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Temperature and emergence effects on the net photosynthesis of two *Zostera noltii* Hornem. morphotypes

J. L. Pérez-Lloréns[1] & F. Xavier Niell[2]
[1]*Departamento de Biología, Facultad de Ciencias del Mar, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain;* [2]*Departamento de Ecología, Facultad de Ciencias, Universidad de Malaga, 29071, Málaga, Spain*

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Abstract

Apparent photosynthetic rates (APS) of two *Zostera noltii* Hornem. morphotypes were measured in air and in water at different temperatures with a closed infra-red gas analysis system (IRGA).

Hyperbolic functions accurately described the photosynthesis-CO_2 relationships when the leaves were exposed to air. The photosynthetic behaviour in water, on the contrary, could not be described by Michaelis type kinetics, due to the existence of a rapid transition from the initial slope to the saturation phase. Both morphotypes (narrow-leaved, NLM and large-leaved, LLM) showed higher APS rates in water than in air, although the highest APS rates, in air as well in water, were recorded for the NLM.

Temperature had a significant influence on the photosynthetic parameters: APS_{max} (maximum photosynthetic rate) decreased (in air and in water) with increased temperature in both morphotypes; compensation points (CP) in air increased at high temperature, especially in the LLM. NLM specimens showed enhanced affinity (lower Km) with increasing temperature in air. On the contrary, Km values in water were not significantly affected by temperature.

The results suggest that NLM specimens are better adapted than the LLM to occur exposed to air. The distributional pattern of the two morphotypes in the Palmones Estuary is discussed on the basis of their photosynthetic behaviour.

Introduction

The main environmental feature of the intertidal zone is a regular exposure to the air. When plants are emersed, they are subject to desiccation and fluctuating temperature regimes. The desiccation effects depend on season and time of day; the irradiance (and desiccation) may be reduced through shading by clouds, water, shore topography and clumping (Lobban *et al.*, 1985); in general, and from an anthropocentric point of view, the plants are stressed. But one of the most interesting questions to answer is: What is the degree of adaptation of aquatic plants to live in air?.

Much classical work has been devoted to measuring the capacity of seaweeds to survive desiccation (Muenscher, 1915; Isaac, 1933, 1935; Biebl, 1938, 1962; Feldman, 1951). In the literature on emersion effects, several studies have examined the relationship between desiccation and photosynthetic rates. Positive effects of desiccation (loss of 10–50% of the just emersed water) on photosynthesis rates of intertidal brown algae have been reported (Johnson *et al.*, 1974; Quadir *et al.*, 1979; Johnston & Raven, 1986a), showing that high intertidal species had higher photosynthetic rates in air than in water. However, decreasing photosynthetic rates following desiccation have also been reported for many algae (Bidwell & Craigie, 1963; Quadir *et al.*, 1979; Beer & Eshel, 1983).

Little work on emersion effects on seagrass photosynthesis has been reported. However Cooper & McRoy (1988) and Cooper (1989), using carbon isotopic techniques, reported that intertidal populations of the seagrasses *Phyllospadix torreyi* Watson, *P. serrulatus* Rupr. ex Aschers. and *Zostera marina* L. were probably incorporating some atmospheric carbon dioxide during emersed photosynthesis.

The study of air exposure and temperature effects on the photosynthetic rate of two *Zostera noltii* morphotypes is the major aim of this work; one morphotype (NLM) is emersed daily and its narrow leaves are exposed to air desiccation, and the other (LLM) is emersed only rarely and its leaves are erect and wider than those of the morphotype living in the high intertidal. It seemed likely that differences between these morphotypes are not just morphological and some particular physiological performances could be observed. Drew (1979) compared the photosynthetic rates in water at different irradiances of wide-leaved (subtidal) and narrow-leaved (intertidal) forms of *Z. marina*, showing the smaller seagrass the higher rates of net photosynthesis than did leaves of the larger form. Also McMillan (1984) reported a higher tolerance to high temperatures a narrow-leaved seagrass morphotype.

Similar cases of polymorphism to that found in *Zostera noltii* have been reported in other seagrasses. Thus, Setchell (1920) noted that plants of *Z. marina* with the narrowest leaves were found in shallower water, on firmer substrate, and plants in the intertidal zone, on soft substrate, always had the widest leaves. Drew (1979) and Harrison (1982) also noted this polymorphism in *Z. marina* and *Z. japonica*. den Hartog (1970) recorded that certain species of *Halodule* are more likely to occur in shallow, inshore habitats, and that the narrow-leaved plants of *Halodule* seem to be the most ubiquitous and more likely to be exposed to air at low tides. The same author reported a diversity of habitats for *Halophila ovalis*, treated as a polymorphic taxon, and noted that plants with small, flexible leaves occurred in sites exposed at low tides, while plants with stiff erect and large leaves occurred in the lower part of the intertidal belt. Furthermore, Nienhuis (personal communication) found a morphological variation in *Z. noltii* from the Mauritanian coast, depending on their bathymetric location. den Hartog (1970) reported that in non-tidal waters (in the French Mediterranean and in Ringkobing Fjord, Denmark) the generative axes of *Z. noltii* are often considerably elongated and the leaves are usually wider than those of specimens from the intertidal belt.

Material and methods

Specimens of the seagrass *Zostera noltii* were collected at low tide in the Palmones Estuary, Southern Spain (36° 11′ N, 5° 27′ S). The two morphotypes of *Z. noltii* dealt with in this paper differ in leaf dimensions and in their location in the estuary in relation to tidal height. Large-leaved plants (LLM) occur at 0.10–0.25 m (height above lowest astronomical tide) and their leaf dimensions are up to 45 cm × 2–2.5 mm. Narrow-leaved plants (NLM) are located at 0.75–0.90 m, at the edges of the marsh channels, and their leaf dimensions are up to 15–20 cm × 1–1.5 mm. The length of time in emersion depends on their distributional pattern: the narrow-leaved plants are emersed 50–70% of the time while the large-leaved plants are out of the water only 5% of the time (Pérez-Lloréns, 1991).

After collection, plants were kept cool and transported to the laboratory in a polyethylene icebox within 5 hours of collection. Before any experimental work, plants were maintained for 1 to 3 days in filtered seawater (pH = 8.3) (Whatman GF/C) under aeration at a constant temperature of 16 °C and photon flux density of 150 μE m^{-2} s^{-1} (Solar LFD 18 W tube).

To study the disappearance of inorganic carbon in the water, healthy leaves were placed in a water-jacketed glass chamber (Browse, 1979) containing 75 ml of filtered seawater (19‰ Cl) in equilibrium with air at 400 $\mu l\,l^{-1}$ gas phase $CO_{2(g)}$. The volume of the gas phase was 60 ml. In the measurements of APS by exposed seagrass, leaves were suspended in the water-jacketed glass chamber and weighed before and after each experiment so that the rate of water loss from the leaves could be calculated. The air was pumped at a constant flow rate (200 ml min^{-1}) through the glass chamber. Leaves were exposed to a saturating photon flux density of 950 $\mu E\ m^{-2}\ s^{-1}$ (400–700 nm) (Jiménez *et al.*, 1987) generated by a Reflecta slide projector (FLECTA 150W halogen lamp). A magnetic stirrer provided constant mixing. The leaves had an acclimation time of 20 minutes before starting the incubations.

The change in the CO_2 partial pressure in the gas phase of the chambers was determined with a closed infra-red gas analysis system (IRGA, ADC model MKIII) connected to a strip chart recorder to obtain continuous $CO_{2(g)}$ concentration-time curves. The system was calibrated by using CO_2 from a gas cylinder with known CO_2 concentration (Sestak *et al.*, 1977). Incubations were carried out in air as well in water at 15, 20, 23, 25, 27, 30 and 35 °C for 120 minutes. CO_2 compensation points were directly obtained; this value corresponds to the lowest CO_2 concentration attainable at which the net photosynthetic rate is zero (Edwards & Walker, 1983; Halliwell, 1984; Johnston & Raven, 1986a).

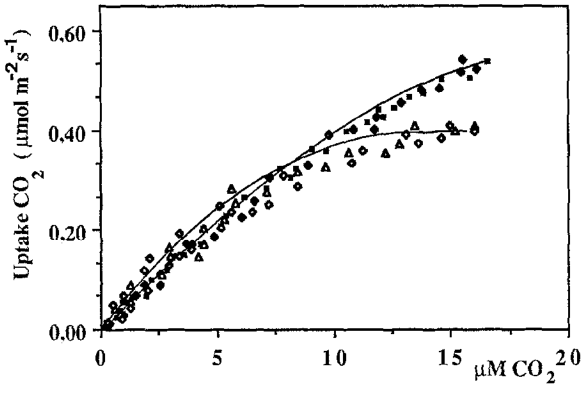
In experiments with submerged seagrass, inorganic carbon abstraction by the leaves will cause that aquaeus and (because they are in equilibrium) the gasaeus CO_2 concentrations to fall. However, the IRGA will underestimate photosynthesis whenever bicarbonate is present, because CO_2 will be generated from bicarbonate as the dissolved inorganic carbon pool shifts toward a new equilibrium. The problem is to estimate the change in total inorganic carbon (TIC) content of the system. Seawater from Palmones Estuary, (pH 8.3) had a concentration of 13.3 μM of CO_2 and 2500 μM of HCO_3^-. Seawater is not well buffered itself, and a buffer (10 mM Tris-HCl) was added to overcome the problems associated with the pH-dependent equilibrium among the dissolved inorganic carbon species. Henry's Gas law provided the basic tool to calculate the partial pressure of CO_2 (HCO_3^- and CO_3^{-2} concentrations) during the incubations in water (Riley & Skirrow, 1965; Stum & Morgan, 1970; Riley & Chester, 1977; Johnston & Raven, 1986b). The method we used has sources of error. One among them is that the concentration of CO_2 in the gas phase decreases during the experiments due to the uptake by plants, and the proportion of oxygen increases. This can enhance photorespiration in C_3 plants giving an overestimation of the compensation point; C_4 and C_4-like plants do not present this problem; Bidwell & McLachlan (1985) and Johnston & Raven (1986b) suggested that photorespiration is suppressed in macroalgae but nothing is known about seagrasses.

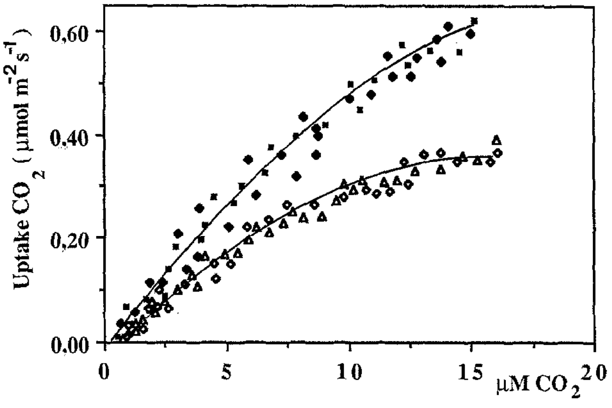
APS rates were calculated as the slopes of the recorded curves (CO_2 concentration-time). The model used to describe the photosynthetic behaviour of the leaves in water is a double linear model composed by two straight lines; the first one (initial slope) includes those values of APS for TIC (total inorganic carbon) concentrations lower than the TIC saturation point, and the second one has been defined for TIC concentrations higher than TIC saturation point. TIC saturation point (TIC_k) could be defined in the same way as the saturation onset parameter (E_k) for light (see Kirk, 1983), i.e. the value of TIC corresponding to the point of intersection between the extrapolated linear part of the photosynthesis-carbon curve and the horizontal line at APS_{max}. APS rates in air were fitted to equation (13.41) of Edwards & Walker (1983) (equation 1).

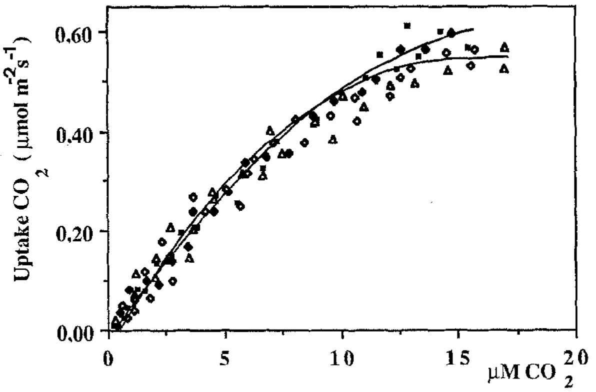
$$APS = APS_{max} \times \frac{[CO_2] - CP}{[CO_2] + Km}, \qquad \text{(Eq. 1)}$$

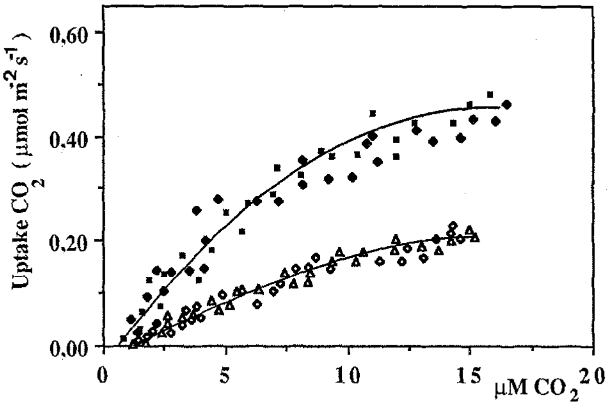
where:

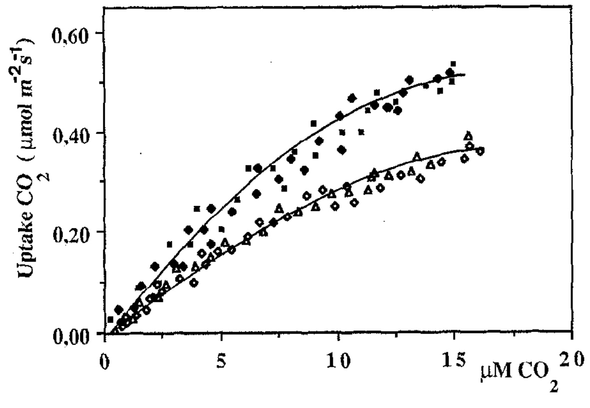
APS = net photosynthetic rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$);
CP = compensation point for CO_2 (μM);
Km = half-saturation constant (μM).

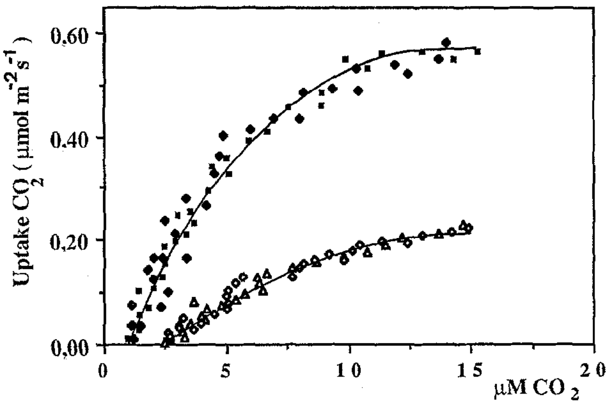












Results

Exposed leaves

Results of the emersion experiments are presented in Fig. 1 (a–g) and in Fig. 3. (a–d) Water losses were determined at 15 and 35 °C as percentage of dehydration. As expected, the highest desiccation value (15%) was observed at 35 °C.

Data showed significant fitting (χ^2 test, $p < 0.01$) to the Edwards & Walker (1983) model

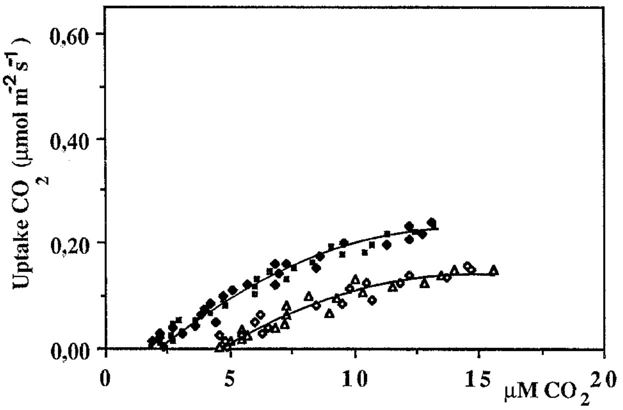


Fig. 1. The rate of apparent photosynthetic CO_2 assimilation by two morphotypes of *Zostera noltii* in air as a function of CO_2 concentration, at different temperatures (a: 15 °C, b: 20 °C, c: 23 °C, d: 25 °C, e: 27 °C, f: 30 °C, g: 35 °C) and at PFD of 950 μE m^{-2} s^{-1}. Results of two experiments depicted with different signatures (solid symbols correspond to NLM; open symbols to LLM). Solid lines are predicted by Edwards & Walker (1983) equation.

(equation 1). Values of APS_{max} Km, and CP have been obtained using a computer assisted iterative process (Elsevier-Biosoft ENZFITTER program) for this equation.

Atmospheric CO_2 concentrations did not saturate photosynthesis when the plants were exposed. Uptake rates obtained at the highest atmospheric CO_2 concentrations (400 ppm) have been designated as APSobs.

Temperature had a significant influence on the kinetic parameters calculated. APS_{max} decreases with increasing temperature in both morphotypes (Kruskall-Wallis test, $p<0.1$), although NLM specimens reached higher values than did the LLM plants (U-test, $p<0.1$) (Fig. 3a). No significant influence of temperature was observed in the APSobs values of NLM up to 35 °C (Fig. 3b). The influence was significant for the LLM plants, with a decrease in the APSobs values, with increasing temperature (Kruskall-Wallis test, $p<0.1$). The highest APSobs values were shown by the NLM at 25 °C. APS rates decreased considerably at 35 °C in both morphotypes.

Km is an inverse estimation of the affinity for the substrate. The affinity of NLM increased with temperature (Kruskall-Wallis test, $p<0.05$) (Fig. 3c). In contrast, it decreased in LLM up to 27 °C. The LLM showed higher affinity for CO_2 at lower temperatures (15–25 °C) than did the NLM (U-test, $p<0.05$).

CO_2 compensation points increased with increasing temperature in both morphotypes (Kruskall-Wallis test, $p<0.05$) (Fig. 3d). LLM specimens showed the highest values from 23 °C upwards (U-test, $p<0.01$).

Submerged leaves

Results corresponding to this set of experiments are presented in Fig. 2 (a–f) and in Fig. 4 (a–d). APS rates versus TIC were fitted to the double linear model described above. Saturation was observed at 2–2.5 mM of TIC.

Temperature had a significant influence (Kruskall-Wallis test, $p<0.1$) on the APS_{max} values reached by NLM, showing a decrease with increasing temperature. No significant effect was observed for the LLM specimens (Fig. 4a).

The affinity was affected by temperature in both morphotypes (Kruskall-Wallis test, $p<0.1$), showing variable behaviour and with the highest affinity occurring in the NLM at 23–25 °C (U-test, $p<0.1$) (Fig. 4b). No significant differences between the two morphotypes were found at higher temperatures.

Temperature had a significant influence (Kruskall-Wallis test, $p<0.1$) on the compensation point in both morphotypes (Fig. 4c), but the trend was not so clear as in the data from seagrass exposed to air. No significant differences were observed between the morphotypes.

Initial slopes (conductance to inorganic carbon m s^{-1}) decreased significantly with increasing temperature values for the NLM specimen (Fig. 4d); on the contrary, a maximum at 27 °C was found in the LLM plants, although the temperature had no significant influence in this latter morphotype. NLM plants showed higher values of initial slope at low temperatures (15–25 °C).

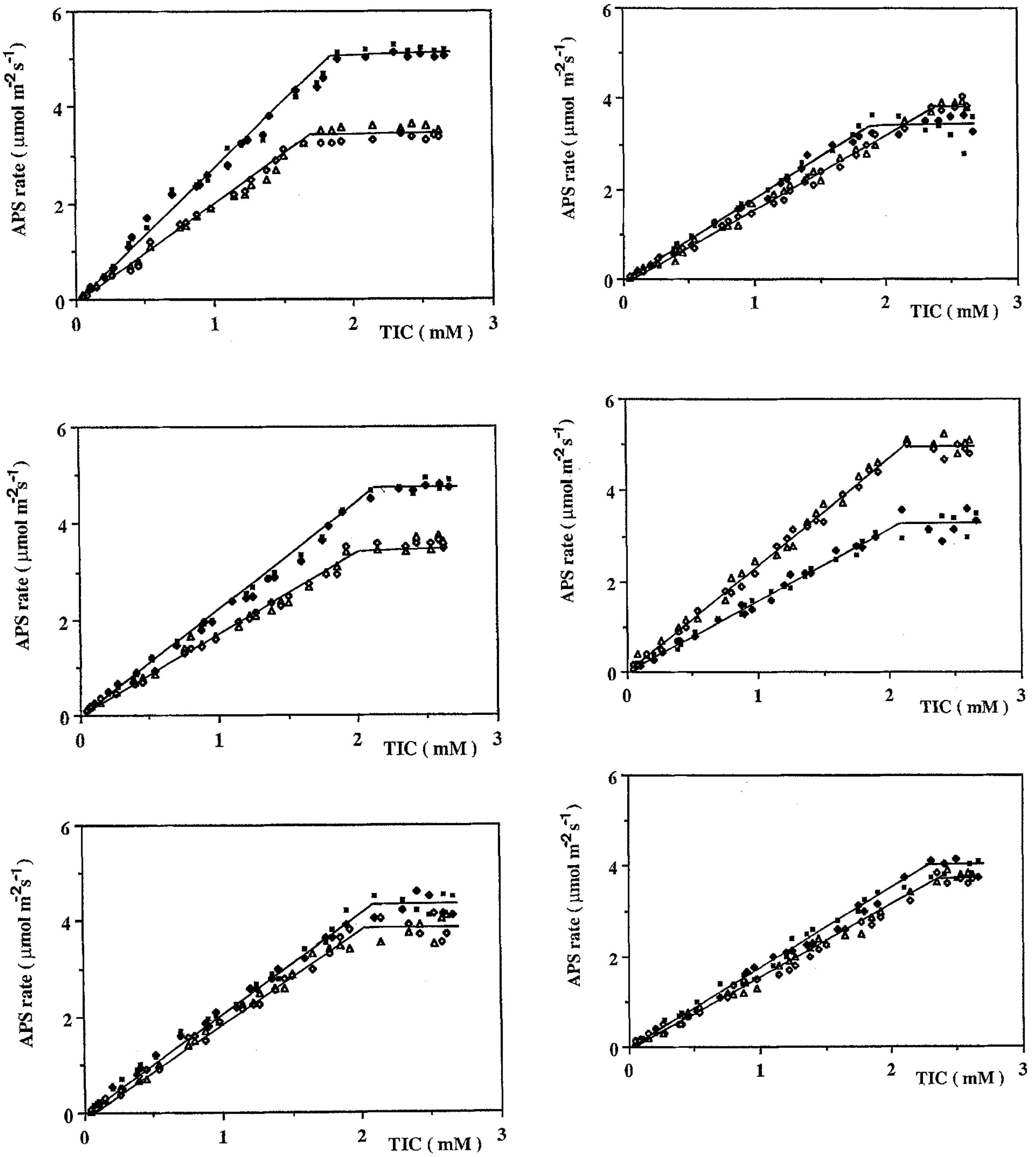


Fig. 2. APS rates by two morphotypes of *Zostera noltii* in water as a function of the total inorganic carbon at different temperatures (a: 15 °C, b: 20 °C, c: 23 °C, d: 25 °C, e: 27 °C, f: 30 °C) and at PFD of 950 μE m^{-2} s^{-1}. Results of two experiments depicted with different signatures (solid symbols correspond to NLM; open symbols to LLM).

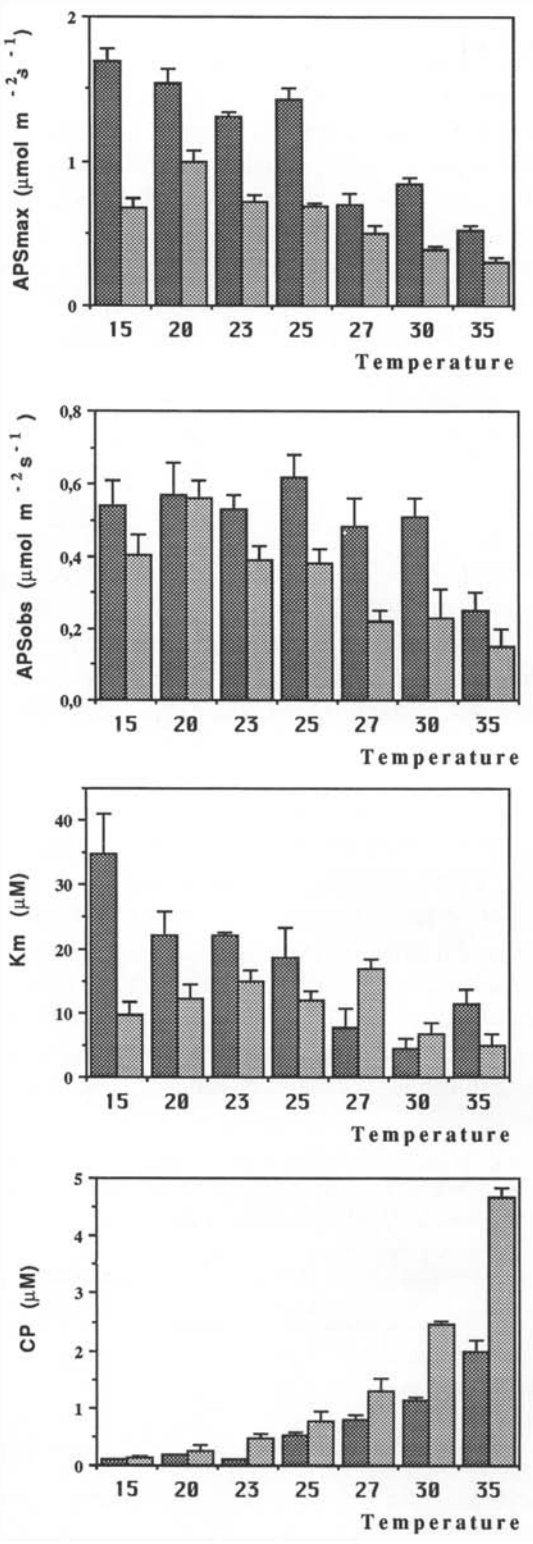


Fig. 3. a- Predicted CO_2 uptake rates (APS_{max}), b- maximum observed uptake rates ($APSo_{bs}$), c- half saturation constants (Km), d- CO_2 compensation points (CP) of two *Zostera noltii* morphotypes measured in air (NLM, darker bars; LLM, lighter bars) at different temperatures and PFD of 950 μE m^{-2} s^{-1} s.d. given as vertical bars.

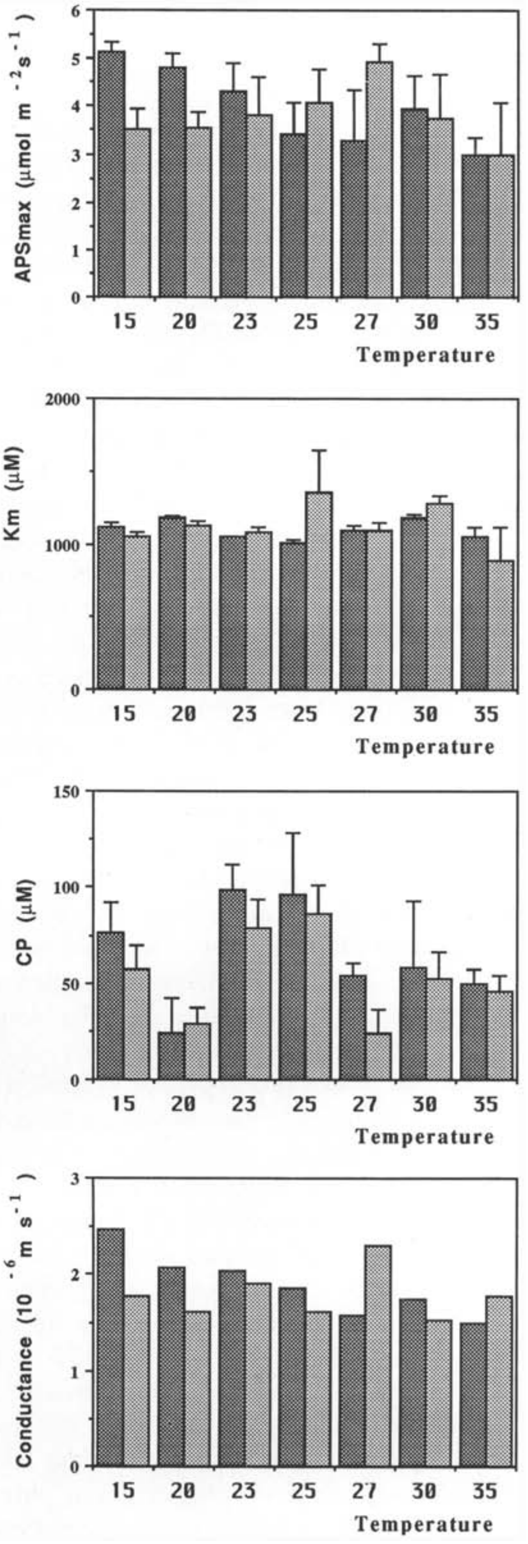


Fig. 4. a- Maximum APS rates (APS_{max}), b- half saturation constants (Km), c- TIC compensation points (CP), d- initial slopes (conductance) of two *Zostera noltii* morphotypes measured in water (NLM, darker bars; LLM, lighter bars) at different temperatures and PFD of 950 μE m^{-2} s^{-1} s.d. given as vertical bars...

Discussion

Two main aspects will be discussed, the first one being the fitting of the photosynthetic curves and the second one is the trend in the photosynthetic behaviour of the two morphotypes.

Hyperbolic functions (Michaelis-Menten type) have been employed by several authors to describe the photosynthesis-CO_2 concentration relationships for aquatic plants (Lucas & Dainty, 1977; Raven & Beardall, 1981; Beer & Eshel, 1983; Sand-Jensen & Gordon, 1984, 1986; Johnston & Raven, 1986a). A fit to equation (13.41) of Edwards & Walker (1983) was tried for data obtained both in air and in water. This equation fitted the data obtained for exposed leaves, but it did not describe in an accurate way the results obtained for submerged leaves. APS_{max} and Km are overestimated due to the existence of a rapid transition from the initial slope to the saturation phase. Instead of this model, a double linear model, providing the best fit to the experimental results, has been employed as described in the material and methods section.

Smith & Walker (1980) and Johnston & Raven (1986a) studied the photosynthetic behaviour of several aquatic plants in water. It could not be described on the basis of Michaelis-Menten kinetics which were introduced to describe simple cell-free enzyme assays which are often single-step reactions. When this approach is applied to *in vivo* photosynthesis certain complications arise. The uptake of inorganic carbon can be separated into two phases, the transport of carbon to the carboxylating enzyme (see Kerby & Raven, 1984) and the fixation of inorganic carbon by the enzyme RUBISCO. The degree to which each is rate-limiting will influence the shape of the photosynthesis-carbon curve. In a totally transport controlled system, the shape will be that of a Blackman type curve, linear initially, followed by a rapid transition to saturation. At the other extreme, when the relationship between photosynthetic rate and inorganic carbon is enzyme-controlled, the curve will be a rectangular hyperbola (Smith & Walker, 1980; Johnston & Raven 1986a; Larkum *et al.*, 1989). This former kinetic (Blackman-type kinetic) has been observed in *Zostera noltii* from Palmones Estuary in the submergence experiments. It appears that the photosynthetic process could be controlled by CO_2 or HCO_3^- transport to the carboxylating enzyme.

APS rates obtained in water are higher than those for exposed leaves (Figs 3a and 4a), in spite of the much lower diffusion resistance to CO_2 in air than in water. This fact could constitute indirect evidence that *Zostera noltii* is able to take up HCO_3^- ions, although the most accurate method to investigate HCO_3^- use by plants would be either an analysis of a pH profile of photosynthesis at a constant concentration of inorganic carbon (Raven, 1970; Beer *et al.*, 1977; Beer & Waishel, 1979; Benedict *et al.*, 1980; Sand-Jensen & Gordon, 1984; Millhouse & Strother, 1986) or to show that inorganic carbon-dependent photosynthesis at high pH occurs faster than uncatalysed HCO_3^- to CO_2 conversion can occur (Briggs, 1958; Lucas, 1975). If the results of Cooper & McRoy (1988) are accepted, this means by implication that submerged plants with more positive $\delta^{13}C$ values are likely to be HCO_3^- users.

APS rates showed by *Zostera noltii* in water are similar to those reported by Drew (1979) in *Cymodocea nodosa*, *Phyllospadix torreyi*, *Zostera angustifolia* and *Z. marina* and higher than those he recorded in *Posidonia oceanica* and *Halophila stipulacea* (Table 1). APS rates in air were lower than those found by Johnston & Raven, (1986a) in the

Table 1. Maximum photosynthetic rates of various seagrasses. (* at different temperatures; NLM = Narrow-leaved morphotype, LLM = Large-leaved morphotype).

Seagrass species	APS_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	References
Cymodocea nodosa	5.00	Drew, 1979
Posidonia oceanica	1.89	Drew, 1979
Zostera marina	3.33	Drew, 1979
	1.67	Dennison & Alberte, 1985
Zostera angustifolia	4.17	Drew, 1979
Halophila stipulacea	2.08	Drew, 1979
Phyllospadix torreyi	6.11	Drew, 1979
Zostera noltii (NLM)	3.00–5.15*	This paper
(LLM)	2.98–4.94*	This paper

intertidal brown seaweed *Ascophyllum nodosum* (13 μmolC m^{-2} s^{-1}). Although few reports on assimilation of CO_2 from air known for seagrasses (Cooper & McRoy, 1988; Cooper, 1989), some experiments in this way could, in the future, establish if the accommodation to the air is just a physical question or an intrinsic characteristic of seagrasses (and seaweeds) growing in the intertidal zone.

Zostera noltii plants growing in the intertidal mudflats of the Palmones Estuary are subjected to a high Spring and Summer temperatures when they occur in emersion (Pérez-Lloréns, 1991). According to our results, the NLM specimens seem to be better adapted to live in exposed zones than the LLM. This idea could be supported by the photosynthetic behaviour of the former morphotype when exposed to air: enhanced affinity for CO_2 with increasing temperature, lower CO_2 compensation points, slight decrease in APS_{max} until 25 °C, with higher APS_{max} values than the LLM at high temperatures. In this way, McMillan (1984) reported a higher tolerance to high temperatures in the narrow-leaved seagrass morphotypes. This better adaptation of the NLM plants to tolerate air exposure could explain the distributional pattern of these plants in the Palmones Estuary: they occur at the edges of channels remaining emersed for long periods, reducing the effects of desiccation by clumping.

Submergence of both morphotypes, especially the LLM, are subjected to several factors that will influence their photosynthetic capacity. LLM leaves are fully covered by macro and microscopic epiphytes. Sand-Jensen (1977) reported that epiphytes reduced eelgrass photosynthesis by up to about 31% at optimum light conditions and ambient HCO_3^- concentrations, becoming even greater at low light intensities. The water from Palmones Estuary is muddy, corresponding to type 9 of the Jerlov classification (1976). The high light attenuation coefficient (0.5–3 m^{-1}) is due to the mud removal by tides and to the high phytoplankton densities which contributes to the depletion of TIC. Phytoplankton are regarded to be more efficient than submersed macrophytes in taking up inorganic carbon (Allen & Spence, 1981), but considering the high TIC level in Palmones Estuary (up to 3 mM), light seems more likely than TIC to be the photosynthetic limiting factor for the autotrophs in this area (Pérez-Lloréns, 1991). These factors will produce important deviations from the results obtained in the laboratory. Moreover, the method used to measure photosynthetic rates requires cutting the plant to produce leaves segments. It may also alter the response of the plants due to a wounding response resulting in increased respiration (Hatcher, 1977) or elimination of the translocation pattern or active sink region (Penot & Penot, 1979).

The results presented in this work show that net photosynthesis rates in the NLM plants are higher than those for LLM plants. The question is why *Zostera noltii* plants with the highest APS rates (NLM) are smaller than plants with lower uptake rates (LLM). A possible answer to this question could be a higher rate of dark respiration in leaves and / or root-rhizomes in NLM than in LLM. Another source of differential carbon loss is the excretion of low molecular weight compounds. Excretion rates vary from < 1 to 10% of carbon fixed photosynthetically by *Z. marina* as a function of temperature and light, representing an incomplete adaptation of the submersed plants to a totally aqueous medium (Wetzel & Allen, 1970; Wetzel, 1975; Zieman & Wetzel, 1980) but further work is needed to test this hypothesis.

The question mentioned above could have a photomorphogenic response basis. As was pointed out in the introduction, foliar polymorphism has been observed in aquatic angiosperms such as *Halodule* (den Hartog, 1970), *Potamogeton* (Spence *et al.*, 1973), *Halophila* (Lipkin, 1979), *Zostera noltii* (Nienhuis, personal communication), *Zostera muelleri* (Strother, personal communication) as well as in seaweeds (Duncan & Foreman, 1980; Lapointe *et al.*, 1981; Algarra & Niell, 1987). Duncan & Foreman (1980) found that stipe elongation in *Nerocystis* could be controlled by the phytochrome system. The elongation is favoured by a high R/FR ratio (the ratio increases with depth, Fig. 5) and it is inhibited by a decrease in this ratio as the growing seaweed

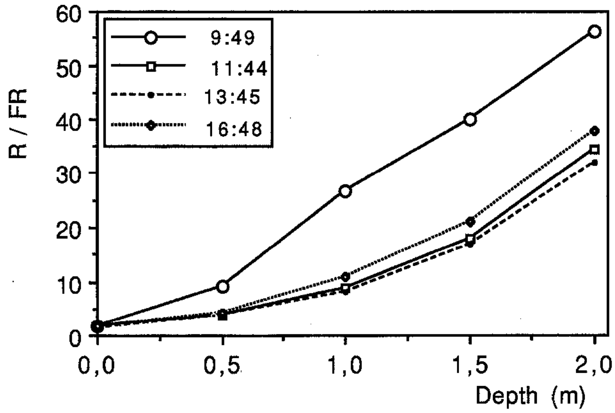


Fig. 5. Variation of the R/FR ratio with depth and time (hours) in the Palmones Estuary. (Data from López-Figueroa *et al.*, inedit).

reaches the water surface. This photomorphogenic response would allow *Zostera noltii* plants growing in the deepest zones to elongate their leaves to the water surface, thus increasing the amount of captured light.

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