

# Effect of shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera noltii*

Fernando G. Brun\*, Juan J. Vergara, Gabriel Navarro, Ignacio Hernández, J. Lucas Pérez-Lloréns

Departamento de Biología, Area de Ecología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain

**ABSTRACT:** The effects of macroalgae blooms on seagrasses were assessed by shading *Zostera noltii* Hornem. with *Ulva rigida* C. Agardh mats under laboratory and field conditions. In the laboratory, where there was no direct contact between *U. rigida* and *Z. noltii*, leaf, rhizome, and root elongation rates, as well as gross production, declined as a function of *U. rigida* layers, causing a mobilization of non-structural carbohydrates in both above- and belowground tissues to meet carbon demands. However, when shading was performed in the field, where direct contact exists between *Z. noltii* and *U. rigida*, *Z. noltii* responses were not proportional to the number of *Ulva* layers. Elongation rates and gross production were reduced by *U. rigida* shading, with the lowest values under 2 *Ulva* layers, while there were no significant differences between controls and 4 *U. rigida* layers. This suggests another *Ulva* effect occurs besides shading. To test the likely effect of dissolved organic carbon (DOC) derived from *U. rigida*, *Z. noltii* plants were cultured under light limitation with radioactive dissolved organic carbon (DO<sup>14</sup>C) released by *U. rigida*. Plants cultured under a full DO<sup>14</sup>C load showed a significant enhancement of growth. The DO<sup>14</sup>C disappeared from the culture medium during the first 4 d of culture as a linear function of external DO<sup>14</sup>C concentration. This was coupled to a linear increase of radioactive particulate organic carbon (PO<sup>14</sup>C) in aboveground tissues, while a substantial part of this PO<sup>14</sup>C was allocated in belowground tissues. Overall, the PO<sup>14</sup>C incorporated in *Z. noltii* plants represented ca. 20 to 25 % of the DO<sup>14</sup>C which had disappeared. Therefore, a net transfer of DO<sup>14</sup>C from *U. rigida* to *Z. noltii* has been documented. Other additional possibilities, such as a light quality effect or other kind of signals (i.e. growth factors), are discussed.

**KEY WORDS:** Eutrophication · Heterotrophic DOC uptake · Dissolved organic carbon · Seagrass · Non-structural carbohydrates · Light reduction

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## INTRODUCTION

In the last decades, a tendency towards increased nutrient loadings from industrial and domestic wastewater, agricultural run-off and other sources (i.e. eutrophication) have been observed in many coastal waters (Schramm & Nienhuis 1996, Cloern 2001). Eutrophication is the most likely cause of seagrass decline worldwide (Cambridge & McComb 1984, Cambridge et al. 1986, Borum & Sand Jensen 1996, Short & Burdick 1996). High concentrations of nitrate

and/or ammonium in the water column can be directly toxic to seagrasses (Burkholder et al. 1992, 1994, van Katwijk et al. 1997, Brun et al. 2002). In addition, seagrass decline following eutrophication also relies on indirect effects, by prompting growth of several groups of marine primary producers (benthic macroalgae, epiphytic macro- and microalgae and phytoplankton) (Hemminga & Duarte 2000, Hauxwell et al. 2001). The outcome (i.e. the dominant group) will largely depend on water residence time in the system. Thus, in shallow estuaries or in semi-enclosed bays with medium to

\*Email: fernando.brun@uca.es

high water turnover rates, macroalgae are likely the main primary producers (Harlin 1995, Valiela et al. 1997). Proliferation of opportunistic macroalgal species (mainly pleustophytic or epiphytic chlorophyta) reduces the light available to seagrasses below them. Since the compensation irradiance for seagrass growth is higher than for most ephemeral macroalgae (Enriquez et al. 1996), such light reduction may result in a negative carbon balance. The capacity to accumulate carbohydrates in the rhizomes allows seagrasses to support brief periods of shading (Peralta et al. 2002, Brun et al. 2003a), but these reserves cannot impede mortality when light reduction occurs over longer time spans (Gordon et al. 1994, Onuf 1996), specially in 'small' seagrasses with a low storage capacity (i.e. *Zostera noltii*).

Most of the studies on the effects of light reduction on seagrass survival and growth use neutral density screens to simulate such light conditions (Philippart 1995, Short et al. 1995, Lee & Dunton 1997, Moore & Wetzel 2000, Peralta et al. 2002). However, the major light-absorbing components (gilvin, tripton, phytoplankton and macroalgae) in the aquatic system are not optically neutral (Kirk 1983), which becomes evident under a dense blanket of ephemeral macroalgae. In fact, thalli of *Ulva* sp. arranged in canopies preferentially absorb the blue and red regions of the light spectrum, resulting in a progressively green-enriched spectrum as light passes through the mats (Vergara et al. 1998). Photosynthesis and growth of seagrasses are probably affected by light quality (i.e. by the ratio between the photosynthetic active radiation and the photosynthetic usable radiation [PAR and PUR, respectively] (Morel et al. 1987), as well as by the red:far red ratio (Tomasko 1992, Rose & Durako 1994, Terrados 1997) as in *Ulva* sp. (López-Figueroa & Niell 1989, López-Figueroa & Rüdiger 1990). Thus, a more realistic approach can be achieved by using 'natural filters' in studying the response of seagrasses to light reduction imposed by overgrowing algae. Additionally, other effects of macroalgae on seagrasses should not be discarded, such as the alteration of turbulent regimes and sediment conditions, excretion of dissolved organic carbon (DOC) or other kind of signals (i.e. hormones) by *Ulva* sp. mats.

In a previous study, the effect of neutral screens on *Zostera noltii* growth and production has been assessed (Peralta et al. 2002). The aim of this work was to study the effect of overgrowing *Ulva rigida* mats on dynamic properties, growth, elemental composition (C, N, P) and non-structural carbohydrates of the seagrass *Z. noltii*. By combining laboratory studies and *in situ* manipulative experiments in different seasons (winter and spring), we also checked the degree of extrapolation of the laboratory experiments to field conditions.

Furthermore, the results derived from experiments suggested that there might be another interaction between *U. rigida* and *Z. noltii* distinct from shading. Thus, the growth and  $^{14}\text{C}$  allocation in *Z. noltii* cultured with labelled  $\text{DO}^{14}\text{C}$  from *U. rigida* has been assessed.

## MATERIALS AND METHODS

**Sampling site and plant collection.** Specimens of the seagrass *Zostera noltii* Hornem. and the chlorophyte *Ulva rigida* C. Agardh, both for laboratory experiments and for *in situ* manipulative studies, were collected from intertidal muddy beds at Los Toruños, a salt marsh ecosystem of 773 ha situated in the Cadiz Bay Natural Park (36° 30' N, 6° 10' W) (see Brun et al. 2003b for further physical and geographical information). The system can be considered as mesotrophic (sensu Nixon 1995), with nutrient concentrations in the sampling site varying broadly on a seasonal and tidal basis. Maximum concentrations are usually found in the winter, with values ( $\mu\text{M}$ ) up to 1.4 ( $\text{NO}_2^-$ ), 12 ( $\text{NO}_3^-$ ), 42 ( $\text{NH}_4^+$ ) and 1.5 ( $\text{PO}_4^{3-}$ ) (González-Gordillo 1999, Tovar et al. 2000). Plants of *Z. noltii* were gathered carefully to keep belowground structures intact, and transported to the laboratory within 15 min of collection at ambient temperature. Upon arrival, and before any experimental setup, *Z. noltii* plants and *U. rigida* blades were rinsed in seawater and visible epiphytes were removed by gently scraping. Plants included in experiments were weighed before transplantation. For laboratory studies, plants were pre-incubated for 2 d in the experimental setup (see below).

**Shading by *Ulva rigida* canopies in the laboratory.** The effect of *U. rigida* canopies on the growth and internal composition (C, N, P and non-structural carbohydrates) of *Zostera noltii* was assessed. For standardization, 5 apical plants (those exhibiting the apical dominance of a large, branched plant) consisting of 1 shoot (4 to 5 leaves) with 2 rhizome internodes, and the corresponding roots, were transplanted in an agared substratum filling a glass crystalliser (Peralta et al. 2000b). Prior to transplantation, shoots were weighted and measured, and marked at 1 cm above the bundle sheath to estimate growth rates using the punching method (Zieman 1974, as modified by Peralta et al. 2000a). The substratum consisted of an agar-solidified root-rhizome layer (2% agar w/v) with natural seawater as the solvent. The crystalliser with the plants was placed inside clear perspex cylinders (8 cm diameter, 900 ml) filled with filtered (Whatman GF/C) natural seawater. Fitted inside the jars, just above the water surface, *U. rigida* discs (8 cm diameter) were arranged in canopies (0, 2, 4 and 8 layers) sandwiched between 2 petri dishes with a layer of seawater, which

was daily replenished. Those thalli damaged by excess light or temperature were replaced. In this experimental design there was no a direct contact between *U. rigida* and *Z. noltii* culture medium. Dark PVC jars were used to prevent light entering from sites other than the top. Light attenuation in each treatment was measured with a flat quantum sensor (LI-COR LI-192 SA) according to Vergara et al. (1998). Incident photon flux ( $I_0$ ) was  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  (control), while the light levels attained under 2, 4 and 8 *U. rigida* layers were 25, 10 and 1%  $I_0$ , respectively. Seawater was gently air-bubbled. Phosphate ( $2 \mu\text{M}$ ) and nitrate ( $8 \mu\text{M}$ ) were added daily and seawater was renewed every 2 d. Nine jars, 3 controls (petri dishes without *U. rigida* mats) and 3 treatments (2, 4 and 8 *U. rigida* layers)  $\times$  2 replicates, were placed into a 40 l aquarium filled with distilled water and connected to a temperature-controlled ( $18^\circ\text{C}$ ) thermostatic bath by a closed circuit. Halogen lamps (Sylvania, HI-SPOT ES 50, 5 cm diameter) individually lit each jar. Light was supplied on a 12:12 h light:dark cycle. After 16 d culture, the plants were harvested for further analyses (see 'Plant analysis' below).

**Manipulative field experiments.** A rather similar approach was designed in the field, although in this case *Ulva rigida* thalli directly covered *Zostera noltii* plants. It allowed us to make a comparison with the results obtained in laboratory. The experiment was carried out twice, in spring and winter, to assess likely seasonal differences. Four apical shoots were marked in the laboratory and placed in the aged substratum (as above). The crystallisers (8) containing the plants were transported to the experimental *Z. noltii* bed ( $150 \text{ m}^2$ ) and fixed on the sediment. This meadow occurs in a shallow intertidal lagoon (30 cm depth at low tide) where drifting *Ulva* sp. mats usually accumulate. The proximity of this site to the lab allowed us to make a daily inspection of the field experimental devices. Each crystalliser was placed into a white plastic cage ( $13 \times 13 \times 10 \text{ cm}$ , 1 cm mesh size), and fixed on the sediment. The top of the cages was covered by a variable number of *U. rigida* blades ( $13 \times 13 \text{ cm}$ ): 0 (control), 2, 4 and 8 layers. To avoid *U. rigida* thalli being lost, cages were covered with a grid of fishing line. The enclosures (8 = 2 replicates  $\times$  4 treatments) were attached to plastic rods, which were sunk into the sediment (Vergara et al. 1998). The light attenuation was similar to that described in the laboratory experiment at 100, 25, 10 and 1%  $I_0$ , although the incident photon flux was higher, with a maximum value at midday in winter of ca. 1000 to  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and 1200 to  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  in spring. After 14 d, plants were removed, transported to the laboratory and measured (see 'Plant analysis' below).

***Zostera noltii* culture with labelled dissolved organic carbon ( $\text{DO}^{14}\text{C}$ ).** Results from the previous experiments suggested a probable direct interaction between *Ulva rigida* and *Z. noltii*, besides the effects caused by light reduction. Thus, a new experiment was designed to test the hypothesis of resource transference from *U. rigida* to *Z. noltii*. Plants of *Z. noltii* were cultivated at different levels of labelled  $\text{DO}^{14}\text{C}$  excreted by *U. rigida*, and under a limited photosynthetic capacity (i.e. applying a light level close to the light compensation point for *Z. noltii*). Net production, external  $\text{DO}^{14}\text{C}$  disappearance and labelled particulate organic carbon ( $\text{PO}^{14}\text{C}$ ) appearance in *Z. noltii* tissues were assessed.

For *Ulva rigida* DOC production, specimens collected at Los Toruños salt marsh were carefully washed in the laboratory and acclimated for 3 d to experimental conditions. *U. rigida* discs of 1.5 cm diameter were cultured at a high density ( $5 \text{ g dry weight [DW] l}^{-1}$ ) in 10 l of filtered (Millipore  $0.22 \mu\text{m}$ ) seawater. To stimulate DOC release by *U. rigida* (Gordillo et al. 2001), a saturating light intensity ( $200$  to  $230 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 18:6 h light:dark photoperiod, with soft bubbling and without nutrient addition, was applied. Following this acclimation period, *U. rigida* specimens were transferred to a new culture medium containing radioactive dissolved inorganic carbon ( $\text{DI}^{14}\text{C}$ ). Before transferring *U. rigida*, seawater was acidified down to pH 5 with HCl (37 %) and bubbled with  $\text{N}_2$  for 1 h to increase the ratio of labelled:non-labelled DIC. Before adding  $\text{DI}^{14}\text{C}$ , the pH was adjusted to pH 9 with NaOH 10 N.  $\text{DI}^{14}\text{C}$  was added to the culture medium as radioactive sodium bicarbonate (NEN, NEC086H) at a final concentration of ca.  $100 \mu\text{Ci l}^{-1}$  ( $222000$  disintegrations  $\text{min}^{-1}$  [ $\text{dpm ml}^{-1}$ ]). DOC production was monitored daily at the beginning and end of the day (Shimatzu TOC-5050 analyser). Once maximum DOC had been measured (the 5th day, see Fig. 7), seawater (10 l) was filtered (Millipore  $0.22 \mu\text{m}$ ), acidified down to pH 3.5 and bubbled with  $\text{N}_2$  for 24 h to remove total DIC ( $\text{DI}^{14}\text{C} + \text{DIC}$ ). Afterwards, pH and DIC were adjusted to typical seawater values (pH 8.3; DIC 2.2 mM).

Five apical plants of *Zostera noltii* were transplanted to each triplicate in an agar-solidified substratum as described above. Plants had been previously weighted and labelled, and epiphytes removed. Treatments were designed by culturing plants of *Z. noltii* in seawater with 3 different initial  $\text{DO}^{14}\text{C}$  concentrations by mixing seawater from *Ulva rigida* culture with filtered seawater (SW) at different ratios ( $\text{DO}^{14}\text{C}:\text{SW}$ ): 1:0; 0.5:0.5; and 0.25:0.75, which corresponded to initial  $\text{DO}^{14}\text{C}$  values of 237, 131 and  $53 \text{ dpm ml}^{-1}$ . These treatments were assumed to be proportionate to the possible DOC production by a different number of *U. rigida* layers (2, 4 and 8). Two additional control treat-

ments were performed: plants cultured in a DO<sup>14</sup>C-free medium, and a complete DO<sup>14</sup>C medium over the agar phase without plants.

The culture was carried out under controlled temperature (20°C) and photoperiod (14:10 h light:dark) at low light (15 to 25  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), close to the light compensation point for this species (Peralta et al. 2000a, 2002). Plants and seawater were sampled every 2 d, and jars were supplemented with 0.2  $\mu\text{M}$  phosphate and 2  $\mu\text{M}$  nitrate to prevent nutrient depletion. Total radioactivity (DIC<sup>14</sup>C and DO<sup>14</sup>C, addition of NaOH 10 N) and DO<sup>14</sup>C (addition of H<sub>3</sub>PO<sub>4</sub>) were measured. The experiment lasted 10 d.

One plant was randomly sampled from each jar, separated into above- and belowground tissues and weighed for the assessment of net production (g fresh weight [FW] plant<sup>-1</sup> d<sup>-1</sup>). Above- and belowground parts were processed for measuring labelled PO<sup>14</sup>C according to Lewis et al. (1982) and Modigh et al. (1998). Tissues were soaked in diluted HCl (0.1 N) to remove adsorbed carbonates, and then into 10 ml of concentrated nitric acid (65%) for 48 h. One ml of the acid extract was diluted with 9 ml Tris buffer (0.75 M), and 1 ml of this solution was diluted in 4 ml of scintillation liquid (Ultima Gold<sup>TM</sup>). Radioactivity was counted in a WALLAC 1410 liquid scintillation counter. Data were expressed as dpm g FW<sup>-1</sup> for tissue samples and as dpm ml<sup>-1</sup> for water samples.

**Plant analysis.** After each experimental procedure, plants were harvested and parts which had appeared after marking were separated from those present at the time of marking to estimate shoot, rhizome and root elongation rates (cm d<sup>-1</sup> plant<sup>-1</sup>) (Brun et al. 2002). Plastochrone interval (time interval between the formation of 2 consecutive leaves from the same meristem) and loss time (time elapsed to lose the oldest leaf) were calculated using the punching method. Leaf losses (those found free-floating in the jars during the experiments) were expressed as cm d<sup>-1</sup> plant<sup>-1</sup>. Any new tissue produced (leaves, rhizomes or roots) was dried at 60°C during 24 h to estimate DW. Production was expressed as mg DW plant<sup>-1</sup> d<sup>-1</sup>. The tissue nutrient concentration was measured in pooled samples of ground dry tissue (above- and belowground parts). Internal P concentration was quantified by the persulphate digestion method (APHA 1992). Other tissue subsamples were used to determine C and N concentration using a CHN elemental analyser (Perkin-Elmer 240 C). 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 (Hubber & 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 & Willis 1954).

**Statistical analysis.** Initially, differences between treatments were tested by a hierarchical (nested) ANOVA. The levels of variability were the jars for each treatment (n = 2 to 3) and the plants within each jar (n = 5). However, because no significant differences in the experimental units (jars) were found, the factor 'jar' was discarded in further analysis and 1-way ANOVA was applied, using the pooled data from every jar in the new ANOVA test (Zar 1984, Underwood 1997). Previously, to the use of pooled data, we followed the post-hoc recommendation of Underwood (1997) to reduce the risk of making a Type I or II error. This procedure increased the power of the ANOVA test because of the larger number of degrees of freedom associated with the pooled mean-square (Underwood 1997). Normality of data and homogeneity of variances were previously tested (Zar 1984), and data were log-transformed when necessary and tested again for normality and homogeneity of variances. Multiple post-hoc comparisons between means were assessed by the Tukey procedure (Zar 1984). The relationship between DO<sup>14</sup>C disappearance rate and initial DO<sup>14</sup>C concentration was checked by means of a linear regression, tested by ANOVA. In all cases, the significance level was set at 5% probability.

## RESULTS

### Laboratory experiment

Elongation rates (leaf, rhizome and root), leaf loss rate and total plant production were significantly affected by light availability (Fig. 1, Table 1). All plants showed positive leaf elongation rates, but increased numbers of overlying *Ulva rigida* layers resulted in decreased leaf elongation rates and enhancement of leaf losses. Thus, when leaf net changes (i.e. gains minus losses) were considered, only control plants showed positive growth (0.15 cm plant<sup>-1</sup> d<sup>-1</sup>). The plastochrone interval ranged between 6 (control) and 10.8 d (8 layers), but no significant differences were detected (data not shown; Table 1). Rhizome and root elongation rates also diminished with shading. No root elongation was recorded in plants grown under 4 and 8 *U. rigida* layers. Control plants exhibited the highest gross production, with leaves, rhizomes and roots accounting for 48, 34 and 18% of total production, respectively. In contrast, the lowest value was recorded in plants underneath 8 *U. rigida* blades, with leaves and rhizomes accounting for 69 and 31%, respectively.

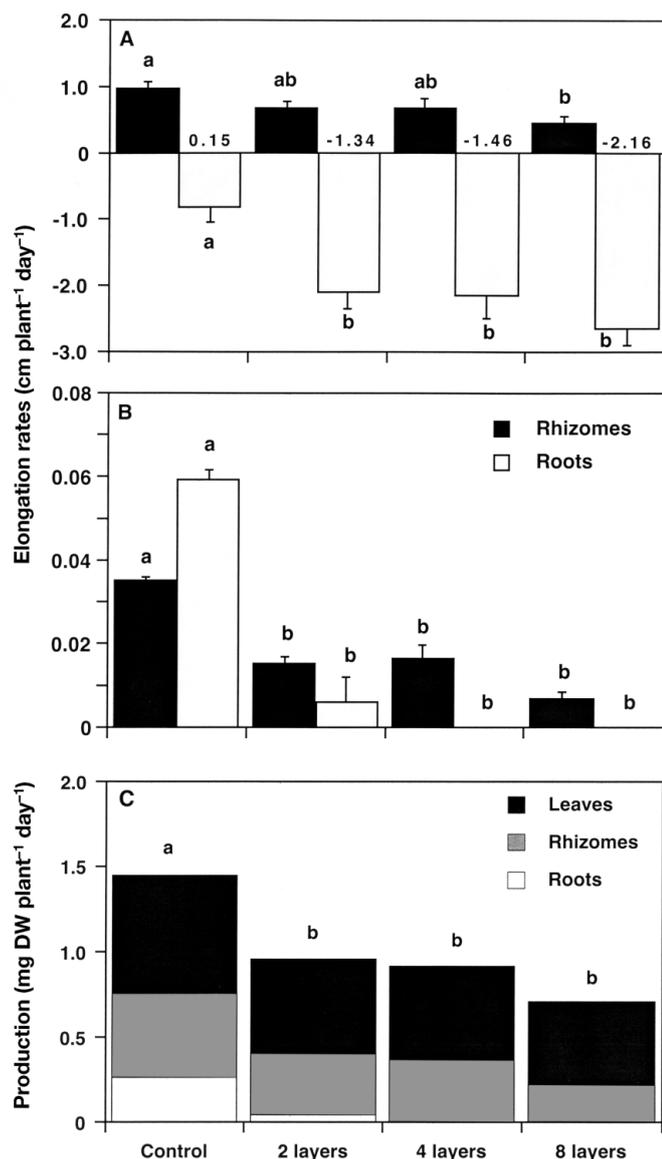


Fig. 1. *Zostera noltii*. Effects of *Ulva rigida* shading on (A) leaf elongation and loss rates, (B) rhizome and root elongation rates, and (C) gross production in the laboratory. Filled bars in (A) represent gross elongation rates, and empty bars represent leaf loss rates. Numbers within the graph denote net elongation rates. Different letters on bars indicate significant differences ( $p < 0.05$ ) among means (mean  $\pm$  1 SE;  $n = 10$  to 15)

Nutrient content (C, N and P) and atomic ratios (C:N and N:P) were little affected by light, exhibiting values above the critical levels stated for N (1.8%) and P (0.2%) in leaves and belowground tissues (Duarte 1990) (Table 2).

Sucrose content in the control and in plants under 2 *Ulva rigida* layers was similar to that in the initial plants, and was ca. 5 times higher in belowground parts than in

Table 1. *Zostera noltii*. Statistical results of 1-way ANOVA, examining the effects of light reduction by *Ulva rigida* canopies and labelled dissolved organic carbon (DO<sup>14</sup>C) uptake in the variables measured. ns = no significant differences; \* $p < 0.05$ ; \*\* $p < 0.001$ ; \*\*\* $p < 0.0001$

Variable	df treatments/ df error	F (p)
<b>Leaf elongation rate</b>		
Laboratory	3/36	4.1(*)
Spring (field)	3/28	3.1(*)
Winter (field)	3/28	5.2(*)
<b>Leaf loss rate</b>		
Laboratory	3/36	8.6(**)
Spring (field)	3/28	5.2(*)
Winter (field)	3/28	1.6(ns)
<b>Rhizome elongation rate</b>		
Laboratory	3/36	13.1(***)
Spring (field)	3/28	3.5(*)
<b>Root elongation rate</b>		
Laboratory	3/36	5.5(*)
Spring (field)	3/28	3.1(*)
<b>Leaf production</b>		
Laboratory	3/36	3.27(*)
Spring (field)	3/28	3.1(*)
<b>Rhizome production</b>		
Laboratory	3/36	10.9(***)
Spring (field)	3/28	3.5(*)
<b>Root production</b>		
Laboratory	3/36	22.8(***)
Spring (field)	3/28	3.1(*)
<b>Total production</b>		
Laboratory	3/36	20.7(***)
Spring (field)	3/28	4.3(*)
<b>Plastochrone index</b>		
Laboratory	3/36	2.8(ns)
Spring (field)	3/28	2.9(ns)
<b>Leaf loss time</b>		
Laboratory	3/36	2.3(ns)
Spring (field)	3/28	4.1(*)
<b>Net growth (DO<sup>14</sup>C uptake)</b>		
Laboratory	3/56	3.1(*)
<b>Mean aboveground radioactivity</b>		
Laboratory	3/56	39.5(***)
<b>Mean belowground radioactivity</b>		
Laboratory	3/56	3.7(*)

aboveground ones (Table 2). Under increased shading (4 and 8 *Ulva* layers), there was a sharp reduction in the rhizome roots' sucrose content, and a slight increase in that of shoots, resulting in similar levels in both parts. This acute sucrose drop in belowground tissues was not paralleled in C content, decreasing the sucrose:C ratio (Table 2). Starch represented a low proportion (1:10) of non-structural carbohydrates. Aboveground parts exhibited a similar starch content in control plants as in plants sampled in the field (Table 2). However, there was a strong reduction in belowground tissues (about 60%). Increased shading resulted in a further decline of starch content in shoots and belowground parts.

Table 2. *Zostera noltii* under *Ulva rigida* canopies. Biochemical changes of *Z. noltii* in carbon, nitrogen, phosphorus, non-structural carbohydrate and sucrose (suc):total carbon ratio under laboratory and field experiments. Layers: number of *U. rigida* layers in the canopy. DW: dry weight

	Sucrose (mg g <sup>-1</sup> DW)	Starch (mg g <sup>-1</sup> DW)	C:N (%)	N:P (%)	Suc:C (%)
<b>Laboratory trial</b>					
<b>Aboveground</b>					
Initial	41.93	7.03	15.8	13.8	4.99
Control	45.51	5.85	16.8	12.7	5.92
2 layers	45.76	3.97	15.7	12.1	6.43
4 layers	46.13	2.52	14.8	13.3	6.77
8 layers	51.72	2.43	15.9	16.3	7.21
<b>Belowground</b>					
Initial	167.2	12.70	41.9	5.99	21.9
Control	145.4	8.09	29.2	7.52	19.9
2 layers	158.1	4.65	22.8	10.1	24.4
4 layers	46.13	2.96	26.1	10.0	6.91
8 layers	51.71	2.15	28.9	10.2	7.64
<b>Spring field trial</b>					
<b>Aboveground</b>					
Initial	52.1	6.21	14.2	12.0	7.02
Control	58.3	4.83	14.5	23.0	8.43
2 layers	23.4	4.21	11.3	17.5	3.42
4 layers	35.1	4.56	11.4	14.8	5.18
8 layers	24.2	3.62	11.9	16.9	3.44
<b>Belowground</b>					
Initial	128	11.5	22.7	8.3	19.4
Control	49.3	8.75	16.8	10.3	7.67
2 layers	8.12	5.13	12.8	12.3	1.38
4 layers	23.4	9.24	14.0	9.65	4.0
8 layers	7.73	6.41	14.1	10.4	1.25
<b>Winter field trial</b>					
<b>Aboveground</b>					
Initial	105	6.14	11.9	23.4	12.8
Control	63.1	8.72	13.6	17.5	7.07
2 layers	26.6	6.97	13.6	21.0	2.89
4 layers	28.4	8.12	14.5	18.1	3.03
8 layers	14.3	5.21	14.7	18.3	1.58
<b>Belowground</b>					
Initial	93.3	14.1	15.9	20.9	13.2
Control	94.2	12.8	16.2	24.4	12.9
2 layers	52.3	7.24	22.1	16.5	6.7
4 layers	35.7	8.38	19.6	10.9	4.7
8 layers	21.4	7.72	20.4	12.4	2.7

### Field experiments

#### Spring

Light availability significantly affected leaf, rhizome and root elongation rates, loss rate and gross production (Table 1), but the response differed both quantitatively and qualitatively to that found in the laboratory (Fig. 2). Only plants grown under 2 *Ulva rigida* layers exhibited significantly lower elongation rates and higher loss rates than control plants. Under such conditions, leaf losses exceeded leaf gains, resulting in a negative growth rate ( $-1.82 \text{ cm plant}^{-1} \text{ d}^{-1}$ ; Fig. 2A).

With the exception of this treatment, net growth rate of shoots declined (but not significantly) from control to plants grown under 8 *U. rigida* layers. The plastochrone interval ranged between 3.3 (control) and 7.8 d (8 layers), but no significant differences were observed (data not shown, Table 1). Treatments affected rhizome and root elongation rates, with a significant reduction under 2 and 4 *U. rigida* layers with respect to control plants, but not beneath 8 *U. rigida* layers (Fig. 2B). Gross plant production paralleled leaf elongation rates. There were no significant differences between control plants and those grown under 4 *U. rigida* layers, reaching the lowest gross plant production under 2 *U. rigida* layers (Fig. 2C).

As in the laboratory experiment, nutrient (C, N and P) contents were scarcely affected by *Ulva rigida* shading (Table 2). The sucrose content in belowground tissues of initial plants was higher than in shoots, although differences were much lower than in the laboratory trial. The sucrose content decreased drastically in belowground parts of the control plants, reaching similar values to shoot sucrose content (Table 2). This decreased further in the above- and belowground parts of plants shaded by *U. rigida*, reaching minimum values under 2 and 8 *U. rigida* layers. Although starch represented a low ratio of non-structural carbohydrates, there was a slight decrease of shoot starch content with increasing shading. The lowest starch content in rhizomes' roots was mainly detected in plants grown under 2 *U. rigida* layers (Table 2). This pattern for

sucrose and starch in belowground parts paralleled the response of leaf elongation rates and total production to *U. rigida* shading.

#### Winter

The response to light treatments is only reported for shoots, since the experimental period was not long enough to give accurate estimates of rhizome and root elongation rates. Shoot elongation rates and leaf losses showed the same pattern as in spring (Fig. 3). Minimum elongation rates and maximum loss rates were

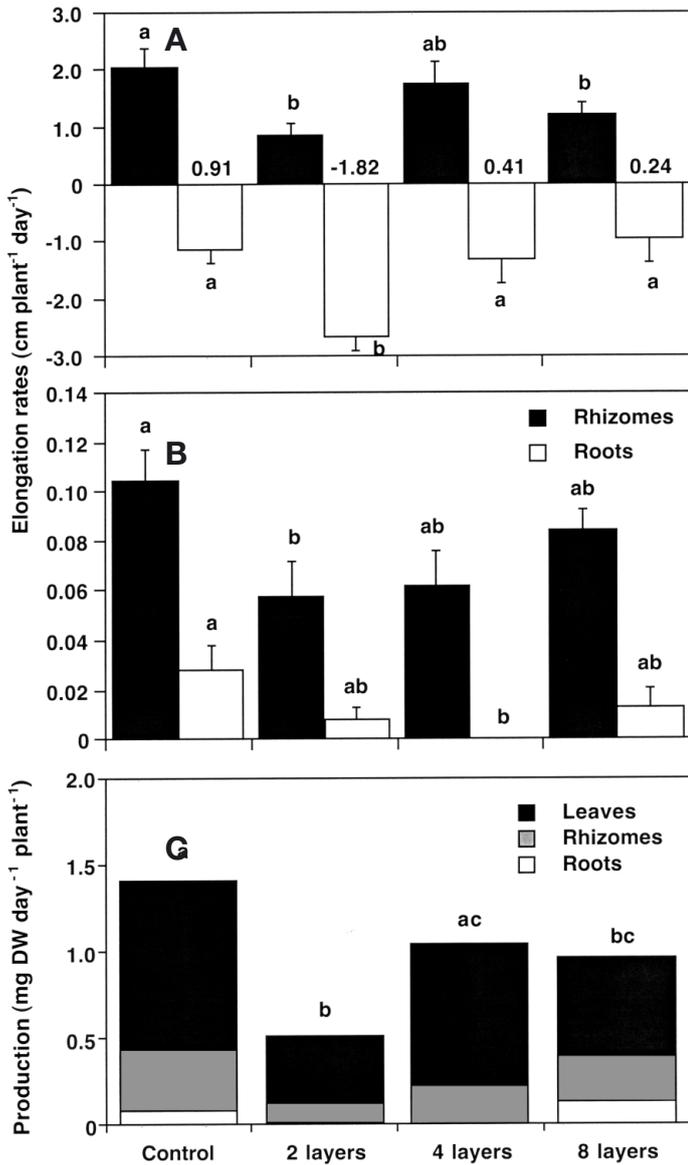


Fig. 2. *Zostera noltii*. Effects of *Ulva rigida* shading on (A) leaf elongation and loss rates, (B) rhizome and root elongation rates, and (C) gross production in the spring trial. Filled bars in (A) represent gross elongation rates and empty bars leaf loss rates. Numbers within the graph denote net elongation rates. Different letters on bars indicate significant differences ( $p < 0.05$ ) among means (mean  $\pm$  1 SE;  $n = 8$ )

measured under 2 *Ulva rigida* layers. In contrast, there were no differences in the rest of the shading treatments when compared to the control.

Non-structural carbohydrates (sucrose and starch) showed a similar pattern to that observed in the laboratory and in the spring field experiment (Table 2). In this case, sucrose content was similar in above- and below-ground parts for initial plants, and there was a slight re-

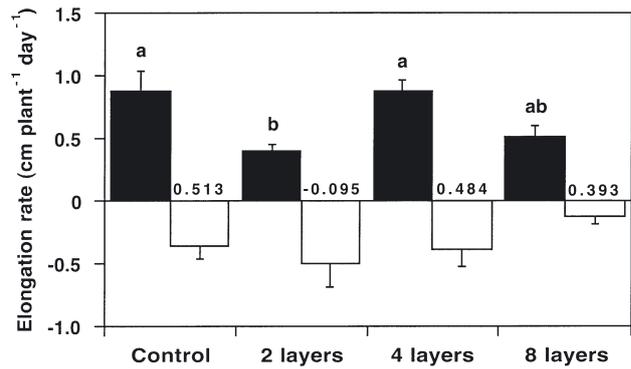


Fig. 3. *Zostera noltii*. Effects of *Ulva rigida* shading on leaf elongation and loss rates in the winter trial. Filled bars represent gross elongation rates and empty bars leaf loss rates. Numbers within the graph denote net elongation rates. Different letters on bars indicate significant differences ( $p < 0.05$ ) among means (mean  $\pm$  1 SE;  $n = 8$ )

duction in aboveground parts for the controls (Table 2). *Ulva rigida* shading caused sucrose to decline in both above- and belowground parts. The starch content was similar in initial and control plants, while shading caused a reduction in the belowground parts (Table 2).

### DO<sup>14</sup>C incorporation experiment

*Ulva rigida* was cultured with labelled DI<sup>14</sup>C for 5 d under experimental conditions that stimulated DOC release. The DOC concentration increased with time to values of ca. 60 mg l<sup>-1</sup>. In addition, a diel pattern was observed, with minima at the beginning of the light period and maxima at the end of the day (Fig. 4).

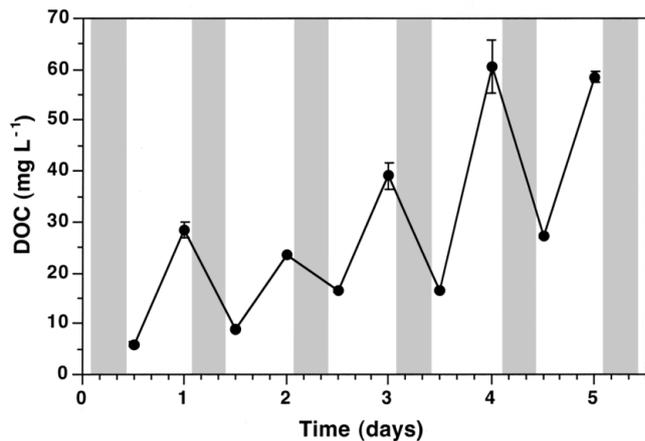


Fig. 4. Time-course evolution of stimulated dissolved organic carbon (DOC) release during *Ulva rigida* culture with labelled dissolved inorganic carbon (DI<sup>14</sup>C). Shaded bars represent the dark period of culture (mean  $\pm$  1 SE;  $n = 4$ )

Seawater enriched with DOC (and with  $\text{DO}^{14}\text{C}$ ) from the *Ulva rigida* culture, where  $\text{DI}^{14}\text{C}$  was eliminated by acidification, was used to grow *Zostera noltii* under  $\text{DO}^{14}\text{C}$  loads resembling those caused by a variable number of *U. rigida* layers. Net growth rate of *Z. noltii* was affected by DOC load, being highest under a full DOC load (Fig. 5), suggesting that DOC affects plant production.

Fig. 6A represents  $\text{DO}^{14}\text{C}$  disappearance with time from the external medium of the cultures (0 to 10 d). The  $\text{DO}^{14}\text{C}$  concentration decreased only slightly in the control treatment (full load without plants), while there was a significant decrease in  $\text{DO}^{14}\text{C}$  concentration in all other treatments. This decrease was nearly linear during the first 4 d. The  $\text{DI}^{14}\text{C}$  levels measured, coming presumably from  $\text{DO}^{14}\text{C}$  bacterial degradation and/or *Zostera noltii* respiration, were quite low in comparison with  $\text{DO}^{14}\text{C}$  concentration (a maximum mean value for  $\text{DI}^{14}\text{C}$  of 17 dpm  $\text{ml}^{-1}$  in the control without plants, and a minimum of 2 dpm  $\text{ml}^{-1}$  in the highest  $\text{DO}^{14}\text{C}$  dilution assayed (data not shown).

Part of the disappeared  $\text{DO}^{14}\text{C}$  was recovered as  $\text{PO}^{14}\text{C}$  in the plants (Fig. 6B,C).  $\text{PO}^{14}\text{C}$  accumulated in the aboveground parts during the first 4 d, and the amount depended on the  $\text{DO}^{14}\text{C}$  loads. This accumulation was fairly well maintained along the experiment (Fig. 6B).  $\text{PO}^{14}\text{C}$  also accumulated in belowground parts during the first period (4 d). However, there were no significant differences among the  $\text{DO}^{14}\text{C}$  treatments (Fig. 6C). In both parts, control plants only exhibited a baseline level for  $\text{PO}^{14}\text{C}$ .

The  $\text{DO}^{14}\text{C}$  disappearance rate in seawater and the  $\text{PO}^{14}\text{C}$  appearance rate in biomass were computed from data of Fig. 6 during the linear phase (0 to 4 d),

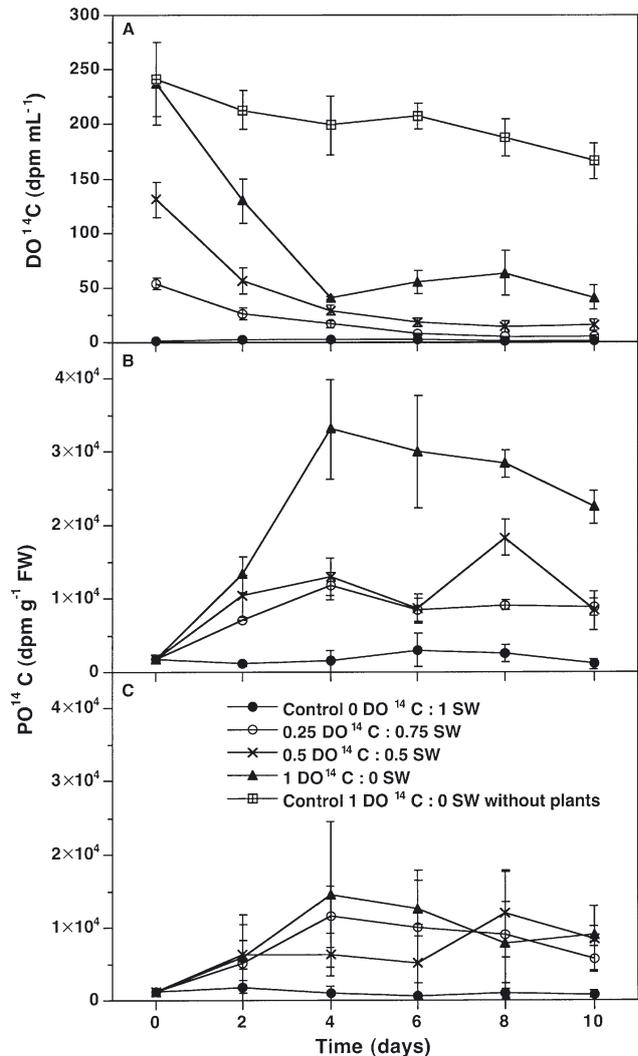


Fig. 6. *Zostera noltii*. (A) Changes in the concentration of labelled dissolved organic carbon ( $\text{DO}^{14}\text{C}$ ) over time, and appearance of radioactive particulate organic carbon ( $\text{PO}^{14}\text{C}$ ) in (B) above- and (C) belowground tissues. Data are means of 3 separate samples  $\pm$  1 SE. SW: seawater; FW: fresh weight; dpm: disintegrations  $\text{min}^{-1}$

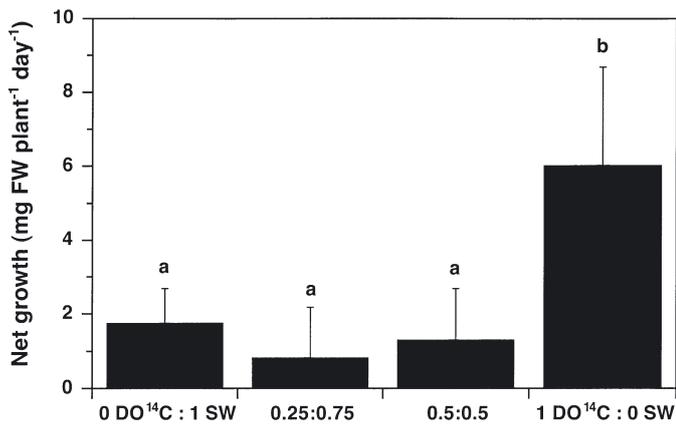


Fig. 5. *Zostera noltii*. Effect of the labelled dissolved organic carbon ( $\text{DO}^{14}\text{C}$ ) load on net growth. Different letters on bars indicate significant differences ( $p < 0.05$ ) among means. Data are means of 15 separate plants  $\pm$  1 SE. FW: fresh weight; SW: seawater

and are represented in Fig. 7A. The  $\text{DO}^{14}\text{C}$  disappearance rate was a linear function of the initial  $\text{DO}^{14}\text{C}$  concentration ( $r^2 = 0.95$ ,  $p < 0.01$ ), while the  $\text{PO}^{14}\text{C}$  appearance rate in the seagrass tissues represented ca. 20 to 25% of the  $\text{DO}^{14}\text{C}$  disappeared from the water (please note different axis units in Fig. 7). While aboveground parts were affected by initial  $\text{DO}^{14}\text{C}$  loads, there was an accumulation of  $\text{PO}^{14}\text{C}$  in belowground parts irrespective of the  $\text{DO}^{14}\text{C}$  load. Fig. 7B represents the mean  $\text{PO}^{14}\text{C}$  content of plants submitted to different initial  $\text{DO}^{14}\text{C}$  loads along the experiment. While there was an increase of  $\text{PO}^{14}\text{C}$  in aboveground tissues, belowground parts showed a

PO<sup>14</sup>C accumulation that was not affected by external DO<sup>14</sup>C concentration. Both above- and belowground parts showed higher PO<sup>14</sup>C levels than the control plants, considered as a baseline level.

## DISCUSSION

Nowadays, eutrophication has arisen as one of the most important human-induced events affecting seagrass meadows (Short & Wyllie-Echevarria 1996, Valiela et al. 1997, Hemminga & Duarte 2000).

One of the known effects is the overgrowing of *Ulva* mats over seagrasses causing light reduction, which was assessed in this laboratory experiment. Light reduction caused decreased leaf, rhizome and root elongation rates, followed by diminished production. This phenomenon has been widely recorded in previous studies, independently of the nature of the screen used (Philippart 1995, Short & Burdick 1995, Longstaff & Denninson 1999, Longstaff et al. 1999, Moore & Wetzel 2000, Havens et al. 2001, Nelson & Lee 2001, Ruíz & Romero 2001, Peralta et al. 2002, Brun et al. 2003a). Non-structural carbohydrates were mobilized, both in above- and belowground tissues, to meet carbon demands under light limitation (Alcoverro et al. 1999, Ruíz & Romero 2001, Peralta et al. 2002, Brun et al. 2003a,b), with the capacity of sucrose formation and exportation restricted in aboveground tissues, and sink strength stimulated in belowground tissues, under low light (Brun et al. 2003a).

However, the response to *Ulva rigida* shading in the field, where a direct contact existed between *Zostera noltii* plants and *U. rigida* canopies, differed to that found in the laboratory. Hence, *U. rigida* canopies affected elongation rates and production by *Z. noltii*, but the response was not proportional to shading. As an example, growth and production under 4 *U. rigida* layers was higher than under 2 *Ulva* layers (Figs. 2 & 3). This unexpected result indicated that there might be (an)other *U. rigida* effect(s) on *Z. noltii*.

One possibility is a light quality effect. Light passing through *Ulva rigida* canopies is not optically neutral; it is green- (Vergara et al. 1997) and far red-enriched, as the red:far red (R:FR) ratio decreases through *U. rigida* canopies (Salles et al. 1996). Phytochrome responses have been reported in the seagrass *Halodule wrightii* (Tomasko 1992), and usually in land plants. The main

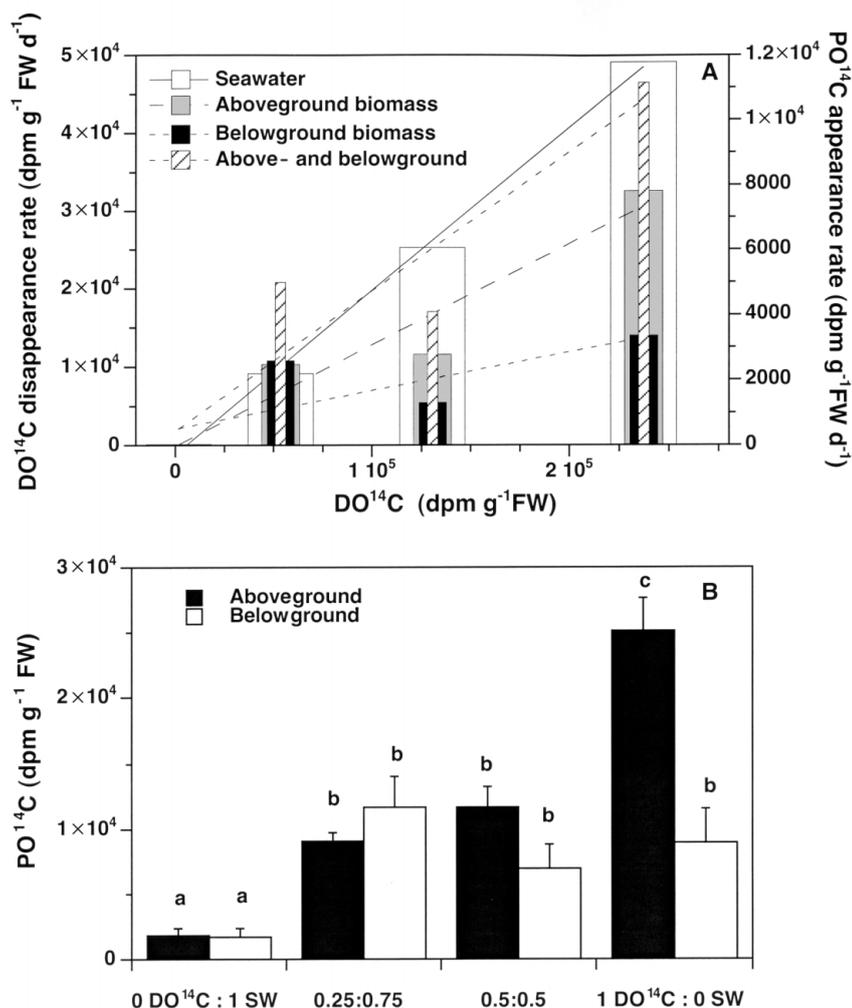


Fig. 7. *Zostera noltii*. (A) Rate of labelled dissolved organic carbon (DO<sup>14</sup>C) disappearance in seawater and labelled particulate organic carbon (PO<sup>14</sup>C) appearance in tissues, during the linear uptake phase (0 to 4 d). (B) Mean total PO<sup>14</sup>C in tissues over time. Different letters on bars indicate significant differences ( $p < 0.05$ ) among means. Data are means of 15 separate plants  $\pm$  1 SE. SW: seawater; FW: fresh weight; dpm: disintegrations min<sup>-1</sup>

morphological features described to change in plants subjected to phytochrome action are the lengths of the different modules (rhizomes, leaves and tillers; Weller et al. 1994, Schmitt et al. 1995). Our results, however, show that internode and leaf lengths remained unchanged or diminished (data not shown), making phytochrome effects unlikely. This lack of a phytochrome effect could also be due to restricted light levels for photosynthesis under *U. rigida* canopies, restricting *Zostera noltii* growth, in addition to the green-enriched light spectrum that may affect photosynthesis capacity, as light is less usable for photochemical reactions (Vergara et al. 1997). Furthermore, this green filter could also act in the laboratory and in the field in a different way, as halogen lamps do not

have the same spectral composition (poorer in blue wavelengths) than natural sunlight.

A second explanation involves some kind of resource/signal interaction between *Zostera noltii* and *Ulva rigida* canopies. Macroalgae can release a variable amount of their daily fixed carbon as DOC (Khailov & Burlakova 1969, Otsuki & Wetzel 1974, Pregnall 1983). This DOC is usually composed of low molecular weight molecules like carbohydrates, organic nitrogen, polyphenols and halocarbons (Sieburth 1969, Wetzel & Penhale 1979, Nightingale et al. 1995), which can be used as a source for plant growth. On the other hand, some bacteria associated to macroalgae can produce extracellular auxins and cytokinins that could affect marine vegetation (Maruyama et al. 1989, Koch & Durako 1991, Terrados 1995).

The culture of *Zostera noltii* under different levels of DOC released from *Ulva rigida* indicated a significant effect on growth, at a limited light level for photosynthesis (close to the light compensation point for this species). Besides enhanced growth, the  $\text{DO}^{14}\text{C}$  that disappeared from external medium accumulated in aboveground tissues as a function of DOC loads and a fraction of  $\text{PO}^{14}\text{C}$  allocated to belowground tissues.

A bi-directional transfer of DOC between epiphytes and seagrass leaves has been reported in *Phyllospadix scouleri* and *Zostera marina* (Harlin 1973, Smith & Penhale 1980). In both studies, the quantitative relevance of DOC uptake was minimized in comparison to the carbon fixed by photosynthesis. However, the data presented here show that the addition of DOC released from *Ulva rigida* increases net growth, as a surplus of carbon, for *Z. noltii* maintenance under low light conditions. In this sense, *Z. marina* production was unaffected under 2 cm of macroalgal canopy height after 3 mo (Havens et al. 2001). More insights in the possible uptake of DOC by seagrasses come from axenic cultures of *Thalassia testudinum* (Durako 1988) and *Ruppia maritima* (Rose & Durako 1994), where the reduction in root production in an axenic enriched sucrose medium was explained as a possible nutrient sufficiency with a reduced allocation to nutrient-absorptive root tissues.

The rates of DOC uptake found in this experiment were comparable to those found for *Zostera marina* (range of 24 to 48%). Moreover, a linear kinetic uptake was described for *Z. marina* (Smith & Penhale 1980), which agrees fairly well with the linear DOC uptake recorded in this experiment. A part of the existing  $\text{DO}^{14}\text{C}$  in the medium can be recycled as  $^{14}\text{CO}_2$  or as  $\text{DO}^{14}\text{C}$  release from *Z. noltii* plants, bacteria and epiphytes, since culture was not axenic, and the addition of DOC may have stimulated bacterial and heterotrophic epiphyte growth (Penhale & Smith

1977, Neckles et al. 1994). However  $\text{DI}^{14}\text{C}$  levels were low throughout the experiment. This process (recycled  $^{14}\text{CO}_2$ ) only would affect the late stage of the culture.

The data presented here should be extrapolated to field populations with caution. The normal DOC concentration in coastal waters is typically  $<5 \text{ mg l}^{-1}$  (Sharp et al. 1993), while under field and experimental bloom conditions the values recorded are habitually  $<20 \text{ mg l}^{-1}$  (Packard et al. 2000). Higher values ( $>300 \text{ mg l}^{-1}$ ) of DOC than that used in this experiment ( $60 \text{ mg l}^{-1}$ ) have been recorded in laboratory experiments of macroalgae degradation (Alber & Valiela 1994). Therefore, the heterotrophic growth of seagrasses can be a punctual phenomenon in locations where there is a high macroalgae accumulation and a high water residence time, allowing DOC to accumulate and to be used by seagrasses.

In conclusion, as a general trait, *Zostera noltii* decreased its productivity and elongation rates when subjected to overlying *Ulva rigida* mats, but not in a proportional way. Non-structural carbohydrates were mobilized both in above- and belowground tissues to cope with carbon demands. An uptake of DOC released by *U. rigida* was found, allowing positive elongation rates and maintenance production in *Z. noltii* under low light conditions. Other *U. rigida* effects, such as light quality (i.e. phytochrome responses), are unlikely to have occurred in this experiment, and other signals (hormones, growth factors) are not discarded and need further experimental research.

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