PHOTOSYNTHESIS AND INORGANIC CARBON ACCUMULATING MECHANISMS IN MARINE INTERTIDAL MACROALGAE

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by

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DECLARATION

I hereby declare that the research was carried out by myself, that the thesis is my own composition and that the work has not been submitted for any degree other than that of Doctor of Philosophy at the University of Newcastle upon Tyne.

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The ultimate source of all metabolic energy in our planet is the sun and photosynthesis is essential for maintaining all forms of life on earth.

D.O. Hall and K.K. Rao.

CONTENTS

SUMMARY

The characteristics of photosynthesis and inorganic carbon accumulation were investigated in two species of marine intertidal macroalgae: *Porphyra umbilicalis* and *Ulva lactuca*.

Both species have a similar mid-intertidal location, although *P.umbilicalis* grows on bare rock faces while *U.lactuca* is found submersed in rock pools. The similar morphology of the species allows a direct comparison of both the gas exchange and the light response characteristics.

For *P.umbilicalis* the low light compensation and saturation points are comparable to the light requirements of shade adapted terrestrial plants. Photosynthetic capacity is greater in seawater although the efficiency of light utilization is no higher than that in air. In the summer population, however, the photosynthetic response is reduced. In the absence of any corresponding effect on the the light requirements, this can be related in part to seasonal variation in the total chlorophyll concentration. Even after acclimation the summer population can be photoinhibited by periods of excess PAR.

P.umbilicalis is capable of using both HCO_3^- and CO_2 as a substrate for photosynthesis. The maximum photosythetic capacity is greater in air, and corresponds to a higher affinity for CO_2. The gas exchange characteristics are O_2-insensitive indicating low photorespiratory activity in this species. The suppression of RuBPc-oxygenase may be attributable to an effective method of inorganic carbon uptake.

The mechanism in *P.umbilicalis* involves the diffusive uptake of CO_2, supplied directly or by the Carbonic Anhydrase (CA) dependent catalysis of HCO_3^-, external to the plasma membrane. An intracellular CA maintains the rate of transfer of inorganic carbon to the site CO_2 fixation in the chloroplast.

U.lactuca shows very similar light response characteristics to those of *P.umbilicalis*. The low light-requirements are the same as for the summer population, while the decrease in the V_{max} of the response is again independent of an effect on the photosynthetic efficiency. The change in the capacity corresponds to a seasonal variation in the total chlorophyll content and corresponding ratio of chlorophyll a:b. Photoinhibition of the photosynthetic response significantly alters the characteristics of the light response, even though this species has the ability to maintain efficient rates of carbon fixation.

In *U.lactuca* the mechanism of inorganic carbon accumulation involves the direct uptake of both HCO_3^- and CO_2, independent of any external CA activity. CO_2 will diffuse across the plasma membrane, while HCO_3^- must be actively transported. Internal CA activity modifies the photosynthetic capacity of this species although more than one possible location is proposed for this enzyme.

The difference in the characteristics of photosynthesis and inorganic carbon accumulation may be determined by the specific habitat of the two species. *P.umbilicalis*, more often exposed, shows a greater photosynthetic efficiency in air and a higher affinity for CO_2. In contrast, the photosynthetic system in *U.lactuca* appears to be adapted to longer periods of submersion. In relation to the inorganic carbon accumulating mechanism, there is evidence that those marine intertidal macroalgae more often exposed have a greater affinity for CO_2, dependent to a degree on the activity of an external CA.

GENERAL INTRODUCTION

INTRODUCTION

The intertidal environment

Marine intertidal macroalgae form a distinct group of plants that inhabit rocky shores, attached to the solid substrates that form the boundaries of the oceans. This boundary or intertidal zone is the area of the shore between the upper and lower limits of the tides which are caused by the gravitational pull of the sun and moon. Spring and neap tides form the extremes of a range of tidal amplitudes that occur over a two week cycle. The pattern is related to the alignment of the sun and the moon.

The habitat is characterized by large variations in environmental factors which influence the ecophysiology of marine intertidal macroalgae. The effect of these conditions is believed to determine the vertical distribution of the different species, either directly or indirectly.

Periods of emersion and submersion over a range of tidal elevations, result in temporal variations in irradiance, inorganic carbon supply, salinity and temperature. Temperature differences between air and seawater occur as a result of the high specific heat capacity of water. These changes may have a direct effect on metabolism, as well as affecting evaporation. Desiccation increases the concentration of salts at the plant surface and during evaporation of seawater in rockpools, while lowered levels of salt results from freshwater run off and rainfall. In contrast the concentration in seawater remains fairly constant.

Light levels, measured as photosynthetically active radiation (PAR) fluctate considerably, on both a seasonal and diurnal basis. The maximum PAR levels of up to 2000 μmol photon m^{-2} s^{-1} received by emersed macroalgae will be limited both by changes in atmospheric conditions and during periods of submersion. Although there may be a decrease in the quantity of light, the quality will not be significantly different at the depth encountered by most intertidal algae.

Probably of most significance however, is the variation in the supply of inorganic carbon for photosynthesis. During exposure macroalgae are dependent on CO_2 in air, as are terrestrial plants. When submerged a high concentrations of inorganic carbon is available, bound as HCO_3^- in seawater. Although there is evidence that marine intertidal macroalgae may be able to use both CO_2 and HCO_3^-, the nature and distribution of the photosynthetic mechanisms involved have not been fully elucidated.

Supply of inorganic carbon

Intertidal algae grow in conditions where the supply of inorganic carbon for photosynthesis is as gaseous carbon dioxide (CO_2) in air, and in the hydrated forms of bicarbonate and carbonate in seawater (HCO_3^- and CO_2^{3-}).

Although marine algae are primarily aquatic plants all intertidal species are emersed for some period of time, when the only available source of inorganic carbon is gaseous CO_2, at a concentration of around 15 mmol m^{-3} or 33 Pa. The concentration at the plant surface is determined by the occurence of unstirred air created by laminar flow parallel to the surface, and by the presence of a capillary film of water. The movement of inorganic carbon through this boundary layer must be by diffusion. As the diffusion coefficient of CO_2 in the aqueous phase is 10^{-4} times less than that of the gaseous phase, the capillary water film will be significant in limiting uptake. The flux of CO_2 from the bulk phase in air, to the thallus surface is given by Fick's Law :

$$J = \frac{D\ (C_o - C_w)}{\delta}$$

where

J = steady state inorganic carbon flux from the bulk phase to the outer surface of the cell wall.

D = diffusion cofficient for CO_2 in the aqueous phase (mol m^{-3}).

Co = CO_2 concentration in the bulk phase (mol m^{-3}).

Cw = CO_2 concentration at the cell surface during steady state photosynthesis (mol m^{-3}).
δ = thickness of the boundary layer (m).

Under atmospheric conditions, fluxes of CO_2 may be low enough to limit the rate of photosynthesis (Johnston and Raven 1986).

During periods of submersion, marine macroalgae have available all the forms of inorganic carbon present in the carbonate system in seawater. The concentration of dissolved CO_2 in air equilibrated seawater and consequently the total inorganic carbon content of seawater, is dependent on temperature, salinity and pressure. Seawater in equilibrium with atmospheric CO_2 will dissolve the gas in quantities related to the atmospheric concentration and the solubility coefficient. As the CO_2 dissolves it participates in a sequence of equilibria, predominantly pH dependent that result in large quantities bound in the ionic forms that make up the carbonate system. In this sequence dissolved CO_2 in the form of carbonic acid (H_2CO_3) converts to the bicarbonate ion (HCO_3^-), which in turn forms the carbonate (CO_2^{3-}), as the pH of the medium increases. The relative proportions of each ion in seawater is a function of the pH.

The equilibration reactions of the carbonate system are:

$[CO_2]_{aq} = x\ [CO_2]_g$ where x is the solubility coefficient

$CO_2 + H_2O = H_2CO_3$

$H_2CO_3 = HCO_3^- + H^+$

$HCO_3^- = CO_3^{2-} + H^+$

$HCO_3^- = CO_2 + OH^-$ (Skirrow 1975)

The equilibria between H_2CO_3 and HCO_3^-, and HCO_3^- and CO_3^{2-} are instantaneous, while the hydration of CO_2 is slower, with the equilibrium in favour of CO_2. Direct dehydroxylation of HCO_3^-, giving rise to CO_2 and OH^-, also occurs. The rate of reaction is considered important especially at high pH, in terms of the supply of free CO_2 required for photosynthesis. Under ambient conditions (35‰

salinity, pH 8.2 and a total inorganic carbon concentration of 2.2 mol m-3) the predominant carbon species is HCO_3^- (greater than 90%) with the remainder as CO_2^{3-}. Any CO_2 available under these conditions comes from the direct dehydroxylation of HCO_3^-. The maximum rate of supply, as calculated by Miller and Colman (1980), takes account of the reaction rates of the equilibria between H_2CO_3 and HCO_3^- and the direct dehydroxylation of HCO_3^-. It also assumes that any CO_2 formed is instantaneously removed preventing rehydration; that the reaction is not catalysed by external Carbonic Anhydrase (CA) and that the HCO_3^- in the unstirred layer around the surface of the thallus is in equilibrium with that in the bulk phase. Even in aqueous systems the supply of inorganic carbon to the plant may be limited by a boundary or unstirred layer. The turbulent flow of the bulk medium changes to laminar flow parallel to the surface of the thallus, necessitating movement of inorganic carbon by diffusion. As in air the flux of this carbon species can be determined using Ficks Law.

Inorganic carbon use

Since ambient seawater pH dictates that more than 90% of the total inorganic carbon (TIC) is as HCO_3^- it has been suggested that marine macroalgae may be able to use this form as a carbon supply for photosynthesis. The methods employed to test this theory in blue-green algae (Miller and Colman 1980), microscopic green algae (Beardall and Raven 1981), and other freshwater algae (Birmingham, Coleman and Colman 1982) have been used to investigate a similar ability in marine macroalgae (Johnston and Raven 1986b; Colman and Cook 1985; Beer and Eshel 1983b).

As a consequence of the properties of the carbonate system in seawater, which prevent direct comparison of CO_2 and HCO_3^- utilization, a number of indirect methods have been developed. These are based on the pH dependent equilibria that characterize the system and can be analysed using the known dissociation and rate constants.

Indirect methods which compare the photosynthetic efficiency in relation to HCO_3^- and CO_2 uptake are based on

varying the pH and TIC concentration of a closed carbonate system. At a low pH CO_2 will predominate and its rate of removal from the system during photosynthesis can be compared to that under conditions of additional inorganic carbon supply in the form of HCO_3^-, or in the absence of CO_2 at higher pH. For all methods the depletion of each species can be calculated from the remaining TIC concentration and pH. The importance of the inorganic carbon form as a substrate for photosynthesis is determined by analysis of the concentration-response curve.

A range of HCO_3^- concentrations can be generated by two methods. By varying the pH, so that at values of 8.0 and above the concentration of CO_2 is constant while that of HCO_3^- varies in proportion to CO_2^{3-}, or by the addition of amounts of $NaHCO_3$ to carbon free seawater at a stable buffered pH. For CO_2 a range of concentrations can be achieved by varying the pH of a closed system from pH 9.0 to 5.0 during which the TIC remains constant, or by the addition of CO_2 saturated water to buffered seawater at pH 5.0. Taking into account the contribution of each form of inorganic carbon to the observed rates of photosynthesis, these methods have been used to show the relative importance of HCO_3^- and CO_2 as a source of inoganic carbon for photosynthesis (Sand-Jensen and Gordon 1984; Beer and Shragge 1987; Holbrook, Spencer, Reiskind and Davis 1987).

The pH drift method involves measuring the depletion of inorganic carbon from a carbonate system calculated from the change in pH that occurs during the experimental period. Removal of one of the carbon species results in an adjustment in their relative proportions and this re-equilibration, with a concomitant pH change, can be used to determine the uptake from any system of known alkalinity (the sum of HCO_3^- and CO_2^{3-} anion concentration). Alternatively the remaining TIC may be measured directly by acid stripping, where the HCO_3^- in the seawater sample is converted to CO_2 by the addition of HCl and the concentration measured using an infra-red gas analyser. Johnston and Raven (1986b) point out that one problem with pH drift experiments, also found by other workers, involves

a change in the pH without a parallel uptake of inorganic carbon. In addition all these methods are based on the assumption that the pH of the experimental media has no direct effect on the metabolic processes involved.

A further method involves a comparison of the observed rates of photosynthetic oxygen evolution of a plant under ambient conditions, with the rate that could be supported solely by CO_2 formed from the uncatalysed conversion of HCO_3^- and CO_3^{2-} (Cook, Lanaras and Colman 1986). This method is only valid in situations where the activity of any external Carbonic Anhydrase (CA) is absent or completely suppressed, to prevent the conversion of HCO_3^- to CO_2 at the plant surface.

Brechignac, Andre and Gerbaud (1980) working on the red macroalgae *Chondrus cripus* measured the rate of HCO_3^- uptake using an experimental system that could detect an imbalance in the concentration of each of the major carbon species, by measuring a gradient generated during photosynthesis. The observed rate of flux within the system, resulting from the transformation of CO_2 to HCO_3^-, corresponds to the rate of HCO_3^- consumption by the macroalgae. The flux of carbon within the system was maintained by the input of gaseous CO_2 at rate equivalent to inorganic carbon depletion over the time period. The positive relationship between CO_2 and HCO_3^- would be absent if CO_2 was the species used.

Evidence from the various indirect methods is not conclusive but begins to show the importance of HCO_3^- as a source of inorganic carbon for photosynthesis. However, in certain situations such as when emersed it is apparent that CO_2 is more likely to be the form that is taken up. One method that can be used to distinguish the inorganic carbon species that are taken up is the inorganic carbon isotope disequilibrium technique (ICID). This analysis is based on the relatively slow equilibration between pools of HCO_3^- and CO_2. Following addition of ^{14}C, as either $^{14}CO_2$ or $H^{14}CO_3^-$ photosynthetic incorporation of the label into acid stable products will be determined by the time course of the return to equilibrium. The relative specific activity

of the two pools at any moment in time can be calculated, and compared to the rate of ^{14}C fixation to determine whether CO_2 or HCO_3^- serve as the source of inorganic carbon for photosynthesis (Espie and Colman 1986; Johnston 1990; Espie, Owttrim and Colman 1986).

While marine intertidal macroalgae may have the ability to transport both HCO_3^- and CO_2 into cells, the form of inorganic carbon fixed by the primary carboxylase enzyme RuBPco (Ribulose Bisphosphate carboxylase-oxygenase) is always CO_2.

Photosynthesis in air and seawater

Numerous studies concerned with the characterization of photosynthesis in intertidal macroalgae have compared the response during emersed and submersed conditions. The investigations have been concerned with inorganic carbon uptake, patterns of zonation, the relationship between morphology and photosynthesis and the effects of temperature and desiccation.

Bidwell and Craigie (1963) found that *Fucus vesiculosus* exhibited lower rates of net photosynthesis in air than in seawater and concluded that the higher capacity in seawater was maintained by utilization of HCO_3^- as a source of inorganic carbon. For a range of intertidal macroalgae the rates of photosynthesis in air and seawater appear to be related to zonation (Johnson, Gigon, Gulman and Mooney 1974). For the high tidal species *Porphyra perforata*, *Fucus disticus* and *Iridea flaccida* the rates of net photosynthesis in air far exceeded the rates in seawater, while the reverse is true for the lower tidal species *Ulva expansa* and *Priontis lanceolata*. They concluded that plants exposed for 50-80% of their life are able to continue growth under emersed conditions rather than just tolerate these periods. A similar correlation between the zonation and emersion was discussed by Quadir, Harrison and De Wreede (1979). A comparison of *Fucus disticus* (high intertidal), *Ulva fenestra* (mid to low intertidal) and *Iridaea cordata* (low intertidal) showed *Fucus* to have a greater photosynthetic capacity in air than

water (air/water ratio of 1.6) while the emersed rates of net photosynthesis of *Ulva* and *Iridaea* were lower than those during submersion (air/water ratio of 0.7 and 0.4 respectively).

Oates and Murray (1983) investigated photosynthetic capacity of two intertidal fucoids, *Hesperophycus harveyanus* and *Pelvetia fastigiata*, both part of the mid intertidal rocky shore community but found in separate zones. The higher tidal fucoid *Hesperophycus* showed increased rates of net photosynthesis in air, with respect to the net rate in seawater. The submersed rate for *Pelvetia* was comparable to that of *Hesperophycus* while the emersed rate was only slightly enhanced (air/water ratios of 1.4 and 4.4 for respectively).

A simple comparison of the maximum rates of net photosynthesis of *Ascophyllum nodosum* in air and seawater agreed with the previous suggestion that macroalgae located at the upper reaches of the shore have greater air/water ratios (Johnston and Raven 1986a). Both rates were saturated at similar light levels, although the use of Michaelis-Menten kinetics revealed that atmospheric concentrations of CO_2 may not saturate photosynthesis.

Later work by Oates (1985;1986) on two intertidal saccate macroalgae *Colpomenia peregrina* and *Halosaccion americanum* caused him to refute the correlation between the capacity of macroalgae to maintain higher emersed rates of photosynthesis and their position on the shore. For *Colpomenia* the rate of net photosynthesis in air was lower than than that in seawater. In comparison to other studies, the photosynthetic capacity of this macroalgae was low which Oates attributed to the low surface area to volume ratio of this species. Oates (1985) proposed that the rates in air are related to water content rather than vertical zonation. In addition following analysis of the results of his study and others, he dismissed the relationship between zonation and the relative rates of photosynthesis as many of those in air appear to be enhanced by desiccation (1986).

Johnson et al (1974) measured the maximum rates of net photosynthesis after appreciable amounts of drying. Half-maximum rates were maintained even after a considerable water loss of between 40-90% in the five species observed. The net rate of photosynthesis in *Fucus disticus* was greatest after 20% water loss while the lower intertidal species *Ulva fenestra* and *Iridaea cordata* maintained a positive carbon balance above a water loss of 70% and 35% respectively (Quadir, Harrison and DeWreede 1979). Later work on *Ulva faciata* agreed with the results above (Beer and Eshel 1983b). The reason for the apparent increase in the rate of net photosynthesis during desiccation is believed to be due to the enhanced uptake of gaseous CO_2 as the capillary film of water on the surface of the plant is reduced.

Affinity for inorganic carbon

Analysis of the relationship between photosynthesis and inorganic carbon concentration has provided more definitive evidence of the nature of the gas exchange mechanism in marine macroalgae. The apparent efficiency of inorganic carbon use can by quantified using values for the maximum rate of net photosynthesis (saturated rate or V_{max}) and the concentration of substrate required to obtain half this maximum rate, $K_{0.5}$. These kinetic parameters calculated from the response of photosynthesis to inorganic carbon concentration in experimental systems are related to the biochemical mechanisms that govern the characteristic photosynthetic physiology.

A comparison of the concentration-response of *Ascophyllum nodososum* in air and seawater revealed that this macroalgae is able to use both CO_2 and HCO_3^- as a source of inorganic carbon for photosynthesis. Even in the presence of a lower TIC concentration, higher rates were observed in air (Johnston and Raven 1986b). High rates of photosynthesis in air have also been measured for *Fucus vesiculosus* and *Enteromorpha compressa*, both of which appear to be saturated by air levels of CO_2. The concentration response of photosynthesis measured in

seawater for a range of marine macroalgae suggests that they differ in their ability to use the inorganic carbon available. The natural HCO_3^- concentration of around 2.0 mol m^{-3} was either saturating or close to saturating for a number of marine macroalgae including *Enteromorpha compressa* and *Ascophyllum nodosum* (Sand-Jensen and Gordon 1984; Beer and Shragge 1987; Johnston and Raven 1986b). In a number of other marine macroalgae maximum rates were not achieved without TIC concentrations of well above 2.5 mol m^{-3} and the corresponding $K_{0.5}$ and K_m values of between 1.1 and 15.5 mol m^{-3} indicate a very low substrate affinity. These values however, may be substantially exaggerated by the effect of diffusion limitation on inorganic carbon availability associated with the presence of an unstirred layer. This complication also arose in the analysis of the HCO_3^- response of *A.nodosum*, where the Hill-Whittingham equation which incorporates a permeability constant to account for diffusion resistance, gave a better fit to the data than the Michaelis-Menten equation (Johnston and Raven 1986b).

The ability of marine macroalgae to use HCO_3^- and CO_2 can be quantitatively compared using the $K_{0.5}$(TIC) for the two forms of inorganic carbon. Kinetic analysis of the concentration-response curve at pH 5.0 and 8.0 allows a direct comparison of $K_{0.5}(CO_2)$ and $K_{0.5}(HCO_3^-)$ as these conditions dictate the almost exclusive presence of one of the two forms. *Codium decorticatum* and *Udotea flabellum*, two members of the Chlorophyta showed distinctly different responses under these conditions (Reiskind, Seamon and Bowes 1989). Both had similar maximum rates at pH 8.0, and at pH 5.0 for *C.decorticatum*, while that for *U.flabelllum* was substantially lower. For both species the $K_{0.5}(HCO_3^-)$ values were greater than the $K_{0.5}(CO_2)$ values although there again appears to be a significant degree of diffusion limitation that may not have been considered. Overall the results suggest that *U.flabellum* has a greater affinity for (or better ability to use) the available inorganic carbon than *C.decorticatum* and it would appear that CO_2 is the preferred form. Sand-Jensen and Gordon (1984) studied six

marine macroalgae in order to examine their ability to use HCO_3^- and CO_2. For these species the kinetics of inorganic carbon use were determined by varying the pH between 7.0 and 10.0 at constant TIC concentration and calculating the proportions of the individual ions as a function of the pH. Although natural seawater concentrations were saturating and an increase in the CO_2 concentration did not alter the observed rates, the calculated $K_{0.5}(CO_2)$ were low in comparison to those for $K_{0.5}(HCO_3^-)$. The observation that macroalgae have a greater affinity for CO_2 than for HCO_3^-, is reported by other studies which have compared the kinetics of inorganic carbon uptake by a variety of methods (Johnston and Raven 1986b; Reiskind, Seamon and Bowes 1989). However, in comparison to freshwater plants which have similar values for $K_{0.5}(CO_2)$, the apparent affinity of marine macroalgae for HCO_3^- is substantially higher which is consistent with the high and constant availabilty of this form of inorganic carbon in seawater. (Sand-Jensen and Gordon 1984).

Although it would appear that CO_2 is the preferred source of inorganic carbon for photosynthesis, under certain conditions the evidence for inorganic carbon uptake appears to contradict this. Many macroalgae are substrate saturated in seawater where the concentration of CO_2 is insignificant. Even when observed rates were well below saturation an increase in the CO_2 due to a decrease in the pH from 8.2 to 7.3 did not give rise to the dramatic increase in photosynthesis that would be expected if photosynthesis was dependent solely on direct CO_2 uptake (Holbrook *et al.* 1988). In addition to the low CO_2/HCO_3^- ratio the availabilty of this inorganic carbon form in seawater is limited by a combination of the lower diffusion coefficient of CO_2 in seawater than in air, and the presence of an unstirred layer over the surface of the plant. Cook, Lanaras and Colman (1986) calculated the maximum rate of supply of CO_2 from the uncatalysed or spontaneous dehydroxylation of HCO_3^- in seawater. For a variety of marine macroalgae the photosynthetic capacity determined in the absence of an external CA exceeded the

maximum rate of supply of CO_2 in the medium by 6-24 fold at pH 8.0 and 19-101 fold at pH 9.2. These results are evidence that in some species HCO_3^- must be taken up as a source of inorganic carbon for photosythesis in addition to the diffusive entry of CO_2.

The characteristics of inorganic carbon use in marine macroalgae appears to be related to the availability of inorganic carbon in seawater. In addition there is evidence that under natural conditions when the availability of inorganic carbon is low, such as during long periods of exposure, the efficiency of inorganic carbon use increases. Surif and Raven (1989) investigated a number of marine macrolgae collected over a range of sublittoral to eulittoral positions. Although the maximum rates in seawater were higher for the eulittoral species, a more important correlation was observed in relation to the half saturated values for inorganic carbon use. The results showed that for the Fucales, the higher tidal species had a greater affinity for the available inorganic carbon, and are close to substrate saturation under natural conditions, while *Halydris siliquosa* and the *Laminaria* species, normally always submersed will be only half saturated under these concentrations.

Although it appears that the affinity for HCO_3^- could be the result of metabolic adaptation to the high concentration of this ion in seawater, it is possible that this increased affinity for inorganic carbon is not purely an increase in the ability to use HCO_3^-. There is evidence that changes in the photosynthetic capacity of marine macroalgae exposed to high levels of CO_2 results in a reduced capacity for photosynthesis in air as well as seawater. Johnston and Raven (1990) found that *Fucus vesiculosus* cultured at 5.0 kPa CO_2 (300 times that of air) had lower rates of photosynthesis, and a reduced ability to use not only HCO_3^- in seawater at pH 8.0 but also CO_2, available in air and at pH 5.5.

Other important physiological characteristics of photosynthesis in marine macroalgae are taken as a measure of the efficiency of the carbon fixation system. The CO_2

compensation point and the degree of oxygen inhibition are dependent on the competitive interaction of the two possible substrates for RuBPco, determined by kinetic analysis of the inorganic carbon response. The oxygenase activity of RuBPco which initiates the photorespiratory carbon cycle (PCOC), affects the balance between the photosynthetic CO_2 fixation and respiratory CO_2 release, of which the CO_2 compensation point is a function. The values obtained for C_3 plants are characteristically high due to the effects of photorespiratory gas exchange. In air the compensation points for a range of macroalgae were uniformly low over the temperature range of 0 - 30°C (Coughlan and Tattersfield 1977), and in general marine macroalgae have been shown to have low O_2-insensitive CO_2 compensation points and little light dependent O_2 uptake. Values for the compensation point of *Ascophyllum nodosum* in air and seawater were similar and comparable to values obtained for C_4 terrestrial plants (Johnston and Raven 1986b; 1987). The low compensation point of *Ulva faciata* was insensitive to both O_2 concentration and temperature, an indication that photorespiration is absent (Beer and Israel 1986; Colman 1984).

For six species investigated some oxygen inhibition of photosynthesis was evident at concentrations of HCO_3^- below those in natural seawater (Holbrook *et al.* 1988). The significance of the effect of an increase in the oxygen concentration at 0.5 mol m^{-3} can be seen as an increase in the $K_{0.5}$(TIC). This correlation is also evident in the comparison of the the two species of Chlorophyta. *C.decorticatum* has a relatively high CO_2 compensation point and showed significant levels of O_2 inhibition of V_{max} in addition to a comparatively lower affinity for HCO_3^- and CO_2 than those of *U.flabellum*. The results suggest that the greater efficiency of the photosynthetic system in this species, defined by the absence of RuBPco oxygenase activity, is dependent on a high apparent affinity for inorganic carbon use (Reiskind, Seamon and Bowes 1989).

A relationship between the available inorganic carbon source and the CO_2 compensation point has also been

observed by Surif and Raven (1989). For a range of marine intertidal macroalgae the measured CO_2 compensation points were related to both the affinity of each species for inorganic carbon, determined in part by the degree of exposure, and on the availability of inorganic carbon. The measured range of values at pH 8.0 were lower for all species than those at pH 5.0 indicating an ability to use HCO_3^-. It is also evidence that the affinity for HCO_3^-, although lower in most cases than that for CO_2, is quantitatively more important in determing the photosynthetic efficiency, due to the high HCO_3^-/CO_2 ratio in seawater.

The kinetic analysis of the concentration-response of photosynthesis in marine macroalgae can be used to define some important characteristics. High values for the K_m of RuBPco in macroalgae have been reported by Colman and Cook (1985) and Beer and Israel (1986). The values are greater than those for terrestrial plants, but at the lower end of the range for microalgae (Yeoh, Badger and Watson 1981). The kinetic analysis reveals that the substrate affinity measured in vitro is lower than the apparent affinity of inorganic carbon uptake. In order to maintain saturation of the carboxylase and suppress oxygenase activity, when the CO_2 of the external medium is at most 0.5 mol m^{-3}, a mechanism must be in operation which serves to accumulate and concentrate CO_2 at the active site of RuBPco. The kinetic parameters do not support the occurrence of a purely diffusive entry of CO_2, nor the supply of CO_2 produced from the spontaneous dehydroxylation of HCO_3^- to facilitate inorganic carbon accumulation.

The comparable photosynthetic characteristics of C_4 metabolism are mediated by a biochemical concentrating mechanism that involves the formation of a sequence of four carbon organic acids such as malate and aspartate, which act as CO_2 transporters between the site of CO_2 uptake, the spongy mesophyll cells, and the site of CO_2 fixation by RuBPco in the chloroplasts of the bundle sheath cells.

Early work by Kremer and Krüppers (1977) showed that with short term labelling during photosynthesis in $H^{14}CO_3^-$

more than 90% of the label was incorporated into phosphorylated compound after just 2-5 seconds, 3-phosphoglycerate being the most predominantly labelled assimilate. Incorporation of the ^{14}C into C_4 acids accounted for less than 10% of the label. From data for the activity and distribution of the carboxylating enzymes, RuBPco appeared to be the primary carboxylase in all species investigated, while two additional carboxylases, PEPc and PEPck, were minor components of the total enzyme activity. There is considerable intraspecific variation in the level of activity of these two enzymes, the latter more significant in the Phaeophyceae (Ferron and Coudret 1984). The activity of the beta-carboxylase PEPck has been further investigated to ascertain whether it plays a similar role in carbon concentration in marine macroalgae, as PEPc does in C_4 terrestrial plants (Kremer 1980). It has also been suggested that PEPck may be involved in dark CO_2 fixation similar to that which occurs in CAM plants. The titratable acidity value of around 10.0 mol m^{-3} has been shown by Johnston and Raven (1986), but this is an order of magnitude less than the those reported for terrestrial CAM plants and probably represents recycled, respired CO_2 (Griffiths 1988). Both PEPc and PEPck have been found in appreciable quantities in a Phaeophyte *Dilophus guineensis* and the Rhodophyte *Laurencia papilosa* (Holbrook *et al.* 1988). Malate levels showed diel fluctations in *L. guineensis* but the amplitude of the day/night difference was too small to make a substantial contribution to daytime CO_2 fixation. The suggestion that PEPck functions as a carboxylase enzyme during the day or night is not supported by the kinetics data for this enzyme (Johnston and Raven 1987) and there does not always appear to be a correlation between malate levels and PEPck activity (Ferron and Coudet 1984).

Uptake of inorganic carbon

An efficient gas exchange system, like that involving C_4 metabolism provides a higher inorganic carbon concentration inside the cell, during steady state

photosynthesis, than can be accounted for by passive CO_2 and HCO_3^- distribution maintained by pH and electrochemical gradients. In marine macroalgae the general absence of a biochemical concentrating mechanism means that the primary fixation of CO_2 occurs via RuBPco. The photosynthetic characteristics observed, and the evidence confirming the ability of macroalgae to utilize HCO_3^-, indicate a mechanism of carbon acquisition that is physical in its nature.

The characteristics, mechanism and regulation of inorganic carbon concentration in microalgae have been investigated using a variety of techniques. The results reveal the complexity and the plasticity of the process, both in terms of the important components and their relationship to the available source of inorganic carbon.

Unicellular green microalgae including *Chlamydomonas reinhardtii* and *Chlorella* species appear to use both HCO_3^- and CO_2 although kinetic analysis indicates that CO_2 is the preferred carbon source (Marcus, Volokita and Kaplan 1984; Hogetsu and Miyachi 1979). It has also been shown that the mechanism requires a number of interactive components. The comparison of photosynthesis at two pH values in the presence of the CA inhibitor Acetazolamine (AZ) suggests that external CA activity is required when HCO_3^- is the ion present (Moroney, Husic and Tolbert 1985). In addition the membrane permeable inhibitor Ethoxzolamide (EZ), reduced the photosynthetic affinity for inorganic carbon at both pH 5.0 and 8.0, indicating that a second component, an internal CA is also required. Although free external CO_2 or CO_2 formed from extracellular catalysis crosses the plasma membrane, at the physiological pH of cytoplasm most of the inorganic carbon will be in the form of HCO_3^-. The internal CA converts the accumulated HCO_3^- to CO_2 for fixation by RuBPco.

It has been shown that the occurrence and activity of the concentrating mechanism is inducible and regulated by the availability of CO_2 during growth. While air grown cells are able to use both CO_2 and HCO_3^-, those adapted to high levels of CO_2 have a requirement for inorganic carbon

in this form. The reduction in the photosynthetic efficiency, or the apparent lack of concentrating capacity of high CO_2 grown cells, and an O_2-sensitivity that is evident from photorespiratory characteristics, can be related to a change in the ability to actively accumulate inorganic carbon. It is also concomitant with lower levels of both external and internal levels of CA (Imamura, Tsuzuki, Shiraiwa and Miyachi 1983). The accumulating mechanism in air grown cells gives rise to a 40 fold increase in internal inorganic carbon concentration, enhanced by the inhibition of the internal CA by EZ. High CO_2 grown cells are also able to accumulate inorganic carbon although both the rate and steady state concentrations are reduced. Following addition of EZ it is evident that the process in these cells still has a requirement for internal CA (Badger, Kaplan and Berry 1980). It is therefore suggested that the lower efficiency of photosynthesis and corresponding lower affinity for the substrate is a result of suppression of the transport of inorganic carbon into the cells. Part of this appears to be related to the reduction in external CA activity, although this protein is almost certainly not the only component of the concentrating mechanism that is involved. Work with mutant strains of *Chlamydomonas reinhardtii* led Moroney *et al*. (1987) to conclude that external CA activity alone cannot account for the difference in the characteristics of air grown cells. The level of accumulation seen following inhibition of external CA at high pH suggest that other proteins such as a HCO_3^- transporter may be involved.

Spalding, Spreitzer and Ogren (1983a) also conclude that a component other than CA was involved in the CO_2 concentrating system, using a *C.reinhardtii* mutant deficient in internal CA. Although these cells were capable of accumulating inorganic carbon above the level of the external medium, photosynthesis in the mutants at atmospheric concentrations of CO_2 was greatly reduced. The resultant characteristics including O_2 inhibition and an increased CO_2 compensation point were independent of the affinity for inorganic carbon uptake and only related to

the supply of CO_2 to the carboxylase, a result mimicked by the wild type cells treated with EZ. A second mutant with normal levels of internal CA activity but with a reduced capacity to transport inorganic carbon, also showed reduced rates of photosynthesis at atmospheric levels of CO_2. Uptake of inorganic carbon by this mutant was comparable to passive distribution of CO_2 and HCO_3^-. Only when EZ was added preventing further transfer of inorganic carbon to the site of fixation, did any internal accumulation occur, although the concentrations were still below those of the wild type cells. Spalding *et al.* (1983b) concluded that the requirement for high CO_2 in these two mutants results from a deficiency in both internal CA and an additional component. The CO_2 concentrating mechanism operates via mediated active transport of inorganic carbon followed by the conversion of a large internal pool of inorganic carbon pool to CO_2 by intracellular CA activity.

Studies have shown that other green microalgae have similar methods of accumulating inorganic carbon. *Scenedesmus obliquus* cells grown under air levels of CO_2 can use both CO_2 and HCO_3^- and contain high levels of both external and internal CA. In contrast, cells grown at high CO_2 concentration lack CA activity but are able to maintain higher rates of photosynthesis even without HCO_3^-. Aeration of high CO_2 grown cells with air levels of CO_2 gave rise to the sequential development of external and internal CA activity, and a decrease in the requirement for free CO_2 alone. This is evidence that although CA is not essential for photosynthesis, it can modify the response of the cells to the available inorganic carbon (Findenegg 1976). However, in *Chlorella* species the biochemical nature of the inorganic carbon uptake is unclear, with some species using only CO_2 and others, where external CA is present, using both CO_2 and HCO_3^-. In these species the enzyme would facilitate uptake of inorganic carbon by conversion to CO_2, but independent of the method of uptake, the activity of an internal CA has been shown to determine the apparent affinity for bicarbonate (Miyachi, Tzuzuki, and Avramova 1983).

There is however no evidence of internal CA activity in the cyanobacteria *Anabaena variabilis* and *Synechococcus* species (Badger and Andrews 1982) In these cells inorganic carbon accumulation via uptake of both HCO_3^- and CO_2 results in internal levels which can be as high as 1000 times greater that of the external medium (Badger and Andrews 1982). Suppression by inhibitors and stimulation by increased Na^+ levels indicates that the process is dependent on active transport at the plasma membrane. Although there is little or no detectable external CA activity the membrane permeable inhibitor EZ inhibits the uptake of inorganic carbon to a degree (Kaplan, Badger and Berry 1980). The absence of an internal CA is overcome by the very high internal concentration capable of supply CO_2 at a rate that saturates the carboxylase enzyme RuBPco.

As with *Chlamydomonas reinhardtii*, the CO_2 concentration during growth determines the photosynthetic characteristics of these cells. The effect of the induction of a mechanism can be seen as a change in the affinity of photosynthesis for inorganic carbon although the maximum capacity is unaffected. This is concomitant with a faster uptake rate and higher steady state internal inorganic carbon concentration in air grown cells. This greater capacity for transport appears to be the basis of the increased ability to accumulate inorganic carbon and maintain efficient photosynthesis (Kaplan, Badger and Berry 1980).

As with CA activity the location and function of the additional components such as those involved with active transport, is unclear. *Porphyridium purpureum* and *Phaeodactylum tricornutum* both show an increased affinity for HCO_3^- use in the presence of Na^+, inferring the existence of active transport across at least one membrane Patel and Merrett 1986, Dixon Patel and Merrett 1987). Air grown cells of *Porphyridium* have high levels of CA that can be suppressed by elevation of the CO_2 concentration, with all the measurable activity intracellular. Inhibitor studies have indicated that, as in other mechanisms, the activity of this enzyme determines the affinity for

inorganic carbon under alkaline conditions but it is of less significance if CO_2 is available (Dixon, Patel and Merrett 1987). In comparison, *Phaeodactylum* was found to have very low total CA activity all of which is internal, and a low affinity for CO_2. Stimulation of HCO_3^- uptake even at high concentration suggests the occurence of Na^+ dependent active HCO_3^- transport at the plasma membrane (Patel and Merrett 1986).

In contrast, the acid tolerant unicell *Chorella saccharophila* exhibits both a low CO_2 compensation point and a low $K_{0.5}$(CO2) at pH 4.0, where only CO_2 is available. Active transport of HCO_3^-, believed to maintain high internal inorganic carbon levels, could be present at the chloroplast envelope. This would serve to increase the concentration gradient between the external source and the cytoplasmic sink, allowing both rapid fluxes at the plasma membrane and accumulation of a large internal pool of HCO_3^- within the chloroplast stroma. Saturating concentrations of CO_2 at the site of fixation could occur by either catalysed or uncatalysed dehydroxylation. Kaplan, Badger and Berry (1980) have suggested a common system of inorganic carbon accumulation in *Chlorella* and *Chlamydomonas* implying a requirement for components involved in both active transport at the chloroplast envelope and intracellular CA activity within the chloroplast.

That active transport could occur at either the chloroplast envelope and/or the plasma membrane was proposed by Raven and Glidwell (1978). They studied the CO_2 accumulation of the green algae *Hydrodictium americanum* and showed that increased active uptake of HCO_3^- across the plasma membrane of air grown cells occurs in parallel with decreased CO_2 compensation concentrations. At the lower pH higher rates of photosynthesis were maintained by the diffusive entry of CO_2 in combination with HCO_3^- transport at the chloroplast envelope. They also propose that the above system could account for the photosynthetic capacity of marine intertidal macroalgae during periods of emersion and submersion.

Mechanisms of inorganic carbon accumulation

From the work on unicellular algae, it is believed that the active transport of the inorganic carbon across either the plasma membrane or the chloroplast envelope occurs via an electrogenic pump, powered by ATP, in the form of a primary active transport system or a co-transport mechanism (secondary active transport). Evaluation of the possible mechanisms of inorganic carbon accumulation are complicated by the seemingly anomalous utilization of several carbon species, and location of the components of active transport. A model proposed by Kaplan (1985) for *Anabaena variabilis* included a system that accounts for the apparent ability of this organism to remove both HCO_3^- and CO_2 from the external medium, and the competitive nature of the uptake of these two sources of inorganic carbon. Although CA activity was not detectable in *A. variabilis*, the use of CA inhibitors resulted in a reduced capacity for CO_2 uptake in comparison to HCO_3^-. The model includes a CA-like moiety as a component of an HCO_3^- porter system present in the plasma membrane. CO_2 arriving at this site can be converted to HCO_3^- by the moiety and then transported into the cytoplasm. This same mechanism may account for the apparent ability of other algae to utilize both HCO_3^- and CO_2, although evidence suggests that the species accumulated is HCO_3^-.

Following HCO_3^- transport, the decarboxylation of HCO_3^- to CO_2, the species of carbon required by RuBPco, liberates near stoichiometric quantities of OH^-. To prevent pH changes within the cell, the alkalinity must be disposed of via OH^- efflux or neutralization. A mechanism proposing that HCO_3^- influx is mediated by an HCO_3^-/OH^- antiport system was rejected following work on *Chara corallina* (Lucas 1976). The results indicate that HCO_3^- and OH^- are transported across the membrane independently, and on spatially distinct carriers, a situation that results in altered membrane potentials.

Kaplan, Zenvirth, Reinhold and Berry (1982) found that the membrane potentials of *A. variabilis* varied in response to the supply of HCO_3^- to CO_2 depleted cells. By combining

inhibitor studies and phenyl-^{3}H(TPP$^+$)distribution analysis they were able to propose two models that could explain active transport. The first, a primary electrogenic pump for HCO_3^- and the second, an H^+/HCO_3^- co-transporter, the driving force for which is generated by a proton pump sensitive to HCO_3^- concentrations. The data did not provide decisive evidence for either mechanism, and it has been suggested by Lucas (1983) that various species, including macroalgae, may have evolved different means by which they energize the mechanism.

Later work by Kaplan (1985) elaborates on the possible mechanisms involved in active HCO_3^- transport, following the discovery of a Na^+ stimulated electrogenic pump in *A. variabilis*. The effect of the presence of Na^+ on the $K_{0.5}(HCO_3^-)$ of active transport led Kaplan to propose several possible areas of Na^+ involvement in the process. A primary HCO_3^- pump and a secondary Na^+/H^+ antiport system would provide a means of neutralizing the OH^- produced by dehydroxylation, although the mechanism of Na^+ entry is unclear. Alternatively the primary pump might be an Na^+ efflux pump, while HCO_3^- enters via HCO_3^-/Na^+ symport. The primary pump would maintain low internal levels of Na^+ and allow a HCO_3^- accumulation ratio of 1000, assuming a stoichiometry of 1:1 Na^+ to HCO_3^-, although the method of pH regulation by this system is unclear. The third model proposed involves Na^+ binding to the HCO_3^- porter at the membrane which serves to mediate the substrate affinity of the system. In the absence of Na^+, CO_2 is removed from the external medium and converted to HCO_3^- by the CA-like moiety, while in the presence of Na^+, HCO_3^- affinity is increased and its uptake enhanced. This model allows for the cycling of OH^- between the cytoplasm and the CA-like moiety at the plasma membrane.

In all the studies the operation of the electrogenic pumps suggests the involvement of an HCO_3^- stimulated membrane ATPase. As active uptake occurs only in the light, the energy requirement appears to be dependent on light activated processes such as cyclic and non-cyclic photophosphorylation during photosynthesis, or the

pseudocyclic photophosphorylation of the Mehler reaction (Brechignac and Andre 1984; 1985), all of which produce ATP.

The significance of the mechanisms proposed for microalgae have yet to be evaluated in terms of importance to inorganic carbon concentration in marine macroalgae.

Smith and Bidwell (1987) have found evidence that in *Chondrus crispus* both periplasmic and intracellular CA activity is involved in the photosynthetic uptake of inorganic carbon. Enzyme extracts from a number of species analysed by Graham and Smillie (1976) showed measurable activities of CA although the values were low in comparison to those in Pea extracts. Their procedure did not determine whether the CA was located external or internal to the plasma membrane. A similar study carried out by Cook, Lanaras and Colman (1985) assayed for the extracellular enzyme only, but there was no evidence of activity in any of the species tested. Internal CA appears to be present in all macroalgae so far investigated and as in microlgae facilitates the supply of CO_2 to RuBPco by the catalytic conversion of the internal pool of inorganic carbon, present as HCO_3^-. Treatment with the permeable inhibitor EZ inhibits intracellular CA activity, decreasing the rate of photosynthesis and in most cases resulting in an increase in the oxygen sensitivity or the Warburg effect (Reiskind, Seamon and Bowes 1989; Smith and Bidwell 1989). Both these studies found evidence, using the impermeable inhibitor AZ, that external CA is not an absolute requirement of the photosynthetic system but that it modifies the dependence of the process on CO_2 as the inorganic carbon source. The $K_{0.5}$(TIC) values of high intertidal Fucoid species suggest a greater affinity for HCO_3^- than in the intermediate species *Halidris siliquosa* and lower intertidal Laminariales (Surif and Raven 1989). This can be directly related to higher levels of CA, a correlation between HCO_3^- use and CA activity that is also evident in marine microalgae. In addition the Fucales showed a greater photosynthetic capacity at high pH.

CA dependent HCO_3^- use relies on the external catalysis of HCO_3^- to CO_2 by the extracellular enzyme, which then passively diffuses through the plasmamembrane. This mechanism does not exclude the probability that external CO_2 also contributes to internal levels of inorganic carbon but, as previously stated, an internal CA is ubiquitous in enhancing the final stage of fixation. In addition, HCO_3^- uptake may occur independently of CA activity. Smith and Bidwell (1989a) used inhibitors of CA, of the band 3 anion exchange protein and of a Na^+/K^+ exchange protein. The results showed no evidence that either an active or facilitated mechanism of HCO_3^- uptake operates in *Chondrus crispus*.

Even though both internal and external CA is present in *Ulva faciata*, Beer and Israel (1990) suggest that the thallus surface pH of this species facilitates active HCO_3^- uptake across the plasma membrane. A measured value of 10.0 gives rise to an extracellular layer in which CO_2 in the bulk medium would be converted to HCO_3^- and transported into the cell by means of HCO_3^-/H^+ symport or HCO_3^-/OH^+ antiport. The measured low internal pH and membrane potential means that the H^+ accumulation must be active, in the form of an H^+ pump. However, this mechanism also shows some requirement for external CA and it may be that in air the surface pH of 10.0 prevents adequate CO_2 diffusion. CA catalysis would increase the supply of HCO_3^- from CO_2 and allow inorganic carbon accumulation during emersion. The two phase increase in pH suggests that stimulation of the proton pump may be dependent on an initial uptake of CO_2 by diffusion. Therefore any CA activity would be of only marginal importance.

These mechanisms of inorganic carbon uptake in marine macrolgae appear to be analogous to those in the unicellular species. However as yet there is no direct evidence that internal accumulation of inorganic carbon above that of the external medium, occurs in macroalgae. Smith and Bidwell (1989a; 1989b) have experimented with both thallus sections and isolated chloroplasts, for use with the silicon oil centrifugation technique. In both

studies it was found that in *Chondrus crispus*, the internal inorganic carbon concentrations were no greater than could be accounted for by passive diffusion. The levels were unaffected by AZ or EZ inhibition, further evidence that in this species there is no active uptake of HCO_3^- at the plasma membrane, resulting in the build up of a pool within the cytoplasm as seen in *Chlamydomonas reinhardtii*.

But there may be additional biochemical processes that serve to maintain the efficiency of inorganic carbon use between uptake and fixation. Inhibition of photosynthesis by EZ does not increase the O_2-sensitivity of the carboxylase in *Udotea flabellum*, as it does in *Codium decorticatum* (Reiskind, Seamon and Bowes 1989). In this case saturation of CO_2 fixation appears to be maintained by the high activity of a ß-carboxylase which could concentrate carbon as malate and aspartate. The transport of these two organic acids across the chloroplast membrane would account for one mode of the active transport that appears to be important in microalgae, in addition to the HCO_3^- use mediated by internal CA. As previously discussed, there is much evidence to verify that as in terrestrial plants RuBPco is the primary carboxylase in marine macroalgae. However, as measurable activities of both PEPck and PEPc are present in more species than previously believed, this mechanism may be of some importance .

Light and photosynthesis

Emersed macroalgae receive the same quantity and quality of light as terrestrial plants. Photosynthetically active radiation (PAR) between 350 and 700 nm measured as μmol photon m^{-2} s^{-1} varies between 0 and 2000 in full sunlight.

Submersed macroalgae receiving PAR passing through water may experience changes in the quality and quantity of light, resulting from the absorption and scattering of the photons; ie attenuation. A value that describes the effect of these processes, the attentutation coefficient, can be used to calculate the change of irradience with depth:

$$\text{Attenuation coefficient, } k\ (m^{-1}) = \frac{\log_e I_1 - \log_e I_2}{d_2 - d_1}$$

if I_1 and I_2 are the irradience at depths d_1 and d_2, the irradience at depth z is

$$\log_e Iz = \log_e I_o - k.z$$

where I_o is the irradience at the surface of the water.

As light penetrates water, attenuation of the wavelenghs that make up white light occurs. Wavelenghs in the infra-red region (>700) are absorbed by the first metre of seawater so that the remaining light is PAR. The depth to which the remaining wavelenghs penetrate increases with decreasing wavelengh until around 400 nm where the absorption and scattering of the ultraviolet end of the spectrum increase sharply. While the transmittance of light of specific wavelenghs is constant this is not so for white light. As the intensity of the more strongly attenuated wavelenghs decreases with depth, the quantity of light is reduced even though it becomes enriched in the wavelengths that penetrate further (Dring 1981). However, the most critical factor governing photosynthesis in marine intertidal macroalgae is light intensity (Ramus, Beale, Mauzerall and Howard 1979).

The relationship between PAR and photosynthesis can be determined from the light intensity-response curve. The characteristic shape of this curve reflects two important phases. The initial slope or light limited response and the transition to a light saturated response. Light limitation represents the photochemical step of photosynthesis. Light saturation of the process is regulated by the rate of the enzymic reaction. The efficiency of light utilization is termed the apparent quantum ratio, and is a measure of the ratio of mol photon incident/mol O_2 evolved. The value can be calculated using least square regression analysis of the light limited portion of the slope. The light compensation point, where net photosynthesis is zero, reflects the balance between respiration, photorespiration and

photosynthesis. This intensity can be determined from the algebraic solution of the regression line. The point of saturation (I_k) can also be quantitatively determined from the intersect of the lines representing the light limited and light saturated rates of photosynthesis (Arnold and Murray 1980).

Marine intertidal macroalgae have been characterized as shade plants. These characteristics include low light compensation and saturation points, high photosynthetic capacity at low intensity and high accessory pigment to chlorophyll a ratios.

A linear relationship between photosynthesis and light intensity was measured for a variety of species of Chlorophyta (Arnold and Murray 1980). Compensation points were between 1.4 and 6.1 μmol photon m^{-2} s^{-1}, and saturation points between 50.3 and 75.5 μmol photon m^{-2} s^{-1}. These were calculated as described previously and are considerably lower than those reported for other species, normally visually estimated. Similar low compensation intensities have been observed for Rhodophyte, Chlorophyte and Phaeophyte species (Reiskind, Seamon and Bowes 1989; Krist 1981; Oates and Murray 1983). However the light saturation points appear to be more variable. It has also been suggested that there may be a relationship with morphology. Although several of the Chlorophyte species were thin sheet-like or filamentous forms, similar low values for I_k were measured for both the thick intertidal saccate forms (Oates 1985; 1986), and the Phaeophytae *Hesperophycus harveyanus* and *Pelvetia fastigiata* (Oates and Murray 1983).

Many of these studies have shown that the efficiency of light utilization during photosynthesis in air is reduced, seen as higher light saturation and compensation points. Reduced V_{max} values for the response of three Fucoid species in air corresponded to higher values for I_k (Oates 1985, Oates and Murray 1983). However, a fourth related species did not show this correlation, and a higher capacity in air was reported for *Ascophyllum nodosum* (Oates 1986, Johnston and Raven 1986a).

It has been proposed that the requirements for light saturation are habitat related with levels for *Fucus* and other eulittoral species of 500 μmol photons m^{-2} s^{-1}, 200 for mid-intertidal species and 100 or less for those in deep water (Coutinho and Zingmark 1987). There is also evidence for seasonal variation in the efficiency of light utilization. King and Schramm (1976) investigating the effect of light intensity on a seasonal basis, found a general tendency for light compensation to be lower in winter. *Gracilaria tikvahiae* needed a higher light level for saturation in summer while the light compensation point remained the same (Penniman and Mathieson 1985). Although the increase in the irradience requirements of the light intensity-response appear to reduce the overall efficiency of photosynthesis, it may be that they reflect some degree of adaptation or acclimatization. The ability to absorb large quantities of light without a corresponding capacity to utilize this energy, by both photochemical and enzymatic processes, will be detrimental. The adaptations apparent in summmer may be dependent on longer periods of high intensity irradience in comparison to winter months. Similarly, more eulittoral species are exposed for longer periods of time under conditions when the light intensity is not attenuated by the water column.

Regression analysis of the light limited rate and the maximum rate or capacity suggests that these characteristics are also related to morphology (Arnold and Murray 1980). A higher photosynthetic efficiency and capacity was recorded for both the thin sheet like *Ulva* species and the tubular *Enteromorpha intestinalis*; in comparison to the thick optically dense *Codium* sp. Similar conclusions were drawn by King and Schramm (1976). Their study also suggests that short lived annuals rather than perenials, and eulittoral in contrast to sublittoral species, are more productive in terms of light utilization.

THe light limited rate of photosynthesis, or the photosynthetic efficiency, is defined by the apparent quantum ratio. The theoretical value of 8.0 is based on the number of μmol photon absorbed, although for most studies

the ratio is calculated from incident light, and as a consequence the reported values are greater than 8.0. In constrast, a disregard of the Kok effect is believed to lead to an under estimation of the quantum ratio (Sharp, Matthews and Boyer 1984). A non linear response to light intensity observed between 5 and 11 μmol photon m^{-2} s^{-1} results from the partial suppression of dark respiration in the light. In view of this phenomenom, quantum ratios must be measured when respiration is constant in the dark, at low O_2 concentrations which inhibit the light sensitive part of the process, or at light levels that saturate the suppression. A value of 12.24 was reported for *Ascophyllum nodosum* (Johnston and Raven 1986a). Few other studies have measured apparent quantum ratios. Arnold and Murray (1980) have showed a correlation with macroalgal morphology. The optically dense species having higher quantum ratios than the distinct group of species with thin simple sheet like morphology. Oates (1985; 1986) measured light intensity responses for two saccate macroalgae. In both air and seawater the photosynthetic efficiency was related to the light requirements but there was little correlation with the absolute capacity achieved. It appears that although the photochemical system may be modified in response to environmental conditions, this does not always result in a decrease in the overall capacity. There is also strong evidence that photosynthetic capacities are generally greater in summer, which may a reflect changes in light intensity-response of marine intertidal macroalgae.

Any changes in the photosynthetic characteristic will be mirrored by changes in the photosynthetic pigments of these plants. Although intertidal macroalgae are defined as shade plants these characteristics vary in response to light levels (Dring 1981). Changes in the light intensity response can be directly attributed to alteration of the chlorophyll/accessory pigment concentration of the light harvesting system of photosynthesis. In terrestrial plants variations in light intensity result in photoacclimation. The adaptation from shade to sun plants is characterized by

a decrease in the total chlorophyll content and in the ratio of chlorophyll a:b (Dring 1981).

Studies with macroalgae have showm that the same effect on chlorophyll content and pigment ratio occurs in response to irradience transitions. *Ulva faciata* and *Ulva rotunda* both showed a relationship between chlorophyll a and b content and a:b ratio. This indicates a strict regulation of pigment composition by light. The reduction in the chlorophyll a content of *U.faciata* decreased the photosynthetic efficiency (Henley and Ramus 1989a; Lapointe and Tenore 1981). However, Ramus *et al.* (1976) concluded that adaptation from a shade to sun species resulted in a decrease in the total pigment concentration, with little of no variation in the accessory pigment/chlorophyll a ratio. They suggest that, for intertidal macroalgae, while light intensity may vary dramatically, the colour of the habitat remains fairly constant. This is in contrast to the sublittoral habitat were attenuation results in colour variation as well as a decrease in the total photon flux.

Of the two possible strategies of light acclimation, intertidal macroalgae appear to favour manipulation of the total concentration of pigments. However, any increase in photosynthesis in relation to total pigment concentration may be reduced by self-shading. It has been shown that only optically translucent genera such as *Porphyra* and *Ulva* will benefit fully from this method of adaptation (Ramus, Beale and Mauzerall 1976).

An increase in the pigment concentration can be achieved by the addition of chlorophyll molecules to increase the size of existing photosynthetic units (PSU). Alternatively, the increase in chlorophyll may be due to the build up of new reaction centres. It is possible that both a change in size and number of PSUs occurs in response to variation in light intensity (Dring 1981).

The effect of light intensity on the photosynthetic compensation and saturation point, and on the accessory pigment/chlorophyll a content and ratio all reflect strategies of acclimation. Rather than negatively affecting the photosynthetic response, it may be that they are of

some benefit. An increase in the light requirements suggests a mechanism that is able to regulate the amount of light absorbed in response to environmental fluctations. One such modification is the increase in the concentrations of pigments when light levels are low, which facilitate optimal light absorption. When the intensity increases a similar level of light utilization will require a comparatively smaller light harvesting complex. Without this degree of control the absorption of light energy, above that which can be processed by the reactions of photosynthesis, may be inhibitory. *Ulva rotunda* maintained at 2000 μmol photon m^{-2} s^{-1} was less efficient at 180 μmol photon m^{-2} s^{-1}, and conversely plants maintained at 180 μmol photon m^{-2} s^{-1} were unable to utilize fully light levels of 2000 μmol photon m^{-2} s^{-1}. This was attributed to a change in the RuBPco content or in the capacity for electron transport. The latter may be due to an effect on the concentration of the rate limiting components of the system, or to photoinhibition (Henley and Ramus 1989b).

These adaptations appear to be correlated with seasonal, morphological and habitat variations, all of which are interrelated. Light levels in excess of those required for saturation will occur more frequently during the summer months. The suseptibility to photoinhibition increases in relation to seasonal variations in temperature and resultant desiccation. Both these factors will have a greater effect on thin, sheet like species, with little or no self shading of chlorophyll, and on the higher intertidal species that are subject to longer periods of exposure. Therefore the strategies outlined above will be of significance in reducing the effect in marine intertidal macroalgae.

Photoinhibition of photosynthesis occurs when the rate of light absorption exceeds the rate of energy consumption. Three bases for photoinhibition have been proposed (Osmond 1981). An imbalance between the energy transfer to the reaction centres and energy transfer to the reducers will occur when: shade plants are transferred to sun, due to the disproportionate content of light harvesting pigments to

energy transducers; if the transducers system becomes disrupted, ie by temperature or water stress; and finally if the effective functioning of the transducer system is prevented by changes in the concentration of CO_2 or O_2.

Conditions in the intertidal environment are such that marine intertidal macroalgae may be subjected to one or all of these conditions. It therefore seems inevitable that photoinhibition of photosynthesis will occur. Therefore changes in the light intensity-response of photosynthesis on a seasonal basis may be the result of both acclimation or photoinhibition of the process.

Photoinhibition

Although photoinhibition of photosynthesis in marine intertidal macroalgae seems to be inevitable, few studies have investigated its effect. Light intensity-response curves are measured under ambient substrate, water and O_2 level over short periods of time. The absence of any reduction in the rate, at light intensities in excess of saturation, has been taken as evidence for the absence of this phenomenon. Under natural conditions however, environmental stress and/or long periods of exposure at irradiences of up to 2000 μmol photon m^{-2} s^{-1} occur frequently. Any inhibition of the photosynthetic process would have a significant effect on the overall capacity of intertidal macroalgae.

Evaluation of photoinhibition in a sun and shade species of *Porphyra* indicated that both plants were affected by high light intensities. Even under an optimum temperature and water regime the intertidal species *P.perforata* exhibited photoinhibition at one third of that seen in the subtidal species *P.nerocystis* (Herbert and Waaland 1988). Light intensity-response curves measured for a number of subtidal macroalgae have shown reductions in V_{max} that can be attributed to photoinhibition (Coutinho and Zingmark 1987). This study also concludes that the effect varies on a seasonal basis. Exposure of the red subtidal macroalgae *Polyneura hilliae* to high light intensities greatly reduced photosynthetic rates

(Nultsch, Pfau and Huppertz 1989). Recovery of the capacity was dependent on the length of the photoinhibitory treatment. The effect was only fully reversed after a period of low light, rather than complete darkness. The action spectrum for photosythesis in this species showed that the photoinhibitory wavelenghs are absorbed by the photosynthetic pigments. This indicates that photoinhibition occurs within Photosystem II (PS II). Until recently photoinhibition was seen as damage to the photosynthetic apparatus. It is now believed to be an adaptation to light, in the form of mechanisms to prevent such damage (Krause 1981; Sibald and Vidaver 1987).

Photoinhibition is primarily a disruption of the electron transport chain in the thylakoid membrane. The initial effect has been traced to the reaction centre of PS II resulting in inactivation of the primary electron acceptor QB (Krause 1981). It was also suggested that photoinactivation of the carboxylase RuBPco may also contribute to photoinhibition in vivo. Wavelenghs of 420 nm and above (blue light) increased the photoinhibitory effect seen in *Polyneura hilliae*. This light appeared to be absorbed by non-photoreceptors, and may represent an additional site that is sensitive to high light intensities.

The degree of photoinhibitory damage depends on the physiological state of the plant, the environmental conditions and the duration of the exposure to high light intensities (Krause 1981). Interaction of these parameters form the basis of susceptibility but the effect appears to be regulated by both protective mechanisms and the rate of the recovery/repair processes. Four main protective mechanisms have been proposed:

1. Energy consuming processes such as photorespiration which maintain electron transport along the chain. Herbert and Waaland (1988) considered photorespiratory protection from photoinhibition. They found that photorespiration rates were higher in the photoinhibition-sensitive species but also that under low O_2 photoinhibition was reduced.

2. Various reaction systems are proposed to reduce the damage by preventing the formation or reaction of free radicals. Recent work by Demmig (1987) proposes a mechanism of non-radiative dissipation of excess excitation energy to prevent photo-oxidation. The process occurs at the pigment bed and involves the formation of the carotenoid zeaxanthin from the xanthophyll cycle, dissipating energy via the formation of chemical bonds. Although this results in a degree of photoinhibiton in the form of reduced photosynthetic efficiency, photo-oxidation is prevented while there are sufficient precursors to synthesise zeaxanthin. An increase in the chlorophylla a/carotenoid ratio of *Ulva rotunda*, in response to increasing irradience, is believed to be part of the protective mechanism in this species (Henley and Ramus 1989a).
3. Long term acclimation to excess light which, as previously discussed, could result in changes in the photosynthetic characteristics such as light harvesting and pigment composition. It may also rely on effective carbon fixation, such as that maintained by the inorganic carbon concentrating mechanism of microalgae.
4. An increase in the rate of radiationless thermal dissipation of energy from the photosynthetic pigments to the reaction centres. Fork, Bose and Herbert (1986) investigated radiationless transitions in both a red macroalga and higher plants. In the higher plants energy was dissipated by the radiationless transition involving the production of heat. This was seen as a pH dependent quenching of fluoresence that is brought about by increased thermal de-excitation. A second mechanism was exclusive to the macroalga. Excess excitation energy at PS II was regulated by an increase in the transfer to PS I, termed the State 1-State 2 transition. This results from phosphorylation of the components of PS II causing a dissociation of the reaction centre and the peripheral chlorophyll complex within the thylakoid membrane (Andersson and Anderson 1988).

Photoinhibition resistance can be evaluated in terms of the rate of damage and repair. High and low light grown

cyanobacteria, and intertidal and subtidal *Porphyra* species have been compared to determine the physiological basis of this resistance (Samuelsson, Lonneborg, Gustafsson and Oquist 1987; Bose, Herbert and Fork 1988; Herbert 1990). *P.perforata* the intertidal macroalgae was less inhibited than the subtidal species *P.nerocystis*. Fluorescence characteristics and the rate of oxygen evolution indicated that this resistance was based on a greater capacity for recovery, than seen in the latter species (Bose, Herbert and Fork 1988). Samuelsson *et al*. (1987) offered a similar explanation for the difference in susceptibility of high and low grown *Anacystis nidulans*. Differences in the rate of recovery of photosynthesis following photoinhibition are thought to depend on the rate of turnover of the proteins of PS II such as QB.

Later work on the two *Porphyra* species contradicts this theory. Herbert (1990) proposed that the photoinhibition resistance of *P.perforata* results from reduced damage rather than increased repair of the electron transfer during photosynthesis. The desiccation that is likely to occur in conjuction with photoinhibitory conditions would almost certainly limit *de novo* protein synthesis. The only effective means of limiting photoinhibition under these conditions appears to be to prevent the initial damage.

As yet it is unclear as to the importance of photoinhibiton of photosynthesis in marine intertidal macroalgae. The various protection mechanisms proposed, and the balance between recovery/repair appear to be of importance to this group of plants which are subjected to large fluctations in environmental conditions.

SECTION 1 - Photosynthetic characteristics of P.umbilicalis and U.lactuca

INTRODUCTION

Photosynthesis in marine intertidal macroalgae can be characterized in terms of the relationship between CO_2 fixation, inorganic carbon concentration and light intensity.

Analysis of the concentration-response curves for HCO_3^- and CO_2, with respect to Michaelis-Menten kinetics gives a measure of the ability of macroalgae to utilise the two forms of inorganic carbon available. The relationship between the rate of photosynthesis and the substrate concentration can be described by two parameters. V_{max} is defined as the maximum rate of the response. This may be limited at the level of saturation by the activity of the carboxylation enzyme RuBPco, or by saturation of inorganic carbon transport across membranes. The $K_{0.5}(IC)$ is the inorganic carbon concentration at which the photosynthetic rate is half the V_{max}. It is a measure of the affinity of the photosynthetic process for available carbon, calculated from the part of the response limited by the supply of substrate. This initial rate is directy related to the concentration of inorganic carbon in the external medium but may be affected by the diffusion resistance imposed by the unstirred layer. The shape of the concentration response curve is governed by the two limiting processes described above. If the relationship between photosynthesis and inorganic carbon is enzyme mediated it will be represented by a characteristic rectangular hyperbola, and Michaelis-Menten kinetics will be adequate to describe the relationship. If the system is controlled by inorganic carbon uptake mediated by diffusion the curve shows a characteristically rapid transition to saturation. Analysis using the Hill-Whittingham equation which incorporates a permeability constant (P_u) to account for diffusion resistance, will give a more accurate description of the concentration-response curve (Johnston and Raven 1986a).

These parameters determined from physiological studies provide some evidence as to the biochemical nature of the ability of marine macroalgae to utilize HCO_3^- or CO_2.

Although a number of studies have compared the photosynthetic capacity in air and seawater, few have analysed the kinetics of the concentration-response. The ratio of the maximum net rates in air and seawater appear to be related to the degree of exposure of intertidal macroalgae (Johnson, Gigon, Gulmon and Mooney 1974; Quadir, Harrison and Dewreede 1979). However, it is not possible to define the nature of carbon aquisition from their results. The kinetic analysis of photosynthesis in *Ascophyllum nodosum* shows a similar comparison although saturation is not achieved at atmospheric CO_2 concentrations (Johnston and Raven 1986a).

Evidence that HCO_3^- use is important has come from a study investigating a variety of red and brown macroalgae. The observed rates of photosynthesis, in the absence of an external CA, could not be supported solely by CO_2 formed by the spontaneous dehydration of HCO_3^- in the external medium. In all the macroalgae tested the rates exceeded the maximum CO_2 supply rate by 6-24 times at this pH, evidence that HCO_3^- must be taken up as a source of carbon for photosynthesis, in addition to the diffusive entry of CO_2 (Cook, Lanaras and Colman 1985). Values for V_{max} close to the concentration of HCO_3^- in seawater support the theory that this form of inorganic carbon is important in maintaining the rates of photosynthesis observed for marine macroalgae in seawater. This does not however exclude the possibility that CO_2 is the form that is taken up. A low $K_{0.5}(HCO_3^-)$ also indicates a high affinity for HCO_3^-, although that for CO_2 is normally higher. The ratio of 2.0 for the CO_2/HCO_3^- affinity of marine maccrophytes is better in comparison to 5.4 for freshwater species. This is consistent with the high and constant concentration of HCO_3^-, in comparison to that of CO_2, in the carbonate system of seawater (Sand-Jensen and Gordon 1984).

Values for $K_{0.5}(CO_2)$ can be determined from the concentration-response curves measured in air and in seawater at low pH, where the predominant form of inorganic carbon is CO_2. The results for *Codium decorticatum* and *Udotea flabellum* showed that the $K_{0.5}$(DIC) and (HCO_3^-)

determined at pH 8.0, were higher than the corresponding values at pH 5.5. However, the initial rate of uptake and corresponding CO_2 affinity measured at the lower pH was decreased by a degree of diffusion limitation in one of the species (Reiskind, Seamon and Bowes 1989).

Further evidence suggests that the mechanisms of inorganic carbon acquisition in marine intertidal macroalge may be phenotypically controlled. The high affinity for HCO_3^- may be due to metabolic adaptation to an environment where there is a high and constant concentration of this form of inorganic carbon (Sand-Jensen and Gordon 1984). Growth under conditions of reduced CO_2 concentration in seawater failed to alter the CO_2 compensation points of *C.decorticatum* or *U.flabellum* (Reiskind, Seamon and Bowes 1989). The V_{max} of the photosynthetic response of *Fucus serratus* grown under conditions of high CO_2 was suppressed in air and in seawater and there was an increase in both the $K_{0.5}(CO_2)$ and the CO_2 compensation point (Johnston and Raven 1990).

Analysis of concentration-response curves measured under conditions of high and low O_2 do not usually show any significant levels of oxygenase activity by RuBPco. At seawater levels of HCO_3^- most macroalgae show little O_2 inhibition of photosynthesis and only when the TIC concentration is limiting is there any evidence of light dependent O_2 uptake. These findings are confirmed by relatively low CO_2 compensation concentrations in marine macroalgae (Cook and Colman 1987; Holbrook *et al.* 1987; Reiskind, Seamon and Bowes 1989).

Determination of the kinetic parameters of photosynthesis in marine macroalgae has shown that the affinity for HCO_3^- is high and that it is an important source of inorganic carbon in seawater. However, the affinity for CO_2 in normally higher and it is possible that this is the predominant form of inorganic carbon taken up both in air and seawater. In addition the observed rates showed little or no response to a change in the oxygen concentration. From this it has been concluded that some mechanism of inorganic carbon acquisition was operating.

This resulted in an internal CO_2 concentration that was sufficient to suppress the oxygenase activity of the primary carboxylase. The apparent high affinity of the uptake process, higher than the apparent $K_m(CO_2)$ for RuBPco is indicative of the activity of an inorganic carbon concentrating mechanism.

The relationship between the rate of photosynthesis and the PAR (the light intensity-response curve) shows the characteristics that are determined by the light dependent components of the photosynthetic system. The V_{max} is a measure of the maximum capacity of the system to utilize PAR. The initial slope or light limited part of the curve can be used to calculate the quantum ratio or efficiency of the process. Physiological parameters such as the light compensation and light saturation are also defined by the response.

Marine macroalgae are shade plants with relatively low light saturation points and correspondingly low light compensation points (Reiskind, Seamon and Bowes 1989). The characteristics of the light response have been shown to vary on a seasonal basis and in relation to periods of exposure (King and Shramm 1976; Oates and Murray 1986). These physiological parameters are dependent on the arrangement of the light harvesting apparatus. The concentration of total pigment, and accessory pigment/chlorophyll a ratio found in the photosynthetic units is related to light absorption (Dring 1981). A shift in the light response curve with an increase in the light saturation point and decrease in the initial slope was measured in *Fucus serratus* grown under high CO_2. This suggests that suppression of the carbon concentrating mechanism has reduced the photosynthetic efficiency of this species (Johnston and Raven 1990). The ability to concentrate inorganic carbon may alleviate photoinhibiton and subsequent photosynthetic damage, under conditions where the inorganic carbon supply would otherwise be insufficient to maintain adequate rates of light energy utilization.

This section aims to analyse the general photosynthetic characteristics of *Porphyra umbilicalis* and *Ulva lactuca* and to assess the significance of these parameters in terms of the possible mechanisms of inorganic carbon uptake.

Experiments were carried out with the two species to measure and compare:

1. The substrate concentration-response of photosynthesis in air and seawater for both winter and summer populations; values for the V_{max} and $K_{0.5}$ calculated from the Hill-Whittingham or Michaelis-Menten equations.
2. The effect of O_2 on the concentration-response of photosynthesis in air and seawater.
3. The light intensity-response of photosynthesis in air and seawater for both the summer and winter populations; values for the light utilization and photosynthetic efficiency.

MATERIALS AND METHODS

Plant material.

Porphyra umbilicalis and *Ulva lactuca* were collected from St. Mary's Island, North Tyneside at low tide. The material was maintained in a cold room in tanks of filtered aerated seawater for a maximum of 7 days, at 8^{oC} and 100 μmol photon m^{-2} s^{-1} PAR over a 12 hour photoperiod. Preliminary measurements showed no decline in the photosynthetic capacity of the plants over this period.

Measurement of photosynthetic O_2 evolution in seawater.

Rates of photosynthetic oxygen evolution were measured polarographically with a water-jacketed oxygen electrode (Hansatech DW1), maintained at 12^{oC} by a thermostatically

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Measurement of photosynthetic O_2 evolution in seawater.

Rates of photosynthetic oxygen evolution were measured polarographically with a water-jacketed oxygen electrode (Hansatech DW1), maintained at 12^{oC} by a thermostatically controlled water bath. Saturating light, 500 μmol photon m^{-2} s^{-1} photosynthetically active radiation (350-700 nm PAR), was supplied by a high intensity light source (Hansatech LS2). Strips of plant material attached to stainless steel grids were held stationary within the electrode chamber, perpendicular to the light source, during measurements. CO_2-free seawater was prepared by acidifying filtered seawater with HCl to a pH below 3.0, before sparging for 5 hours with CO_2-free air (passed through a carbasorb column). The pH of the seawater was then adjusted by the addition of 50 mol m^{-3} (N-(2-Hydroxyethy)piperazine-N'-3-propanesulfonic acid) EPPS (pH 8.0), and freshly prepared NaOH solution. The appropriate inorganic carbon concentrations were obtained by the addition of successive aliquots of $NaHCO_3$ solution to CO_2-free seawater in the chamber.

Measurements of photosynthetic CO_2 assimilation in air.

Rates of photosynthetic CO_2 assimilation were measured using an Infra Red Gas Analyser (ADC Type 225 mk 3).The assimilation chamber was maintained at 12^{oC} using a thermostatically controlled water bath. With the IRGA in differential mode the rate of photosynthesis, calculated from the depletion of the CO_2 in the gas stream leaving the

chamber, was measured. CO_2 concentrations between 0 and 100 mmol m^{-3} were supplied by a mass flow controller (Brooks Instruments) and CO_2 diluted with O_2 or N_2 (BOC). Discs of plant material attached to stainless steel grids were held perpendicular to the high intensity light source, at 500 μmol photon m^{-2} s^{-1} PAR and rinsed in seawater following each reading. CO_2 compensation points were calculated from regression of the rates of photosynthesis at CO_2 concentations between 0 and 2.5 mmol m^{-3}.

The response of photosynthesis to inorganic carbon

The response of photosynthesis to inorganic carbon concentration was measured in seawater and air at 500 μmol photon m^{-2} s^{-1} PAR and concentrations of 0 to 100 mmol m^{-3} CO_2 and 0 to 10 mol m^{-3} HCO_3^- respectively. The response of photosynthesis to oxygen concentration was determined by measuring the concentration response in seawater sparged with N_2, air or O_2 to give oxygen concentrations of 1%, 21% and 42% respectively.

The kinetic analysis of the concentration-response curve

The relationship between concentration of substrate and photosynthesis can be defined in terms of the maximum rate of response (V_{max}) and the concentration at which this response reach half the maximum rate ($K_{0.5}$). For the photosynthetic response measured in air the curve for the rate of CO_2 assimilation in relation to CO_2 concentration closely resembled Michaelis-Menten kinetics and was analysed using the non-linear regression programme PEST (Paterson, Weyers and A'Brook 1988). The relationship between O_2 evolution in seawater is more complex and normally limited by the substrate supply. In this case the response curve is analysed using the Hill-Whittingham equation which takes into account the available substrate concentration by incorporating a diffusion coefficient into the equation (Hill and Whittingham 1955; MacFarlane and Raven 1985).

The response of photosynthesis to PAR

The response of photosynthesis to PAR was measured in seawater and air at saturating concentrations of inorganic carbon and PAR levels of 0 to 2000 μmol photon m^{-2} s^{-1}. The relationship between PAR and photosynthesis was determined from the light intensity-reponse curve. Values for light compensation and photosynthetic efficiency (Quantum Ratio) were obtain using regression analysis of the light limited response. The light saturated portion of the curve was used to define the light saturation point and the maximum capacity (V_{max}) of the system.

The extraction and determination of chlorophyll content.

The quantitative determination of the chlorophyll a content of *P.umbilicalis* and the chlorophyll a and b content of *U.lactuca* was determined using the methods described by Shoaf and Liam (1976). Discs of plant material of 100mg fresh weight were incubated in 7.0 ml of Dimethyl sulfoxide at 60^{oC} for 15 minutes. The extract was made up to 10.0 ml and the absorbance determined at 663 nm (chlorophyll a) and 645 nm (chlorophyll b). Chlorophyll concentrations were calculated using the equation for extraction in 90% acetone (Arnon 1949).

RESULTS

Inorganic carbon concentration-response

For intertidal marine macroalgae both CO_2 and HCO_3^- are possible sources of inorganic carbon for photosynthesis. Kinetic analysis of the HCO_3^- response curves, using the Hill-Whittingham equation, takes into account the effect of diffusion resistance on inorganic carbon supply. The CO_2 concentration response can be adequately described by the Michaelis-Menten kinetics. The values obtained from the concentration-response of these two intertidal species are shown in Table 1.

Oxygen evolution in *Porphyra umbilicalis* was only saturated at 3.0 mol m^{-3} HCO_3^-, above the concentration found in seawater. For CO_2 assimilation the air equilibrium concentration of 15 mmol m^{-3} was substantially below the 60 mmol m^{-3} required for saturation (Figs 1 & 2). Under natural concentrations the submersed rate of net photosynthesis would be around 2.5 fold greater than that during exposure. However, for *P.umbilicalis* the value for V_{max} in air (3.62 μmol O_2 m^{-2} s^{-1}) was greater than that in seawater (3.02 μmol CO_2 m^{-2} s^{-1}), with substrate $K_{0.5}$ values of 32 mmol m^{-3} and 572 mmol m^{-3} respectively (Table 1 & 2).

In constrast, the rate of photosynthesis in *Ulva lactuca* was saturated with respect to the HCO_3^- concentration in seawater but at a lower rate than *P.umbilicalis* (1.80 μmol O_2 m^{-2} s^{-1}). The capacity was greater than that achieved in air at the equilibrium CO_2 concentration (75 μmol CO_2 m^{-2} s^{-1}) which is saturated at a concentration of above 60 mmol m^{-3} CO_2 (Figs 3 & 4). Kinetic analysis of the responses gave a similar value of V_{max} in air as in seawater (1.78 μmol O_2 m^{-2} s^{-1}) with corresponding values for the substrate $K_{0.5}$ of 21 mmol m^{-3} and 572 mmol m^{-3} respectively (Table 1 & 2).

For both species the concentration-response curves showed a marked seasonal variation. For *P.umbilicalis* in summer the photosynthetic rate was reduced at the seawater HCO_3^- concentration, with a V_{max} of 2.15 μmol O_2 m^{-2} s^{-1}

TABLE 1. The kinetic parameters describing the relationship between photosynthesis and HCO_3^- concentration for *P.umbilicalis* and *U.lactuca*. Values obtained for the winter and summer populations, using the least squares programme, represent the mean of five replicates.

	V_{max} μmol O_2 m^{-2} s^{-1}	$K_{0\cdot5}$(TIC) mol m^{-3}	Pu 10^{-6} m s^{-1}
P.umbilicalis			
Winter	3.07	572	1.39
Summer	2.15	427	2.41
U.lactuca			
Winter	1.78	595	1.6
Summer	1.12	540	0.6

TABLE 2. The kinetic parameters describing the relationship between photosynthesis and CO_2 concentration for *P.umbilicalis* and *U.lactuca*. Values obtained for the winter and summer populations, using the non-linear regession programme, represent the mean of five replicates.

	V_{max} μmol CO_2 m^{-2} s^{-1}	$K_{0\cdot5}(CO_2)$ mmol m^{-3}
P.umbilicalis		
Winter	3.62	35
Summer	1.92	8
U.lactuca		
Winter	1.79	21
Summer	0.78	5

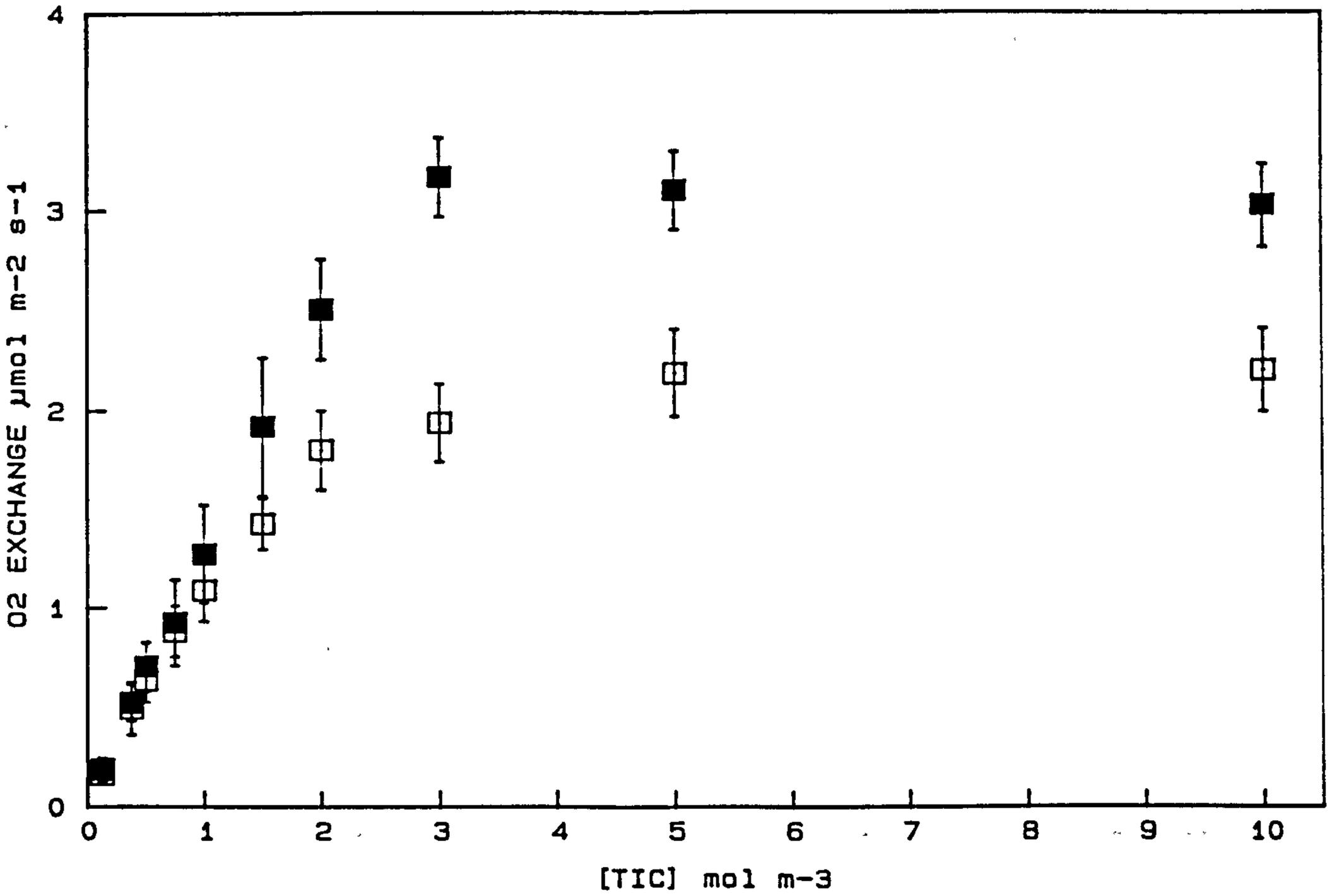

Figure 1. Rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of HCO_3^- concentration. Data for the summer (□) and winter (■) population represents the mean of five replicates ± SE.

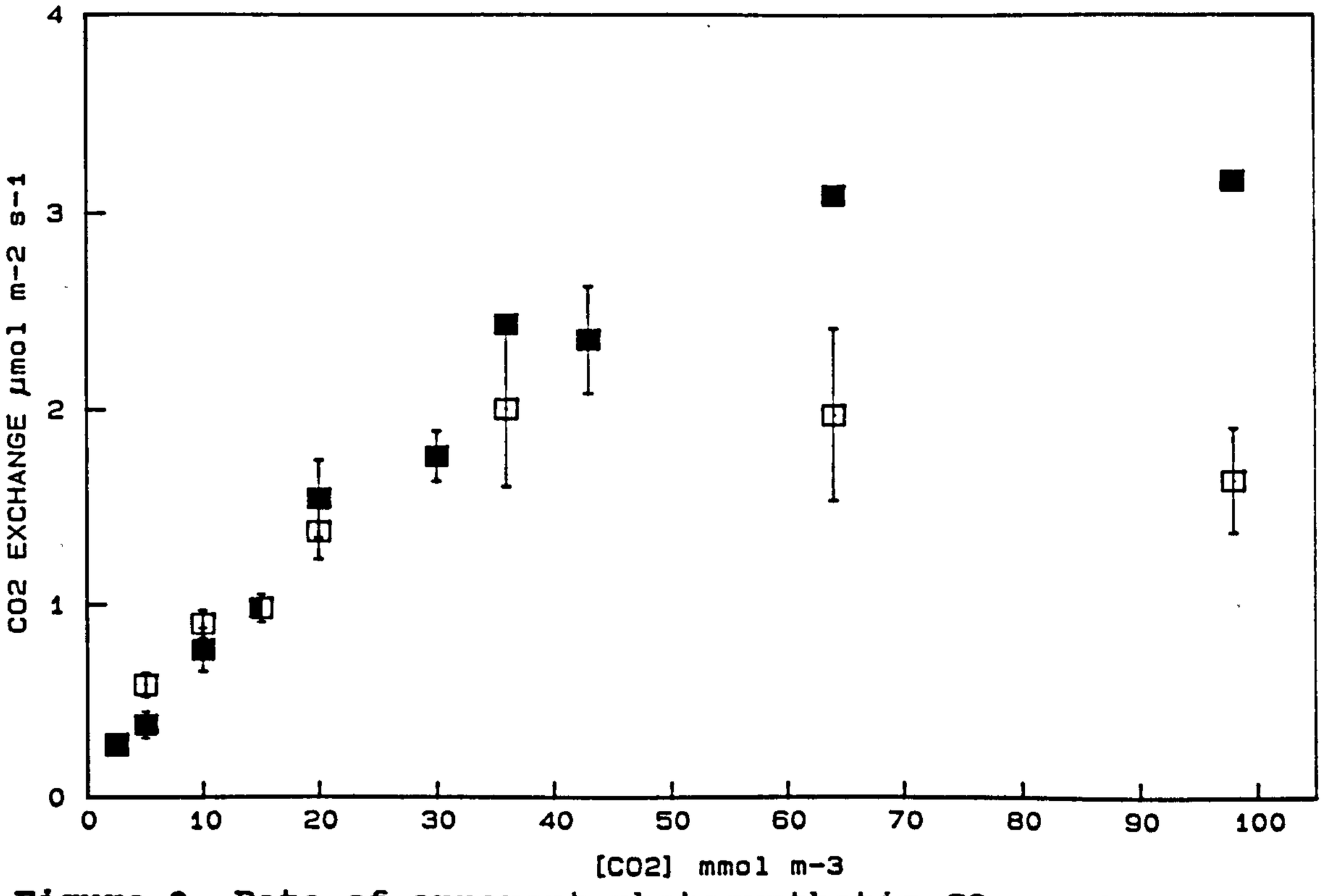

Figure 2. Rate of apparent photosynthetic CO_2 exchange by *P.umbilicalis* in air as a function of CO_2 concentration. Data for the summer (□) and winter (■) population represents the mean of five replicates ± SE.

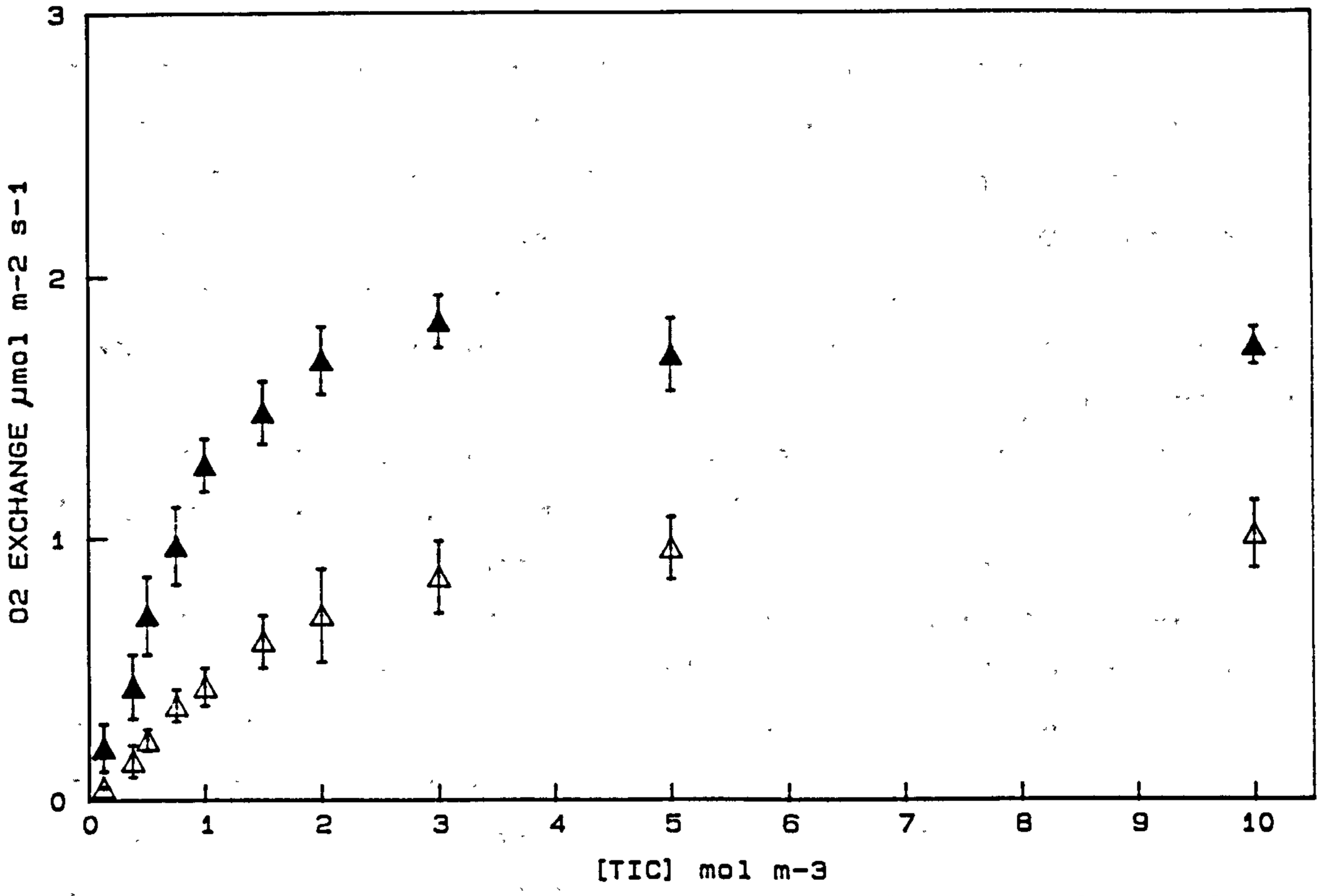

Figure 3. Rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of HCO_3^- concentration. Data for the summer (Δ) and winter (▲) population represents the mean of five replicates ± SE.

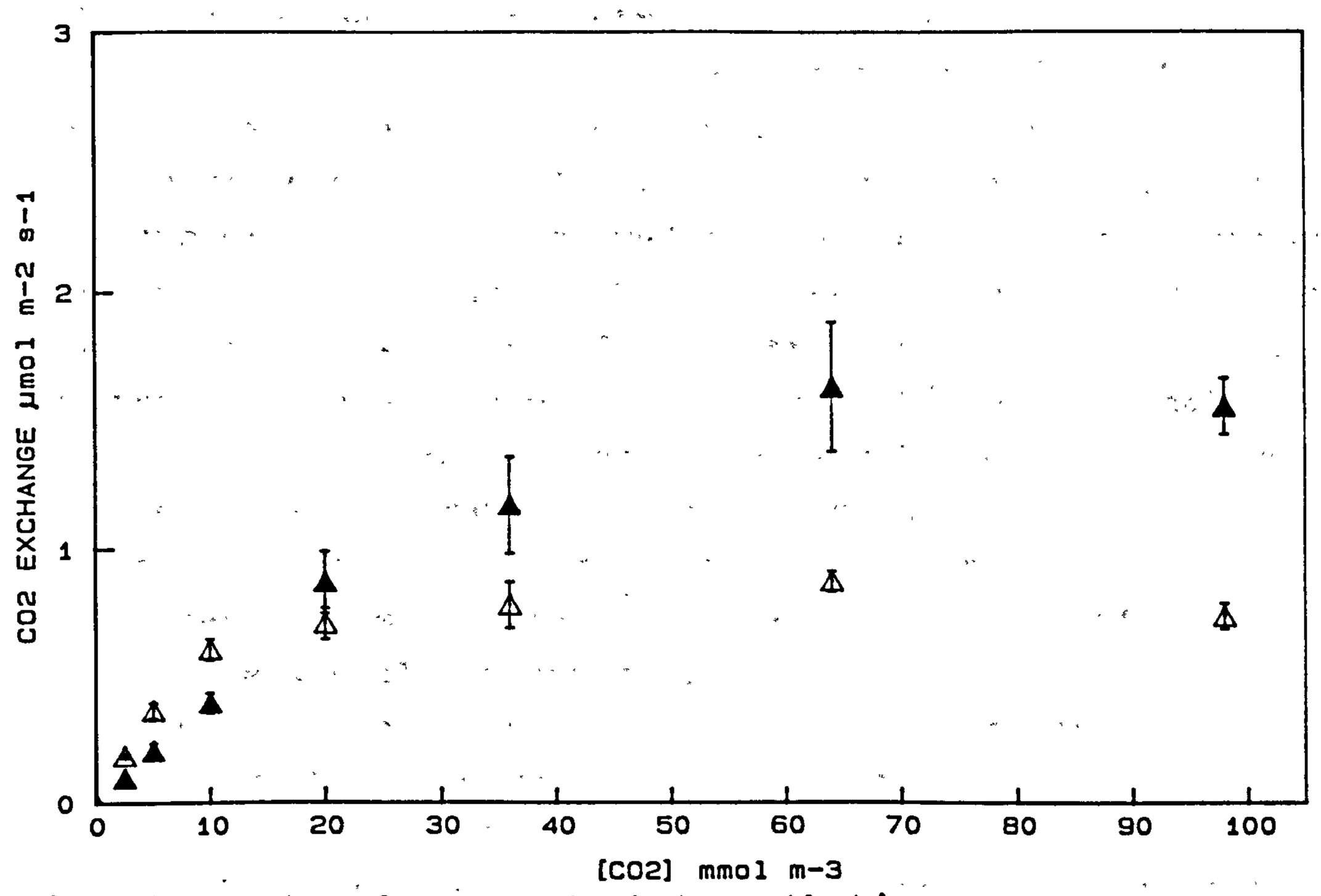

Figure 4. Rate of apparent photosynthetic CO_2 exchange by *U.lactuca* in air as a function of CO_2 concentration. Data for the summer (Δ) and winter (▲) population represents the mean of five replicates ± SE.

(Fig 1). However, saturation occurred at 2.5 mol m^{-3} HCO_3^- as the apparent affinity for inorganic carbon was increased ($K_{0\cdot5}$ (TIC) of 427 mol m^{-3}). The same characteristics could be seen in the response to CO_2, the greatest change being the increase in CO_2 affinity with a $K_{0\cdot5}(CO_2)$ of 8 mmol m^{-3} (Table 2). This gave rise to a better than expected rate of net photosynthesis at the air equilibrium CO_2 concentration in comparison to the winter population, even though the V_{max} was lower (1.92 μmol CO_2 m^{-2} s^{-1}; Fig 2).

As with *P.umbilicalis* the summer population of *U.lactuca* showed a lower photosynthetic capacity overall (Figs 3 & 4). In seawater both the V_{max} and net rate at the seawater HCO_3^- concentration were lower. There was no change in the inorganic carbon affinity or in the concentration at saturation (Table 1). When exposed the rate achieved at the air equilibration CO_2 concentration was maintained by the increase in affinity for CO_2 ($K_{0\cdot5}$ (CO_2) 5.2 mmol m^{-3}) even though the V_{max} was lower than in the winter population (Table 2).

Effect of O_2 on the concentration-response

The photosynthetic response in seawater of the two species was determined over a range of dissolved O_2 concentrations (Figs 5 & 6). Measurements carried out on the summer populations showed that as for the concentration response curves (Figs 1 & 3) both species were saturated at the natural seawater HCO_3^- concentrations of 2.0 mol m^{-3}, so that the rates achieved at 5.0 mol m^{-3} were maximal. In addition the control responses for *P.umbilicalis*, measured at the air equilibration concentration of 21% O_2, had a higher affinity for inorganic carbon (107 mmol m^{-3} TIC) than that determined previously, although the diffusion resistance was also greater (Table 3).

Apparent photosynthesis in *P.umbilicalis* was insensitive to O_2 concentrations of between 1% and 42% with V_{max} values of 2.18 and 2.14 μmol O_2 m^{-2} s^{-1} respectively. These rates were not significantly higher than that of the control (Figs 5 & 6). In *U.lactuca* oxgyen sensitivity was not evident from the photosynthetic capacities (V_{max} values

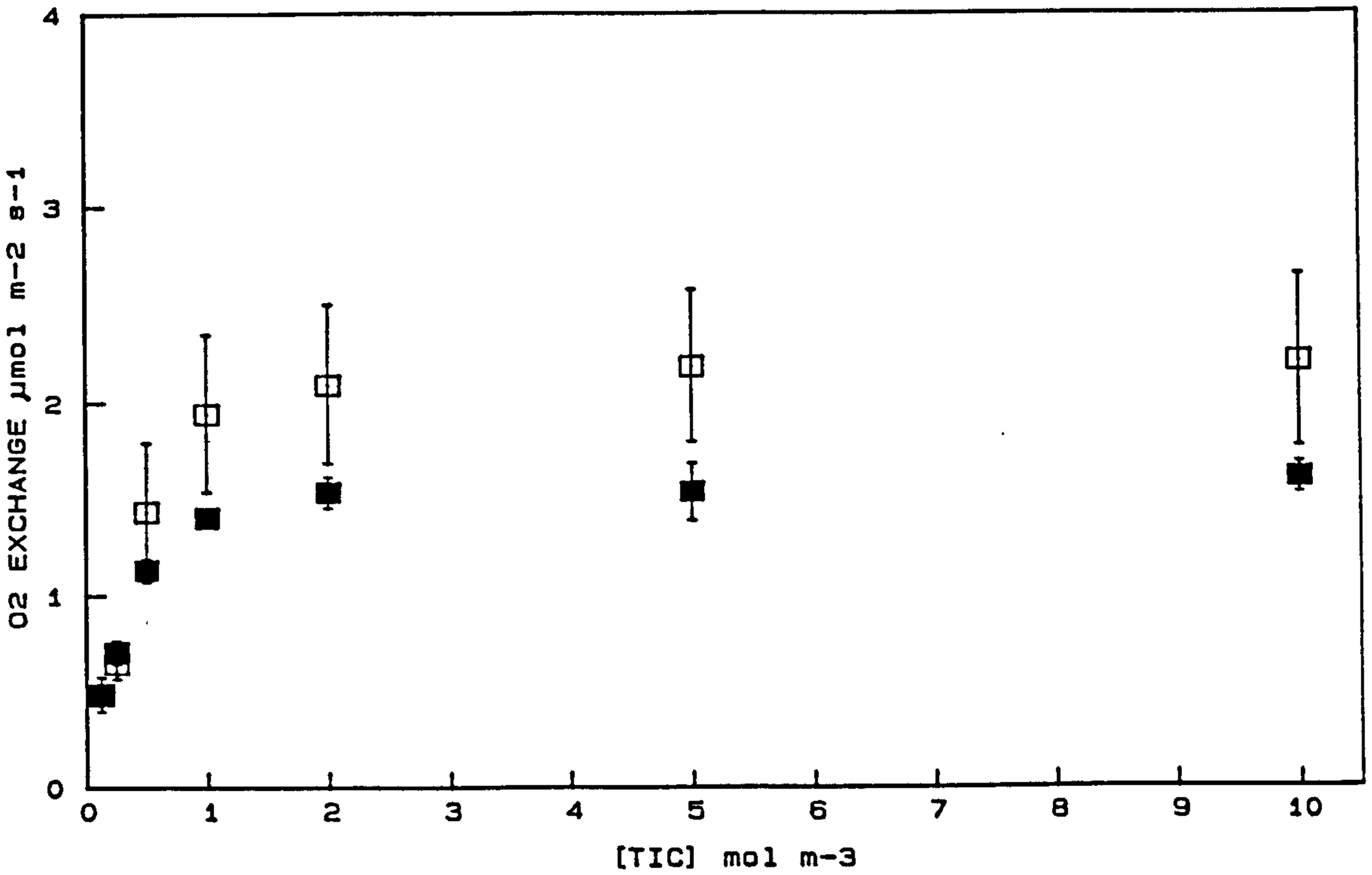

Figure 5. Rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of HCO_3^- concentration at pH 8.0. Response measured at 1% (□) and 21% (■) air equilibrium O_2 concentration represents the mean ± SE of three replicates.

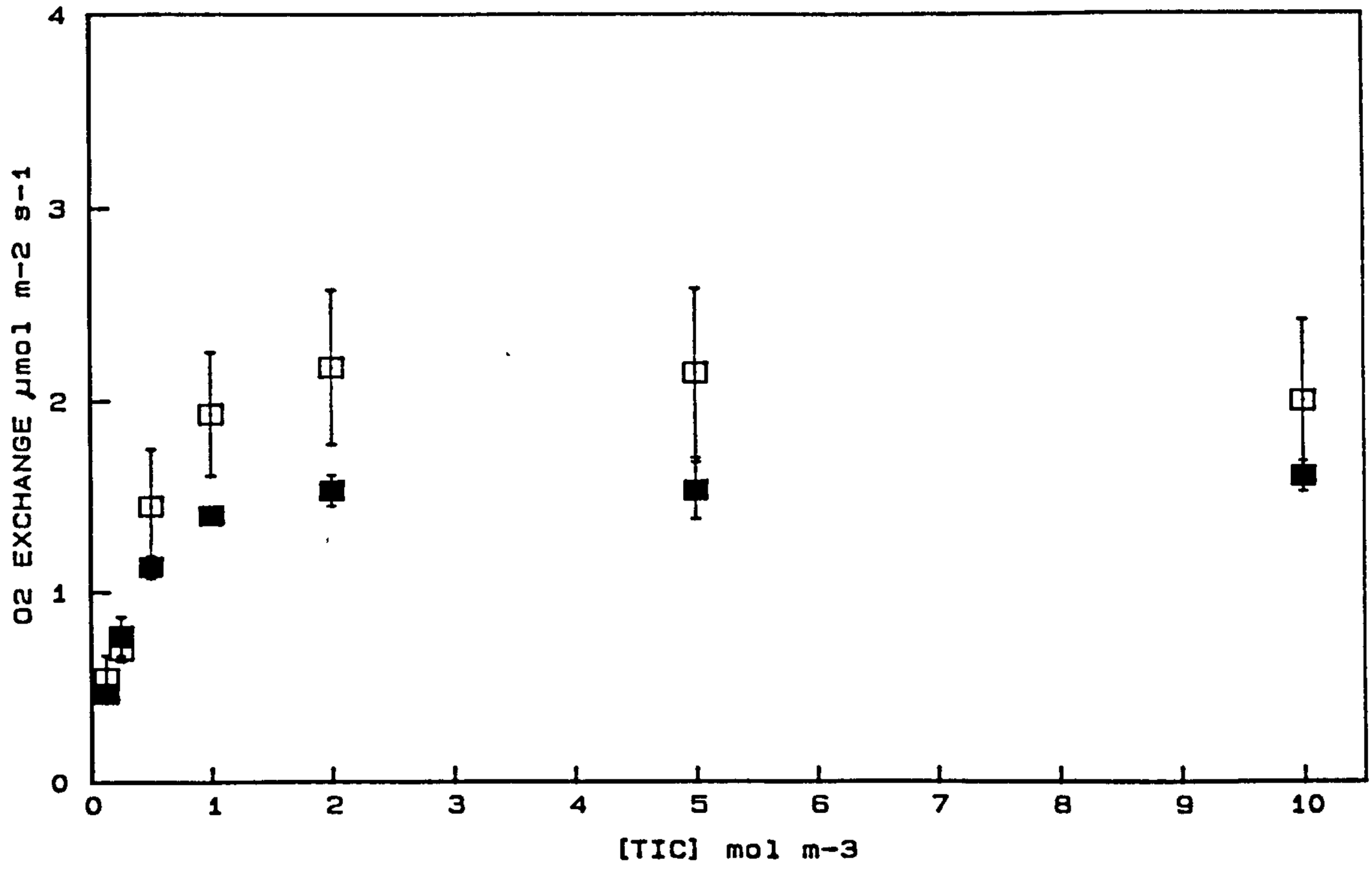

Figure 6. Rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of HCO_3^- concentration at pH 8.0. Response measured at 21% (■) and 42% (□) air equilibrium O_2 concentration represents the mean ± SE of three replicates.

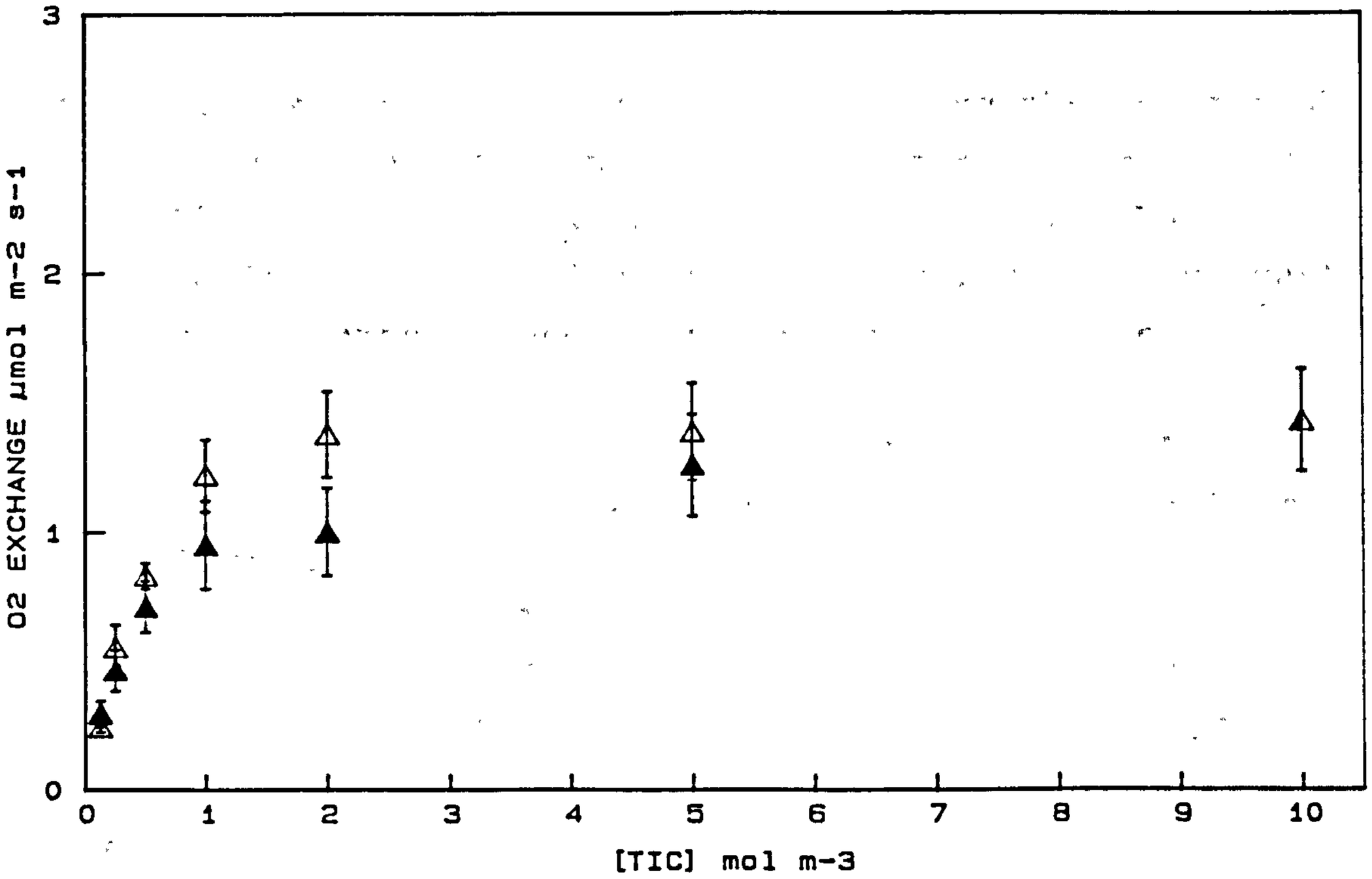

Figure 7. Rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of HCO_3^- concentration at pH 8.0. Response measured at 1% (Δ) and 21% (▲) air equilibrium O_2 concentration represents the mean ± SE of three replicates.

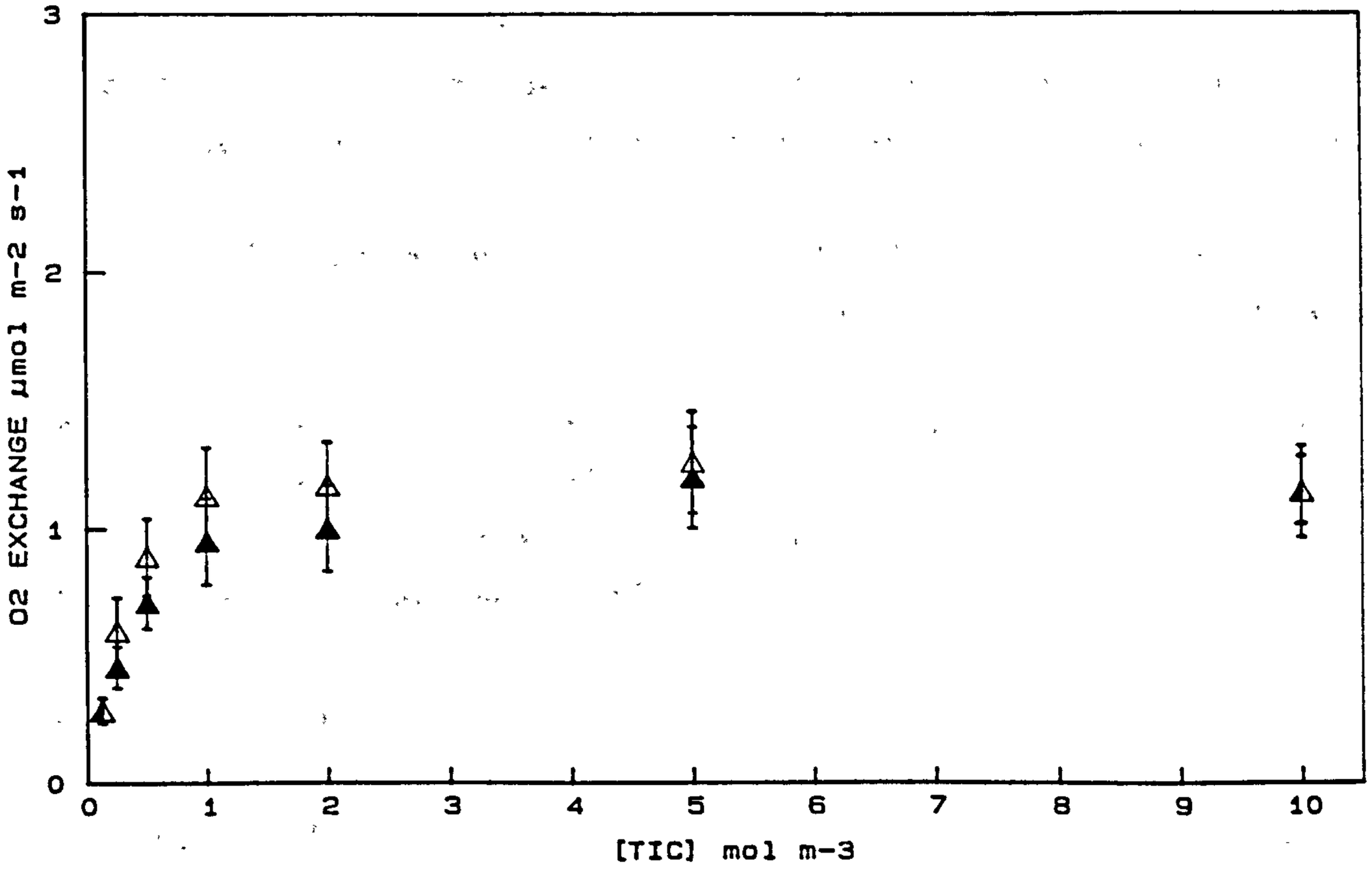

Figure 8. Rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of HCO_3^- concentration at pH 8.0. Response measured at 21% (▲) and 42% (Δ) air equilibrium O_2 concentration represents the mean ± SE of three replicates.

Table 3. The kinetic parameters describing the relationship between photosynthesis and HCO_3^- concentration measured under 1%, 21% and 42% O_2 in seawater at pH 8.0. Values obtained for *P.umbilicalis* and *U.lactuca*, using the least squares programme, represents the mean of five replicates.

$[O_2]$	V_{max} μmol O_2 m^{-2} s^{-1}	$K_{0.5}$(TIC) mol m^{-3}	Pu 10^{-6} m s^{-1}
P.umbilicalis			
1%	2.18	119	3.8
21%	1.61	107	4.8
42%	2.14	137	2.7
U.lactuca			
1%	1.45	106	2.5
21%	1.27	219	1.9
42%	1.19	499	2.8

TABLE 4. The characteristics of the relationship between photosynthesis and light intensity in *P.umbilicalis* and *U.lactuca* in air and seawater. Data for the winter and summer populations represents the mean of five replicates.

Response in seawater	*P.umbilicalis*		*U.lactuca*	
	Winter	Summer	Winter	Summer
LCP (μmol photon m^{-2} s^{-1})	0.3	3.6	3.0	5.5
LSP (μmol photon m^{-2} s^{-1})	200	200	100	100
P_{max} (μmol O_2 m^{-2} s^{-1})	3.25	2.34	1.85	1.17
AQR (μmol photon μmol O_2^{-1})	42	60	61	52
Response in air				
LCP (μmol photon m^{-2} s^{-1})	1.7	6.6	7.9	8.7
LSP (μmol photon m^{-2} s^{-1})	200	200	100	100
P_{max} (μmol CO_2 m^{-2} s^{-1})	1.65	1.30	0.95	0.56
AQR (μmol photon μmol O_2^{-1})	47	62	122	100

of between 1.45 and 1.19 μmol O_2 m^{-2} s^{-1}(Figs 7 & 8). In contrast, the affinity for inorganic carbon showed a 4 fold decrease with $K_{0.5}$(TIC) values of 106, 219, and 499 mol m^{-3} at 1, 21, 42% O_2 respectively (Table 3).

Light intensity-response

The response of photosynthesis to PAR was measured under saturating HCO_3^- and CO_2 concentrations (Table 4). *P.umbilicalis* showed a higher maximum rate of light saturated photosynthesis in seawater (3.28 μmol O_2 m^{-2} s^{-1}) than in air (1.72 μmol O_2 m^{-2} s^{-1}; Figs 9 & 10). In seawater light saturation occurred at 200 μmol photon m^{-2} s^{-1} with a light compensation point of 0.3 μmol photon m^{-2} s^{-1} (Fig 9). These values were 200 and 1.7 μmol photons m^{-2} s^{-1} respectively when measured in air (Fig 10). The photosynthetic efficiency of light utilization is expressed as the apparent quantum ratio. In *P.umbilicalis* there was little difference in the ability to utilize the light energy available under submerged or exposed conditions (42 and 47 mol photon mol O_2^{-1}; Figs 11 & 12). The summer population again had a decreased light saturated rate of 2.34 μmol O_2 m^{-2} s^{-1} in seawater and 1.30 μmol O_2 m^{-2} s^{-1} in air (Figs 9 & 10). Under both conditions the quantum requirement increased to 60 mol photons mol O_2^{-1} (Figs 11 & 12). Light compensation points were higher in the summer population although the light saturation points were the same in seawater and in air (Table 4).

For *U.lactuca* the light saturated rate of photosynthesis in air of 1.0 μmol O_2 m^{-2} s^{-1} was around half the rate of 1.90 μmol O_2 m^{-2} s^{-1} in seawater (Figs 13 & 14). The light compensation point of 3 μmol photons m^{-2} s^{-1} in seawater, compared to 7 μmol photons m^{-2} s^{-1} in air, while the values for light saturation were similar. The decrease in the quantum ratio (71 and 122.2 mol photons mol O_2^{-1} respectively) indicates that photosynthetic efficiency was greater during submersion (Figs 15 & 16). The same trend was evident in the summer population of *U.lactuca*, with light saturated rates of 1.27 μmol O_2 m^{-2} s^{-1} in seawater and 0.57 μmol O_2 m^{-2} s^{-1} in air (Figs 13 & 14).

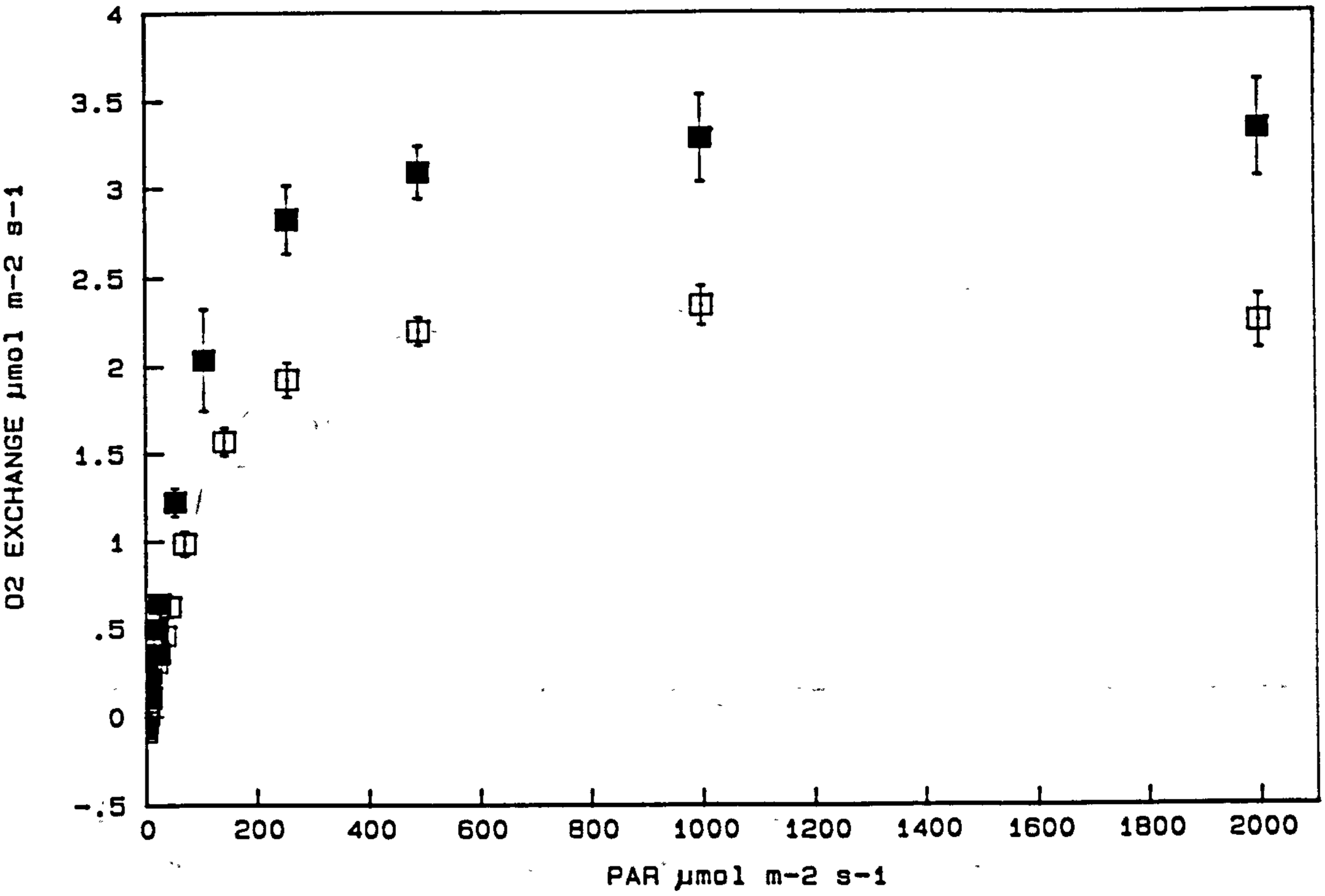

Figure 9. Rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of light intensity (PAR). Data for the winter (■) and summer (□) population represents the mean ± SE of five replicates.

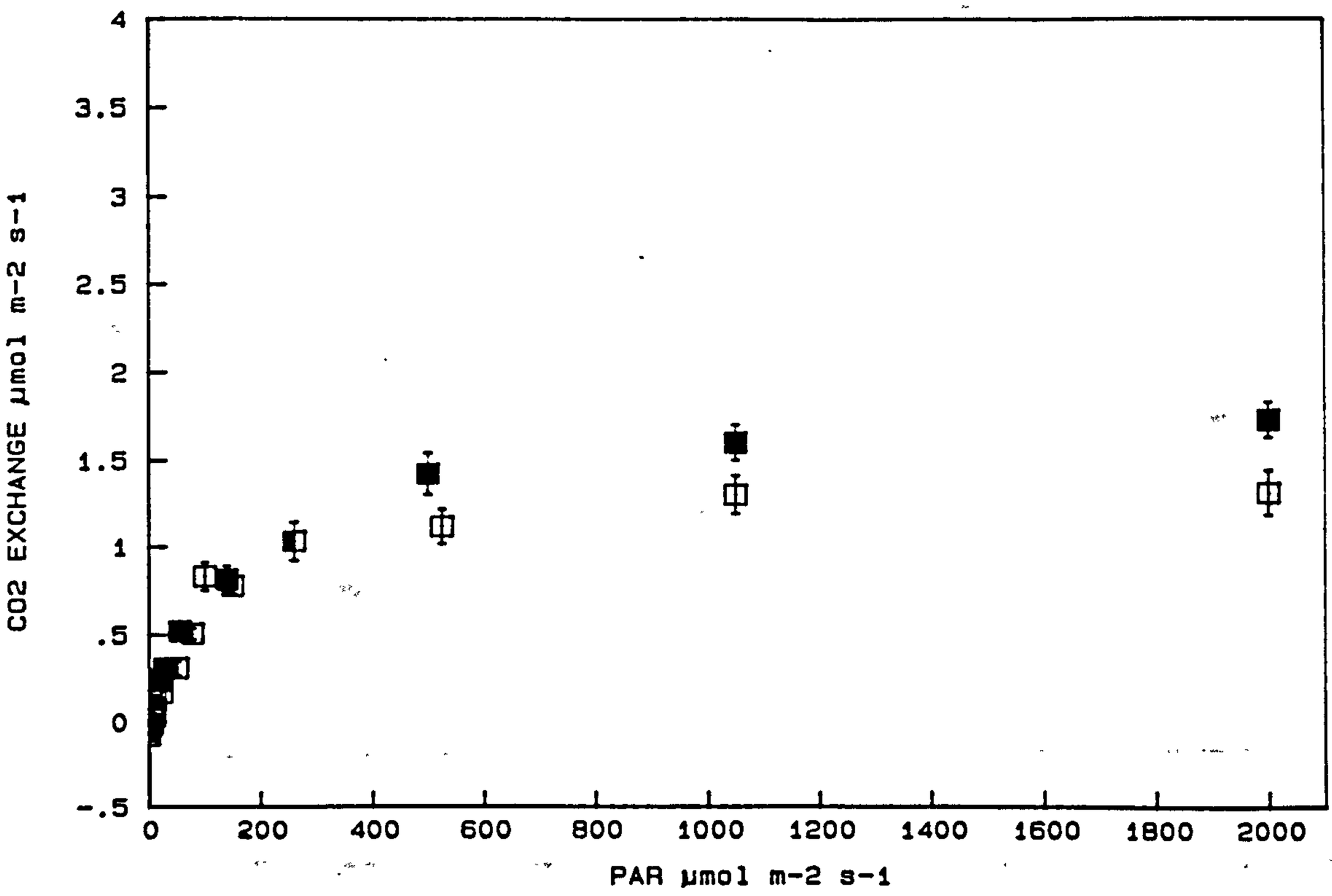

Figure 10. Rate of apparent photosynthetic CO_2 exchange by *P.umbilicalis* in air as a function of light intensity (PAR). Data for the winter (■) and summer (□) population represents the mean ± SE of five replicates.

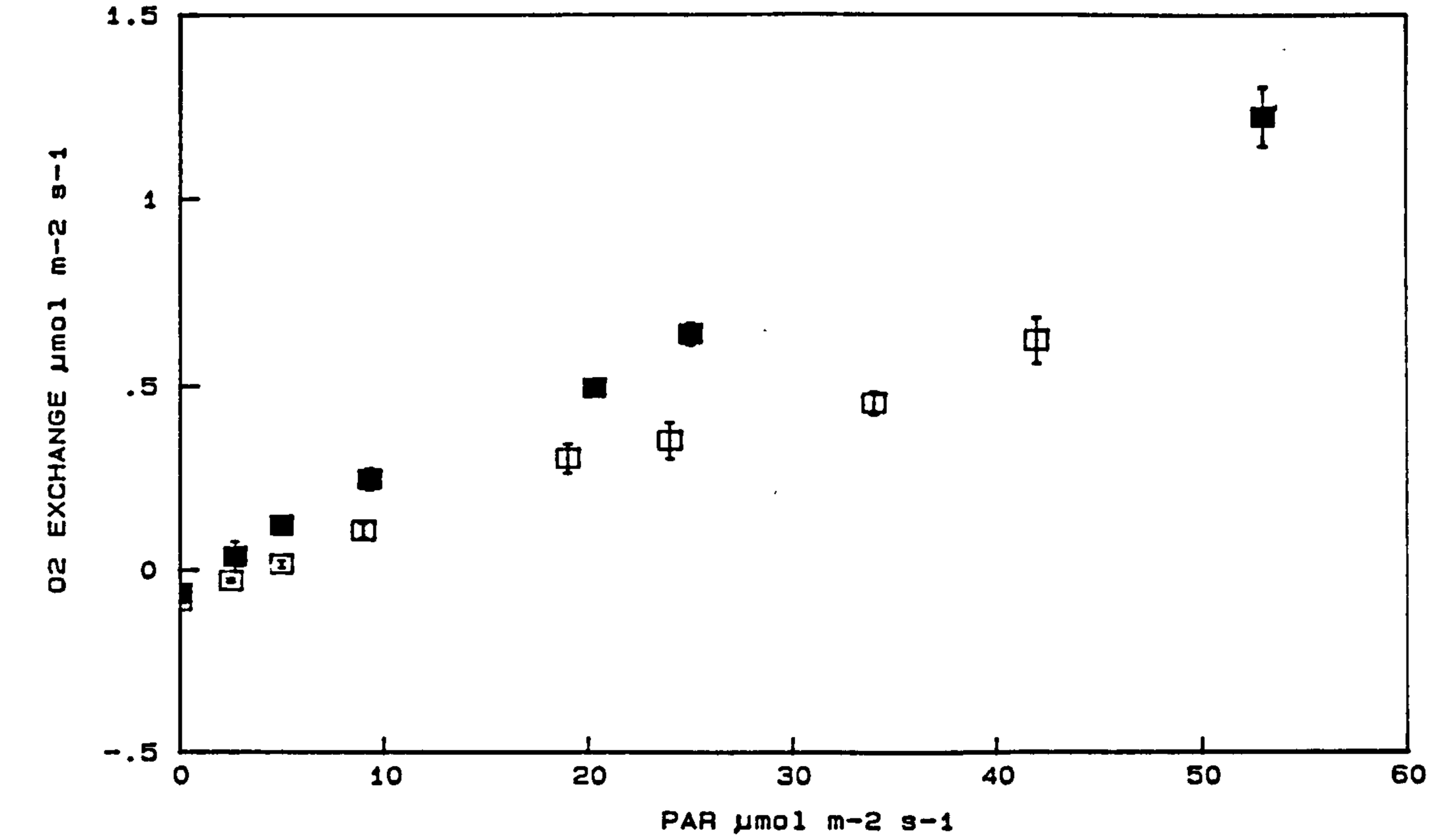

Figure 11. Rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of limiting PAR. Value for the light compensation point (LCP) and apparent quantum ratio (AQR) for the winter (■) and summer (□) population calculated from the mean ± SE of five replicates.

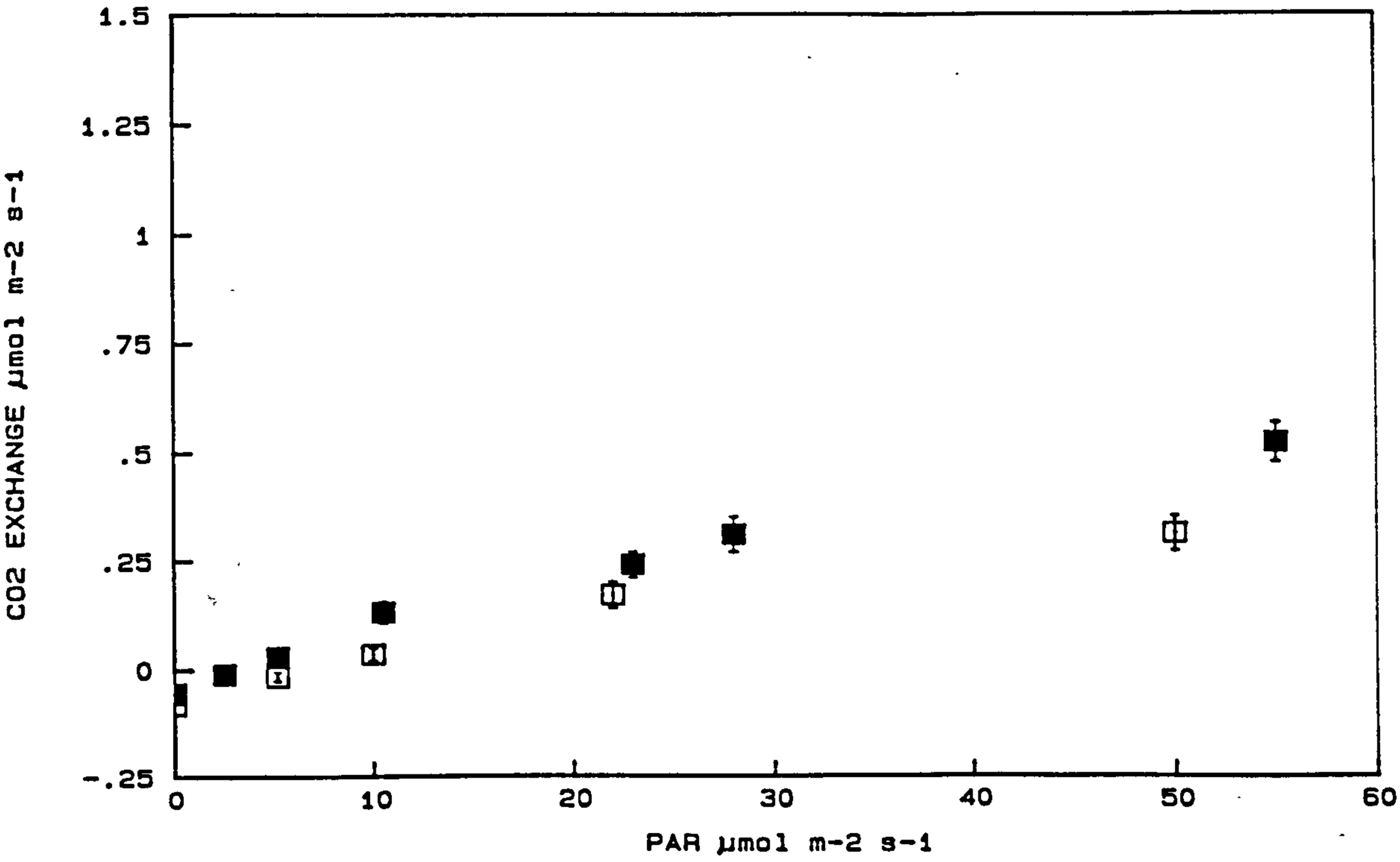

Figure 12. Rate of apparent photosynthetic CO_2 exchange by *P.umbilicalis* in air as a function of limiting PAR. Value for the light compensation point (LCP) and apparent quantum ratio (AQR) for the winter (■) and summer (□) population calculated from the mean ± SE of five replicates.

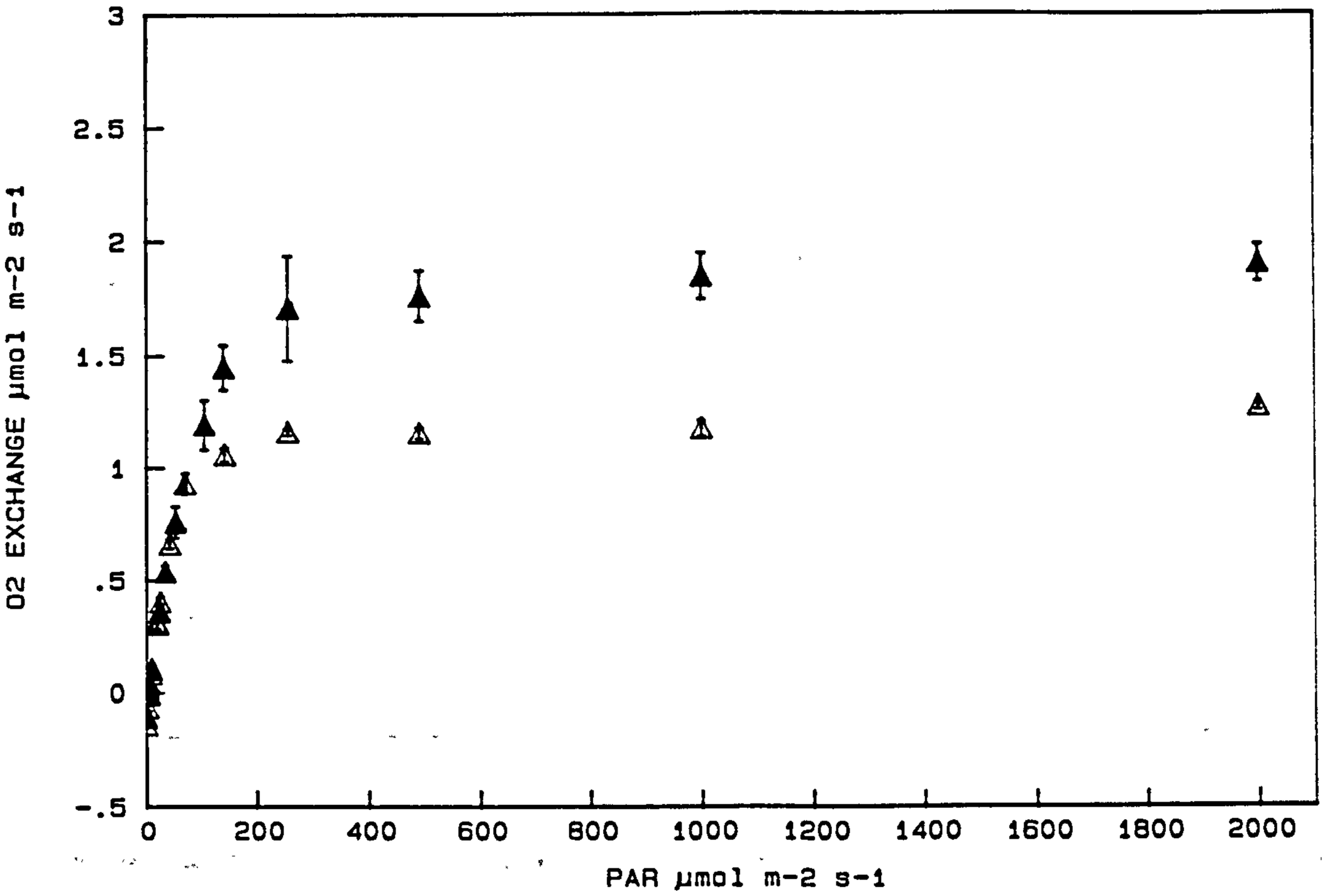

Figure 13. Rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of light intensity (PAR). Data for the winter (▲) and summer (Δ) population represents the mean ± SE of five replicates.

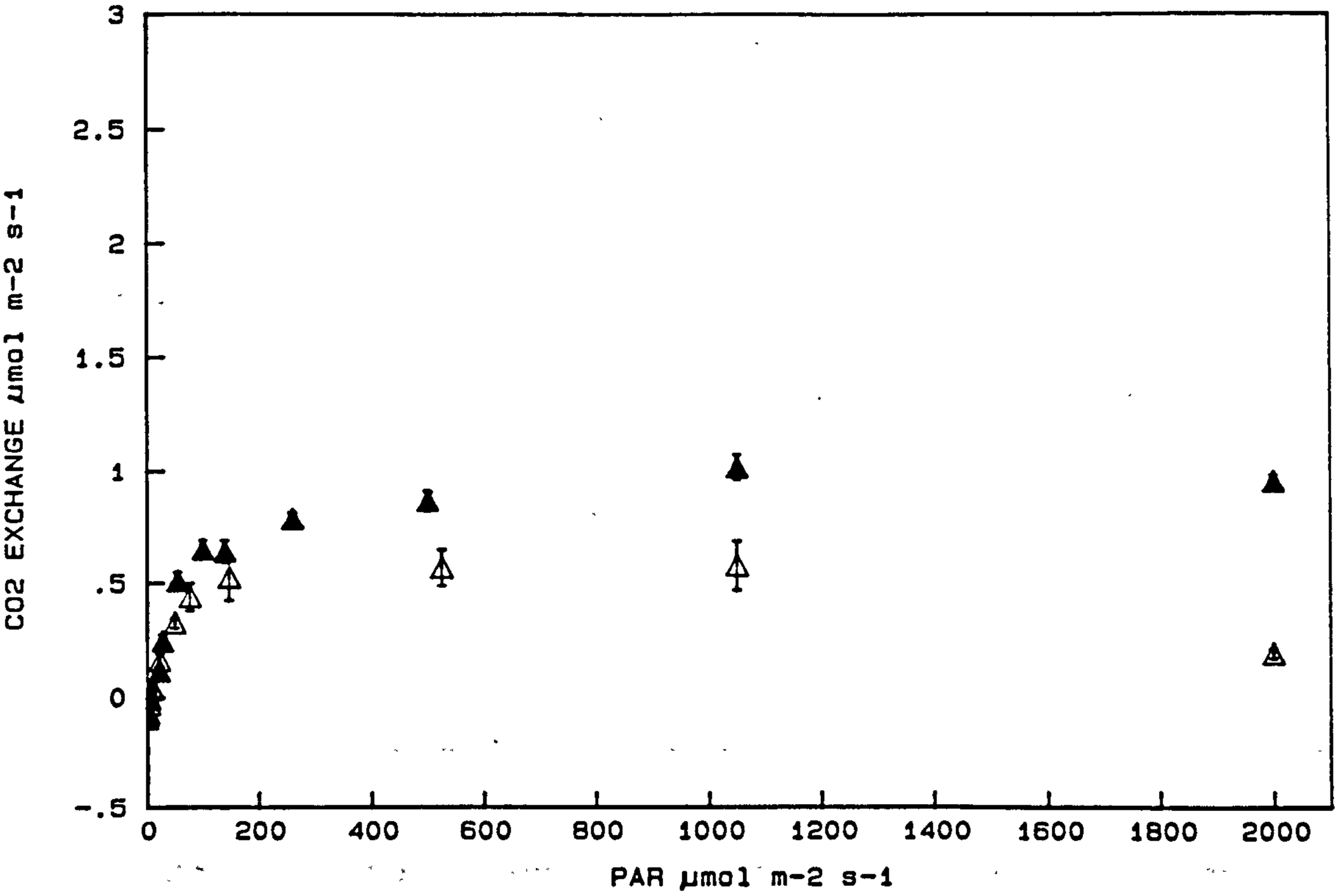

Figure 14. Rate of apparent photosynthetic CO_2 exchange by *U.lactuca* in air as a function of light intensity (PAR). Data for the winter (▲) and summer (Δ) population represents the mean ± SE of five replicates.

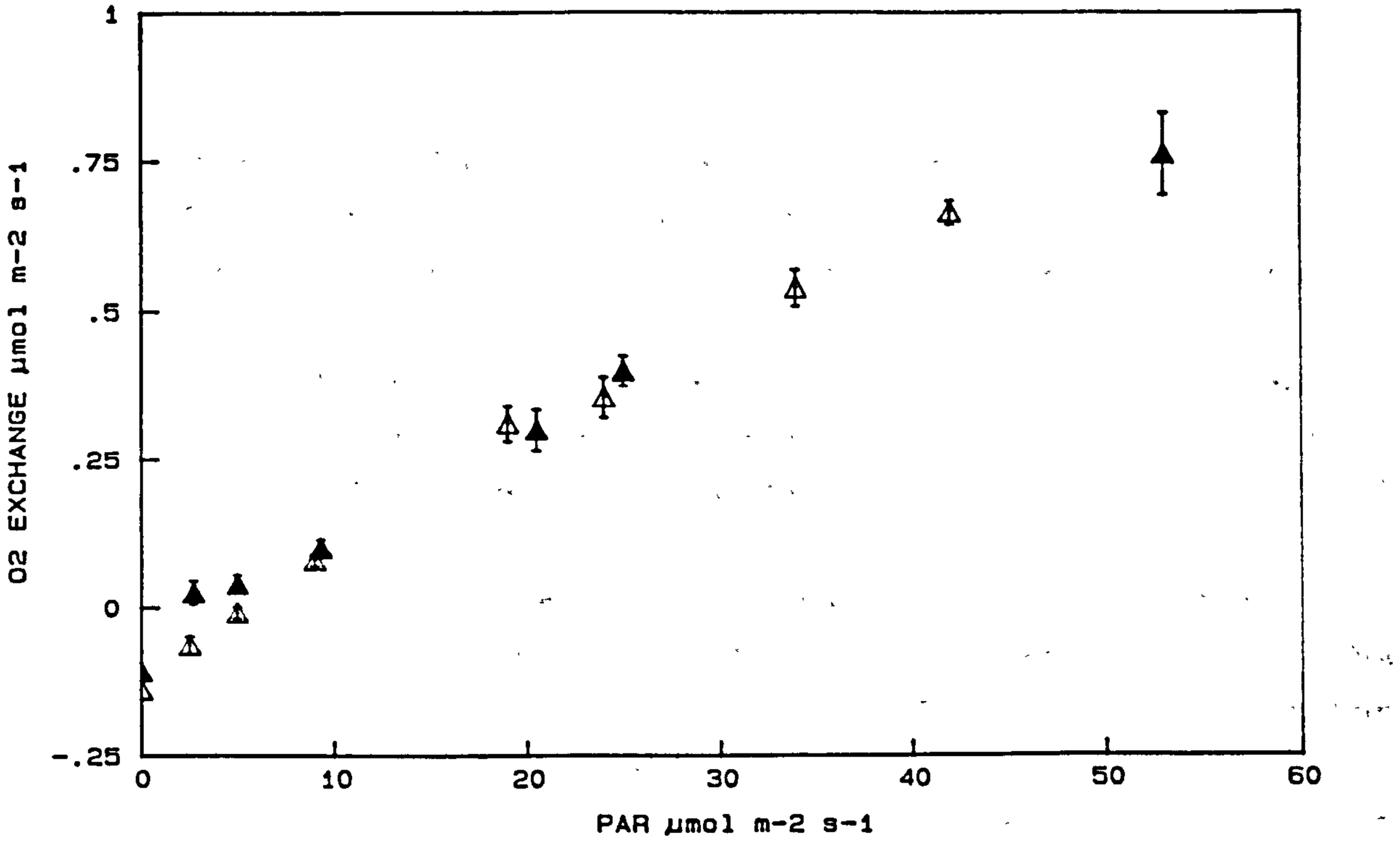

Figure 15. Rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of limiting PAR. Values for the light compensation point (LCP) and apparent quantum ratio (AQR) for the winter (▲) and summer (Δ) population calculated from the mean ± SE of five replicates.

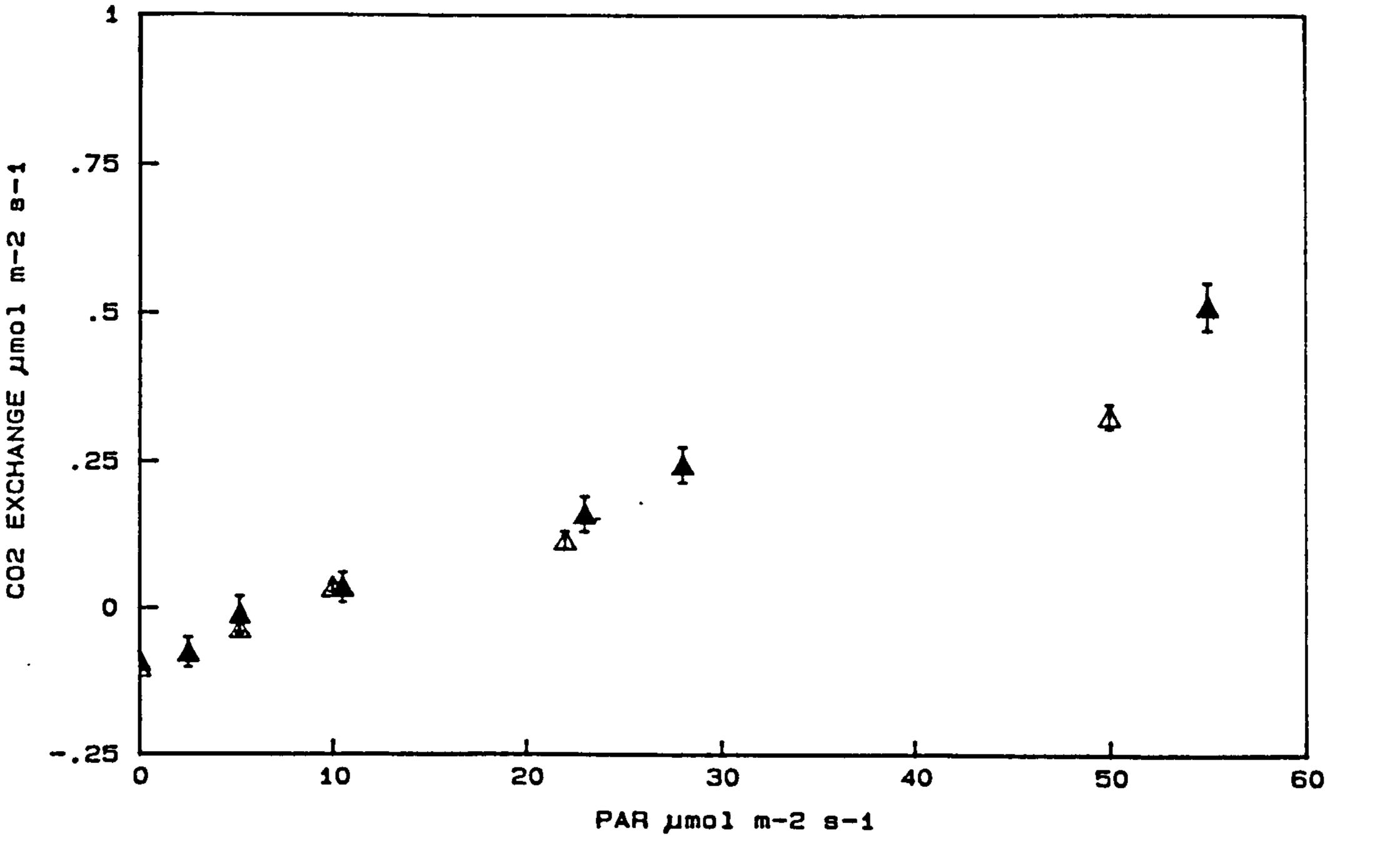

Figure 16. Rate of apparent photosynthetic CO_2 exchange by *U.lactuca* in air as a function of limiting PAR. Values for the light compensation point (LCP) and apparent quantum ratio (AQR) for the winter (▲) and summer (Δ) population calculated from the mean ± SE of five replicates.

Seasonal variation in chlorophyll content

The seasonal variation in the concentration of chlorophyll a and b with the ration of chlorophylls a:b is shown in Table 5.

Only chlorophyll a is present in *P.umbilicalis*. The highest concentration was present in December (1.29 μg mg fwt^{-1}) and this progressively decreased to 1.01 μg mg fwt^{-1} in April and 0.82 μg mg fwt^{-1} in July.

For *U.lactuca* the highest total pigment concentration and greatest ratio of chlorophyll a:b occured in April. The total concentration of 2.48 μg mg fwt^{-1} is greater than for *P.umbilicalis*, and the ratio of a:b was 1.82. In June the total concentration was only 1.62 μg mg fwt^{-1}, although the ratio of a:b was 1.89, indicating a decrease in chlorophyll a. During December the total concentration 1.12 μg mg fwt^{-1} with a ratio of 1.72 showed that the concentration of chlorophyll a had again increased.

Table 5. The seasonal variation in the concentration and ratio of chlorophyll a and chlorophyll b in *P.umbilicalis* and *U.lactuca*. The calculated values represent the mean (± SD) of five replicates.

	Chlorophyll content (μg mg fwt^{-1})		
	Chl. a	Chl.b	Ratio a:b
P.umbilicalis			
December	1.29 (0.18)	-	-
April	1.01 (0.09)	-	-
July	0.82 (0.23)	-	-
U.lactuca			
December	1.34 (0.17)	0.59 (0.13)	1.72
April	1.58 (0.33)	0.87 (0.20)	1.82
July	1.06 (0.17)	1.56 (0.09)	1.89

DISCUSSION

The concentration-response curves for photosynthesis in air and seawater of the two species reflects contrasting characteristics of HCO_3^- and CO_2 use as a substrate for the carbon reduction cycle. Marine intertidal macroalgae which undergo periods of submersion and exposure are believed to be able to use both forms of inorganic carbon although the biochemical processes involved in the uptake are as yet uncertain.

Porphyra umbilicalis has a greater maximum photosynthetic capacity in air than in seawater, although this is only achieved at a CO_2 concentration well above that of air equilibrium. A comparison of the rates obtained under natural conditions shows that photosynthetic carbon fixation during periods of submersion will be greater. Unlike for *Ulva lactuca* this is not as a consequence of saturation at the seawater HCO_3^- concentration. These findings are in contrast to those of Johnson et al (1974) who calculated that *P.umbilicalis* achieved an air/water ratio under natural conditions of 2.84. However for *U.lactuca* saturation of the HCO_3^- response in seawater at 2.0 mol m^{-3} does give rise to a maximum net rate which is more than double that observed at the air equilibrium CO_2 concentration. This value is comparable to the air/water ratio of 0.66 shown for *Ulva expansa* in the same study. Beer and Shragge (1987) also found that for *Enteromorpha compressa* the saturated rate at 2.5 mol m^{-3} HCO_3^- was two times greater than that in air at 15 mmol m^{-3} CO_2. It has been suggested that there is a positive correlation between the ratio of the net rates of photosynthesis in air and seawater and the degree of exposure in *Fucus distichus, Ulva expansa, and Iridaea flaccida*, three intertidal macroalgae from various heights on the shore (Quadir, Harrison and DeWreede 1979). The characteristic response of *U.lactuca* and *P.umbilicalis* in this study are comparable to that of *Ulva fenestra*, the mid intertidal species.

The two macroalgal species in this study do show considerable seasonal variation. The photosynthetic rates

at seawater HCO_3^- concentration are greatly reduced in summer. In contrast an increased affinity for CO_2 was apparent from the reduction in the concentration required for saturation. This maintains the air equilibrated CO_2 rates equivalent to, or slightly higher than, those of the winter population. There have been few published reports to date of seasonal variation in photosynthetic capacity. Smith and Bidwell (1987) found a marked difference in the absolute rates of inorganic carbon uptake in experiments carried out during December and June. It is probable that both temperature and light levels contribute to relative changes in the total concentration of the carboxylase RuBPco or in the activity of this enzyme.

Kinetic analysis of the concentration-response defines parameters that quantitatively characterize the efficiency and absolute capacity of the biochemical process involved. As pointed out by Johnston and Raven (1986a), the theory is complicated when applied to an in vivo situation such as photosynthesis. The process can be divided into two stages, uptake and transfer of inorganic carbon from the bulk phase, and fixation by the carboxylase enzyme. Both steps will influence the shape and corresponding values given by the curve. Michaelis-Menten kinetics, applied to the CO_2 response in the marine macroalgae, appear to adequately describe photosynthesis measured during exposure (Johnston and Raven 1986a). The diffusion of CO_2 in water is 10,000 times slower than that in air, in addition to the presence of an unstirred boundary layer. Significant limition of the substrate supply in seawater means that the concentration-response curve no longer reflects the metabolic control of the process. Under these conditions it is necessary to define the diffusion resistance imposed (Pu: m^{-2} s^{-1}) when calculating the kinetic parameters, by using the Hill-Whittingham equation.

The analysis shows that under natural conditions both *U.lactuca* and *P.umbilicalis* appear to achieve higher rates of photosynthesis in seawater than in air. These values, however, are not directly related to the absolute capacity of the process under submerged and exposed conditions. With

P.umbilicalis it is evident that a higher maximum rate is achieved in CO_2 than with HCO_3^- in seawater. In contrast there is little difference in the corresponding capacities determined for *U.lactuca*. Although both species are located at a mid-tidal zone on the shoreline, *U.lactuca* is found always at least partially submerged in rookpools while *P.umbilicalis* is attached to exposed rocks. The results for *U.lactuca* and *P.umbilicalis* are consistent with the observation that plants from higher up the shore are capable of higher ratios of exposed/submerged rates of photosynthesis (Oates and Murray 1983).

An indirect measure of the inorganic carbon affinity in air and seawater for each of the species is the concentration required for saturation. Values for $K_{0\cdot5}(CO_2)$ and $K_{0\cdot5}(TIC)$ are more direct indicators of the preferred form of inorganic carbon. As with *P.umbilicalis*, many species appear to be substrate limited in seawater, most requiring TIC concentrations above 2.5 mol m^{-3} (Holbrook *et al.* 1988) and in some cases concentrations of between 5.0 and 10.0 mol m^{-3} (Reiskind, Seamon and Bowes 1989). In constrast, the results for *U.lactuca* and for *Enteromorpha compressa* (Beer and Shragge 1987) show saturation at 2.0 mol m^{-3} HCO_3^-, although for the latter species substantially increased rates were achieved by the addition of CO_2. In air *E.compressa* required a concentration of around 15 mmol m^{-3} CO_2, while the response curves for both *Fucus serratus* and *Ascophyllum nodosum* suggest that the concentration required by these two species may be rather higher. Both *U.lactuca* and *P.umbilicalis* have $K_{0\cdot5}(CO_2)$ values above the concentration of CO_2 in air and the affinities are lower than that calculated for *F.serratus* in air (Johnston and Raven 1990). From their study of this species grown under two different concentrations of CO_2 there is evidence that the inorganic carbon uptake mechanism may be regulated by CO_2. In contrast to *F.serratus*, a high intertidal species, the characteristic affinities of *U.lactuca* and *P.umbilicalis* could be attributed to the longer periods of complete submersion encountered by these two species.

The higher affinity for CO_2 of the summer population may be an adaptation or acclimatization brought about by changes in environmental conditions. Both reduced carboxylation rates or an increase in photoinhibition, dependent on changes in temperature and light intensity, may be alleviated to a degree by the higher substrate affinity. The results show that although the maximum capacity of both species was reduced during the summer months the apparent increase in affinity serves to maintain rates at the air CO_2 equilibrium concentration comparable to those of the winter population.

In seawater the affinity for inorganic carbon was determined from the bicarbonate-response kinetics (Table 1). The values do not indicate any differences in the ability of the two species to use this form of inorganic carbon and the values are similar to those for a number of other species investigated. Sand-Jensen and Gordon (1984) determined $K_{0.5}$(TIC) of between 0.54 and 0.80 mol m^{-3} for a number of marine macroalgae, including *U.lactuca* (0.60 mol m^{-3}). As with the affinity for CO_2 there is some evidence that environmental factors may determine the ability to use HCO_3^- as defined by the $K_{0.5}$(TIC). Eulittoral phaeophte macroalgae, such as *Fucales*, have a greater affinity for HCO_3^- than the sublittoral *Laminariales* which, with $K_{0.5}$(TIC) values as high as 2.0 mol m^{-3} would be only half saturated at seawater HCO_3^- concentrations (Surif and Raven 1990). Values of $K_{0.5}$(TIC) calculated for *U.lactuca* and *P.umbilicalis* in this study (0.57 and 0.60 mol m^{-3} respectively) are comparable to those for the species that undergo relatively long periods of exposure. In contrast, some studies suggest that the affinity for inorganic carbon in seawater is much lower. Values of up to 3.20 mol m^{-3} were determined for a variety of marine macroalgae which when calculated using the Michaelis-Menten equation were substantially higher (Holbrook *et al.* 1988). These results stress the importance of taking into account the degree of difussion resistance when characterizing photosynthesis. The effect of diffusion limitation is an important factor governing inorganic carbon uptake and can be used to

investigate in more detail HCO_3^- and CO_2 use in marine intertidal macroalgae.

The affinity for the substrate is a function of not only the activity of the uptake processes, but also of the rate of formation of the enyzme substrate complex. RuBPco is capable of binding O_2 as well as CO_2. The substrate specificity of the reaction will be affected by changes in the temperature and O_2 concentration, both of which indirectly affect the CO_2/O_2 solubility ratio.

Neither *U.lactuca* nor *P.umbilicalis* respond to a change in the O_2 concentration. Under the experimental conditions the values calculated for V_{max} suggest the absence of O_2 inhibition in these species. This is in agreement with the results of Beer and Israel (1986), although Kremer (1980) found inhibition of up to 47% of the rate measured in air for both *U.lactuca* and *P.umbilicalis*. *Enteromorpha compressa*, saturated at the seawater HCO_3^- concentration, is insensitive to variations in the O_2 concentration between 1% and 35% in air (Beer and Shragge 1987) and for both *Codium decorticatum* and *Udotea flabellum* the rates of net photosynthesis at 1% O_2 are not significantly higher than at 21% O_2 (Reiskind, Seamon and Bowes 1988). Three of the five species studied by Holbrook *et al.* (1988) appear to be sensitive to an increase in the oxygen concentration especially when the inorganic carbon concentration is below saturation. Kremer (1980) showed a greater degree of apparent O_2 inhibition in Rhodophyte and Chlorophyte macroalgae in comparison to Phaeophyte species. He attributes this insensitivity to a higher activity of PEPc in the latter group, although in this study photorespiration appears to be of some importance in all the species investigated.

In addition to the expected decrease in the maximum rate of photosynthesis as a result of oxygenase activity of RuBPco, this reaction must also have an effect on the apparent substrate affinity of the process. This can be seen as a change in both the $K_{0.5}$(TIC) and in the CO_2 compensation point. Although for *U.lactuca* there is no decrease in capacity, the $K_{0.5}$(TIC) shows a significant

increase over the range of O_2 concentrations. The only other similar analysis shows that for *Chondrus crispus* there is no difference in the $K_{0.5}(CO_2)$ under 1% and 21% O_2 (Colman and Cook 1985). The results for *U.lactuca* suggest a change in the CO_2 affinity, which should also be seen as an increase in the CO_2 compensation point.

The temperature sensitivity of RuBPco activity is well researched but there is some evidence to suggest that the effective substrate specificity may reflect more that just a changes in the CO_2/O_2 solubility ratio, which is inversely related to temperature (Brooks and Farquhar 1985). Although inhibition of photosynthesis at air levels of O_2 increases in proportion to the solubility ratio of CO_2/O_2, if the temperature is increased while the ratio is kept constant inhibition still occurs (Ku and Edwards 1977). It has been suggested that the O_2 sensitivity may denote changes in the characteristic of the primary carboxylase/oxygenase, which would affect both the capacity and efficiency of photosynthesis. If the RuBPoxygenase activation energy is higher than that of the carboxylase then an increase in temperature would favour the oxygenase reaction. If the activation energies and the the ratio of the Vmax carboxylase/Vmax oxygenase is constant, an increase in the respective K_m ratio could result in as apparent change in the affinity. It is believed, however, that most of the effect of temperature is largely due to the changes in solubility.

The observed response of photosynthesis in *P.umbilicalis* and *U.lactuca*, with respect to O_2 concentration and seasonally varying temperature regimes may be dependent on one or more of these effects. In addition to the dependence on substrate concentration the response will be influenced considerably by the light levels. Substrate saturated light response curves can be used to define the limitation imposed by the light reaction on the overall process.

Most macroalgae can be classified as shade adapted plants in terms of the low light saturation and light compensation points. As in other studies there is a change

in the light requirements between air and seawater. In both *P.umbilicalis* and *U.lactuca* however, this is only evident as an increase in the light compensation point while the saturation point remains constant. A general trend is found in the two intertidal species *Heserophycus harveyanus* and *Pelvetia fastigiata* investigated by Oates and Murray (1983). During exposure both species require higher light compensation and saturation levels for photosynthesis and the efficiency of light utilization and maximum capacities are lower than in seawater (44 and 14% for *H.harveyanus* and *P.fastigiata* respectively). The former species undergoes longer periods of exposure and has a higher light compensation and saturation point in comparison to the mid-tidal *P.fastigiata*, although the submersed maximum rates are similar. Oates (1985;1986) found the same trend in the response of *Halosaccion americanum*, while for *Colpomenia perigrina* the light saturated emersed rate is acheived at considerably lower levels of PAR. It is evident from the most of these studies that light utilization is more effective during submersion. It has also been proposed that the requirements for light saturation are habitat related and values of between 100 and 200 for *U.lactuca* and *P.umbilicalis* respectively, are again consistent with those for mid intertidal species (Lüning 1981). The values for *P.umbilicalis* are comparable to those reported by Smith and Berry (1986), and similar to the red species *Chondrus crispus* (Brechignac and Andre 1985). The most direct measure of the efficiency of light utilization is that of the quantum ratio (mol photon mol O_2^{-1}). In this study only the apparent quantum ratio can be calculated, as the light levels only represent incident PAR. Values as low as 12.24 mol photons mol O_2^{-1} have been reported for *Ascophyllum nodosum* (Johnston and Raven 1986a) indicating a better efficiency of light utilisation than for either *U.lactuca* or *P.umbilicalis*. The values for the two species in air and seawater suggest that *P.umbilicalis* is better adapted to longer periods of exposure than *U.lactuca*.

Seasonal variation in the light response is represented by the decrease in the maximum rates acheived although

there is little or no effect on the apparent quantum ratio under light limitation. The overall reduction in capacity is greater for the response measured in air than in seawater. The ability to maintain photosynthetic efficiency is consistent with the fact that the light compensation or saturation points of both *P.umbilicalis* or *U.lactuca* are similar in summer and winter. The results also show that the concentration and ratio of photosynthetic pigments varies on a seasonal basis (Table 5). For both *P.umbilicalis* and *U.lactuca* the lowest concentration of chlorophyll occurs in summer, and increases towards the winter months. For *U.lactuca* the ratio of chlorophyll a:b follows the same pattern. A decrease in the total pigment concentration which corresponds to a decrease in the maximum capacity, expressed on an area basis, indicates a reduction in the number of photosynthtic units (Dring 1981).As for other studies there is no evidence that light levels in excess of those required for saturation result in photoinhibition. It is evident that seasonal variation in photosynthetic capacity cannot be explained in terms of a reduction in the efficiency of light harvesting or electron transport. The overall reduction in the capacity may reflect a reduction in the capacity of the light harvesting apparatus. It may also be the result of an effect on the rate of turnover or rate of catalysis of the carboxylase enzyme RuBPco.

The alternate periods of exposure and submersion and subsequent variation in substrate and light levels are reflected by characteristics that maintain photosynthesis over a wide range of conditions. These characteristics also suggest that a number of specific and important steps are involved in the mechanism of inorganic carbon accumulation.

SECTION 2 - The role of carbonic anhydrase in the uptake of inorganic carbon

INTRODUCTION

The kinetics of inorganic carbon uptake in marine intertidal macroalgae are consistent with an ability to photosynthesize in both seawater and air, where HCO_3^- and CO_2 are possible sources of inorganic carbon for photosynthesis. Substrate affinities calculated from the concentration-response curves suggest that CO_2 is used most efficiently, but there is little indication as to the nature of the mechanism from the photosynthetic characteristics determined in Section 1.

Studies using microalgae have shown that the ability to use HCO_3^- and CO_2 can be modified by the external conditions, and related substrate concentration, during growth. The induction of this ability can be seen as changes in the levels, activity and in some cases the distribution of the enzyme Carbonic Anhydrase (CA) associated with these cells (Badger, Kaplan and Berry 1980; Findenegg 1976; Imamura, Tsuzuke, Shiraiwa and Miyachi 1983). Assays for CA activity using either intact cells or cell homogenate, in combination with inhibitor treatments, have been used to determine the location and possible role of these isoenzymes as part of a carbon concentrating mechanism.

CA can be inhibited by sulphonamides which bind at a site close to the metal cofactor Zn^+. They act by replacing the reactant H_2O molecule or OH^- ion so that although CO_2 can bind, it cannot be hydrated. The two sulphonamides most widely use are Acetazolamide (AZ) and Ethoxzolamide (EZ), of which the latter is more lipid soluble. As a consequence EZ is believed to be more easily taken up by the cells. Concentrations as low as 8×10^{-6} mol m^{-3} and 6×10^{-6} mol m^{-3} of AZ and EZ respectively have been shown to inhibit CA in cell free extracts (Moroney, Husic and Tolbert 1985), while a somewhat greater value of 10^{-3} mol m^{-3} was reported by Dixon, Patel and Merrett (1987). It has been shown, however, that sulphonamides also have a direct effect on photosystem II, which would result in a decrease in the response of photosynthesis (Lonergon and Sargent 1985). It

is apparent from both this and other studies that the concentrations of AZ and EZ required to inhibit inorganic carbon uptake are well below those found to affect the processes of photosystem II (Moroney, Husic and Tolbert 1985).

Much of the evidence for the role of CA has come from microalgal studies. AZ and EZ have been used widely to investigate the consequences of altered CA activity on the inorganic carbon uptake and accumulation. By using the two inhibitors it is possible to attribute differences in the response to an effect on the activity of either the internal or external enzyme. For *Chlamydomonas reinhardtii* treatment with the inhibitors AZ and EZ gave rise to changes in both inorganic carbon uptake and accumulation. The results are consistent with the hypothesis that CO_2 is the form of carbon that crosses the plasma membrane. External CA is required to replenish CO_2 from HCO_3^-, while internal CA has an important role in the transport process (Moroney, Husic and Tolbert 1985). External CA catalysis of HCO_3^- is not universal in microalgae. Some species have been shown to actively transport this ion across the plasma membrane (Dixon, Patel and Merrett 1987). Even when this is the case treatment with EZ indicates that a CA located internally is required to maintain substrate saturation of the primary carboxylase. This is irrespective of the mechanism of inorganic carbon transport across the plasma membrane. Suppression of the internal enzyme results in the build up of an internal pool of HCO_3^-, consistent with an important role for at least one CA in the inorganic carbon concentrating mechanism in microalgae (Badger, Kaplan and Berry 1980).

To date there has been little attempt to make a similar assessment of the activity CA in marine macroalgae. One of the problems that arise is a discrepancy over the properties of the two inhibitors. There appears to be difference of opinion as to the degree of permeability of AZ, which complicates the ability to distinguish between the roles of the internal and external enzymes (Smith and Bidwell 1987; Surif and Raven 1989).

Early studies have shown that the CA is widely distributed among species in all macroalgal groups, in addition to marine phytoplankton (Graham and Smillie 1976). The assay, carried out on tissue homogenate did not distinguish between the presence of internal and external CA. In a later study the same assay performed with whole plants showed that for a range of Rhodophyte, Chlorophyte and Phaeophyte species there was little or no external catalysis, indicating that most or all of the activity may be attributed to the an enzyme located internally within the cytoplasm or chloroplast (Cook, Lanaras and Colman 1986). It has been suggested that, as for *Anabena variabilis*, the observed inhibition of inorganic carbon uptake following treatment with CA inhibitors, in the absence of any detectable enzyme activity, could be due to the presence of a membrane bound CA-like moiety involved as a component of a HCO_3^- porter (Kaplan 1985).

Inhibitor studies have shown that the intracellular enzyme appears to be present in all macroalgae so far investigated. As in microalgae the enzyme functions as a catalyst of the HCO_3^- dehydroxylation reaction. Unlike in the unicellular species, there is as yet no evidence that macroalgae can accumulate internal pools of HCO_3^- above the concentration of the external medium, although the enzyme is important in maintaining the characteristic photosynthetic efficiencies (Smith and Bidwell 1989a,b). The initial uptake of inorganic carbon may also be dependent on HCO_3^- use and inhibitor studies have been used to indentify the the activity of an extracellular enzyme in macroalgae (Smith and Bidwell 1987; 1989; Reiskind Seamon and Bowes 1988). For a range of macroalgae a relationship between total activity and habitat has been observed (Giordano and Maberley 1989), while the levels of external CA activity show a correlation with HCO_3^- affinity (Surif and Raven 1989). Modification of the photosynthetic characteristics of *Ascophyllum nodosum* during growth at elevated CO_2 levels demonstrated a shift in the gas exchange physiology comparable to the response in

microalgae. Changes in the levels of CA activity were not determined in this study (Johnston and Raven 1990).

The aim of this section is to determine the location of the enzyme CA and its physiological function in regulating the transfer of inorganic carbon to the site of fixation. From the results it may be possible to determine whether CA activity is an important part of a mechanism of inorganic carbon concentration that confers C_4-like photosynthetic characteristics on marine intertidal macroalgae. Comparison of the effect of the two inhibitors was used to define the location and relative importance of intracellular and extracellular CA in *P.umbilicalis* and *U.lactuca*. This was assesssed in terms of changes in the kinetics of inorganic carbon uptake in both air and seawater.

Experiments were carried out with the two species to determine and compare:

1. An effective inhibitor concentration of AZ and EZ.
2. The effect of inhibition on the characteristic inorganic carbon concentration-response in air and seawater.
3. The effect of AZ and EZ on the CO_2 compensation point in air under varying concentrations of O_2.

MATERIALS AND METHODS

Plant material

Porphyra umbilicalis and *Ulva lactuca* were collected and maintained as described in Section 1.

Measurement of photosynthetic O_2 evolution in seawater

Rates of photosynthetic oxygen evolution were measured in seawater at the appropriate pH and inorganic carbon concentration as decribed in Section 1.

Measurements of photosynthetic CO_2 assimilation in air

Rates of photosynthetic CO_2 assimilation were measured using an Infra Red Gas Analyser as described in Section 1.

Inhibitors of Carbonic Anhydrase activity

The effect of the Carbonic Anhydase inhibitors AZ and EZ on the rate of apparent photosynthesis was determined in both seawater and air. The inhibitors were dissolved in 0.1 and 1.0 mol m-3 NaOH solution and diluted to the appropriate concentration. Plants were treated with either Acetazolamide (membrane impermeable) or Ethoxzolamide (membrane permeable) added to 5.0 mol m^{-3} DIC buffered seawater at pH 8.0 and kept under 500 μmol photon m^{-2} s^{-1} PAR for 15 minutes before analysis. The appropriate inhibitor concentration was maintained during photosynthetic measurements, to prevent reversal of the inhibition.

RESULTS

Effect of AZ and EZ concentration on photosynthesis

The inhibition of CA activity by the two sulphonamide inhibitors AZ and EZ was determined from the maximum rates of photosynthesis measured at saturating substrate concentration and light intensity, in seawater and in air (Figs 1-4).

At 5 mol m^{-3} HCO_3^- the maximum rate of photosynthesis for *Porphyra umbilicalis* of 2.21 μmol O_2 m^{-2} s^{-1} was reduced by 40% at 10 mmol m^{-3} AZ (0.69 μmol O_2 m^{-2} s^{-1}), and further inhibited by 100 mmol m^{-3} AZ (0.25 μmol O_2 m^{-2} s^{-1}), by more than 85% (Fig 1). With EZ, although the maximal inhibition at a concentration of 100 mmol m^{-3} is similar to that with AZ, a concentration as low as 1 mmol m^{-3} was capable of inhibiting the maximum net rate by 60%, while the rate of 0.38 μmol O_2 m^{-2} s^{-1} at 10 mmol m^{-3} EZ is little above that at 100 mmol m^{-3}, the highest inhibitor concentration.

Both AZ and EZ have a similar effect on photosynthetic assimilation of CO_2 in air (Fig 2). The control rates in air are lower than those measured in seawater, as seen in Section 1. The degree of inhibition in air, even at 100 mmol m^{-3} was less than in seawater, rates decreasing from a maximum of 1.58 μmol CO_2 m^{-2} s^{-1} to between 0.50 and 0.60 μmol CO_2 m^{-2} s^{-1}, a reduction of around 65%. Unlike in seawater AZ and EZ had comparable effects on photosynthesis in air.

In contrast, the membrane impermeable inhibitor AZ had no effect on the rate of photosynthesis in *U.lactuca*. In both seawater and air there is no inhibition at any concentration (Figs 3 & 4). EZ did suppress the maximum rate of 1.41 μmol O_2 m^{-2} s^{-1} in seawater by 50% at 1 mmol m^{-3} (0.72 μmol O_2 m^{-2} s^{-1}) and by greater than 90% at 100 mmol m^{-3} EZ. At this concentration the rate of 0.12 μmol O_2 m^{-2} s^{-1} was comparable to that measured for the response to EZ in air. Under conditions of CO_2 supply the lower concentrations of EZ had less effect on the photosynthetic capacity than in seawater. This was also evident from the

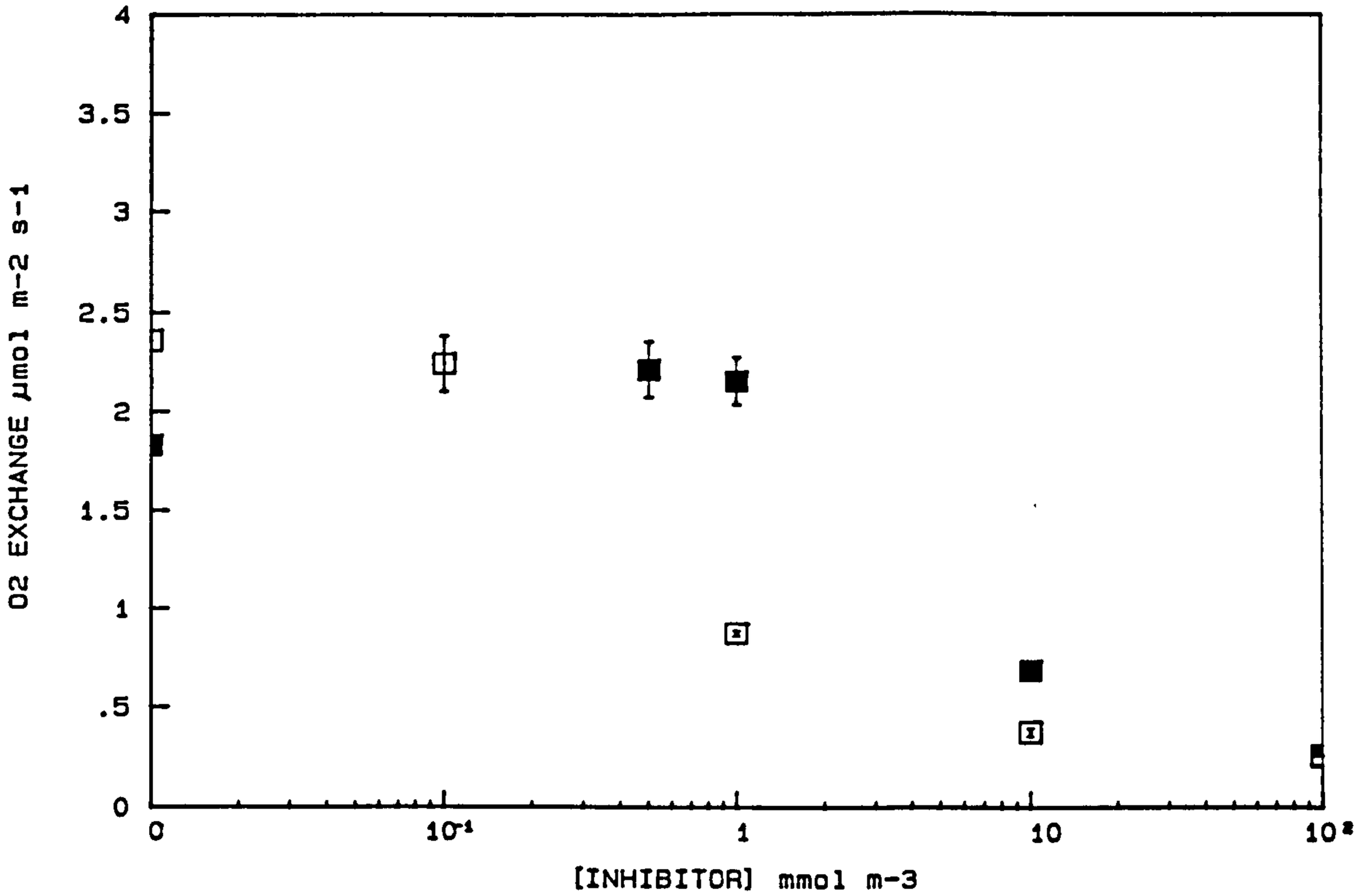

Figure 1. Effect of increasing concentrations of AZ and EZ on the maximum rate of apparent O_2 exchange by *P.umbilicalis* in seawater. Data represents the mean ± SE of four replicates. AZ treated; (■); EZ treated (□).

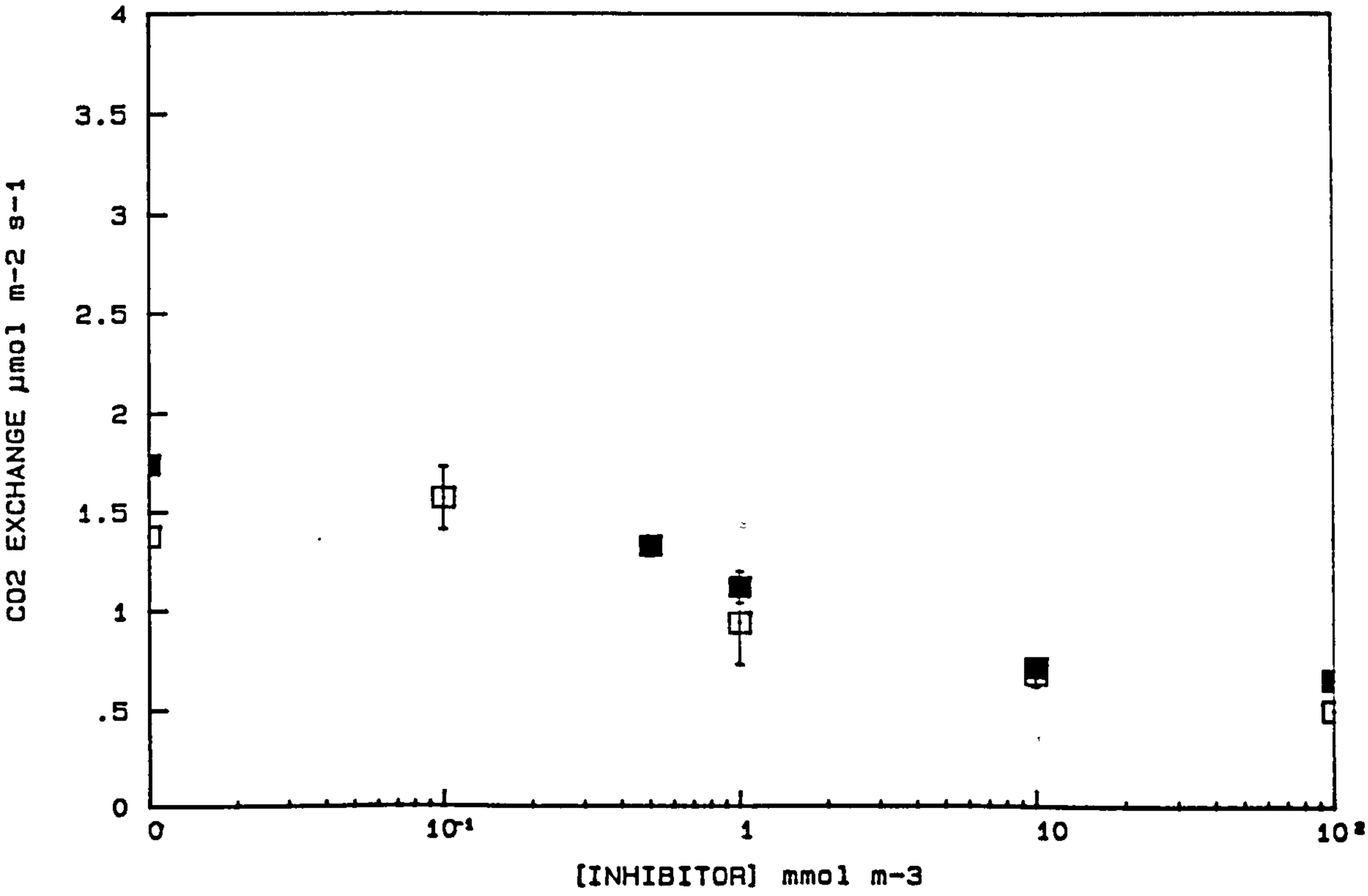

Figure 2. Effect of increasing concentrations of AZ and EZ on the maximum rate of apparent CO_2 exchange by *P.umbilicalis* in air. Data represents the mean ± SE of four replicates. AZ treated (■); EZ treated (□).

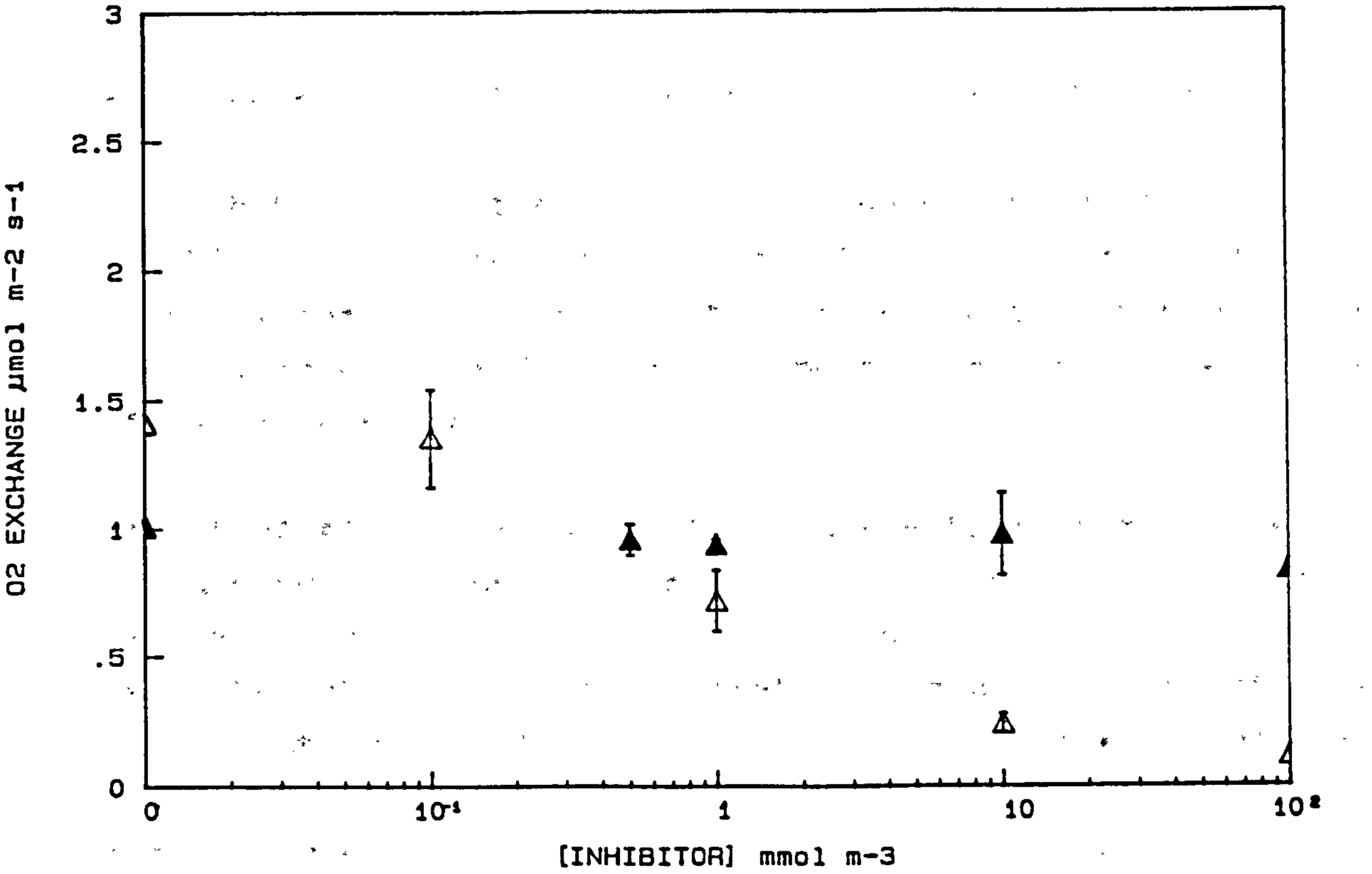

Figure 3. Effect of increasing concentrations of AZ and EZ on the maximum rate of apparent O_2 exchange by *U.lactuca* in seawater. Data represents the mean ± SE of four replicates. AZ treated; (▲); EZ treated (Δ).

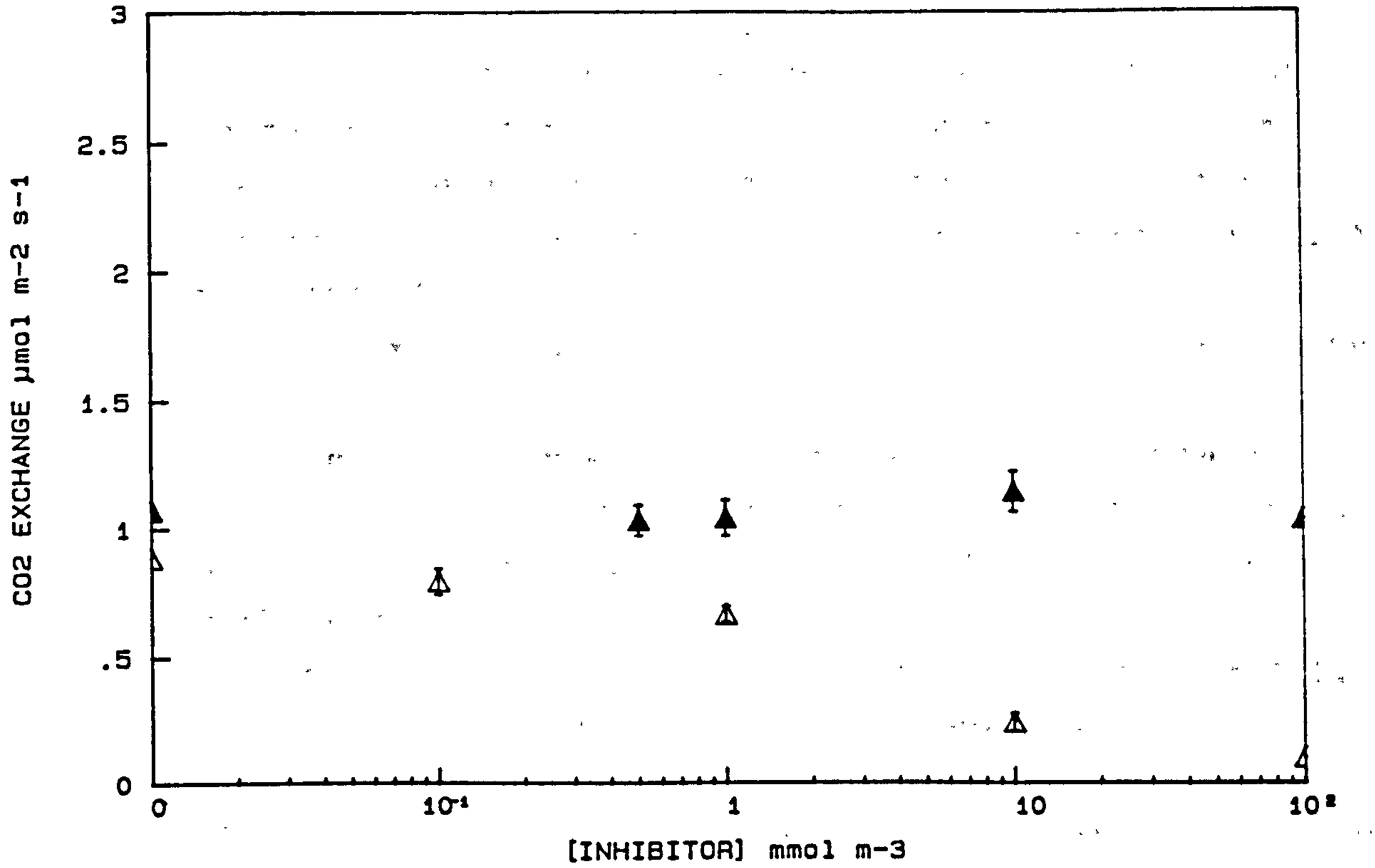

Figure 4. Effect of increasing concentrations of AZ and EZ on the maximum rate of apparent CO_2 exchange by *U.lactuca* in air. Data represents the mean ± SE of four replicates. AZ treated (▲); EZ treated (Δ).

response of *P.umbilicalis* measured in air with both AZ and EZ.

With *U.lactuca* the differential sensitivity to the two inhibitors, in comparison to the effect on *P.umbilicalis*, suggests that the mode of action of these two sulphonamides is very specific and appears to be determined by the degree of membrane permeability.

Effect of AZ and EZ on the HCO_3^- concentration-response

A concentration of 5 mmol m^{-3} inhibitor was found to partially inhibit photosynthetic oxygen evolution, as described above. This concentration was used to investigate the effect on the affinity for HCO_3^- in seawater (Figs 5 & 6). For *P.umbilicalis* both AZ and EZ significantly altered the characteristic response. The maximum rate of 2.38 μmol O_2 m^{-2} s^{-1} at concentrations of 5 mol m^{-3} HCO_3^- and above was not significantly greater than that measured during inhibition by AZ. However, as the response was linear the rates achieved were directly related to the substrate concentration. HCO_3^- at 10 mol m^{-3}, was insufficient to saturate the response and at the lower substrate concentrations the rates were greatly reduced in comparison to the control. A similar effect was seen with 5 mol m^{-3} EZ. Again the response was linear, although the uptake process appears to be limited to a greater degree by EZ. A maximum rate of only 1.00 μmol O_2 m^{-2} s^{-1} at the highest HCO_3^- concentration still represented an inhibition greater than 65%.

The linear response that characterized the uptake inhibition in *P.umbilicalis* was not observed in *U.lactuca* (Figs 7 & 8). As previously seen (Figs 3 & 4) AZ did not alter the photosynthetic capacity of *U.lactuca* and consequently there was no change in the affinity for TIC. In contrast, EZ caused a significant decrease in the photosynthetic capacity which decreased from 2.01 to 0.45 μmol O_2 m^{-2} s^{-1}, an inhibition of greater than 80%. Unlike for *P.umbilicalis*, the rate was substrate saturated at between 0.25 and 0.5 mol m^{-3} TIC and the response

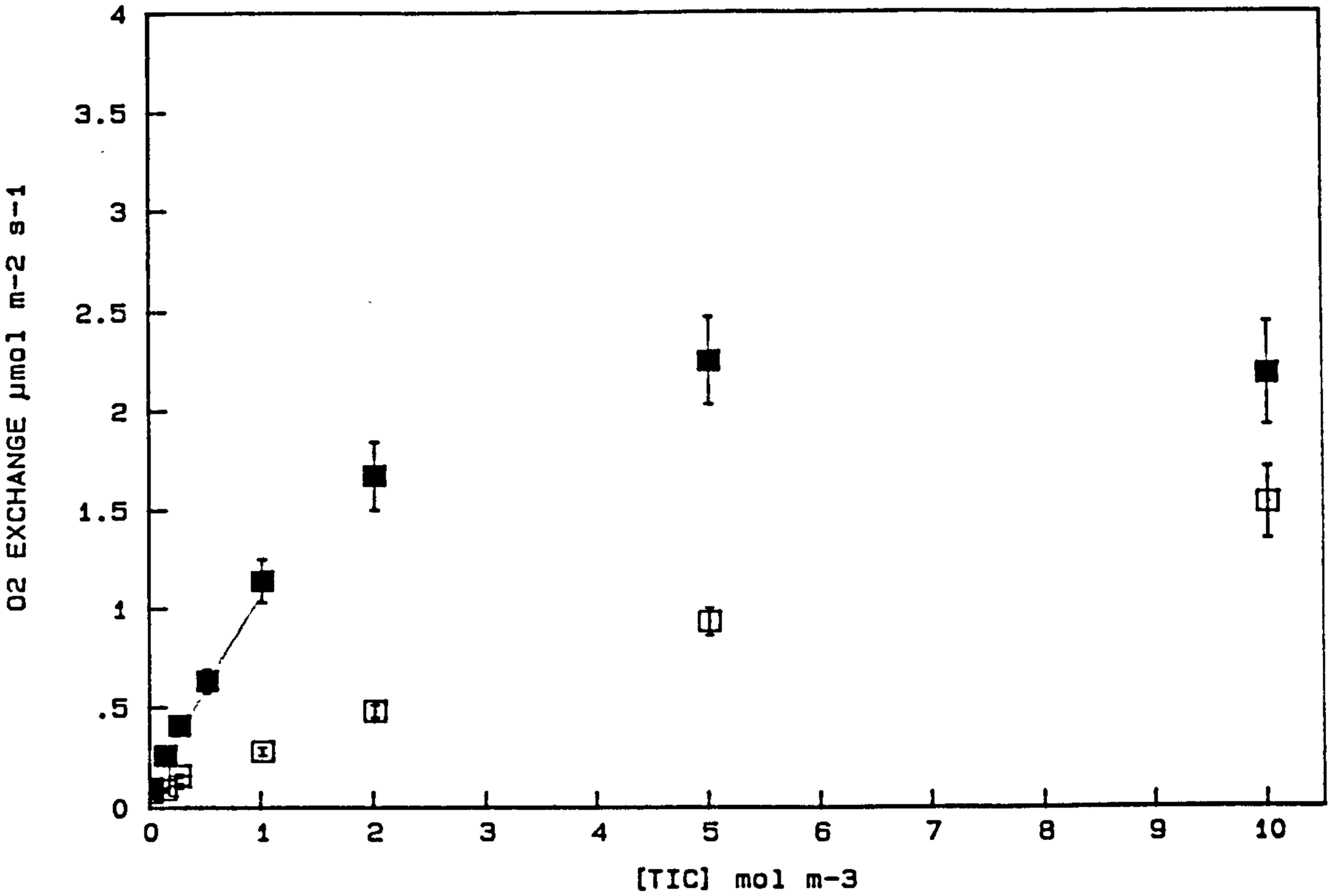

Figure 5. Effect of 5 mmol m^{-3} AZ on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater, as a function of TIC concentration. Data represents the mean ± SE of four replicates. Control (■); AZ treated (□).

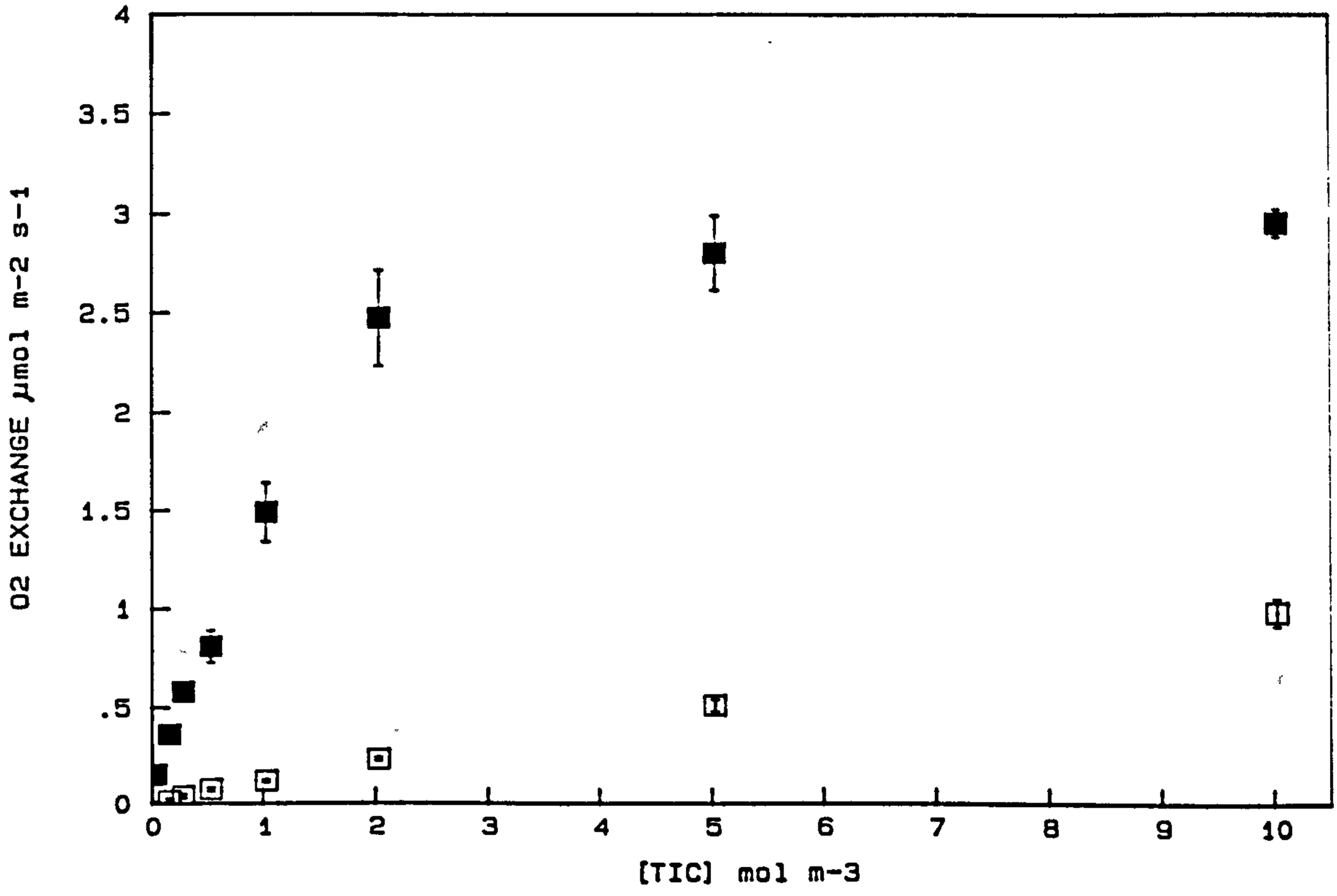

Figure 6. Effect of 5 mmol m^{-3} EZ on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater, as a function of TIC concentration. Data represents the mean ± SE of four replicates. Control (■); EZ treated (□).

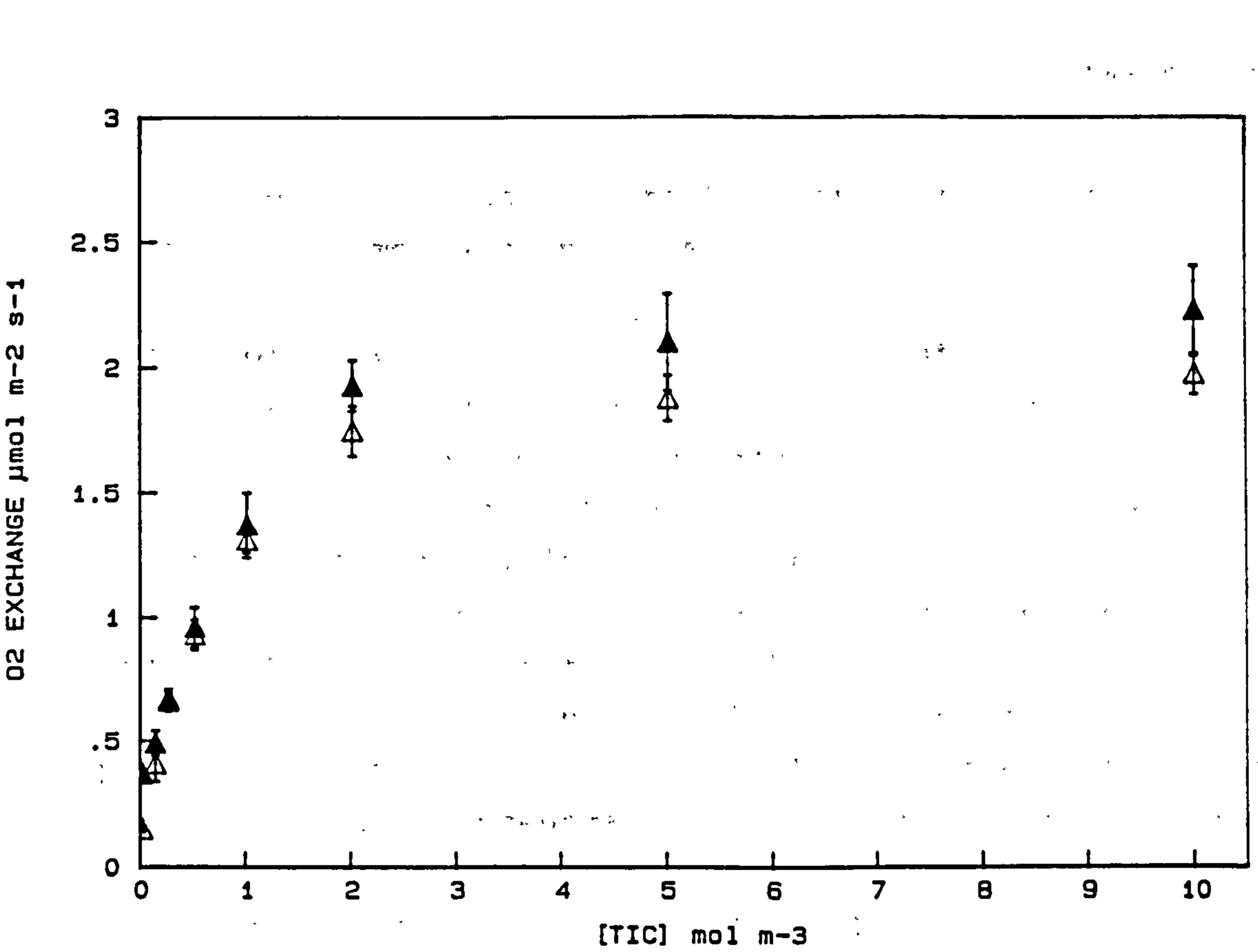

Figure 7. Effect of 5 mmol m^{-3} AZ on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater, as a function of TIC concentration. Data represents the mean ± SE of four replicates. Control (▲); AZ treated (Δ).

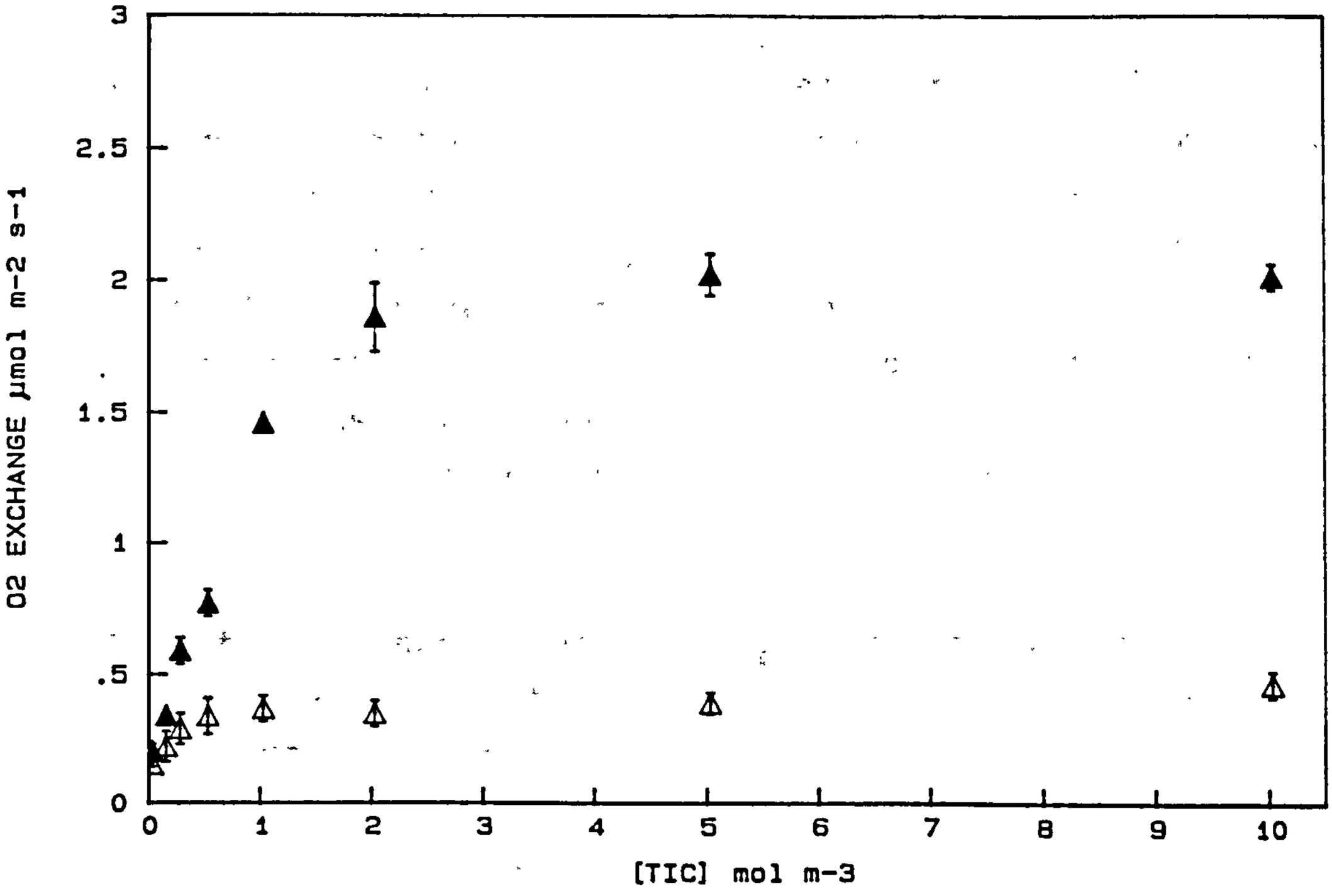

Figure 8. Effect of 5 mmol m^{-3} EZ on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater, as a function of TIC concentration. Data represents the mean ± SE of four replicates. Control (▲); EZ treated (Δ).

maintained the kinetic characteristics of active transport or an enzyme mediated process.

Effect of AZ and EZ on the CO_2 concentration-response

The regulation of inorganic carbon uptake in air by CA activity was investigated by measuring the effect of AZ and EZ on the CO_2 concentration-response of *P.umbilicalis* and *U.lactuca*. As was evident from the results for *P.umbilicalis* in seawater, both the permeable and impermeable inhibitor modified the photosynthetic charactistics, resulting in a linear response directly proportional to the external substrate concentration. At the highest CO_2 concentration of 98 mmol m^{-3} the maximum rates achieved were 2.11 and 1.45 μmol CO_2 m^{-2} s^{-1} with AZ and EZ respectively (Figs 9 & 10). This is consistent with the effect observed in seawater, where EZ caused a considerably greater degree of suppression than AZ.

The CO_2 concentration-response of *U.lactuca* remained unaltered in the presence of AZ. As in seawater, there was no significant difference in either photosynthetic efficiency or capacity (Fig 11). In contrast EZ inhibition was as effective in air as in seawater (Fig 12). The maximum saturated rate of 1.79 μmol CO_2 m^{-2} s^{-1} was reduced to 0.84 μmol CO_2 m^{-2} s^{-1}, an inhibition of more than 50% and the affinity for CO_2 was lower than that of the control. As in seawater the response maintained the characteristics of an uptake process that is actively mediated. As for *P.umbilicalis*, the photosynthetic response in air is less sensitive to inhibition of CA than in seawater.

Effect of AZ and EZ on the CO_2 compensation point

The values for the CO_2 compensation point measured at CO_2 concentrations of 2.0 mmol m^{-3} and below, were low in comparison to those for most C_3 species. This indicates that there was little or no photorespiratory activity in either *P.umbilicalis* or *U.lactuca*. Values of between 0.085 and 0.30 mmol m^{-3} for *P.umbilicalis* measured at 12^{o}C, were insensitive to changes in the concentration of O_2 between

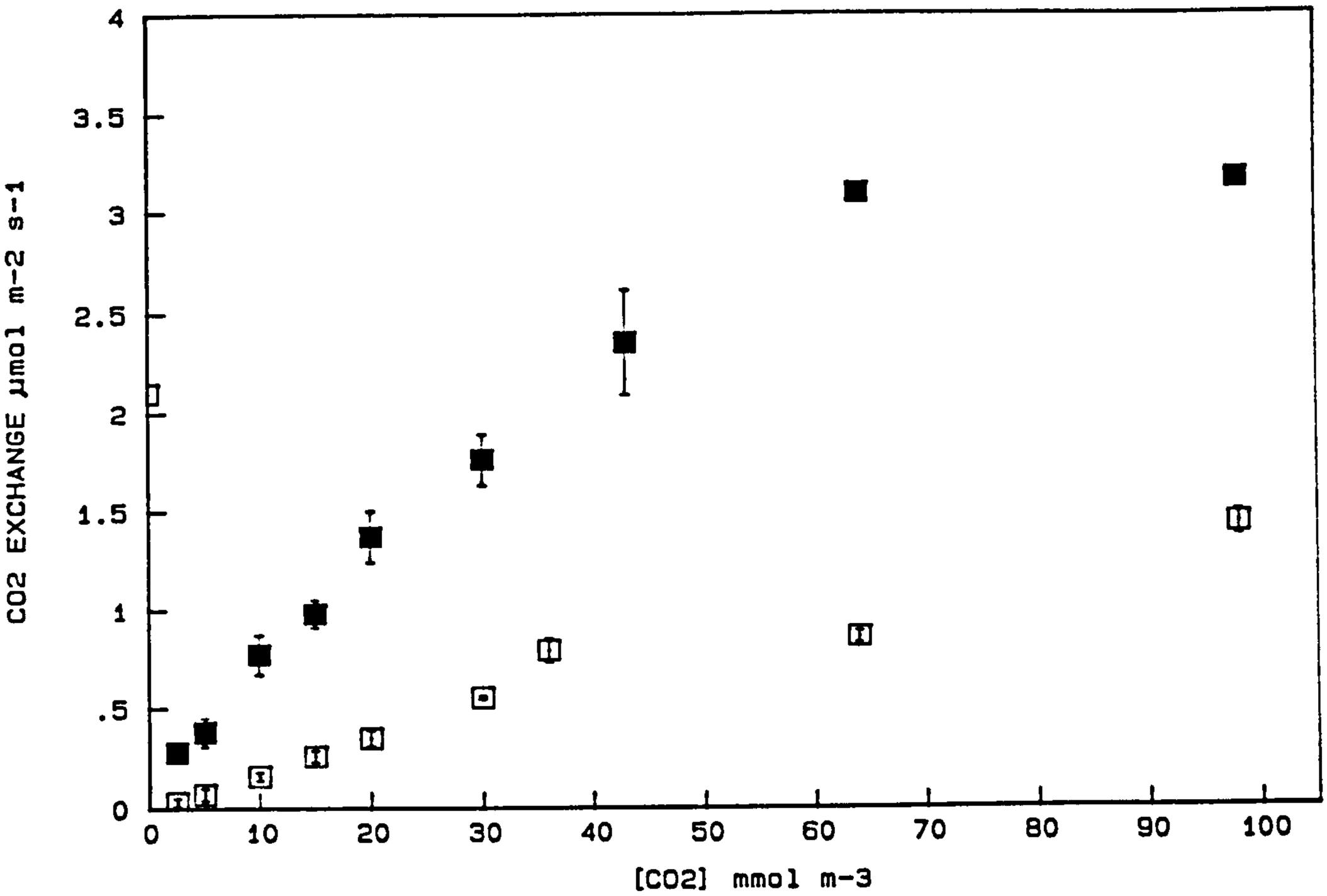

Figure 9. Effect of 5 mmol m^{-3} AZ on the rate of apparent photosynthetic CO_2 exchange by *P.umbilicalis* in air, as a function of CO_2 concentration. Data represents the mean ± SE of four replicates. Control (■); AZ treated (□).

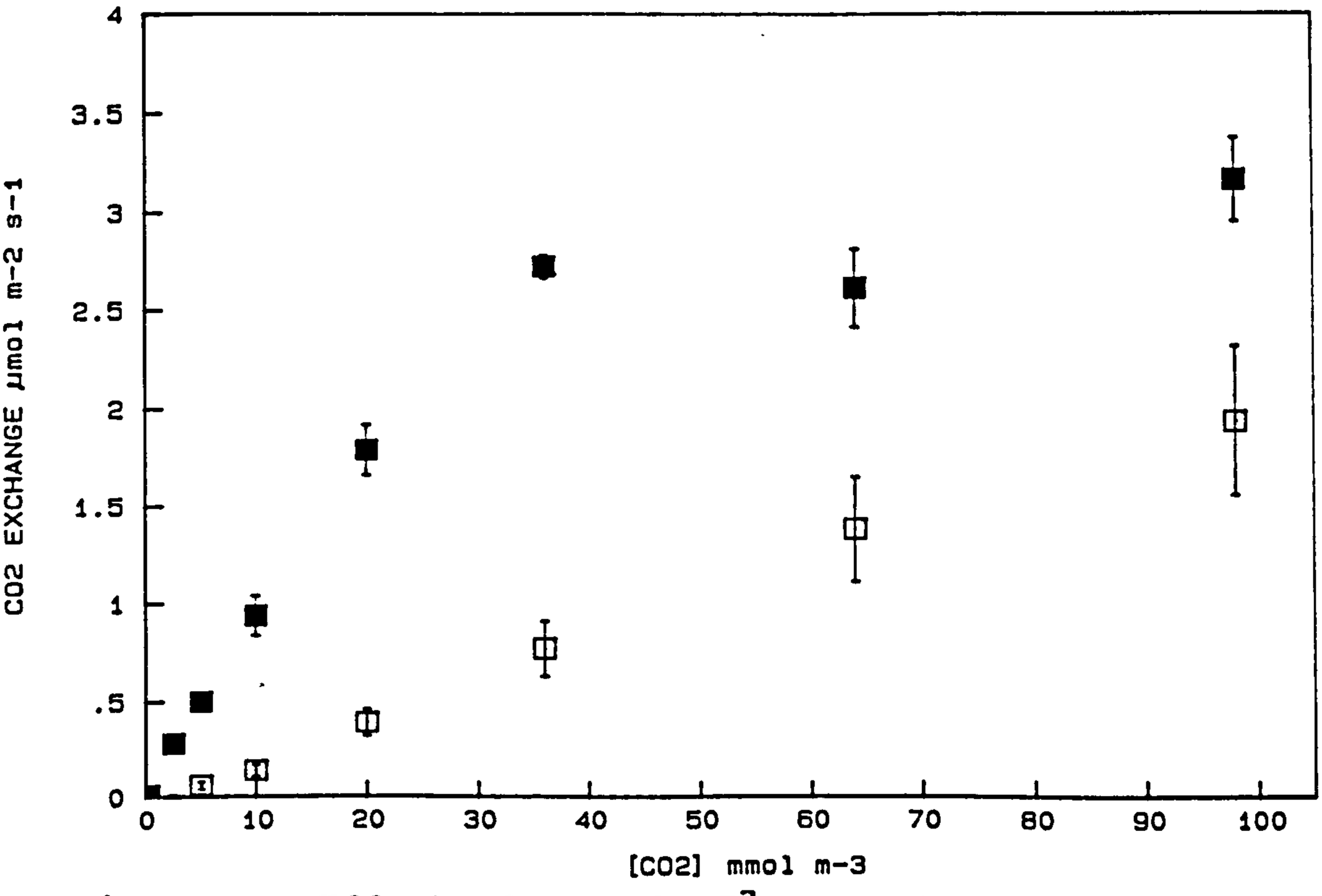

Figure 10. Effect of 5 mmol m^{-3} EZ on the rate of apparent photosynthetic CO_2 exchange by *P.umbilicalis* in air, as a function of CO_2 concentration. Data represents the mean ± SE of four replicates. Control (■); EZ treated (□).

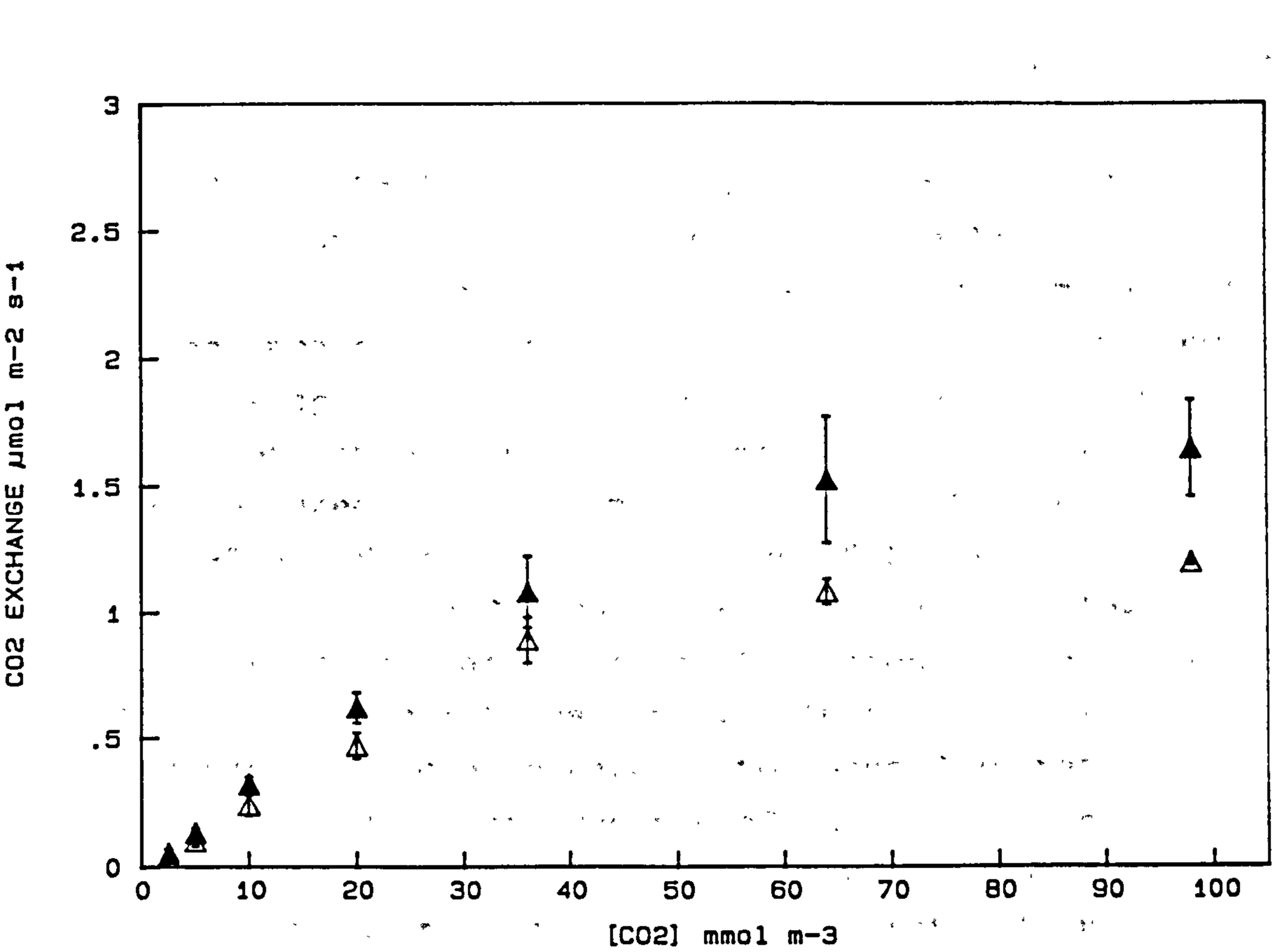

Figure 11. Effect of 5 mmol m^{-3} AZ on the rate of apparent photosynthetic CO_2 exchange by *U.lactuca* in air, as a function of CO_2 concentration. Data represents the mean ± SE of four replicates. Control (▲); AZ treated (Δ).

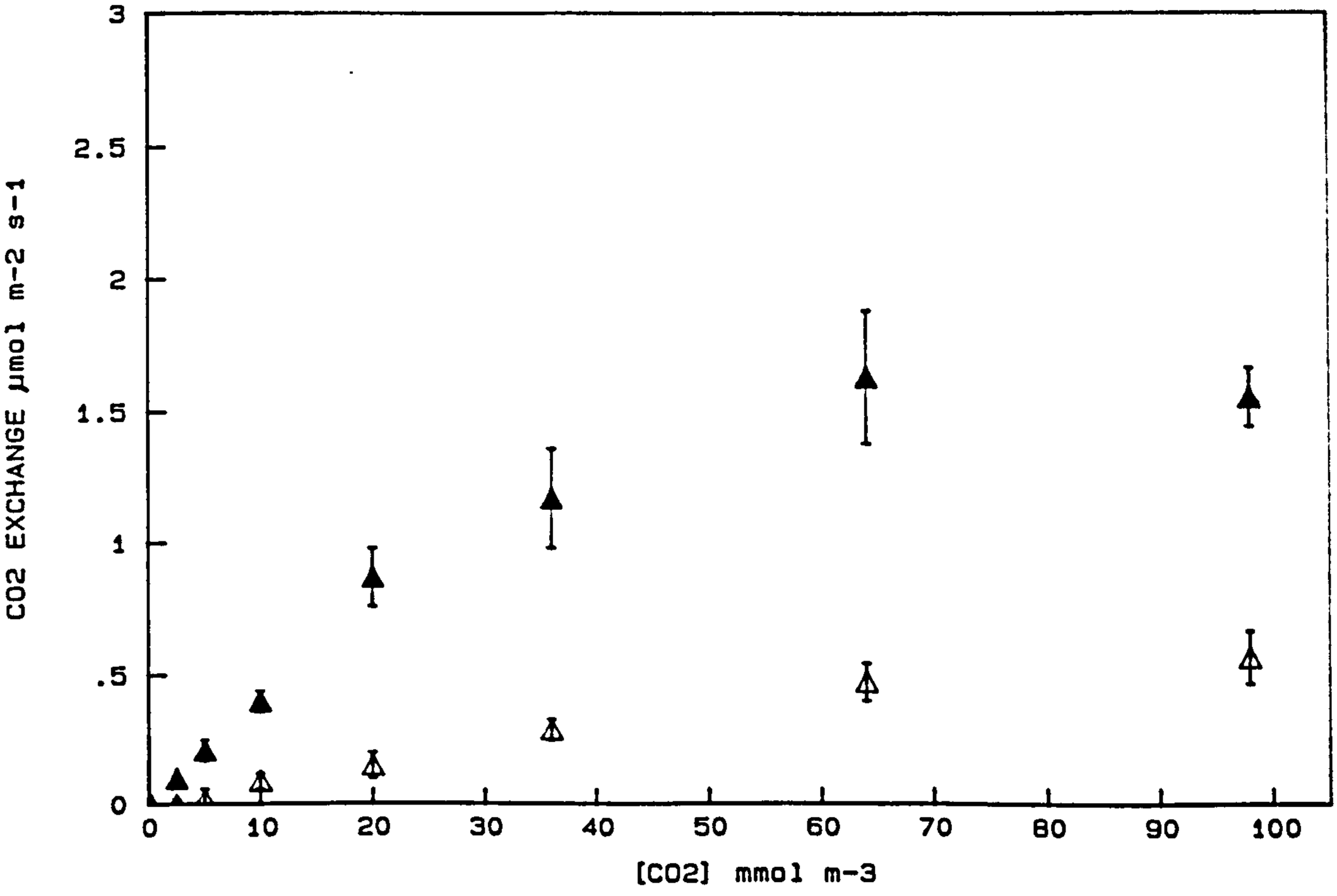

Figure 12. Effect of 5 mmol m^{-3} EZ on the rate of apparent photosynthetic CO_2 exchange by *U.lactuca* in air, as a function of CO_2 concentration. Data represents the mean ± SE of four replicates. Control (▲); EZ treated (Δ).

1% and 21%. Following addition of either AZ or EZ, the compensation points increased to 0.56 mmol m^{-3} at 21% O_2 (Figs 13 & 14). In addition there was a concomitant effect on the apparent substrate affinity, defined as an decrease in the slope of the response. Uncharacteristically, the effect of inhibition on the compensation points was greater when measured at 1% O_2 in air (0.85 and 1.20 mmol m^{-3} CO_2 with AZ and EZ respectively; Figs 15 & 16).

For *U.lactuca* CO_2 compensation points are low and insensitive to changes in the O_2 concentration (Figs 17-20). The concentrations for *U.lactuca* of between 0.34 and 0.47 mmol m^{-3} were comparable to those measured for *P.umbilicalis* in the presence of the inhibitors. As expected AZ had no effect on the compensation concentration nor was there any change in the substrate affinity. Following treatment with EZ the compensation point increased to 0.90 and 1.28 mmol m^{-3} under 1% and 21% O_2 respectively. In contrast to the effect on *P.umbilicalis*, there was no apparent change in the substrate affinity in conjunction with an apparent increase in the oxygenase activity.

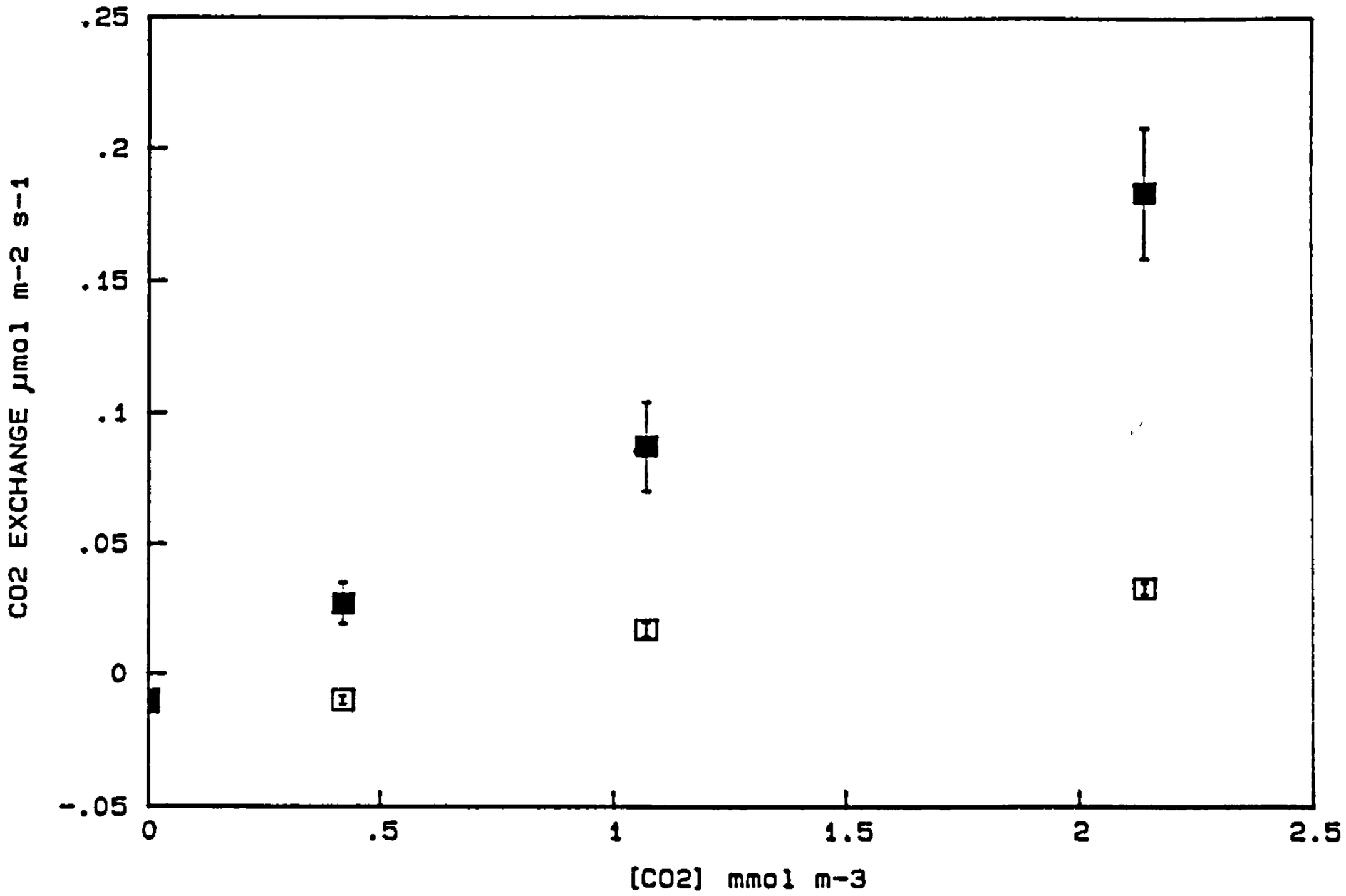

Figure 13. Effect of 5 mmol m^{-3} AZ on the rate of apparent CO_2 exchange by *P.umbilicalis* in air. Response measured at limiting CO_2 concentration and 21% O_2 represents the mean ± SE of four replicates. Control (■); AZ treated (□).

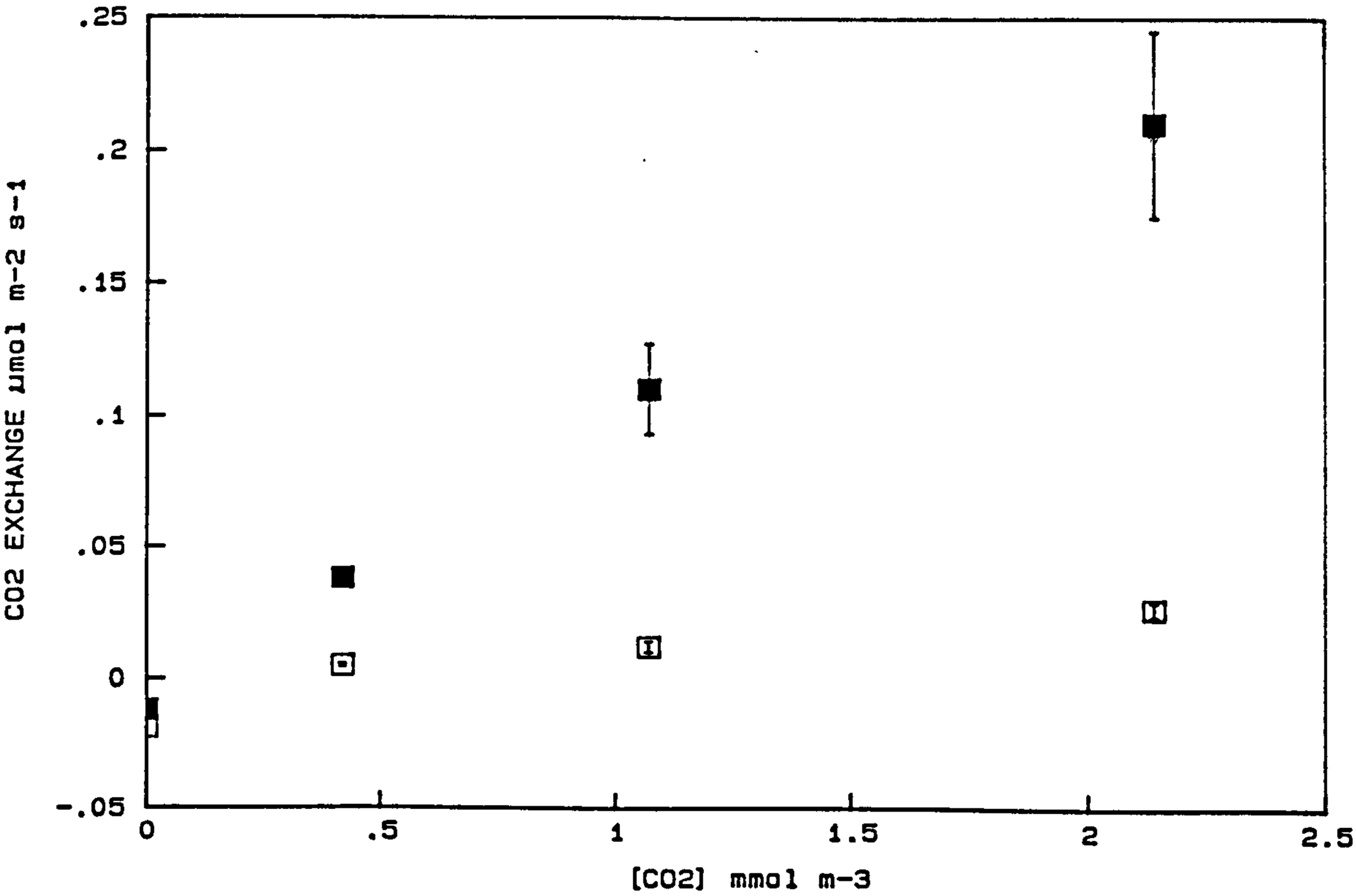

Figure 14. Effect of 5 mmol m^{-3} EZ on the rate of apparent CO_2 exchange by *P.umbilicalis* in air. Response measured at limiting CO_2 concentration and 21% O_2 represents the mean ± SE of four replicates. Control (■); EZ treated (□).

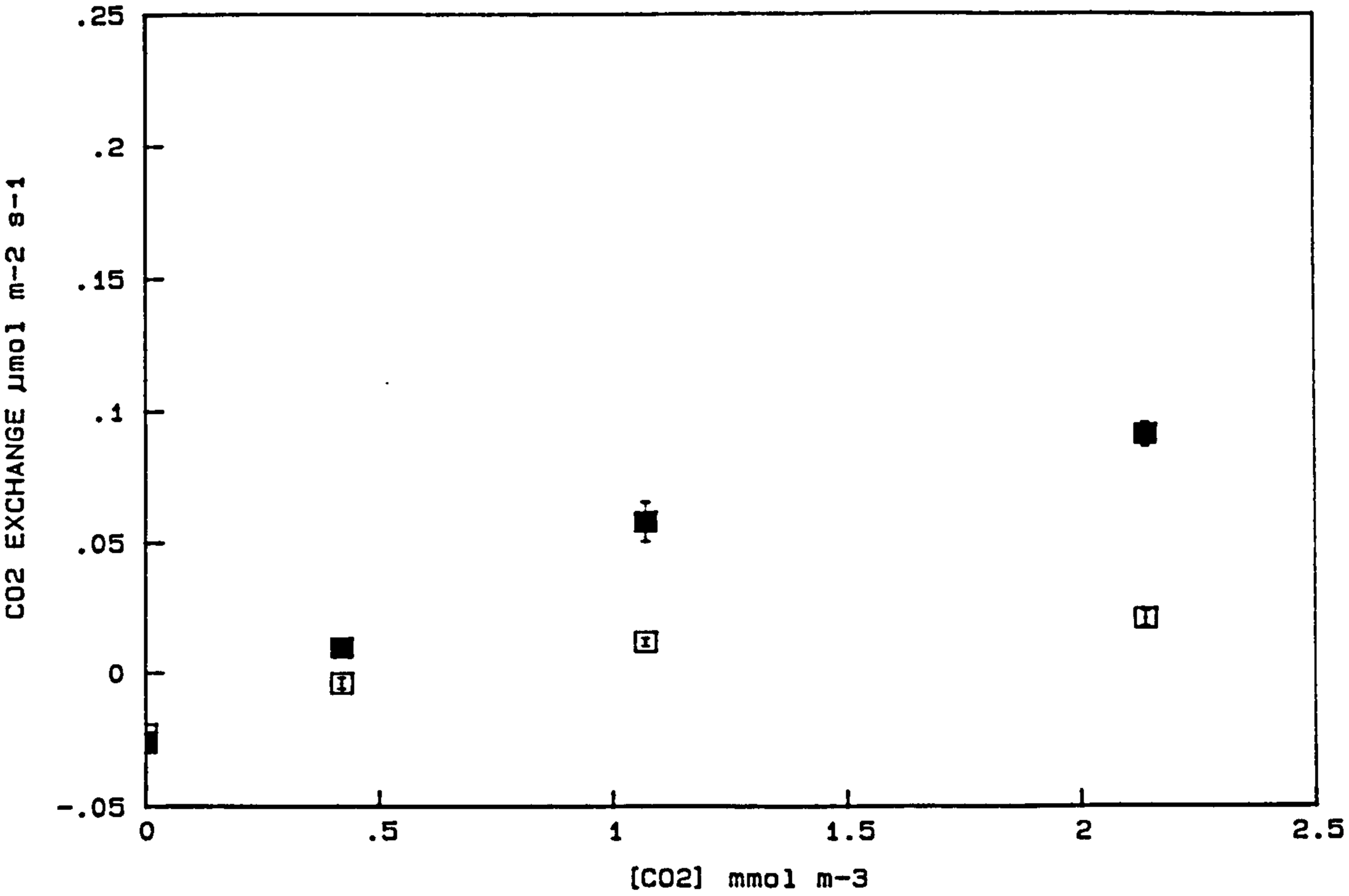

Figure 15. Effect of 5 mmol m^{-3} AZ on the rate of apparent CO_2 exchange by *P.umbilicalis* in air. Response measured at limiting CO_2 concentration and 1% O_2 represents the mean ± SE of four replicates. Control (■); AZ treated (□).

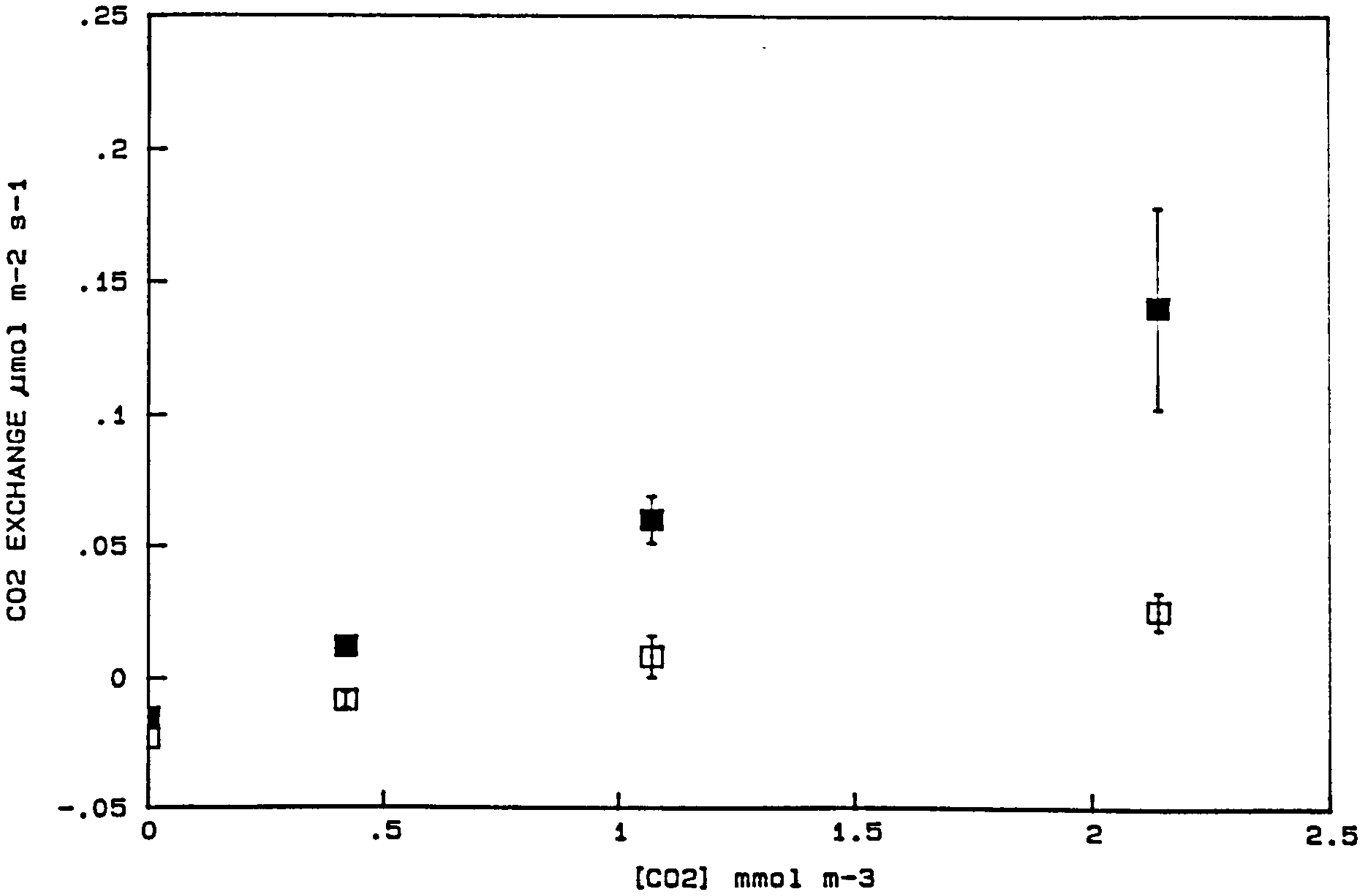

Figure 16. Effect of 5 mmol m^{-3} EZ on the rate of apparent CO_2 exchange by *P.umbilicalis* in air. Response measured at limiting CO_2 concentration and 1% O_2 represents the mean ± SE of four replicates. Control (■); EZ treated (□).

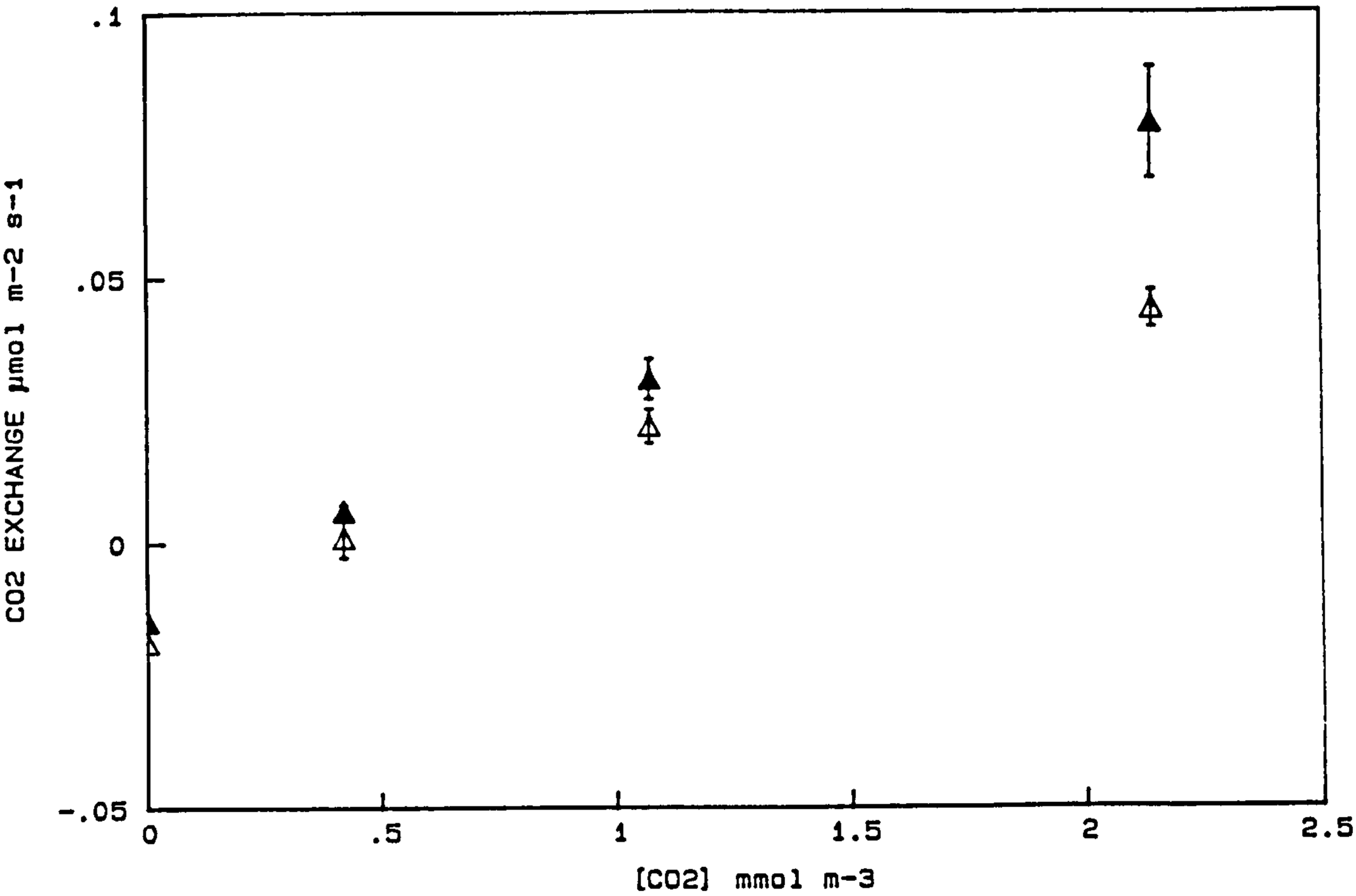

Figure 17. Effect of 5 mmol m^{-3} AZ on the rate of apparent CO_2 exchange by *U.lactuca* in air. Response measured at limiting CO_2 concentration and 21% O_2 represents the mean ± SE of four replicates. Control (▲); AZ treated (Δ).

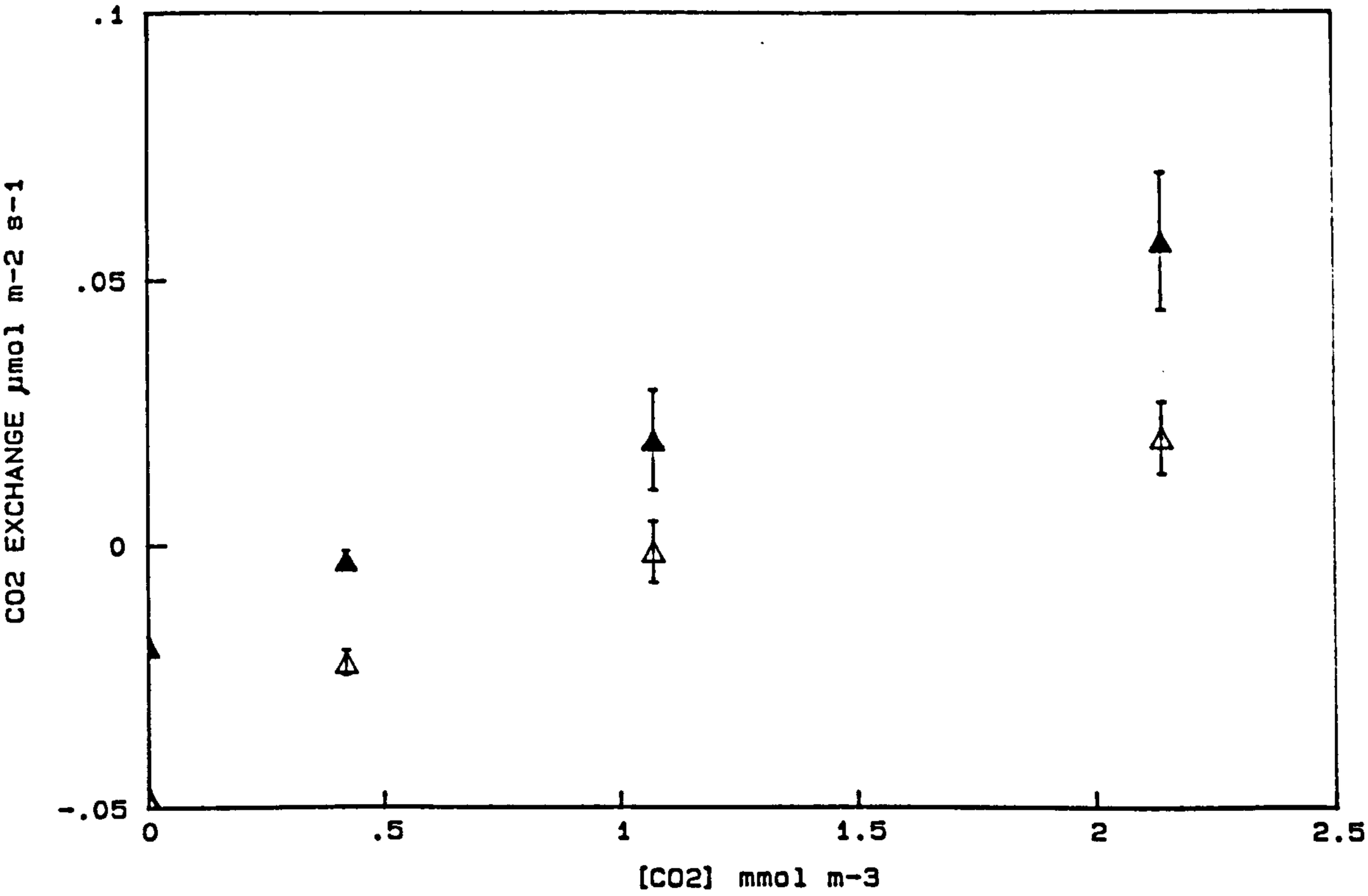

Figure 18. Effect of 5 mmol m^{-3} EZ on the rate of apparent CO_2 exchange by *U.lactuca* in air. Response measured at limiting CO_2 concentration and 21% O_2 represents the mean ± SE of four replicates. Control (▲); EZ treated (Δ).

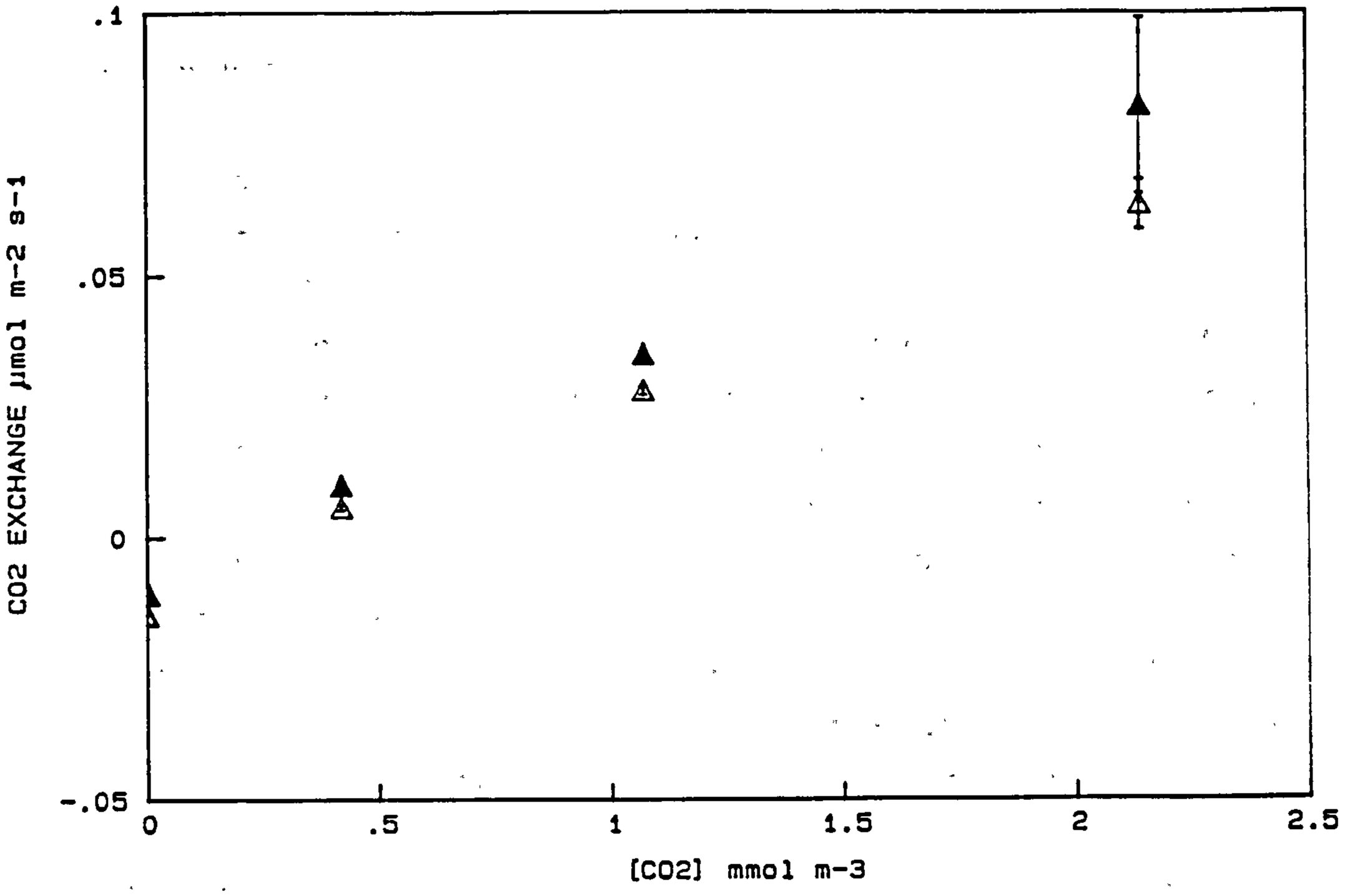

Figure 19. Effect of 5 mmol m^{-3} AZ on the rate of apparent CO_2 exchange by *U.lactuca* in air. Response measured at limiting CO_2 concentration and 1% O_2 represents the mean ± SE of four replicates. Control (▲); AZ treated (Δ).

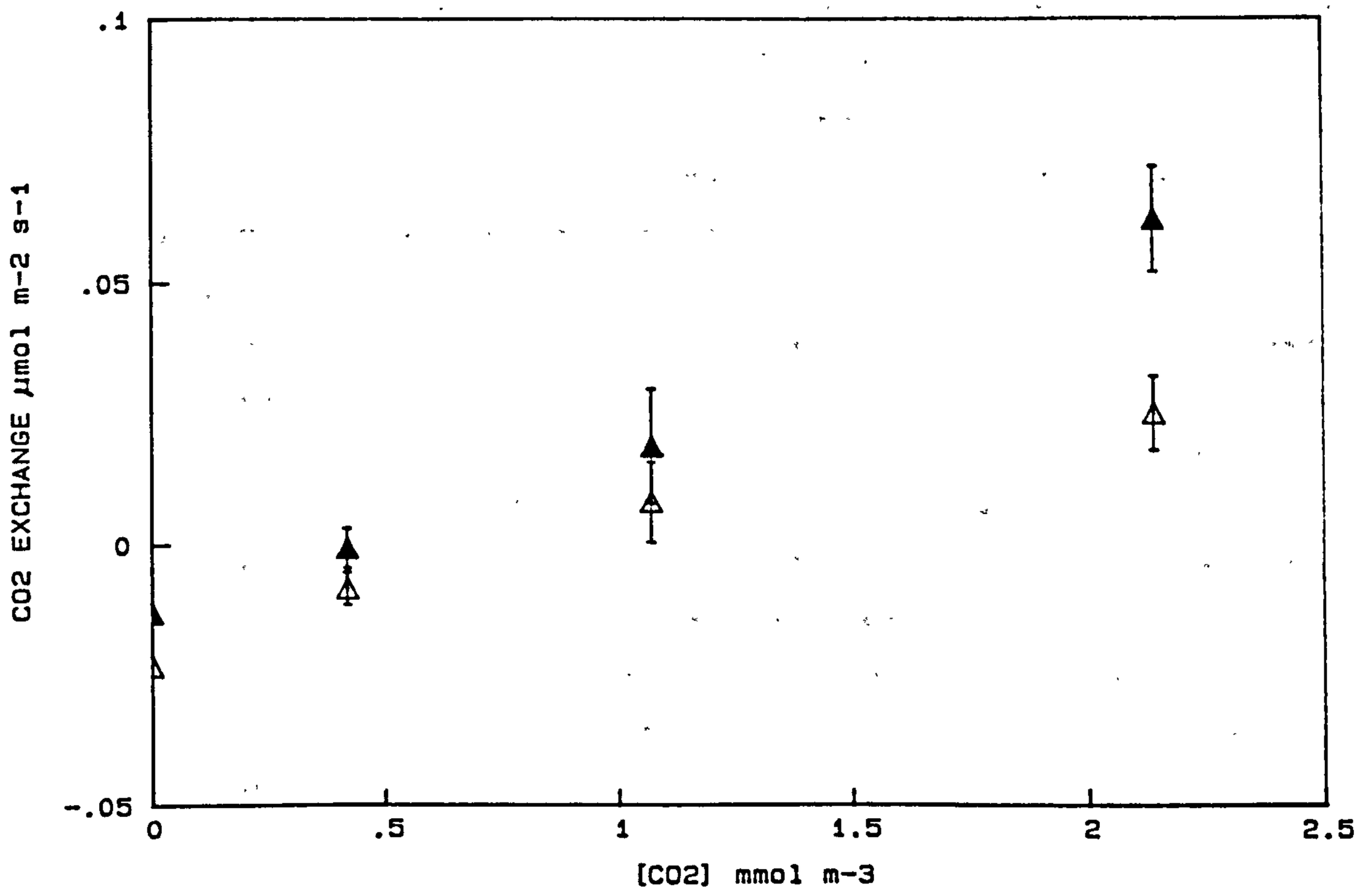

Figure 20. Effect of 5 mmol m^{-3} EZ on the rate of apparent CO_2 exchange by *U.lactuca* in air. Response measured at limiting CO_2 concentration and 1% O_2 represents the mean ± SE of four replicates. Control (▲); EZ treated (Δ).

DISCUSSION

Carbonic anhydrase is believed to have an important role in the mechanism of inorganic carbon accumulation in intertidal macroalgae.

In microalgae the inhibitor concentrations that effect CA activity in cell free extracts are relatively low. The concentrations required to produce the initial phase of inhibition in *Chlamydomonas reinhardtii* were around 100 times higher than these reported I_{50} values, suggesting there is a large excess of extracellular CA above that required for inorganic carbon uptake (Moroney, Husic and Tolbert 1985). These inhibitor concentrations are lower than those reported for macroalgae. Both *Ulva lactuca* and *Porphyra umbilicalis* show 50% inhibition at a concentration of 5 mmol m^{-3} AZ and EZ and saturation of the effect 100 mmol m^{-3}. Lower values of inhibition are reported for both *Chondrus crispus* (Smith and Bidwell 1987) and for *Ulva faciata* (Beer and Israel 1990). The effect for *C.crispus* was saturated at 2 mmol m^{-3}, although for this species the percentage inhibition at this concentration only reaches a maximum of 30% even when CO_2 is limiting.

In general, it appears that the response to AZ is more rapid but less effective than for EZ, an observation that suggests variation in the regulation of the mechanism of inorganic carbon uptake (Beer and Israel 1990). One explanation is that AZ is unable to penetrate the plasma membrane, and so will only inhibit an exteral CA (Moroney, Husic and Tolbert 1985). The consistent difference in the effect of AZ on *U.lactuca* and *P.umbilicalis* can be explained by the inability of this compound to reach the CA located intracellularly, in contrast to the results with EZ. Therefore the efficacy of AZ inhibition can be used to determine the presence of an extracellular or cell-wall bound enzyme, as in *P.umbilicalis*. The absence of inhibition by AZ in *U.lactuca* is in contrast to the results for *U.faciata* which showed a 50% reduction in the rate of photosynthesis although the maximum inhibition only occurs following the addition of EZ (Beer and Israel 1990).

For both *U.lactuca* and *P.umbilicalis* the residual photosynthetic capacity after treatment with 100 mmol m^{-3} EZ appears to be dependent solely on the diffusion of CO_2, maintaining the rate at around 10% of the maximum. This apparent requirement for an internal CA is evident when both CO_2 and HCO_3^- were supplied, although for *P.umbilicalis* the inhibition is alleviated to a degree by the availability of CO_2 in air. This was also apparent for inorganic uptake during AZ inhibition in air. The results suggest that at least one methods of inorganic carbon use in marine macroalgae relies on the ability to passively take up CO_2 by diffusion.

From the response to the non-permeable inhibitor AZ, it is evident, however, that the maximum photosynthetic capacity in *P.umbilicalis* is primarily dependent on the activity of an external CA. There was little or no additional reduction in rate when both the internal and external enzyme was inhibited by EZ. Two explanations for this are possible, that the intracellular activity is insignificant or absent, or that inorganic carbon uptake mediated by the external CA is the rate limiting step of the process in *P.umbilicalis*. For *U.lactuca* inorganic carbon uptake does not appear to be regulated by an external enzyme, but in the absence of any internal CA activity following EZ addition, the photosynthetic capacity was reduced as in *P.umbilicalis*.

There has been little other work to compare the differential mode of action of these two inhibitors or indeed their effect on inorganic carbon uptake in marine intertidal macroalgae. *U.faciata* showed a greater reduction in the photosynthetic HCO_3^- response following addition of EZ, but was still inhibited to a degree by AZ (Beer and Israel 1990). The results for *C.crispus* suggest inhibition of the internal CA, in addition to that at the cell surface, brought about a significant decrease in the photosynthetic response. The conclusions from both these studies are comparable to the results for *U.lactuca* and *P.umbilicalis*.

Further distinctions between the mechanism in the two species can be determined from the shape of the concentration-response curves obtained following inhibitor treatment. For *P.umbilicalis* the photosynthetic response is directly proportional to the concentration of substrate, independent of the carbon species avalailable. However, unlike the apparent similarity between the effect of the two inhibitors on the photosynthetic capacity, it is evident that the affinity for inorganic carbon in seawater is limited to a greater degree by EZ. In contrast to the observed response in air, the inhibition of both the external and internal enyzmes further decreased the affinity and corresponding capacity. These results are consistent with the previous conclusion that CO_2 is the form of inorganic carbon that is transferred across the plasma membrane. In air the concentration of CO_2 was sufficient to maintain a substantial rate of photosynthesis dependent on passive diffusion, although this was well below that achieved in the presence of external CA. During exposure under natural conditions, the activity of this enzyme appears to increase the rate of diffusion.

In seawater the rate of CO_2 diffusion was also governed to some extent by the activity of the internal CA. This enzyme may serve to increase the concentration gradient of CO_2 required for uptake under low CO_2 conditions. CO_2 supply by diffusion would normally, however, be insignificant in comparison to the rate of CO_2 produced by external catalysis of HCO_3^-.

As predicted there is no significant change in the HCO_3^- or CO_2 response of *U.lactuca* measured following AZ treatment. With EZ, although there was a decrease in the photosynthetic capacity in both air and seawater, the rates were not directly proportional to the external substrate concentration and showed the characteristics of substrate saturated uptake. Under both HCO_3^- and CO_2 conditions the kinetic response was saturated at a lower concentration than in the controls and achieved only a fraction of the maximum capacity. Unlike in *P.umbilicalis*, inhibition did not alter the affinity for inorganic carbon, as the

calculated $K_{0.5}$ values were similar and inorganic carbon uptake did not increase linearly with external substrate concentration. The mechanism appears to be facilitated by an actively mediated step. Inhibition by EZ could directly affect the initial uptake of inorganic carbon, or alter the capacity of the system by inhibition of an internal CA.

CO_2 uptake by *P.umbilicalis* is consistent with the mechanism suggested for *C.crispus* (Smith and Bidwell 1989a). Their model proposed that CO_2 crosses the plasma membrane, so that HCO_3^- use must be dependent on external CA. Internal CA also enhances the rate of carbon fixation either by facilitating transport of CO_2 to the active site, or by maintaining a CO_2 concentration gradient by increasing the rate of HCO_3^- formation.

In contrast it has been stated by Beer and Israel (in press) that their work with *U.faciata* strongly suggests that HCO_3^- uptake is probable, although CO_2 use at times is not excluded. They propose a mechanism which at least in seawater, relies on an HCO_3^-/OH^- antiport system that could be initially stimulated by the uptake of CO_2. This model could explain the observed response of *U.lactuca* in this study.

The measured CO_2 compensation points are further evidence for the operation of a mechanism that concentrates inorganic carbon at the site of fixation. They represent the balance between photosynthetic uptake and the photorespiratory/respiratory release of CO_2. Values of between 0 and 10 $\mu l\ l^{-1}$ (0 to 0.43 mmol m^{-3}) are characteristic of plants which have a C_4-like metabolism while values of around 50 $\mu l\ l^{-1}$ (2.14 mmol m^{-3}) denote C_3 metabolism. The values for reported for marine intertidal macroalgae are low, although they do show some sensitivity to temperature (Couglan and Tattersfield 1977). While CO_2 compensation points are related to the level of photorespiration, any increase in the values may not be solely due to an increase in this process.

For *P.umbilicalis* the CO_2 compensation point and carboxylation efficiency was consistantly lower under 21% O_2. Both AZ and EZ inhibition affected the balance of the

gas exchange, resulting in an increase in the CO_2 compensation point and a decrease in the carboxylation efficiency. The alteration of the carboxylation efficiency suggests that the effect on the compensation point can be attributed to an overall decrease in the apparent affinity for inorganic carbon. The same effect was shown by shape of the HCO_3^- and CO_2 concentration-response curves. As there was little evidence of any O_2 sensitivity of the response even at such low levels of CO_2, it is unlikely that the effect can be attributed to a direct effect on CO_2 fixation by RuBPco.

In contrast, there was little or no decrease in carboxylation efficiency of *U.lactuca* and consequently no change in the relative affinity for the two substrates, although inhibition by EZ caused a substantial increase in the CO_2 compensation point. This denotes a change in the overall activity of an enyzme mediatiated process involved in gas exchange, as was evident from the HCO_3^- and CO_2 concentration-response curve. Inorganic carbon uptake at the plasma membrane, in the two species appears to determine the substrate by two significantly different mechanisms.

There also appears to be a considerable effect of pH and/or substrate on the CO_2 compensation point of both microalgae and macroalgae. Several species of freshwater microalgae had compensation points as high as 30 μl l^{-1} (1.28 mmol m^{-3}) when measured in acid media (Birmingham and Colman 1979). The same pattern is evident for macroalgae where an increase in the CO_2 compensation points corresponded to a higher proportion of CO_2 at pH 5.5. The values also appear to be lower for higher intertidal species which are more frequently exposed (Surif and Raven 1989). The CO_2 compensation values determined for *P.umbilicalis* and *U.lactuca* follow this same trend.

From the results it is apparent that the major difference in inorganic carbon uptake between *P.umbilicalis* and *U.lactuca* is in the operation of an external CA. In *P.umbilicalis* the ability to use HCO_3^- in seawater relies on external catalysis by CA at the surface. A similar

requirement in air may increase the rate of transfer of inorganic carbon through the capillary film of water. In contrast an external CA does not facilitate either HCO_3^- or CO_2 use in *U.lactuca*. This absence of inhibition by AZ is in contrast tothe results for *U.faciata* which was sensitive to this inhibitor. In this latter species transport of inorganic carbon may involve a CA-moiety at the plasma membrane, as in *Anabaena variabilis* (Kaplan 1985).

Internal CA activity was demonstrated for both *P.umbilicalis* and *U.lactuca*. Within the cytoplasm this enzyme would increase the rate of HCO_3^- formation, giving rise to a concentration gradient that would facilitate CO_2 uptake across the plasma membrane. A second enzyme required to convert HCO_3^- to CO_2 at the site of fixation is believed to have a chloroplastic location. Either of these two roles would explain the observed effect of EZ on the rate of photosynthesis in *P.umbilicalis*.

The response in *U.lactuca* maintains the characteristics of an active uptake mechanism, even after addition of EZ. In contrast to the effect on *P.umbilicalis*, the decreased capacity cannot be attribute to uptake of CO_2 by diffusion. The response may reflect one of two possible mechanisms; active transport of HCO_3^- and CO_2 at the plasma membrane regulated by a concentration gradient dependent on the activity of internal CA, or the active uptake by a process involving a CA-like moiety that is sensitive to EZ.

Although this inhibitor study highlights some differences in the roles of internal and external carbonic anhydrase activity in the two species, there is still no conclusive evidence as to which form of inorganic carbon is taken up, or the biochemical nature of the accumulation process in marine intertidal macroalgae.

SECTION 3 - Inorganic carbon affinity: preference for HCO_3^- or CO_2

INTRODUCTION

The assimilation of inorganic carbon can be modified by the inhibition of the functional CA enzymes, as shown in Section 2. The results from this section have shown that the activity of both an external and internal enzyme play a role in the mechanism that determines the photosynthetic efficiency and capacity in marine intertidal macroalgae.

The properties of the carbonate system are such that at certain pH values the TIC will be almost exclusively CO_2 or HCO_3^-. Under these conditions it is possible to investigate the importance of the substrate concentration in regulating inorganic carbon uptake.

In this section the photosynthetic response for the two intertidal species was determined under conditions of varying pH and inhibitor treatment. The initial experiments compared the observed rates of photosynthesis at high and low pH. This method indicates whether HCO_3^- ions can be used, but does not show whether HCO_3^- or CO_2 is the carbon species that crosses the plasma membrane.

To date most of the work concerned with defining the mechanisms of inorganic carbon accumulation has been carried out with microalgae. For *Chlamydomonas reinhardtii* measured at pH 5.5 the maximum rates of photosynthesis achieved were higher than those at pH 8.0. Neither AZ and DBI (dextran-bound AZ) had any effect on the $K_{0.5}(CO_2)$ or the internal concentration of inorganic carbon (Moroney, Husic and Tolbert 1985). The membrane permeable inhibitor EZ both inhibited fixation and increased the $K_{0.5}(CO_2)$. In contrast, at pH 8.0 where most of the inorganic carbon is as HCO_3^-, the CO_2 affinity was reduced by AZ and DBI, but to a much greater extent by EZ. From these results it was concluded that CO_2 is the species that crosses the plasma membrane. Extracellular CA is required to maintain the supply of CO_2 from HCO_3^-. Intracellular CA also appears to have an important function in the uptake mechanism, seemingly independent of the method of inorganic carbon uptake into the cell. In the chloroplast this enzyme may rely on active transport of HCO_3^- from the cytoplasmic pool

to the chloroplast stroma, where the decarboxylase function is believed to occur. It is evident from this work that the diffusive uptake of CO_2 from the bulk medium is sufficient to maintain high rates of assimilation in the presence of an internal CA. Inhibition of this enzyme results in the accumulation of inorganic carbon, taken up by diffusion. By contrasting the differential effects of the two inhibitors at varying pH it was possible to define in some detail the role of the CA in the mechanism of inorganic carbon in this species.

A subsequent analysis of the role of this enzyme was carried out with wild type cell of *Chlamydomonas reinhardtii* and mutant cells deficient in internal CA. The responses of the two types of cell were compared at pH 5.1 and 7.3 (Moroney, Husic and Tolbert 1987). In addition both wild type and mutant cells were grown under conditions of high CO_2 to determine which of the CA enzymes, if any, would be induced. At an external concentration of 5% CO_2 the substrate affinity was still dependent on internal CA. This confirmed the conclusion of the earlier study, that the activity of this enzyme limits the rate of CO_2 fixation at both high and low pH (Moroney, Husic and Tolbert 1985). The major proportion of CA located externally, was only present in air grown cells. Even the mutant strain, which lack an internal CA, maintain the ability to transport inorganic carbon across the plasma membrane. The results suggest that induction of the mechanism causes changes in the activity of one or more proteins in addition to CA. These could include a HCO_3^- transporter at either the chloroplast or plasma membrane.

Similar studies combining pH dependent restrictions on substrate supply with CA inhibition, have confirmed that the physiological characteristics are not dependent solely on CA activity. Dixon, Patel and Merrett (1987) found that at pH 5.0 both AZ and EZ had little effect on the CO_2 affinity of *Porphyridium purpureum*. At pH 8.0 the $K_{0.5}(CO_2)$ increased dramatically when treated with EZ. Photosynthetic rates measured at high pH were 3 times greater than those at low pH, showing that the cells have a greater capacity

to use the HCO_3^- rather than the CO_2. In the absence of any detectable extracellular CA activity the results were consistent with an active uptake of HCO_3^- across the plasma membrane. The subsequent decarboxylation of HCO_3^- however, requires CA located internally, as the uncatalysed rate of CO_2 production would be insufficient to maintain optimum levels of CO_2 fixation.

In contrast, similar analysis of *Chlorella saccharophila* revealed a preference for the uptake of CO_2 (Beardall 1981; Gehl, Cook and Colman 1987). Higher rates of assimilation occurred at the lower pH values although the $K_{0.5}(CO_2)$ and compensation point were independent of the pH of the external medium. When CO_2 is available uptake would be enhanced by the presence of a CO_2 concentration gradient across the plasma membrane. Transport of HCO_3^- across the chloroplast membrane followed by CA catalysed decarboxylation would maintain this gradient. However, as the pH increased the photosynthetic characteristics appeared to be dependent on active uptake at the plasma membrane.

For marine macroalgae evaluation of the carbon concentrating mechanism from a combination of the effect of pH and inhibitors, has provided less conclusive evidence for the role of CA. This is due mainly to the difficulty of measuring directly the internal concentration of inorganic carbon. Johnston and Raven (1986b) used similar techniques to investigate HCO_3^- utilization in *Ascophyllum nodosum*. The photosynthetic capacity at high and low pH, the pH compensation points and an estimation of uncatalysed rate of inorganic carbon supply, all indicated that this species could take up HCO_3^-. Few studies have presented any direct evidence of the ability of macroalgae to accumulate the high internal levels of inorganic carbon found in microalgae. Smith and Bidwell (1989a; 1989b) have attempted to measure the internal HCO_3^- concentration in *Chondrus crispus*, using both chopped thallus and isolated chloroplasts with the silicon oil centrifugation technique. They found no internal accumulation of HCO_3^- above that which could be accounted for by diffusion. Beer *et al.*

(In press) determined the internal inorganic carbon levels by measuring the photosynthetic O_2 release into pH 5.5 medium, minus the contribution made by uptake of the external substrate. This species was found to possess a mechanism that concentrated inorganic carbon internally when CO_2 in the external medium was low. The pH increase in unbuffered medium indicates that this ability depends on the uptake of HCO_3^-. Further investigations, possibly using inhibitor treatment, are needed to determine the mechanism by which this process is mediated.

The aim of this section was to investigate the nature of the mechanism of inorganic carbon uptake. As for the studies described above the methods made use of the properties of the carbonate system. A change in the pH alters the proportion of CO_2 and HCO_3^- while the TIC remains constant. Thus at pH 5.5, 77% of the TIC is present as CO_2 and only 1% at pH 8.0. It may be however, that some of the response could be due directly to the altered external pH, and so further measurements were carried out at pH 7.5 and 8.5. At these two values the proportion of TIC as CO_2 decreases from 32% to 0.2%, with little effect on the percentage of HCO_3^-.

In combination with the use of CA inhibitors, basic comparisons of the photosynthetic response in relation to pH, were used to define any preference for the form of inorganic carbon and the relative importance of internal and external CA. Experiments in the previous section were devised to investigate the effect of of partial inhibition of CA on the photosynthetic capacity. In this section higher concentrations of AZ and EZ were used (100 mmol m^{-3}) so as to maximally inhibit CA activity and distinguish between catalysed and uncatalysed inorganic carbon uptake. In addition the maximum concentration of TIC was increased (up to 60 mol m^{-3}) to determine at which concentration inhibition would be overcome.

Experiments were carried out with *P.umbilicalis* and *U.lactuca* to measure and compare:

1. The effect of pH in terms of substrate supply, on both the photosynthetic efficiency and capacity.

2. The role of CA in determing the substrate specificity and affinity of the inorganic carbon concentrating mechanism.

MATERIALS AND METHODS

Plant material

Porphyra umbilicalis and *Ulva lactuca* were collected and maintained as described in Section 1.

Measurement of photosynthetic O_2 evolution in seawater

Rates of photosynthetic oxygen evolution were measured polarographically as described in Section 1.

The pH of the seawater was then adjusted by the addition of 50 mol m^{-3} EPPS (N-(2-Hydroxyethy)piperazine-N'-3-propanesulfonic acid pH 8.5 and 8.0), 50 mol m^{-3} MOPS (3-[N-Morpholino]propanesulphonic acid pH 7.5), or 50 mol m^{-3} MES (2[N-Morpholino]ethanesulfonic Acid pH 5.5) and freshly prepared NaOH solution. The appropriate inorganic carbon concentrations between 0 and 60 mol m^{-3} were obtained by the addition of sucessive aliquots of concentrated $NaHCO_3$ solution.

Inhibitors of Carbonic Anhydrase activity.

The effect of Carbonic Anhydase inhibitors AZ and EZ on the net rate of photosynthesis was determined at pH 5.5 and 8.0 or 7.5 and 8.5. The inhibitors were prepared and applied as described in Section 2.

RESULTS

Photosynthetic capacity measured at pH 5.5 amd pH 8.0

The photosynthetic capacity of *Porphyra umbilicalis* at pH 8.0 was saturated at 2.0 mol m^{-3} TIC, where the proportion of CO_2 is maximally only 1.2% (Fig 1). At pH 5.5 the proportion of CO_2 is substantially higher (77%) which corresponds to a decrease in the concentration of HCO_3^-. The photosynthetic capacity was not significantly different at the two pH values. As the TIC concentration was increased to 5.0 mol m^{-3} the CO_2 concentration is around 3.80 mol m^{-3}. There was a significant decrease in the maximum rate (2.85 to 2.25 μmol O_2 m^{-2} s^{-1}) suggesting that this level of CO_2 was inhibitory (Fig 1).

In contrast, for *Ulva lactuca*, although the photosynthetic capacity at pH 8.0 was saturated by 2.0 mol m^{-3} TIC, at pH 5.5 there was a substantial reduction in the rates achieved at both levels of TIC (0.53 and 0.38 μmol O_2 m^{-2} s^{-1} at 1.0 and 2.0 mol m^{-3} TIC respectively; Fig 2).

Substrate concentration-response at pH 8.0 and pH 5.5

For *P.umbilicalis* the response at pH 5.5 showed an increase in substrate affinity with $K_{0.5}$(TIC) values of 144 and 87 mmol m^{-3} at pH 8.0 and pH 5.5 respectively (Fig 3). The photosynthetic capacity or V_{max} was around 2.30 μmol O_2 m^{-2} s^{-1} for both pH values, but the greater substrate affinity at pH 5.5 gave rise to saturation of the rate at 1.0 mol m^{-3} TIC in comparison to 2.0 mol m^{-3} TIC at pH 8.0. As shown in Figure 1, at pH 5.5 an increase in the TIC levels to concentrations of 5.0 mol m^{-3} and above inhibited the response. This was reduced to only 1.3 μmol O_2 m^{-2} s^{-1} at 10 mol m^{-3} TIC. One important parameter determined from the Hill-Whittingham equation is Pu. This is a measure of the diffusion resistance which for *P.umbilicalis* was 1.9 x 10^{-6} m s^{-1} at pH 8.0 and 5.0 x 10^{-6} m s^{-1} at pH 5.5.

The results for *U.lactuca* show a greater effect on the response in relation to pH. There was a decrease in both the affinity and maximum capacity as the concentration of CO_2 increased (Fig 4). At pH 8.0 the $K_{0.5}$(TIC) is 67 mol

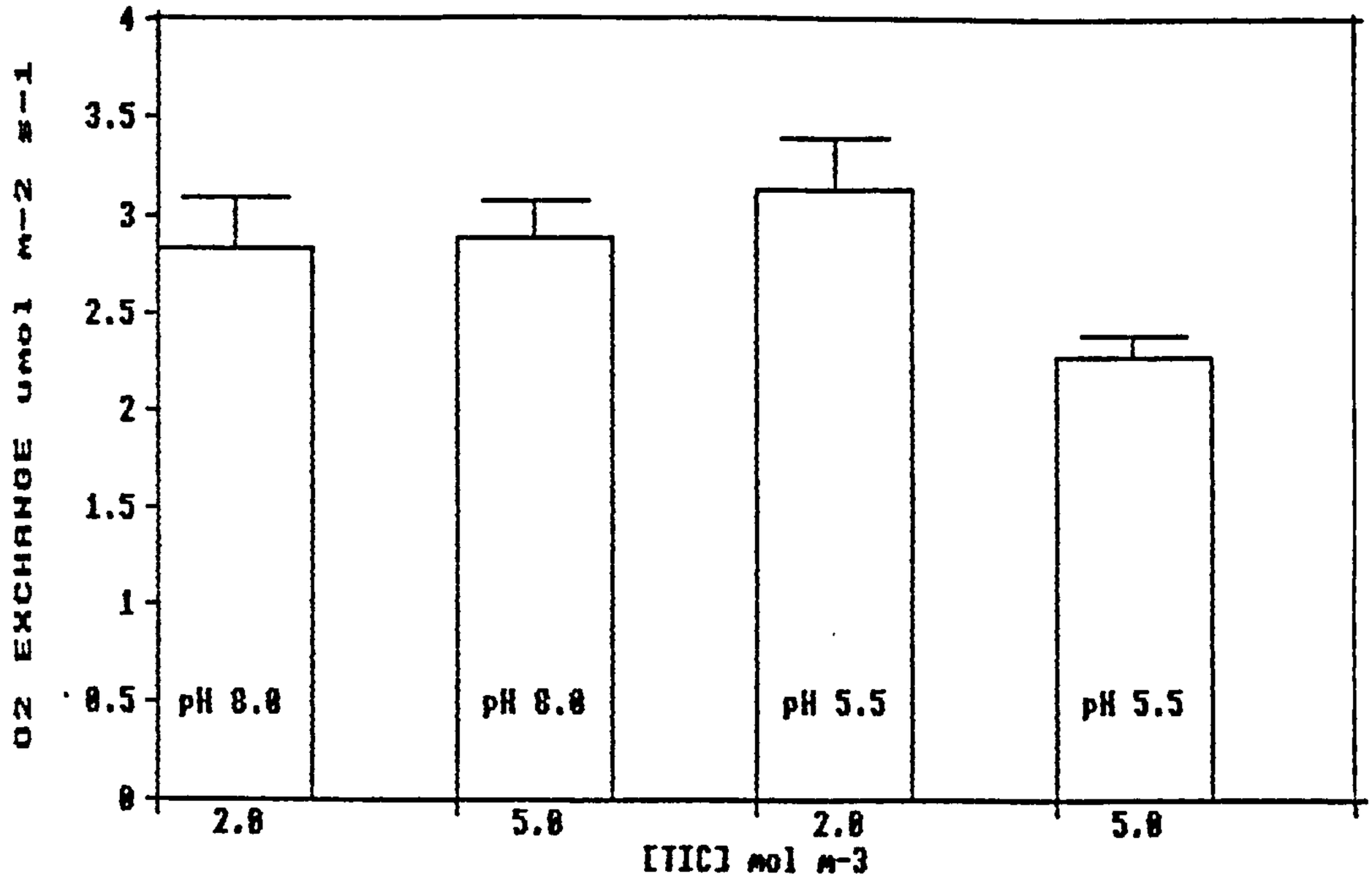

Figure 1. Effect of pH dependent substrate supply on the capacity for apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater, measured at 2.0 and 5.0 mol m^{-3} TIC. Data represents the mean ± SE of three replicates.

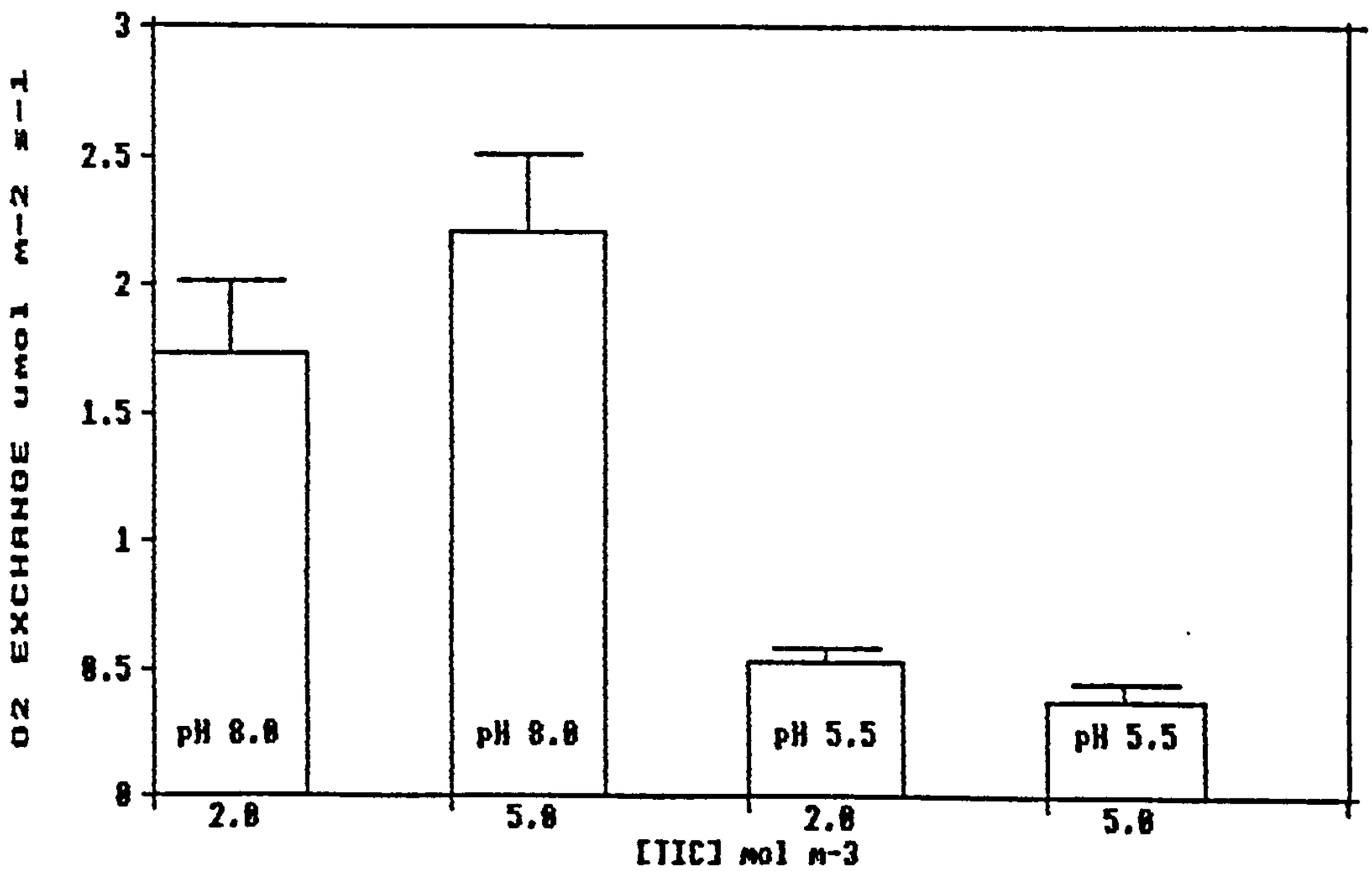

Figure 2. Effect of pH dependent substrate supply on the capacity for apparent photosynthetic O_2 exchange by *U.lactuca* in seawater, measured at 2.0 and 5.0 mol m^{-3} TIC. Data represents the mean ± SE of three replicates.

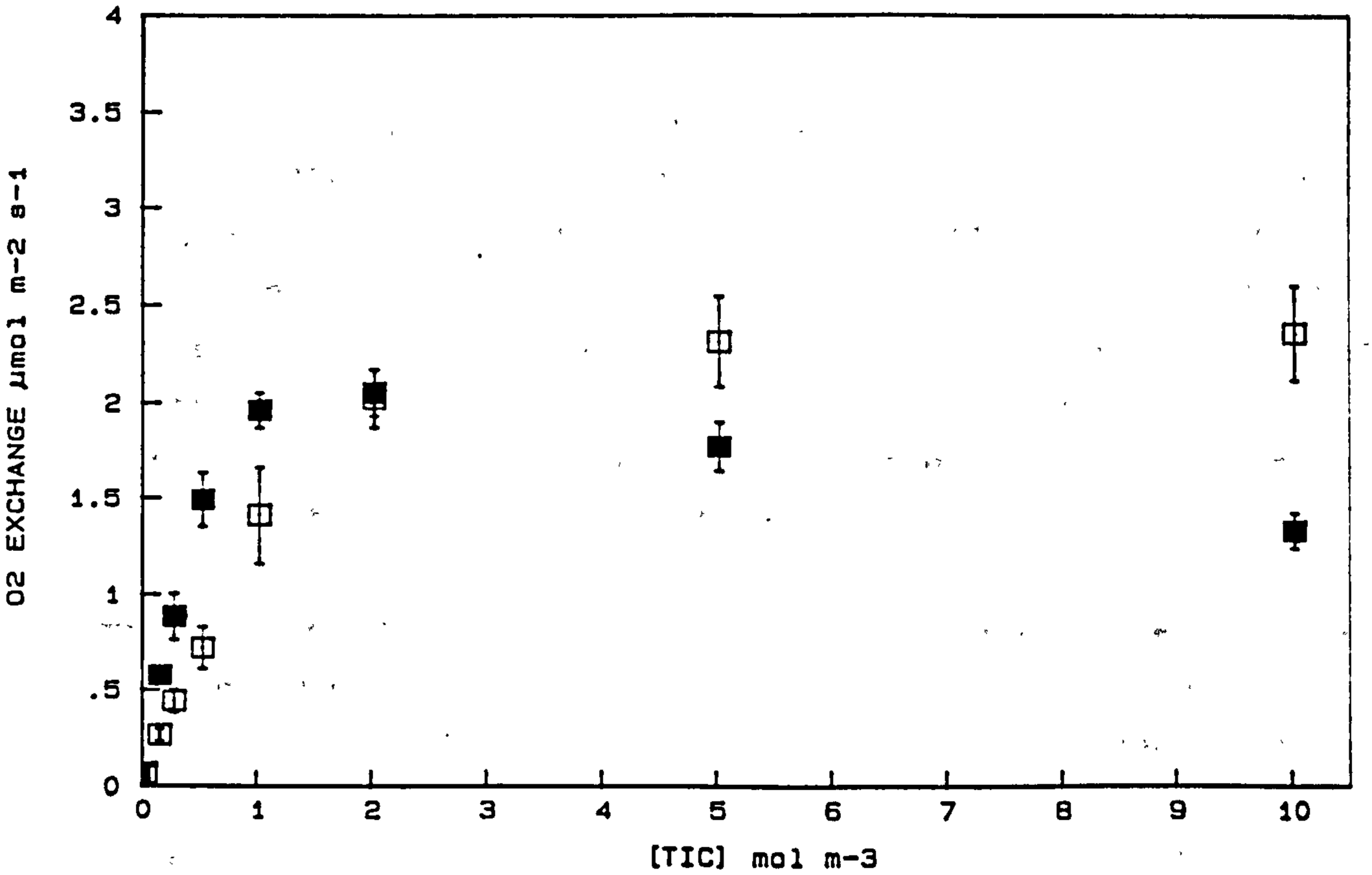

Figure 3. Rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of TIC concentration measured at pH 8.0 and pH 5.5. Data represents the mean ± SE of three replicates.
pH 8.0 (□); pH 5.5 (■).

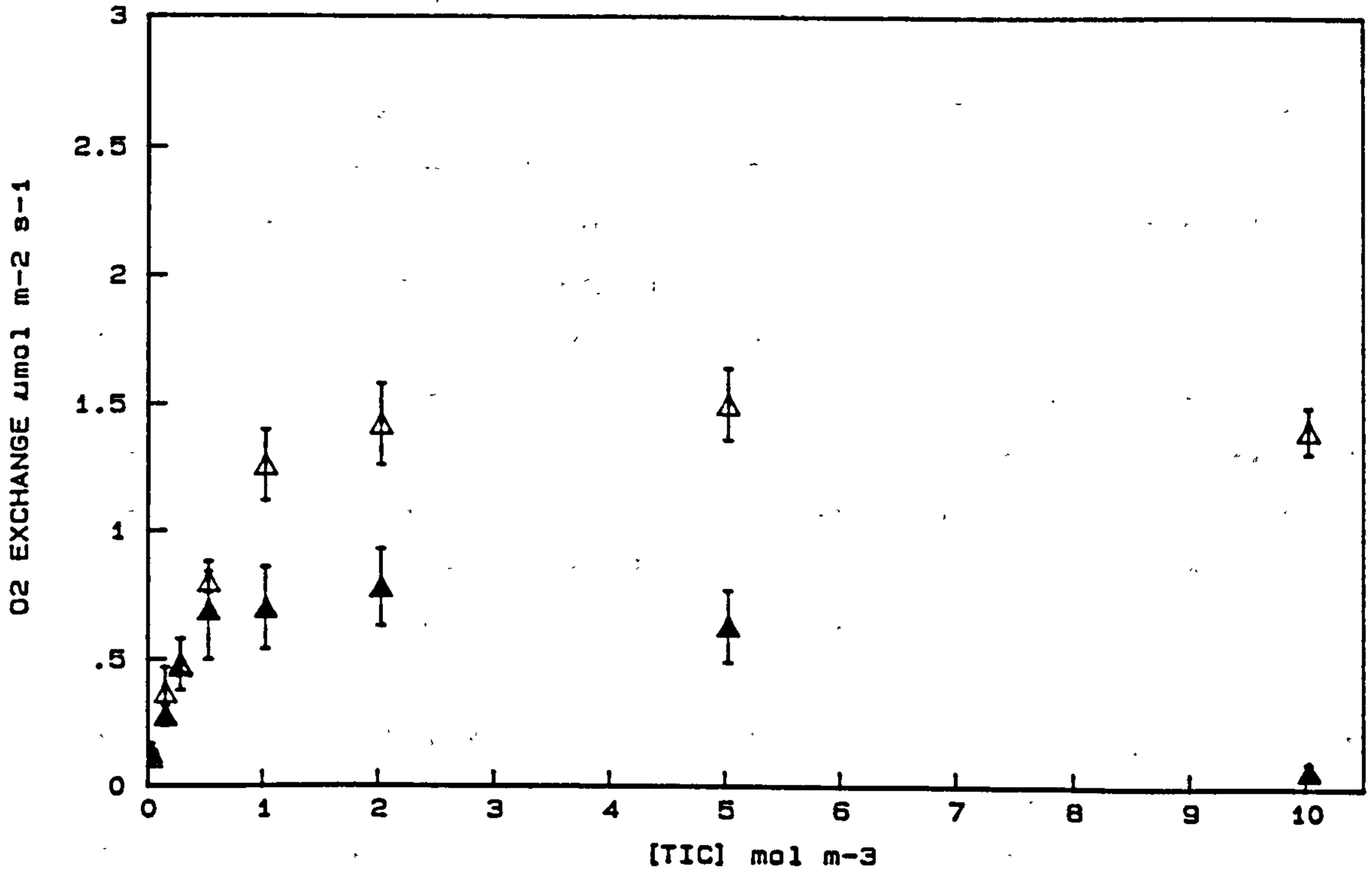

Figure 4. Rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of TIC concentration measured at pH 8.0 and pH 5.5. Data represents the mean ± SE of three replicates.pH 8.0 (Δ); pH 5.5 (▲).

m^{-3} with a V_{max} of 1.47 μmol O_2 m^{-2} s^{-1}, saturated at 2.0 mol m^{-3} DIC. At pH 5.5 the $K_{0.5}$(TIC) is 127 mol m^{-3} and the V_{max} only 0.84 μmol O_2 m^{-2} s^{-1}. Saturation occurred at the same substrate concentration as at pH 8.0. In addition there was a further decrease in the photosynthetic capacity as the concentration of CO_2 at pH 5.5 increased. The diffusion resistance was lower at the higher pH increasing from 2.0 to 14.0 m^{-2} s^{-1} at pH 5.5.

The effect of 100 mmol m^{-3} AZ on photosynthesis at pH 5.5

For *P.umbilicalis* at pH 8.0 the maximum rate or capacity of 1.87 μmol O_2 m^{-2} s^{-1} was almost completely inhibited by 100 mmol m^{-3} AZ (Fig 5). This resulted in a rate of 0.31 μmol O_2 m^{-2} s^{-1} under conditions where the concentration of CO_2 was around 0.02 mol m^{-3}. At pH 5.5 the control rate was similar to that at pH 8.0 but, importantly, inhibition by 100 mmol m^{-3} AZ was not as severe. The rate of 1.28 μmol O_2 m^{-2} s^{-1} coincided with the higher proportion of free CO_2 present at this pH (Fig 5).

The full concentration-response of *P.umbilicalis* under the same high AZ concentration also reveals that at pH 5.5 this inhibitor has little effect. This is in contrast to the concentration-response following AZ inhibition at pH 8.0 (Section 2-Figure 1). The presence of high levels of CO_2 at pH 5.5 appeared to alleviate the requirement for external CA in the uptake of inorganic carbon (Fig 6). There was no significant effect on the response with the maximum capacity of 1.6 μmol O_2 m^{-2} s^{-1} saturated at 2.0 mol m^{-3}. At 5.0 mol m^{-3} TIC and above, both the control and inhibitor treated concentration-response was inhibited by the high CO_2 concentration present (Fig 6). This is consistent with the results shown in Figure 3.

The effect of 100 mmol m^{-3} EZ on photosynthesis at pH 5.5

The increased affinity for CO_2 was again evident from the response to inhibition by 100 mmol m^{-3} EZ. The rates achieved following inhibition, of between 0.16 and 0.28 μmol O_2 m^{-2} s^{-1} at 1.0 and 2.0 mol m^{-3} TIC represent 20% of the maximum capacity. In comparison, inhibition by 100 mmol

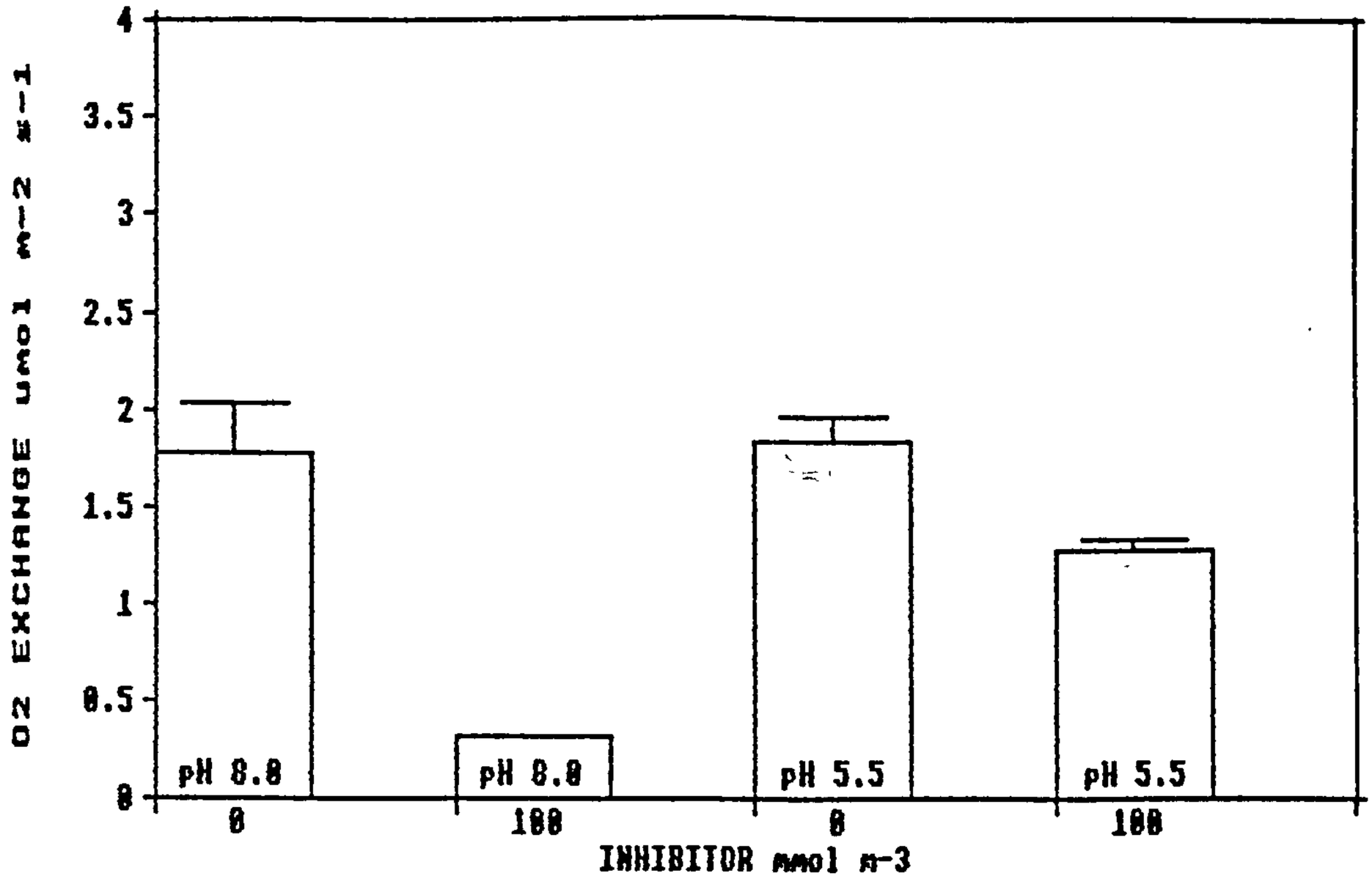

Figure 5. pH dependence of the effect of 100 mmol m^{-3} AZ on the capacity for apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater. Data represents the mean ± SE of three replicates.

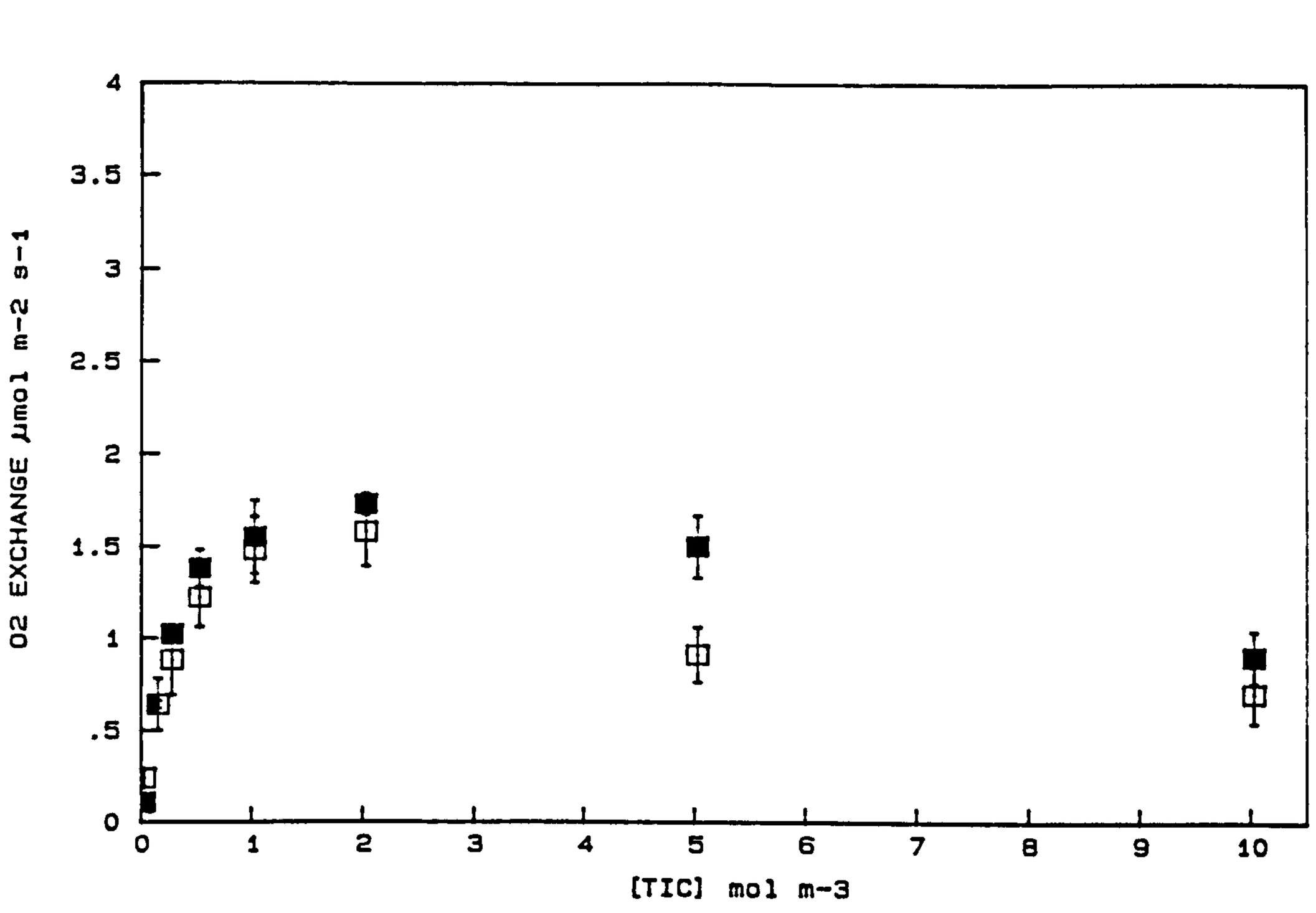

Figure 6. Effect of 100 mmol m^{-3} AZ on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of TIC concentration at pH 5.5. Data represents the mean ± SE of three replicates.
Control (■); AZ treated (□).

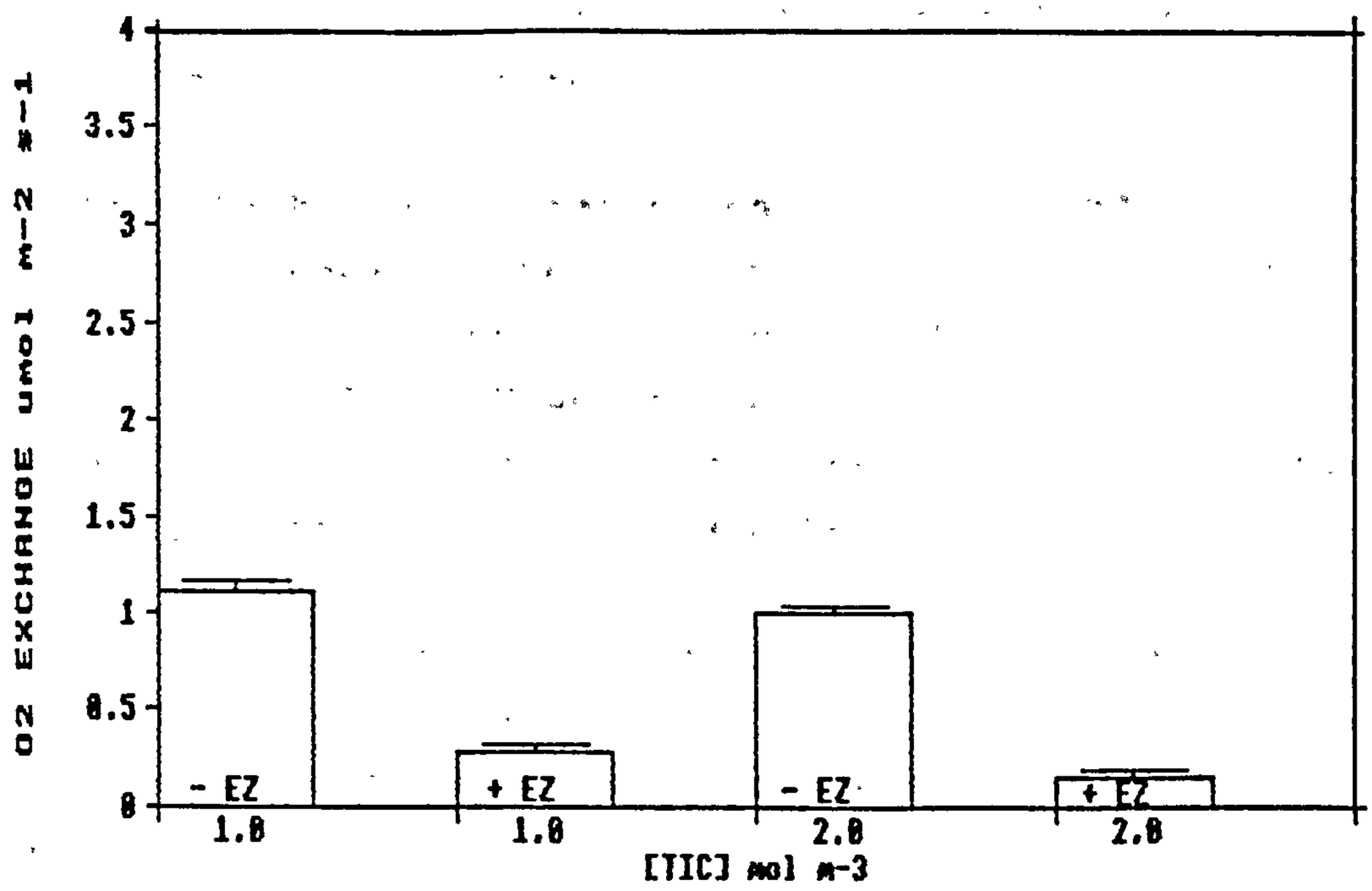

Figure 7. Effect of 100 mmol m^{-3} EZ on the capacity for apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater at pH 5.5. Data represents the mean ± SE of three replicates.

m^{-3} AZ at pH 5.5 gave rates that were not significantly different to the controls.

Photosynthetic characteristics at pH 7.5 and pH 8.5

Differences in the proportion of the carbon species at pH 7.5 and pH 8.5 are less distinct than at the two more extreme pH values. However the results still showed a significant variation in the concentration-response. It is unlikely that there would have been any direct effect of pH on photosynthesis at values between 7.5 and 8.5. Any differences in the responses reflect the altered proportions of the available inorganic carbon species.

AZ inhibition at pH 7.5 decreased the concentration response of *P.umbilicalis* (Fig 8). The curve showed a substrate limited rate of inorganic carbon uptake at a concentration below 20 mol m^{-3} TIC. Above this level the substrate saturation of the rate resulted in a maximum capacity equivalent to that of the control. At pH 8.5 the inhibition resulted in a linear response to inorganic carbon concentrations below 10 mol m^{-3}. Saturation was achieved around 25 mol m^{-3} TIC and again the maximum rate was comparable to that of the control (Fig 9). At the lower pH it is evident from both the substrate limited and substrate saturated rates that the higher level of CO_2 is significant.

The response to EZ was similar, although the effect of this inhibitor was greater than that of AZ (Figs 10 & 11). As above, it was evident that the substrate affinity of *P.umbilicalis* was higher at pH 7.5 than at 8.5 and this was reflected by the effect of the CA inhibitor under these conditions. At pH 7.5 the response was saturated by 5.0 mol m^{-3} TIC and at this concentration the rate was equal to that of the control (Fig 10). At pH 8.5 the inhibited response required a concentration of 40.0 mol m^{-3} TIC to saturate inorganic carbon uptake. In contrast to the response at pH 7.5, the rate at 5.0 mol m^{-3} TIC was less than 12% of the control (Fig 11). As shown previously, at pH 7.5 the level of CO_2 at concentrations of 10 mol m^{-3} TIC

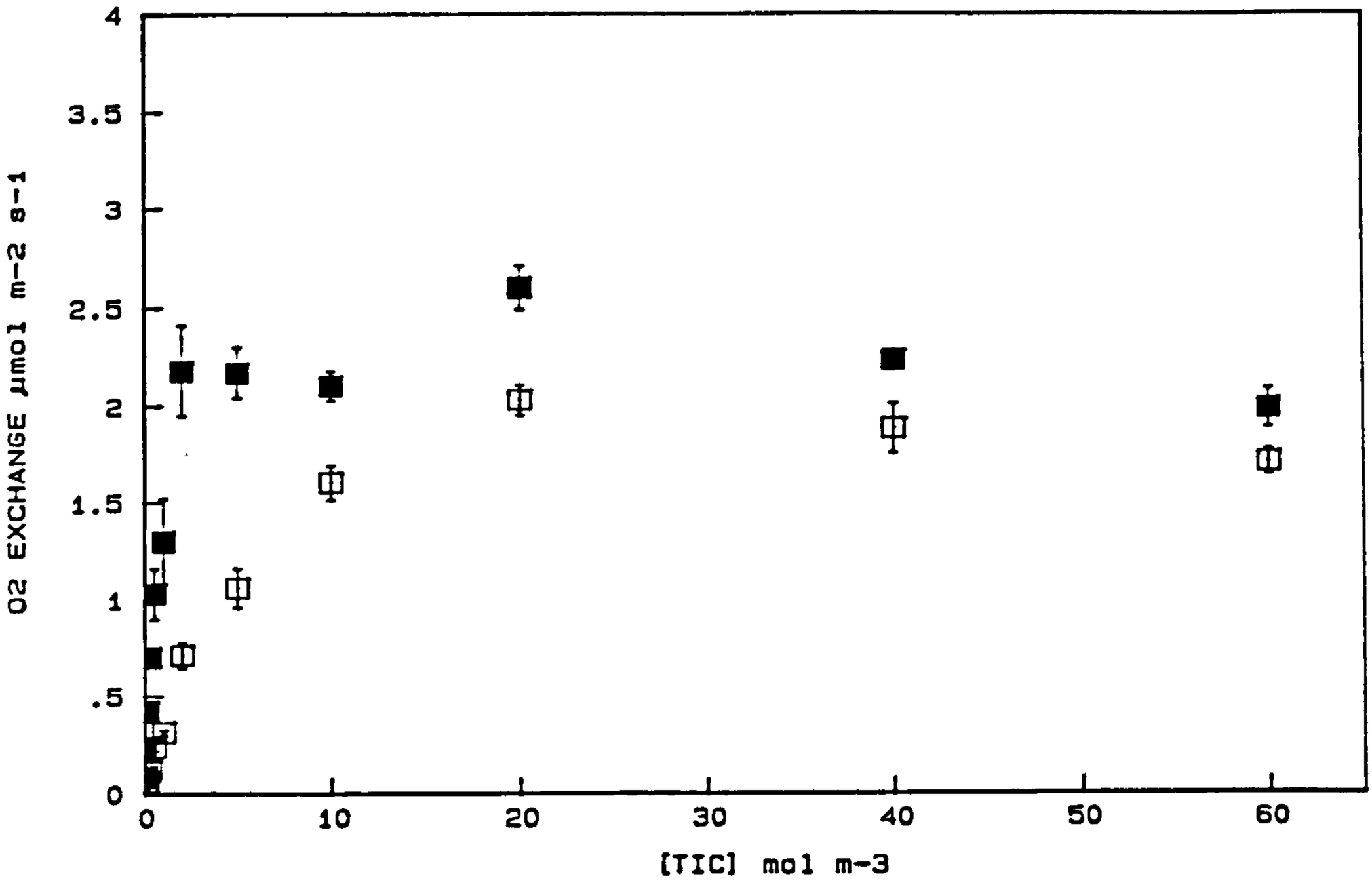

Figure 8. Effect of 100 mmol m^{-3} AZ on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of the TIC concentration at pH 7.5. Data represents the mean ± SE of three replicates. Control (■); AZ treated (□).

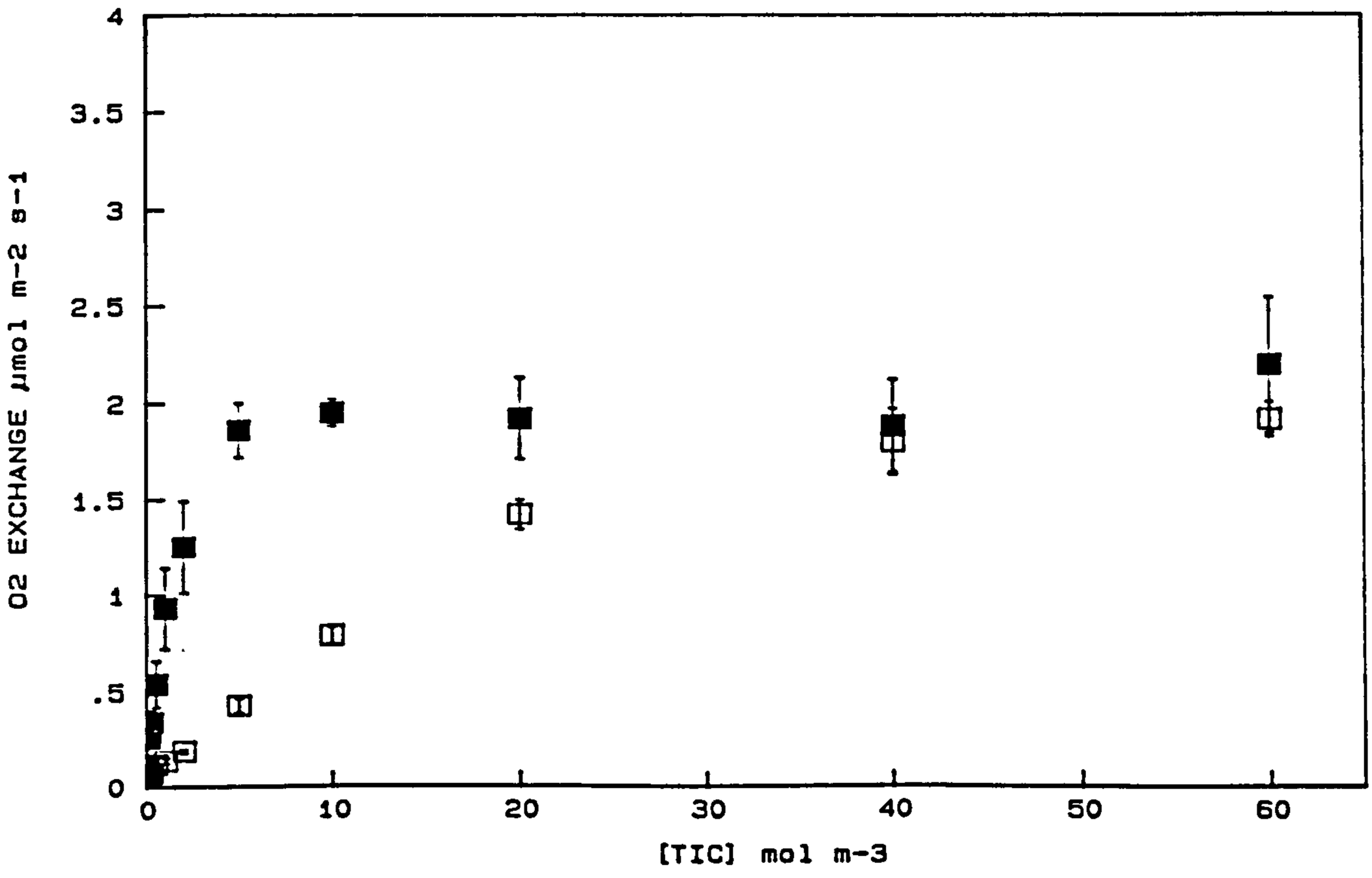

Figure 9. Effect of 100 mmol m^{-3} AZ on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of the TIC concentration at pH 8.5. Data represents the mean ± SE of three replicates. Control (■); AZ treated (□).

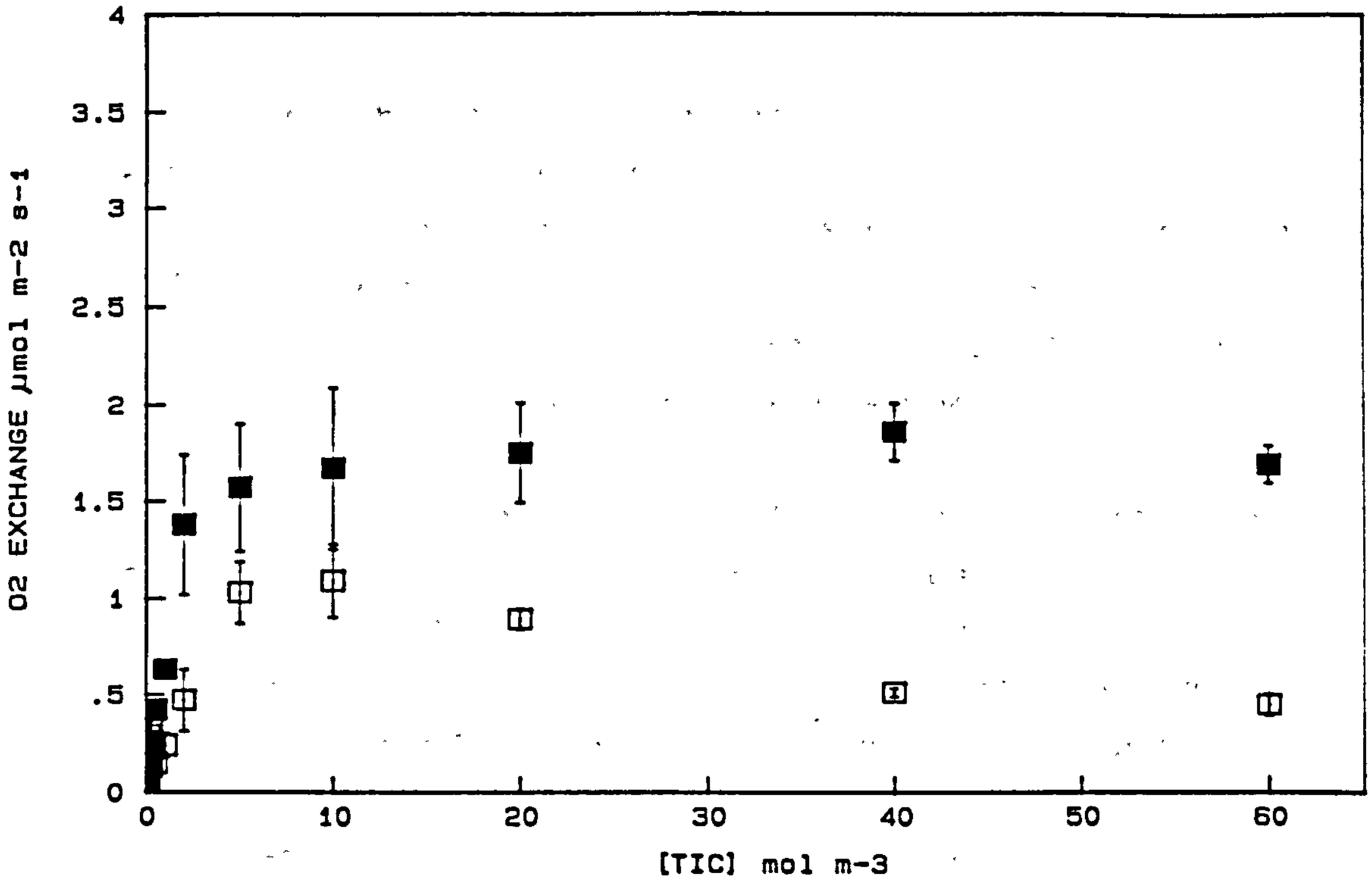

Figure 10. Effect of 100 mmol m^{-3} EZ on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of the TIC concentration at pH 7.5. Data represents the mean ± SE of three replicates. Control (■); EZ treated (□).

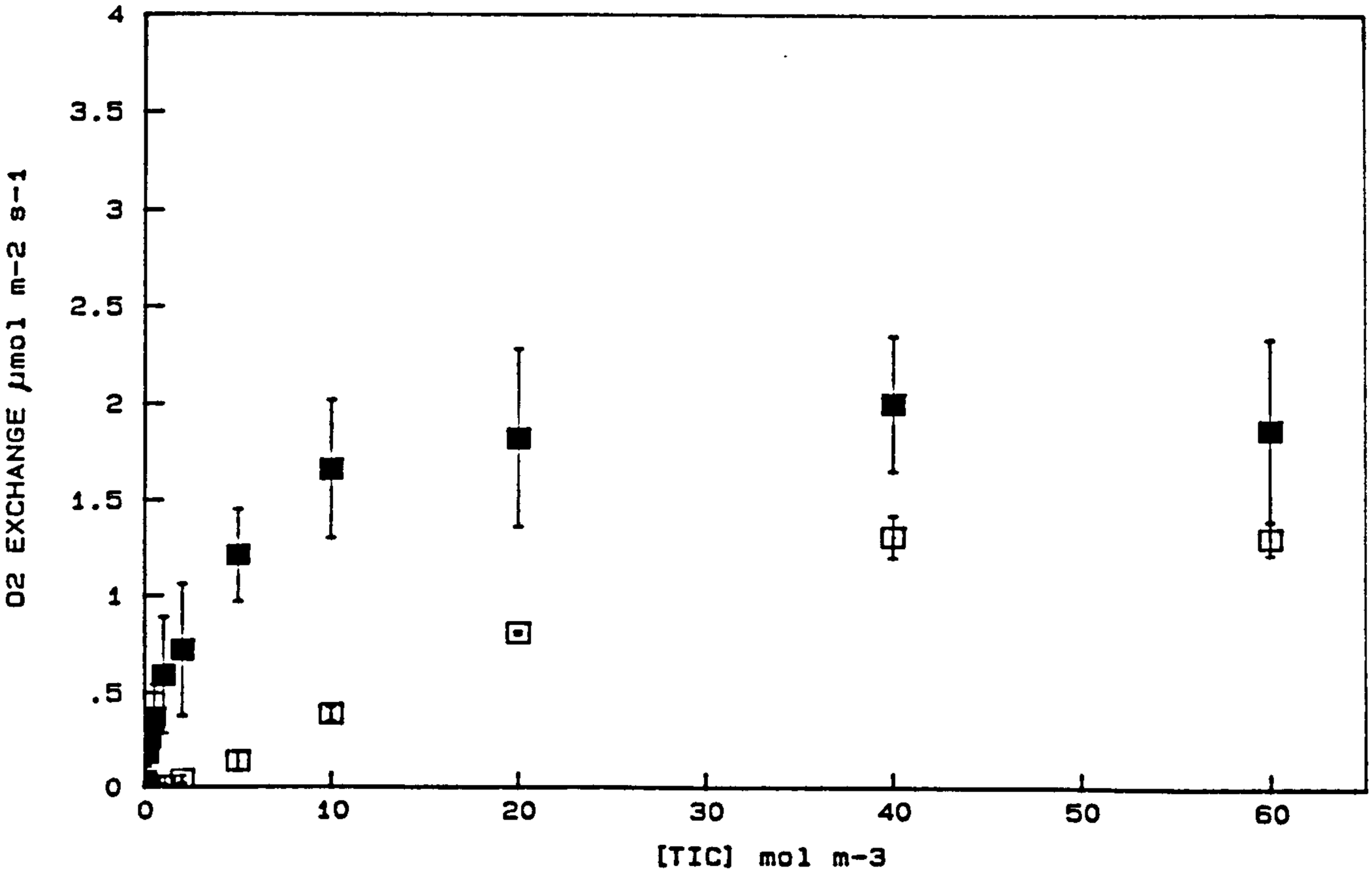

Figure 11. Effect of 100 mmol m^{-3} EZ on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater as a function of the TIC concentration at pH 8.5. Data represents the mean ± SE of three replicates. Control (■); EZ treated (□).

and above inhibited the inorganic carbon concentration-response of photosynthesis (Figs 6 & 9).

For *U.lactuca* both the maximum rate and the apparent affinity for the substrate was greater than at the lower pH value, as was evident for *P.umbilicalis*. As expected addition of AZ did not inhibit the photosynthetic response at either pH 7.5 or 8.5 (Figs 12 & 13).

EZ inhibition drastically reduced the photosynthetic response with little difference between the inhibited rates at either pH (Fig 14 & 15). At 10.0 mol m^{-3} TIC the rates were less than 15% of the maximum and at the highest concentration of 60 mol m^{-3} TIC were only around 30% of the control. Saturation of the response at pH 7.5 occurred at 20 mol m^{-3} TIC, while at pH 8.5 the response remains linear. Unlike *P.umbilicalis*, the effect of an increase in the substrate concentration on the EZ inhibited reponse did not reflect the higher concentration of CO_2 available. It is possible, however, that the apparent degree of inhibition may be due in part to the narcotic effect, which would mask the response to an increase in the levels of CO_2.

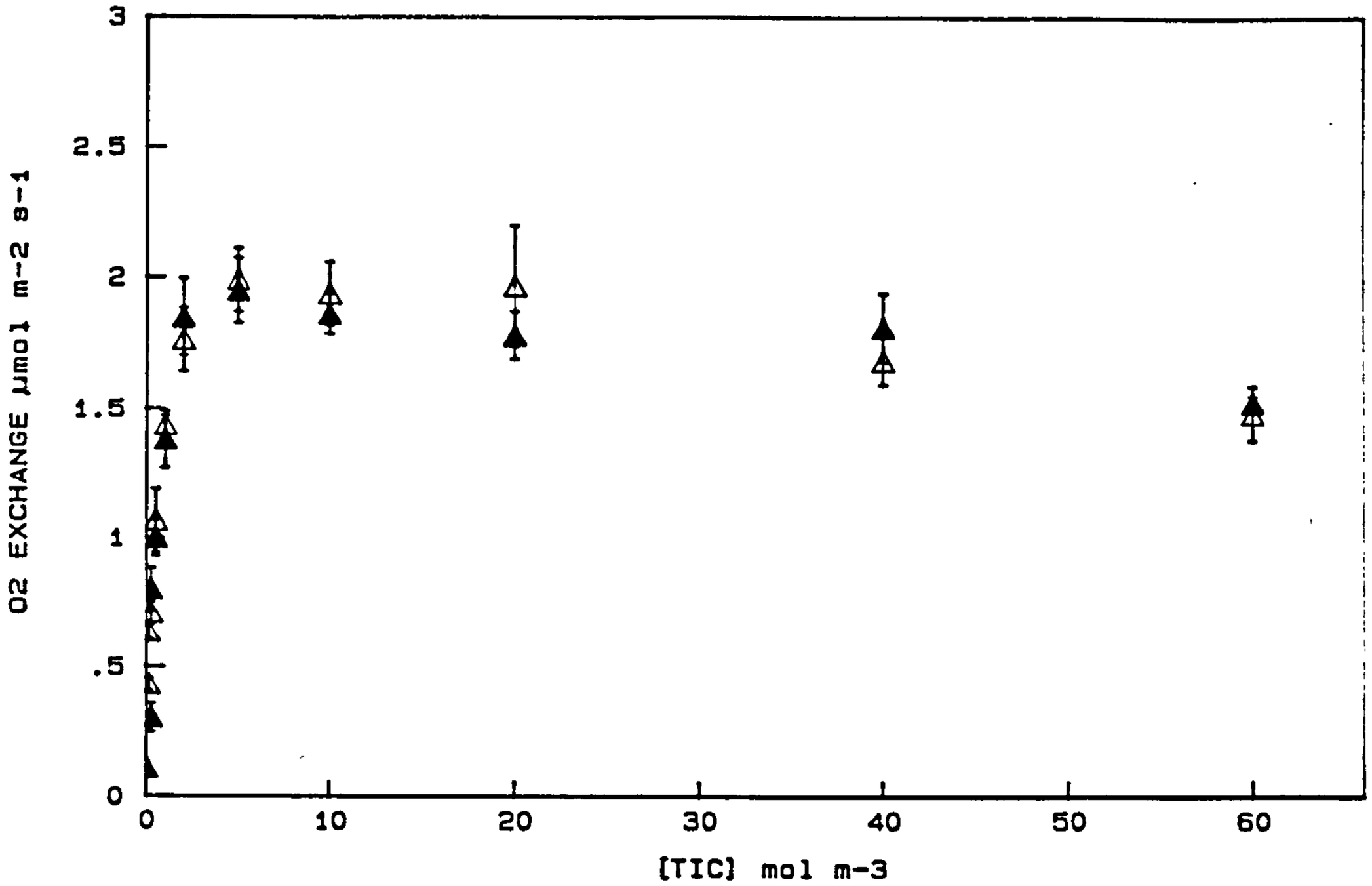

Figure 12. Effect of 100 mmol m^{-3} AZ on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of the TIC concentration at pH 7.5. Data represents the mean ± SE of three replicates. Control (▲); AZ treated (Δ).

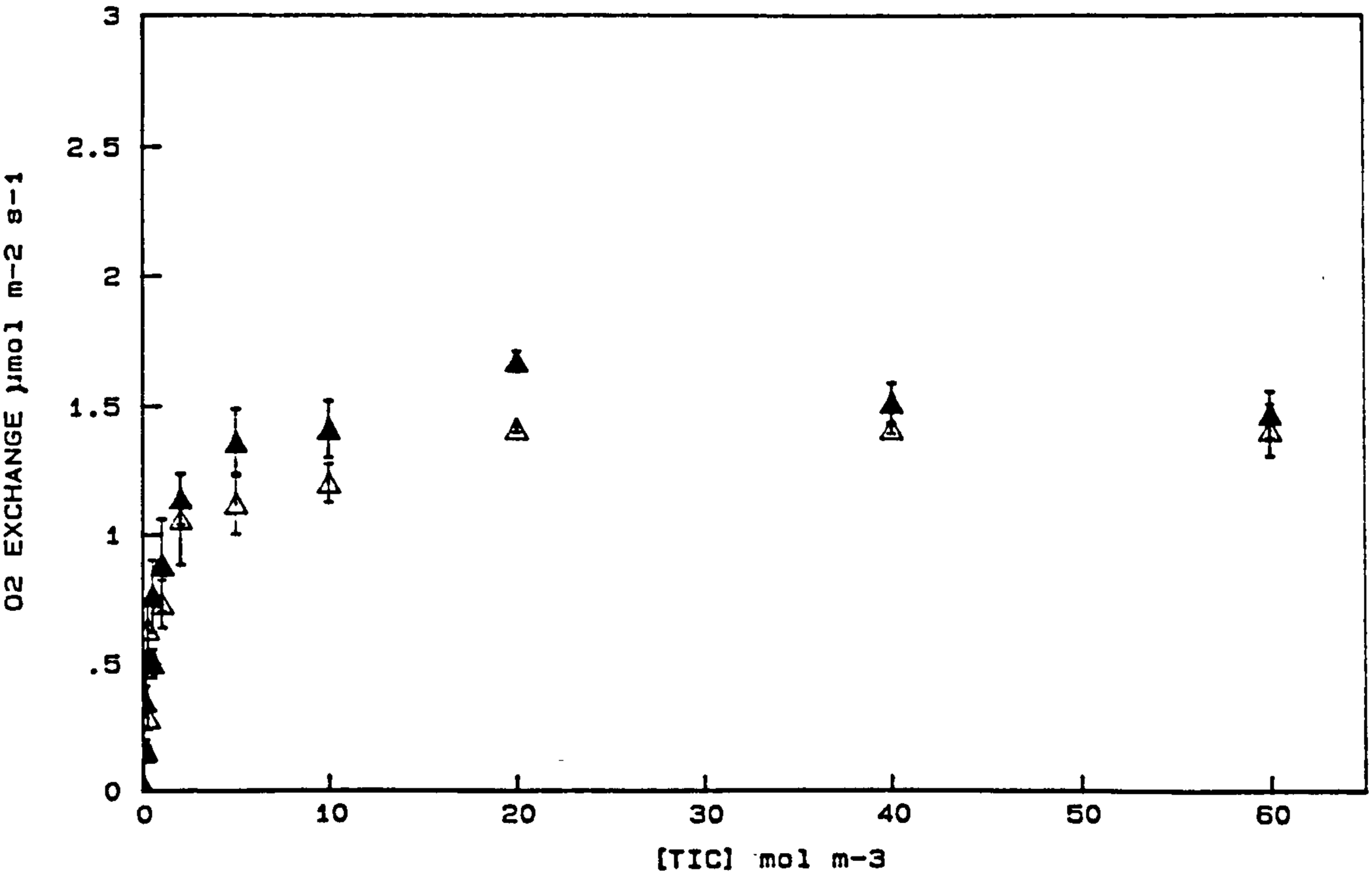

Figure 13. Effect of 100 mmol m^{-3} AZ on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of the TIC concentration at pH 8.5. Data represents the mean ± SE of three replicates. Control (▲); AZ treated (Δ).

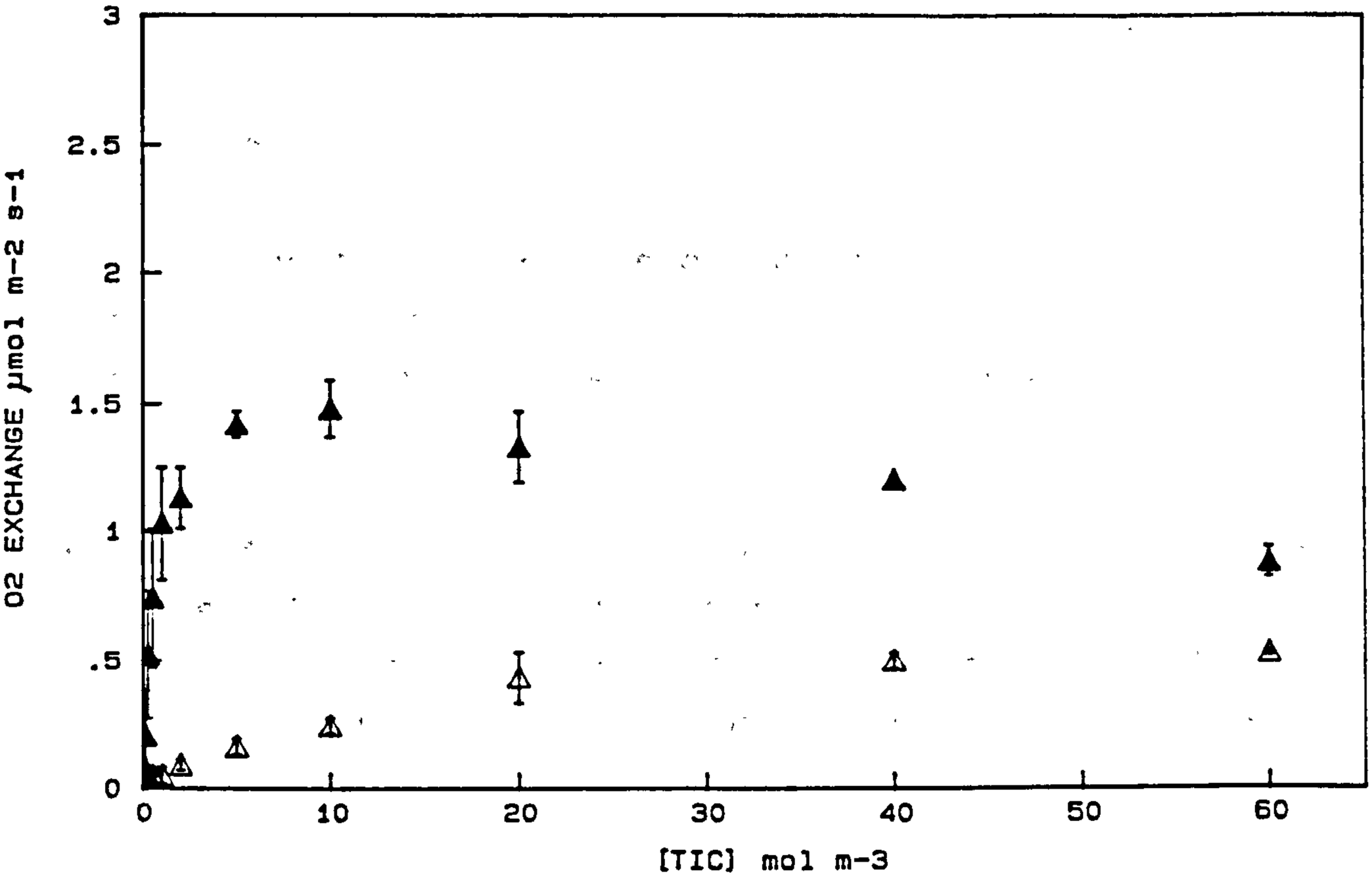

Figure 14. Effect of 100 mmol m^{-3} EZ on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of the TIC concentration at pH 7.5. Data represents the mean ± SE of three replicates. Control (▲); EZ treated (Δ).

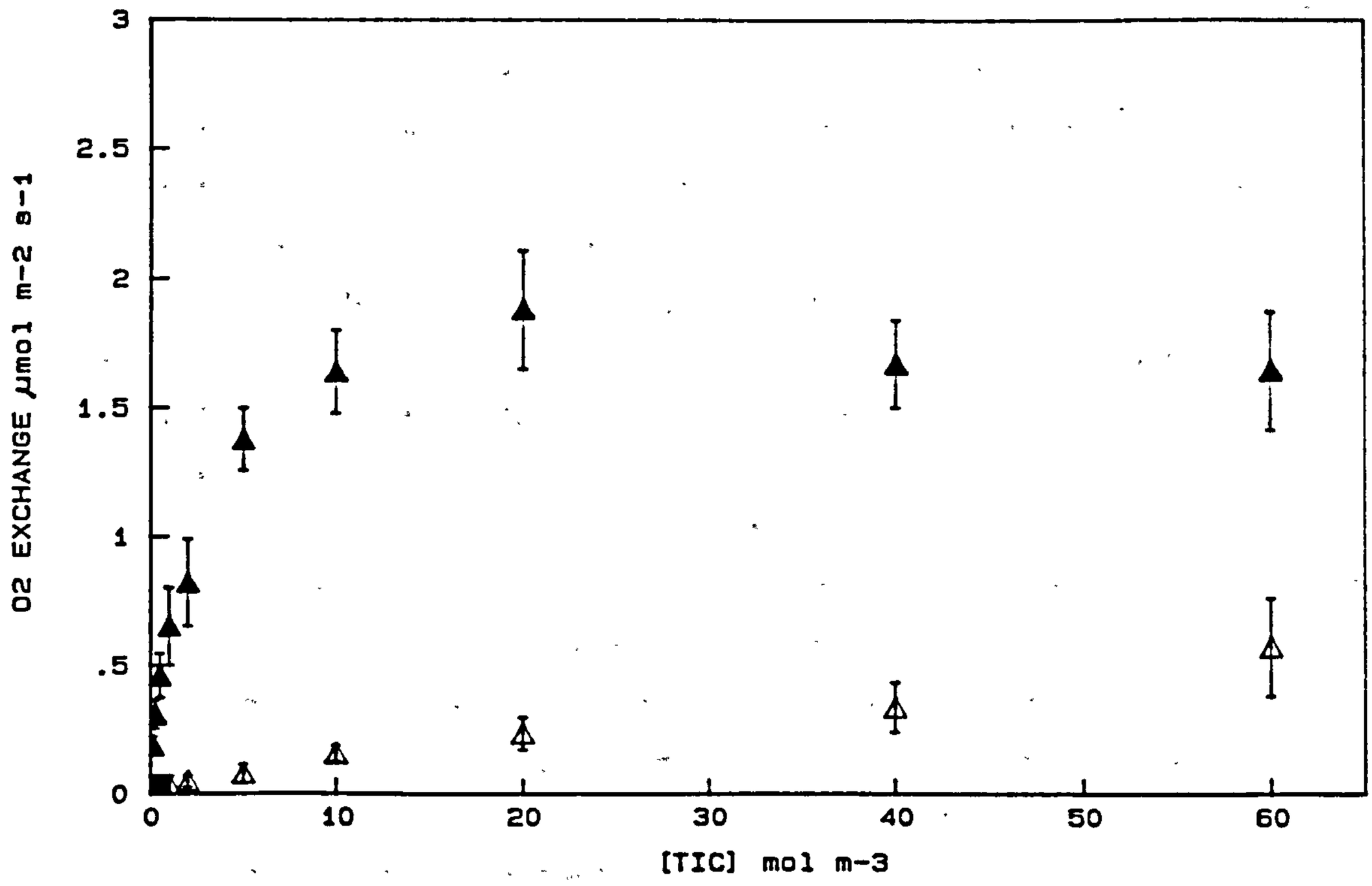

Figure 15. Effect of 100 mmol m^{-3} EZ on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater as a function of the TIC concentration at pH 8.5. Data represents the mean ± SE of three replicates. Control (▲); EZ treated (Δ).

DISCUSSION

Using the properties of the carbonate system, it is possible to investigate the nature of the inorganic carbon uptake mechanisms, in terms of the preferred carbon source or a requisite for CA activity.

The photosynthetic capacity of *P.umbilicalis* measured at pH 5.5 and 8.0 shows that either CO_2 or HCO_3^- may be utilized (Figs 1 & 3). In contrast, for *U.lactuca* it is evident that the maximum rate of fixation is limited when CO_2 is the predominant ion, indicating that HCO_3^- may be the form of inorganic carbon taken up (Figs 2 & 4). It is possible however, that the results for *U.lactuca* reflect a sensitivity to the change in pH *per se*.

For *P.umbilicalis* there appeared to be no difference in the ability to use CO_2 and HCO_3^-. At both pH 5.5 and 8.0 the saturated rates and substrate concentration required to achieve these are similar. Analysis of the substrate concentration-responses, however, shows a greater affinity for CO_2 than for HCO_3^- (Fig 3). In contrast, the $K_{0.5}$(TIC) values for *U.lactuca* denotes that the affinity for HCO_3^- is greater, and the photosynthetic capacity at pH 5.5 reflects an inability to use CO_2 effectively (Figs 2 & 4). In addition the concentration-response reveals a significant degree of inhibition when the levels of CO_2 are high. Although this effect can be seen in the response of *P.umbilicalis*, the degree of inhibition is substantially greater in *U.lactuca*. The narcotic effects of high CO_2 have been pointed out by a number of studies, although there was no explanation for this effect (Jones and Osmond 1973; MacFarlane and Raven 1985; Robe and Griffiths 1988).

Few studies have sought to characterize the nature of carbon accumulation in *P.umbilicalis*. The most relevent work for comparison is that of Smith and Bidwell (1989a;b) carried out with the red macroalgae *Chondrus crispus*. The data supports the view that this species acquires inorganic carbon for photosynthesis as CO_2. In contrast there have been a number of studies carried out on various *Ulva*

species, the latest of which supports the hypothesis that direct HCO_3^- uptake is important (Beer and Israel 1990).

The $K_{0.5}$ values for *Codium decorticatum* and *Udotea flabellum* at pH 8.0, calculated using the Michaelis-Menten equation, are well above those determined for *U.lactuca* and *P.umbilicalis* by this study (Reiskind, Seamon and Bowes 1989). The former values would be considerably lower if they were corrected for diffusion resistance. At pH 5.5 *U.flabellum* shows a very high affinity for CO_2, with a $K_{0.5}(CO_2)$ value that approximates to that of free CO_2 in seawater. The corresponding maximum rate is less than half that when HCO_3^- is available. For *C.decorticatum* the equivalent concentration is 35 times greater than that of CO_2 in seawater. From the shape of the response it appears that an even greater degree of diffusion resistance may be restricting the inorganic carbon uptake at this pH. The concentration-response curves for *U.lactuca* and *P.umbilicalis* were analysed using the Hill-Whittingham equation which defines the degree of diffusion resistance inherent in the response. From this it is possible to determine the boundary layer thickness and the maximum rate of uptake by passive diffusion of CO_2. The Pu values determined suggest that potentially both species have a similar ability to take up HCO_3^-. In *U.lactuca* the degree of resistance to CO_2 uptake is much greater than for HCO_3^-, which is consistent with the earlier observations in this study.

The apparent capacity of *P.umbilicalis* to use both HCO_3^- and CO_2 provides some insight into the mechanism of inorganic carbon uptake. The investigation was taken one step further, by using inhibitors of internal and external CA, to determine the role of these isoenzymes in determining the photosynthetic characteristics.

The differential effect of AZ at the pH 5.5 and 8.0 shows that HCO_3^- use is dependent on an external CA, while the uptake of CO_2 is not reliant on the activity of this enzyme (Fig 5). The concentration-response measured at pH 5.5 shows no significant decrease in photosynthetic efficiency or capacity following inhibition of the

extracellular enzyme. There are three possible explantions for these results: that AZ is inactive at this pH and so has no effect on the enzyme; that external CA is not required for the uptake of CO_2; or at this pH there is some stimulation of the enzyme activity, and CA is not completely inhibited. Many other studies have investigated the effects of AZ at pH 5.5, but as for *P.umbilicalis* the results obtained do not directly confirm that the inhibitor was effective. If we assume however, that AZ does completely inhibit external CA, then CO_2 uptake is not affected by inactivation of this enzyme. It appears that, at this pH, the process of direct CO_2 uptake is capable of maintaining observed rates of photosynthesis (Fig 6).

In contrast, the membrane permeable inhibitor EZ reduced the photosynthetic capacity at pH 5.5 to about 20% of the maximum, inhibiting both an internal and external CA (Fig 7). The substrate concentrations used were lower than 3.0 mol m^{-3}, to eliminate any inhibitory effect of high levels of CO_2 evident from the earlier concentration response curves (Figs 2-6). From these results it is possible to prove two important points; if EZ is inhibitory at this pH it is likely that AZ would also be functional; also that the two inhibitors are specific in their mode of action, inhibiting either the external or both external and internal enzymes. In addition, there is no evidence for an increase in the activity of CA that would results in an incomplete inhibition of the enzyme. The remaining rate appears to be proportional to the diffusive uptake of CO_2 rather than a residual component of an active uptake process.

It is evident from this study that in *P.umbilicalis* the prerequisite for an external CA can be overcome by the increase in CO_2, but that an internal CA is required to maintain the maximum capacity in both predominantly HCO_3^- and CO_2 conditions. Thus it appears that HCO_3^- use depends on the dehydroxylation by CA at the cell surface and that only CO_2 is able to cross the plasma membrane. This system of inorganic carbon uptake is analagous to that proposed for *Chlamydomonas reinhardtii* (Moroney, Husic and Tolbert

1985). Their results using AZ and EZ at pH 5.5 and 8.0 are consistent with CO_2 uptake at the plasma membrane supplied as free CO_2, or by the catalysed conversion of external CA. Internal CA is required to transfer inorganic carbon to the site of fixation probably facilitated by HCO_3^- transport across the chloroplast membrane.

This mechanism does not appear to be universal among macroalgae. Reiskind, Seamon and Bowes (1989) used similar experimental conditions with *C.decorticatum* and *U.flabellum*. They found that at pH 5.5 the addition of EZ did not severely inhibit photosynthesis in either species, and even at pH 8.0 this inhibitor only reduced the maximum rate of *U.flabellum* by only 39%. It was not possible to ascertain much more about the mechanism of inorganic carbon uptake in the absence of a response to AZ. Work by Beer and Israel (1990) with *U.faciata* strongly suggests a mechanism involving the uptake of the HCO_3^- ion. Under natural conditions this is facilitated by a very high surface pH, although they do not exclude the possibility of CO_2 use under certain circumstances.

The effect of differences in the substrate concentration at pH 7.5 and 8.5 was less pronounced than that between pH 5.5 and pH 8.0. At the less extreme values the proportions of inorganic carbon as HCO_3^- is similar, while at pH 7.5 the concentration of CO_2 is 10 times greater than at pH 8.5. Under more alkaline conditions there is an increase in CO_2^{3-}, while CO_2 is absent. The advantage of using less extreme pH values was that the results were less likely to be complicated by the effect of pH on metabolism, although there was still some evidence of the high CO_2 narcosis found at pH 5.5.

For *P.umbilicalis* a general comparison of the response at pH 7.5 and 8.5 shows that the increase in CO_2 gives rise to a greater affinity for inorganic carbon. As a consequence there is marked difference in the effect of the inhibitors on the substrate limited response at the two pH values. At the concentrations of AZ and EZ used, the enyzme activity was completely inhibited, and the rates of photosynthesis were directly proportional to the external

concentration. Initially the difference in levels of CO_2 determines the difference in the photosynthetic response. Concentrations of inorganic carbon well in excess of that required for saturation were high enough to overcome the dependence on the external CA to supply CO_2 from the bulk medium. These levels also reduced the observed requirement for an internal enzyme to transfer CO_2 to the site of fixation (Section 2-Figure 6). The final photosynthetic capacities achieved at pH 8.5 and 60 mol m^{-3} TIC were also equal to that of the controls, following both AZ and EZ inhibition. However, at pH 7.5 it appears that EZ inhibition may result in the build up of a high internal concentration of inorganic carbon that could explain the narcotic effects seen throughout this section.

With *U.lactuca* the increase in the concentration of CO_2 also effects the apparent substrate affinity. In contrast to *P.umbilicalis*, this does not appear to reflect a higher affinity for CO_2. The effect of EZ inhibition was to substantially decrease the photosynthetic response, and the inhibition was not alleviated by an increase in the substrate concentration. Conversely, high levels of inorganic carbon, predominantly CO_2, significantly inhibit the maximum capacity. As for *P.umbilicalis*, this may reflect a high internal concentration of inorganic carbon that result in inhibition of photosynthesis *per se*. These results qualify the earlier conclusions that there is no efficient mechanism of direct CO_2 use in *U.lactuca*.

The conclusions from this section confirm that two distinct mechanisms of inorganic carbon accumulation have evolved in *P.umbilicalis* and *U.lactuca*.

In the previous section it is evident that HCO_3^- and CO_2 use by *P.umbilicalis* is dependent on the activity of one or more CA enzymes. Inhibition of the external activity substantially reduces the initial transfer across the plasma membrane. Addition of the permeable inhibitor also affects CA located internally, increasing the effect on the photosynthetic response. The results from this section confirm that inorganic carbon uptake relies on both an external and internal CA, and that the catalysed conversion

of HCO_3^- at the plasma membrane may be the rate limiting step. They also show that the affinity of CO_2 is higher that that of HCO_3^-. This confirms that in *P.umbilicalis*, CO_2 is the preferred carbon species and that external CA serves to increase the rate of CO_2 uptake. The synergistic effect of EZ inhibition indicates that the transfer of substrate to the carboxylase is aided by the activity of an internal CA. As discussed in Section 2, it is not possible to determine the exact location or corresponding function from these results.

In contrast for *U.lactuca*, although there is some evidence of enhanced photosynthetic response at pH 7.5 the earlier results show that the affinity for HCO_3^- is greater than for CO_2. It is also evident that this species is unable to to directly use CO_2 effectively. As demonstrated in the previous section, inorganic carbon uptake is independent of any external CA activity, although as for *P.umbilicalis*, there is an important requirement for this enzyme located internally. Uptake of inorganic carbon by *U.lactuca* appears to be facillitated by direct HCO_3^- use which would require active transport across the plasma membrane. It is possible that the same mechanism may also regulate CO_2 uptake, possible involving a CA-like moiety as part of the process. The requirement for an internal CA however, appears to be similar to that for *P.umbilicalis*.

INTRODUCTION

Various mechanisms of inorganic carbon accumulation in microalgae have been proposed. These include HCO_3^- uptake facilitated by external CA as for *Chlamydomonas reinhardtii* (Moroney, Husic and Tolbert 1987), or direct HCO_3^- use as in *Porphyridium purpureum* (Dixon, Patel and Merrett 1987). In both these systems there is evidence of CA located internally. This enzyme is also required to facilitate the decarboxylation of HCO_3^- to provide CO_2 at the site of fixation by RuBPco. In contrast, *Chlorella saccarophilla* shows a preference for direct uptake of CO_2 (Gehl, Cook and Colman 1987).

Few studies have investigated the biochemistry of inorganic carbon accumulation in marine macroalgae. Inhibitor studies have shown that in most species photosynthesis is mediated by the activity of an internal CA. In addition, the uptake of inorganic carbon may be dependent on the activity of an external CA. None of these studies present any direct evidence of which species of inorganic carbon is taken up, or of the role of both internal and external CA.

A recent study has attempted to investigate inorganic carbon accumulation in *Ascophyllum nodosum* using the inorganic carbon isotope disequilibrium technique (Johnston 1990). This technique is based on the relatively slow equilibration between the CO_2 and HCO_3^- pools in seawater. Addition of a known quantity of ^{14}C label, as either $H^{14}CO_3^-$ or $^{14}CO_2$, will disrupt the equilibrium. From the theoretical calculations described by Espie and Colman (1986) it is possible to calculate the specific activity (SA) of each pool over the time course of the re-equilibration. Photosynthetic incorporation of the ^{14}C will reflect the specific activity of the species of inorganic carbon taken up. A comparison of the theoretical time course and the observed time course of ^{14}C incorporation will indicate whether HCO_3^- or CO_2 is the major species crossing the plasma membrane at a given pH and whether the uptake is dependent on CA.

There are however limitations to the technique. Firstly, the photosynthesizing alga must be substrate saturated so that any additional inorganic carbon added (such as that used to initiate the disequilibrium) does not affect the rate of photosynthesis. This may be a problem when using CA inhibitors. Secondly, the specific activity of the internal inorganic carbon should be equal to that in the bulk medium. If macroalgae do accumulate inorganic carbon there may not be complete equilibration of the specific activity of the internal and external substrate. Both these limitations will be accentuated by the occurrence of a thick unstirred layer at the plant surface.

Although this technique has been used successfully with both microalgae and cell suspension(Espie, Owttrim and Colman 1986) it is less suitable for macrophytic species. The method requires rapid and repeated sampling over the time course of the disequilibrium. With microalgae this can be accomplished by using an eppendorf repeater pipette in combination with the silicon oil centrifugation technique. The results presented in this section were obtained using the simplest form of this experiment. As a complete time course of ^{14}C incorporation is impractical due to the nature of the material, macroalgal tissue was sampled at a specific point during the time course. The rates of incorporation were then compared to the theoretical incorporation of HCO_3^- and CO_2 uptake, at the same point in time.

The aim of this section was to determine the relationship between the observed photosynthetic characteristics of *P.umbilicalis* and *U.lactuca*, described in the previous sections, and the species of inorganic carbon transported across the plasmamembrane.

Experiments were carried out using the inorganic carbon istopic disequilibrium technique to measure and compare:

1. The methods of inorganic carbon use at pH 7.5 and pH 8.5, with respect to direct CO_2 and HCO_3^- use and CA mediated HCO_3^- use.
2. The effect of the CA inhibitors AZ and EZ on the characteristic methods of inorganic carbon uptake.

MATERIALS AND METHODS

Isotopic disequilibrium technique

Tissue discs of *P.umbilicalis* and *U.lactuca* were equilibrated in the electrode chamber in seawater at pH 7.5 or 8.5 and 5.0 mol m^{-3} TIC. Rates of photosynthetic oxygen evolution were measured polarographically as described in Section 1. The appropriate pH and TIC concentrations of 5.0 or 26 mol m^{-3} were achieved as described in Section 3. The temperature was maintained at 13^{oC} and the light intensity at 500 μmol photon m^{-2} s^{-1} PAR. When a steady state rate of photosynthesis was achieved isotopic disequilibrium was initiated by the addition of 2 μl of $NaH^{14}CO_3$ or $^{14}CO_2$ prepared from 2 μl of $NaH^{14}CO_3$ in 10 μl of 5.0 mol m^{-3} acetate buffer at pH 4.35. Immediately, two 50 μl samples of the medium were withdrawn and added to 250 μl of 0.1 mol m^{-3} NaOH, from which the total specific activity was calculated. Exactly 30 seconds from initiation of the disequilibrium the tissue disc was removed and placed immediately in liquid nitrogen to prevent any further reactions. The algal tissue was solubilized using the method described by Lobban (1974). Frozen tissue discs were placed in 500 μl of 70% $HClO_4$ and 1000 μl of 30% H_2O_2, heated to 60^{oC} until dissolved. The mixture degassed on cooling and was then decanted to give two 750 μl portions. The specific activity samples and tissue solutions were counted following the addition of 4.0 mls of Ecoscint (Mensura Technology Limited).

Inhibiton of Carbonic Anhydrase

The Carbonic Anhydrase inhibitors AZ or EZ were added to the electrode chamber after the control rate of photosynthesis was measured. Following an equilibration period of 5 minutes the rate of photosynthesis was again recorded before initiation of the isotopic disequilibrium. Inhibitor concentrations of 5.0 mmol m^{-3} were prepared as described in Section 2.

Theoretical calculations

Values for ^{14}C incorporation were calculated using the fortran programme written by A.M. Johnston, based on the theoretical equations described by Espie and Colman (1986). The values are adjusted so that the SA of the TIC following $H^{14}CO_3^-$ and $^{14}CO_2$ addition are initially equal.

RESULTS

The half-life ($t_{½}$) of inorganic carbon isotope disequilibrium is governed by both pH and temperature. Equilibrium is established most slowly at pH 7.5 and 10°C, where the value for $t_{½}$ is 90 seconds. At 30°C the value falls to only 10 seconds and at both pH 5.0 and pH 10.0 the rate is instantaneous and independent of temperature. Following initiation of ionic disequilibrium using a ^{14}C label, the specific activity of the TIC components can be calculated for any point during the disequilibrium.

Theoretical time courses for ^{14}C incorporation during photosynthesis, measured at 13°C show a characteristic reponse at pH 7.5 (Figs 1-2) and at pH 8.5 (Figs 3-4). At pH 7.5 the distinct lag in the incorporation of $^{14}CO_2$ following $H^{14}CO_3^-$ initiation of the disequilibrium reflects the initial small exchange of label between the HCO_3^- and CO_2 pool, which becomes linear with time (Fig 1). In contrast $H^{14}CO_3^-$ incorporation is always linear as the label in this pool is high. In the presence of CA the rate of incorporation is just below that predicted for direct HCO_3^- use, as the enzyme catalysed equilibration of the ^{14}C is almost instantaneous.

When the isotopic disequilibrium is initiated with $^{14}CO_2$, the label in this carbon species can be incorporated rapidly but the rate decreases with time as the ^{14}C equilibrates with the HCO_3^- pool (Fig 2). $H^{14}CO_3^-$ incorporation is low, shows an initial lag phase and is below that the rate predicted for CA dependent catalysis.

At pH 8.5 $H^{14}CO_3^-$ incorporation following $H^{14}CO_3^-$ initiation are similar, while the rate of incorporation of $^{14}CO_2$ is increased (Fig 3). This reflects the fact that the SA of CO_2 is higher because the total pool size is smaller at this pH. Following $^{14}CO_2$ initiation the response is reversed, with lower rates of $^{14}CO_2$ incorporation with respect to pH 7.5 as a result of the smaller pool size (Fig 4). In contrast, as the pool size of HCO_3^- is greater the low concentration of label result in almost no

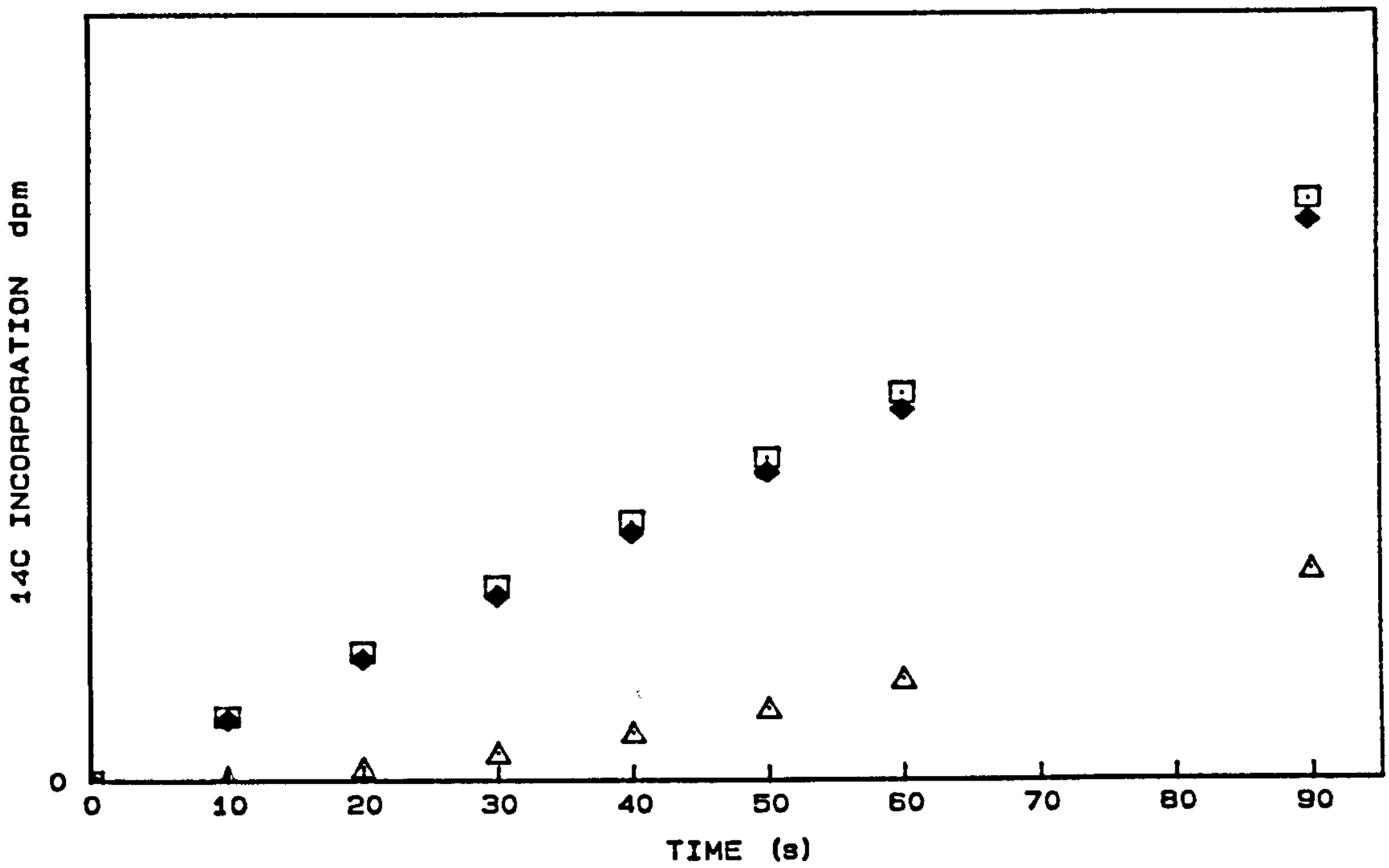

Figure 1. Theoretical time course for the photosynthetic incorporation of ^{14}C during the period of isotopic disequilibrium at pH 7.5, initiated by $H^{14}CO_3^-$. Rate of ^{14}C incorporation at a constant rate of photosynthesis dependent on direct HCO_3^- uptake (□); direct CO_2 uptake (Δ); CA dependent equilibration (◆).

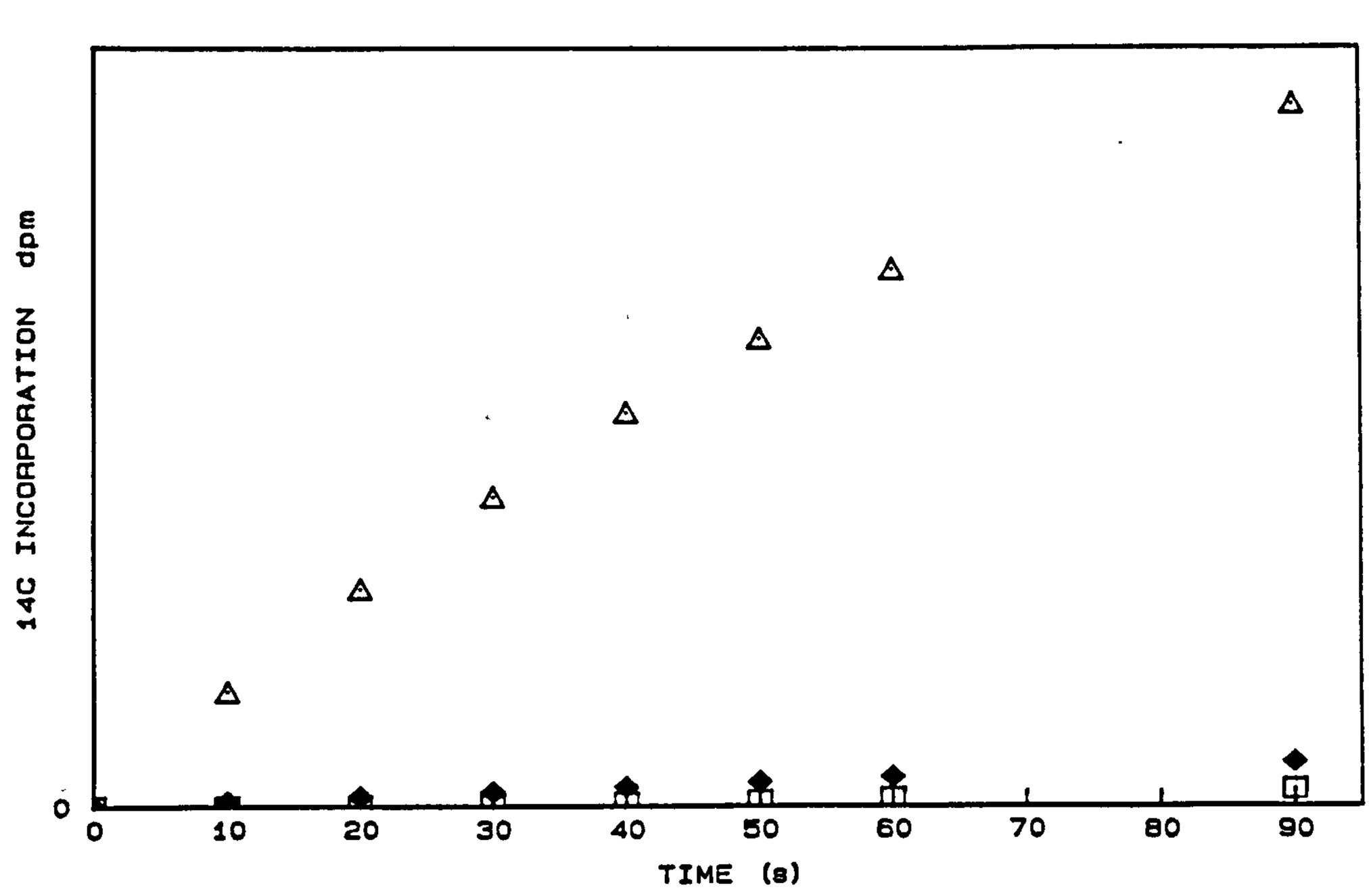

Figure 2. Theoretical time course for the photosynthetic incorporation of ^{14}C during the period of isotopic disequilibrium at pH 7.5, initiated by $^{14}CO_2$. Rate of ^{14}C incorporation at a constant rate of photosynthesis dependent on direct HCO_3^- uptake (□); direct CO_2 uptake (Δ); CA dependent equilibration (◆).

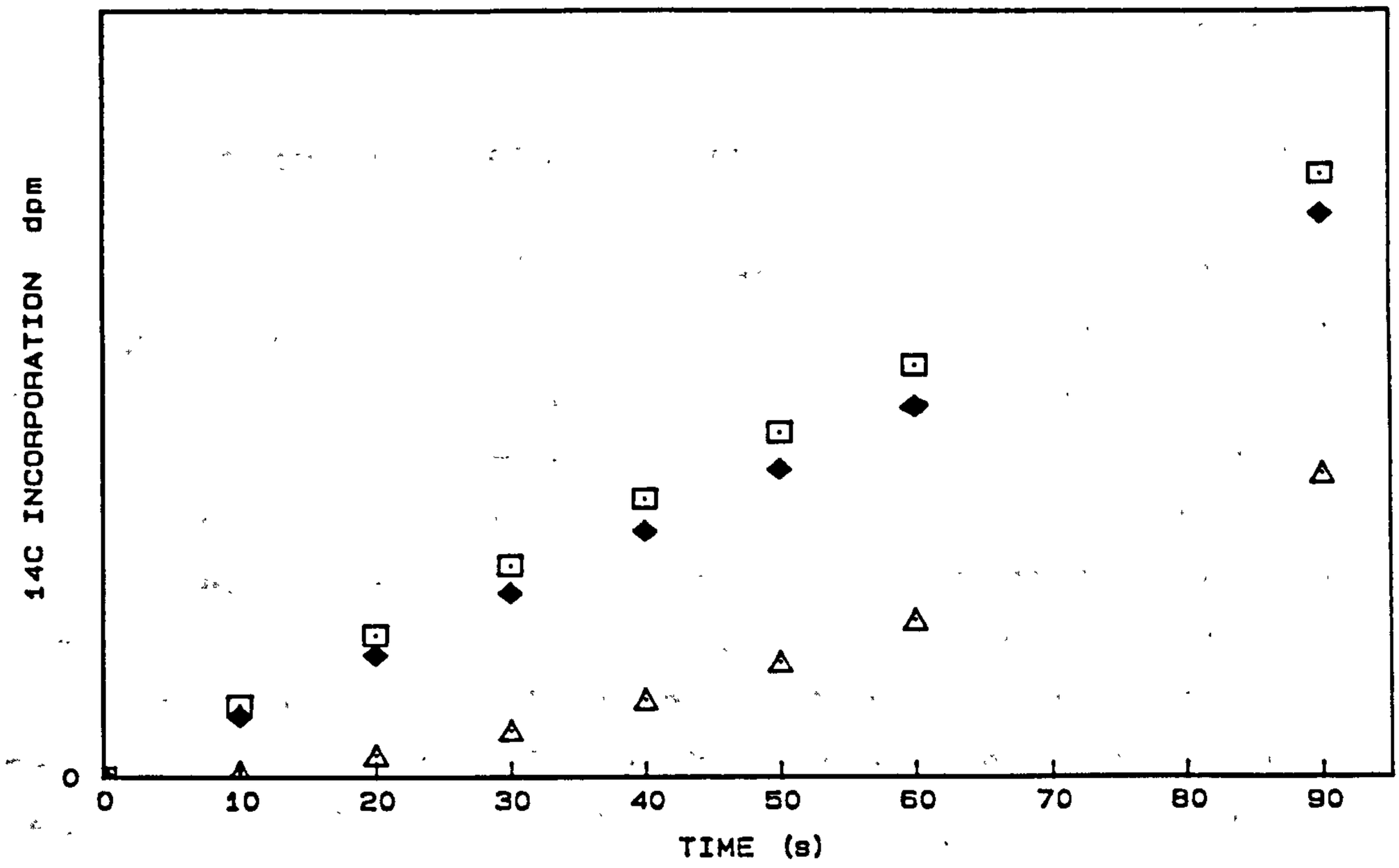

Figure 3. Theoretical time course for the photosynthetic incorporation of ^{14}C during the period of isotopic disequilibrium at pH 8.5, initiated by $H^{14}CO_3^-$. Rate of ^{14}C incorporation at a constant rate of photosynthesis dependent on direct HCO_3^- uptake (□); direct CO_2 uptake (Δ); CA dependent equilibration (◆).

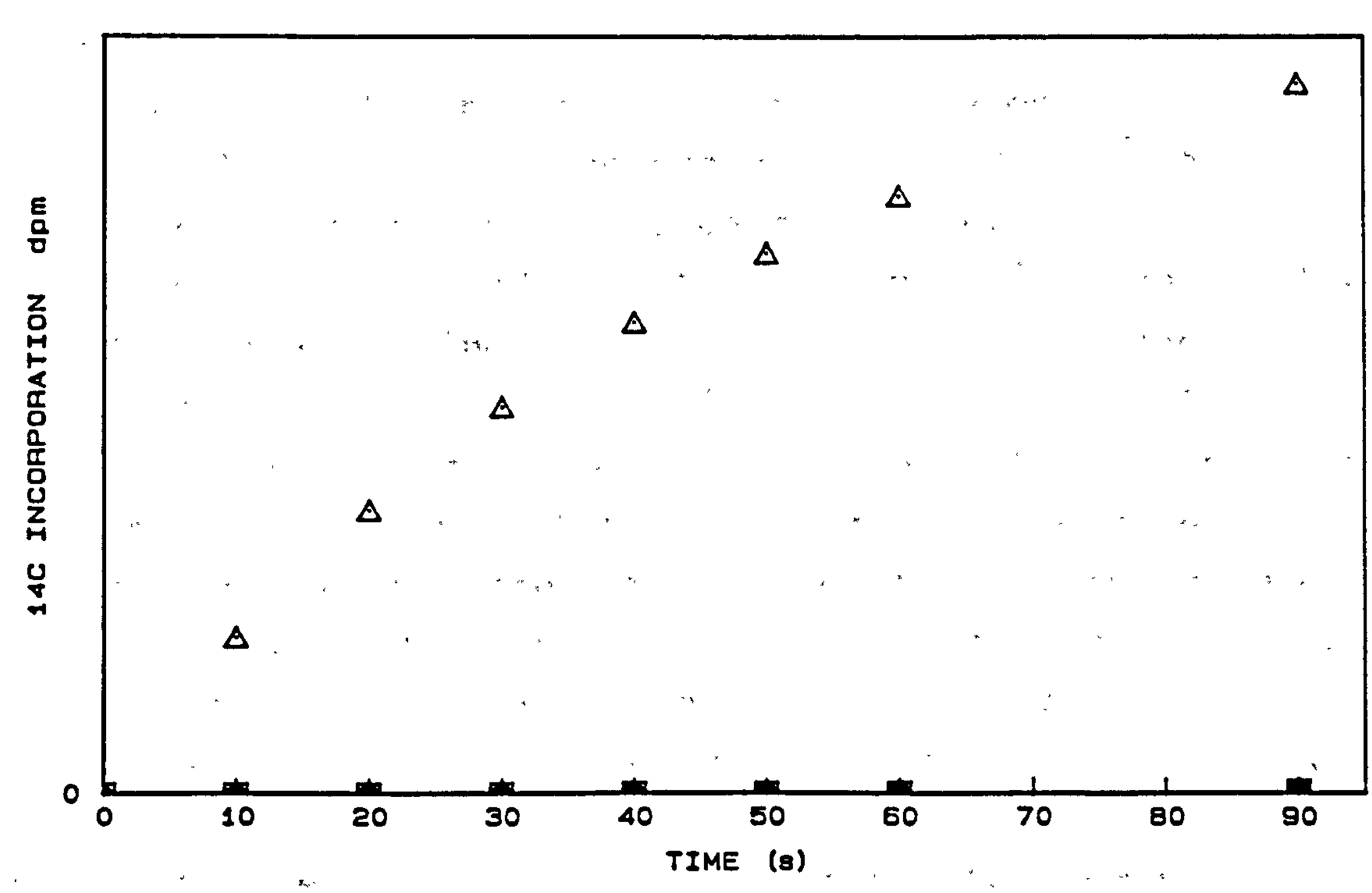

Figure 4. Theoretical time course for the photosynthetic incorporation of ^{14}C during the period of isotopic disequilibrium at pH 8.5, initiated by $^{14}CO_2$. Rate of ^{14}C incorporation at a constant rate of photosynthesis dependent on direct HCO_3^- uptake (□); direct CO_2 uptake (Δ); CA dependent equilibration (◆).

incorporation of $H^{14}CO_3^-$ either directly, or mediated by CA.

Incorporation of ^{14}C into acid stable products of photosynthesis in *P.umbilicalis* and *U.lactuca* was measured 30 seconds after initiation of the isotopic disequilibrium. These values were compared to the theoretical values, at this time, for ^{14}C incorporation if either CO_2 or HCO_3^- are used directly or if the uptake is dependent on the inter-conversion of CO_2 and HCO_3^- catalysed by CA (Tables 1 & 2).

The data presented in Tables 1 and 2 represents the parameter determined for each treatment and represents the mean (± SD) of three replicates. The control photosynthetic rate (PS), measured prior to initiation, is followed by the observed SA of the inorganic carbon pool (dpm) recovered from the alga, after 30 seconds of incubation (OBS). For each treatment the theoretical value for incorporation with respect to the initial SA(TIC), is represented as the SA (in dpm) for direct HCO_3^- use (^{14}B), direct CO_2 use (^{14}C) and CA mediated HCO_3^- respectively (^{14}CA). It is therefore possible to see how the measured SA correlates to that expected when the inorganic carbon uptake occurs via one or more of these specific processes, and so determine the characteristic mechanism for each species.

For *U.lactuca* at pH 7.5, the observed ^{14}C incorporation of 2895 dpm following $H^{14}CO_3^-$ initiation approaches the value of 6942 expected if CA is operating (Table 2). However, the predicted incorporation of ^{14}C by direct HCO_3^- is similar (6084 dpm). Direct CO_2 use alone can be disregarded, as the predicted value of 1087 dpm is below the observed SA value of 2895 dpm. The value of 10960 dpm following $^{14}CO_2$ initiation was closer to the theoretical CA catalysed value of 5864 dpm than that predicted for CO_2 use (124596 dpm). In relation to direct use of substrate the observed rate was well above that predicted for HCO_3^- use (917 dpm), so the direct uptake of CO_2 may have occurred.

At pH 8.5 the ratio of CO_2 to HCO_3^- is lower than at pH 7.5, although initially the specific activity of CO_2 is higher at this pH. The observed ^{14}C incorporation after $H^{14}CO_3^-$ initiation (6902 dpm) is comparable to both CA

TABLE 1 & 2. The observed and theoretical values for incorporation of ^{14}C into acid stable products 30 seconds after initiation of isotopic disequilibrium. The data, presented as dpm is the mean ± SE of three replicates for *P.umbilicalis* (Table 1) and *U.lactuca* (Table 2).

TABLE 1.

	pH 7.5		pH 8.5	
	Initiated with:		Initiated with:	
	$H^{14}CO_3^-$	$^{14}CO_2$	$H^{14}CO_3^-$	$^{14}CO_2$
Control				
PS	1.60 (0.13)	1.87 (0.18)	1.59 (0.12)	1.81 (0.17)
OBS	1105 (1396)	1968 (681)	4492 (4172)	3776 (193)
^{14}B	5574 (418)	883 (88)	5527 (427)	1616 (151)
^{14}C	813 (61)	119863 (118670)	1228 (95)	1155884 (107854)
^{14}CA	5292 (397)	5642 (559)	4819 (373)	5469 (511)
AZ (5 mmol m^{-3})				
PS	1.66 (0.10)	1.79	0.75 (0.05)	0.83
OBS	8330 (4138)	38112	6743 (2425)	18523
^{14}B	5299 (315)	847	2584 (168)	740
^{14}C	773 (45)	114941	574 (37)	529303
^{14}CA	5031 (299)	5410	2253 (147)	2504
EZ (5 mmol m^{-3})				
PS	1.82 (0.20)	1.72 (0.86)	0.39	0.27 (0.15)
OBS	1581 (222)	69353 (15802)	388	17276 (16407)
^{14}B	3794 (644)	813 (408)	1352	239 (129)
^{14}C	864 (94)	110307 (55337)	300	171014 (92640)
^{14}CA	5502 (612)	5192 (2605)	1179	809 (438)

TABLE 2.

	pH 7.5 Initiated with: $H^{14}CO_3^-$		pH 8.5 Initiated with: $H^{14}CO_3^-$	
		$^{14}CO_2$		$^{14}CO_2$
Control				
PS	2.34 (0.11)	1.94 (0.41)	2.30 (0.50)	2.09 (0.19)
OBS	2895 (1947)	10960 (2368)	6902 (2282)	14760 (7025)
^{14}B	6086 (2075)	917 (194)	7961 (1675)	1863 (172)
^{14}C	1087 (52)	124596 (26344)	1771 (368)	133322 (12331)
^{14}CA	7072 (337)	5864 (1241)	6942 (1450)	6307 (584)
AZ (5 mmol m^{-3})				
PS	1.46 (0.57)	2.13 (0.11)	1.71 (0.19)	1.47 (0.03)
OBS	4549 (66)	166233 (42794)	6210 (522)	35959 (14315)
^{14}B	4150 (2532)	1011 (59)	5911 (642)	1318 (28)
^{14}C	679 (267)	137229 (8103)	1313 (143)	942675 (1738)
^{14}CA	4411 (1730)	6459 (382)	5154 (560)	4459 (93)
EZ (5 mmol m^{-3})				
PS	1.13 (0.09)	1.29 (0.44)	1.35 (0.51)	1.95 (0.26)
OBS	1179 (472)	36715 (7304)	1415 (965)	36114 (11697)
^{14}B	3596 (272)	613 (211)	4934 (1582)	1738 (228)
^{14}C	525 (40)	83114 (28676)	1221 (416)	1242845 (1738)
^{14}CA	3503 (390)	3912 (1350)	4794 (1633)	6472 (1365)
EZ (100 mmol m^{-3})				
PS	0.42 (0.06)	0.29 (0.01)	0.13 (0.06)	0.10 (0.05)
OBS	99 (12)	11508 (10337)	142 (171)	2859 (865)
^{14}B	1322 (180)	140 (4)	425 (233)	88 (40)
^{14}C	193 (26)	18644 (901)	94 (52)	62521 (29095)
^{14}CA	1256 (170)	878 (43)	371 (203)	296 (138)
EZ (100 mmol m^{-3}) HCO_3^- (26 mol m^{-3})				
PS	1.56 (0.28)	1.40 (0.06)	0.38 (0.24)	0.26 (0.08)
OBS	9210 (9897)	234238 (129542)	1567 (662)	22794 (3808)
^{14}B	4983 (887)	1569 (1593)	1275 (756)	203 (78)
^{14}C	726 (130)	213034 (216160)	281 (165)	178052 (51667)
^{14}CA	4731 (843)	10027 (10176)	989 (500)	802 (245)

catalysed and direct HCO_3^- uptake (6942 and 7961 dpm respectively), rather than CO_2 use (1771 dpm; Table 2). The value observed following $^{14}CO_2$ initiation was greater than HCO_3^- or CA catalysed incorporation although lower than that predicted for CO_2 use.

In the presence of 5.0 mmol m^{-3} AZ there was no significant inhibition of the rate of photosynthesis in *U.lactuca*, consistent with the results in Sections 1 & 2. Consequently ^{14}C incorporation was also unaffected by the CA inhibitor. The theoretical values for either CA dependent or direct HCO_3^- use following $H^{14}CO_3^-$ initiation, were closest to the observed rate at pH 7.5 (Table 2). It must be noted, however, that in the presence of AZ the enzyme catalysed rate would be reduced and so incorporation must have been dependent on direct HCO_3^- uptake. The results following $^{14}CO_2$ initiation showed that the rate closest to that for direct CO_2 use. At pH 8.5 the observed values reflected a similar pattern of inorganic carbon uptake (Table 2).

In contrast, 5.0 mmol m^{-3} EZ inhibited the rate of photosynthesis and the corresponding ^{14}C incorporation. Only direct HCO_3^- uptake (3596 dpm) could have accounted for the observed values following $H^{14}CO_3^-$ initiation (1179 dpm) while only direct CO_2 uptake (11508 dpm) could have achieved the rate observed following $^{14}CO_2$ addition (18644 dpm; Table 2). Similar conclusions can be drawn from the results obtained at pH 8.5.

However, after treatment with 100 mmol m^{-3} EZ, it was evident that the observed ^{14}C incorporation was dependent on direct CO_2 use, regardless of the species of ^{14}C used to initiate the disequilibrium, or of the pH. When excess HCO_3^- was added to overcome the effect of EZ inhibition of CA the results reflected the pH of the media. The dpm value obtained with $H^{14}CO_3^-$ initiation (9210) was greater than either of those predicted for either CO_2 or HCO_3^- use alone (726 & 4983 dpm respectively). The incorporation during $^{14}CO_2$ addition (234238 dpm) was only comparable to that for CO_2 use (213034 dpm). At pH 8.5 the photosynthetic rates did not show any increase in response to the excess HCO_3^-

(Table 2). Values for ^{14}C incorporation when CA was maximally inhibited showed the same pattern of inorganic carbon uptake as at pH 7.5, although in comparison the rates were now limited by the substrate supply (22795 dpm).

For *P.umbilicalis* the results are not as conclusive, as lack of time prevented the extensive treatments carried out with *U.lactuca* (Table 1). Photosynthetic incorporation of ^{14}C following initiation by $H^{14}CO_3^-$ appeared to be similar to that for *U.lactuca* (1105 dpm). The value reflected a CA mediated or direct HCO_3^- uptake (5292 & 5574 dpm respectively; Table 1). The result for $^{14}CO_2$ initiation also corresponded to an enzyme catalysed incorporation although there was no evidence of HCO_3^- use. At pH 8.5 the pattern of ^{14}C incorporation again appeared to be mediated by CA, although the values were higher than at pH 7.5 (Table 1).

Inhibition by 5.0 mmol m^{-3} AZ was ineffective at pH 7.5 (Table 1). The rates of photosynthesis and the corresponding rates of ^{14}C incorporation are higher than at pH 8.5, but they were also higher than those of the controls. Because of this it was difficult to establish which of the theoretical values could account for the observed incorporation rates at either pH.

The effect of 5.0 mmol m^{-3} EZ showed the same pH dependence that was evident for AZ. The ^{14}C incorporation following $H^{14}CO_3^-$ initiation at both pH 7.5 and 8.5 were close to the value for direct CO_2 use. This was also reflected by the oberved rates following $^{14}CO_2$ initiation of the disequilibrium.

DISCUSSION

The results from the previous chapters have shown that the mechanisms of inorganic carbon accummulation in *P.umbilicalis* and *U.lactuca* have different characteristics.

P.umbilicalis has a higher affinity for CO_2 (Sections 1 and 3). Uptake of inorganic carbon appears to be rate limited at the plasma membrane and it can be concluded from the inhibitor studies that this step of the mechanism is regulated by the activity of an external CA (Section 2).

In contrast the affinity of *U.lactuca* for CO_2 is similar to that for HCO_3^- (Section 3). Although HCO_3^- use is apparent, there is no evidence that this ability is dependent on the activity of external CA (Section 2 and 3).

Four possible mechanisms of inorganic carbon acquisition have been proposed for marine intertidal macroalgae (A.M.Johnston, personal discussion):

1. CO_2 use alone.
2. CO_2 use and CA dependent HCO_3^- use.
3. CO_2 use, CA dependent HCO_3^- use and direct HCO_3^- use.
4. CO_2 use and direct HCO_3^- use.

The inorganic carbon isotope disequilibrium technique has been used to attempt to identify which species of inorganic carbon are transported across the plasma membrane (Johnston In Press). The results presented in this section, obtained using the simplest form of this method, give some indication as to possible methods of inorganic carbon accumulation in *P.umbilicalis* and *U.lactuca*.

The control results for *U.lactuca* show little pH dependence in terms of rates of photosynthesis or ^{14}C incorporation. This is consistent with the results from Section 3. Following $H^{14}CO_3^-$ initiation of the disequilibrium it is not possible to distinguish between a CA mediated HCO_3^- use or direct HCO_3^- uptake. Following $^{14}CO_2$ initiation the resultant incorporation can be accounted for solely by CO_2 uptake. The rates of photosynthesis and ^{14}C incorporation after addition of AZ are no different from those of the controls. If the uptake of HCO_3^- was dependent on external CA activity these

results would not be expected. It is possible that the concentration of AZ was too low to inhibit external CA, but the results in Section 3 have shown that even a concentration of 100 mmol m^{-3} had no effect on the rate of photosynthesis in *U.lactuca*. In view of this it appears that this species is able to use both CO_2 and HCO_3^- directly.

In the absence of an external CA, any observed affects of EZ on photosynthesis and ^{14}C incorporation can be attributed to inhibition of an internal CA. A concentration of 5.0 mmol m^{-3} inhibitor did not alter the pattern of ^{14}C incorporation although CO_2 uptake appeared to be slightly enhanced. Following addition of 100 mmol m^{-3} EZ there appears to a significant change in the mechanism of inorganic carbon uptake. The rate of photosynthesis and ^{14}C incorporation during $H^{14}CO_3^-$ initiation are low and correspond to the theoretical maximum for uptake solely as CO_2. This is also evident from the observed rates following $^{14}CO_2$ initiation. As would be expected, the effect of EZ is greater at pH 8.5. If photosynthesis under these conditions is dependent on CO_2 uptake the rate will be directly proportional to the external concentration. At pH 8.5, 83% of the TIC is as HCO_3^- and 0.2 % as CO_2, while at pH 7.5 the relative proportions, especially of CO_2, are greater (95% and 3% respectively). This pH dependence is even more marked when the TIC concentration was increased to 26 mol m^{-3}. At pH 7.5 this concentration appeared to overcome the effect of EZ inhibition on internal CA. The results suggest that at least some of this ^{14}C incorporation occurred as a result of direct HCO_3^- use in addition to CO_2 uptake. At pH 8.5 EZ inhibition of photosynthesis was not alleviated by the high TIC concentration. In addition, the observed rates are well below those predicted for ^{14}C incorporation by CO_2 uptake, suggesting that either the substrate concentration is limiting, or an additional process was inhibited.

As indicated in the results section, the data for *P.umbilicalis* is less conclusive. For the controls ^{14}C incorporation following $H^{14}CO_3^-$ initiation of the disequilibrium could be accounted for by either direct

HCO_3^- uptake or HCO_3^- use mediated by CA. In contrast to *U.lactuca* the incorporation following $^{14}CO_2$ initiation appears to be related to CA catalysis rather than direct CO_2 use. This dependence of inorganic carbon on the activity of an external CA was concluded in both Section 2 and 3.

Inhibition of external CA by 5.0 mmol m^{-3} AZ was pH dependent, as there was little effect on the rate of photosynthesis at pH 7.5 in contrast to that at 8.5. This may be due to a differential sensitivity of CA at the each pH, or due to the indirect effect of higher substrate concentration of both HCO_3^- and CO_2 at pH 7.5. The same effect of pH and substrate concentration was apparent in Section 3. It can be explained in terms of the calculated substrate affinities, which show a preference for CO_2 in this species. The rates of ^{14}C incorporation following $H^{14}CO_3^-$ do not reflect any change in the mechanism of inorganic carbon uptake, following AZ inhibition, although after $^{14}CO_2$ initiation ^{14}C incorporation no longer appears to be mediated by CA.

The results are similar for the treatment with 5.0 mmol m^{-3} EZ. Again, the effect of the inhibitor on the rate of photosynthesis was pH dependent. Photosynthesis was inhibited to a greater degree at pH 8.5 and as shown in Section 2, EZ has a greater effect than AZ. The observed rates of ^{14}C incorporation suggest that the mechanism has been altered but the only conclusion that can be drawn is that it is not totally dependent on CO_2 use under these conditions. This is not unexpected as this concentration EZ is only partially inhibitory (Section 2). Additional investigations using higher concentration of both AZ and EZ are required before any further conclusions can be drawn. It is apparent that CA dependent HCO_3^- use occurs in *P.umbilicalis*, while the importance of either direct HCO_3^- or CO_2 use is as yet unclear. With respect to the pH dependence of the response following inhibition, evident in both Section 2 and 3, it is probable however that direct CO_2 use is of significance to this species.

Few other studies have attempted to define which of the four possible mechanisms operate in marine intertidal macroalgae. Beer *et al.* (In press) have shown that *Ulva faciata* is capable of direct HCO_3^- use. This species was found to have a surface pH of 10.0. In the absence of an external CA, the CO_2 concentration in the unstirred layer would be below the CO_2 compensation point, while that of HCO_3^- would be higher than the bulk medium. Based on this observation, it was concluded that HCO_3^- must be taken up during submersion. In accordance with the results for *U.lactuca*, CO_2 use was not excluded. It has been suggested that inorganic carbon uptake occurs in two phases, initial transport of CO_2 which stimulates the mechanism that facilitates HCO_3^- uptake.

These observations are in contrast to the results for *Ascophyllum nodosum* obtained by using the inorganic carbon disequilibrium technique, which show that a CA is involved in the mechanism of inorganic carbon assimilation in this species (Johnston In press). In addition, the results of this section define a mechanism of inorganic carbon accumulation in *P.umbilicalis* analagous to that proposed for *Chondrus crispus* (Smith and Bidwell 1989a) which revealed the absence of any active or facilitated transport of HCO_3^-. As in *P.umbilicalis*, CO_2 derived from the HCO_3^- catalysed by eternal CA passively diffuses across the plasma membrane, while intracellular CA also enhances fixation in this species.

SECTION 5 - Photoinhibition under high PAR: effect of varying O_2 regimes

INTRODUCTION

For marine macroalgae in the intertidal environment carbon metabolism may be limiting for the consumption of energy. Under high levels of PAR, the quantum ratio may be below optimum and more photons are absorbed than required to drive photosynthesis. Such conditions of excess light energy induce processes that inhibit photosynthesis, termed photoinhibition.

Environmental conditions where the primary processes of photosynthesis may be disrupted, such as during desiccation or reduced substrate levels, will increase photoinhibition although it can be initiated solely by high levels of PAR over extended periods of time. The imbalance between the transfer of excitation energy to the photochemical reaction centres and from these reaction centres to the electron transport chain reflects a disruption of the thylakoid membrane, and of the inherent photosynthetic processes. The term photoinhibition is also applied to a number of reversible mechanisms which are believed to protect against the permanent damage caused by photo-oxidation.

The occurrence of photoinhibition in macroalgae when emersed seems almost inevitable. Most of the studies investigating this process in macroalgae have been carried out with subtidal species. Photoinhibition at light intensities above 500 μmol photon m^{-2} s^{-1} was evident in a number of species including *Ulva curvata* and *Porphyra rosengurtii*. As expected the effect was greatest at midday, and showed an increase between February and March (Coutinho and Zingmark 1987). Both the duration of the exposure and the characteristic wavelength affects the photoinhibitory reduction of photosynthesis. For the subtidal species *Polyneura hilliae* the degree to which the process is reversible depends on the duration of the treatment. The effect of the each wavelengh was presented as an action spectrum for the response. In this species red light above 700 nm was ineffective, while the range between 680 and 570 nm caused a significant decrease in photosynthetic oxygen evolution. Below 420 nm an inhibition of up to 100% was

attributed to photodamage, that may not be irreversible (Nultsch, Pfau and Huppertz 1990). The greater effect of this blue light could also be the result of photoinactivation of RuBPco, in addition to disruption of the electron transport chain.

Photoinhibition in an intertidal species of *Porphyra perforata* has been compared to that in the subtidal species *Porphyra nerocystis* (Herbert and Waaland 1988; Herbert 1989). Although both species showed a significant degree of photoinhibition, the response to excess PAR was lower in *P.perforata*. This resistance was evaluated in terms of the rate of damage and repair of the components of photosynthesis. There were however, contradictory conclusions as to whether the reduced susceptibility of *P.perforata* results from a decrease in the initial damage or from a more rapid rate of repair (Bose, Herbert and Fork 1988).

Possible mechanisms that would suppress the apparent photoinhibition need to be evaluated, in relation to the characteristic photosynthetic capacity and efficiency of these plants. Photorespiration is one of the mechanisms that has been proposed to alleviate photoinhibition. In the absence of adequate concentration of CO_2, fixation of O_2 by RuBPco would maintain the flow of electrons along the transport chain. Additionally O_2 can be used as a terminal electron acceptor in the process termed the Mehler reaction.

There is little evidence of a clear relationship between photorespiration and photosynthesis. Photorespiration rates were higher in the photoinhibition sensitive species of *Porphyra*. At a reduced O_2 concentration the effect of high light was greater than under normal levels of O_2 (Herbert and Waaland 1988). Photoinhibition of PS II appears to be oxygen independent, while it has been proposed that an effect on PS I could involve O_2 (Satoh 1970). It is therefore possible, that in contrast to the proposed protective role of O_2 uptake, a proportion of the photoinhibitory response could be attributed to an effect on PS I.

There are other important photosynthetic characteristics which should be considered in relation to photoinhibition. Few studies have investigated the effect of high light intensities over longer time periods. Under natural conditions intertidal macroalgae are subjected to both short bursts and longer periods of irradiances up to 2000 μmol photon m^{-2} s^{-1}. In addition these fluctations will, at times, coincide with periods of exposure of up to five hours. Over this time, most species will experience both desiccation and as a consequence alteration of the supply of inorganic carbon. Water loss will also affect the rate of metabolism and impair the efficiency of any protection and recovery/repair processes that modify the degree of photoinhibitory damage. Any reduction of the photosynthetic capacity will also make marine intertidal macroalgae more susceptible to the effects of excess light. The apparent suppression of photoinhibition may be achieved by the operation of an effective mechanism of inorganic carbon accumulation.

The aim of this section was to evaluate the response of *Porphyra umbilicalis* and *Ulva lactuca* to levels of PAR equivalent to full sunlight over substantial time periods. The treatments were carried out in seawater at saturating or subsaturating concentrations of HCO_3^- and O_2 concentration of 1% and 21% air equilibrium. This approach was taken to allow a comparison of photoinhibitory responses under optimal conditions, in the absence of any secondary effects of desiccation and temperature.

Experiments were carried out with *P.umbilicalis* and *U.lactuca* to measure and compare:

1. Photoinhibition of photosynthesis following incubation under a high level of PAR; values for the apparent quantum ratio, V_{max} ,light saturation and compensation point were calculated as in Section 1.
2. The effect of the concentration of O_2 on photoinhibition of photosynthesis, determine from the above parameters.

MATERIALS AND METHODS

Plant material

Porphyra umbilicalis and *Ulva lactuca* were collected and maintained as described in Section 1.

Measurement of the light intensity-response in seawater

Rates of photosynthetic oxygen evolution in relation to light intensity were measured in seawater as described in Section 1.

The intensity of the light was increase incrmentally between 0 and 2000 μmol photon m^{-2} s^{-1} PAR, following an initial induction period of 10 minutes at 50 μmol photon m^{-2} s^{-1}. The response was measured before and after the photoinhibitory treatments described below.

Photoinhibitory treatments

Strips of plant material were maintained in seawater at pH 8.0 and 12^{o}C for 3 hours. Light levels of 2000 μmol photon m^{-2} s^{-1} PAR were provided by a high intensity light source (LS2 Hansatech). Concentrations of 2.5 and 5.0 mol m^{-3} TIC were achieved by the addition of $NaHCO_3$ solution to seawater. The seawater solutions were bubbled with either air or N_2 to provide O_2 concentrations of 21% and 1% respectively.

RESULTS

The light intensity-response of photosynthesis

Photoinhibition of photosynthesis was determined from the response of photosynthesis to PAR. For both species the optimum photosynthetic characteristics were obtained from the light intensity-response curves. These were compared to those calculated from the reponse measured following incubation under 2000 μmol photon m^{-2} s^{-1} PAR for 3 hours.

The response for *P.umbilicalis* measured at 21% O_2 and 5.0 mol m^{-3} TIC is shown in Figure 1. The light compensation and saturation points, determined from regression analysis of the initial rate, were 4 and 100 μmol photon m^{-2} s^{-1} respectively. The photosynthetic efficiency or slope of the initial response, expressed as the apparent quantum ratio was 68 μmol photon μmol O_2^{-1}. At saturating PAR the response achieved a V_{max} of 1.35 μmol O_2 m^{-2} s^{-1}.

Light intensity-response curves were also measured under varying concentration of substrate and oxygen concentration. The characteristics described above were similar at 2.0 and 5.0 mol m^{-3} TIC and were unaffected by changes in the O_2 concentration (measured at 1% or 21% O_2 in air; Figs 1-4). The light compensation points were between 2 and 4 μmol photon m^{-2} s^{-1} PAR and saturation of the V_{max} occured at 100 μmol photon m^{-2} s^{-1}.

The photosynthetic efficiencies, expressed as the apparent quantum ratio (AQR), were between 58 and 76 μmol photon μmol O_2^{-1}. The corresponding capacity or V_{max} values were between 1.35 and 1.60 μmol O_2 m^{-2} s^{-1}.

For *Ulva lactuca* the control responses are similarly independent of the concentrations of substrate and O_2 present (Figs 5-8). The light compensation points were between 14 and 20 μmol photon m^{-2} s^{-1}, and saturation occurred at between 100 and 125 μmol photon m^{-2} s^{-1}. Photosynthetic efficiencies of 86 to 100 were slightly lower than for *P.umbilicalis*, as were the V_{max} values (0.70 - 1.25 μmol O_2 m^{-2} s^{-1}).

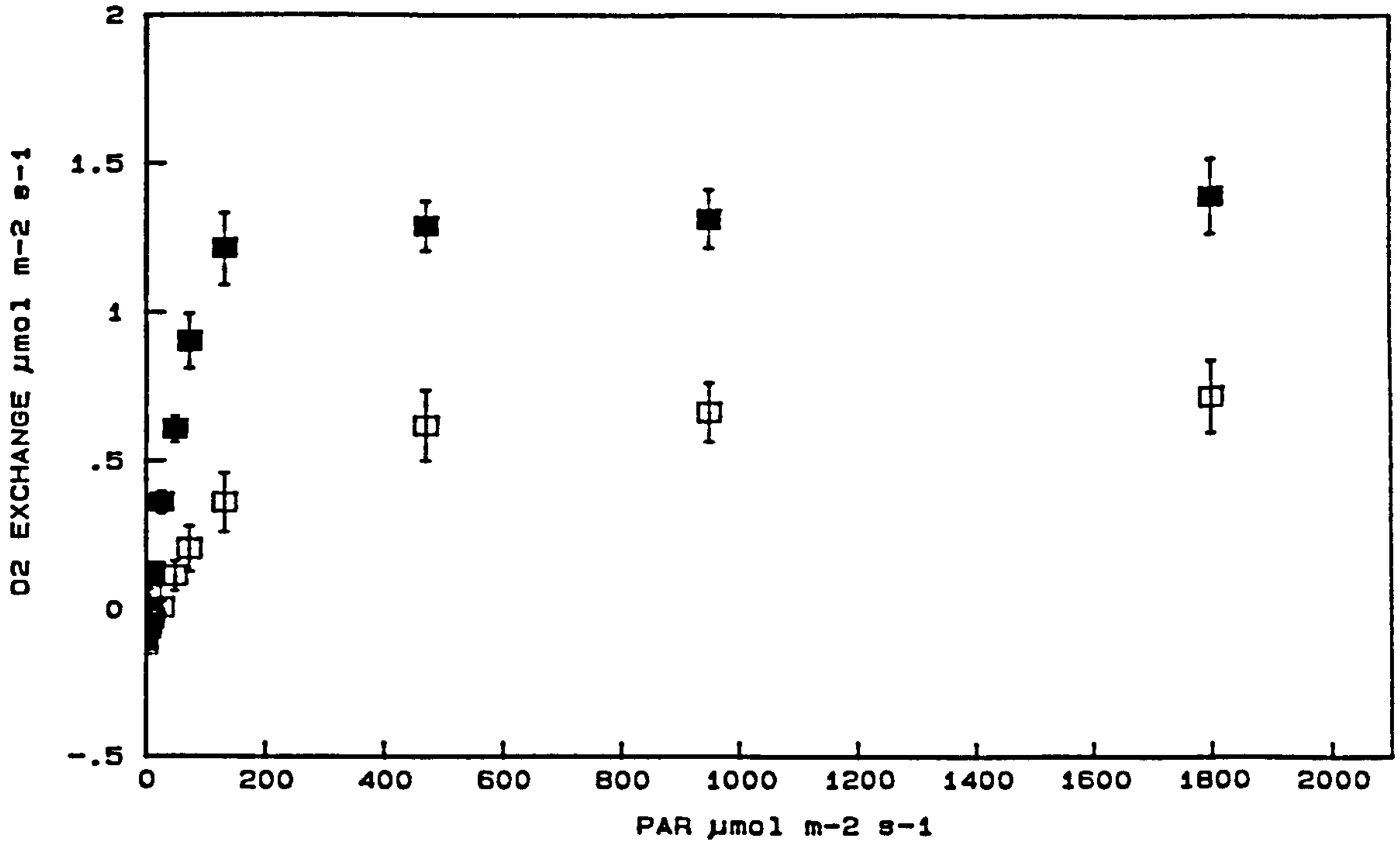

Figure 1. Effect of exposure to 2000 μmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater, as a function of light intensity (PAR). Response measured at 5.0 mol m^{-3} TIC and 21% O_2 represents the mean ± SE of four replicates. Control (■); high PAR treated (□).

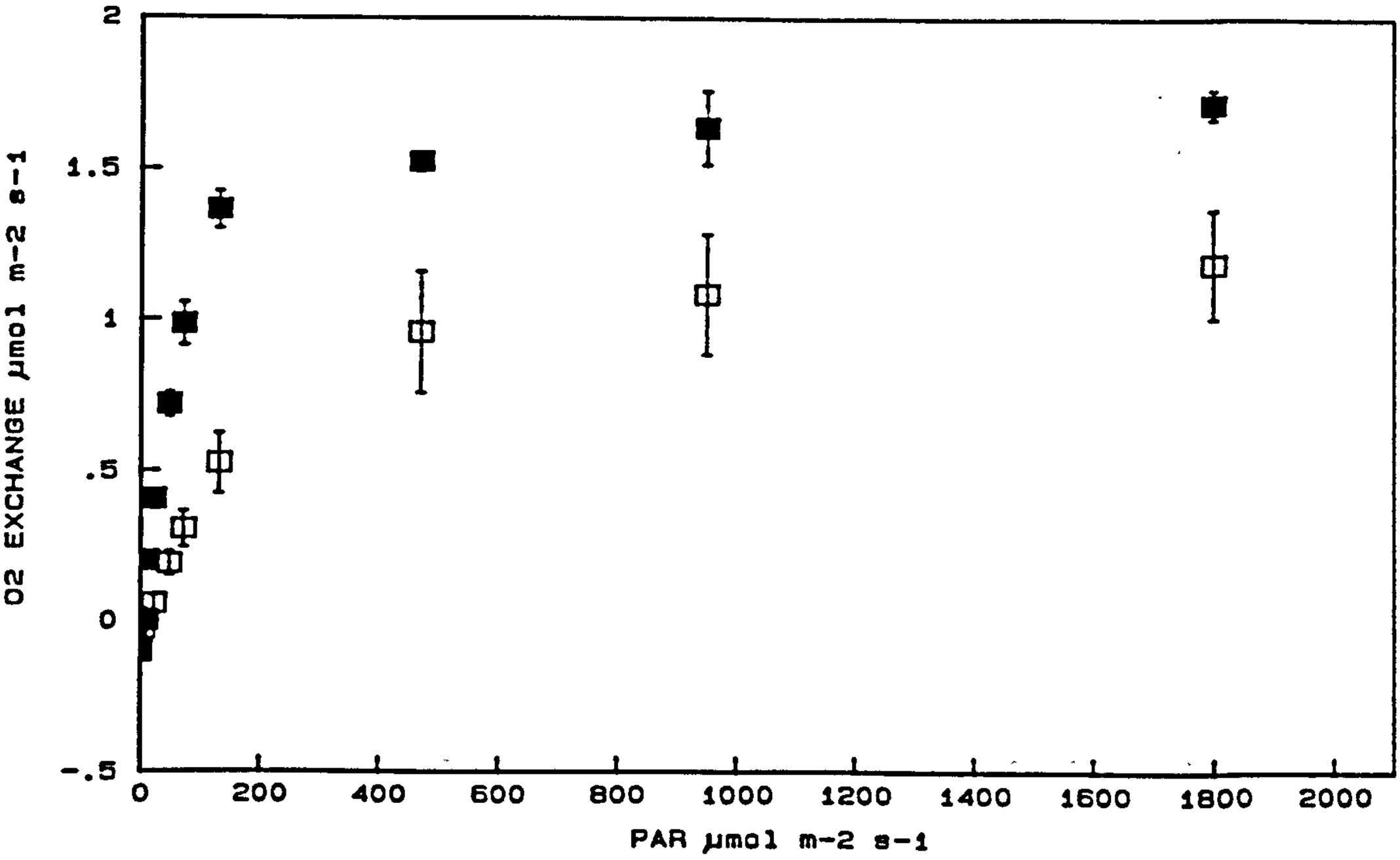

Figure 2. Effect of exposure to 2000 μmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater, as a function of light intensity (PAR). Response measured at 5.0 mol m^{-3} TIC and 1% O_2 represents the mean ± SE of four replicates. Control (■); high PAR treated (□).

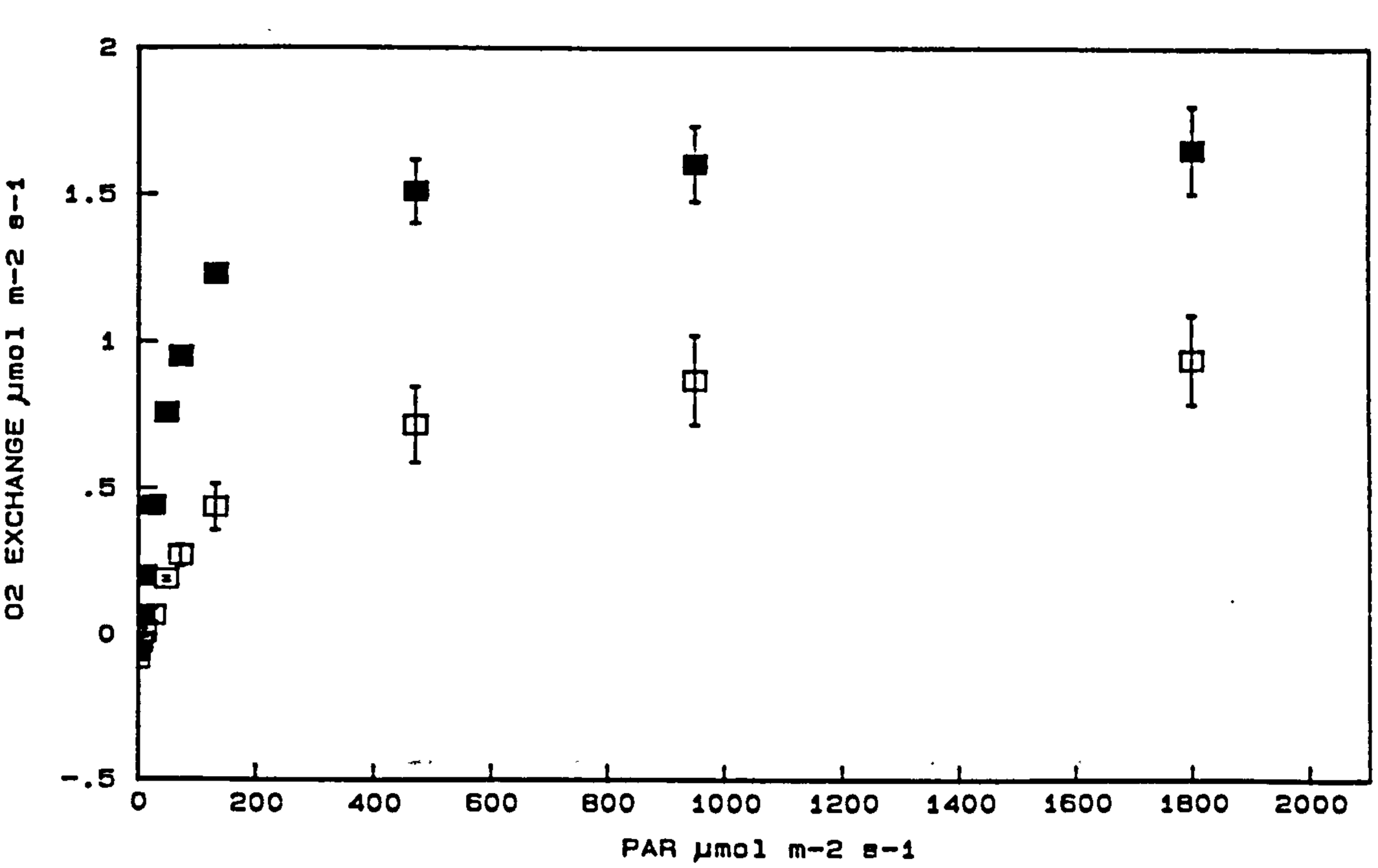

Figure 3. Effect of exposure to 2000 μmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater, as a function of light intensity (PAR). Response measured at 2.0 mol m^{-3} TIC and 21% O_2 represents the mean ± SE of four replicates. Control (■); high PAR treated (□).

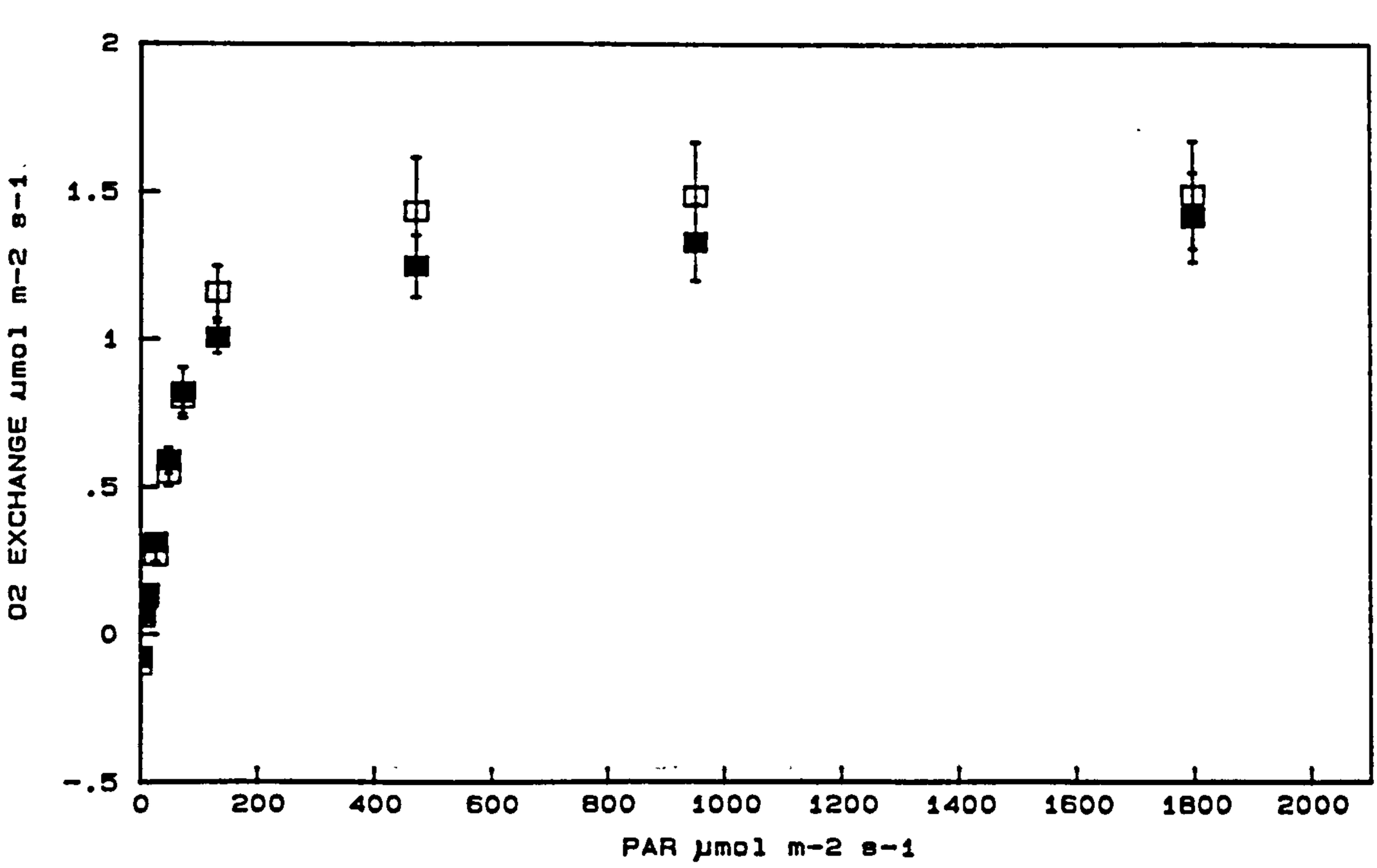

Figure 4. Effect of exposure to 2000 μmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *P.umbilicalis* in seawater, as a function of light intensity (PAR). Response measured at 2.0 mol m^{-3} TIC and 1% O_2 represents the mean ± SE of four replicates. Control (■); high PAR treated (□).

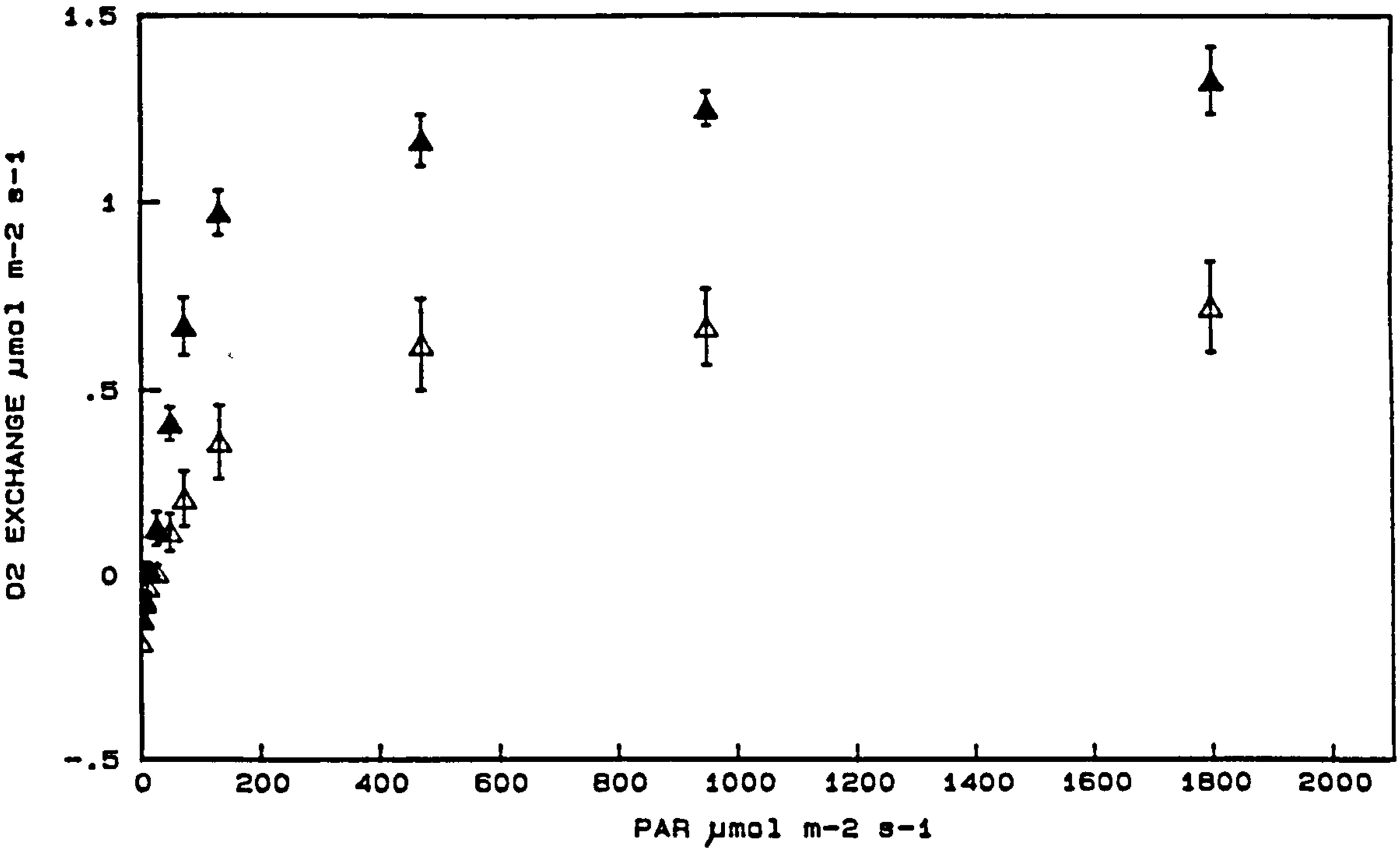

Figure 5. Effect of exposure to 2000 µmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater, as a function of light intensity (PAR). Response measured at 5.0 mol m^{-3} TIC and 21% O_2 represents the mean ± SE of four replicates.
Control (▲); high PAR treated (Δ).

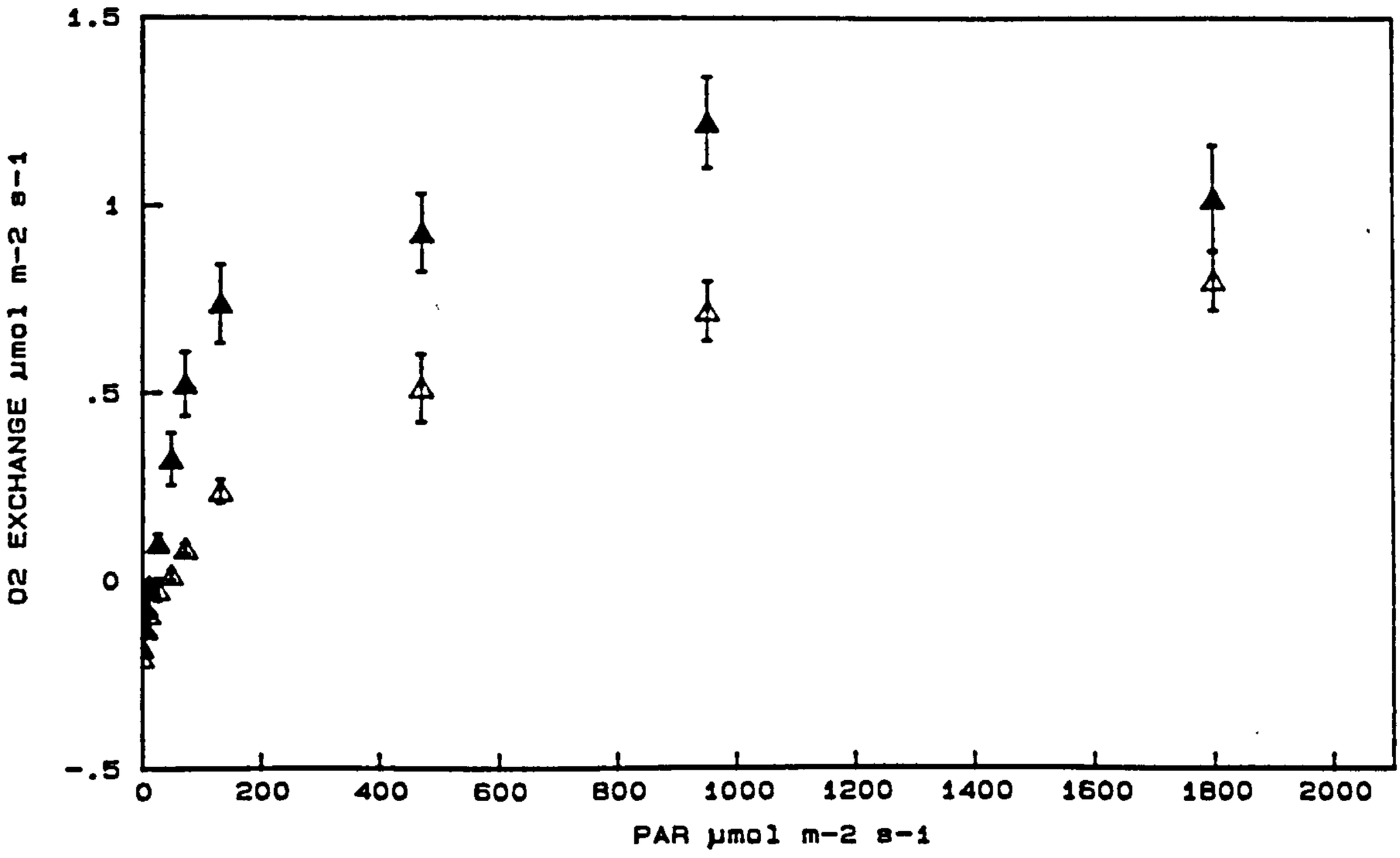

Figure 6. Effect of exposure to 2000 µmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater, as a function of light intensity (PAR). Response measured at 5.0 mol m^{-3} TIC and 1% O_2 represents the mean ± SE of four replicates.
Control (▲); high PAR treated (Δ).

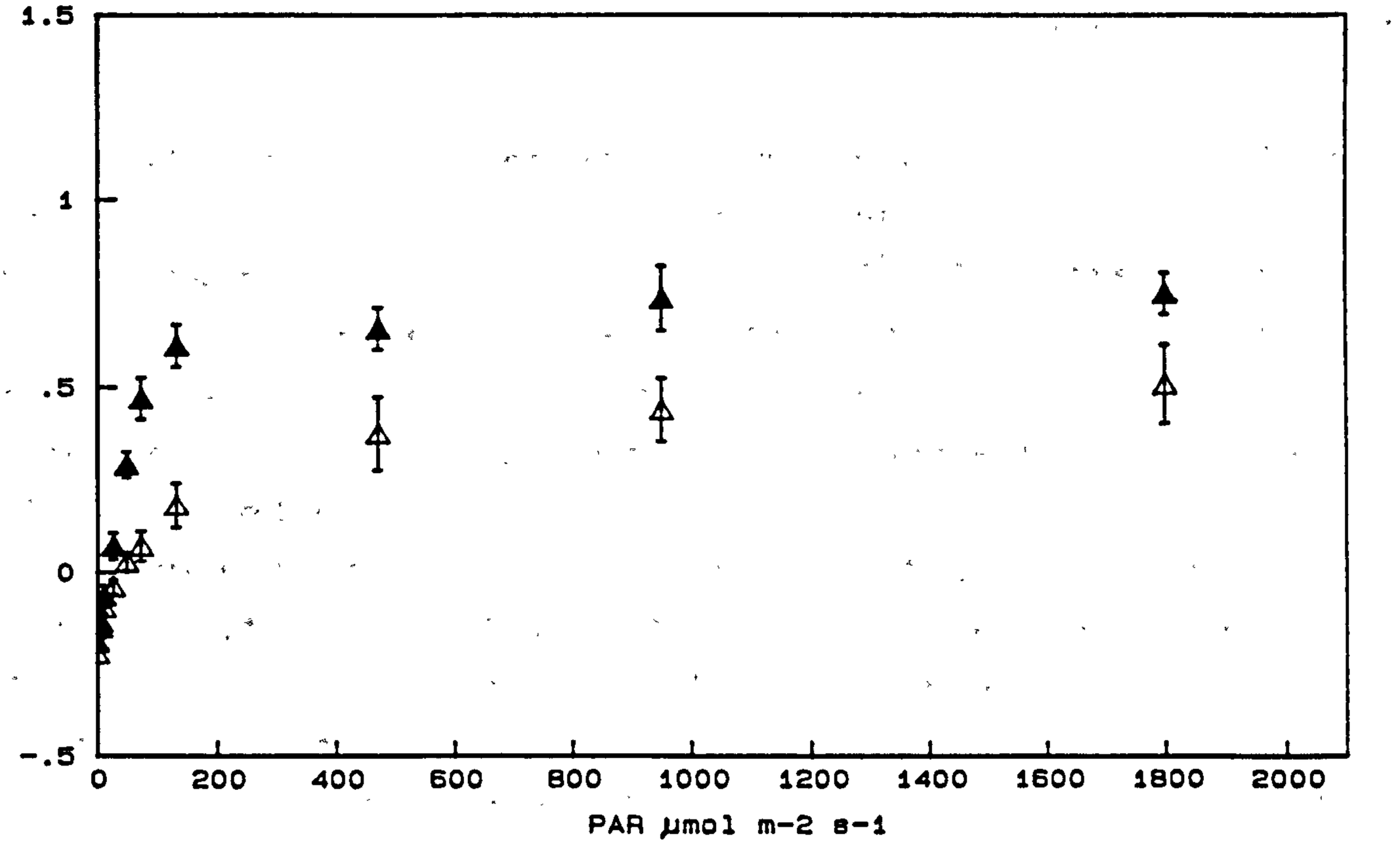

Figure 7. Effect of exposure to 2000 µmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater, as a function of light intensity (PAR). Response measured at 2.0 mol m^{-3} TIC and 21% O_2 represents the mean ± SE of four replicates.
Control (▲); high PAR treated (Δ).

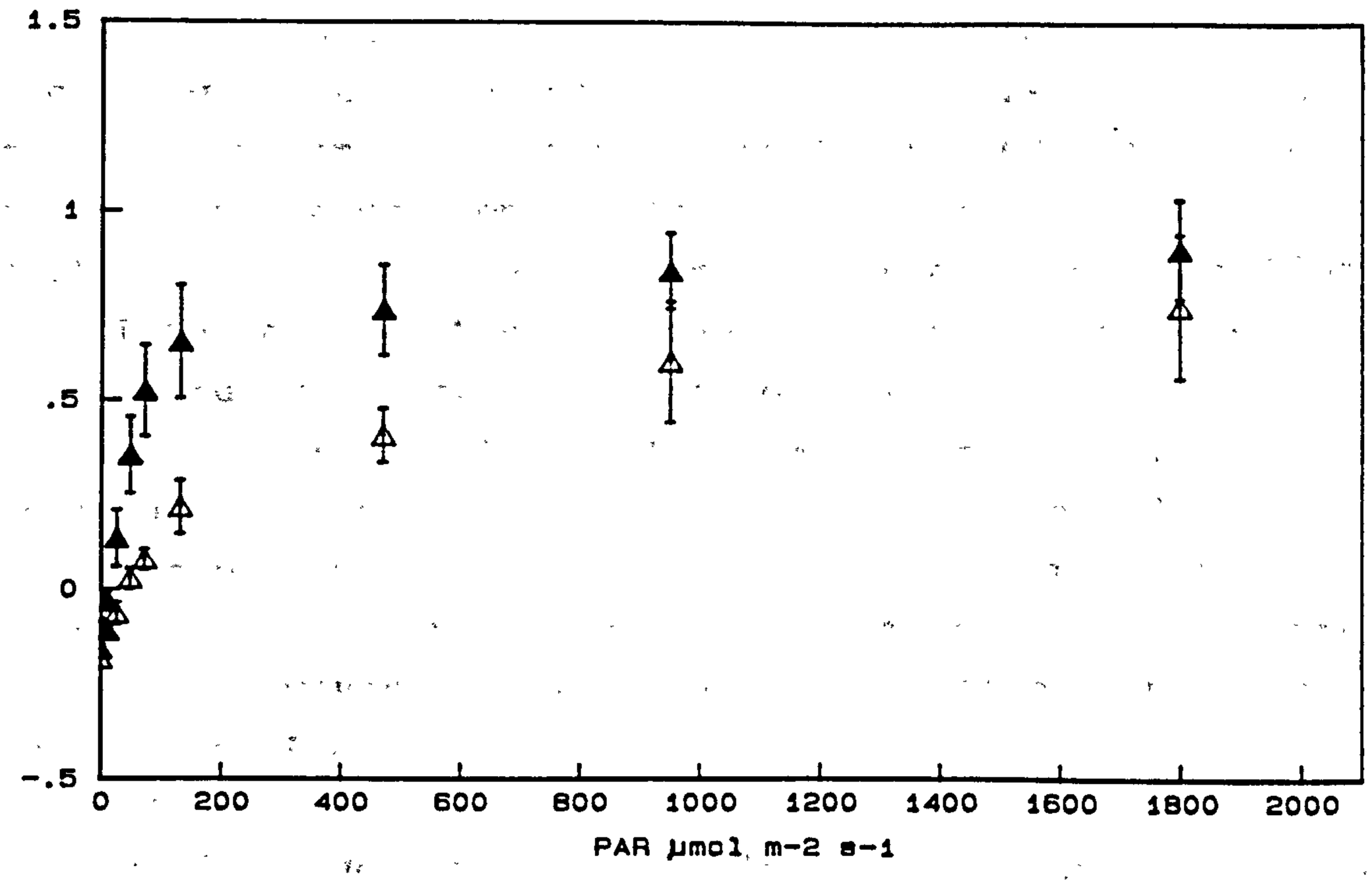

Figure 8. Effect of exposure to 2000 µmol photon m^{-2} s^{-1} (3 hrs) on the rate of apparent photosynthetic O_2 exchange by *U.lactuca* in seawater, as a function of light intensity (PAR). Response measured at 2.0 mol m^{-3} TIC and 1% O_2 represents the mean ± SE of four replicates.
Control (▲); high PAR treated (Δ).

Dark respiration rates were measured at the zero light intensity. For *P.umbilicalis* the rates of O_2 assimilation were between 0.08 and 0.10 μmol O_2 m^{-2} s^{-1}. Rates of dark respiration can be calculated from the algebraic solution of the regression line for the response under light limitation. In this species these values were lower than the observed rates.

Respiratory O_2 assimilation rates for *U.lactuca* of between 0.18 and 0.22 μmol O_2 m^{-2} s^{-1} were higher than for *P.umbilicalis* but similar to those calculated from the regression line.

These control photosynthetic responses were compared to those obtained following photoinhibitory treatments.

Photoinhibition of the photosynthetic response

Following exposure to 2000 μmol photon m^{-2} s^{-1} for 3 hours, considerable photoinhibition of the light response of *P.umbilicalis* was evident. There was however, no significant difference in the effects measured under the various O_2 and substrate concentrations (Figs 1-4). The light requirements increased from 2-4 to 12-22 μmol photon m^{-2} s^{-1} for compensation and from 100 to 200 μmol photon m^{-2} s^{-1} for saturation. Photoinhibition reduced the photosynthetic efficiency by more than a third, resulting in AQR values of between 176 and 218 μmol photon μmol O_2^{-1}. The maximum capacity of the response was inhibited by as much as 52% (rates of 0.65-1.00 μmol O_2 m^{-2} s^{-1}). Dark respiration rates were not significantly affected by photoinhibition, although those calculated from the regression line were closer to those measured in the dark. There was however, no evidence of photoinhibition of the response of *P.umbilicalis*, measured at 2.0 mol m^{-3} TIC and 1% O_2 (Fig 4).

For *U.lactuca*, significant levels of photoinhibition of the response were evident although, as for *P.umbilicalis*, the effect on photosynthesis was independent of the O_2 and substrate concentration (Figs 5-8). The light compensation point increased from 14-20 to 40 μmol photon m^{-2} s^{-1} and saturation of the response required 200 to 230 μmol photon

m^{-2} s^{-1}. Photosynthetic efficiencies were reduced by more than half, giving AQR values of between 118 and 270 μmol photon μmol O_2^{-1}. Values for V_{max} of between 0.40 and 0.73 μmol O_2 m^{-2} s^{-1} represent an inhibition of up to 48% of the maximum capacity. Again the observed rates of dark respiration were not affected by the photoinhibitory treatment (0.16 to 0.20 μmol O_2 m^{-2} s^{-1} assimilated).

DISCUSSION

Photoinhibition results from the interception of light fluxes in excess of those required for the process of photosynthesis. Although this results in a reduction of the overall response, it is due in part to a number of reversible protective mechanisms that are believed to prevent the occurrence of irreversable photo-oxidative damage.

Irradiances of 2000 μmol photon m^{-2} s^{-1} PAR were photoinhibitory for both *Porphyra umbilicalis* and *Ulva lactuca*. There is also evidence of photoinhibition in a number of subtidal macroalgal species (Coutinho and Zingmark 1987). Studies have compared the response of a subtidal and intertidal species of a genus to attempt to elucidate differences in the degree of susceptibility (Herbert and Waaland 1988; Herbert 1990).

P.umbilicalis and *U.lactuca* were exposed to high irradiance levels for a period of three hours. Rates of photosynthesis measured after this treatment showed up to 52% photoinhibition of the maximum capacity in both these species. Even after only one hour some effect was apparent (data not shown). There is however, little change in the rates of dark respiration which suggests the response is as a result of a specific effect on the photochemical steps of photosynthesis, rather than an overall effect on metabolism. For the subtidal species *Polyneura hilliae*, three hours at 200 μmol photon m^{-2} s^{-1} was sufficient to cause a 50% reduction in the maximum capacity, while at 400 μmol photon m^{-2} s^{-1} rate reduced by 78% (Nultsch, Pfau and Huppertz 1989). A second experiment also showed that the duration of the photoinhibitory light levels also determined the degree of inhibition. With short term treatments at 2000 μmol photon m^{-2} s^{-1}, *Porphyra nerocystis*, a subtidal species, was inhibited around three times faster that the intertidal *Porphyra perforata*. The treatment was carried out over a 100 minute period although after 60 minutes the photosynthetic response of *P.nerocystis* had fallen to zero. In contrast, there was a

20 minute lag phase in the effect on *P.perforata*, and the only 40% inhibition was evident after the first 60 minutes (Herbert and Waaland 1988). As for *P.perforata*, the photosynthetic capacities of the photoinhibited responses of *P.umbilicalis* and *U.lactuca* indicate that these intertidal species exhibited some degree of resistance to the effect of high irradience levels.

From the light intensity-response curves measured for the two intertidal species it is evident that an increase in the light requirements occurred in both species. The light compensation points, a measure of the threshold value of light utilization, were increased by photoinhibition. The effect was greater in *U.lactuca* although the control values for this species were consistently higher than that for *P.umbilicalis*, as shown in Section 1. The increase in the light saturation point is a related effect of the treatment. While a level of 100 μmol photon m^{-2} s^{-1} PAR was adequate to saturate the response in the controls, the curve of the photoinhibited response shows that light levels of two times greater are required to saturate the substantially reduced V_{max}. It is evident from these parameters that photoinhibition reduces the efficiency of the photosynthetic response as well as the capacity. A substantial increase in the AQR for both *P.umbilicalis* and *U.lactuca* quantifies the reduced ability to utilize the incident light energy. This is consistent with the overall increase in the light required to stimulate and maintain the photochemical reactions of photosynthesis, even when the overall rate of fixation is low. The whole thallus absorption spectra, measured for photoinhibited plants of *P.preforata* and *P.nerocyctis*, were unchanged in relation to the controls. This demonstrates that the decline in efficiency could not be attributed to self shading chloroplast movement or photo-oxidation of pigments (Herbert and Waaland 1988).

The primary site of photoinhibitory disruption has been attributed to damage to the primary electron acceptor QB. The degree of susceptability however, is thought to depend on the relative rates of damage and repair. The

inactivation of this component of the photosynthetic apparatus reduces the efficiency of electron transfer from the reaction centre of Photosystem II, to the the electron transport chains that generate reductants for the carboxylation reaction. The high energy state of the thylakoid membranes is also believed to stimulate the operation of a number of other photo-protective mechanisms, in order to reduce the damage to QB. These mechanisms however, result in an overall decrease in the light response of photosynthesis.

The magnitude of the response to excess PAR, in both *P.umbilicalis* and *U.lactuca*, was independent of the substrate concentration and level of O_2 present. Photoinhibition has been shown to occur in response to limitation of carbon fixation (Osmond 1980). From the control response it was evident that both 2.0 and 5.0 mol m^{-3} TIC was saturating for the two species. Even at the lower substrate concentration it appears that cycling of photosynthetic intermediates, maintained by an effective mechanism of carbon accumulation, may prevent further inhibition in *P.umbilicalis* and *U.lactuca*.

In addition, the degree of inhibition was similar under low and ambient O_2 concentrations. These results suggest that any photoinbitory effects were independant of O_2, and that photo-protection involving oxygen as an electron acceptor, or as a substrate for RuBPco was minimal. The resistance to photoinhibition in *P.umbilicalis* exposed to high PAR at low sustrate and O_2 concentrations is difficult to explain. Although the treatment was repeated it seems that this result should be taken as an anomaly.

In contrast, *P.nerocystis* and *P.perforata* treated at 2000 μmol photon m^{-2} s^{-1} under low O_2 showed less inhibition than during the same light treatment in air saturated seawater. Rather than having a photo-protective role, O_2 contributed to the observed effects. Oxidative reactions can form part of the terminal stages of photoinhibition, resulting in photobleaching of chlorophylls and carotenoids. This reaction results in irreversible damage to the photosynthetic apparatus, in

contrast to the effects caused by the photo-protective mechanisms, which are reversible. Resistance to photoinhibiton of photosynthesis has been investigated by a number of studies. In *Polyneura hilliae*, recovery of the photosynthetic ability was dependent of the duration of exposure to excess photon fluxes (Nultsch, Pfau and Huppertz 1990). A greater capacity for recovery is the basis of the proposed mechanism, which makes *P.perforata* more resistant to photoinhibition that *P.nerocystis* (Bose, Herbert and Waaland 1988). Later work by Herbert (1990) however, suggests that resistance in the intertidal species, in terms of a reduced rate of photoinhibitory damage in constrast to an increase in the rate of repair, is more feasible.

There appears to be some difference between *P.umbilicalis* and *U.lactuca* in the degree of susceptibity to photoinhibition, under the conditions of these experiments. This is evident from the greater effect on the light compensation and saturation point although the changes in V_{max} and AQR are similar for the two species. It has been pointed out however, that any mechanisms believed to confer resistance must be considered in relation to other interactive effects. Factors such as the duration of exposure and subsequent alteration of metabolism will influence the two species to a different degree. As no measure of the rate or extent of recovery was made for *P.umbilicalis* and *U.lactuca*, it is not possible to determine the nature of the photoinhibitory effect in these two species.

In view of the fact that marine intertidal macroalgae appear to be shade adapted plants (Section 1-Discussion) photoinhibition seems inevitable. Fewer PS II units, with larger antennae, in relation to PS I, increases the absorption cross section and therefore the efficiency under low light. This seemingly benificial modification of the photosynthetic apparatus, must be balanced against the greater susceptibility to the photoinhibitory effects expected in the event of excess PAR. More importantly, other distinctive characteristics of the photosynthetic

system in these plants must also be considered, in relation to the mechanisms proposed.

In marine intertidal macroalgae photoinhibition may represent an important adaptation to the intertidal environment, that has not yet been evaluated. In combination with desiccation resistance and the operation of an effective mechanism of carbon accumulation, photoinhibition may determine the ability of the plants to exist in the extreme environment of the intertidal zone.

CONCLUSIONS

CONCLUSIONS

This study investigates photosynthesis and mechanisms of inorganic carbon accumulation in two species of marine intertidal macroalgae. By using a range of physiological and biochemical techniques it was possible to determine the the photosynthetic characteristics and understand more about the method of inorganic carbon uptake in both *Porphyra umbilicalis* and *Ulva lactuca*.

These two species occur at the same tidal elevation, although *P.umbilicalis* grows on bare rock faces and *U.lactuca* is submersed in rockpools. The two species have a similar morphology which justifies the physiological comparison of the gas exchange and light response characteristics.

P.umbilicalis shows an ability to use both HCO_3^- and CO_2 as the substrate for photosynthesis. The V_{max} is higher when CO_2 is available, while the rates at natural levels of HCO_3^- and CO_2 reveal that productivity will be greater when submersed. The gas exchange characteristics of this species show little oxgyen sensitivity over the range of 1% to 42% air equilibrated O_2 concentration. By comparing both the $K_{0.5}$(TIC) and the V_{max} it is evident that the oxygenase activity of RuBPco is largely suppressed.

Analysis of the inorganic carbon concentration-reponse indicates the preferred inorganic carbon species in air and in seawater. The photosynthetic $K_{0.5}$(TIC) of *P.umbilicalis*, measured in seawater at pH 5.5 can be compared to the that at pH 8.0. These two values show that the affinity for CO_2 is greater than for HCO_3^-, consistent with the greater capacity in air, under elevated CO_2 concentrations.

These characteristics vary seasonally, with a substantial increase in the affinity for CO_2 in summer. This maintains the rate of photosynthesis under substrate limitation at a higher than expected rate, as the V_{max} for both HCO_3^- and CO_2 use is lower than in winter.

Low light compensation and light saturation points denote light requirements comparable to shade adapted

terrestrial plants. The light requirements are similar in both air and seawater and while there is an increase in the compensation point in the summer population, there is no corresponding effect on the light saturation point. The maximum capacity of the light intensity-response in *P.umbilicalis* is greater in seawater than in air, but this is not reflected by any difference in the photosynthetic efficiency. The higher level of PAR in summer result in a decrease in the total content of chlorophyll a in *P.umbilicalis*. This adaptation is consistent with the decrease in the V_{max} of the photosynthetic system. The effect can be attributed to a decrease in the number, rather that a decrease in overall size of the PS units as there is no corresponding loss of photosynthetic efficiency.

A high level of PAR over an extended period of time results in photoinhibition of *P.umbilicalis* even under optimum conditions. In the summer population, already acclimatized to a degree by increased light levels, excess PAR significantly alters the photosynthetic characteristics. Higher light compensation and saturation points are evident and both the maximum capacity and corresponding photosynthetic efficiency is reduced.

Susceptibility to photoinhibition appears to be related to a number of interelated factors. In marine intertidal macroalgae some resistance may be attributed to the occurrence of an effective mechanism of inorganic carbon accumulation.

Various methods of inorganic carbon uptake have been proposed for marine intertidal macroalgae. Most involve a combination of direct HCO_3^- and CO_2 use, or CA mediated HCO_3^- use. By using the sulphonamide inhibitors AZ and EZ it is possible to selectively inhibit CA. This highlights some important characteristics of the inorganic carbon accumulating mechanism in *P.umbilicalis*.

Inhibition of the external CA significantly alters the substrate concentration-response. The decrease in photosynthetic capacity, and reduced substrate affinity suggest that effective inorganic carbon accumulation is

dependent on the activity of this enzyme. The linear substrate concentration-response is evidence that the observed rates of photosynthesis are maintained by passive diffusion. The effect of external CA inhibition is similar in air and seawater, suggesting this enzyme mediates both HCO_3^- and CO_2 use.

At pH 5.5 however, the concentration of CO_2 in seawater is sufficient to alleviate the requirement for internal CA. In contrast, HCO_3^- will be converted to CO_2 and take up by diffusion, in addition to any available free CO_2. In air however, the role of this enzyme is less clear, although the same reaction would give rise to an concentration gradient in the surface film of water maintaining a supply of HCO_3^- at the plant surface.

Inhibition by EZ further decreases the photosynthetic response indicating inhibition of internal CA. The effect is less severe in air than in seawater, while the response in seawater is consistent with a lower concentration of free CO_2 available for direct use, in the absence of CA activity. It is apparent from these responses that internal CA activity governs the rate of CO_2 diffusion. The affinity for inorganic carbon is primarily dependent on external CA, although internal CA determines the overall capacity.

The direct relationship between rates of photosynthesis and external CO_2 concentration is evident from a comparison of the response to range of seawater pH values. Analysis of the response curve at pH 5.5 shows that *P.umbilicalis* has a higher affinity for CO_2 than HCO_3^-. At pH 8.0 and above the inhibition of external CA only is sufficient to modify the inorganic carbon affinity. Below pH 8.0 this affinity is governed to a greater extent by the availability of CO_2. Consequently, inorganic carbon uptake at pH 5.5 is no longer dependent on the activity of the external enzyme. Although the response is maintained by diffusion, the activity of internal CA has an effect on the maximum capacity. In the presence of adequate levels of CO_2 the photosynthetic capacity can equal that achieved by CA dependent inorganic carbon uptake. There is however some evidence that high levels of diffusive CO_2 uptake, in the

absence of CA activity, results in inhibition of photosynthesis *per se*.

Direct analysis of the inorganic carbon species taken up by *P.umbilicalis*, using the inorganic carbon disequilibrium technique, confirms that HCO_3^- use is mediated by an external CA although direct CO_2 use is also of importance. It is also a possibility that direct HCO_3^- use, in addition to that for CO_2, can occur. This mechanism will be unimportant unless the substrate concentration is very high.

The results of this study suggest that inorganic carbon acquisition in *P.umbilicalis* depends primarily on CA mediated HCO_3^- use, and some direct CO_2 use. The characteristic kinetic relationship between external inorganic carbon concentration and the rate of photosynthesis is a function of the activity of an external CA. This enzyme has been shown to determine the affinity of the photosynthetic response for the available substrate. In contrast, the absolute capacity of the inorganic carbon uptake is mediated to a degree by the activity of a CA located internally.

A high affinity for CO_2, and the requisite for external CA suggests that HCO_3^- may be converted to CO_2 which then passes across the plasma membrane, presumably by diffusion. Within the cell, internal CA increases the rate of transfer of inorganic carbon to the site of fixation in the chloroplast. Located within the cytoplasm, this enzyme may catalyze the rate of conversion of CO_2 to HCO_3^-. The reaction would give rise to a concentration gradient, facilitating the uptake of CO_2 and increasing the rate of formation of a cytoplasmic pool of HCO_3^-. In contrast, the enzyme may catalyze the reverse reaction, converting HCO_3^- to CO_2. Within the cytoplasm the CO_2 formed would diffuse across the plasma membrane. Alternatively, active transport of HCO_3^- into the chloroplast and subsequent catalysis by CA, would also supply CO_2 for photosynthesis.

Ulva lactuaca also shows an ability to use both HCO_3^- and CO_2. Substrate saturation of the HCO_3^- response occurs at the natural seawater concentration of HCO_3^-, in contrast

to the requirement for CO_2 in air, so that productivity will be greater when submerged. The summer population of *U.lactuca* is characterized by a reduced photosynthetic capacity in response to HCO_3^- and CO_2 supply, although there is a significant increase in the affinity for CO_2. Both changes in temperature and light levels may determine the photosynthetic characteristics of this population.

In *U.lactuca*, acclimation to higher irradiance levels in summer is reflected by a decrease in both the total chlorophyll content and the ratio of chlorophyll a:b. The light intensity response of this species shows that the light compensation point is higher than that of *P.umbilicalis*, but the level of PAR required for saturation is lower, and constant when measured on a seasonal basis.

The summer population of *U.lactuca* has a reduced photosynthetic efficiency and capacity in air, although the light requirements are similar. The decrease in the maximum rate occurs in the absence of an effect on the photosynthetic efficiency. As in *P.umbilicalis* this can be attributed to a decrease in the number of PS units, in addition to any effect on RuBPco activity.

Analysis of the substrate concentration-response of *U.lactuca* shows that the affinity for HCO_3^- is significantly greater than for CO_2. At pH 5.5, both the V_{max} and $K_{0.5}$(TIC) are lower than at pH 8.0. In addition there is evidence of the narcotic effect of high levels of CO_2.

The photosynthetic response of *U.lactuca* is unaffected by the addition of the non-permeable inhibitor AZ. This indicates that, in contrast to *P.umbilicalis*, inorganic carbon uptake is not dependent on the activity of an external CA. The results obtained using the inorganic carbon disequilibrium technique are consistent with an ability to use HCO_3^- directly, although CO_2 use is not excluded.

In contrast, the effect of the permeable inhibitor is of some importance. Partial inhibition of internal CA results in a greatly decreased photosynthetic capacity in this species. The shape of the concentration-response,

however, reveals the effect to be significantly different to that in *P.umbilicalis*. While the saturated rate or V_{max} is considerably lower, the uptake of inorganic carbon shows the characteristics of an actively mediated response. The substrate affinity for HCO_3^- and for CO_2 is high and does not reflect uptake mediated solely by diffusion. Under these conditions it is evident that although the photosynthetic reponse is affected by partial inhibition of internal AZ, active uptake of the available substrate is still of greater importance than the diffusive uptake of CO_2.

Complete inhibition of internal CA significantly alters this characteristic response. The direct relationship between the external HCO_3^- concentration and the rate of photosynthesis indicates the diffusive uptake of substrate. Direct CO_2 use is consistent with the results obtained using the inorganic carbon disequilibrium technique, although at the greatly elevated substrate concentration there is evidence of some passive HCO_3^- uptake. The rates at pH 7.5 and 8.5 do not show the same correlation with the change in concentration that would be expected if only CO_2 is diffusing across the plasma membrane. This contrasts with the response in *P.umbilicalis* is, however, easily explained in terms of the lower CO_2 affinity of this species.

The results of this study suggest that inorganic carbon acquisition in *U.lactuca* is dependent on direct HCO_3^- and CO_2 use. CO_2 uptake can occur by diffusion, while the transport of HCO_3^- across the plasma membrane must be actively mediated. It is also possible that this same mechanism may regulate a proportion of the CO_2 transferred. Inorganic carbon uptake in air and seawater is, however, significantly dependent on the activity of CA located internally. The photosynthetic response following partial inhibition suggests that although the substrate affinity of the response is unaffected, the overall capacity is limited. In addition the effect is not overcome by an increase in CO_2 diffusion. One possible role for the internal enzyme is a CA-like moiety involved as a component

of the HCO_3^- porter system at the plasma membrane as in *Anabaena* (Kaplan 1983). This would explain the apparent ability of this enzyme to regulate both HCO_3^- and CO_2 use.

The effect of higher concentration of EZ could be attributed to a more complete inhibition of the CA-like moiety or an effect on a second possible CA, located within the cytoplasm or chloroplast. As in *P.umbilicalis* this enzyme may determine the overall capacity of the system by regulating the levels of one or more pools of inorganic within the cell. Catalysis of the interconversion of HCO_3^- to CO_2 would serve to both increase the supply of substrate to the carboxylase and maintain a concentration gradient, assisting HCO_3^- uptake. In contrast, catalysis of the reverse reaction would serve to increase the rate of HCO_3^- accumulation within the cytoplasm, whilst maintaining a concentration gradient of CO_2 across the plasma membrane.

While *P.umbilicalis* and *U.lactuca* have a very similar morphology this study has shown the photosynthetic characteristics and related physiological mechanisms are not directly comparable. Although both species are able to use both HCO_3^- and CO_2 as substrates for photosynthesis, the inorganic carbon uptake occurs by two different methods. In *P.umbilicalis* the mechanism involves the direct CO_2 use and CA dependent HCO_3^- use, while for *U.lactuca* it involves direct HCO_3^- and CO_2 use. Both mechanisms are mediated by the activity of an internal CA, but there is insufficient evidence to conclude whether the location and role of this enzyme is the same in the two species.

This and other studies have provided some evidence for inorganic carbon accumulation in marine macroalgae. With the experimental techniques used, it has not yet been possible to obtain the same degree of detail achieved by studying the mechanism in microalgae. In addition there is little correlation between environmental factors and the various mechanisms elucidated so far. Surif and Raven (1989) found higher levels of external CA, as a percentage of the internal enzyme, in species that experience longer periods of emersion. This is also evident for the two species investigated in this study. They also state that

the ability to concentrate inorganic carbon appears to be more efficient in higher intertidal species, determined to a degree by HCO_3^- availability and facilitated by the activity of external CA. Similar observations from this study suggest that this ability may also be determined by the affinity for CO_2.

In conclusion, it is evident that the strategy and regulation of inorganic carbon accumulation in marine intertidal macroalgae is both significant and complex, depending on environmental conditions and habitat preference. The characteristics presented here suggest that *P.umbilicalis*, more frequently exposed, has adapted to use either HCO_3^- with the aid of an external CA, or take up CO_2. In contrast, the rockpool habitat of *U.lactuca*, with a more consistent HCO_3^- supply, has resulted in direct HCO_3^- use and a reduced affinity for CO_2.

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