PHOTOSYNTHESIS AND RESPIRATION IN *ELODEA CANADENSIS* MICHX.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

Philip Stanley SIMPSON

September 1981

Philip Stanley SIMPSON

PHOTOSYNTHESIS AND RESPIRATION IN ELODEA CANADENSIS MICHX

Oxygen effects on photosynthesis and respiration in detached leaves of Elodea canadensis were determined over a range of conditions which the plant experiences in shallow water. Its photosynthesis was inhibited by O_2 under all light, temperature, CO_2 and pH conditions but this was not caused solely by accelerated dark respiration. These effects were comparable to those described in terrestrial C_3 plants. The enhancement of O_2 inhibition of photosynthesis by high $^3O_2$, pH and low CO_2 suggests that similar field conditions could lead to major losses in photosynthesis and might explain some of the biomass losses under still water conditions. Compared to the filamentous algae Cladophora glomerata and Spirogyra sp., its photosynthesis was impaired, especially at high pH and low CO_2. It is thought that these algae may photosynthetically increase the pH and reduce CO_2 so that they out-compete this vascular macrophyte. Such a consideration may help to explain the reduction in E. canadensis biomass in some nutrient rich water.

Leaves of different insertion level showed distinct differences in photosynthesis, dark respiration and O_2 inhibition of photosynthesis that appeared similar to age-related phenomena in terrestrial plants. Moreover, these patterns changed with season, in a manner that might be expected if age were contributing to these differences. The reduced inhibition in summer apical leaves may be of especial relevance to the plant's carbon budget under high pH and low CO_2, since it implies reduced carbon loss through photorespiration. This is of benefit whether or not seasonal differences in photorespiration are caused by the relative youth of summer specimens or by environmental triggers.

Light saturation and compensation points of leaves were reduced in the winter and with low insertion level. Such a reduction suggests light adaptation to both seasonal and spatial changes in light availability within a weed bed. The seasonal studies revealed little evidence of physiological dormancy in E. canadensis but did indicate slight thermal adaptation of photosynthesis in the summer which might permit the plant to make use of daytime temperature rises.

The results of investigations suggest that E. canadensis is a plant that is flexible in its response to light availability yet inflexible in terms of temperature adaptation. It is also poorly adapted to competition for inorganic carbon sources. These aspects of physiology are discussed in relation to the ecology of the plant.

C O N T E N T S

1. INTRODUCTION

This thesis is concerned with the photosynthetic and respiratory physiology of Elodea canadensis, with respect to the environmental factors that may influence its performance. The aquatic habitat, despite inferences to the contrary, can impose severe limitations on a submersed plant's photosynthesis. Various factors might interact to reduce its photosynthetic performance, in some instances through photorespiration. Therefore, in the Introduction I shall outline the process of photorespiration, factors that affect it, the aquatic habitat and its possible effects on photosynthesis and respiration, as well as possible methods of amelioration of the limitations placed upon submersed aquatic plants by the environment. Finally there will be an outline of the thesis aims and a brief plan of its format and contents.

The Mechanism of Photorespiration

Photorespiration is the light dependent O_2 uptake and CO_2 evolution that occurs primarily in photosynthetic tissues of higher plants which fix CO_2 via the Calvin cycle, which is now referred to as the C_3 pathway of photosynthesis (Chollet and Ogren, 1975). It is explained metabolically by glycolate biosynthesis in the chloroplasts, followed by its metabolism in the peroxisomes and mitochondria of leaves, the total sequence being termed the glycolate pathway (Tolbert, 1971).

Various phenomena are interpreted as evidence for the presence of photorespiration. These are a high CO_2 compensation point of 40 - 50 ppm at 21% O_2, CO_2 evolution into CO_2- free air, reversible O_2 inhibition of apparent photosynthesis, and a post illumination burst of CO_2 evolution or O_2 uptake. They have been observed in plants which fix CO_2 exclusively via the Calvin cycle (Goldsworthy 1970; Jackson and Volk, 1970; Zelitch, 1971). These plants are

known as C_3 species and during photosynthesis the first step is the carboxylation of RuBP, catalysed by the enzyme RuBP carboxylase, and results in the formation of 3-PGA. The 3-C sugar thus produced is then reduced to triose phosphate and undergoes a complex series of reactions to regenerate the CO_2 acceptor and provide the net carbon fixed in photosynthesis (Bassham and Calvin, 1957).

Glycolate appears to be an early product of photosynthesis. The synthesis of this 2-C acid and photorespiration are both enhanced by O_2 and are repressed by CO_2, whilst photosynthesis is stimulated by CO_2 and inhibited by O_2 (Goldsworthy, 1970; Jackson and Volk, 1970; Zelitch, 1971). Although several mechanisms have been proposed to explain glycolate synthesis the currently favoured one is based on the fact that RuBP carboxylase also catalyses the oxidation of RuBP to P-glycolate and PGA by molecular O_2 (Andrews et al., 1973; Bowes and Ogren, 1972; Bowes et al., 1971; Lorimer et al., 1973). The P-glycolate is then converted to glycolate in the chloroplast by P-glycolate phosphatase (Richardson and Tolbert, 1961). Thus CO_2 and O_2 appear to compete for RuBP at the active site of RuBP carboxylase since O_2 is a competitive inhibitor with respect to CO_2 in the carboxylase reaction and CO_2 is a competitive inhibitor with respect ot O_2 in the oxygenase reaction (Badger and Andrews, 1974; Bowes and Ogren, 1972; Laing et al., 1973, 1974; Ogren and Bowes, 1971).

Therefore O_2 inhibition of photosynthesis (the Warburg effect), and the balance between photosynthesis and photorespiration is seemingly based upon the double enzymatic functions of RuBP carboxylase (Bowes and Ogren, 1972) and the competition between CO_2 and O_2 for RuBP which determines the relative rates of photosynthesis and photorespiration.

Thus high CO_2 and/or low O_2 promotes carboxylation and photosynthesis whilst low CO_2 and/or high O_2 favour oxygenation and therefore glycolate synthesis and photorespiration.

Once produced, the glycolate is subsequently oxidised to CO_2 via the glycolate pathway (Tolbert, 1971). Glycolate is excreted from the chloroplast into the peroxisome. Here glycolate is oxidised to glyoxylate by the enzyme glycolate oxidase which has a relatively low affinity for O_2 and required 20% O_2 in the air for rapid activity. Oxygen is utilised and H_2O_2 is also produced, which could then react nonenzymatically with glycolate to produce CO_2, formate and water. However, the peroxisome contains an excess of catalase, an enzyme which decomposes H_2O_2 to water and O_2 and thus prevents nonenzymatic oxidation of glycolate. Kisaki and Tolbert (1967) suggest that the importance of this catalase mechanism is revealed by the lack of CO_2 evolution, and glycine accumulation observed when glycolate was fed to isolated peroxisomes. The glycolate is then aminated to glycine by peroxisomal transaminases, and the glycine transferred from the peroxisome to the mitochondrion. Here 2 moles of glycine are converted to one mole each of serine and CO_2 by the enzyme serine hydroxymethyltransferase. It is this conversion which is considered to be the major source of CO_2 evolution in photorespiration. Serine can then be further metabolised in the peroxisome to produce glycerate which is phosphorylated and re-enters the Calvin cycle to be converted to sucrose (Tolbert, 1971).

Several explanations have been proposed to account for O_2 inhibition of photosynthesis. Back reactions involving molecular O_2, narcosis, photooxidation and enzyme inhibition have all been considered (Turner and Brittain, 1962; Zelitch, 1971). However, Zelitch (1971) considers

that stimulation of photorespiration is the most important factor involved in O_2 inhibition of photosynthesis.

Factors Affecting Photorespiration

Photorespiration is enhanced by increased O_2 concentration, high temperatures, high light intensities, low CO_2 concentration and high pH (Chollet and Ogren, 1975; Goldsworth, 1970; Jackson and Volk, 1970; Zelitch, 1971). In the natural situation many of these factors will of course vary together, and their effects on photorespiration may be compounded.

Dark respiration in leaves is saturated at about 2% O_2, whilst photorespiration is not saturated at 100% O_2 (Forrester et al., 1966; Jackson and Volk, 1970). All the manifestations of photorespiration i.e. the post illumination burst of CO_2, CO_2 evolution into a CO_2-free atmosphere, CO_2 compensation point and O_2 inhibition of photosynthesis are enhanced with increased O_2 (Jackson and Volk, 1970). This enhancement of photorespiration with increased O_2 is explained on the basis of two observations. The first is the production of glycolate from RuBP, which is catalysed by the enzyme RuBP carboxylase† and produces P-glycolate and 3-PGA under conditions of high O_2 and/or low CO_2 due to the competitive effect on the enzyme, as already described (Andrews et al., 1973; Bowes and Ogren, 1972; Bowes et al., 1971; Lorimer et al., 1973). The second is that the flavoprotein glycolate oxidase, which is responsible for the formation of glyoxylate, has a relatively low affinity for O_2 and requires 20% O_2 for rapid activity in vivo and is further stimulated by 60-100% O_2 (Tolbert, 1971).

Increased temperature causes a differential increase in the rate of photorespiration with respect to photosynthesis. Such an increase has

---

† because of its dual enzymatic role, it is now known as RuBP carboxylase-oxygenase.

been demonstrated for both the CO_2 compensation point (Jolliffe and Tregunna, 1968; Laing et al., 1974) and the O_2 inhibition of photosynthesis (Jolliffe and Tregunna, 1968; Ku et al., 1977). The increased ratio of photorespiration to photosynthesis has been explained by the changing kinetic properties of RuBP carboxylase with changes in temperature. At high temperature the affinity of the enzyme for CO_2 is reduced whilst its affinity for O_2 is slightly increased. Such an alteration in the relative affinities of the enzyme for CO_2 and O_2 at elevated temperatures increases the ratio of oxygenase activity relative to carboxylase activity and thereby increases the rate of photorespiration (Laing et al., 1974). It has also been suggested that the temperature effects on photorespiration could be caused by a higher activation energy for the oxygenase reaction compared to the carboxylase reaction (Badger and Andrews, 1974), but this was not found to be the case (Laing et al., 1974).

Increased CO_2 reduces photorespiration (Jolliffe and Tregunna, 1968; Forrester et al., 1966; Ku et al., 1977; Laing et al., 1974) and this is explained on the basis of competition between O_2 and CO_2 for the same site on RuBP carboxylase (Andrews et al., 1971; 1973; Ogren and Bowes, 1971; Bowes et al., 1971). Photorespiration is also increased by the increased pH of the bathing medium and this effect has been described for isolated chloroplasts (Dodd and Bidwell, 1971; Heber et al., 1976; Robinson et al., 1977). Such an increase could be explained because increased pH causes a shift in the equilibrium between CO_2 and HCO_3^- towards HCO_3^- and thus reduces the CO_2 concentration with consequent less successful competition of CO_2 with O_2 for RuBP carboxylase. Alternatively it could be explained because of the high pH optimum for the oxygenase reaction (pH 9.3-9.5) of RuBP carboxylase compared to that of the carboxylase reaction (pH 7.8) (Andrews et al., 1973).

Photorespiration is increased with increased irradiance (Jackson and Volk, 1970; Zelitch, 1971) and may be explained simply on the basis that photorespiratory glycolate is derived from RuBP. Thus light is necessary to drive the Calvin cycle to provide the precursor of glycolate. Alternatively, Andrews et al. (1973) propose that the high light stimulation of photorespiration could be dependent upon the high pH optimum of the RuBP oxygenase, the chloroplast stroma becoming increasingly alkaline with increased light as CO_2 is removed and fixed in photosynthesis.

Photosynthesis and Respiration in the Aquatic Habitat

It is thought that the aquatic environment may offer more equable conditions for plant growth than the terrestrial environment. Temperature fluctuations are much less violent than in the aerial environment (Sculthorpe, 1967), and submersion in water often provides protection from freezing during the winter. However, the relatively high winter temperatures experienced may allow respiration to exceed photosynthesis, while the relatively low summer temperatures may retard growth (Westlake, 1971). The potential light intensity experienced by the plant may be considerably reduced in the aquatic environment because reflection removes approximately 10% of the incident light, light is attenuated by the water itself, and further reduced by suspended and dissolved matter (Sculthorpe, 1967; Westlake, 1971). In addition the light actually reaching the photosynthetic tissue of a submerged plant can be reduced by epiphyte encrustations (Sand-Jensen, 1977), silt or marl deposition (Wetzel, 1960). Self shading may also be important in dense weed beds (Sculthorpe, 1967).

It has also been suggested that photosynthetic gaseous exchange is more favourable in water than on land because CO_2 is more soluble in water than either O_2 or N_2, and often forms over 1% of the volume of

gas dissolved in water compared with only about 0.03% in the atmosphere (Hutchinson, 1957; Westlake, 1971). In addition, CO_2 is supplemented by HCO_3^-, often in substantial amounts in most natural waters. However, the coefficients of molecular diffusion are about 10,000 times slower in water than in air (Hutchinson, 1975; Westlake, 1971) and there is always a stagnant (or near stagnant) layer of water around the leaf which may offer considerable resistance to the uptake of CO_2 and/or HCO_3^-, and the release of photosynthetically produced O_2. The thickness of the boundary layer depends upon the rate of water flow (Smith, 1975), and increased flow rate does increase photosynthesis of aquatic plants, presumably in part at least as a result of a decreased boundary layer (Westlake, 1967).

The water surrounding photosynthesising aquatic plants may be subject to rapid fluctuations in quality, as a result of photosynthesis. This will be especially so in dense weed beds when surface irradiance is high. Photosynthetically induced changes include supersaturation of the water with O_2, reduced availability of CO_2, and HCO_3^-, and increased pH (Bamforth, 1962; Brown et al., 1974; Dale, 1957; Ganf, 1974; Goulder, 1970; Moss, 1969; Van et al., 1976; Westlake, 1971). In addition the temperature of the water surrounding submersed plants can be raised relative to that outside the weed bed due to enhanced absorption of radiant energy and subsequent retarded dissipation of the energy due to restricted water movement within the plant stand (Dale, 1957; Dale and Gillespie; 1977). All of the above conditions enhance photorespiration (Goldsworthy, 1970; Jackson and Volk, 1970; Zelitch, 1971), and may be expected to do so in aquatic plants that exhibit this phenomenon.

Recent investigations have established the presence of photorespiration and related O_2 effects on photosynthesis and CO_2 compensation points in a number of aquatic plants (Brown et al., 1974; Bowes et al., 1977a; Helder et al., 1974; Hough, 1974, 1976; Hough and Wetzel, 1972, 1978; Jana and Choudhuri, 1979; Kutyurin et al., 1964; Prins and Wolff, 1974; Søndergaard, 1979; Tolbert and Osmond, 1976; Van et al., 1976) although some of the photorespiratory effects reported are not as strong as those expected from typical C_3 plants. To date all the species investigated have been found to have low photosynthetic rates (about 1 to 5% that of terrestrial plants) and their photosynthesis is inhibited by O_2. Despite low photosynthetic rates, submersed vegetation is capable of rapid growth, probably because relatively little of their photosynthate is required for support compared to terrestrial plants. This is reflected by the low dry weight to fresh weight ratio of submersed vegetation. Photorespiratory CO_2 release has been shown to be low in Hydrilla verticillata (Bowes et al., 1977b) and Najas flexilis (Hough, 1974). Hough (1974) suggests that submersed plants may photorespire less than terrestrial ones because they are generally exposed to lower O_2 concentrations, light and temperature. Alternatively he suggests that photorespiration may be higher than that actually measured but high diffusive resistance to photorespiratory CO_2 release and retention of some of this CO_2 in gas lacunae may permit extensive refixation and thereby underestimate the true magnitude of photorespiration. It is also thought that some aquatic plants are able to adapt their photosynthetic physiology by changing the proportions of photosynthetic and photorespiratory enzymes in response to low CO_2 conditions, and thereby reduce photorespiratory loss (Bowes et al., 1977a)

Carbon dioxide compensation points of aquatic plants, taken as a group, show wide variation from 0 in Myriophyllum spicatum (Stanley and Naylor, 1972) to 85 µl CO_2 l^{-1} in Egeria densa under 21% O_2 (Brown et al., 1974). There would also appear to be intraspecific variations in CO_2 compensation point. E. canadensis has been shown to have a low O_2- insensitive, CO_2 compensation point (Hough, 1979) and the results of Brown et al. (1974) have also been quoted as evidence that this species has a low compensation point (Benedict, 1978; Hough, 1979). However, examination of their data reveal that this is not actually the case. Whilst E. canadensis did indeed have the lowest CO_2 compensation point of the three species investigated, at 21% O_2 its CO_2 compensation point was approximately 30 ppm. This is much greater than the compensation point of < 5-10 ppm characteristic of C_4 plants, but more akin to the C_3 plants whose compensation point is commonly within the range 40-50 ppm (Jackson and Volk, 1970). In addition, Brown et al. (1974) found that the CO_2 compensation point was O_2-sensitive and only reduced to zero at 0.6% O_2.

Despite some of these uncertainties about both the nature and extent of photorespiration in aquatic macrophytes there is no doubt that it can be a factor of potential importance in aquatic plant productivity. An afternoon depression of photosynthesis is often seen in submersed aquatic plants and has been described for H. verticillata (Van et al., 1976), N. flexilis (Hough, 1974) and E. canadensis (Hartman and Brown, 1967; Hough, 1979). It has been argued that as the day progresses there will be an increase in light intensity, O_2 concentration and possibly a decrease in CO_2 availability which could together enhance photorespiration and hence explain the afternoon depression of photosynthesis (Hough, 1974).

Dale (1957) describes the effect of environment on the growth of E. canadensis. Specimens from shallow water under strong light had a characteristically crowded appearance at the terminal portions of the shoot due to shortened internodes, and leaf length was also restricted. He explains that the surface of the water was choked with strong growth, the water circulated little, was saturated with O_2 and up to 8°C warmer than the surrounding water. With hindsight it is known that these conditions stimulate photorespiration and could help explain the observed retarded growth which Dale observed.

Aspects of Physiology and Anatomy that could Ameliorate the Effects of Photorespiration

There are a number of ways in which the restrictions placed upon the plant by photorespiration and its associated O_2 effects on photosynthesis might be reduced. These are 1. Active C_4 metabolism, which has been suggested by high ^{14}C fixation into C_4 acids (Benedict and Scott, 1976; Brown et al., 1974; De Groote and Kennedy, 1977). 2. Adapation to reduced CO_2 conditions by altering levels of photosynthetic enzymes, thereby reducing photorespiration (Bowes et al., 1977a, Salvucci et al., 1979). 3. Active HCO_3^- utilization as an inorganic carbon source for photosynthesis through carbonic anhydrase activity (Sculthorpe, 1967; Steemann Nielsen, 1960). 4. Gas movement and storage within the lacunar system (Raven, 1970).

1. Active C_4 Metabolism

On the basis of radioactive isotope studies it appears that RuBP carboxylase and the Calvin cycle account for most of the inorganic carbon entering aquatic plants (Andrews and Abel, 1979; Browse et al., 1977, 1979; Hough, 1974: Stanley and Naylor, 1972; Winter, 1978). In contrast, there are some reports of heavy labelling into malate (Benedict and Scott, 1976; Brown et al., 1974; De Groote and Kennedy, 1977) and this had led to suggestions that aquatic plants may be intermediate

with respect to photosynthesis between the high photorespiratory C_3 plants and low photorespiratory C_4 plants. However, whilst preliminary studies on Eg. densa indicated high ^{14}C fixation into C_4- acids (Brown et al., 1974), when experiments were repeated at natural CO_2 concentrations the distribution of ^{14}C fixed into PGA, sugar phosphates and sucrose indicated that the Calvin cycle was the primary carboxylation mechanism (Browse et al., 1977). This suggested that the previously reported high ^{14}C fixation into malate might have been induced by very low experimental CO_2 concentrations with consequent suppression of Calvin cycle activity.

In addition, pulse-chase experiments on E. canadensis indicated that whilst a ^{14}C exposure period of 2 seconds resulted in 45% of the label in C_4- acids, there was little subsequent turnover of this label (De Groote and Kennedy, 1977). A similar absence of turnover of ^{14}C in the malate pool of Eg. densa has also been reported (Browse et al., 1980). The lack of turnover in both these species suggests that little of the organic acid produced was involved in a C_4- type of carbon supply to the Calvin cycle. Indeed, Browse et al. (1980) propose that malate synthesis in Eg. densa is not related to the synthetic or energy fixing roles of photosynthesis but may be required to balance excess cation uptake in the light.

A major diagnostic feature of all plants with the C_4 pathway of photosynthesis is the 'Kranz' leaf anatomy. In C_4 plants such as maize, the vascular tissue is surrounded by a concentric layer of large bundle sheath cells containing numerous starch filled chloroplasts. This layer is in turn surrounded by more rings of mesophyll cells (Chollet and Ogren, 1975). The mesophyll cells initially fix CO_2 into C_4- acids and these are then transported to the bundle sheath cells where they are

decarboxylated and the CO_2 fixed in to RuBP via the Calvin cycle. This typical C_4 system in its complete anatomical-metabolic form has not evolved in any aquatic vascular plants (including E. canadensis) tested to date (Hough and Wetzel, 1977).

2. Adaptations of Physiology

Under certain conditions some submersed aquatic plants show a reduced CO_2 compensation point and altered levels of photosynthetic enzymes (Bowes et al., 1979). The reduced CO_2 compensation point, which was correlated with an increase in the proportion of PEP carboxylase relative to RuBP carboxylase, as reported by Bowes and co-workers in summer samples of H. verticillata, was apparently linked to triggers such as increased daylength, elevated temperature and reduced CO_2 availability. Similarly, reduced levels of photorespiration have been reported in summer samples of N. flexilis (Hough, 1974) and E. canadensis (Søndergaard, 1979). These lower levels in the summer compared to winter or autumnal ones may not necessarily be related to environmental triggers, but could be indirect and caused by the relative age of the samples. However, reduced photorespiration, whether induced by the environment or age, would still be advantageous to the plant in still water conditions.

Aging effects on photosynthetic and photorespiratory processes are well documented in terrestrial plants (e.g. Catsky et al., 1976; Hodgkinson, 1974; Kisaki et al., 1973). They have also been inferred in aquatic plants from observations on seasonal variation in photorespiration (Hough, 1974), and demonstrated for leaves of different age in Vallisneria spiralis (Jana and Choudhuri, 1979).

3. HCO_3^- Utilization

Active HCO_3^- utilization in aquatic plants may give the plant a competitive advantage in alkaline waters (Hutchinson, 1957, 1975; Sculthorpe, 1967). It may also help to ameliorate the effects of reduced CO_2 supply at high pH in photorespiring plants by acting as a 'CO_2- pump' to increase the CO_2 concentration at the site of carboxylation thereby enabling better competition of CO_2 for RuBP carboxylase. Whilst many aquatic plants are reputedly able to utilise HCO_3^- as a carbon source in photosynthesis (Sculthorpe, 1967; Steemann Nielsen, 1960), the evidence for this ability is equivocal since most investigations were based upon the plants ability to raise the pH of the water in unbuffered media. It was assumed that if the plant could raise the pH of the medium by photosynthesis, to a pH level at which free CO_2 concentration was negligible, then HCO_3^- was being utilized. However, pH rises in themselves cannot indicate either photosynthesis (Thomas and Tregunna, 1967) or HCO_3^- utilization (Raven, 1970). Indeed, other investigations have indicated that E. canadensis, Eg. densa and Lagarosiphon major are unable to use HCO_3^- (Brown et al., 1974). Carr (1969) found this ion practically useless to Ceratophyllum demersum. Van et al. (1976) reinvestigated the active species of CO_2 used in photosynthesis in aquatic plants. They found that at saturating free CO_2 at pH 4 and pH 8 there was no indication of HCO_3^- use. At subsaturating free CO_2, the photosynthetic rate was higher at pH 8 than at pH 4. This type of enhancement would normally be taken to indicate HCO_3^- use, but it could also result from increased affinity of the carboxylating enzyme for CO_2 at high pH and HCO_3^- concentrations (Bowes et al., 1975).

4. Gas Movements and Storage in the Lacunar System

Gas lacunae are of widespread occurrence in aquatic macrophytes (Sculthorpe, 1967) and it is thought that gas movement and storage within the lacunar system could help with problems of restricted CO_2 and O_2 exchange (Raven, 1970). In terrestrial plants, photosynthetically produced O_2 diffuses into the atmosphere, whilst in submersed plants there is a tendency for this gas to preferentially diffuse into the intercellular lacunae because of the greater resistance to diffusion of gases in water (e.g. Hough, 1974; Hough and Wetzel, 1972; Sculthorpe, 1967). Sculthorpe (1967) reports that the rate of downward diffusion is governed by the steepness of the concentration gradient and the frictional resistance of the diaphragms, but suggests that, on average, the rate is probably about 4% of that in air. However, this figure is based on the rate of diffusion in the rhizome of Menyanthes trifoliata, as measured by Coult (1964) and thus the validity of extrapolating to all aquatic vascular plants is doubtful. None the less, photosynthetically evolved oxygen is probably made available to respiring root tissues as these may be in anaerobic sediments (Arber, 1920; Sculthorpe, 1967).

In a similar manner, respiratory CO_2 probably also diffuses through the lacunal system and becomes available to photosynthesising tissue. Indeed, both Lobelia dortmanna (Wium-Anderson, 1971) and Litorella uniflora (Sǿndergaard and Sand-Jensen, 1979) are able to photosynthesise $^{14}CO_2$ fed to their roots. However, whilst gas lacunae are present in E. canadensis, the presence of a two cell thick diaphragm at each node (Figure 1.1) may make its morphology unfavourable for rapid gas transport. As a consequence CO_2 and O_2 are unlikely to diffuse through the lacunae in sufficient quantities to significantly affect the photosynthetic performance of the plant (J.W. Eaton, personal communication).

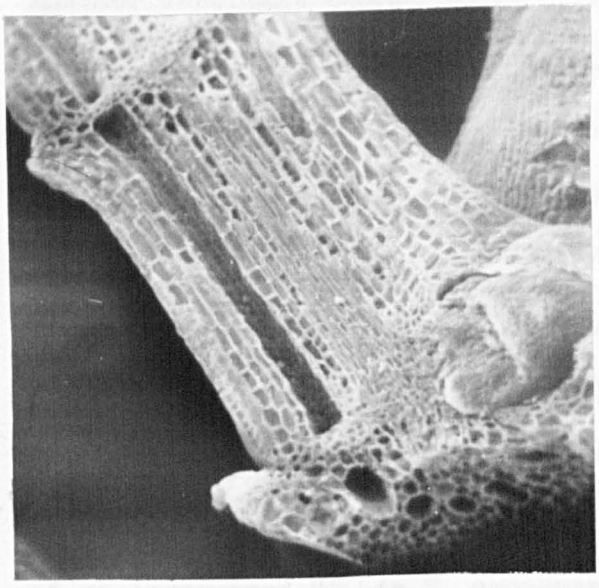
Figure 1.1 Gas lacunae in E. canadensis.

a. and b. Longitudinal section of stem with gas channels interrupted by a multicellular diaphragm, x45 and x90 respectively.

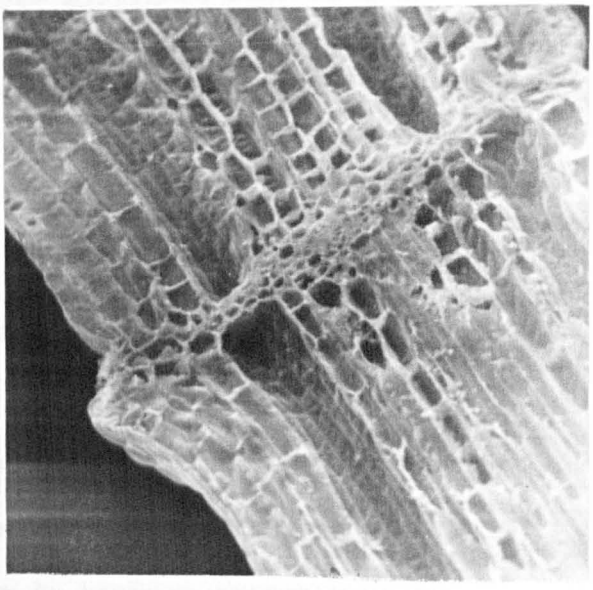
c. Transverse section of stem with gas channels, x90.

d. and e. Transverse section of stem in nodal region showing the surface of the diaphragm. Note the air spaces at the corners of the cells, x350 and x800 respectively.

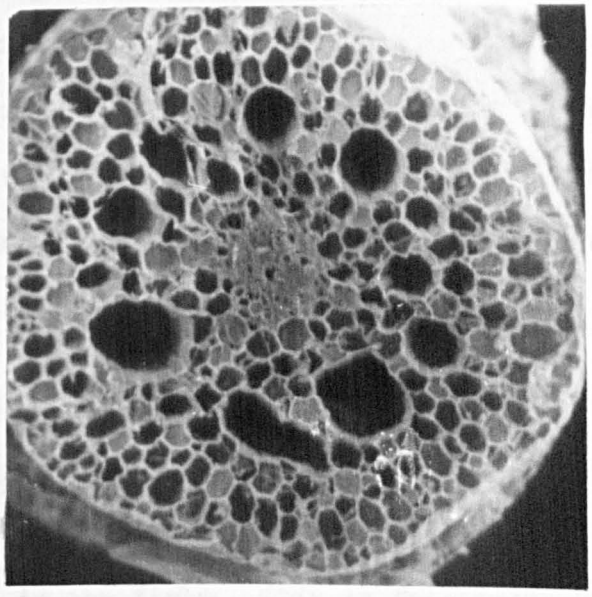
Sections were cut under water and left standing in distilled water for approximately 2 hours to help remove cell contents. They were then dehydrated by immersion for successive 30 minute periods in acetone/water mixtures containing 20, 40, 60, 80, 90, 100 and 100% acetone. Sections were critical point dried (Polaron E3000 Critical Point Drying Apparatus using liquid CO_2) and glued onto a stub with Durafix. The specimens were coated with 60% gold/palladium (NGN. SG-2, 12 inch Coating Unit) and viewed with a Cambridge Stereoscan Mk. IIA Electron Microscope using an accelerating voltage of 20 kv.



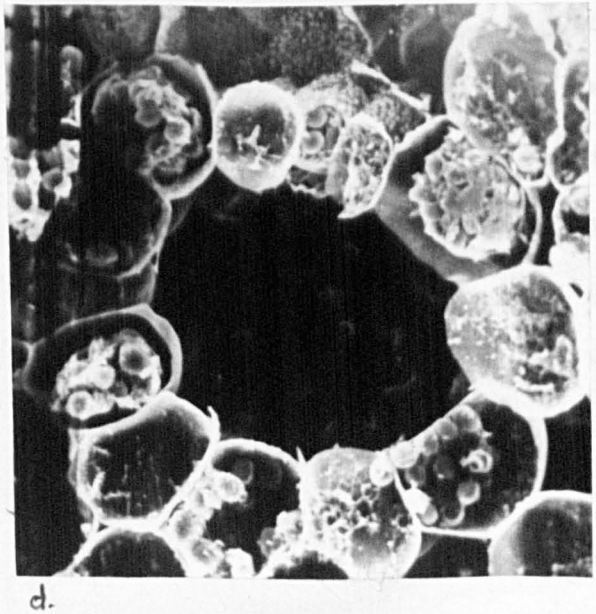
a.



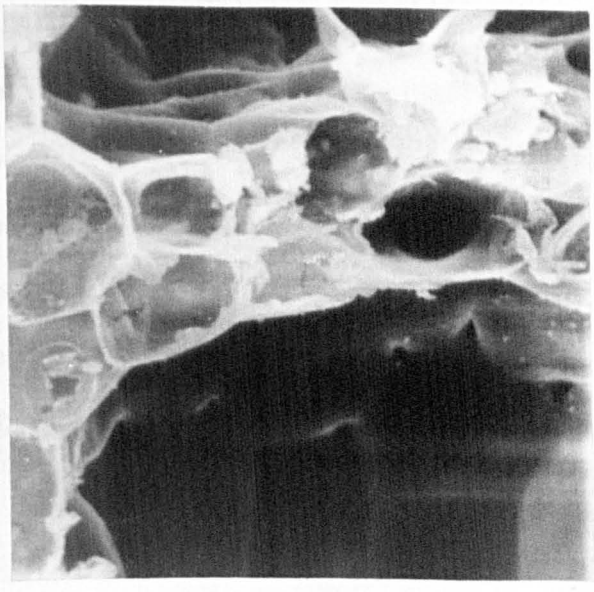
b.



c.



d.



e.

Another important aspect of the lacunar system is that it might allow refixation of photorespired CO_2. The low CO_2 evolution in the light by aquatic plants has been explained by effective refixation of photorespired CO_2 due to the gas filled lacunar system (Hough, 1974; Hough and Wetzel, 1972, 1978; Søndergaard, 1979). It is thought that 50-67% of the photorespired CO_2 may be refixed in N. flexilis (Hough, 1974) and 50-70% in L. dortmanna and Li. uniflora (Søndergaard, 1979). These species contain large lacunae which run the length of the leaves and allow extensive refixation. Refixation of photorespired CO_2 in E. canadensis is however probably minor because this species has relatively few, small gas lacunae in the leaves which are only two cell layers thick (Søndergaard, 1979). However, whether the photorespired CO_2 is lost from the plant or recycled, considerable photosynthetic assimilatory power is lost during photorespiration.

Leaving aside the possible ways in which the plant can ameliorate the effects of low CO_2 conditions on its photosynthetic metabolism, laboratory measurements may over estimate photosynthetic performance of aquatic plants in the field. This is especially so with rapid flow rates of rapidly stirred conditions which reduce the thickness of boundary layers and thereby enhance exchange. Westlake (1967) found that increased flow rate caused a great increase in photosynthesis of aquatic plants. Therefore it seems likely that laboratory experiments would over estimate photosynthesis and possibly underestimate photorespiration in the field because of the artificially favourable degree of water movement occurring in the laboratory apparatus, as compared with the field.

While the morphology of E. canadensis is probably not conducive to rapid gas transport and refixation of respired CO_2 (see above), excised leaves were used to circumvent these, probably minor effects. Removal of leaves in this way probably did not reduce photosynthetic rates since they are comparable to those found by Van et al., (1976). It would appear that the rates found by these workers are the highest reported for submersed angiosperms.

Aims of Research

Over a period of several years, summer growths of E. canadensis in the Leeds and Liverpool Canal have been observed in many cases to be followed by sudden drastic reductions in biomass (J. W. Eaton, personal communication). During such reduction, tissue analysis suggested no evidence of N or P deficiency, the water was clear, the main growth zone of the weed beds was in the top 0.5m of the canal and therefore the growing tips were receiving abundant light. Water conditions changed markedly and on warm, sunny days O_2 concentration was 150-200% saturation, pH 9-10 and the temperature up to 5°C higher than the open water so that the temperature experienced by the plant was a maximum of 25°C. Since these conditions favour photorespiration in terrestrial plants, and this process has been suggested as a possible cause of the afternoon depression of photosynthesis in aquatic plants (Hough, 1974), one of the aims was to determine whether such conditions could possibly help explain the observed biomass decrease in E. canadensis under still conditions.

Another change noted in the Leeds and Liverpool Canal in recent years has been the gradual replacement of E. canadensis, as the dominant submersed plant, by the filamentous alga Cladophora glomerata in sections

receiving nutrient rich River Douglas water. Reductions in macrophyte biomass and even complete replacement by filamentous and planktonic algae have been observed elsewhere. This replacement has been attributed to algal shading of the macrophytes to below light compensation with consequent suppression and decline in growth (Phillips et al., 1978). Thus another aim was to determine the relative photosynthetic performance of E. canadensis, with respect to its filamentous algal competitors, in relation to light and other environmental factors that affect photosynthesis.

While seasonal fluctuations in the aquatic environment may not be as severe as in the terrestrial one (see Introduction Section 'Photosynthesis and respiration in the aquatic habitat') there is never the less significant variation in environmental conditions that could affect photosynthesis and respiration. In addition, some macrophytes evidently exhibit seasonal and age related effects on their photosynthetic metabolism. They might also be expected to show other adaptations to their environment e.g. to the light conditions experienced within a weed bed, which will be reduced compared to that experienced by the apex because of self shading, epiphytes and silt deposition. For these reasons, the effect of season was determined for leaves of different age to ascertain how such factors might affect the photosynthetic performance of E. canadensis.

Thus work was undertaken to determine the environmental effects on the photosynthesis of E. canadensis, and specifically to relate these effects to its ecology and weed bed conditions. In this context, it is important to include comparisons between the performance of this macrophyte and its algal competitors, especially as these appear to be linked with macrophyte decline in some sections of the canal.

For the sake of clarity, the thesis has been divided into several sections, each of which is written as a self-contained paper. Each covers a different aspect of the photosynthetic and respiratory physiology of E. canadensis.

The results of some preliminary method testing are given in Section 2, with particular reference to the effects of laboratory holding conditions on the physiology of E. canadensis. It is considered particularly vital to test these to avoid the introduction of experimentally induced artefacts that could invalidate extrapolation to the field situation.

In Section 3 the effect of environmental factors on the photosynthetic and respiratory performance of E. canadensis leaves are described. Major consideration is given to the photosynthetically induced changes of the water surrounding aquatic plants e.g. supersaturation of the water with O_2, reduced availability of CO_2 and HCO_3^- and increased pH. Such conditions drastically reduce photosynthesis in this plant and enhance photorespiration, as indicated by the O_2 inhibition of apparent photosynthesis. Results are discussed in relation to the possibility that this may help to explain the reduction in biomass of this plant under still water conditions.

The effect of leaf position on the photosynthetic and respiratory metabolism of E. canadensis is described in Section 4. As distance from the apex increases, the light saturation and compensation points declines. This is probably the result of adaptation to available light, but may reflect the light availability at the time of leaf formation and development. The patterns of change in apparent photosynthesis, dark respiration and O_2 inhibition of photosynthesis with leaf position are

characteristic of age-related effects on the photosynthesis of terrestrial plants, but are different depending upon whether the rate is expressed per unit chlorophyll or per unit leaf area.

In the light of these results, investigations into seasonal variation of photosynthesis and respiration were performed on leaves of known insertion level. Section 5 describes the seasonal variation in photosynthesis and respiration of 1cm and 6cm excised leaves to light, temperature and O_2. It also compares glycolate oxidase activity, a key photorespiratory enzyme, of these leaves with time of year. The results show that during the summer light saturated photosynthesis, light saturation and compensation points were highest. There was no difference in the temperature optimum for either photosynthesis or dark respiration in winter and summer specimens. In contrast, O_2 inhibition of apparent photosynthesis and glycolate oxidase activity was least in the summer. This suggests that there may be some amelioration of photorespiratory loss in the summer, at a time when this process would otherwise proceed at a high rate.

The results of a study of the inter-relationship between season and leaf insertion level on irradiance response and O_2 inhibition of photosynthesis are given in Section 6. All leaves showed a reduction in light saturation and compensation points in the winter and this probably resulted from adaptation to seasonal insolation. Oxygen inhibition of photosynthesis exhibited a general trend of increased magnitude to a level at a certain distance below the apex and then declined to the oldest leaves. However, the peak of inhibition was closest to the apex in winter leaves and may reflect the relatively greater age of these leaves in winter compared to corresponding summer ones.

Considering the severe limitation placed upon the photosynthesis of E. canadensis by high O_2, low CO_2 and high pH conditions (Section 3), the photosynthesis of this macrophyte is compared with that of the filamentous algae C. glomerata and Spirogyra sp. in Section 7. These two algae are often found intermingled with E. canadensis and have been implicated in the decline of other macrophytes. Results show that both these algae photosynthesise more effectively than E. canadensis under high O_2, high pH and low CO_2 conditions. This suggests that these algae may be responsible for the decline of E. canadensis in some stretches of the Leeds and Liverpool Canal, because their photosynthesis could alter water conditions so much that E. canadensis is out-competed.

While in the field situation there will be undoubted interaction between environmental factors, age and leaf position, it is necessary to separate out these effects in the thesis. However, I shall attempt to draw together this interplay of factors in the final discussion (Section 8) and relate the physiology of this macrophyte to its ecology.

In this section, the results of some preliminary investigations and method testing are described. These investigations were performed to eliminate the introduction of experimentally induced artefacts which could otherwise lead to misinterpretations and invalidate extrapolation to the field situation. The results of investigations are used to substantiate the adoption of standard methods and treatment of the plant material.

2. SOME NOTES ON PRELIMINARY INVESTIGATIONS INTO THE PHOTOSYNTHESIS OF ELODEA CANADENSIS

ABSTRACT

The effect of various laboratory holding conditions and experimental techniques on O_2 exchange characteristics of Elodea canadensis Michx. were investigated. The results indicate that the conditions and techniques employed did not significantly affect subsequent recorded rates.

There was no evidence of substantial bicarbonate use for photosynthesis in E. canadensis, despite earlier claims that this species is able to use it. In contrast, there was a distinct preference for CO_2 and photosynthesis was drastically reduced with increased pH. These results are discussed in the light of previous claims.

INTRODUCTION

Laboratory holding conditions may affect the metabolism of aquatic plants. Brown et al. (1974) noted a rapid decline in photosynthesis of Elodea canadensis Michx. after collection from the field and recently Jana and Choudhuri (1979) found that photosynthetic rates of Vallisneria spiralis were significantly affected because of premature aging of plant material.

Whilst E. canadensis was collected fresh each week during the course of this present study it was considered necessary to test the effects of various laboratory holding conditions and experimental techniques on the O_2 exchange characteristics of this species. The aim of this was to eliminate or reduce experimental artefacts that could lead to misinterpretation. The factors investigated were epiphyte removal, leaf number, time from collection and leaf excision, temperature preincubation period, holding temperature, diurnal variation, buffer type and its concentration.

Bicarbonate utilization is assumed to occur in E. canadensis (Sculthorpe, 1967; Steemann Nielsen, 1960), yet recent studies suggest that this ability is very limited (Brown et al., 1974). Therefore the results of investigations into the effect of pH on photosynthesis and the possible role of HCO_3^- in photosynthesis are also included since its utilization may confer a competitive advantage to an aquatic plant in neutral or alkaline waters (Hutchinson, 1957; 1975; Sculthorpe, 1967).

MATERIALS AND METHODS

Plant Material

Samples of E. canadensis were collected from the Leeds and Liverpool Canal, near Aintree, Merseyside (O.S. 393990), by grapnel at unshaded positions from clear, unpolluted water of average depth 1m. Boat movements in this section averaged 1-2 per day in summer and less than 1 per day in winter. Where possible only undamaged mainstems were collected. In all instances only stems growing vertically in the water column were collected. These were transported to the laboratory in canal water where they were immediately placed in large glass tanks. They were maintained in the laboratory in canal water at 15°C under a 16 hour photoperiod, 54 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance. Oxygen exchange rates were measured within 4 days of collection.

Photosynthetic and Respiratory Rate Determination

Unless otherwise indicated photosynthetic and respiratory rates of E. canadensis were measured as O_2 evolution in the light or O_2 uptake in the dark, at 20°C using a Clarke-type O_2 electrode, Rank Brothers, Cambridge. Temperature was controlled using a Churchill cooler/heater unit. The reaction vessel was illuminated with a 250W projector bulb to saturating incident light of 290 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance, measured by Lambda meter.

The three leaves of a whorl were excised by gently pulling them from the stem using forceps. They were placed directly in the oxygen electrode in $3cm^3$ of Forsberg medium II (Forsberg, 1965), modified by omission of Na_2SiO_3 and carbon sources. Buffer components were varied to cover the required pH range and were normally 50 mM NaCitrate-NaH_2PO_4 for pH 5 to 6, and 50 mM Tris-HCl for pH 7 to 9. The buffers Tris-HNO_3, Tris-H_2SO_4, Hepes, Tricine and Bes were also employed at pH 7.5 to investigate possible depression of oxygen exchange rates caused by the buffer solution usually employed. Photosynthesis was initiated by injection of $0.1cm^3$ of $NaHCO_3$ solution to give a final concentration of 2.4 mM HCO_3^- which approximated to that present in the canal water. In experiments at higher HCO_3^- concentrations, the buffering capacity of the added HCO_3^- was compensated for by addition of predetermined amounts of 0.2 M HCl.

Rates of O_2 exchange were calculated from the slope of the recorded changes in dissolved O_2 and, in all cases reported here O_2 exchange was determined under 10 ± 0.5 mg O_2 l^{-1}. Treatments were usually run in triplicate.

The method of Arnon (1949) was used for all chlorophyll determinations i.e. chlorophyll was extracted by grinding leaf samples with 80% acetone in an iced mortar and pestle. Extracts were made up to $5cm^3$ with ice cold 80% acetone and centrifuged to remove cell debris. The absorbance of supernatants at 645 and 663 nm in a 10mm light path cell were measured using a Unicam SP600 spectrophotometer. A matched cell containing 80% acetone acted as the blank. The chlorophyll content of the extracts, in mg cm^{-3}, were calculated by inserting the relevant absorbance values in the following equations:-

$$\text{Chl. a} = (0.0229 \times \text{Abs}_{645}) - (0.00468 \times \text{Abs}_{663})$$

$$\text{Chl. b} = (0.127 \times \text{Abs}_{663}) - (0.00269 \times \text{Abs}_{645})$$

$$\text{Chl. (total)} = (0.0202 \times \text{Abs}_{645}) + (0.00802 \times \text{Abs}_{663})$$

After centrifuging and before determination of absorbance, the samples were stored in an ice bath and covered with several layers of aluminium foil to exclude light. The interval between measuring the absorbance of the first sample and the last was usually less than 30 minutes. During this time no denaturation of the chlorophyll occurred, since repeat determination of absorbance on the first sample gave the same value. In most instances background absorbance proved negligible.

RESULTS AND DISCUSSION

The effect of Brushing to Remove Epiphytes

Older leaves of *E. canadensis*, particularly during the winter, were encrusted with epiphytes. These epiphytes were removed by gently brushing the leaves with a fine art brush and rinsing in several changes of water. Microscopical examination revealed no damage and the remaining presence of few epiphytes. The effect of such brushing was tested using non-epiphytised apical leaves. Photosynthetic O_2 evolution and O_2 uptake in the dark were not significantly different for brushed and non-brushed leaves (Table 2.1).

Table 2.1 The effect of leaf brushing on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of apical E. canadensis leaves.

	Light-saturated Photosynthetic Rate ($\mu g\ O_2$/mg Chl. min)	Rate of Dark Respiration ($\mu g\ O_2$/mg Chl.min)
non-brushed	21.1 ± 1.4	3.6 ± 0.3
brushed	21.7 ± 1.4	4.0 ± 0.5

Mean of four replicates ± standard deviation

The effect of Number of Leaves on Oxygen Exchange

The number of leaves in the reaction vessel did not significantly affect the apparent photosynthetic O_2 evolution or O_2 uptake in the dark (Table 2.2). However the leaves excised from 6cm below the apex had lower O_2 exchange rates than 1 cm excised leaves (see also Table 2.3) and could reflect the type of age-related changes that have been reported for terrestrial plants (e.g. Catsky et al., 1975). It was therefore decided that only 3 leaves would be used in oxygen exchange rate determinations so that they could be taken from the same whorl and therefore have experienced the same growth conditions and be approximately the same age.

The effects of Time from Collection

Apparent photosynthetic O_2 evolution and dark respiratory O_2 uptake of E. canadensis leaves remained relatively stable for seven days after collection of the plant material, but photosynthesis was reduced on day 10 in both 1cm and 6cm excised leaves (Table 2.3). This contrasts with the rapid decline in photosynthesis after 24 hours previously noted for E. canadensis (Brown et al., 1974). It was therefore decided that

Table 2.2 The effect of leaf number in the reation vessel on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 1cm and 6cm excised leaves of E. canadensis

Number of Leaves	Light-saturated Photosynthetic Rate ($\mu g\ O_2$/mg Chl. min)		Rate of Dark Respiration ($\mu g\ O_2$/mg Chl. min)	
	1 cm	6 cm	1 cm	6 cm
1	28.4 ± 1.2	20.8 ± 2.3	5.6 ± 0.5	4.9 ± 0.4
2	28.7 ± 1.4	20.9 ± 0.9	5.8 ± 0.8	5.0 ± 0.4
3	28.1 ± 1.1	21.4 ± 2.4	6.0 ± 0.8	4.7 ± 0.1
4	27.7 ± 1.1	21.3 ± 2.5	5.4 ± 0.6	4.7 ± 0.1
5	28.6 ± 1.3	21.3 ± 1.9	4.8 ± 0.4	4.9 ± 0.2
6	28.9 ± 0.5	21.1 ± 2.1	4.4 ± 0.6	4.6 ± 0.1

Mean of three replicates ± standard deviation

Table 2.3 The effect of time from collection on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 1cm and 6cm excised leaves of E. canadensis.

Time (days)	Light-saturated Photosynthetic Rate ($\mu g\ O_2$/mg Chl. min)		Rate of Dark Respiration ($\mu g\ O_2$/mg Chl. min	
	1 cm	6cm	1 cm	6cm
0	29.0 ± 1.3	24.9 ± 1.0	5.6 ± 0.5	4.2 ± 0.9
2	29.9 ± 2.0	25.5 ± 0.7	5.9 ± 1.0	4.4 ± 0.3
4	28.6 ± 1.5	24.2 ± 1.8	5.3 ± 0.3	4.6 ± 0.4
7	29.6 ± 2.4	24.4 ± 1.9	5.6 ± 0.7	4.1 ± 0.6
10	21.8 ± 1.2	16.4 ± 0.9	5.6 ± 0.7	3.7 ± 0.3

Mean of three replicates ± standard deviation

photosynthetic and respiratory rate determinations should be made within 4 days of collection to avoid any decline in rates induced either by the laboratory holding conditions or by aging of the specimens. Such premature aging induced by laboratory holding conditions has recently been demonstrated in an aquatic angiosperm (Jana and Choudhuri, 1979).

The effect of Time from Leaf Excision

Apparent photosynthetic and dark respiratory rates of excised E. canadensis leaves remained stable for 95 minutes but declined thereafter (Table 2.4). This decline was not caused by a reduction in nutrient levels in the assay solution because replenishing the medium did not restore metabolic rates. The decline was probably due to reduced physiological activity of the leaves and thus measurements were completed within 90 minutes of leaf excision. However this length of time was only necessary when determining the photosynthetic response of leaves to irradiance. In all other experiments measurements were completed within 30 minutes from excision.

The effect of Incubation Time on Temperature Response

At 15°C and 20°C there was no change, but at 25°C and above apparent photosynthetic and dark respiratory rates were initially high, but declined to lower stable rates within 15 to 30 minutes (Table 2.5). This decline was probably associated with enzymes equilibrating to the temperature.

It was considered that the 25-30°C temperature optima for both apparent photosynthesis and dark respiration obtained after 30 minutes probably reflected the true optimum temperature for these processes rather than

Table 2.4 The effect of time from leaf excision on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 1cm and 6cm excised leaves of E. canadensis.

Time (min)	Light-saturated Photosynthetic Rate (μg O_2/mg Chl. min)		Rate of Dark Respiration (μg O_2/mg Chl. min)	
	1cm	6cm	1cm	6cm
5	27.3 ± 0.4	16.6 ± 0.6	4.1 ± 0.4	2.9 ± 0.5
20	27.9 ± 1.1	17.1 ± 0.9	4.2 ± 0.5	3.1 ± 0.9
35	27.8 ± 0.8	16.7 ± 1.5	4.0 ± 0.2	2.5 ± 0.5
50	27.3 ± 0.6	16.8 ± 0.9	4.0 ± 0.3	2.4 ± 0.4
65	27.4 ± 0.2	16.5 ± 1.1	3.8 ± 0.2	2.7 ± 0.5
95	27.4 ± 0.4	16.3 ± 1.8	4.3 ± 0.5	3.1 ± 1.0
120	26.4 ± 0.8	14.3 ± 2.1	4.5 ± 0.5	3.3 ± 1.0

Mean of three replicates ± standard deviation

Table 2.5 The effect of preincubation time on the temperature response of apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 2cm excised leaves of E. canadensis.

Time (min)	Light-saturated Photosynthetic Rate (μg O_2/mg Chl. min)					Rate of Dark Respiration (μg O_2/mg Chl. min)				
	15°C	20°C	25°C	30°C	35°C	15°C	20°C	25°C	30°C	35°C
0	13.8 ± 0.5	24.2 ± 0.4	32.9 ± 0.5	35.3 ± 0.2	35.0 ± 2.1	2.7 ± 0.4	4.4 ± 0.8	7.1 ± 0.4	8.4 ± 0.9	10.9 ± 0.9
15	13.7 ± 0.4	24.2 ± 0.4	32.3 ± 0.3	32.3 ± 1.8	29.0 ± 2.1	2.9 ± 0.5	4.9 ± 0.9	7.2 ± 0.5	7.7 ± 0.3	7.3 ± 1.2
30	14.1 ± 0.1	23.9 ± 0.2	31.5 ± 0.4	31.2 ± 1.5	26.6 ± 1.7	2.9 ± 0.5	4.9 ± 0.9	7.2 ± 0.4	7.3 ± 0.1	6.6 ± 0.8
45	14.0 ± 0.3	24.4 ± 0.5	31.4 ± 0.4	30.4 ± 1.7	26.6 ± 1.2	2.8 ± 0.5	5.1 ± 0.6	7.2 ± 0.4	6.9 ± 0.7	6.6 ± 0.8
60	14.5 ± 0.8	23.7 ± 0.6	31.0 ± 0.6	30.1 ± 0.5	26.7 ± 0.9	2.8 ± 0.2	4.9 ± 0.8	7.2 ± 0.3	6.8 ± 0.7	6.6 ± 0.8
120	14.5 ± 0.4	23.8 ± 0.6	30.8 ± 0.4	30.7 ± 1.5	25.5 ± 0.5	3.3 ± 0.3	5.2 ± 0.8	8.1 ± 1.0	7.2 ± 1.0	6.3 ± 0.7
180	14.7 ± 0.8	22.8 ± 0.9	30.6 ± 1.3	30.8 ± 0.6	25.6 ± 1.4	3.2 ± 0.8	5.2 ± 1.0	6.4 ± 0.7	7.4 ± 0.7	6.3 ± 0.8
360	13.6 ± 0.6	23.6 ± 0.9	30.6 ± 0.3	30.5 ± 0.5	25.4 ± 1.5	3.3 ± 0.3	4.6 ± 0.8	6.9 ± 0.3	7.7 ± 0.5	6.8 ± 0.8
1200	13.9 ± 0.5	23.9 ± 0.6	30.6 ± 0.8	30.4 ± 0.6	25.2 ± 1.3	3.3 ± 0.9	2.9 ± 0.4	7.3 ± 0.8	6.2 ± 0.3	5.8 ± 0.5

Mean of three replicates ± standard deviation

the higher transitory temperature optima found with short incubation times. Thus in experiments using temperatures higher than the 15°C laboratory holding conditions, all specimens were preincubated for 30 minutes at the appropriate temperature before rate determination.

The effect of Laboratory Holding Conditions

Throughout the course of the study E. canadensis samples were maintained under standard laboratory holding conditions (15°C, 16:8 hour photoperiod, 54 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance). As some reports in the literature suggest that E. canadensis exhibits winter dormancy (e.g. Sculthorpe, 1967), the effect of standard holding conditions were tested on winter specimens to determine if such conditions might break 'dormancy' and give higher metabolic rates than might otherwise be expected.

Comparisons between winter plants held under standard conditions and ones held under more realistic winter field conditions (5°C, 8:16 hour photoperiod, 36 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance) indicated that there was no significant effect of such holding conditions on photosynthetic and respiratory response to temperature (Table 2.6). Thus it is unlikely that the relatively short exposure (maximum 4 days) of winter plants to the elevated temperature and extended photoperiod during laboratory holding had any effect on the outcome of investigations.

The effect of Time of Day

There was evidence of diurnal effects on apparent photosynthesis of E. canadensis leaves (Table 2.7). At 24.00 hours the percentage reduction in apparent photosynthesis was 11% compared to the maximum rate achieved at 12.00 hours. However, the physiological activity of the material was very low, even for winter specimens, and this might indicate a greater potential for diurnal effects in more active material.

Table 2.6 The effect of laboratory holding conditions on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 2cm excised leaves of E. canadensis.

Holding Temp. (°C)	Light-saturated Photosynthetic Rate (μg O_2/mg Chl. min)					Rate of Dark Respiration (μg O_2/mg Chl. min)				
	15°C	20°C	25°C	30°C	35°C	15°C	20°C	25°C	30°C	35°C
5	17.7 ± 1.4	36.4 ± 1.5	39.6 ± 1.7	40.4 ± 2.5	30.8 ± 1.3	2.6 ± 0.1	5.1 ± 0.4	5.1 ± 1.1	5.3 ± 0.2	2.8 ± 0.4
15	17.4 ± 0.5	36.9 ± 1.1	40.8 ± 1.6	41.2 ± 2.1	32.3 ± 0.8	2.7 ± 0.1	4.8 ± 0.2	5.3 ± 0.7	5.6 ± 0.6	3.2 ± 0.4

Mean of three replicates ± standard deviation

Table 2.7 The effect of time of day on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 3cm excised leaves of E. canadensis.

Time of Day	Light-saturated Photosynthetic Rate ($\mu g\ O_2$/mg Chl. min)	Rate of Dark Respiration ($\mu g\ O_2$/mg Chl. min)
10.00	17.2 ± 0.7	4.9 ± 0.5
12.00	16.9 ± 0.5	4.8 ± 0.4
15.00	16.6 ± 0.4	4.9 ± 0.3
18.00	16.0 ± 0.3	4.9 ± 0.3
21.00	15.2 ± 0.6	4.8 ± 0.3
24.00	14.8 ± 0.3	4.7 ± 0.2
03.00	15.1 ± 0.7	5.0 ± 0.3
06.00	16.7 ± 0.3	4.6 ± 0.3
09.00	16.4 ± 0.2	4.9 ± 0.2
12.00	17.8 ± 0.3	4.9 ± 0.2
15.00	17.2 ± 0.6	4.8 ± 0.3

Mean of three replicates ± standard deviation

Therefore in all experiments, rate determinations were always completed within the middle 8 hours of the photoperiod. In addition experimental treatments were randomised to avoid possible complications due to diurnal effects.

The Role of HCO_3^- as the Carbon Source in Photosynthesis

Many submersed aquatic plants are able to 'use' HCO_3^- as a carbon source in photosynthesis (Sculthorpe, 1967; Steemann Nielsen, 1960) and yet photosynthesis declines with increased pH (Brown et al., 1974; Kadono, 1980; Shiyan and Merezhko, 1972; Steemann Nielson, 1960; Van et al., 1976). Such a decline may indicate a preference for free CO_2 as the carbon source in photosynthesis (Kadono, 1980; Raven, 1970; Van et al., 1976) since free CO_2 declines with increasing pH whilst HCO_3^- increases.

Controversy exists about the role of HCO_3^- in photosynthesis of aquatic plants. Early claims of HCO_3^- utilization as a carbon source in photosynthesis were based upon pH increases in unbuffered media but such increases in themselves cannot indicate either photosynthesis (Thomas and Tregunna, 1967) or HCO_3^- utilization (Raven, 1970). Recent studies have suggested that there is little or no HCO_3^- utilization in E. canadensis (Brown et al., 1974) and therefore investigations were performed on E. canadensis in an attempt to clarify the role of HCO_3^- in photosynthesis. If this were so the use of HCO_3^- as a carbon source would give the plant a competitive advantage over non-HCO_3^- users in alkaline waters where HCO_3^- is a major inorganic carbon source (Hutchinson, 1957; 1975; Sculthorpe, 1967).

The data in Table 2.8 shows that photosynthesis of both 1cm and 6cm leaves under 0.5mM total inorganic carbon was greatest at low pH and declined rapidly above pH 6 to give very low photosynthetic rates at pH 9. In contrast, photosynthesis was constant over the same pH range when 0.5 mM free CO_2 was employed. The decline under constant total inorganic carbon is consistent with reports of similar reductions in other aquatic macrophytes (Brown et al., 1974; Kadono, 1980; Shiyan and Merezhko, 1972; Steemann Nielsen, 1960; Van et al., 1976). The fact that in the present investigation photosynthetic rates were restored to high levels by increasing the concentration of free CO_2 to that encountered at low pH indicates that pH per se was not affecting photosynthesis but was acting through and causing a decline in free CO_2.

The effect of Tris-HCl buffer concentration, pH 7.5, on oxygen exchange of E. canadensis was tested and did not cause any significant depression of photosynthesis except at a concentration of 100 mM, i.e. twice the concentration used in all investigations (Table 2.9). Neither did Tris-HCl cause any depression of oxygen exchange when compared with several other buffers (Table 2.10). These results, together with the fact that there was no significant difference between photosynthesis at pH 7 under 50 mM NaCitrate-NaH_2PO_4 (28.7 ± 0.8 μg O_2 mg $Chl^{-1} min^{-1}$) and that at pH 7 under 50 mM Tris-HCl (27.7 ± 1.1 μg O_2 mg $Chl^{-1} min^{-1}$) suggest that the decline in photosynthesis observed with increasing pH was not caused by the buffer but by pH induced changes in the proportion of CO_2 and HCO_3^- as carbon sources for photosynthesis.

Table 2.8 The effect of pH on apparent photosynthetic O_2 evolution under constant total inorganic carbon (TC) or constant free CO_2 (FC) concentrations of 1cm and 6cm excised leaves of E. canadensis.

pH	Light-saturated Photosynthetic Rate (μg O_2/mg Chl. min)			
	1cm		6cm	
	0.5 mM TC	0.5 mM FC	0.5 mM TC	0.5 mM FC
5	45.1 ± 3.3	45.1 ± 3.3	39.7 ± 1.4	39.7 ± 1.4
6	42.8 ± 3.9	45.4 ± 3.6	34.9 ± 1.2	37.1 ± 0.8
7	38.8 ± 1.0	44.3 ± 1.9	32.6 ± 1.3	38.9 ± 0.8
8	9.7 ± 1.0	44.2 ± 3.0	10.7 ± 1.6	38.9 ± 3.4
9	1.6 ± 0.3	44.0 ± 2.7	1.2 ± 0.2	32.5 ± 0.9

Mean of three replicates ± standard deviation

Table 2.9 The effect of Tris-HCl, pH 7.5, on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 1cm and 6cm excised leaves of E. canadensis.

Conc. (mM)	Light-saturated Photosynthetic Rate ($\mu g\ O_2$/mg Chl. min)		Rate of Dark Respiration ($\mu g\ O_2$/mg Chl. min)	
	1cm	6cm	1cm	6cm
12	38.5 ± 3.0	27.1 ± 1.3	7.0 ± 1.0	4.5 ± 0.7
25	39.4 ± 1.9	28.3 ± 2.3	6.6 ± 1.0	5.2 ± 0.3
37	37.8 ± 1.6	28.2 ± 2.3	6.8 ± 1.0	5.2 ± 0.2
50	39.6 ± 3.1	28.9 ± 2.1	7.1 ± 1.1	4.2 ± 0.7
75	39.5 ± 1.9	25.3 ± 2.2	8.5 ± 1.2	4.1 ± 0.5
100	32.5 ± 2.4	12.1 ± 2.0	9.5 ± 0.5	3.8 ± 0.5

Mean of three replicates ± standard deviation

Table 2.10 The effect of different buffers on apparent photosynthetic O_2 evolution and O_2 uptake in the dark of 1cm and 6cm excised leaves of E. canadensis.

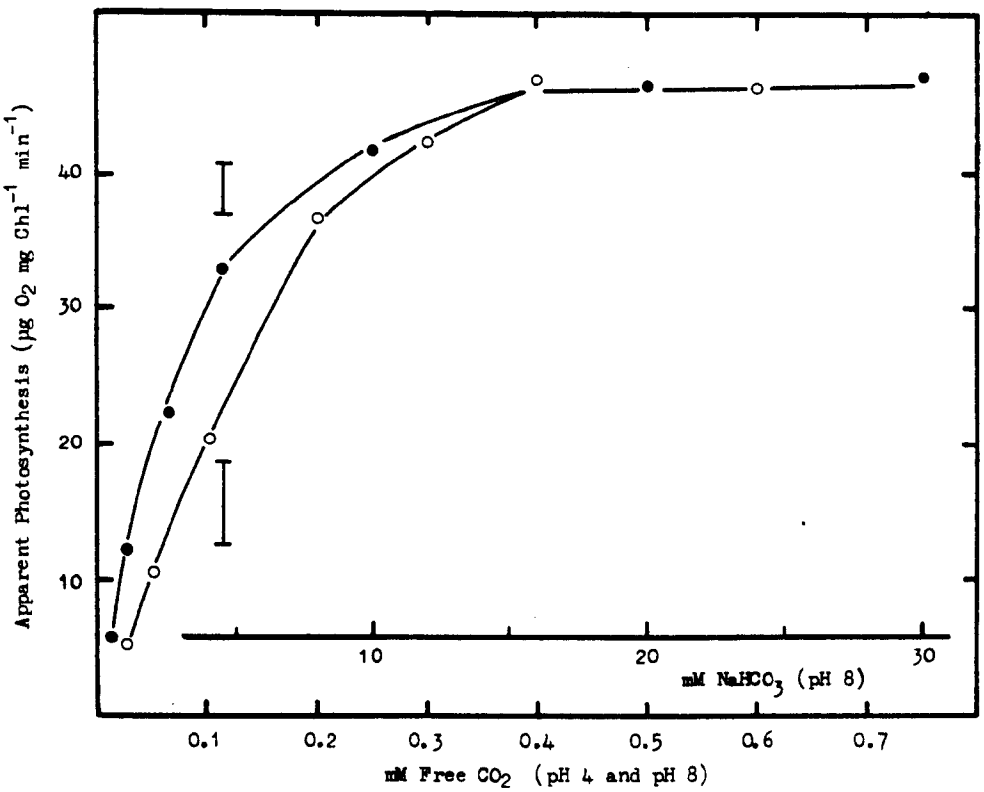
Buffer	Light-saturated Photosynthetic Rate ($\mu g\ O_2$/mg Chl. min)		Rate of Dark Respiration ($\mu g\ O_2$/mg Chl. min)	
	1cm	6cm	1cm	6 6cm
Tris-HCl	37.1 ± 2.7	28.6 ± 0.4	5.9 ± 1.1	4.6 ± 0.5
Tris-HNO_3	37.1 ± 1.2	28.9 ± 2.6	6.7 ± 0.5	4.9 ± 0.5
Tris-H_2SO_4	38.1 ± 1.1	28.2 ± 1.1	6.6 ± 0.7	4.9 ± 0.4
Hepes	37.8 ± 1.1	29.8 ± 1.9	5.7 ± 1.0	4.1 ± 0.2
Tricine	36.5 ± 1.7	29.7 ± 1.3	6.1 ± 1.3	3.9 ± 0.2
Bes	36.8 ± 0.7	28.4 ± 2.4	7.3 ± 0.2	4.8 ± 0.4

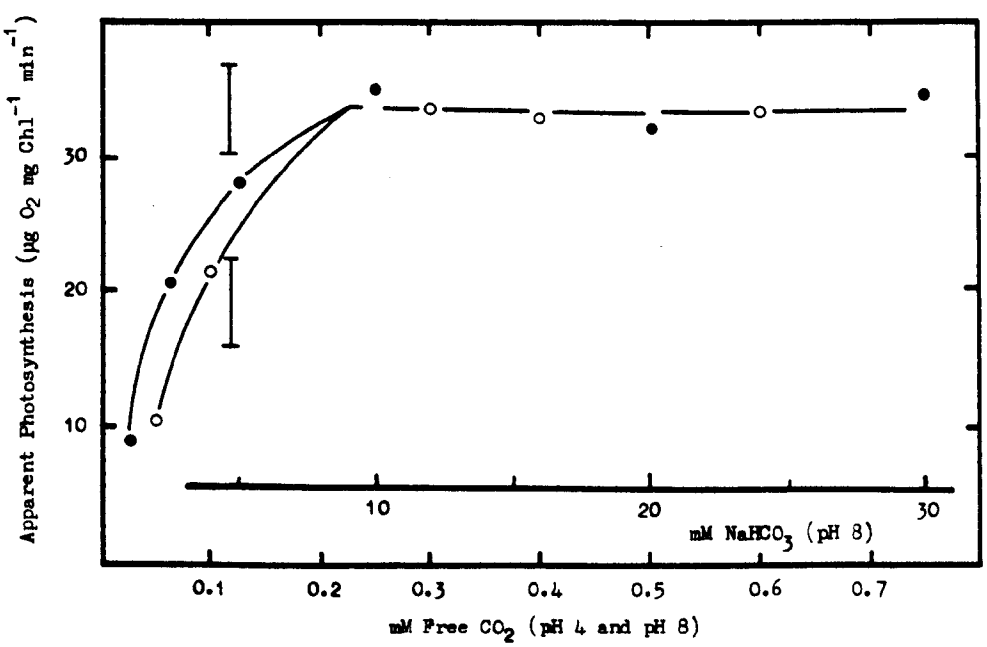
Mean of three replicates ± standard deviation

Kadono (1980) has used the ratio of photosynthesis at pH 8.5 : pH 5 as an indication of the ability of an aquatic plant to utilize HCO_3^- as a carbon source in photosynthesis. A high ratio (0.3 to 0.5) was characteristic of plants able to use HCO_3^- whilst a low ratio (< 0.1) was characteristic of non-HCO_3^- users. Values of photosynthesis at pH 8.5 in 1cm and 6cm E. canadensis leaves were estimated by interpolation of the graphed data contained in Table 2.8. These were 4.0 and 3.5 $\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$ respectively, and gave a ratio of 0.09 in both types of leaf and therefore confirms the suggestion that E. canadensis has a poor ability to use HCO_3^- in photosynthesis (Brown et al., 1974).

Another method of determining the ability of a plant to utilize HCO_3^- for photosynthesis is to compare its photosynthesis at pH 4 with that at pH 8. If photosynthesis is enhanced at pH 8 compared to that at pH 4 for a given subsaturating CO_2 concentration then HCO_3^- utilization is inferred (Raven, 1970; Steemann Nielsen, 1960). In Figure 2.1, photosynthetic rates of 1cm and 6cm E. canadensis leaves as a function of free CO_2 concentration, are compared at pH 4 and pH 8. It was assumed that at pH 4 free CO_2 comprised 99% of the total inorganic carbon whilst at pH 8 it was only 2.5% (Hutchinson, 1957). For each leaf the maximum photosynthetic rates were similar at pH 4 and pH 8 but the HCO_3^- concentration required to saturate photosynthesis at pH 4 was 0.4 and 0.2 mM compared with 16 and 8 mM at pH 8 in 1cm and 6cm leaves respectively. In each type of leaf the free CO_2 concentration required to saturate photosynthesis was similar. There was no evidence that high pH per se had a direct effect on photosynthesis especially as the photosynthetic rate at subsaturating CO_2 concentrations at pH 8 was greater than at pH 4.

Figure 2.1 Photosynthetic rates of 1 cm (top) and 6 cm (bottom) excised leaves of E. canadensis at pH 4 (o) and pH 8 (•) as a function of the free CO_2 concentration. For reference the total inorganic carbon ($NaHCO_3$) concentration is also shown. The assay solution was a modified Forsberg medium No. II containing 50 mM NaCitrate-NaH_2PO_4 at pH 4 or 50 mM Tris-HCl at pH 8. Oxygen exchange was determined at 20°C at a PAR irradiance of 290 $\mu E\ m^{-2}s^{-1}$. All data points represent the mean of triplicate determinations. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


Apparent Photosynthesis (μg O_2 mg Chl^{-1} min^{-1})
40
30
20
10
10
20
30
mM $NaHCO_3$ (pH 8)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
mM Free CO_2 (pH 4 and pH 8)


Apparent Photosynthesis (μg O_2 mg Chl^{-1} min^{-1})
30
20
10
10
20
30
mM $NaHCO_3$ (pH 8)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
mM Free CO_2 (pH 4 and pH 8)

This type of enhancement is usually attributable to HCO_3^- supplementing free CO_2 use (Raven, 1970; Steemann Nielsen, 1960). However, the affinity of RuBP carboxylase for CO_2 is increased at high pH and HCO_3^- concentrations (Bowes et al., 1975) and this fact may provide an alternative explanation for enhancement (Van et al., 1976). Indeed the apparent $Km(CO_2)$ was reduced with increased pH and 1cm E. canadensis leaves had an apparent $Km(CO_2)$ of 125 μM at pH 4 which was reduced to 70 μM at pH 8. A similar trend has been reported in three other aquatic macrophytes (Van et al., 1976).

Thus the lack of conclusive evidence for HCO_3^- use in E. canadensis together with the distinct preference for CO_2 as the carbon source in photosynthesis (Table 2.8) and the high concentration of HCO_3^- required to saturate photosyntheses (Figure 2.1) would suggest that this species does not have a competitive advantage under the high pH conditions which often occur in the Leeds and Liverpool Canal.

CONCLUSIONS AND ADOPTION OF STANDARD PROCEDURE FOR MEASUREMENTS

Investigations into the effect of laboratory holding conditions and experimental techniques indicated that these did not significantly affect O_2 exchange characteristics of E. canadensis leaves. Removal of epiphytes by gentle brushing of the leaves and rinsing in several changes of water did not significantly affect subsequent rates (Table 2.1). Neither did the number of leaves in the reaction vessel (Table 2.2), but since O_2 exchange rates of older leaves were less than those of younger ones, only the three leaves of a whorl were used so that they were of similar age.

Oxygen exchange rates were stable for 7 days after collection (Table 2.3) and for at least 95 minutes after leaf excision (Table 2.4). However, short temperature preincubation times at temperatures above 20°C resulted in high initial O_2 exchange rates which declined to stable levels within 15 to 30 minutes of experiencing that temperature (Table 2.5).

The O_2 exchange characteristics of winter samples maintained under 15°C, 16 : 8 hour photoperiod, 54 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance was not significantly different to those held under 5°C, 8 : 16 hour photoperiod, 34 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance (Table 2.6). However the photosynthesis of winter specimens of E. canadensis was reduced by 11% at 24.00 compared to 12.00 hours (Table 2.7) therefore suggesting the presence of diurnal effects on the photosynthesis of this plant.

Photosynthetic rates of E. canadensis declined above pH 6 to give very low readings at pH 9, when 0.5 mM total inorganic carbon was present. However rates remained high over the whole pH range if the supply of free CO_2 was maintained at 0.5 mM (Table 2.8). It would appear that the decline is associated with a pH induced decrease in CO_2 concentration at high pH. Results also suggest that there is a strong preference for CO_2 as the carbon source in photosynthesis and the value of 0.09 for the ratio of photosynthesis at pH 8.5 : pH 5 suggests that E. canadensis has a poor ability to use HCO_3^-. The decline at high pH under constant total inorganic carbon was not due to either the buffer or its concentration since 50 mM Tris-HCl did not cause a reduction in oxygen exchange compared with lower concentrations (Table 2.9) or other buffers (Table 2.10).

Photosynthesis at a given subsaturating free CO_2 concentration was greater at pH 8 that at pH 4 (Figure 2.1). This type of enhancement is usually ascribed to HCO_3^- use in photosynthesis but could also be caused by the increased affinity of RuBP carboxylase for CO_2 at high pH and HCO_3^- concentrations. This may be so in E. canadensis since the apparent $Km(CO_2)$ of photosynthesis was reduced with increased pH. Thus no evidence of substantial HCO_3^- use was found in this species.

The standard procedure adopted for collection, laboratory maintenance and physiological measurements were:-

1. Plant samples were collected by grapnel from an unpolluted, non-shaded stretch of the Leeds and Liverpool Canal near Aintree, Merseyside (O.S. 393990). Only undamaged main stems, growing vertically in the water column were collected. They were transported to the laboratory in canal water, the journey time was approximately 20 minutes.
2. Plant material was maintained for up to 4 days in large glass tanks containing canal water at 15°C. Plants were exposed to a 16 hour photoperiod of 54 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance no matter what season of the year.
3. Prior to leaf excision, plants were incubated for 30 minutes at the appropriate temperature. The three leaves of a whorl were excised by gently pulling them from the stem with a pair of forceps. If necessary epipytes were removed by gentle brushing and rinsing in several changes of water.
4. The brushed, excised leaves were placed directly into the O_2 electrode which contained a modified Forsberg medium II including suitable buffers (50 mM NaCitrate-NaH_2PO_4 for pH 5 to 6, or 50 mM Tris-HCl for pH 7 to 9), and unless otherwise indicated ca. 9.5 mg $O_2\ l^{-1}$.

The electrode was sealed and photosynthesis initiated by injecting concentrated $NaHCO_3$ to give a final concentration of 2.4 mM total inorganic carbon.

5. Rates of O_2 exchange were calculated from the slope of the recorded changes in dissolved O_2. The stated O_2 concentration represented the mid-point of the range (up to $\pm$ 0.5 mg O_2 l^{-1}) over which the rate was determined. Apparent photosynthesis was measured as O_2 evolution in the light, and dark respiration as O_2 uptake after the O_2 electrode had been covered with several layers of close fitting aluminium foil to exclude light.

6. Rate determinations were always completed within the middle 8 hours of the photoperiod, and experimental treatments were randomised to avoid possible complications due to diurnal effects on O_2 exchange.

7. The leaves were removed from the O_2 electrode after O_2 exchange rate determinations, which was usually within 30 minutes of excision, except in the case of irradiance response investigations which were completed within 90 minutes of excision. They were then rinsed and stored in Forberg medium II at 10°C until the evening when chlorophyll content was determined by the method of Arnon (1949) as already described.

Other methods or modifications special to each part of the study are introduced in the appropriate methods sections e.g. the glycolate oxidase assay is described in Section 5.

During the summer months water bodies may be exposed to supersaturation with O_2, increased pH, elevated temperatures and increased surface irradiances. These conditions have been shown to retard growth of Elodea canadensis, and their effects have been suggested as the likely cause of the afternoon depression of photosynthesis in submersed water plants. Therefore, this section describes the results of investigations into the effects of environmental conditions that arise during the summer on the photosynthesis and respiration of E. canadensis, and attempts to relate these results to observations of this plant's performance in the Leeds and Liverpool Canal.

3. THE INFLUENCE OF ENVIRONMENTAL FACTORS ON APPARENT PHOTOSYNTHESIS AND RESPIRATION OF THE SUBMERSED MACROPHYTE ELODEA CANADENSIS

(This section of the thesis has recently been published in Plant, Cell and Environment, 3, 415-428 (1980), but in the thesis, reference will only be made to the thesis section rather than to the actual published article).

ABSTRACT

Oxygen effects on apparent photosynthetic and dark respiratory O_2 exchange rates of detached leaves of Elodea canadensis Michx. (Hydrocharitaceae) were determined over a range of conditions which the submersed plant is likely to experience in shallow water. Apparent photosynthesis is inhibited by O_2 under all the experimental regimes of light, temperature, CO_2 concentration and pH. This inhibition is not caused solely by an accelerated rate of dark respiration, and the observed variations in O_2 inhibition are comparable to O_2 effects on photosynthesis and photorespiration of terrestrial C_3 plants. Percentage inhibition of apparent photosynthesis is enhanced by high O_2 and also by low CO_2. These results indicate that high O_2, high pH and low CO_2 conditions could cause major losses in photosynthetic activity under field conditions. This may account for some of the losses in biomass that are observed under still water conditions.

INTRODUCTION

It is often thought that the aquatic environment provides more equable conditions for plant growth than does the terrestrial one. Submersion in water normally provides protection from freezing during the winter (Westlake, 1971) and temperature fluctuations are much less violent than in the aerial environment (Sculthorpe, 1971). It is sometimes suggested that photosynthetic gaseous exchange is more favourable in water than on

land, because CO_2 is more soluble in water than is O_2 (Hutchinson, 1957). It is true that 1 m^3 of pure water in equilibrium with air at S.T.P. contains 25 mMoles CO_2 and 442 mMoles O_2, and that the CO_2 is supplemented by HCO_3^-, often in substantial amounts in most natural waters, whereas the same volume of air has only 15 mMoles CO_2 but 9353 mMoles O_2. However, this advantage is in practice counter-balanced by the much lower coefficients of molecular diffusion of both gases in water than in air (Hutchinson, 1975; Westlake, 1971), and by the lower mobility of the medium in all but fast-flowing waters. As a consequence, O_2 and CO_2 disequilibria can build up in the medium surrounding photosynthesizing waterplants to extents without parallel in terrestrial vegetation.

The plant-induced changes in the body of water are likely to be disadvantageous to the assimilating plant, especially in dense stands of submersed aquatic plants under conditions of high surface irradiance. Such plant-induced changes include supersaturation of the water with O_2, reduced availability of CO_2 and HCO_3^-, increased pH and also increased temperature (Bamforth, 1962; Brown et al., 1974; Dale and Gillespie, 1977; Ganf, 1974; Goulder, 1970; Moss, 1969: Van, et al., 1976). These are conditions which are conducive to high rates of photorespiration in those plants which exhibit this phenomenon (e.g. Goldsworthy, 1970; Jackson and Volk, 1970; Zelitch, 1971). Whilst estimates of the magnitude of photorespiration vary, in terrestrial plants it may account for 50% or more of the carbon assimilation (Zelitch, 1971), and clearly can be a phenomenon of potential importance in plant productivity.

Recent investigations have established the presence of photorespiration and related O_2 effects on apparent (net) photosynthesis and CO_2

compensation points of aquatic plants (Brown et al., 1974; Helder et al., 1974; Hough, 1974, 1976; Hough and Wetzel, 1972, 1978; Kutyurin et al., 1964; Prins and Wolff, 1974; Søndergaard, 1979; Tolbert and Osmond, 1976; Van et al., 1976). It has been suggested by Hough (1974) that submersed plants may photorespire less than terrestrial ones because the maxima reached by O_2 concentrations, light and temperature all tend to be lower in water than air. On the other hand, Hough (1974) also points out that diffusive losses of photorespired CO_2 may be rather slow from aquatic plants, and that some of the CO_2 may be retained in the gas lacunae, thereby permitting extensive refixation, leading to underestimation of the true magnitude of photorespiration. However, Elodea canadensis Michx. (Hydrocharitaceae) has few gas lacunae in the leaves, which are only two cell layers thick, and as a consequence, the refixation in this species is probably minor (Søndergaard, 1979).

E. canadensis may attain large standing crops in shallow, slow flowing waters, and although there have been isolated reports of O_2 effects on its CO_2 compensation point, photosynthesis and light-mediated CO_2 evolution into CO_2 free air (Brown et al., 1974; Kutyurin et al., 1964; Søndergaard, 1979), the possible combined effects of O_2 and other plant-induced changes in the water and consequent effects on the plant's photosynthetic productivity have not been determined.

This present study was initiated, first to investigate the effects of plant-induced changes in the water on photosynthetic productivity of E. canadensis. A second aim was to determine whether such effects could be involved in sudden, drastic reductions in E. canadensis biomass observed over a number of years in the Leeds and Liverpool Canal, Merseyside, during periods when light, temperature and nutrient supply

appeared otherwise favourable for cont inuing growth of the population (J.W. Eaton, personal communication). During such periods the water was clear, and the main growth zone of the weed beds was in the top 0.5m of the canal, so the growing tips of E. canadensis received abundant light. Water conditions in this zone changed drastically, especially on calm, sunny days. The O_2 concentration rose from normal 90-100% saturation to 150-200% saturation, pH rose from pH 7.0 - 7.5 to pH 9 - 10, and the temperature was up to 5°C higher than in the open water, reaching a maximum of 25°C. Since these were the field conditions within which growth and sudden collapse of biomass were observed, the experiments reported here were designed to investigate the photosynthetic and respiratory behaviour of the plant within these ranges of O_2, pH and temperature.

MATERIALS AND METHODS

Plant Material

Samples of E. canadensis were collected from the Leeds and Liverpool Canal, near Aintree, Merseyside (O.S. 393990). Plants were kept at 15°C in canal water under a 16 hour photoperiod, 54 $\mu E\ m^{-2}s^{-1}$ (PAR). Photosynthetic and respiratory oxygen exchange rates were made within 4 days of collection. Preliminary experiments indicated that the decline in oxygen exchange of detached E. canadensis shoots after 24 hours noted by Brown et al. (1974) was not evident in these specimens, and that rates remained constant for seven days.

Epiphytes were removed, when necessary, by lightly brushing leaves and rinsing them in several changes of water prior to oxygen exchange rate determinations. Tests using non-epiphytised apical leaves showed that this treatment did not affect subsequent recorded rates.

Photosynthetic and Respiratory Rate Determination

Unless otherwise indicated, photosynthetic and respiratory rates were measured as oxygen evolution or uptake at 20°C using a Clark-type O_2 electrode (Rank Brothers, Cambridge). The reaction vessel was illuminated with a 250-W projector bulb to a saturating incident light intensity of 290 $\mu E\ m^{-2}s^{-1}$ (PAR). Light intensity was varied, when necessary, by altering the distance between the light source and the reaction vessel.

Three leaves from a whorl 3cm below the apex were excised and directly placed in the oxygen electrode in 3 cm^3 of Forsberg medium II (Forsberg, 1965), modified by omission of Na_2SiO_3 and carbon sources. Buffer components were varied to cover the required pH range, i.e. 50 mM NaCitrate-NaH_2PO_4 for pH 5 to 6, 50 mM Tris-HCl for pH 7 to 9. Preliminary studies at the junction between these two buffers pH ranges, indicated no significant difference in photosynthetic rate with buffer. Photosynthesis was immediately initiated by injection of 0.1 cm^3 of $NaHCO_3$ solution, to give a final concentration of 2.4 mM HCO_3^-, which approximated to that present in the canal water. In experiments at higher HCO_3^- concentrations, the buffering capacity of the added HCO_3^- was compensated for by addition of predetermined amounts of 0.2 M HCl. All treatments were run in triplicate.

The buffered solutions were sparged with appropriate gases (N_2 and/or O_2) to give the desired initial O_2 concentration, prior to immersion of the leaf samples and injection of HCO_3^- solution.

Rates of O_2 exchange were calculated from the slope of the recorded changes in dissolved O_2, the stated O_2 concentration representing the mid-point of the range over which the rate was determined. This range

was up to ± 0.5 mg O_2 l^{-1}. To avoid any possible complications due to diurnal effects on oxygen exchange rates, rate determinations were always completed in the middle 8 hours of the photoperiod, and experimental treatments were randomised. The method of Arnon (1949) was used for all chlorophyll determinations.

Data Analysis

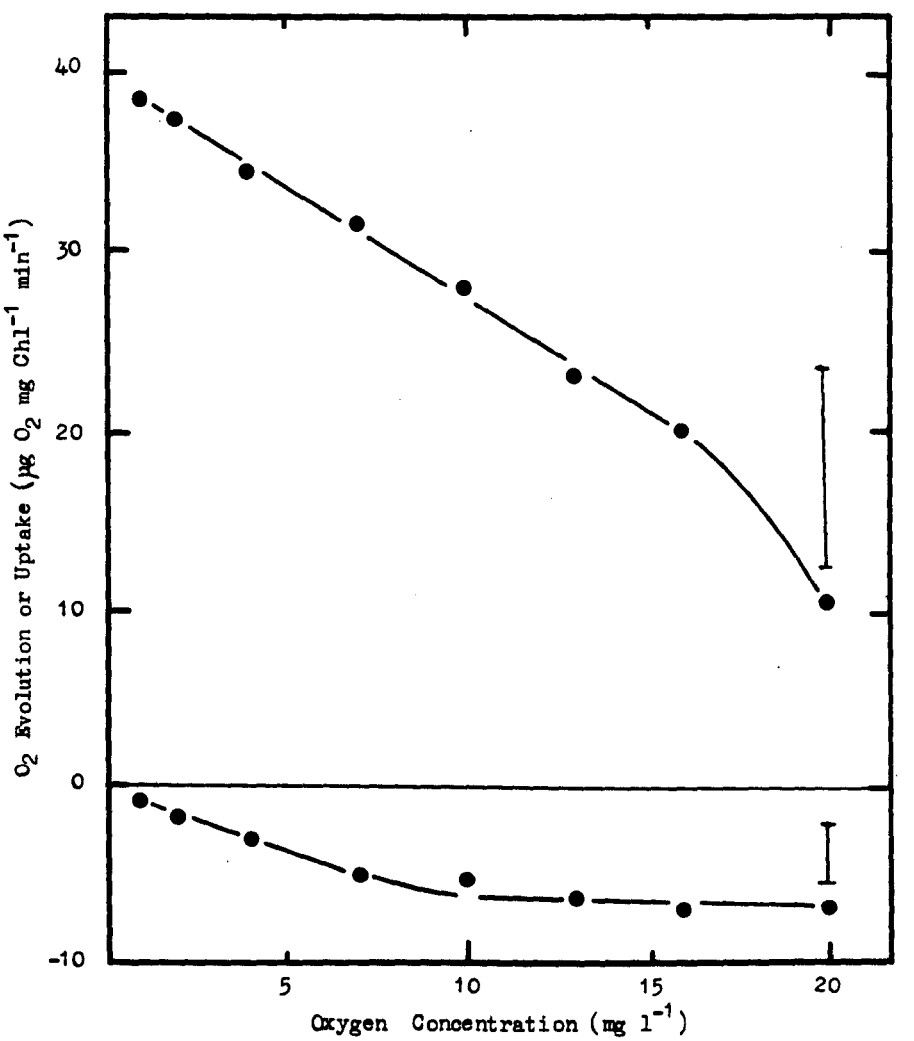
The significance of differences between means of the triplicated experimental treatments was tested by analysis of variance, and subsequent conservative comparison of means by Tukey's Least Significant Difference /LSD (Tukey)/ or Orthogonal Least Significant Difference /LSD (Orthogonal)/, as appropriate (Winer, 1971). The analyses were performed on raw, untransformed data except where noted otherwise, using the University of Liverpool ZIF Mk. 6 statistical package, on computer. Results for the 95% probability level ($P \leq 0.05$) are shown as bars on Figures 3.1 to 3.9. The nature and application of each individual test is given in figure captions, and all the results discussed subsequently are statistically significant on the above criteria.

RESULTS AND DISCUSSION

The effect of Oxygen Concentration on Oxygen Exchange

At pH 7.5 and in the presence of 2.4 mM HCO_3^- the light-saturated photosynthetic O_2 evolution decreased linearly with increasing O_2 concentration up to 16 mg O_2 l^{-1} (Figure 3.1). Above this O_2 concentration (approximately 175% air-saturated at 20°C) the departure of apparent photosynthetic O_2 evolution from linearity may be due to the presence of O_2 bubbles in the medium causing an underestimation of O_2 evolution. Similar problems with O_2 evolution measurements under supersaturated conditions have been reported (e.g. Brown et al., 1974; Dromgoole, 1978).

Figure 3.1 Apparent photosynthetic O_2 evolution, and O_2 uptake in the dark by E. canadensis leaves at different O_2 concentrations. The assay solution was a modified Forsberg medium No. II containing 2.4 mM total inorganic carbon and 50 mM Tris-HCl, pH 7.5. Oxygen exchange was determined at 20°C at a PAR irradiance of 290 $\mu E\ m^{-2}s^{-1}$ or in darkness. All data points in this and subsequent figures represent the mean of triplicate determinations. Bars indicate LSD (Tukey) at $P \leq 0.05$ for comparing any one mean on a line with any other mean on the same line.


40
30
20
10
0
-10
5
10
15
20
O_2 Evolution or Uptake (μg O_2 mg Chl^{-1} min^{-1})
Oxygen Concentration (mg l^{-1})

Dark respiratory O_2 uptake increased with increasing external O_2 concentration (Figure 3.1). Whilst dark respiration in most plants is unaffected by an atmospheric O_2 concentration above 2% (Jackson and Volk, 1970), the O_2 consumption rates of aquatic plants have been shown to increase with increasing dissolved O_2 concentration within the range 1.2 - 17 mg O_2 l^{-1} (Owens and Maris, 1964).

Dark respiration may be inhibited in the light in most plants (e.g. Jackson and Volk, 1970; Zelitch, 1971) but the existence of relatively high CO_2 compensation points in some aquatic plants at 1% O_2 (Van et al., 1976) has been used to argue that dark respiration may continue in the light (Benedict, 1978). However, the CO_2 compensation point of E. canadensis has been shown to fall to almost zero at low O_2 concentrations (Brown et al., 1974) and by a similar argument it could be suggested that light suppresses dark respiration in this species. Whether or not this is so, the observed increase in dark respiration rate with increased O_2 in this present study never equalled the observed decrease in apparent photosynthetic O_2 evolution. It would thus appear that the observed decrease in apparent photosynthetic O_2 evolution with increasing O_2 cannot be wholly accounted for by a concomitant increase in dark respiratory O_2 uptake, even supposing that this process continues in the light.

Although several mechanisms have been proposed to account for O_2 inhibition of photosynthesis, often termed the Warburg effect, Zelitch (1971) considers that the most important is the stimulation of photorespiration.

The effect of Temperature on Oxygen Exchange

Preliminary studies (data not presented here but see Table 2.5) showed

that above 20°C photosynthetic and respiratory oxygen exchange were initially high, but declined to lower stable rates within 15 to 30 minutes. All the data presented were obtained after 30 minute incubation.

Increased external O_2 concentration resulted in a decreased apparent photosynthetic O_2 evolution rate at all experimental temperatures (Figure 3.2). Oxygen evolution and external O_2 concentration showed a linear relationship which permitted extrapolation to zero O_2 for calculation of the O_2 inhibition of apparent photosynthesis data. Increasing O_2 concentration also resulted in increasing dark respiratory O_2 uptake rates at all temperatures, although again this increase in respiratory rate was far too small to explain all the observed decrease in apparent photosynthetic O_2 evolution. Photosynthetic and respiratory rates were determined for two additional temperatures at 10 mg O_2 l^{-1} (Figure 3.2) and on the basis of these results and other similar experiments (see Section 5) temperature optima for both apparent photosynthesis and dark respiration appeared to be between 25°C and 30°C.

Increasing temperature and O_2 both resulted in increased inhibition of photosynthesis (Figure 3.3a). Such an increase in O_2 inhibition of photosynthesis is explainable on the basis of the greater increase in RuBP oxygenase activity with increasing temperature reported for spinach (Badger and Andrews, 1974) and soybean (Laing et al., 1974) Apparently there was no marked upward displacement of the temperature optimum for photosynthesis at low compared with more natural O_2 tensions. These results agree with some reports for terrestrial plants where photosynthesis was compared at low and at atmospheric concentrations of O_2 and maximum O_2 inhibition was found at the temperature of maximal photosynthesis (e.g. Hofstra and Hesketh, 1969; Ku and Hunt, 1973; Ku et al., 1977).

Figure 3.2 Apparent photosynthetic O_2 evolution, and O_2 uptake in the dark by E. caradensis leaves at different temperatures under 2 (○), 5 (△), 10 (●) and 15 (□) mg O_2 l^{-1}. Other assay conditions were as described for Figure 3.1. Bars indicate LSD (Tukey) at $P \leq 0.05$ for comparisons between O_2 concentrations at any one temperature, the data points at 35 and 40°C being excluded.

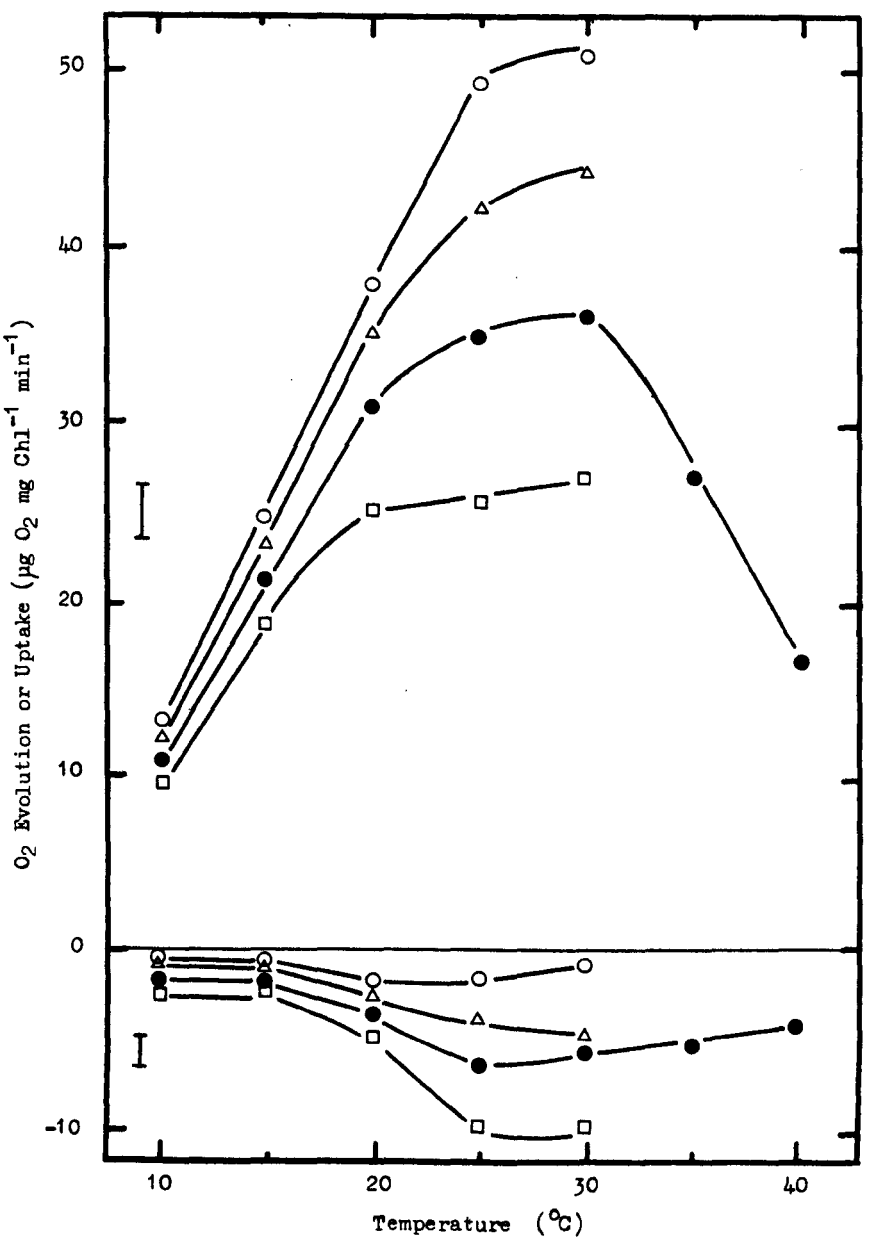
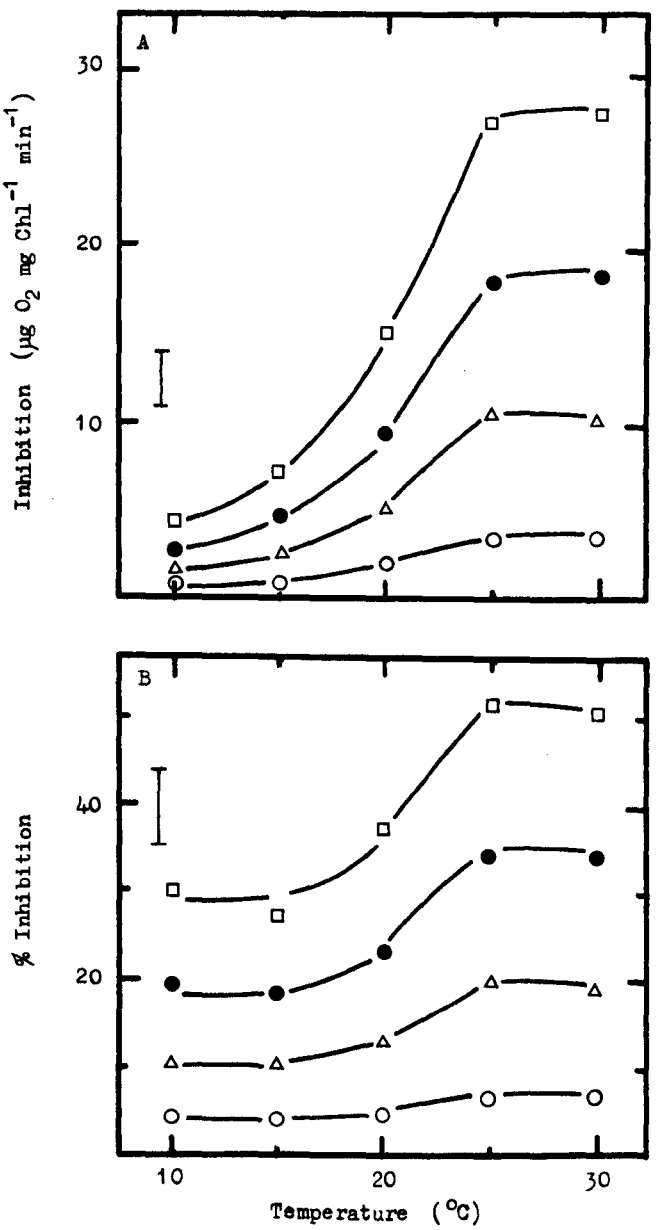

50
40
30
20
10
0
-10
10
20
30
40
Temperature (°C)
O2 Evolution or Uptake (µg O2 mg Chl-1 min-1)

Figure 3.3 The effect of temperature on A: O_2 inhibition of apparent photosynthesis, and B: percentage inhibition of apparent photosynthesis by O_2 in E. canadensis leaves under 2 (○), 5 (△), 10 (●) and 15 (□) mg O_2 l^{-1}. Inhibition is expressed relative to the estimated rate at zero O_2. Assay conditions were as described in Figure 3.2. Bars indicate LSD (Tukey) at $P \leq 0.05$ for comparisons between O_2 concentrations at any one temperature.


A
B
30
20
10
40
20
10
20
30
Inhibition (μg O_2 mg Chl^{-1} min^{-1})
% Inhibition
Temperature (°C)

However, the present results disagree with other reports (e.g. Hew et al., 1969; Jolliffe and Tregunna, 1968; Pearson and Hunt, 1972) in which maximal inhibition was found about 10 to 15°C higher than maximal photosynthesis. The present study casts no light on the reasons for this descrepancy.

Percentage inhibition of apparent photosynthesis by O_2 (Figure 3.3b) increased with increasing O_2, but apparently represented a constant fraction of apparent photosynthesis at 10 to 15°C and only increased significantly above 15°C.

The effect of CO_2 on Oxygen Exchange

Oxygen increased the apparent Km (HCO_3^-) for photosynthesis from 1.2 mM at 2 mg O_2 l^{-1} to 1.9 mM at 10 mg O_2 l^{-1} but did not alter the maximal velocity (Figure 3.4), indicating that O_2 was a competitive inhibitor of photosynthesis with respect to CO_2. Similar types of response have been described for terrestrial plants in response to CO_2 concentration at atmospheric and low O_2 tensions (e.g. Jolliffe and Tregunna, 1968; Ku et al., 1977; Servaites and Ogren, 1978). Increased O_2 again increased dark respiratory O_2 uptake, but not as much as it inhibited apparent photosynthesis, except at the two highest HCO_3^- concentrations which were 6-12 times greater than those experienced by the plant in the field.

Oxygen inhibition of apparent photosynthesis remained relatively constant over the HCO_3^- range 0.15 mM - 2.4 mM, and thereafter declined (Figure 3.5). In contrast, percentage inhibition of apparent photosynthesis by O_2 exhibited an exponential-type decline from 54% at 0.15 mM to 3% at 12 mM HCO_3^-. Similar results have been reported for terrestrial crop plants (e.g. Jolliffe and Tregunna, 1968; Ku et al., 1977). However, the

Figure 3.4 Apparent photosynthetic O_2 evolution, and O_2 uptake in the dark by E. canadensis leaves at different HCO_3^- concentrations under 2 (○) and 10 (●) mg O_2 l^{-1}. Other assay conditions were as described for Figure 3.1. Bars indicate LSD (Orthogonal) at $P \leqslant 0.05$ for comparisons between O_2 concentrations at any one HCO_3^- concentration.

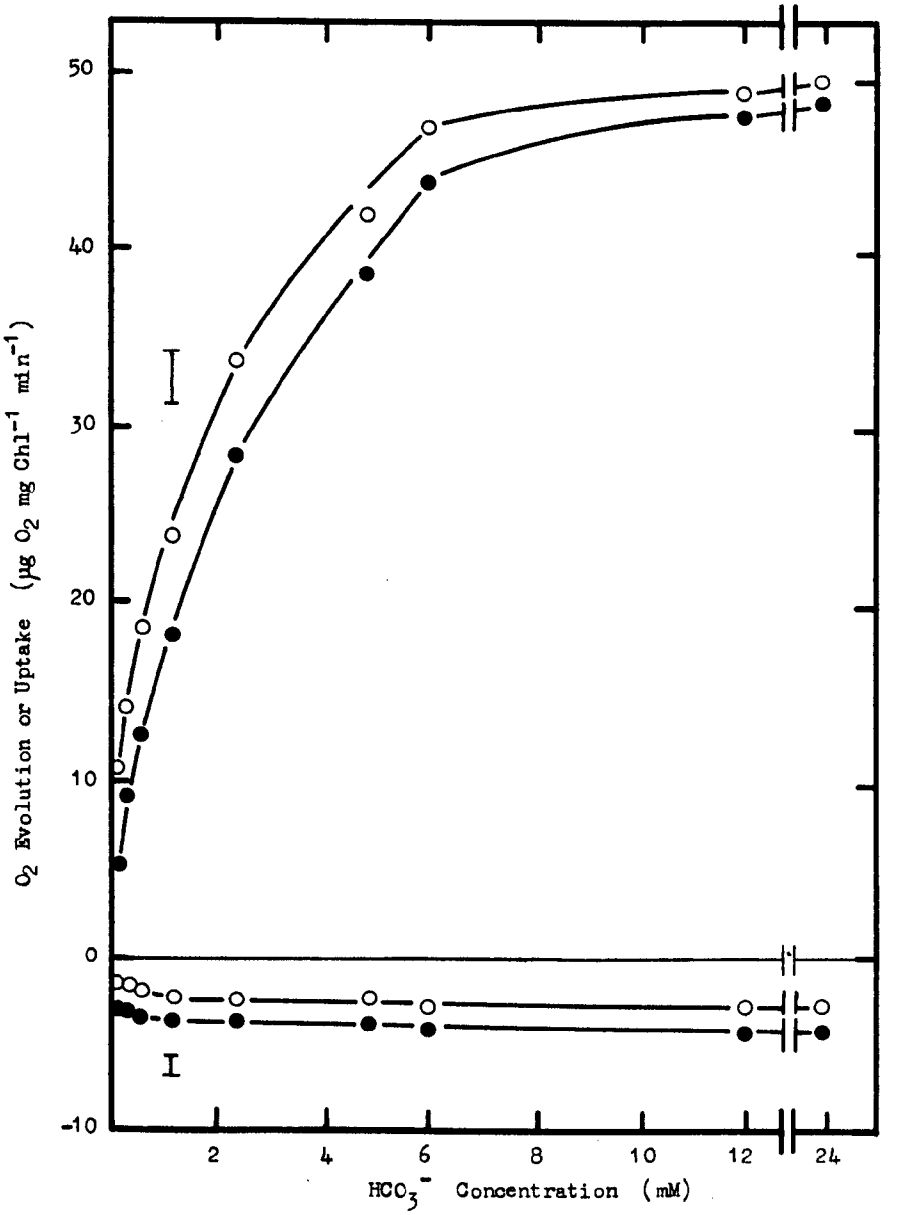
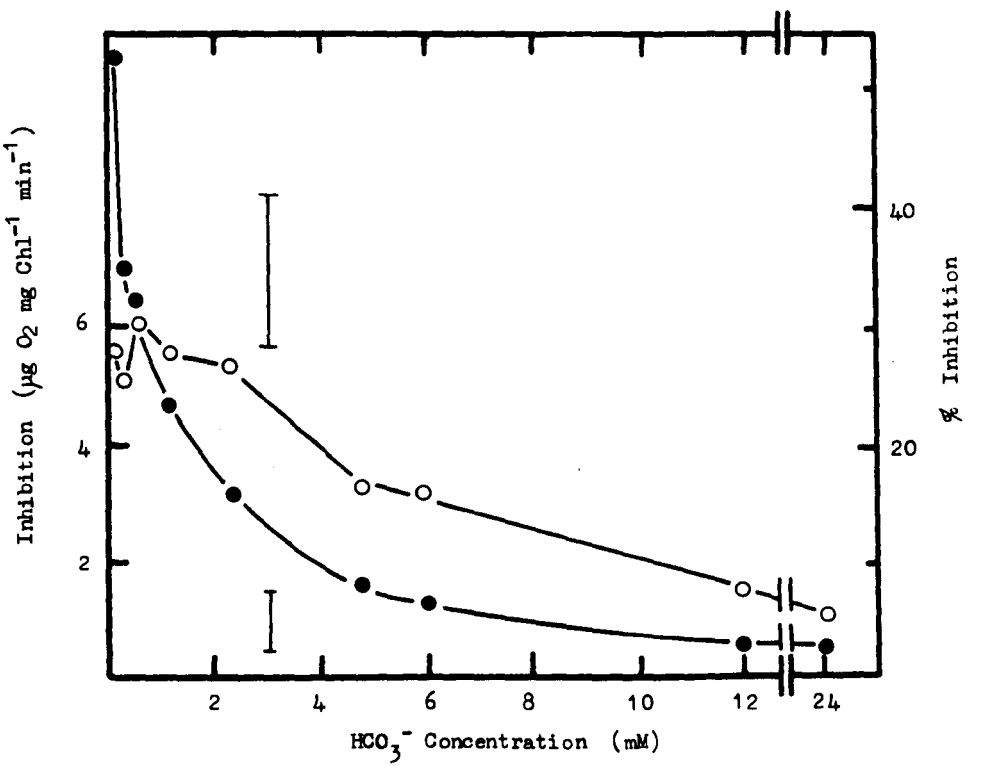

50
40
30
20
10
0
-10
2
4
6
8
10
12
24
O_2 Evolution or Uptake (μg O_2 mg Chl^{-1} min^{-1})
HCO_3^- Concentration (mM)
I
I

Figure 3.5 The effect of HCO_3^- concentration on O_2 inhibition of apparent photosynthesis (○) and percentage inhibition of photosynthesis by O_2 (●) in E. canadensis leaves. Assay conditions were as described in Figure 3.4. Bars indicate LSD (Tukey) at $P \leq 0.05$ for comparing any one mean on a line with any other mean on the same line.


Inhibition (μg O_2 mg Chl^{-1} min^{-1})
% Inhibition
HCO_3^- Concentration (mM)
2
4
6
8
10
12
24
20
40

observed decrease in percentage inhibition with increasing HCO_3^- concentration differs from the report of Søndergaard (1979) that in E. canadensis an increase in free CO_2 concentration from a non-saturating 160 μM to near saturation at 900 μM (at pH 5) did not alter the relative rates of photosynthesis at high and at low O_2 concentrations. No reason for this discrepancy can be found.

The repression of O_2 inhibition of photosynthesis by either increasing the HCO_3^- concentration or decreasing O_2 (Figure 3.4) fits a current theory on the mechanism of O_2 inhibition of photosynthesis, i.e. that O_2 competes with CO_2 for the same site on RuBP carboxylase (Andrews et al., 1971, 1973; Ogren and Bowes, 1971; Bowes, et al., 1971).

The effect of pH on Oxygen Exchange

At constant total inorganic carbon (2.4 mM) the light-saturated photosynthetic O_2 evolution was maximal at pH 5 to 6 and declined thereafter to give very low rates at pH 9 (Figure 3.6). These results are consistent with those in other reports of a decrease in photosynthetic rates of submersed aquatic macrophytes as the pH of the bathing medium increased (Brown et al., 1974; Shiyan and Merezhko, 1972; Steemann Nielsen, 1960; Van et al., 1976).

Oxygen inhibition of photosynthesis was greatest at pH 7.5 whilst percentage inhibition of photosynthesis by O_2 increased from 3% at pH 5 to 45% at pH 9 (Figure 3.7). The observed increase in percentage inhibition of photosynthesis by O_2 is consistent with reports for isolated chloroplasts (Dodd and Bidwell, 1971; Robinson, et al., 1977) As the pH of the bathing medium increases, at constant inorganic carbon, the equilibrium between CO_2 and HCO_3^- shifts towards HCO_3^-, whilst the effect on O_2 is negligible. Thus the data in Figure 3.7 do not show

Figure 3.6 Apparent photosynthetic O_2 evolution, and O_2 uptake in the dark by E. canadensis leaves at different pH values under 2 (o) and 10 (•) mg O_2 l^{-1}. Assay conditions were as described for Figure 3.1 except that the assay solution was buffered with 50 mM NaCitrate-NaH_2PO_4 at pH 5 and 6, and 50 mM Tris-HCl at pH 7 to 9. Bars indicate LSD (Orthogonal) at $P \leq 0.05$ for comparisons between O_2 concentrations at any one pH. Analysis of the raw O_2 evolution data only, showed positive correlation between residuals and the treatment effects, but square root transformation yielded significant results. For reasons of consistency, the raw data are plotted.

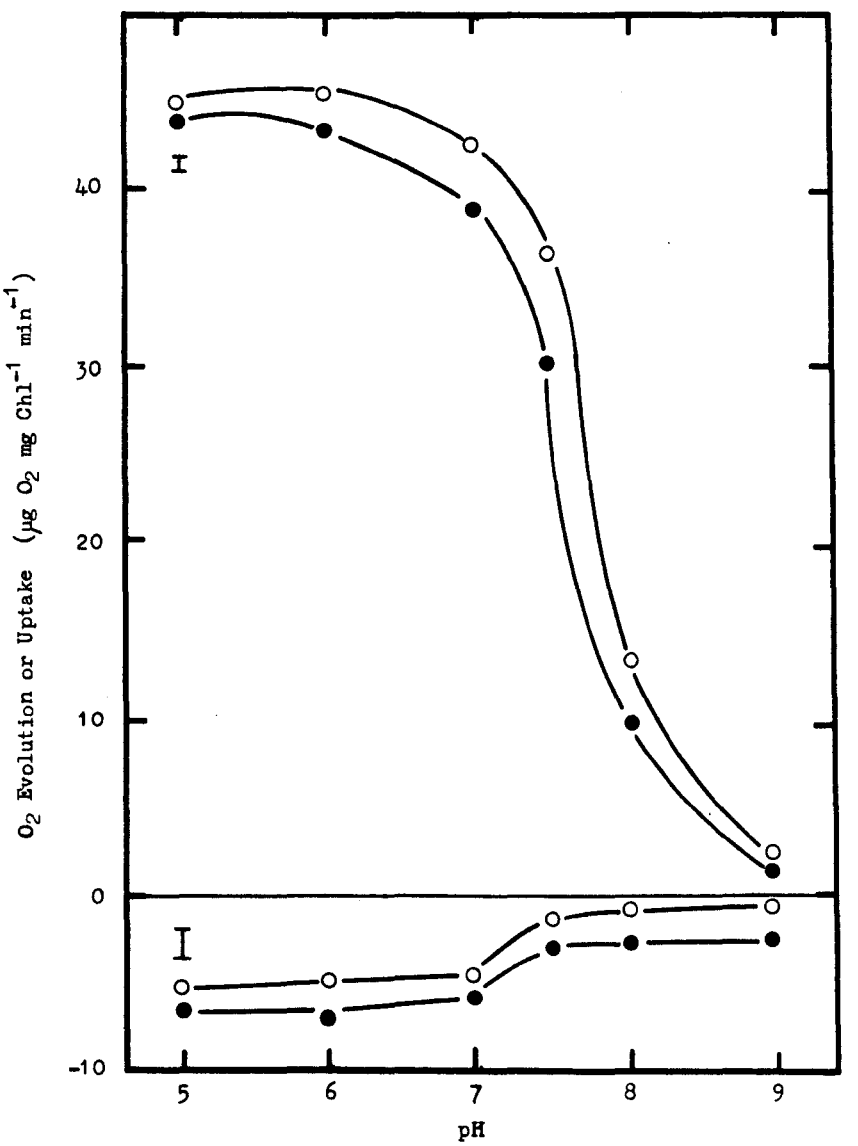
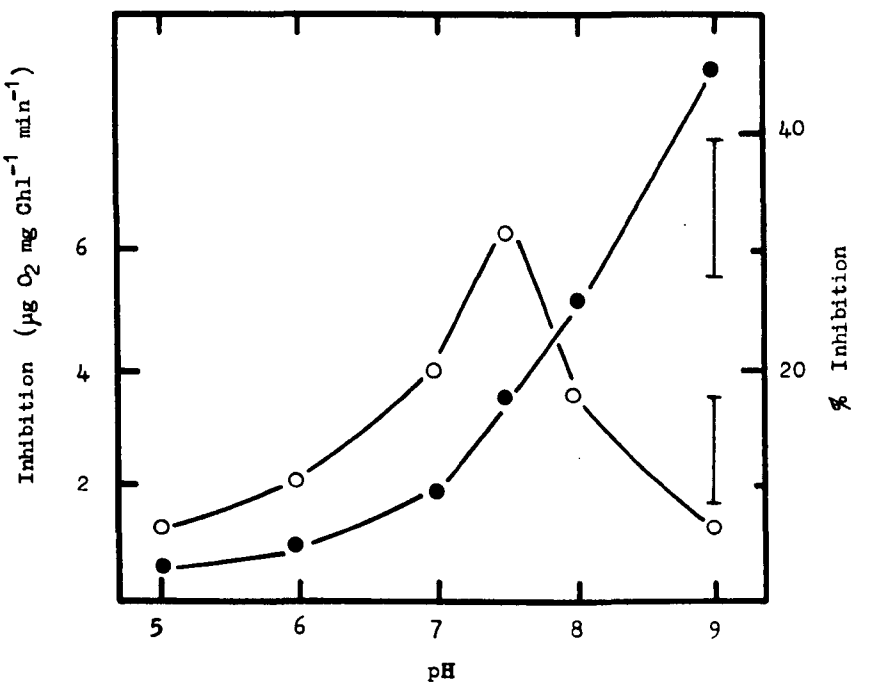

O_2 Evolution or Uptake (μg O_2 mg Chl^{-1} min^{-1})
40
30
20
10
0
-10
I
I
5
6
7
8
9
pH

Figure 3.7 The effect of pH on O_2 inhibition of apparent photosynthesis (o) and percentage inhibition of photosynthesis by O_2 (•) in E. canadensis leaves. Assay conditions were as described in Figure 3.6. Bars indicate LSD (Tukey) at $P \leq 0.05$ for comparing any one mean on a line with any other mean on the same line.


Inhibition (µg O_2 mg Chl^{-1} min^{-1})
2
4
6
5
6
7
8
9
pH
20
40
% Inhibition

that O_2 is intrinsically more inhibitory at high pH, but demonstrate the effect of reduced availability of CO_2 at high pH and consequent less successful competition of CO_2 with O_2 for RuBP carboxylase.

The effect of Light on Oxygen Exchange

Photosynthesis was light-saturated at an irradiance of 290 $\mu E\ m^{-2}s^{-1}$ (PAR) at both 2 and 10 mg $O_2\ l^{-1}$ (Figure 3.8). Oxygen inhibition of photosynthesis increased with increasing irradiance up to that required to saturate photosynthesis and thereafter remained constant (Figure 3.9). In contrast, percentage inhibition of apparent photosynthesis by O_2 remained constant over the irradiance range used.

Whilst the observed trend of increasing O_2 inhibition of photosynthesis up to light saturation is in agreement with recent reports (e.g. Holmgren and Jarvis, 1967; Ku and Hunt, 1977; Ku et al., 1977; Servaites and Ogren, 1978), the constant percentage inhibition found here contrasts with the decreasing percentage inhibition with rising irradiance reported elsewhere for E. canadensis (Kutyurin et al., 1964), potato (Ku et al., 1977) and alfalfa (Ku and Hunt, 1977). However, the inhibition irradiance relationship is greatly affected by the concentration of CO_2 and O_2 used (Bjorkman, 1973; Servaites and Ogren, 1978, Turner and Brittain, 1962). It should therefore be noted that whilst the total CO_2 concentration used by Kutyurin et al., (1964) was lower than in the present study (0.8 mM compared with 2.4 mM), the concentration of free CO_2 would have been approximately 3 times greater due to the lower pH of their experimental medium (pH 5.6 compared with pH 7.5 in the present study). Such consideration could explain the different findings for E. canadensis.

Figure 3.8 Apparent photosynthetic O_2 evolution, and O_2 uptake in the dark by E. canadensis leaves at different PAR irradiances under 2 (o) and 10 (•) mg O_2 l^{-1}. Other assay conditions were as described for Figure 3.1. Bars indicate LSD (Orthogonal) at $P \leqslant 0.05$ for comparisons between O_2 concentrations at any one PAR irradiance.

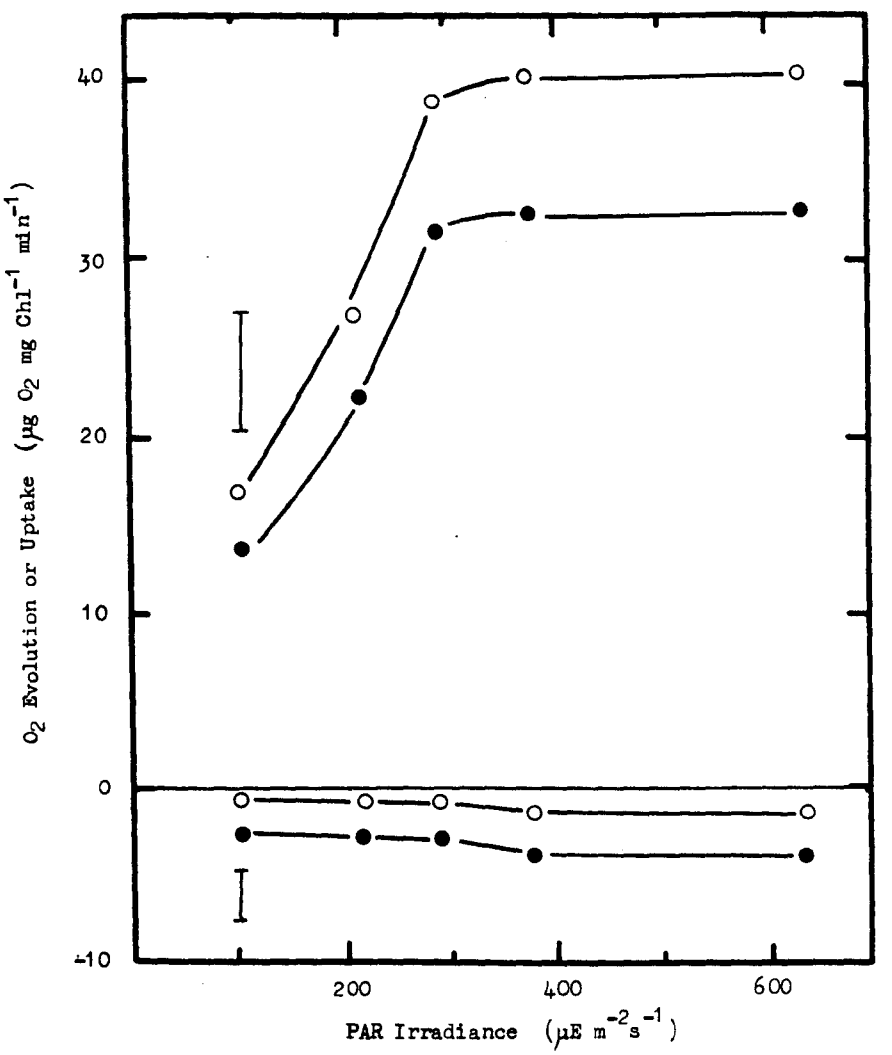
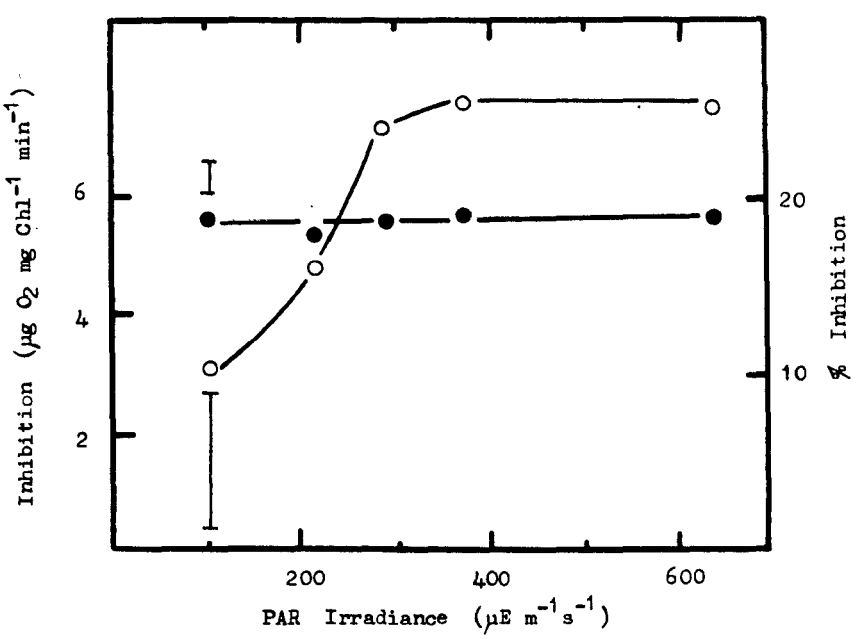

40
30
20
10
0
-10
200
400
600
O2 Evolution or Uptake (μg O2 mg Chl-1 min-1)
PAR Irradiance (μE m-2s-1)

Figure 3.9 The effect of irradiance level on O_2 inhibition of apparent photosynthesis (o) and percentage inhibition of photosynthesis by O_2 (●) in E. canadensis leaves. Assay conditions were as described in Figure 3.8. Bars indicate LSD (Tukey) at $P \leq 0.05$ for comparing any one mean on a line with any other mean on the same line.


Inhibition (μg O2 mg Chl-1 min-1)
6
4
2
20
10
% Inhibition
200
400
600
PAR Irradiance (μE m-1s-1)

Ecological Implications

The observed laboratory changes in O_2 inhibition of photosynthesis and percentage inhibition of photosynthesis by O_2 in detached E. canadensis leaves with O_2 concentration (Figure 3.1), O_2 concentration and temperature (Figure 3.3), CO_2 concentration (Figure 3.5), pH (Figure 3.9) are consistent with the process of photorespiration occurring in E. canadensis.

Of particular relevance, in terms of the plant's productivity, is the high level of inhibition of photosynthesis at high pH (Figure 3.7) and at high O_2 tensions (Figures 3.1 and 3.3). It would appear, on the basis of these laboratory studies, that the high O_2 tensions and high pH conditions which develop in dense stands of submersed waterplants (e.g. Brown et al., 1974; Sculthorpe, 1967; Van et al., 1976), especially in calm weather conditions, may severely limit the photosynthetic performance of the plants especially if they are exposed to high pH and O_2 conditions for extended periods of time, and photorespire.

Comparing the conditions of E. canadensis stands in the Leeds and Liverpool Canal (see Introduction) with those studied experimentally, it will be noted that at 20°C a rise of O_2 from 10 to 15 mg l^{-1} reduced photosynthesis in the laboratory from 30.8 to 25.2 µg O_2 mg Chl^{-1} min^{-1} (Figure 3.2). The effect of rising pH was more severe. At 10 mg O_2 l^{-1}, a rise from pH 7.5 to pH 9.0 reduced photosynthesis from 30.2 to 1.5 µg O_2 mg Chl^{-1} min^{-1} (Figure 3.6). Bearing in mind the fact that in the field O_2 and pH rises occur simultaneously and often exceed the experimental limits quoted, the laboratory results indicate the likelihood of severe limitation of photosynthesis under these field conditions. Such reductions in the rate of gaseous exchange could have very significant effects on the balance of respiration, photorespiration and photosynthesis

and consequently on field growth of species such as E. canadensis. It must also be remembered that temperatures can exceed 20°C and light intensities are probably saturating in the surface water layer for lengthy periods and both these factors would tend to aggravate inhibition. Even so, the laboratory results may still underestimate the extent of inhibition in the field because of the artificially favourable degree of water movement occurring in the laboratory apparatus, as compared with the field.

Photosynthetic and respiratory metabolism of aquatic vegetation are generally stimulated by rising water flow rate (e.g. Dromgoole, 1978; Westlake, 1967), and for this reason rates determined in the rapidly stirred conditions of this present study are probably much greater than would normally be attained in slowly flowing non-turbulent field conditions. Consquently the greater boundary layer resistances in the field will limit CO_2 supply at high pH and also aggravate O_2 inhibition of photosynthesis more severely than these laboratory studies would suggest.

An afternoon depression of apparent photosynthesis has been demonstrated in several submersed angiosperms (e.g. Hartman and Brown, 1967; Van et al., 1976). It has been argued that the progressive increase in light intensity and O_2 tension, and the possible decrease in CO_2 availability, which occur in daytime, may progressively increase photorespiration through the day, and hence explain this afternoon depression of apparent photosynthesis (Hough, 1974). The strong dependence of photosynthesis on O_2 and CO_2, which these results demonstrate, support this possibility.

There appear to be a number of ways in which the limitations imposed upon the plant by photorespiration and associated O_2 effects on photosynthesis might be ameliorated. One possibility is the utilization of HCO_3^- as an

inorganic carbon source for photosynthesis through carbonic anhydrase activity (e.g. Sculthorpe, 1967; Steemann Nielsen, 1960); another is through active C_4-metabolism suggested by high ^{14}C fixation into C_4-acids (Brown et al., 1974; De Groote and Kennedy, 1977). However, there is controversy about the role of HCO_3^- in the photosynthesis of aquatic plants. Earlier claims of HCO_3^- utilization were based upon pH increases in unbuffered media, but these increases cannot in themselves be taken to indicate either photosynthesis (Thomas and Tregunna, 1967) or HCO_3^- utilization (Raven, 1970). More recent studies showed no evidence of HCO_3^- utilization in three aquatic macrophytes including E. canadensis (Brown et al., 1974). Egeria densa has been reported to exhibit high ^{14}C fixation into C_4-acids (Brown et al., 1974), but when experiments were repeated at natural CO_2 concentrations (Browse et al., 1977), the distribution of ^{14}C fixed into phosphoglyceric acid, sugar phosphates and sucrose indicated that the Calvin cycle was the primary carboxylation mechanism, and that the predominant ^{14}C fixation into malic acid, noted in previous resports on Hydrocharitacean species, might have been caused by very low experimental CO_2 concentrations and consequent supression of Calvin cycle activity. In addition, pulse-chase experiments of E. canadensis indicated that whilst a ^{14}C exposure period of 2 seconds resulted in 45% of the label being in C_4-acids, there was little subsequent turnover of this label in the C_4-acids (De Groote and Kennedy, 1977). This suggests that little of the organic acid produced was involved in a C_4-type of carbon supply to the Calvin cycle. It therefore seems that neither HCO_3^- utilization nor C_4-metabolism significantly ameliorate the effects of CO_2 depletion and O_2 elevation on E. canadensis.

A further way in which problems of restricted CO_2 and O_2 exchange might be ameliorated could be through gas movement and storage within the lacunar system (Raven, 1970). Indeed, both Lobelia dortmanna (Wium-Anderson, 1971) and Littorella uniflora (Sønderg aard and Sand-Jensen, 1979) have been shown to photosynthesize $^{14}CO_2$ fed to their roots. However, although gas lacunae do occur in E. canadensis, its morphology is unfavourable to lacunar gas movement and storage. As a consequence, CO_2 and O_2 are unlikely to diffuse through the lacunae in sufficient quantities to significantly affect the photosynthetic performance of the plant (J.W. Eaton, personal communication), and lacunar storage and refixation of photorespired CO_2 is probably minor (J.W. Eaton, personal communication; Sønderg aard, 1979).

It therefore seems likely that under field conditions of rising O_2 and pH, the photosynthetic performance of E. canadensis will be progressively impaired, and that under extreme conditions apparent photosynthesis may cease, even though temperature and light are near-optimal.

A review of the recent literature on aquatic photosynthesis reveals little mention of the effect of leaf position on photosynthesis, yet reference to the importance of such factors abound in the literature on terrestrial plants. Preliminary studies (see Section 2) showed that the photosynthesis of older leaves was less than that of younger apical leaves. Therefore this section describes the effect of leaf position on oxygen exchange characteristics of Elodea canadensis. Rates are expressed in terms of both chlorophyll and leaf area, since units of expression might also affect interpretation.

4. THE INFLUENCE OF LEAF POSITION ON APPARENT PHOTOSYNTHESIS AND RESPIRATION OF THE SUBMERSED MACROPHYTE ELODEA CANADENSIS

ABSTRACT

The effect of leaf position on apparent photosynthesis, photosynthetic light saturation, O_2 inhibition of photosynthesis and dark respiration has been determined for detached leaves of Elodea canadensis Michx. (Hydrocharitaceae). Distinct patterns of change of apparent photosynthesis and dark respiration with leaf position were found when rates were expressed per unit chlorophyll and per unit leaf area. The patterns of change of O_2 inhibition of photosynthesis were similar on both chlorophyll and leaf area bases. These patterns are discussed in relation to known aging effects on photosynthesis, photorespiration and dark respiration in terrestrial plants. Light saturation and compensation points for photosynthesis were highest in apical leaves and were reduced further down the plant. This trend is discussed in relation to adaptation to light gradients in natural populations of aquatic plants.

INTRODUCTION

Submersed macrophytes live in a light climate which varies greatly in both space and time. Absorption by the water causes a decrease in light availability with depth, which is especially rapid in turbid or coloured waters (Hutchinson, 1957; Sculthorpe, 1967). The seasonal cycle of insolation supplies more light in summer than in winter, a difference which increases with latitude and is very marked in temperate areas. The plant experiences these environmental gradients, but modifies its experience of them by its own growth. Thus a plant growing upwards from the base of the water column produces its first leaves in deeper, and therefore less brightly illuminated water than

its later leaves. Apical growth progressively shades the older parts of the stem below, and this effect is accentuated when branching or dense plant stands develop. Leaves may become colonised by epiphytes or encrusted with marl over a period of time. The light reaching the plant's photosynthetic tissues is thereby reduced, and the effect is greatest in older, and therefore, at least on the main stem, lower leaves. Superimposed on these influences are the seasonal cycle of insolation, and the aging of the leaves themselves, which again in the case of the mainstems, will be greatest in lower leaves.

It is therefore not surprising that submerged macrophytes show great variations in photosynthetic rate with depth, season and age of plant, and that in some cases their photosynthetic behaviour is adapted to reduce the deleterious effects of light attenuation at some depths and times of year. Early workers incubated plant samples of similar age at different depths, and found that photosynthetic rate depended upon incident light, water turbidity and the species investigated, but was generally maximal between 0 and 5 m depth (e.g. Meyer and Heritage, 1941; Meyer et al., 1943; Ruttner, 1926; Schomer, 1934). More recent studies using plant samples incubated at their depths of natural occurrence have confirmed that maximal photosynthesis usually occurs in the upper part of the water column. Adams et al. (1974) concluded that in Myriophyllum spicatum the depth distributions of biomass and light were major factors causing depth variation in photosynthesis, and that tissue aging with depth was not of prime importance. However Ikusima (1965) attributed depth difference in photosynthesis in Vallisneria asiatica to a 'gradient of physiological activity inherent to different depths of the community.'

Seasonal variations in photosynthetic and photorespiratory capacity are also reported (Bowes et al., 1977a; Hough, 1974; Søndergaard, 1979). These variations may in part be aging effects, only indirectly linked to environmental variation through winter growth resulting in a higher average leaf age than in summer. In terrestrial plants, photosynthesis and photorespiration increase to a maximum at maturity, and later decline during senescence, whereas respiration is rapid at first and diminishes at maturity, though it may increase again at senescence (e.g. Aslam et al., 1977; Catsky et al., 1976; Mokronosov and Nekrasova, 1977). It appears possible that similar aging effects could play a part in depth differences in photosynthesis of aquatic plants.

Adaptation to light attenuation with depth has been inferred from reduced light saturation points and more efficient utilization of light by deep water plants compared with shallow water ones, as well as from morphological differences (Adams et al., 1974; Bowes et al., 1977b; Gessner, 1938; Ikusima, 1966; Spence and Chrystal, 1970). Such adaptation may be independent of aging effects, and may counteract to some extent the disadvantage of diminishing light with depth.

Here the variation of photosynthetic and respiratory behaviour is described for mainstem leaves with depth and leaf age in actively growing summer plants of Elodea canadensis. Seasonal variations in these metabolic features are reported in thesis Section 5 and a more detailed account of the plant's response to photosynthetic stress conditions is given in thesis Section 3.

MATERIALS AND METHODS

Plant Material

Samples of E. canadensis were collected from the Leeds and Liverpool

Canal, near Aintree, Merseyside (O.S. 393990) in June, 1979. The population sampled was green, actively growing, of healthy appearance and there were few epiphytes especially on the most apical 15 cm section. The shoots had not reached the water surface and were vertical in the water column. These samples were maintained in the laboratory in canal water at 15°C, under a 16 hour photoperiod with illumination at 54 $\mu E\ m^{-2}s^{-1}$ (PAR). When necessary, epiphytes were removed by gently brushing the leaves and rinsing in several changes of water.

Photosynthetic and Respiratory Rate Determination

Photosynthetic and respiratory rates of leaves were measured as O_2 evolution or uptake using a Clarke-type O_2 electrode (Rank Brothers, Cambridge). The light source was a 250-W projector bulb, and the intensity was varied by altering the distance between the light source and the reaction vessel. During tests to determine the response of leaves to irradiance, the leaves were first exposed to randomised lower light intensities and then to higher light intensities. At these high light intensities (light saturation point and above) the leaves were exposed to successively increasing light. This pattern of exposure was employed to minimise the risk of light damage to the photosynthetic apparatus and any subsequent effects on rates, although there was no evidence of any such damage.

Leaf samples comprised the three leaves of a whorl, and were taken from undamaged mainstems only. These samples were placed directly into the assay solution, which was a modified Forsberg Medium No. II (Forsberg, 1965), modified by omission of Na_2SiO_3, and containing: 2.4 mM total inorganic carbon; 50 mM Tris-HCl, pH 7.5; and 2 or 10 mg $O_2\ l^{-1}$ achieved by sparging with N_2 or O_2 gases as appropriate. Oxygen exchange was

determined at 20°C in the light or in darkness. All treatments were run in triplicate.

Since interpretation can be affected by the units in which results are expressed (see e.g. Malkina, 1976; Mokronosov and Nekrasova, 1977), metabolic rates were calculated on both a total chlorophyll and a leaf area basis. Leaf areas were measured under a microscope at x10 magnification after completion of the experiments. Chlorophylls a and b, and total chlorophyll were determined by the method of Arnon (1949).

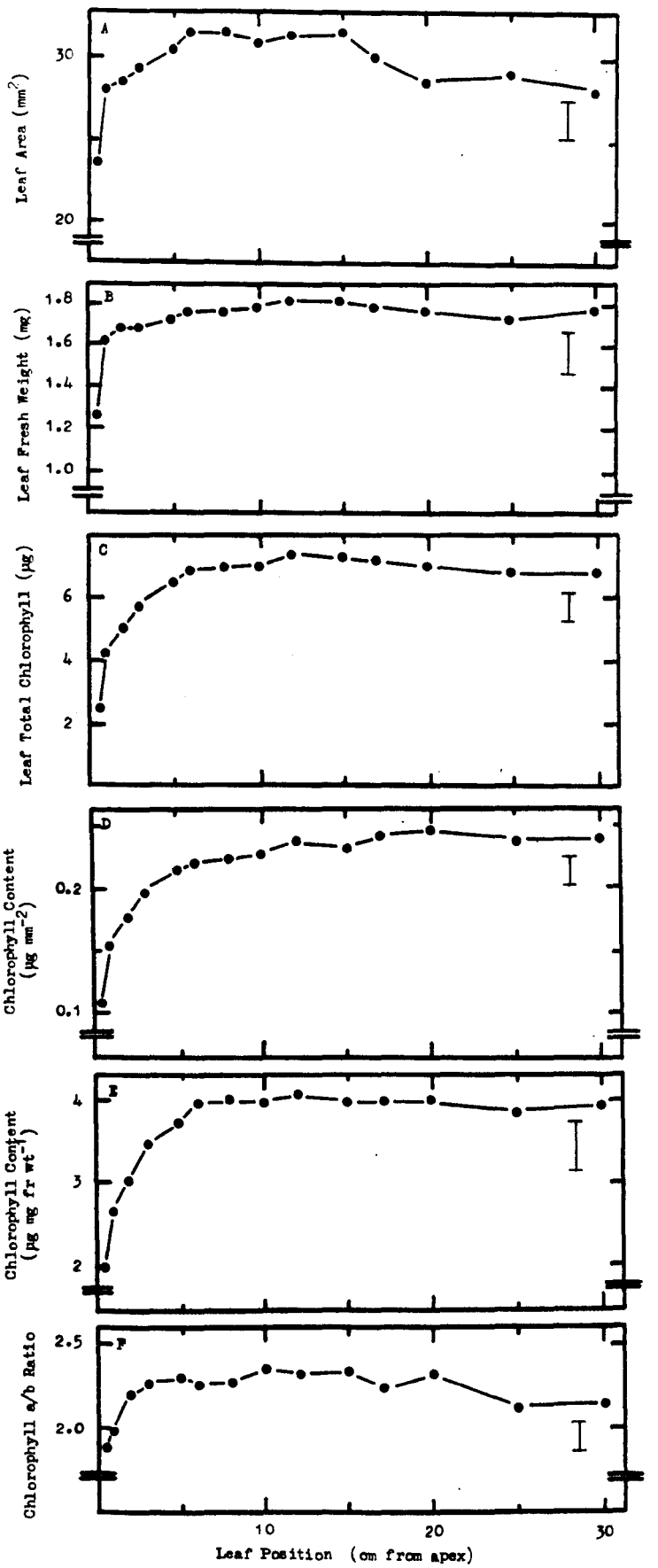
RESULTS AND DISCUSSION

Chlorophyll and Leaf Area

Differences in chlorophyll content and leaf weight and area with leaf position are shown in Figure 4.1. Chlorophyll per unit leaf area and per unit fresh weight increased from the apex downwards to a maximum from 5 - 6 cm onwards. Leaf area and fresh weight also increased between the apex and 5 - 6 cm, presumably due to expansion of the maturing laminae. Between 6 - 15 cm area was maximal. Below this the leaves were distinctly smaller.

During the lifespan of a single leaf, the normal pattern is for an early phase of leaf expansion and increase in chlorophyll concentration to be followed by a mature phase of stable size and pigment concentration, and finally a phase of declining pigment concentration as the leaf senesces (Sestak et al., 1975; Treharne et al., 1968). The E. canadensis samples showed all these features except the final decline of chlorophyll content, perhaps because the plants were collected in June from a vigorously growing population in which no parts had started to decline.

Figure 4.1 A: Leaf area, B: leaf fresh weight, C: leaf total chlorophyll, D and E: leaf chlorophyll content, and F: chlorophyll a/b ratio in E. canadensis. Data points represent the mean of at least six replicates. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


A
Leaf Area (mm²)
30
20
B
Leaf Fresh Weight (mg)
1.8
1.6
1.4
1.2
1.0
C
Leaf Total Chlorophyll (µg)
6
4
2
D
Chlorophyll Content (µg mm⁻²)
0.2
0.1
E
Chlorophyll Content (µg mg fr wt⁻¹)
4
3
2
F
Chlorophyll a/b Ratio
2.5
2.0
10
20
30
Leaf Position (cm from apex)

Sestak (1977) reports that in general the earliest and latest leaves are smaller at maturity than the intermediate ones. This variation probably explained the smaller size of the oldest leaves in E. canadensis, which would have developed at an early stage in the plant's seasonal growth.

The ratio of chlorophyll a/b showed the increase, steady-state and then decline with distance from the apex previously reported for ontogenesis of a single leaf (Sestak, 1977; Sestak et al., 1975).

Chlorophyll a/b ratio can vary in response to light intensity. In general there is an inverse relationship, the proportion of chlorophyll b increasing as the light intensity is reduced (Boardman, 1977; Egle, 1960; Seybold and Egle, 1937). A greater proportion of chlorophyll b in aquatic plants has been considered as a chromatic adaptation to the aquatic environment because this pigment improves the utilization of blue-green light (Seybold and Egle, 1937). Indeed the chlorophyll a/b ratio has been shown to be reduced with increasing depth, and thus diminishing light, in Hydrilla verticillata (Van et al., 1977) and in M. spicatum (Marcus, 1980) and has reduced with depth in mature E. canadensis leaves (Table 4.1). The chlorophyll a/b ratio for E. canadensis leaves (Figure 4.1 and Table 4.1) is of the same order as that reported for other aquatic plants, and is much less than the ratio of 4.4 for emersed aquatic plants and sun adapted plants (Seybold and Egle, 1937). However as the decreased chlorophyll a/b ratio has two explanations i.e. a natural aging progression and chromatic adaptation, it is not clear which is operating here with E. canadensis.

Table 4.1 The relationship between leaf position and leaf chlorophyll content, chlorophyll a/b ratio, light compensation point (L.C.P.), light saturation point (L.S.P.), photosynthesis and respiration in E. canadensis

Leaf Position (cm below the apex)	Chlorophyll Content ($\mu g/mm^2$)	Chlorophyll a/b Ratio	L.C.P. ($\mu E/m^2.s$)	L.S.P. ($\mu E/m^2.s$)	Light-saturated Photosynthetic Rate ($\mu g\ O_2/mg$ Chl. min)	Rate of Dark Respiration ($\mu g\ O_2/mg$ Chl. min)	Dark Respiration (percent of light-saturated photosynthesis)
0.5	0.11 ± 0.009**	1.87	23	350	40.9 ± 3.0*	7.9 ± 0.6*	19.3
1	0.15 ± 0.009	1.96	22	320	36.1 ± 1.6	7.7 ± 0.4	21.3
5	0.21 ± 0.009	2.29	17	290	29.8 ± 1.2	3.9 ± 0.2	13.1
10	0.23 ± 0.006	2.34	15	280	27.0 ± 1.2	4.2 ± 0.4	15.6
20	0.25 ± 0.008	2.32	10	270	23.3 ± 0.8	4.0 ± 0.4	17.2
30	0.24 ± 0.008	2.14	10	240	20.0 ± 1.8	5.3 ± 0.4	26.5

** Mean of nine replicates ± standard deviation

* Mean of three replicates ± standard deviation

Photosynthesis and Respiration

Apparent photosynthesis and dark respiration per unit chlorophyll were greatest in the youngest apical leaves (Figure 4.2). They both declined rapidly down to a leaf position 6 cm below the apex, which has been shown to be the position of maximum chlorophyll content, chlorophyll a/b ratio, leaf area and leaf fresh weight. Below 6 cm the rate of decline in apparent photosynthesis at 10 mg O_2 l^{-1} was more gradual so that in the oldest leaf studied (30 cm from the apex) the photosynthetic rate was approximately half that at 0.5 cm. This pattern was essentially the same at both subsaturating and saturating irradiances (Figure 4.3). The rate of dark respiration at 10 mg O_2 l^{-1} did not change with leaf position below 5 cm (Figure 4.2).

The pattern of differences in apparent photosynthesis of E. canadensis leaves conforms to the general trend reported by Mokronosov and Nekrasova (1977) for aging in potato leaves, when their data was expressed on a unit chlorophyll basis. These authors suggested that the decrease in apparent photosynthesis per unit chlorophyll with age may be associated with accumulation of photosynthetically inactive forms of free chlorophyll, or an increase in the amount of antenna chlorophyll in the photosynthetic unit. If the decrease in apparent photosynthesis reported here for E. canadensis is associated with an increase in the amount of antenna chlorophyll this could be advantageous to the plant in combating the deleterious effects of attenuation of light by water and plant self-shading in natural stands.

There were pronounced differences in O_2 inhibition of apparent photosynthesis per unit chlorophyll with leaf position (Figure 4.2B). Inhibition was slight in the immature apical leaves, increased to a

Figure 4.2 The relationship between leaf position and A: apparent photosynthetic O_2 evolution, and O_2 uptake in the dark under 2 (o) and 10 (•) mg O_2 l^{-1}, and B: oxygen inhibition of apparent photosynthesis in E. canadensis leaves when rates are expressed per unit chlorophyll. The assay solution was a modified Forsberg medium No. II containing 2.4 mM total inorganic carbon and 50 mM Tris-HCl, pH 7.5. Oxygen exchange was determined at 20°C at a PAR irradiance of 290 µE $m^{-2}s^{-1}$ or in darkness. All data points in this and subsequent figures represent the mean of triplicate determinations. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.

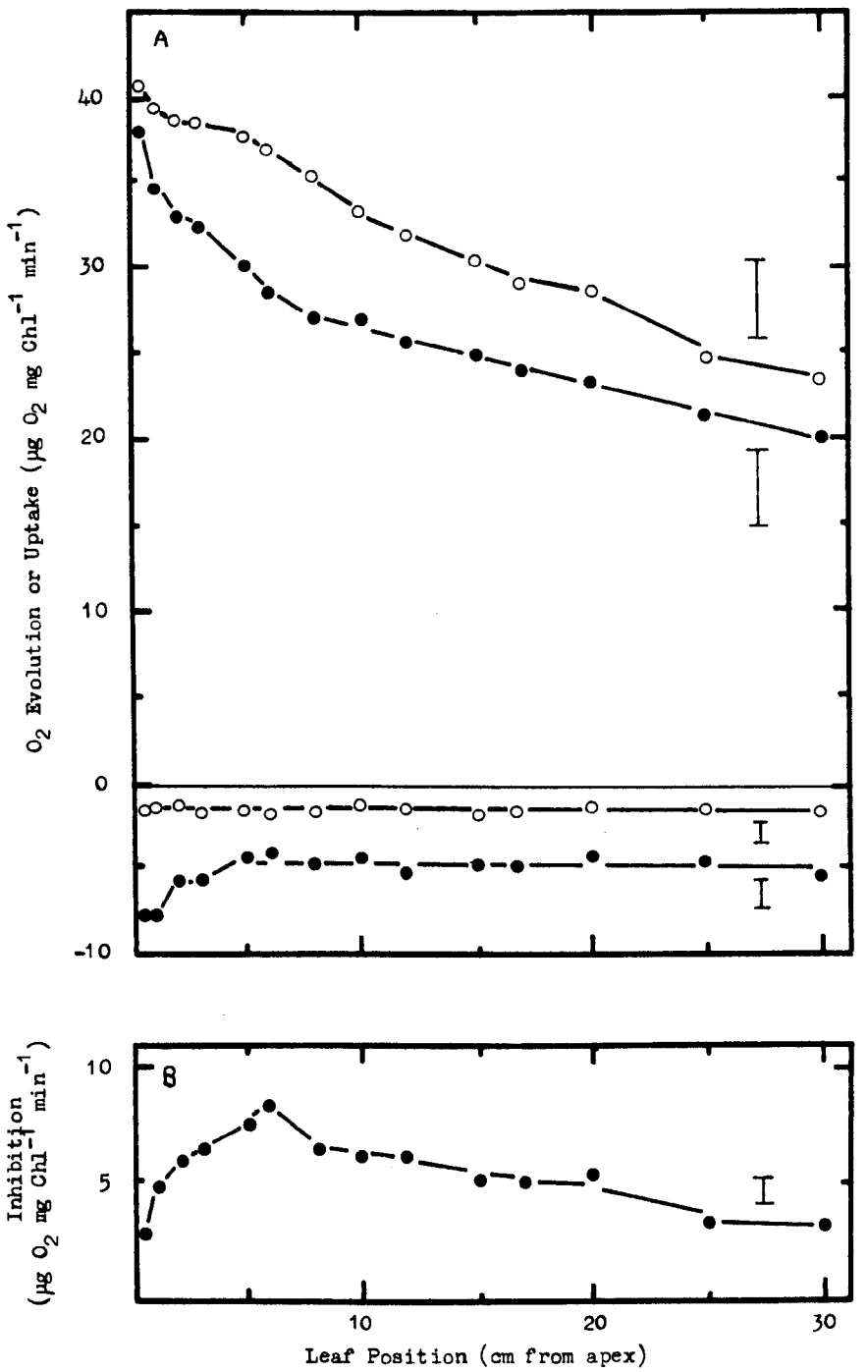
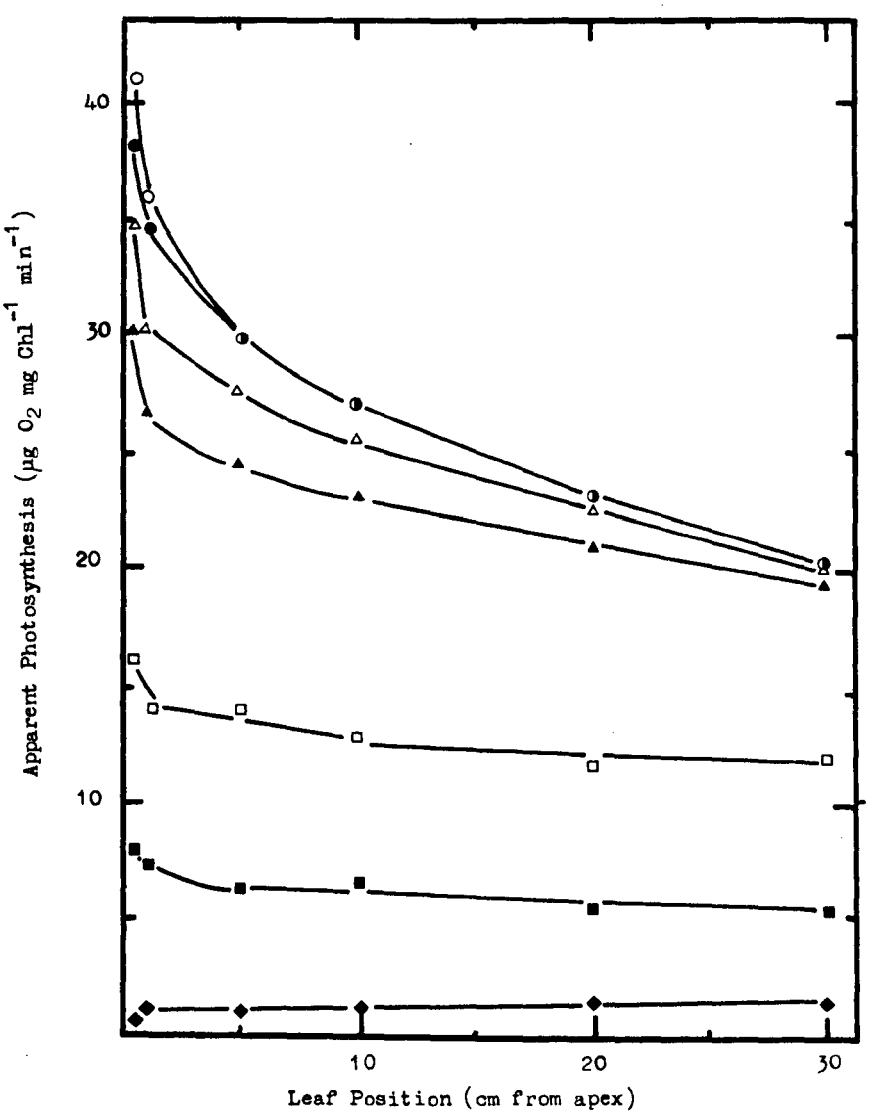

A
40
30
20
10
0
-10
O_2 Evolution or Uptake ($\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$)
B
10
5
Inhibition ($\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$)
10
20
30
Leaf Position (cm from apex)

Figure 4.3 The relationship between leaf position and apparent photosynthetic O_2 evolution in E. canadensis leaves under 10 mg O_2 l^{-1} when rates are expressed per unit chlorophyll. Oxygen exchange was determined at a PAR irradiance of 25 (◆), 55 (■), 104 (□), 220 (▲), 254 (△), 290 (●) or 380 (○) μE m^{-2} s^{-1}. Other assay conditions were as described in Figure 4.2.


40
30
20
10
Apparent Photosynthesis (μg O_2 mg Chl^{-1} min^{-1})
10
20
30
Leaf Position (cm from apex)

maximum 6 cm below the apex, and declined gradually thereafter.

Oxygen inhibition of apparent photosynthesis has been demonstrated in submersed aquatic plants including E. canadensis, and can be used to indicate the magnitude of photorespiration (see thesis Section 3). The use of O_2 inhibition of photosynthesis to estimate photorespiration assumes that mitochondrial (dark) respiration is unaffected by changes in O_2 concentration. This may be so for terrestrial plants (Jackson and Volk, 1970) but is not the case in aquatic plants (Dromgoole, 1978; Owens and Maris, 1964; Westlake, 1967). However, because dark respiration is likely to be reduced in the light (Jackson and Volk 1970; Mangat et al., 1974), and because the increase in dark respiration with increased O_2 is less than the increase in apparent photosynthesis in all but the youngest leaves (Figure 4.2), it was considered that O_2 inhibition of apparent photosynthesis could provide an estimate of photorespiration in E. canadensis.

The pattern of differences of O_2 inhibition of photosynthesis with leaf insertion level conforms with changes of photorespiration with leaf aging reported in bean (Catsky et al., 1976), mustard (Cornic et al., 1970), poplar (Dickmann et al., 1975), lucerne (Hodgkinson, 1974) and tobacco (Kisaki et al., 1973). The differences reported here for E. canadensis are also consistent with the possibility of aging being a factor in the observed seasonal changes in photorespiratory metabolism of the plant (Søndergaard, 1979), as has been suggested for H. verticillata (Bowes et al., 1977a) and Najas flexilis (Hough, 1974).

When apparent photosynthetic and dark respiratory rates are expressed on a leaf area basis (Figure 4.4), a different pattern is observed. Apparent photosynthetic O_2 evolution was least in the young apical

leaves and increased to a maximum 3 cm below the apex. Apparent photosynthesis then declined gradually with distance through to the oldest leaf studied at 30 cm. Whilst it will be shown later that the photosynthetic rates of the 0.5 cm and 1 cm leaves were not quite light saturated by 290 μE $m^{-2}s^{-1}$ PAR irradiance, a similar relationship between leaf position and photosynthetic rate was obtained at both subsaturating and saturating irradiances (Figure 4.5). In contrast to photosynthesis, dark respiration was relatively stable for leaves sampled from all positions 0.5 - 30 cm below the apex, when expressed on a per unit leaf area basis (Figure 4.4).

The observed trends in apparent photosynthesis per unit leaf area with leaf position and age are similar to those described for terrestrial plants (e.g. Aslam et al., 1977; Catsky et al., 1976; Dickmann et al., 1975; Kisaki et al., 1973; Mokronosov and Nekrasova, 1977).

The relationship between photosynthesis per unit area and leaf position reported here for E. canadensis is similar to that between photosynthesis per unit weight and depth of occurrence in Ceratophyllum demersum (Carr, 1969) and V. asiatica (Ikusima, 1965), when all plant material was incubated under the same experimental conditions. Thus it could be that differences in photosynthesis of aquatic plants with depth can be caused by the differing physiological conditions of material with depth and age, as well as by light attenuation. In view of the differences in the relationship between leaf position and photosynthesis which are obtained by expressing results on a per unit chlorophyll and a per unit leaf area basis in E. canadensis

Figure 4.4 The relationship between leaf position and A: apparent photosynthetic O_2 evolution, and O_2 uptake in the dark under 2 (o) and 10 (•) mg O_2 l^{-1}, and B: oxygen inhibition of apparent photosynthesis in E. canadensis leaves when rates are expressed per unit leaf area. Assay conditions were as described in Figure 4.2. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.

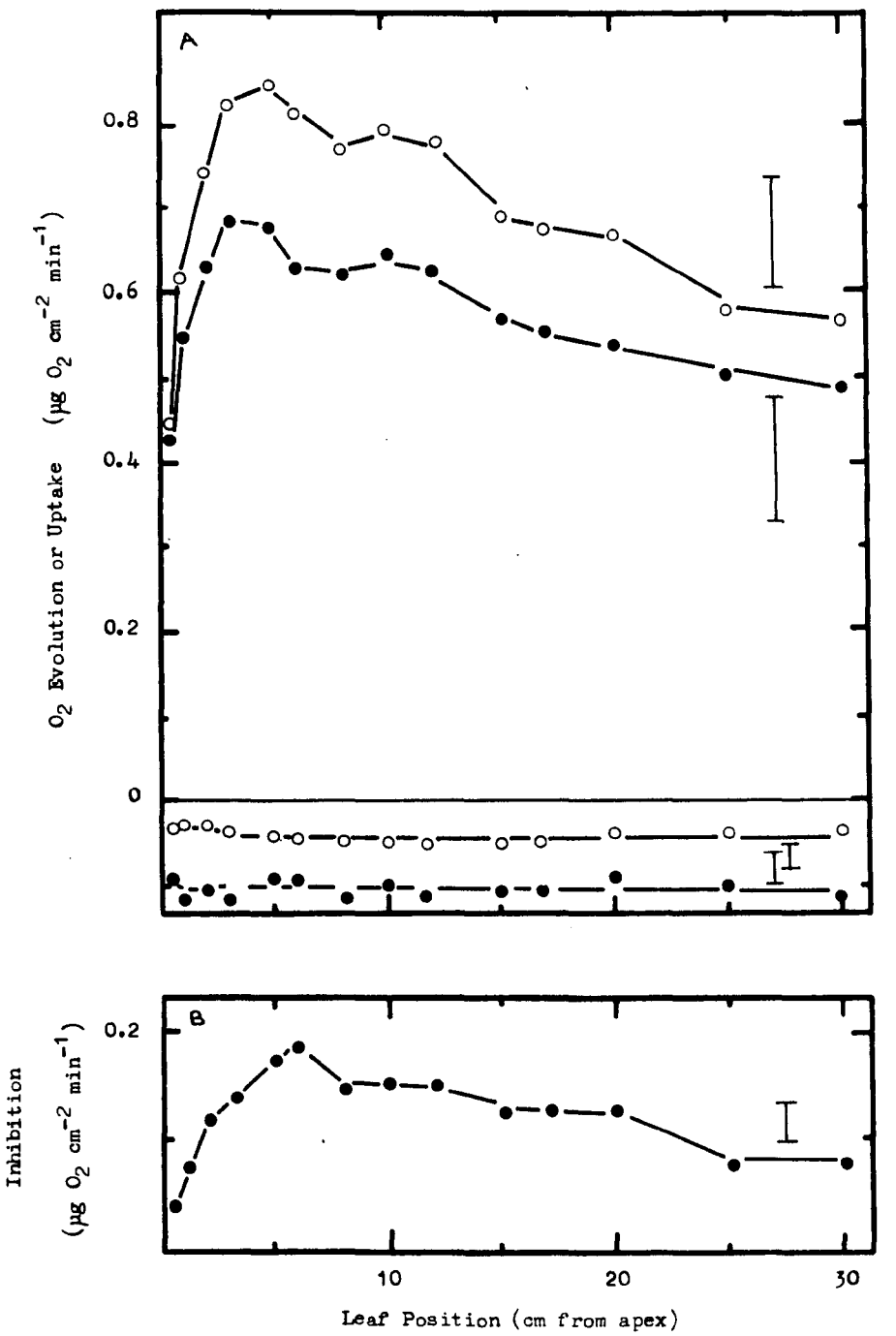
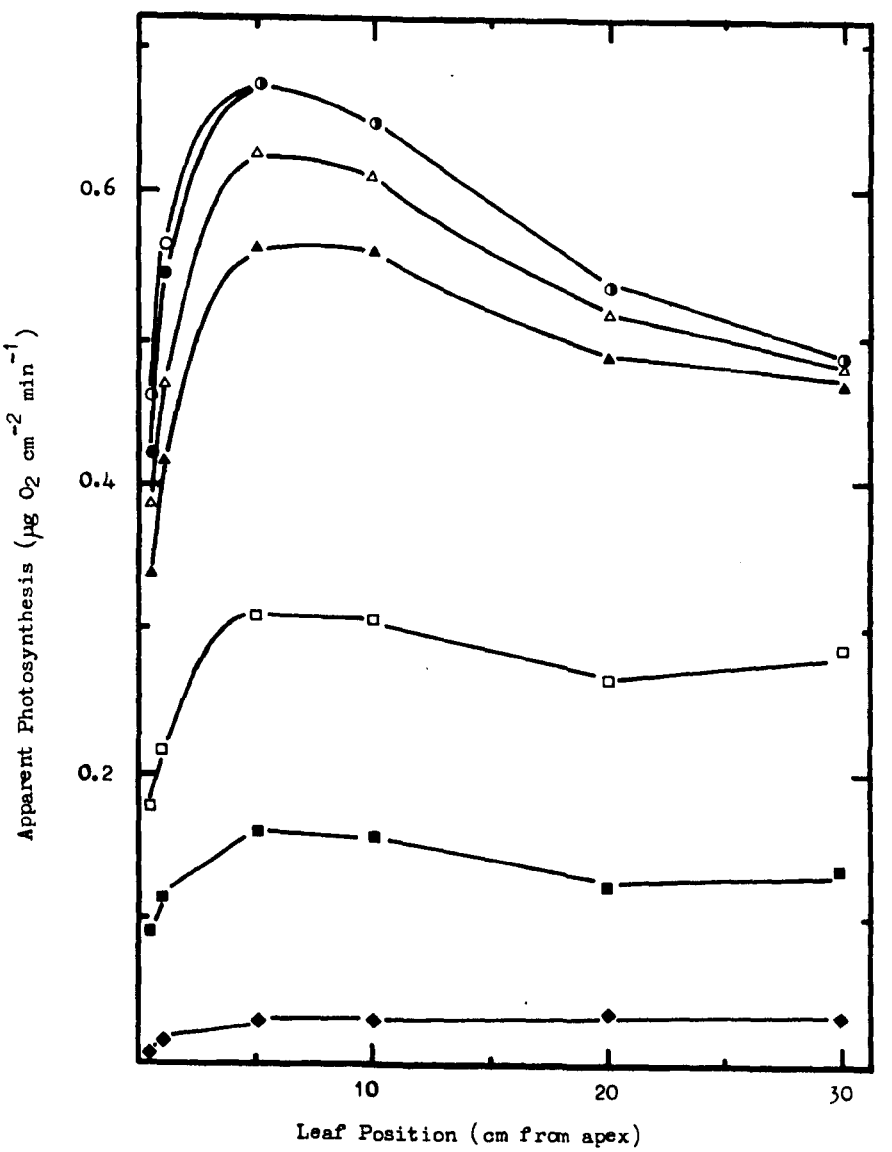

A
0.8
0.6
0.4
0.2
0
O_2 Evolution or Uptake ($\mu g\ O_2\ cm^{-2}\ min^{-1}$)
B
0.2
Inhibition
($\mu g\ O_2\ cm^{-2}\ min^{-1}$)
10
20
30
Leaf Position (cm from apex)

Figure 4.5 The relationship between leaf position and apparent photosynthetic O_2 evolution in *E. canadensis* leaves under 10 mg O_2 l^{-1} when rates are expressed per unit leaf area. Oxygen exchange was determined at a PAR irradiance of 25 (◆), 55 (■), 104 (□), 220 (▲), 254 (△), 290 (●) or 380 (○) $\mu E\ m^{-2} s^{-1}$. Other assay conditions were as described in Figure 4.2.


0.6
0.4
0.2
10
20
30
Apparent Photosynthesis (μg O_2 cm^{-2} min^{-1})
Leaf Position (cm from apex)

(compare Figures 4.2 and 4.3 with 4.4 and 4.5), it is conceivable that the depth differences reported by Carr (1969) and Ikusima (1965) would have been interpreted differently if they had been expressed in terms of pigment rather than plant weight.

Inhibition of photosynthesis by O_2 showed the same pattern whether expressed per unit area (Figure 4.4B) or per unit chlorophyll (Figure 4.2B).

Light Saturation Curves

Light saturation curves of apparent photosynthesis per unit chlorophyll and per unit area of E. canadensis leaves are presented in Figures 4.6 and 4.7 respectively. Light saturation points were highest in apical leaves, lower and rather similar in leaves from 5, 10 and 20 cm and markedly less in 30 cm leaves. Light compensation points were derived by regression analysis of the data points and showed a similar trend to that of light saturation points, being highest in apical leaves, intermediate in leaves from 5 and 10 cm, and least in the 20 and 30 cm leaves (Table 4.1).

Studies on aquatic macrophytes have shown that light adaptations can result from growth under different natural light regimes (Adams et al., 1974; Gessner, 1938; Ikusima, 1966), or be induced in mature specimens by growth under new conditions (Bowes et al., 1977b; Spence and Chrystal, 1970). It is not clear whether the depth differences in light saturation and compensation points reported here for E. canadensis are due to adaptation of young leaves to prevailing conditions at the time of growth and leaf expansion (which would have been spring) or are the result of re-adaptation by mature leaves as

Figure 4.6 Apparent photosynthetic O_2 evolution by E. canadensis leaves at different PAR irradiances under 10 mg O_2 l^{-1} when rates are expressed per unit chlorophyll. Leaves were excised from positions 0.5 (o), 1 (●), 5 (△), 10 (▲), 20 (□) or 30 (■) cm below the apex. Other assay conditions were as described in Figure 4.2.

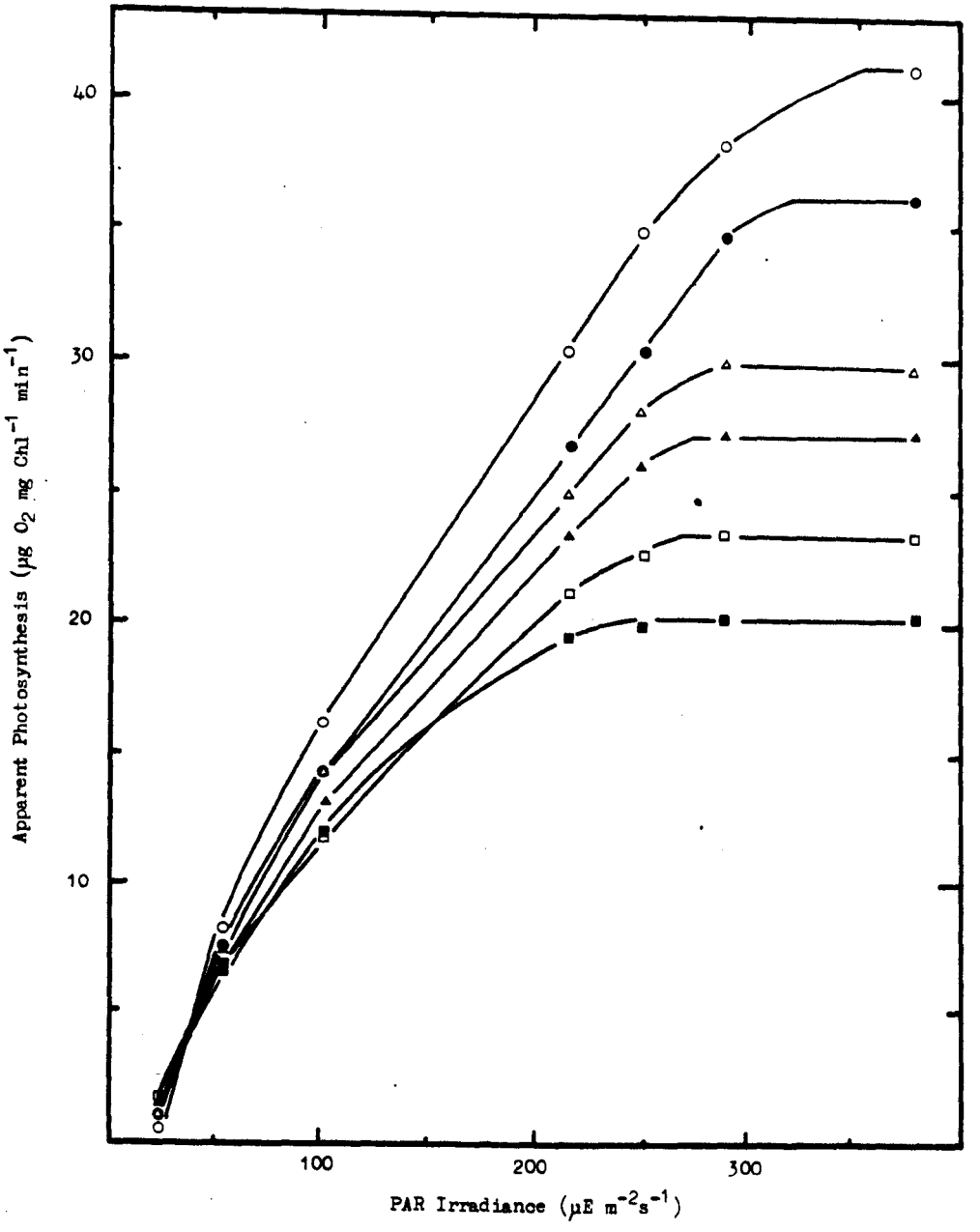
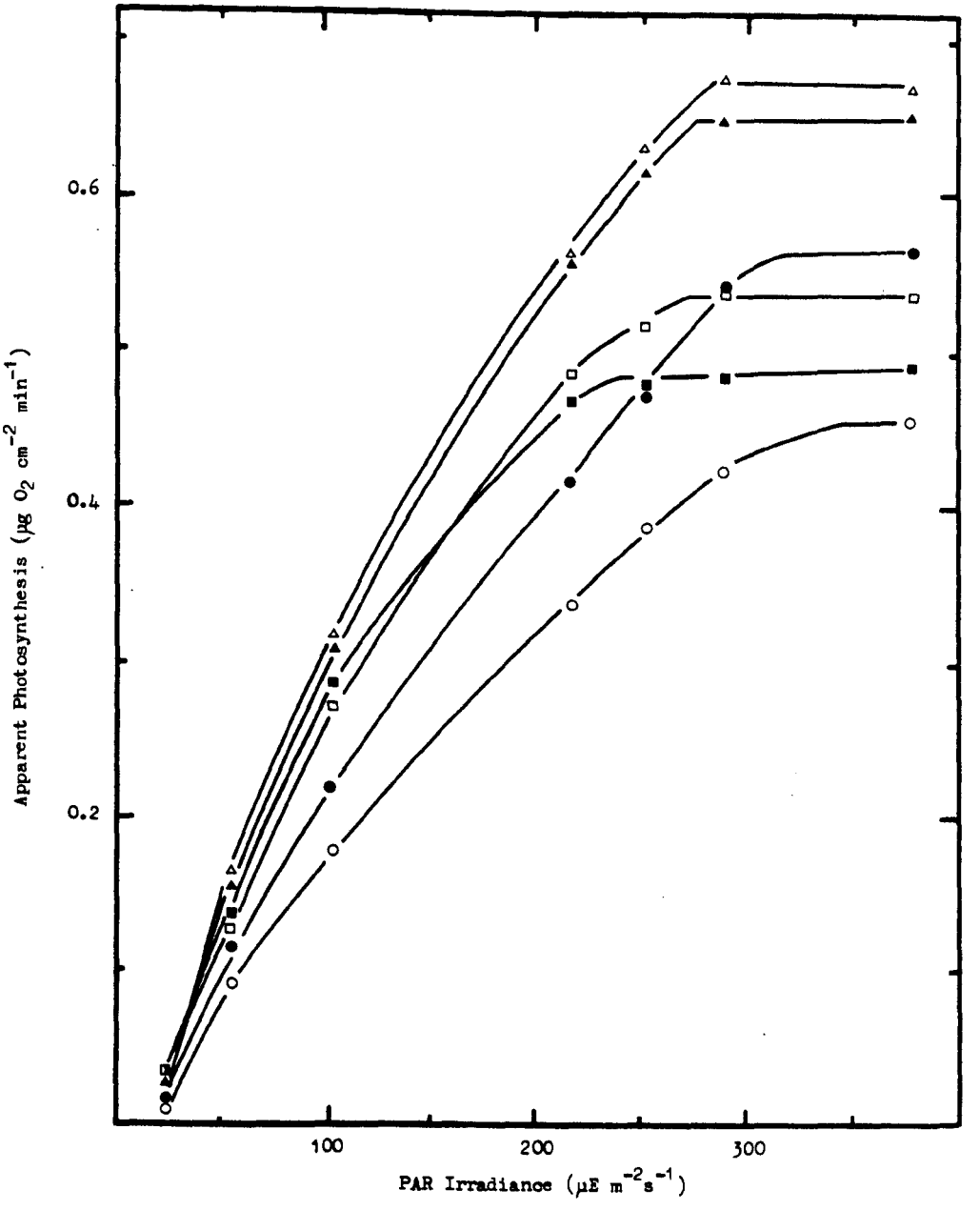

40
30
20
10
100
200
300
Apparent Photosynthesis (μg O_2 mg Chl^{-1} min^{-1})
PAR Irradiance (μE $m^{-2}s^{-1}$)

Figure 4.7 Apparent photosynthetic O_2 evolution by E. canadensis leaves at different PAR irradiances under 10 mg O_2 l^{-1} when rates are expressed per unit leaf area. Leaves were excised from positions 0.5 (o), 1 (●), 5 (△), 10 (▲), 20 (□) or 30 (■) cm below the apex. Other assay conditions were as described in Figure 4.2.


0.6
0.4
0.2
Apparent Photosynthesis (μg O_2 cm^{-2} min^{-1})
100
200
300
PAR Irradiance (μE $m^{-2}s^{-1}$)

light conditions change in the weed bed. However, such a distinction may be academic because light availability will be reduced with depth, and such reduction could result from several factors e.g. light attenuation by water and plant material (Hutchinson, 1957; Sculthorpe, 1967), epiphyte encrustation (Sand-Jensen, 1977) or marl deposition (Wetzel, 1960).

In June 1978, samples of E. canadensis were obtained from adjacent shaded and non-shaded sections of the Leeds and Liverpool Canal, Maghull. The shaded site was under the Westway Bridge (in between Bridges 14 and 15), the growth of E. canadensis was sparse, its leaves were deep green and surface light intensity was 7000 lux. In contrast, the growth of E. canadensis in an adjacent non-shaded section was dense, leaves were bright green and the surface light intensity was greater than 50 000 lux.

The irradiance response of photosynthesis in 1 cm and 5 cm excised leaves of E. canadensis from shaded and non-shaded sites were compared and the data obtained presented in Table 4.2. In both instances the light-saturated photosynthetic rate, light compensation and light saturation points were less in 5 cm leaves than 1 cm leaves. This presumably reflects the reduction in light availability with depth. However, light compensation points were reduced in the E. canadensis samples obtained from the shaded region compared to the non-shaded region. Thus E. canadensis evidently shows adaptation to growth under different light regimes, as has been described for other species (see above for references).

Adaptations to low light intensities have been regarded as being primarily related to energy conservation (e.g. Bjorkman et al., 1972; Spence and Chrystal, 1970). However the data presented in Table 4.1

Table 4.2 The effect of shading on apparent photosynthetic O_2 evolution, light compensation point (L.C.P.) and light saturation point (L.S.P.) of 1 cm and 5 cm excised leaves of E. canadensis

PAR Irradiance ($\mu E\ m^{-2} s^{-1}$)	Apparent photosynthesis ($\mu g\ O_2$/mg Chl. min)			
	1 cm		5 cm	
	non-shaded	shaded	non-shaded	shaded
0	-4.4 ± 0.6	-4.2 ± 0.5	-3.0 ± 0.4	-2.8 ± 0.2
25	1.5 ± 0.2	1.5 ± 0.3	1.7 ± 0.2	1.3 ± 0.2
55	5.9 ± 0.3	8.4 ± 1.0	6.1 ± 0.1	6.3 ± 0.5
105	13.3 ± 0.4	16.6 ± 1.2	12.5 ± 0.9	12.2 ± 0.7
220	22.2 ± 0.7	23.4 ± 0.5	24.8 ± 1.0	21.6 ± 1.4
254	25.7 ± 0.3	25.2 ± 0.8	28.6 ± 0.8	23.9 ± 0.8
290	30.4 ± 1.7	25.0 ± 1.0	30.0 ± 0.6	23.8 ± 0.9
380	34.1 ± 1.1	25.1 ± 1.0	30.4 ± 0.8	23.9 ± 0.9
630	36.5 ± 1.2	24.9 ± 1.2	30.2 ± 1.5	23.9 ± 0.9
L.C.P.	21.4	18.1	12.8	10.6
L.S.P.	450	250	290	250

Mean of three replicates ± standard deviation

show that whilst dark respiration is indeed reduced with increasing distance from the apex, when dark respiration is expressed as a percentage of light saturated photosynthesis, the most shade adapted leaves (20 and 30 cm below the apex) have respiration which is as high as, or higher than, the apical leaves.

Large differences are seen in the photosynthetic response to irradiance for leaves of different insertion level when rates are expressed in terms of pigment and area (Figures 4.6 and 4.7 respectively). When expressed in terms of pigment, maximal rates were seen in apical leaves, and declined rapidly to a leaf position 5 cm below the apex. Below 5 cm the rate of decline was more gradual. This pattern contrasted to that of low photosynthesis in apical leaves, which rose to a maximum in mature leaves and thereafter declined steadily through to the oldest leaves, if rates were expressed on an area basis. Such differences in photosynthetic rate with leaf position could be related to aging effects on photosynthesis and have already been discussed in this context.

CONCLUSIONS

Leaf area, fresh weight and chlorophyll a/b ratio of *E. canadensis* leaves varied with leaf position, and all showed a general pattern of early increase followed by a mature phase characterised by stability, and finally a phase of decline. Leaf chlorophyll content exhibited the first two phases i.e. increase to maturity and stability, but did not show the final decline, perhaps because the plants were collected from a vigorously growing population in which no plants had started to decline. Conditions during growth, aging phenomena and light adaptation are all likely determinants of these patterns.

The variations in photosynthesis, dark respiration and O_2 inhibition of apparent photosynthesis with leaf position in E. canadensis conform to patterns described for leaf aging in terrestrial plants, and are most probably related to aging effects in the aquatic species. However, variation in photosynthesis with leaf position showed different patterns, depending on whether rates were expressed in terms of pigment or leaf area. Apparent photosynthesis and respiration were greatest in apical leaves and declined with increasing age and distance from the apex when rates were expressed on a per unit chlorophyll basis. If however rates were based on unit area, apparent photosynthesis was least in apical leaves, rose as leaves were maturing and then declined gradually thereafter. Dark respiration remained relatively stable. Oxygen inhibition of apparent photosynthesis showed essentially the same pattern whether rates were on a pigment or area basis. Inhibition was least in apical leaves, rose during maturation and declined gradually thereafter. These patterns conformed to those described in the literature on terrestrial plants.

The light saturation and compensation points for photosynthesis were greatest in the apical leaves, and decreased down the plant. These differences could be adaptations to compensate for light attenuation by water, plant material and epiphyte encurstations in natural plant stands. Further, they could benefit the whole plant, especially if some of the carbon fixed by the older, more shaded leaves contributes to new growth of the plant.

Having established that leaf position, and thus leaf age affects the photosynthetic and respiratory physiology of Elodea canadensis, this next section includes both young and mature leaves. It describes the effect of season on the photosynthesis and respiration of 1 cm and 6 cm excised leaves with respect to light and temperature. Seasonal variations in O_2 inhibition of apparent photosynthesis and glycolate oxidase activity are also described.

5. SEASONAL TRENDS IN APPARENT PHOTOSYNTHESIS AND RESPIRATION OF THE SUBMERSED MACROPHYTE ELODEA CANADENSIS

ABSTRACT

The light saturation point of detached young leaves of Elodea canadensis Michx. (Hydrocharitaceae) was greatest in summer months, when field insolation was at its height. The temperature optimum of apparent photosynthesis and dark respiration did not change seasonally. There was little evidence of dormancy in winter, but in summer the leaves showed thermal adaptation of apparent photosynthesis to temperatures between 10-20°C. Mature leaves were generally less active than young ones, but showed the same seasonal patterns of response to light and temperature. Results are discussed in relation to adaptation to seasonal changes in the natural environment.

Both glycolate oxidase activity and oxygen inhibition of apparent photosynthesis were greatest in young apical leaves in the winter, and showed a marked reduction in summer. The possible advantages of reduced potential for photorespiration in summer are discussed in relation to the environmental conditions occurring that season.

INTRODUCTION

In temperate climates, seasonal patterns of growth in the aquatic plant community have been linked with the annual cycle of temperature and insolation (Sculthorpe, 1967; Westlake, 1975). A typical feature is low biomass in winter, during the period of low light and temperature, with dormancy often inferred, but rarely investigated experimentally. Increasing light and temperature in the spring allow accelerating growth. As biomass increases, self-shading may become pronounced, producing great variations in light availability over short intervals

of space and time. With such varying conditions, it would be of advantage to an aquatic plant to be capable of adapting its photosynthetic characteristics to make best use of the available light. Indeed light adaptation has been reported for many aquatic macrophytes (e.g. Adams et al., 1974; Bowes et al., 1977b; Gessner, 1938; Ikusima, 1966; Spence and Chrystal, 1970), and adaptation to light of a particular intensity does not necessarily preclude subsequent re-adaptation, for plants in general, should conditions change (Boardman, 1977). One of the aims of this research was to measure the extent to which light saturation characteristics of young and mature Elodea canadensis leaves varied during a yearly cycle.

Temperature optima for photosynthesis in aquatic plants appear capable of variation. For example Ceratophyllum demersum from Lake Ohakuri, New Zealand had a photosynthetic temperature optimum of 20°C (Carr, 1969), whereas specimens from a Florida lake had an optimum of 28.5°C, the difference being attributed by Van et al., (1976) to adaptation to higher temperatures in the latter waterbody. Dormancy has also been reported in a number of aquatic species (Sculthorpe, 1967). Therefore another aim of the present study was to test for seasonal adaptations to temperature in E. canadensis, either in the form of winter dormancy or annual variation in temperature optimum of photosynthesis.

Photorespiration has been demonstrated in many submersed macrophytes, and in general the rates obtained during the summer tend to be less than those for autumn or winter (Bowes et al., 1977a; Hough, 1974; Søndergaard, 1979). Such a reduction in photorespiration during the summer could be valuable to the plant when such plant-induced changes

in water quality in dense stands as increased O_2, temperature and pH, and reduced CO_2 (Bamforth, 1962; Brown et al., 1974; Dale and Gillespie, 1977; Van et al., 1976) would all tend to accelerate photorespiratory loss of assimilate. E. canadensis has been shown to exhibit photorespiration (Brown et al., 1974; Hough, 1979; Kutyurin et al., 1964; Søndergaard, 1979; thesis Section 3), and any reduction in potential for photorespiration during the summer months would have obvious benefit for this plant. It was for this reason that the potential for photorespiration, as indicated by O_2 inhibition of apparent photosynthesis and by glycolate oxidase activity, were determined through a yearly cycle.

There is considerable difference in physiological activity between young and mature leaves of E. canadensis (thesis Section 4) and Vallisneria spiralis (Jana and Choudhuri, 1979). The present study therefore included both types of leaf.

MATERIALS AND METHODS

Plant Material

Samples of E. canadensis were collected from the Leeds and Liverpool Canal, near Aintree, Merseyside (O.S. 393990) at intervals from December 1977 to January 1979. These samples were maintained in the laboratory in canal water at 15°C, under a 16 hour photoperiod with illumination at 54 $\mu E\ m^{-2}s^{-1}$(PAR). Physiological measurements were made on the material within 4 days of collection.

When necessary, epiphytes were removed by gently brushing the leaves and rinsing in several changes of water. Preliminary tests showed that such treatment did not significantly affect recorded rates.

Photosynthetic and Respiratory Rate Determination

Photosynthetic and respiratory rates of leaves were measured as O_2 evolution or uptake using a Clarke-type O_2 electrode (Rank Brothers, Cambridge). The light source was a 250-W projector bulb, and the intensity was varied by altering the distance between the light source and reaction vessel. During tests to determine the response of leaves to irradiance, the plant material was first exposed to randomised lower light intensities and then to higher light intensities. At these high light intensities (light saturation point and above) the plant material was exposed to successively increasing light intensities. This pattern of exposure was designed to minimise the risk of light damage to the photosynthetic apparatus and any subsequent effects on rates although no evidence could be found for such effects, since O_2 exchange at lower light intensities was the same before and after exposure to the maximum irradiance used.

Temperature was controlled by circulating water from a Churchill cooler/heater of the appropriate temperature through the outer jacket of the electrode.

Prior to leaf excision, sprigs of E. canadensis were incubated at the appropriate temperature for 30 minutes. Leaf samples comprised the three leaves of a whorl excised from either 1 cm or 6 cm below the apex. These were placed directly into the assay solution, which was a modified Forsberg Medium No. II (Forsberg, 1965), modified by omission of Na_2SiO_3, and containing 50 mM Tris-HCl, pH 7.5, and 2 or 10 mg O_2 l^{-1}, achieved by sparging with N_2 or O_2 gases as appropriate. Photosynthesis was initiated by injecting 0.1 cm^3 of $NaHCO_3$ solution, to give a final concentration of 2.4 mM total

inorganic carbon, which approximated to that present in canal water. All treatments were run in triplicate.

Glycolate Oxidase Assay

Glycolate oxidase activity was measured as O_2 uptake using a method based on that of Frederick et al., (1973). Samples of approximately 50 leaves were ground in a chilled pestle and mortar using 5 cm^3 of an extraction medium of 50 mM Tris-HCl, pH 8, containing 10 mM $MgCl_2$, 1 mM EDTA, 0.1 mM FMN and 2% w/v insoluble polyvinylpyrrolidone. Aliquots of the resultant homogenates were taken for chlorophyll determinations and the remaining homogenates were centrifuged at 500 g for 10 minutes to remove cell debris. The supernatants were used for enzyme assay. Glycolate oxidase activity was determined by measuring the dark O_2 uptake by a Clarke-type electrode, in the presence of 14 mM sodium glycolate at 25°C. All the reported rates are corrected for endogenous dark O_2 uptake of the extracts, measured in the reaction vessel before injection of glycolate.

Chlorophyll Determination

The method of Arnon (1949) was used for chlorophyll determinations.

RESULTS AND DISCUSSION

Light-responses Curves

Seasonal changes in apparent photosynthetic and dark respiratory response to light intensity of leaves excised 1 cm below the apex are shown in Figure 5.1 and Table 5.1. In general, the light saturation point of apparent photosynthesis was lowest in winter and increased in spring to give maximal values in mid-summer. Thereafter

Figure 5.1 Seasonal patterns of apparent photosynthetic O_2 evolution and O_2 uptake in the dark by E. canadensis leaves, excised 1 cm below the apex, at different PAR irradiances. The assay solution was a modified Forsberg medium No. II containing 2.4 mM total inorganic carbon and 50 mM Tris-HCl, pH 7.5. Oxygen exchange was determined at 20°C, in the light or in darkness, under 10 mg O_2 l^{-1}. All data points in this and subsequent figures represent the mean of triplicate determinations.

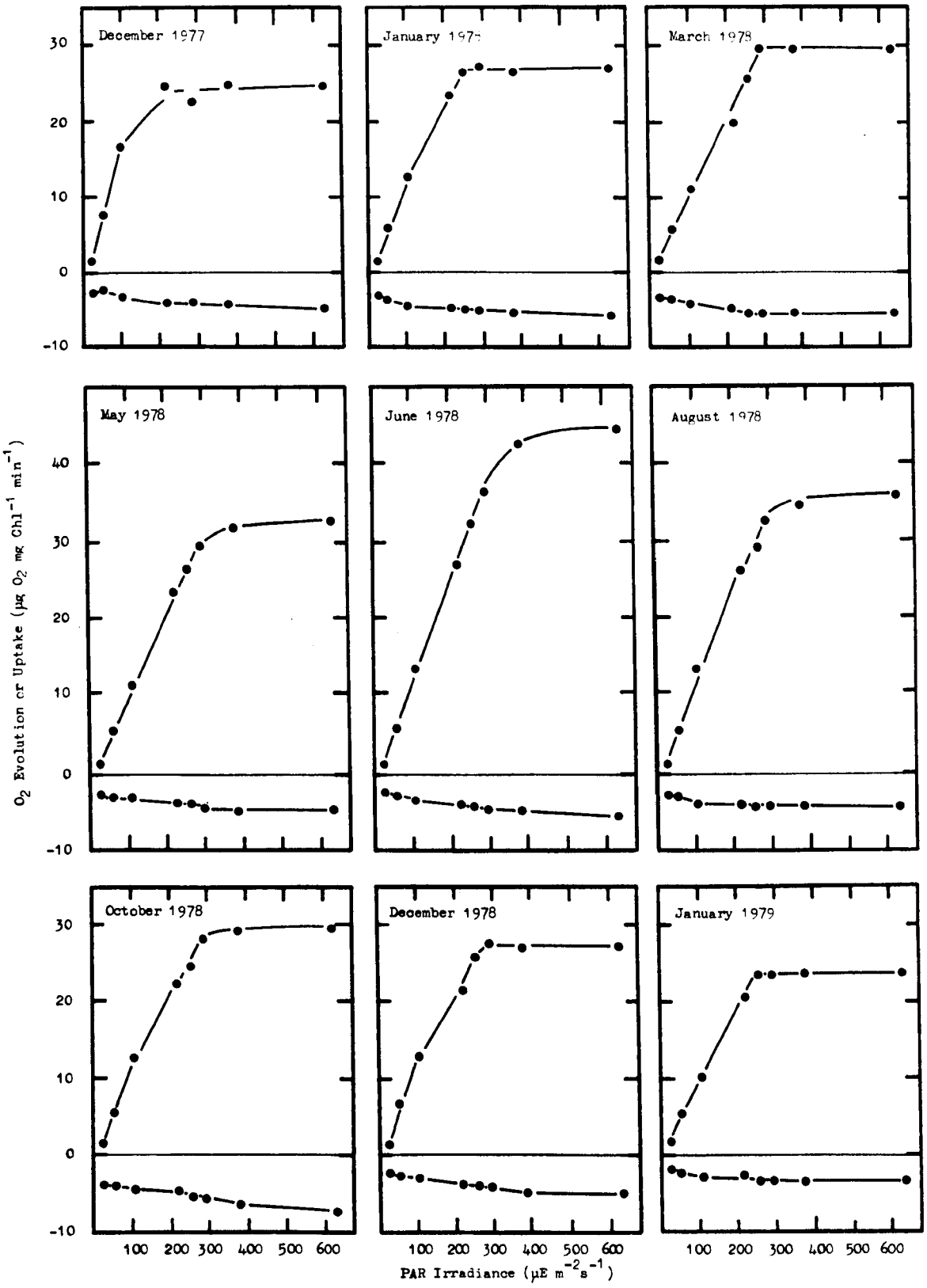

December 1977
January 1978
March 1978
May 1978
June 1978
August 1978
October 1978
December 1978
January 1979
30
20
10
0
-10
40
100 200 300 400 500 600
O2 Evolution or Uptake (μg O2 mg Chl-1 min-1)
PAR Irradiance (μE m-2s-1)

Table 5.1 The effect of time of year on light compensation point (L.C.P.), light saturation point (L.S.P.), light-saturated photosynthetic rate and dark respiration rate of E. canadensis leaves at 20°C. Leaves were excised from 1 cm below the apex.

Date Sample Collected	L.C_2P. ($\mu E/m^2.s$)	L.S_2P. ($\mu E/m^2.s$)	Light-saturated Photosynthetic Rate ($\mu g\ O_2$/mg Chl. min)	Rate of Dark Respiration ($\mu g\ O_2$/mg Chl. min)	Dark Respiration (percent of light-saturated photosynthesis)
07.12.77	14.5	220	24.7 ± 2.4	4.9 ± 0.8	19.8
09.01.78	11.2	250	27.1 ± 1.9	5.8 ± 0.5	21.4
06.03.78	14.8	290	29.5 ± 2.3	5.5 ± 0.4	18.6
15.05.78	14.8	400	32.6 ± 1.9	4.6 ± 0.3	14.1
26.06.78	14.6	510	44.0 ± 1.4	5.4 ± 1.0	12.3
08.08.78	15.8	400	35.4 ± 1.9	4.6 ± 0.3	12.9
09.10.78	14.9	330	29.2 ± 1.7	7.2 ± 0.5	24.6
04.12.78	10.8	290	27.1 ± 2.1	5.1 ± 0.3	18.8
15.01.79	11.3	250	23.5 ± 1.0	3.6 ± 0.3	15.3

Mean of three replicates ± standard deviation

there was a decline which extended through autumn to restore the low winter levels. Light compensation point of apparent photosynthesis showed a similar, though less pronounced trend. The mean light compensation point of mid-winter 1 cm leaves (December and January) was 11.9 $\mu E\ m^{-2}s^{-1}$ whilst that of summer 1 cm leaves (May, June and August) was 15.1 $\mu E\ m^{-2}s^{-1}$. Dark respiratory O_2 uptake increased slightly with increasing light intensity, but tended to stabilise when light saturation point was reached.

Adaptation to different light regimes have been documented for aquatic plants e.g. E. crispa can exhibit characteristics of sun or shade adapted species depending upon whether they are in an open or shaded environment (Gessner, 1938). Re-adaptation to light has been demonstrated for mature specimens of Hydrilla verticillata when grown under new conditions (Bowes et al., 1977b). Depth differences in photosynthetic response to light have been demonstrated in Myriophyllum spicatum (Adams et al., 1974) and Vallisneria denseserrulata (Ikusima, 1966). The seasonal change in light saturation characteristics reported here for E. canadensis could result from adaptation to low light intensity in the winter when insolation is low and light intensity can be further reduced by ice cover. Increased insolation in the spring and summer induces adaptation to higher light intensities in the new growth.

Research by Bjorkman et al. (1972), and Spence and Chrystal (1970) suggest that adaptations to low light intensities are primarily related to energy conservation. However the data presented in Tables 5.1 and 5.2 indicate that dark respiration rates in both 1 cm and 6 cm leaves were relatively constant throughout the year and yet

respiration was proportionately greater in the shade adapted winter samples because of the lower photosynthesis in that season. Thus in 1 cm leaves the maximum dark respiration values in December 1977 and June 1978 were 4.9 and 5.4 $\mu g\ O_2\ mg\ Chl^{-1} min^{-1}$ respectively, whereas they were 20% and 12% of the respective light saturated photosynthetic rate. Similar comparisons can be made with the 6 cm leaves whose activity is lower than the 1 cm leaves. This generally lower activity of mature leaves is discussed in thesis Section 4.

The seasonal pattern of response of the 6 cm leaves (Figure 5.2 and Table 5.2) generally followed that of the 1 cm leaves. However the light saturation point was distinctly lower in the summer months, whilst the mean summer light compensation point was only marginally depressed (14.5 $\mu E\ m^{-2}s^{-1}$ in 6 cm leaves compared to 15.1 $\mu E\ m^{-2}s^{-1}$ in 1 cm leaves). Reduced light intensity with depth may be caused by several factors e.g. light attenuation by water and plant material (Hutchinson, 1957; Sculthorpe, 1967), epiphyte encrustation (Sand-Jensen, 1977) and marl deposition (Wetzel, 1960). The lower light saturation point of the 6 cm leaves compared with the summer 1 cm leaves may be regarded as an adaptation to reduced light with depth, though in this case the plant material as collected from the field had little epiphyte or marl encrustation so light attenuation was probably mainly by water and self-shading.

The lower magnitude of light saturated photosynthesis in the 6 cm leaves may be related to aging effects which have been well documented for terrestrial plants (e.g. Aslam *et al.*, 1977; Dickmann *et al.*, 1975).

Figure 5.2 Seasonal patterns of apparent photosynthetic O_2 evolution and O_2 uptake in the dark by E. canadensis leaves, excised 6 cm below the apex, at different PAR irradiances. Assay conditions were as described in Figure 5.1.

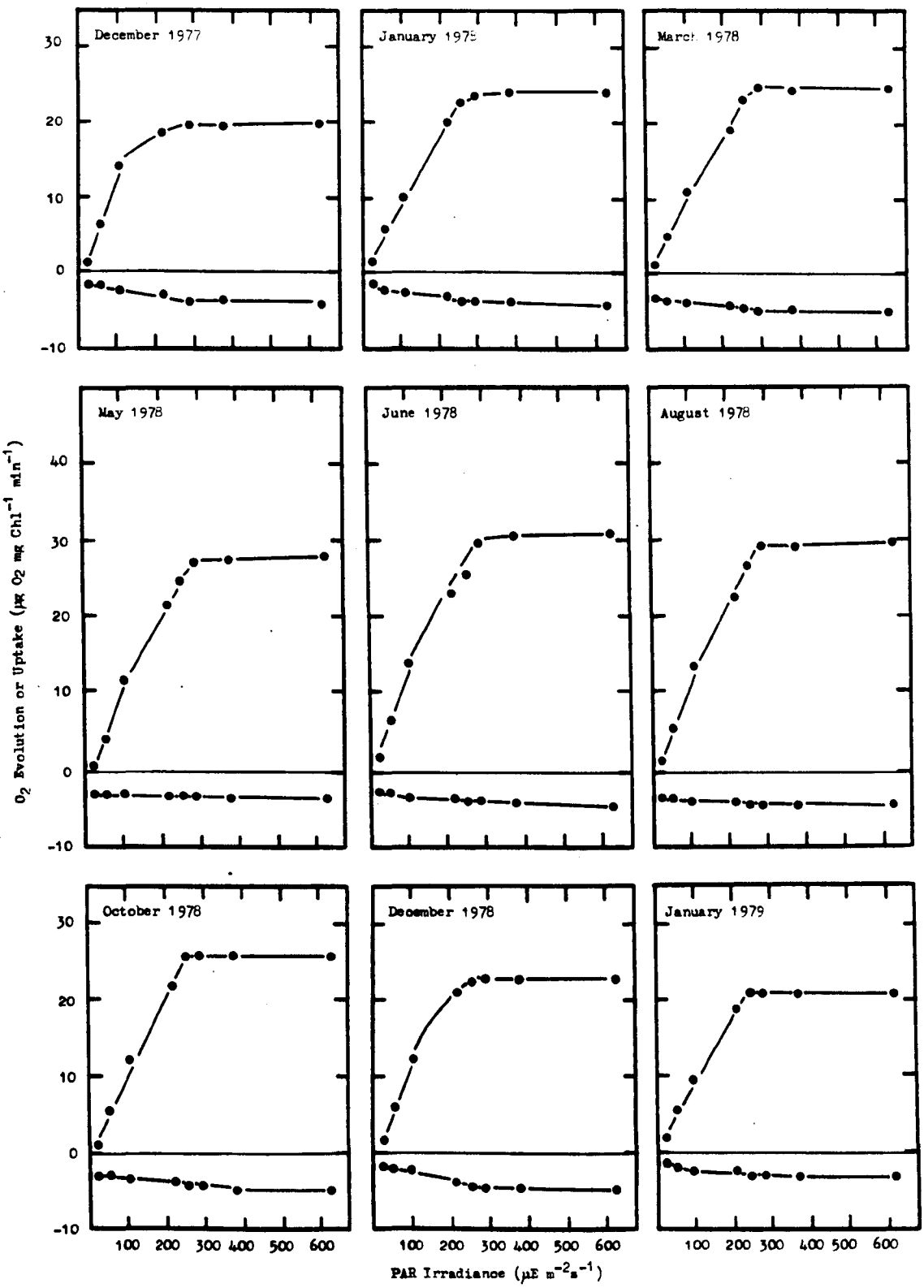

December 1977
January 1978
March 1978
May 1978
June 1978
August 1978
October 1978
December 1978
January 1979
30
20
10
0
-10
40
100 200 300 400 500 600
O2 Evolution or Uptake (μg O2 mg Chl-1 min-1)
PAR Irradiance (μE m-2s-1)

Table 5.2 The effect of time of year on light compensation point (L.C.P.), light saturation point (L.S.P.), light-saturated photosynthetic rate and dark respiration rate of E. canadensis leaves at 20°C. Leaves were excised from 6 cm below the apex.

Date Sample Collected	L.C.P. ($\mu E/m^2.s$)	L.S.P. ($\mu E/m^2.s$)	Light-saturated Photosynthetic Rate ($\mu g\ O_2/mg$ Chl. min)	Rate of Dark Respiration ($\mu g\ O_2/mg$ Chl. min)	Dark Respiration (percent of light-saturated photosynthesis)
07.12.77	13.9	220	19.7 ± 0.9	4.2 ± 0.4	21.3
09.01.78	9.3	260	23.9 ± 1.7	4.2 ± 0.2	17.6
06.03.78	14.9	290	24.4 ± 2.1	5.0 ± 0.3	20.5
15.05.78	16.5	290	27.6 ± 1.2	3.6 ± 0.1	13.0
26.06.78	14.7	330	30.8 ± 2.6	4.4 ± 0.5	14.3
08.08.78	12.1	290	29.6 ± 0.6	4.6 ± 0.5	15.5
09.10.78	11.9	250	25.4 ± 0.3	5.1 ± 0.6	20.1
04.12.78	10.6	260	22.3 ± 1.8	4.9 ± 0.3	21.9
15.01.79	11.5	240	20.4 ± 0.4	3.5 ± 0.3	17.2

Mean of three replicates ± standard deviation

Temperature-response Curves

Throughout the period of this study field samples of the plant material were maintained under standard laboratory holding conditions (15°C, 54 $\mu E\ m^{-2}s^{-1}$ (PAR), 16:8 hour photoperiod) for a maximum of 4 days before physiological measurements were completed. Comparisons between winter plants (data not presented here, but see Table 2.6) held under these conditions and ones held under more realistic winter field conditions (5°C, 36 $\mu E\ m^{-2}s^{-1}$ (PAR), 8:16 hour photoperiod) indicated that there was no significant effect of such holding conditions on photosynthetic and respiratory response to temperature. Thus it is unlikely that the relatively short exposure of winter plants to the elevated temperature and extended photoperiod during laboratory holding had any effect on the outcome of investigations into seasonal response to temperature.

Temperature response curves of apparent photosynthesis and dark respiration for 1 cm and 6 cm leaves are shown in Figures 5.3 and 5.4 respectively. Temperature optima for both apparent photosynthesis and respiration were between 25°C and 30°C and no seasonal variation or difference between 1 cm and 6 cm leaves could be discerned. The maximal rate was higher in the summer than in winter, and so exhibited a seasonal trend which was similar to, but less marked than that of the light saturation curves. Photosynthetic and respiratory rates were both slightly less in 6 cm leaves than in 1 cm leaves, but the annual mean proportions of respiration to apparent photosynthesis were almost the same (16.6% and 15.7% respectively).

The relatively high temperature optimum of 27.5°C, averaged for 1 cm and 6 cm leaves throughout the sampling period, is considerably above

Figure 5.3 Seasonal patterns of apparent photosynthetic O_2 evolution and O_2 uptake in the dark by E. canadensis leaves, excised 1 cm below the apex, at different temperatures. Assay conditions were as described in Figure 5.1 except that photosynthetic rates were determined at a PAR irradiance of 290 $\mu E\ m^{-2} s^{-1}$.

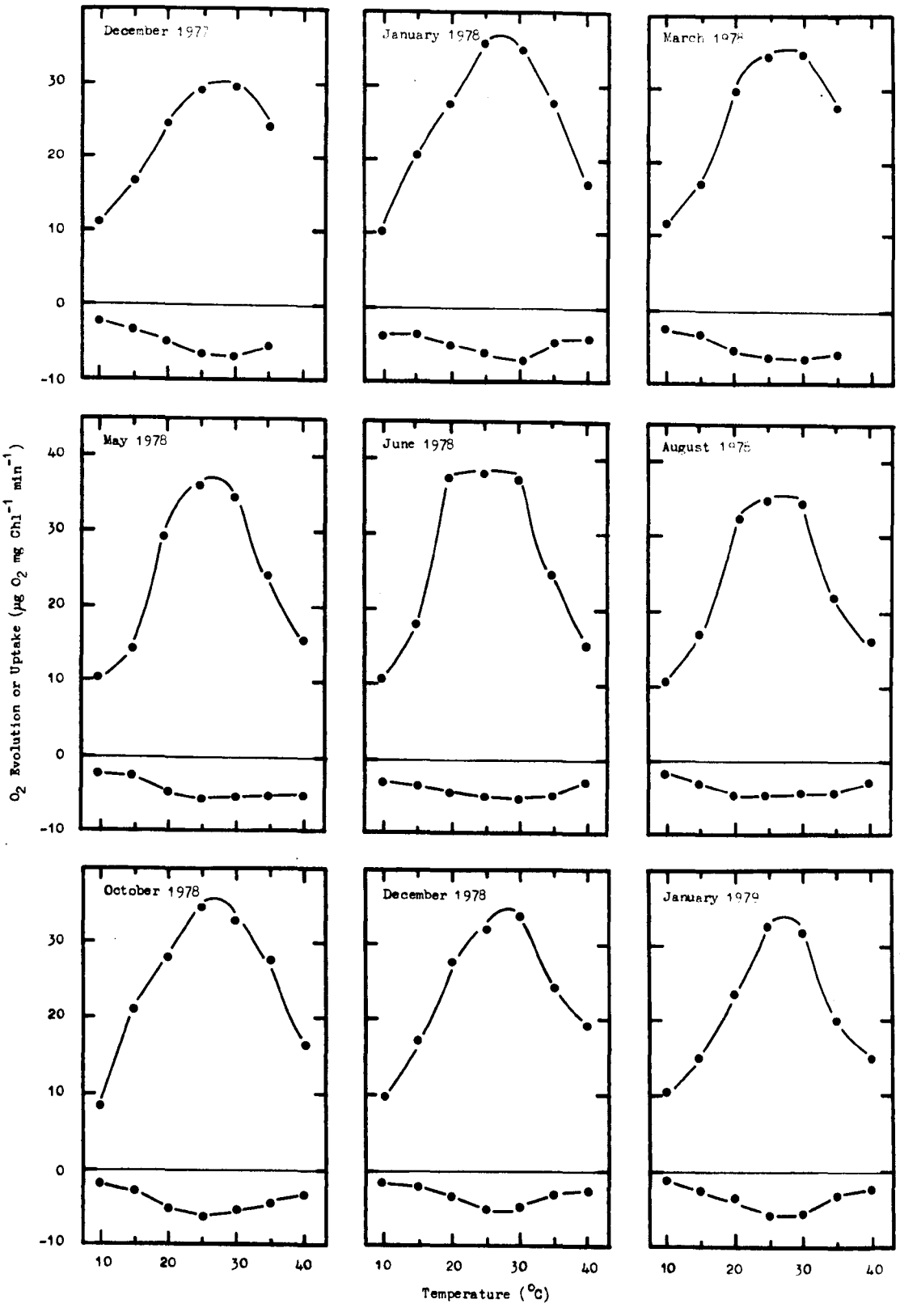
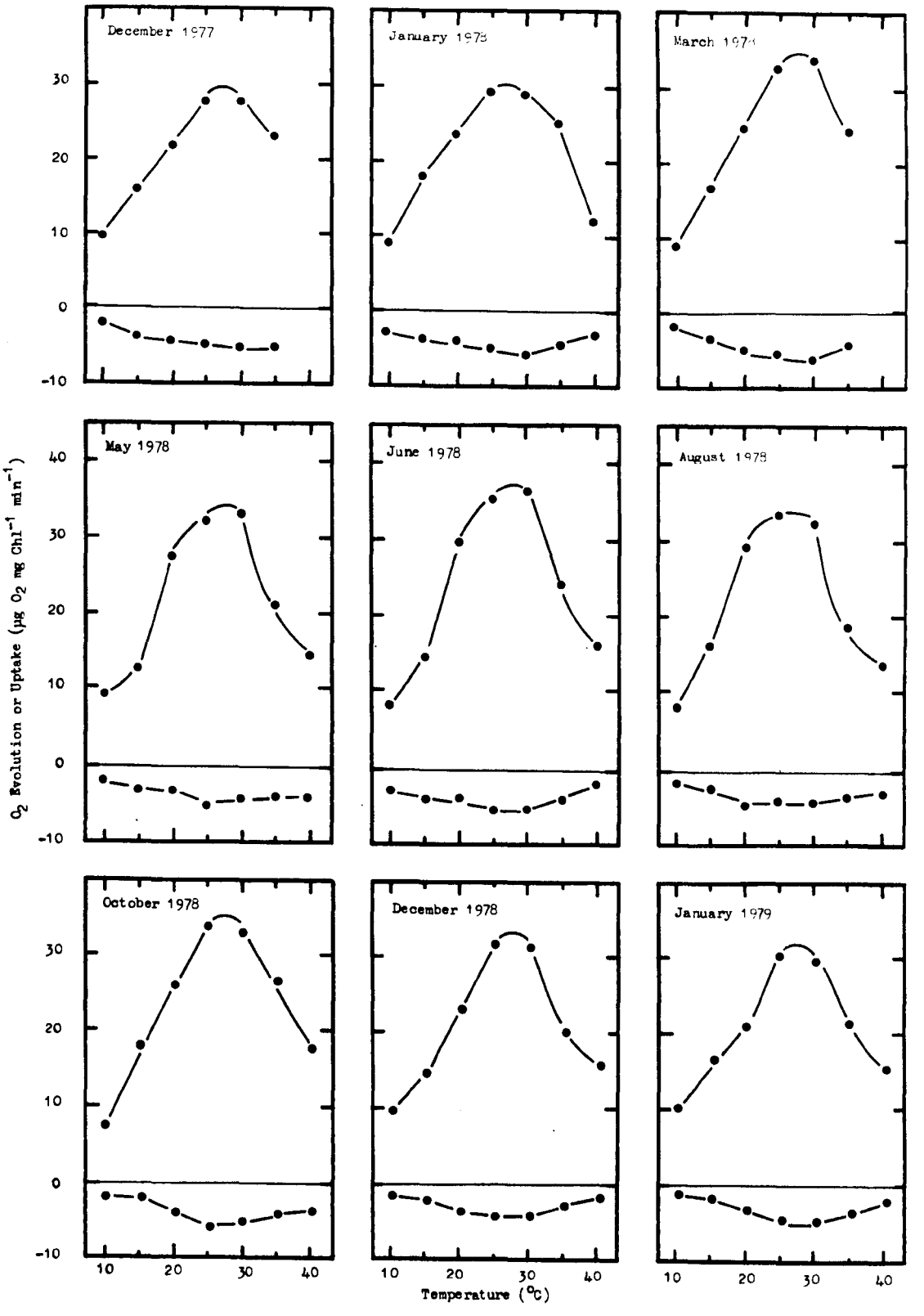

December 1977
January 1978
March 1978
May 1978
June 1978
August 1978
October 1978
December 1978
January 1979
O_2 Evolution or Uptake ($\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$)
Temperature (°C)

Figure 5.4 Seasonal patterns of apparent photosynthetic O_2 evolution and O_2 uptake in the dark by E. canadensis leaves, excised 6 cm below the apex, at different temperatures. Assay conditions were as described in Figure 5.3.


December 1977
January 1978
March 1978
May 1978
June 1978
August 1978
October 1978
December 1978
January 1979
O_2 Evolution or Uptake ($\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$)
Temperature (°C)

the normal daytime water temperatures in the Leeds and Liverpool Canal, where measurements indicate an annual mean over 5 years of 11-12°C, with usually less than 30 days each summer above 20°C. It is however in accordance with the rapid net assimilation rate reported for E. canadensis at high temperature by Allen (1973), and may be a reflection of the species' origin in North American waters where summer conditions are generally warmer.

The E. canadensis collected in winter conformed to published descriptions of 'winter buds' i.e. leaves were closely crowed together at the stem apices, and lower parts of stems were often leafless (Sculthorpe, 1967). Such material showed little evidence of physiological dormancy. The mean apparent photosynthetic rate of midwinter 1 cm leaves (December and January) at 10°C was 10.2 $\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$, compared with a summer (May, June and August) mean of 10.3 $\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$. Nor was there any marked seasonal difference in the less active 6 cm leaves, which had winter and summer means of 9.4 and 8.5 $\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$ respectively.

The E. canadensis population in the Leeds and Liverpool Canal may therefore be able to respond to short periods of mild and sunny weather in winter by increasing assimilation and counteracting to some extent the general seasonal decline in biomass. The canal is shallow (mean depth of 1 - 1.5 m) and so not strongly temperature buffered, and daytime water temperature usually exceeds 7°C on 5 - 10 days within each December to January period (J.W. Eaton, personal communication). Field observations showed that during mild periods, overwintering plants produced new, bright green growth at the stem

apices, which contrasted conspicuously with the dark green or black older stems.

The photosynthetic rates of winter samples at 20°C were markedly less than those of summer ones. The Q_{10} for apparent photosynthesis over the 10 - 20°C range was least in the winter plants and increased in the spring to give maximal values in midsummer (Table 5.3). Values then dropped slightly and a second autumn peak was evident in the October samples before low winter values were restored. The high Q_{10} in October for both 1 cm and 6 cm leaves could be due to a relatively high water temperature in that month, which was accompanied by re-growth of E. canadensis at the sampling site, and also in other parts of the Leeds and Liverpool Canal (Eaton et al., 1981).

The Q_{10} for apparent photosynthesis over the 10 - 20°C range averaged 2.51 in December - January and 3.17 in May - August for 1 cm leaves, and 2.33 and 3.38 respectively for 6 cm leaves. Winter plants were therefore less able to take advantage of high temperatures than were summer plants, and so might be considered to be showing slight dormancy. However, this effect appears to have no ecological significance, as temperatures of 20°C never occur in the canal for at least 15 weeks either side of midwinter. These findings contrast with reports of dormancy in E. canadensis in midwinter (Sculthorpe, 1967), but are in agreement with those of Boylen and Sheldon (1976) who measured net assimilation rates 10 - 20% of summer values under ice cover, and of Haag and Gorham (1977) who suggest that the plant is quiescent rather than dormant in midwinter.

Table 5.3 The effect of time of year on photosynthetic rates (10°C and 20°C) and Q_{10} for apparent photosynthesis (range 10 - 20°C) of E. canadensis leaves. Leaves were excised from 1 cm and 6 cm below the apex.

Date Sample Collected	1 cm leaves			6 cm leaves		
	Photosynthetic Rate (μg O_2/mg Chl. min)		Q10	Photosynthetic Rate (μg O_2/mg Chl. min)		Q10
	10°C	20°C		10°C	20°C	
07.12.77	11.2 ± 1.5	24.8 ± 1.3	2.21	9.7 ± 0.9	21.5 ± 1.5	2.22
09.01.78	10.2 ± 1.0	27.3 ± 1.8	2.68	9.0 ± 0.9	23.5 ± 1.8	2.61
06.03.78	11.4 ± 1.1	29.4 ± 1.9	2.58	8.8 ± 0.3	24.7 ± 2.0	2.81
15.05.78	10.6 ± 1.3	29.4 ± 0.9	2.77	9.4 ± 0.5	27.2 ± 1.1	2.89
26.06.78	10.3 ± 0.2	36.1 ± 1.1	3.51	8.2 ± 0.7	29.7 ± 2.4	3.62
08.08.78	10.0 ± 0.5	32.3 ± 1.2	3.23	8.0 ± 0.6	29.1 ± 0.3	3.64
09.10.78	8.2 ± 1.1	28.0 ± 2.5	3.41	7.3 ± 1.1	25.5 ± 0.2	3.49
04.12.78	9.7 ± 1.1	27.4 ± 1.9	2.80	9.3 ± 1.0	22.4 ± 2.0	2.41
15.01.79	10.0 ± 1.4	23.3 ± 1.1	2.33	9.7 ± 1.0	20.3 ± 0.5	2.09

Mean of three replicates ± standard deviation

The vigorous summer response of apparent photosynthesis to temperature rises above 10°C may enable the plant to take advantage of daytime temperature increases. These can be up to 5°C between dawn and early afternoon in the bulk water of the canal, and up to 10°C in plant stands where water circulation is badly retarded by the density of the vegetation.

Oxygen Inhibition of Apparent Photosynthesis

Seasonal changes in oxygen inhibition of apparent photosynthesis, percentage inhibition of apparent photosynthesis by O_2, and glycolate oxidase activity are shown in Figures 5.5 and 5.6 for 1 cm and 6 cm excised leaves respectively. For reference the apparent photosynthetic rate at 10 mg O_2 l^{-1} and 25°C, field temperature, and difference in dark respiratory rate under 2 compared with 10 mg O_2 l^{-1} are also included.

Maximum rates of apparent photosynthesis at 10 mg O_2 l^{-1} occurred in the summer months in both 1 cm and 6 cm excised leaves, at the approximate time of maximum water temperature in the canal. Oxygen inhibition of apparent photosynthesis was greatest in the 1 cm leaves in winter, and declined to lower levels in summer (Figure 5.5c). There was no such seasonal trend in 6 cm leaves (Figure 5.6c).

Oxygen inhibition of photosynthesis (the Warburg effect) may be used to indicate the magnitude of photorespiration, provided that dark respiratory rates are unaffected by O_2 concentrations above 2% (Jackson and Volk, 1970). Whilst this may be so in terrestrial plants, it is not the case for aquatic plants (e.g. Dromgoole, 1978; Owens and Maris, 1964; Westlake, 1967). However as dark respiration is likely to be reduced in the light (Mangat et al., 1974), and in all instances in this study, O_2 inhibition of apparent photosynthesis exceeded the

Figure 5.5 The effect of season on A: apparent photosynthetic O_2 evolution under 10 mg O_2 l^{-1}, B: surface water temperature of the canal, C: O_2 inhibition of apparent photosynthesis (●) and O_2 enhancement of dark respiration (○), D: percentage inhibition of apparent photosynthesis by O_2, and E: glycolate oxidase activity of E. canadensis leaves excised 1 cm below the apex. Assay conditions for O_2 exchange rates were as described in Figure 5.1 except that rates were determined at 25°C, and apparent photosynthesis at a PAR irradiance of 290 μE $m^{-2}s^{-1}$. Inhibition and enhancement are expressed for rates at 10 relative to those at 2 mg O_2 l^{-1}. Glycolate oxidase activity was measured as the dark O_2 uptake at 25°C in the presence of 14 mM glycolate in an assay medium of 50 mM Tris-HCl, pH 8.0, containing 10 mM $MgCl_2$, 1 mM EDTA, and 0.1 mM FMN. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.

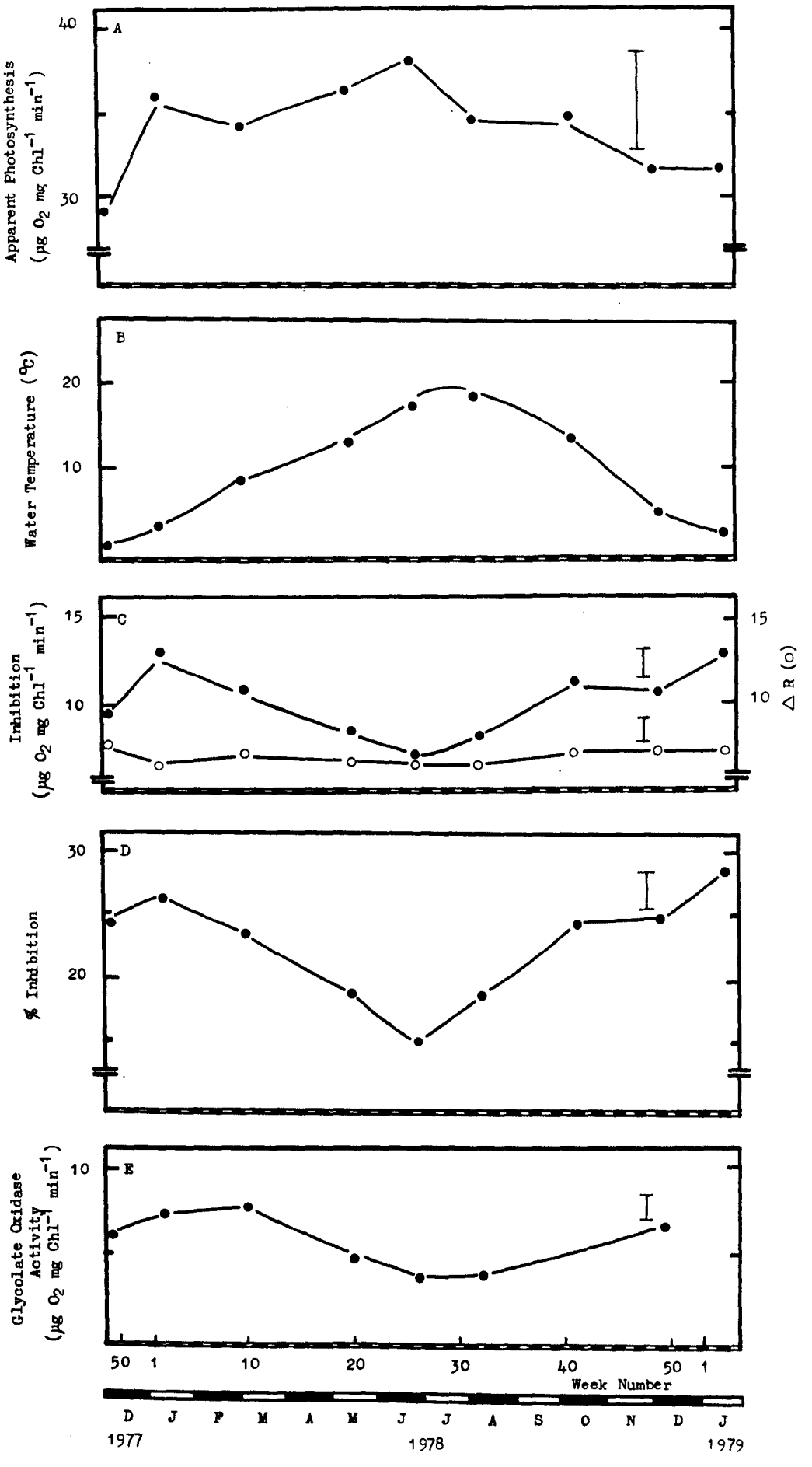
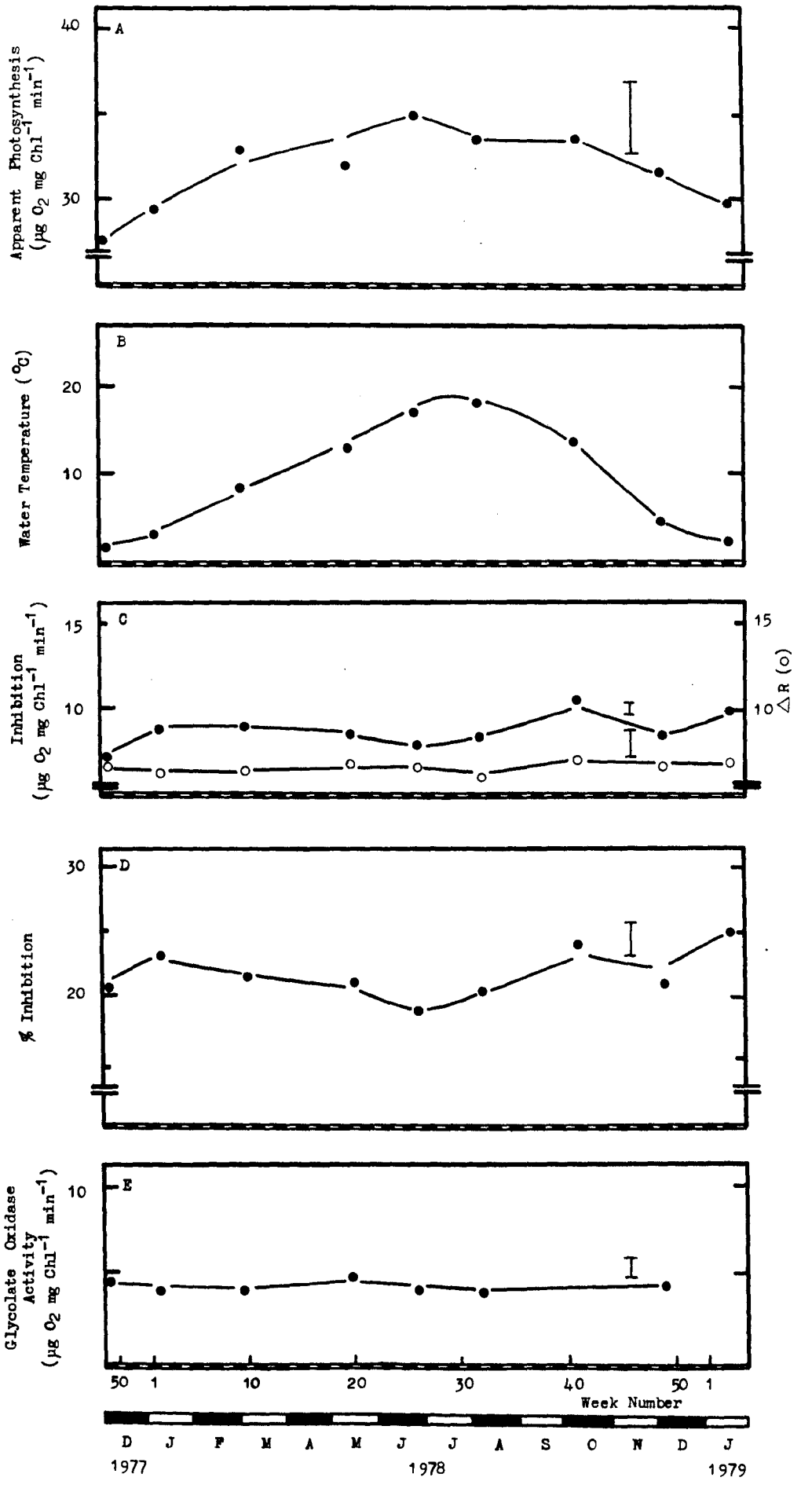

A
Apparent Photosynthesis (μg O2 mg Chl-1 min-1)
40
30
B
Water Temperature (°C)
20
10
C
Inhibition (μg O2 mg Chl-1 min-1)
15
10
ΔR (○)
D
% Inhibition
30
20
E
Glycolate Oxidase Activity (μg O2 mg Chl-1 min-1)
10
50 1 10 20 30 40 50 1
Week Number
D J F M A M J J A S O N D J
1977
1978
1979

Figure 5.6 The effect of season on A: apparent photosynthetic O_2 evolution under 10 mg O_2 l^{-1}, B: surface water temperature of the canal, C: O_2 inhibition of apparent photosynthesis (●) and O_2 enhancement of dark respiration (○), D: percentage inhibition of apparent photosynthesis by O_2, and E: glycolate oxidase activity of E. canadensis leaves excised 6 cm below the apex. Assay conditions were as described in Figure 5.5. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


A
Apparent Photosynthesis
(μg O2 mg Chl-1 min-1)
40
30
B
Water Temperature (°C)
20
10
C
Inhibition
(μg O2 mg Chl-1 min-1)
15
10
15
10
ΔR (○)
D
% Inhibition
30
20
E
Glycolate Oxidase
Activity
(μg O2 mg Chl-1 min-1)
10
50 1 10 20 30 40 50 1
Week Number
D J F M A M J J A S O N D J
1977
1978
1979

increase in dark respiration with rising O_2 (Figures 5.5C and 5.6C), it appears that the Warburg effect can be used to provide an approximate estimate of the magnitude of photorespiration.

The 1 cm leaves obtained during winter months of slow growth are likely to be correspondingly older than leaves from the same position in the summer months of fast growth, whereas those taken from 6 cm are likely to be relatively mature and physiologically stable at all times of the year. Thus, variation in leaf age near the stem apex could be responsible for the seasonal patterns of O_2 inhibition of apparent photosynthesis reported here. Such aging effects on photorespiration are best known in terrestrial crop species (e.g. Catsky et al., 1976, Hodgkinson, 1974; Kisaki et al., 1973) but have been domonstrated in E. canadensis (Søndergaard, 1979), H. verticillata (Bowes et al., 1977a) and Najas flexilis (Hough, 1974).

Bowes et al. (1977a) cite evidence that H. verticillata varies its photosynthesis : photorespiration ratio, a summer reduction in CO_2 compensation point being associated with increased net (apparent) photosynthesis and decreased photorespiration. They further noted a shift in RuBP/PEP carboxylase, with PEP carboxylase becoming predominant in the summer. Such a shift could explain the changes in photosynthesis and photorespiration in H. verticillata, but whether such changes in RuBP/PEP carboxylase ratios, photosynthesis and photorespiration can be linked solely to environmental triggers such as daylength and temperature, must remain debatable. The relationship may be indirect, and related to the varying growth and maturation state of the apical segments used. Mokronosov and Nekrasova (1977) found that in juvenile potato leaves, PEP carboxylase provided 80 - 90% of all CO_2 fixation, but RuBP carboxylase activity increases with age,

and as the leaf matured PEP carboxylase activity became negligible. High PEP carboxylase and other enzyme activity associated with the C_4 acid cycle also occurs in young tobacco leaves (Kisaki et al., 1973).

However, whether reduced potential for photorespiration in the summer is due to age-related effects or is triggered by environmental factors does not affect the fact that such a reduction is likely to be advantageous to the plant in that season. Under conditions of high surface irradiance, plant-induced O_2 supersaturation, depletion of CO_2 and HCO_3^-, increased pH and temperature occur in the water bathing submerged waterplant stands (e.g. Bamforth, 1962; Brown et al., 1974; Dale and Gillespie, 1977; Van et al., 1976). Such conditions are conducive to high rates of photorespiration generally (Jackson and Volk, 1970; Zelitch, 1971), and in E. canadensis in particular (thesis Section 3). Clearly, a reduced potential for photorespiration in leaves exposed to such conditions will be advantageous to the plant in curtailing its fixed carbon losses.

The seasonal pattern of glycolate oxidase activity of 1 cm leaves of E. canadensis paralleled that of O_2 inhibition of apparent photosynthesis (Figure 5.5E). In 6 cm leaves glycolate oxidase activity again paralleled that of O_2 inhibition of apparent photosynthesis, but in this instance there was no pronounced seasonal trend (Figure 5.6E). Regression analysis of glycolate oxidase activity (G) upon O_2 inhibition of apparent photosynthesis (I) for all 1 cm leaf data and the mean of 6 cm leaf data yielded a positive correlation, $G = 3.29 + 1.09I$ ($r = +0.87$). Glycolate oxidase is an important enzyme of the photorespiratory glycolate pathway of higher plants. Its activity is associated with active photorespiration, and is much higher in C_3 plants than in C_4 plants (Jackson and Volk, 1970; Tolbert, 1971). The glycolate

oxidase activity reported here for E. canadensis during the winter months, is similar to that of H. verticillata being approximately 15% of that found in spinach, a typical C_3 plant (Van et al., 1976).

The experimental conditions for winter samples could have been more conducive to higher levels of O_2 inhibition of apparent photosynthesis than for summer samples because they were more fully light saturated. However, whilst it may be true that photorespiration increases with increasing light intensity (Jackson and Volk, 1970; Zelitch, 1971), percentage inhibition of apparent photosynthesis by O_2 in E. canadensis is unaffected by irradiance under similar experimental conditions to those used here (thesis Section 3). Thus, as O_2 inhibition of apparent photosynthesis and percentage inhibition of apparent photosynthesis by O_2 showed very similar seasonal patterns (Figures 5.5C and D and 5.6C and D), it is unlikely that the seasonal trends described are accentuated by the lower light saturation point of winter samples.

CONCLUSIONS

The light saturation point of apparent photosynthesis in E. canadensis exhibited a seasonal pattern of variation adapted to environmental changes, being greatest in summer when insolation was high, and lowest in winter when insolation was least.

By contrast, the temperature optimum for photosynthesis and dark respiration did not vary seasonally. The optimum was above the temperatures normally occurring in the field in summer, indicating perhaps an inflexible, genetic adaptation to the warmer summer conditions in the North American waters from which this species was introduced into Britain. There was no evidence of dormancy in winter, and the light-saturated rate of photosynthesis at $10^{o}C$ showed no

perceptible variation throughout the year. Stands of E. canadensis were probably held in a quiescent state by low temperature and/or light availability, but seemed well able to exploit brief periods of warm, bright weather even in midwinter by temporarily increased photosynthesis. The photosynthetic Q_{10} between 10 - 20°C showed seasonal adaptation, passing from a minimum in winter to a maximum in summer. This probably had no significance for the plant's winter performance, but may have permitted more active photosynthetic response to daytime temperature rises in summer.

Mature leaves were generally less active than young ones, in photosynthesis and respiration, as reported in more detail in thesis Section 4, but the two types of leaf showed similar seasonal light and temperature responses.

Oxygen inhibition of photosynthesis and activity of the photorespiratory enzyme glycolate oxidase, were lowest in the summer months for 1 cm leaves, but did not vary seasonally in 6 cm leaves. This pattern may be a reflection of the relatively young age of 1 cm leaves in the summer, or an adaptation induced by environmental conditions. It is likely to be advantageous to the plant under the high temperature, O_2 and pH and low CO_2 conditions which occur in dense plant stands in summer, as it will curtail loss of carbon through photorespiration in the tips of the growing shoots.

In view of the fact that Elodea canadensis shows adaptations to seasonal variations in light availability and has reduced photorespiration, as indicated by reduced O_2 inhibition of photosynthesis, in the summer, this next section describes the effect of leaf position on these two phenomena.

6. SEASONAL TRENDS IN APPARENT PHOTOSYNTHESIS AND RESPIRATION IN LEAVES OF DIFFERENT INSERTION LEVEL OF THE SUBMERSED MACROPHYTE ELODEA CANADENSIS

ABSTRACT

The responses of apparent photosynthesis and dark respiration in detached leaves of Elodea canadensis Michx. (Hydrocharitaceae), taken from different insertion levels, to light and oxygen concentration were determined under standard laboratory conditions between June 1978 and June 1979. The light-saturated photosynthetic rates, light saturation and light compensation points were greatest in the summer months. Their magnitude was greatest in apical leaves and was reduced as distance from the apex increased. These trends are discussed in relation to photosynthetic adaptation to irradiance and known aging effects on photosynthesis of both terrestrial and aquatic plants.

Oxygen inhibition of apparent photosynthesis exhibited a general trend of increasing magnitude to a level at a certain distance below the apex, and thereafter declined to the oldest leaves. The level of inhibition in summer apical leaves was much less than in corresponding winter leaves. These patterns are discussed in relation to aging effects on the photorespiratory metabolism of leaves and the possibility of amelioration of photorespiratory loss during summer.

INTRODUCTION

In the aquatic environment many factors may interplay to affect the photosynthetic rate of submersed macrophytes. Plant-induced changes in the water e.g. supersaturation with O_2, increased temperature,

increased pH and reduced CO_2 (Bamforth, 1962; Brown et al., 1974; Dale and Gillespie, 1977; Van et al., 1976) may exert their effect on the photosynthetic activity of plants. The effect of these conditions may be caused by altering the ratio of photosynthesis to photorespiration. All the above plant-induced changes create conditions favouring theflow of carbon through the photorespiratory glycolate pathway (Jackson and Volk, 1970; Zelitch, 1971), and these conditions may be expected to occur more frequently in the summer when growth is rapid and densities high (e.g. Sculthorpe, 1967).

Photorespiration is of wide occurrence in the plant kingdom and has been demonstrated in many aquatic plants, including E. canadensis (Brown et al., 1974; Hough, 1979; Kutyurin et al., 1964; Søndergaard, 1979; thesis Section 3). The magnitude of photorespiration in some aquatic plants has been shown to be lower in summer than in autumn (Bowes et al., 1977a; Hough, 1974; Søndergaard, 1979). However, it is not certain whether this lower summer activity is similar to that ascribed to aging effects on photorespiration reported for terrestrial plants (e.g. Catsky et al., 1976; Hodgkinson, 1974; Kisaki et al., 1973) or whether it is caused by environmental triggers affecting the RuBP/PEP carboxylase ratio (Bowes et al., 1977a). Despite this uncertainty, there is no doubt that a reduced potential for photorespiration in the summer would be advantageous to an aquatic plant exposed to conditions that would otherwise favour this process. In this study the extent of photorespiration (indicated by O_2 inhibition of apparent photosynthesis) was monitored for E. canadensis leaves of different insertion levels at intervals over a period of one year.

Aquatic plants may also exhibit adaptation to light of different intensities (e.g. Adams et al., 1974; Bowes et al., 1977b; Gessner, 1938; Ikusima, 1966; Spence and Chrystal, 1970). Available light will vary with season, and also with change in plant biomass and epiphyte encrustation which will determine self-shading. It was for these reasons that light saturation characteristics of E. canadensis leaves of different insertion levels were monitored through the same one year cycle.

MATERIALS AND METHODS

Plant Material

Samples of E. canadensis were collected from the Leeds and Liverpool Canal, near Aintree, Merseyside (O.S. 393990) on six occasions between June 1978 and June 1979. These samples were maintained in the laboratory in canal water at 15°C, under a 16 hour photoperiod with illumination at 54 $\mu E\ m^{-2}s^{-1}$ (PAR). All physiological measurements were made within 4 days of collection. When necessary, epiphytes were removed by gently brushing the leaves and rinsing in several changes of water.

Photosynthetic and Respiratory Rate Determination

Photosynthetic and respiratory rate of leaves were measured as O_2 evolution or uptake using a Clarke-type O_2 electrode (Rank Brothers, Cambridge). The light source was a 250-W projector bulb, and the intensity was varied by altering the distance between the light source and the reaction vessel. In tests on the irradiance response of leaves, the leaves were exposed first to randomised lower light intensities and then to higher light intensities. At

these higher light intensities (light saturation point and above) the leaves were exposed to successively increasing light. This pattern of exposure was employed to minimise the risk of light damage to the photosynthetic apparatus, although no such damage was evident at the highest light intensities, because the same O_2 exchange rates were obtained at lower light intensities both before and after exposure to high light intensities.

Leaf samples comprised the three leaves of a whorl, and were taken from undamaged mainstems only from positions 0.5, 1, 5, 10, 20 and 30 cm below the apex. These samples were placed directly into the assay solution, which was a modified Forsberg Medium No. II (Forsberg, 1965), modified by omission of Na_2SiO_3, and containing: 2.4 mM total inorganic carbon; 50 mM Tris-HCl, pH 7.5; and 2 or 10 mg O_2 l^{-1} achieved by sparging with N_2 or O_2 gases as appropriate. Oxygen exchange was determined at 20°C in the light or darkness. All treatments were run in triplicate.

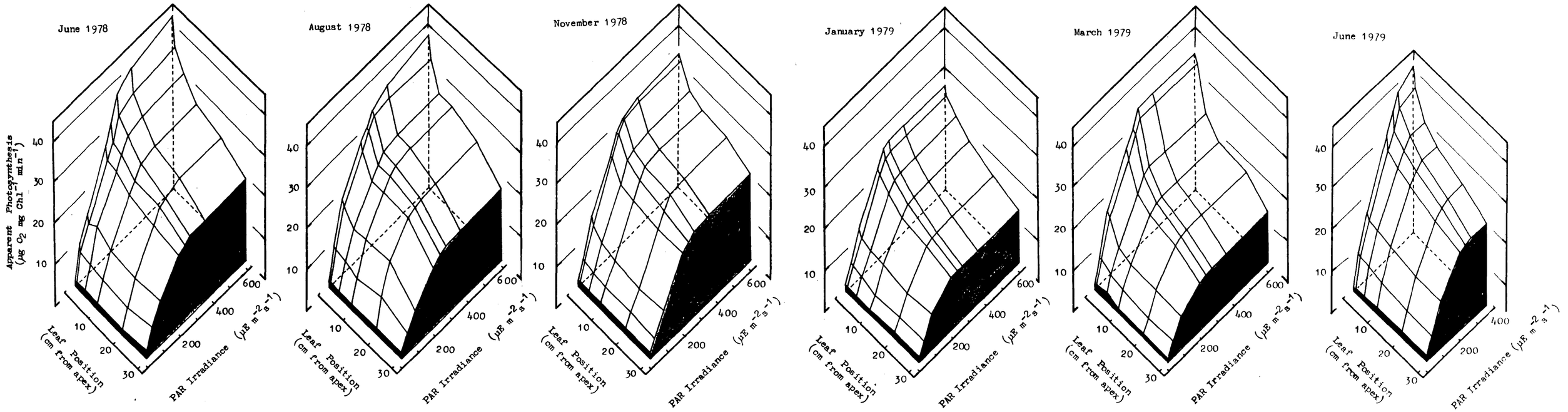
Leaf chlorophyll content was determined by the method of Arnon (1949).

RESULTS AND DISCUSSION

Light Saturation Curves

The apparent photosynthetic response curves of E. canadensis leaves to light intensity for leaves excised from positions of 0.5 to 30 cm below the apex and sampled at intervals between June 1978 and June 1979 are shown in Figure 6.1. The general pattern in summer samples was one of rapid light-saturated photosynthesis and high light saturation point in the apical leaves, with a gradual reduction in both of these

Figure 6.1 Seasonal patterns of apparent photosynthetic O_2 evolution in *E. canadensis* leaves of different insertion level to different PAR irradiances. The assay solution was a modified Forsberg medium No. II containing 2.4 total inorganic carbon and 50 mM Tris-HCl pH 7.5. Oxygen exchange was determined in the light under 10 mg O_2 l^{-1}. All data points in this and subsequent figures represent the mean of triplicate determinations.


June 1978
August 1978
November 1978
January 1979
March 1979
June 1979
Apparent Photosynthesis (μg O2 mg Chl-1 min-1)
10
20
30
40
Leaf Position (cm from apex)
10
20
30
PAR Irradiance (μE m-2 s-1)
200
400
600

parameters as distance from the apex increased. These high summer values were reduced as winter approached, until January when the lowest light saturation points and light-saturated photosynthetic rates were recorded. Thereafter they increased again to restore high values in the following summer (Figure 6.1 and Tables 6.1 and 6.2). Light compensation points were derived by regression analysis of the data and showed a similar trend to that of light saturation points, being generally high in summer apical leaves and reduced in subapical and winter leaves (Table 6.3).

Photosynthetic adaptation to light has been reported for many aquatic plants, including E. crispa (Gessner, 1938), Hydrilla verticillata (Bowes et al., 1977b), Myriophyllum spicatum (Adams et al., 1974) and Vallisneria denseserrulata (Ikusima, 1966). The reduction in the irradiance required to saturate apparent photosynthesis with increasing distance from the apex (Figure 6.1 and Table 6.1) may be an adaptation to reduced light availability with depth, which results from light attenuation by water and plant material (Hutchinson, 1967; Sculthorpe, 1967), epiphyte encrustation (Sand-Jensen, 1977) and marl deposition (Wetzel, 1960). The winter reduction in light saturation point may be an adaptation to generally reduced insolation during winter months. During sampling, care was taken to obtain plants from approximately the same position in the water column, so that depth differences in irradiance were not superimposed on seasonal differences in insolation. However, light attenuation with depth could further reduce the available light to field winter plants in deeper water. The canal is shallow (mean depth 1 - 1.5m) and the plants sampled had growing tips at a depth of 10 - 20 cm, avoiding horizontally growing surface plants in the summer. In general,

Table 6.1 Seasonal effect on light saturation point of E. canadensis leaves of different insertion level

Leaf Position (cm below the apex)	Light Saturation Point ($\mu E\ m^{-2}s^{-1}$)					
	June 1978	August 1978	November 1978	January 1979	March 1979	June 1979
0.5	510	500	340	260	290	350
1	440	320	320	250	290	320
5	340	290	300	250	270	290
10	340	280	290	250	250	280
20	340	250	290	240	240	270
30	290	250	260	220	230	240

Table 6.2 Seasonal effect on light saturated photosynthetic rate of E. canadensis leaves of different insertion level

Leaf Position (cm below the apex)	Light-saturated Photosynthetic Rate ($\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$)					
	June 1978	August 1978	November 1978	January 1979	March 1979	June 1979
0.5	42.1 ± 2.3	37.1 ± 2.6	32.5 ± 2.6	25.5 ± 1.5	33.7 ± 2.2	40.9 ± 3.1
1	35.3 ± 1.6	32.0 ± 1.8	31.6 ± 0.4	23.6 ± 1.0	31.4 ± 1.0	36.1 ± 1.6
5	30.5 ± 3.3	25.5 ± 0.2	26.2 ± 0.6	20.4 ± 0.4	22.0 ± 2.3	29.5 ± 1.0
10	25.9 ± 1.3	25.0 ± 0.9	24.4 ± 1.1	17.6 ± 1.0	17.7 ± 3.1	27.1 ± 1.0
20	24.1 ± 1.3	22.4 ± 1.2	23.6 ± 2.0	15.3 ± 1.4	15.6 ± 1.0	23.3 ± 0.8
30	19.7 ± 1.1	17.1 ± 1.1	20.8 ± 2.3	13.0 ± 1.6	12.2 ± 0.5	20.3 ± 1.7

Mean of three replicates ± standard deviation

Table 6.3 Seasonal effect on light compensation point of E. canadensis leaves of different insertion level

Leaf Position (cm below the apex)	Light Compensation Point ($\mu E\ m^{-2}s^{-1}$)					
	June 1978	August 1978	November 1978	January 1979	March 1979	June 1979
0.5	16.7	19.9	19.6	14.2	21.2	23.2
1	15.2	18.8	13.4	11.3	16.2	21.7
5	13.6	15.3	12.6	11.5	13.6	17.2
10	13.0	15.6	11.1	12.9	10.5	14.9
20	11.6	14.1	10.5	9.9	11.9	10.6
30	10.0	5.7	12.5	7.8	4.5	10.7

leaves on the main growing stem were bright green and intact down to approximately 30 cm, but below this distance were blackened and eroded especially in winter.

The slower light-saturated photosynthetic rate in lower leaves (Figure 6.1 and Table 6.2) may be related to aging effects on photosynthesis. In terrestrial plants leaf insertion level, and thus leaf age, affects photosynthetic and respiratory metabolism. Reported patterns vary and appear to depend on the units of expression. One pattern is increased photosynthetic activity up to full leaf expansion, and thereafter a steady decline, with greatest dark respiratory rates in apical leaves, when rates are expressed per unit leaf area (Dickmann et al., 1975; Kisaki et al., 1973). Another pattern is high photosynthetic activity in young leaves followed by a steady decline to senescence when rates are expressed per unit chlorophyll (e.g. Mokronosov and Nekrasova, 1977). It seems probable that similar aging effects will occur in aquatic plants, and may explain the reduced photosynthetic activity of E. canadensis leaves with increased distance from the apex.

Changes in response of dark respiratory rate to the preceding light intensity of leaves excised atdifferent distances from the apex are shown in Figure 6.2. In general increased light intensity caused an increased dark respiratory rate, but tended to stabilise when the light saturation point of photosynthesis was reached. Apical leaves exhibited the greatest dark respiratory O_2 uptake, and this gradually declined with increased distance from the apex. This decline could be related to aging effects on metabolism in a manner similar to that discussed for apparent photosynthesis. Dark respiration was also

Figure 6.2 Seasonal patterns of dark respiratory O_2 uptake in *E. canadensis* leaves of different insertion level to the preceding PAR irradiance. Assay conditicns were as described in Figure 6.1.

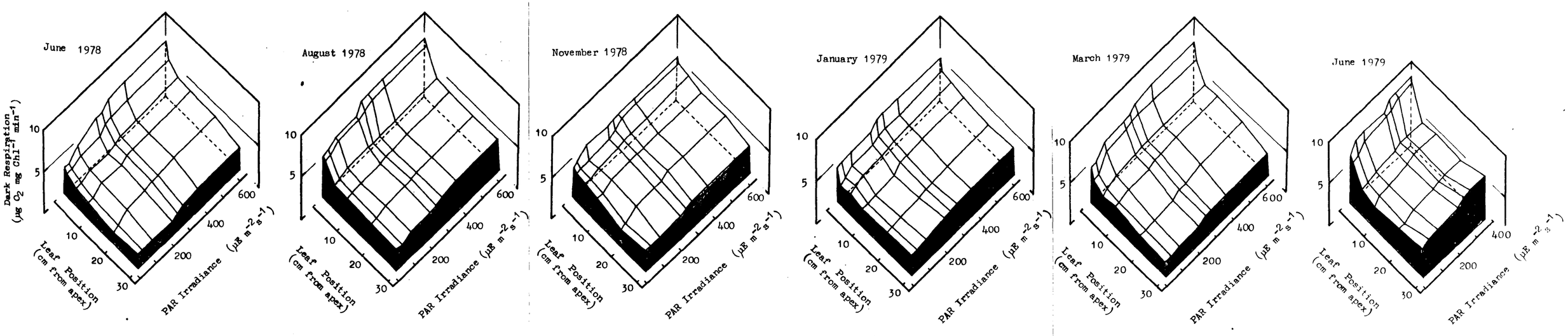

June 1978
August 1978
November 1978
January 1979
March 1979
June 1979
Dark Respiration (μg O2 mg Chl-1 min-1)
10
5
Leaf Position (cm from apex)
10
20
30
PAR Irradiance (μE m-2 s-1)
200
400
600

Table 6.4 Seasonal effect on dark respiration of E. canadensis leaves of different insertion level exposed to saturating irradiance before rate determination

Leaf Position (cm below the apex)	Dark Respiration Rate ($\mu g\ O_2\ mg\ Chl^{-1}\ min^{-1}$)					
	June 1978	August 1978	November 1978	January 1979	March 1979	June 1979
0.5	6.6 ± 0.6	7.0 ± 0.5	4.9 ± 0.6	5.2 ± 0.4	7.1 ± 1.1	8.0 ± 0.6
1	4.5 ± 0.5	6.4 ± 0.6	4.7 ± 0.9	3.6 ± 0.3	5.2 ± 0.2	7.7 ± 0.4
5	3.5 ± 0.5	3.6 ± 0.2	4.0 ± 0.5	3.5 ± 0.3	3.5 ± 0.5	3.9 ± 0.2
10	3.4 ± 0.2	3.4 ± 0.2	3.7 ± 0.7	3.2 ± 0.6	3.5 ± 0.5	4.2 ± 0.4
20	3.5 ± 0.8	3.6 ± 0.5	3.9 ± 0.5	2.9 ± 0.6	3.3 ± 0.6	4.1 ± 0.3
30	2.6 ± 0.2	3.7 ± 0.4	3.5 ± 0.2	3.2 ± 0.2	2.6 ± 0.2	5.3 ± 0.4

Mean of three replicates ± standard deviation

slightly reduced in winter leaves compared with summer ones and this reduction may be related to aging, winter leaves being proportionately older than leaves of the same insertion in summer (Table 6.4)

Oxygen Inhibition of Apparent Photosynthesis

The use of oxygen inhibition of photosynthesis to indicate the magnitude of photorespiration relies upon the assumption that dark respiration is unaffected by O_2 concentrations above 2% (Jackson and Volk, 1970). Whilst this may be valid for terrestrial plants, it is not necessarily so for aquatic plants (e.g. Dromgoole, 1978; Owens and Maris, 1964; Westlake, 1967; thesis Section 3). However, as the increase in dark respiratory O_2 uptake caused by increasing the O_2 concentration from 2 to 10 mg l^{-1} was less than the O_2 inhibition of apparent photosynthesis in all cases except the most apical leaves sampled in the summer, O_2 inhibition of apparent photosynthesis was taken here as an indicator of the magnitude of photorespiration. (See Section 3 for a more detailed discussion).

Differences in O_2 inhibition and percentage inhibition of photosynthesis are shown in Figures 6.3 and 6.4 respectively. There were marked changes in O_2 inhibition of apparent photosynthesis with both leaf position and season. In general inhibition was greatest some distance below the apex, and rather less both near the apex and towards the base of the plant. This pattern of change in O_2 inhibition of photosynthesis with leaf insertion level conforms with aging effects on photorespiration in many terrestrial crop plants which generally have low photorespiration in young leaves, increasing to a maximum in fully expanded mature leaves and then declines to

Figure 6.3 Seasonal patterns of O_2 inhibition of apparent photosynthesis in E. canadensis leaves of different insertion level. Inhibition is expressed for rates at 10 relative to those at 2 mg O_2 l^{-1}. Assay conditions were as described in Figure 6.1 except that rates were determined at a PAR irradiance of 290 μE $m^{-2}s^{-1}$. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.

For reference, the enhancement of dark respiration (o) caused by 10 mg O_2 l^{-1} is included for comparison with the data on O_2 inhibition of apparent photosynthesis (●).

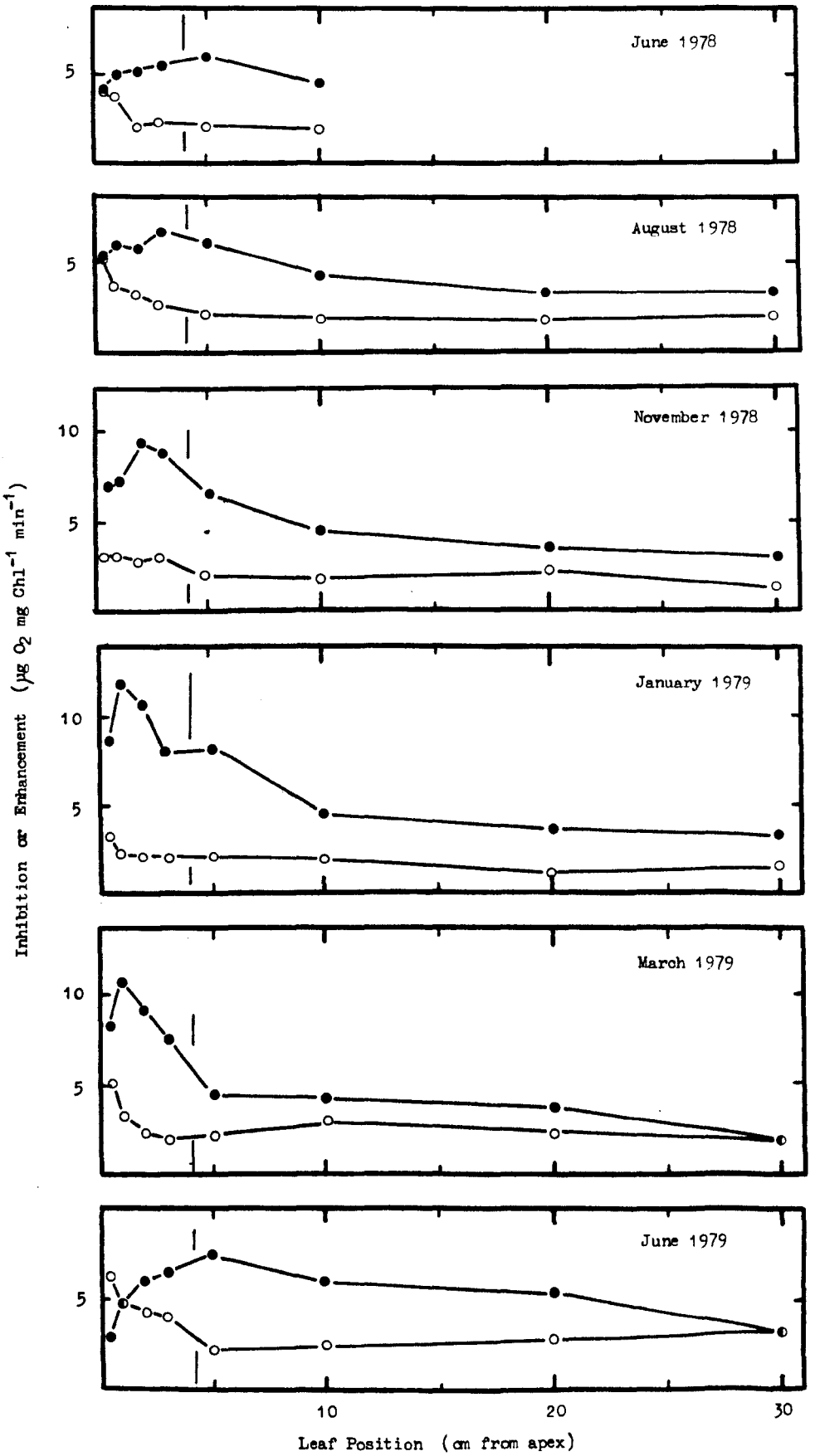
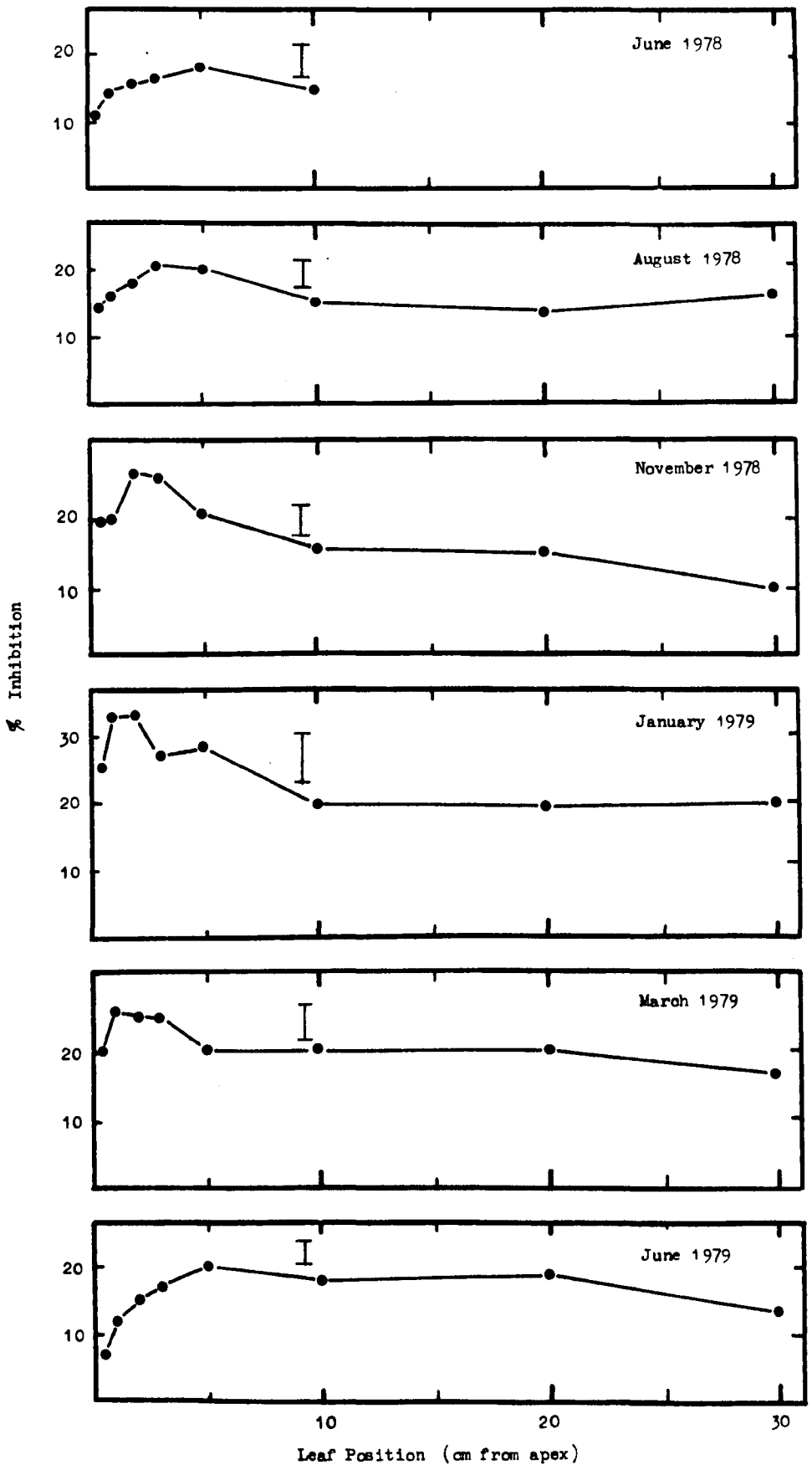

June 1978
August 1978
November 1978
January 1979
March 1979
June 1979
5
10
10
Inhibition or Enhancement (μg O2 mg Chl-1 min-1)
10
20
30
Leaf Position (cm from apex)

Figure 6.4 Seasonal patterns of percentage inhibition of photosynthesis caused by O_2 in E. canadensis leaves of different insertion level. Assay conditions were as described in Figure 6.3. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


June 1978
August 1978
November 1978
January 1979
March 1979
June 1979
% Inhibition
Leaf Position (cm from apex)
10
20
30

senescence (Catsky et al., 1976; Hodgkinson, 1974; Kisaki et al., 1973), and is also consistant with the possibility of aging contributing to seasonal changes in photorespiration of aquatic plants (Bowes et al., 1977a; Hough, 1974; Søndergaard, 1979). The gradual shift in the position of greatest O_2 inhibition of photosynthesis towards the apex in the winter may again be because apical leaves in the winter are proportionately older than leaves of the same insertion level in the summer.

Winter apical leaves of E. canadensis exhibit higher O_2 inhibition of photosynthesis than summer apical leaves. Similar trends in photorespiratory metabolism can be inferred from the report of Søndergaard (1979) that the CO_2 compensation point of E. canadensis doubled between May and September. Hydrilla verticillata exhibited a high CO_2 compensation point in the winter, and a low one in summer (Bowes et al., 1977a). In this latter instance the low CO_2 compensation point corresponded to an increase in the proportion of PEP carboxylase compared with RuBP carboxylase which could explain some of the reduced photorespiratory activity by a shift from C_3 to C_4 metabolism. However, immaturity has been associated with high PEP carboxylase activity in terrestrial plants (Kisaki et al., 1973; Mokronosov and Nekrasova, 1977) and some of the seasonal shift in RuBP and PEP carboxylase activity noted by Bowes et al., (1977a) for H. verticillata could be caused by the varying maturation state of the apical segments used.

Reduced photorespiratory activity in the summer may be caused by such environmental triggers as daylength and temperature, as suggested

by Bowes et al. (1977a), or may be a reflection of the relatively young average age of such leaves at that time of the year. In either case, it is likely to confer advantages on the plant under summer conditions of high irradiance, high temperature, high O_2 and low CO_2 (e.g. Bamforth, 1962; Brown et al., 1974; Dale and Gillespie, 1977; Van et al., 1976). Such conditions stimulate deleterious loss of carbon through the photorespiratory glycolate cycle (Jackson and Volk, 1970; Zelitch, 1971), and any reduction in the potential for such loss must be advantageous to plants exposed to such conditions during their main growing season.

CONCLUSIONS

Light-saturated photosynthetic rates, and light saturation and compensation points of E. canadensis leaves were greatest in the summer months when insolation was high, and least in the winter when insolation was low. The magnitude of these parameters was greatest in the apical leaves and was reduced as the distance from the apex increased. The trends in light saturation and compensation points may be the result of photosynthetic adaptation to seasonal changes in insolation, and in light attenuation caused by water, plant material, epiphyte and marl encrustations in natural plant stands. Such adaptations could benefit the plant by permitting photosynthesis under low winter irradiances and allowing carbon fixation to occur in the older, more shaded leaves, thereby either reducing the respiratory drain of these shaded leaves on the rest of the plant or permitting them to contribute some fixed carbon to the new growth.

Oxygen inhibition of apparent photosynthesis was greatest some distance below the apex, and rather less both near the apex and

towards the base of the plant. Such differences may be related to aging effects similar to those reported for terrestrial plants. This inhibition was also least during the summer months, and may be a reflection of the relatively young age of such leaves in the summer, or an adaptation induced by environmental conditions. In either case, it may indicate some control of the potential for carbon loss through the photorespiratory cycle. Such control is likely to be advantageous to the plant under summer conditions of high temperature, irradiance, O_2 and low CO_2 which would otherwise tend to stimulate deleterious carbon loss during the main growing season.

Interspecific competition for light has often been cited as a major factor controlling plant distribution and competitive success in natural waters. However, the photosynthetic performance of Elodea canadensis is severely limited by high O_2, high pH and low CO_2 conditions, all of which develop in the water surrounding photosynthesising aquatic plants. Therefore in this section, the photosynthetic performance of E. canadensis and two of its algal competitors are compared to ascertain whether factors other than competition for light might help to explain reductions of E. canadensis biomass in nutrient rich sections of the Leeds and Liverpool Canal in South Lancashire.

7. COMPARATIVE STUDIES ON THE PHOTOSYNTHESIS OF THE SUBMERSED MACROPHYTE ELODEA CANADENSIS AND THE FILAMENTOUS ALGAE CLADOPHORA GLOMERATA AND SPIROGYRA SP.

ABSTRACT

The photosynthetic and respiratory responses of Elodea canadensis Michx., Cladophora glomerata Kütz and Spirogyra sp. to oxygen, temperature, HCO_3^- concentration, pH and irradiance were determined. Photosynthesis was inhibited by O_2 in all three species under all conditions, and inhibition was greatest in E. canadensis. This inhibition was not caused solely by an accelerated rate of dark respiration, and this suggests that photorespiration may be an important factor controlling productivity, particularly in E. canadensis.

The photosynthetic performance of E. canadensis was impaired much more than was that of either of the filamentous algae under conditions of high pH and low CO_2 concentrations. The possibility of these algae increasing the pH and reducing the CO_2 content of the water as a result of their photosynthesis with consequent deleterious effects on macrophyte photosynthesis is discussed. Such a plant-induced change in water quality could give C. glomerata a competitive advantage over E. canadensis, and be a factor in the replacement of the vascular plant by the alga in some waters.

INTRODUCTION

The aquatic environment experiences rapid diurnal changes in many factors that may affect the photosynthesis of submersed vegetation. Such changes include daytime supersaturation of the water with O_2, reduced availability of CO_2, increased pH and also increased

temperature (e.g. Bamforth, 1962; Brown et al., 1974; Dale and Gillespie, 1977; Van et al., 1976). The ability of a plant to photosynthesise effectively in such changing conditions may influence its competitive success and therefore affect species composition of a water body.

Some reports suggest that interspecific competition for light, CO_2 and nutrients affects species composition, and that light availability may be an important factor. The dominance of Hydrilla verticillata over native aquatic vegetation in Florida has been attributed to its low light requirement for photosynthesis (Van et al., 1976). Compared to native species, H. verticillata required less light to half-saturate photosynthesis, and had a lower light compensation point. This enabled it to outcompete native species during regrowth from the sediment and allowed photosynthesis in dense weed mats when light was limiting. A similar correlation between photosynthetic responses to light by three exotic macrophytes and their ability to displace native vegetation has also been reported in New Zealand lakes (Brown et al., 1974).

Competition between algae and macrophytes has also resulted in changing species composition of waterbodies. The disappearance of Najas marina from Upton Broad was correlated with the appearance of a large Spirogyra community and shading of the macrophyte by this alga was thought to be the main factor (Phillips et al., 1978). Similarly Cladophora glomerata Kütz has gradually replaced vascular macrophytes in a nutrient enriched river (Lund and Bolas, 1974). Such a displacement is usually associated with eutrophication of the water which increases nutrient supply and allows growth of epiphytic and

filamentous algae, which subsequently shade the macrophytes to below compensation (Phillips et al., 1978). In nutrient-poor waters, vascular rooted plants can obtain nutrients from mud through roots, whereas algae may be limited to nutrients from the water. When nutrient supply in the water is increased, the competitive advantage of vascular plants is lost, and algal growth is stimulated. However epiphytic algae have been shown to reduce eelgrass photosynthesis by limiting CO_2 supply as well as by shading them (Sand-Jensen, 1977).

In recent years the species composition of the submersed vegetation in parts of the Leeds and Liverpool Canal in South Lancashire has changed from a community dominated by Elodea canadensis Michx. to one dominated by the filamentous alga C. glomerata sometimes with Spirogyra spp. intermingled. The stretch affected receives nutrient-rich water through a feeder at the upstream end. The water immediately surrounding actively photosynthesising algae is often supersaturated with O_2 and has a high pH value which is often locally in excess of pH9. High O_2, high pH and correspondingly low CO_2 conditions stimulate photorespiration in E. canadensis and severely limit its photosynthetic activity (thesis Section 3). Thus the main aim of this work was to determine whether differential photosynthetic performance of E. canadensis and the filamentous algae C. glomerata and Spirogyra sp. under simulated field conditions could explain the decline in E. canadensis biomass noted in the field.

MATERIALS AND METHODS

Plant Material

Samples of E. canadensis, C. glomerata and Spirogyra sp. were collected from the Leeds and Liverpool Canal, near Aintree, Merseyside

(O.S. 393990). The total inorganic carbon content of the water was 2 mM. The apices of the E. canadensis stems were within the top 25 cm of the water column, with C. glomerata and Spirogyra sp. intermingled. Therefore all three species came from the same depth and probably experienced the same water conditions. Plants and algae were maintained in the laboratory in canal water at 15°C under a 16 hour photoperiod, 54 $\mu E\ m^{-2}s^{-1}$ (PAR) irradiance. Photosynthetic and respiratory oxygen exchange rates were measured within 4 days of collection.

Photosynthetic and Respiratory Rate Determination

Unless otherwise indicated photosynthetic and respiratory rates of E. canadensis and the filamentous algae were measured as O_2 evolution or uptake at 20°C using a Clarke-type O_2 electrode (Rank Brothers, Cambridge). O_2 exchange was determined for the 3 leaves of a whorl excised 3 cm below the apex of E. canadensis, and for 0.5 cm cut lengths of C. glomerata and Spirogyra filaments that had been washed several times to remove cell debris. The light source was a 250-W projector bulb and the reaction vessel was illuminated to 290 $\mu E\ m^{-2}s^{-1}$ PAR irradiance. The intensity was varied when necessary by altering the distance between the light source and reaction vessel. During tests to determine the response of specimens to irradiance, the plant material was first exposed to randomised lower light intensities and then to higher light intensities. Above light saturation point the plant material was exposed to successively increasing light intensities. This pattern of exposure was designed to minimise the risk of light damage to the photosynthetic apparatus although there was no evidence of such damage.

Temperature was controlled by circulating water of the appropriate temperature through the outer jacket of the elctrode. All specimens were incubated for 30 minutes at the relevant temperature before O_2 exchange rates were determined.

Photosynthetic and respiratory rates were measured in 3 cm^3 of a Forsberg medium No. II (Forsberg, 1965) modified by omission of Na_2SiO_3 and carbon sources. Buffer components were varied to cover the required pH range i.e. 50 mM NaCitrate-NaH_2PO_4 for pH 5 to 6, 50 mM Tris-HCl for pH 7 to 9. Photosynthesis was initiated by injecting 0.1 cm^3 $NaHCO_3$ solution to give a final concentration of 2.4 mM HCO_3^- which approximated to that present in the canal water. In experiments at higher HCO_3^- concentrations, the buffering capacity of the added HCO_3^- was compensated for by addition of predetermined amounts of 0.2 M HCl. All treatments were run in triplicate.

The buffered solutions were sparged with N_2 and/or O_2 gases to give the desired initial O_2 concentration before immersion of plant samples and injection of HCO_3^- solution.

The method of Arnon (1949) was used for all chlorophyll determinations.

Oxygen Inhibition of Photosynthesis

Oxygen inhibition of apparent photosynthesis is expressed as the rate of photosynthesis at 10 mg O_2 l^{-1} relative to the rate at 2 mg O_2 l^{-1} and was used in this study to indicate the extent of photorespiration. Such use normally assumes that dark respiration rates are unaffected by O_2 concentrations above 2% (Jackson and Volk, 1970). However dark respiration is affected by O_2 concentration in aquatic plants (Dromgoole, 1978; Westlake, 1967; thesis Section 3) but in most

instances in this study the enhancement of dark respiration caused by increased O_2 was insufficient to account for the observed inhibition of photosynthesis. The exceptions were at 12 mM HCO_3^- concentration in E. canadensis and Spirogyra, pH 5 and 6 in C. glomerata and Spirogyra and pH 5, 6 and 9 in E. canadensis. As high CO_2 conditions do not favour photorespiration it was considered that O_2 inhibition of photosynthesis could be used to provide an estimate of photorespiration.

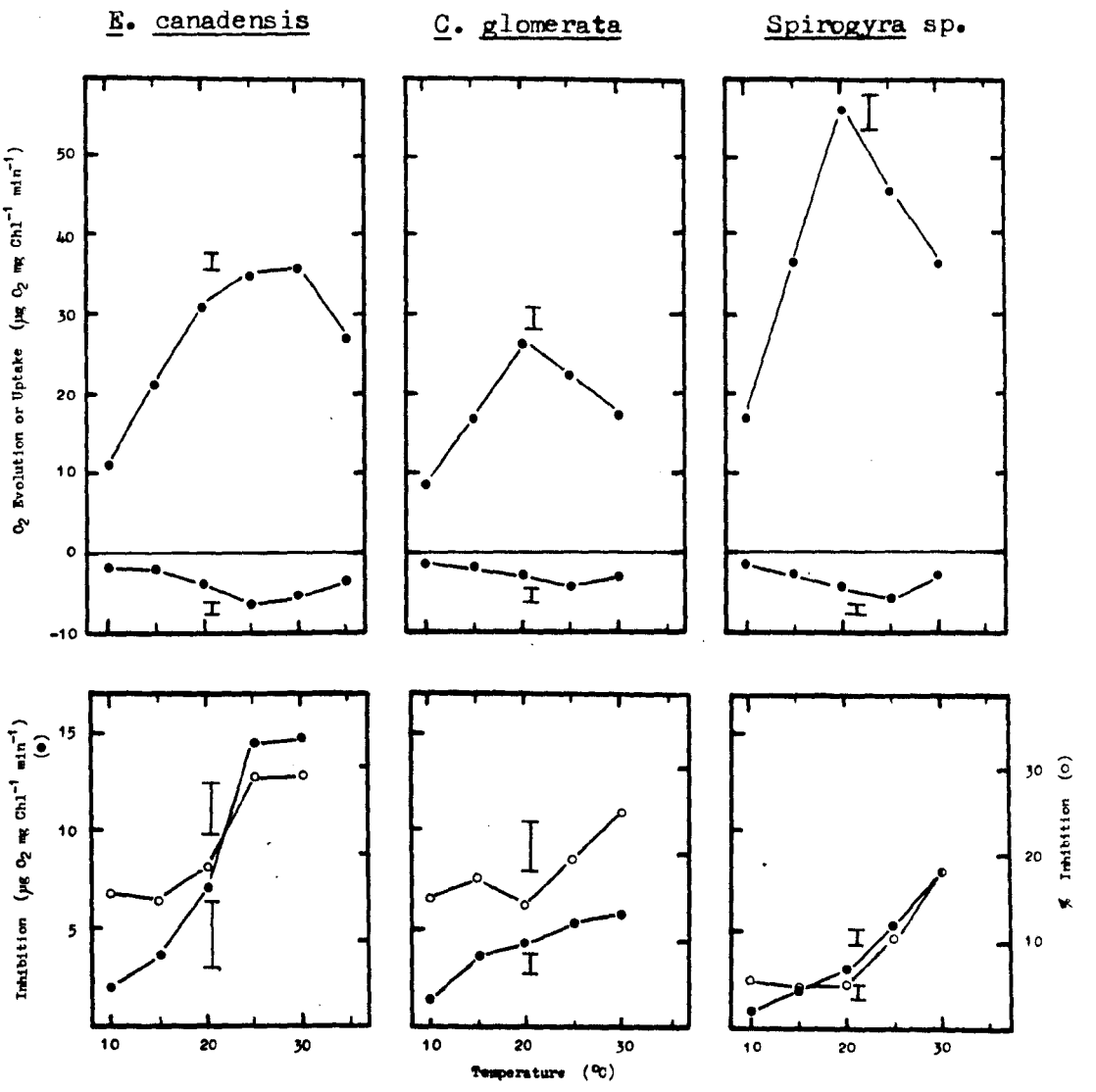
RESULTS AND DISCUSSION

The effect of Temperature on Oxygen Exchange

The photosynthetic and respiratory responses of E. canadensis, C. glomerata and Spirogyra sp. to temperature are shown in Figure 7.1. Elodea canadensis had the highest temperature optima for photosynthesis and dark respiration, these being between 25° and 30°C for both processes. The optimum temperatures for photosynthesis in both C. glomerata and Spirogyra sp. were somewhat lower, at approximately 20°C andtheir optima for dark respiration about 25°C in both cases.

The photosynthetic temperature optimum reported here for C. glomerata corresponds to optimum temperature requirements of 18°C reported elsewhere (Bellis and McLarty, 1967, Whitton, 1970) and is only slightly less than the upper temperature tolerance limits of 23.5°C derived from field observations (Wong et al., 1978) and of 24-25°C from laboratory investigations (Whitton, 1967). Cladophora glomerata has been shown to exhibit seasonal periodicity, with intense vegetative growth occurring twice yearly at the onset and return of intermediate (15 ± 4°C) water temperatures (Bellis and McLarty, 1967; Thurman and Kuehne, 1952). The relatively high photosynthetic

Figure 7.1 The effect of temperature on apparent photosynthetic O_2 evolution and O_2 uptake in the dark (top), and oxygen inhibition of apparent photosynthesis (bottom) of E. canadensis, C. glomerata and Spirogyra sp. The assay solution was a modified Forsberg medium No. II containing 2.4 mM total inorganic carbon and 50 mM Tris-HCl, pH 7.5. Oxygen exchange was determined under 10 mg O_2 l^{-1} and 290 μE $m^{-2}s^{-1}$ PAR irradiance. Inhibition is expressed as the rate at 10 mg O_2 l^{-1} relative to that at 2 mg O_2 l^{-1}. All data points in this and subsequent figures represent the mean of triplicate determinations. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


E. canadensis
C. glomerata
Spirogyra sp.
O2 Evolution or Uptake (µg O2 mg Chl-1 min-1)
50
40
30
20
10
0
-10
Inhibition (µg O2 mg Chl-1 min-1) (●)
15
10
5
% Inhibition (○)
30
20
10
10
20
30
Temperature (°C)

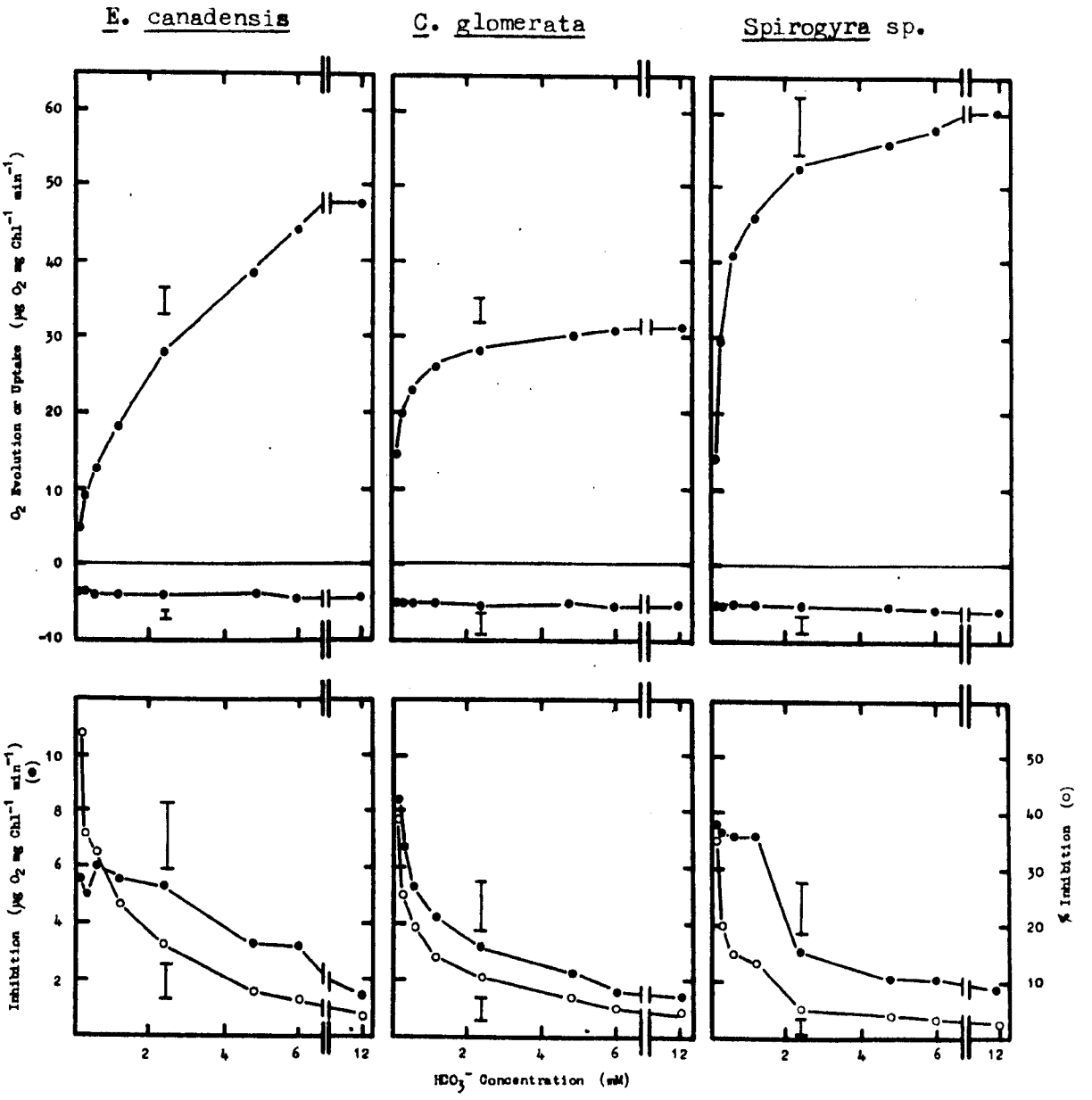
temperature optimum of 25-30°C for E. canadensis is in accordance with the rapid net assimilation reported for this species at high temperature (Allen, 1973) and may give it a competitive advantage over species with lower temperature optima during hot summers in temperate waters. However, the normal daytime temperatures in the Leeds and Liverpool Canal are considerably lower than the optimum for E. canadensis and measurements indicate an annual mean over 5 years of 11-12°C with usually less than 30 days each summer above 20°C. Thus this potential advantage may be of little actual value in this species.

Increased temperature resulted in increased O_2 inhibition of apparent photosynthesis in all three species (Figure 7.1). This inhibition was maximal in E. canadensis at the same temperature as the optimum for photosynthesis, but the maximum inhibition attained in C. glomerata and Spirogyra sp. were at 15°C higher than the temperature optimum for photosynthesis. Both types of relationship between the optimum temperatures for O_2 inhibition and photosynthesis have been reported in terrestrial plants where maximum O_2 inhibition has been found at the temperature of maximal photosynthesis (e.g. Ku and Hunt, 1973; Ku et al., 1977) and also at 10-15°C higher than the maximal photosynthesis (e.g. Jolliffe and Tregunna, 1968; Pearson and Hunt, 1972).

The effect of CO_2 on Oxygen Exchange

Under saturating CO_2 concentrations, apparent photosynthesis was greatest in Spirogyra sp. least in C. glomerata and intermediate in E. canadensis (Figure 7.2). However, the apparent Km(HCO_3^-) values for photosynthesis in Spirogyra sp. and C. glomerata were 0.2 and 0.3 mM respectively, which were much lower than the value of 1.9 mM in

Figure 7.2 The effect of HCO_3^- concentration on apparent photosynthetic O_2 evolution and O_2 uptake in the dark (top), and oxygen inhibition of apparent photosynthesis (bottom) of E. canadensis, C. glomerata and Spirogyra sp. at 20°C. Other assay conditions were as described for Figure 7.1. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


E. canadensis
C. glomerata
Spirogyra sp.
O2 Evolution or Uptake (μg O2 mg Chl-1 min-1)
Inhibition (μg O2 mg Chl-1 min-1) (●)
% Inhibition (○)
HCO3- Concentration (mM)

E. canadensis. These large differences in apparent Km for photosynthesis suggest that the two filamentous algae were better adapted to maintain photosynthetic activity in low CO_2 concentrations than was E. canadensis.

Photosynthetic use of CO_2 at low ambient CO_2 concentrations may be facilitated by the enzyme carbonic anhydrase which catalyses the reversible hydration of CO_2 ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$). In general submersed aquatic plants have very low carbonic anhydrase activity compared with terrestrial plants (Van et al., 1976) and also with floating-leaved, freefloating and emergent macrophytes (Weaver and Wetzel, 1980). In contrast various algal types have much greater (10 to 20 times) the carbonic anhydrase activity when grown at atmospheric levels of CO_2 than when grown in air supplemented with 1% CO_2 (Nelson et al., 1969) or 5% CO_2 (Graham et al., 1971; Ingle and Colman, 1975). High carbonic anhydrase activity in algae at low CO_2 concentrations may help to explain the low apparent Km(HCO_3^-) for photosynthesis found here and may also put such species at a competitive advantage in the low CO_2 conditions that develop in water surrounding vigorously photosynthesising plants.

In all three species O_2 inhibition of apparent photosynthesis was greatest at low HCO_3^- concentration and was reduced with increased HCO_3^- concentration (Figure 7.2). Percentage inhibition was greatest in E. canadensis at 0.15 mM HCO_3^-, being 54% compared with 39% in C. glomerata and 35% in Spirogyra sp. Inhibition then showed an exponential-type decline to give similar, very low (3-4%) levels

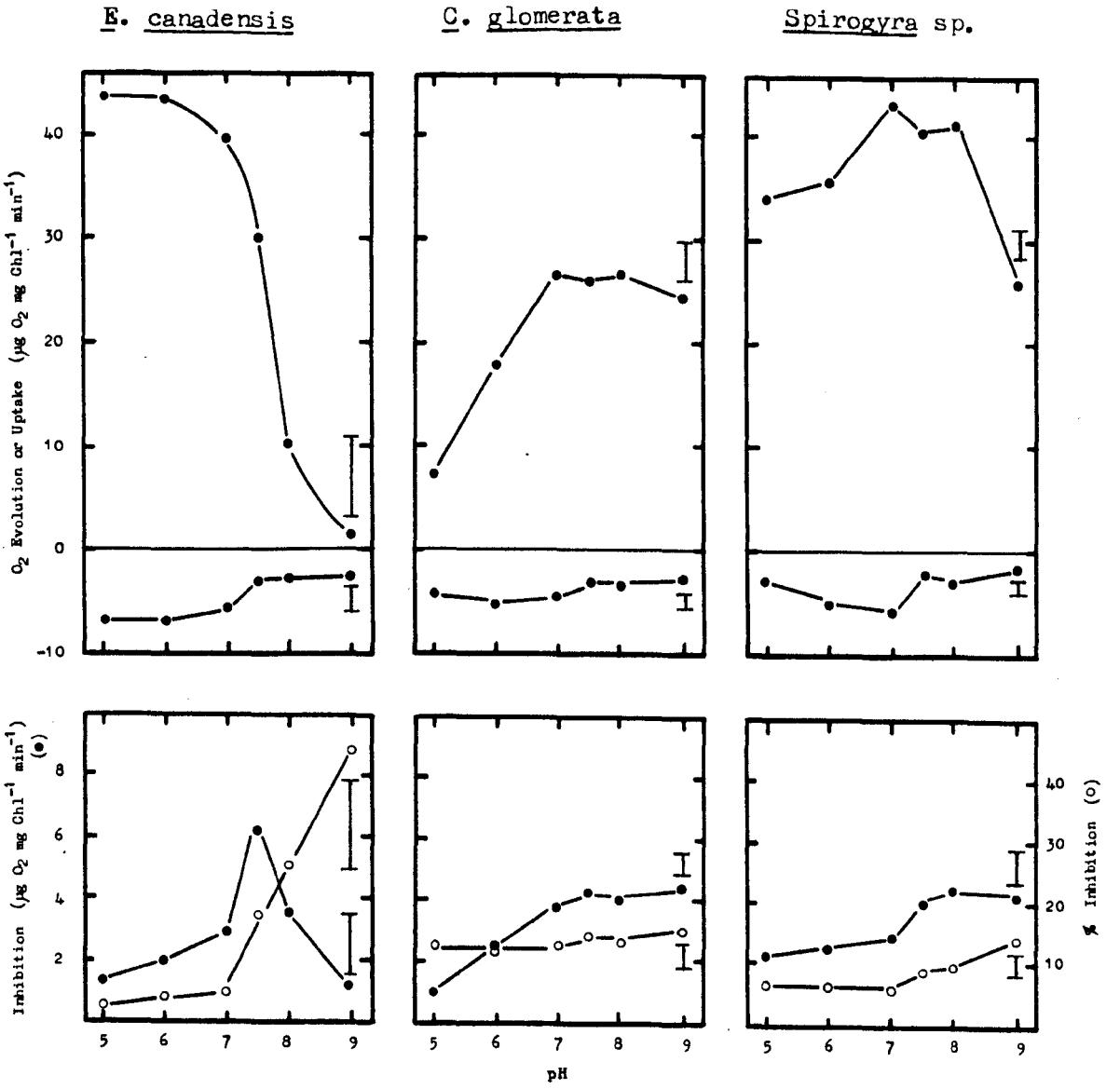
in all three species. This inhibition was consistently greater in E. canadensis than in the algae over the whole range of HCO_3^- concentrations tested. At 2 mM HCO_3^-, which approximates to the HCO_3^- concentration in the Leeds and Liverpool Canal, inhibition was 18% compared to 10% and 6% in C. glomerata and Spirogyra sp. respectively (Figure 7.2).

The effect of pH on Oxygen Exchange

The light saturated photosynthetic rate of E. canadensis was maximum at pH 5 to 6 and declined thereafter to give very low rates at pH 9 (Figure 7.3). Similar pH effects on photosynthesis have been reported in other macrophytes (Brown et al., 1974; Shiyan and Merezhko, 1972; Steemann Nielsen, 1960; Van et al., 1976). In contrast the two filamentous algae showed maximum photosynthesis above neutrality, and exhibited a decline in photosynthesis above pH 8. Photosynthetic rates of C. glomerata were very low at pH 5 and if left in these conditions for longer than 15 minutes photosynthetic O_2 evolution became negligible. In addition, if samples were left in low pH buffered solutions overnight, cells were bleached the following day. This poor tolerance exhibited by C. glomerata to low pH conditions has been noticed in laboratory studies where growth only occurred within the pH range 7 to 9 (Bellis, 1968) and can be inferred from field observations which indicate that this alga is restricted to natural waters having an average pH between 7 and 9 (Blum, 1956; Mason, 1965).

Oxygen inhibition of photosynthesis was low at low pH in E. canadensis and rose to a maximum at pH 7.5. Thereafter it declined. In C. glomerata and Spirogyra sp. O_2 inhibition increased with increased pH up to pH 7.5 and pH 8 respectively and remained stable thereafter

Figure 7.3 The effect of pH on apparent photosynthetic O_2 evolution and O_2 uptake in the dark (top), and oxygen inhibition of apparent photosynthesis (bottom) of E. canadensis, C. glomerata and Spirogyra sp. at 20°C. Other assay conditions were as described for Figure 7.1 except that the assay solution was buffered with 50 mM NaCitrate-NaH_2PO_4 at pH 5 and 6, and 50 mM Tris-HCl at pH 7 to 9. Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


E. canadensis
C. glomerata
Spirogyra sp.
O2 Evolution or Uptake (µg O2 mg Chl-1 min-1)
Inhibition (µg O2 mg Chl-1 min-1) (●)
% Inhibition (○)
pH

(Figure 7.3). The effect of increased pH on percentage inhibition of photosynthesis was greatest in E. canadensis and resulted in an increase from 3% at pH 5 to 45% at pH 9. The effect of similar pH rises on percentage inhibition in C. glomerata and Spirogyra sp. were far less severe and only resulted in an increase from 12 to 15% and 6 to 14% respectively.

The observed increases in percentage inhibition of photosynthesis with increasing pH are consistent with reports for isolated chloroplasts (Dodd and Bidwell, 1971; Robinson et al., 1977) and are explicable in terms of reduced CO_2 at high pH and consequent less successful competition of CO_2 with O_2 for RuBP carboxylase.

The relatively greater photosynthesis and lower inhibition of photosynthesis shown at high pH by the filamentous algae compared with E. canadensis may put such algae at an advantage under the high pH conditions which develop in water surrounding vigorously photosynthesising water plants.

Some uncertainty exists about the nature and existence of photorespiration in algae (Cheng and Colman, 1974; Chollet and Ogren, 1975; Tolbert, 1974). Many algae produce glycolate and possess the necessary enzymes of the photorespiratory glycolate pathway (Merret and Lord, 1973; Tolbert, 1974) but O_2 effects on photosynthesis and photorespiration are generally lower in algae than in conventional C_3 plants (Raven and Glidewell, 1978; Tolbert, 1974) and could be reduced by glycolate excretion thereby reducing available glycolate for photorespiration (Colman et al., 1974; Merret and Lord, 1973), partial limitation of an enzymatic step of the glycolate pathway

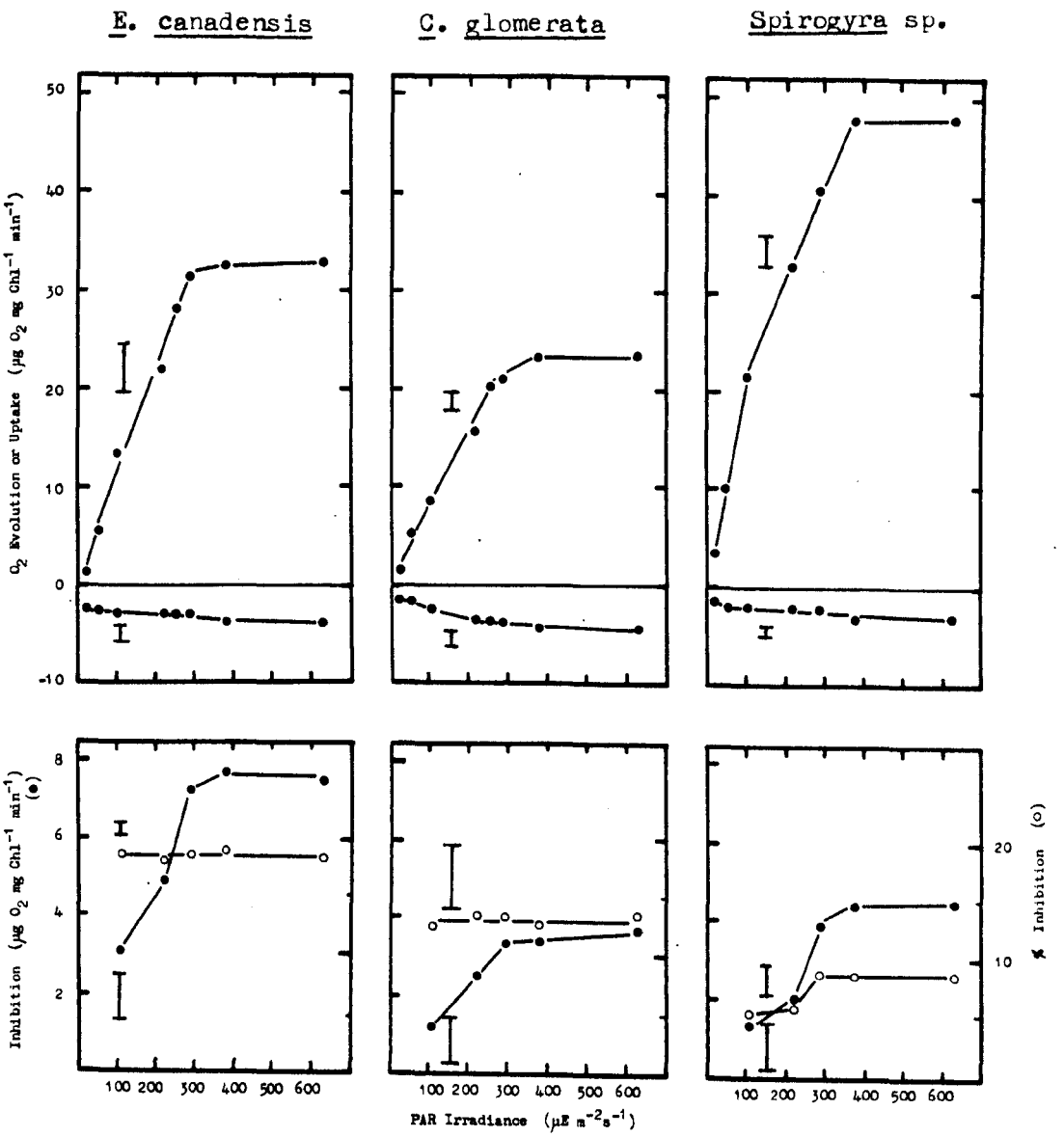
(Bruin et al., 1970) and/or efficient refixation of photorespired CO_2 (Goldsworthy, 1970; Raven and Glidewell, 1978). However glycolate excretion and refixation must still reduce the plant's assimilatory potential. Some workers have concluded that conventional photorespiration does not exist in algae (Bidwell, 1977; Lloyd et al., 1977) but their inability to detect CO_2 evolution in illuminated chlorophyte algae may be explained by efficient reassimilation (Raven and Glidewell, 1978). However, in most instances in this present study, with the exception of low pH and high CO_2 conditions i.e. ones that do not stimulate photorespiration, O_2 inhibition of photosynthesis was much greater than the enhancement of dark respiration caused by similar increases in O_2 concentration. Thus photorespiration may operate in these algae, although at a reduced rate compared to other C_3 plants.

The effect of Light on Oxygen Exchange

Photosynthesis was saturated in E. canadensis at 290 $\mu E\ m^{-2}s^{-1}$ and at 380 $\mu E\ m^{-2}s^{-1}$ in C. glomerata and Spirogyra sp. (Figure 7.4). However both algae and macrophytes are capable of photosynthetic adaptation to different light intensities (e.g. Adams et al., 1974; Bowes et al., 1977; Jørgensen, 1969; Spence and Chrystal, 1970; thesis Sections 5 and 6) and therefore isolated determinations of light saturation point may not give a good indication of differences of significance in determining competitive success.

Oxygen inhibition of photosynthesis increased with increasing irradiance to light saturation. A similar pattern was evident for percentage inhibition in Spirogyra sp. but inhibition represented a constant fraction of photosynthesis over the range 100 to 630 $\mu E\ m^{-2}s^{-1}$

Figure 7.4 The effect of irradiance level on apparent photosynthetic O_2 evolution and O_2 uptake in the dark (top), and oxygen inhibition of apparent photosynthesis (bottom) of E. canadensis, C. glomerata and Spirogyra sp. at 20°C. Other assay conditions were as described for Figure 7.1 Bars indicate LSD at P = 0.05 for comparing any one mean on a line with any other mean on the same line.


E. canadensis
C. glomerata
Spirogyra sp.
O2 Evolution or Uptake (µg O2 mg Chl-1 min-1)
Inhibition (µg O2 mg Chl-1 min-1) (●)
% Inhibition (○)
PAR Irradiance (µE m-2s-1)
50
40
30
20
10
0
-10
8
6
4
2
20
10
100 200 300 400 500 600

irradiance in E. canadensis and C. glomerata. Percentage inhibition was greatest in E. canadensis, intermediate in C. glomerata and least in Spirogyra being approximately 18, 13 and 8% respectively at light saturation (Figure 7.4).

Ecological Implications

In terms of photosynthetic performance, E. canadensis was much less effective than the filamentous algae C. glomerata and Spirogyra sp. under conditions of high O_2, high pH and low CO_2. On the basis of these laboratory studies, photosynthesis of E. canadensis would be most severely limited under the high O_2, high pH and consequent low CO_2 conditions that develop in water bodies as a result of plant photosynthesis at high surface irradiances (e.g. Bamforth, 1962; Brown et al., 1974; Van et al., 1976), especially if exposed to such conditions for extended periods of time.

The limitations imposed on an aquatic plant by photorespiration and associated O_2 effects on photosynthesis may be reduced by a number of mechanisms. These include HCO_3^- utilisation through carbonic anhydrase (Sculthorpe, 1967; Steemann Nielsen, 1960), active C_4 metabolism (Brown et al., 1974; De Groote and Kennedy, 1977) and gas movement and storage within the lacunar system (Hough and Wetzel, 1977; Raven, 1970; Sø ndergaard and Wetzel, 1980). However, amelioration by such mechanisms may be insignificant in E. canadensis and has been discussed in more detail elsewhere (thesis Sections 1 and 3). In contrast chlorophyte algae probably have a 'CO_2-pump' to increase the CO_2 concentration at the site of photosynthesis (Raven and Glidewell, 1978) and this may help to explain the low levels of O_2 inhibition of photosynthesis in C. glomerata and Spirogyra sp. In addition the

inducible carbonic anhydrase which operates at low levels of CO_2 in algae (Graham et al., 1971; Ingle and Colman, 1975; Nelson et al., 1969) compared with low levels of this enzyme in E. canadensis (Weaver and Wetzel, 1980) may help to explain the generally better performance of C. glomerata and Spirogyra sp. in low CO_2 and high pH conditions.

The involvement of filamentous algae in macrophyte decline has been inferred from laboratory and field investigations. Photosynthetic ^{14}C uptake of Najas marina was reduced by 33% when placed under a Spirogyra community and the appearance of this alga correlated with the disappearance of N. marina in Upton Broad (Phillips et al., 1978). Spirogyra has also been responsible for retarded growth and disintegration of some Ceratophyllum shoot apices after entanglement around them (Goulder, 1968). The gradual replacement of macrophytes by C. glomerata in a nutrient enriched river has also been reported (Bolas and Lund, 1974). Such macrophyte decline has recently been attributed to nutrient enrichment causing increased growth of epiphytic and filamentous algae which then shade the macrophytes to such an extent that they cannot compensate for respiration (Phillips et al., 1978). However shading by algae may not be the only factor involved and epiphytic algae have been shown to limit eelgrass photosynthesis by reducing light intensity and acting as a barrier to CO_2 uptake (Sand-Jensen, 1977).

The results reported here suggest another way in which filamentous algae can be put at an advantage over a vascular competitor. Plant-induced high O_2, high pH and low CO_2 conditions in the water put vascular macrophytes like E. canadensis under severe photosynthetic stress whilst the filamentous algae C. glomerata and Spirogyra sp.

are less affected. The algae themselves may create conditions in which they can continue to photosynthesise and grow, but E. canadensis cannot, and hence the latter is outcompeted.

8. GENERAL DISCUSSION

As already has been pointed out in the Introduction, it was necessary in the main part of the thesis to separate out the interaction between environmental factors, age and leaf position on the photosynthesis and respiration of Elodea canadensis. In this general discussion I hope to draw together the physiological aspects of E. canadensis ecology and interpret these in relation to field conditions. To this end, the results of laboratory investigations into the effects of environmental factors on the photosynthesis and respiration of E. canadensis and its algal competitors will be discussed first. This will be followed by a discussion of the adaptability of E. canadensis to certain environmental variables, with particular reference to light, temperature and CO_2 availability. Only minor reference will be made to the available literature since this has been referred to extensively in the relevant thesis sections. It should also be remembered that the results reported for E. canadensis and the inferences made do not necessarily apply to other, and especially to non-hydrocharitacean species.

These studies provide strong evidence for the occurrence of photorespiration in the submersed macrophyte E. canadensis. This is evidenced by the inhibition of photosynthesis caused by O_2, and it cannot be explained solely on the basis of increased dark respiration in response to increased O_2 content of the experimental medium. Oxygen inhibition of apparent photosynthesis in E. canadensis is enhanced by increased O_2 concentration (Figure 3.1), increased temperature (Figure 3.3), reduced CO_2 concentration (Figure 3.5), increased pH (Figure 3.7) and increased light (Figure 3.9).

As a consequence of the artificially favourable degree of water movement in the laboratory apparatus, the thickness of the boundary layer next to the leaf is reduced, and this may facilitate uptake of CO_2 by the leaf and removal of photosynthetically produced O_2 from the leaf as compared with more static conditions in the field. This will be of especial significance in dense weed beds in slow moving or still water. Such a situation would favour photosynthesis and underestimate the importance of O_2 inhibition of photosynthesis in intact field plants. Nonetheless, the relatively low level of photosynthesis and high O_2 inhibition of photosynthesis that occurs in E. canadensis under low CO_2 concentrations (Figures 3.4 and 3.5), high pH (Figures 3.6 and 3.7) and high O_2 tensions (Figures 3.1, 3.2 and 3.3) indicate that the plant's photosynthesis may be severely limited under similar field conditions. Such adverse conditions do develop in the water surrounding aquatic plants, especially in calm weather conditions as a result of their photosynthesis (e.g. Brown et al., 1974; Sculthorpe, 1967; Van et al., 1976). It is also interesting to note that similar conditions to those described here have been implicated in retarded growth of E. canadensis by Dale (1957).

As a result of photosynthesis, a rise in O_2 and pH occurs simultaneously with a reduction in CO_2, and these changes often exceed the experimental limits investigated. Therefore the laboratory results probably underestimate the degree of limitation of photosynthesis which E. canadensis will experience under field conditions where all the deleterious factors operate in unison, in some cases to severe degree, and their effects are enhanced by local retardation of water movement

in dense plant stands. It is of interest that a similar suppression of photosynthesis and enhanced photorespiration with increased O_2 tensions and reduced CO_2 availability has been proposed as an explanation for the afternoon depression of photosynthesis which is observed in many macrophytes (Hough, 1974). The data in Section 3 are consistent with this proposal and suggest that a sustained depression of photosynthesis, similar to that observed on warm, bright afternoons could drastically reduce the photosynthetic performance and possibly help explain marked reductions in E. canadensis biomass under still water conditions.

A decline in macrophyte biomass in nutrient rich waters is often associated with increased populations of epiphytic and filamentous algae. One theory to explain the demise of the macrophytic population has been put forward by Phillips et al. (1978). They suggest that in nutrient limited conditions, rooted aquatic plants are at an advantage because they can obtain nutrients from the sediment (e.g. Bristow and Whitcombe, 1971; Toetz, 1974). However, this advantage is lost as the nutrient supply in the water increases, thereby allowing algal growth to occur with subsequent shading of vascular macrophytes to below light compensation. As a consequence vascular plant growth declines, even to the extent of being totally replaced by the filamentous algae.

The present study suggests another contributing factor, at least in the decline of E. canadensis when in competition with filamentous algae, may be the relatively poor photosynthetic performance of this vascular species as compared with its algal competitors under low CO_2 conditions. The comparative studies on E. canadensis, Cladophora

glomerata and Spirogyra sp. indicate that the photosynthesis of E. canadensis is most severely limited by low CO_2 conditions (Figure 7.2) and high pH (Figure 7.3). The filamentous algae may themselves increase the pH of the water and reduce the CO_2 content to such an extent that E. canadensis is placed under severe photosynthetic stress whilst the algae are little affected. Such a situation could lead to macrophytes being outcompeted under field conditions with subsequent decline and even replacement by the better-adapted algae.

The physiological basis of this competitive advantage, under high pH and consequent low CO_2 conditions, might be the possession of a CO_2 concentrating mechanism in C. glomerata and Spirogyra sp. It is probable that Chlorophyte algae possess a CO_2-pump which increases the level of CO_2 at the site of photosynthesis (Raven and Glidewell, 1978). In addition some algae have an inducible carbonic enhydrase which operates at low levels of CO_2 (e.g. Graham et al., 1971; Ingle and Colman, 1975). Both these factors may help to explain the generally better photosynthesis and low O_2 inhibition of photosynthesis in C. glomerata and Spirogyra sp. under low CO_2 and high pH conditions.

In contrast, photosynthesis of E. canadensis is low under high pH and low CO_2 conditions. This may reflect its inability, or at most very limited ability to use HCO_3^- as a carbon source in photosynthesis, as indicated by its preference for free CO_2 in photosynthesis (Figures 3.6 and 7.3, Table 2.8) and the low ratio of photosynthesis at pH 8.5 to that at pH 5 (Section 2). It may also be because of the lack of a suitable CO_2 concentrating mechanism. Carbon dioxide could be

concentrated either by carbonic anhydrase or by C_4-acid metabolism as typified by terrestrial C_4 plants. However, carbonic anhydrase levels are generally very low in submersed aquatic plants, including E. canadensis (Weaver and Wetzel, 1980) and high ^{14}C fixation into C_4-acids in macrophytes is evidently not associated with the synthetic or energy fixing roles of photosynthesis (Browse et al., 1980; see also Introduction, and discussions of Sections 3 and 7). The lacunar system of E. canadensis seems too small in volume and too constricted in morphology to be of significant value in storing or transporting CO_2. Thus amelioration by bicarbonate use, possession of a CO_2-pump or concentrating mechanism and lacunar gas transport are unlikely in E. canadensis.

It is however possible that the decrease in O_2 inhibition of photosynthesis noted in summer specimens of E. canadensis (Sections 5 and 6) might help to reduce the effects of low CO_2, high pH and O_2 on its photosynthesis by reducing photorespiration and thereby improving the plant's carbon budget. This would be the case whether the summer reduction in O_2 inhibition of photosynthesis, and thus photorespiration, is induced by environmental triggers such as low CO_2, increased daylength or temperature, as suggested by Bowes et al. (1977a) or is a reflection of the relative youth of plant samples in the summer as compared with ones in the winter. To clarify this, it would be instructive if induced aging experiments were performed on such samples to determine directly the effect of age on photosynthesis, photorespiration and the proportions of photosynthetic and photorespiratory enzymes.

Bowes et al. (1977a) interpreted the reduction in photorespiration

and the shift in RuBP carboxylase : PEP carboxylase ratio of Hydrilla verticillata as a response to elevated temperature and extended photoperiod. However the results are open to other interpretations since these conditions would also enhance growth. Therefore apical sections of a given length from either high temperature or extended photoperiod would be younger than equivalent stem sections from a lower temperature or shorter photoperiod. Thus the changing photosynthetic and photorespiratory metabolism of H. verticillata may have been a product of age-related phenomena rather than the result of environmental triggers affecting metabolism.

Leaves of E. canadensis exhibit adaptation to available light in the field. This adaptation takes the form of reduced light compensation and light saturation points in leaves of low insertion level (Table 4.1) and winter 1 cm and 6 cm excised leaves (Tables 5.1 and 5.2 respectively). In addition, reduced light saturation and compensation points are evident in winter leaves of low insertion when compared with leaves of similar position in the summer (Tables 6.1 and 6.3). Therefore E. canadensis apparently shows adaptation to available light in terms of season and depth. However, it is not clear whether depth differences in light compensation and saturation points are due to adaptation of young leaves to the prevailing conditions at the time of leaf growth and expansion or result from re-adaptation by mature leaves in response to changing conditions in the weed bed.

Clearly, it would be instructive if laboratory investigations were performed on mature specimens of E. canadensis to ascertain whether they were capable of re-adaptation similar to that described for

H. verticillata (Bowes et al., 1977b) and Potamogeton polygonifolius and P. obtusifolius (Spence and Chrystal, 1970). Another approach might be to look at young leaves of deep sideshoots i.e. leaves much younger than mainstream leaves but at the same depth. However, such a distinction might be academic since light availability will be reduced with depth and may result from light attenuation by water, plant self shading, epiphyte growth, marl or silt deposition (Hutchinson, 1957; Sculthorpe, 1967). Such adaptations may be of benefit to the whole plant especially if they permit older leaves to reduce their respiratory drain on younger more active leaves or even allow them to contribute fixed carbon to the new growth of the plant.

The results of various investigations indicated that the photosynthetic activity of old E. canadensis leaves were less than those of young or mature leaves (Sections 4, 5 and 6), and such differences could be the result of age-related phenomena, similar to those described for terrestrial plants. Other age-related effects on the photosynthetic physiology of E. canadensis can be inferred from the differences observed in O_2 inhibition of photosynthesis with leaf position (Figures 4.2 and 4.4), season (Figures 5.5 and 5.6) and the interaction of season with leaf position (Figures 6.3 and 6.4). The likely benefits of the reduction in photorespiration in young leaves has already been discussed above.

Besides the seasonal variation and adaptation exhibited in the light response of photosynthesis and O_2 inhibition of photosynthesis, there was also an indication of thermal adaptation in summer specimens of E. canadensis. This is evidenced by the increased Q_{10} for apparent photosynthesis (range 10-20°C) in summer samples (Table 5.3). Such an

adaptation may enable the plant to take advantage of diurnal temperature increases which are up to 10°C in plant stands where water circulation is retarded by the dense plant growth. In contrast there was no evidence of a summer change in the temperature optimum for photosynthesis which averaged 27.5°C during the annual cycle. Nor was there any evidence of physiological dormancy in winter samples, even though photosynthesis was reduced in such plants compared to those in the summer. Lack of dormancy may enable the plant to take advantage of short mild spells in the winter by increasing assimilation and counteracting to some extent the general seasonal decline in biomass.

It thus appears that E. canadensis is capable of modifying its photosynthetic and respiratory responses to the environment, particularly with respect to light availability, a factor which is often cited as controlling plant distribution.

The experimental results suggest that E. canadensis is a plant that is flexible in its light requirements as indicated by adaptations of leaves with season and leaf insertion level. This is apparently supported by the range of light saturation points reported in the literature despite the fact that some authors seem to regard it as a species of restricted light range (e.g. Brown et al., 1974; Hartman and Brown, 1967). Its light saturation point is variable and is reported to be as low as 40 $\mu E\ m^{-2}s^{-1}$ (Allen, 1973), a factor which apparently permitted net assimilation at very low spring light intensities (Haag and Gorham, 1977). However Brown et al. (1974) consider E. canadensis to be a sun adapted species because its photosynthesis was not light saturated at the highest light intensity

investigated, 65 W $m^{-2}min^{-1}$. In contrast to the adaptability shown by E. canadensis to light, temperature adaptations are restricted, and whilst this species has a high potential at high temperature, its realisation of this potential is limited by the rarity of temperatures approaching its optimum in Britain. In addition it seems to be poorly adapted to competition for organic carbon especially at high pH. Therefore E. canadensis is probably very successful in warm water, rich in CO_2, but outcompeted where CO_2 becomes critical.

Thus, environmental factors such as high O_2, low CO_2 and high pH may severely limit the photosynthesis of this plant with little hope of physiological or morphological amelioration. The effect of such environmental factors when aggravated by competition with filamentous algae, which are relatively little affected, may be especially severe on E. canadensis and could lead to its decline in nutrient rich waters.

9. ACKNOWLEDGMENTS

I would like to thank the Science Research Council for the provision of a Postgraduate Studentship, and also Professor D. H. Jennings for allowing me to undertake this work in his department. My thanks also go to the members of the Department of Botany for their support and advice, all of which has been invaluable.

Special thanks go to Dr. J. W. Eaton, for supervising the work and giving much constructive criticism, both during the research and thesis preparation; to Dr. K. Hardwick and Dr. D. A. Thurman for helpful discussions on plant physiology; to Dr. G. Skirrow for advice on bicarbonate equilibria in natural waters; to Dr. A. M. Mortimer for advice on statistical analyses; Mr. C. J. Veltkamp for assistance with the Scanning Electron Microscope; and Mr. A. Tollett for photographic work.

Finally, very special thanks go to my wife, Cathie, for her neverending patience.

10. REFERENCES

Adams, M.S. Titus, J. & McCracken, M. (1974) Depth distribution of photosynthetic activity in a Myriophyllum spicatum community in Lake Wingra. Limnology and Oceanography, 19, 377-389.

Allen, E.D. (1973) An ecophysiological study of the effects of thermal discharges on the submersed macrophytes of Lake Wabamun. M.Sc thesis, University of Alberta, Edmonton, Alberta.

Andrews, T.J. & Abel, K.M. (1979) Photosynthetic carbon metabolism in seagrasses. ^{14}C labelling evidence for the C_3 pathway. Plant Physiology, 63, 650-656.

Andrews, T.J., Lorimer, G.H. & Tolbert, N.E. (1971) Incorporation of molecular oxygen into glycine and serine during photorespiration in spinach leaves. Biochemistry, 10, 4777-4782.

Andrews, T.J., Lorimer, G.H. & Tolbert, N.E. (1973) Ribulose diphosphate oxygenase. I. Synthesis of Phosphoglycolate by fraction I protein of leaves. Biochemistry, 12, 11-18.

Arber, A. (1920) Water plants. Cambridge University Press. 436 pp.

Arnon, D.I. (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. Plant Physiology, 24, 1-15.

Aslam, M., Lowe, S.B. & Hunt, L.A. (1977) Effect of leaf age on photosynthesis and transpiration of cassava (Manihot esculenta). Canadian Journal of Botany, 55, 2288-2295.

Badger, M.R. & Andrews, T.J. (1974) Effects of CO_2, O_2 and temperature on a high-affinity form of ribulose diphosphate carboxylase - oxygenase from spinach. Biochemical and Biophysical Research Communications, 60, 204-210.

Bamforth, S.S. (1962) Diurnal changes in shallow aquatic habitats. Limnology and Oceanography, 7, 348-353.

Bassham, J.A. & Calvin, M. (1957) The path of carbon in photosynthesis. Prentice-Hall, New Jersey. 104 pp.

Bellis, V.J. (1968) Unialgal cultures of Cladophora glomerata (L.) Kützz II. Response to calcium-magnesium ratio and pH of the medium. Proceedings of the 11th Conference of Great Lakes Research, 11-15.

Bellis, V.J. & McLarty, D.A. (1967) Ecology of Cladophora glomerata (L.) Kützz in southern Ontario. Journal of Phycology, 3, 57-63.

Benedict, C.R. (1978) Nature of obligate photoautotrophy. Annual Review of Plant Physiology, 29, 67-73.

Benedict, C.R. & Scott, J.R. (1976) Photosynthetic carbon metabolism of a marine grass. Plant Physiology, 57, 876-880.

Bidwell, R.G.S. (1977) Photosynthesis and light and dark respiration in freshwater algae. Canadian Journal of Botany, 55, 809-818.

Björkman, O. (1973) Comparative studies on photosynthesis in higher plants. Photophysiology, 8, 1-63.

Björkman, O., Boardman, N.K., Anderson, J.M., Thorne, S.W., Goodchild, D.J. & Pyliotis, N.A. (1972) Effect of light intensity during growth of Atriplex patula on the capacity of photosynthetic reactions, chloroplast components and structure. Carnegie Institution of Washington Year Book, 71, 115-134.

Blum, J.L. (1956) The ecology of river algae. Botanical Review 22, 291-341.

Boardman, N.K. (1977) Comparative photosynthesis of sun and shade plants. Annual Review of Plant Physiology, 28, 355-377.

Bolas, P.M. & Lund, J.W.G. (1974) Some factors affecting the growth of Cladophora glomerata in the Kentish Stour. Water Treatment Exam., 23, 25-51.

Bowes, G., Ogren, W.L. & Hageman, R.H. (1971) Phosphoglycolate production catalysed by ribulose diphosphate carboxylase. Biochemical and Biophysical Research Communications, 45, 716-722.

Bowes, G. & Ogren, W.L. (1972) O_2 inhibition and other properties of ribulose 1, 5-diphosphate carboxylase. Journal of Biological Chemistry, 247, 2171-2176.

Bowes, G., Ogren, W.L. & Hageman, R.H. (1975) pH dependence of the Km(CO_2) of ribulose 1, 5-diphosphate carboxylase. Plant Physiology, 56, 630-633.

Bowes, G., Holaday, A.S., Van, T.K. & Haller, W.T. (1977a) Photosynthetic and photorespiratory carbon metabolism in aquatic plants. In : Proceedings of the Fourth International Congress on Photosynthesis, (Edited by D.O. Hall, J. Coombs and T. W. Goodwin) 289-298.

Bowes, G., Van. T.K. Garrard, L.A. & Haller, W.T. (1977b) Adaptation to low light levels by Hydrilla. Journal of Aquatic Plant Management, 15, 32-35.

Boylen, C.W. & Sheldon, R.B. (1976) Submergent macrophytes: growth under winter ice cover. Science, 194, 841-842.

Bristow, J.M. & Whitcombe, M. (1971) The role of roots in the nutrition of aquatic vascular plants. American Journal of Botany, 58, 8-13.

Brown, J.M.A., Dromgoole, F.I., Towsey, M.W. & Browse, J. (1974) Photosynthesis and photorespiration in aquatic macrophytes. In : Mechanisms of Regulation of Plant Growth (Ed. by R.L. Bieliski, A.R. Ferguson and M.M. Cresswell), pp 243-249. Wellington: R.Soc New Zealand.

Browse, J.A., Dromgoole, F.I. & Brown, J.M.A. (1977) Photosynthesis in the aquatic macrophyte Egeria densa. I. $^{14}CO_2$ fixation at natural CO_2 concentrations. Australian Journal of Plant Physiology, 4, 169-176.

Browse, J.A. Brown, J.M.A. & Dromgoole, F.I. (1979) Photosynthesis in the aquatic macrophyte Egeria densa II. The effects of inorganic carbon conditions on ^{14}C-fixation. Australian Journal of Plant Physiology, 6, 1-9.

Browse, J.A., Brown, J.M.A. & Dromgoole, F.I. (1980) Malate synthesis and metabolism during photosynthesis in Egeria densa Planch. Aquatic Botany, 8, 295-305.

Bruin, W.J., Nelson, E.B. & Tolbert, N.E., (1970) Glycolate pathway in green algae. Plant Physiology, 46, 386-391.

Carr, J.L. (1969) The primary productivity and physiology of Ceratophyllum demersum. II. Micro primary productivity, pH, and the P/R ratio. Australian Journal of Marine and Freshwater Research, 20, 127-142.

Catsky, J., Ticha, I. & Solarova, J. (1976) Ontogenetic changes in the internal limitations to bean-leaf photosynthesis I. Carbon dioxide exchange and conductances for carbon dioxide transfer. Photosynthetica, 10, 394-402.

Cheng, K.H. & Colman, B. (1974) Measurements of photorespiration in some microscopic algae. Planta, 115, 207-212.

Chollet, R. & Ogren, W.L. (1975) Regulation of photorespiration in C_3 and C_4 species. Botanical Review, 41, 137-179.

Colman B., Miller, A.G. & Grodzinski, B. (1974) A study of the control of glycolate excretion in Chlorella. Plant Physiology, 53, 395-397.

Cornic, G., Mousseau, M.& Monteny, B. (1970) Importance de la photorespiration dans le bilan photosynthétique au cours de la croissance foliare. Oecologia Plantarum, 5, 355-363.

Coult, D.A. (1964) Observations of gas movement in the rhizome of Menyanthes trifoliata, L., with comments on the role of the endodermis. Journal of Experimental Botany, 15, 205-218.

Dale, H.M. (1957) Development studies on Elodea canadensis MICHX. II experimental studies on morphological effects of darkness. Canadian Journal of Botany, 35, 51-64.

Dale, H.M. & Gillespie, T.J. (1977) The influence of submersed aquatic plants on temperature gradients in shallow water bodies. Canadian Journal of Botany, 55, 2216-2225.

DeGroote, D. & Kennedy, R.A. (1977) Photosynthesis in Elodea canadensis Michx. Four carbon acid synthesis. Plant Physiology, 59, 1133-1135.

Dickmann, D.I., Gjerstad, D.H. & Gordon, J.C. (1975) Developmental patterns of CO_2 exchange, diffusion resistance and protein synthesis in leaves of Populus X euramericana. In : Environmental and Biological Control of Photosynthesis (Ed. by R. Marcelle), pp 171-181. Dr. Junk, The Hague.

Dodd, W.A. & Bidwell, R.G.S. (1971) The effect of pH on the products of photosynthesis in $^{14}CO_2$ by chloroplast preparations from Acetabularia mediterrania. Plant Physiology, 47, 779-783.

Dromgoole, F.I. (1978) The effects of oxygen on dark respiration and apparent photosynthesis of marine macro-algae. Aquatic Botany, 4, 281-297.

Eaton, J.W., Murphy, K.J. and Hyde, T.N. (1981) Comparative trials of herbicidal and mechanical control of aquatic weeds in canals. Proceedings of a Conference on Aquatic Weeds and their Control, pp 105-116. Association of Applied Biologists, Oxford.

Egle, K. (1960) Menge und verhältnis der pigmente. In : Encyclopedia of Plant Physiology (Ed. by W. Ruhland), 5(1), 445-496. Springer-Verlag, Berlin.

Frederick, S.E., Gruber, P.J. & Tolbert, N.E. (1973) The occurrence of glycolate dehydrogenase and glycolate oxidase in green plants: An evolutionary survey. Plant Physiology, 52, 318-323.

Forsberg, C. (1965) Nutritional studies of Chara in axenic cultures. Physiologia Plantarum, 18, 275-290.

Forrester, M.L., Krotkov, G. & Nelson, C.D. (1966) Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. Plant Physiology, 41, 422-427.

Ganf, G.G. (1974) Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda). Journal of Ecology, 62, 611-629.

Gessner, F. (1938) Die Beziehung zwischen Lichtintensitat und Assimilation bei submersen Wasserpflanzen. Jahrbuch fur Wissenschaftliche Botanik, 86, 491-526.

Goldsworthy, A. (1970) Photorespiration. Botanical Review, 36, 321-340.

Goulder, R. (1968) Aspects of primary production in certain gravel pits at Brandesburton. PhD. Thesis. University of Hull, 153 pp.

Goulder, R. (1970) Daytime variations in the rates of production by two natural communities of submerged freshwater macrophytes. Journal of Ecology, 58, 521-528.

Graham, D., Atkins, C.A., Reed, M.L., Patterson, B.D. & Smillie, R.M. (1971) Carbonic anhydrase, photosynthesis and light-induced changes In: M.D. Hatch, C.B. Osmond and R.A. Slatyer (Eds), Photosynthesis and Photorespiration. Wiley-Interscience, New York. pp 267-274.

Haag, R.W. & Gorham, P.R. (1977) Effects of thermal effluent on standing crop and net production of Elodea canadensis and other submerged macrophytes in Lake Wabamun, Alberta. Journal of Applied Ecology, 14, 835-851.

Hartman, R.T., & Brown, D.L. (1967) Changes in internal atmosphere of submersed vascular hydrophytes in relation to photosynthesis. Ecology, 48, 252-258.

Heber, U., Andrews, T.J. & Boarman, N.K. (1976) Effects of pH and oxygen on photosynthetic reactions of intact chloroplasts. Plant Physiology, 57, 277-283.

Helder, R.J., Prins, H.B.A. & Schuurmans, J. (1974) Photorespiration in leaves of Vallisneria spiralis. Proceedings Koniklijke Nederlandse Academie van Wetenschappen, 77C, 338-344.

Hew, C.S., Krotkov, G. & Canvin, D.T. (1969) Effects of temperature on photosynthesis and CO_2 evolution in light and darkness by green leaves. Plant Physiology, 44, 671-677.

Hodgkinson, K.C. (1974) The influence of partial defoliation on photosynthesis, photorespiration and transpiration by lucerne leaves of different ages. Australian Journal of Plant Physiology, 1, 561-578.

Hofstra, G. & Hesketh, J.D. (1969) Effect of temperature on the gas exchange of leaves in the light and dark. Planta, 85, 228-237.

Holmgren, P. & Jarvis, P.G. (1967) Carbon dioxide efflux from leaves in light and dark. Physiologia Plantarum, 18, 557-573.

Hough, R.A. (1974) Photorespiration and productivity in submersed aquatic vascular plants. Limnology and Oceanography, 19, 912-927.

Hough, R.A. (1976) Light and dark respiration and release of organic carbon in marine macrophytes of the Great Barrier Reef region, Australia. Australian Journal of Plant Physiology, 3, 63-68.

Hough, R.A. (1979) Photosynthesis, respiration and organic carbon release in Elodea canadensis Michx. Aquatic Botany, 7, 1-11.

Hough, R.A. & Wetzel, R.G. (1972) A ^{14}C-assay for photorespiration in aquatic plants. Plant Physiology, 49, 987-990.

Hough, R.A. & Wetzel, R.G., (1977) Photosynthetic pathways of some aquatic plants. Aquatic Botany, 3, 297-313.

Hough, R.A. & Wetzel, R.G. (1978) Photorespiration and CO_2 compensation point in Najas flexilis. Limnology and Oceanography, 23, 719-725.

Hutchinson, G.E. (1957) A Treatise on Limnology. Vol. I. Geography, Physics and Chemistry. John Wiley & Sons, New York.

Hutchinson, G.E. (1975) A treatise on Limnology. Vol. III. Limnological Botany. John Wiley & Sons, New York.

Ikusima, I. (1965) Ecological studies on the productivity of aquatic plant communities I. Measurement of photosynthetic activity. Botanical Magazine, Tokyo, 78, 202-211.

Ikusima, I. (1966) Ecological studies on the productivity of aquatic plant communities II. Seasonal changes in standing crop and productivity of a natural submerged community of Vallisneria denseserrulata. Botanical Magazine, Tokyo, 79, 7-19.

Ingle, R.K. & Colman, B. (1975) Carbonic anhydrase levels in blue-green algae. Canadian Journal of Botany, 53, 2385-2387.

Jana, S. & Choudhuri, M .A. (1979) Photosynthetic, photorespiratory and respiratory behaviour of three submersed aquatic angiosperms. Aquatic Botany, 7, 13-19.

Jackson, W.A. & Volk, R.J. (1970) Photorespiration. Annual Review of Plant Physiology, 21, 385-432.

Jolliffe, P.A. & Tregunna, E.B. (1968) Effect of temperature, CO_2 concentration and light intensity on oxygen inhibition in wheat leaves. Plant Physiology, 43, 902-906.

Jørgensen, E.G. (1969) The adaptation of plankton algae IV. Light adaptation in different algal species. Physiologia Plantarum. 22, 1307-1315.

Kadono, Y. (1980) Photosynthetic carbon sources in some Potamogeton species. Botanical Magazine, Tokyo, 93, 185-194.

Kisaki, T. & Tolbert, N.E. (1969) Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. Plant Physiology, 44, 242-250.

Kisaki, T., Hirabayashi, S.& Yano, N. (1973) Effect of age of tobacco leaves on photosynthesis and photorespiration. Plant and Cell Physiology, 14, 505-514.

Ku, S.B. & Hunt, L.A. (1973) Effects of temperature on the morphology and photosynthetic activity of newly matured leaves of alfalfa. Canadian Journal of Botany, 51, 1907-1916.

Ku, S.B. & Hunt, L.A. (1977) Effect of temperature on photosynthetic-irradiance response of newly matured leaves of alfalfa. Canadian Journal of Botany, 55, 872-879.

Ku, S.B. Edward, G.E. & Tanner, C.C. (1977) Effects of light, carbon dioxide, and temperature on photosynthesis, oxygen inhibition of photosynthesis, and transpiration in Solanum tuberosum. Plant Physiology, 59, 868-872.

Kutyurin, V.M., Ulubekova, M.V. & Nazarov, N.M. (1964). The influence of light and oxygen on photosynthesis and respiration in aquatic plants. Soviet Plant Physiology, 11, 822-829. /Fiziologiya Rastenii, 11, 965-973 (1964)/

Laing, W.A., Ogren, W.A., & Hageman, R.H. (1973) Ribulose diphosphate carboxylase regulation of an oxygenase response in soybean photosynthesis. Plant Physiology, Supplement, 51, 40.

Laing, W.A., Ogren, W.L. & Hageman, R.H. (1974) Regulation of soybean net photosynthetic CO_2 fixation by the interaction of CO_2, O_2 and ribulose 1, 5-diphosphate carboxylase. Plant Physiology, 54, 678-685.

Lloyd, N.D.H., Canvin, D.T. & Culver, D.A. (1977) Photosynthesis and photorespiration in algae. Plant Physiology, 59 936-940.

Lorimer, G.H., Andrews, T.J. & Tolbert, N.E. (1973) Ribulose diphosphate oxygenase II. Further proof of reaction products and mechanism of action. Biochemistry, 12, 18-23.

Malkina, I.S. (1976) Changes in the light curves of photosynthesis with aging in the leaf of Norway Maple. Soviet Plant Physiology, 23, 208-213. [Fiziologiya Rastenii, 23, 247-253 (1976)]

Mangat, B.S., Levin, W.B. & Bidwell, R.G.S. (1974) The extent of dark respiration in illuminated leaves and its control by ATP levels. Canadian Journal of Botany, 52, 673-681.

Marcus, B.A. (1980) Relationship between light intensity and chlorophyll content in Myriophyllum spicatum L. in Canadice Lake (New York, U.S.A.). Aquatic Botany, 9, 169-171.

Mason, C.P. (1965) The ecology of Cladophora glomerata in farm ponds. Ecology, 16, 421-429.

Merret, M.J. & Lord, J.M. (1973) Glycollate formation and metabolism by algae. New Phytologist, 72, 751-767.

Meyer, B.S., Bell, F.H., Thompson, L.C. & Clay, E.I. (1943) Effect of depth of immersion on apparent photosynthesis in submerged vascular aquatics. Ecology, 24, 393-399.

Meyer, B.S. & Heritage, A.C. (1941) Effect of turbidity and depth of immersion on apparent photosynthesis in Ceratophyllum demersum. Ecology, 22, 17-22.

Mokronosov, A.T. & Nekrasova, G.F. (1977) Ontogenetic aspect of photosynthesis (on the example of the potato leaf). Soviet Plant Physiology, 24, 366-372. [Fiziologiya Rastenii, 24, 458-465 (1977)]

Moss, B. (1969) Vertical heterogeneity in the water column of Abbot's Pond. 1. The distribution of temperature and dissolved oxygen. Journal of Ecology, 57, 381-396.

Nelson, E.B., Cenedella, A. & Tolbert, N.E. (1969) Carbonic anhydrase levels in Chlamydomonas. Phytochemistry, 8, 2305-2306.

Ogren, W.L. & Bowes, G. (1971) Ribulose diphosphate carboxylase regulates soybean photorespiration. Nature, 230, 159-160.

Owens, M. & Maris, P.J. (1964) Some factors affecting the respiration ofsome aquatic plants. Hydrobiologia, 23, 533-543.

Pearson, C.J. & Hunt, L.A. (1972) Effects of pretreatment temperature on carbon dioxide exchange in alfalfa. Canadian Journal of Botany, 50, 1925-1930.

Phillips, G.L., Eminson, D. & Moss, B. (1978) A mechanism to account for macrophyte decline in progressively eutrophicated waters. Aquatic Botany, 4, 103-126.

Prins, H.B.A. & Wolff, R.W. (1974) Photorespiration in leaves of Vallisneria spiralis: the effect of oxygen on the carbon dioxide compensation point. Proceedings Koniklijke Nederlandse Akademie van Wetenschappen. 77C, 239-245.

Raven, J.A. (1970) Exogenous inorganic carbon sources in plant photosynthesis. Biological Reviews, 45, 167-221.

Raven, J.A. & Glidewell, S.M. (1978) C_4 characteristics of photosynthesis in the C_3 alga Hydrodictyon africanum. Plant, Cell and Environment 1, 185-197.

Richardson, K.E. & Tolbert, N.E. (1961) Phosphoglycolic acid phosphatase. Journal of Biological Chemistry, 236, 1285-1290.

Robinson, J.M., Gibbs, M. & Cotler, D.N. (1977) Influence of pH upon the Warburg effect in isolated intact spinach chloroplasts. I. Carbon dioxide photoassimilation and glycolate synthesis. Plant Physiology, 59, 530-534.

Ruttner, F. (1926) Über die Kohlensäureassimilation einiger Wasserpflanzen in verschiedenen Tiefen des Lunzer Untersees. Internationale Revue Gesampten Hydrobiologie und Hydrographie 15, 1-30.

Salvucci, M., Holaday, S. & Bowes, G. (1979) Enzymes associated with C_4 acid metabolism in submersed aquatic macrophyte (SAM) species. Plant Physiology, Supplement, 63, 52.

Sand-Jensen, K. (1977) Effects of epiphytes on eelgrass photosynthesis. Aquatic Botany, 3, 55-63.

Schomer, H.A. (1934) Photosynthesis of water plants at various depths in lakes of north-eastern Wisconsin. Ecology, 15, 217-218.

Sculthorpe, C.D. (1967) The Biology of Aquatic Vascular Plants. Edward Arnold, London.

Servaites, J.C. & Ogren, W.L. (1978) Oxygen inhibition of photosynthesis and stimulation of photorespiration in soybean leaf cells. Plant Physiology, 61, 62-67.

Sestak, Z. (1977) Photosynthetic characteristics during ontogenesis of leaves I. Chlorophylls. Photosynthetica, 11, 367-448.

Sestak, Z., Catsky, J., Solarova, J., Strnadova, H. & Ticha, I. (1975) Carbon dioxide transfer and photochemical activities as factors of photosynthesis during ontogenesis of primary bean leaves. In: Genetic Aspects of Photosynthesis (Ed. by Yu.S. Nasyrov & Z. Sestak) pp 156-166. Dr. Junk, The Hague.

Seybold, A. & Egle, K. (1937) Lichfeld und Blattfarbstoff. Planta, 26, 491-526.

Shiyan, P.N. & Merezhko, A.I. (1972) Effect of hydrogen ion concentration on photosynthesis and radiocarbon metabolism in aquatic plants. Hydrobiological Journal, 8, 23-29.

Simpson, P.S., Eaton, J.W. & Hardwick, K. (1980) The influence of environmental factors on apparent photosynthesis and respiration of the submersed macrophyte Elodea canadensis. Plant, Cell and Environment, 3, 415-423.

Smith, I.R. (1975) Turbulence in lakes and rivers, F.B.A. Scientific Publication, No. 29.

Søndergaard, M. (1979) Light and dark respiration and the effect of the lacunal system on refixation of CO_2 in submerged aquatic plants. Aquatic Botany, 6, 269-283.

Søndergaard, M. & Sand-Jensen, K. (1979) Carbon uptake by leaves and roots of Litorella uniflora (L.) Aschers. Aquatic Botany, 6, 1-12.

Søndergaard, M. & Wetzel, R.G. (1980) Photorespiration and recycling of CO_2 in the submersed angiosperm Scirpus subterminalis. Terr. Canadian Journal of Botany, 58, 591-598.

Spence, D.H.N. & Chrystal, J. (1970) Photosynthesis and zonation of freshwater macrophytes II. Adaptability of species of deep and shallow water. New Phytologist, 69, 217-227.

Stanley, R.A. & Naylor, A.W. (1972) Photosynthesis in Eurasian watermilfoil (Myriophyllum spicatum L.). Plant Physiology, 50, 149-151.

Steemann Nielsen, E. (1960) Uptake of CO_2 by the plant. In: Encyclopedia of Plant Physiology. Vol. 5, Part 1. (Ed. by W. Ruhland). pp 70-84. Springer-Verlag, Berlin.

Thomas, E.A. & Tregunna, E.B. (1968) Bicarbonate ion assimilation in photosynthesis by Sargassum muticum. Canadian Journal of Botany, 46, 411-415.

Thurman, M.H. & Kuehne, R.A. (1952) An ecological study of Cladophora glomerata (Chlorophyceae) near Dallas. Field Lab, 20, 26-28.

Toetz , D.W. (1974) Uptake and translocation of ammonia by freshwater hydrophytes. Ecology, 55, 199-201.

Tolbert, N.E. (1971) Microbodies-peroxisomes and glyoxysomes. Annual Review of Plant Physiology, 22, 45-74.

Tolbert, N.E. (1974) Photorespiration. In: Algal Physiology and Biochemistry (Ed. W.D.P. Stewart), pp. 475-504. Blackwell, Oxford.

Tolbert, N.E. & Osmond, C.B. (editors) (1976) Photorespiration in Marine Plants. CSIRO, Melbourne and University Park Press, Baltimore.

Treharne, K.J., Cooper, J.P. & Taylor, T.H., (1968) Growth response of orchard-grass (Dactylis glomerata L.) to different light and temperature environments II. Leaf age and photosynthetic activity. Crop Science, 8, 441-445.

Turner, J.S. & Brittain, E.G. (1962) Oxygen as a factor in photosynthesis. Biological Reviews, 37, 130-170.

Van, T.K., Haller, W.T. Bowes, G. (1976) Comparison ofthe photosynthetic characteristics of three submersed aquatic plants. Plant Physiology, 58, 761-768.

Van, T.K., Haller, W.T., Bowes, G. & Garrard, L.A. (1977) Effects of light quality on growth and chlorophyll composition in Hydrilla. Journal of Aquatic Plant Management, 15, 29-31.

Weaver, C.I., & Wetzel, R.G. (1980) Carbonic anhydrase levels and internal lacunar CO_2 concentrations in aquatic macrophytes. Aquatic Botany, 8, 173-186.

Westlake, D.F. (1967) Some effects of low velocity currents on the metabolism of aquatic macrophytes. Journal of Experimental Botany, 18, 187-205.

Westlake, D.F. (1971) Waterplants and the aqueous environment. Biology and Human Affairs, 36, 1-20.

Westlake, D.F. (1975) Macrophytes In: River Ecology (Ed. by B.A. Whitton) pp 106-128 Blackwell Scientific, London, Edinburgh, Melbourne.

Wetzel, R.G. (1960) Marl encrustations on hydrophytes in several Michigan lakes. Oikos, 11, 223-236.

Whitton, B.A. (1967) Studies on the growth of Riverain Cladophora in culture. Archiv für Mikrobiologie, 58, 21-29.

Whitton, B.A. (1970) Biology of Cladophora in freshwaters. Water Research, 4, 457-476.

Winer, B.J. (1971) Statistical principles in experimental design. McGraw-Hill, New York, Second Edition.

Winter, K. (1978) Short-term fixation of ^{14}Carbon by the submerged aquatic angiosperm Potamogeton pectinatus. Journal of Experimental Botany, 29, 1169-1172.

Wium-Anderson, S. (1971) Photosynthetic uptake of free CO_2 by the roots of Lobelia dortmanna. Physiologia Plantarum. 25, 245-248.

Wong, S.L. Clark, B., Kirby, M. & Kosciuw, R.F. (1978) Water temperature fluctuations and seasonal periodicity of Cladophora and Potamogeton in shallow rivers. Journal of Fisheries Research Board of Canada, 35, 866-870.

Zelitch, I. (1971) Photosynthesis, photorespiration and plant productivity. Academic Press, London and New York.