Norwegian University
of Life Sciences

**Master's Thesis 2021 60 ECTS**
Faculty of Environmental Science and Natural Resource Management

# Short-term effects of substrate type on the growth and survival of transplanted eelgrass (*Zostera marina*)

## An experimental case study in Horten harbor


Embla Vildalen Uleberg
Master of Science in Natural Resource Management

# Preface

This master thesis marks the end of my master's degree in Natural Resource Management at the Norwegian University of Life Sciences (NMBU).

I want to give a special thanks to my two supervisors Jonathan Colman (NMBU) and Ingvild Størdal (NGI). Ingvild came up with the idea of testing eelgrass reestablishment in different substrate types after the harbour remediation project in Horten. I appreciate the enthusiasm, good discussions, and close follow up all the way. Furthermore, a thanks to Thor-Øyvind Ødegård and Anleggsdykk AS for assisting with the equipment and diving to help conduct the experiment in the harbour and to Horten Havnevesen for assisting with their boat during the fieldwork. I also want to give a thanks to Kjetil Flydal (UIO) for helping in the practical fieldwork and to Håkon Gregersen (Norconsult) for assisting with eelgrass transplanting advice. A special thanks needs to be directed to Diress Tsegaye Alemu (UIO) for assisting with the statistical part of the thesis. Also, a big thanks to Miljøringen for awarding me with the student scholarship and to Anders Bergsli in the project "Ren indre havn" that contributed financially to the master thesis fieldwork.

Lastly, I want to give a big thanks to my family and friends for their support and especially to my mom for helping me during the writing process and for letting me build the wooden crates in her basement during the first pandemic lockdown.

Norwegian University of Life Sciences, Ås. 01.06.2021
Embla Vildalen Uleberg

## Abstract

Eelgrass (*Zostera marina*) provides important functions in marine ecosystems as feeding habitat and nurseries for fish and several other species. Degradation of coastal areas caused by human activities threatens these vital ecosystems resulting in loss of biodiversity. In the inner Horten harbour, there is an ongoing remediation project of contaminated sediments where eelgrass has been registered. Remediation methods include dredging and capping, both highly intrusive for the marine environment. It was therefore of interest to investigate the effects of substrate type and transplant methods for the reestablishment of eelgrass in relation to the remediation project. In summer 2020, an experiment was conducted in Horten harbour testing the growth and survival of transplanted eelgrass in substrate types representing a difference in grain size, grain structure and silt/clay content. One sandy sediment type from a natural deposit (Natural substrate), one sediment type made from crushed mineral material (Machine substrate) and one muddy sediment type with a high silt/clay content (Control substrate). Each of the substrates were allocated into two of six 1.2 m x 0.8 m wooden crates (plots) placed near intact eelgrass meadows in about 3 m depth, providing one replicate for each substrate type. Eelgrass was then transplanted by single plants into one half of the crate, while patches were transplanted into the other half. Survival and growth were sampled 5 months after transplantation by measuring number and length of plants, respectively. Growth and survival were compared for the two transplant methods by visual inspection of underwater videos and pictures. Biodiversity (i.e., fish, starfish, and other organisms) associated with each substrate was also registered. In addition, time in air was registered during transplanting to test whether this factor also affects eelgrass growth and survival. The results show better growth in Natural substrate compared to Machine substrate, while no significant difference was found for number of individuals. Although not tested empirically, visual inspection supported better survival and growth for transplanted patches compared to single plants. There was a positive correlation between “time in air” and “growth”, although further research is needed. A higher number of species were observed in plots allocated in Natural substrate compared to Machine substrate which confirms a positive correlation between biodiversity and eelgrass survival and growth. These results provide knowledge about an effective return of biodiversity in the Norwegian coast after similar harbour remediation projects or other activities that damage eelgrass. In future restoration projects with eelgrass, this information can provide guidelines for a cost- and time-effective methodology, as substrate type and transplantation technique clearly influence eelgrass survival and growth.

# Sammendrag

Ålegress (*Zostera marina*) bidrar til viktige funksjoner i marine økosystemer som beiteområde og habitat for ungfisk og flere andre arter. Degradering av kystområder forårsaket av menneskelige aktiviteter truer disse vitale økosystemene som resulterer i tap av biologisk mangfold. I Horten indre havn er det et pågående opprydningsprosjekt av forurensede sedimenter der ålegress er registrert. Opprydningsmetoder inkluderer mudring og tildekking, begge svært forstyrrende for det marine miljøet. Det var derfor av interesse å undersøke effekten av substrattype og transplantasjonsmetoder for reetablering av ålegress i sammenheng til oppryddingsprosjektet. Sommeren 2020 ble det utført et eksperiment i Horten havn som testet overlevelse og vekst av transplantert ålegress i tre ulike typer substrater (dvs. Natur, Maskin og Kontroll). Disse substrattypene representerer forskjell i kornstørrelse, kornstruktur og silt/leirinnhold. Et sandig substrat fra en naturlig avsetning (Natursubstrat), et substrat laget av knust mineralmateriale (Maskinsubstrat) og et gjørmete substrat med høyt silt/leirinnhold (Kontroll substrat). Hvert av substratene ble fordelt i to av seks 1,2 m x 0,8 m trekasser plassert nær intakte ålegressenger i omtrent 3 m dybde, som gav en replikant for hvert substrat. Ålegress ble deretter transplantert på to måter, enkeltplanter i den ene halvdelen versus tuer i den andre halvdelen av kassen. Overlevelse og vekst ble undersøkt 5 måneder etter transplantasjon ved å måle antall planter og lengde på planter henholdsvis. Vekst og overlevelse ble sammenlignet for de to transplantasjonsmetodene gjennom visuell inspeksjon av undervannsvideoer og bilder. Biodiversitet (dvs. fisk, sjøstjerne og andre organismer) assosiert med hvert substrat ble også registrert. I tillegg ble tid i luft mellom transplantasjon og når plantene kom i vannet igjen målt for å teste om dette er en faktor som også påvirker ålegress vekst og overlevelse i forbindelse med transplantasjon. Resultatene viser bedre vekst i Natursubstrat sammenlignet med Maskinsubstrat, men varierte ikke for antall individer. Den visuelle inspeksjonen av transplantasjonsmetode viste bedre overlevelse og vekst for transplanterte tuer sammenlignet med enkeltplanter. Tid i luft viste en positiv korrelasjon mellom «tid» og «vekst», men mer forskning er nødvendig her. Flere arter ble observert i kassene med Natursubstrat sammenlignet med Maskinsubstrat noe som bekreftet en positiv sammenheng mellom biologisk mangfold og ålegress overlevelse og vekst. Disse resultatene gir kunnskap om en effektiv tilbakeføring av biologisk mangfold langs norskekysten etter lignende opprydningsprosjekter eller andre aktiviteter som forringer ålegress. I fremtidige restaureringsprosjekter med ålegress kan denne informasjonen gi retningslinjer for en kostnads- og tidseffektiv metodikk, da substrattype og transplantasjonsmetode tydelig påvirker ålegressoverlevelse og vekst.

# Table of contents

# 1. Introduction

Loss of marine ecosystems due to human activities and effects of climate change is a widespread and well-known problem. Seagrasses are important coastal marine habitats, as they provide ecosystem services such as filters for inorganic nutrients from urban and agricultural run-off (Asmala et al., 2019), stabilize sediments and prevent erosion (Gacia & Duarte, 2001; Orth et al., 2006) and enhance biodiversity by providing shelter, foraging and nursery grounds for numerous species (Boström & Bonsdorff, 1997; Green & Short, 2003; Fredriksen et al., 2005; Warren et al., 2010; García et al., 2019). Seagrasses also reduce the effects of climate change by sequestering carbon (Duarte & Middelburg, 2005; Kennedy et al., 2010; Duarte et al., 2011).

Seagrass ecosystems, including eelgrass (*Zostera marina*), are under high pressure in coastal areas all over the world (Duarte, 2002; Baden et al., 2003; Jørgensen & Bekkby, 2013). Threats include multiple stressors and a combination of direct and indirect threats caused mainly by human activities (Rehra et al., 2014). Changes in land and sea use is the main driver causing fragmentation of eelgrass habitats worldwide (Huges et al., 2002; Reed & Hovel, 2006; Waycott et al., 2008; Livernois et al., 2017; Riera et al., 2020). Other activities causing threats to eelgrass include dredging, depositing, industrial activities, and boat traffic (Short & Wyllie-Echeverria, 1995). Indirect threats include eutrophication, sedimentation, turbidity, and urban and agricultural runoff into coastal areas where eelgrass thrive (Short & Wyllie-Echeverria, 1995; Moore & Orth, 1996; Saunders et al., 2017; Chao et al., 2021). In addition, global warming is causing an altering threat to eelgrass by increased water temperatures, run-off, eutrophication, and particle pollution (Orth et al., 2006).

Environmental contamination often ends up in the coastal seabed due to run-off and other sources from industrial activities (SFT, 2000). Hazardous substances like persistent organic pollutants (POPs) can remain in the environment for a long time and can therefore be harmful to marine organisms that live in these areas (Miljødirektoratet, 2016). Conventions like *European Water Framework Directive (EU, 2000)*, the *Marine Strategy Framework (EU, 2008)*, the *Convention for Protection of the Marine Environment of the North-East Atlantic (OSPAR, 1992)* and the *Helsinki Convention* (Helcom, 2014) laid the foundation for an action plan by the Norwegian Government to clean up contaminated seabed along the Norwegian coast (St.meld.nr.14, 2006-2007).

Common methods used for removing or containing contamination in the seabed include dredging and capping. Reducing the risk posed by contamination in coastal areas is important to improve water quality, ecological state and restore biodiversity (St.meld.nr.12, 2001-2002). Simultaneously, these methods are highly intrusive in a marine environment and disturb the benthic flora and fauna in the process (Sabol et al., 2005). In Horten, there is an ongoing harbour remediation project using dredging and capping in areas where eelgrass has been registered. Several studies have reported that the natural re-establishment of eelgrass often fails despite water quality improvements (Duarte et al., 2009; Leschen et al., 2010; Carstensen et al., 2011; Boström et al., 2014; Moksnes et al., 2018). This underlines the need for facilitating reestablishment of eelgrass after "improvement measures". Restoration of eelgrass could provide a faster return of biodiversity in these coastal areas where water quality improvement measures have been made (Orth et al., 2020). In Norway's present harbour remediation projects, the focus is mainly on improvement of chemical water quality, and the biological aspect is not specifically included.

To include a key biological aspect in harbour remediation projects in areas supporting or suitable for eelgrass, there is a need for knowledge about the substrate types suitable for both capping and facilitating good conditions for eelgrass reestablishment afterwards. Several studies have reported a variation in eelgrass growth and survival based on sediment type (Van Katwijk & Hermus, 2000; Jung-Im & Kun-Seop, 2003; Van Katwijk & Wijgergangs, 2004; Jarvis & Moore, 2014; Zhang et al., 2015). Results from these studies show that sediment grain size and content of silt and clay are important for the survival of eelgrass. Furthermore, a higher content of silt and clay support greater growth rates for eelgrass after transplanting (Jung-Im & Kun-Seop, 2003; Zhang et al., 2015). In addition, the stability of the eelgrass root system has proven to be higher in sediment types containing smaller fractions (Van Katwijk & Hermus, 2000). Lower root stability is linked to lower survival rates, as the plants are less resilient to physical disturbance in the environment (Van Katwijk & Hermus, 2000). Thus, to optimize substrate types used in Norwegian capping projects towards the successful colonization by eelgrass, more information on sediment grain size, fraction and silt content is necessary.

Several transplanting methods have been developed with various results of success in eelgrass restoration projects (Eriander et al., 2016). Therefore, the choice of transplant method is also an important factor for eelgrass growth and survival in connection with restoration. Transplanting single shoots with the horizontal rhizome method is reported to facilitate restoration success in numerous studies (Orth et al., 1999; Jung-Im & Kun-Seop, 2003; Leschen et al., 2010; Eriander et al., 2016). Simultaneously, a study by van Keulen and co-workers (2003) show that the unit size of the plants plays a vital role in the survival of transplanted seagrass. By planting patches of individual plants still clung together within some substrate, the survival rate is reported to be higher, as it supports higher root stabilization (Van Keulen et al., 2003). Nevertheless, the method of transplanting single plants is more widespread in the field of eelgrass restoration. Simultaneously, the method is more time-consuming and can therefore be more costly in larger restoration projects (Busch et al., 2010). To compare the two methods in both practicality and eelgrass reestablishment success, both methods were included in this study.

This master thesis aims to test the effects on eelgrass growth and survival in substrate types representing a difference in grain size, grain structure and silt/clay content (i.e., Natural, Machine and Control). In addition to test whether transplanting patches or single plants facilitate better eelgrass growth and survival. Time between transplanting and when the plants entered the water was registered to test whether this can affect the growth of transplanted eelgrass. Lastly, biodiversity (i.e., fish, starfish, and other organisms) associated with each substrate type was registered to test the correlation between eelgrass density and number of species. This generated the following hypotheses:

1. Natural substrate facilitates better growth and survival of eelgrass after transplanting.
2. Transplanting eelgrass in patches provides better survival compared to transplanting single plants.
3. More time in air will have a negative effect on growth and survival of the transplanted eelgrass.
4. Biodiversity will increase with the increasing density of eelgrass.

Entering the year of 2021, and the United Nations years of nature restoration (UN, 2019), this project can contribute as a knowledge base for more successful eelgrass restoration projects. In testing eelgrass growth and survival in connection with common substrate types used for capping, this study aims to contribute new knowledge towards restoring a key habitat for biological diversity along the Norwegian coast. Hopefully, this information can contribute to more cost-and time effective methods that also involve eelgrass restoration.

# 2. Methods

## 2.1 Study site

The study was conducted in the inner Horten harbour (Figure 1). Horten is located in the southeast of Norway in the county of Vestfold and Telemark. The area outside Bromsjordet was used as the donor site (59°26’09.6” N 10°29’15.2” E), and the experiment site was placed outside the peninsula of Vealøs (59°25’25.4” N 10°28’28.6” E) (Figure 1).

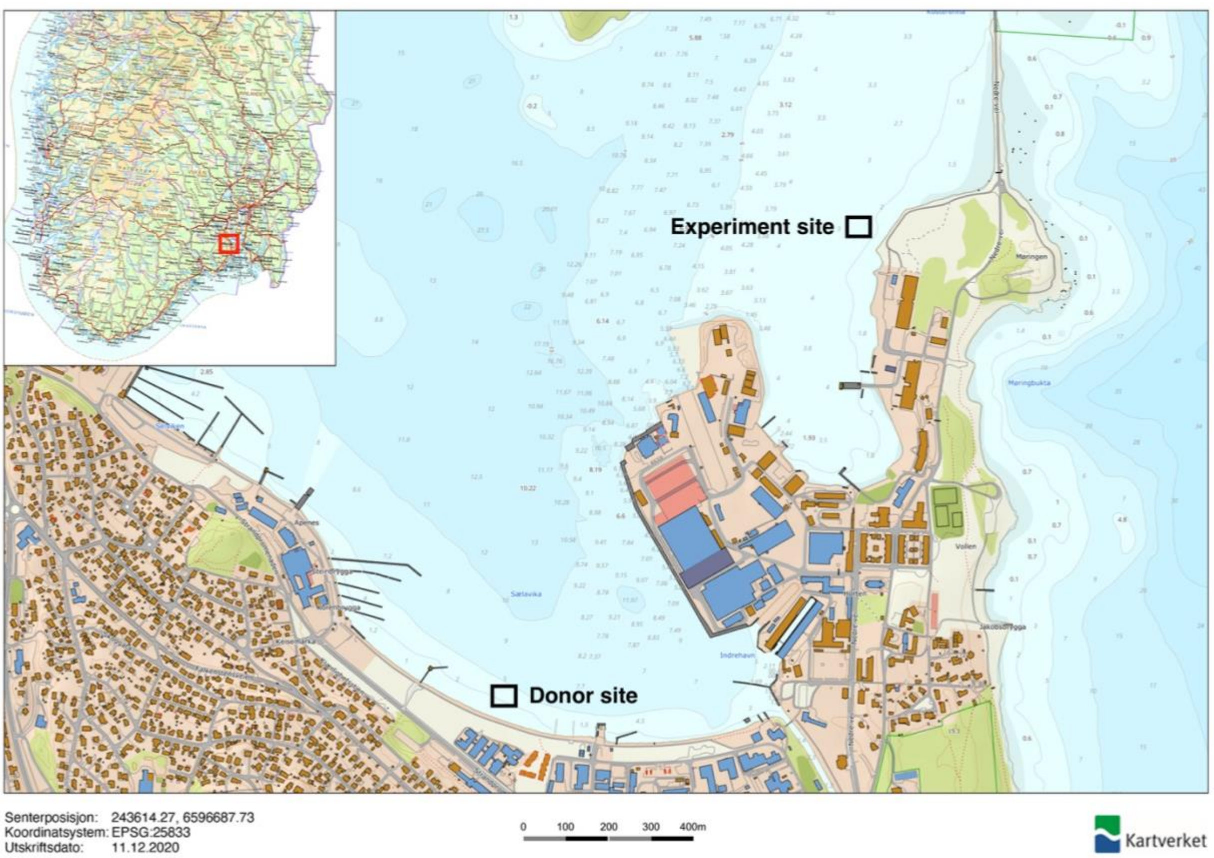



**Figure 1**: Map of Horten inner harbour. The donor site and the experiment site are marked with a square (Kartverket, 2021).

The background for the choice of this area was the ongoing capping project to reduce contamination in the sediments within Horten. The project is called “Ren Indre Havn” or “Clean inner harbour”. The contamination in Horten inner Harbour is caused by earlier industrial activities, run off from landfills and urban discharge, resulting in high levels of contaminants in the seabed (Forsvarsbygg, 2000; NGI, 2016). During a five-year period, the contaminant levels in the sediments have been mapped in detail, plans for dredging and disposal of contaminated sediments was conducted, and capping of the seabed were designed (NGI, 2016). Dredging and capping activities occurred from November 2019 to December 2020, and turbidity was continuously measured during the dredging, disposal and capping to prevent large spread of pollution. Several eelgrass meadows have been registered in the area, both from *Naturbase* (see section 2.5) and during fieldwork for this study. Therefore, it was a good location to test reestablishment of eelgrass in connection with the harbour remediation project in Horten.

## 2.2 Study species

Two species of eelgrass inhabit the Norwegian waters. The more common *Zostera marina* and the rare *Zostera noltii* (Lid & Lid, 2005). Eelgrass in Norway can form large underwater meadows on sand or mud bottom in shallow sea areas all over the Norwegian coast (Bekkeby et al., 2011). Eelgrass are perennial angiosperms and can reproduce both vegetative (asexual) and by seed dispersal (sexual) depending on their environmental conditions (Phillips et al., 1983). The plant has horizontal rhizomes with many small roots that function both as an anchor to the bottom sediments and for nutrient uptake (Phillips et al., 1983). From the rhizome, the plant can reproduce vegetative by shooting new, genetically identical individuals (Lid & Lid, 2005). Sexual reproduction happens as some shoots develop flowers, both female and male, that pollinate through seed dispersal (Lid & Lid, 2005). The pollination season is from April to September (Lid & Lid, 2005).

Eelgrass thrive in water temperatures below $25^0$ C. Increased water temperatures, exceeding $25^0$ C, result in lower eelgrass growth rates or plant mortality (Orth & Moore, 1982; Hammer et al., 2018). Scandinavian eelgrass prefers water temperatures around $10\text{-}20^0$ C for optimal growth (Nejrup & Pedersen, 2008). The leaves can be from 15-100 cm long and the plant thrives from 2-10 meters depth depending on light availability (Lid & Lid, 2005).

As eelgrass depends on photosynthesis to grow, turbidity and sedimentation can affect eelgrass growth and survival by reducing the light availability (Moore & Orth, 1996; Saunders et al., 2017). The minimum light requirements for eelgrass, expressed as percentage of surface irradiance (% SI), lie between 9.6 (% SI) to 35.7 (% SI), with an average of 20.4 (% SI) (Kun-Seop et al., 2007; Chao et al., 2021). Studies show that eelgrass can survive exceeded turbidity levels in shorter periods, but prolonged reduction in light availability will result in damage or loss of eelgrass (Giesen et al., 1990; Chao et al., 2021). According to a study on turbidity levels and light availability registered those values below 50 Nephelometric Turbidity Unit (NTU) did not affect eelgrass productivity negatively based on light availability (Chao et al., 2021).

## 2.3 Experiment setup

An *in-situ* experiment was conducted in Horten harbour summer 2020. The experimental setup included transplanting of eelgrass into three different test substrates: Natural, Machine and Control (detailed information about the substrates are presented in section 2.4). Six wooden crates were filled with the substrate types leaving two replicates for each substrate. Each wooden crate was further divided into two halves with a wooden plank in the middle. Ten single plants were transplanted on one side of the plot and about six patches of eelgrass (24-42 plants) on the other side (Figure 2). The plants were collected from a donor site in Horten harbour, transplanted into the substrate types and transported to the experiment site. The wooden crates were placed at approximately 3 m depth under water. The eelgrass was observed monthly throughout the summer season from April to September. At the end of the experiment, growth and survival was measured to test which substrate and transplanting technique was more optimal for reestablishment of eelgrass.

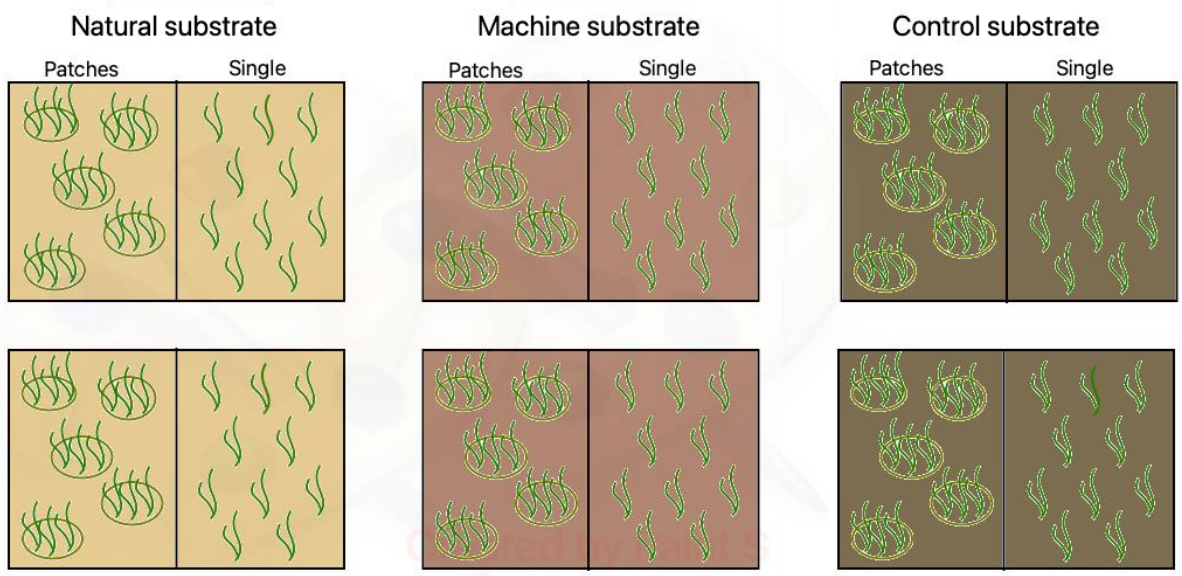



**Figure 2:** Illustration of the experimental setup in Horten harbour. Two wooden crates allocating each of the three substrate types. From the left: The Natural substrate, the Machine substrate, and the Control substrate. Patches of eelgrass were transplanted on one side and single plants on the other side.

## 2.4 Materials

The substrates were 0-8 mm crushed mineral material (Machine substrate), 0-8 mm Natural sand (Natural substrate) and Control substrate collected from Horten harbour at the donor site. The Natural and Machine substrates were the same materials used for capping in the harbour remediation project in Horten (COWI, 2020). These substrate types were chosen for this experiment because they were used in the remediation, but also because they were expected to be suitable for eelgrass reestablishment. The substrates were approved for capping by NGIs design and according to requirements set by the guideline M-411 from the Norwegian Environmental Agency (Miljødirektoratet, 2017). The criteria for approval are based on the content of total organic carbon (TOC), metals, organic environmental contaminants in the material, and grain size of capped seabed and materials in the cap (Miljødirektoratet, 2017; NGI, 2020).

The Natural substrate was collected from Svelviksand AS located in Hurum. The material is extracted from a natural sand deposit and undergoes no other processing than sieving. It was stored on-site before being transported by boat to Horten inner harbour (NGI, 2020). The Natural substrate contains grains of granite, gneiss, silt, sand, and clay and has a d50 value of 0.9 mm, meaning that 50% of the grains are smaller than 0.9 mm (Appendix A-1). The grains are cubic, and the cones are round-edged (Appendix A-1 (NGI, 2020).

The Machine substrate was produced by Veidekke Industri AS dep. Skoppum pukkverk located in Skoppum. The petrographic examination of the Machine substrate shows a 100% content of the rock rhombic porphyry. The main difference from the Natural substrate is the fraction structure and size. The material is made of crushed rock deposits, and the grain structure is 71% cubic sharp-edged, 29% chipped and 0% round edged (NBTL, 2018). The purpose of the petrographic examination is to document the aggregate according to the Norwegian Product Standards. The standard used in this specific test was NS-EN 932-3 (NBTL, 2018). The d50 value of the Machine substrate is 2.5 mm (Appendix A-2).

The Control substrate was analysed by NIVA in 2011, prior to the remediation project in Horten. The sediments were described as grey silty sediments from 0-5 cm deep with a thin brown top layer. Below 5 cm, the sediments changed to grey clay (NIVA, 2011). Sediment texture analysis of samples (0-5 cm deep) showed a content of 66,66% silt, 25,87% sand and 7,48% clay in the Control substrate with a d50 value of 0.058 (Appendix A-3).

### 2.5 Fieldwork

The fieldwork included inspection of the donor and experimental site. The next step was collection and transplantation of eelgrass into the test substrates and placing the wooden crates at the experimental site. The last step in the fieldwork included registrations through the summer and data collection at the end of the experiment.

As this project wanted to test different substrate types, the substrates needed to be within a confined space. Therefore, six wooden crates were built prior to the fieldwork. The wooden crates were built with the diameter of 1.2 x 0.8 x 0.4 m. Two wooden crates were built for each of the three substrate types. Sustainable materials were used to prevent further pollution into the harbour. Planks without preservative treatment, biodegradable ropes and fabric made from jute.

## Inspection of the sites

The registered eelgrass in Horten inner harbour indicates a value A – very important, with a total area of 870 000 m$^2$ according to *Naturbase* (Naturbase, 2020). Outside Bromsjordet, an eelgrass meadow was mapped 06-09-2007 (Figure 3). The meadow was registered as "Large eelgrass meadow with middle to high density vegetation of *Zostera marina"*.

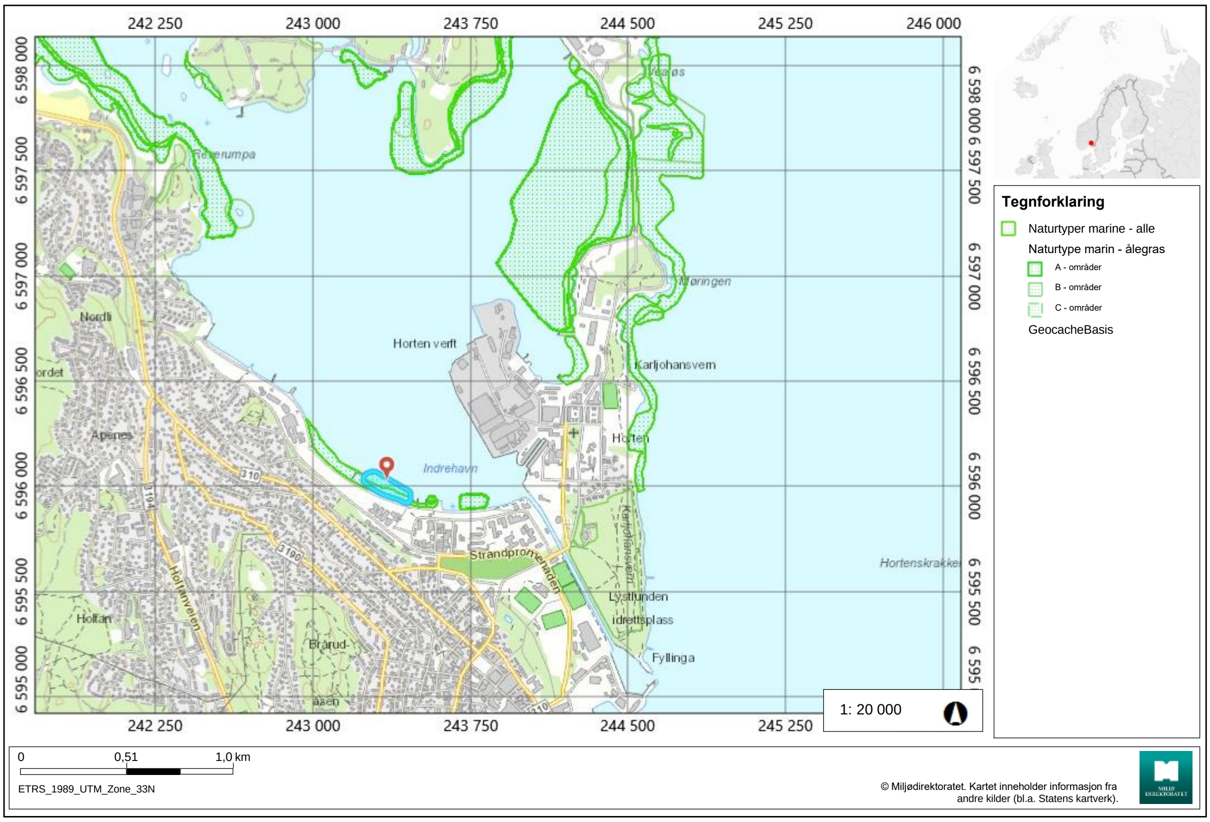



**Figure 3:** Eelgrass meadow registered from *Naturbase* in 2007 marked with a blue circle and a red dot. ID BM00041822 (Naturbase, 2020).

This meadow was mapped again on April 14, 2020, with snorkelling and drop camera. The registration showed a well-established eelgrass meadow with a medium to high-density vegetation from 0-2 meters depth (Appendix A-4). Due to the density of the meadow, this area was chosen as the donor site. In addition, extraction of 5,76 $m^2$ of plants from this area was assumed not to affect the meadow negatively.

To ensure a suitable site for the experiment, the site was also inspected by snorkelling and with a Go-pro camera. The site was a shallow area with scattered eelgrass meadows. The occurrence of eelgrass confirmed that conditions for eelgrass growth existed at the site. Less dense meadows could indicate that the experiment site was more wave exposed than the donor site. Both wave exposure and light availability affect eelgrass growth as mentioned above. Therefore, it was decided to place the crates at approximately 3 meters depth to avoid too much disturbance from waves, while ensuring light availability for the plants.

### Transplantation of eelgrass

The transplanting took place on April 17 and April 27, 2020, shortly after receiving permission from the county governor in Vestfold and Telemark (FMVT) to extract plants from the donor site (Appendix A-10).

The methods used for transplanting eelgrass (single vs. patches) was instructed by biologist Håkon Gregersen from Norconsult, Norway's largest multidisciplinary consultancy firm in the Nordic region (Norconsult, 2021). The techniques are described in the Swedish manual on restoration of nature (Moksnes et al., 2016).

Eelgrass was collected from the seabed by divers from the diver company "*Anleggsdykk*". The divers were instructed to remove plants with around 15 cm of sediments with a shovel to ensure intact roots. The plants were placed into a steel box on the seafloor and the box was lifted onto the boat. From there, representative plants were picked out, rinsed, and planted by hand 2-5 cm into the experimental plots. Ten single plants with approximately 20 cm length, with rhizomes around 10 cm were chosen. The rhizome was placed horizontally into the sediment with the shoot pointing upwards to simulate natural growth (Figure 4). Patches were planted in the other half of each plot by moving intact groups of 4-7 individual plants, with some remaining substrate holding them together, on top of the test substrate. The patches were a size of about 20 $cm^2$ with 5-10 cm of sediment following. Photos from the transplanting day are shown in Figure 4.


**Figure 4:** A) The six wooden crates onboard the boat. B) Transplantation of single eelgrass plants. C) Eelgrass patches (left half of plot) and single plants (right half of plot) after transplanting. D) The wooden crate lowered into the sea by the onboard crane.

The following parameters were registered during transplanting: Time of transplanting (TimeP), time of when the crates enter the water (TimeW), calculated time in air (TIA), number of plants (single plants and patches), height of leaves (single plants) and comments (Table 1). The TIA was recorded between transplantation and when the crates were lowered into the water at the experimental site. It was assumed that higher amounts of TIA can cause a higher disturbance level for the plants like dehydration. The relationship between TIA and growth were analysed to test the influence on eelgrass using this method. Due to the short timeframe for this experiment, height of plants in the patches were not registered. Therefore, the transplant method was evaluated visually based on videos from the underwater drone.

**Table 1:** Registration during transplanting. TimeP = time planted, TimeW = time when entering the water, TIA = time in air.

| Date | Plot | Substrate | TimeP | TimeW | TIA | Number | Height | Comments |
|---|---|---|---|---|---|---|---|---|
| 17.04 | NT1 | Natural | 15.10 | 15.30 | 20 | 24-42 | Not registered | Strong sun and wind. Air temperature: 9,7 Co, water temperature: 6,5 Co, wind: 3 m/s. Air humidity: 27%, precipitation: 0 mm. Dehydration problems for the plants |
| 17.04 | NE1 | Natural | 13.48 | 15.30 | 102 | 10 | 20 | |
| 17.04 | NT2 | Natural | 14.39 | 15.45 | 66 | 24-42 | Not registered | |
| 17.04 | NE2 | Natural | 14.08 | 15.45 | 97 | 10 | 20 | |
| 17.04 | MT1 | Machine | 14.24 | 15.52 | 88 | 24-42 | Not registered | |
| 17.04 | ME1 | Machine | 14.10 | 15.52 | 102 | 10 | 20 | |
| 17.04 | MT2 | Machine | 14.24 | 15.49 | 85 | 24-42 | Not registered | |
| 17.04 | ME2 | Machine | 14.09 | 15.49 | 100 | 10 | 20 | |
| 27.04 | KT1 | Control | 13.15 | 15.45 | 150 | 24-42 | Not registered | Rainy and cold<br>Air temperature: 6,4 C°, water temperature: 8,5 C°, wind: 8,1 m/s. Air humidity: 91%, precipitation: 10,6 mm<br>No dehydration of the plants |
| 27.04 | KE1 | Control | 13.30 | 15.45 | 135 | 10 | 20 | |
| 27.04 | KT2 | Control | 13.15 | 16.00 | 165 | 24-42 | Not registered | |
| 27.04 | KE2 | Control | 13.45 | 16.00 | 135 | 10 | 20 | |

The plots were transported to the experiment site by boat and lowered into the water by an onboard crane at approximately 3,5 meters depth (Appendix A-5). Before immersing, the plants were hydrated with seawater and the plots were marked with letters and numbers for identification. For example, Natural substrate, plot 1 was market with NE1 on the single plant side and NT1 on the side with patches. A buoy was attached to each plot with the number of plot and contact information. To inform the public and avoid potential damage to the site, a poster was also put up on shore with more detailed information about the project (Appendix A-6).

## Monitoring of environmental conditions

Two environmental factors known to affect eelgrass growth and survival are turbidity and temperature. These parameters were monitored during the summer.

Data on turbidity used in the present study was collected by PEAB for the "Ren Indre Havn" project, with a turbidity sensor. Turbidity was monitored as a condition in the permit from the FMVT (Appendix A-7). The trend through April showed low levels of turbidity ranging from 0 to 42.5 NTU with a mean value of 2.3 NTU (Appendix A-8). Some high values exceeding 200 NTU were registered. These values were removed from the dataset, as they most likely are errors caused by a foreign object blocking the sensor. As the turbidity device was used for measuring turbidity from the harbour remediation project, the registrations were affected by this and could not be used for showing the trends in turbidity at the experiment site. Due to lack of data from the rest of the summer, the turbidity trend is based on registrations from April. That said, the permit from FMVT included a stop of the dredging and capping work with turbidity levels exceeding 15 NTU (Appendix A-7). As the project did not have any stops during the summer, assumptions can be made that turbidity levels did not exceed 15 NTU. In addition, did the harbour remediation project use 1 NTU as the background value for Horten inner harbour over the whole period (Størdal, 2021). As registrations show low levels of turbidity below 50 NTU, one can conclude that the registered levels of 0-8 NTU during daytime did not affect light availability for the transplanted eelgrass (see section 2.2) (Appendix A-8).

Air/water temperature was collected from a weather station in Horten harbour. The data are published on the website *hortenhavn.no* (Hortenhavn, 2020). Air temperature was collected as an average for each day, and water temperature was collected at noon each day. Trends in mean air and water temperatures from April 17 to September 29 in Horten inner harbour show air temperature varied between $4^0$ C - $23,6^0$ C and the water temperature data varied between $6,1^0$ C – $22,5^0$ C with an average of $15,38^0$ C (Appendix A-9). Therefore, the trend in temperatures showed values within the range of not causing stress for the plants in the experiment.

## 2.6 Data collection

The season's growth and survival data used in the analyses was collected on September 29, 2020, by a diver. Each plot was observed monthly from April to September using the underwater drone Chasing GO1 Mini. Video uptakes from the underwater drone were used to collect information about transplant method (single vs. patches), algae growth and fauna associated with the transplanted eelgrass. The fieldwork took place on a boat anchored in the middle of the wooden plots. The underwater drone was controlled from the boat using a handheld control. All data was collected *in situ* to avoid damaging the plants and allow for future monitoring of the experiment.

Parameters to quantify eelgrass growth and survival were based on the biological quality elements for eelgrass in the guideline 02:2018 from The Water Directive framework (Appendix B-1) and various of studies referenced below. The guideline uses the parameters of depth index, plant density and amount of algae growth for measuring eelgrass condition (Directive, 2018). Some studies on eelgrass use the parameters of biomass (Huntington &

Boyer, 2008; Zhang et al., 2016), leaf area index (Eriander, 2017), shoot density (Moore & Orth, 1996; Eriander, 2017), number of plants (Nejrup & Pedersen, 2008) and leaf height (Huntington & Boyer, 2008) to quantify growth and survival. As the plants could not be harvested, biomass and leaf index were excluded from this experiment. In addition, depth index was not relevant to use as a parameter as the plants were in a confined space and at equal depths. Based on this, height of leaves, number of plants and amount of algae coverage (see below) were chosen as parameters for health, growth, and survival.

### Substrate type

Growth and survival in the different substrate types were measured using the parameters change in height of plants and change in number of plants, respectively. The change was calculated by measuring height and number of single plants on the transplanting day and again at the end of the experiment.

The data collection at the end of the experiment was done by a diver from *Anleggsdykk as* the underwater drone did not provide videos with adequate quality for this purpose. The diver used full divers' equipment, a dry suit, and an air tank. The diver was also filming with a handheld camera in addition to the GoPro attached to the helmet. Sampling included filming each plot, counting the number of plants, and measuring the length of leaves in the single plants-side of the plots (NE1, NE2, ME1, ME2, KE1, KE2). The registrations from the result collection day are listed in Appendix B-2.

### Transplant method

Data on eelgrass growth and survival based on transplant method was collected from videos by the underwater drone. The empiric data (height and number of plants) were not collected separately for each of the patches, as time did not allow for this in the field. The analysis of the two transplanting methods is therefore based on photos and semi-quantitative analysis of growth and survival by visually comparing the abundance of plants on each side of the plots.

### Algae growth

Algae growth was estimated by the videos from the underwater drone. To determine the amount of algae growth on eelgrass, classification from Guideline 02:2018 was used (Directive, 2018). The amount of algae growth on individual leaves was determined subjectively in both single transplanted plants and patches and registered in four different classes: 1 = Low amount, 2 = Scattered occurrence (coverage <15%), 3 = Normal occurrence (coverage 15%-50%), and 4 = dominating occurrence (coverage >50%) (Appendix A-11).

### Biodiversity

Biodiversity was estimated using the videos from the underwater drone. Biodiversity was only tested for substrate type, as sampling was done for each plot and not at the level of each half of each plot. Organisms observed were grouped into three: Fish, starfish, and other organisms. Other organisms were mainly different types of molluscs observed in the plots or on the eelgrass.

## 2.7 Statistical analysis

The statistical analysis of eelgrass growth and survival in substrate type is based on single transplanted plants. The analysis of biodiversity associated with substrate type are based on a combination of both single plants and patches. The analysis of TIA is based on both single plants and patches. No statistical analysis was performed on the comparison of transplant methods due to lack of empiric data.

The two replicates of each substrate as shown in Figure 2 were put together into one treatment variable in the analysis giving three levels (i.e., substrate type: Control, Natural and Machine). A one-way ANOVA was used to test which substrate type provides better conditions for "growth" and "survival" of transplanted single eelgrass. In this study, "growth" and "survival" relate to changes in height and number of single transplanted plants, respectively. It is assumed that results showing height less than the starting height (ca. 20 cm) indicate a "negative growth", as during the growing seasons, leaves can still be worn down, broken, grazed by herbivores or rot, and thus decrease in height unless growing well enough to overcome these challenges. Height greater than the start indicates a "positive growth" during the experimental period. Results showing less than 10 plants at the end of the experiment indicate a negative survival rate, and results showing more than 10 plants at the end of the experiment indicate a positive survival rate.

Similar analysis was used to compare the number of observed faunae among the substrate types for each species group separately (i.e., fish, starfish, and other organisms).

A two-way ANOVA was also used to compare the interaction of the transplanting method (single vs. patch) and substrate type (Natural, Machine and Control) in relation to TIA of eelgrass. This analysis helps to understand if more or less TIA indirectly affects eelgrass growth.

For all analyses described above, a post hoc test was conducted using Tukey-HSD to determine which specific treatment levels differed from each other. All statistical analyses were performed using R statistical computing environment, version 4.0.3 (Team, 2021).

## 3. Result

### 3.1 Effect of substrate type

#### Change in plant height

A significantly better growth was found for single plants of eelgrass transplanted in Natural (p=0.04) and Control (p=0.01) substrate compared to Machine substrate (Figure 5). However, there was no significant difference in growth for eelgrass transplanted in Natural compared to Control substrate (p=0.79) (Figure 5).

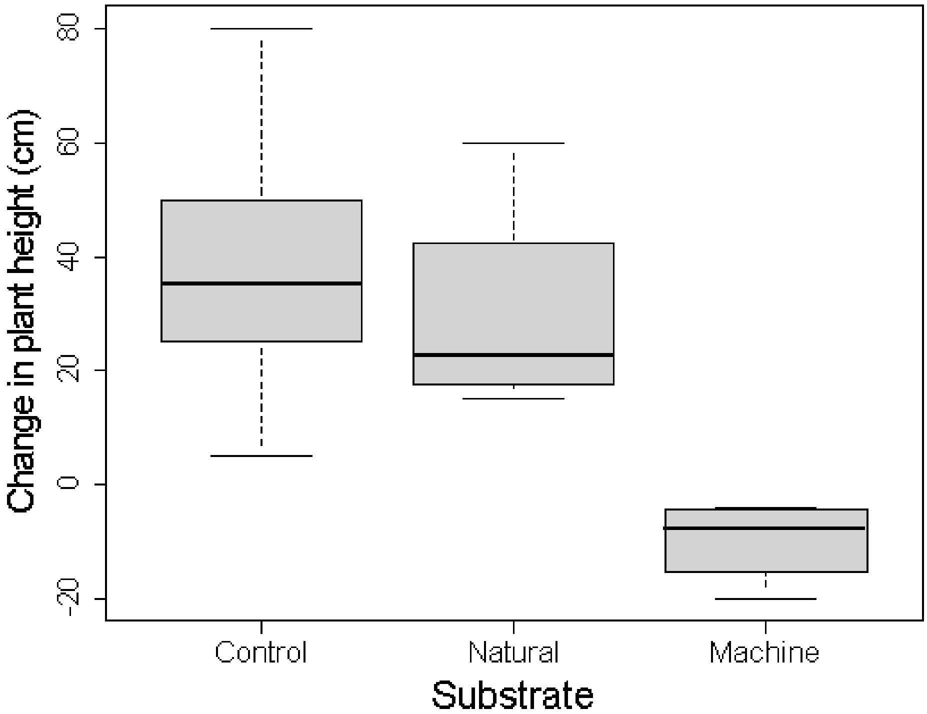



**Figure 5:** Change in eelgrass height for the three substrate types (Control, Natural and Machine). The grey zones are the interquartile range (IQR) and represent 25% of the dataset above (Q3) and under (Q1) the median value (the black horizontal line). And the whisker represents the values that fall outside the Q1 and Q3 (outliers) with the lowest and the highest values in each end. The vertical bars indicate a 95% confidence interval.

The height for plants ranged between 25-100 cm, 35-80 cm, and 9-16 cm for Control, Natural and Machine substrates, respectively (Appendix B-2). The upper quartile (Q3) of the "Control" box shows a value of 50 cm indicating that 75% of the plants had up to 50 cm change in height from April to September. The lower quartile (Q1) for the "Control" box shows a value of 23 cm indicating that 25% of the plants had up to 23 cm change in height from April to September. The Q3 for "Natural" shows a value of 42 cm, and a value of 19 cm for the Q1. The "Machine" box has a Q3 value of -3 cm and a Q1 value of -18. The negative number represents a negative change in plant height, i.e., the plant height decreased in the period April to September. Plants in both Natural and Control substrates had positive change in plant height. Plants in the Control substrate had the largest positive change in individual plant height relative to the other substrates, although the analysis shows no significant difference between Natural and Control substrates (Figure 5).

Figure 5 shows a median value of 38.2 cm for the "Control" box and the mean value is 35 cm indicating a non-normal distributed dataset as the mean and the median values are different. The median value for the "Natural" box is 30 cm and the mean value is 22.5 cm. The median value for "Machine" is -10 cm and the mean value is -8 cm.

### Change in plant number

Plant number showed no significant difference between single plants transplanted in Natural (5 plants) and Machine (4 plants) substrate ($p=0.99$) (Figure 6). Eelgrass transplanted into the Control (24 plants) substrate showed the highest change in number of plants, significantly more than Natural ($p <0.01$) and Machine substrate ($p <0.01$).

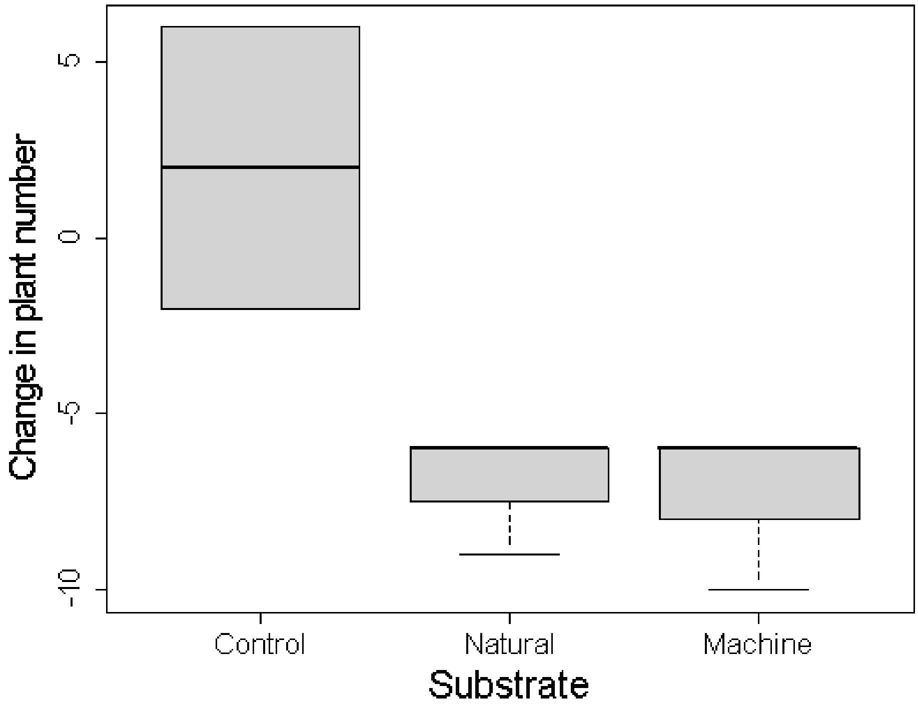



**Figure 6:** Change in eelgrass number for the three substrate types (Control, Natural and Machine). The grey zones are the interquartile range (IQR) and represent 25% of the dataset above (Q3) and under (Q1) the median value (the black horizontal line). And the whisker represents the values that fall outside the Q1 and Q3 (outliers) with the lowest and the highest values in each end. The vertical bars indicate a 95% confidence interval.

Figure 6 shows a mean and a median value of 2 plants for the "Control" box. The initial number of plants were 20 plants in the two plots in April and 24 plants in September, indicating a change of two additional plants in each plot during the experiment period.

In this dataset, the mean and the median are the same, indicating a normal distributed dataset. The mean and median value for "Natural" is -7.5 as the plant number decreased from 20 to 5 plants in the two plots from April to September. The mean and median value for "Machine" is -8 as the plant number decreased from 20 to 4 plants. The negative median values illustrate a decrease in number of plants during the experiment.

## 3.2 Effect of transplant method

Growth and survival for single transplanted plants vs. transplanted patches are illustrated in Table 2 (Machine), 3 (Natural), and 4 (Control) below. The photos are a representative selection from April, July, August, and September.

Photos from April show eelgrass directly after transplanting. The difference in number of plants is visible with less plants in the single-plants side of the crates compared to the patches. The substrate following the patches in the transplanting are also visible in the photos. Photos from July show more healthy green leaves for both single plants and patches compared to photos from April. A higher plant density is observed in the plots allocating the Control substrate (Table 4) for both patches and single plants compared to plants planted in the Machine (Table 2) and Natural substrate (Table 3).

In the photos from August, a continuous high plant density can be observed for the patches for all substrate types. Photos of single transplanted plants in Machine substrate (Table 2) show low plant density. Single plants in Natural substrate (Table 3) show also low plant density, although more plants compared to plants in Machine substrate. Single plants in the Control substrate (Table 4) show the highest plant density. Some small fish can be observed in plots allocating Natural and Control substrate from both July and August.

Observations of the photos from September show low plant density for all the substrates and high plant mortality. The highest plant mortality rate was observed in plots with Natural and Machine substrate for both single plants and patches. For patches and single plants in Control substrate, a lower plant mortality rate can be observed, and a higher plant density. A lower visibility in photos from September indicates a high amount of particles in the water this day. Although, clear pits can be observed in plots with Natural and Machine substrate at the end of the experiment. In addition, a silt layer on the leaves can be observed on the plants in all substrate types.

Observations of the patches show a higher plant density in Control substrate throughout the experiment (i.e., August, July, and September) compared to the beginning (April). For patches in Natural and Machine substrate, the photos show a positive growth and survival for patches in July and August compared to the transplanting day in April.

**Table 2:** Single plants vs. patches of eelgrass in Machine substrate

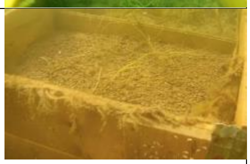
| | Machine 1 | Machine 2 |
|---|---|---|
| **April** Single plants |  |  |
| Patches |  |  |
| **July** Single plants |  |  |
| Patches |  |  |
| **August** Single plants |  |  |
| Patches |  |  |
| **September** Single plants |  |  |
| Patches |  |  |

**Table 3:** Single plants vs. patches of eelgrass in Natural substrate

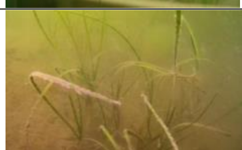
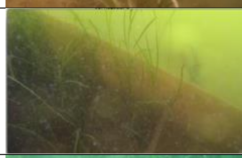
| | Natural 1 | Natural 2 |
|---|---|---|
| **April** Single plants |  |  |
| Patches |  |  |
| **July** Single plants |  |  |
| Patches |  |  |
| **August** Single plants |  |  |
| Patches |  |  |
| **September** Single plants |  |  |
| Patches |  |  |

**Table 4:** Single plants vs. patches of eelgrass in Control substrate

| | **Control 1** | **Control 2** |
|---|---|---|
| **April**<br><br>Single plants |  |  |
| Patches |  |  |
| **July**<br><br>Single plants |  |  |
| Patches |  |  |
| **August**<br><br>Single plants |  |  |
| Patches |  |  |
| **Septemb**<br><br>Single plants |  |  |
| Patches |  |  |

## 3.3 Algae growth

Results from the video uptake showed low algae growth on the eelgrass in all three substrate types for both patches and single transplanted plants. Therefore, the score value for algae growth was set to 1 (low or no algae growth) for all plots indicating healthy eelgrass (Appendix B-3).

### 3.4 Biodiversity

Quantified fauna associated with the different substrates (single and patches) varied between plots (Figure 7). The highest number of fish was observed during May to August in Natural and Control substrate, while Machine substrate had the lowest. A relatively higher number of starfish was observed in May and September for the Machine substrate compared to Natural. One starfish was observed in the Natural substrate in April, and no starfish observed in the Control substrate. Other organisms observed varied slightly in the different substrates. Associated with Machine substrate, three individuals of molluscs were observed, in Natural substrate one individual was observed, and in the Control substrate, one individual of mollusc was observed during the experimental period.

Total number of organisms observed also varied between the substrate types (i.e., Natural, Machine and Control) (Figure 7-D). For starfish and other organisms, there is no significant difference in the number of organisms among the three substrates (p= >0.05). A significantly higher (p= <0.05) number of fish was observed in the Natural and Control substrate compared to the Machine substrate, but no significant difference between Control Natural substrates (p= >0.05) (Figure 7-D).

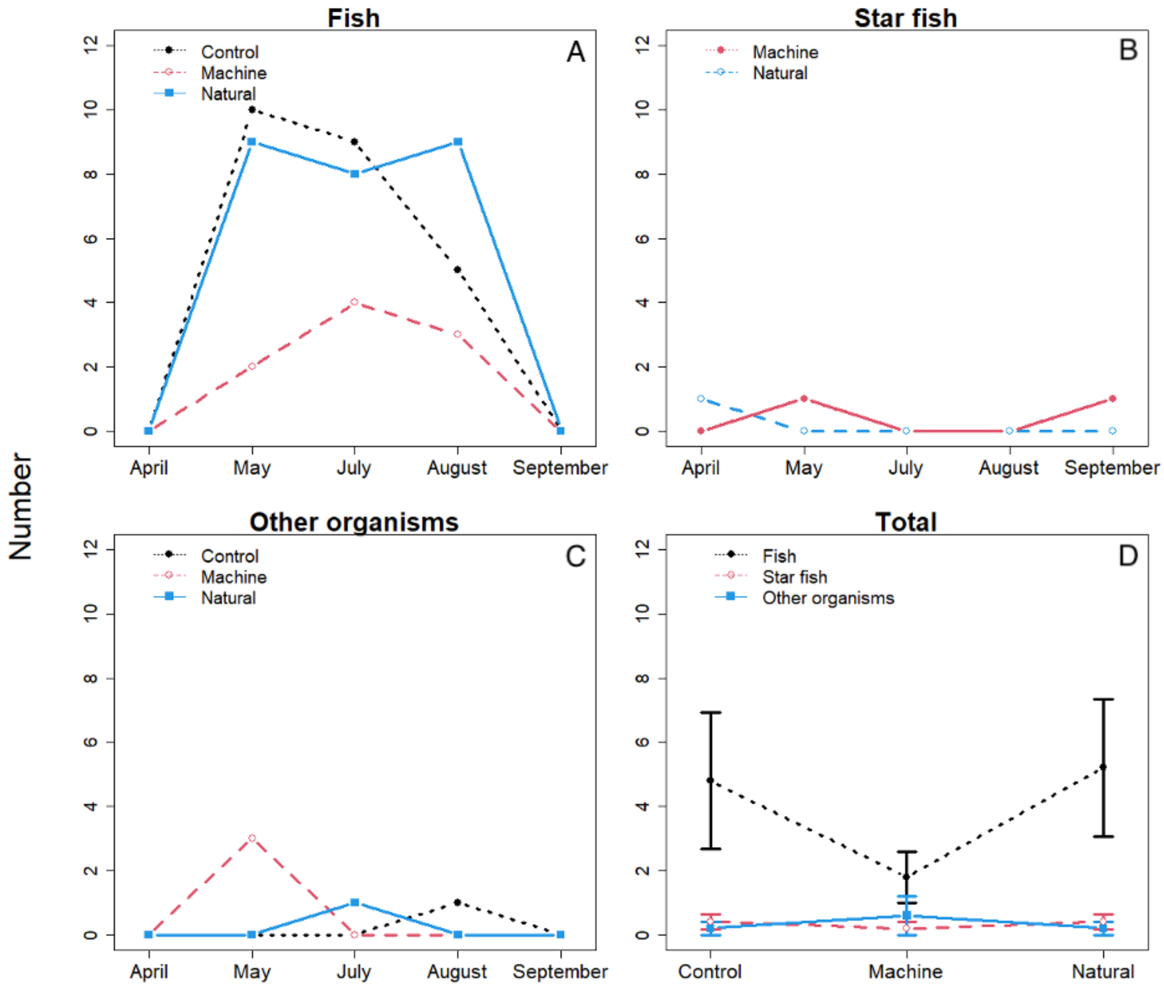



**Figure 7:** Biodiversity in eelgrass from April to September in the Natural, Machine and Control substrate. A) Number of fish. B) Number of starfish. C) Number of other organisms. D) Total number of organisms. The vertical bars represent the range in number (lowest to highest) and indicate 95% confidence interval. The point in the middle of the vertical bars represents the median value.

## 3.5 TIA and growth

The analysis for TIA shows more time spent in air for eelgrass transplanted (single and patches) in Control substrate compared to the Natural and the Machine substrates. The analysis shows that growth (change in height) increases with increased TIA, indicating a positive relationship between growth and TIA regardless of substrate type (Figure 8).

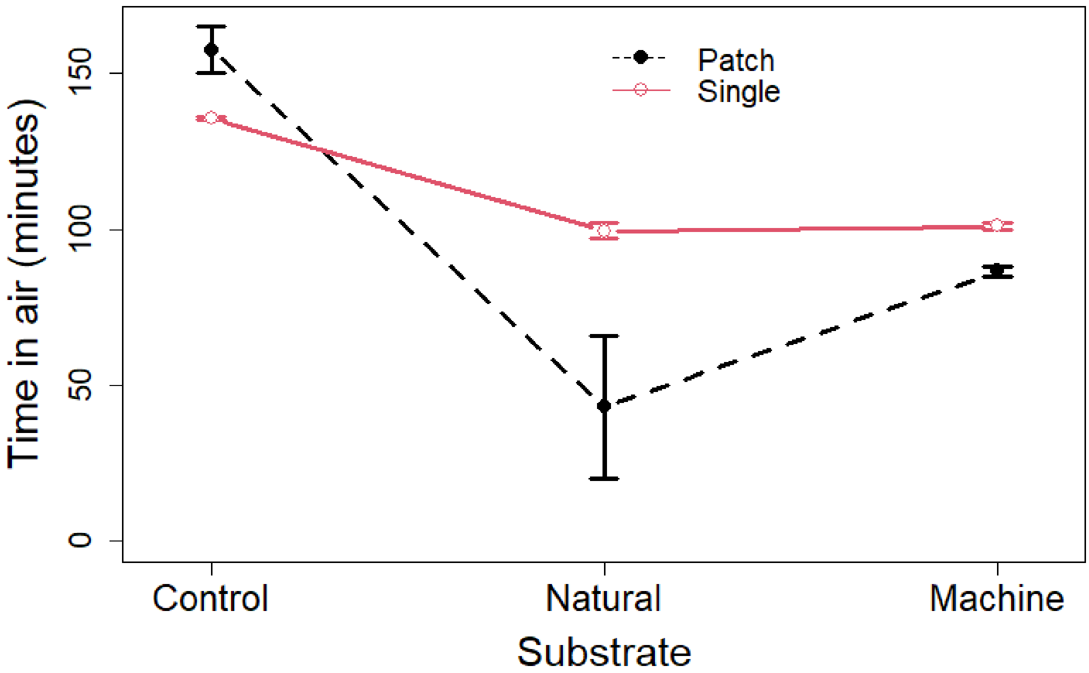



**Figure 8:** TIA in relation to substrates (Control, Machine and Natural) and transplanting method (patch vs. single). The vertical bars represent the range in time (lowest to highest) and indicate 95% confidence interval. The point in the middle of the vertical bars represents the median value.

TIA was found to be significantly lower ($p = <0.05$) for patches transplanted in the Natural substrate (43 min) compared to the Machine substrate (86,5 min) and the Control substrate (157,5 min). TIA for single plants was found to be significantly higher ($p = <0.05$) for plants in Control substrate (135 min) compared to both Natural (100 min) and Machine substrate (100 min) (Figure 8). No significant difference ($p = 0.56$) in TIA was found for single plants between Natural and Machine substrates. Plants in the Control substrate experienced overall most TIA for both patches and single transplanted plants.

# 4. Discussion

The aim of the current experiment was to investigate eelgrass growth and survival in three various substrate types using two different transplanting methods. The initial hypotheses were whether natural substrate enables better growth and survival for eelgrass after transplantation. Whether transplantation patches of eelgrass provide better survival compared to single plants was visually evaluated. This study was also able to include a practical aspect of transplanting onboard a boat, namely higher TIA induced higher growth rates of transplanted eelgrass. Importantly, and according to expectations for this important habitat type, a higher number of species was linked to higher densities of eelgrass, even though the scale was very small. Overall, there was better growth in Natural and Control substrate compared to Machine substrate, supporting the initial hypotheses.

## 4.1 Effect of substrate type

The present study conducted in Horten inner harbour shows a significant difference in eelgrass growth and survival for the substrate types (Figure 5 and 6). These results indicate that the type of substrate plays a vital role for eelgrass growth and survival. The substrate types used in the present study represent a variety of silt/clay content in addition to grain size and structure. The Control substrate contains 74,14% of silt and clay. The exact percentage of silt and clay content was not tested for in the Natural and Machine substrate. The petrographic analysis shows less silt/clay content in the Natural substrate compared to the Control substrate. The Machine substrate contains the lowest amount of silt/clay. The highest growth and survival found in the Control and Natural substrate, would indicate that substrate types containing more silt and clay have a positive effect on eelgrass growth and survival. Previous studies also show that substrate types containing a higher percentage of silt and clay, around 75%, facilitate better eelgrass growth and survival (Jung-Im & Kun-Seop, 2003; Van Katwijk & Wijgergangs, 2004; Zhang et al., 2015). Therefore, substrate types containing around 75% silt and clay may facilitate good growth conditions, and substrate types containing more or less than 75% may facilitate lower growth conditions.

The grain size also varied between the three substrate types. The smallest grain size was found in the Control substrate (d50= 0.058 mm), followed by Natural substrate (d50= 0.9 mm), and then Machine substrate (d50= 2.5 mm). The grain size in the substrate has also been shown to affect microbial activity, with positive correlation between grain size and absence of bacteria in the substrate (Zhang et al., 1998). Results in this experiment suggest that the presence of bacteria also affects the growth of eelgrass. The Machine substrate contained the largest grains in addition to the lowest content of silt and clay. This combination has been shown to cause a higher exchange of porewater associated with a lower nutrient content in the substrates (Haluna et al., 2002). Thus, lower growth and survival in Machine substrate may be due to the large grain size causing low microbial activity in addition to low availability of nutrients in the substrate. Based on this, it is suggested that the Machine substrate provided too low growing conditions for the plants to overcome the natural challenges of grazing, and other disturbances in the environment which resulted in the negative change in plant number and height (Figure 5 and 6).

In reestablishment projects, the plants can experience a “shock” in the first period after transplanting due to conditional changes in the environment. Some studies show that after the transplanting shock, the eelgrass shoot density slowly increases as the plants establish into the new substrates (Jung-Im & Kun-Seop, 2003; Fishman et al., 2004). One explanation for the reduced growth and survival of plants transplanted into Machine and Natural substrate compared to plants in the Control substrate is that they may have experienced a higher transplant shock due to major changes in environmental conditions.

The experiment site was a shallow and wave exposed area (Appendix A-5). At the end of the experiment, a few large storms occurred causing large pits in some of the plots with Natural and Machine substrate (Table 2, 3 and 4). The pits could have occurred due to the strong wave exposure in this period. High plant mortality can be observed in Table 2, 3 and 4 for plants in both the Machine and the Natural substrate. This can be explained by the location of these plots or by the grain size and structure. The plots could have been placed in more exposed areas causing higher disturbance for the plants. On the other hand, the substrate could contain a grain size facilitating lower plant root stability. With lower root stability, the plants are less resilient to disturbance in the environment like wave exposure (Van Katwijk & Hermus, 2000). Based on the theory of grain size and root stability, assumptions can be made that the Control substrate would facilitate higher root stability in the time of the storm events as a higher plant survival was found in this substrate.

On the practical side of the experiment, the three substrate types had a large variety in texture due to the substrate composition as discussed above. The texture of the Natural substrate made it easier for transplanting and to shape and pack around the roots. The Control substrate with its high content of silt and clay made the substrate compact and hard to transplant into. This could also be explained by the fact that this substrate was wet during transplanting. The Machine substrate had a porous texture that made it difficult not to damage the roots when planting. Knowledge about textures of the substrates and the suitability for transplanting is another factor that can provide better guidance for eelgrass reestablishment.

Importantly, the difference between the three substrate types is not only the substrate composition and grain size, but also the grain structure (Appendix A-1, A-2, A-3). The Natural substrate contains round edged grains, and the Machine substrate contains mostly sharp-edged grains, and none of the grains were round edged, as it is made of crushed rock deposit. There is no analysis of the grain structure of the Control substrate. It is still reasonable to suggest that it contains round edged grain structure as the Control substrate originating from a natural deposit. The grain structure may cause an effect on the microbial activity in the substrates and furthermore affect the growth of eelgrass. As no research has been found to study this effect of grain structure, this is only speculation and further research is needed here.

## 4.2 Effect of transplant method

The technique of transplanting single plants was developed in 1996 by Orth and co-workers (Orth et al., 1999). It is a well-established method with a high success rate according to several studies on eelgrass restoration (Orth et al., 1999; Leschen et al., 2010; Eriander et al., 2016). Observations made from the photos presented in Table 2, 3 and 4 show a higher survival rate for transplanted patches compared to single transplanted plants for all substrate types. This could simply be caused by the lower number of plants planted for single plants. About 24 - 42 plants were transplanted in total with the patches, and 10 single plants on the other side of the plot. The initial visual evaluation indicate that more plants have survived for the patches may be caused by this arbitrarily from the start. Still, a positive change in eelgrass density within the patches can be observed through the summer. More plants can be observed at the end of the experiment (August) compared to April indicating a positive growth and survival rate for eelgrass transplanted as patched. In September, a high mortality rate can be observed for all plots, although patches in the Control substrate show a higher eelgrass density compared to the beginning of the experiment.

Other explanations for a higher survival and growth observed for patches from Table 2, 3 and 4 could be due to the facilitation of better root stability. The patches were transplanted as an intact turf without tearing apart each plant. Approximately 10-15 cm of substrate also followed within each patch. This could result in higher root stability, as more plants were transplanted together. In addition, this method may have caused less disruption of the natural growth compared to transplanting single plants. This theory is supported by the study from Van Keulen and co-workers (Van Keulen et al., 2003). With the natural substrate following, the patches also could have experienced less of a transplanting shock. The single plants got transplanted by tearing apart each plant, rinsing off all original substrate and transplanted some centimetres apart from each other. This could result in lower growth and survival for the single plants.

TIA for the transplanted single plants showed a positive correlation between "time" and "growth" (Figure 8). The single plants experienced more TIA compared to transplanted patches as the single plants were transplanted before the patches and the plots entered the water at the same time. Both single plants and patches transplanted in the Control substrate experienced overall most TIA (Figure 8) and the highest growth and survival (Figure 5 and 6). With more time spent in air between transplanting and when the eelgrass was placed in the sea, one would assume a negative impact on the growth and survival of the plant, as eelgrass are water plants. Therefore, there are other factors that might be more important for regulating growth after transplantation. On the first day of transplanting into Natural and Machine substrate, the weather was sunny and windy compared to the second day when transplanting into Control substrate. The weather on this day was rainy and cold. In addition, the test substrates (Natural and Machine) were dry at the time of transplanting compared to the Control substrate as this substrate was collected from the seafloor. These conditions may have caused higher dehydration of the plants in the test substrates. The plants in the Control substrate did not experience dehydration, and this may have been a more contributing factor for growth and survival compared to TIA. However, this hypothesis needs further research.

Other studies have added an additional step in the transplantation scheme by keeping the plants in bags or coolers with seawater before transplanting (Davis & Short, 1997; Jung-Im & Kun-Seop, 2003). This keeps the plant alive for longer. However, it is also more work and more time consuming. By using the method of direct transplanting, the method is simplified.

On the other hand, by using the method of direct transplanting, more transplanters are needed in the transplantation step that can result in a higher cost. Suggestions here is to use volunteers or students to assist in the transplanting to save time, and to share knowledge about the importance of restoration of nature.

### 4.3 Eelgrass and biodiversity

Biodiversity data showed a high number of juvenile fish, a few molluscs, and a few starfish observed within the wooden crates. The total number of observed species was higher within eelgrass transplanted in Natural and Control substrate (Figure 7). A higher number of observed species in the substrate types associated with higher eelgrass density confirms the positive correlation between biodiversity and eelgrass survival and growth. Several studies on successful eelgrass reestablishment projects report a return of marine species and ecosystems (Orth et al., 2020). In other words, a successful eelgrass reestablishment leads to a more rapid recovery of marine ecosystems.

The highest number of fish observed from the videos was in May, and July. Although, a high number of fish can also be observed in July. In April and September, no fish was observed (Figure 7). As the diver did the data collection in the beginning (April) and the end of the experiment (September), it is assumed that the reason for this distribution of results is due to the presence of the diver affecting the presence of the fish. Therefore, to collect data on pelagic species associated with eelgrass, an underwater drone might be preferred in addition to using one method to ensure consistency in the results.

The number of juvenile fish observed from Figure 7 showed many individuals within the small area of eelgrass in the plots (0,96 $m^2$). Up to 10 individuals were observed in one wooden crate within 5 minutes of video. To observe this quantity of fish in the small area of eelgrass that this experiment represents suggests that even small-scale eelgrass meadows play an important role in supporting biodiversity. In a study by NIVA (NIVA, 2014). The biological diversity was found to be great for both high density and low-density eelgrass meadows. From a management perspective, the present experiment shows that it is important to protect and reestablish both large and small-scale eelgrass meadows to sustain marine biodiversity.

## 4.4 Critic of the methodology

This experiment took place *in situ*. Therefore, the experiment site was chosen due to the low disturbance level. In addition, information on buoys and a poster on land was created to minimize disturbance from by-passers. Still, some boat traffic did occur. This could have caused disturbance in the experiment and resulted in a lower success rate compared to an area with no boat traffic. That said, disturbances will also be found in natural eelgrass meadows. Therefore, the transplanted eelgrass should tolerate some level of disturbance to represent a realistic reestablishment.

The availability of light can also cause stress for the transplanted eelgrass. Therefore, measures to ensure light availability for the transplanted eelgrass were done prior to and during the experiment. The evaluations of the experiment site attempted to optimize light availability and at the same time avoid high wave exposure. Still, by moving plants growing in 1-2 meters depth to 3,5 meters depth, reduction in light availability will occur as the depth increases. This could be a factor affecting the restoration success rate, yet not this experiment, as all plots were at the same depth. A Swedish study (Eriander, 2017) transplanted eelgrass from a shallow donor meadow with hight light availability to an area with low light availability and found an adaptation for the plant into the new environment when exposed to environmental conditions with less light. The results from Table 2, 3 and 4 show healthy, growing eelgrass after a few months, especially for the patches, indicating that the transplanted eelgrass in Horten harbour did adjust to environmental conditions with less light availability. This information is useful when choosing donor sites and transplanting sites in future restoration projects.

Monitoring of turbidity was also done as measure to ensure light availability for the transplanted eelgrass. The monitoring showed low turbidity levels (Appendix A-8). Nevertheless, photos in Table 2 (Machine substrate), 3 (Natural substrate) and 4 (Control substrate) show a particle layer on the leaves in the end of the experiment. A particle layer on the leaves can affect the photosynthesis by reduction in light reaching the leaf surface (Chao et al., 2021). Towards the end of the project, when capping was performed, the turbidity is normally at its highest. The observed particle layer confirms the suspension of increased particle load towards the end of the experiment. Increased particle load may also be due to erosion of seabed from storm events. Erosion of the seabed may also be caused by heavy boat traffic. Therefore, it is suggested to consider low disturbance levels, turbidity and wave exposure when choosing experimental sites in future experiments.

Transplanting patches of eelgrass was performed by several people at the same time, as this method was easier to perform compared to transplanting single plants. Single plants needed to be transplanted by one person as the method were more complicated. Transplanting single plants is therefore more time consuming, which can lead to a higher cost in large-scale restoration projects. Thus, the method of transplanting patches should result in more cost-efficient restoration projects. Simultaneously, by using this method, the contaminated seabed also gets transferred with the plants. This restoration experiment was of such small scale that the polluted substrate following each patch was assumed to not cause an impact on contaminant levels at the experiment site. On the other side, this method could cause problems with pollution in large-scale restoration projects in polluted harbour areas.

Using the underwater drone Chasing GO1 Mini to register data was time efficient and a low-cost method compared to using a diver. Especially for collecting data on biodiversity, algae growth and for comparing the two transplant methods. The drone was easy to maneuverer, although the drone did cause some damage to the plants. Additionally, the video quality was not sufficient to estimate length of leaves and number of plants. The quality varied also with turbidity; high turbidity provided videos with little information. For these reasons, the final data collection in September was done by a diver. Note, the diver had no experience working with underwater plants, nor scientific experiments like this, so the accuracy of the results registered by the diver might have been suboptimal.

Working in the field, everything does not always go as planned. Therefore, empiric data on transplant method was not collected. A semi-quantitative method, using videos from the underwater drone, was the only method available for comparison of the two transplantation techniques. Future research should obviously collect empiric data for testing transplanting methods.

Lastly, critique can be aimed at the duration of the project. Restoration of marine ecosystems have been shown to take between five and twenty-five years (Borja et al., 2010). The timeframe of a master thesis was too short to observe the long-time effects of the restoration. Continuing the experiment for several seasons will likely provide more time for the transplanted eelgrass to establish into the new environment and overcome the first transplanting shock. That again will give more reliable results on the growth and survival rate of transplanted eelgrass in the different substrates.

## 5. Conclusion

Physical disturbance in the coastal marine environment caused by human activities result in loss of important ecosystems. The focus to improve water quality as a measure to bring back marine life falls short, as the methods of dredging and capping of contaminated seabed causes further disturbance of important habitats like eelgrass. Also, the natural reestablishment of eelgrass often fails despite improvement of water quality. Therefore, the need for facilitating eelgrass reestablishment after such physical disturbance is crucial for the future of coastal biodiversity, especially in light of “harbour remediation projects”.

This study showed that the Natural substrate facilitated more successful growth rates for eelgrass compared to the Machine substrate, but not a significantly higher number of single plants. The method of transplanting patches resulted in higher growth and survival of transplanted eelgrass compared to single transplanted plants, regardless of substrate type, yet this was not empirically tested. TIA showed a positive correlation between TIA and growth. Although further research is needed whether TIA influences growth or not, as several other factors may have played a more vital role.

The Control substrate facilitated the highest growth and survival of the three substrate types used in this experiment. This may be explained by the substrate composition i.e., silt and clay content in addition to grain size. Substrate types more suitable for eelgrass reestablishment in general are finer substrate types with a high silt/clay content. A greater transplanting shock may have caused lower growth and survival for eelgrass transplanted in Natural and Machine substrate compared to the Control substrate because of differences in the physical properties of the substrate types.

Continuing the experiment for a longer period can provide more reliable results as the eelgrass establishes into the new environment. For similar remediation projects like the one in Horten harbour, natural substrate such as sand from Svelviksand AS is recommended to use in the upper layers of capping when eelgrass reestablishment is the desired outcome, as this substrate type facilitates higher growth and survival compared to the Machine substrate.

From a management perspective, it is important to protect and reestablish both large-, and small-scale eelgrass meadows to sustain marine biodiversity. Biodiversity observations showed higher abundance in eelgrass transplanted in Natural and Control substrates. This confirms a positive correlation between biodiversity and eelgrass survival and growth. Hopefully, this information can provide insight into cost-and time-effective remedies in future eelgrass restoration projects when the substrate type and transplant method influence eelgrass survival and growth.

# 6. References


Asmala, E., Gustafsson, C., Krause-Jensen, D., Norkko, A., Reader, H., Staehr, P. & Carstensen, J. (2019). Role of Eelgrass in the Coastal Filter of Contrasting Baltic Sea Environments. *Estuaries and Coast*, 42 (1): 1882-1895. doi: https://doi.org/10.1007/s12237-019-00615-0.

Baden, S., Gullström, M., Lundén, B., Pihl, L. & Rosenberg, R. (2003). Vanishing Seagrass (*Zostera marina, L.*) in Swedish Coastal Waters. *Ambio*, 32 (5): 374-377. doi: 10.1579/0044-7447-32.5.374.

Bekkeby, T., Bodvin, T., Fridjof, E. M., Bøe, R., Olsen, H. & Rinde, E. (2011). *Nasjonalt program for kartlegging og overvåking av biologisk mangfold - marint. Sluttrapport for perioden 2007-2010.* Rapport L.NR. 6105-2011: NIVA, Havforskningsintituttet og NGU. Available at: https://niva.brage.unit.no/niva-xmlui/handle/11250/215287 (accessed: 14.01.2020).

Borja, Á., Dauer, D. M., Elliott, M. & Simenstad, C. A. (2010). Medium- and Long-term Recovery of Estuarine and Coastal Ecosystems: Patterns, Rates and Restoration Effectiveness. *Estuaries and Coast*, 33 (6): 1249–1260. doi: 10.1007/s12237-010-9347-5.

Boström, C. & Bonsdorff, E. (1997). Community structure and spatial variation of benthic invertebrates associated with *Zostera marina* (L.) beds in the northern Baltic Sea. *Journal of Sea Research*, 37 (1-2): 153-166. doi: 10.1016/S1385-1101(96)00007-X.

Boström, C., Baden, S., Bockelmann, A., Dromph, K., Fredriksen, S., Gaustafsson, C., Krause-Jensen, D., Møller, T., Laurentius Nielsen, S., Olesen, B., et al. (2014). Distribution, structure and function of Nordic eelgrass (*Zostera marina*) ecosystems: implications for coastal management and conservation. *Aquatic conservation: Marine and freshwater ecosystems*, 24 (3): 410–434. doi: 10.1002/aqc.2424.

Busch, K. E., Golden, R. R., Parham, T. A., Karrh, L. P., Lewandowski, M. J. & Naylor, M. D. (2010). Large-Scale *Zostera marina* (eelgrass) Restoration in Chesapeake Bay, Maryland, USA. Part I: A Comparison of Techniques and Associated Costs. *Restoration Ecology*, 18 (4): 490–500. doi: 10.1111/j.1526-100X.2010.00690.x.

Carstensen, J., Sanchez-Camacho, M., Duarte, C. M., Krause-Jensen, D. & Marba, N. (2011). Connecting the Dots: Responses of Coastal Ecosystems to Changing Nutrient Concentrations. *Environmental Science & Technology*, 45 (21): 9122-9132. doi: dx.doi.org/10.1021/es202351y.

Chao, L., Yan-Hao, Z., Xiao-Xiao, W., Yu-Shan, J., Wen-Tao, L. & Pei-Dong, Z. (2021). Changes in survival and growth in response to different combinations of turbidity and duration in eelgrass *Zostera marina* plants. *Estuarine, Coastal and Shelf Science*, 249 (February 5): 107108. doi: https://doi.org/10.1016/j.ecss.2020.107108.

COWI. (2020). *Vurdering av egenhet av erosjonssikringsmasser fra Skoppum pukkverk etter M-411*. NOT 02. Available at: http://projects.cowiportal.com/ps/A126650/Documents/Externals/03 Prosjektdokumenter/Vurdering av tildekkingsmasser/Skoppum/NOT002_Skoppum_Vurdering av tildekkingsmasser_v02.docx (accessed: 26.10.2020).

Davis, R. C. & Short, F. T. (1997). Restoring eelgrass, *Zostera marina L.,* habitat using a new transplanting technique: The horizontal rhizome method. *Aquatic Botany*, 59 (1-2): 1-15. doi: 10.1016/S0304-3770(97)00034-X.

Directive, T. W. F. (2018). *Klassifisering av miljøtilstand i vann. Økologisk og kjemisk klassifiseringssystem for kystvann, grunnvann, innsjøer og elver*. Veileder 02:2018. Vann fra fjell til fjord. Available at: https://www.vannportalen.no/veiledning/klassifiserings/.

Duarte, C. M. (2002). The future of seagrass meadows. *Environmental Conservation*, 29 (2): 192–206. doi: 10.1017/S0376892902000127.

Duarte, C. M. & Middelburg, J. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeoscinces discussions*, 2 (1): 1-8. doi: https://doi.org/10.5194/bg-2-1-2005.

Duarte, C. M., Conley, D. J., Carstensen, J. & Sánchez-Camacho, M. (2009). Return to Neverland: Shifting Baselines Affect Eutrophication Restoration Targets. *Estuaries and Coasts*, 32 (1): 29–36. doi: 10.1007/s12237-008-9111-2.

Duarte, C. M., Kennedy, H., Marbà, N. & Hendriks, I. (2011). Assessing the capacity of seagrass meadows for carbon burial: Current limitations and future strategies. *Ocean & Coastal Management*, 83: 1-7. doi: 10.1016/j.ocecoaman.2011.09.001.

Eriander, L., Infantes, E., Olofsson, M., Olsen, J. L. & Moksnes, P.-O. (2016). Assessing methods for restoration of eelgrass (*Zostera marina L.*) in a cold temperate region. *Journal of Experimental Marine Biology and Ecology*, 479 (June 2016): 76–88. doi: http://dx.doi.org/10.1016/j.jembe.2016.03.005.

Eriander, L. (2017). Light requirements for successful restoration of eelgrass (*Zostera marina L.*) in a high latitude environment – Acclimatization, growth and carbohydrate storage. *Journal of Experimental Marine Biology and Ecology*, 496 (C): 37-48. doi: http://dx.doi.org/10.1016/j.jembe.2017.07.010.

EU. (2000). *Establishing a framework for Community action in the field of water policy*. Directive 2000/60/EC of 23 October 2000: The European Parliament and the Council of the European Union. Available at: http://data.europa.eu/eli/dir/2000/60/2014-11-20 (accessed: 22.11.2020).

EU. (2008). *Establishing a framework for community action in the field of marine environmental policy*. Marine Strategy framework Directive 2008/56/EC of june 17: The European Parliament and the Council of the European Union. Available at: http://data.europa.eu/eli/dir/2008/56/oj (accessed: 18.01.2020).

Fishman, J. R., Orth, R. J., Marion, S. & Bieri, J. (2004). A Comparative Test of Mechanized and Manual Transplanting of Eelgrass, *Zostera marina*, in Chesapeake Bay. *Restoration Ecology*, 12 (2): 214-219. doi: https://doi.org/10.1111/j.1061-2971.2004.00314.x.

Forsvarsbygg. (2000). *Kartlegging av forurensing i indre havn, Horten*. In Johnsen, A. & Ljønes, M. (eds). FFI/RAPPORT-2000/02206: Forsvarets forskningsinstitutt. Available at: https://www.horten.kommune.no/_f/p1/ib5144b1f-550c-48fe-9fc1-5a837e69264c/2000-kartlegging-av-forurensning-i-indre-havn-horten-utgitt-av-ffi1.pdf (accessed: 07.10.2020).

Fredriksen, S., Christie, H. & Sæthre, B. A. (2005). Species richness in macroalgae and macrofauna assemblages on *Fucus serratus L.* (Phaeophyceae) and *Zostera marina L.* (Angiospermae) in Skagerrak, Norway. *Marine Biology Research*, 1 (1): 2-19. doi: 10.1080/17451000510018953.

Gacia, E. & Duarte, C. M. (2001). Sediment Retention by a Mediterranean Posidonia oceanica Meadow: The Balance between Deposition and Resuspension. *Estuarine, Coastal and Shelf Science*, 52 (4): 505–514. doi: 10.1006/ecss.2000.0753.

García, V., Ignacio, B. & Diaz-Tapia, P. (2019). Biodiversity of epiphytic macroalgae (Chlorophyta, Ochrophyta, and Rhodophyta) on leaves of *Zostera marina* in the northwestern Iberian Peninsula. *Anales del Jardín Botánico de Madrid*, 76 (1): e078. doi: https://doi.org/10.3989/ajbm.2502.

Giesen, B. J. T. W., Van Katwijk, M. M. & Den Hartog, C. (1990). Eelgrass condition and turbidity in the Dutch Wadden Sea. *Aquatic Botany*, 37 (1): 71-85. doi: https://doi.org/10.1016/0304-3770(90)90065-S.

Green, E. P. & Short, F. T. (2003). *World Atlas of seagrasses*. UNEP World Conservation Monitoring Centre: University of California Press, Berkeley, USA.

Haluna, Z., Terrados, J., Borum, J., Kamp-Nielsen, L., Duarte, C. M. & Fortes, M. D. (2002). Experimental evaluation of the effects of siltation-derived changes in sediment conditions on the Philippine seagrass *Cymodocea rotundata*. *Journal of Experimental Marine Biology and Ecology*, 279 (1-2): 73-87. doi: https://doi.org/10.1016/S0022-0981(02)00366-0.

Hammer, K. J., Borum, J., Hasler-Sheetal, H., Shields, E. C., Sand-Jensen, K. & Moore, K. A. (2018). High temperatures cause reduced growth, plant death and metabolic changes in eelgrass *Zostera marina*. *Marine Biology Research*, 604 (October 2018): 121-132. doi: https://doi.org/10.3354/meps12740.

Helcom. (2014). *Convention on the protection of the marine environment of the baltic sea area, 1992*. Helsinki Convention. Available at: https://helcom.fi/about-us/convention/ (accessed: 18.01.2021).

Hortenhavn. (2020). *Webkamera*. Available at: https://www.hortenhavn.no/webkamera/horten/#indreHavn (accessed: 14.04.2020).

Huges, J., E. , Deegan, L., A. , Wyda, J., C., Weaver, M., J.  & Wright, A. (2002). The Effects of Eelgrass Habitat Loss on Estuarine Fish Communities of Southern New England. *Estuaries and Coast*, 25 (2): 235-249. doi: 10.1007/BF02691311.

Huntington, B., E.  & Boyer, K., E. (2008). Effects of red macroalgal (Gracilariopsis sp.) abundance on eelgrass *Zostera marina* in Tomales Bay, California, USA. *Marine Biology Research Series*, 367 (1): 133-142. doi: 10.3354/meps07506.

Jarvis, J. C. & Moore, K. A. (2014). Effects of Seed Source, Sediment Type, and Burial Depth on Mixed-Annual and Perennial *Zostera marina L.* Seed Germination and Seedling Establishment. *Estuaries and Coasts*, 38 (3): 964–978. doi: 10.1007/s12237-014-9869-3.

Jung-Im, P. & Kun-Seop, L. (2003). Site-specific success of three transplanting methods and the effect of planting time on the establishment of *Zostera marina* transplants. *Marine Pollution Bulletin*, 54 (8): 1238–1248. doi: 10.1016/j.marpolbul.2007.03.020.

Jørgensen, N. M. & Bekkby, T. (2013). Historical and present distribution of *Zostera marina* in the high north (Troms County, northern Norway) – a decline over the last century. *Botanica Marina*, 56 (5-6): 425–430. doi: 10.1515/bot-2013-0040.

Kartverket. (2021). *Norgeskart utskrift*. Horten: Kartverket. Available at: https://www.norgeskart.no/#!?project=norgeskart&layers=1002&zoom=12&lat=6596928.54&lon=243405.09 (accessed: 18.01.2021).

Kennedy, H., Beggins, J., Duarte, C. M., Fourqurean, J. W., Holmer, M., Marbà, N. & Middelburg, J. J. (2010). Seagrass sediments as a global carbon sink: Isotopic constraints. *Global Biogeochem*, 24 (4). doi: 10.1029/2010GB003848.

Kun-Seop, L., Sang Rul, P. & Young Kyun, K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology and Ecology*, 350 (1-2): 144-175. doi: 10.1016/j.jembe.2007.06.016.

Leschen, A. S., Ford, K. H. & Evans, N. T. (2010). Successful Eelgrass (*Zostera marina*) Restoration in a Formerly Eutrophic Estuary (Boston Harbor) Supports the Use of a Multifaceted Watershed Approach to Mitigating Eelgrass Loss. *Estuaries and Coasts*, 33 (6): 1340–1354. doi: 10.1007/s12237-010-9272-7.

Lid, J. & Lid, D. T. (2005). *Norsk Flora*. 7. utgave ed.: Det norske samlaget.

Livernois, M., Grabowski, J., Poray, A., Gouhier, T., Huges, A. R., O´Brien, K., Yeager, L. & Fodrie, F. J. (2017). Effects of habitat fragmentation on *Zostera marina* seed distribution. *Aquatic Botany*, 142 (September 2017): 1-9. doi: http://dx.doi.org/10.1016/j.aquabot.2017.05.006.

Miljødirektoratet. (2016). *Persistente organiske miljøgifter (POPs) - faktaark*. M-571. Available at: https://www.miljodirektoratet.no/globalassets/publikasjoner/m571/m571.pdf (accessed: 11.11.2020).

Miljødirektoratet. (2017). *Testprogram for tildekningsmasser - forurenset sjøbunn*. M-411. Available at: https://www.miljodirektoratet.no/publikasjoner/2016/september-2016/testprogram-for-tildekkingsmasser/ (accessed: 26.10.2020).

Moksnes, P.-O., Gipperth, L., Eriander, L., Laas, K., Cole, S. & Infantes, E. (2016). *Handbok för restaurering av ålgräs i Sverige – Vägledning*. Havs- och vattenmyndigheten rapport 2016:9. Available at: https://www.gu.se/sites/default/files/2020-06/1586990_handbok---lgr--srestaurering.pdf (accessed: 06.11.2020).

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 Coast. *Estuaries and Coasts*, 41 (6): 1712–1731. doi: https://doi.org/10.1007/s12237-018-0382-y.

Moore, K. A. & Orth, R. J. (1996). *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. *Marine ecology progress series*, 142 (1): 247-259. doi: 10.3354/meps142247.

Naturbase. (2020). *Faktaark*. Available at: https://faktaark.naturbase.no/?id=BM00041822 (accessed: 07.10.2020).

NBTL. (2018). *Tilslagsprøving iht. NS-EN standarder. 0-4 mm fra Skoppum Pukkverk.* Prøvingsrapport P 18167A: Norsk betong- og tilslagslaboratorium AS - Kontrollrådet.

Nejrup, L. B. & Pedersen, M. F. (2008). Effects of salinity and water temperature on the ecological performance of *Zostera marina*. *Aquatic Botany*, 88 (3): 239-246. doi: 10.1016/j.aquabot.2007.10.006.

NGI. (2016). *Helhetlig tiltaksplan for forurensede sedimenter i Horten Indre havn*. Horten indre havn - helhetlig tiltaksplan. DOK.NR. 20150201-01-R. Available at: https://www.horten.kommune.no/_f/p1/idc0e47d2-e754-426b-9da9-2d05c46cb65a/horten-indre-havn-helhetlig-tiltaksplan_rev11.pdf (accessed: 07.10.2020).

NGI. (2020). *Vurdering av 0-8 mm masser fra Svelviksand etter krav i M-411*. Horten Ren Indre havn - Miljøteknisk og geoteknisk oppfølging i anleggsfasen. Dok.Nr. 20190573-01-R: NGI (accessed: 26.10.2020).

NIVA. (2011). *Prøvetaking og kartlegging av miljøgifter i sjøsedimenter i Horten Indre Havn i 2011*. FBSE-2011/20. Available at:

https://www.horten.kommune.no/_f/p1/i0ec92d8e-ed8e-4d35-88cc-6b132290a62f/2011_proevetaking_og_kartlegging_av_miljoegifter_i_sjoesedimenter_i_horten_indre_havn_i_2011_utgitt_av_niva.pdf (accessed: 10.03.2021).

NIVA. (2014). *Hva bestemmer egenskaper og økologisk funksjon i ålegrasenger?* Rapport L.NR.6747-2014: Niva og Havforskningsinstituttet. Available at: https://imr.brage.unit.no/imr-xmlui/handle/11250/282947 (accessed: 16.03.2021).

Norconsult. (2021). *Om oss*. Available at: https://www.norconsult.no/om-oss/om-norconsult/.

Orth, R. J. & Moore, K. A. (1982). The Biology and Propagation of *Zostera marina*, Eelgrass, in the Chesapeake Bay, Virginia. *Special Reports in Applied Marine Science and Ocean Engineering (SRAMSOE)*, 265. doi: https://doi.org/10.21220/V5R443.

Orth, R. J., Harwell, M. C. & Fishman, J. R. (1999). A rapid and simple method for transplanting eelgrass using single, unanchored shoots. *Aquatic Botany*, 64 (1): 77-85. doi: https://doi.org/10.1016/S0304-3770(99)00007-8.

Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L. J., Hughes, R., Kendrick, G. A., Kenworthy, W. J., Olyarnik, S., et al. (2006). A global crisis for seagrass ecosystems. *BioScience*, 56 (12): 987-996. doi: 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Orth, R. J., Lefcheck, J. S., McGlathery, K. J., Aoki, L., Luckenbach, M. W., Moore, K. A., Oreska, M. P. J., Snyder, R., Wilcox, D. J. & Lusk, B. (2020). Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. *Science advances, applied ecology*, 6 (41): eabc6434. doi: 10.1126/sciadv.abc6434.

OSPAR. (1992). *Convention for the protection of the marine environment of the North-east Atlantic*. The Ospar commision. Available at: https://www.ospar.org/site/assets/files/1290/ospar_convention-1.pdf (accessed: 03.11.2020).

Phillips, R., Grant, W. & McRoy, P. (1983). Reproductive strategies of eelgrass (*Zostera marina L.*). *Aquatic Botany*, 16 (1): 1-20. doi: 10.1016/0304-3770(83)90047-5.

Reed, B. J. & Hovel, K. A. (2006). Seagrass habitat disturbance: how loss and fragmentation of eelgrass *Zostera marina* influences epifaunal abundance and diversity. *Marine ecology progress series*, 326 (1): 133-143. doi: 10.3354/meps326133.

Rehra, A. P., Williams, G. D., Tolimieri, N. & Levin, P. S. (2014). Impacts of Terrestrial and Shoreline Stressors on Eelgrass in Puget Sound: An Expert Elicitation. *Coastal management*, 42 (3): 246-262. doi: 10.1080/08920753.2014.904195.

Riera, R., Vasconcelos, J., Baden, S., Gerhardt, L., Sousa, R. & Infantes, E. (2020). Severe shifts of *Zostera marina* epifauna: Comparative study between 1997 and 2018 on the Swedish Skagerrak coast. *Marine Pollution Bulletin*, 158 (September 2020): 111434. doi: https://doi.org/10.1016/j.marpolbul.2020.111434.

Sabol, B., Shafer, D. J. & Lord, E. (2005). Dredging Effects on Eelgrass (*Zostera marina*) in a New England Small Boat Harbor. *Journal of marine environmental engineering*, 7 (1): 57-81. doi: https://doi.org/10.21236/ada438020.

Saunders, M. I., Atkinson, S., Klein, C. J., Weber, T. & Possingham, H. P. (2017). Increased sediment loads cause non-linear decreases in seagrass suitable habitat extent. *PLoS ONE*, 12 (11): e0187284. doi: https://doi.org/10.1371/journal.pone.0187284.

SFT. (2000). *Miljøgifter i norske fjorder - ambisjonsnivåer og strategi for arbeidet med forurenset sjøbunn*. TA1774. Available at: https://www.miljodirektoratet.no/publikasjoner/publikasjoner-fra-

klif/2000/desember/miljogifter-i-norske-fjorder--ambisjonsnivaer-og-strategi-for-arbeidet-med-forurenset-sjobunn/ (accessed: 11.11.2020).
Short, F. T. & Wyllie-Echeverria, S. (1995). Natural and human-induced disturbance of seagrasses. *Environmental Conservation*, 23 (1): 17-27 doi: https://doi.org/10.1017/S0376892900038212.
St.meld.nr.12. (2001-2002). *Rent og rikt hav*: Miljøverndepartementet. Available at: https://www.regjeringen.no/no/dokumenter/stmeld-nr-12-2001-2002-/id195387/?ch=1.
St.meld.nr.14. (2006-2007). *Sammen for et giftfritt miljø - forutsetninger for en tryggere fremtid*. Oslo: Miljøverndepartementet. Available at: https://www.regjeringen.no/no/dokumenter/Stmeld-nr-14-2006-2007-/id441267/?ch=13 (accessed: 11.11.2020).
Størdal, I. (2021). *Turbidity in Horten* (06.05.2021).
Team, R. C. (2021). *A language and environment for statistical computing*. Available at: https://www.R-project.org/ (accessed: 11.01.2021).
UN. (2019). *United Nations Decade on Ecosystem Restoration (2021–2030)*. Seventy-third session. Agenda item 14: United Nations. Available at: https://undocs.org/pdf?symbol=en/A/RES/73/284.
Van Katwijk, M. M. & Hermus, D. C. R. (2000). Effects of water dynamics on *Zostera marina*: transplantation experiments in the intertidal Dutch Wadden Sea. *Marine ecology progress series*, 208 (1): 107-118. doi: 10.3354/meps208107.
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. *Aquatic Botany*, 80 (1): 1–12. doi: 10.1016/j.aquabot.2004.04.003.
Van Keulen, M., Paling, I. E. & Walker, C. J. (2003). Effect of Planting Unit Size and Sediment Stabilization on Seagrass Transplants in Western Australia. *Restoration Ecology*, 11 (1): 50-55. doi: 10.1046/j.1526-100X.2003.00036.x.
Warren, M. A., Gregory, R. S., Laurel, B. J. & Snelgrove, P. V. R. (2010). Increasing density of juvenile Atlantic (Gadus morhua) and Greenland cod (G. ogac) in association with spatial expansion and recovery of eelgrass (*Zostera marina*) in a coastal nursery habitat. *Journal of Experimental Marine Biology and Ecology*, 394 (1): 154-160. doi: https://doi.org/10.1016/j.jembe.2010.08.011.
Waycott, M., Duarte, C. M., Carruthers, T., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L. J., Hughese, R. A., et al. (2008). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *PNAS*, 106 (30): 12377-12381. doi: https://doi.org/10.1073/pnas.0905620106.
Zhang, C., Palumbo, A. V., Phelps, T. J., Beauchamp, J. J., Brockman, F. J., Murray, C. J., Parsons, B. S. & Swift, D. J. P. (1998). Grain size and depth constraints on microbial variability in coastal plain subsurface sediments. *Geomicrobiology Journal*, 15 (3): 171-185. doi: https://doi.org/10.1080/01490459809378074.
Zhang, P.-D., Liu, Y. S., Guo, D., Li, W. T. & Zhang, Q. (2016). Seasonal Variation in Growth, Morphology, and Reproduction of Eelgrass *Zostera marina* on the Eastern Coast of the Shandong Peninsula, China. *Journal of Coastal Research*, 32 (2): 315-322. doi: https://doi.org/10.2112/JCOASTRES-D-14-00117.1.
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

experiment in Swan Lake on the eastern coast of Shandong Peninsula, China. *Aquatic Botany*, 122 (2015): 15-19. doi: http://dx.doi.org/10.1016/j.aquabot.2015.01.001.

# 7. Appendix

SVELVIKSAND

## Svelviksand AS

Fjordveien 21b
3490 Klokkarstua

32792300
hurum@svelviksand.no

Verket

1111-CPR-0028

Sikteanalyse, tørrsikt iht NS-EN 933-1

Nr: 19524 Uttak:15.01.2020 Registrert: 15.01.2020 14:44 Laborant: Runar Fagerli

Prøvenr:

Produkt: Støpesand 0/8 mm

Notat: Prøve tatt under lasting av Falksund til Horten Havn

Uttakssted: Båt

Kontakt: Svelviksand AS-Prøve

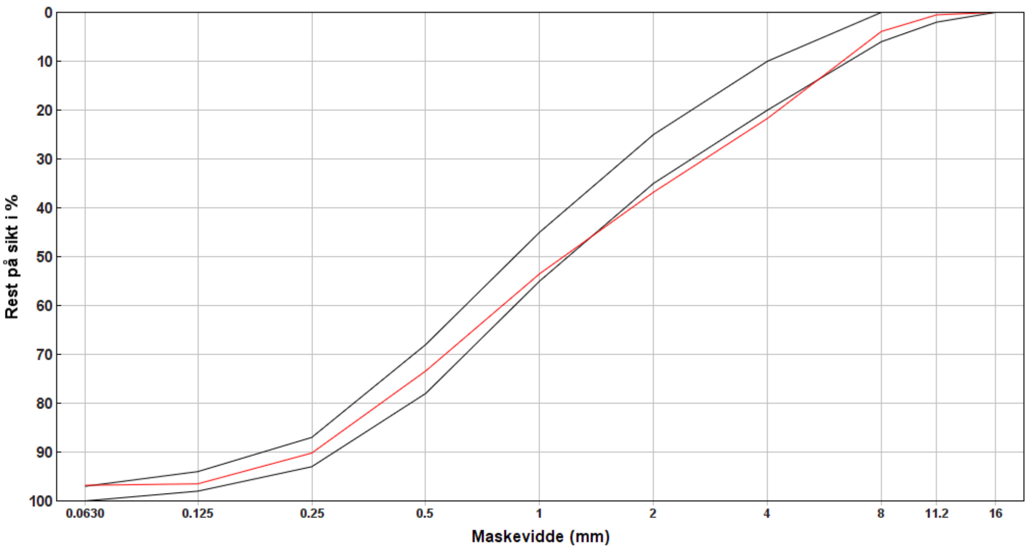
Vekt, fuktig: 899 gr.

FM: 3.28

Vekt, tørr: 865 gr.

Fuktighet: 3.93 %

| Maskevidde (mm) | bp | 0.0630 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 11.2 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rest på sikt (gr.) | 864 | 837 | 835 | 780 | 635 | 463 | 318 | 188 | 34 | 4 | 0 |
| Rest på sikt (%) | 99.88 | 96.76 | 96.53 | 90.17 | 73.41 | 53.53 | 36.76 | 21.73 | 3.93 | 0.46 | 0 |




Støpesand 0/8 mm er iht følgende standarder:
NS-EN 12620

QR koder til ytterligere dokumentasjon av produktet

Ytelseserklæring NS-EN12620

CE dokument NS-EN12620

labark

Labark Laboratoriesystem - www.labark.com

**Figure A-1:** Particle distribution of 0/8mm "støpesand", the Natural substrate, from Svelviksand AS.

# Korngradering

**Materialet tilregnes bruk etter standard: NS-EN 13242**

| | | | |
|---|---|---|---|
| Testet i henhold til | NS-EN 933-1 | Forekomst | **Skoppum** |
| Identifikasjon av prøven | **0/8 mm Ga 75** | Operatør | Johnsen |
| Dato mottatt/uttatt | 27.01.2020 | Dato utført | 31.01.2020 |
| Lab pr. nr. | Uke 5.2020 | Lastrada pr. nr. | 4 |
| Produktliste Produksjon | | Uttak i | Produksjon |
| Prosjekt | | Kunde | |
| Merknad | | | |
| Prøveuttak | | | |

Metode (angi)
- [x] Vasking og sikting
- [ ] Tørsikting

Total tørr masse $M_1$ = 941,1

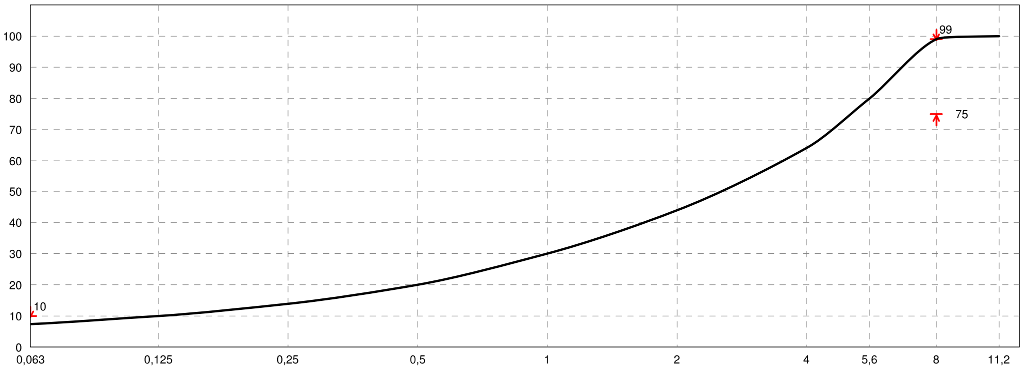
Tørr masse etter vasking $M_2$ = 872,8

Tørr masse av Finstoff fjernet ved vasking $M_1 - M_2$ = 68,3

Vanninnhold (%) NS 1097-5 = 3,6

| | | 941,1 | Gjennomgang | | |
|---|---|---|---|---|---|
| Sikteåpning (mm) | På sikt, akumulert (g) | Prosentandel (%) | Akumulert Prosentandel (%) | Min (%) | Max (%) |
| | | | | 100,0 | |
| 11,2 | | | 100 | | |
| 8 | 14,1 | 1,0 | 99 | 75,0 | 99,0 |
| 5,6 | 184,2 | 19,0 | 80 | | |
| 4 | 338,8 | 16,0 | 64 | | |
| 2 | 531,0 | 20,0 | 44 | | |
| 1 | 660,8 | 14,0 | 30 | | |
| 0,5 | 751,0 | 10,0 | 20 | | |
| 0,25 | 811,6 | 6,0 | 14 | | |
| 0,125 | 847,0 | 4,0 | 10 | | |
| 0,063 | 870,2 | 2,5 | 7,5 | | 10,0 |
| < 0,063 | 70,4 | 7,5 | | | |
| Sum | 940,6 | 100.0 | 0,1 | *< 1% Prosentsats materiale tapt* | |

Samfengt tilslag 0/8 Ga 75




| | |
|---|---|
| 31.01.2020 | [signature] |
| Dato | Signatur |

**Figure A-2:** Particle distribution of 0/8mm “Ga 75”, the Machine substrate, from Skoppum pukkverk.

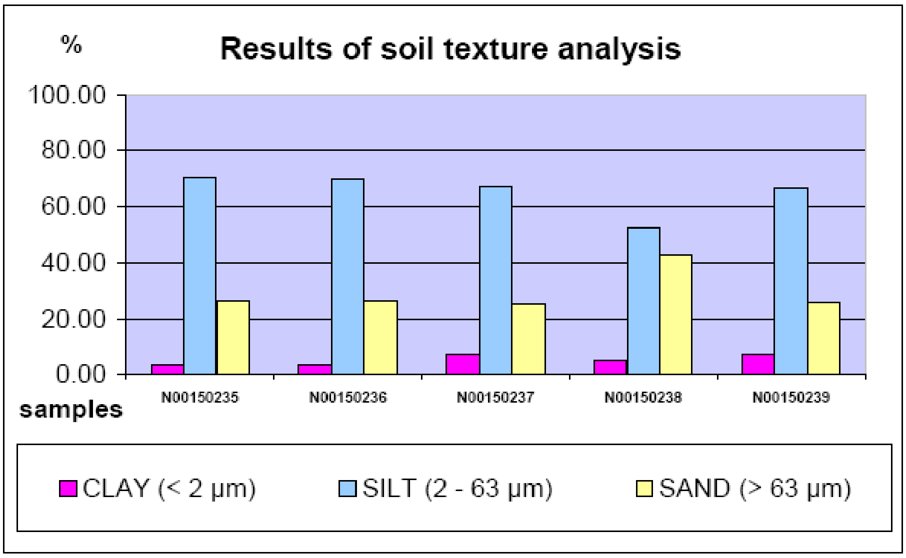
**ALS Laboratory Group**
ANALYTICAL CHEMISTRY & TESTING SERVICES

ALS Czech Republic, s.r.o., Na Harfě 336/9, 190 00 Praha 9

ALS Czech Republic, s.r.o., Laboratory Česká Lípa **Annex No. 2 to the Test Report No.: PR1122812**
Bendlova 1687/7, CZ-470 03 Česká Lípa, Czech Republic

## RESULTS OF SOIL TEXTURE ANALYSIS

| Sample label: | | N00150235 | N00150236 | N00150237 | N00150238 | N00150239 |
|---|---|---|---|---|---|---|
| Lab. ID: | | 029 | 030 | 031 | 032 | 033 |
| Gross sample weight | [g] | 18.15 | 19.35 | 13.76 | 18.97 | 11.89 |
| CLAY (< 2 µm) | [%] | 3.18 | 3.65 | 7.32 | 5.18 | 7.48 |
| SILT (2 - 63 µm) | [%] | 70.53 | 69.87 | 67.40 | 52.27 | 66.66 |
| SAND (> 63 µm) | [%] | 26.29 | 26.49 | 25.28 | 42.55 | 25.87 |




**Test method specification: CZ_SOP_D06_07_120** Grain size analysis using the wet sieve analysis using laser diffraction (fraction from 2 µm to 63 mm) Fraction > 0.063 mm determined by wet sieving method, other fractions determined from the fraction "< 0.063mm" by laser particle size analyzer using liquid dispersion mode. Fractions "**Sand >63 µm**", "**Silt 2-63 µm**" and "**Clay <2 µm**" evaluated from measured data. DUPL= duplicite analyse.

**Test specification, deviations, additions to or exclusions from the test specification:**

Page: 3 / 4

276

**Figure A-3:** Soil texture analysis from *ALS laboratory Group* of the soil from Horten inner harbour: The Control substrate.

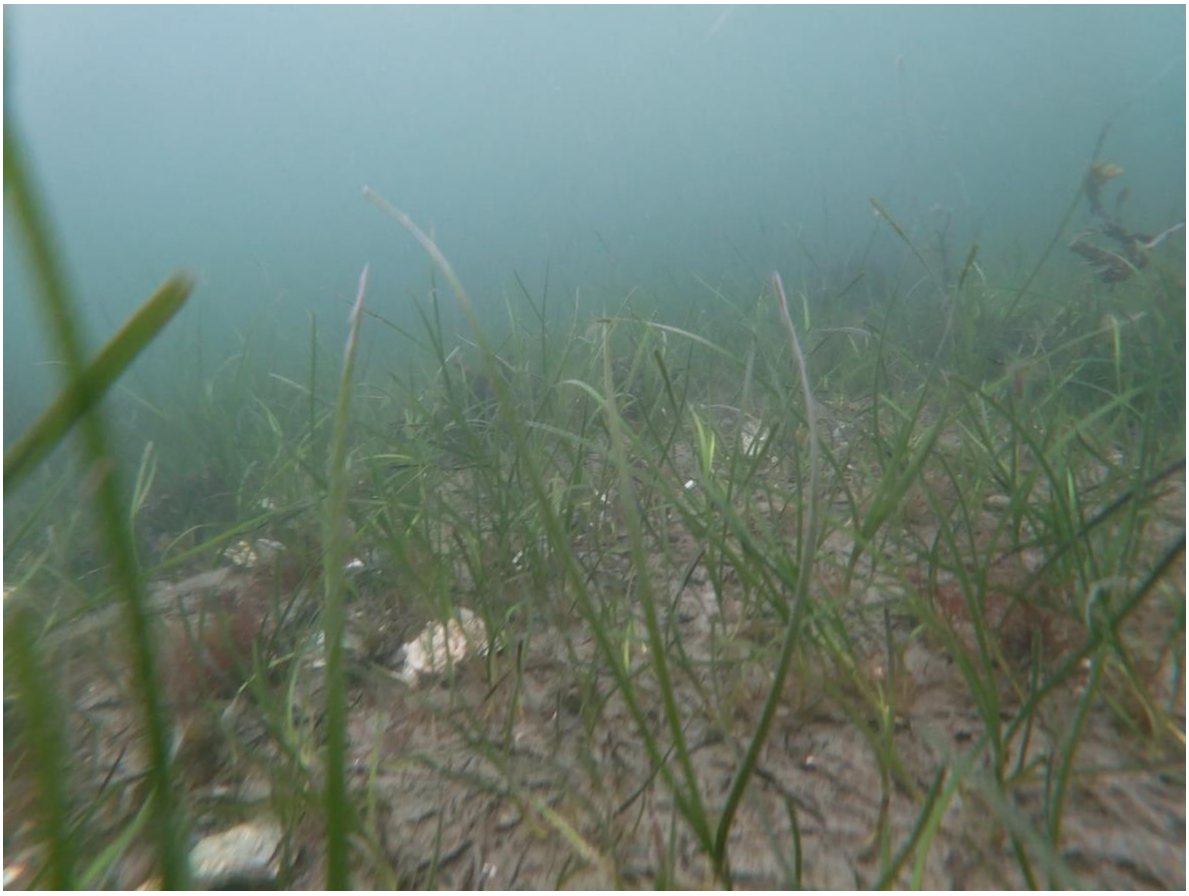


**Figure A-4:** Photo of the eelgrass meadow at the donor site.

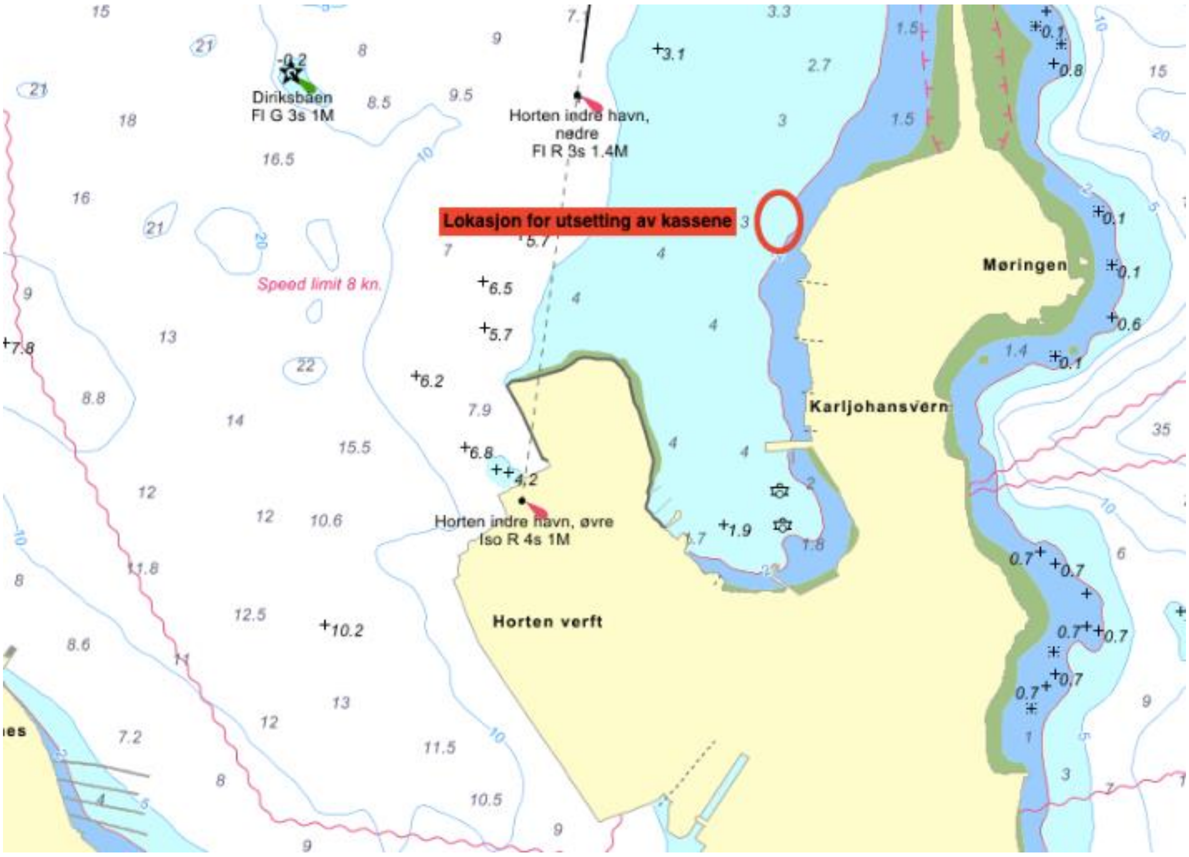



**Figure A-5:** Location of the plots at the experiment site, outside the peninsula Vealøs.

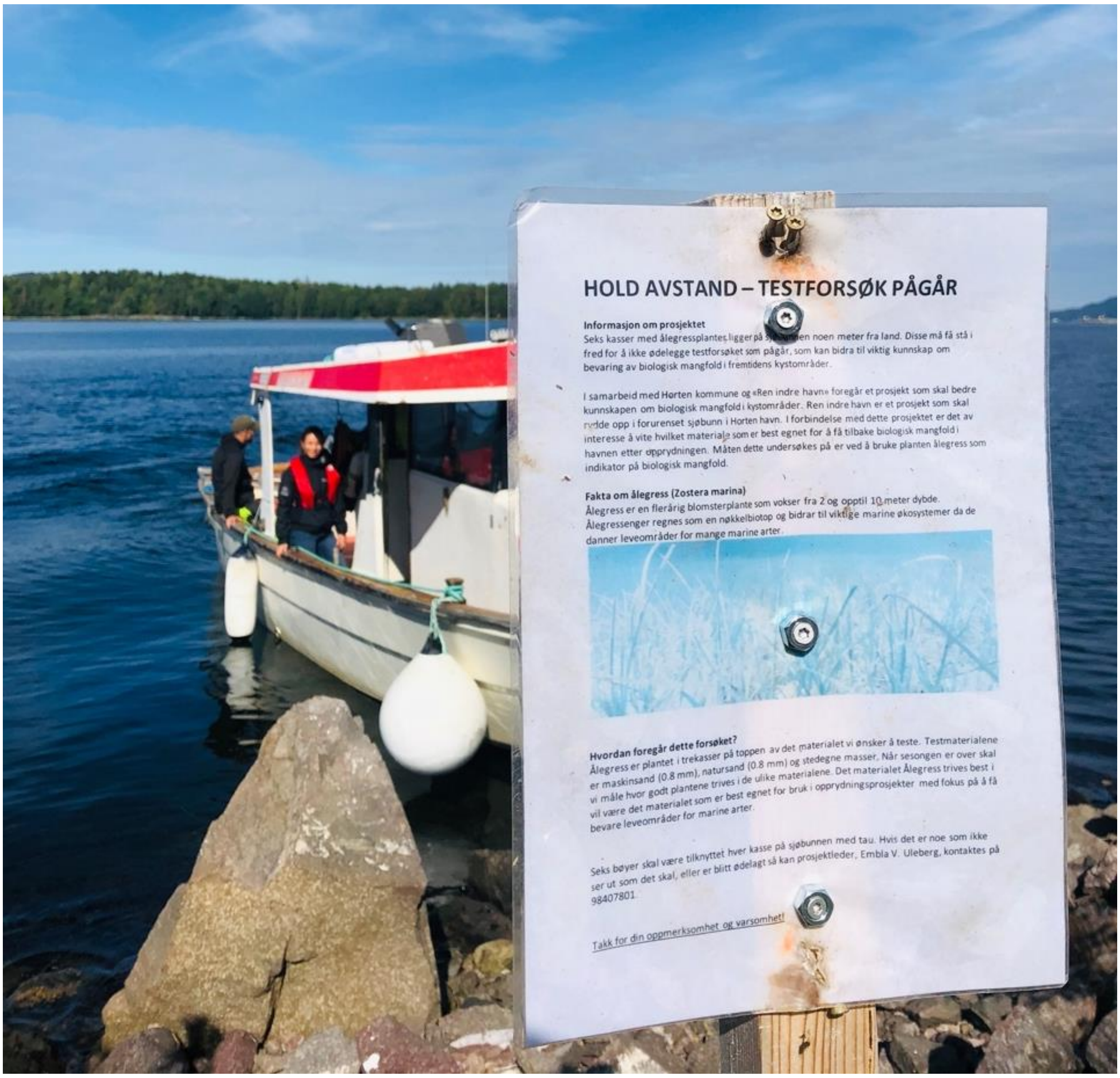


**Figure A-6:** The poster on shore with information about the experiment.

herunder avfallsforskriften[5]. Farlig avfall som kan identifiseres og sorteres ut skal leveres til godkjent mottak.

# 9 Utslippskontroll

## 9.1 Måleprogram

Det skal gjennomføres spredningskontroll av mudringen, deponeringen og tildekkingen i henhold til et måleprogram, utformet i tråd med gjeldende retningslinjer (M-350, TA-2624). Turbiditet skal måles i henhold til norsk standard (NS 9433:2017). Måleprogrammet skal inngå i virksomhetens internkontroll og en miljørisikovurdering skal ligge til grunn for programmet. Kontrollen skal være tilstrekkelig til å avdekke eventuell spredning av forurensning i forbindelse med gjennomføring av tiltakene. Resultatene fra kontrollen skal dokumenteres. Loggføring skal som et minimum inneholde måleresultat, eventuelle overskridelser og korrigerende tiltak. Endringer av måleprogrammet skal oversendes Fylkesmannen.

Under anleggsperioden skal det måles turbiditet ved et tilstrekkelig antall stasjoner til å gi et representativt bilde på partikkelspredning ut av områdene og for å beskytte sensitive områder mot nedslamming. Målerne skal være plassert slik i forhold til dyp og strømretning, at de på best mulig måte fanger opp spredning av forurensede partikler ut av tiltaksområdet og/eller inn i sårbare områder. Turbiditeten skal måles kontinuerlig så lenge arbeidet pågår. Det skal også benyttes referansestasjoner og/eller bakgrunnsmålinger som er representative for normal turbiditet i områdene.

Følgende alarmgrenser skal benyttes ved mudring:

- i tiltaksområder: 15 NTU over referansenivå i 1 time
- utenfor tiltaksområder: 15 NTU over referansenivå i 30 minutter

Følgende alarmgrenser skal benyttes ved tildekking:

- utenfor tiltaksområder: 20 NTU over referansenivå i 4 timer

Følgende alarmgrenser skal benyttes ved deponering av forurensede masser i sjødeponi:

- 15 NTU over referansenivå i 30 minutter

Overskridelse av alarmgrensene skal medføre at arbeidene stanses, årsaksforholdene avklares og nødvendige avbøtende tiltak gjennomføres. Også innenfor de fastsatte tidsrammene plikter tiltakshaver å redusere spredningen så langt dette er mulig uten urimelige kostnader. Dersom overskridelser skyldes arbeidene, skal tiltakshaver dokumentere miljøgiftinnholdet i spredt materiale og arbeidene kan ikke starte opp igjen før turbiditeten er nede på stabile nivåer under grenseverdien. Rutiner og eventuelle tiltak skal beskrives i internkontrollen. Tidspunkt, årsak og varighet av eventuelle hendelser skal loggføres.

Under anleggsperioden skal det benyttes sedimentfeller og passive prøvetakere ved et tilstrekkelig antall stasjoner til å gi et representativt bilde på spredning av forurensning ut av tiltaksområdet. Målerne skal være plassert slik at de på best mulig måte fanger opp spredning av forurensning ut av tiltaksområdet. Det skal utføres kjemisk analyse av prøvene med en frekvens som angitt i måleprogrammet vedlagt søknaden.

---

[5] Forskrift om gjenvinning og behandling av avfall (avfallforskiften) av 01.06.2004, nr. 930

**Tillatelse nr**. 2019.0462.T Side 9 av 12
Tillatelse gitt: 28.05.2019

**Figure A-7:** Terms about monitoring of turbidity in the permit from FMVT.

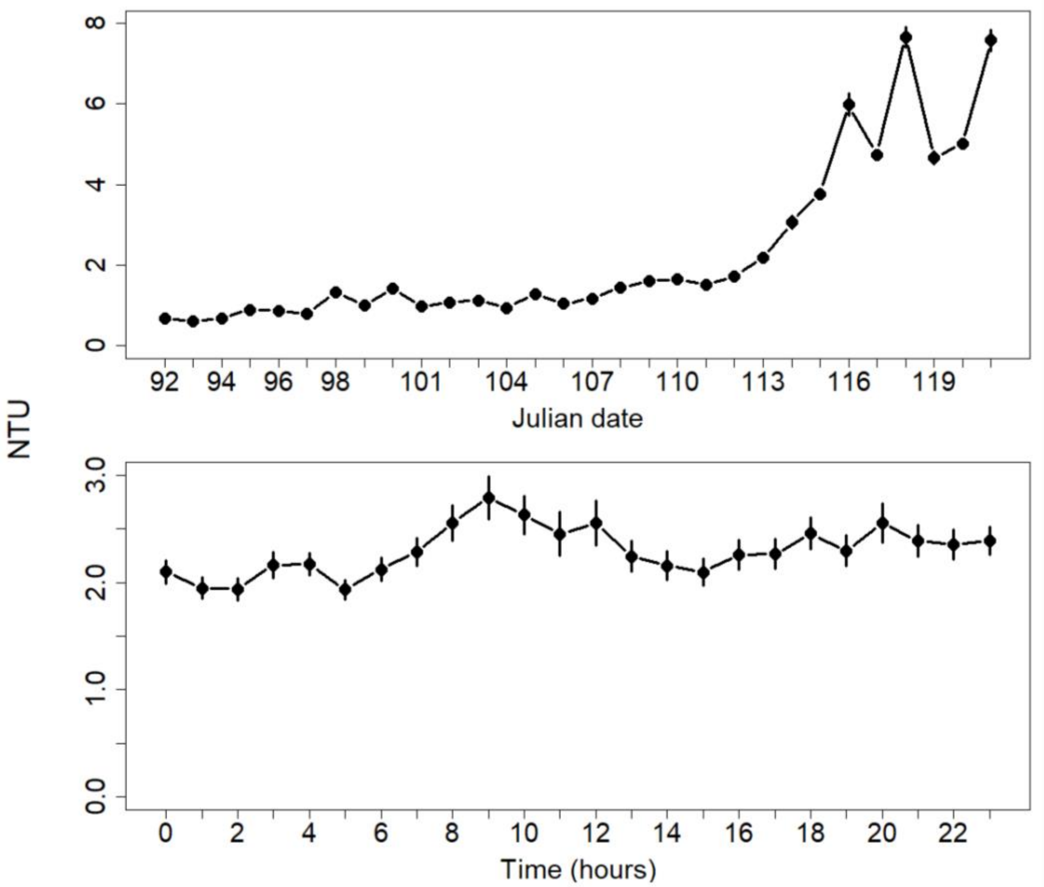



**Figure A-8:** Trend in turbidity from April 2020 expressed as NTU and presented in Julian date. The above figure shows the trend in NTU every day during April month, and the below figure shows the average NTU value of every hour.

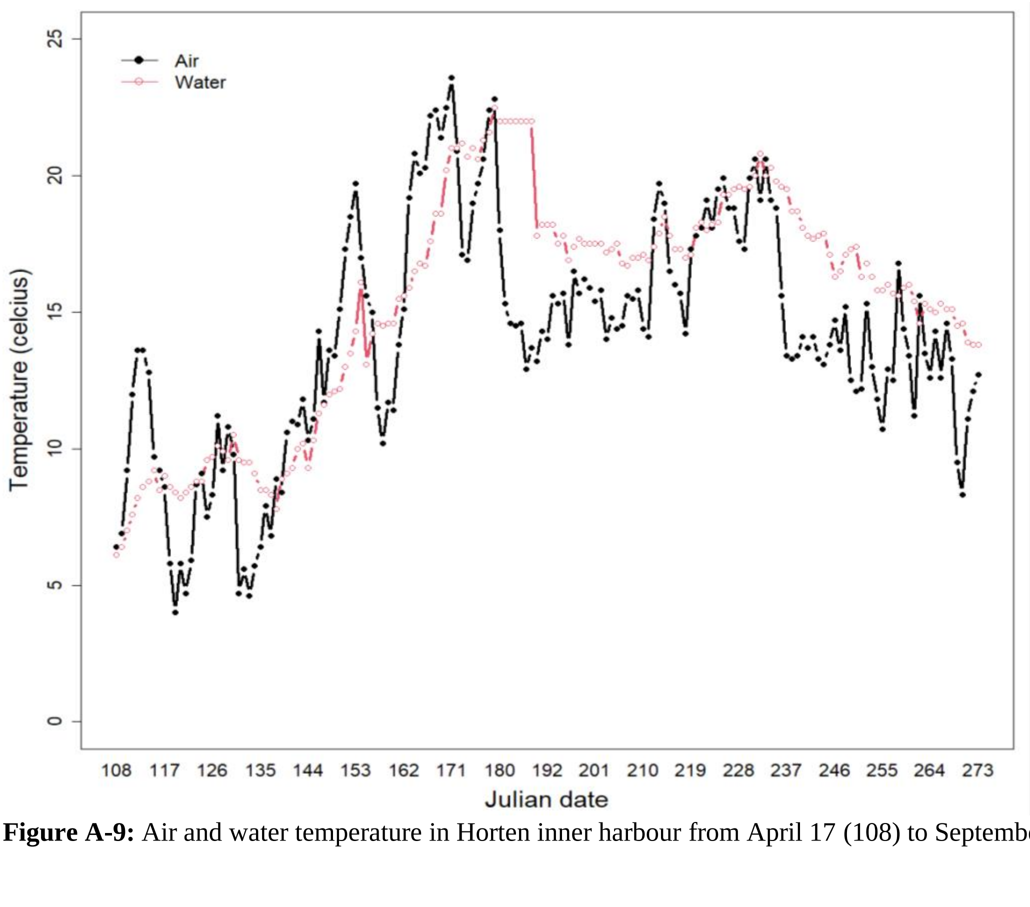



**Figure A-9:** Air and water temperature in Horten inner harbour from April 17 (108) to September 29 (273)

**From:** Holth, Tor Fredrik <fmvetfh@fylkesmannen.no>
**Sent:** mandag 27. januar 2020 09:13
**To:** Ingvild Fladvad Størdal <Ingvild.stordal@ngi.no>
**Subject:** SV: Masteroppgave NINA - Ålegressenger

**Ang. flytting av 1-5 m3 forurenset sediment i Horten Indre havn – MSc-oppgave ålegressenger**

Fylkesmannen har vurdert om tiltaket omfattes av forurensningsforskriften kapittel 22 *Mudring og dumping i sjø og vassdrag* og fordrer en tillatelse. Vi mener den beskrevne innsamlingen av materiale, og formålet med den, er av et omfang og hensikt som kan betegnes som en normal aktivitet i sjø og dermed ikke omfattes av forskriftens forbud, jf. § 22-2 bokstav d. Det må derfor gjøres en vurdering av tiltaket etter forurensningsloven.

Vår vurdering etter forurensningsloven omfatter både innsamling og utplassering av sediment: Fylkesmannen har vurdert tiltaket til å være av et omfang som ikke utgjør noen risiko for å kunne medføre nevneverdig forurensningsmessig skade eller ulempe, jf. forurensningsloven § 8, 3. ledd. Tiltaket kan dermed finne sted uten tillatelse etter forurensningsloven § 11. Vurderingen etter forurensningsloven § 8, 3. ledd, er å anse som en lovanvendelse, og ikke et enkeltvedtak som kan påklages.

Det kreves heller ikke særskilte tillatelser for flytting av ålegress i Horten Indre havn etter regelverk vi forvalter, men det kan være lurt også å avklare med Horten kommune.

Lykke til med prosjektet, vi ser frem til interessante resultater!

Med vennlig hilsen
**Tor Fredrik Holth**
senioringeniør
Fylkesmannen i Vestfold og Telemark
Telefon: 33 37 11 92
E-post: fmvetfh@fylkesmannen.no
Web: www.fylkesmannen.no/vt

**Figure A-10:** Permission from FMVT, Tor Fredrik Holth, to move eelgrass from the donor site

**Table B-1:** Table of quality elements in biological parameters in coastal waters (Directive, 2018)

**Tabell 9.1** Oversikt over kvalitetselementer, parametre og indekser i klassifiseringssystemet for kystvann.

| | Biologiske kvalitetselementer | | | | Kjemiske- og fysisk-kjemiske kvalitets-elementer som støtter de biologiske elementene. | | | Støtteparametre i sedimenter | | Hydro-morfologiske Kvalitets-elementer som støtter de biologiske kvalitets-elementene |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plante-plankton | Makro-alger | Ålegress | Bløtbunns-fauna | Fysiske | Nærings-salter | Vannregion-spesifikke stoff | Organisk innhold | Korn-fordeling | |
| Para-meter/ indeks | Klorofyll a | Nedre vokse-grense: MSMDI<br>Fjære-samfunn: RSLA, RSL | Nedre vokse-grense, tetthet og mengde begroings-alger | Artsmangfold: ES100, H'<br>Ømfintlighet: ISI2012 og NSi<br>Sammensatt indeks: NQI1 | Siktdyp<br>Tempera-tur<br>Salinitet<br>Oksygen | Nitrat + nitritt, Fosfat, Total fosfor<br>Total nitrogen, Ammonium | Grense-verdier for stoffer utover de priorit-erte. Se forøvrig kap. 11. | TOC og evt. glødetap | Sedi-ment-fraksjon | % påvirkning av substrat<br>Dyp<br>Struktur og substrat av kystsone<br>Struktur av tidevanns-sone Strøm og eksponer-ing |

**Table B-2:** Registration of result data (number and height) on September 29.

| Date | Plot | Substrate | Number | Height | Comments |
|---|---|---|---|---|---|
| 29.09 | NT1 | Natural | Not registered | Not registered | Higher plant density (survival) of patches compared to single plants |
| 29.09 | NE1 | Natural | 1 | 80 | High plant mortality<br>The plot has moved from August to September |
| 29.09 | NT2 | Natural | Not registered | Not registered | Higher plant density (survival) of patches compared to single plants |
| 29.09 | NE2 | Natural | 4 | 45, 40, 35 | High plant mortality |
| 29.09 | MT1 | Machine | Not registered | Not registered | Higher plant density (survival) of patches compared to single plants |
| 29.09 | ME1 | Machine | 4 | 16, 15, 9 | High plant mortality<br>Pits in the sand (due to the storm?) |
| 29.09 | MT2 | Machine | Not registered | Not registered | Higher plant density (survival) of patches compared to single plants |
| 29.09 | ME2 | Machine | 0 | 0 | High plant mortality |
| 29.09 | KT1 | Control | Not registered | Not registered | Higher plant density (survival) of patches compared to single plants |
| 29.09 | KE1 | Control | 8 | 60, 40, 50, 50, 50 | Less plant mortality |
| 29.09 | KT2 | Control | Not registered | Not registered | Higher plant density (survival) of patches compared to single plants |
| 29.09 | KE2 | Control | 16 | 100, 80, 60, 70, 25 | Less plant mortality |

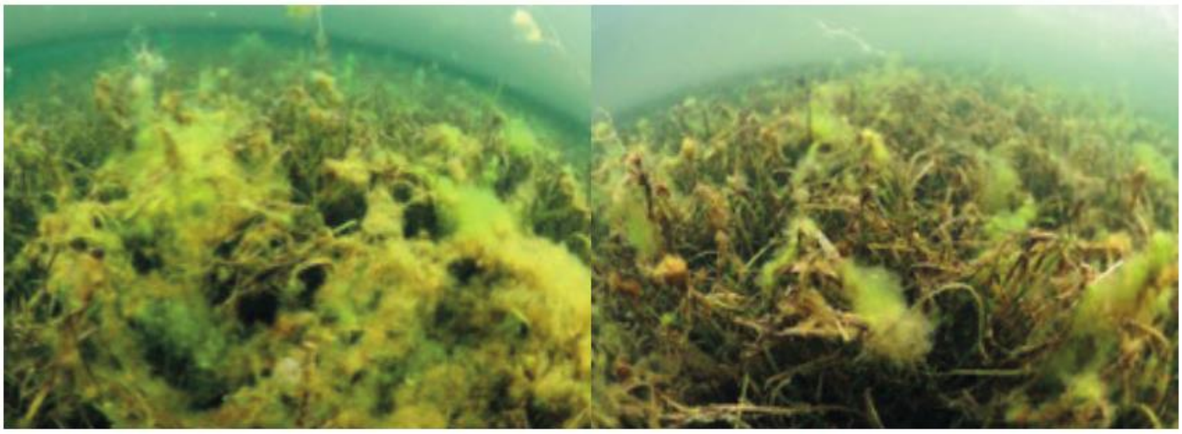


**Figure A-11:** Photos of algae growth in eelgrass from the Guideline 02:2018 (Directive, 2018). On the left: dominant occurrence of algae coverage (>50%). On the right: normal occurrence of algae coverage (15%-50%).

**Table B-3:** Score value for the amount of algae growth on eelgrass by substrate

| **Date** | **Substrate** | **Score*** |
|---|---|---|
| 29.09 | Natural 1 | 1 |
| 29.09 | Natural 2 | 1 |
| 29.09 | Machine 1 | 1 |
| 29.09 | Machine 2 | 1 |
| 29.09 | Control 1 | 1 |
| 29.09 | Control 2 | 1 |

*1 = low amount of algae growth, 2 = scattered occurrence (coverage <15%) 3 = normal occurrence (coverage 15% - 50%) 4 = dominating occurrence (coverage >50%) (Directive, 2018)

**Norges miljø- og biovitenskapelige universitet**
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway