bed and to early-morning/evening, when light is low. The data from other investigations, usually sampled from the water surface, suggest that this is true for many communities of submerged aquatic plants (Goulder, 1970; Unni 1972; Brown *et al.*, 1974; Van *et al.*, 1976; Pokorny *et al.*, 1984). The afternoon lull in photosynthesis found by many workers and ascribed by Hough (1974) to increased photorespiration, could easily be a result of carbon limitation, since the importance of increasing pH has not been fully realised. Carbon limitation of photosynthesis has been described for many species growing in the field (Adams *et al.*, 1978; Hough & Fornwall, 1988; Rattray *et al.*, 1991; Maberly, 1993), including species capable of utilising HCO_3^- (Hough & Putt, 1988; Madsen & Maberly, 1991) and has been suggested to be of utmost importance to submerged plants (Adams, 1985). However, there must be balance between carbon and light limitation, dependent on which is more restrictive. The point where light becomes more important will be very much influenced by the source of photosynthetic carbon, with those plants utilising HCO_3^- less influenced by CO_2^* availability. The effect of nutrient availability on growth can not be ignored (Raven, 1981; Hough & Putt, 1988), but access to minerals within the sediment may to a large extent reduce limitation.

Floating-leaved and emergent species are obviously not affected by carbon concentrations and light attenuation in the same way as submerged plants and although plants of this type may persist for longer than submerged species in highly eutrophic lakes, ultimately they are often lost (Lachavanne, 1982 & 1985; Balls *et al.*, 1989; Bales *et al.*, 1993). Some species have a large proportion of their photosynthetic parts submerged and must grow to the surface before breaking it, eg. *Potamogeton natans* L. and *Hippurus*

vulgaris L. Although they are often classed as floating-leaved or emergent, they can be affected by the same factors as submerged plants during the early parts of their life cycle.

One of the consequences of HCO_3^- use by the polar leaf mechanism, particularly in calcium-rich waters, is the deposition of a layer of marl (crystalline $CaCO_3$) over the adaxial leaf surface. Since the mechanism involves a pH increase at the adaxial surface greater than that driven by photosynthesis alone, marl will precipitate in this position before anywhere else. It is suggested here that the patterning of the precipitate is characteristic of plants taking up HCO_3^- by the polar leaf mechanism and is closely associated with those cells involved in the process (Plates 6 to 14). These marl deposits can build into a substantial layer and attenuate incident light (Losee & Wetzel, 1983), although it is likely that leaves utilising HCO_3^- are in a climate of excess light (see above). Other consequences of marl precipitation may be of advantage to the plant. The permanently high pH may adversely affect the growth of epiphyton, which attenuates more light than marl (Losee & Wetzel, 1983), or favour slower-growing green algae and cyanobacteria over diatoms, the latter being poor users of HCO_3^-.

Physical disruption of epiphyton growth may also occur (Plate 15). On the plant surfaces the marl will adsorb phosphate from the water column to form apatite, and, once the marl reaches the sediment, it will bind phosphate molecules and reduce recycling of phosphorus back into the water column, even under conditions of anoxia (Boström & Pettersson, 1982; Löfgren & Ryding, 1985a & b). Since internal recycling in shallow lakes often contributes greatly to the supply of phosphorus to the open water (Osborne & Phillips, 1978; Moss *et al.*, 1986), and hence to phytoplankton, a reduction in recycling would serve to stabilise plant dominance. The reduction of phosphorus concentrations in calcium-rich Lake Krankesjön, Sweden and hence stabilisation in the clear water phase (Blindow *et al.*, 1993), was probably due to adsorption onto the marl of *Chara tomentosa* L. This was reflected in the P concentration of the uncleaned plants (Blindow, 1992). It may be the case that increased nutrients favour species such as *Potamogeton pectinatus* and

Myriophyllum spicatum, which utilise HCO_3^- by mechanisms other than the creation of acid zones and therefore do not precipitate marl in the same way. One of the effects of eutrophication is the production of highly organic muds, either by increased macrophyte or phytoplankton productivity, which encourage recycling of P. A low degree of nutrient recycling confers a high degree of resistance to change on an ecosystem (Carpenter *et al.*, 1992). It is suggested that a layer of $CaCO_3$ spread over the sediment surface will reduce internal loading in hypereutrophic lakes without sediment removal and may be an engineering answer with a biological analogue. A similar technique, bringing naturally $CaCO_3$-rich sediment to the surface to cover more organic mud, has been tested with promising results on P concentration in the overlying water (Klapper, 1992). This technique should only be employed in calcium-rich waters, where plants possessing the polar-leaf or acid-alkali band mechanisms, once established, can continue to seed the sediment with marl and thus remove P from the water column.

Flow must also be very important to submerged plants in the field (Raven, 1970 & 1981). Increasing flow will not only reduce the build-up of extreme conditions by replenishing the water within the weedbed, but will also reduce the boundary layer surrounding the leaves and increase CO_2^* supply (Westlake, 1967; Smith & Walker, 1980; Black *et al.*, 1981; Madsen & Søndergaard, 1983; Søndergaard, 1988). The effect of increasing flow on those species which rely on the creation of acid zones within the boundary layer, where HCO_3^- is converted to CO_2^* (ie. some members of the Characeae, Hydrocharitaceae and Potamogetonaceae), is not known, but these species typically form beds in slow flowing environments. Field studies indicate that increasing flow, up to moderate levels, is beneficial to plant growth (Ham *et al.*, 1981; Nilsson, 1987; Boeger, 1992), but that high velocities can be detrimental (Brooker *et al.*, 1978; Nilsson, 1987; Chambers *et al.*, 1991), with plants well rooted into the sediment able to survive at higher velocities (Boeger, 1992). For the waterbodies in question, shallow lakes with large

populations of submerged macrophytes, flows would be expected to be very low (Losee & Wetzel, 1988 & 1993).

Another aspect of water movement is turbulence, which is of great importance to many non-motile phytoplankon species, as they depend largely on continual resuspension to maintain their position within the water column. The reduced flow found within weedbeds is bound to lead to the sedimentation of such algae, in the same way that it causes accumulation of inorganic material (Schiemer & Prosser, 1976; Petticrew & Kalff, 1992). This may explain the lower numbers of phytoplankton found in plant beds and sometimes attributed to allelopathy (Fitzgerald, 1969, Wium-Andersen *et al.*, 1982). Further, if submerged macrophytes occupy a large proportion of a lake, non-motile (or non-buoyant) phytoplankton populations will be severely disadvantaged, particularly if the plants extend to the water surface, since the fetch in the plant free areas will be reduced. Conversely, if plants are sparse, turbulence will be greater and the risk of uprooting increased, this effect being more pronounced in highly-productive lakes with loose, poorly structured sediment (Schiemer & Prosser, 1976; Moss & Timms, 1989). This may in part explain the loss of water-lilies once protective submerged plants have disappeared, but increased wave damage to the floating leaves will occur also. Boat-induced turbulence has been implicated in the loss of water-lilies from a eutrophic river in the Netherlands (Vermaat & de Bruyne, 1993). Once the protection given by the still water around weedbeds is lost, floating non-rooted plants such as *Lemna*, are likely to be disadvantaged by increasing wave action. A further problem for sparse populations of submerged plants is that the higher turbulence will increase resuspension of sediment and algae and worsen the light climate (Lohammar, 1966; Schiemer & Prosser, 1976; Blindow *et al.*, 1993). This population of inorganic and algal particles may then be deposited onto the surfaces of the plants, greatly increasing the rate of epiphyton development (Schiemer & Prosser, 1976; van Dijk, 1993). In the shallow, eutrophic lake Veluwe with low macrophyte

biomass, colonisation and deposition contributed more to the development of periphyton on slides than did the growth of algae (van Dijk, 1993).

Mathematical models have been used to argue the theoretical existence of alternative stable communities (May, 1977; Scheffer, 1990; Schffer *et al.*, 1993), but equal environmental conditions between the two states are required for their existence in nature, except for those parameters influenced by the resident organisms (Sousa & Connell, 1985). These alternatives should not be defined by nutrient levels and climate alone, but also by morphometry. The reduced effect of wind-induced turbulence on algal suspension and disturbance of plants could explain the restriction of plants to the dyke and not the open water of Cockshoot Broad (Moss, 1990). Differences in water level may cause the switch from plant to phytoplankton dominance through increased suspension of algae and reduced available light for the plants (Blindow, 1992; Blindow *et al.*, 1993). The survival of the water-lilies in Hudson Bay, Norfolk and not in the more open, but connected Hoveton Great Broad (Timms & Moss, 1984), could be due to less wind disturbance, with the higher zooplankton populations a product of the plants' survival and not necessarily the cause. That is not to say that these quoted examples do not represent alternative stable states, but that, when dealing with natural systems, defining "alternative" in a myriad of environmental variables is very difficult. Morphometry does appear to be of great importance in the functioning of lakes (Fee *et al.*, 1992), particularly small, shallow ones (Jeppesen *et al.*, 1990a; Scheffer *et al.*, 1993; Moss *et al.*, in press). In such lakes, plants can potentially fill the majority of the water column and maintain a clear-water state at nutrient concentrations associated with plankton dominance in larger (deeper) lakes (Jeppesen *et al.*, 1990a; Scheffer *et al.*, 1993; Moss *et al.*, in press), through a suite of stabilizing mechanisms (Figure 7.1). Once these have been lost, a catastrophic change to a phytoplankton-dominated state is likely. In agreement with other workers (Jeppesen *et al.*, 1990a; Scheffer *et al.*, 1993; Moss *et al.*, in press) it is suggested here that the principles

determining the limnology of shallow lakes should be regarded as somewhat different from those for deep lakes.

7.2 Conclusions.

In an earlier study (Birch, 1990), it was found that the fast, caulescent growth-form of *E. nuttallii* permitted it to outgrow epiphyton colonisation, keeping the most active region (the shoot tip) largely epiphyton free. This finding appears difficult to reconcile with the results reported here. The difference must lie in the rate of growth of the plant. If a plant is under favourable conditions and not severely limited by light or carbon, then rates of growth and extension will be high and epiphyton colonisation will be outgrown. However, if photosynthesis is limited by carbon and especially if the switch to HCO_3^- utilisation has occurred, then the rate of extension will be low (Chapter 4) and dense growths of epiphyton may be found in the region of maximum activity, especially in eutrophic waters where algal growth rates are not greatly retarded by nutrient supply limitations. This will set up a positive feedback serving to increase epiphyton density in this vital region. It is therefore suggested that for epiphyton to be a major cause of plant loss in the field, the growth rate of the plants must first be limited by carbon restriction. This could occur through an initial nutrient-mediated increase in productivity of the plants themselves, or of associated meta- and epiphyton, leading to increased pH levels within the weedbeds. Once the plants are thus restricted the stage is set for epiphyton to exert its direct influence (Figure 7.2). A situation where the plants are limited by CO_2^*, with a possible switch to HCO_3^- use, is most likely to occur in waters of moderately high DIC and pH, since such waters have small reserves of CO_2^* but plentiful HCO_3^-. It is from just such waters that catastrophic plant loss has been described (eg. Benson-Evans *et al.* 1967; Mason & Bryant, 1975; Jupp & Spence, 1977; Moss 1981; Hough & Fornwall, 1988; Bales *et al.* 1993). Further, those fertilisation experiments conducted in high

DIC/pH waters resulted in reduced plant growth, or plant loss, as a result of increased epiphyton (Moss, 1976; Eminson & Phillips, 1978; Daldorph & Thomas, 1991), whilst that conducted in low pH/DIC water did not (Balls *et al.*, 1989). In low DIC waters, high productivity can lead to rapid depletion of CO_2^* to levels which arrest further photosynthesis (Svedäng, 1992) but the alternative HCO_3^- reserves are not available.

The mechanism proposed here would apply to species typical of shallow eutrophic waters other than *E. nuttallii*. It is noted that the species most frequently described as being eutophication tolerant are *Potamogeton pectinatus* and *Myriophyllum spicatum*, which both acquire HCO_3^- by mechanisms other than the creation of acid zones (Salvucci & Bowes, 1983; Steemann-Nielsen, 1947) and are particularly efficient at HCO_3^- utilisation (Maberly & Spence, 1983).

Seasonal variations in the relative attenuation of light by the water column and epiphyton must also be considered. As expected, the contribution of the epiphyton to light attenuation is small when an overlying large column of water is present, but increases as the depth of overlying water is reduced, either by growth of plants towards the surface or by rooting depth (Vermaat & de Bruyne, 1993). Whilst the absolute proportions depend on the light attenuated by the water column and the thickness of the epiphyton, there are three possible ways in which epiphyton can exert its influence. Firstly, even though its contribution to total attenuance may be small, early in the growing season when light levels are lower, epiphyton can delay the onset of net photosynthesis by keeping the plants below their light compensation points until later in the year (Phillips *et al.*, 1978; Vermaat & De Bruyne, 1993). Many species can overcome this effect to some extent by extending up into the water column where light is stronger, using reserves from storage organs (tubers or turions), or by etiolation. However, the overall effect is to reduce the growing season for the plants. Secondly, epiphyton can reduce the light incident on the leaves throughout the growing season (Vermaat & De Bruyne, 1993) and reduce growth of the plant by shading and disruption of CO_2^* supply, so that weedbeds are smaller and a reduced number of

propagules are produced. Propagule size may also be reduced. The size of the tubers of *P. pectinatus* determines the initial growth of the plant (Vermaat & Hootsmans, 1991b) and the tubers are more important to overwintering than seed production (Van Wijk, 1989). Hence, the growth that can be sustained to obtain improved light conditions is lower with smaller tubers, as is the maximum rooting depth. In other species, the size of the vegetative organs varies, as does dependence on vegetative survival (Sculthorpe, 1985), but presumably a reduced standing crop of plants would lead to lower seed production. As argued above, it is probably failure to establish in substantial numbers and hence failure to establish the self-stabilising mechanisms, that causes the loss of submerged macrophytes. The third manner in which epiphyton can disrupt plant growth is by reducing the light incident on the plants throughout the growing season and hence the depth to which they colonise the lake basin (Sand-Jensen & Søndergaard, 1981; Sand-Jensen & Borum, 1984; Sand-Jensen, 1990). This applies only to isoetid plants, which cannot extend upwards through the water column to better light conditions. Extension of elodeid plants growing in deeper waters is common (Maberly, 1993).

Seasonal changes in the number of macroinvertebrate grazers such as snails and chironomid larvae (Bell, 1976; Brönmark, 1989; Botts, 1993), which serve to clear the plants of tall and filamentous epiphyton (Underwood & Thomas, 1990; Underwood *et al.*, 1992; Underwood, in press), should also be considered. Although the extent of grazing depends on the species present, there is a tendency for numbers to increase towards the autumn and epiphyton numbers to be reduced at this time (Bell, 1976).

It must be stressed at this point that the mechanism proposed here is applicable for shallow lakes only. In deep lakes with a small littoral zone, macrophytes do not have the potential to occupy the majority of the water column and thus stabilise their dominant position (Scheffer *et al.*, 1993). Under such cicumstances, phytoplankton populations can increase and adversely affect the macrophytes in the littoral, although epiphyton may play a role there also.

In conclusion, it is suggested that epiphyton can only exert its detrimental effects on the growth of elodeid plants if the growth rate of the host plant is first reduced by carbon limitation. If the plants are not limited in such a way then their growth form gives them a distinct advantage over epiphyton colonisation. But, if dense growths of epiphyton occur in the vital zone near to the shoot apex a positive feedback loop is set up and the host plant's growth is severely affected by epiphyton shading (Figure 7.2). Colonisation of the upper shoot is likely to occur in early summer when light intensities, macrophyte growth and water pH are high, turbulence is low, carbon limitation predominates, and macroinvertebrate populations not fully established. Poor growth at this time will lead to reduced input into overwintering propagules, with consequences for establishment the following year (Figure 7.2). If in any one year insufficient plants become established, with epiphyton interfering in the establishment phase also, the protective self-stabilising mechanisms will be lost and a relatively sudden switch from macrophyte to phytoplankton dominance will occur in the water body.

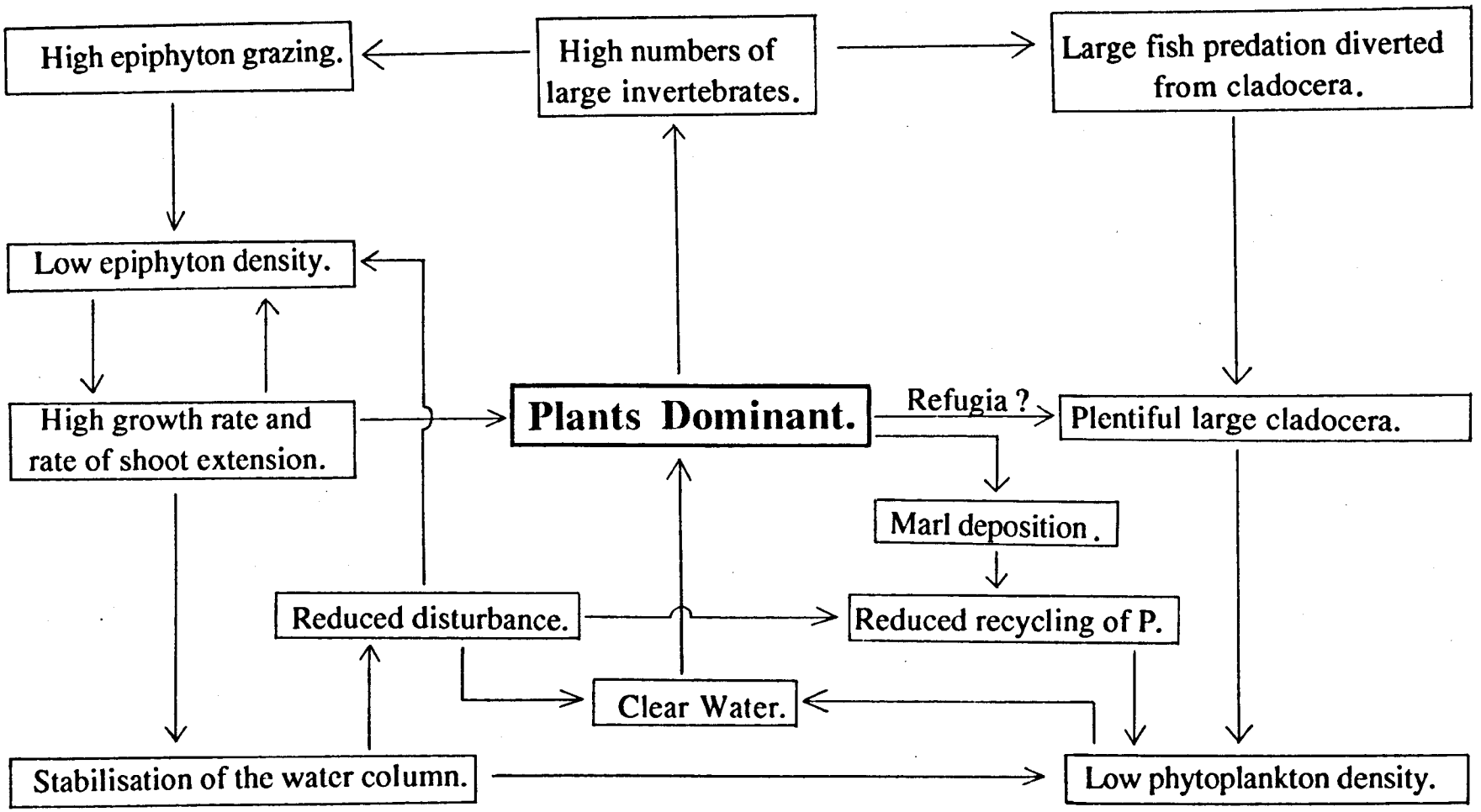


Figure 7.1 Flow diagram showing mechanisms which serve to stabilise macrophyte dominance.

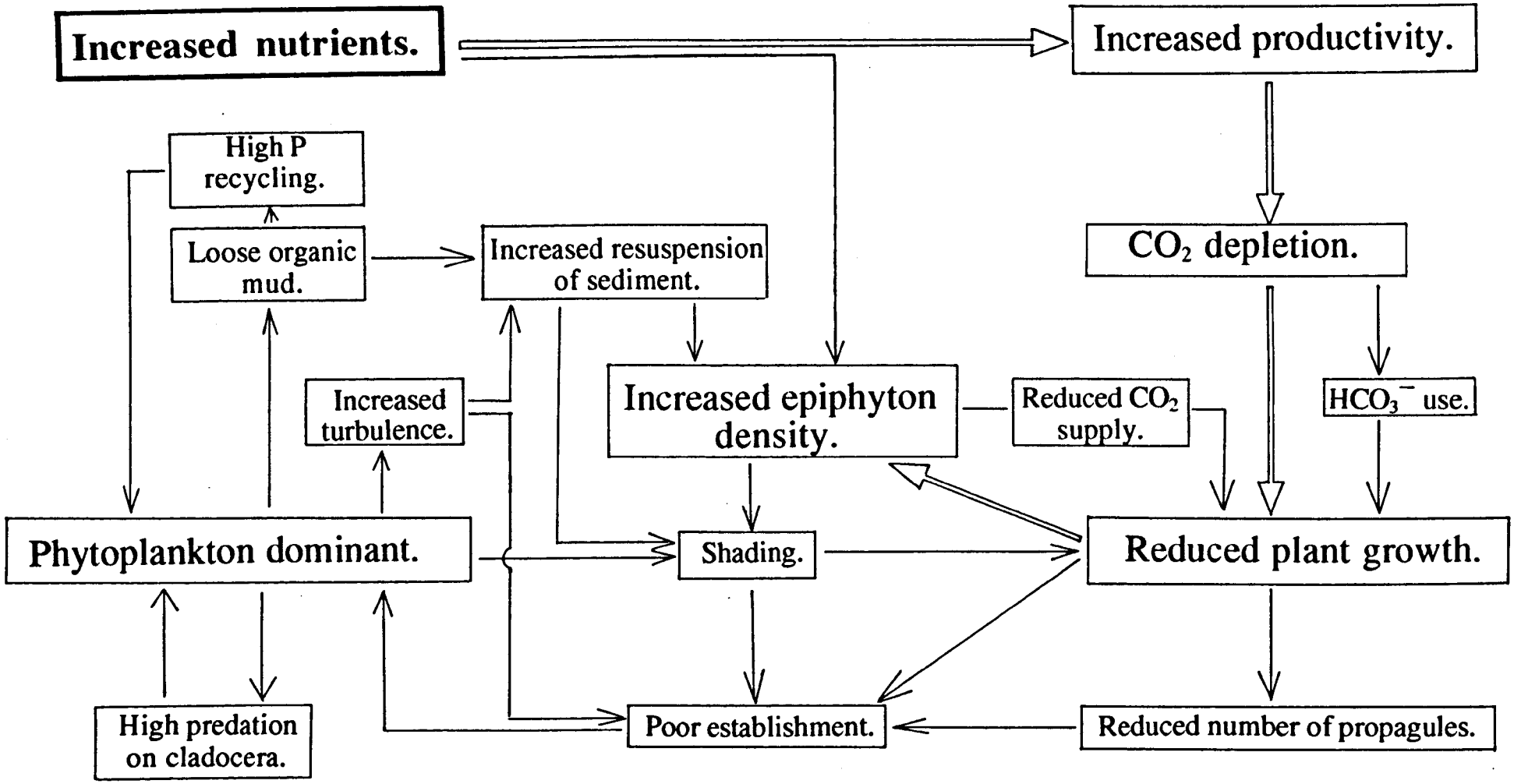


Figure 7.2 Flow diagram showing proposed mechanism whereby increased nutrient loading leads to macrophyte loss.

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Appendix 1 Growth of *E. nuttallii* in different rooting substrate.

In order to ensure good growth of *E. nuttallii* in culture, a simple test was conducted to establish the performance of the plant with different rooting substrates.

Method.

One clean, healthy shoot cut to 10 cm length was planted into one of twenty brown plastic beakers, height 7·5 cm, diameter 7 cm, filled with either canal sediment (collected as described in Section 2.1.3) or soaked John Innes Seed Compost. These beakers, totalling ten per treatment, were then placed into two glass aquaria filled with 20 l each of twice filtered canal water. The beakers, five of each treatment, were randomised in position within the aquaria. The plants were then cultured for 14 days at 20 °C and approximately 100 $\mu E\ m^{-2}\ s^{-1}$. At the end of the culture period the plants were harvested, and their total length measured.

Results and Discussion.

The mean length of the plants grown in sediment was 16·62 cm (s.e. = 1·04), and the mean for those grown in the compost was 14·28 cm (s.e. = 0·61). A t-test indicated that the difference in the total length of the plants from the two substrates was significant at the 10% level, $t = 1·941$, $p = 0·068$. At this level of significance there was a larger chance of making a type one error than is usually acceptable, ie. the rooting medium made no difference to the growth of the plants. However, as the plants rooted into canal sediment grew well and the sediment was collected from a stretch of canal where *E. nuttallii* was plentiful, it was decided to use canal sediment as a rooting medium.

Appendix 2 Diurnal changes in physiology – preliminary experiments.

Prior to the investigation described in Section 2.3.1.7, where diurnal influences on the rate of oxygen exchange were studied, two experiments were conducted on a larger time-scale.

Methods

A stock of freshly collected and cleaned plants was stored in twice filtered canal water at 20°C± 2°C, light intensity 100 μE m^{-2} s^{-1} on a 16:8 (light:dark) cycle. From this stock, samples were taken for measurements of photosynthetic and respiratory rates, as described in Section 2.2.1, over a temporal scale in the following ways.

i) Paired samples taken at various times over the whole 16 hour photoperiod, as well as one hour before, and after the period of illumination and the mean for each pair plotted against the time that the sample was taken. In this experient, all the plants were stored in a single bucket (5 l approximately).

ii) Samples, clumped into two-hour long groups, taken over the whole photoperiod, as well as two hours before the illumination period and two hours after the period. Here the plants were stored in a number of separate glass jars (capacity 2 l) and only one random sample taken from each jar.

These experiments were carried out at a time of year when day length in the field was similar to that in culture, with the time that the lights turned on and off corresponding approximately with dawn and dusk.

Results and Discussion.

The photosynthetic rates were rather low and variable in these experiments (Figures A2.2 and A2.2), indicating that the plants were not in an optimal physiological state.

Figure A2.1 The effect of time of day on the measured oxygen evolution in the light and uptake in the dark of *E. nuttallii* over a diurnal period, each point calculated from paired samples taken at that time.

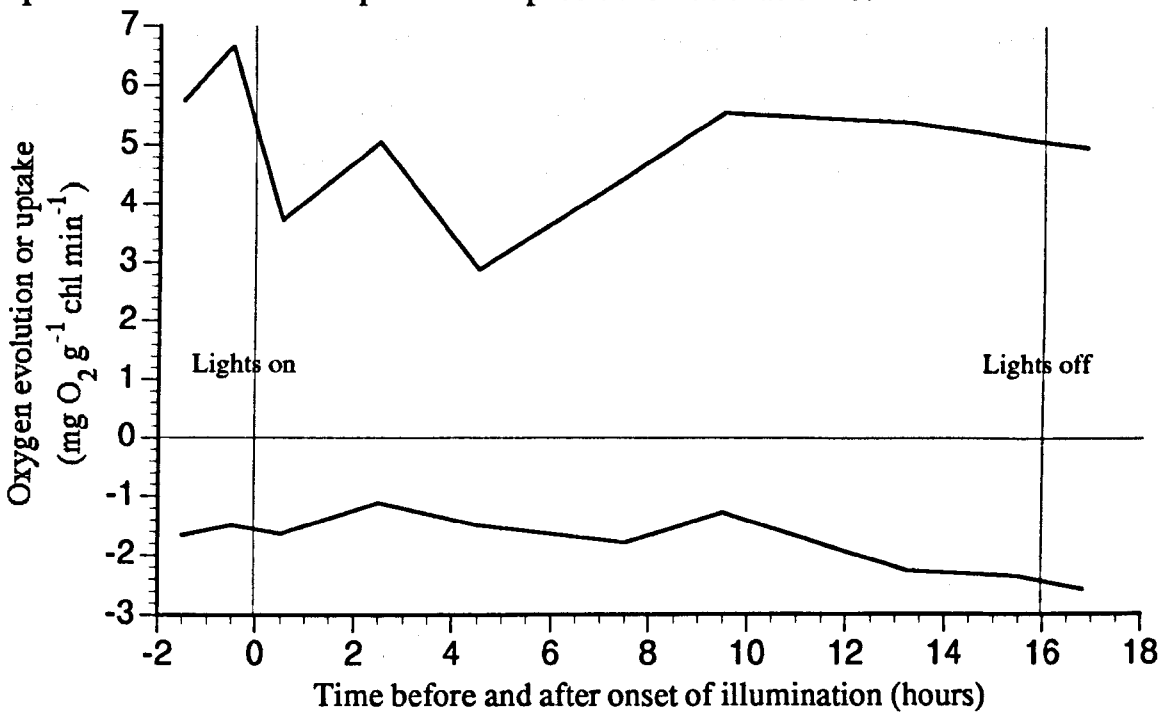
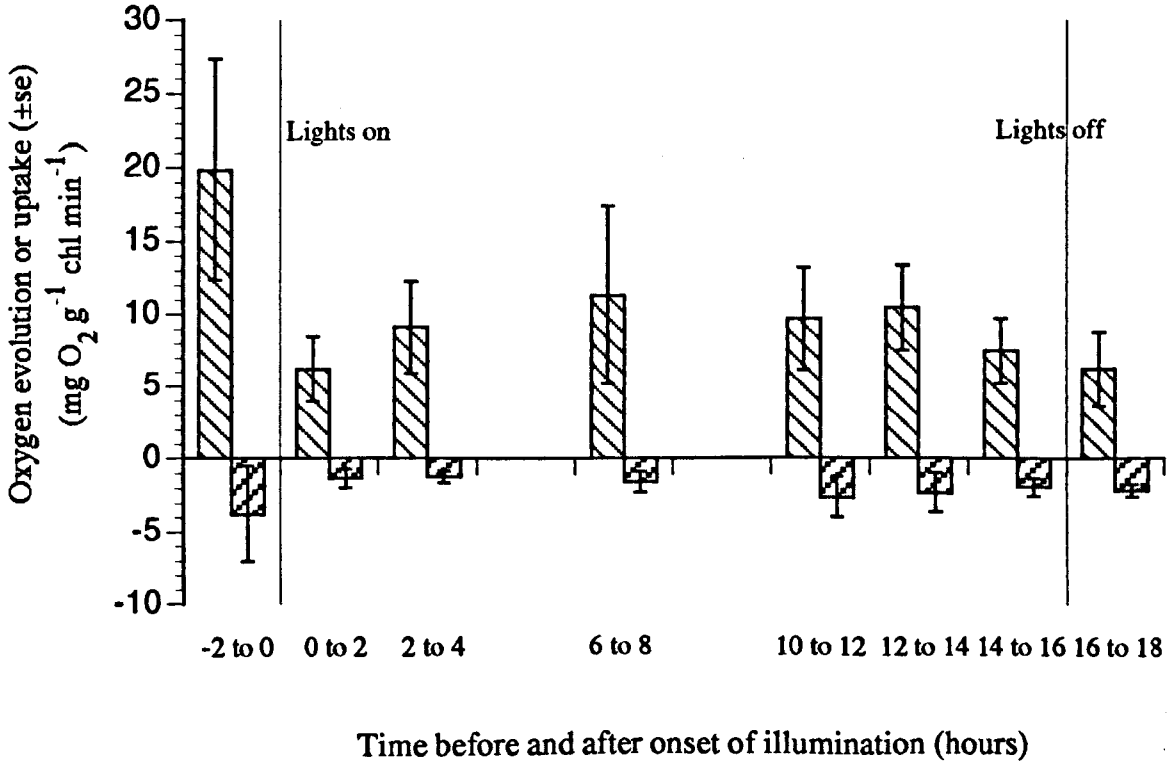


Figure A2.2 The effect of time of day on the measured oxygen evolution in the light and uptake in the dark of *E. nuttallii* over a diurnal period, each point calculated as the mean (± se) of the samples taken over that time period.



However, they appeared to show the expected pattern with rates maximal prior to the onset of illumination, and dropping during the day. A similar pattern of high rates of photosynthesis in the early morning, becoming reduced as the day progresses, has been reported from the field (Goulder, 1969; Pokorny *et al.*, 1984). The third experiment (described in Section 2.3.1.7) concentrated on this shortened time period.

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