Is the vegetative development of the invasive chlorophycean, *Caulerpa taxifolia*, favored in sediments with a high content of organic matter?

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Abstract

We performed an *in situ* experiment raising the concentration of organic matter in sediment relative to the natural concentration, and quantified biomass development of the invasive alga, *Caulerpa taxifolia*, and native *C. prolifera*. Our objective was to determine whether vegetative development of *C. taxifolia* is favored in sediments with a high content of organic matter. The number of stolon apices and fronds, and the total dry weight of *C. taxifolia* at the end of the experiment increased relative to initial values in the “natural” sediment, while those in the “organic matter-enriched” sediment did not change. As a result, the vegetative development of *C. taxifolia* in the “organic matter-enriched” sediment was marginally lower than that in the sediment with natural concentration of organic matter. The contention that the vegetative development of the invasive *C. taxifolia* is favored in sediments with a high content of organic matter is not sustained. Additionally, we wanted to evaluate whether the presence of an external source of dissolved organic carbon (DOC) would facilitate the survival of *Caulerpa taxifolia* and *C. prolifera* under conditions unfavorable for biomass development. To this end we performed two laboratory experiments (one under light-saturated and another under light-limited conditions) where we maintained fragments of *C. taxifolia* and *C. prolifera* in the presence of different low molecular weight DOC compounds and assessed the survival of the two species. Mortality of *Caulerpa* fragments occurred under light-limited conditions only and showed no relationship to the presence of any of the low molecular weight DOC compounds tested. Our results, therefore, do not provide support for the contention that the presence of low molecular weight DOC compounds could contribute to alleviating negative effects associated with light-limited conditions.

Keywords: *Caulerpa prolifera*; *Caulerpa taxifolia*; growth; sediment organic matter.

Introduction

The introduction of exotic species is a major driver of biodiversity loss (Sakai et al. 2001). When the introduc-

tion of a species modifies the abundance and/or the spatial distribution of native species and, therefore, the structure and function of the ecosystem, this exotic is considered invasive. Eight of the 85 species of macrophytes considered as exotic in the Mediterranean marine flora show an invasive behavior (Boudouresque and Verlaque 2002).

The introduction of the chlorophyte *Caulerpa taxifolia* (Vahl) C. Agardh in the Mediterranean Sea is an invasion event that has received major attention from the scientific community. Since 1984, *C. taxifolia* has invaded 131 km² of bottom surface in six Mediterranean countries (data for 2000, Meinesz et al. 2001). Comparison of invaded and uninvaded *Posidonia oceanica* L. Delile meadows suggests that *C. taxifolia* may interfere with the vegetative development of the seagrass (Villèle and Verlaque 1995, Dumay et al. 2002), and reduce algal diversity within the invaded communities (Verlaque and Fritayre 1994, Balata et al. 2004).

The monitoring of *Caulerpa taxifolia*'s invasion suggests that *Posidonia oceanica* meadows with high shoot density are more resistant to *C. taxifolia* invasion than low shoot density meadows (Meinesz et al. 1993, Villèle and Verlaque 1995, Jaubert et al. 1999, 2003), a contention that has received experimental support (Ceccherelli and Cinelli 1999). The invasion of *C. taxifolia* progressed fast in localities affected by urban sewage, where *P. oceanica* had already experienced significant mortality (Chisholm et al. 1997, Jaubert et al. 1999), which suggested that sediment conditions associated with urban pollution (i.e., anoxia, high content of organic matter and hydrogen sulfide) could promote the growth of *C. taxifolia* (Chisholm et al. 1997, Jaubert et al. 2003). Indeed, *C. taxifolia* is able to acquire inorganic phosphorus and the amino acid valine from the sediment through its rhizoids (Chisholm et al. 1996). Additionally, *C. taxifolia* photosynthesis did not meet respiratory demand below 24–29 m depth (Chisholm and Jaubert 1997), which is not consistent with the presence of this species at depths up to 99 m (Belsher and Meinesz 1995), and leads to the suggestion that *C. taxifolia* is able to meet part of its carbon demand through the assimilation of external organic carbon (Chisholm and Jaubert 1997).

Caulerpa prolifera (Forsskal) Lamouroux is present on tropical and sub-tropical coasts, and it is very common in the Mediterranean Sea (Guiry 2006). It has a clonal, coenocytic architecture, similar to that of *C. taxifolia*. *C. prolifera* usually grows in shallow muddy substrata in sheltered bays, coastal lagoons and port areas, and it is also common on dead mattes of *Posidonia oceanica* (Meinesz 1980, Ballesteros 1989, Terrados and Ros 1995, Ribera et al. 1997, Vicens 1999, Ballesteros et al. 2001). The habitat of the native *C. prolifera*, therefore, appears similar to those where the invasion of *C. taxifolia*

may be favored, i.e., muddy, anoxic sediments with high content of organic matter.

The first goal of this study was to evaluate whether sediments with an increased concentration of organic matter would provide a better substratum for the vegetative development of *Caulerpa taxifolia* than sediments with “natural” concentrations of organic matter. To this end we performed an *in situ* experiment where we increased the content of organic matter of the sediment relative to natural concentrations, and assessed the development of biomass of the invasive *C. taxifolia* and the native *C. prolifera*.

External organic carbon has not been considered to play a significant role in the total carbon balance of macroalgae given the low concentration of dissolved organic carbon in sea water (Kremer 1981). However, the organic matter content of marine sediments can be high (Bourcier 1989, Delgado et al. 1999, Ruiz et al. 2001), leading to high pools of dissolved organic carbon (DOC) in sediment pore water (Burdige and Homstead 1994, Köster and Meyer-Reil 2001). External glucose and acetate at mM concentration allowed *Ulva lactuca* L. to maintain an intact photosynthetic apparatus and survive during long periods of darkness or low-light in laboratory conditions (Markager and Sand-Jensen 1990). Hence, it can be surmised that the presence of external sources of DOC associated with sediments enriched with organic matter might facilitate the survival of other macroalgae under conditions unfavorable to biomass development.

The second goal of this study was to determine whether the presence of an external source of DOC would facilitate the survival of *Caulerpa taxifolia* and *C. prolifera* under conditions unfavorable to biomass development (light limitation). We performed two laboratory experiments (one under light-saturated and another under light-limited conditions) where we maintained fragments of *C. taxifolia* and *C. prolifera* in the presence of different low molecular weight DOC compounds and assessed the survival of the two species. An external DOC-facilitated survival of *C. taxifolia* under light-limited conditions could provide, partly at least, a mechanistic explanation for the fast progression of the invasion of this species in sediments affected by urban pollution with dead or dying seagrasses (Chisholm et al. 1997, Jaubert et al. 2003).

Materials and methods

Caulerpa taxifolia and *C. prolifera* material used in the experiments was collected haphazardly during the summer of 2004 by SCUBA divers in Cala D’Or (N 39°22.164', E 3°13.887'), a small bay in the southeast of Mallorca island, where *C. taxifolia* has been present since 1992 (Meinesz et al. 2001). The “organic matter-enriched” sediment was prepared two months before the initiation of the *in situ* experiment by mixing natural sediment collected in the submerged part of a beach adjacent to the *Posidonia oceanica* meadow where the experiment was to be performed with an equal volume of *C. taxifolia* and *P. oceanica* leaves collected in the *P. oceanica* meadow, and maintaining the mixture at room temperature in the laboratory until the start of the experiment. Natural

sediment was collected at the same time and kept in similar conditions. Three samples of each type of sediment were collected, dried at 60°C over 48 h, weighed, and burned at 450°C for 4 h to estimate the amount of organic matter as percentage of sediment dry weight (DW). Additional samples of sediment were collected and pore water was extracted by forcing N₂ to flow through the sample. Five-ml of Whatman® (Maidstone, UK) GF/C filtered pore water were fixed with 50 µl of NaOH 1 M and 50 µl of Zn (CH₃-COO)₂ 1 M and frozen for later sulfide analysis (Cline 1969).

The day before the initiation of the *in situ* experiment, *Caulerpa taxifolia* and *C. prolifera* were collected, and the fragments to be planted in plant window boxes were selected. Each selected fragment contained at least one stolon apex. The numbers of fronds and stolon apices of each fragment were counted, fresh weights were determined, and the fragments were haphazardly assigned to each treatment and replicate. Sixty-three fronds of *C. taxifolia* and 59 fronds of *C. prolifera* additional to those of the fragments were measured to estimate the average initial length of the fragment fronds of each species. Nineteen additional fragments of both *C. taxifolia* and *C. prolifera*, encompassing the range of sizes of those used in the experiment were selected, weighed fresh, and dried at 60°C over 48 h to estimate dry weight and to obtain a fresh weight-dry weight ratio for the fragments of each species.

The *in situ* experiment started on 8 July 2004 by filling 12 plastic plant window boxes (internal dimensions: 11.5×37×13 cm) with sediment (6 with “natural” sediment and 6 with “organic matter-enriched” sediment) and planting in them fragments of either *Caulerpa taxifolia* or *C. prolifera* with the aid of 2–3 plastic cable ties placed around the stolon of each fragment. Care was taken so that each plant window box contained a similar number of fragments, stolon apices and fronds, and algal fresh weights. Thus, the number of replicates for each combination of sediment type and *Caulerpa* species was 3. The plant window boxes were placed in a sand gap at a depth of 6 m in a *Posidonia oceanica* meadow invaded by *C. taxifolia*; they were buried in the sand so that the brims of the boxes were at the level of the sediment surface.

The *in situ* experiment ended on 20 September 2004 when the plant window boxes were dug out and the extant algal material in each of them was placed inside a plastic bag and transported to the laboratory. The plant window boxes were transported to the laboratory and a sediment sample was collected from each to estimate the content of organic matter and the concentration of sulfide in sediment pore water. The numbers of fronds and stolon apices present in each window box at the end of the experiment were counted, and the lengths of the fronds were measured (exactly 30 fronds were measured in those window boxes in which the number of extant fronds was >30). Total fresh weight of extant *Caulerpa* in each window box was determined and dried at 60°C over 48 h to estimate its dry weight.

The content of organic matter of the sediment increased (*t*-test, *p*<0.05) from 1.7±0.07 (SE)% of sediment DW for the “natural” sediment to 3.5±0.37 (SE)%

of sediment DW for the “organic matter-enriched” sediment. Sulfide concentration in sediment pore water was higher (t -test, $p < 0.05$) in the “organic matter-enriched” sediment [46.8 ± 19.41 (SE) μM] than in the “natural” sediment [0.1 ± 0.06 (SE) μM]. The content of organic matter at the end of the *in situ* experiment was still higher (t -test, $p < 0.05$) in the organic matter-enriched sediment [2.3 ± 0.26 (SE)% DW] than in the natural sediment [1.6 ± 0.18 (SE)% DW], but the concentrations of sulfide in sediment pore water were similar (t -test, $p > 0.05$) [natural: 1.4 ± 0.66 (SE) μM ; organic matter-enriched: 0.9 ± 0.39 (SE) μM].

The significance of differences in the number of stolon apices and fronds, and both the fresh and dry weights of *Caulerpa* between window boxes filled with natural sediment and those filled with organic matter-enriched sediment were tested using a t -test for independent samples. Differences between initial and final number of stolon apices and fronds, average frond length, and total fresh and dry weight in natural and organic matter-enriched sediments were tested using a t -test for dependent samples. Finally, differences between natural and organic matter-enriched sediment in the number of stolon apices and fronds, average frond length, and total fresh and dry weight at the end of the experiment were tested using a t -test for independent samples.

The *Caulerpa* material to be used in the laboratory experiments was collected on two different days (9 July 2004 for the light-saturated conditions, 20 September 2004 for the light-limited conditions) and it was maintained at 25°C and a 14 h light:10 h darkness photoperiod until fragments of fronds were produced. The fragments of *C. taxifolia* were obtained by cutting the apical part of fronds [fragment length: 3.8 ± 0.1 (SE) cm] while those of *C. prolifera* were produced by cutting the frond stalk near its insertion in the stolon [i.e., the fragments contained both the blade and the stalk; fragment length: 7.4 ± 0.1 (SE) cm]. We used full fronds of *C. prolifera* as previous studies showed that 4-cm-long fragments of this species, which included only the blade, did not survive fragmentation while fragments of the same length but including the stalk had 100% survivorship (Smith and Walters 1999). Large-size and persistent branching of *C. taxifolia* fronds at the tips prevented us from using fragments of length similar to those of *C. prolifera*. Previous studies, however, showed 100% survivorship of *C. taxifolia* fragments of only 2 cm length (Smith and Walters 1999). Ten additional fragments of each species similar to those used in the experiment were cut and dried at 60°C over 48 h to calculate a frond length:dry weight ratio and estimate the initial dry weight of each fragment. Each fragment was placed inside a 50-ml polyethylene culture tube containing 10 ml of dry beach sand and filled to the 50-ml level with seawater (seawater and sand collected from the same location as the algal material).

One ml of either sodium acetate, sodium lactate, D-glucose, L-glutamic acid, β -alanine, L-alanine, glycine, or L-serine concentrated solutions was injected into the sand phase of each culture tube to achieve a nominal concentration of 294 μM in pore water (if all the injected solution remained within sand interstices). We cannot

exclude the possibility that a fraction of the injected solution might have moved to the water phase. If all the injected solution had moved to the water phase, the nominal concentration of the added compound would be 44 μM . The amino acids chosen were those reported to be most abundant in *Posidonia oceanica* sediments, where total dissolved free amino acid concentration varies between 105 μM and 436 μM in interstitial water, while the concentration in the water column is lower than 2 μM (Jorgensen et al. 1981). In an additional treatment, we injected 1 ml of seawater to control for experimental manipulations. Seawater inside the culture tubes was changed weekly, and the different organic carbon compounds were supplied again according to treatments. The initial number of replicates for each treatment varied between 7 and 8.

Caulerpa fragments were maintained for 18 days (light-saturated conditions) or 25 days (light-limited conditions) at 25°C, and each culture tube was manually agitated every 2–3 days for 15 s to break gradients in the water phase. Photon flux density inside the incubation chamber varied between 187 and 298 $\mu\text{mol m}^{-2} \text{s}^{-1}$, depending on the particular position. Saturation photon irradiance for *C. taxifolia* photosynthesis varies between 44 and 166 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Gacia et al. 1996, Chisholm et al. 2000), while that of *C. prolifera* varies between 11 and 112 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Terrados and Ros 1992). Light-saturated and light-limited conditions were achieved by maintaining the fragments under 14 h light:10 h dark and 1.5 h light:22.5 h dark photoperiod regimes, respectively. At the end of the experiments the fragments were dried at 60°C for 48 h to estimate their dry weights. The specific growth rate (SGR) of each *Caulerpa* fragment (i.e., the average instantaneous rate of production of mass relative to the initial mass of the fragment) was calculated as

$$\text{SGR} = \frac{W_f - W_0}{(T_f - T_0)W_0}$$

where W_0 and W_f are the initial and final dry weights of the fragment, respectively, and $(T_f - T_0)$ is the duration of the experiment. Differences in SGR between *Caulerpa* fragments in light-saturated and light-limited conditions were tested using 2-way ANOVAs with “species” and “external organic carbon” as fixed factors. Prior to analysis the homogeneity of variances of data was tested using the Bartlett’s test.

Results

The number of stolon apices and fronds, and the total fresh weight and dry weight per plant window box of both *Caulerpa taxifolia* and *C. prolifera* at the initiation of the *in situ* experiment were not different between the natural sediment and the organic matter-enriched sediment (t -test for independent samples; Figure 1, Table 1). The number of stolon apices and fronds, and total dry weight of *C. taxifolia* in natural sediments tended to increase from the initiation to the end of the experiment (Figure 1a,c,e, Table 1). However, these variables did not change

Figure 1 *Caulerpa taxifolia* and *C. prolifera*: number of stolon apices (a, b), fronds (c, d), and total dry weight (e, f) per plant window box, and frond length (g, h) at the beginning and end of a 74-day growth period in “natural” and “organic matter-enriched” sediment. Error bars represent mean +1 SD. OM=organic matter.

Table 1 *Caulerpa taxifolia* and *C. prolifera*: biomass development in natural and organic matter-enriched sediments: assessment of differences between algal material planted in each sediment type at the initiation of the experiment, and after 74 days.

Species	Variable	Natural vs. o.m.-enriched, initial		Initial vs. final, natural sediment		Initial vs. final, o.m.-enriched sediment		Natural vs o. m.-enriched, final	
		t-test for independent samples		t-test for dependent samples		t-test for dependent samples		t-test for independent samples	
<i>C. taxifolia</i>	Number of stolon apices per window box	t=0.32, df=4, p=0.77		t=3.40, df=2, p=0.08		t=0.00, df=2, p=1.00		t=2.36, df=4, p=0.08	
	Number of fronds per window box	t=0.72, df=4, p=0.51		t=3.39, df=2, p=0.08		t=2.00, df=2, p=0.18		t=1.92, df=4, p=0.13	
	Total dry weight (g) per window box	t=0.27, df=4, p=0.81		t=2.83, df=2, p=0.11		t=0.95, df=2, p=0.44		t=2.05, df=4, p=0.11	
	Average frond length (cm)	NA		t=5.75, df=2, p=0.03		t=12.89, df=2, p=0.01		t=0.12, df=4, p=0.91	
<i>C. prolifera</i>	Number of stolon apices per window box	t=2.00, df=4, p=0.12		t=22.52, df=2, p=0.002		t=8.60, df=2, p=0.01		t=1.07, df=4, p=0.34	
	Number of fronds per window box	t=0.14, df=4, p=0.89		t=8.69, df=2, p=0.01		t=5.40, df=2, p=0.03		t=0.79, df=4, p=0.47	
	Total dry weight (g) per window box	t=0.77, df=4, p=0.48		t=10.87, df=2, p=0.01		t=2.42, df=2, p=0.14		t=0.66, df=4, p=0.54	
	Average frond length (cm)	NA		t=0.76, df=2, p=0.53		t=0.82, df=1, p=0.56		t=1.17, df=4, p=0.32	

Average frond lengths at the initiation of the experiment were calculated before the assignment of algal material to treatments. Bold font indicates significant differences.

NA: not applicable; o.m.: organic matter.

in the organic matter-enriched sediment. Hence, the number of stolon apices and fronds, and total dry weight of *C. taxifolia* at the end of the experiment tended to be lower in the organic matter-enriched sediment than in the natural sediment. The average frond length of *C. taxifolia* decreased from 12.4 cm at the beginning of the experiment to 8.2 cm at the end, but there were no differences between natural and organic matter-enriched sediment at any time (Figure 1g, Table 1).

Caulerpa prolifera experienced large losses of biomass during the *in situ* experiment, both in natural and organic matter-enriched sediments (Figure 1f), and the number of stolon apices and fronds, and total dry weight were not different between the two sediment types at the beginning and end of the experiment (Figure 1b,d,f, Table 1). The average length of *C. prolifera* fronds was not different between the two types of sediments either (Figure 1h).

Mortality of *Caulerpa* fragments maintained in the laboratory occurred under light-limited conditions only. The mortality of *C. prolifera* fragments (two of seven replicates in the β -alanine and L-alanine treatments, and one of seven replicates in the control, D-glucose, and L-glutamic acid treatments) was accompanied by indications of gametogenesis (cf. Meinesz 1979a, Clifton and Clifton 1999). Mortality of *C. taxifolia* fragments occurred in only two of seven replicates in the D-glucose treatment.

The SGR of the fragments of *Caulerpa prolifera* under light-saturated conditions was higher than that of *C. taxifolia* (Figure 2, Table 2), but there was no significant difference in fragment SGRs among the various low molecular weight DOC compounds. When light availability was reduced to only 1.5 h per day, fragments of *C. prolifera* experienced a net loss of mass, resulting in negative values of SGR while values for *C. taxifolia* were low-positive or negative (Figure 3). Again, the presence of different low molecular weight DOC compounds did not seem to affect the SGR of the fragments (Figure 3, Table 2).

Discussion

Our results do not support the contention that sediments with a higher content of organic matter than those with natural concentrations provide a more favorable substratum for the vegetative development of *Caulerpa taxifolia*. Indeed, the number of stolon apices and fronds, and total dry weight at the end of the *in situ* experiment increased relative to initial values in the natural sediment, while those in the organic matter-enriched sediment did not change. The final vegetative development attained by *C. taxifolia* in the organic matter-enriched sediment was marginally lower than that in the natural sediment. The concentration of dissolved sulfide in pore water was higher in organic matter-enriched sediment than in natural sediment at the initiation of the experiment and this high concentration of sulfide might have affected *C. taxifolia* growth negatively, for sulfide is toxic to marine macrophytes (Goodman et al. 1995, Erskine and Koch 2000, Holmer and Bondgaard 2001, Koch and Erskine 2001, Halun et al. 2002). *C. prolifera* experienced high biomass losses in both types of sediments, which prevents us

Figure 2 *Caulerpa taxifolia* and *C. prolifera*: specific growth rates of fragments maintained in the presence of different low molecular weight dissolved organic carbon compounds under light-saturated conditions. Error bars represent mean +1 SD.

Figure 3 *Caulerpa taxifolia* and *C. prolifera*: specific growth rates of fragments maintained in the presence of different low molecular weight dissolved organic carbon compounds under light-limited conditions. Error bars represent means +1 SD.

Table 2 *Caulerpa taxifolia* and *C. prolifera*: analysis of variance of the specific growth rates of fragments growing in the presence of different low molecular weight DOC compounds under light-saturated and light-limited conditions.

Conditions	Source of variation	df	MS
Light-saturated	Species	1	0.0043*
	Carbon source	8	0.0002 ^{ns}
	S×C	8	0.0003 ^{ns}
	Error	120	0.0006
	Total	137	
Light-limited	Species	1	0.0097***
	Carbon source	8	0.0001 ^{ns}
	S×C	8	0.0001 ^{ns}
	Error	100	0.0002
	Total	117	

*: $p < 0.05$; ***: $p < 0.001$; ^{ns}: not significant.

from determining whether the vegetative development of this species is promoted in sediments with high content of organic matter.

Oxygen measurements in the rhizosphere of *Caulerpa taxifolia* maintained in aquaria suggest that the rhizoids of this species might release oxygen to the sediment at least during daylight hours (cf. Chisholm and Moulin 2003). However, the same study provides indirect evidence of the release of photosynthetic products to the sediment and of the promotion of sulfate reduction and nitrogen fixation in the rhizosphere of *C. taxifolia*. The large reduction in the concentration of sulfide in the pore water of the organic matter-enriched sediment during the *in situ* experiment might have been driven by the release of oxygen by *C. taxifolia* rhizoids, but sulfide diffusion driven by a strong concentration gradient and chemical oxidation might have been a significant process too, for the reduction in sulfide concentration in pore water also occurred in the sediment where *C. prolifera* was planted and decayed rapidly.

The large mass losses that *Caulerpa prolifera* experienced in both natural and organic matter-enriched sediments suggest that this species was more sensitive to the experimental manipulations than *C. taxifolia*. The experiment was performed between July and October, that is, within the vegetative growth period of both species (Meinesz 1979b, Meinesz et al. 1995, Terrados and Ros 1995). High sensitivity to the experimental manipulations, which involved mechanical fragmentation of thalli and planting in the sediment, could be interpreted as lower viability of thallus fragments produced by mechanical force. The viability of the fragments of *C. prolifera* is lower than those of *C. taxifolia*. Lower viability of thallus fragments produced by mechanical fragmentation, such as that produced naturally by waves and currents, suggests lower dispersal and invasive capacities in *C. prolifera*. Indeed, fronds of this species are less robust when fragmented mechanically than those of *C. taxifolia* (Smith and Walters 1999).

External glucose and acetate at mM concentrations allow *Ulva lactuca* to maintain an intact photosynthetic apparatus and survive during 41 days of darkness or low-light laboratory conditions (Markager and Sand-Jensen 1990). Although the relevance to field conditions is not clear, these observations suggest the importance of organic carbon uptake in maintaining an intact photosynthetic apparatus during light-limited conditions, rather than as a source of carbon for growth (op. cit.). Our results show that the presence of low molecular weight DOC compounds external to *Caulerpa taxifolia* and *C. prolifera* fragments did not promote the growth of specimens maintained in the laboratory under light-saturated and light-limited conditions. The SGRs of fragments with no DOC compounds added (controls) were not different from the SGRs of fragments exposed to the different DOC compounds. The amount of carbon added to the culture tubes during the experiments was only 3% to 13% of the average carbon gain of *C. prolifera* fragments in light-saturated conditions, and 4% to 18% of the average carbon loss in light-limited conditions (considering an average ash content of 13.5% of DW and an average carbon content of 33.6% of DW for *C. prolifera*;

cf. Terrados and Ros 1995). These calculations suggest that the amount of carbon added might not have been enough to increase fragment growth significantly had it been completely assimilated by *C. prolifera* fragments, and the same likely holds for *C. taxifolia*.

Mortality of *Caulerpa* fragments occurred under light-limited conditions only and showed no relationship to the presence of any of the low molecular weight DOC compounds. Mortality of *C. prolifera* fragments was associated with the morphological changes that occur when this species reproduces sexually (Meinesz 1979a, Clifton and Clifton 1999) and it occurred in both control fragments and in fragments exposed to some of the DOC compounds. In the case of *C. taxifolia*, two events of fragment mortality were recorded and only when D-glucose was present. These *C. taxifolia* mortality events were not associated with any morphological change that could be linked to sexual reproduction, as described for the genus *Caulerpa* (Goldstein and Morrall 1970, Clifton and Clifton 1999, Žuljević and Antolić 2000). Our results, therefore, do not provide support for the hypothesis that the presence of low molecular weight DOC compounds could contribute to alleviating negative effects associated with light-limited conditions, as has been suggested for *Ulva lactuca* (Markager and Sand-Jensen 1990).

Our experiments showed, however, different patterns in the response of *Caulerpa taxifolia* and *C. prolifera* to the experimental conditions. First, the SGRs of the fragments of *C. prolifera* were higher than those of *C. taxifolia* under light-saturated conditions. Second, the SGRs of *C. prolifera* fragments under light-limited conditions were negative and similar in magnitude to the values recorded under light-saturated conditions, while the SGRs of *C. taxifolia* fragments under light-limited conditions were minimal. This pattern was not affected by the presence of low-molecular weight DOC compounds. Could this be interpreted as *C. taxifolia* being more resistant to light-limiting conditions than *C. prolifera*? Previous knowledge on the influence of light availability on the photosynthetic rate of both species does not suggest this to be the case, since compensation irradiance (I_c) and saturation irradiance (I_k) of *C. taxifolia* at water temperatures above 20°C vary between 6 and 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 44 and 309 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Gacia et al. 1996, Chisholm and Jaubert 1997, Chisholm et al. 2000), while I_c of *C. prolifera* varies between 5 and 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and I_k varies between 19 and 97 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Terrados and Ros 1992). Given that the *Caulerpa* material used in the light-saturated and light-limited conditions was collected on different dates, that the experiments were not performed simultaneously, and that they were not repeated, it is not possible to determine whether the different patterns of response of the two species are driven by species-specific differences (i.e., the contention that *C. taxifolia* is more resistant to light-limited conditions than *C. prolifera*) or by fragment-specific differences (i.e., a higher amount of photosynthetic products stored inside the *C. taxifolia* fragments collected in September compared to that of fragments collected in July).

In summary, the results of the experiments performed do not provide support to the hypothesis that vegetative development of *Caulerpa taxifolia* is favored in sediments

with high loads of organic matter. Hence, the hypothesized higher invasive capacity of *C. taxifolia* in these types of substrata is not sustained. The presence of low molecular weight DOC compounds does not seem to affect the viability of fragments of either of the species under light-limited conditions. The patterns of response in the *in situ* experiment suggest that the invasive *C. taxifolia* might be more resistant to mechanical fragmentation than native *C. prolifera*, and this might be of relevance to the differing invasive capabilities.

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