



## Effects of filamentous macroalgae mats on growth and survival of eelgrass, *Zostera marina*, seedlings

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### ABSTRACT

A laboratory experiment was conducted to assess the effect of filamentous algae mats on the performance of seedlings of the eelgrass, *Zostera marina*. The seedlings were covered by three levels (3, 6 and 9 cm) of natural (*Chaetomorpha linum*) and imitation algae mats and it was hypothesised that the effects of the natural algae on seedling growth may be more severe because of the metabolic demands of the algae. Results show that coverage by both *C. linum* and imitation algae significantly reduced seedling growth and increased allocation of resources to above ground tissues. No clear effects of algae type on seedling performance were found and since there were no differences in oxygen or sulphide concentrations between the treatments, we attribute the reductions in seedling growth to algal shading. In a comparison with other studies it is shown that lower turbulence, higher temperature, organically enriched sediments and longer duration of exposure than applied here may result in oxygen depletion thereby intensifying the negative effects of light attenuation by algal mats on seedling performance.

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### 1. Introduction

In Danish waters, eelgrass (*Zostera marina* L.) has experienced population decline over the last decades, and it is estimated that the present distribution area constitutes approximately 20–25% of the area in 1901 (Rasmussen, 1977; Boström et al., 2003). A major reduction was caused by the wasting disease epidemic of the 1930s (Rasmussen, 1977), but reduced water clarity associated with high nutrient loadings has led to further decline, in particular of deep water populations after 1970 (Boström et al., 2003; Frederiksen et al., 2004; Krause-Jensen et al., 2008).

Nutrient loading was maximal around 1980 and has since declined, however, water clarity is still low, anoxic events are frequent and the recolonisation of former eelgrass habitats is slow (Petersen and Hjorth, 2010). The expansion of established eelgrass beds through clonal growth occurs at a maximum rate of 45 cm yr<sup>-1</sup> and is insufficient for recolonisation of large areas away from extensive source populations (Olesen and Sand-Jensen, 1994). Re-establishment of former vegetated areas therefore relies primarily on sexual reproduction, seed dispersal and seedling survival.

Eelgrass plants can produce large numbers of seeds when conditions are favourable, and recolonisation can be fast where

seedbanks, which often well exceed 1000 seeds m<sup>-2</sup>, are available (Olesen, 1999; Plus et al., 2003; Greve et al., 2005; Orth et al., 2006b; Jarvis and Moore, 2010). Although eelgrass seeds are negatively buoyant and >80% of the seeds remain within 5 m of the parent population (Orth et al., 1994), seeds can disperse several kilometres on floating reproductive shoots allowing for colonisation of distant habitats suitable for seagrass growth (Harwell and Orth, 2002; Erfemeijer et al., 2008). Also, seed germination rates can be high (40–90%, Churchill, 1983; van Katwijk and Wijgengangs, 2004), but even in areas where light conditions and physical exposure should support eelgrass growth, high seedling mortality rates have been observed (Hootsmans et al., 1987; Olesen, 1999; Jarvis and Moore, 2010). Seedling establishment and survival is therefore often considered a major bottleneck for recolonisation by seeds (Valdemarsen et al., 2010). The high seedling mortality has been attributed to unfavourable chemical and physical conditions, but complex biotic interactions such as macrofauna activity and coverage by drifting macroalgae mats may also reduce seedling survival (Orth et al., 2006a; Valdemarsen et al., 2010).

Blooms of ephemeral macroalgae are a well-known consequence of eutrophication in coastal waters and have repeatedly been connected with increased seagrass mortality (den Hartog, 1994; Sugimoto et al., 2007; Martinez-Lüscher and Holmer, 2010; Valdemarsen et al., 2010). The algae often accumulate in sheltered areas suitable for seagrass growth and some species assemble in mats with densities of more than 250 g DW m<sup>-2</sup> (Rasmussen et al., 1993; Valiela et al., 1997). In extreme cases densities of more than a kg DW m<sup>-2</sup> have been found (Sfriso et al., 1993). Depending on type

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and density, the algal mats will not only physically affect underlying seagrasses (i.e. by shading and increase of the diffusive boundary layer), but high rates of algal production and respiration may also significantly alter the biochemical conditions present within and below the mat (Krause-Jensen et al., 1999; Hauxwell et al., 2001; Holmer and Nielsen, 2007).

In dense algal mats, where little light will penetrate and exchange of water is limited, algal respiration and sediment oxygen consumption may result in reduced oxygen concentrations within mats and around the meristematic region of rooted seagrass (Krause-Jensen et al., 1996). As the leaves of *Z. marina* are highly permeable to oxygen, hypoxic conditions will cause oxygen loss due to passive diffusion, thereby reducing the supply of oxygen to be used in rhizome and root respiration (Borum et al., 2006). Insufficient supply of oxygen results in the less efficient anaerobic respiration imposing a strong sink on carbon resources and possibly resulting in the formation of toxic metabolites such as ethanol (Zimmerman et al., 1989; Pulido and Borum, 2010). Furthermore, insufficient oxygen supply to the root tips can result in intrusion of toxic sulphide and other reduced compounds inhibiting metabolic processes (Goodman et al., 1995; Holmer and Bondgaard, 2001; Frederiksen and Glud, 2006; Borum et al., 2006; Holmer and Nielsen, 2007).

In this laboratory study we examined the impact of mats formed by the ephemeral alga *Chaetomorpha linum* on eelgrass seedlings. The objectives were to assess the influence of algal mat thickness on seedling growth and survival, and treatments with imitation algae were used to evaluate whether measured changes in seedling performance could be ascribed to physical or biochemical effects of algal presence.

We hypothesised that seedlings, due to their small size, are highly vulnerable to even moderate cover by ephemeral algae, and that drifting algal mats therefore may increase seedling mortality imposing a barrier to eelgrass colonisation. We expected seedling growth to decrease with increasing mat thickness and that this effect would be more pronounced in treatments of natural algae compared to imitation algae, due to additional effects of algal metabolism on oxygen concentrations.

## 2. Methods

### 2.1. Experimental design

Growth response and survival of eelgrass (*Z. marina*) seedlings to cover by filamentous macroalgae was studied by growing seedlings in aquaria with three different thicknesses of macroalgae (*C. linum*) and artificial structures (green Spectra® fibre fishing line, 0.28 mm) imitating the algae (Table 1). The Spectra fibre was chosen as imitation algae as these have roughly the same diameter and structure as the natural *C. linum*, and to ensure comparable mat thickness both algae types were held in place by thin wire pins. The amount of artificial algae was adjusted to approximate the light extinction measured within the *C. linum* mats. For eelgrass, light intensity rather than spectral composition is important for the photosynthetic activity (Mvungi et al., 2011), hence we assumed that differential spectral attenuation between *C. linum* and imitation algae would not be important.

Eelgrass seedlings and sediment were collected in mid June 2010 in Aarhus Bay (56.2° N, 10.3° E), Denmark (depth 0.5–1.0 m; water temperature 18 °C; salinity 22 PSU). Seedlings were identified as small shoots with 2–5 narrow (1–2 mm) leaves and no rhizome present. In addition, many of the collected plants still had the seed coat attached to the hypocotyle. The collected seedlings ( $7.9 \pm 2.3$  SD cm height) were gently cleaned from epiphytes and dead leaves, and stored in seawater overnight.

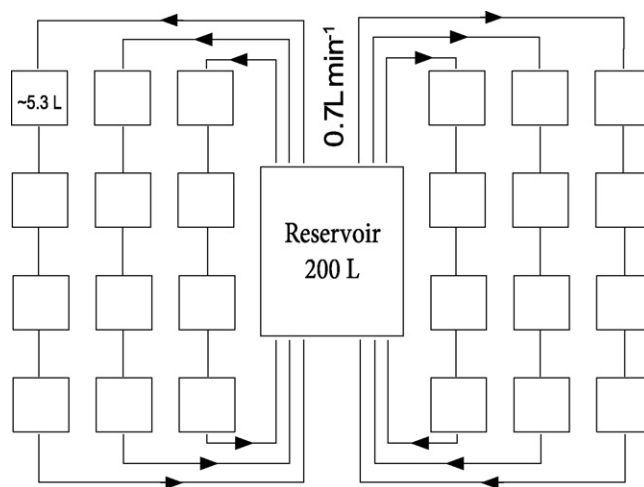


Fig. 1. Schematic drawing of experimental setup and water flow.

Before transplantation to the aquaria, the fresh weight of all seedlings was measured. Four seedlings were then planted in each aquarium ( $L 20 \text{ cm} \times W 14 \text{ cm} \times H 19 \text{ cm}$ ; volume 5.3 L) in a layer of 4 cm of sieved (2 mm) natural sediment with low organic matter content ( $\text{LOI } 0.29 \pm 0.07 \text{ SD \% DW}$ ) and 4 L of 20 PSU artificial seawater (Marinemix professional, HW Wiegand GmbH). To minimise nutrient limitation a single grain of Osmocote® plant nutrition was added at the roots of each plant. Treatments consisted of three densities of either *C. linum* or imitation algae cover (Table 1). The amount of algae applied to the aquaria represented low to intermediate algal coverage ( $17\text{--}84 \text{ g DW m}^{-2}$ ) relative to maximum densities observed in situ in a Danish embayment ( $100\text{--}250 \text{ g DW m}^{-2}$ , Rasmussen et al., 1993) and covered the seedlings completely at the highest algal density (Table 1). Three replicas of each treatment and, for statistical reasons, two times three replicas of controls without algae were distributed in a randomised block design with six blocks of four aquaria.

The water in the aquaria was circulated between six series of four connected aquaria and a 200 L reservoir at a relatively high rate of  $0.7 \text{ L min}^{-1}$  by six Eheim Universal 1250 aquarium pumps (Fig. 1). This water exchange rate was chosen to imitate the relatively turbulent conditions expected to exist in coastal areas due to wind and current velocities. To minimise exchange between surface water and water within the mats, the inlet ( $\varnothing 8 \text{ mm}$ ) of each aquarium was placed in the upper corner and an overflow outlet was placed 3 cm higher in the opposite corner. Water movement in the aquaria was turbulent rather than directional and flow velocity was therefore estimated as the average time taken for a dye (Rhodamin B) to pass through an aquarium (Webster and Valett, 2006). The dye was injected into the inlet of an aquarium and water samples were subsequently taken from the outlet and measured spectrophotometrically. The first samples were taken every 20 s and intervals were gradually increased until no more dye could be detected. Estimated average velocities of water passing through the aquaria ranged between  $0.10$  and  $0.13 \text{ cm s}^{-1}$  and were expected to be lower at the bottom than at the surface due to the main water flow direction from inlet to outlet.

The aquaria were placed in a temperature regulated room kept at 20 °C, and irradiance, provided by fluorescent tubes (Philips TL5 HO, 39W, 830/840), was  $97 \pm 12$  (SD)  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  just below the water surface, corresponding to near saturating levels for *Z. marina* (Olesen and Sand-Jensen, 1993). The photoperiod was set to a 16 h light, 8 h dark cycle corresponding to natural spring conditions and the seedlings were allowed to grow for two weeks before harvest. This treatment period is within range of the plastochrone interval

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