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Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem.

G. Peralta*, J.L. Pérez-Lloréns, I. Hernández, J.J. Vergara

Division of Ecology, Faculty of Environmental and Marine Sciences, University of Cádiz, 11510 Puerto Real, Cádiz, Spain

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Abstract

The growth vs. irradiance response of the seagrass *Zostera noltii* from Cadiz Bay Natural Park (southwestern Spain) was characterised. Plants were exposed along 14 days to different light treatments (1%, 7%, 42% and 100% surface irradiance, SI), using shade screens in an outdoor mesocosm. Growth at 100% SI (1.6 mg DW plant $^{-1}$ day $^{-1}$) was lower than that at 42% SI (2.4 mg DW plant $^{-1}$ day $^{-1}$), suggesting photoinhibition. The minimum light requirement estimated was 0.8 mol photons m $^{-2}$ day $^{-1}$ (2% SI). Light availability affected the pattern of plant development and the overall plant growth. The contribution of the apical shoots to the aboveground production was nearly constant (c.a. 1.13 cm plant $^{-1}$ day $^{-1}$) regardless of the light level (except at 1% SI). In contrast, recruitment and growth of lateral shoots arising from the main rhizome axes accounted for the observed differences in aboveground growth. Rhizome branching was only observed at 42% SI. The possibility of a light threshold for rhizome branching could explain the seasonality of shoot recruitment, as well as the observed decrease in shoot density along depth (or light) gradients in seagrass meadows. Carbon demands at low irradiances (1% and 7% SI) were partially met by mobilization of carbohydrate reserves (sucrose in belowground and starch in aboveground parts). Plant nitrogen content decreased with increasing light, especially in belowground parts, reaching critical levels for growth.

Keywords: Branching; C/N ratio; Growth rate; Light; Nonstructural carbohydrates; Plant architecture; Seagrass

* Corresponding author. Present address: Netherlands Institute of Ecology, P.O. Box 140, 4400 AC Yerseke, The Netherlands. Tel.: +31-113-5777470; fax: +31-113-573616.

E-mail address: peraltaG@cemo.nioo.knaw.nl (G. Peralta).

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1. Introduction

Light is the main factor controlling the production and growth of seagrass meadows (Zieman and Wetzel, 1980). In many coastal areas, human and/or natural deterioration of underwater light availability often results in large-scale losses of seagrasses (Short and Wylie-Echeverria, 1996). Plant responses to light include a variety of adaptations at physiological (photosynthesis, nutrient uptake) and biochemical (pigments, nutrient quota, carbohydrates) levels (Wiginton and McMillan, 1979; Grice et al., 1996; Lee and Dunton, 1997; Alcoverro et al., 1999; Moore and Wetzel, 2000). Such responses are translated into alterations of growth rate and plant architecture (i.e., individual morphological features) and, finally, on meadow morphological characteristics (i.e., canopy height and/or shoot density) and distribution (Backman and Barilotti, 1976; West et al., 1990; Fitzpatrick and Kirkman, 1995; Krause-Jensen et al., 2000). Therefore, the understanding of seagrass decline relies largely on the knowledge of processes occurring at smaller biological scales.

Experimental studies on seagrass shoot responses to light are relatively common (see above-cited references). However, those focussed on belowground (rhizome–roots) growth and branching pattern (architecture) are either scarce (Olesen and Sand-Jensen, 1993), or observational and poorly documented (Hemminga and Duarte, 2000). Seagrass architectural features and space occupation are determined by clonal growth (Duarte, 1991a), which largely relies on rhizome branching (Hemminga and Duarte, 2000). In addition to the space occupation role of the rhizomes, the aboveground/belowground biomass ratio become very important for seagrass carbon budgets because belowground respiratory tissues rely on photosynthates provided by shoots. This is particularly critical for growth in low-light environments. To what extent seagrasses are able to respond to reduced light with acclimations in the biomass partitioning between shoots and root–rhizomes is still unknown (Hemminga, 1998). It has been suggested that the length of time a seagrass species can survive below their minimum light requirements is related to its ability to store carbohydrates, especially in the rhizomes (Czerny and Dunton, 1995; Kraemer and Alberte, 1995; Alcoverro et al., 1999). The storage capacity and the clonal integration (sensu Hartnett and Bazzaz, 1983) is largely seagrass-size dependent (Hemminga and Duarte, 2000). Small species, like *Zostera noltii*, have presumably lower capacities than those with thick and long-lived rhizomes (Tomasko and Dawes, 1989; Marbà and Duarte, 1998), conferring a very limited tolerance to light deprivation episodes.

In Cadiz Bay Natural Park (south-western Spain), declines in subtidal and lower-intertidal meadows of the seagrass *Z. noltii* were parallel to industrial and tourism development of the surrounding coastal areas (Seoane, 1965; Muñóz-Pérez and Sánchez de Lamadrid, 1994). Human-induced (e.g., regular dredging, land disturbance), in addition to wind- or tidal-driven sediment resuspension frequently result in pulsed, short-lived, turbidity episodes. The decline of these seagrass beds could be self-accelerated since the annual shoot recruitment of populations relies mainly on rhizome branching and plant propagules from nearby well-established meadows (i.e., "donor meadows") (Peralta et al., 2000a; Brun, 1999). Thus, the understanding of the species-specific response to light is an important tool for environmental managers in the establishment of management guidelines for protection and restoration of *Z. noltii* beds from this Natural Park.

The aim of this work was to evaluate the effect of light availability on architectural, dynamic and biochemical (C, N, nonstructural carbohydrates) characteristics in plants of *Z. noltii* Hornem. by simulating transient light reductions (2 weeks) in an outdoor mesocosm experiment.

2. Materials and methods

2.1. Plant material

Intact vegetative plants of *Z. noltii* were collected from a low intertidal monospecific bed at the Rio San Pedro Inlet (Cádiz Bay Natural Park), Spain (36°30′ N, 6°10′ W), in autumn. Plants were collected carefully to keep belowground structures intact, and transported to the laboratory within 15 min of collection in an ice chest. Plants were gently washed free of sediments, sorted, placed in a clear aquarium and maintained under natural irradiance for 24 h prior to the start of the experiments. For standardization, transplant units (onward plants), consisting of a single apical shoot (four to five leaves) with two rhizome internodes and associated root, were selected.

2.2. Microcosm design

The effects of light reduction on the growth and biochemical composition of *Z. noltii* were determined in an outdoor mesocosm at the roof of the Faculty of Marine Sciences (500 m from the sampling point). The experimental set up consisted of eight clear Plexiglas cores (four light levels × two replicates). In each core (8 cm diameter and 24 cm height), four plants were transplanted in a biphasic medium consisting of an agar-solidified root–rhizome layer (seawater with 2% agar w/v, bottom compartment) with overlying 900 ml of filtered (Whatman GF/C) seawater (37) (Peralta et al., 2000a). The biphasic medium was not fertilized. Seawater was air-bubbled and renewed every 2 days to prevent excessive microalgae growth and nutrient depletion. Temperature was kept at 18.5 °C by placing the cylinders in a 50-l glass aquarium supplied with distilled water continuously pumped from a 50-l reservoir provided with a heating and a cooling unit. The experiment lasted 14 days.

The cores were covered with a variable number of neutral density screens to reduce the surface irradiance (SI) (attenuation coefficient = 0.29 screen^{-1}). The eight cores were allocated randomly inside the aquarium, setting four groups ($n = 2$ each): one control (100% SI) and three experimental levels (1% SI, 7% SI and 42% SI).

Photosynthetically active radiation (PAR; 400–700 nm) was recorded continuously using a LI-192 SA flat sensor connected to a L-1000 data logger (LI-COR) adjacent to the experimental site. The irradiance was measured at 30-min intervals and integrated daily (I_d, mol photons m^{-2} day^{-1}).

2.3. Plant measurements

Architectural properties of plants were measured at the start of the experiment (Table 1). Individual plants were weighted prior to transplantation. Fresh weight (FW)–dry

Table 1
Architectural and dynamic properties of *Z. noltii* plants estimated in this work

Properties	Abbreviation	Units
Architectural		
Plant weight	PW	mg DW plant $^{-1}$
Leaves per shoot	NL	number of leaves shoot $^{-1}$
Leaf length	LL	cm
Leaf width	LW	mm
Rhizome internode number	IN	number of internodes plant $^{-1}$
Rhizome internodal length	IL	cm
Roots per plant	RN	number of roots plant $^{-1}$
Root length	RL	cm
Dynamic		
Growth rate	GR	mg DW plant $^{-1}$ day $^{-1}$
Leaf elongation rate	LER	cm plant $^{-1}$ day $^{-1}$
Leaf appearance rate	LAR	leaves plant $^{-1}$ day $^{-1}$
Leaf loss rate	LLR	cm plant $^{-1}$ day $^{-1}$
Internode elongation rate	IER	cm day $^{-1}$
Internodal appearance rate	IAR	internodes day $^{-1}$
Root elongation rate	RER	cm day $^{-1}$
Root appearance rate	RAR	roots day $^{-1}$

Plant is defined as the group of leaves, rhizome and roots developed from each transplant unit (see Materials and Methods).

weight (DW) and length (L)–DW relationships for shoots, rhizomes and roots were determined in separate specimens to estimate growth on a mass (weight) basis.

All plants (32) were marked for growth estimations according to Zieman (1974) as modified by Peralta et al. (2000a,b). At the end of the experiment, plants were carefully

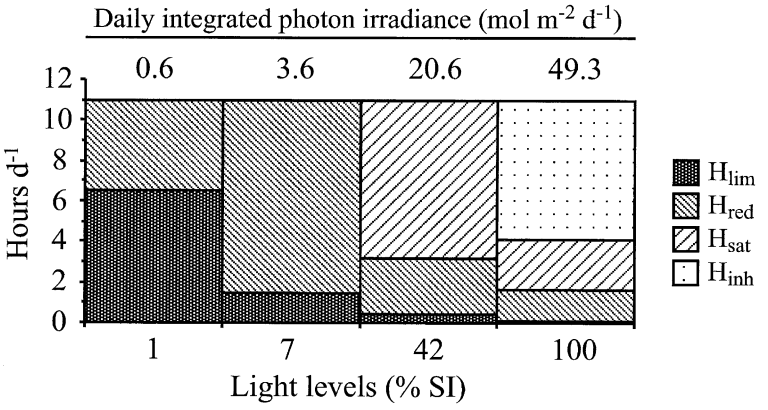

Fig. 1. Daily irradiance distribution based on the photosynthetic parameters of *Z. noltii* (I_c: compensation irradiance; I_{sat}: saturation irradiance; I_{inh}: photoinhibitory threshold fixed at 1000 μmol photons m $^{-2}$ s $^{-1}$). At each light level, the categories to divide the daylight period were H_{lim} (for $I<I_c$), H_{red} (for $I_c<I<I_k$), H_{sat} (for $I_k<I<I_{inh}$), and H_{inh} (for $I>I_{inh}$). The means of daily integrated photon irradiance are indicated on the top of columns.

retrieved and separated into root, rhizome and leaves for biomass measurements. In addition to the above architectural properties, the appearance of new leaves, roots and rhizome internodes, and the length of the new portion of leaves and rhizomes were noted. That allowed the estimation of the dynamic properties shown in Table 1. Rhizomes, roots and shoots (initial and final samples) were oven-dried (60 °C) until constant weight to determine the dry weight of each fraction. Subsamples of all fractions were powdered and stored for nutrient content analysis. Tissue C and N were determined using a Perkin-Elmer 240 CNH elemental analyzer. For carbohydrate determination, soluble sugars were extracted in boiling 80% ethanol. The extracts were evaporated to dryness at room temperature, redissolved in distilled water and analyzed spectrophotometrically using a resorcinol assay standardized to sucrose (Huber and Israel, 1982). Starch was extracted from the ethanol-insoluble fraction overnight in 1 N NaOH and analyzed spectrophotometrically using an anthrone assay standardized to sucrose (Yemn and Willis, 1954).

Plant net growth rate vs. daily integrated irradiance (GR vs. I_d) data were fitted to an homologous equation to that proposed by Platt et al. (1980) for photosynthesis vs. irradiance (P vs. I) when photoinhibition accounts:

$$\mathrm{GR} = \left[\mathrm{GR_S}\left(1 - \exp\left(\frac{-\alpha I_d}{\mathrm{GR_S}}\right)\right)\exp\left(\frac{-\beta I_d}{\mathrm{GR_S}}\right)\right] - \mathrm{MR}$$

$$\mathrm{GR_{max}} = \mathrm{GR_S}\left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{\beta}{\alpha+\beta}\right)^{\frac{\beta}{\alpha}}$$

where GR and GR_S are the plant net growth rate and the coefficient to estimate the maximum growth rate (GR_{max}) (mg DW plant^{-1} day^{-1}), respectively; α and β are the light-limited slope and the photoinhibition coefficient of the GR vs. I_d curve, respectively; I_d is the daily integrated irradiance (mol photons m^{-2} day^{-1}) and MR is the maintenance rate or weight loss rate in continuous darkness (mg DW plant^{-1} day^{-1}). Curve fitting and estimation of parameters GR_S, α, β and MR were performed by an iterative procedure (Solver from Excel, Microsoft©), according to the minimum squares procedure (Zar, 1984). The compensating and the optimum daily integrated irradiance for growth ($I_{d,c}$ and $I_{d,o}$, respectively) were defined as:

$$I_{d,c} = \frac{\mathrm{MR}}{\alpha} \quad \text{and} \quad I_{d,o} = \frac{\mathrm{GR_S}}{\alpha}\ln\left(\frac{\alpha+\beta}{\beta}\right).$$

2.4. Statistics

Means and standard errors of all variables were calculated for each light level. The leaf loss rate, and the internode and root appearance rates data violated the assumption of homocedasticity, even when data were log-transformed. In these cases, the effect of the light treatment was tested using the nonparametric Kruskall–Wallis test (Zar, 1984) followed by the nonparametric Tukey test (Zar, 1984). For other variables, the effect of the light treatment was analyzed by one-way ANOVA (Zar, 1984), followed by the parametric Tukey test. In all cases, the significant level was set at 5% probability.

3. Results

3.1. Light treatment

During the experiment, the daily average integrated photon irradiance at the surface of the mesocosm was 49.3 mol m^{-2} day^{-1} (100% SI) with a photoperiod of 11:13 (L/D). The filters reduced this value to 20.6 (42% SI), 3.6 (7% SI) and 0.64 (1% SI) mol photons m^{-2} day^{-1}, respectively. Estimated photosynthetic parameters for this species in autumn (compensation irradiance, $I_c = 21$; saturation irradiance, $I_k = 330$ μm and photoinhibitory

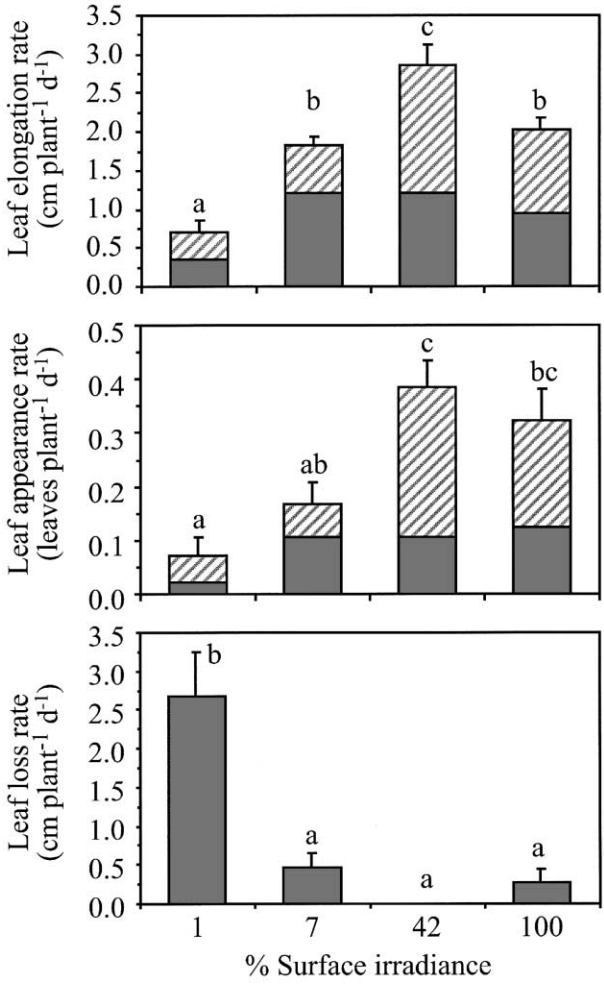

Fig. 2. Leaf dynamic properties of *Z. noltii* as a function of light availability. For each column, the striped compartment represents the contribution of lateral shoots. Different letters on columns indicate significant differences ($P < 0.05$) among means. Error bars represent SE ($n = 8$).

threshold, $I_{inh} = 1000$ μmol m^{-2} s^{-1}; Peralta, 2000) were useful to roughly divide the daylight period into four categories: *I*-limited (H_{lim} for $I < I_c$), *I*-reduced (H_{red}, for $I_c < I < I_k$), *I*-saturated (H_{sat}, for $I_k < I < I_{inh}$), and *I*-photoinhibited (H_{inh}, for $I > I_{inh}$) photosynthesis. On this basis, only control plants and those grown at 42% SI received saturating irradiances, in contrast to those cultured at 7% or 1% SI, which always grew under reduced or limited irradiances (Fig. 1).

3.2. Architectural and dynamics properties

No significant differences ($P > 0.05$) were observed for the architectural properties in response to light treatments. Mean leaf, rhizome internode and root lengths were 12.5, 2.2 and 3.0 cm, respectively, and average leaf width was 1.5 mm. In contrast, most of the leaf dynamic properties were significantly affected by light availability. Thus, maximum elongation rate (LER, 2.9 cm $plant^{-1}$ day^{-1}), appearance rate (LAR, 0.39 leaves $plant^{-1}$ day^{-1}) and minimum leaf loss rate (LLR, 0.02 cm $plant^{-1}$ day^{-1}) were recorded mainly at 42% SI (Fig. 2). The contribution of the apical shoots (meristem) to the

Table 2
Statistical analysis for the effects of light on architectural and dynamic properties of *Z. noltii* plants

Variable	Degrees of freedom	*F*	*H*
Growth rate	3	31.1***	
Leaf elongation rate	3	24.4***	
Leaf appearance rate	3	9.5***	
Leaf loss rate			19.7***
Internode elongation rate	3	19.4***	
Internode appearance rate			19.7***
Root elongation rate	3	33.6***	
Root appearance rate			22.5***
C content			
Leaves	3	42.8***	
Rhizome–root	3	5.2*	
N content			
Leaves	3	15.2**	
Rhizome–root	3	20.3***	
C/N ratio			
Leaves	3	20.9***	
Rhizome–root	3	22.2***	
Sucrose			
Leaves	3	2.4^{ns}	
Rhizome–root	3	7.6*	
Starch			
Leaves	3	10.4*	
Rhizome–root	3	1.4^{ns}	

When parametric test were possible, results of one-way ANOVA are represented by the *F*-values. In case of nonparametric tests, the results of the Kruskall–Wallis test are represented by the *H* value.

* $P < 0.05$.

** $P < 0.005$.

*** $P < 0.001$.

^{ns} not significant.

LER and LAR was nearly constant (1.13 cm plant^{-1} day^{-1} and 0.11 leaves plant^{-1} day^{-1}, respectively) regardless the light level, excepting at 1% SI (0.4 cm plant^{-1} day^{-1} and 0.02 leaves plant^{-1} day^{-1}). Thus, the recruitment of new lateral shoots (as well as apical meristems, see below) during the experiment could explain the observed pattern in LER and LAR. Overall, the recruitment of lateral shoots contributed ca. 30–75% of the total LER and LAR values. Leaf plastochrone interval (LPI) was unaffected by light regime (10–12 days) (data not shown). However, only two plants (from the initial eight) receiving 1% SI generated new leaves from the apical shoots.

Rhizome internodal elongation rate (IER) was significantly affected ($P < 0.001$, Table 2) by the light treatment, with minimum values (0.03 cm day^{-1}) in plants grown at 1% SI, and maximum ones (0.16 cm day^{-1}) at 42% SI (Fig. 3). Secondary rhizomes (i.e., lateral axis arising from the main rhizome), with their corresponding apical shoots, developed only in plants subjected to 42% SI. Such rhizome branching accounted for ca. 8% of the total IER. The rhizome internodal appearance rate (IAR) was also affected by the light treatment (Table 2, Fig. 3). The plants grown at 1% SI exhibited the minimum IAR (0.08 internodes day^{-1}) and the maximum values were detected at 42% SI (0.16 internodes day^{-1}). This latter treatment was the only one where secondary rhizomes appeared, and its contribution to the total IAR was about 27%.

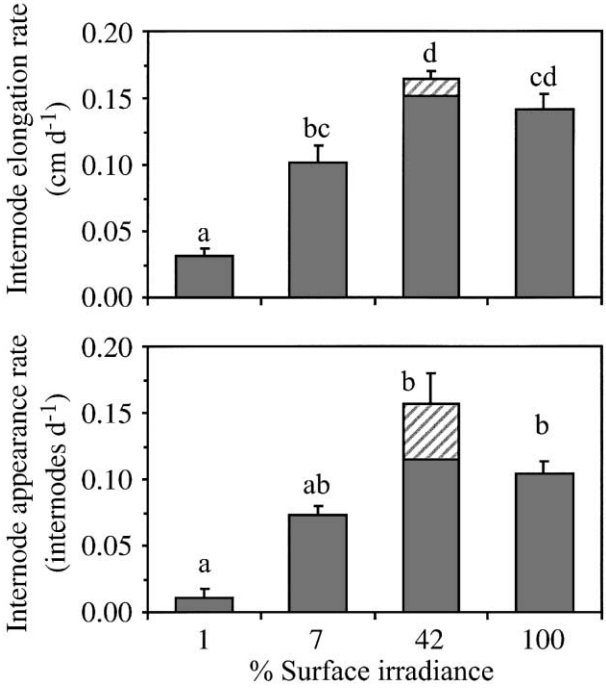

Fig. 3. Internode dynamic properties of *Z. noltii* as a function of light availability. For each column, the striped compartment represents the contribution of secondary rhizomes. Letters on columns as in Fig. 2. Error bars represent SE ($n = 8$).

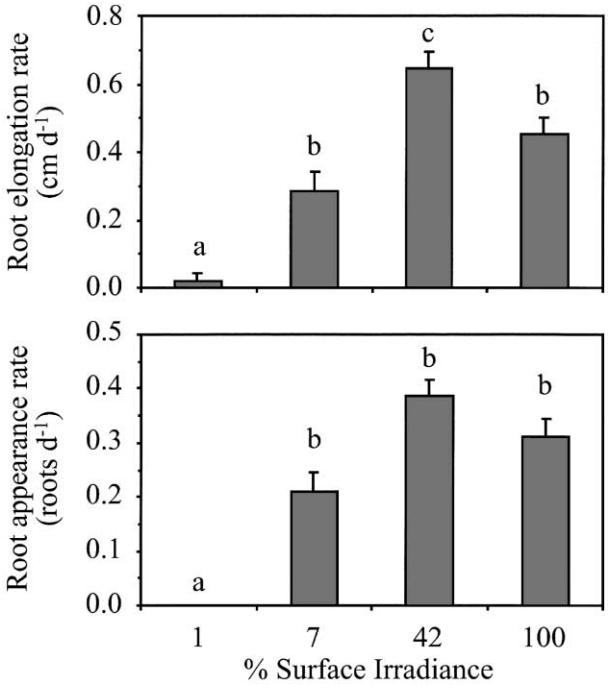

Fig. 4. Root dynamic properties of *Z. noltii* as a function of light availability. Letters on columns as in Fig. 2. Error bars represent SE ($n = 8$).

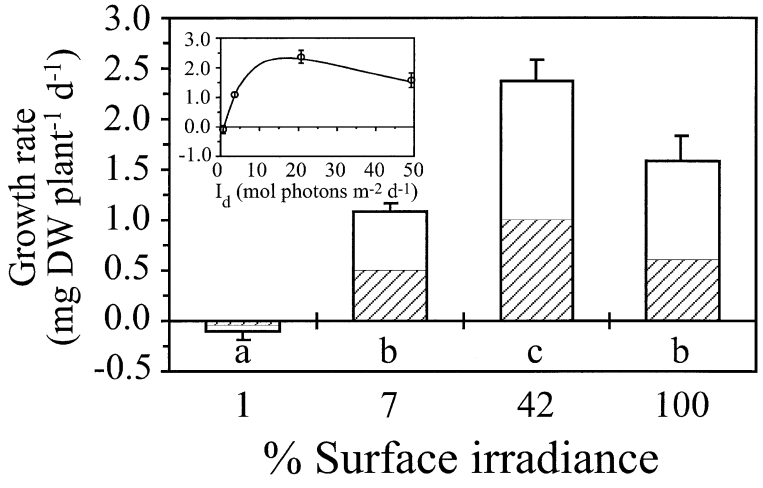

Fig. 5. Growth rate of *Z. noltii* plants as a function of light availability. For each column, the striped compartment represents the contribution of belowground modules. Letters under columns indicate significant differences ($P < 0.05$) among means. The inset shows the relationship between the plant growth rate and the daily integrated photon irradiance (I_d). The line fits the data to the theoretical model. Error bars represent SE ($n = 8$).

The light treatment also affected RAR and RER values (Table 2). Plants grown at 1% SI did not develop any new roots (RAR = 0 roots day $^{-1}$), and only one plant showed positive root elongation rates (RER) (Fig. 4). Plants exposed to 42% SI showed higher RER values than those at 7% and 100% SI.

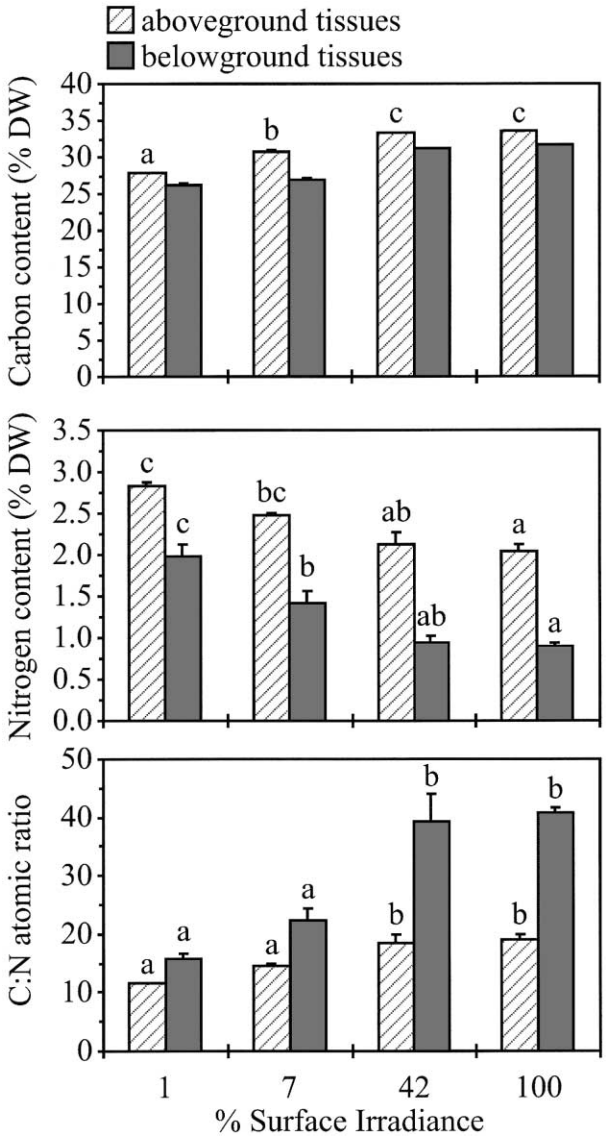

Fig. 6. Nutrient contents and C/N ratio in aboveground and belowground tissues of *Z. noltii* as a function of light availability. For each module (aboveground and belowground), different letters on columns indicate significant differences ($P < 0.05$) among means. Error bars represent SE ($n = 8$).

In agreement with the results detailed above, plant growth rate on weight basis (GR) was significantly affected by light availability ($P < 0.001$, Table 2) (Fig. 5). A value of 2.4 mg DW plant^{-1} day^{-1} was recorded at 42% SI followed by control plants (100% SI, 1.6 mg DW plant^{-1} day^{-1}), whereas negative growth was observed at 1% SI (-0.09 mg DW plant^{-1} day^{-1}). The relative contribution of leaves to the total plant growth increased with light availability, ranging from 55% (at 7% SI) to 61% (control). The relationship between the plant growth rate and the daily integrated photon irradiance followed a saturation model with photoinhibition (Fig. 5, inset). Estimated values for compensating and optimum daily integrated photon irradiances for growth were 0.8 and 16.9 mol photons m^{-2} day^{-1}, respectively, which corresponded to 2% and 34% of the SI.

3.3. Tissue nutrient content (C, N) and nonstructural carbohydrates

Tissue C and N contents were significantly higher ($P < 0.001$) in shoots than in rhizome–roots (Fig. 6), and were significantly affected by the light treatment (Table 2). Above- and belowground C content decreased at reduced light levels, whereas the

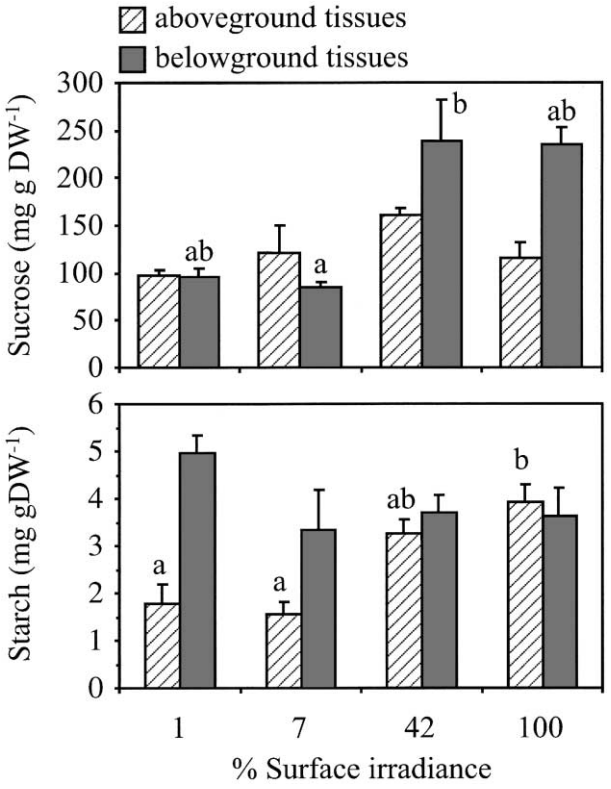

Fig. 7. Nonstructural carbohydrates in aboveground and belowground tissues of *Z. noltii* as a function of light availability. Letters on columns as in Fig. 6. Error bars represent SE ($n = 8$).

opposite pattern was observed for internal N content, resulting in decreased C/N atomic ratios. Thus, internal N content was accumulated in rhizome–roots and, at lower extent, in leaves, when growth diminished (7% SI) or ceased (1% SI). The sucrose content of aboveground and belowground parts was much higher than the starch content (Fig. 7). Sucrose levels of belowground structures, as well as starch content of aboveground parts, were significantly affected by the light treatment (Table 2), with higher contents (ca. 250 sucrose mg g^{-1} DW and 4 starch mg g^{-1} DW) at the two highest light levels (100% SI and 42% SI). At low light levels, the sucrose content of belowground parts and the starch levels of the aboveground tissues decreased drastically compared to higher light levels (ca. 50%).

4. Discussion

In seagrasses, growth–irradiance relationships have been less studied than photosynthesis–irradiance relationships. The response of seagrass growth to irradiance has been described by hyperbolic functions indicating saturation kinetics (Olesen and Sand-Jensen, 1993 for *Z. marina*; Vermaat and Verhagen, 1996 for *Z. noltii*) or by linear models (Short et al., 1995 for *Z. marina*). However, we observed that the net growth rate of *Z. noltii* was maximum at intermediate light levels (42% SI or 20.6 mol photons m^{-2} day^{-1}), and decreased at 100% SI. This decrease was not associated to negative effects of UV on plant growth, since the glass and Plexiglas used absorb most of the UV radiation (Jagger, 1967). Therefore, the growth pattern was fitted to a homologous equation to that proposed by Platt et al. (1980) for photosynthesis–irradiance when photoinhibition accounts. As far as we know, this growth response has not been previously reported in seagrasses. However, the photodamage of the photosystem II after 120 min exposure at 1000 μmol photons m^{-2} s^{-1} is a phenomenon reported for other species, as *Halophila ovalis* (Ralph and Burchett, 1995), and plants at 100% SI suffered irradiances above 1000 μmol photons m^{-2} s^{-1} during more than 6 h day^{-1}. This circumstance is relatively common during spring tides for scattered patches with low shoot density occurring at high intertidal locations (Brun, 1999). At lower intertidal positions, irradiance decreases easily below 50% SI, not only because of light attenuation by the water column (attenuation coefficients ranged from 1.1 to 3.5 m^{-1}; Brun, unpublished data), but also for an increased self-shading within more dense beds (Brun, 1999). In fact, the optimum growth rate recorded at 42% SI is consistent with the biomass distribution gradient at the donor site, where maximum biomass values are found at 1 m depth below the mean high water level (MHWL), in a system with 2.5 m of tidal range. At this depth, the average light levels oscillated between 3% and 33% at high tide, and 100% SI at low tide (Brun, 1999). It means that water column reduces between 10% and 52% the time-integrated irradiance reaching the plants growing at 1 m below MHWL. A similar distribution pattern has been also reported for other species such as the small specie *Halodule wrightii* and the larger one *Thalassia testudinum*, both located at depths of 1.2 m, and receiving around 50% SI (Czerny and Dunton, 1995; Lee and Dunton, 1997).

All transplants of *Z. noltii* survived along the 2 weeks experiment, showing positive elongation and appearance rates (i.e., gross growth rate) regardless the light treatment.

However, when tissue losses were taken into account, or when calculations were performed on weight basis (i.e., net growth rate), plants maintained at 1% SI showed a negative growth. The compensation point ($I_{d,c}$) derived from the net growth vs. daily integrated irradiance curve (GR–I_d) for *Z. noltii* was 0.8 mol photons m^{-2} day^{-1}. Olesen and Sand-Jensen (1993) reported a value of 1.07 mol photons m^{-2} day^{-1} for *Z. marina* under similar experimental conditions, and Moore et al. (1997) estimated values of 1.2 and 7.5 mol photons m^{-2} day^{-1} (at 25 °C) in *Z. marina* depending on the meadow location. The lower photosynthetic–nonphotosynthetic biomass ratio of *Z. marina* compared to *Z. noltii*, as well as the higher temperature used by Moore et al. (1997), would largely explain the higher light requirement of *Z. marina* to balance the daily C demand (Hemminga, 1998).

Overall, the minimum light requirement reported for *Z. noltii* in this study (2% SI) is below the average value of 11% SI given by Duarte (1991b) for long-term seagrass survival, and the 4–36% SI given by Dennison et al. (1993). Effects of agar substratum in such disagreement were neglected since previously, there were no significant differences observed between growth of *Z. noltii* in agar or in natural sediments (Peralta et al., 2000b). Three facts must be considered for the reduced minimum light requirement reported in this work. First, unlike the minimum seagrass light requirements based on maximum depth distribution, the values reported for *Z. noltii* were based on the effective light availability reaching individual shoots. That is, it did not account for usual effects occurring in natural environments like self-shading (i.e., reduced plant density) or short-term light deprivation (i.e., no clouds were observed during the experimental period). Second, *Z. noltii* can tolerate severe (below 2% SI), but short-lived (2 weeks), light reductions because of its ability to store and mobilise carbohydrates (see below). However, the survival period of seagrass below its minimum light requirements is shorter in small species (with low storage capacity) than in the larger ones (Bulthuis, 1983; Longstaff et al., 1999; Lee and Dunton, 1997). That means that not only the minimum light requirements but also the term and periodicity of light deprivation events may limit the distribution of small seagrasses into deeper places. Third, when possible, and for intersite comparison purposes, light requirements for seagrass growth should be given as absolute values (e.g., time-integrated) rather than in relative units (e.g., % SI) since small differences on percent SI (i.e., 2–4% SI) may be due to differences on latitude, tides and/or local meteorological characteristics rather than to differences in time-integrated photon-flux. In addition, another reason for the large differences in light requirements is that light measurements are often discrete, and do not account for the effect of seasonal or pulsed changes in light availability (Moore et al., 1997), and/or the effect of the tidal range (Koch and Beer, 1996).

Experimental studies of light availability effects on seagrasses either do not look at the whole plant response or, in examining whole plants, scarcely quantify architectural or growth responses and their relation with internal constituents. In this study, light availability affected the pattern of plant development and the overall plant production. The contribution of the apical shoots to the aboveground production was nearly constant (c.a. 1.13 cm $plant^{-1}$ day^{-1}) regardless the light level, except under severe light limitation. In contrast, recruitment and growth of lateral shoots arising from the main rhizome axes, and from the newly formed secondary rhizomes (only at 42% SI),

accounted for the observed differences in aboveground production. Vermaat and Verhagen (1996) reported that, in natural meadows of *Z. noltii*, leaf growth rate (different shoot types were not considered) was largely constant above a low tide irradiance of 5 mol photons m^{-2} day^{-1}, but dropped rapidly below that value. Their study also observed that rhizomes started to branch above a threshold low tide irradiance of about 15 mol photons m^{-2} day^{-1}, a value close to the 20.6 mol photons m^{-2} day^{-1} that plants at 42% SI received in our study. The possibility of the existence of a light threshold for rhizome branching could explain the seasonality of ramet recruitment described for numerous species (Marbà et al., 1996), and the production of several annual cohorts in *Z. noltii* populations (Vermaat and Verhagen, 1996). On the other hand, the reduced rhizome branching at lower light levels would also explain the observed decrease in shoot density along depth (that is light) gradients in numerous seagrass meadows (Bulthuis, 1983; Abal et al., 1994; Gordon et al., 1994; Krause-Jensen et al., 2000). The reduction of shoot density in response to decreased light availability is a well-known response of seagrasses to reduce self-shading, and therefore, to enhance light-harvesting efficiency (Hemminga and Duarte, 2000).

Seagrass architectural features and space occupation are determined by clonal growth (Duarte, 1991a), which largely relies on rhizome branching (Hemminga and Duarte, 2000). In large species like *Cymodocea nodosa*, branching is considered to be a tightly regulated process by apical meristem activities (i.e., "apical dominance"; Terrados-Muñoz, 1995), which probably involves hormone regulation. However, apical dominance in small species like *Z. noltii* is expected to be weaker since it has been positively related to the rhizome diameter (Hemminga and Duarte, 2000). Hormonal regulation aside, the linkage between the degree of rhizome branching and light availability in *Z. noltii* could be via the amount of available photosynthates, once the growth of the apical shoot was saturated. This could explain the asymptotic growth rate of apical shoots at irradiances above 1% SI, and the similar growth pattern (i.e., bell-shaped) observed for rhizomes, roots and lateral shoots. As described for *Z. marina* (Alcoverro et al., 1999), the apical shoot seems to be the primary sink for reduced carbon withdrawn from belowground tissues when the availability of photosynthates is limited.

The decrease in C content of shoots and belowground parts of *Z. noltii* maintained under severe (1% SI) and limited (7% SI) light reduction was a smooth mirror of those observed for nonstructural carbohydrates. As described for other seagrasses (Touchette and Burkholder, 2000a), sucrose was the primary storage compound in *Z. noltii*. Under light limitation, concentration of sucrose in rhizomes is expected to decrease as a result of (1) in situ consumption to meet the respiratory demands, (2) reduced translocation of photosynthate from leaves because of the lowered pool (Kraemer and Alberte, 1993; Zimmerman and Alberte, 1996; Lee and Dunton, 1997), and (3) the contribution to maintain new growth in aboveground tissues. In addition, anaerobiosis in the belowground parts (see below) would reduce the sucrose content, either by reducing translocation from the shoots (Zimmerman et al., 1995) or by increasing the sucrose synthase activity in rhizome–roots to sucrose utilization (Touchette and Burkholder, 2000a). On the other hand, the higher sucrose concentration in belowground parts under higher irradiances (42% SI and 100% SI) indicated an effective translocation from the aboveground photosynthetic tissues (Moriarty et al., 1986).

Like rapid growth plants, *Z. noltii* had a reduced storage of starch compared with the sucrose storage (Taiz and Zeiger, 1991). Despite the fact that sucrose was the main storage compound, starch was also affected by the light availability. Under light limitation, this carbohydrate was not accumulated in shoots as a result of a restricted photosynthetic C fixation. However, in contrast to sucrose, in belowground tissues, starch concentration did not decrease under low light. Similar responses have been recorded in *Z. marina* (Burke et al., 1996; Zimmerman and Alberte, 1996) and *H. ovalis* (Longstaff et al., 1999). In *Z. marina*, starch is probably not mobilized in anoxic roots (Smith et al., 1988; Zimmerman and Alberte, 1996). Thus, the maximum starch concentrations found in rhizome–roots at low light levels (the dried material was pooled for the analysis) may be a consequence of anaerobiosis in belowground tissues (personal observation). Such anoxic episodes are due to a decreased photosynthesis rate and, consequently, to restricted basipetal flow of oxygen (Larkum et al., 1989).

Shoot nitrogen content of *Z. noltii* was higher than in belowground N tissues regardless of the light level. Nitrogen content of plant tissues decreased with increasing light. Similar responses have been observed in laboratory experiments for several species (Longstaff and Dennison, 1999) as well as in seasonal studies of *Z. noltii* beds (Pérez-Lloréns and Niell, 1993; Vermaat and Verhagen, 1996; Brun, 1999). The above-cited pattern may be explained by dilution processes (Stocker, 1980), owing to a faster utilisation than uptake, so that stored N resources are gradually diluted during growth. In our study, the decrease in N content was more acute in belowground parts, reaching the nitrogen level considered critical for belowground tissues (1% DW; Pedersen and Borum, 1993). Since no N was added to the substratum (agar), part of N resources needed to sustain the high production rates of belowground tissues must be translocated from leaves (Iizumi and Hattori, 1982). The higher N content found under low light conditions is explained by the reduced growth rates. Moreover, in *Z. marina*, the decline in available energy and carbohydrates coincide with a decline in nitrate reductase activity (Touchette and Burkholder, 2000b) that would reduce the capacity of nitrate assimilation favouring the storage of this N source.

In conclusion, *Z. noltii* showed the highest growth rates and rhizome branching at intermediate light levels. The possibility of a light threshold for rhizome branching could explain the seasonality of shoot recruitment, as well as the observed decrease in shoot density along depth (i.e., light) gradients in numerous seagrass meadows. Minimum light requirement for 2 weeks was estimated as 0.8 mol photons m^{-2} day^{-1} (2% SI). Such low light requirement was maintained by C mobilization.

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