DIS-method Instruction manual

the fieldwork company

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Introduction

This manual describes the Dispenser Injection Seeding (DIS) method, an effective and science-based method for seed-based restoration of intertidal *Zostera marina* (eelgrass) in the Dutch Wadden Sea. It is based on multi-year research by the Fieldwork Company and the University of Groningen, and the result of an iterative approach to develop a method that results in high plant emergence rates in the field that is suitable for scaling up seagrass restoration.

For the Dutch Wadden Sea specifically, research showed that the optimal seeding strategy is 25 injections per m^2. Plants emerge when seeding at a depth between 2 and 4 cm into the sediment, although a seeding depth of 4 cm yields significantly more plants. However, the optimal seeding strategy will differ between locations, as it depends on the abiotic and biotic conditions of the system in which you work. Ideally, one injection yields one plant, hence the number of seeds per injection is between 3 and 5 seeds, depending on seed viability.

Seed-based seagrass restoration – the process

Seedbearing *Z. marina* shoots are collected from a donor population in the Wadden Sea when most seeds are ripe. A donor population is selected based on the quality and density of the meadow. No more than 1% of the present seagrass is collected from the donor meadow to ensure the collection is sustainable and the impact on the donor meadow is minimal.

After collection, the donor material is processed in aerated saltwater tanks with the same salinity as the donor meadow. 4-mm sieves are installed in the bottom half of the processing tanks, which helps to separate the raw plant material from the seeds. During the following 6-8 weeks of processing, the seagrass spathes will decay, releasing the negatively-buoyant seeds; the seeds subsequently fall through the 4-mm sieve onto the floor of the processing tanks. Regular water changes are conducted throughout the processing period. Once the spathes are all devoid of seeds, the seeds and other plant material that have fallen through the sieve are collected, after which the seeds are cleaned from dead organic matter and mudsnails (*Peringia ulvae*) based on seed size and density. The seed cleaning methodology is updated and improved on an annual basis.

Cleaned seeds are kept in controlled storage facilities to reduce winter losses, as seeding in the autumn or winter can result in excessive seed burial or seed wash-out due to winter storms in the Wadden Sea. To keep seeds healthy whilst preventing germination, seeds are stored in highly saline water (45-50 ppt), at low temperature (c. 5°C), in complete darkness. Seeds are spread out in a layer that is one seed thick (i.e. no stacking of seeds) and water is constantly circulated over the seeds to prevent stagnant water.

During storage, the seeds are continuously treated against *(Halo)Phytophthora* with a 0.5-ppm copper sulphate ($CuSO_4$) solution. The temperature, salinity, and concentration of copper in the system are monitored during storage. To minimise impacts on health and the environment, apply appropriate safety precautions and measures to use and dispose of $CuSO_4$ as per local regulations – the material safety data sheet can be found on the web.

To determine seed quality, the seed viability is determined by counting ripe and unripe seeds in storage followed by germination tests.

Seeding generally takes place at the end of March to reduce winter losses as described above, after which regular monitoring takes place in the field to determine the success of the seeding campaign.

DIS-method: caulking gun adjustments

Materials

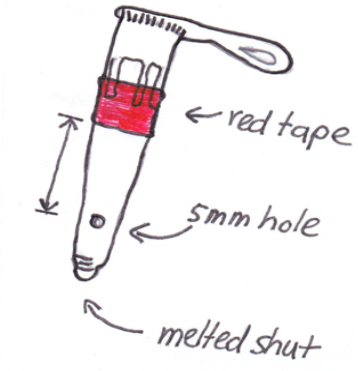
- Caulking gun
- Caulking tubes
- Nozzles
- Red tape
- Drill
- Plier-type rivet gun
- Blind rivet nut
- Bolt and nut

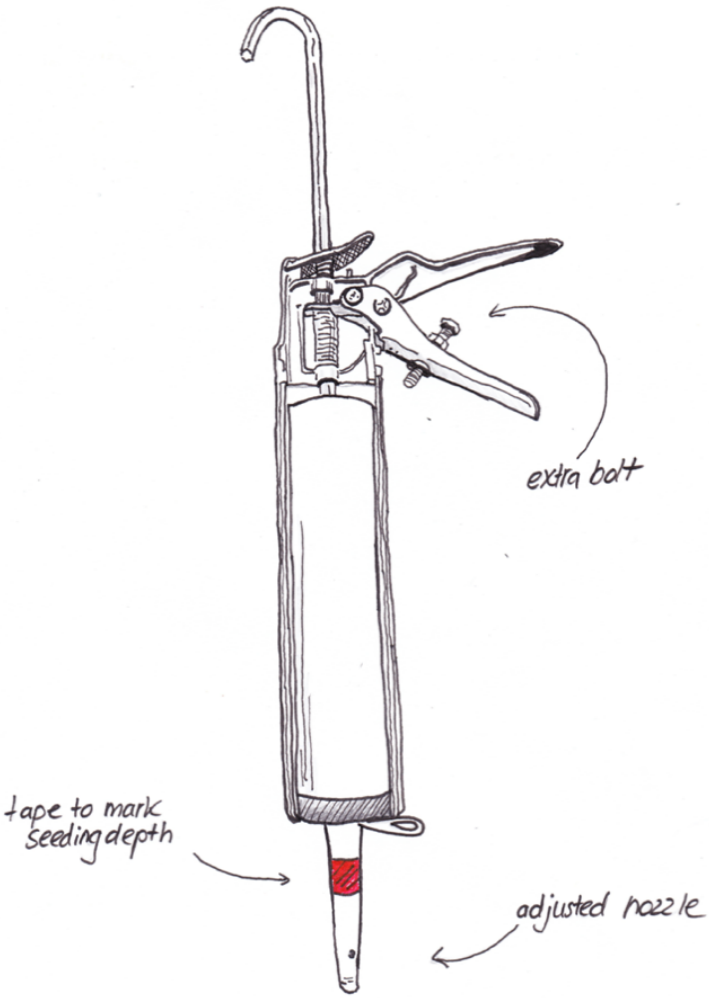
Adjusted nozzle

A nozzle with a standard open tip at the end will become clogged when injecting the seed-mud mixture into the sediment, causing seeds to be spilled on top of the sediment when retracting the nozzle from the sediment. This can be avoided by closing the opening of the nozzle tip and drilling a new 5-mm hole on the side of the nozzle. The adjusted nozzle creates a vacuum in the sediment opening which keeps the injected material in the sediment after injection. To mark the intended seeding depth, we apply a piece of red tape.

Adjustments to a regular nozzle are:

- Nozzle tip is melted shut
- 5-mm hole on the side
- Tape at a fixed distance from the centre of the hole, dependent on your intended seeding depth.





Adjusted caulking gun

An extra bolt on the handle is added to be able to adjust the amount of seed-mud mixture injected into the sediment. It is a bolt screwed through a blind rivet nut. With the extra nut on the bolt, the amount of seed-mud mixture per injection can be adjusted. The adjustment depends on the required number of seeds per injection. See ‘Calibrating injections’ for this procedure.

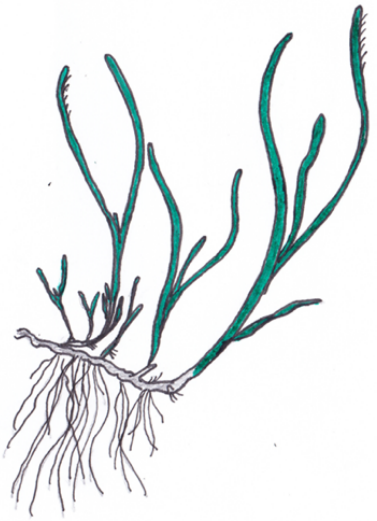
Caulking gun type

At The Fieldwork Company, we use a standard caulking gun with a metal frame, 310 mL tubes and universal 115 mL plastic nozzles.

Seed-mud mixture

Materials required

- Sediment (local clay/silt)
- Saltwater (local salinity)
- Buckets
- Whisks & scoops
- Freshwater shocked seeds
- Measuring cup



Instructions

The seed-mud mixture is prepared two days before seeding. For intertidal seagrass restoration in the Wadden Sea, we use locally collected mudflat sediment (median grain size of 24 µm, 15% OM – hereafter referred to as mud) which is sieved with a 2-mm mesh size before mixing with seeds. Sieving the mud is necessary to remove benthic animals and large particles, and to create a homogenous mixture for the proper functioning of the DIS caulking gun. The mud should consist of fine muddy clay with a low percentage of sand, having the consistency of Greek yoghurt before adding any seeds. You can test whether the mud has the correct consistency by taking a scoop: the mud should stick to the scoop, and not drip or fall off. Add saltwater if the mud is too dry; if the mud is too wet, reduce water content by leaving the mud overnight in a sieve with a 1-mm mesh to release moisture. To prevent anoxia, do not store mud for more than 5 days before mixing it with the seeds. Store the mud in a cold environment without placing a lid on the buckets.

If local mud cannot be collected (e.g. due to local biosecurity regulations or a lack of mud available in the area), you can instead use specific clay powder types. When mixed with water in the correct ratio, this creates suitable mud with the right consistency in which seagrass seeds can germinate. Contact us if you need additional information on this and to learn about the specific clay powder types that have been tested for seagrass seeds in the lab and the field.

To obtain the right seed density in the seed-mux mixture, we add a set amount of seeds to a set amount of sediment. The volume of *Z. marina* seeds added to the mixture depends on seed quality which varies between years.

To calculate the total required volume of seed-mud mixture, the ideal seeding density (injections/m^2) is multiplied by the total seeding area (m^2) to obtain the total number of injections needed. This is subsequently multiplied by the injection volume. For instance, if the seeding density is 25 injections / m^2 and the seeding area is 100 m^2, the total number of injections required is 2500 injections. If one injection has a volume of 2 mL, then you require 2500*2 = 5000 mL of mud. We usually prepare 10% more mixture than required for the work to compensate for any unexpected losses in the field.

Once seeds are added to the mix, we gently mix the seeds into the mud with bare hands or whisks to prevent damaging the seeds while mixing, and preferably store the seed-mud mixture in cooled conditions before seeding.

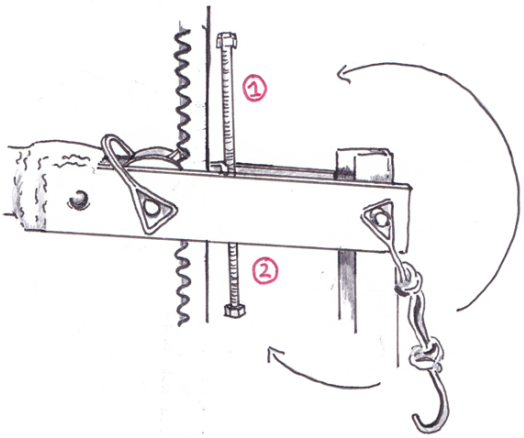
(Re)filling & calibration

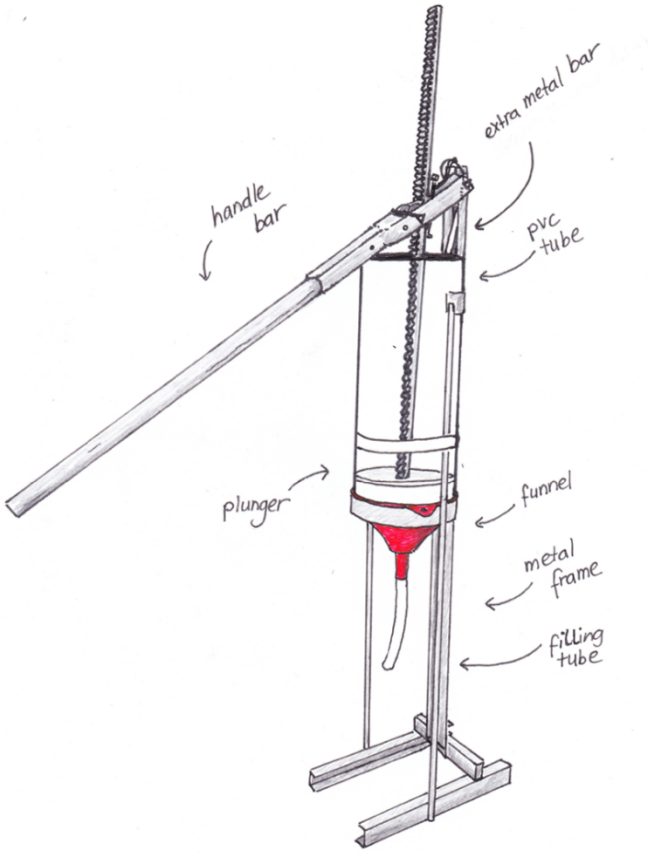
Materials

- Buckets with seed-mud mixture
- Filling machine
- Shovels or scoops
- Caulking tubes with endcaps
- Crates
- 0.5 mm sieve
- Saltwater (local salinity)
- Rinsing brushes
- Metal bars
- Cooler box

Instruction

For filling up many (>200) caulking tubes, we use a custom-made filing machine. This filling machine consists of a 200-mm wide PVC tube connected to a funnel and a tube, which are fastened to a metal bar frame. A plunger pushes the mixture through the PVC tube into the caulking tubes. The handle on the filling machine works both ways: (1) for pushing down and (2) pulling the plunger up by attaching the hook (see figure below). The filling tube helps to fill the caulking tubes from the bottom to the top. It is important to fill the tubes with 300 mL seed-mud mixture without air bubbles.





After loading the caulking tubes, we store and transport them in crates under cooled conditions. Before refilling empty caulking tubes, we use a metal bar to push back the endcap in the caulking tubes and clean the caulking tubes with local seawater and rinsing brushes.

Calibrating injections

We calibrate the caulking guns to 200 injections per caulking tube by adjusting the nut. As the caulking tube contains 300 mL of seed-mud mixture, this results in 1.5 mL per injection containing a set average number of seeds per injection. The seed density per injection can be checked by injecting into a sieve with a mesh size of $\leq$ 0.5 mm, rinsing the mixture with saltwater, and counting the seeds that are present in the sieve. Repeat this procedure several times per caulking gun during calibration until the required number of seeds per injection is reached.

Seeding

Field equipment

- Crates with caulking tubes and endcaps
- DIS caulking guns & adjusted nozzles
- Spare nozzles
- Spare bolts for the DIS caulking guns
- Red tape
- 1-m^2 metal frames (10 cm x 10 cm grid)
- Marking poles
- Hammers & drills
- Labels
- Measuring tape or laser gun
- Fieldwork-proof trolley
- GPS or dGPS
- Handheld transceivers for communication
- Tidal chart
- Waders
- Gloves
- Raincoat
- Safety and emergency equipment

Instructions

Seeding takes place during low tide. Before seeding starts, mark your seeding plot with marked poles and GPS, measuring tape, hammers, and drills as needed. Use a 1-m frame to guide your seeding work and start in the upper left corner of the frame. Press the nozzle into the sediment at a slight angle and use the red tape to inject to the right depth. Make sure the 5-mm hole in the nozzle is directed towards your body. Check when withdrawing that the injected seed-mud mixture stays in the sediment. Count the number of injections you make with one caulking tube ensure the nozzle is not clogged, and that the mixture is injected correctly.

Once a 1-m^2 area has been seeded, flip the frame towards you and repeat the procedure, working in a backwards manner so that you do not walk on seeded areas. Repeat the procedure until the total intended area has been seeded.

References

Govers LL, Heusinkveld JHT, Gräfnings MLE, Smeele Q, van der Heide T (2022) Adaptive intertidal seed-based seagrass restoration in the Dutch Wadden Sea. PLoS ONE 17(2): e0262845

Govers LL, van der Zee EM, Meffert JP, van Rijswick PCJ, Man in 't Veld WA, Heusinkveld JHT, van der Heide T (2017) Copper treatment during storage reduces *Phytophthora* and *Halophytophthora* infection of *Zostera marina* seeds used for restoration. Scientific Reports 7, 43172; doi: 10.1038/srep43172

Gräfnings MLE, Heusinkveld JHT, Hoeijmakers DJJ, Smeele Q, Wiersema H, Zwarts M, van der Heide T Govers LL (2022) Optimizing seed injection as a seagrass restoration method. Restoration Ecology dec 2022; doi 10.111/rec.13851

Safety & precautions

Engage with local authorities and stakeholders regarding potential permit requirements in your restoration area before starting any of the fieldwork described above.

Before planning your seeding campaign, ensure you have checked the tide chart to plan starting and ending time of seeding and check local weather conditions.

Wear protective clothing and take proper safety measures when working in the field.

Make sure to always keep an eye on your surroundings, your co-workers and volunteers, respect local wildlife and leave nothing and nobody behind.

Disclaimer

The optimal seeding strategy with the DIS method varies between locations due to differences in biotic and abiotic conditions. The specific seeding strategy that works in the Dutch Wadden Sea may not be the optimal seeding strategy for your system. Hence, to identify the optimal seeding strategy for your system, systematic testing of the injection density, injection depth, and number of seeds per injection should be conducted.

More information

Contact The Fieldwork Company if you need more information or advice on seagrass collecting, processing, seed cleaning, storing seeds and treating them with copper sulphate, seeding in the field and monitoring.

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