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A new marine measure enhancing *Zostera marina* seed germination and seedling survival

Ana I. Sousa[a,b,*], Thomas Valdemarsen[a], Ana I. Lillebø[b], Lisbeth Jørgensen[a], Mogens R. Flindt[a]

[a] Institute of Biology, University of Southern Denmark, 5230 Odense M, Denmark
[b] Department of Biology & CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

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A B S T R A C T

Seagrass global distribution has declined in the last decades due to many causes, and the implementation of recovery programmes as well as the development of new restoration techniques are needed. This work describes the development of an innovative restoration measure to enhance *Zostera marina* (eelgrass) seed germination and seedling survival in sediments inhabited by lugworms (*Arenicola marina*) and its validation in mesocosm experiments. The technique consists of placing 3 cm thick biodegradable coconut fibre mats (membrane) in the surface sediment to exclude the negative effects of sediment reworking (burial of seeds and destabilization/burial of seedlings). Two different flume mesocosm experiments were setup to test for: i) the effect of membranes on burial of *Z. marina* seeds; ii) the effect of membranes on survival and growth of *Z. marina* seedlings. The experiments were run for 8 and 10 weeks, respectively. Results show that the membrane was effectively preventing critical burial of *Z. marina* seeds as all seed mimics placed on the surface initially were recovered from 0 to 4 cm depth in the plots with membrane, while in the absence of the membrane, all seeds were buried to below the critical depth of 5–6 cm. The membrane also significantly enhanced the survival of *Z. marina* seedlings. The initial seedling density was in both cases $30/m^2$ and the final density was $26.0 \pm 3.3/m^2$ with membrane *versus* $8.0 \pm 1.6/m^2$ without membrane. This new marine restoration measure showed to be effective on the reduction of the physical stress imposed by sediment reworking lugworms on *Z. marina* recovery, as a membrane keeps seeds at optimal depth for germination and protects seedlings from burial and erosion. In comparison to other measures, this new restoration technique is a low-tech nature-based solution. The results clearly show that this restoration technique can support *Z. marina* recovery through seeds and seedling protection. In this way, this technique contributes to decrease *Z. marina* vulnerability and increase its natural recovery potential and stability.

1. Introduction

Seagrasses are highly productive constituents of coastal ecosystems, contribute to many ecological processes and provide important ecosystem functions and services (Cullen-Unsworth and Unsworth, 2013; Duarte et al., 2013a). For instance, seagrasses increase sediment stability (Amos et al., 2004; Barbier et al., 2011; Christianen et al., 2013), immobilize dissolved inorganic nutrient by growth related uptake during the growth season (Flindt et al., 1999, 2016), function as ecological engineers (Bos et al., 2007; Canal-Vergés et al., 2016), and contribute to climate regulation through carbon storage and sequestration (Fourqurean et al., 2012; Duarte et al., 2013a,b). However, various pressures threaten seagrasses all over the world, resulting in declining global distribution in the last decades (Orth et al., 2006a; Waycott et al., 2009; Short et al., 2011).

Concerns about these widespread losses have led to research efforts to elucidate the threats and processes that adversely affect seagrass meadows and their recovery, and to evaluate seagrass vulnerability (Valdemarsen et al., 2010; Grech et al., 2012). Namely, physical disturbance processes have enhanced seagrass decline and/or hampered recovery, with sediment reworking by benthic macrofauna being one of the most studied (Valdemarsen et al.,

* Corresponding author. Present address: Department of Biology & CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.
E-mail address: anaisousa@ua.pt (A.I. Sousa).

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2010, 2011; Canal-Vergés et al., 2010, 2014; Suykerbuyk et al., 2012; Govers et al., 2014; Kuusemäe et al., 2016).

Lugworms, *Arenicola marina* L., have been identified as conspicuous ecosystem engineers in intertidal habitats. *A. marina* indirectly modifies the biogeochemistry of the surrounding sediment through bioturbation processes, such as sediment reworking and bioirrigation (Kristensen et al., 2012). The sediment reworking activity of lugworms is especially critical in *Z. marina* and *Z. noltei* in a recovery situation where the meadows are premature (low in density), as it leads to burial of seeds (particularly *Z. marina*, Valdemarsen et al., 2011); burial, destabilization or uprooting of entire shoots and seedlings (Valdemarsen et al., 2010, 2011; Suykerbuyk et al., 2012); and changes the physical structure of the sediment matrix (Wendelboe et al., 2013). In mature seagrass beds big individuals of lugworms are excluded by the high biomass of rhizomes and roots making it difficult for the polychaetes to create and maintain their burrows (Goerlitz et al., 2015). Only small individuals manage to coexist in high density meadows, which consequently reduces the biomass of lugworms and their negative impact on the seagrasses in dense beds. So the antagonistic impact of lugworms on seagrass distribution is primarily occurring during seagrass recovery where the seagrasses try to expand into areas occupied by the lugworm.

The effects of *A. marina* sediment reworking are critical during seagrass recovery, where seeds must be positioned above the critical depth to ensure a successful germination. This critical depth for *Z. marina* seeds, ensuring that hypocotyls can reach the surface, was shown to be up to 5–6 cm sediment depth at Odense Fjord (Greve et al., 2005). Nevertheless, other critical depths have been suggested for *Z. marina* seed germination at other systems, ranging from 1 to 8 cm (e.g. Moore et al., 1993; Granger et al., 2000; Morita et al., 2010). A previous work (Valdemarsen et al., 2011) estimated that *A. marina* at densities of 5–10 ind/m^2 can bury surface deposited seeds below this critical depth and thus prevent seagrass recovery. In contrast, other bioturbating species may have positive effects for seagrass recovery (Blackburn and Orth, 2013), such as other polychaetes (e.g. *Amphitrite ornata*, *Clymenella torquata*), which only bury seeds to 1–2 cm depth. This protects seeds from predation, losses by wave and current enforced dispersal, and is optimal for germination (Blackburn and Orth, 2013). In addition, *Z. marina* seedling establishment is determined by physical sediment-seedling interactions, being positively affected by seed burial (2–3 cm depth) which reduces seed loss due to sediment disturbance (Marion and Orth, 2012). Still, bioturbation was suggested to play an important role in the maintenance of seagrass landscape pattern through limiting the seagrass bed margins, thus influencing the spatial heterogeneity (Townsend and Fonseca, 1998).

Seagrass restoration programmes and protection plans have over the last decades been implemented, to either reverse seagrass decline or protect remaining seagrass meadows. The global success of these seagrass revegetation and restoration programmes performed worldwide since the 1940′s is reviewed in a recent work by van Katwijk et al. (2016). This work highlights the importance of removal of threats prior to replanting programs and shows that large-scale planting increases trial survival (through the spread of risks to overcome variability and through an increase in population growth rate by enhancing selfsustaining feedback). The restoration success is also dependent on a cautious site-selection and on choosing the proper technique (van Katwijk et al., 2016).

Table 1 summarizes relevant examples of implemented recovery measures, its strengths & success, and challenges & failures. These examples include nutrient load reductions followed by passive recovery (e.g. Lillebø et al., 2011a,b) or combined with human interventions (de Jonge et al., 2000), namely seagrass transplantation (Paling et al., 2009; van Katwijk et al., 2009), seed addition actions (Orth et al., 2012) and application of live blue mussel beds and/or shells (Bos and van Katwijk, 2007; Suykerbuyk et al., 2012) to stabilize the surface sediment. As expected, some measures were more effective and successful for seagrass recovery than others, which depended on the former mentioned crucial aspects for successful recovery. Regarding a similar nature-based solution approach, pilot transplantation experiments with fibre mats were performed before with *Amphibolis antarctica* seedlings and propagules, but its success was limited and dependent on a careful handling and calm weather (Kirkman 1998).

Following the global trend, *Z. marina* distribution in Odense Fjord (Denmark) decreased about 90% from 1983 to 2005. This decline was mainly attributed to high nutrient loading to the fjord and eutrophied conditions from the 1960s to 2000s (Petersen et al., 2009). Even though nutrient loading has been reduced since 1990 (about 40%), leading to water quality improvements (improved benthic light availability and reduced frequency of hypoxic events and algal blooms), *Z. marina* meadows have never recovered (Valdemarsen et al., 2010; Lillebø et al., 2011). Different physical stressors were identified as critical for *Z. marina* recovery in Danish coastal ecosystems. In many estuaries, where *Z. marina* had declined (e.g. Odense Fjord, Limfjorden and Roskilde Fjord) it was observed that the large areas previously covered with *Z. marina* had been colonized by *A. marina*. Experiments showed that sediment reworking by this 'ecosystem engineer' prevented *Z. marina* recovery through uprooting and/or burial of *Z. marina* seedlings and seeds (Valdemarsen et al., 2010, 2011), suggesting that lugworm exclusion might be essential for *Z. marina* recovery. Therefore, different methods to exclude *A. marina* can be applied. Volkenborn and Reise (2006) excluded *A. marina* by placing polyethylene nets with 1 mm mesh size, approximately 5 cm beneath the sediment surface. A different exclusion method consisted on the application of shells below *Z. noltei* transplanted square blocks, and reduced by 80% the adult lugworm density, allowing the *Z. noltei* adult plants' growth (Suykerbuyk et al., 2012).

It has been acknowledged by several authors (e.g. Greve et al., 2005; Jarvis and Moore 2010; Valdemarsen et al., 2011; Jarvis et al., 2014) that after a considerable decline, *Z. marina* recovery depends on vegetative reproduction and sexual reproduction, but mainly on the latter, with seed density, seed viability and seedling establishment being critical factors. Therefore, techniques to prevent disturbances of the *Z. marina* seed bank and enhance seedling survival are critical in restoration projects, rather than focusing only on a later life-stage. In several large Danish coastal ecosystems it appears that lugworms are preventing eelgrass recolonization (Valdemarsen et al., 2010, 2011; Canal-Verges et al., 2016; Kuusemae et al., 2016). Having seagrass restoration as an ultimate goal, the objective of this study was to evaluate under controlled experimental conditions if the burial, uprooting and destabilization of *Z. marina* seeds and seedlings by *A. marina* could be diminished by placing membranes in the surface sediment. In this study we tested whether 3 cm thick biodegradable coconut fibre mats could exclude *A. marina*, and thus potentially be used as a new measure to promote seagrass recovery from seeds and seedlings. The study included two annular flume mesocosm experiments, where coconut fibre mats were placed in the surface sediment in lugworm inhabited sediment. In experiment 1 we tested if coconut fibre mats exclude *A. marina* and in this way prevent the burial of *Z. marina* seeds through sediment reworking; and in experiment 2 we tested if survival and growth of *Z. marina* seedlings was enhanced by coconut fibre mats, through *A. marina* exclusion.