

Annual variation in leaf photosynthesis and leaf nutrient content of four Mediterranean seagrasses

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Abstract

We examine here the annual variation in leaf photosynthesis and nutrient status of four temperate seagrass species: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii*, and *Zostera marina*. We also examine the relationship between leaf photosynthesis and seasonal plant growth and productivity. The four seagrass species examined co-occurred in a shallow protected cove on the NE Spanish Mediterranean coast. The results presented give evidence of large annual variation in the leaf physiological status of these four seagrasses, as well as the occurrence of significant differences in the seasonal pattern among species. We found two contrasting seasonal patterns characterized by optimal leaf photosynthesis in summer (represented by *C. nodosa*), and winter-autumn (represented by *P. oceanica* and *Z. noltii*). These contrasting patterns corresponded with a differential plant dependence on climate fluctuations (described by temperature and irradiance) vs. species-specific nutrient status (described by leaf nutrient content). As plant nutrient status results from species-specific differences in nutrient requirements, nutrient uptake efficiency, and internal nutrient economy, we conclude that the variation among species found in leaf nutrient content derives from species-specific responses to environmental forcing. Consequently, we can infer that an important fraction of the seasonal variability that seagrasses show may be related to species-specific responses. We also found significant differences among species in the coupling between leaf photosynthesis and plant growth and productivity. Both aspects, species-specific responses to environmental forcing and species-specific coupling between leaf photosynthesis and plant growth may result in differential seasonal patterns of growth and productivity among seagrass species.

Keywords: leaf photosynthetic status; nutrient status; plant growth; seagrasses; seasonality.

Introduction

Seagrasses form extensive meadows in shallow coastal waters, which support a significant fraction of global marine primary production (Duarte 1989, Hillman et al. 1989, Larkum et al. 1989, Duarte and Chiscano 1999). Seagrass meadows experience considerable annual fluctuations in biomass and productivity (Zieman 1975, Duarte 1989, van Tussenbroek 1995, Alcoverro et al. 1995, Marbà et al. 1996, Cebrián et al. 1997, Agawin et al. 2001), resulting in important annual changes in the energy balance and the habitat provided to the associated biota of the coastal ecosystem. The extent of this seasonal variability varies broadly among seagrass meadows (Duarte 1989).

The evidence of strong regulation of temperate seagrass seasonality by climate fluctuations derives largely from empirical correlations between seasonal variation in light and temperature and plant growth (e.g., Sand-Jensen 1975, Jacobs et al. 1982, Phillips et al. 1983, Wiium-Andersen and Borum 1984, Kentula and McIntire 1986, Pérez and Romero 1992, Olesen and Sand-Jensen 1994, Alcoverro et al. 1995). Temperate and tropical seagrasses display, however, a wide range of seasonal responses, even when located at similar latitudes (e.g., Keddy and Patriquin 1978, Duarte 1989, Alcoverro et al. 1995, Marbà et al. 1996, Agawin et al. 2001). This observation suggests that the regulation of annual fluctuation in seagrass biomass and productivity is more complex and still poorly elucidated, and cannot be explained by a simple direct and positive association with seasonal variation in temperature and irradiance. Other factors related to local environmental conditions (e.g., local resource availability, sediment conditions), or to species-specific seagrass responses to environmental variation need to be considered (Pirc 1986, Dennison and Alberte 1986, Evans et al. 1986, Williams 1988, Masini et al. 1995, Marbà et al. 1996, Cebrián et al. 1997, Agawin et al. 2001).

The annual variation in seagrass photosynthetic and nutrient status was monitored monthly, from September 1991 until July 1993 in a shallow protected cove on the NE Spanish Mediterranean coast, where all four seagrass species present in Europe co-occur, in order to assess the coherence of their variation when exposed to similar seasonal forcing.

We examined seasonal variation in leaf photosynthesis of the four seagrass species growing in the NW Mediterranean: *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* Aschers., *Zostera noltii* Horn., and *Z. marina* L. Of these, *P. oceanica* and *C. nodosa* are dominant in sheltered bays, *Z. noltii* occurs in shallow, protected bays and lagoons, and *Z. marina*, which is probably introduced from the Atlantic Ocean, is restricted to brackish lagoons and some sheltered coves (den Hartog 1970). Leaf photosynthesis is described by four parameters derived from

the photosynthetic light response curve: maximum photosynthetic rate at saturating light (P_{max}), dark respiration rate (R), photosynthetic efficiency at subsaturating light (α) and light compensation point (I_c). We also described the seasonal variation in chlorophyll content, tissue C, N, P, leaf light absorption and the specific leaf area (SLA [$\text{cm}^2 \text{g dw}^{-1}$]). We compared annual patterns of variation of these physiological and morphological descriptors and their coupling with seasonal variability in climate fluctuations. We also tested the relationship between the variation in seagrass leaf physiological status and seagrass growth, as described by the relative growth rate (RGR), leaf elongation rate (LER), crop growth rate (CGR), and leaf turnover rate (PB).

Materials and methods

The four seagrass species examined co-occurred in a shallow protected cove (Cala Jonquet, ≈ 3.8 ha) on the NE Spanish Mediterranean coast ($42^\circ 18.26' \text{ N}$, $3^\circ 18.11' \text{ E}$). *Cymodocea nodosa* and *Zostera noltii* develop a mixed seagrass meadow in the shallow subtidal zone (< 1.5 m deep, about 2000 m^2), *Zostera marina* forms two small patches (2 m in diameter) next to this mixed meadow, and *Posidonia oceanica* grows deeper (2 to 16 m) as a continuous, monospecific meadow, covering an area of about 1.1 ha. Daily surface irradiance was obtained from a station 21 km south of the study site (Experimental Research Station Mas Badía, St. Pere Pescador, Spain), and surface water temperature was measured weekly at a station 27 km south of the study site (L' Estarit, Spain; Pascual unpublished data). Both data sets were averaged by month. We used the values recorded in these nearby stations to describe large-scale climate fluctuations.

Samples of leaf material were collected monthly at the depth of maximal shoot density for each species, between 0.25 and 5 m, and carefully transported to the laboratory in refrigerated sea water, within 3 h of collection. Samples for the physiological descriptions were collected from November 1991 to May 1993, while growth measurements were conducted between September 1991 and July 1993. Two patches of *Zostera marina* were found in April 1992, and followed subsequently, resulting in a shorter observational period for this species. The sizes of the sampling areas were approximately: 0.8 m^2 for *Cymodocea nodosa* and *Zostera noltii*, 0.75 m^2 for *Posidonia oceanica*, and 0.3 m^2 for *Z. marina*. On every sampling visit, for the growth and biomass determinations we collected: 3 cores of 20 cm diameter, randomly pushed 20 cm into the sediment within the mixed meadow of *C. nodosa* and *Z. noltii*; and 50 to 100 shoots of each of the larger species, *P. oceanica* and *Z. marina*. In addition, we randomly collected in the same area 20 to 30 shoots of *C. nodosa* and *Z. noltii*, and 10 to 15 shoots of *P. oceanica* and *Z. marina* for the physiological descriptions. More extensive descriptions of the sampling methods for the growth determinations are provided in Marbà et al. (1996), and Cebrián et al. (1997).

We used four parameters derived from the photosynthetic light response curve (P vs. E) to describe leaf pho-

tosynthesis: (1) photosynthetic rate at saturating light (P_{max} , $\text{mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$); (2) the slope of the linear increment in photosynthetic rate at subsaturating photon irradiance (photosynthetic efficiency, α , $\text{mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$); (3) dark respiration rate (R , $\text{mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$); and (4) light compensation point, the irradiance at which photosynthesis and respiration are balanced (I_c , $\mu\text{mol m}^{-2} \text{ s}^{-1}$). We also measured monthly variability in leaf nutrient content (C, N, P) and chlorophyll (a and b) concentration. The P vs. E curves were determined by incubating epiphyte-free photosynthetic tissues with a fully developed pigment composition (the second youngest leaf for *Posidonia oceanica* and *Zostera marina*, and the first or second leaf for *Cymodocea nodosa* and *Zostera noltii*), in clear glass BOD bottles exposed to different light intensities. We measured the photosynthetic activity through changes in dissolved oxygen concentration following the recommendations of Littler (1979), Littler and Arnold (1980) and Kemp et al. (1990), particularly with regard to incubation time, mixing, and sample size (fresh weight relative to bottle volume). The plant tissues (≈ 150 mg fresh weight) were placed in the 280 ml BOD bottles, the bottles filled with filtered ($0.45 \mu\text{m}$) seawater using a siphon to prevent bubble formation and to reduce variance in the oxygen concentration among replicates. Changes in dissolved oxygen were measured using a Clarke-type electrode (sensor 2112) with a built-in stirrer, connected to an Orbisphere oxymeter (Model 2610, Pryde Measurement Pty Ltd, Switzerland). We used four reference bottles (i.e., without tissue) incubated in parallel to four (*P. oceanica* and *Z. marina*) or three (*C. nodosa*, *Z. noltii*) replicate bottles per species (i.e., with tissue) representing a total of 18 bottles per incubation when the four species were present. Only *P. oceanica* maintained sufficient leaves over the year to perform monthly P vs. E curves; the remaining three species lost virtually all leaves during the adverse periods of the year (cf. Marbà et al. 1996). We incubated the leaves at 20°C in an incubator-shaker (model MKXD, LH Fermentation LTT, Maidenhead, UK). Stirring was provided by continuous orbital shaking using 1 cm diameter glass sphere attached to the bottle lid with fishing line. Incubation time ranged between 45 min and 4 h, and was scaled to the rate of oxygen change to ensure accurate estimates of oxygen production in the light and consumption in the dark, avoiding bubble formation and over-saturation. Illumination was provided by cool-white fluorescent tubes placed above the bottles. The P vs. E curves were derived from consecutive measurements on the same set of bottles, from darkness through increasing photon flux densities (0, 15, 35, 70, 150, 300, 425, 500 and $750 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Photosynthetic efficiency (α , cf. Kirk 1983) was estimated from the P vs. E curve as the initial slope calculated by linear least-squares regression analysis (e.g., Ledermann and Tett 1981). Dark respiration rate (R) was estimated as the intercept of the regression equation on the ordinates, and the light compensation point (I_c), the irradiance at which photosynthesis and respiration are balanced, calculated as the intercept of the regression equation on the x axis.

All P vs. E curves were constructed from data collected at 20°C , the mean water temperature during the growth

period (May to September), to ensure comparability of the resulting parameters reflecting the physiological status of the plants rather than the direct effect of temperature on photosynthesis. The selected temperature of 20°C represents the optimum temperature for photosynthesis for these seagrasses, as assessed experimentally (P vs. E curve determinations for a range of temperatures between 10 and 35°C [cf. Enríquez et al. 1995]).

We measured leaf light absorption (leaf absorptance), SLA ($\text{cm}^2 \text{dw}^{-1}$), chlorophyll (*a+b*) and nutrient (C, N, P) concentrations on the same leaf segments used to construct P vs. E curves. Light absorption was measured spectrophotometrically, using the opal glass technique developed for intact leaves by Shibata (1959) (cf. Enríquez et al. 1994). Fragments of leaves were mounted between microscope slides in a Shimadzu (Hadano, Japan) UV-2100 dual-beam scanning spectrophotometer containing an opal glass unit. Light absorption was measured at 1 nm intervals across the PAR range (between 380 and 750 nm). The correction for non-pigment absorption was made using bleached leaves as a reference in the spectrophotometer. Leaves were bleached by extracting photosynthetic pigments in acetone for 24 hours. Shibata's technique may overestimate absorptance if residual- and back-scattering are important. The attenuation (apparent absorbance) obtained was corrected by subtracting absorbance at 750 nm to exclude residual scattering. In our study, absorbance at 750 nm was in all cases very low, confirming low levels of back- and residual-scatter. Chlorophyll *a* and *b* concentrations were measured spectrophotometrically following pigment extraction with 80% acetone in a homogenized suspension (Dennison 1990). Carbon and nitrogen contents were determined in duplicate sub-samples using a Carlo-Erba CHN analyzer (Elemental Analysis Instruments, Okehampton, UK), and phosphorus content was measured colorimetrically following tissue digestion in boiling concentrated acid (Koroleff 1983).

We described seagrass growth and productivity using the following parameters: relative growth rate (RGR, $\text{mg dw g dw}^{-1} \text{day}^{-1}$) as the main descriptor of instantaneous growth in traditional growth analysis (cf. Blackman 1919, Evans 1972); leaf elongation rate (LER, $\text{mm shoot}^{-1} \text{day}^{-1}$) as a descriptor of absolute leaf growth; leaf turnover rate (PB day^{-1}) as a common descriptor for seagrasses; and crop growth rate, as a descriptor of total production per unit ground area ($\text{CGR, g dw m}^{-2} \text{d}^{-1}$). The calculations of the former parameters were derived from Marbà et al. (1996) and Cebrián et al. (1997).

We used the coefficient of variation to compare the variability of the physiological and morphological leaf descriptors over the observational period. Differences among species in the physiological and morphological leaf descriptors were assessed using one-way analysis of variance (ANOVA test, species as a fixed factor with four levels). Pearson correlation analysis was used to examine the association between different leaf descriptors, such as absorptance and chlorophyll density, and cross correlation analyses were used to examine the associations between leaf physiological status, plant growth and seasonal fluctuations. We also used cross correlation analyses to compare annual patterns in leaf physiological status among species.

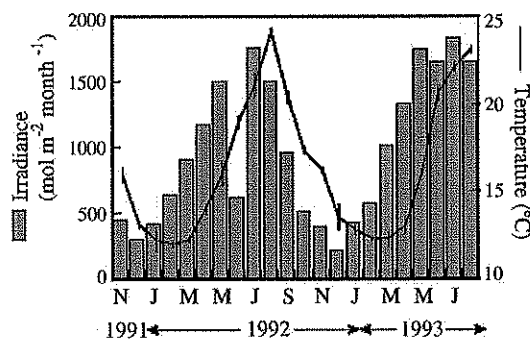


Figure 1 Monthly variation in surface sea temperature (solid line) and solar irradiance (vertical bars) during the sampling period (November 1991 to August 1993).

Results

Water temperatures ranged from a minimum of 12°C in February, and a maximum of 23.2°C in September (Figure 1), with an annual average of 15.1°C. The photon irradiance received at the water surface ranged from 269 $\text{mol m}^{-2} \text{month}^{-1}$ in December to 1824 $\text{mol m}^{-2} \text{month}^{-1}$ in July 1993. The irradiance in June 1992 was anomalously low, about half of that in June 1993 (Figure 1), as a consequence of a persistent storm period.

Ambient nutrient concentrations were relatively low (annual average 1.1 $\mu\text{mol l}^{-1}$ of nitrate, 1.4 $\mu\text{mol l}^{-1}$ of ammonium and 0.3 $\mu\text{mol l}^{-1}$ of phosphate, Vidondo and Duarte 1995). There were significant differences in the annual average nutrient concentration and chlorophyll density (normalized to projected leaf area) among species (ANOVA, $p < 0.005$; $\text{DF} = 3, 177$; $F(\text{nitrogen}) = 9.06$; $F(\text{phosphorus}) = 17.4$; $F(\text{chlorophyll}) = 4.98$; Figure 2). *Posidonia oceanica* leaves showed the lowest phosphorus content, and the highest chlorophyll density (Figure 2), as well as the lowest chlorophyll *a/b* ratio. *Cymodocea nodosa* leaves showed the highest nitrogen concentration and a high phosphorus concentration similar to that in *Zostera marina* (Figure 2). *Zostera noltii* leaves showed the lowest chlorophyll density, displaying the lowest light absorption values, and the highest chlorophyll *a/b* ratios (Figure 2). There were no significant differences in chlorophyll density among *Z. marina*, *Z. noltii* and *C. nodosa* leaves (ANOVA, $p > 0.1$, $\text{DF} = 2, 118$; $F = 1.3$; Figure 2). Both *Zostera* species showed a significant positive correlation between light absorption and chlorophyll density ($r_{Z. noltii} = 0.45$, $r_{Z. marina} = 0.71$, $p < 0.001$), which was not significant for the remaining, highly pigmented, species. *Z. marina* showed the highest pigment- and weight-specific absorption (data not shown), thereby possessing the highest efficiency in light absorption per unit of pigment and biomass, among the four species examined.

The photosynthetic performance differed significantly among species for some descriptors, but showed remarkable similarities for others (Figure 2). Annual average P_{max} was similar for *Posidonia oceanica*, *Cymodocea nodosa* and *Zostera marina* ($\approx 7 \text{ mg O}_2 \text{ g dw}^{-1} \text{h}^{-1}$, ANOVA, $p > 0.5$; $\text{DF} = 2, 136$; $F = 0.66$), and was about twice as high for *Zostera noltii* ($13 \text{ mg O}_2 \text{ g dw}^{-1} \text{h}^{-1}$, ANOVA, $p < 0.001$; $\text{DF} = 3, 177$; $F = 11.05$). The maximum photo-

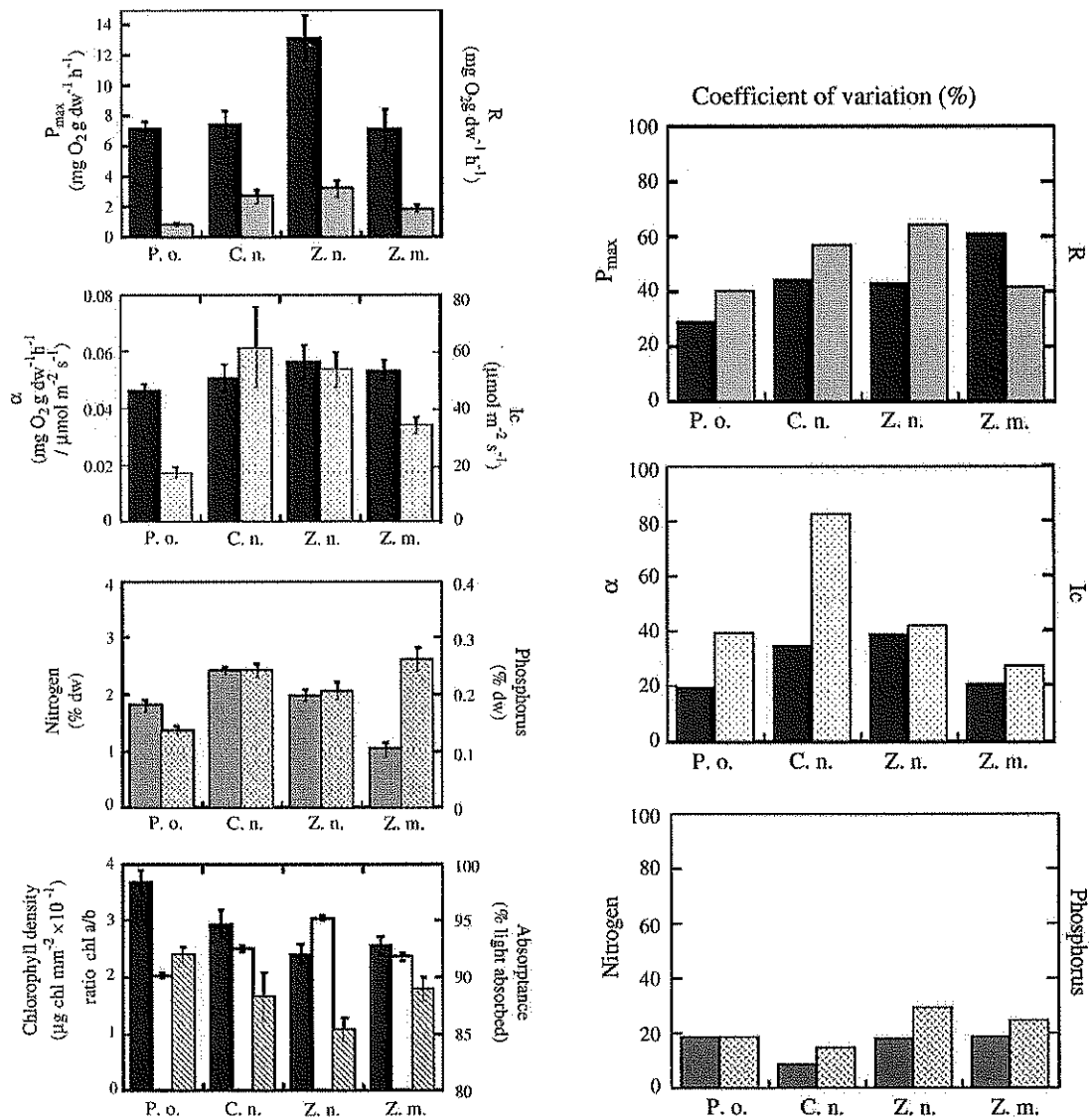


Figure 2 Average \pm SE values of seagrass physiological descriptors and seagrass tissue descriptors.

P_{max} and R are expressed as $\text{mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$; α as $\text{mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$; I_c as $\mu\text{mol m}^{-2} \text{ s}^{-1}$; chlorophyll a+b as $\mu\text{g mm}^{-2} \times 10^{-1}$; absorbance as % of incident light absorbed; and nutrient content as % of dry weight. Left-right bars correspond to left-right axis labels. Ratio chlorophyll a/b corresponds to the white bars. P.o., C.n., Z.n. and Z.m. refer, respectively, to *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii*, and *Zostera marina*.

synthetic rates were shown by *Z. noltii* ($24 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$) in winter, and the minimum by *C. nodosa* and *Z. marina* ($<3 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$) in winter and spring, respectively (Figure 3). The photosynthetic efficiency (α) was remarkably similar among species (ANOVA, $p > 0.5$, $DF=3, 177$; $F=0.22$, Figure 2) and displayed the lowest coefficients of variation among the photosynthetic parameters (Figure 3). *Z. noltii*, and *C. nodosa* exhibited the maximum dark respiration rates ($>6.5 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$) and the highest average annual rates ($>2.5 \text{ mg O}_2$

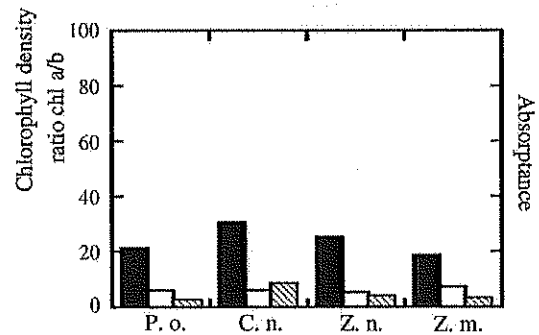


Figure 3 Coefficients of variation reflecting the magnitude of annual fluctuation in physiological descriptors (P_{max} , α , R, I_c) and tissue characteristics [SLA, chlorophyll density (ratio chl a/b) and nutrient content].

Left-right bars correspond to left-right axis labels. P.o., C.n., Z.n. and Z.m. refer, respectively, to *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii*, and *Zostera marina*.

g $\text{dw}^{-1} \text{h}^{-1}$). The dark respiration rate (R) showed significant differences among species (ANOVA, $p < 0.001$, $\text{DF} = 3$, 177 ; $F = 7.31$, Figure 2). The minimum dark respiration rate ($0.25 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$) was exhibited by *P. oceanica*, which also showed the lowest annual average rate ($\approx 1 \text{ mg O}_2 \text{ g dw}^{-1} \text{ h}^{-1}$) (Figure 2). These differences among species resulted in parallel differences in the light compensation point (ANOVA, $p > 0.001$, $\text{DF} = 3$, 177 ; $F = 7.9$, Figure 2), which were the highest for the small and shallow-growing species, *C. nodosa* and *Z. noltii* ($> 50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), and lowest for the deep-growing species *P. oceanica* ($< 20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). *Z. marina* showed intermediate average light compensation points. *C. nodosa* displayed the largest variability in I_c (maximum of $207 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and minimum of $23 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), indicating that this species exhibited the widest plasticity in light acclimation among the four seagrasses examined.

Leaf nutrient and chlorophyll content changed little throughout the year (coefficients of variation $< 30\%$ for the four species, Figure 3), in contrast, leaf photosynthesis showed large variation (Figure 3). The photosynthetic efficiency (α) showed the lowest annual variability among the physiological descriptors, being rather uniform throughout the year for *Zostera marina* (Figures 3 and 4). *Cymodocea nodosa* exhibited the highest variation in P_{max} and I_{c1} , and *Zostera noltii* in dark respiration rate (R) and α (Figure 4). The largest seagrass species examined, *Posidonia oceanica*, showed the lowest annual variation in leaf photosynthetic rates.

The annual pattern of variation in leaf photosynthetic performance differed broadly among species. Maximum P_{max} occurred in winter and late autumn for *Posidonia oceanica* and *Zostera noltii* leaves (Figure 3), and in spring and summer for *Cymodocea nodosa* and *Zostera marina* (Figure 4), respectively. All four species showed minimum dark respiration rates in summer (late June–July) and two maxima, in winter (December–January), and early spring (March–April) (Figure 4). A winter maximum for *C. nodosa* dark respiration rates was observed in 1991–1992 (December), but not in 1992–1993, when the plant lost all green leaves. The summer respiration minima coincided with the maximum water temperatures, and allowed the leaves to display minimal annual values in light compensation point. These I_c minima coincided for *P. oceanica*, *C. nodosa* and *Z. marina* with their maximum biomass density (cf. Marbà et al. 1996, and Cebrián et al. 1997), and maximum leaf area index (LAI [$\text{m}^2 \text{ m}^{-2}$]) values of: 5.3, 2, and 13.2, respectively.

Cross correlation analysis was used to examine the relationships between leaf photosynthetic performance and annual changes in irradiance and water temperature. P_{max} of *Cymodocea nodosa* showed significant and positive correlation with both irradiance and water temperature (Figure 5a), with a lag of about one month for irradiance. P_{max} of *Posidonia oceanica* and *Zostera noltii* were significantly, but negatively, correlated with irradiance with the same lag (one month). P_{max} of *P. oceanica* showed, in addition, a significant and negative correlation with temperature (Figure 5a). In contrast, *Z. marina* P_{max} showed no association with light and water temperature

variation (Figure 5a). The photosynthetic efficiency (α) of *P. oceanica* and *C. nodosa* was significantly correlated with irradiance, and that of *Z. noltii*, and *Z. marina* with water temperature (Figure 5b). The respiration rates (at 20°C) tended to decrease with light and *in situ* temperature for all species, although these relationships were only significant for *C. nodosa*, *Z. noltii*, and *Z. marina* (Figure 5c).

The relationship between leaf photosynthesis and leaf nutrient content, chlorophyll content, and SLA, differed among seagrass species (Figure 6). Leaf photosynthesis for *Cymodocea nodosa*, the species with the highest average nutrient concentration, was independent of nutrient and chlorophyll content (Figure 6). In contrast, *Posidonia oceanica* and *Zostera noltii*, the species with the lowest nutrient content, showed leaf photosynthesis closely associated with both leaf nutrient and chlorophyll content. Two particularly strong relationships were found, (1) between phosphorus content and leaf photosynthesis for *P. oceanica*, and (2) between nitrogen content and leaf photosynthesis for *Z. noltii* (Figure 6). *Zostera marina* displayed very weak relationships, limited to a significant and negative association between P_{max} and phosphorus content (Figure 6).

Chlorophyll content showed a significant and positive correlation coefficient with P_{max} for only *Zostera noltii* leaves. SLA was significantly and positively related to P_{max} for *Cymodocea nodosa*, and to R for *Zostera marina*. SLA also showed for *C. nodosa* and *Z. noltii* a significant, but negative, association with R, and with α for *Z. noltii*. (data not shown). Chlorophyll content and SLA showed no association with the photosynthetic rates of the highly pigmented leaves of *Posidonia oceanica*.

We used cross correlation analysis to examine the coherence among the species in their annual variation in leaf photosynthesis and leaf morphological descriptors (Table 1). This analysis revealed a close coherence for *Posidonia oceanica* and *Zostera noltii* in the annual variation in leaf photosynthesis (P_{max} and R) and leaf chlorophyll and nutrient content (Table 1). In contrast, the annual variation in the photosynthetic rates (P_{max} and α) of *Cymodocea nodosa* was the reverse of that for *Z. noltii* (P_{max}) and *P. oceanica* (α) (Table 1). *C. nodosa* and *Z. noltii* also showed the reverse pattern of temporal variation in leaf nitrogen content (Table 1). The temporal pattern of variation in the properties examined of *Zostera marina* showed a weak coupling with the other three species.

Annual averages by species in RGR, LER, CGR and PB showed that both *Zostera* species displayed the highest specific growth rates (RGR, Table 2). *Posidonia oceanica* and *Z. marina*, showed the highest leaf elongation rates (LER), and the highest productivity per surface area (total crop growth rate, CGR). *P. oceanica* also showed the lowest leaf turnover rate (PB) (Table 2). A more extensive seasonal growth description for these four Mediterranean seagrasses can be found in Marbà et al. (1996) and Cebrián et al. (1997).

We found a significant coupling between plant growth and leaf photosynthetic performance. The P_{max} of *Cymodocea nodosa* and *Zostera noltii* was significantly correlated with LER, RGR and CGR (positively for *C. nodosa* [Figure 7]), and negatively for *Z. noltii* [Figure 7]). The

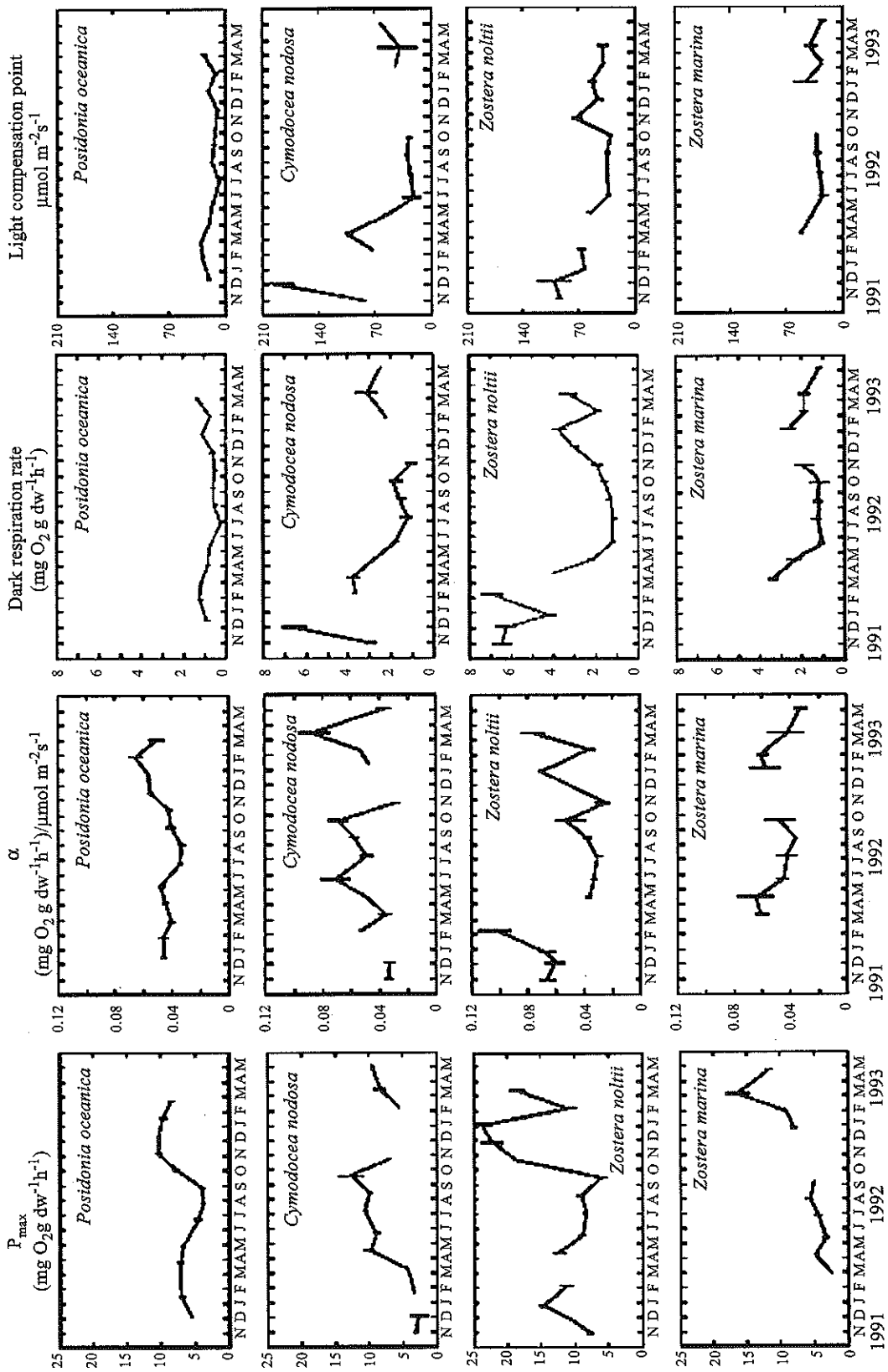


Figure 4 The time course of seasonal change in seagrass leaf physiological descriptors for the four Mediterranean seagrasses examined: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii*, and *Zostera marina*. Bars represent the standard error of the mean. Broken lines indicate periods of the year with absence of green leaf material on the plants.

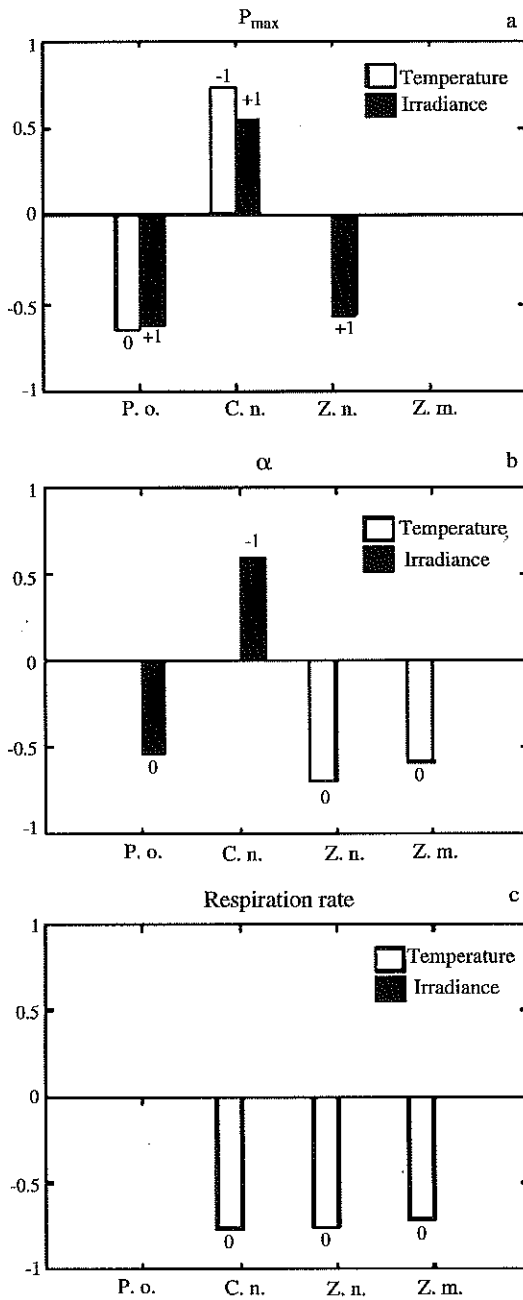


Figure 5 Cross correlation between the physiological descriptors and water temperature and solar irradiance. Vertical axis label=correlation coefficient. Only significant values are represented. Numbers above and below the bars represent time lag in months. P.o., C.n., Z.n. and Z.m. refer, respectively, to *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii*, and *Zostera marina*.

dark respiration rate of *C. nodosa* and *Z. noltii* also showed significant and negative correlation with LER, RGR and CGR (Figure 7). No significant correlation was observed between leaf photosynthesis and plant growth for *Posidonia oceanica* and *Zostera marina*, with the exceptions of a significant positive association between

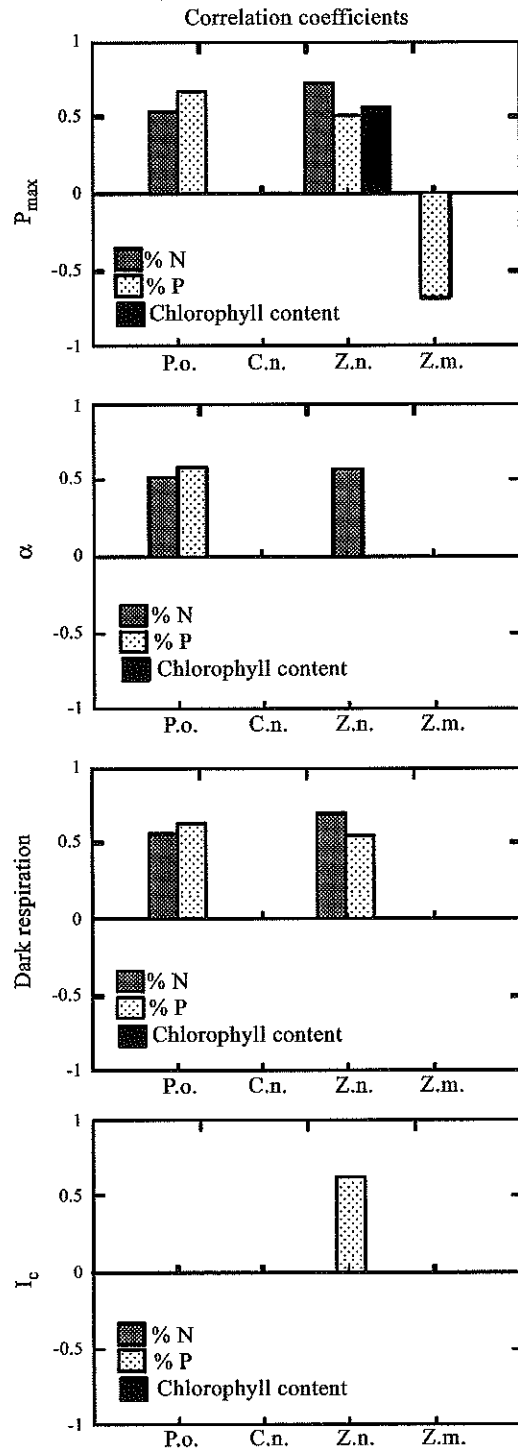


Figure 6 Significant Pearson correlation coefficients ($p < 0.05$) between physiological descriptors and tissue descriptors (nitrogen, phosphorus, and chlorophyll a+b content). Vertical axis label=correlation coefficient. Non significant values not shown. P.o., C.n., Z.n. and Z.m. refer, respectively, to *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii*, and *Zostera marina*.

Table 1 Cross correlations between seasonal changes in leaf photosynthetic and nutrient descriptors among the four seagrass species.

Variable	<i>P. oceanica</i> <i>C. nodosa</i>	<i>P. oceanica</i> <i>Z. noltii</i>	<i>P. oceanica</i> <i>Z. marina</i>	<i>C. nodosa</i> <i>Z. noltii</i>	<i>C. nodosa</i> <i>Z. marina</i>	<i>Z. noltii</i> <i>Z. marina</i>
P_{max}	-0.44 ^{N.S.} lag (month):0	0.72* lag:0	0.49 ^{N.S.} lag:1	0.71* lag:5	-0.22 ^{N.S.} lag:1	0.58* lag:1
α	-0.56* lag:-1	0.33 ^{N.S.} lag:1	0.46 ^{N.S.} lag:-1	-0.41 ^{N.S.} lag:-1	-0.61* lag:1	0.36 ^{N.S.} lag:1
R	0.32 ^{N.S.} lag:-1	0.58* lag:0	0.59* lag:0	0.84* lag:-1	0.28 ^{N.S.} lag:0	0.34 ^{N.S.} lag:0
t_c	0.19 ^{N.S.} lag:0	0.26 ^{N.S.} lag:0	-0.59* lag:0	0.80* lag:-1	0.21 ^{N.S.} lag:0	0.24 ^{N.S.} lag:0
Chl (a+b) density	0.77* lag:0	0.71* lag:0	-0.36 ^{N.S.} lag:2	0.89* lag:0	0.54* lag:-1	-0.51 ^{N.S.} lag:-1
SLA	0.60* lag:-2	-0.47 ^{N.S.} lag:3	0.50 ^{N.S.} lag:1	0.59* lag:0	-0.51 ^{N.S.} lag:-1	-0.32 ^{N.S.} lag:0
N	0.59* lag:3	0.81* lag:0	0.67* lag:-1	-0.64* lag:3	-0.56* lag:2	0.79* lag:-1
P	0.64* lag:4	0.58* lag:-1	-0.58* lag:2	-0.38 ^{N.S.} lag:0	0.42 ^{N.S.} lag:3	-0.70* lag:3

* Indicates significant cross correlations ($p < 0.05$), and ^{N.S.} non-significant correlations. Time lag in months also indicated (1=30 days).

P_{max} and leaf turnover rate (PB) in *P. oceanica* (Figure 7), and between α and PB in *Z. marina* (Pearson correlation coefficient, $r=0.72$, $p < 0.05$).

Discussion

The results presented reveal substantial temporal variation in the leaf photosynthesis of the four Mediterranean seagrasses examined. They also provide evidence of significant differences among species in the relationship between leaf photosynthesis and large-scale forcing factors (e.g., temperature and irradiance). Our descriptions of leaf photosynthesis reflect leaf physiological status, rather than the realized photosynthetic rates the leaves may exhibit *in situ*. Spatial and temporal variation of *in situ* photosynthetic rates has been addressed in the past (e.g., Dennison and Alberte 1986, Pirc 1986, Murray and Wetzel 1987, Pérez and Romero 1992, Herzka and Dunton 1997, Plus et al. 2001). However, the approach

employed here allowed us to specifically document temporal changes in the potential photosynthetic rates at 20°C (in the range of leaf photosynthetic optimum), discriminating the photoacclimatory seagrass response from the direct effect of irradiance and temperature on the realized seagrass photosynthetic rates.

We found two contrasting patterns, one characterized by maximum photosynthetic capacity at 20°C in summer, represented by *Cymodocea nodosa*, and a second pattern characterized by maximum photosynthetic capacity during the cold season (winter and autumn) represented by *Posidonia oceanica* and *Zostera noltii* (Table 1). The significant and positive association found in this study between leaf photosynthesis of *C. nodosa* and water temperature and irradiance (Figure 6) is consistent with the finding that the maximum productivity (leaf-blade, horizontal rhizome and root production rates) of this species occurred in summer (Cebrián et al. 1997). Similarly, the significant and negative association found here between leaf photosynthesis of *P. oceanica* and *Z. noltii*

Table 2 Annual averages \pm SE and coefficients of variation (C.V.) of seagrass growth descriptors.

Species	RGR (mg dw g dw ⁻¹ day ⁻¹)	LER (mm shoot ⁻¹ day ⁻¹)	CGR (g dw m ⁻² day ⁻¹)	Leaf PB (day ⁻¹)
<i>Posidonia oceanica</i>	6.82 \pm 0.96 C.V.=0.45	0.66 \pm 0.07 C.V.=0.36	2.16 \pm 0.28 C.V.=0.41	0.28 \pm 0.03 C.V.=0.37
<i>Cymodocea nodosa</i>	4.74 \pm 1.52 C.V.=1.24	0.22 \pm 0.05 C.V.=0.95	1.30 \pm 0.5 C.V.=1.49	0.56 \pm 0.05 C.V.=0.32
<i>Zostera noltii</i>	10.65 \pm 0.01 C.V.=0.90	0.40 \pm 0.06 C.V.=0.61	1.11 \pm 0.26 C.V.=0.87	0.59 \pm 0.04 C.V.=0.23
<i>Zostera marina</i>	9.70 \pm 0.97 C.V.=0.32	1.13 \pm 0.32 C.V.=0.90	3.46 \pm 0.93 C.V.=0.93	0.61 \pm 0.05 C.V.=0.27

RGR is the specific growth rate; LER is the leaf elongation rate; CGR is the crop growth rate (total production per unit ground area); and leaf PB is the leaf turnover rate.

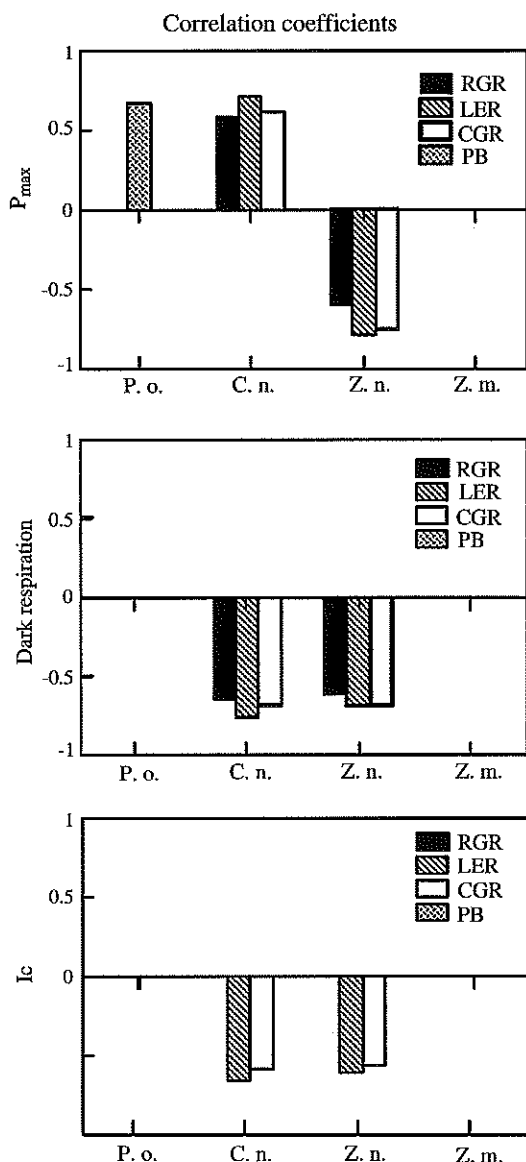


Figure 7 Significant Pearson correlation coefficients ($p < 0.05$) between physiological descriptors and plant growth. Vertical axis label=correlation coefficient. We used as plant growth descriptors: specific growth rate (RGR), leaf elongation rate (LER), crop growth rate (CGR), and leaf turnover rate (PB). Non significant values not shown. P.o., C.n., Z.n. and Z.m. refer, respectively, to *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii*, and *Zostera marina*.

and light and temperature, is also consistent with the finding that the maximum leaf productivity of these species occurred in winter and spring for *P. oceanica*, and in autumn for *Z. noltii* (Cebrián et al. 1997). In contrast, the peak of productivity observed for *Zostera marina* in summer (Cebrián et al. 1997) was not consistent with its maximum photosynthetic capacity at 20°C, measured in early spring 1993.

An important fraction of the temporal variance in the photosynthetic rates of the species examined in this

study was associated with factors other than light and temperature. Seasonal changes in light and temperature affect seagrass growth (e.g., Orth and Moore 1986, Pérez and Romero 1992, Zimmerman et al. 1994, Alcoverro et al. 1995, Marbà et al. 1996, Masini et al. 1995, Cebrián et al. 1997, Agawin et al. 2001), but the timing and the magnitude of plant responses can be species-dependent or related to other local factors. These local factors may be related, among others, to site-specific variation in: (1) light availability (e.g., light attenuation within seagrass canopy [Plus et al. 2001, Enríquez et al. 2002]), (2) sediment status (e.g., redox potential [Alcoverro et al. 1995, Enríquez et al. 2001]), (3) periphyton growth (Cambridge et al. 1986, Shepherd et al. 1989), (3) local variation in resource availability (nutrient and dissolved inorganic carbon), or (4) other abiotic or biotic perturbations (e.g., freshwater influxes associated with storms, grazing losses). The importance of taking into account species-specific responses to environmental forcing to explain seagrass seasonality has been pointed out previously (e.g., Evans et al. 1986, Marbà et al. 1996, Cebrián et al. 1997, Agawin et al. 2001). Supporting evidence based on the seasonal variation patterns of plant growth and productivity may be masked by species-specific characteristics (e.g., differences in carbon storage capacity that allows some species to sustain growth during adverse periods (*Posidonia oceanica*, Alcoverro 1995), or differences in loss rates due to species-specific differences in mortality and recruitment rates (Marbà et al. 1996). The examination of seasonal variation in leaf photosynthesis, which represents a lower level of plant integration, supports again the conclusion that species-specific responses should be considered in explaining seagrass seasonality.

The significant and positive relationships observed in our study between leaf photosynthetic rate at 20°C and leaf nutrient content for *Posidonia oceanica* and *Zostera noltii* suggest nutrient regulation of the photosynthetic performance of these species. Both species contained leaf nutrient concentrations that remained at levels believed to indicate nutrient deficiency (Duarte 1991) during the summer (Figure 4). In contrast, leaf photosynthesis of *Cymodocea nodosa*, the species that showed the highest leaf nutrient content, appears mainly regulated by seasonal changes in light and temperature. *C. nodosa* does not appear to experience nutrient deficiency at the study site. Likewise, *Zostera marina* showed an intermediate degree of association between leaf photosynthesis and seasonal variation in both nutrient content (P_{max}) and water temperature (R and α). This species, which is probably introduced from the Atlantic Ocean, appears, in this area, to be under suboptimal physiological conditions, and may be the species most sensitive to the higher water temperatures and the lower nutrient availability of the Mediterranean Sea. However, leaves of *Z. marina* showed the highest efficiency in absorbing light (maximum pigment and weight specific absorption values, data not shown) among the four seagrasses. This species also exhibited the highest leaf growth rate (LER) and productivity per ground area (CGR). According to this, *Z. marina* has the ability to compete with the other three species for space and/or other resources (e.g.,

light). Nevertheless, the weak acclimation of this species to Mediterranean conditions can explain the limited expansion of *Z. marina* in this area and why it was restricted to two small patches.

As *Cymodocea nodosa* and *Zostera noltii* were growing in the same meadow, under similar environmental resource availability, differences in their leaf nutrient status will mainly reflect species-specific differences in their ability to acquire and use these resources. For instance, *C. nodosa* develops much longer and denser roots than *Z. noltii* (Marbà et al. 1996), which allows this species to access nutrient pools deeper down into the sediments. *C. nodosa* also has larger and more long-lived rhizomes than those of *Z. noltii*, which confers on this species a higher capacity to store and recycle nutrients, compared to the limited storage capacity of the small rhizomes of *Z. noltii*. In addition, nutrient requirements are also species-dependent, and may be related to species-dependent ability to invest carbon gain into growth (RGR). The higher specific growth rate (RGR) of *Z. noltii* (Table 2) suggests greater nutrient demands than *C. nodosa*. Consequently, the lower nutrient concentration found in leaves of *Z. noltii* may have been insufficient to support balanced growth, and may reflect a low competitive ability of *Z. noltii* relative to *C. nodosa* in this area.

Posidonia oceanica leaves showed, as well, low nutrient content and low RGR, which may also be indicative of nutrient deficiency. Nevertheless, *P. oceanica* is a long-lived, large, and slow-growing species with remarkably small nutrient requirements compared to the other fast-growing species. In addition, *P. oceanica* supports long-lived tissues and massive rhizome and root biomass compared with the other three species. Thus, the higher capacity to store and recycle nutrients may confer *P. oceanica* a considerable ability for internal resource economy among the four Mediterranean seagrasses. Furthermore, *P. oceanica* showed little temporal variation in the leaf photosynthetic rates at 20°C (CV of P_{\max} <30%), and leaf nutrient content (CV of nutrient concentration <20%). This result is in agreement with the low seasonal growth variability (Marbà et al. 1996) and the absence of seasonality in the below-ground biomass (horizontal rhizome and roots [Cebrián et al. 1997]) found for this species. Accordingly, nutrient deficiency may not be, necessarily, the primary controlling factor for the physiological status of *P. oceanica* leaves. Other factors, both external (e.g., water temperature, periphyton growth, sediment conditions) or internal (e.g., internal carbon economy, canopy structure [LAI]), need to be considered to account for the seasonal variation in leaf photosynthesis in *P. oceanica*.

The four species exhibited leaf respiration rate minima in summer, which resulted in minimum annual values in light compensation point (I_c). I_c minima occurred a month earlier for the three shallow-growing species than for *Posidonia oceanica*, and coincided with the peak in biomass density for *P. oceanica*, *Cymodocea nodosa* and *Zostera marina* (cf. Marbà et al. 1996, and Cebrián et al. 1997) and the maximum LAI values for the same species (5.3, 2.0, and 13.2 m² m⁻² respectively). Light availability may have been strongly reduced at the peak of biomass density for the large species *P. oceanica* and *Z. marina*,

especially for *Z. marina*, because this species had the highest biomass density in summer (Cebrián et al. 1997), and the highest LAI among the four seagrasses studied. Light attenuation and differences in leaf photoacclimation within seagrass canopy (Enríquez et al. 2002) may in part explain the weak relationship found for *Z. marina*, between leaf photosynthesis and environmental factors, and the small temporal variation in leaf photosynthetic rates at 20°C also found in this study for *P. oceanica*. Light attenuation coefficient (kd- m⁻¹) within seagrass canopy appears as an important parameter to be considered while examining variation in leaf photosynthetic performance of large seagrasses.

Finally, our study revealed significant species-specific differences in the coupling between leaf photosynthetic rates at 20°C and seagrass growth. Only the smallest species, *Cymodocea nodosa* and *Zostera noltii*, showed significant relationships between leaf photosynthesis and plant growth descriptors. Increasing leaf respiration rates were associated with reductions in plant specific growth rate, leaf elongation rate, and total production in *C. nodosa* and *Z. noltii*. On the other hand, increasing maximum photosynthetic rates at 20°C resulted in a significant increment in plant growth rates only for *C. nodosa*. For the other three species, *Posidonia oceanica*, *Zostera marina*, and *Z. noltii*, we did not find significant association between leaf photosynthetic rates and plant growth rates. Plant growth is regulated by multiple factors and the photosynthetic light response curve is not necessarily an adequate descriptor of net carbon input and plant growth for all species. Species-specific differences in carbon allocation to non-photosynthetic tissue and in leaf self-shading within the canopy can result in species-dependent coupling between plant growth and leaf photosynthetic performance.

In summary, we conclude that temporal variability in the photosynthetic status of temperate seagrasses is not a simple function of seasonal climate fluctuations, but involves species-specific responses and local environmental variability. Species-specific variation in the photoacclimatory leaf responses, and differential nutrient requirements of the plants, may result in species-specific differences in the degree of coupling between leaf photosynthetic rates and environmental factors. Plant nutrient requirements have, however, a complex control involving external factors (e.g., nutrient availability) and internal factors, such as nutrient uptake efficiency and internal nutrient economy. The results also support previous suggestions (Duarte 1991) that increasing species size and life span increases the capacity to buffer seasonal changes, limiting the dependence of the plants on external environmental fluctuations.

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