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Short communication

Restoration of seagrass habitats: Effects of artificial and natural sediments on the development of transplanted eelgrass (*Zostera marina*)

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ABSTRACT

Near-shore areas face multiple stressors, effects of climate change, coastal construction and contamination. Although capping the seabed in these areas with mineral masses can reduce the impact of legacy contaminants in sediment, it can also result in the loss of flora and sessile fauna, both of which are vital components of near-shore ecosystems. Eelgrass (*Zostera marina*) is essential to marine near-shore areas as it supports biodiversity and mitigates the effects of climate change. Therefore, it would be beneficial to modify the top layer of caps to facilitate the reestablishment of these ecosystems when capping near-shore areas. This study describes results from an in situ, six-month field experiment conducted to compare increase in leaf length over the growing season and survival of eelgrass transplanted in two commercially available substrates (Natural sand and Crushed stone) and indigenous sediment (i.e., indigenous control sediment) in a capping project in Horten Inner harbour, Norway. Similar leaf length increase was found in Natural sand and Indigenous control sediment, both significantly higher compared to Crushed stone substrate. Survival was highest in our case in the Indigenous control sediment (120 %), with no significant difference between Crushed stone (20 %) and Natural sand substrates (25 %). These findings emphasize the importance of selecting appropriate substrate for successful seagrass restoration.

1. Introduction

Marine near-shore areas face various challenges, including effects of climate change and coastal construction. Climate change leads to temperature changes, increased rainfall, floods, erosion, and sedimentation, which increases pollution and affects chemical and biological processes in the sea, such as coastal hypoxia, deoxygenation and acidification (Doney, 2010; Hillebrand and Kunze, 2020). Construction in these areas adds pressure, along with the legacy and ongoing contamination of marine sediment, especially in urban areas and harbours (Airoldi and Beck, 2007). Such plethora of ecosystem stressors have altered the dynamics, structure, and function of marine ecosystems, influencing biodiversity, stability, and resilience (O'Leary et al., 2017). As coastal urban areas continue to expand, anthropogenic activity in near-shore areas is expected to further intensify (Fan et al., 2019).

To mitigate effects of legacy contamination in marine sediment, capping of the contaminated seabed with mineral masses is often used, e.g., sand, gravel, and stone (e.g., Lampert and Reible, 2009). This reduces flux of toxic component to the water and thus, negative effects on marine life (Eek et al., 2008). It also smothers flora and sessile fauna on the seabed, alters structure and function of marine benthic ecosystems (Näslund et al., 2012), and consolidates muddy areas (Flindt et al., 2022). Previous research demonstrated that natural colonization of eelgrass after physical disturbances typically is slow or unsuccessful (e.g., Moksnes et al., 2018).

Eelgrass meadows are high-functional near-shore ecosystems. These ecosystems provide services that mitigate negative impacts of climate change by filtering nutrients from run-off, the sedimentation of particles, and reducing erosion by stabilizing sediments, as well as provide nurseries and other habitats benefiting biodiversity and global fisheries

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(Nordlund et al., 2016; Cullen-Unsworth and Unsworth, 2016). Unfortunately, these habitats have been rapidly disappearing over the last century (Orth et al., 2006), highlighting the need to better understand how to facilitate eelgrass restoration, survival and growth. Several studies have investigated the effect of different environmental parameters on eelgrass growth and survival (Van Katwijk and Wijgergangs, 2004; Zhang et al., 2015). Sediments with higher silt and clay content tend to support better growth after transplantation of single eelgrass plants (Zhang et al., 2015). Sheltered locations and reduced sediment mobility can also have a positive effect on eelgrass survival (Van Katwijk and Hermus, 2000).

There is lack of data on which substrate parameters are most effective in facilitating eelgrass reestablishment. We examined the survival and leaf length increase of eelgrass plants transplanted in two commercially available substrates. The two substrates are named "Crushed stone" and "Natural sand", where the main difference is that the former is prepared by grinding large stone down to the desired size at a quarry, while the latter consisted of sand extracted in its original form from a quarry. The “Natural sand” is thus already subjected to natural degradation and rounding of the grains. Both substrates have a grain size distribution of 0–8 mm. These were compared to an "Indigenous control sediment”, which is locally obtained.

2. Material and methods

2.1. Study site

The experiment was conducted in Horten inner harbour, Vestfold and Telemark, Norway (see Fig. S1, Supplementary materials). Eelgrass plants were harvested from a donor site located south of the harbour (59°25’25.4” N, 10°28’28.6” E). The experimental site was established north-east in the harbour (59°26’09.6” N, 10°29’15.2” E) (see Fig. S1, Supplementary materials). Eelgrass was observed at the experimental site and the location was thus assumed to suitable for eelgrass. Horten inner harbour is a small, naturally sheltered bay (3.8 km^2) with land areas to the west, east, and south, and two large islands to the north. The bay is relatively shallow, with a maximum depth of approx. 27 m, and is connected to the Oslo fjord in the north.

2.2. Substrate types

The experiment aimed to evaluate the suitability of Crushed stone and Natural sand as substrates for eelgrass growth. Crushed stone was produced by mechanically grinding stone extracted from a stone quarry at Veidekke Industri AS, department Skoppum, resulting in grain sizes of 0 and 8 mm, and a d_{50}-value of 2.8 mm (Norsk Betong- og Tilslagslaboratorium [NBTL], 2018). The grain size distributions for all three substrates are shown in Table 1. The petrographic examination of Crushed stone found 100 % rhombic porphyry rock with 71 % cubic sharp-edged, 29 % chipped and 0 % round-edged grains (NBTL, 2018).

Svelviksand AS, Hurum, mined the Natural sand from a natural sand quarry. There were no further alterations to this substrate. The size distribution was between 0 and 8 mm (Table 1), with a d_{50}-value of 0.8 mm. The substrate contained darker rock types, including granite, gneiss, silt, sand, and claystone, and its grains were mostly cubic with rounded edges. The indigenous control sediment, collected from the same location as the plants (59°25’25.4” N, 10°28’28.6” E) in Horten Inner harbour, consisted of grey, sandy mud with shell fragments and plant debris, containing 6 % total organic matter content (TOC_{63}) (Hess et al., 2020) and a d50-value of approximately 0.06 mm (Norwegian Geotechnical Institute [NGI], 2016). There was no pre-treatment of the substrates/sediment prior to use in the experiment.

Table 1
Percent grain size distribution for the three substrate types.

Grain size (μm)	Crushed stone (N = 5)	Natural sand (N = 5)	Indigenous control sediment (N = 4)
< 2	5.36	1.0	6.8
2–63	65.4	2.3	50.8
> 63	29.3	96.8	42.4

2.3. Experimental setup and data collection

To test the substrates for re-establishing eelgrass, individual plants collected from a donor site were transplanted into crates containing all three substrates. Eelgrass was collected over two days in April (2021–04–17 and 2021–04–27), and the transplantation was performed the same day as retrieval of plants. Crates were transported and placed into the experimental site. The donor site was a robust eelgrass ecosystem with an area of approx. 16 500 m^2. The methods for transplanting followed Moksnes et al. (2016); a diver collected turfs by digging ca. 15 cm into the sea floor with a hand-held shovel. Care was taken to ensure intact roots on the plants. Patches of sediment with plants were taken to the work boat and carefully separated from the sediment by rinsing with salt water. Ten individual healthy plants, 20 cm in length with 10 cm rhizomes, selected for each treatment and transplanted by hand. We placed the rhizome horizontally into a 5 cm deep hole in the substrates made with an upward bend at the deepest section to ensure anchoring of the root. We anchored the rhizome in this upward bend. Two replicate treatments with 10 plants each were prepared for each substrate. The substrate was contained in 1.2 × 0.8 × 0.5 m wooden crates filled with 0.35 m of substrate up to 0.15 m below the top of the crate. We placed the crates in a random grid pattern at a depth of 3.5 m at a site selected because of existing patches of eelgrass, suggesting good conditions for growth and survival.

Leaf length increase and survival of eelgrass in the different substrates was registered monthly for all crates from April (2020–04–17) to September (2020–09–16) using an underwater drone (See Fig. S2, Supplementary materials). Leaf length was also measured by a diver during the final September sampling, and leaf length increase over the growing season was calculated as the difference between the plant lengths at transplantation and at the end of the experiment. Survival was recorded as the number of plants in each crate at the end of the experiment. Growth is usually assessed as production per time. Loss and death of leaves over the growing season will influence the accuracy of this parameter as a representation of growth. Loss and death of leaves was not recorded in the current experiment and thus represents a limitation in the data set. However, leaf length increase and survival as registered in the dataset described in the current manuscript are common biological quality elements to describe the success of eelgrass restoration.

2.4. Data analysis

We tried a mixed model with crate as a random factor and a one-way ANOVA to test which sediment type resulted in higher “leaf length increase” and “survival” of transplanted eelgrass. However, there was lack of normality due to small number of replicates for both analyses. Thus, we used a non-parametric Kruskal-Wallis (KW) test to determine whether the median scores of the three substrates (i.e., indigenous control sediment, Natural sand and Crushed stone) had an effect on changes in plant number or leaf length, respectively. Post-hock tests were performed using Dunn Test because the KW tests showed significant effects. Analyses were done in R version 4.1.0 (R Core Team, 2021).

3. Results and discussion

The results highlight the importance of using appropriate substrate for successful eelgrass restoration after capping or other physical disturbances.

Based on the KW-test (Chi-squared = 12.294, df = 2, p = 0.002), the survival of eelgrass transplanted into both commercial test-substrates was lower compared to survival of eelgrass in the indigenous control sediment. There were no differences between the two commercial substrates (p = 0.939; see also Fig. 1A). In the Natural sand substrate, five out of initially 20 plants survived (25 %), while four out of 20 plants survived in Crushed stone substrate (20 %). This shows that neither of the two commercially available substrate types supported good survival of the transplanted eelgrass. For Indigenous control sediment, there was an increase of four plants, to 24 plants (120 %), at the end of the experiment. The survival in Indigenous control sediment was significantly higher compared to the survival in both Natural sand (p = 0.010) and Crushed stone substrate (p = 0.011) (see Fig. 1A).

The Indigenous control sediment was taken from the harbour area where the field experiment was performed. Most of the grains in the Natural sand substrate were larger than 63 μm, thus fewer of the fine clayey and silty grains were present (Table 1). Eelgrass can grow in different types of sediment, including gravel, sand, or mud (Borum et al., 2004), however, a relative high share of silt and clay, around 75 %, can facilitate eelgrass survival (Zhang et al., 2015). Physical sediment-seedling interactions have been identified as a key process for eelgrass recruitment following restoration (Marion and Orth, 2010). The lack of fine-grained particles in the Natural sand substrate likely led to a larger loss of individual plants, reducing the survival rates. In the Natural sand substrate, clay and silt made up 3.3 % of the grain weight, while in the Crushed stone and Indigenous control sediment, they account for 71 % and 58 %, respectively (Table 1).

Large-scale field experiments have shown strong interactions between sediment and seagrass ecosystems. The ecosystems prevent sediment erosion and enhance sedimentation of small particles (Van Katwijk et al., 2010; van der Heide et al., 2021). Inadequate sediment anchoring has been identified as a key factor for low recovery of eelgrass after physical disturbances (Jiang et al., 2022). The results from our experiment reinforce the significance of the influence sediment type has on eelgrass survival. They indicate that a sufficient quantity of natural and fine-grained particles was necessary for the survival of transplanted individuals and for establishing resilient eelgrass meadows.

Plant height at the start and end of the field experiment showed that leaf length increase was lowest for plants transplanted into Crushed stone substrate (Fig. 1B). Height ranged between 25 and 100 cm for plants in the indigenous control sediment, 35–80 cm in Natural sand substrate, and 9–16 cm in Crushed stone substrate. The KW test showed a significant effect of substrate type on plant height change (Chi-squared = 8.937, df = 2, p = 0.011), indicating significantly higher leaf length increase in both the Natural sand (p = 0.009) and Indigenous Control sediment (p = 0.040) compared to Crushed stone (Fig. 1B), yet no effect between Natural sand and Indigenous control sediment (p = 0.519; Fig. 1B). Both the Indigenous control sediment and Natural sand substrate have undergone wear and natural degradation, which resulted in rounded grains lacking sharp edges. The Crushed stone substrate, on the other hand, consisted of cubic sharp-edged grains that have been chipped (NTBL, 2018). The low increase in leaf length observed in the Crushed stone substrate may thus be due to a shift in resources from leaf length increase to repair of root lesions caused by sharp edges in the Crushed stone substrate. Stressful conditions can reduce plant growth in *Z. marina* by draining carbon reserves and allocating energy to defence and repair processes instead of growth (Moreno-Marin et al., 2018). As our study did not include microscopic investigation of roots and investigation of resource allocation, this cannot be confirmed as the reason for the observed lower growth in the crushed stone substrate.

Our hypothesis that eelgrass would grow equally well in Natural sand and Indigenous control sediment was proven wrong, as indigenous substrate showed higher survival. The hypothesis was that the presence of fine grains (< 63 μm) in the indigenous substrate would be the most important parameter supporting growth, as reported by Zhang et al. (2015). Moreover, the results show that substrates with sharp-edged grains, such as those produced from crushed stone, can reduce eelgrass growth and survival. As shallow coastal areas face significant pressure in the coming years, it is important to minimize the negative impact of interventions such as for instance capping, and if needed, identify how to best facilitate for reestablishment of locally important ecosystems, such as seagrass.

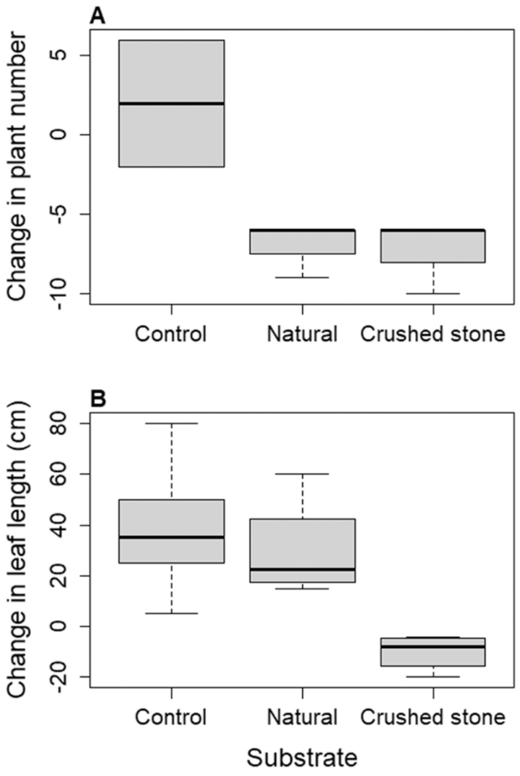

Fig. 1. Survival and leaf length increase of eelgrass plants registered as change in plant number (A) and height (B) from start to end of the experiment in three types of substrates: Control (indigenous sediment), Natural sand, and Crushed stone. Non-overlapping grey boxes are significantly different ($p < 0.05$).

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CRediT authorship contribution statement

Conceptualization (IFS, EU, JC), methodology (IFS, EU, JC), validation (IFS, JC), formal analysis (DT), investigation (IFS, EU, JC, DT), data curation (EU, DT), writing – original draft (IFS, EU, JC, DT), writing review & editing (IFS, JC, DT), visualization (EU, DT), supervision (IFS, JC), project administration (IFS), funding acquisition (EU).

Declaration of Competing Interest

We declare that there are no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquabot.2023.103677.

References

Airoldi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe. Oceanogr. Mar. Biol.: Annu. Rev. 45, 345–405.

Borum, J., Duarte, C.M., Krause-Jensen, D., Greve, T.M., 2004. European seagrasses: an introduction to monitoring and management. The MandMS project, ISBN 87–89143-21–3.

Cullen-Unsworth, L.C., Unsworth, R.K.F., 2016. Strategies to enhance the resilience of the world's seagrass meadows. J. Appl. Ecol. 53, 967–972.

van der Heide, T., Temmink, R.J.M., Fivash, G.S., Bouma, T.J., Boström, C., Didderen, K., Esteban, N., Gaeckle, J., Gagnon, K., Infantes, E., van de Koppel, J., Lengkeek, W., Unsworth, R., Christiansen, M.J.A., 2021. Coastal restoration success via emergent trait-mimicry is context dependent. Biol. Conserv. 264, 109373.

Doney, S.C., 2010. The growing human footprint on coastal and open-ocean biogeochemistry. Science 328, 1512–1516.

Eek, E., Cornelissen, G., Kibsgaard, A., Breedveld, G.D., 2008. Diffusion of PAH and PCB from contaminated sediments with and without mineral capping; measurement and modelling. Chemosphere 71, 1629–1638.

Fan, D., Xu, Y., Wu, Y., Lee, G., 2019. Coastal environmental changes under increasing anthropogenic impacts: an introduction. Anthr. Coasts 2. https://doi.org/10.1139/anc-2019-0011.

Flindt, M.R., Oncken, N.S., Kuusemae, K., Lange, T., Aaskoven, N., Winter, S., Sousa, A.I., Rasmussen, E.K., Canal-Verges, P., Connolly, R.M., Kristensen, E., 2022. Sand capping stabilizes muddy sediment and improves benthic light conditions in eutrophic estuaries: laboratory verification and the potential for recovery of eelgrass (Zostera marina). J. Sea Res. 181, 102177.

Hess, S., Alve, E., Andersen, T.J., Joranger, T., 2020. Defining ecological reference conditions in naturally stressed environments – how difficult is it? Mar. Environ. Res. 156, 104885.

Hillebrand, H., Kunze, C., 2020. Meta-analysis on pulse disturbances reveals differences in functional and compositional recovery across ecosystems. Ecol. Lett. 23, 575–585.

Jiang, Z., Liu, S., Cui, L., et al., 2022. Sand supplementation favors tropical seagrass Thalassia hemprichii in eutrophic bay: implications for seagrass restoration and management. BMC Plant Biol. 22. https://doi.org/10.1186/s12870-022-03647-0.

Lampert, D.J., Reible, D., 2009. An analytical modelling approach for evaluation of capping of contaminated sediments. Soil Sediment Contam. 18, 470–488.

Marion, S.R., Orth, R.J., 2010. Innovative techniques for large-scale seagrass restoration using Zostera marina (eelgrass) seeds. Restor. Ecol. 18, 514–526.

Moksnes, P.O., Gipperth, L., Eriander, L., Laas, K., Cole, S., Infantes, E., 2016. Handbok för restaurering av ålgräs i Sverige – Vägledning. Havs- och Vatten Rapp. 2016, 9.

Moksnes, P.O., Eriander, L., Infantes, E., Holmer, M., 2018. Local regime shifts prevent natural recovery and restoration of lost eelgrass beds along the swedish west cost. Estuaries Coasts 41, 1712–1731.

Moreno-Marin, F., Brun, F.G., Pedersen, M.F., 2018. Additive response to multiple environmental stressors in the seagrass *Zostera marina* L. Limnol. Oceanogr. 63, 1528–1544.

Näslund, J., Samuelsson, G.S., Gunnarsson, J.S., Nascimento, F.J.A., Nilsson, H.C., Cornelissen, G., Schaanning, M.T., 2012. Ecosystem effects of materials proposed for thin-layer capping of contaminated sediments. Mar. Ecol. Prog. Ser. 449, 27–39.

NBTL, 2018. Tilslagsprøving iht. NS-EN standarder. 0–4 mm fra Skoppum Pukkverk (In Norwegian). Prøvingsrapport P-18167A.

NGI, 2016. Horten Indre havn - Helhetlig tiltaksplan. Helhetlig tiltaksplan for forurensede sedimenter i Horten Indre havn (In Norwegian). Dok. nr. 20150201–01-R/Rev. 1.

Nordlund, L.M., Koch, E.W., Barbier, E.B., Creed, J.C., 2016. Seagrass ecosystems services and their variability across genera and geographical regions. PLOS One. https://doi.org/10.1371/journal.pone.0163091.

O'Leary, J., Micheli, F., Airoldi, L., Boch, C., De Leo, G., Elahi, R., Ferretti, F., Graham, N.A.J., Litvin, S.Y., Low, N.H., Lummis, S., Nickols, J., Wong, J., 2017. The resilience of marine ecosystems to climatic disturbances. BioScience 67, 208–220.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystem. BioScience 56, 987–996.

R Core Team, 2021. R: A language and environment for statistical computing. Available at: ⟨https://www.R-project.org/⟩ (Accessed 25 May 2021).

Van Katwijk, M.M., Hermus, D.C.R., 2000. Effects of water dynamics on *Zostera marina*: transplantation experiments in the intertidal Dutch Wadden Sea. Mar. Ecol. Prog. Ser. 208, 107–118.

Van Katwijk, M.M., Wijgergangs, L.J.M., 2004. Effects of locally varying exposure, sediment type and low-tide water cover on *Zostera marina* recruitment from seed. Aquat. Bot. 80, 1–12.

Van Katwijk, M.M., Bos, A.R., Hermus, D.C.:R., Suykerbuyk, W., 2010. Sediment modification by seagrass beds: Muddification and sandification induced by plant cover and environmental conditions. Estuar. Coast. Shelf Sci. 89, 175–181.

Zhang, Q., Liu, J., Zhang, P.D., Liu, Y.S., Xu, Q., 2015. Effect of silt and clay percentage in sediment on the survival and growth of eelgrass *Zostera marina*: Transplantation 45 experiment in Swan Lake on the eastern coast of Shandong Peninsula, China. Aquat. Bot. 122, 15–19.