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# Effects of light and biomass partitioning on growth, photosynthesis and carbohydrate content of the seagrass *Zostera noltii* Hornem

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**Abstract**

Plants of the seagrass *Zostera noltii* were cultured in the laboratory (mesocosms) for two weeks to assess the effect of above: below-ground (AG/BG) biomass ratios and light on growth, photosynthesis and chemical composition. Experimental plant units (EPUs) with different proportions between AG and BG biomass were obtained from plants of the same size (containing 6 shoots and 5 internodes) by excising 0–5 shoots. The EPUs maintained the proportions in AG/BG biomass ratios during the experiment. While growth rate was unaffected by biomass partitioning at high light, maximum growth at low light was recorded in plants with low AG/BG ratios. The production of shoots and rhizomes showed a compensatory morphological response depending on the initial AG/BG proportions regardless of the light level. While shoot production, estimated as shoot appearance rate, was high at low AG/BG ratios and minimal under high AG/BG values, rhizome production, estimated as internode appearance rate and internode elongation rate, was maximal under high AG/BG proportions and decreased towards lower AG/BG ratios. This rhizomatic response was observed for secondary rhizomes and not for primary ones. In contrast to morphological response, no significant differences were detected in maximum electron transport rates (ETRm) among the different shoots in the plant. However, mean values of ETRm in plants were affected by biomass partitioning and light. EPUs grown in low light increased the sucrose stored in shoots as the AG/BG biomass ratios decreased; however, EPUs grown at high light showed no effect of biomass partitioning on sucrose levels. In conclusion, shoots excision by experimental manipulation caused a compensatory morphological response in plants while photosynthetic performance remained almost unaffected.


*Keywords:* Apical dominance; Biomass partitioning; Clonal integration; Growth; Light; *Zostera noltii*

## 1. Introduction

Seagrasses are aquatic angiosperms that may complete their life-cycle fully submerged in seawater (Arber, 1920; Hemminga and Duarte, 2000). They represent an ecologically important structuring element of the ecosystem and a major source of primary production in shallow waters worldwide (Hemminga and Duarte, 2000). The possession of a root–rhizome system offers seagrasses competitive advantages compared to seaweeds: (1) anchorage in soft substrata, (2) assurance of a nutrient supply from sediment pool, and (3) resource

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storage (Hemminga, 1998). However, since below-ground tissues are heterotrophic (i.e. sinks) they depend exclusively on light-driven basipetal translocation of photosynthates and oxygen (Smith et al., 1984; Kraemer and Alberte, 1993; Zimmerman and Alberte, 1996).

Plant responses to light encompass a mixture of adaptations at biochemical (Wiginton and McMillan, 1979; Alcoverro et al., 1999; Moore and Wetzel, 2000; Brun et al., 2003a) and physiological (Abal et al., 1994; Grice et al., 1996) levels. Furthermore such adaptations frequently affect growth rate and plant architecture (i.e., individual morphological features), meadow morphological characteristics (i.e., canopy height, shoot density, above to below-ground (AG/BG) biomass ratios, etc.) and seagrass distribution (Backman and Barilotti, 1976; West, 1990; Fitzpatrick and Kirkman, 1995; Krause-Jensen et al., 2000; Brun et al., 2002, 2003b; Peralta et al., 2002, 2003).

Experimental studies on seagrass shoot responses to light are relatively common (see references cited above). In comparison, those focussed on below-ground (rhizome–roots) growth and branching pattern (plant architecture) are scarce (Olesen and Sand-Jensen, 1993; Peralta et al., 2002; Brun et al., 2006b) or observational and poorly documented (Hemminga and Duarte, 2000; Brun et al., 2003b, in press). Besides space occupation role of the rhizomes, the biomass partitioning (i.e. AG/BG ratio) becomes essential for seagrass carbon budgets because below-ground tissues are the main storage compartments but, as heterotrophic tissues, they rely on photosynthates provided by shoots. Thus, several studies have revealed that the seagrass *Zostera noltii* has a relatively high capacity to cope with environmental stress by adapting its morphology (Vermaat and Verhagen, 1996; Peralta et al., 2002, 2005, 2006; Brun et al., 2003c, 2006b). However, morphological plasticity has several costs and risks, since it may increase the fitness of the phenotype to some environmental stresses but may reduce the fitness in other underlying traits (de Witt et al., 1998). For instance, a great reduction in the AG/BG ratio of *Z. noltii* was recorded as a response to human-induced increase in hydrodynamic conditions. This change in biomass partitioning improved the anchoring capacity of the plants but may imbalance the source/sink ratio for carbon, and thus, to increase the vulnerability of plants under sudden low light conditions (Peralta et al., 2005). As light is the main driving force involved in this process, the benefit of having a root–rhizome system becomes a drawback when the quantity of light reaching the canopy decreases and the sediment becomes anaerobic (Hemminga, 1998). To what extent seagrasses are able to respond to reduced light through changes in the biomass partitioning between shoots and root–rhizomes is still unknown (Hemminga, 1998).

The response of a seagrass plant (usually composed by a variable number of shoots and internodes) to light may depend on the number of shoots recruited by the plant, because connected shoots share resources among them (Tomasko and Dawes, 1989; Marbà et al., 2002). Furthermore, apical shoot influences the nutrient status of adjacent-connected shoots due to its higher metabolic activity and hormone levels (Phillips, 1975; Cline, 1997). Consequently, the interrelation between both clonal traits (clonal integration and apical dominance) may modify the response of the whole plant and individual elements (shoots and internodes) against the same environmental stimulus. The acclimation of *Z. noltii* to environmental stress involves several plant-properties (apical dominance, clonal integration, and plant morphological variability) at different organization levels (leaves, shoots, and whole plant) that must be considered when studying acclimation processes.

The aim of this work was to assess the combined effect of light and AG/BG biomass ratios (artificially generated) on growth, photosynthetic estimators and biochemical composition of the seagrass *Z. noltii* taking into account the apical dominance and the clonal integration. The results showed a compensatory morphological response between above (AG) and below-ground (BG) parts that can be an important adaptation to cope with variable AG/BG ratios.

## 2. Materials and methods

### 2.1. Plant material

*Z. noltii* Hornem. plants were collected from an intertidal sand bed at El Bajo de la Cabezuela, Cádiz (36° 32′ N, 6° 15′ W) in summer when *Z. noltii* is in its maximum growth phase (Brun et al., 2003a). Plants were gathered carefully to keep below-ground parts intact and transported to the laboratory in an ice-chest. Previous to any experimental manipulation, plants were gently rinsed in seawater and visible epiphytes were removed by scraping. Eighty plants, with 6 shoots and 5 internodes, were selected and acclimated to the laboratory conditions in a clear aquarium for 24–48 h. All plants were sized and weighted on fresh basis (FW). Twenty plants, of the 80, were randomly chosen as representative of the initial experimental conditions (initial plants).

### 2.2. Experimental design

The effects of light availability and biomass partitioning (i.e. above to below-ground biomass ratio, AG/BG)

on growth, photosynthesis and biochemical composition of *Z. noltii* were determined using a two-way factorial design [light (2 levels, saturating vs. limiting) × AG/BG biomass ratios (6 levels)] in a mesocosms experiment.

The different AG/BG ratios were obtained experimentally by excising 0 to 5 shoots and keeping intact the 5 internodes. Thus, "experimental plant units" (EPUs) ranged from 6 shoots with 5 internodes (highest AG/BG ratio, treatment "6") to 1 shoot with 5 internodes (lowest AG/BG ratio, treatment "1"). Treatment "6" is considered as a control since EPUs conserved the same AG/BG ratios than those plants collected in the field. Shoots were removed from distal (older most) to apical (younger most) parts of the plant, maintaining always the apical shoot. Thirty (6 contrasting AG/BG ratios × 5 replicates) of the 60 EPUs were grown under light-saturated conditions and the other 30 under light-limited levels. All EPUs were individually weighted before transplantation and tagged. All plants were carefully transplanted into aquaria according to the light treatment. Aquaria were filled with a layer of sediment and 15 L of natural seawater collected from El Bajo de la Cabezuela where plants were gathered. The sediment was washed thoroughly with 5–10% sodium hypochlorite and rinsed with tap water to reduce the amount of organic matter. Seawater was also filtered with cellulose paper. Nutrients (2.0 μM ammonium and 0.66 μM phosphorus, final concentration) were added to aquaria twice a week to prevent nutrient limitation. Water was homogenised with an air-pumping system.

EPUs were grown for 14 days in an incubation chamber (D-1400-3BL, ASL) at 18 °C. Plants in high light conditions were exposed to a saturating light level of 165 μmol photon $m^{-2}$ $s^{-1}$, while plants in low light were grown at 25 μmol photon $m^{-2}$ $s^{-1}$. Light was supplied with fluorescent tubes (Philips TLD 18w/865). The limiting light level was achieved by using neutral density covers under the light source. Light intensity was measured with a LI 193 SA (LiCor) quantum meter mounted with a 4π PAR (400 to 700 nm) sensor.

### 2.3. Plant growth and biomass partitioning

After 14 days, EPUs were collected, sized, split into above and below-ground biomass, and weighted to determine the dynamic growth parameters of plants: net growth rate (GR, mg FW $plant^{-1}\cdot d^{-1}$), shoot appearance rate (SAR, no. $shoots\cdot plant^{-1}\cdot d^{-1}$), internode appearance rate (IAR, no. $internodes\cdot plant^{-1}\cdot d^{-1}$) and internode elongation rate (IER, $cm\cdot plant^{-1}\cdot d^{-1}$) for main and secondary rhizome axes, according to Peralta et al. (2002).

Before weighting, the second innermost leaf of every shoot on each EPU ($n>60$) was taken for fluorescence measurements (see below). Afterwards, 3 EPUs of the 5 replicates per treatment (previously split in above and below-ground parts) were randomly chosen and dried to a constant weight (48 h at 60 °C in an oven), ground into powder using a mortar and pestle and stored for non-structural carbohydrate determinations. The other 2 EPUs of each treatment were immediately frozen with liquid $N_2$ and conserved at −80 °C for protein determination.

### 2.4. Photosynthetic response

Seagrass photosynthetic estimators were determined by calculating the quantum yield of photosynthesis using measurements of chl*a* fluorescence (for details on this technique see (Krause and Weis, 1991)). The measurements were made with a PAM-2000 (Walz Effeltrich, Germany). Quantum yield was determined through measurements on the second innermost leaf of every shoot in 8 initial plants at the beginning of the experiment (6 shoots × 8 plants, $n=48$) and in all EPUs at the end of the experiment (all shoots × 60 plants, $n>60$).

A leaf clip (Diving-LC, Walz) connected with fibre optics to the fluorometer was mounted in the middle part of the leaf, at 5 cm approximately from the base. A 5 s weak far-red pulse was applied to oxidise the electron transport chain (Hanelt, 1998), after which the shutter of the clips was closed. After 5 min of dark acclimation, the ground fluorescence ($F_0$) was estimated, followed by a saturating pulse to measure maximum fluorescence (Fm), allowing calculation of the variable fluorescence ($Fv=Fm-F_0$) and the maximum quantum yield of PSII (Fv/Fm). Rapid light curves (RLCs), were also performed by subjecting seagrass leaves to a series of increasing PAR levels from darkness up to 3000 μmol $photon\cdot m^{-2}\cdot s^{-1}$, using the PAM halogen lamp as the light source (Ralph et al., 1998; White and Critchley, 1999; Bischof et al., 2000; Malta et al., 2005). At each irradiance, the fluorescence (Ft) was measured, followed by a saturating light pulse to measure the maximum fluorescence (Fm′), and the effective quantum yield of PSII [$\Delta F/Fm'=(Fm'-Ft)/Fm'$] was calculated. Electron transport rate (ETR) was calculated according to (Beer et al., 1998) as $ETR=\Delta F/Fm'\cdot I_{PAR}\cdot A\cdot 0.5$; where $I_{PAR}$ is the incident irradiance, $A$ is the absorptance of the sample (set as 0.80, mean value obtained from measurements in *Z. noltii* leaves) and the factor 0.5 assuming that both photosystems absorb equal amount of photons. Maximum ETR (ETRm) values

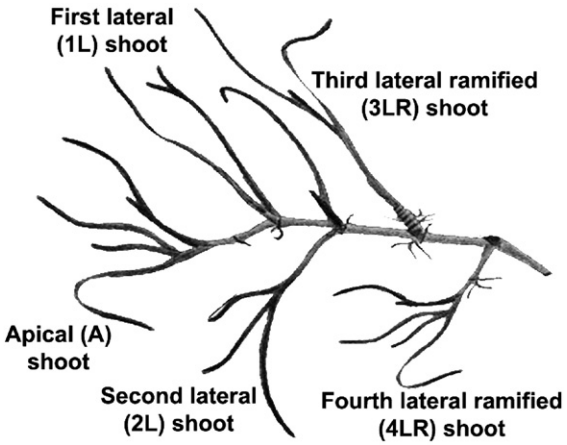


Fig. 1. *Zostera noltii*. Major morphological features in *Z. noltii* plant. For further information see Brun et al. (2006a).

were obtained from the average maximum values above saturating irradiance from ETR vs. irradiance curves after fitting the data to the model of Platt et al. (1980) with photoinhibition using the least-square non-linear regression.

The photosynthetic study was carried out measuring fluorescence in all shoots of every plant. To reduce any within-shoot variability, measurements were done in the middle part of the second innermost leaf of every shoot. Values were grouped further according to different shoot types observed (i.e. apical (A), first lateral (1L), second lateral (2L), third lateral ramified (3LR)) (Fig. 1) in order to study the role of shoot distribution on ETRm. To evaluate the effect of light and AG/BG ratios on *Z. noltii* photosynthesis, the ETRm and Fv/Fm from shoots of the same experimental treatment (light and AG/BG ratio) were pooled obtaining mean values of ETRm and Fv/Fm.

### 2.5. Biochemical composition

After drying (48 h at 60 °C in an oven), above and below-ground parts of the EPUs selected for carbohydrate determination were individually powdered using a mortar and pestle. Soluble sugars were extracted in boiling 80% ethanol. The extracts were evaporated to dryness at room temperature, re-dissolved in distilled water and analysed with a spectrophotometer, by resorcinol assay standardized to sucrose (Huber and Israel, 1982). Starch was extracted from the ethanol-insoluble fraction after overnight in 1 N NaOH and analysed with a spectrophotometer, by anthrone assay standardized to sucrose (Yemm and Willis, 1954). Wet samples, frozen at −80 °C, were powdered with a mortar and resuspended in ice-cold extraction buffer (50 mM Tris at 7.5 pH), sonicated and spun (see Brun et al., 2003a). Supernatants were frozen in liquid $N_2$ and stored for determining the total soluble protein content. Soluble protein concentration was determined according to Bradford (1976) with bovine serum albumin (BSA) as a standard. Protein content was measured in fresh tissues; however, results were transformed to dry weight. Data are expressed on dry weight bases.

### 2.6. Statistical analysis

A two-way ANOVA was used to test the effect of light and biomass partitioning (AG/BG ratio) on growth, fluorescence and metabolic compounds. A one-way ANOVA was used to test the effect of shoot distribution on fluorescence (ETRm). Post-hoc comparisons were assessed by the Tukey HSD test. In all cases, the significance level was set at 0.05. Heteroscedastic data were transformed using a square root or log transformation but when the transformation was not possible the

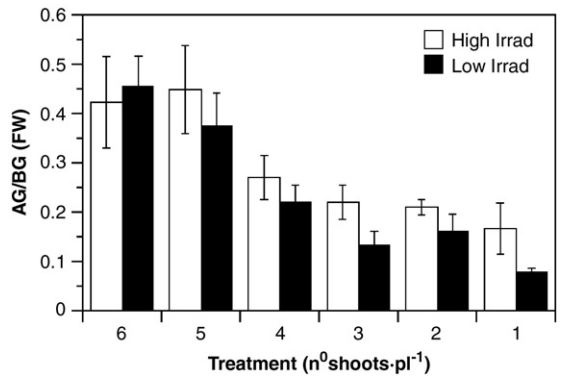


Fig. 2. *Zostera noltii*. Above:below-ground (AG/BG) fresh biomass ratios in EPU. Data represent mean±standard error.

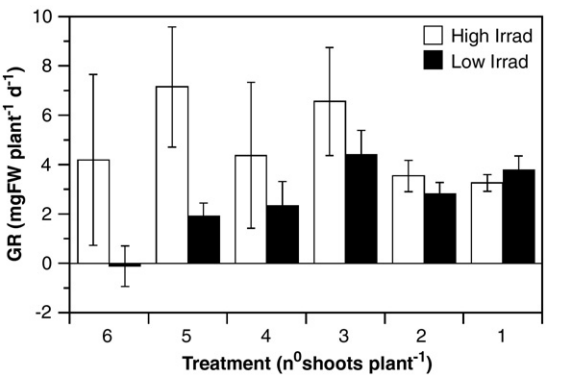


Fig. 3. *Zostera noltii*. Net growth rate (GR) in EPUs. Data represent mean±standard error.

Table 1
Statistical results of the two-way ANOVA analysis, examining the effects of the biomass partitioning and irradiance in *Zostera noltii* dynamic growth parameters (plant net growth rate (GR), shoot appearance rate (SAR), internode appearance rate (IAR) and internode elongation rate (IER) for main (1) and secondary (2) rhizome axes), fluorescence measurements, sucrose, starch and protein content

| Variables | *df* treat / *df* error | *F*(*p*) |
|---|---|---|
| AG/BG (FW) | | |
| AG/BG | 5/39 | 14.29* |
| Light | 1/39 | 3.48 (n.s.) |
| AG/BG × Light | 5/39 | 0.43 (n.s.) |
| GR No Parametric | | |
| AG/BG | 5/39 | 1.24 (n.s.) |
| Light | 1/39 | 6.82* |
| AG/BG × Light | 5/39 | 0.92 (n.s.) |
| SAR | | |
| AG/BG | 5/39 | 4.02* |
| Light | 1/39 | 4.58* |
| AG/BG × Light | 5/39 | 0.55 (n.s.) |
| IAR 1 | | |
| AG/BG | 5/48 | 1.38 (n.s.) |
| Light | 1/48 | 0.99 (n.s.) |
| AG/BG × Light | 5/48 | 0.70 (n.s.) |
| IAR 2 | | |
| AG/BG | 5/48 | 5.78* |
| Light | 1/48 | 7.05* |
| AG/BG × Light | 5/48 | 2.19 (n.s.) |
| IER 1 | | |
| AG/BG | 5/48 | 1.63 (n.s.) |
| Light | 1/48 | 0.14 (n.s.) |
| AG/BG × Light | 5/48 | 1.72 (n.s.) |
| IER 2 Non Param | | |
| AG/BG | 5/48 | 6.11* |
| Light | 1/48 | 0.61 (n.s.) |
| AG/BG × Light | 5/48 | 0.98 (n.s.) |
| ETRm shoot type | | |
| Initial plants | 7/35 | 1.93 (n.s.) |
| High light plants | 9/15 | 0.99 (n.s.) |
| Low light plants | 8/8 | 0.66 (n.s.) |
| Mean ETRm Log transf | | |
| AG/BG | 6/268 | 13.72* |
| Light | 1/268 | 9.19* |
| AG/BG × Light | 6/268 | 1.09 (n.s.) |
| Fv/Fm | | |
| AG/BG | 6/284 | 4.29* |
| Light | 1/284 | 0.20 (n.s.) |
| AG/BG × Light | 6/284 | 0.40 (n.s.) |
| Sucrose AG | | |
| AG/BG | 4/29 | 50.56* |
| Light | 1/29 | 1.11 (n.s.) |
| AG/BG × Light | 4/29 | 1.95 (n.s.) |
| Sucrose BG | | |
| AG/BG | 5/36 | 9.66* |
| Light | 1/36 | 0.001 (n.s.) |
| AG/BG × Light | 5/36 | 0.36 (n.s.) |
| $Sucr_{AG}/Sucr_{BG}$ root transf | | |
| AG/BG | 4/29 | 1.53 (n.s.) |
| Light | 1/29 | 5.57* |
| AG/BG × Light | 4/29 | 1.01 (n.s.) |
| Starch AG Log transf | | |
| AG/BG | 4/30 | 2.21 (n.s.) |
| Light | 1/30 | 3.96 (n.s.) |
| AG/BG × Light | 4/34 | 0.71 (n.s.) |
| Starch BG Non Param | | |
| AG/BG | 6/41 | 1.54 (n.s.) |
| Light | 1/41 | 0.82 (n.s.) |
| AG/BG × Light | 6/41 | 0.50 (n.s.) |
| Protein AG | | |
| AG/BG | 6/30 | 4.05* |
| Light | 1/30 | 0.97 (n.s.) |
| AG/BG × Light | 6/30 | 0.36 (n.s.) |
| Protein BG | | |
| AG/BG | 6/30 | 1.46 (n.s.) |
| Light | 1/30 | 0.28 (n.s.) |
| AG/BG × Light | 6/30 | 1.18 (n.s.) |

A one-way ANOVA analysis was set to examine the effect of shoot position in ETRm values of *Z. noltii* in initial plants and EPUs. Significant level was set at $\alpha = 0.05$ except for non-parametric test where the level was set at 0.10. n.s = no significant differences; (*) $p < 0.05$; *df* = degrees of freedom.

data were analysed using a Kruskal–Wallis test. Results are expressed as mean ± standard error.

## 3. Results

### 3.1. Growth

The AG/BG ratios established in EPUs at the beginning of the culture were maintained up to the end of the experiments (Fig. 2). EPUs grown at saturating irradiances reached higher net growth rates (GR) than those grown at limiting irradiances (Fig. 3). There was no significant trend between GR and biomass partitioning under saturating irradiances. However, GR in EPUs

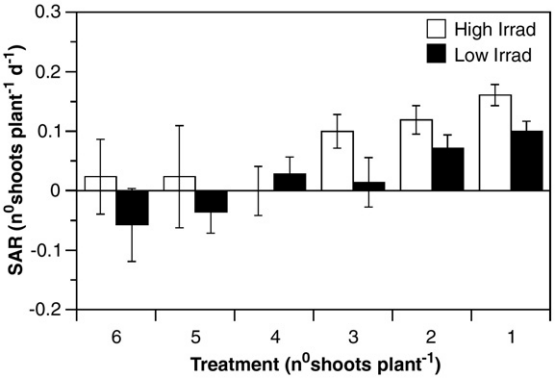


Fig. 4. *Zostera noltii*. Shoot appearance rate (SAR) in EPUs. Data represent mean ± standard error.

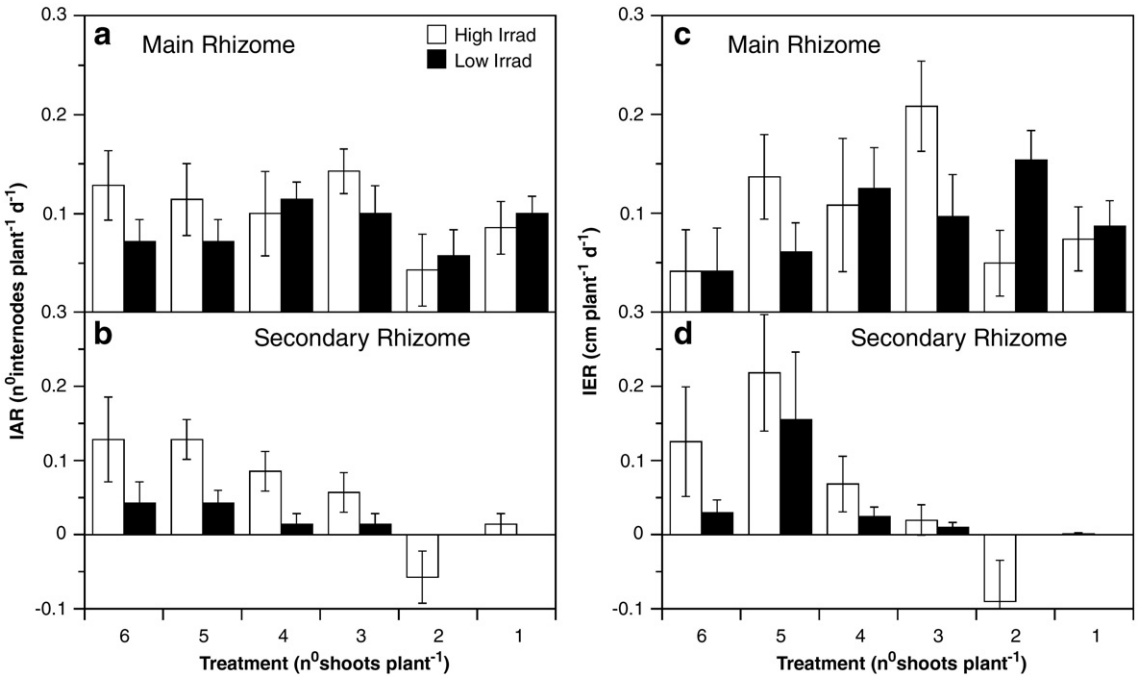


Fig. 5. *Zostera noltii*. Internode appearance rates (IAR) for (a) primary and (b) secondary rhizomes, and internode elongation rates (IER) for (c) primary and (d) secondary rhizomes in EPUs. Data represent mean ± standard error.

cultured at limiting light decreased as the number of shoots per plant increased, especially in plants with 6 shoots that exhibited negative GR values (Fig. 3, Table 1).

Overall, the shoot appearance rate (SAR) was higher in EPUs grown under saturating light than in those grown at limiting irradiances (Fig. 4, Table 1). Regardless of the light growing conditions, SAR was always higher in EPUs with low biomass allocated to above-ground parts (i.e. treatments 1, 2 and 3). In contrast, EPUs with high AG/BG ratios (treatments 5 and 6) grown under limiting light presented net shoot losses (Fig. 4).

Rhizome formation rates (IAR and IER) of the main rhizome were not significantly affected either by light conditions or by biomass partitioning treatments (Fig. 5a and c, Table 1). However, a clear decrease was recorded for IAR and IER in secondary rhizomes (Fig. 5b and d, Table 1) as the biomass allocated in above-ground parts decreased (i.e. from treatment 6 to 1). In addition, IAR and IER values of secondary rhizome axes were generally higher in plants grown at saturating light levels (Fig. 5b and d, Table 1).

### 3.2. Fluorescence

The ETRm obtained from the different shoot types showed no significant differences in relation to the

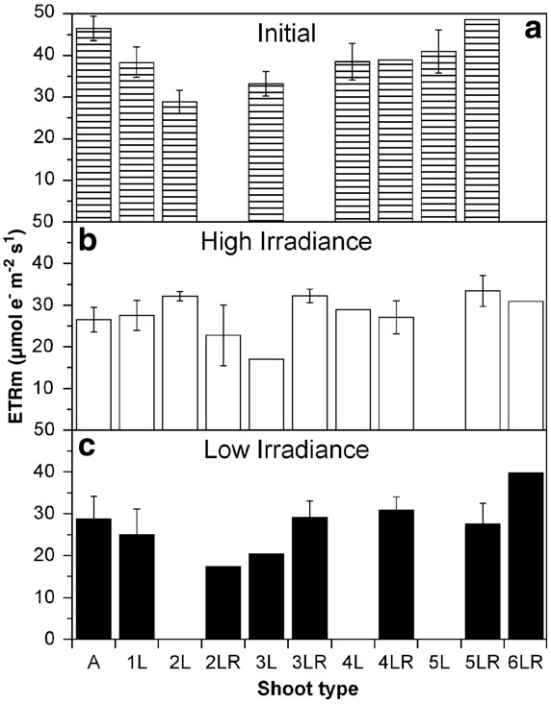


Fig. 6. *Zostera noltii*. ETRm values in the different shoot types. (a) Initial plants, (b) EPUs at high light, and (c) EPUS at low light. The *x*-axis represents the different types of shoots in a plant from apical (A), to first lateral (1L), second lateral (2L) and so on. Branching is indicated as R. Data represent mean ± standard error.

light growing conditions (Fig. 6b and c, Table 1). Nevertheless, ETRm values measured before the experiment (initial plants) decreased from apical (46.5±2.9 μmol $e^-$ $m^{-2}$ $s^{-1}$) to immediate neighbouring shoots (1L, 2L, 3L) (34.2±2.0 μmol $e^-$ $m^{-2}$ $s^{-1}$) and enhanced again in those shoots located far enough from the apical shoot (4L, 4LR, 5L, 5LR) (40.2±2.9 μmol $e^-$ $m^{-2}$ $s^{-1}$) (Fig. 6a).

Mean values of ETRm and Fv/Fm (values obtained when all shoot types were pooled) of initial plants and EPUs as a function of different AG/BG ratios grown at two light conditions are plotted in Fig. 7a and b, respectively. Values of ETRm were slightly lower after the incubations than initial plants (Fig. 7a) and significantly affected by biomass partitioning and light (Table 1). Thus, EPUs grown under saturating light presented higher ETRm values (31.5±1.3 μmol $e^-$ $m^{-2}$ $s^{-1}$) than those cultivated at low irradiance (28.6±1.6 μmol $e^-$ $m^{-2}$ $s^{-1}$). The Fv/Fm values ranged between 0.7–0.8 and were only affected by biomass partitioning (Fig. 7b, Table 1).

### 3.3. Sucrose and starch

Non-structural carbon reserves (sucrose and starch) in above and below-ground parts were quantified at the beginning and at the end of the experiments. In general,

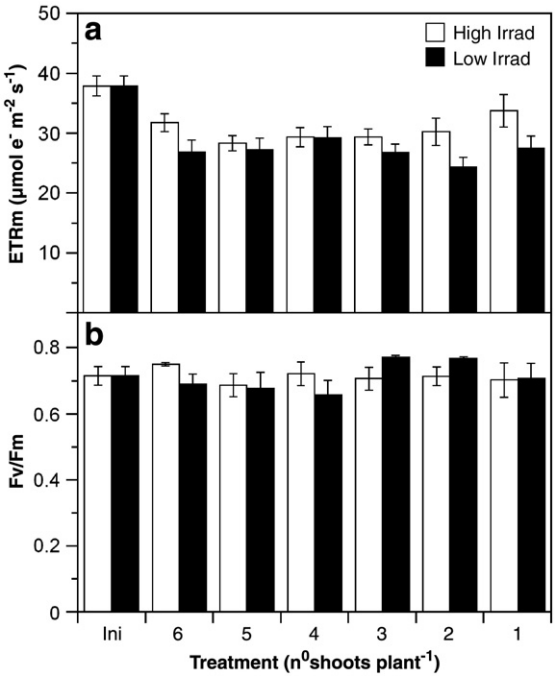


Fig. 7. *Zostera noltii*. ETRm (a) and Fv/Fm (b) mean values from initial plants and from EPUs. Data represent mean±standard error.

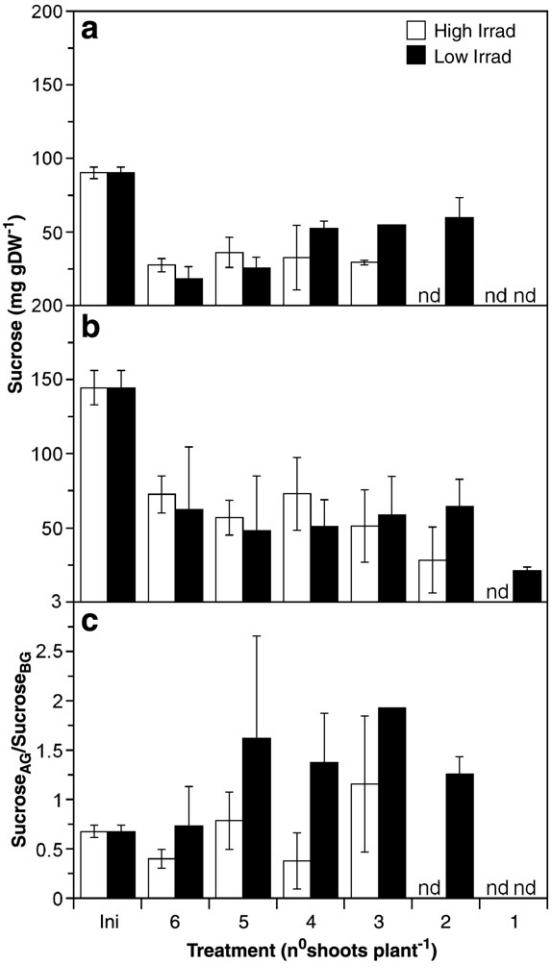


Fig. 8. *Zostera noltii*. Sucrose concentration in (a) above and (b) below-ground tissues from initial plants and from EPUs. (c) Ratio of sucrose content between AG and BG parts. Data represent mean±standard error. The lack of columns (n.d., not determined) is due to the low amount of sample obtained.

sucrose content was higher in below-ground structures than in shoots either at the beginning (90.4±3.8 mg g $DW^{-1}$ for shoots and 144.7±11.7 for below-ground tissues) or at the end of the incubations (49.9±8.3 mg g $DW^{-1}$ for shoots and 67.3±10.4 for below-ground tissues) (Fig. 8a and b). A drop in sucrose content was recorded at the end of the experiment in all the samples (Fig. 8a and b). No clear pattern was observed at the end of the experiment in the sucrose content of below-ground parts depending either on the light conditions or the biomass partitioning (Fig. 8b, Table 1). However, the lowest content in shoots was found in EPUs with the highest AG/BG ratios (i.e. treatments 5 and 6 grown in low irradiance) (Fig. 8a, Table 1). The $sucrose_{AG}$/$sucrose_{BG}$ ratio was higher in limiting than in saturating

light (Fig. 8c, Table 1) but was not significantly affected by biomass partitioning treatments; however, the $sucrose_{AG}/sucrose_{BG}$ ratio seemed to increase as the number of shoots per plant decreased at low light (Fig. 8c). Neither irradiance nor biomass partitioning affected significantly the starch content in above and below-ground parts (5.19±0.57 mg g $DW^{-1}$ for shoots and 3.61±0.23 for below-ground) (Fig. 9a and b, respectively; Table 1) although, in leaves, the starch content in EPUs grown under limiting light was slightly higher than that measured in plants growing under saturating light. Overall, starch content was one order of magnitude lower than sucrose.

### 3.4. Soluble protein

Soluble protein content of above and below-ground parts were quantified at the beginning and at the end of the experiments (Fig. 10a and b). In general, soluble protein concentration was higher in above than in below-ground parts. The leaf protein content in EPUs where none shoot was removed (i.e. treatment "6", control plants) was similar to that of the initial plants. However, an increasing trend in protein content was observed as the proportion of shoots per plant decreased

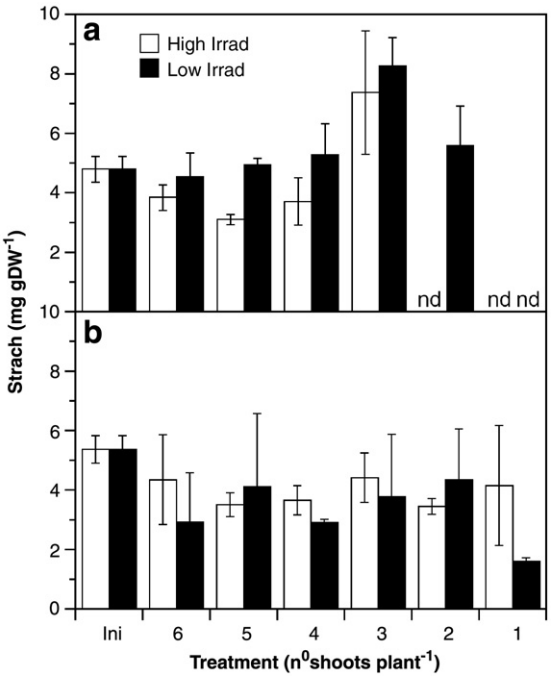


Fig. 9. *Zostera noltii*. Starch, concentration in (a) above and (b) below-ground parts in EPUs. Data represent mean±standard error. The lack of columns (n.d., not determined) is due to the low amount of sample obtained.

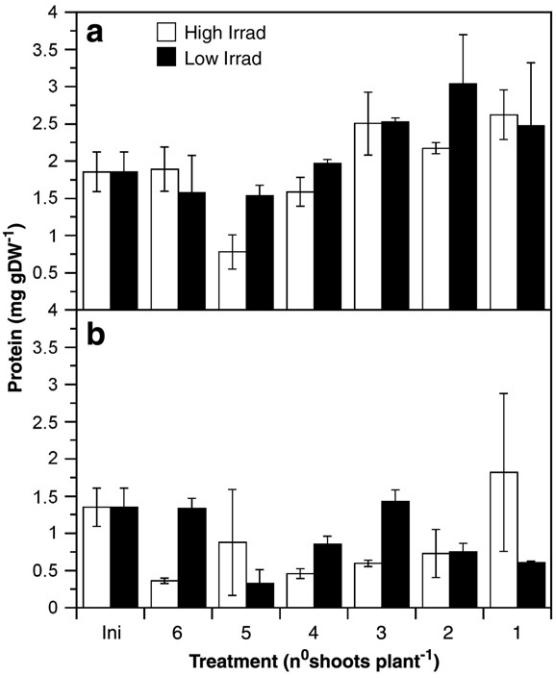


Fig. 10. *Zostera noltii*. Soluble protein concentration in (a) above and (b) below-ground parts in EPUs. Data represent mean±standard error.

(from treatment 5 to treatment 1) regardless of the growing light conditions (Fig. 10a; Table 1). No significant trend was recorded in protein content of below-ground parts in relation to light growing conditions or biomass partitioning (Fig. 10b, Table 1).

## 4. Discussion

Light and biomass partitioning (AG/BG ratios) are two key factors for seagrass viability (Hemminga and Duarte, 2000). Light is one of the main driving force that regulates seagrass survival and fitness (Hemminga and Duarte, 2000); while, AG/BG biomass ratio becomes especially important in stress events, such as low light availability, when the synthesis of photosynthates is limited and stored reserves become important for energy plant requirements (Hemminga, 1998).

Reductions in light levels have been related with drops in growth and production rates in many seagrasses (Hemminga and Duarte, 2000). Particularly, Peralta et al. (2002) determined a reduction in leaf, internode, and root elongation and appearance rates for *Z. noltii* at low light levels. Our results agree with these findings, since the whole plant net growth rate (GR) (Fig. 3), as well as the individual modules growth rates (SAR, IAR, IER) (Figs. 4 and 5), were lower in low light.

Under limiting light conditions plants bearing few shoots (i.e. low AG/BG ratios) had higher growth (GR) and shoot recruitment rates (SAR) than plants with a higher proportion of biomass allocated into above-ground structures (i.e. high AG/BG ratios) (Figs. 2 and 3). This result may be associated with the "free-sharing" of resources among different plant modules (i.e. clonal integration), indicating that active shoots are sinks that deplete the resources previously stored in the below-ground system. Thus, the sharing of stored reserves increased with the number of connected shoots, resulting in reduced growth rates.

The apical shoot was always present in EPUs. Thus, in plants with low AG/BG ratios, the existence of an apical shoot might imply a preferential translocation of resources to feed this highly demanding shoot as it has been previously demonstrated in some seagrass species (Marbà et al., 2002; Malta et al., 2006). Moreover, apical dominance besides affecting the nutrient status of the neighbouring shoots (Phillips, 1975; Marbà et al., 2002) seemed to affect the photosynthetic performance of such shoots (Fig. 6a), resulting in an improvement in the photosynthetic capacity of apical shoot over the neighbouring ones. Thus, ETRm values from shoots of initial plants (Fig. 6a) showed an interesting trend that could suggest a kind effect mediated by apical dominance. That is, values decreased from the apical shoot (A) towards the nearest shoots (1L and 2L) enhancing again as the distance from the apical shoot did increase. In this way, shoots far enough from apical presented again high ETRm values indicating a release of the apical influence or a possible secondary apical dominance process in apical shoots of secondary rhizomes (shoot 5LR in Fig. 6a). Thus, the energy and resources driven to the highly active apical modules are, in certain way, compensated by the photosynthates they produce since apical shoots are the most active photosynthetic tissues.

At saturating irradiances, carbon fixation exceeds leaf respiration typically by a factor around 5 (Hemminga, 1998). However, when below-ground biomass is considered, this factor decreases due to the surplus of respiratory demand that rhizomes and roots represent (Touchette and Burkholder, 2000). Despite the respiratory burden that below-ground biomass represents, rhizomes are the main reservoir of soluble carbohydrates and support growth and maintenance of green tissues during periods of low photosynthetic production (Touchette and Burkholder, 2000). A significant translocation of carbohydrates may occur within seagrass tissues under certain conditions. For instance, in *Zostera marina* and *Z. noltii*, sucrose mobilisation in rhizome–roots system is stimulated in winter and under low light conditions but acropetal translocation is inhibited under prolonged anaerobic conditions (Zimmerman et al., 1995; Alcoverro et al., 1999; Brun et al., 2003a). The carbohydrate content recorded in initial plants was within the range of those reported previously for this species (Brun et al., 2003a). Concentrations of sucrose and starch in initial plants were higher in rhizomes than in leaves confirming the reservoir role of below-ground tissues. At the end of the experiments, there was an overall decrease in sucrose concentration (compared to initial conditions) regardless of the light and AG/BG ratio treatments. Sucrose drop in below-ground tissues (53%) was higher than in leaves (44%). At low light, the sucrose drop was lower in leaves corresponding to low AG/BG biomass treatments compared to those from treatments with higher AG/BG ratios. This fact could indicate that carbohydrate reserves in rhizomes must be reallocated towards the few standing shoots. Accordingly, the $\text{sucrose}_{AG}/\text{sucrose}_{BG}$ ratio tended to increase as AG/BG biomass ratio decreases, especially at low light. In contrast, at high light there was no significant trend in EPUs with different AG/BG ratios. This could be due to the fact that plants at high light can produce more photosynthates and are less dependent of below-ground reserves than EPUs grown at low light.

The argument of resource sharing among different shoots could also be applied for nitrogen (N). Nitrogen can be stored as diverse organic forms such as amino acids and proteins (Udy and Dennison, 1997; Udy et al., 1999). Soluble protein content increased as the AG/BG ratio of plants decreased whereas in non-excised (control) EPUs the leaf protein content remained unaffected. This fact indicates that plant growth was not N-limited along the experiment. In addition, such preferential allocation of N into leaves could favour the synthesis of photosynthetic components (chloroplast membrane complexes, Calvin cycle enzymes...) and, therefore, could result in an enhancement of photosynthesis and growth. However, whereas shoot production was clearly stimulated at low AG/BG biomass ratios, photosynthetic estimators did not show the same tendency among AG/BG biomass treatments (Fig. 7a). Therefore, changes in morphology and metabolic compounds do not have to be accompanied by changes in photosynthetic rates (Alcoverro et al., 1999).

A slight decrease in mean ETRm was observed at the end of the experiment compared to initial conditions (Fig. 7a), especially under low light conditions. It could reflect some sort of acclimation response to cope with the stress suffered by plants under the experimental conditions. Nevertheless, the values of Fv/Fm remained high, ranging between 0.7–0.8, in all treatments at the

end of the experiment, regardless of the biomass allocation and the light treatment, (Fig. 7b) indicating a good physiological status of the photosynthetic apparatus (Enríquez et al., 2002). This fact may be the result of a low (or nearly null) effect of light/biomass treatments or, alternatively, a full acclimation of the photosynthetic apparatus to the new experimental conditions after 14 days (Huner et al., 1998).

In conclusion, the morphological response of *Z. noltii* involved a compensatory mechanism in the building-up and partitioning of plant biomass depending on its AG/BG biomass ratio. That is, the production of photosynthetic tissues (shoots) was stimulated in plants with low AG/BG biomass ratios, whereas heterotrophic tissues (rhizomes and roots) were preferentially produced in plants with larger portion of biomass allocated into shoots. This compensatory mechanism was higher in low light conditions indicating the special importance of biomass partitioning under stressing conditions. Under low light levels, sucrose and protein allocation to apical shoots in plants with low AG/BG biomass ratios suggest a preferential distribution of resources directed to feed the high metabolic demands of apical shoots as well as to stimulate the shoot recruitment. Thus, the acclimatory response of *Z. noltii*, especially at low light conditions, seemed to be modulated by clonal traits, that is, the dominance of apical shoot over nearest ones and clonal integration of resources among different modules or plant parts.

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